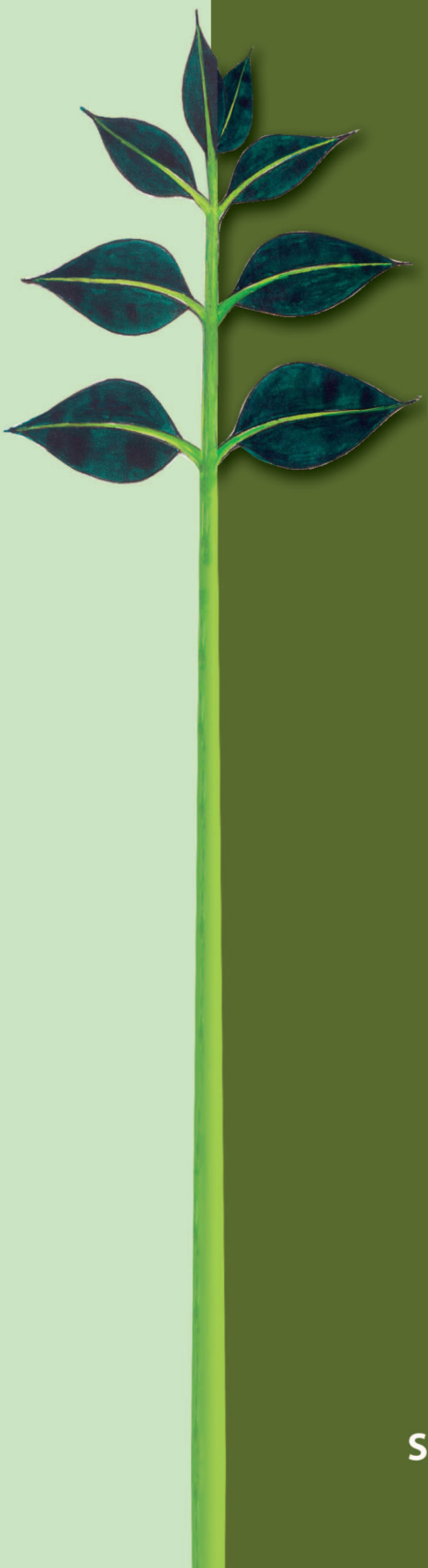
Vol. 15 Issue 2 December 2017

ISSN: 1682-8348 (Print), 2312-8038 (Online)



SAARC Journal of Agriculture

SAARC Agriculture Centre

www.sac.org.bd

SAARC JOURNAL OF AGRICULTURE (SJA)

Volume 15, Issue 2, 2017
ISSN: 1682-8348 (Print), 2312-8038 (Online)

Published by

SAARC Agriculture Centre (SAC)
BARC Complex, Farmgate, Dhaka-1215, Bangladesh
Phone: 880-2-8141665, 8141140; Fax: 880-2-9124596
E-mail: saarcjournal@yahoo.com, Website: http://www.banglajol.info/index.php/SJA/index

Printed at
Natundhara Printing Press, 277/3, Elephant Road, Dhaka-1205, Bangladesh
Cell: 01711019691, 01911294855, Email: natundhara2014@gmail.com

ISSN: 1682-8348 (Print), 2312-8038 (Online)

SAARC JOURNAL OF AGRICULTURE

VOLUME 15 ISSUE 2 DECEMBER 2017

SAARC

SAARC Agriculture Centre

www.sac.org.bd

Dr. Bhim Bahadur Khatri
Senior Scientist and Coordinator
National Potato Research Programme
Nepal Agricultural Research Council
Khumaltar, Lalitpur, Nepal

Dr. Muhammad Musa
Deputy Director (Research)
Directorate of Director General of Agricultural Research
Ayub Agricultural Research Institute
Jhang Road, Faisalabad-38800, Pakistan

Dr. Badar Naseem Siddiqui
Department of Agriculture Extension & Communication
PMAS-Arid Agricultural University
Rawalpindi, Pakistan

Dr. WART Wickramaarachchi
Senior Program Specialist (PS &PD)
SAARC Agriculture Centre
Dhaka, Bangladesh

Dr. P.B. Dharmasena
National Consultant
Food and Agriculture Organization
Sri Lanka

CONTENTS

SAARC J. Agri., 15(2): 1-18 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35155*

RESTRICTION ENDONUCLEASE ANALYSIS OF THE GENOMES OF DIFFERENT ISOLATES OF CHICKEN ANEMIA VIRUS AMPLIFIED BY POLYMERASE CHAIN REACTION

S.M.Z.H. Chowdhury[1*], A.R. Omar[2], A. Ideris[2], H. Bejo[2] and A.A. Jamaluddin[3]

[1]Livestock Division, Bangladesh Agricultural Research Council, Farmgate, Dhaka-1215, Bangladesh
[2]Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia

ABSTRACT

Four DNA fragments (fragments A, B, C and D) covering the whole genome of chicken anemia virus (CAV) were amplified enzymatically by polymerase chain reaction (PCR) using four pairs of oligonucleotide primers. The DNA fragments were amplified from each of nine CAV isolates including eight Malaysian isolates and one European isolate (Cux-1). For all nine CAV isolates, fragment A (1518 bp) was digested with one restriction enzyme, *Eco*130I (*Sty*I); fragment B (926 bp) with three enzymes, *Eco*130I (*Sty*I), *Hpa*II and *Mbo*I separately; fragment C (675 bp) with also three enzymes, *Bsu*RI (*Hae*III), *Hin*fI, and *Hpa*II separately; and the fragment D (552 bp) with one enzyme, *Eco*RI. Enzyme digested products of different fragments were separated by agarose gel or polyacrylamide gel electrophoresis. Each of the eight-enzymatic reactions differentiated at least two isolates except the *Hpa*II digestion of fragment C where no isolate was distinguished. The overall restriction endonuclease (RE) analysis separated four isolates (BL-1, BL-2, BL-4 and BL-5) in one group and the rest five isolates (SMSC-1, SMSC-2, 3-1, BL-3 and Cux-1) were differentiated from each other and also from the group of four isolates, based on the number of restriction site differences and the fragments generated by different enzymatic digestions. The study revealed that RE analysis could be used to identify and differentiate CAV isolates based on the number of restriction site differences. The study showed that more isolates, even the isolates from the same poultry farm, could be differentiated with proper genomic diversity after RE analysis of more genome fragments compared to that of single genome fragment.

Keywords: Chicken anemia virus; polymerase chain reaction; restriction endonuclease analysis.

[*] Corresponding author e-mail: ziqrul06@yahoo.com

Received: 28.08.2017 *Accepted: 20.11.2017*

INTRODUCTION

Chicken anaemia virus (CAV) is an economically important avian pathogen with a worldwide distribution (AboElkhair et al., 2014; De Herdt et al., 2001; Farkas et al., 1998, Ganar et al., 2017; Ledesma et al., 2001; McNulty 1991; Olszewska-Tomczyk et al., 2016; Rehman et al., 2011; Stanislawek and Howell, 1994; Zhou et al., 1996). The virus causes aplastic anaemia and generalised lymphoid atrophy with a concomitant immune suppression characterised by secondary bacterial, viral infections or vaccination failures (Adair, 2000; Bülow et al., 1983; Engstrom and Luthman 1984; Rosenberger and Cloud 1989; Schat, 2003). The mortality is usually between 5% and 10%, but up to 60% has been recorded (Coombes and Crawford 1996; McNulty 1991). The virus is small, non-enveloped, spherical, 23 to 26 nm in diameter, containing a circular single-stranded DNA genome of 2.3 kb (Coombes and Crawford 1996; Li and Cui, 2007; McNulty 1991; Noteborn et al., 1991; Pope 1991; Zhang et al., 2012).

CAV is a member of genus *Gyrovirus* in the family *Anelloviridae* (Breitbart, 2015). The CAV isolates originating from different parts of the world belong to the same serotype and produce the same pathogenic effects in experimentally inoculated chicks (McNulty 1991). Later a study proposed a possible second serotype (Spackman, et al., 2002a,b). However, studies have been carried out to characterize the virus based on its DNA sequence (Noteborn et al., 1992; Todd et al., 1992; Tham and Stanislawek, 1992). Both Southern analysis and restriction mapping showed only minor differences on the CAV genomes of the field isolates from United States (Noteborn et al., 1992). However, Todd et al. (1992) differentiated CAV isolates by restriction endonuclease (RE) analysis of PCR-amplified DNA. They used three enzymes (*Hae*III, *Hinf*I, and *Hpa*II) for digestion of only one 675 bp PCR-amplified fragment from different CAV genomes. Genetic variations of different CAV isolates were also detected by other workers using RE analysis of PCR-amplified CAV genome fragments (Oluwayelu et al., 2005; Nayabian and Mardani, 2013; van Santen et al., 2001). Both type specific and common DNA sequences can be detected among various CAV isolates. However, analysis of more genome fragments with different restriction enzymes can differentiate different CAV isolates more appropriately than that of single genome fragment and this information could be important in epidemiological point of view. In this paper, we investigated for the first time the molecular differences of the genomes of different CAV isolates by restriction endonuclease analysis of PCR-amplified DNA fragments covering the whole CAV genome.

MATERIALS AND METHODS

Viruses

Five CAV isolates, BL-1, BL-2, BL-3, BL-4, and BL-5, isolated recently at University Putra Malaysia (UPM); three CAV isolates, SMSC-1, SMSC-2 and 3-1, isolated at Veterinary Research Institute (VRI), Malaysia, and an European CAV

isolate Cux-1, kindly provided by Dr. K.A. Schat, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, were used for the study.

Cells and cell culture

MDCC-MSB1 cells, a cell line derived from a Marek's disease lymphoma, were kindly provided by the Director, VRI, Ipoh, Perak, Malaysia. The cell culture was maintained following the methods described by Chowdhury et al. (2002). For each isolate, two ml of the stock virus were inoculated into 2 x 10^6 cells in a 75-cm2 flask containing 18 ml RPMI 1640 media. One flask was also maintained as an uninoculated control. The infected and control cells were harvested 48-72 hours after infection.

DNA extraction

DNA was extracted from uninfected and CAV infected MDCC-MSB1cells harvested at 48-72 hours post infection following the procedures described by Chowdhury et al. (2002) with minor modification and stored at -20^0C until use.

Amplification of DNA fragments by polymerase chain reaction (PCR)

The following DNA fragments were amplified by PCR from the whole genome of CAV for analysis by restriction endonulease enzymes (Figure 1a). The nucleotide (nt) positions used here are based on Cux-1 sequence (Noteborn et al., 1991).

(i) Fragment A: 1518 bp fragment (nt 2317-1515). Two oligonucleotide primers flanking fragment A were as follows. Forward primer (CAV5): 5′- ATC GAA TTC CGA GTG GTT ACT ATT CC -3′ (nt 2317-23) and the reverse primer (CAV6): 5′- GAA GGA TCC CTC ATT CTT AGT GGC -3′ (nt 1515-1492) (Soiné et al., 1993).

(ii) Fragment B: 926 bp fragment (nt 1463-69). The primers flanking fragment B were as follows. Forward primer (CAV 9): 5′- GAC ACA TTG AAA CCC GCT TT -3′ (nt 1463-1482) and the reverse primer (CAV 10): 5′- GCG ATT CGT CCA TCT TGA CT -3′ (nt 69-50) (Todd et al., 1996).

(iii) Fragment C:675 bp fragment (nt 844-1519). The primers flanking fragment C were as follows. Forward primer (CAV 13): 5′- GAC TGT AAG ATG GCA AGA CGA GCT C -3′ (nt 844-868) and the reverse primer (CAV 14): 5′- GGC TGA AGG ATC CCT CAT TC -3′ (nt 1519-1500) (Todd et al., 1992).

(iv) Fragment D: 552 bp fragment (nt 2074-306). The primers designed based on Cux-1 sequence (Noteborn et al., 1991), were used for amplifying fragment D. Forward primer (CAV15): 5′- GTA ATG AAG AGC GAT GCA TGG GC- 3′ (nt 2074-2096) and the reverse primer (CAV6b): 5′- CCA TTT TCG AAA CGT CAC TTT CGC- 3′ (nt 306-283).

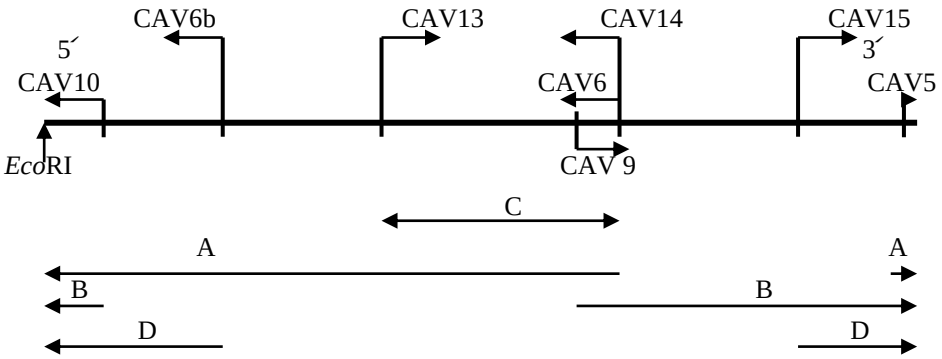


Figure 1a. Amplification strategy of different fragments from CAV genome (2319 bp) based on Cux-1 sequence (M55918). The circular CAV genome is represented by a straight line, whose both ends are connected.

The fragment A and fragment B covers the whole CAV genome. Fragment C falls within the fragment A at the 3′-end, and fragment D includes part of fragment B (at 3′-end) and part of fragment A (at 5′-end) (Figure 1a). All oligonucleotide primers were synthesized by Operon Technologies, Inc., USA. For amplification of all of the above fragments, PCR's were carried out in 50 µl reaction mixture containing distilled water, PCR buffer (1x), $MgCl_2$ (2.5 mM), dNTP mixture (0.3 mM each), forward and reverse primers (30 pmole each), *Taq* DNA polymerase (2.0 unit) and DNA template (0.2 to 0.5 µg). The thermal cycling profiles were as follows: initial 5 min incubation at 94°C, followed by 40 cycles of 94°C for 1 min, 60°C for 1.5 min, 72°C for 2 min; a final incubation at 72°C for 10 min and cooling at 4°C. The PCR products were run on 1 to 1.5% agarose gel electrophoresis. After staining with ethidium bromide, the specific bands were excised and purified by Geneclean kit (BIO 101, Inc., USA) following the supplied instructions.

Analysis of amplified DNA fragments by restriction endonuclease enzymes

For all nine isolates of CAV, the fragment A (1518 bp) was digested with one restriction enzyme, Eco130I (StyI); the fragment B (926 bp) with three enzymes, Eco130I (StyI), HpaII and MboI separately; the fragment C (675 bp) with three enzymes BsuRI (HaeIII), HinfI, and HpaII; and the fragment D (552 bp) with one enzyme, EcoRI. All enzymatic digestions were performed separately in a 20 µl reaction mixture containing buffer (1x) for individual enzyme as recommended by the manufacturer (MBI Fermentas, Lithuania), enzyme (15-25 units), distilled water (if any) and the purified DNA fragment. The reaction mixture was incubated at 37°C for 10-14 hours.

Agarose gel electrophoresis

After incubation, the resulting digested products, in case of fragment A/StyI were run in 2% agarose gel electrophoresis and in case of fragment D/EcoRI were run in 2.5%

agarose gel electrophoresis, and the gels were stained in ethidium bromide (1 μg ml^{-1}). For fragment A/StyI, 1 kb DNA ladder (MBI Fermentas, Lithuania) and for fragment D/EcoRI, 50 bp DNA ladder, ready to use (MBI Fermentas, Lithuania) was used as molecular size markers. The separated DNA fragments in the gel were visualised by using a UV transilluminator and photographs were taken.

Sodium dodecyl sulphate polyacrylamide gel electrophesis (SDS-PAGE)

The fragment B digested products with each of *Sty*I, *Hpa*II and *Mbo*I, and fragment C digested products with each of *Hae*III, *Hin*fI, and *Hpa*II were separated by SDS-PAGE. For the experiment, 12% separating or resolving gel and 4% stacking gel were prepared and electrophoresis was conducted at 44-48 V in a Mini-Protein® II Electrophoresis Cell (BIO-RAD, USA) following the instructions of the manufacturer. The run was maintained up to 1.5 cm from the bottom level, which took about 4 hours. Fragments generated by digesting the ϕX174 DNA (replicative form) with *Hae*III were used as size markers. After electrophoresis, the gel was stained with ethidium bromide (1μg ml^{-1}). The separated DNA bands in the gel were visualised by the UV transilluminator and photographs were taken.

RESULTS

PCR amplification of different fragments

Analysis by agarose gel electrophoresis indicated single DNA fragment in all sets of PCR reactions. The PCR with primers flanking fragments A, B, C and D, generated expected DNA fragments of around 1500 bp, 900 bp, 700 bp and 550 bp, respectively, for all nine isolates.

Restriction endonuclease analysis

A total of six restriction enzymes (EcoRI, HaeIII, HinfI, HpaII, MboI, and StyI) were used for analysis of different amplified fragment DNAs covering the whole CAV genome. The restriction sites found for all enzymes with regard to different PCR amplified fragments of different isolates are shown in table 1.

Fragment A (1518 bp)

Fragment A was treated with the restriction endonuclease, *Sty*I. The restriction map with *Sty*I for fragment A is shown in figure 1b. *Sty*I cleaved the 1518 bp fragment A from Cux-1 DNA at only one site, producing two fragments, A1 (1071 bp) and A2 (447 bp) (Figure 1b). The CAV isolates, SMSC-2, 3-1, BL-1, BL-2, BL4 and BL-5 produce the same profiles as Cux-1. However, the fragment A from isolates SMSC-1 and BL-3 remained undigested indicating absence of *Sty*I site. The *Sty*I digested products of fragment A, separated by 2% agarose gel, for all the nine isolates are shown in figure 2.

Table 1. Restriction endonuclease analysis of different PCR-amplified DNAs specified by different CAV isolates

DNAs	Enzy-me	Site	Isolates								
			SMSC-1	SMSC-2	3-1	BL-1	BL-2	BL-3	BL-4	BL-5	Cux-1
Frag. A	*Sty*I	1	-	+	+	+	+	-	+	+	+
Frag. B	*Sty*I	1	+	-	+	-	-	+	-	-	+
		2	+	+	+	+	+	+	+	+	+
	*Hpa*II	1	+	+	+	+	+	+	+	+	+
		2	-	+	+	+	+	-	+	+	+
		3	+	+	+	+	+	+	+	+	+
		4	+	+	+	+	+	+	+	+	+
		5	+	-	-	-	-	+	-	-	-
	*Mbo*I	1	+	+	+	+	+	+	+	+	+
		2	+	+	-	+	+	+	+	+	+
		3	+	-	+	+	+	+	+	+	+
Frag. C	*Hae*III	1	-	+	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+	+	+
		3	-	+	+	+	+	+	+	+	+
		4	+	+	+	+	+	+	+	+	+
		5	-	+	+	+	+	+	+	+	+
		6	-	-	+	-	-	-	-	-	-
	*Hin*fI	1	+	+	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+	+	+
		4	+	+	-	+	+	+	+	+	-
	*Hpa*II	1	+	+	+	+	+	+	+	+	+
		2	+	+	+	+	+	+	+	+	+
		3	+	+	+	+	+	+	+	+	+
Frag. D	*Eco*RI	1	-	-	+	-	-	-	-	-	+

'+' indicates presence and '-' indicates absence of restriction site.

Frag. = Fragment.

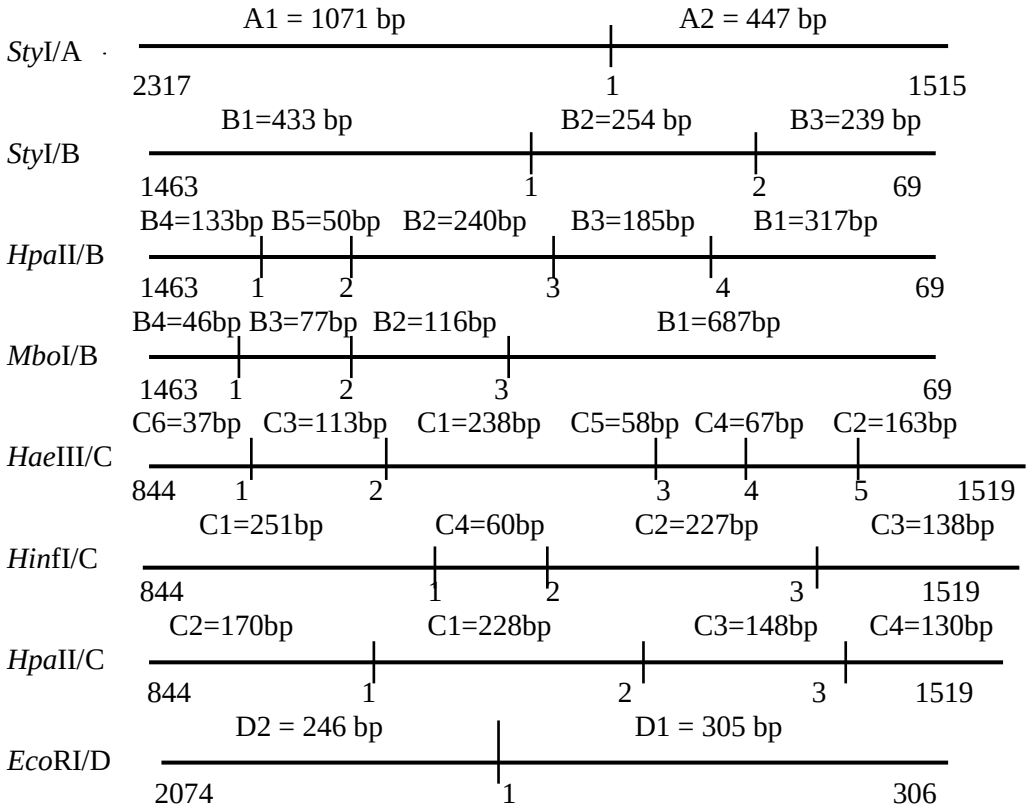


Figure 1b. Restriction map of different CAV fragments with respect to their enzyme(s). The position of restriction sites in the respective fragments and the internal fragment size (in bp) were predicted from the Cux-1 sequence (M55918).

Fragment B (926 bp)

Fragment B was treated with three restriction endonucleases, *Sty*I, *Hpa*II and *Mbo*I. The restriction maps with these enzymes for the fragment B are depicted in figure 1b. The enzyme *Sty*I cleaved the 926 bp fragment B from Cux-1 DNA at two sites producing three different fragments (433 bp, 254 bp and 239 bp) (Figure 1b). Similar cleavage pattern were also found in SMSC-1, 3-1 and BL-3 isolates. On the other hand, the *Sty*I site 1 was absent in the rest five isolates (SMSC-2, BL-1, BL-2, BL-4 and BL-5) leading to the generation of the fragment of 687 bp and 239 bp (calculated based on figure 1b, *Sty*I/B) and absence of the fragments B1 (433 bp) and B2 (254 bp). Electrophoresis in gels containing 12% acrylamide provided effective separation of the DNA fragments generated by *Sty*I digestion of the fragment B (Figure 3, *Sty*I/B).

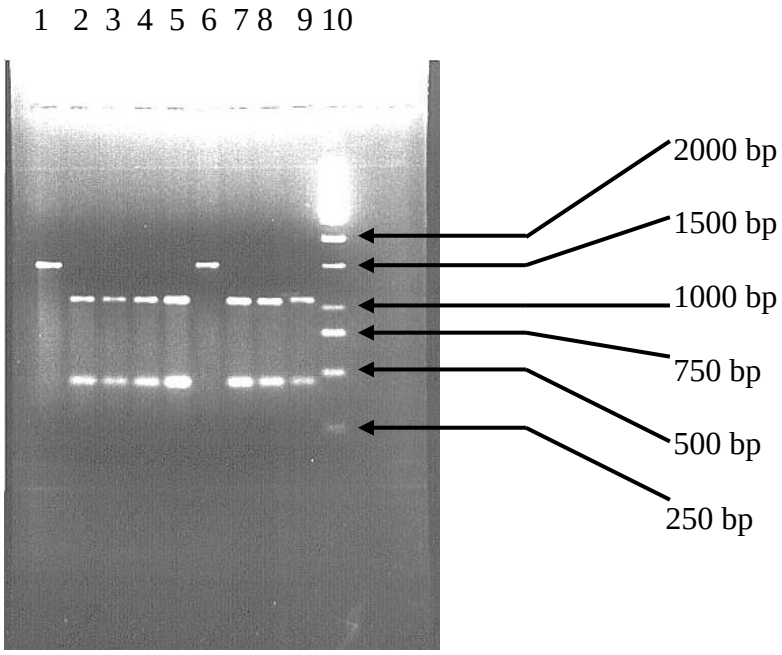
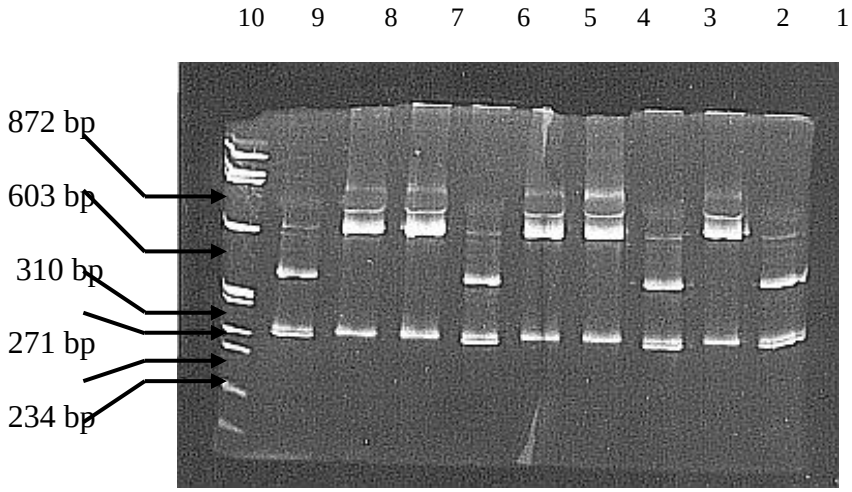


Figure 2. Restriction endonuclease (*Sty*I) analysis of 1518 bp fragment A amplified by PCR from DNAs specified by CAV isolates SMSC-1 (Lane 1), SMSC-2 (Lane 2), 3-1 (Lane 3), BL-1 (Lane 4), BL-2 (Lane 5), BL-3 (Lane 6), BL-4 (Lane 7), BL-5 (Lane 8) and Cux-1 (Lane 9). Lane 10- 1 kb DNA size marker. Restriction fragments were separated by 2% agarose gel.



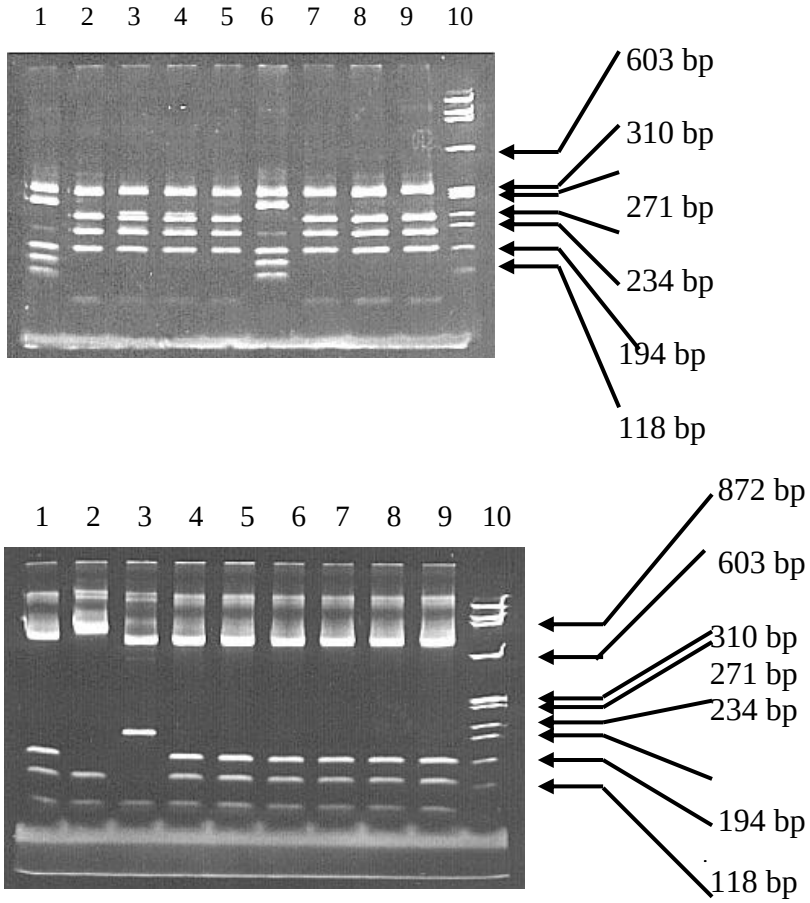


Figure 3: Restriction endonucleases (*Sty*I, *Hpa*II, *Mbo*I) analysis of 926 bp fragment B amplified by PCR from DNAs specified by CAV isolates SMSC-1 (Lane 1), SMSC-2 (Lane 2), 3-1 (Lane 3), BL-1 (Lane 4), BL-2 (Lane 5), BL-3 (Lane 6), BL-4 (Lane 7), BL-5 (Lane 8) and Cux-1 (Lane 9). Fragments generated by digesting the ϕX174 DNA (replicative form) with *Hae*III were used as size marker (Lane 10). Restriction fragments were separated by SDS-PAGE.

The enzyme *Hpa*II cleaved fragment B from Cux-1 DNA at four sites generating five different DNA fragments of 317 bp, 240 bp, 185 bp, 133 bp and 50 bp (Figure 1b). The fragment B of SMSC-2, 3-1, BL-1, BL-2, BL-4 and BL-5 isolates also showed similar pattern of fragments as Cux-1 after digestion with *Hpa*II. The fragment B of SMSC-1 and BL-3 isolates, after digestion with *Hpa*II also produced five fragments. Out of these five fragments, the pattern of the two fragments, B1 (317 bp) and B4 (133 bp), was similar to that of other seven isolates, but the pattern of the rest three

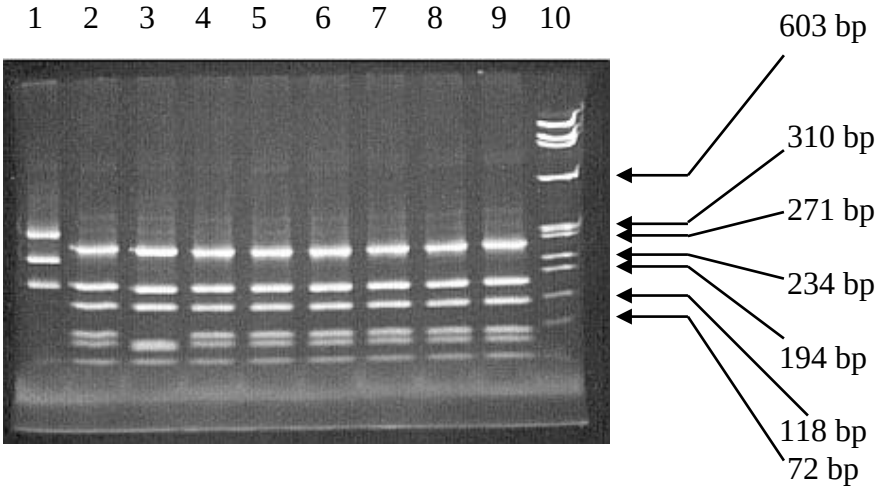
fragments was different from that of other isolates. The site 2 was absent in SMSC-1 and BL-3 which leads to the creation of the fragment of 290 bp (calculated based on figure 1b, *Hpa*II/B) and absence of the fragments, B2 (240 bp) and B5 (50 bp). There was one additional site in fragment B3 (185 bp) of the SMSC-1 and BL-3 isolates, that led to the formation of two additional fragments below the fragment B4 (133 bp). The DNA fragments generated by *Hpa*II digestion of fragment B were effectively separated by SDS-PAGE with 12% acrylamide (Figure 3, *Hpa*II/B).

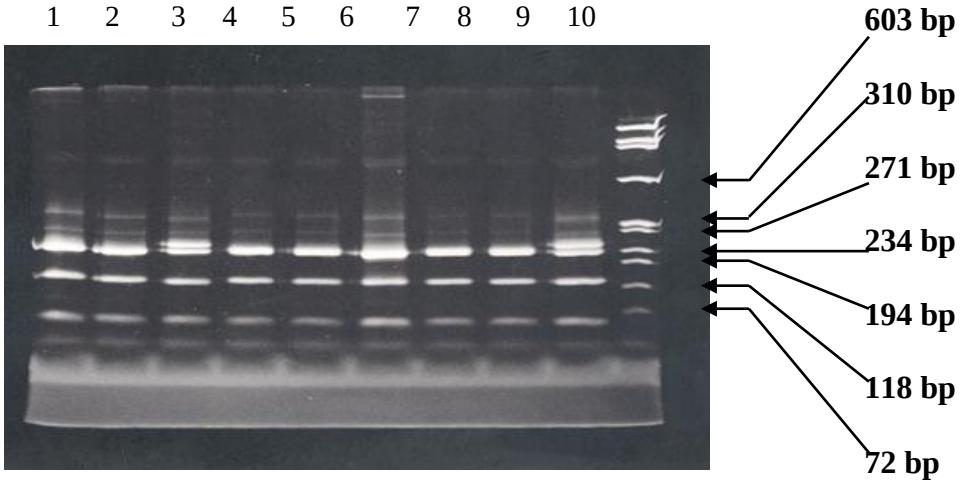
The enzyme *Mbo*I cleaved fragment B from Cux-1 DNA at three sites producing four different fragments, B1 (687 bp), B2 (116 bp), B3 (77 bp) and B4 (46 bp) (Figure 1b). The same pattern of fragments was also produced by digestion with *Mbo*I of the fragment B DNAs from SMSC-1, BL-1, BL-2, BL-3, BL-4 and BL-5 isolates. The difference was found in SMSC-2 and 3-1 isolates, both of which produced three fragments instead of four. In case of SMSC-2, the *Mbo*I site 3 was absent in fragment B that resulted in the absence of the fragments, B1 (687 bp) and B2 (116 bp). Instead, there was creation of an 803 bp fragment (calculated on the basis of figure 1b, *Mbo*I/B). The other two fragments, B3 (77 bp) and B4 (46 bp), had the same pattern with Cux-1 and other isolates except 3-1 where only fragment B4 had the similarity. While in fragment B of 3-1, the *Mbo*I site 2 was absent that resulted in the generation of a 193 bp fragment (calculated on the basis of figure 1b, *Mbo*I/B) and absence of the fragments B2 (116 bp) and B3 (77 bp). The fragment B4 (46 bp) had similar pattern with all other isolates, and the fragment B1 (687 bp) had also same pattern with other isolates except SMSC-2. The generated DNA fragments in case of all isolates were separated effectively by SDS-PAGE with 12% acrylamide. Figure 3 (*Mbo*I/B) displays the *Mbo*I digested products of fragment B for all isolates.

Fragment C (675 bp)

Fragment C was treated with three restriction endonucleases, *Hae*III, *Hin*fI and *Hpa*II. The restriction map with *Hae*III for fragment C is shown in figure 1b. *Hae*III cleaved the 675 bp fragment C from Cux-1 DNA at five sites producing six different fragments. Other isolates, except SMSC-1 and 3-1, showed similar restriction profiles as Cux-1 after digestion of their fragment C DNAs with *Hae*III. The enzyme digested fragment C DNA of SMSC-1 isolate producing only three fragments instead of six. The *Hae*III sites 1, 3 and 5 were absent in fragment C of SMSC-1. The absence of site 1 led to the absence of fragments C3 (113 bp) and C6 (37 bp) and the formation of a 150 bp fragment (calculated based on figure 1b, *Hae*III/C). The absence of site 3 reflected with the creation of a 296 bp fragment and absence of the fragments C1 (238 bp) and C5 (58 bp) (calculated based on figure 1b, *Hae*III/C). While the absence of site 5 resulted in the deletion of fragments, C2 (163 bp) and C4 (67 bp) and the formation of a fragment of 230 bp (calculated based on figure 1b, *Hae*III/C). In case of 3-1, after *Hae*III digestion of fragment C DNA, seven fragments were produced instead of six, out of which five fragments were seen in the gel. Here, all five *Hae*III sites like Cux-1 and other six isolates were present, but there was an additional

*Hae*III site (site 6) in fragment C4 (67 bp) which was cleaved to produce a fragment that was merged with fragment C5 (58 bp) and a small fragment that was undetectable in the gel. Electrophoresis in gels containing 12% acrylamide provided effective separation of DNA fragments generated by *Hae*III digestion of fragment C (Figure 4, *Hae*III/C).





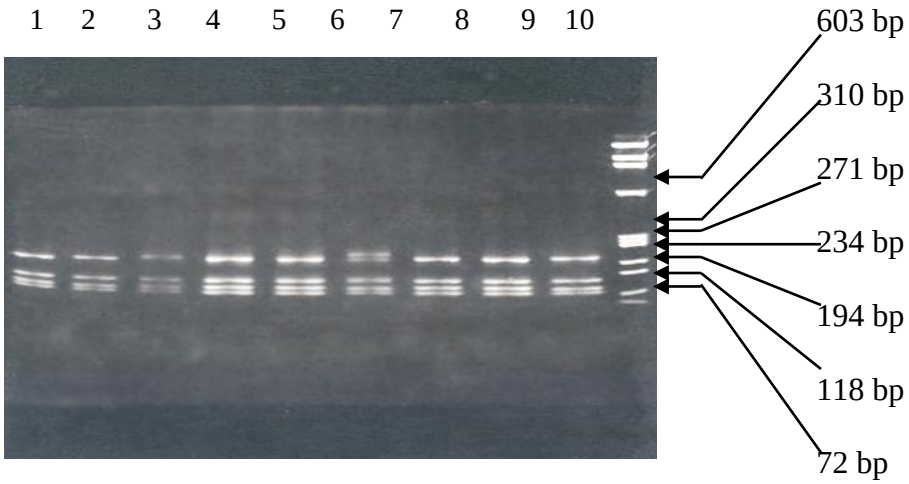


Figure 4: Restriction endonuclease (*Hae*III, *Hin*fI, *Hpa*II) analysis of 675 bp fragment C amplified by PCR from DNAs specified by CAV isolates SMSC-1 (Lane 1), SMSC-2 (Lane 2), 3-1 (Lane 3), BL-1 (Lane 4), BL-2 (Lane 5), BL-3 (Lane 6), BL-4 (Lane 7), BL-5 (Lane 8) and Cux-1 (Lane 9). Fragments generated by digesting the ϕX174 DNA (replicative form) with *Hae*III were used as size marker (Fermentas) (Lane 10). Restriction fragments were separated by SDS-PAGE.

*Hin*fI cleaved fragment C from Cux-1 DNA at three sites producing four different fragments, C1 (251 bp), C2 (227 bp), C3 (138 bp) and C4 (60 bp) (Figure 1b). Similar profiles as Cux-1 was also showed by digestion with *Hin*fI of the fragment C from 3-1 isolate. Whereas, other seven isolates exhibited a different profile producing five fragments, out of which three fragments were visible. There was one additional site in fragment C1 (251 bp) producing two fragments, of which the big one had almost the same mobility with fragment C2 (227 bp) showing a prominent band, the other one was so small that could not be visible in the acrylamide gel. The pattern for C3 and C4 fragments is similar to that in Cux-1 and 3-1 isolates. The *Hin*fI digested products of fragment C were separated by SDS-PAGE with 12% acrylamide (Figure 4, *Hin*fI/C).

*Hpa*II also cleaved fragment C from Cux-1 DNA at three sites producing four different fragments of 228 bp, 170 bp, 148 bp and 130 bp (Figure 1b). The fragment C from all other isolates after digestion with *Hpa*II also showed the same restriction profiles as Cux-1. SDS-PAGE with 12% acrylamide provided effective separation of the *Hpa*II digested products of fragment C (Figure 4, *Hpa*II/C).

Fragment D (552 bp)

Fragment D was treated with the restriction endonuclease, *Eco*RI. The restriction map with *Eco*RI for fragment D is depicted in figure 1b. *Eco*RI cleaved the 552 bp fragment D from Cux-1 DNA at only one site, producing two fragments, D1 (305 bp) and D2 (247 bp). Similar digestion pattern was found in 3-1 isolate. Whereas, all other isolates remained undigested exhibiting the original fragment indicating absence of *Eco*RI site. The DNA fragments generated after *Eco*RI digestion of fragment D were separated effectively by 2.5% agarose gel and displayed in figure 5.

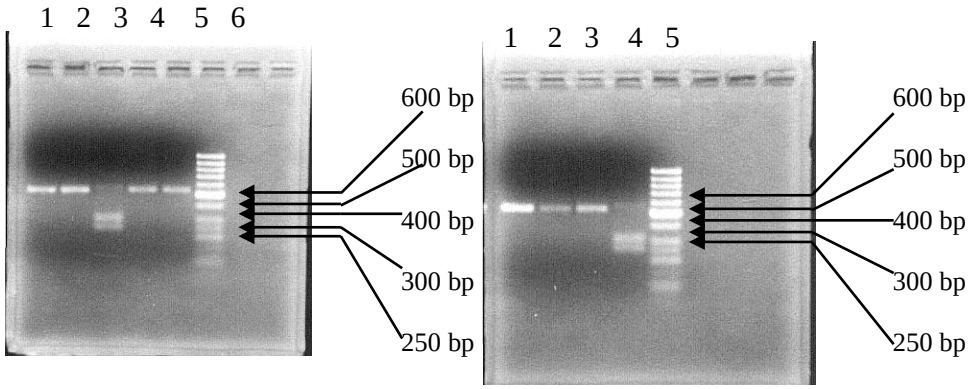


Figure 5: Restriction endonuclease (*Eco*RI) analysis of 552 bp fragment D amplified by PCR from DNAs specified by CAV isolates SMSC-1 (Lane 1), SMSC-2 (Lane 2), 3-1 (Lane 3), BL-1 (Lane 4), BL-2 (Lane 5) in A, and BL-3 (Lane 1), BL-4 (Lane 2), BL-5 (Lane 3), Cux-1 (Lane 4) in B. Lane 6 in A and Lane 5 in B indicate 50 bp DNA marker. Restriction fragments were separated by 2.5% agarose gel.

DISCUSSION

This is the first time that the whole CAV genomes of different CAV isolates were characterized by restriction endonuclease (RE) analysis. In this study, the PCR technology was first used to amplify different DNA fragments encompassing the whole CAV DNA. Then the enzymatic technology was applied with different restriction endonucleases to find out the molecular differences among the Malaysian local field isolates as well as a standard reference isolate (Cux-1) of CAV. Todd et al. (1992) differentiated 14 CAV isolates by RE analysis of PCR-amplified 675-bp fragment that encompasses the first half of the largest open reading frame (VP1). The present study, first time differentiated the Malaysian field CAV isolates as well as the European isolate, Cux-1, by RE analysis of four PCR-amplified DNA fragments encompassing the whole CAV genome.

The primers selected for amplifying different fragments were based on Cux-1 sequence (Noteborn et al., 1991). These primers were specifically hybridized with the DNAs specified by Malaysian CAV isolates and effectively amplified the different DNA fragments from the CAV genomes with similar pattern as Cux-1 isolate. These procedures further confirm the Malaysian isolates as CAV, though these isolates have been confirmed earlier by other tests including PCR (Chowdhury et al., 2002).

The *Sty*I digestion of fragment A and *Hpa*II digestion of fragment B, each of which differentiated two isolates (SMSC-1 and BL-3) from other seven CAV isolates. The *Sty*I digestion of fragment B separated five isolates (SMSC-2, BL-1, BL-2, BL-4 and BL-5) from other four isolates. The enzyme *Mbo*I differentiated SMSC-2 and 3-1 isolates from other seven isolates after digestion of fragment B. These two isolates also do not exhibit the same profiles, whereas the other seven isolates have the same *Mbo*I profiles. *Hae*III digestion of fragment C distinguished two isolates (SMSC-1 and 3-1) from the other seven isolates, though these two isolates also do not have the same restriction profiles. In case of *Hin*fI digestion of fragment C and *Eco*RI digestion of fragment D, two isolates (3-1 and Cux-1) were differentiated in each case from the other seven isolates. The enzyme *Hpa*II could not distinguish any of the nine isolates after digestion of fragment C.

The results of restriction endonuclease analysis of different genome fragments revealed similar restriction profiles for four isolates (BL-1, BL-2, BL-4 and BL-5) in all enzymatic digestions (Table 1), indicating that these four isolates belong to one group and probably possess the same genomic characteristics. These isolates showed similar restriction profiles with Cux-1 isolate when digested by *Sty*I (fragment A), *Hpa*II, *Mbo*I and *Hae*III, and were differentiated from Cux-1 isolate when the fragment B was digested by *Sty*I.and fragment C was digested by *Hin*fI.

SMSC-1 and BL-3 isolates always exhibited the same restriction profiles with different enzymatic digestions of different fragments, indicating maximum similarity between these two isolates (Table 1). However, only *Hae*III digestion of fragment C differentiated SMSC-1 from BL-3, implying that these two are separate isolates (Table 1). These two isolates showed the same profiles as Cux-1 when digested with *Sty*I (fragment B), *Mbo*I and *Hpa*II (fragment C), but were different from Cux-1 when digested with *Sty*I (fragment A), *Hpa*II (fragment B), *Hin*fI and *Eco*RI. SMSC-1 isolate also could be differentiated from Cux-1 by *Hae*III, while BL-3 isolate in this case, produced the same profile as Cux-1.

The SMSC-2 isolate showed the same restriction profiles as Cux-1 isolate by *Sty*I (Fragment A), *Hpa*I and *Hae*III. This isolate was differentiated from Cux-1 by *Sty*I (fragment B), *Mbo*I, and *Hin*fI. The 3-1 isolate exhibited the same profiles with Cux-1 in maximum enzymatic digestions, indicating highest similarity between these isolates. However, 3-1 isolate was differentiated from Cux-1 by *Mbo*I digestion of fragment B and *Hae*III digestion of fragment C (Table 1).

The results of restriction endonuclease analysis demonstrated that the isolates, SMSC-1, SMSC-2, 3-1, BL-3 and Cux-1 are different from each other and also from the group of the above four isolates (Table 1). The present study revealed that the restriction enzyme analysis differentiated CAV isolates, though obtained from the same poultry farm at the same time. The group of four isolates (BL-1, BL-2, BL-4 and BL-5) and the BL-3 isolate were isolated from same broiler farm. However, the restriction profiles differentiated BL-3 from the group of four isolates, when digested by *Sty*I (fragments A and B) and *Hpa*II (fragment B). SMSC-1 and SMSC-2 isolates were also obtained from same broiler farm; these two isolates were found to be different from each other in five of the eight enzymatic digestions (Table 1).

The present findings and the findings of Todd et al. (1992) support the view that CAV isolates can be differentiated with number of restriction site differences occurring between isolates, though these isolates are antigenically and pathologically indistinguishable (McNulty et al., 1990; Connor et al., 1991; Yuasa and Imai, 1986). However, the findings of Todd et al. (1992) were confined with only 675 bp fragment of the CAV genome that is equivalent to fragment C of the present study. The three enzymes (*Hae*III, *Hin*fI and *Hpa*II) used by them were also used in the present study for the fragment (675 bp/fragment C). Todd et al. (1992) made seven groups of CAV isolates based on the restriction site differences after restriction endonuclease analysis (with *Hae*III, *Hin*fI and *Hpa*II) of the 675 bp fragment from 14 CAV isolates derived from seven different countries (UK, Ireland, Germany, Sweden, USA, Japan and Australia). When compared with the CAV groupings made by Todd et al. (1992), the present findings with three enzymatic digestions (with *Hae*III, *Hin*fI and *Hpa*II) of the 675 bp fragment (fragment C) provided the following groupings for the isolates of the present study. SMSC-1 fell in group 7 to which placed Australian IMP 704 isolate (Todd et al., 1992). SMSC-2, BL-1, BL-2, BL-3, BL-4 and BL-5 isolates fell in group 2 which placed Japanese TK 5803, Sweden 1/80 and 1/91 isolates (Todd et al., 1992). Cux-1 isolate fell in group 1, which substantiated the finding of Todd et al., (1992), where also placed Japanese Gifu-1 isolate. Only 3-1 isolate of the present study did not fall in any of the group made by Todd et al. (1992) and for this isolate we are creating a new group, i.e. group 8 in addition to the groups made by Todd et al. (1992). CAVs were also differentiated by RE analysis of single genome fragments by other investigators (Nayabian and Mardani, 2013; Oluwayelu et al., 2005; van Santen et al., 2001).

In the present study, different PCR-amplified genome fragments covering the whole CAV genome, after digestion with different restriction enzymes, differentiated more isolates than the single genome fragment digested by the three enzymes (*Hae*III, *Hin*fI and *Hpa*II). Therefore, restriction endonuclease analysis with different fragments of the genome under the present study gave proper genomic diversity than that with the single genome fragment dealt with by Todd et al. (1992).

Different restriction endonucleases recognise only small portion of sequences from the whole genome. Based on this, it is difficult to assess the actual sequence diversity

between CAV isolates. Therefore, it is better to perform sequencing to determine the actual sequence diversity. However, detection of sequence for epidemiological purposes only may not be practical for many diagnostic laboratories, since sequence analysis is expensive and needs sophisticated and costly equipments. Instead, restriction endonuclease analysis may be at least an alternative method to detect molecular differences.

CONCLUSION

The study revealed that restriction endonuclease analysis could be used to identify and differentiate CAV isolates based on the number of restriction site differences, though these isolates are antigenically indistinguishable. Restriction endonuclease analysis of different genome fragments could also be used to differentiate isolates obtained from the same poultry farm. The analysis showed that more isolates could be distinguished with proper genomic diversity after restriction endonuclease analysis of more genome fragments compared to that of single genome fragment. The first time present findings may be most useful for epidemiological purposes.

ACKNOWLEDGEMENT

This work was supported by IRPA grant no. 01-02-04-T002, Ministry of Science, Technology and Environment, Malaysia.

REFERENCES

AboElkhair, M., Abd, A.G., and Metwally, A.E.Y. (2014). Molecular characterization of chicken anemia virus circulating in chicken flocks in Egypt. *Advances in Virology*, 2014, ID 797151, 6 pages. http://dx.doi.org/10.1155/2014/797151.

Adair, B.M. (2000). Immunopathogenesis of chicken anemia virus infection. *Developmental & Comparative Immunology*, 24 (2-3), 247-255.

Breitbart, M. (2015). Move Genus Gyrovirus from the family Circo viridae to the family Anelloviridae. ICTV taxonomy history for chicken anemia virus, London UK (2015).

Bülow, V.V., Fuchs, B., Vielitz, E., and Landgraf, H. (1983). Frühsterblichkeitssyndrom bei Küken nach infektion mit dem Virus der Marekschen Krankheit (MDV) und einem Anämie-Erreger (CAA) (Early mortality syndrome of chickens after dual infection with Marek's disease virus (MDV) and chicken anaemia agent (CAA)). *Zentralbl Veterinärmed*, B 30, 742-750.

Chowdhury, S.M.Z.H., Omar, A.R., Aini, I., Hair-Bejo, M., Jamaluddin, A.A., Kono, Y., Darus, A., and Yatim, H.M. (2002). Isolation, identification and characterization of chicken anemia virus in Malaysia. *Journal of Biochemistry, Molecular Biology and Biophysics*, 6 (4), 249-255.

Connor, T.J., McNeilly, F., Firth, G.A., and McNulty, M.S. (1991). Biological characterization of Australian isolates of chicken anaemia agent. *Australian Veterinary Journal*, 68, 199-201.

Coombes, A.L., and Crawford, G.R. (1996). Chicken anaemia virus: a short review. *World's Poultry Science Journal*, 52, 267-277.

De Herdt, P., Van den Bosch, G., Ducatelle, R., Uyttebroek, E., and Schrier, C. (2001). Epidemiology and significance of chicken infectious anemia virus infections in broilers and broiler parents under nonvaccinated European circumstances. *Avian Diseases*, 45, 706-708.

Engstrom, B.E., and Luthman, M. (1984). Blue wing disease of chickens: signs, pathology and natural transmission. *Avian Pathology*, 13, 1-12.

Farkas, T., Maeda, K., Sugiura, H., Kai, K., Hirai, K., Otsuki, K., and Hayashi, T. (1998). A serological survey of chickens, Japanese quail, pigeons, ducks and crows for antibodies to chicken anaemia virus (CAV) in Japan. Avian Pathology, 27 (3), 316-320.

Ganar, K., Shah, M., Kamdi, B.P., Kurkure, N.V., and Kumar, S. (2017). Molecular characterization of chicken anemia virus outbreaks in Nagpur province, India from 2012 to 2015. *Microbial Pathogenesis*, 102, 113-119.

Ledesma, N., Fehervari, A.T., Casaubon, M.T., Lucio, E., and Ratz, F. (2001). Chicken infectious anemia in Mexico: virus identification and serology survey. *Avian Diseases*, 45, 788-796.

Li, Y.P., and Cui, Z.Z. (2007). Pathogenicity and genomic sequence comparison of a chicken infectious anemia virus field isolate. W*ei Sheng Wu Xue Bao*, 47(5), 894-8. [Article in Chinese]

McNulty, M.S. (1991). Chicken anemia agent: a review. *Avian Pathology*, 20, 187-203.

McNulty, M.S., Connor, T.J., McNeilly, F., McLoughlin, M.F., and Kirkpatrick, K.S. (1990). Preliminary characterization of isolates of chicken anaemia agent from the United Kingdom. *Avian Pathology*, 19, 67-73.

Nayabian, H., and Mardani, K. (2013). Molecular characterization of the chicken anaemia viruses isolated from broiler farms of west Azerbaijan, Iran. *Avian Pathology*, 42 (2), 108-113.

Noteborn, M.H.M., De Boer, G.F., van Roozelaar, D.J., Karreman, C., Kranenburg, O., Vos, J.G., Jeurissen, S.H.M., Hoeben, R.C., Zantema, A., Koch, G., van Ormondt, H., and van der Eb, A.J. (1991). Characterization of cloned chicken anemia virus DNA that contains all elements for the infectious replication cycle. *Journal of Virology*, 65, 3131-3139.

Noteborn, M.H.M., Verschueren, C.A.J., van Roozelaar, D.J., Veldkamp, S., van der Eb, A.J., and de Boer, G.F. (1992). Detection of chicken anaemia virus by DNA hybridisation and polymerase chain reaction. *Avian Pathology*, 21, 107-118.

Olszewska-Tomczyk, M., Swieton, E., Minta, Z., and Smietanka, K. (2016). Occurrence and phylogenetic studies of chicken anemia virus from polish broiler flocks. *Avian Diseases*, 60, 70-74.

Oluwayelu, D.O., Todd, D., Ball, N.W., Scott, A.N., Oladele, O.A., Emikpe, B.O., Fagbohun, O.A., Owoade, A.A., and Olaleye, O.D. (2005). Isolation and preliminary characterization of chicken anemia virus from chickens in Nigeria. *Avian Diseases*, 49(3), 446-50.

Pope, C.R. (1991). Chicken anaemia agent. *Veterinary Immunology and Immunopathology*, 30, 51-65.

Rehman, L.U., Sultan, B., Ali, I., Bhatti, M.A., Khan, S.U., Zaman, K.U., Jahangiri, A.T., Khan, N.U., Iqbal, A., Bakht, J., Swati, Z.A., and Rehman, M.U. (2011). Duplex PCR assay for the detection of avian adeno virus and chicken anemia virus prevalent in Pakistan. *Virology Journal*, 8, 440, doi: 10.1186/1743-422X-8-440.

Rosenberger, J.K., and Cloud, S.S. (1989). The effects of age, route of exposure, and coinfection with infectious bursal disease virus on the pathogenicity and transmissibility of chicken anaemia agent (CAA). *Avian Diseases*, 33, 753-759.

Schat, K.A. (2003). Chicken infectious anemia. In: Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R., Swayne, E., eds. *Diseases of Poultry*. 11th ed. Ames, Iowa, USA: Iowa State University Press; 2003. p. 182–202.

Soiné, C., Watson, S.K., Rybicki, E., Lucio, B., Nordgren, R.M., Parrish, C.R., and Schat, K.A. (1993). Determination of the detection limit of the ploymerase chain reaction for chicken anaemia virus. *Avian Diseases*, 37, 467-476.

Spackman, E., Cloud, S.S., Pope, C.R., and Rosenberger, J.K. (2002a). Comparison of a putative second serotype of chicken infectious anemia virus with a prototypical isolate I. Pathogenesis. *Avian Diseases*, 46, 945-955.

Spackman, E., Cloud, S.S., and Rosenberger, J.K. (2002b). Comparison of a putative second serotype of chicken infectious anemia virus with a prototypical isolate II. Antigenic and physicochemical characteristics. *Avian Diseases*, 46, 956-963.

Stanislawek, W.L., and Howell, J. (1994). Isolation of chicken anaemia virus from broiler chickens in New Zealand. *New Zealand Veterinary Journal*, 42 (2), 58-62.

Tham, K.M., and Stanislawek, W.L. (1992). Detection of Chicken anaemia DNA sequences by the polymerase chain reaction. *Archives of Virology*, 127, 245-255.

Todd, D., Mawhinney, K.A., and McNulty, M.S. (1992). Detection and differentiation of chicken anaemia virus isolates by using polymerase chain reaction. *Journal of Clinical Microbiolgy*, 30, 1661-1666.

Todd, D., Creelan, J.L., Meehan, B.M., and McNulty, M.S. (1996). Investigation of the transfection capability of cloned tandemly-repeated chicken anaemia virus DNA fragments. *Archives of Virology*, 141, 1523-1534.

Van Santen, V.L., Li, L., Hoerr, F.J., and Lauerman, L.H. (2001). Genetic characterization of chicken anemia virus from commercial broiler chickens in Alabama. *Avian Diseases*, 45 (2), 373-88.

Yuasa, N., and Imai, K. (1986). Pathogenicity and antigenicity of eleven isolates of chicken anemia agent (CAA). *Avian Pathology*, 15, 639-645.

Zhang, X., Xie, Q., Ji, J., Chang, S., Liu, J., Chen, F., Ma, J., and Bee, Y. (2012). Complete genome sequence analysis of a recent chicken anemia virus isolate and comparison with a chicken anemia virus isolate from human fecal samples in China. *Journal of Virology*, 86 (19), 10896-7, doi: 10.1128/JVI.01749-12.

Zhou, W., Yang, B., Shen, B., Han, S., and Zhou, J. (1996). A serologic survey of antibody against chicken infectious anemia virus by indirect immunofluorescent assay in domestic poultry in China. *Avian Diseases*, 40, 358-360.

SAARC J. Agri., 15(2): 19-30 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35156*

WILLINGNESS TO PAY A PREMIUM FOR PRODUCE AT DIRECT MARKETING OUTLETS: AN ORDERED PROBIT ANALYSIS

S. Arumugam*

Department of Agricultural, Food and Resource Economics, Rutgers- The State University of New Jersey, New Brunswick, NJ 08901-8520, USA

ABSTRACT

The concept of farm to consumer direct marketing has been popularly known to create opportunities for farmer-consumer relationship and enhance the sustainability of the local farming business. The objective of the present study was to predict the consumers' willingness to pay for fresh produce in the direct marketing outlets and agritourism activities. An Internet survey relating to direct marketing and agritourism was conducted to understand the characteristics of consumers. A total of 1,134 participants completed the survey from Delaware, New Jersey, and Pennsylvania. Based on their responses, an ordered probit model was developed at a low premium (1-5 percent), medium premium (6-10 percent) and high premium (11percent and above) to predict respondents' willingness to pay a premium for produce sold at direct marketing outlets. The estimated results show that consumers' willingness to pay more to help preserve farmland or local business is highly significant. However, we discovered an inverse relationship for the Mid-Atlantic fresh greens shoppers. On average, as the travel distance increases, the likelihood of paying a higher premium decrease based on each additional mile they travel. The results of the ordered probit model will help all relevant stakeholders from the Mid-Atlantic States to promote direct marketing and agritourism industry in the region and enhance their knowledge of the industry.

Keywords: Consumers survey, ordered profit analysis, willingness to pay, farmers to consumers, direct marketing outlets.

INTRODUCTION

Direct-to-consumer sales outlets such as roadside stands, farmers' markets, pick-your-own, community supported agriculture, on-farm stores are directly connected to consumer demand for locally-grown foods (Henderson and Linstrom, 1982). In the

* Corresponding author e-mail: sa856@sebs.rutgers.edu

Received: 05.09.2017 *Accepted: 17.10.2017*

U.S.A small and medium growers have limited land and capital resources which affect the economic viability of the small farm business. Agritourism and direct marketing are used by the farmers to supplement farm income. Agritourism and eco-tourism may include a wide range of farm-related products and services that are educational, interactive, or recreational in nature (Surendran and Sekar, 2010; Tew and Barbieri, 2012; Koutsouris et al., 2014; Chiu et al., 2016). Other direct marketing methods also able to break common obstacles such as farm size, delivery logistics, limited marketing budget, and labor constraints etc. Hence, small and medium-sized farmers make use these direct marketing activities to enhance farm income(Tew and Barbieri, 2012; Koutsouris et al., 2014; Chiu et al., 2016). The direct marketing outlets allow farmers to sell their products directly to their targeted consumers, rather than having their goods pass through several hands before it reaches the ultimate consumer, as it often has. Also, producers can capture a large portion of the product margin by selling directly to the consumer, and consumers know that they can get locally-grown fresh, high quality produce at affordable price along with other factors, including the shopping atmosphere, environmental consciousness, appearance, and variety compared to the identical product retailed in supermarkets (Brown, 2003; Onyango et al., 2015).Consumers also derive cultural and social benefits from direct contacts with farmers, visits to farm and nature (Surendran and Sekar, 2011).

By using direct marketing, producers can cut out the "middleman" in a lot of their operations and eliminate additional expenditures on services such as packaging, storing, transporting, and marketing the goods. Media coverage also reflects contradiction between direct marketing outlets and supermarkets. Further, federal nutrition programs that support purchases from direct marketing venues (e.g. Farmers' Market Nutrition Program, Senior Farmers' Market Nutrition Program) notice quality of produce to be as good (or better), and/or prices to be affordable at the direct market outlets compared to grocery stores (McCormack et al., 2010). The flexibility allows farmers to determine their own product mix and to balance this production between consumer demand and individual talents for produce marketing. Producers who can raise specialty crops such as flowers, snow peas, or peppers, have successfully used direct farmers to consumer marketing to provide products during special seasons or to special ethnic groups (Govindasamy et al., 2015).

Many studies have concentrated on outcomes and benefits of farmers to consumers direct marketing and agritourism activities (Tew and Barbieri, 2012; Kline et al., 2016). However, very few have addressed Willingness to Pay a Premium price (WTP) for traditional produce at Direct Marketing Outlets (Balogh et al., 2016; Dominique et al., 2016). The WTP is often used in determining the market potential of farm/environmental activities (Surendran and Sekar, 2011; Onyango et al., 2015). In most of these studies, researchers have hypothesized that consumers WTP are influenced by socio- demographic factors such as age, education, income, gender, marital status and number of children in the family (Surendran and Sekar, 2010; Govindasamy et al., 2014; Balogh et al., 2016).To encourage direct marketing, it is

crucial to explore participant's interests, preferences and needs pertaining to these activities and opportunities. The purpose of this research is to determine how well farmer to consumer direct markets serve the needs of the consumer by providing an overview of characteristics of direct marketing patrons.

METHODOLOGY

An Internet survey pertaining to direct marketing and agritourism was conducted in June and July 2010to document the characteristics of consumers, who buy at farmer-to-consumer direct market outlets and/or visit agritourism operations in the Mid-Atlantic States. A total of 1,134 participants completed the survey from Delaware, New Jersey, and Pennsylvania. Of the questions asked, respondents acuity about direct marketing outlets and agritourism activities and their willingness to pay a premium for produce at direct marketing outlets were used in the model. From a panel set, the respondents were randomly selected by a survey research company (Sampling International, LLC, and Shelton, CT). Nearly 2,594 members who were registered with this panel, accessed the survey (952 from NJ, 309 from DE, and 1,384 from PA). However, 1,134members met the screener criteria and began the questionnaire (424 from NJ, 133 from DE, and 577 from PA), with 993 respondents completing the study (122 from DE, 364 from NJ, and 507 from PA). Likely respondents were screened and asked to participate if they were: 1) primary food shopper for the household; 2) age 18 and older, and 3) had previously attended agritourism and direct marketing events or activities. Survey questions were pre-tested to a sample of 93 randomly selected Survey Sampling International, LLC panelists.

Ordered Probit analyses of willingness-to-pay (WTP)

The levels of willingness to pay for fresh produce by consumers are of at most importance for farmers operating a direct market platform. The Ordered Probit model implemented is selected over OLS (Ordinary Least Squares) because the nature of the dependent variable is categorical and will provide a greater generality of the purchase likelihoods. The WTP model here can be interpreted as a latent variable that observes the cause of what influences decisions. Three categories of the WTP are estimated using the model: willing to pay a low premium (1-5percent), willing to pay a medium premium (6-10 percent) and willing to pay a high premium (11percent and above) for the fresh greens sold at direct farmer markets. The probability of the categories is estimated under a normal curve calculated as (Greene and Hensher, 2010):

$$\text{Prob [y=1]= } \Phi(-\beta' x), \quad (1)$$

$$\text{Prob [y=2]=} \Phi(\mu - \beta' x) - \Phi(\beta' x) \quad (2)$$

$$\text{Prob [y=3]=} 1 - \Phi(\mu - \beta' x) \quad (3)$$

Where μ is the threshold parameter, Φ is the cumulative normal and x is the vector of independent variables. The threshold parameters are adjusted to make probabilities match sample proportions and do not follow discrete normal or logistic distribution (Greene and Hensher, 2010). WTP here is driven by the extent to which utilities change with regards to the individual consumption choices. If the consumers' WTP falls within a certain range, the numeric value that is assigned to it, reflects the category of individuals' willingness-to-pay. The summary descriptive statistics of explanatory variables are shown in table 1.

Table 1. Descriptive statistics

Variable	Description	Mean Units/ Percentage	SD Units/ Percentage
WTPi *(Dependent Variable)*	WTPi=1 if the respondent is willing to pay a low premium; / WTPi=2 - Medium premium; WTPi=3 - High premium for fresh greens sold at direct markets.	1.02	0.77
RESI_1	1 if the respondent lives at the current location for less than a year; 0=otherwise	0.01	0.23
RESI_3	1 if the respondent lives at the current location for one to three years; 0=otherwise	0.14	0.35
HOME_GR	1 if the respondent has a garden at home; 0= otherwise	0.50	0.50
WTP_HELP	1 if the respondent is willing to pay higher prices to preserve farmland and local agricultural producers; 0= otherwise	0.86	0.34
AG_HELP	1 if the respondent believes that agriculture will help maintain open space/greenery; 0=otherwise	0.96	0.18
ORGANIC	1 if the respondent is willing to buy certified organic fresh fruits and vegetables; 0= otherwise	0.69	0.46
GMO	1 if the respondent is willing to buy genetically modified fresh fruits and vegetables; 0= otherwise	0.18	0.38
QUAL	1 if the respondent thinks that quality of fresh produce sold at direct outlets is better; 0=otherwise	0.94	0.23
PRICE	1 if the respondent thinks that price of fresh produce sold at direct outlets is better; 0=otherwise	0.58	0.49
G_Q	1 if the respondent is a male who thinks the quality of fresh produce sold at direct market outlet is better; 0=otherwise	0.23	0.42
G_P	1 if the respondent is a male who thinks the price of fresh produce sold at direct market outlet is better; 0=otherwise	0.13	0.33

Variable	Description	Mean Units/ Percentage	SD Units/ Percentage
MKTING_B	1 if the respondent has first learned direct outlets through billboard or roadside sign; 0=otherwise	0.38	0.49
MKTING_S	1 if the respondent has first learned direct outlets through sign at the market's entrance; 0=otherwise	0.52	0.50
MKTING_P	1 if the respondent has first learned direct outlets through newspaper; 0=otherwise	0.46	0.50
MKTING_M	1 if the respondent has first learned direct outlets through friends/family/word-of-mouth; 0=otherwise	0.75	0.44
OFM_SP	Average spending per visit at direct markets other than OFM	18.66	20.47
VAL_ADD	1 if the respondent thinks it is not important to purchase value-added products (for example: jams, honey, baked goods) when deciding to visit an agritourism location; 0= otherwise	0.61	0.49
GENDER	1 if the respondent is a male; 0=otherwise	0.26	0.44
AGE_M65	1 if the respondent is over 65 years old; 0=otherwise	0.01	0.29
ETH_WHT	1 if the respondent's ethnicity is White/Anglo; 0=otherwise	0.88	0.32
INC_80	1 if the respondent has annual income between US$ 60,000-79,999 before taxes for year 2009; 0=otherwise	0.19	0.39
INC_100	1 if the respondent has annual income between US$ 80,000-US$ 99,999 before taxes for year 2009; 0=otherwise	0.14	0.35
INC_M100	1 if the respondent has annual income more than US$ 100,000 before taxes for year 2009; 0=otherwise	0.20	0.40
AVG_DIS	Average miles traveled to direct outlets	6.73	4.61
DIST_NJ	Average miles of New Jersey residences traveled to direct outlets	2.37	4.30
EDU-2YRC	1 if the respondent has a two-year college or technical degree education: 0=otherwise	0.26	0.44
EDU_4YRC	1 if the respondent has a four-year college education: 0=otherwise	0.30	0.46
EMP_RE	1 if the respondent is currently retired: 0=otherwise	0.16	0.37
EMP_SE	1 if the respondent is currently self-employed: 0=otherwise	0.01	0.28

The Ordered Probit model is developed as

$WTP_i = \beta_0 + \beta_1$ HOME_GRO$+\beta_2$ WTP_HELP $+\beta_3$ AG_HELP $+\beta_4$QUAL
$+\beta_5$ PRICE$+\beta_6$ G_Q$+\beta_7$ G_P $+\beta_8$ ORGANIC $+\beta_9$ GMO
$+\beta_{10}$ MKTING_M$+$ β_{11} MKTING_B$+\beta_{12}$ MKTING_S $+\beta_{13}$ MKTING_P
$+\beta_{14}$ OFM_SP$+\beta_{15}$ VAL_ADD $+\beta_{16}$ RESI_1$+\beta_{17}$ RESI_3 $+\beta_{18}$ AVG_DIST
$+\beta_{19}$ DIST_NJ + β_{20} Gender$+$ $+\beta_{21}$ AGE_M65 $+\beta_{22}$ ETH_WHT $+\beta_{23}$ INC_80
$+\beta_{24}$ INC_100$+\beta_{25}$ INC_M100 $+\beta_{26}$ EDU_2YRC $+\beta_{27}$ EDU_4YRC $+\beta_{28}$ EMP_SE
$+\beta_{29}$ EMP_RE + ε_i…….....….(4)

RESULTS AND DISCUSSION

Table 2 & 3 provides the results of an Ordered Probit model with estimated coefficients and marginal effects of the selected explanatory variables. The overall model is significant with the McFadden's R-square of 0.04. The correct percentage count is 44 percent, which is estimated over a third of the prediction.

Table 2. Ordered probit parameter estimates of WTP at direct market outlets

Sl. No	Variable	Coefficient	Standard Error	Marginal Change		
				Willing to pay a low premium 1-5%	Willing to pay a medium premium 6-10%	Willing to pay a high premium 11% and above
1	Constant	0.4951	0.2518			
2	RESI_1	0.0710	0.1041	-0.0228	-0.0026	0.0254
3	RESI_3	-0.0707	0.1041	0.0235	0.0011	-0.0246
4	HOME_GR	0.0003	0.0005	-0.0001	0.0000	0.0001
5	WTP_HELP***	0.3101	0.1106	-0.1081	0.0058	0.1023
6	GENDER	0.3472	0.3908	-0.1073	-0.0192	0.1265
7	AGE_M65	-0.0007	0.0005	0.0002	0.0000	-0.0002
8	ETH_WHT**	0.0009	0.0004	-0.0003	0.0000	0.0003
9	ORGANIC	0.0005	0.0005	-0.0002	0.0000	0.0002
10	GMO	-0.0002	0.0005	0.0001	0.0000	-0.0001
11	QUAL	0.0198	0.2497	-0.0065	-0.0004	0.0070
12	PRICE*	-0.1779	0.1020	0.0578	0.0051	-0.0630
13	G_Q	-0.5249	0.3994	0.1841	-0.0144	-0.1697
14	G_P*	0.1779	0.1021	-0.0557	-0.0088	0.0646
15	MKTING_M	-0.1071	0.0860	0.0345	0.0037	-0.0382
16	MKTING_B	0.1420	0.0896	-0.0459	-0.0045	0.0504

Sl. No	Variable	Coefficient	Standard Error	Marginal Change		
				Willing to pay a low premium 1-5%	Willing to pay a medium premium 6-10%	Willing to pay a high premium 11% and above
17	OFM_SP	0.0004	0.0003	-0.0001	0.0000	0.0001
18	VAL_ADD	0.0003	0.0004	-0.0001	0.0000	0.0001
19	INC_80	-0.1228	0.0872	0.0412	0.0012	-0.0424
20	INC_100	0.1007	0.0928	-0.0323	-0.0038	0.0360
21	INC_M10	0.0205	0.0862	-0.0067	-0.0006	0.0072
22	AG_HELP*	-0.0008	0.0005	0.0003	0.0000	-0.0003
23	AVG_DIS**	0.0210	0.0104	-0.0069	-0.0005	0.0074
24	DIST_NJ**	-0.0212	0.0105	0.0070	0.0005	-0.0075
25	MKTING_S	-0.1189	0.0849	0.0388	0.0032	-0.0420
26	MKTING_P	0.0838	0.0812	-0.0274	-0.0022	0.0296
27	EDU_4YRC	-0.0145	0.0801	0.0048	0.0003	-0.0051
28	EMP_SE	0.1257	0.0991	-0.0398	-0.0056	0.0454
29	EMP_RE	-0.1255	0.0990	0.0422	0.0010	-0.0432
30	EDU_2YRC***	-0.2945	0.0848	0.1005	-0.0010	-0.0995

McFadden R^2:0.04 Chi squard: 59.03

Degrees of freedom: 29 Overall Model Significance: 0.00

**** Significant at 1%; **Significant at 5%; * Significant at 10%*

Table 3. Ordered probit model prediction success of the WTP

Actual Value	Predicted			Correct Total
	0	1	2	
0	10	98	6	114
1	9	147	25	181
2	5	95	31	131
Total	24	340	62	426

Number of correct predictions: 188 Percentage of correct predictions: 44%

The results show that the marginal effect for consumers' willingness to pay more to help preserve farmland or local business is highly significant.

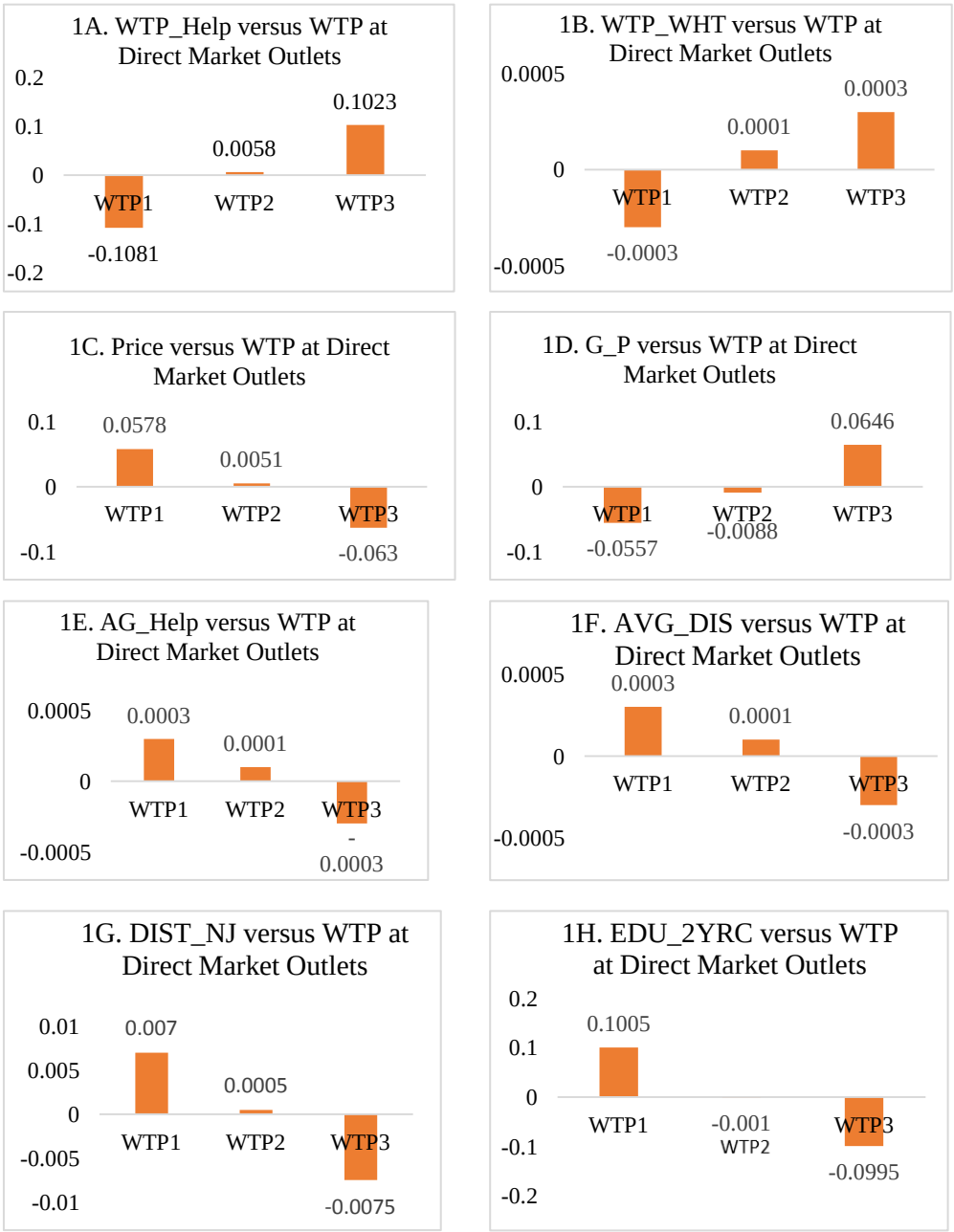


Figure 1 (A-H). Impact of marginal effects on WTP at direct market outlets

As shown in figure 1A, the marginal effect of WTP_HELP (WTP3- high premium) is 0.10, which means that the respondent who values farmland preservation is 10 percent more likely to be willing to pay a high premium for fresh greens sold at direct markets compared to those who think otherwise. A similar result reported that consumers are willing to spend time and money to support local food production (Painter, 2007; Baker, Hamshaw, & Kolodinsky (2009). However, the marginal effect of *WTP_HELP* (WTP_1 low premium) is -0.11 (Figure-1A), which means that the respondent who is preferring to preserve farmland will be around 11 percent less likely to be willing to pay a low premium for fresh greens sold at direct market outlets compared to those who think otherwise. This statistical result is consistent with the theoretical belief that higher the awareness of farmland protection, higher the premium a concerned consumer is willing to pay.

Another environmental awareness related variable, which indicates that agriculture will help maintain open space/greenery, is also significant. Similarly, Williams and Hammitt (2001) and Underhill and Figueroa (1996) studies show that consumers WTP to pay for organic foods is related to the perception of environmentally friendly and supportive of small-scale agriculture and local rural communities. The marginal effect of *AG_HELP* (WTP_1 low premium) is 0.0003 (Figure 1E). Although low in magnitude, the respondent who believes that agriculture will help maintain open space/greenery is more likely to pay a low premium compared to those who do not believe so. On the other hand, the marginal effect of *AG_HELP* (WTP_{3-} high premium) is -0.0003, which means that the respondent who is believes in open space/greenery is less likely to be willing to pay a high premium compared to those who do not believe in open space/greenery. As one can observe from figure 1, greenery awareness is an important concept at a low premium markup.

The price of fresh produce is a crucial factor determining consumer's willingness to pay at direct market outlets. The price variable from the survey maps out individual shopper's attitudes towards the prices of fresh produces in direct market outlets (Figure-1C). The marginal effect of price (WTP_{1-} low premium) is 0.06, which denotes that the respondent who thinks that the prices of produce are better at direct markets are 6 percent more likely to pay a low premium at direct market outlets because they think prices of produce is better at direct market outlets compared to other markers. However, the marginal effect of price (WTP_{3-} high premium) is 0.06, which means that individual shoppers are 6 percent less likely to pay a high premium compared to those who thoughts otherwise. From the above observation, the direct market outlet operators must be tactical at marking the prices of goods if they want to increase their revenues. An interaction term of gender and price was included in this analysis. The marginal effect of *G_P* (WTP_{3-} high premium) is 0.06 (Figure 1D), which means that male shoppers who also think that price of fresh produce is better at direct market outlets are 6 percent more likely to pay a high premium compared to female shoppers who don't think the price is better at direct market outlets as shown in figure-1D. However, in another study, an opposite's relation was reported that

females were more likely to pay a higher price (Brown, 2003).Moving onto demographic variables, the marginal effect of ethnicity WTP_WHT (WTP3- high premium) is 0.0003, which means that Caucasians are more likely to be willing to pay a high premium compared to those of other ethnicities (Figure-1B). They are, however, less likely to be willing to pay a low premium compared to those of other ethnicities. This variable is interestingly discovered, as it has not played much significance in past similar studies. The marginal effect of *ECU_2YRC (*WTP1 -Low Premium*)* is 0.1005 (Figure-1H), which means that a two-year college degree respondent is 10 percent more likely to be willing to pay a low premium compared to those with other educational levels. They are also less likely to be willing to pay a medium and high premium compared to those with other educational levels. This could imply that the magnitude of willing to pay more at direct market outlets are educationally related but will be influenced by other consumer behavior and utility maximization theories.

On average, New Jersey residences are less likely to be willing to pay a higher premium for fresh produces for each additional mile they travel. Looking at the marginal effect of *DIST_NJ* (WTP_{3-} high premium) is -0.01, which means that they will be around 1 percent less likely to be willing to pay a high premium based on each additional mile they travel (Figure-1G). However, we discovered a similar relationship for the mid-Atlantic fresh greens shoppers. On average, as the travel distance increases, the likelihood of paying a higher premium decrease based on each additional mile they travel ((Figure 1F).

CONCLUSION

This study examined the relationships between consumer willingness to pay a premium for direct marketing outlets produce and their economic, demographic, and produce attributes. Based on these results, farmers can develop marketing strategies to increase the profitability of farm business. The results of this study have important implications for the agricultural industry. The understanding of the consumer expectations and demands will assist in the successful placement of the food products in the direct marketing and agritourism outlets. This study may serve as an outreach tool to reach the potential consumers. The findings of this study will also aid industry to develop strategies capable of better anticipating and perhaps bringing about changes in market demand relative to novel products.

REFERENCES

Baker, D., Hamshaw, K., and Kolodinsky J. (2009). Who shops at the market? Using consumer surveys to grow farmers' markets: Findings from a regional market in Northwestern Vermont. *Journal of Extension*, 47(6), 1-9.

Balogh Péter, Dániel Békési, Matthew Gorton, József Popp and Lengyel, P. (2016). Consumer willingness to pay for traditional food products. *Food Policy*, 61, 176-184.

Brown, C. (2003). "Consumers' Preferences for Locally Produced Food: A study in Southeast Missouri. *American Journal of Alternative Agriculture*, 18, 213-224.

Chiu, Hoi. Yan., Chan, Chung-Shing., Marafa, and Lawal, M.(2016). Local perception and preferences in nature tourism in Hong Kong, *Tourism Management Perspectives*, 20, 87–97.

Dominique Gumirakiza J., Kynda, R.C., and Ryan, B. (2016). Bosworth consumer preferences and willingness to pay for bundled fresh produce claims at farmers' markets. *Journal of Food Products Marketing*, 1-19.

Govindasamy Ramu,Surendran Arumugam, Xinling You and Isaac Vellangany. (2015). Willingness to buy organically grown ethnic greens and herbs: A consumers with reference to the east-coast region of United States, Agricultural Economics Research Review, published by Agricultural Economics Research Association, India, 28(2), 213-222.

Govindasamy, Surendran Arumugam and Isaac Vellangany. (2014). The Influence of Country-of-Origin Labeling for Lentils on Consumer Preference: A Study with Reference to Sri Lanka, *The IUP Journal of Marketing Management*, 13(3), 31-43.

Greene, William H., and David, A.H.(2010). Modeling ordered choices: A primer, Cambridge University Press.

Henderson, P.L., and Linstrom, H.R. (1982). Farmer to consumer direct marketing: Selected States, 1979-80. *Statistical Bulletin No. 681*. Washington, D.C.: United States Department of Agriculture, Economic Research Service.

Kline, C., Barbieri, C., and Chantell, L.C. (2016). The influence of agritourism on niche meats loyalty and purchasing, *Journal of Travel Research*, 55(5), 643-658.

Koutsouris, A., Gidarakou, I., Grava, F., and Michailidis, A. 2014. The phantom of agritourism and agriculture symbiosis? A Greek case studies. *Tourism management Perspectives*, 12:94-103.

McCormack, L.A., Laska, M.N., Larson, N.I., and Story, M. (2010). Review of the nutritional implications of farmers' markets and community gardens: A Call for evaluation and research efforts, *Journal of the American Dietetic Association*, 110, 399-408.

Onianwa, O., Mojica, M., and Wheelock, G.(2006). Consumer Characteristics and Views Regarding Farmers Markets: An Examination of On-site Survey Data of Alabama Consumers. *Journal of Food Distribution Research*, 37, 119-125.

Onyango, B., Govindasamy, R., Alsup-Egbers., Clydette, M. (2015). Uncovering success attributes for direct farmers' markets and agritourism in the Mid-Atlantic region of the United States. *International Food and Agribusiness Management Review*, 18(2), 63-78.

Painter Kathleen. (2007). An analysis of food-chain demand for differentiated farm commodities: implications for the farm sector, report to Ag of the Middle July 23, 2007, center for Sustaining Ag & Natural Resources Washington State University.

Surendran, A., and Sekar, C. (2010). An economic analysis of Willingness to Pay (WTP) for conserving the biodiversity, *International Journal of Social Economics, Emerald Group Publishing Limited,* United Kingdom, 37(8), 637-648.

Surendran, A., and Sekar, C. (2011). A Comparative analysis on the socio-economic welfare of dependents of the Anamalai Tiger Reserve (ATR) in India. *Margin: The Journal of Applied Economic Research,* SAGE Publications, Los Angeles/London/New Delhi /Singapore / Washington DC, 5(3), 361-379.

Surendran, A., and Sekar, C. (2010). An economic analysis of willingness to pay (WTP) for conserving the biodiversity", *International Journal of Social Economics,* 37(8), 637-648.

Tew, C., and Barbieria, C.(2012). The perceived benefits of agritourism: The provider's perspective. *Tourism Management*, 33(1), 215-224.

Underhill, Sheila E., and Enrique, E. Figueroa. (1996).Consumer preferences for non-conventionally grown produce. *Journal of Food Distribution Research*, 27, p. 56-66.

Williams, Pamela R.D., and James, K. Hammit. (2001). Perceived risks of conventional and organic produce: pesticides, pathogens, and natural toxins. *Risk Analysis*, 21 (2), 319-330.

SAARC J. Agri., 15(2): 31-43 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35153*

CHANGES IN THE ZINC CONTENT OF SELECTED BANGLADESHI RICE VARIETIES THROUGH MODIFIED PARBOILING AND MILLING METHODS

S.S. Dipti[1*], C. Hotz[2], K.A. Kabir[3], M. Bipul[4]

[1]Grain Quality and Nutrition Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh
[2]Nutridemics, 85 East Liberty Street, Unit 506, Toronto, ON, M6K 3R4, Canada
[3]Consultant, Uttara Sector-7, Dhaka 1230, Bangladesh
[4]GAIN-Bangladesh, North Gulshan-2, Dhaka-1212, Bangladesh

ABSTRACT

Zinc deficiency is prevalent among women and children in Bangladesh, and methods to increase the zinc content of parboiled rice could contribute to its prevention. We quantified the effect of modified parboiling conditions on zinc content and of the degree of milling on zinc and phytate contents of Bangladeshi rice varieties. Parboiling studies varied the conditions used in the local commercial operations, including pre-steaming and soaking times, change of soaking water, and steaming pressure. Milling studies used 10 Bangladeshi varieties at 0% (brown), 2%, 4%, 6%, 8%, and 10% degree of milling. With ambient soaking water, shorter soaking time was observed with a higher zinc content in brown rice, but not in 10% milled rice, and changing soaking water did not modify zinc content in brown or 10% milled rice. Pre-steaming time and open- *vs.* closed-system steaming had no significant effect on brown or 10% milled rice zinc content. Reducing the degree of milling from 10% to 6% or 4% resulted in a mean increase in zinc content of 27% and 47%, respectively, and an increase in phytate content of 35% and 72%, respectively. Zinc content in milled rice did not appear to be significantly affected by the parboiling conditions tested. While lower degree of milling resulted in higher zinc content, it is uncertain whether the higher phytate content would fully negate this increase by decreasing the bioavailable fraction of zinc. Human studies of zinc bioavailability from Bangladeshi rice at different degrees of milling are warranted.

Keywords: Degree of milling, parboiling, phytate, rice

[*] Corresponding author e-mail: sharifadipti@gmail.com

Received: 25.05.2017 *Accepted: 18.10.2017*

INTRODUCTION

In Bangladesh, zinc deficiency is highly prevalent, affecting 45% of preschool children and 57% of non-pregnant, non-lactating women (ICDDRB et al., 2013). However, large-scale programs to prevent zinc deficiency are not currently being implemented. Rice provides 70% of *per capita* caloric intakes (FAO, 2009) in Bangladesh and hence is the most important staple food. Modifications to the zinc content of rice could provide a useful opportunity to deliver larger amounts of dietary zinc to the population.

Most rice in Bangladesh is parboiled and milled, and these post-harvest processes present potential opportunities to increase the zinc content of rice. Parboiling is the process by which rice paddy is hydrated and the starch then gelatinized by hydrothermal treatment, achieved by soaking and steaming. It has long been known that parboiling substantially increases milled rice thiamin content (Hinton, 1948), and the retention of thiamin in milled parboiled rice was shown to be maximized by optimizing the parboiling conditions (Subba and Bhattacharya, 1966). Some information also suggests that modernized methods of commercial parboiling (e.g., hot soaking, pressure steaming) may not only reduce processing time and improve the quality of the parboiled rice product, but also increase the content of some nutrients, including minerals (Ituen and Ukpakha, 2011 and Mazumder et al., 1960). The impact of these parboiling methods on zinc content of rice has not been studied systematically.

The 'degree of milling' of rice, expressed as the percent by weight of the milling fractions removed, is approximately 8-10% for commercially milled rice. Some studies have quantified the retention of zinc in rice at different degrees of milling, but the results are quite variable, ranging from as low as 55% (Pedersen and Eggum,1983) to >90% (Doesthale et al., 1979) for well milled rice. The potential to increase rice zinc content by using lower degrees of milling has not been quantified for rice varieties commonly consumed in Bangladesh.

The objective of this study was to determine the potential impact of modified parboiling conditions and degree of milling on the zinc content of rice using selected Bangladeshi rice varieties.

MATERIALS AND METHODS

Collection of Samples

We conducted studies using Bangladeshi rice varieties to quantify the effect of reduced degree of milling and of modified parboiling processes on rice zinc content. The conditions tested for parboiling and pre-treatment of rice were simulated to reflect the range of conditions used in local small- and large-scale rice mills and that could potentially be optimized without the need for additional equipment. All studies were conducted in the Grain Quality & Nutrition Laboratories at the Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

Parboiling studies

For these studies, open (non-pressurized) and closed (pressurized) steaming systems were used after soaking. The open-steam method Autoclave was used at 100°C temperature but no pressure, and the pressure steam was obtained by placing the soaked paddy in a pressurized autoclave (Pressure 1kg cm^{-2}) with a water vessel to produce steam.

Soaking raw rice paddy in ambient temperature water without pre-steaming, following by open steaming represents conditions commonly used in traditional and semi-automatic rice mills, where batches of paddy are soaked in large tanks using tube well water to achieve adequate hydration, indicated when paddy husks begin to split, typically after 24-72 hours. During this time, soaking water may be changed every 12 hours to avoid microbial growth and fermentation. Soaked paddy is transferred to open tubular vessels, and steam is applied through a perforated pipe inserted in the center. Pre-steaming paddy prior to soaking has accelerates the time to achieve adequate hydration by raising the temperature of the paddy and soaking water. Pre-steaming followed by soaking and open-system steaming is used in small and large commercial mills using manual and semi-automatic operations. Pre-steaming followed by soaking and closed-system steaming is used in the larger commercial automatic mills. In this case, all steps are performed in a closed (pressurized) tubular tank with steam injectors. Our processing methods were designed to replicate these processes, as described below.

The parboiling studies were conducted in two sets. One set using ambient soaking water conditions and the open-steaming system, which considered soaking time and the intermittent changing of soaking water as variables. The second set used pre-steaming followed by soaking and either open- or closed-system steaming, where pre-steaming time and soaking time were considered as variables. All studies used paddy of a common rice variety, BRRI dhan29, derived from one homogenized batch.

Pre-steaming

Pre-steaming was determined by steaming paddy for two or five minutes in an autoclave at 100 C, 0 g cm^{-2}, with a vessel of distilled water placed inside as a source of steam. After two and five minutes pre-steaming water was added. The temperature of the soaking water increased to 37°C followed by 38°C but eventually water temperature became 30°C.

Soaking

Paddy samples (1 kg) were immersed in 2.5 litre distilled, deionized water (Barnstead Fistreem III Glass Still, Model A56220-857, Fistreem International Ltd., UK, and Barnstead E-Pure Ultrapure Water Purification Systems, Model: D4642-33, Thermo Fisher Scientific, USA). For the first studies, paddy was soaked in ambient temperature water (~25 C) for up to 48 hours, with samples drawn at 0, 12, 24, 36 and 48 hours. In a subset of samples, excess water was decanted at 12 hour intervals starting at 12 hours and replaced with fresh water. For the second studies, paddy was pre-steamed prior to soaking in distilled deionized water for 12 hours, with samples drawn at 0, 3, 6, 9 and 12 hours.

Steaming

Open steam parboiling was replicated by putting the soaked paddy samples in a mesh bag and autoclaving (JSAC-40, JS Research Inc, South Korea) at 100 C, 0 kg cm^{-2} for 30 minutes. A vessel of distilled water served as a source of steam. The closed steam parboiling system was replicated similarly but using the autoclave under pressure (10 minutes, reaching 121 C, 1 g cm^{-2}). Parboiled paddy samples were left to cool and then laid out on individual polyethylene sheets; these were partially dried in the laboratory under a fan and drying was completed under the sun until a moisture content of 13-14% was reached. Dried samples were dehusked and milled to 10% degree of milling following procedures described below.

Milling studies

Ten rice varieties were selected to represent popular varieties produced in the two main growing seasons, Boro (irrigated) and Aman (rainfed). Ten rice varieties name are presented in table 1. Nine of the selected varieties were developed and released by BRRI and one is of Indian origin but popular in Bangladesh. For these studies, one standard soaking and parboiling method was used. Ambient temperature soaking water (25 C for 24 hours) followed by parboiling with the open steaming system described above was used.

Dehusking and milling

Outer husks were removed from dried paddy using a Satake Testing Husker (Model THU-35B, Satake Corporation, Hiroshima, Japan) with rubber rollers coated with polyvinyl chloride compound to avoid mineral contamination. The dehusked brown rice was milled using a Grainman tester mill (Model 60-220-50-DT, Grain Machinery Manufacturing Corporation, Miami, FL, USA). Six different degrees of milling were tested: 0%, 2%, 4%, 6%, 8%, and 10%, where 10% represents well-milled, polished rice, and the lower levels represent under milled rice. The degree of milling was calculated as the percent of outer milling fraction removed by weight using equation (1):

% milling degree = 100 – [weight of milled rice (grams) / weight of brown rice (grams)] × 100

For each rice variety, the milling time in seconds needed to achieve each degree of milling was calibrated using 100 grains, in triplicate, and the milling time was then applied to samples. Each sample was milled in 2 × 100 g lots and pooled.

Table 1. Bangladeshi rice varieties used in studies of degree of milling on zinc and phytate content

Variety name	Season	District of origin	Description
BRRI dhan28	Boro	Barisal	One of most highly produced varieties
BRRI dhan29		Gazipur	One of most highly produced varieties
BRRI dhan47		Satkhira	Popular rice variety, salt tolerant
BRRI dhan49		Rangpur	New variety to replace BR11 with better grain quality
BRRI dhan55		Gazipur	New variety
BRRI Hybrid3		Gazipur	One of most highly produced hybrid varieties
BR11	Aman	Rajshahi	One of most highly produced varieties
BR16		Gazipur	Popular rice variety, low glycemic index
BRRI dhan52		Gazipur	New variety
Swarna		Kushtia	Indian variety, popular among rice millers

Analysis of zinc and phytate content

Primary analysis for these samples was performed using atomic absorption spectrophotometry (AAS; Shimadzu Model AA-6800, Shimadzu Corporation, Tokyo, Japan). Samples were digested following an established method (IRRIASL, 2010). Briefly, 300-400 mg of oven-dried sample was weighed into 50 ml Erlenmeyer flasks, to which were added 12 ml each of 1:10 (v:v) 69-72% $HClO_4$ and 65% HNO_3. Duplicate samples were digested on a hotplate to completion (>7 hours), dissolved and made up to 25 ml using 1% HNO_3, and transferred to polypropylene tubes for AAS analysis. Blanks and quality control samples were subjected to the same digestion procedure. A certified standard reference material (SRM1568a, rice flour, National Institute of Standards and Technology, Gaithersburg, MD, USA) and pooled internal control sample of rice grains were included in each run. Intra-run and inter-run CVs were calculated for the standard reference material. In addition, a subset of samples was submitted to a reference laboratory (Waite Analytical Services, Adelaide, Australia) for duplicate analysis by ICP-OES (Wheal et al., 2011). Phytate content was determined using Dionex liquid chromatography at the School of Biological Sciences, Flinders University, Adelaide, Australia. Phytate was extracted using 1.25% H_2SO_4 and 200 mmol /l NaOH in deonized water was used as an eluant (Kim et al., 2007).

Data analysis

For the degree of milling studies, data on zinc content (μg g^{-1}) are presented on a dry weight basis as the mean ± SD of Boro and Aman rice varieties and all varieties combined. For the parboiling studies, data shown are the mean ± SD of duplicate analysis of the same sample. Differences in zinc content by degree of milling were determined by ANOVA with Tukey's post-hoc analysis. For samples that were not pre-steamed, the effect of soaking time and changing of soaking water on the zinc content of 0% and 10% milled samples were determined independently by ANOVA. For samples that were pre-steamed, the independent effects of pre-steaming time and soaking time on zinc content of 0% and 10% milled rice were similarly determined for the open and closed parboiling systems.

RESULTS AND DISCUSSION

Zinc content at different soaking times, with and without changing water

Longer soaking time resulted in an 11% decrease in zinc content in the brown rice samples between 0 and 48 hours ($P < 0.05$) but no significant effect was observed in the 10% milled rice samples. The zinc content of parboiled brown and milled BRRI dhan29 rice produced by different soaking times and with or without changing soaking water are presented in Table 2. There was no significant effect of changing water on the zinc content of rice in either brown or 10% milled rice samples.

For the samples pre-steamed for 2 minutes and 5 minutes, the soaking water temperature initially rose to 37 C and 38 C, respectively, and then decreased to 30 C in both sets of samples. There was a trend towards decreasing zinc content with increasing soaking time for the brown and 10% milled rice samples at either pre-steaming time, but this was only significant for the samples that were subsequently parboiled with closed system steaming ($P<0.05$) but not with open steaming (Table 3). The zinc content of parboiled BRRI dhan29 rice with different pre-steaming and soaking times, followed by open- or closed-system steaming are presented in Table 3. The longer pre-steaming time of 5 minutes compared to 2 minutes did not have a significant effect on zinc content of brown or 10% milled rice in either the open or closed steam systems.

When data were pooled for samples across all soaking and pre-steaming times, there was no overall significant difference between the open and closed steam parboiling methods on the zinc content of brown rice (23.1 ± 0.9 vs 22.6 ± 0.8, respectively; $P > 0.05$, n=20) or 10% milled rice (11.7 ± 0.6 vs 11.9 ± 1.5, respectively; $P > 0.05$, n=20).

Zinc and phytate contents of brown and milled rice by degree of milling

The zinc and phytate contents of brown and milled rice by degree of milling are pre sented in table 4. A step-wise decrease in zinc content was observed with increasing degree of milling for boro and aman parboiled rice varieties alike. Figure 1 shows with all rice varieties combined, zinc content of milled rice was significantly lower than in brown rice starting at 4% degree of milling; the retention of zinc was reduced to approximately three-quarters at 4% degree of milling, two-thirds at 6%, and about half at 10% degree of milling.

Table 2. Zinc content of parboiled brown and milled BRRI dhan29 rice produced by different soaking times and with or without changing soaking water.

Rice type	Treatment	Soaking time (hours) [a]					Soaking time	Water change
		0	*12*	*24*	36	48	*P* [b]	*P* [c]
		Zinc, µg/g [d]						
Brown rice	No water change	24.7 1.6	± 23.7 0.7	± 24.0 1.6	± 22.8 0.5	± 22.1 0.9	±	
	Water changed [e]	-	-	23.7 2.2	± 23.6 0.2	± 22.0 0.1	±*	ns
10% polished	No water change	14.0 1.5	± 12.7 0.3	± 12.3 0.1	± 12.0 1.1	± 11.3 1.1	±	
	Water changed [e]	-	-	13.8 0.1	± 13.7 1.1	± 12.8 1.3	±ns	ns

[a] Rice paddy was soaked in distilled, deionized water at ambient temperature (25 C). All samples were open parboiled in an autoclave (30 minutes, 100 C, 0 g/cm^2).

[b] A statistically significant effect of soaking time was tested for by ANOVA:*, P <0.05; ns = non-significant.

[c] A statistically significant effect of changing water was tested for by ANOVA with all soaking times combined (P <0.05); ns = non-significant.

[d] Zinc content was determined by AAS and is expressed on a dry weight basis. Data are presented as the mean ± SD of duplicate analysis of the same sample.

[e] Soaking water was changed after the 12, 24, and 36 hour time points.

Phytate content was significantly reduced with higher degrees of milling. The greatest reduction in phytate retention (i.e. 39%) occurred between 0% and 2% degree of milling, whereas the reduction in zinc retention was more incremental, with a similar percentage reduction at increasing levels of degree of milling (Figure 1). The phytate: zinc molar ratio, a predictor of zinc bioavailability, tended to decrease with increasing degree of milling. However, this apparent reduction was only statistically significant between brown rice (0% degree of milling) and all other milled forms; the phytate:zinc molar ratio was not significantly different between any degree of milling from 2% to 10% (Table 4).

Table 3. Zinc content of parboiled BRRI dhan29 rice with different pre-steaming and soaking times, followed by open- or closed-system steaming.

		Soaking time (hours) [a]					Soaking time	Pre-steaming time
Rice type	Pre-steaming time (minutes)	0	3	6	9	12	*P* [b]	*P* [c]
		Zinc, µg/g [d]						
Open steaming								
Brown rice	2	24.0 0.6	± 23.8 1.1	± 23.8 1.1	± 21.5 0.1	± 21.4 0.1	±	
	5	23.9 0.1	± 23.6 1.3	± 23.1 1.3	± 23.1 0.1	± 23.1 0.1	± ns	ns
10% polished	2	11.9 1.3	± 11.8 1.4	± 11.7 1.5	± 11.5 1.3	± 11.3 0.1	±	
	5	12.7 0.9	± 12.2 1.1	± 12.1 2.5	± 11.4 0.8	± 10.7 1.2	± ns	ns
Closed steaming								
Brown rice	2	24.0 0.8	± 23.1 0.2	± 22.8 0.1	± 21.8 0.1	± 21.6 0.3	±	
	5	23.3 0.6	± 23.1 0.4	± 22.7 0.1	± 22.3 1.7	± 21.4 0.6	± *	ns
10% polished	2	15.6 1.5	± 12.7 0.5	± 11.3 2.2	± 11.1 0.2	± 10.7 0.8	±	
	5	12.8 0.7	± 12.5 1.3	± 11.0 1.1	± 11.0 0.8	± 10.5 0.8	± *	ns

[a] Rice paddy was soaked in distilled, deionized water at ambient temperature (25 C). All samples were open parboiled in an autoclave (30 minutes, 100 C, 0 g/cm^2).

[b] A statistically significant effect of soaking time was tested for by ANOVA:*, P <0.05; ns = non-significant.

[c] A statistically significant effect of changing water was tested for by ANOVA with all soaking times combined (P <0.05); ns = non-significant.

[d] Zinc content was determined by AAS and is expressed on a dry weight basis. Data are presented as the mean ± SD of duplicate analysis of the same sample.

[e] Soaking water was changed after the 12, 24, and 36 hour time points.

The rice flour SRM was analyzed for zinc content 6 times with each run, over 6 days, for 36 measurements. The zinc content was 19.9 ± 0.3 µg g^{-1}, just within the upper certified range of 19.4 ± 0.5 µg g^{-1}. Reproducibility was very high, with an inter-run CV of 1.6% and a mean intra-run CV of 1.5%. The correlation coefficient for zinc content in a subset of n=60 samples analyzed in the reference laboratory was r=0.96 (P < 0.001).

Table 4. Zinc and phytate contents of brown and milled rice by degree of milling (mean ± SD)

Degree of milling [a]	0%	2%	4%	6%	8%	10%	*P* [b]
Zinc, µg/g dry weight [c]							
Boro rice varieties (n=6)	19.3 ± 1.8 [1]	16.9 ± 1.0 [2]	14.8 ± 0.8 [3]	12.7 ± 0.6 [4]	11.2 ± 0.4 [4,5]	10.5 ± 0.9 [5]	**
Aman rice varieties (n=4)	17.2 ± 3.9 [1]	14.2 ± 2.9 [1,2]	13.1 ± 3.7 [1,2]	11.5 ± 4.0 [1,2]	9.4 ± 4.6 [1,2]	8.3 ± 4.1 [2]	*
All varieties (n=10)	18.5 ± 2.8 [1]	15.8 ± 2.3 [1,2]	14.1 ± 2.4 [2,3]	12.2 ± 2.4 [3,4]	10.5 ± 2.8 [4]	9.6 ± 2.7 [4]	**
Phytate, mg/g dry weight	9.23 ± 1.66 [1]	5.65 ± 0.93 [2]	4.33 ± 0.65 [2,3]	3.45 ± 0.98 [3,4]	2.94 ± 1.15 [3,4]	2.55 ± 0.75 [4]	**
Phytate:zinc molar ratio [d]	49.9 ± 8.2 [1]	35.8 ± 6.3 [2]	31.0 ± 5.2 [2]	28.1 ± 5.7 [2]	28.2 ± 8.9 [2]	26.6 ± 4.1 [2]	**

[a] Degree of milling is expressed as the % polish (outer layers of whole rice grain including bran and germ portions) by weight determined as: 1- [weight of polish/weight of brown rice grain] × 100, in 100 grains.

[b] One-way ANOVA; *P<0.05, **P<0.001

[c] Zinc content was expressed on a dry weight basis as the mean ± SD across varieties. Unlike superscripts indicate means that are significantly different from each other (Tukey's post-hoc, $P < 0.05$).

[d] The phytate zinc molar ratio was calculated as: (mg phytate/660) / (mg zinc/65.4), where 660 is the molecular weight of phytate and 65.4 is the molecular weight of zinc.

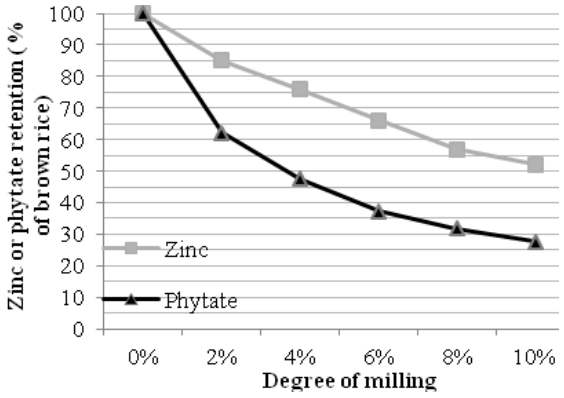


Figure 1. Zinc and phytate retention of rice by degree of milling, as the percent of content in brown rice. Retention of zinc at each degree of milling was determined from the mean of ten rice varieties. Phytate content was analyzed in a subset of samples only with the following sample sizes by degree of milling: 0%, n=9; 6%, n=2; 8%, n=7; 10%, n=9. There were no samples analyzed for phytate content at 4% or 6% degree of milling.

These systematic studies provide quantitative measurements of the magnitude of increase in zinc content that may occur with use of lower degrees of milling of Bangladeshi rice varieties. They also determined that several variables in parboiling conditions have minimal to no effect on rice zinc retention. Longer soaking time was associated with a small significant decrease in zinc retention, but only in brown rice when soaked at ambient temperatures, and in 10% milled or brown rice when pre-steamed followed by closed steam parboiling. Changing the ambient soaking water at 12 hour intervals did not significantly affect rice zinc content. These results fill information gaps with regard to the potential to maximize retention of zinc in parboiled, milled rice products in Bangladesh.

Higher degrees of milling had a linear decreasing effect on the zinc content of parboiled rice, where at the highest level tested (i.e. 10%) zinc content was reduced by half. While some previous studies had observed a negligible (Doesthale et al., 1979) or relatively modest decline (Liang et al., 2008) in zinc content of rice with 10-12% degree of milling, the magnitude of decrease observed in this study of 10 Bangladeshi rice varieties is consistent with the range observed in other studies. A 10-12% degree of milling resulted in retention of zinc of 73% (Villareal et al., 1991) and 55% (Pedersen and Eggum, 1983) compared to brown rice. The reasons for this variability across studies may be attributed to differences in the localization of zinc in the different fractions of the grain as a result of either genetic or environmental variation. In the present study, zinc retention ranged from 35 to 69%, suggesting that a substantial portion of zinc is located in the outer layers in these Bangladeshi varieties.

The degree of milling of commercial rice is typically 8-10% (Kennedy et al., 2002) or more. Using 10% degree of milling as the baseline for current practice, reducing milling to 6% or 4% could result in a 27% or 47% increase in rice zinc content, respectively. While brown rice has the highest zinc content, promoting brown rice consumption may not be considered optimal. Brown rice has an earthy flavor, tougher texture, and longer cooking time than well-milled rice, and hence it may be more difficult to influence consumers to choose brown rice. In contrast, undermilled rice has been found to have greater acceptance among consumers when tested under controlled study conditions (Roberts, 1979 and Billiris et al., 2012) and it may thus be more likely to influence consumers to choose undermilled rice than brown rice. More comprehensive consumer testing would be required to define an acceptable level of under milling.

Zinc bioavailability

Another critical issue with regard to improving the adequacy of dietary zinc intakes is that of zinc bioavailability. While on average, zinc content could increase by 30-50% compared to well-milled rice, the amount of absorbable zinc may not be greater than in well-milled rice due to the higher phytate content. Reducing the degree of milling from a baseline of 10% results, on average, in a higher phytate content, but not in the

phytate: zinc molar ratio, which is a strong predictor of zinc bioavailability in humans (Miller et al., 2007). This indicates that the benefit of a higher rice zinc content at lower degrees of milling may be somewhat minimized by having a lower bioavailability, but this may not be significant. To our knowledge, zinc bioavailablity from rice milled to different degrees has not been studied in humans. In a rat study, the amount of bioavailable zinc from rice was in the order of brown > undermilled > well milled (Hunt et al., 2002). However, adult rats may not be an appropriate model for human zinc absorption [19] as they are known to produce intestinal phytase. Zinc absorption from meals based on 95% extraction vs 80% extraction wheat was measured in human isotopic tracer studies (Rosado et al., 2009). Despite the higher zinc content of the 95% extraction wheat diet, the amount of zinc absorbed (1.6 mg day^{-1}) was similar to that from the 80% extraction wheat (1.5 mg day^{-1}), suggesting that the additional zinc content was not sufficient to compensate for the lower bioavailability.

It was previously demonstrated that modifications to the soaking and steaming conditions of parboiling altered the thiamin content in milled rice (Subba and Bhattacharya, 1966), increasing with longer soaking time and higher temperature. For example, the thiamin content of parboiled milled rice was 28% higher when soaked at 60C than when soaked at room temperature prior to steaming. Based on these studies, it was also suggested that an inward migration of soluble thiamin into the rice endosperm only occurred after steaming, and thus was likely associated with the gelatinization process (Subba and Bhattacharya, 1966). Hence steaming conditions might also modify the migration of nutrients from the outer layers towards the endosperm. We did not consistently observe similar effects of soaking time or pre-steaming time on zinc content of rice (Table 2 and 3). The differences in migration of zinc under parboiling conditions could be related to its solubility. For example while thiamin in rice may be readily soluble in water, zinc may be bound to proteins (Schjoerring et al., 2009) that could limit its migration in the grain.

The effects of pre-steaming time and closed vs open steaming on zinc content were also not important and, as a result, specific recommendations for modifying procedures for the purpose of increasing rice zinc content are not justified.

Increasing the content of bioavailable forms of zinc could provide a useful opportunity to deliver larger amounts of dietary zinc to the Bangladeshi population, which has been shown to have higher rates of deficiency than for other nutrients (ICDDR, B et al., 2013). Studies to consider all possibilities to achieve this in the context of local rice processing systems are important as zinc deficiency is directly associated with increased risk of morbidity and mortality in children due to diarrhea, pneumonia, and malaria (Black et al., 2008). It is also a major risk factor for childhood growth stunting, which is associated with increased risk of death from infectious diseases (Black et al., 2008), impaired cognitive function, lower attained education, and reduced earning potential (Victora et al., 2008).

CONCLUSION

In conclusion, of the post-harvest processing modifications tested here, reducing the degree of milling would have the greatest impact on the zinc content of Bangladeshi rice. Determining the relative bioavailability of zinc at different degrees of milling in human isotopic tracer studies would provide critical information to assess the biological efficacy of this strategy.

ACKNOWLEDGEMENTS

This study was coordinated by the Global Alliance for Improved Nutrition (GAIN). The research was made possible by the generous support of the American people through the US Agency for International Development (USAID). The contents are the responsibility of GAIN and do not necessarily reflect the views of USAID or the United States Government.

REFERENCES

Billiris, M.A., Siebenmorgen, T.J., and Wang, Y.J. (2012). Rice degree of milling effects on hydration, texture, sensory and energy characteristics. Part 2. Cooking using fixed, water-to-rice ratios. *Journal of Food Engineering*, 113, 589-597.

Black, R., Lindsay, H., Bhutta, Z., Caulfield, L., and De Onis, M. (2008). Maternal and child under-nutrition: global and regional exposures and health consequences. *Lancet*, 371, 243-60.

Doesthale, Y. G., Devara, S., Rao, S., and Belavady, B. (1979). Effect of milling on mineral and trace element composition of raw and parboiled rice. *Journal of the Science of Food and Agriculture*, 30, 40-46.

FAO. (2009). Food Balance Sheets [Bangladesh]. [Accessed online, September 23, 2013: http://faostat3.fao.org/faostat-gateway/go/to/download/FB/FB/E].

Hinton, J.J.C. (1948). Parboiling treatment of rice. *Nature*, 4128, p. 913-915.

Hotz, C. (2005). Evidence for the usefulness of animal models, in vitro dialysability, caco-2 cell models, and algorithms to predict zinc bioavailability in humans. *International Journal for Vitamin and Nutrition Research*, 75, 423-435.

Hunt, J.R., Johnson, L.K., and Juliano, B.O. (2002). Bioavailability of zinc from cooked Philippine milled, undermilled, and brown rice as assessed in rats by using growth, bone zinc, and zinc-65 retention. *Journal of Agricultural and Food Chemistry*, 50, 5229-5235.

ICDDR, B, UNICEF, GAIN, and Institute of Public Health and Nutrition. (2013). National Micronutrients Status Survey 2011-12, Final Report, January 2013. Dhaka: ICDDR, B, 2013 [Accessed on September 9, 2014,http://www.icddrb.org/publications/cat_view/10043-icddrb-documents/10058-icddrb-reports-and-working-papers/14275-survey-reports].

IRRI (International Rice Research Institute) Analytical Service Laboratory, Crop and Environmental Services.(2010). Modified ASL digestion procedure for ICP analysis of plant materials. Revision no. 3, QM-AD002-AP02. IRRI, Los Baños, Philippines.

Ituen, E.U.U., and Ukpakha, A.C. (2011). Improved method of par-boiling paddy for better quality rice in Akwa Ibom state of Nigeria. *World Journal of Applied Science Technology*, 3, 31-40

Kennedy, G., Burlingame, B., and Nguyen, N. (2002). Nutrient impact assessment of rice in major rice-consuming countries. International Rice Commission Newsletter volume 51 [Proceedings of the 20th Session of the International Rice Commission, Bangkok, Thailand, 23-26 July, 2002].

Kim, J., Paik, H.Y., Joung, H., Woodhouse, L.R., Li, S., and King, J.C. (2007). Effect of dietary phytate on zinc homeostasis in young and elderly Korean women. *Journal of the American College of Nutrition*, 26, 1-9.

Liang, J., Li, Z., Tsuji, K., Nakano, K., Nout, R.M.J., and Hamer, R.J. (2008). Milling characteristics and distribution of phytic acid and zinc in long-, medium- and short-grain rice. *Journal of Cereal Science*, 48, 83-91.

Mazumder, A.C., Bose, A.N., Ganguli, N.C., and Guha, B.C. (1960). Pilot plant studies on parboiling of rice – III Effect of hot soaking and mechanical drying on the nutritive value of parboiled rice. *Journal of Biochemical and Microbial Technology Engineering*, 2, 447-451.

Miller, L.V., Krebs, N.R., and Hambidge, K.M. (2007). A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. *Journal of Nutrition*, 137, 135-141.

Pedersen, B., and Eggum, B.O. (1983). The influence of milling on the nutritive value of flour from cereal grains. 4. Rice. *Plant Food for Human Nutrition*, 33, 267-278.

Roberts, L.R. (1979). Composition and taste evaluation of rice milled to different degrees. *Journal of Food Science*, 44, 127-129.

Rosado, J.L., Hambidge, K.M., Miller, L.V., Garcia, O.P., Westcott, J., Gonzalez, K., Conde, J., Hotz, C., Pfeiffer, W., Ortiz-Monasterio, I., and Krebs, N.F.(2009). Absorption of zinc from wheat is enhanced by bio-fortification. *Journal of Nutrition*, 139, 1920-1925.

Schjoerring, J.K., Persson, D.P., Hansen, T.H., Laursen, K.H., and Husted, S. (2009). Zinc and iron speciation in the cereal grain. The Proceedings of the International Plant Nutrition Colloquium XVI. University of California, Davis, Davis CA [Accessed online September 8, 2014: www.escholarship.org/uc/item/31h065fh].

Subba Rao, P.V., and Bhattacharya, K.R. (1966). Effect of parboiling on thiamine content of rice. *Journal of Agricultural and Food Chemistry*, 14, 479-482.

Villareal, C.P., Maranville, J.W., and Juliano, B.O. (1991). Nutrient content and retention during milling of brown rices from the International Rice Research Institute. *Cereal Chemistry*, 68, 437-439.

Victora, C.G., Adair, L., Fall, C., Hallal, P.C., Martorell, R., Richter, L., and Sachdev, H.S. (2008). Maternal and child under nutrition: consequences for adult health and human capital. *Lancet*, 371, 441-459.

Wheal, M.S., Fowles, T.O., and Palmer, L.T. (2011). A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. *Anal Methods*, 3, 2854-2863.

SAARC J. Agri., 15(2): 45-5 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35157*

FARM-MADE FEED FOR POLYUNSATURATED FATTY ACIDS (PUFAs) RICH CARP PRODUCTION IN INDIA: A CASE STUDY

B.N. Paul[1*], S.S. Giri[2], S. Chanda[1], S.C. Rath[2] and A.K. Datta[1]

[1]Regional Research Center, ICAR-Central Institute of Freshwater Aquaculture, Rahara, Kolkata-700118, West Bengal, India

[2]ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751002 Odisha, India

ABSTRACT

An 8 months feed demonstration program was conducted in the ponds of Ramakrishna Mission, Belur Math, West Bengal, India. The program aimed at providing hands-on training to trainees at Samaj Sevak Sikshan Mandir (SSSM) on farm-made feed formulation and use in aquaculture. Two ponds were selected, one at Shilpamandira of 0.1 h and fish were fed Feed-1, and another of 0.08 h at Samaj Sevak Sikshan Mandir (SSSM) and fed Feed-2. Indian major carps, *Catla catla* (catla) and *Labeo rohita* (rohu) were stocked at 4500 fish ha^{-1}. The stocking size of rohu was 80-90g and of catla was 220-245g. Locally available low cost feed ingredients, rice bran, mustard oil cake, til oil cake and linseed oil sludge were used to formulate and prepare two farm-made mash feeds (Feed-1 and Feed-2). The fish were fed at 2% of total fish biomass in the ponds and were fed through bag feeding. At the end of the study rohu grew to 1.07 kg and catla to 1.6 kg in Shilpamandira pond (Feed-1), and rohu to1.5 kg and catla to 2.2 kg in Samaj Sevak Sikshan Mandir pond (Feed-2). The net production of fish was 4.9 and 6.8 t ha^{-1} for 8 months with Feed-1 and Feed-2, respectively. Dietary inclusion of linseed-oil-sludge significantly increased the PUFA content in Indian major carps. The feed cost was substantially reduced by replacing mustard oil cake with til oil cake and incorporation of linseed oil sludge, a very low priced ingredient.

Keywords: Omega-3 fatty acids, carp poly culture, linseed oil, unconventional feed

INTRODUCTION

The fisheries sector occupies an important place in the socio-economic development of the country, which envisages livelihood, nutritional security, employment

[*] Corresponding author e-mail: bnpaulcifa@gmail.com

Received: 05.09.2017 *Accepted: 25.10.2017*

generation and export earnings. Indian fisheries occupy the second position in global fish production and second in aquaculture in the world with an annual growth rate of 4.7%, recording 3.2% growth in marine sector and 6.2% in inland sector (Paul and Giri, 2015). The fisheries sector has grown from traditional activity in the early fifties, when India initiated the first five year plan (1950-51 to 1955-56), and now transformed into significant commercial enterprise with impressive growth in production from 0.75 million tons in the 1950s to 10.4 million tons (MT) during 2015-16. The sector has emerged as the largest single employer in the country for more than 14.5million people (DAHAD, 2015-16). About 35% of the Indian population is fish eaters and the per capita consumption is 9.8 kg, whereas present global per capita fish intake is over 20 kg (FAO, 2016) (http://www.fao.org/news/story/en/item/icode/). Inland fisheries have emerged as a major contributor to the overall fish production in the country with a present share of 64.07 % in total fish production. Within inland fisheries there is great shift from captured fisheries to aquaculture and at present freshwater aquaculture shares 80% of total inland fish production in India (DAHD, 2015-16).

The rapid expansions of the aquaculture industry, along with the improvement and change in culture techniques, have increased the demand for fish feeds. Feed cost is considered as the major recurring expenditure in any fish-culture operation. Expenditure on feed alone amounts to 60% of total fish production cost (Paul and Giri, 2015).

Commercial feeds for carp culture are available in the market but small fish farmers are unable to use these feeds because of their high cost. Traditional practice for fish farmers is to use mixture of groundnut oil cake and rice bran or cereals as fish feed. Recently the price of both the ingredients are highly increased which farmers are unable to afford (Rath et al., 2014) to feed the fish.

The Indian Council of Agricultural Research is operating an outreach program on "Fish feeds" among six fisheries research institutes, ICAR-CIFA, Bhubaneswar; ICAR-CIFRI, Barrackpore; ICAR-CIBA, Chennai; ICAR-CMFRI, Kochi; ICAR-DCFR, Bhimtal and ICAR-CIFE, Mumbai. The program has been undertaken to create awareness among the fish farmers across the country on the use of farm-made fish feeds to enhance production and popularization of fish culture in the rural sectors as well besides supporting their livelihood. Being the network leader ICAR-Central Institute of Freshwater Aquaculture under took several farmers awareness programs on fish feeds (Rath et al., 2014), hands-on training on farm feed preparation and feeding demonstration in farmers ponds in Odisha, West Bengal and Karnataka. Use of farm-made feed with locally available feed ingredients in rural aquaculture sectors open a new era for feed based aquaculture. Linseed oil is rich in omega-3 fatty acids and in the present experiment linseed oil sludge has been used as by-product of oil milling industry. The incorporation of linseed oil sludge in farm-made feed would improve the omega-3 fatty acid profile of fish. Keeping in view of the above facts a

feed demonstration program was undertaken at the ponds of Ramakrishna Mission, Belur Math with a view to use the locally available feed ingredients for production of fish enriched with omega-3 fatty acid.

MATERIALS AND METHODS

Pond preparation

Initially ponds were netted out repeatedly for two days with nylon dragnet followed by fry net to take out fish. Both the ponds are located in such places where chances of entry of predator and weed fishes are not there. Pond fertilization was carried out with application of cow dung and single super phosphate at 3 ton ha^{-1} and 75 kg ha^{-1}, respectively as a basal dose one week prior to stocking, with alternating applications every fortnight at 1ton $ha^{-1}month^{-1}$ and 20 kg $ha^{-1}month^{-1}$, respectively (Jena et al., 1999).

Fish maintenance and feeding

Under the Outreach Activity on Fish Feeds, two ponds were selected at Rama Krishna Mission, Belur Math, to provide hands-on training to 30 trainees at Samaj Sevak Sikshan Mandir (SSSM) on farm-made feed. Selected ponds were a) 0.1 ha at Shilpamandira (Feed-1) and b) 0.08 ha at SSSM (Feed-2) under the Sarada Pith unit of Belur Math. Both the ponds were stocked with rohu and catla juveniles at a stocking density of 4500 juveniles ha^{-1} and at a stocking ratio of catla:rohu was 90:10.. The stocking size of rohu was 80-90 g and for catla was 220-245 g. The objective of the program was to train the vocational trainees of RKM Samaj Sevak Sikshan Mandir through learning by doing mode, with a view to focus the utilization of locally available feed ingredients as cheap resources for fish feed making for low cost fish production.

Locally available low-cost feed ingredients were identified by surveying the local oil mills and procured. For the demonstration program two different farm-made feeds were formulated as per nutrient requirement guidelines of NRC (2011) for carps. Two mash feeds were prepared using rice bran, till oil cake, mustard oil cake and linseed sludge in different proportions for feeding the fish (Table 1). Linseed oil sludge is cheaply available in the oil milling industry as one of the by-product of crushing unit. Suitable feed dispensing mechanism were also developed, in which nylon bags were suspended on galvanized wire and connected by a small-pulley so that trainees can provide the feed regularly by standing on the pond side without going into the pond. Fish were fed at 2% of the body weight daily in two divided meals for a period of 8 months. Monthly sampling was done to assess the fish growth, health and calculate the ration requirement. Regular racking of pond water was practiced to release the obnoxious gases from the pond bottom due to accumulation of metabolites.

Table 1. Feed formulations (% as such basis)and proximate composition (% DM basis)

Ingredients	Feed-1	Feed-2
Til oil cake	60	--
Mustard oil cake	--	60
Rice bran	30	30
Linseed oil sludge	10	10
	100	100
Proximate composition		
Dry matter	97.70	97.80
Crude protein	26.32	25.26
Crude Fat	4.61	5.20
Ash	17.78	18.02

Growth parameters

Fishes were weighed at monthly intervals and mortality of fish was recorded. Net weight gain, feed conversion ratio (FCR), specific growth rate (SGR) and percent survival were calculated (Castell and Tiews, 1980).

Proximate composition

Proximate composition of feed and fish tissue samples was analyzed as per AOAC (2005). Water quality parameters such as pH, DO, free CO_2, total alkalinity and temperature were measured employing the method of APHA (2005).

Fatty acid analysis

Pooled samples were extracted for fatty acid analysis by following method of Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared as per Metcalfe et al. (1966). The FAMEs were quantified by injected 1 µl (50:1 split ratio) into (Gas Chromatograph/GC) Perkin Elmer; (CLARUS 480). Fatty acid was quantified through "Total Chrome" software in GC as reported earlier (Paul et al., 2015).

RESULTS AND DISCUSSION

Feed formulation and proximate composition of feed are presented in table 1. The proximate compositions of the feeds are alike ($P>0.05$). The nutrient composition of linseed oil sludge is presented in table 2. The proximate compositions (% DM basis) of linseed sludge was 4.4% crude protein, 19.00% fat and 40.83 % ash. The saturated fatty acids (SFA) was determined as 9.42 % of total fatty acids of the sludge and palmitic acid was the dominating SFA. The MUFA content was 16.83 and oleic acid was the leading fatty acid. The polyunsaturated fatty acid (PUFA) content was 73.76 % of the total fatty acids, where linolenic and linolelaidic acid were the dominant

PUFAs. The water quality parameters are presented in table 3. The water temperature ranged from 21-30^{o}C in two ponds. The water quality parameters like pH, CO_2, total alkalinity (mg l^{-1}) and dissolved oxygen (mg l^{-1}) ranges from 7.4 to 7.8, 4-8, 130-160 and 6.0-8.5 respectively in two different ponds, which is ideal for carp culture (Yadava and Garg, 1992; Hajek and Boyd, 1994, Azad et al. 2004; Swingle, 1969).

Table 2. Proximate composition (% DM basis) and fatty acid composition (% of total fatty acid) of Linseed sludge

Particulars	Linseed sludge
Crude protein	4.40
Crude fat	19.10
Ash	40.80
Fatty acid composition	
C:16 Palmitic acid	9.39
Others	0.03
∑ SFA	9.42
Oleic acid	14.08
Elaidic acid	1.20
Erucic acid	1.45
Others	0.10
∑ MUFA	16.83
Linolelaidic acid	16.83
Linolenic acid	46.05
Others	0.96
∑ PUFA	73.76

Table 3. Hydro biological parameters of ponds with different feed treatments

Particulars	Feed-1	Feed-2
Temperature	21-29^{o}C	22-30 ^{o}C
pH	7.5-7.8	7.4-7.6
CO_2 (ppm)	6-8	4-6
Total alkalinity (ppm)	150-160	130-140
Dissolved oxygen (ppm)	6-8	6-8.5

In the present demonstrated program the variations of total alkalinity were within the productive range for aquaculture ponds which also corroborate with the observations of Wahab et al. (1995) and Kohinoor et al. (1998). Good water quality is characterised by adequate oxygen levels in pond (Chiu (1988). Rahman et al. (1982)

reported that dissolved oxygen content of a productive pond should be 5.0 mg l^{-1} or more.

The fish production performance of different ponds are presented in table 4. The initial stocking size of rohu in two ponds ranged between 83.5 and 87.5 g while catla was 220.0 to 245.5 g. The final average weight of catla and rohu was 1.61 kg and 1.07 kg respectively in the pond of Shilpamandira (Feed-1) whereas respective average weight of catla and rohu in the pond of SSSM (Feed-2) was recorded as 2.20 and 1.51 kg respectively. Survival rate of catla in the two ponds at the time of harvest was recorded to be 88 to 92% while rohu to be 93.3 to 93.91%. The final fish biomass in the pond of Shilpamandira (Feed-1) was registered as 536.30 kg against 586.04 kg in the pond of SSSM (Feed-2), the net weight gain (kg) being 486.44 kg and 544.85 kg respectively. The feed conversion ratio (FCR) was 1.81and while 1.61 in Feed-1 and 2 respectively. The demonstration resulted net production rate of 4.9

Table 4. Fish production in two ponds at Belur math fed on farm-made feeds

Particulars	Feed-1	Feed-2
Pond size (ha)	0.10	0.08
Fish stocked (4500 fish ha^{-1})	450.00	360.00
Initial av. wt.(g) of catla	245.50	220.00
Intial av wt.(g) of rohu	83.50	87.50
Survival at harvest (%): catla	92.00	88.00
Survival at harvest (%) : rohu	93.30	93.910
Final Av. wt.(kg): catla	1.61	2.20
Final Av. wt.(kg) :rohu	1.07	1.51
Total quantity at harvest (kg):		
catla :	74.06	96.80
rohu :	462.24	489.24
Net biomass gain(kg)	486.44	544.85
Total feed intake (kg 8 $month^{-1}$)	880.00	878.00
FCR	1.80	1.60
SGR (%)	1.01	1.13
Net production (t ha^{-1}8 $month^{-1}$)	4.90	6.80
Feed cost (Rs.) kg^{-1}	15.70	18.70
Feed cost (Rs.) kg^{-1} fish production	28.26	29.92

Av. wt = Average weight

and 6.8 t ha^{-1}8 $months^{-1}$ respectively in Feed-1 and 2. At the end of the demonstration the trainees of RKMSSSM developed skill with self confidence to adopt aquaculture practice as one of the avenues to upgrade their livelihood status and scope for self-reliance as well.

Table 5 represents the proximate composition (%w/w basis) of rohu and catla of initial and final samples of both the ponds. The moisture content varies from 69.92 to 74.18 %. The protein content varies from 14.13 to 17.70 %, fat from 2.92 to 4.43% and ash content from 2.62 to 6.05% in catla and rohu of different ponds fed with farm-made feed. The fish carcass composition was higher in both rohu and catla after 8 months of experimental period. The data reported in this experiment is in agreement with the earlier report by Paul et al. (2016)

Table 5. Carcass composition (% w/w basis) of rohu and catla fed with farm-made feed

Particulars	Initial sample		Feed-1		Feed-2	
	Rohu	Catla	Rohu	Catla	Rohu	Catla
Moisture	78.65±0.96	74.44±0.38	74.13±0.94	74.18±0.67	75.29±0.22	69.92±1.11
Protein	12.69±0.30	12.60±0.29	14.13±0.33	14.64±0.04	14.57±0.14	17.70±0.06
Fat	1.77±0.28	2.27±0.06	3.25±0.04	3.85±0.01	2.92±0.07	4.43±0.08
Ash	2.12±0.04	2.41±0.05	3.42±0.07	2.62±0.06	3.82±0.0005	6.05±0.09

Data are presented as Mean± S.E

The fatty acid profile of rohu and catla of initial and final samples of two ponds are presented in table 6. The saturated fatty (SFA) acid content of carcass of rohu at the beginning of the demonstration was 65.88 %, which decreased to 53.57 % and 52.23 % of the total fatty acids, at the end of demonstration, on feeding Feed-1 and Feed-2, respectively. Similarly, in catla the carcass SFA at the beginning was 33.87 % and increased to 61.54 % and 40.72 % with Feed-1 and 2, respectively in the final harvest samples. The predominate fatty acid among SFA was palmitic acid. The palmitic acid was considered as a key to many metabolic processes in fish and other aquatic animals as reported by Ackman and Eaton (1966). In the muscle of rohu, palmitic acid is higher which is in agreement with the earlier report by Paul et al. (2013).

The monounsaturated fatty acid content ranges from 23.67 to 38.76 % in initial carcass sample of catla as well as rohu. However, the MUFA content in harvested sample of rohu and catla were 24.80 to 26.40 and 12.54 to 36.53 %, respectively and the dominating fatty acid of MUFA class is Oleic acid. The polyunsaturated fatty acid (PUFA) content in initial samples were 10.47 to 27.36 % in rohu and catla. The

Table 6. Fatty acid profile of (% of total fatty acid) of rohu and catla

Fatty Acid	Initial Sample		Feed-1		Feed-2	
	rohu	catla	rohu	catla	rohu	catla
Lauric Acid	-	0.22	0.05	0.23	10.23	0.11
Myristic Acid	3.67	5.12	1.10	6.16	2.92	1.89
Pentadeconoic Acid	1.94	1.51	0.24	3.34	2.50	0.92
Palmitic Acid	46.87	19.80	46.08	31.51	25.60	23.63
Heptadeconoic Acid	2.44	2.59	0.22	1.11	4.27	0.90
Stearic Acid	8.16	0.01	4.68	14.27	0.05	10.96
Heneicosanoic Acid		1.81	0.36	2.15	4.25	1.33
Tricosanoic Acid		1.49	0.64	0.31	1.57	0.14
Others	2.80	1.32	0.02	2.46	0.84	0.90
∑ SFA	65.88	33.87	53.57	61.54	52.23	40.72
Palmitoleic Acid	6.21	0.91	0.98	9.89	7.52	4.12
Oleic Acid	15.01	22.67	22.75	-	-	30.4
Eladic Acid	-	11.03	-	-	17.89	-
Eicosanoic Acid	-	2.16	-	-	-	-
Erucic Acid	0.57	0.09	0.78	1.01	0.21	0.91
Others	1.89	1.90	0.29	1.64	0.78	1.10
∑ MUFA	23.67	38.76	24.80	12.54	26.40	36.53
Linoleic Acid	4.66	7.56	12.86	7.97	9.87	13.73
Linolenic Acid	2.70	14.19	6.91	7.09	4.71	4.12
Arachidonic Acid	0.37	1.06	0.02	0.64	0.76	0.92
EPA	1.36	3.95	0.06	2.35	0.99	0.85
DHA	0.70	-	0.89	3.54	3.09	2.15
Others	0.68	0.60	0.91	1.27	1.96	0.97
∑ PUFA	10.47	27.36	21.65	22.86	21.38	22.74
∑ ω3	4.83	17.72	7.96	12.82	9.36	6.30
∑ ω6	5.64	9.64	13.42	10.04	11.57	15.59
ω3: ω6	0.85	1.84	0.59	1.28	0.80	0.40

SFA- Saturated Fatty Acid, MUFA- Monounsaturated Fatty Acid, PUFA-Polyunsaturated Fatty Acid, EPA-Eicosapentaenoic Acid and DHA-Docosahexaenoic Acid.

PUFA content in both the species of the two treatment groups range from 21.38 to 22.86 %. Here the predominant fatty acids are linolenic, linoleic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The EPA content of initial samples was 1.36 to 3.95(%)**.** The EPA content of rohu from both the feed treatment groups ranges from 0.06 to 0.99 % while DHA content in the same species varies from 0.89

to 3.09 (%) in Feed-1 and 2. Value of EPA content in harvested catla of both ponds ranges from 0.85 to 2.35% and DHA content of 2.15 to 3.54 %. Steffens and Wirth (2005) reported that freshwater fish are rich in essential polyunsaturated fatty acid of the n-3and n-6 series, which is in agreement with fillet composition of experimental catla and rohu. Compared to beef and chicken, fish meat contains higher levels of n-3 PUFAs Calder (2004), which are known to be cardio-protective (Sanderson *et al*., 2002) and antithrombotic (Calder, 2004). Fish oils are known to be rich source of essential PUFA of the omega-3 family (Kenari et al., 2009 and Giri et al., 2010). The data on fatty acid profile of catla and rohu as reported in the paper are in agreement with earlier reports (Memon et al., 2011 and Paul et al., 2015). This indicates that the fish reared with farm-made omega-3 feed tailored the fatty acid profile of harvested fish tissue. The feed cost was substantially reduced by replacing mustard oil cake with til oil cake and with incorporation of linseed oil sludge, a very low priced ingredient.

ACKNOWLEDGEMENT

This work is supported by Ministry of Agriculture, Govt. of India under outreach activity on "Fish Feeds". The authors express sincere gratitude to Deputy Director General (Fy.) ICAR and Director ICAR-CIFA for providing necessary facility and taking keen interest to conduct the work. The authors also like to record their deep sense of gratitude with ingenuous regards to Revered Swami Divyananda, Secretary and Swami Shastrarupananda of Saradapitha and Revered Swami Sivakarananda, Principal, Samaj Sevak Sikshan Mandir, Ramakrishna Mission, Belur math for providing infrastructure facilities for the demonstration study at Belur Math, Howrah.

REFERENCES

AOAC. (1995). *Official methods of Analysis*, 18th ed. Association of Official Analytical Chemists, Washinton, D.C.

APHA. (2005). *Standard methods for examination of water and wastewater*, 21st ed. American Public Health Association, Washington, D.C.

Ackman, R.G., and Eaton, C.A. 1966. Some commercial Atlantic herring oils; Fatty acid composition. *Journal of Fisheries Research Board of Canada,* 23, 991-1006.

Azad, M.A.K., Rahman, M.R.Z., Rahman, M.A., Kader, Haque, M.M., and Alam, M.J. 2004. Polyculture of carps, tilapia and pangus using low cost inputs. *Pakistan Journal Biological Science,* 7(11), 1918-1926.

Castell, J.D., and Tiews, K. (1980). Report of the EIFAC, IUNS and ICES working group on *Standardization of Methodology in Fish Nutrition Research*. EIFAC/T36, EIFA/FAO. p. 24.

Calder, P.C. 2004. Long-chain fatty acids and cardiovascular disease: further evidence and insights. *Nutrition Research*, 24, 761-772. Doi: 10.1016/j.nutres.2004.04.008.

Chiu, Y.N. (1988). Water quality management for intensive prawn ponds. In: Y.N. hi, L.M.

Santos and R.O. Juliano (eds), Technical Considerations for the Management and Operation of Intensive Prawn Farm. *Aquaculture Society*, Philippines, p. 102-129.

DAHAD. (2015). Annual Report, 2014-15. Department of Animal Husbandry and Fisheries, Government of India, p.7.

FAO. 2010. FAOSTAT. Food and Agricultural Organization of the United Nations, Rome, Italy.

Folch, J., Less, M., and Stanley, G.H.S. (1957).A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biochemistry*, 226, 497-509.

Giri, S.S., Paul, B.N., Sahoo, S.K., Rangacharyulu, P.V., Rath, S.C., and Mohanty, S.N. (2010). Fish oils and cardio vascular health. *Fishing Chimes*, 30, 37-39.

Hajek, B.F., and Boyd, C.E. (1994). Rating soil and water information for aquaculture. *Aquaculture Engineering*, 13, 115-128.

Jena, J.K., Mukhopadhyay, P.K., and Aravindakshan, P.K. (1999).Evaluation of a formulated diet for raising carp fingerlings in field conditions. *Journal of Applied Icthyology*, 15, 188-192.

Kenari, A.A., Regenstein, J.M., Rezai, M., Tahergorabi, R., Nazari, R.M., Mogaddasi, M., and Kaboli, S.A. (2009). Amino acid and fatty acid composition of cultured Beluga (*Huso huso*) of different ages. *Journal of Aquatic Food Product and Technology*, 18, 245-265.

Kohinoor, A.H.M., Islam, M.L., Wahab, M.A., and Thilsted, S.H. (1998). Effect of mola (*Amblypharyngodon mola* Ham.) on the growth and production of carps in polyculture. *Bangladesh Journal of Fisheries*, 2(2), 111-126.

Memon, N.N., Farah, N.L., Bhanger, M.I., and Balouch, A. (2011). Changes in aftty acid composition in muscle of three farmed carp species (*Labeo rohita, Corrhinus mrigala, Catla catla*) raised under the same conditions. *Food Chemistry*, 126, 405-410.

Metcalfe, L.D., Schmitz, A.A., and Petha, J.R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anaytical Chemists*, 38, 514-515.

NRC. (2011). *Nutrient requirement of Fish and Shrimp*. National Academy Press, Washington D.C.

Paul, B.N., and Giri, S.S. (2015). Freshwater Aquaculture Nutrition Research in India. *Indian Journal of Animal Nutrition*, 32 (2), 113-125.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S., and Giri, S.S. (2015). Fatty acid profile of Indian Major Carp. *Indian Journal of Animal Nutrition*, 32(2), 221-226.

Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S., and Giri, S.S. (2016). Proximate, mineral and vitamin contents of Indian Major Carp. *Indian Journal of Animal Nutrition*, 33(1), 102-107. Doi: 10.5958/2231-6744.2016.00018.9.

Rahman, M.S., Chowdhury, M.Y., Haque, A.K.M.A., and Haq, M.S.(1982). Limnological studies of four ponds. *Bangladesh Journal of Fisheries*, 2-5(1-2), 25-35.

Rath, S.C., Nayak, K.C., Mahanta, K.N., Giri, S.S., Mohanty, S.N. (2014). Carp polyculture by farm feed intervention in farmers pond in Odisha. *Indian Farming*, 64(7), 51-55.

Sanderson, P., Finnegan, Y.E., Williams, C.M., Calder, P.C., Burdge, G.C., Wootton, S.A., Griffin, B.A., Millward, D.J., Pegge, N.C., and Bemelmans, W.J.E. (2002). UK food standards agency alpha-linolenic acid workshop report. *British Journal of Nutrition*, 88, 573-579, doi: 10.1079/BJN2002691.

Steffens, W., and Wirth, M. (2005). Freshwater fish- an important source of N-3 Polyunsaturated fatty acids: A review. *Archives of Polish Fisheries*, 13(1), 5-16.

Swingle, H.S. (1969). Standardization of chemical analysis for waters and pond mud. FAO. *Fisheries Research*, 4(2), 397-421.

Wahab, M.A., Ahmed, Z.F., Haque, M.S., Haque, M.A. and Biswas, B.K. (1995). Effect of frequency of fertilization on the pond ecology and growth of fishes. *BAU Research Progress*, 9, 410-419.

Yadav, N.K., and Garg, S.K. (1992). Relative efficiency of different doses of organic fertilizer and supplement feed utilization under intensive fish farming. *Bioresource Technology*, 42, 61–5.

SAARC J. Agri., 15(2): 57-67 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35161*

CHEMO-INDUCED POD AND SEED MUTANTS IN MUNGBEAN (*Vigna Radiata* L. Wilczek)

M.R. Wani[1*], A.R. Dar[1], A. Tak[1], I. Amin[1], N.H. Shah[1], R. Rehman[1], M.Y. Baba[1], A. Raina[2], R. Laskar[2], M.I. Kozgar[3] and S. Khan[2]

[1]Department of Botany, Abdul Ahad Azad Memorial Govt. Degree College, Bemina, Srinagar-190018, Jammu and Kashmir, India

[2]Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh-202 002, Uttar Pradesh, India

[3]KERB Biotech, Bemina, Srinagar-190018, Jammu and Kashmir, India

ABSTRACT

Mungbean is one of the most important pulse crops due to its nutritive value and maintaining soil fertility through biological nitrogen fixation. Genetic variability is one of the pre-requisite for crop improvement. The present investigation was aimed at to enhance the genetic variability for three quantitative traits viz. pod length, number of seeds per pod and 100-seed weight in M_2 and M_3 generations of mungbean following mutagenesis with ethylmethane sulphonate (EMS), hydrazine hydrate (HZ) and sodium azide (SA). Mean pod length did not differ significantly in most of the mutagenic treatments in M_2. However, significant improvement for the trait was exhibited with lower and moderate concentrations in M_3 generation. The mean number of seeds per pod and 100-seed weight increased with lower and moderate concentrations of the mutagens in M_2, whereas M_3 generation showed a complete positive trend of shift. Long pod and bold seeded mutants may be exploited to increase the number of seeds per pod and seed size leading to increased yield potential. The genotypic coefficient of variation, heritability and genetic advance increased manifold in the treated population for all these traits suggesting that mutagen induced variability has the substantial scope to improve the mungbean crop.

Keywords: Chemical mutagens, bold seeded mutants, enlarged pods, mungbean

INTRODUCTION

Pulses being rich in quality proteins, minerals and vitamins are inseparable ingredient of the diet of majority of Indian population (Siag et al., 2005). Indian population relies on pulses for meeting its protein requirement mainly because of its vegetarian

[*] Corresponding author e-mail: botanyrafiq@gmail.com

Received: 19.09.2017 *Accepted: 10.11.2017*

food habit and high cost of animal-based protein. The country has witnessed a decreasing trend in the per capita availability of pulses from 61 g per day in 1951–1956 to less than 40 g in recent years (Satya Sundaram, 2010). The problem of declining per capita availability can be addressed through rapid improvement in indigenous production levels. Although efforts have been expedited to bring additional area under the cultivation of pulses, it is imperative to increase the production by exploiting the yield potential of existing varieties through genetic manipulation. Mungbean (*Vigna radiata* L. Wilczek), also known as green gram and mung, is native to India where it has been cultivated since ancient times. In India, mungbean was grown over an area of 3.38 million hectares with the production of 1.61 million tons in 2013-14 (www.iipr.res.in/e-pulse-data-book.html). The average seed yield of 474 kg ha^{-1} is far below its presumed potential. It is cultivated mainly as kharif crop, but in southern India where winter is quite mild, it is grown as rabi crop. The crop can withstand drought but is susceptible to water logging. It is usually grown both as pure and mixed crop in different agro-ecological conditions. In addition to its nutritive value, it also has a unique property of maintaining and restoring soil fertility through biological nitrogen fixation (Stevenson and Van Kessel, 1996).

Mutation is an abrupt inheritable qualitative or quantitative change in the DNA sequence which is reflected in the change of sequence of corresponding RNA or protein molecules. Such a change may involve only one base/base pair or more than one base pair of DNA. Mutation breeding has an additional advantage when only one or two traits need improvement in an otherwise well adapted cultivar (Gottschalk, 1986, Joshua, 2000). Particularly, induction of micro-mutations in polygenic system controlling the quantitative traits is important for crop improvement. Several authors (Joshi and Verma, 2004, Khan and Wani, 2005, Singh et al., 2006, Auti, 2012, Bara et al., 2017, Wani, 2017, Patial et al., 2017) have reported in various crops that micro-mutations result in the release of considerable genetic variability in the mutagen treated population. Use of mutations to create genetic variability in the existing gene pool, can be very promising supplementary breeding activity.

Seed yield in pulses is a complex trait and is influenced by many other quantitative traits like fertile branches per plant, pods per plant, seeds per pod and 100-seed weight. Many breeders have so far reported increased seed yield per plant following mutagenesis with physical and chemical mutagens in different pulse crops. Waghmare and Mehra (2000) achieved considerably increased mean seed yield in M_3 generation of *Lathyrus sativus* after treatments with gamma rays and EMS. Dadarwal and Mathur (2015) observed an increased seed yield in urdbean after mutagenic treatments with EMS, DMS and their combination with growth regulators like indole acetic acid (IAA) and gibberellic acid (GA). Similarly, Wani et al. (2012) reported a significant increase in mean seed yield in M_3 and M_4 generations of chickpea following mutagenesis with EMS and SA. The high yielding mutants will play an important role to break the yield constraints in pulse crops particularly mungbean.

The agronomically and nutritionally superior mutants will serve as promising material to plant breeders in future and will economically benefit the resource poor farmers of rainfed areas especially in India.

Keeping above in view, the present study was undertaken to study the genetic basis of various quantitative traits viz. pod length, seeds per pod and 100-seed weight in M_2 and M_3 generations which directly impact the overall yield potential of mungbean.

MATERIALS AND METHODS

A field experiment was conducted during the kharif season of 2005, 2006 and 2007 at University Agricultural Farm, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Uniform and healthy seeds of mungbean (*Vigna radiata* L. Wilczek) var. NM-1 were pre-soaked in distilled water for 9 hours prior to treatment with three chemical mutagens viz. 0.1, 0.2, 0.3% of ethylmethane sulphonate (EMS)- a monofunctional alkylating agent manufactured by Sissco Research Laboratories Pvt. Ltd., Mumbai, India and 0.01, 0.02, 0.03% of hydrazine hydrate (HZ)- a base analogue, manufactured by Qualigens Fine Chemicals, Mumbai and sodium azide (SA)- a respiratory inhibitor, manufactured by Indian Drugs and Pharmaceuticals Ltd., Hyderabad for 6 hours. The healthy, non-dormant and untreated seeds were soaked in distilled water for 15 hours and sown as control. The solutions of EMS and HZ were prepared in phosphate buffer of pH 7, whereas SA solution was prepared in phosphate buffer adjusted to pH 3. Chemically treated seeds were thoroughly washed in running tap water to eliminate the residue mutagens from seed surface. Four hundred seeds for every treatment and control were sown in the field in complete randomized block design (CRBD) to raise M_1 generation. The distance between the seeds in a row and between the rows was kept as 30 cm and 60 cm, respectively. Seeds harvested from individual M_1 plants were sown as M_2 families in three replicates in the field. Seeds from each selected M_2 progeny were bulked by taking an equal amount of seeds from each M_2 progeny and thoroughly mixed. A random sample of this bulk was sown to obtain M_3 progeny. Data collected for pod length (in cm) and the mean for each plant was calculated for pod length, seeds per pods (fully matured pods were threshed and number of seeds per pod was counted) and 100-seed weight (weight of 100 seeds from each plant in g) isolated in M_2 and M_3 generations were subjected to statistical analysis according to Singh and Chaudhary (1985) in order to assess the extent of induced variation. The significance of difference between the means of treated and control population was tested by using least significant difference (LSD) estimated from the error mean square and tabulated ‘T’ value at 5% level of significance.

RESULTS AND DISCUSSION

Success of any plant breeding programme depends on the presence of significant genetic variability, which permits effective selection. In recent years, mutation breeding has been gaining ground for inducing genetic resources (Datta et al., 1993). The direct use of mutations is valuable supplementary approach to plant breeding, particularly when it is desired to improve one or two easily identifiable characters in an otherwise well adapted variety. Induced mutations are thus the ultimate source of genetic variability in crop plants that may be difficult to bring through cross breeding procedures.

Three quantitative traits, namely pod length (cm), number of seeds per pod and 100-seed weight (g) were statistically analyzed to assess the extent of induced variability in M_2 and M_3 generations. Pod mutations with increased length and girth over the control were recorded in M_3 generation. The plants were normal in appearance with comparatively bigger pods. Plant height, primary branches per plant, pod length, seeds per pod and seed yield per plant was significantly increased over the control in these mutants. Data for pod length showed that most of the mutagen treatments were not proficient to induce significant differences in mean pod length in M_2 generation. Also, the mean pod lengths shifted on either side of the control mean (Table 1). However, there was a significant increase in mean pod length with 0.02% (Figure 1b) and 0.03% of EMS treatments and with 0.02% of HZ and SA treatments in M_3 generation.

Figure 1. (a). Pods of var. NM-1 (control)

(b). Mutant isolated from 0.02% EMS in M_3 generation showing increase in pod length and girth over the control

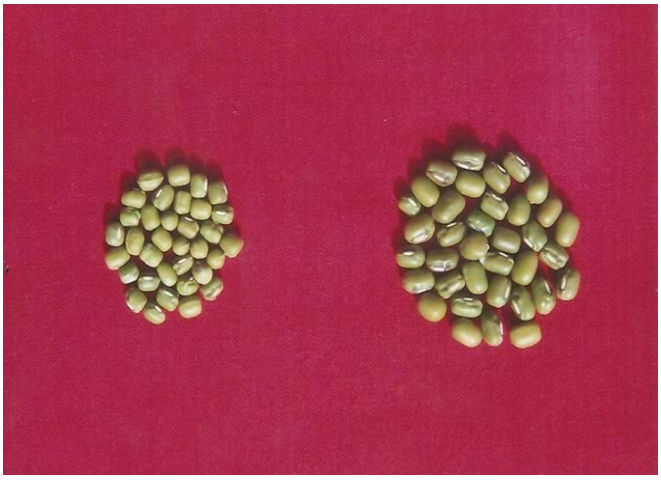


Figure 2. (a). Seeds of var. NM-1 (control)

(b). Bold seeds of the mutant isolated with 0.1% EMS in M_3 generation

Long pod mutant with increased girth is a useful variation and may be exploited to increase the number of seeds per pod and seed size leading to increased seed yield. Sharma and Singh (1992) and Wani et al. (2011) reported long pod mutants with gamma rays, EMS, HZ and SA in mungbean, while Singh and Agarwal (1986) reported long pod mutants with the treatments of EMS, gamma rays and their combination in cluster bean which had increased genetic and yield potential.

For seeds per pod, the mean shifted to both positive and negative directions in M_2, but increased with all the treatments of the mutagens in M_3 generation (Table 2). The increase was significant at lower and moderate concentrations in M_2, which increased further in M_3 generation. However, a reduction with highest mutagenic concentration in all the three mutagens was noticed in M_2 generation. Khan (1985) assumed this depressive effect to be due to high seed sterility induced by higher doses of the mutagens. Similar results were reported by Singh and Chaturvedi (1990) in *Lathyrus sativus* and Singh et al. (2000) in *Vigna mungo*.

The mean 100-seed weight (g) showed a significant improvement over the control with lower and moderate mutagenic concentrations in M_2 generation. However, it decreased at the highest concentration of the mutagens. In M_3, mean 100-seed weight increased significantly with all the treatments (Table 3). The highest increase (bold seeds) was noticed with 0.1% EMS (Figure 2b) in M_3 generation (control mean=3.71; treatment mean= 4.30). Barshile (2006) also recorded bold seeded mutant in 'Vijay' and 'Virat' cultivars of chickpea. The mutant showed vigorous growth, significant increase in leaf area, number of seeds per pod and 100-seed weight over the control. Similarly, Singh et al. (2000) isolated a bold seeded mutant in urdbean following mutagenesis with gamma rays and EMS. This mutant showed vigorous growth and produced more leaves and pods per plant.

Table 1. Estimates of mean values ($\overline{X}$), shift in $\overline{X}$ and genetic parameters for pod length (cm) in M_2 and M_3 generations of mungbean

Treatment	Mean ± S.E.	Shift in $\overline{X}$	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\overline{X}$)
			M_2 Generation			
Control	6.25 ± 0.05	-	4.84	2.34	23.39	2.98
0.1% EMS	6.75 ± 0.10	+ 0.50	9.75	5.20	28.40	7.31
0.2% EMS	6.91 ± 0.11	+ 0.66	11.48	7.18	39.68	11.95
0.3% EMS	6.15 ± 0.08	- 0.10	9.48	5.63	35.29	8.79
LSD (0.05)		0.52				
0.01% HZ	6.17 ± 0.08	- 0.08	9.31	5.37	33.33	8.12
0.02% HZ	6.40 ± 0.07	+ 0.15	8.58	5.18	39.28	8.58
0.03% HZ	5.97 ± 0.10	- 0.28	9.88	4.58	21.42	5.58
LSD (0.05)		0.21				
0.01% SA	6.30 ± 0.07	+ 0.05	6.41	2.89	20.25	3.38
0.02% SA	6.47 ± 0.08	+ 0.22	8.60	4.63	29.03	6.62
0.03% SA	6.37 ± 0.06	+ 0.12	7.19	4.45	38.09	7.25
LSD (0.05)		0.23				
			M_3 Generation			
Control	6.33 ± 0.04	-	4.74	2.23	2.78	22.22
0.1% EMS	6.58± 0.13	+ 0.25	7.16	4.27	29.51	7.25
0.2% EMS	7.17 ± 0.10	+ 0.84	8.97	4.82	28.94	6.77
0.3% EMS	6.67 ± 0.11	+ 0.34	9.28	5.67	32.28	8.50
LSD (0.05)		0.30				
0.01% HZ	6.45± 0.11	+ 0.12	7.09	5.06	23.89	6.12
0.02% HZ	6.78 ± 0.09	+ 0.45	9.77	5.92	41.48	10.15
0.03% HZ	5.97 ± 0.08	- 0.36	8.25	3.47	17.69	3.81
LSD (0.05)		0.25				
0.01% SA	6.52± 0.04	+ 0.19	3.97	2.08	24.45	2.17
0.02% SA	6.61 ± 0.05	+ 0.28	4.34	2.28	27.50	2.68
0.03% SA	6.48 ± 0.06	+ 0.15	4.44	2.36	27.71	3.25
LSD (0.05)		0.22				

PCV (%) = Phenotypic coefficient of variation; GCV (%) = Genotypic coefficient of variation; h^2 (%) = Heritability; GA (%) = Genetic advance

Table 2. Estimates of mean values ($\overline{X}$), shift in $\overline{X}$ and genetic parameters for number of seeds per pod in M_2 and M_3 generations of mungbean

Treatment	Mean ± S.E.	Shift in $\overline{X}$	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\overline{X}$)
			M_2 Generation			
Control	8.93 ± 0.10	-	6.78	2.15	10.08	1.78
0.1% EMS	9.37 ± 0.22	+ 0.44	14.87	9.69	42.47	16.60
0.2% EMS	10.36 ± 0.19	+ 1.43	12.05	7.09	34.61	10.93
0.3% EMS	8.70 ± 0.13	- 0.13	8.86	4.94	31.08	10.86
LSD (0.05)		0.31				
0.01% HZ	9.50 ± 0.15	+ 0.57	11.86	5.95	25.19	7.81
0.02% HZ	10.07 ± 0.19	+ 1.14	12.08	6.88	32.43	10.36
0.03% HZ	8.83 ± 0.16	- 0.10	12.23	4.99	16.39	5.27
LSD (0.05)		0.25				
0.01% SA	9.94 ± 0.14	+ 1.01	9.22	5.41	34.52	8.34
0.02% SA	10.16 ± 0.17	+ 1.23	11.35	7.29	41.35	12.34
0.03% SA	8.66 ± 0.10	- 0.27	6.81	4.41	42.19	7.59
LSD (0.05)		0.29				
			M_3 Generation			
Control	8.27 ± 0.11	-	7.55	3.19	17.95	3.54
0.1% EMS	9.47 ± 0.26	+ 1.20	24.24	21.06	75.52	48.21
0.2% EMS	9.97 ± 0.22	+ 1.70	18.46	15.57	71.09	34.59
0.3% EMS	9.17± 0.18	+ 0.90	15.27	12.92	69.67	32.01
LSD (0.05)		1.12				
0.01% HZ	9.50 ± 0.23	+ 1.23	18.95	14.98	62.34	31.18
0.02% HZ	9.87 ± 0.19	+ 1.60	15.76	12.82	66.12	27.36
0.03% HZ	8.97± 0.26	+ 0.70	12.12	10.92	62.05	28.02
LSD (0.05)		0.83				
0.01% SA	9.36 ± 0.16	+ 1.09	14.41	11.75	66.48	25.32
0.02% SA	9.70 ± 0.14	+ 1.43	11.79	8.58	52.67	16.32
0.03% SA	8.77± 0.20	+ 0.50	10.12	7.67	49.49	14.77
LSD (0.05)		0.69				

PCV (%) = Phenotypic coefficient of variation; GCV (%) = Genotypic coefficient of variation; h^2 (%) = Heritability; GA (%) = Genetic advance

Table 3. Estimates of mean values ($\overline{X}$), shift in $\overline{X}$ and genetic parameters for 100-seed weight (g) in M_2 and M_3 generations of mungbean

Treatment	Mean ± S.E.	Shift in $\overline{X}$	PCV (%)	GCV (%)	h^2 (%)	GA (% of $\overline{X}$)
			M_2 Generation			
Control	3.61 ± 0.02	-	2.90	1.52	27.27	1.99
0.1% EMS	3.87 ± 0.04	+ 0.26	8.53	7.12	69.72	15.69
0.2% EMS	4.01 ± 0.05	+ 0.40	9.43	7.60	65.03	16.26
0.3% EMS	3.51 ± 0.05	- 0.10	9.56	7.50	61.60	15.52
LSD (0.05)		0.15				
0.01% HZ	3.96 ± 0.05	+ 0.35	9.42	7.37	61.15	15.20
0.02% HZ	3.79 ± 0.04	+ 0.18	8.97	6.61	54.52	12.91
0.03% HZ	3.59 ± 0.05	- 0.02	9.82	7.09	52.27	13.54
LSD (0.05)		0.12				
0.01% SA	3.78 ± 0.04	+ 0.17	8.67	5.75	43.92	10.05
0.02% SA	3.82 ± 0.04	+ 0.21	8.56	5.65	43.55	9.83
0.03% SA	3.47 ± 0.05	- 0.14	8.88	5.23	34.73	8.18
LSD (0.05)		0.14				
			M_3 Generation			
Control	3.71 ± 0.01	-	2.62	1.04	15.79	1.09
0.1% EMS	4.30 ± 0.09	+ 0.59	14.88	13.75	83.34	32.73
0.2% EMS	4.49 ± 0.07	+ 0.78	13.67	12.14	78.78	28.44
0.3% EMS	3.91± 0.05	+ 0.20	11.12	10.32	68.23	24.45
LSD (0.05)		0.40				
0.01% HZ	4.38 ± 0.08	+ 0.67	13.26	12.02	82.19	28.66
0.02% HZ	4.11 ± 0.07	+ 0.40	13.14	11.15	72.41	25.11
0.03% HZ	4.06± 0.06	+0.45	12.63	10.97	70.14	23.82
LSD (0.05)		0.31				
0.01% SA	4.02 ± 0.04	+ 0.31	9.08	6.72	54.88	13.15
0.02% SA	4.17 ± 0.05	+ 0.46	10.07	8.15	68.64	18.24
0.03% SA	3.96± 0.07	+0.25	9.63	7.97	65.14	16.82
LSD (0.05)		0.26				

PCV (%) = Phenotypic coefficient of variation; GCV (%) = Genotypic coefficient of variation; h^2 (%) = Heritability; GA (%) = Genetic advance

100-seed weight is a dependable index of measuring yielding ability in pulse crops. 100-seed weight had shown a significant increase (boldness) over the control with most of the mutagenic treatments in both the generations. Bold seeded mutants isolated in various mutagenic concentrations in present study, showed 'gigas' characteristics and vigorous growth and may be utilized in various breeding programs as a donor parent for boldness character as also stated by Wani and Anis (2001) while studying mutagenesis in chickpea. Pawar (2011) has successfully used bold seeded mutants with higher 100-seed weight in cross breeding programmes.

The estimates of genetic parameters revealed a good degree of variability for pod length, seeds per pod and 100-seed weight in both M_2 and M_3 generations. The extent of variability induced by chemical mutagens differed in different traits. The quantitative traits, in general, have complex genetic determination involving large number of genes interacting with one another; consequently, variation in both the directions is expected. Variance level may be less responsive in one trait and highly responsive in other (Sharma, 1995). The phenotypic and genotypic coefficients of variation, heritability and genetic advance increased in all the treatments of mutagens over the control in both the generations for all the traits. High heritability in M_3 generation indicated that the induced variability in mutant population has been fixed by selection. Heritability coupled with genetic advance is more helpful in predicting the effect of selection than the heritability alone because the heritability estimates are subjected to certain estimation errors (Lin et al., 1979).

In this study, the selection for pod length, seeds per pod and 100-seed weight were found to be effective in M_3 generation. Therefore, these traits have high breeding significance in subsequent generations.

CONCLUSION

The narrow genetic base is a serious impediment to breeding progress in mungbean. Induced mutations can help to regenerate and restore the variability, which has been lost in the process of adaptation to various stresses or adaptation during the course of evolution. During the last decade, induced mutations have also been gaining increased importance in plant molecular biology as a tool to identify and isolate the genes and to study their structure and function. Knowledge of genes controlling important agronomic and quality traits is critical for plant breeders to develop proper strategies for efficient breeding programs. These techniques in combination with more efficient screening methods deserve special attention in the days ahead to make mungbean cultivation a promising, remunerative and viable option for pulse growing farmers of India.

REFERENCES

Auti, S.G.(2012).Induced morphological and quantitative mutations in mungbean. *Bioremediation, Biodiversity and Bioavailability*, 6(1), 27-39.

Bara, B.M., Chaurasia, A.K., and Verma P. (2017). Gamma ray effect on frequency and spectrum of chlorophyll mutations in chickpea (*Cicer arietinum* L.) *Journal of Pharmacognosy and Phytochemistry*, 6(3), 590-591.

Barshile. (2006). Induction of genetic variability in *Cicer arietinum* employing sodium azide, ethylmethane sulphonate and gamma radiations. Ph.D thesis, University of Pune, India.

Dadarwal, M., and Mathur, V.L. (2015). Identification of early maturing high yielding mutants in blackgram (*Vigna mungo* L.) Hepper). *Indian Journal of Agricultural Research,* 49(5), 421-426.

Datta, R., Weichsellbaum, R., and Kufe D.W.(1993). Ionizing radiation down regulate histone h_1 gene expression by transcriptional and post transcriptional mechanism. *Radiation Research,* 133, 176-181.

Gottschalk, W.(1986). Experimental mutagenesis in plant breeding. p.81-96. In A.B. Prasad (ed.), Mutagenesis: basic and applied. Print House, Lucknow, India

Joshi, P. and Verma, R.C. (2004). Radiation induced pod and seed mutants in faba bean (*Vicia faba* L.). *Indian Journal of Genetics and Plant Breeding,* 64(2), 155-156.

Joshua, D.C. (2000). Guidelines for successful mutation breeding. p. 21-26. In *Proceedings of DAE-BRNS symposium on the use of nuclear and molecular techniques in crop improvement*, Mumbai, India.

Khan, I.A. (1985). Mutation studies on mungbean (*Vigna radiata* (L.) Wilczek). X. Estimates of genetic variability. *Bangladesh Journal of Scientific Research*, 20(1-4), 189-195.

Khan, S., and Wani, M.R. (2005). Genetic variability and correlations studies in chickpea mutants. *Journal of Cytology and Genetics*, 6, 155-160.

Lin C.Y., Pevzner, I., and Friars, G.W.(1979.Experimental investigation of errors of heritability estimates in index selection. *Canadian Journal of Genetics and Cytology*, 21, 303-308.

Patial, K., Thakur, S.R., Singh K.P., and Thakur A. (2017). Frequency and spectrum of chlorophyll mutations and induced variability in rice bean (*Vigna umbellata* Thunb, Ohwi and Ohashi). *Legume Research*, 40(1), 39-46.

Pawar, S.E. (2011). Impact of mutant varieties of blackgram in realizing improved productivity. *Mutation Breeding Newsletter*, 45, 7-9.

Satya Sundaram, I. (2010). India needs a pulse revolution. *Facts for You* (Dec Issue):10–12

Sharma, B. (1995). Mutation breeding through induced polygenic variability. p. 1210-1219. In *Proc. symposium of genetic research and education: current trends and the next fifty years*, 3. 12-15 February, 1991. New Delhi, India.

Sharma, R., and Singh, V.P. (1992). The influence of altered moisture levels in seeds on the induction of mutations with EMS and gamma rays in mungbean. *Journal of Indian Botanical Society*, 71, 125-128.

Siag, R.K., Gau, R.B., Singh, V., Prakash, V., and Verma, R.S. (2005). Studies on technology transfer through front line demonstration on mungbean semi-arid region of Rajasthan. *Indian Journal of Pulses Research*, 18(1), 64-66.

Singh, M., and Chaturvedi, S.N. (1990). Improvement of yield and quality character of Khesari dhal by use of mutagens. *Mysore Journal of Agricultural Sciences*, 24, 325-330.

Singh, R.K., and Chaudhary, B.D. (1985). Biometrical methods in quantitative genetic analysis. Kalyani Publishers, Ludhiana, India.

Singh, S.P., Singh, N.K., Singh, R.P., and Prasad, J.P. (2006). Mutagenic effect of gamma rays and EMS on nodulation, yield and yield traits on lentil. *Indian Journal of Pulses Research*, 19(1), 53-55.

Singh, V.P., Singh, M., and Lal, J.P. (2000). Gamma ray and EMS induced genetic variability for quantitative traits in urdbean (*Vigna mungo* L.) Hepper). *Indian Journal of Genetics and Plant Breeding*, 60(1), 89-96.

Singh, V.P., and Agarwal, S. (1986). Induced high yielding mutants in cluster bean. *Indian Journal of Agricultural Science*, 56(10), 595-600.

Stevenson, F.C., and Van Kessel, C. (1996). The nitrogen and non nitrogen benefits of pea to eucceeding crops. *Canadian Journal of Plant Science*, 76, 735-745.

Waghmare, V.N., and Mehra, R.B. (2000). Induced genetic variability for quantitative characters in grasspea (*Lathyrus sativus* L.). *Indian Journal of Genetics and Plant Breeding*, 60(1), 81-87.

Wani, A.A., and Anis, M. (2001). Gamma ray induced bold seeded high yielding mutant in chickpea. *Mutation Breeding Newsletter*, 45, 20-21.

Wani, M.R., Khan, S., Kozgar, M.I., and Goyal, S.(2011). Induction of morphological mutants in mungbean (*Vigna radiata* (L.) Wilczek) through chemical mutagens. *The Nucleus*, 48 (3), 243-247.

Wani, M.R., Lone, M.A., Sheikh, S.A., Dar, M.S., Tak, M.A., Ahmad, P., and Khan, S. (2012). Induction and assessment of genetic variability for yield and yield contributing traits of chickpea (*Cicer arietinum* L.). *Journal of Plant Genomics*, 2(1), 28-33.

Wani, M.R. (2017). Induced chlorophyll mutations, comparative mutagenic effectiveness and efficiency of chemical mutagens in lentils (*Lens culinaris* Medik). *Asian Journal of Plant Sciences*, 16, 221-226, doi: 10.3923/ajps.2017

SAARC J. Agri., 15(2): 69-80 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35148*

PHENOTYPIC AND GENOTYPIC SCREENING OF RICE GENOTYPES AT REPRODUCTIVE STAGE FOR SALT TOLERANCE

B. Hossen[1], M.S. Haque[2], K. Miah[1] and M.Z. Tareq[3*]

[1]Department of Agricultural Extension, Khamarbari, Farmgate, Dhaka-1215, Bangladesh
[2]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]Jute Agriculture Experimental Station, Bangladesh Jute Research Institute
Manikganj-1800, Bangladesh

ABSTRACT

Salinity screening of 24 rice genotypes was performed at the reproductive stage for evaluating their salt tolerance level. On the basis of yield and yield components, genotypes were categorized as tolerant, susceptible and moderately tolerant. PBRC-30, Ashfal, Horkuch, STL-20 and Pokkali were found as tolerant while Binadhan-7, S-39 L-11, S-37 L-27, S-37 L-36 and S-37 L-39 were found as susceptible. Selected three SSR markers viz. RM336, RM21 and RM510 were used to determine salinity tolerance. The genetic diversity was ranges from 0.8194 to 0.8854 with an average of 0.8530. The highest PCI value was 0.8742 and the lowest was 0.8004 from RM510 and RM21, respectively. The UPGMA clustering system generated six genetic clusters. The highest genetically dissimilarity of (Cluster 1) vs (Cluster 2 sub-cluster A) and the crossing would be helpful for salt tolerant rice development. Thus, selected SSR primers and genotypes would be useful in marker assisted breeding, quantitative trait loci (QTL) mapping and gene pyramiding in breeding programmed for improvement of rice for salt tolerance.

Keywords: Rice, salinity tolerance, SSR markers, reproductive stage

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for one third of the world's population and occupies almost one-fifth of the total land area covered under cereals (Chakravarthi and Naravaneni, 2006). This staple food ranked first position by production (130 Lac Metric Tons) during the year 2013-14 among all cereals in Bangladesh (BBS, 2013). Over 800 million hectares of land throughout the world are salt affected, either by salinity (397 million ha) or the associated condition of sodicity (434 million ha). Out of 2.85 million hectares of coastal and offshore land of Bangladesh, about 1.0 million hectares are affected by varying degrees of salinity. The coastal saline soils are

* Corresponding author e-mail: zablulbarj@gmail.com

Received: 30.04.2017 *Accepted: 10.09.2017*

distributed unevenly in 64 upazillas of 13 districts, covering portions of eight agro-ecological zones (AEZ) of the country (Seraj and Salam, 2000). Salinity is one of the major constraints for rice production worldwide. Hence, adoption of salt tolerant rice varieties has been considered as one of the strategies to increase rice production in salinity areas. Although soil salinity affects all stages of growth and development of rice plant, but salinity at the reproductive stage depresses grain yield much more than salinity at the vegetative stages. Therefore, screening for salt tolerance at reproductive stages has been considered to be more useful. The use of physiological characters as selection criteria in salt tolerance breeding requires the identification of the contribution of each individual character to salt tolerance (Sabouri et al., 2009). Panicle weight, tiller numbers per plant and harvest index are important agronomic characters for the prediction of rice yield. These yield components are severely affected by salinity (Mojakkir et al., 2015). Breeding for salinity tolerance in rice requires suitable screening techniques and appropriate molecular marker technology (Gregorio et al., 2002). SSR or microsatellite markers are proved to be an ideal method for making genetic maps (Islam, 2004; Niones, 2004), assisting selection procedure (Bhuiyan, 2005) and studying genetic diversity of rice germplasms. Microsatellite marker analysis is promising to identify major gene locus for salt tolerance that can be helpful for plant breeders developing new cultivars. The objective of this study was assessing phenotypic variability of rice genotypes under salt stress at reproductive stage and identification of salt tolerant rice genotypes.

MATERIALS AND METHODS

This study was conducted at the glasshouse and laboratory of Department of Biotechnology, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. A total of 24 traditional and improved rice genotypes were used in the study.

Phenotypic screening

The genotypes were evaluated for their tolerance to salinity under sustained water bath using IRRI standard protocol (Gregorio et al., 1997). At reproductive stage, seedlings were grown in perforated pots which were served as a water tank. Rice yield and yield component data were recorded from the reproductive stage in both normal and salinized conditions. Data were recorded on plant height (cm), days to flowering, days to maturity, number of effective tillers/plant, number of field grains, reduction of number of field grains, number of unfilled grains, total dry mater(g), reduction of total dry matter (g), percent fertility and grain yield (g). These data were recorded following the standard evaluation system of IRRI (IRRI, 1997). MSTATC software was used to perform data analysis on yield and yield components for normal and salinized environments.

Genotypic screening

DNA was extracted using the mini preparation CTAB method (Bhowmik et al., 2009) from 21 days age seedling. Out of 21 microsatellite primers tested three

primers (RM510, RM336 and RM21) were selected for polymorphisms and clear band. These three selected primers were used in final polymerase chain reaction (PCR) amplification for this study.

SSR data analysis

The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 100 base pairs (bp) DNA ladder using Alpha-Ease FC 5.0 software. The number of alleles per locus, major allele frequency, gene diversity and PIC values were calculated using Power Marker version 3.25 (Liu and Muse, 2005). NTSYS-pc was used to construct a UPGMA (unweighted pair group method with arithmetic averages) dendrogram showing the distance-based interrelationship among the genotypes.

RESULTS AND DISCUSSION

Reproductive stage is one of the most sensitive growth stages rice under the saline conditions. Considerable effect due to salinity was observed for most of the traits evaluated during the reproductive stage.

Phenotypic screening

The percent reduction of plant height, total dry matter and number of filled grains of 24 genotypes differed from each other. The per cent reduction in plant height of Pokkali, PBRC-30, PBSAL-656, STL- 15, STL-20, Ashfal, Horkuch, S-39 L-32 and S-39 L-34 were lower (0.8, 6.3, 5.4, 5.0, 3.0, 3.6, 2.8, 4.8 and 2. 7, respectively). On the other hand, Binadhan-7, S-39 L-11, S-39 L-27, S-37 L-14, S-37 L-27, S-37 L-36 and PBSAL-730 showed higher reduction (41.5, 17.2, 33.3, 13.6, 17.9, 13.8 and 13.33, respectively) (Table 1). Salinity stress might inhibit cell division or cell enlargement so that plant height was reduced. Similar result also found by Mojakkir et al. (2015).

Reduction (%) of total dry matter was higher in PBSAL-656 (61.3), Binadhan-7 (75.5), S-39 L-11 (35.8), S-39 L-16 (37.1), S-39 L-27 (57.8), S-37 L-19 (29.8), S-37 L-23 (34.7) and S-37 L-36 (38.3) (Table 1). Similarly per cent reduction of number of filled grains was lower in PBRC-30, PBRC-37, STL-15, STL-20, Ashfal, Horkuch and Pokkali (28.8, 29.9, 16.8, 14.8, 20, 27.3 and 17.7, respectively). Binadhan-7, S-39 L-11, S-39 L-27, S-37 L-19, S-37 L-24, S-37 L-27, S-37 L-36 and S-37 L-39 had higher reduction (81.5, 80.6, 78.4, 71.6, 66.6, 75.1, 63.3, and 65.2, respectively). This is because of loss of biomass production was lower in tolerant genotypes which increased the assimilation and ultimately produced the higher number of grains. Tolerant genotypes showed lower reduction than the susceptible. This result was consistent with the result observed by Islam (2004) who worked with 80 RILs from Pokkali X IR29 cross. He reported that total biomass of tolerant lines was reduced by 49.5% in salinized condition whereas those of susceptible lines were reduced by 64.0%. Most of the genotypes showed higher number of unfilled grains in salinized condition than the normal condition. According to the performance

Table 1. Means of studied traits of 24 rice genotypes under salinized and non salinized conditions NS= Non salinized; S= Salinized; R= Reduction

Sl. No	Variety	Plant height (cm)			Total dry matter (g)			Number of filled grain			No. of unfilled grain		% fertility		Yield/Plant (gm)		1000 seed weight	
		NS	S	% R	NS	S	% R	NS	S	% R	NS	S	NS	S	NS	S	NS	S
1	PBRC – 30	95	89	6.3	14.1	12.2	13.4	288	205	28.8	54	63	84.2	76.6	4.3	1.3	14.8	6.1
2	PBRC – 37	92	84	8.7	8.4	6.8	19.0	300	210	29.9	48	87	86.2	67.6	5.2	3.2	17.3	14.9
3	PBSAL - 656	93	88	5.4	10.8	4.2	61.3	255	165	35.2	30	63	89.5	72.5	4.1	2.2	15.8	13.1
4	PBSAL - 730	90	78	13.3	6.2	5.1	19.0	198	98	50.2	24	73	89.2	57.6	3.1	1.4	15.5	13.9
5	STL – 15	100	95	5.0	9.3	8.1	23.3	260	216	16.8	20	32	92.9	87.0	5.2	3.2	20.0	14.9
6	STL – 20	100	97	3.0	12.2	10.1	17.2	270	230	14.8	48	87	84.9	72.6	6.3	3.9	23.4	17.1
7	Ashfal	110	106	3.6	18.1	16.2	10.5	318	254	20	24	66	92.9	79.3	5.4	4.3	16.9	16.9
8	Horkuch	112	109	2.7	10.3	8.6	16.1	290	211	27.3	26	50	91.8	80.8	5.8	3.8	20.0	17.9
9	Pokkali	125	124	0.8	11.4	9.8	13.8	444	365	17.7	24	45	94.9	89.0	7.5	6.1	16.9	16.6
10	BD – 7	135	79	41.5	23.3	5.7	75.5	660	122	81.5	66	241	90.9	33.6	8.5	1.6	13.1	13.4
11	S-39, L-11	99	82	17.2	10.9	7.0	35.8	495	96	80.6	90	148	84.6	39.2	9.9	1.2	20.1	12.9
12	S-39, L-16	97	90	7.2	9.7	6.1	37.1	360	167	53.6	96	132	78.9	55.8	6.1	2.8	17.1	16.9
13	S-39, L-27	105	70	33.3	13.3	5.6	57.8	480	103	78.4	30	199	94.1	34.2	12.4	2.1	25.8	19.9
14	S-39, L-31	88	81	7.9	12.3	10.9	11.4	270	126	53.3	66	106	80.4	54.3	4.1	1.8	15.0	13.9
15	S-39, L-32	105	100	4.8	9.7	8.3	15.9	288	143	50.5	55	141	83.9	50.2	4.6	2.1	16.0	14.7
16	S-39, L-34	112	109	2.7	13.6	11.2	17.6	280	114	59.3	84	162	76.9	41.3	4.7	1.6	17.1	13.9
17	S-37, L-14	88	76	13.6	7.7	6.1	20.7	195	105	45.9	65	91	75.0	53.8	2.9	1.3	14.9	11.9
18	S-37, L-18	85	78	8.2	6.6	5.6	15.2	270	128	52.5	20	76	93.1	62.8	6.4	1.8	23.5	13.9
19	S-37, L-19	93	81	12.9	13.1	9.2	29.8	550	156	71.6	77	263	87.7	37.2	11.6	3.1	21.0	20.1
20	S-37, L-23	86	78	9.3	9.8	6.4	34.7	336	217	35.2	81	123	80.6	63.9	6.7	4.1	20.1	18.9
21	S-37, L-24	100	88	12.0	7.9	6.2	21.5	294	98	66.6	64	167	82.1	37.0	4.4	1.1	15.1	11.4
22	S-37, L-27	95	78	17.9	10.8	8.3	23.2	320	8o	75.1	65	271	83.1	22.8	7.3	1.6	23.1	19.9
23	S-37, L-36	80	69	13.8	6.3	3.9	38.3	252	93	63.3	90	180	76.7	33.9	4.1	1.3	15.9	14.3
24	S-37, L-39	97	89	8.2	15.8	12.4	21.5	313	110	65.2	91	189	77.6	36.8	5.4	1.5	16.9	13.9
$LSD_{0.05}$		2.114	3.470		1.052	1.189		5.921	4.998		3.917	2.221	1.927	0.933	0.817	0.543	0.744	0.832

of yield/plant in salinized condition, Pokkali, Horkuch, Ashfal, STL-15, STL-20, PBRC-37, S-37 L-19 and S-37 L-23 showed as tolerant (> 3.00) and PBRC-30, PBSAL-730, Binadhan-7, S-39 L-11, S-39 L-34, S-37 L-14, S-37 L-24, S-37 L-27, S-37 L-36 and S-37 L-39 showed as susceptible genotype (≤1.6) (Table 1). Asch et al. (1998) also found that cultivars differed in their salt uptake and the tolerant cultivars had lower salt effect on yield and yield components than the susceptible.

At the reproductive stage, highly significant and positive correlation found between plant height and total dry matter; total dry matter and number of filled grain; plant height and number of filled grain at both salinized and non salinized condition. Grain yield per plant had also the highly significant and positive correlation with plant height, number of filled grains, per cent of fertility and 1000-seed weight. Percent fertility also showed significant and positive correlation with number of filled grain and plant height (Table 2). These results revealed that the higher values increase of such traits have the significant role in the increase of other traits. There was no significant correlation of total dry matter with percent fertility and number of filled grain with 1000-seed weight. Peng et al. (1999) reported that increasing plant height would allow greater biomass production. Zhang et al. (2004) found that increase of plant height was responsible for increase in biomass; so as to increase yield potential. It is crucial to note that Pokkali, PBRC-30, Ashfal, Horkuch and STL-20 genotypes showed higher plant height and total dry matter and also performed as salt tolerant.

Table 2. Correlation of different traits at reproductive stage under salinized and non salinized condition

Trait	Plant height		Total dry matter		No. of filled grain		Fertility (%)		Grain yield/ Plant	
	S	NS	S	NS	S	NS	S	NS	S	NS
Total dry matter	0.572**	0.679**								
No. of filled grain	0.701**	0.578**	0.399**	0.626**						
Fertility (%)	0.583*	0.444**	0.271	0.195	0.835**	0.337*				
Grain yield/ Plant	0.628**	0.305*	0.310*	0.362*	0.915**	0.838**	0.672**	0.407**		
1000-seed wt	0.0337	-0.146	-0.0201	-0.152	0.164	0.134	-0.111	0.332*	0.528**	0.620**

* Significant at 5% level of probability, ** Significant at 1% level of probability

S = Significant and NS = Non-Significant

Genotypic screening

Out of 21 primer pairs tested, three primers were identified as polymorphic. A total of 30 alleles were detected at the loci of three microsatellite markers across 24 rice

genotypes. On average, 25% of the 24 rice genotypes shared a common major allele at any given locus. Diversity exists among three loci tested across 24 rice genotypes, ranged from 0.8194 to 0.8854 with an average of 0.8530. The polymorphism information content (PIC) value is a measure of polymorphism among the genotypes for a marker locus used in linkage analysis. The PIC value of each marker, which can be evaluated on the basis of its alleles, varied for all tested SSR loci (Table 3). The highest PIC value 0.8742 was obtained from RM510 followed by RM336 (0.8390), RM21 (0.8004), respectively. Nejada et al (2010) also found that PIC value varied from 0.56 to 0.88, the highest value belonged to RM8094, while RM8095 showed the lowest PIC value (0.56). The SSR marker RM8094 was found to be superior for analysis of genetic diversity among the markers in the region.

Table 3. Number of genotype, alleles number, gene diversity and polymorphism information content (PIC) value found among 24 rice genotypes for three SSR markers

Primers	Major Allele Frequency	Genotype no.	Allele no.	Gene Diversity	PIC
RM336	0.2500	11.0000	11.0000	0.8542	0.8390
RM510	0.1667	10.0000	10.0000	0.8854	0.8742
RM21	0.3333	9.0000	9.0000	0.8194	0.8004
Mean	0.2500	10.0000	10.0000	0.8530	0.8379

The values of pair-wise comparisons of Nei's (1973) genetic distance (D) between genotypes were computed from combined data for the three primers, ranged from 0.0000 to 1.000 (Table 4). Comparatively higher genetic distance (1.000) was observed between a number of germplasm or germplasm pair. Among them G1/ G 8/ G 9/ G 7 vs G 10/G 11/G 22/ G 23/ G24 were important. The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. Basically this value is an indication of their genetic dissimilarity. Variety pair with higher value is more dissimilar than a pair with a lower value. The lowest genetic distance (0.0000) was found in G1 vs.G8, G1 vs. G9, G8 vs.G9 and G10 vs. G11 etc. Genotypic pair indicating that they are genetically much closer among the genotypes tested. However, potential hybrid line can be produced by inter-varietal crossing based on the genetic dissimilarity value since the more the genetic dissimilarity value the more chance of getting vigorous heterosis in the progeny. Hence microsatellite marker based molecular fingerprinting could serve as a potential basis for the identification of genetically distance genotypes as well as sorting of duplication for morphologically closer genotype.

Table 4. Pair-wise comparisons of Nei's (1973) genetic distance between 24 rice genotypes

OTU	G1	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G2	G20	G21	G22	G23	G24	G3	G4	G5	G6	G7	G8	G9
G1	0.0000																							
G10	1.0000	0.0000																						
G11	1.0000	0.0000	0.0000																					
G12	1.0000	0.6667	0.6667	0.0000																				
G13	1.0000	0.6667	0.6667	1.0000	0.0000																			
G14	0.6667	0.6667	0.6667	1.0000	0.3333	0.0000																		
G15	1.0000	0.6667	0.6667	1.0000	0.6667	0.6667	0.0000																	
G16	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.0000																
G17	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.0000															
G18	1.0000	1.0000	1.0000	0.6667	0.6667	1.0000	1.0000	1.0000	1.0000	0.0000														
G19	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	0.0000													
G2	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	0.6667	0.0000												
G20	1.0000	0.6667	0.6667	0.6667	0.3333	0.3333	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000											
G21	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	0.6667	0.6667	1.0000	0.6667	1.0000	1.0000	1.0000	0.0000										
G22	1.0000	0.3333	0.3333	1.0000	0.6667	0.6667	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.6667	0.0000									
G23	1.0000	0.6667	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.3333	0.0000								
G24	1.0000	0.3333	0.3333	1.0000	0.6667	0.6667	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	0.3333	0.6667	0.0000							
G3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000						
G4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000					
G5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.3333	1.0000	0.0000				
G6	0.6667	1.0000	1.0000	1.0000	0.6667	0.3333	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000			
G7	0.3333	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	0.6667	0.0000		
G8	0.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.3333	0.0000	
G9	0.0000	1.0000	1.0000	1.0000	1.0000	0.6667	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6667	0.3333	0.0000	0.0000

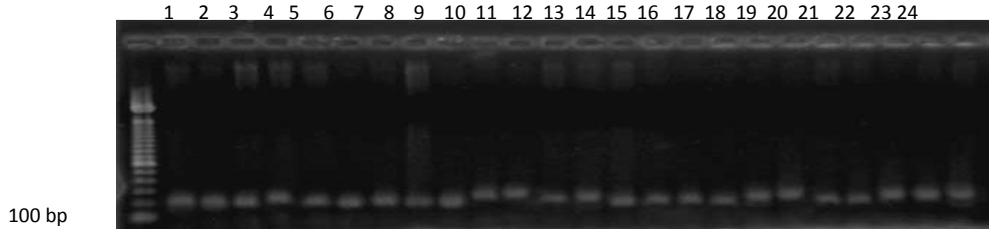


Figure 1. Amplification of DNA segments of tested genotypes using RM336: (Lane 1 to 25 respectively, lKb+; PBRC-30; PBRC-37; PBSAL-656; PBSAL-730; STL-15; STL-20; Ashfal; Horkuch; Pokkali; Binadhan-7, S-39 L-11; S-39 L-16; S-39 L-27; S-39 L-31; S-39 L-32; S-39 L-34; S-37 L-14; S-37 L-18; S-37 L-19; S-37 L-23; S-37 L-24; S-37 L-27; S-37 L-36 and S-37 L-39)

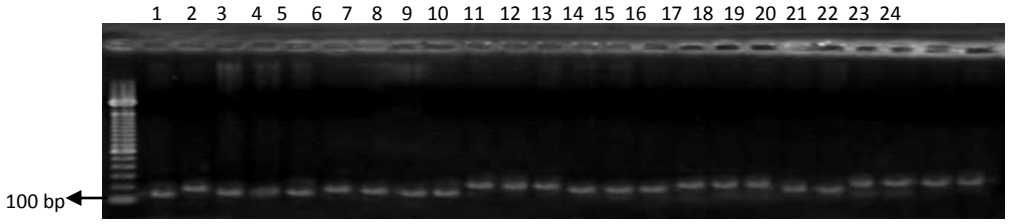


Figure 2. Amplification of DNA segments of tested genotypes using RM510

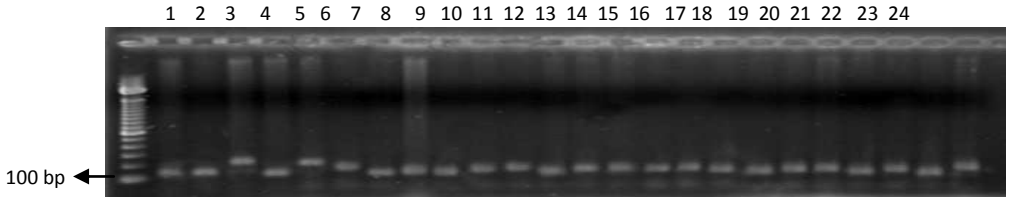


Figure 3. Amplification of DNA segment of tested genotypes using RM21

UPGMA Dendogram

A cluster analysis using UPGMA based on similarity coefficients was done to resolve the phylogenetic relationships among the 24 rice genotypes tested. The UPGMA clustering system generated six genetic clusters with similarity coefficient 11% (Figure 4). Cluster 2 was the biggest group which contained nine genotypes viz. G06, G10, G11, G13, G 14, G20, G22, G23, and G24. This cluster had two separate additional sub-clusters within it, where G10, G11, G22, G23 and G24 were in sub cluster A and G6, G20, G13 and G14 formed sub cluster B. The sub-cluster A were mainly salt susceptible. The cluster analysis revealed that G10, G11, G22 and G24 are closer than G23 while G10, G11 are closer than G22 and G24.

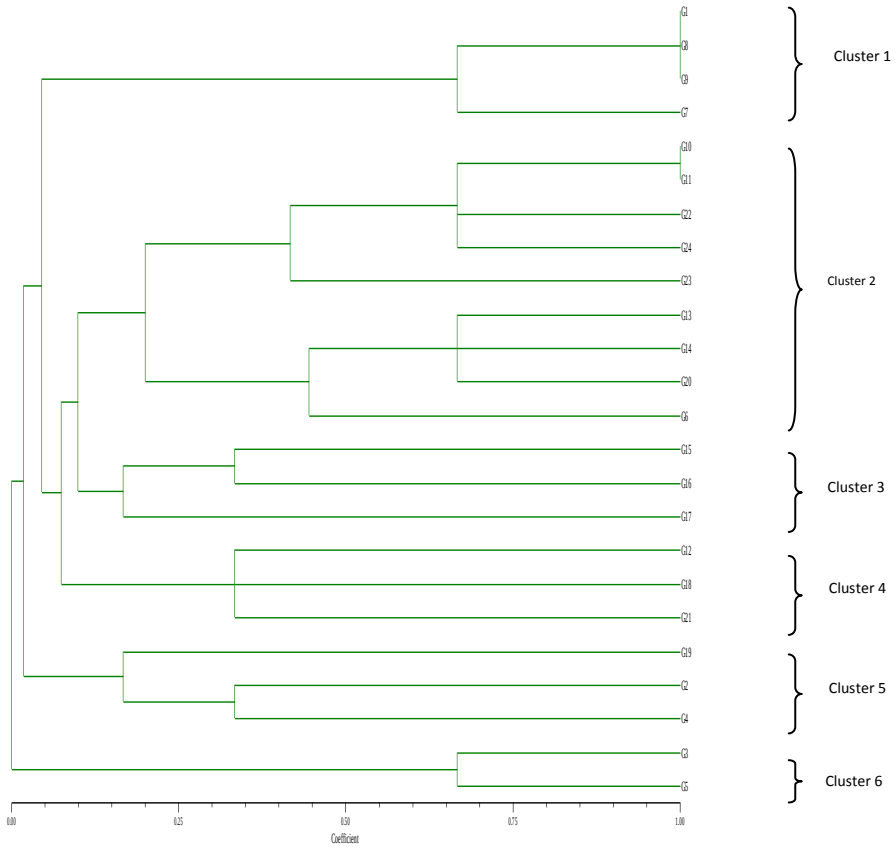


Figure 4. A UPGMA clustering dendogram showing the genetic relationship among 24 rice genotypes based on three SSR markers.

Legend: G1= PBRC-30; G2= PBRC-37; G3= PBSAL-656; G4= PBSAL-730; G5= STL-15; G6= STL-20; G7= Ashfal; G8= Horkuch; G9= Pokkali; G10= Binadhan-7; G11= S-39 L-11; G12= S-39 L-16; G13= S-39 L-27; G14= S-39 L-31; G15= S-39 L-32; G16= S-39 L-34; G17= S-37 L-14; G18= S-37 L-18; G19= S-37 L-19; G20= S-37 L-23; G21= S-37 L-24; G22= S-37 L-27; G23= S-37 L-36; G24= S-37 L-39

In sub-cluster B G13, G14 and G20 are closer than G6. In cluster 1 G1, G8 and G9 formed a sub-cluster and only G7 formed another sub-cluster and also revealed that G1, G8 and G9 are very closer than G7. The genotypes under Cluster 1 were salt tolerant Cluster 3, 4 and 5 contained three genotypes in each cluster. G15 and G16 are closer than G17 (Cluster 3). The three genotypes G12, G18 and G21 were clustered in the same group (Cluster 4). Cluster 5 contains G19, G2 and G4 where G2 and G4 are closer than G19. Cluster 6 was the smallest group which contains G3 and G5. This clustering analysis agreed with the allelic diversity observed among Basmati and Non-basmati long grain indica rice varieties using microsatellite markers (Siwach et al., 2004). DNA fingerprinting and phylogenic analysis of Indian aromatic high quality rice germplasms also showed similar trend (Jain et al., 2004).

CONCLUSION

Salinity screening was done using IRRI standard protocol under non salinized and salinized conditions. Yield and yield components of the germplasms were reduced in saline condition. Pokkali, PBRC-30, Ashfal, Horkuch and STL-20 were found tolerant, on the other hand Binadhan-7, S-39 L-11, S-37 L-27, S-37 L-36 and S-37 L-39 were found susceptible for all the traits. Tolerant genotypes showed higher number of effective tillers/plant, per cent fertility, yield/plant and 1000-seed weight than the susceptible genotypes. Grain yield/plant had the positive and significant correlation with total dry matter, number of filled grains and per cent of fertility.

Out of 21 primers tested three primers (RM336, RM21 and RM510) were selected for genotyping across the 24 rice genotypes tested in this study. Diversity exists among three loci ranged from 0.8194 to 0.8854 with an average of 0.8530. The polymorphism information content (PIC) value was evaluated on the basis of its alleles. The highest PCI value was obtained from RM510 and lowest RM21 respectively, 0.8742 and 0.8004. The values of Nei's (1973) genetic distance between genotypes were calculated from combined data of three primers. The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. This value is an indication of their genetic dissimilarity. Variety pair with higher value is more dissimilar than a pair with a lower value. The lowest genetic distance (0.0000) was found in G1 vs.G8, G1 vs. G9, G8 vs.G9 and G10 vs. G11 etc. indicating that they are genetically much closer among the genotype. The UPGMA clustering system generated six genetic clusters. The biggest cluster 2 was contained nine genotypes with two sub-clusters, viz. sub-cluster A and sub-cluster B. The sub-cluster A contained G10, G11, G22, G23 and G24 on the other hand G6, G20, G13 and G14 formed sub-cluster B. The sub-cluster A were mainly salt susceptible. The salt tolerant genotypes were under Cluster 1 and this contained G1, G8, G7 and G9. The crossing between Cluster 1 vs Cluster 2 sub-cluster A for salt tolerant rice development will be successful due the highest genetically dissimilarity. The genotypes G15, G16 and G17 were under (Cluster 3). The three genotypes G12, G18 and G21 were clustered in the same group (Cluster 4).

Cluster 5 contains G19, G2 and G4 and last clastar (Cluster 6) was contained G3 and G5. Considering both phenotypic and genotypic observations, five genotypes viz. Pokkali, PBRC 30, Ashfal, Horkuch and STL-20 were identified as salt tolerant on the other hand Binadhan-7, S39 L11, S37 L27, S37 L36 and S37 L39 were identified as salt susceptible. The markers (RM336, RM21 and RM510) that were used in this study showed polymorphism, these markers could be proficiently used in tagging salt tolerant genes, in marker-assisted selection and quantitative trait loci (QTL) mapping; and identified salt tolerant rice genotypes could be used in the improvement of salt tolerant rice genotypes.

REFERENCES

Asch, F., Dingkuhn, M., Wittstock C., and Doerffling, K. (1998). Sodium and Potassium uptake of rice panicles as affected by salinity and season in relation to yield and yield components. *Journal of Plant and Soil*, 207, 133-145.

BBS (Bangladesh Bureau of Statistics). (2013). Statistical Yearbook of Bangladesh. Statistics Division, Ministry of Planning. Government of the People's Republic of Bangladesh, p. 189-258.

Bhowmik, S.K., Titov, S., Islam, M.M., Siddika, A., Sultana, S., and Haque, M.S. (2009). Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. *African Journal of Biotechnology*, 8(23), 6490-6494.

Bhuiyan, M.A.R. (2005). Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. MS Dissertation, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Chakravarthi, B.K., and Naravaneni, R. (2006). SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). *African Journal of Biotechnology*, 5 (9), 684-688.

Gregorio, G.B., Senadhira, D. and Mendoza, R.D. 1997. Screening rice for salinity tolerance. IRRI Discussion Paper Series no. 22. Manila (Philippines): International Rice Research Institute.

Gregorio, G.B., Senadhira, D., Mendoza, R.D., Manigbas, R.D., Roxas, N.L., and Guetra, C. Q. (2002). Progress in breeding for salinity and associated abiotic stresses in rice. *Field Crops Research*, 76, 91-101.

IRRI (International Rice Research Institute). (1997). Rice Almanae. IRRI-WARDA-CIAT, Los Baños, Laguna, Philippines.

Islam, M.M. (2004). Mapping salinity tolerance genes in rice (*Oryza satiya* L.) at reproductive stage. Ph. D Dissertation. University of the Philippines Los Banos, College, Laguna, Philippines.

Jain, S., Jain, R.K. and McCouch, S.R. (2004). Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) Germplasm using panels of fluorescently-labeled microsatellite markers. *Theoretical and Applied Genomics*, 109(5), 965-977.

Liu, K. and Muse, S.V. (2005). Power Marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, 21(9), 2128-2129.

Mojakkir, A.M., Tareq, M.Z., Mottalib, M.A., Hoque, A.B.M.Z., and Hossain, M.A. (2015). Effect of salinity at reproductive stage in rice, *International Journal of Business Social and Scientific Research*, 3(1), 7-12.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Science of the United States of America, 70(12), 3321-3323.

Nejada, G.M., Singhb, R.K., Arzanic, A., Rezaiec, A.M., Sabourid, H., and Gregoriob, G.B. (2010). Evaluation of salinity tolerance in rice genotypes. *International Journal of Plant Production*, 4 (3), 153-173.

Niones, J.M. (2004). Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near-isogenic lines. MS Dissertation University of the Philippines Los Banos, College, Laguna, Philippines.

Peng, S., Cassman, K.G., Virmani, S.S., Sheehy, J., and Khush, G.S. (1999). Yield potential trends of tropical rice since the release of IR 8 and the challenge of increasing rice yield potential. *Crop Science*, 39, 1552-1559.

Sabouri, H., Rezai, A.M., Moumeni, A., Kavousi, A., Katouzi, M., and Sabouri, A. (2009). QTL Mapping of physiological traits related to salt tolerance in young rice seedling. *Biologia Plantarum*, 53(4), 657-662.

Seraj, Z.I., and Salam, M.A. (2000).Growing rice in saline soils. The Biotechnology Directory. MacMillan Reference Ltd., Porters South, Crinan street, London.

Siwach, P., Jain, S., Saini, N., Chowdhury, V.K., and Jain, R.K. (2004). Allelic diversity among basmati and non-basmati long-grain *Indica* rice varieties using microsatellite marker. *Journal of Plant Biochemistry and Biotechnology*, 13(1), 25-32.

Zhang, Z.H., Li, P., Wang, L.X., Hu, Z.L., Zhu, L.H., and Zhu, Y.G. (2004). Genetic dissection of the relationships of biomass production and partitioning with yield and yield related traits in rice. *Plant Science*, 167, 1-8.

SAARC J. Agri., 15(2): 81-91 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35154*

EFFECT OF SEED RATE AND ROW SPACING ON GRAIN YIELD OF SORGHUM

M.R. Gondal[1], A. Hussain[1], S. Yasin[1], M. Musa[2*] and H.S. Rehman[2]

[1]Fodder Research Institute, Sargodha, Pakistan
[2]Ayub Agricultural Research Institute, Faisalabad, Pakistan

ABSTRACT

An experiment to investigate the effect of seed rate (5, 7.5, 10, 12.5 and 15 kg ha^{-1}) and row spacing (30, 45 and 60cm) on agronomic characteristics of plants including stem densitym^{-2}, plant height, stem diameter, number of heads m^{-2}, number of heads per plant, number of grains per head, 1000-grain weight and grain yield was conducted using the cultivar "Sorghum 2011" for two years 2016 and 2017. Seed rates and row spacing had significant effect on plant height, plant density m^{-2}, number of heads m^{-2}, number of grains per head and grain yield. Row spacing had non-significant effect on stem diameter, number of heads per plant and 1000-grain weight. Row spacing at 30 cm produced the highest number of plants m^{-2} and plant height. Plant height increased with increase in seed rate in all the row spacing. Stem diameter decreased with increase in the seed rate and row spacing. Narrow row spacing (30 cm) and low seeding rate (5 kg ha^{-1}) produced the maximum grain yield consistently during both years. Lower yields were recorded in the treatments having greater row spacing (60 cm) and higher seed rates (7.5, 10, 12.5 & 15 kg ha^{-1}). Higher seed rates and wider row spacing induced morphological changes rendering plants to lodging.

Keywords: Sorghum bicolor, seed rate, row spacing, grain yield.

INTRODUCTION

Seed rate and row spacing are important factors for crop establishment technique that affects the crop stand and other yield parameters in different crops. Maintenance of optimum planting density is always a big problem to the farmers. Lower plant density results in higher weed infestation, poor radiation use efficiency and lower yields. On the other hand, dense plant population may cause lodging, poor light penetration in the canopy, reduction of photosynthesis due to shading of lower leaves and serious reduction in the yield (Lemerle et al., 2004; Lemerle et al., 2006). Similarly, plant population, on the basis of row spacing and seed rate, affects the crop stand,

* Corresponding authors e-mail: mum96@hotmail.com

Received: 06.06.2017 *Accepted: 20.10.2017*

agronomic plant characteristics and the yield in sorghum crop (McMurray, 2004; McRae et al., 2008).

Tillering is an important morphological component of grain sorghum (*Sorghum bicolor* L. Moench) development because it affects light capture, water use, grain yield, plant competition and other physical and biological processes (Krishnareddy et al., 2006). It has also been reported that increase in plant population results in decrease in number of tillers (Pawlowski et al, 1993; Caliskan et al., 2007), plant height (Ayub et al., 2003) and stem diameter (Caravetta et al., 1990) but grain yield per unit area increased (Caliskan et al., 2007).

Row spacing affects the crop yield potential (Staggenborg, 1999; Bryant et al., 1986). Reducing the distance between rows improves weed control (Walker & Buchanan, 1982) by increasing crop competition and reducing light transmission to the soil (Andrade et al., 2002). Narrow row spacing results in higher grain yields in soybean (De Bruin & Pederson, 2008) and in other crops (Stickler & Laude, 1960). Narrow row spacing resulting in higher yield is explained by the improved light interception (Steiner, 1986) and decreased plant to plant competition between plants (De Bruin & Pederson, 2008). Johnson et al. (2005) reported reduction in total weed density in 30cm apart rows of peanut (*Arachis hypogea*) as compared to the weed density at greater spacing.

Grain sorghum is commonly cultivated in rows with 60 to 75cm spacing, but with the development new production technology and introduction of new herbicides has opened a new window of opportunity to test narrower row spacing for grain production of sorghum. Determining the optimum seed rate is essential to get the proper crop stand and maximum yield (Cox, 1996; Widdicombe & Thelen, 2002) of sorghum crop. The aim of this research was to study the effect of seed rate and row spacing on different attributes of sorghum in order to get maximum grain yield of sorghum crop.

MATERIALS AND METHODS

This study was conducted at experimental field of Fodder Research Institute (FRI), Sargodha, Pakistan during the years 2015 and 2016 to investigate the proper seed rate with relation to row spacing of new variety "Sorghum 2011". The experiment was laid out in split plot design keeping row spacing (30, 45 & 60 cm apart) in main plots and seeding rate (5, 7.5, 10, 12.5 & 15 kg ha^{-1}) in sub-plots measuring 2.7m x 5m with three replications. The experiment was sown on 2nd week of July during both years. Sowing was done with the help of hand drill. First irrigation was given after 25 days of sowing. The subsequent irrigations were adjusted according to the climatic conditions and need of the crop. Fertilizer @ 57-57-57 NPK kg ha^{-1} was applied during both years.

Data on plant height, stem diameter, stem density (number of plants)m^{-2}, number of headm^{-2}, number of heads $plant^{-1}$,numbers of grains $head^{-1}$, 1000-grain weight and grain yield ha^{-1} were recorded.

The trend of data collected during two years was found similar. So, the data were averaged. The data averaged on two years were analyzed statistically and differences among the treatment means were compared by using the least significant difference (LSD) test at 0.05 probability level with the help of M-Stat C programme.

RESULTS AND DISCUSSION

Significant differences among row spacing, seed rates and interaction of both the variables were found for number of grains per head, number of headsm^{-2}, stem density m^{-2} and stem diameter in the combined results of two years (Table 1).

Plant height increased with increasing seed rate and decreasing row spacing. The combined effect of row spacing and seed rate, the interaction, had no effect on plant height (Table 1). Snider et al. (2012) also reported the effect of seeding rate on the plant height to be significant but contrasting effects at different sites. Higher seeding rate may stimulate plant height due to internode elongation (Schmitt & Wulff, 1993). The results indicated that besides genetic constitution, row spacing and seed rate also control growth behavior of sorghum plant. Data also revealed that plant height decreased with increased row spacing and decreased seed rate that probably due to plant competition for light. The maximum plant height 313.11cm was recorded with seed rate of 15 kg ha^{-1} while maximum plant height (309.87cm) was recorded at 30cm row spacing. Similar results of row spacing on plant height have been reported by others (Srivastava et al., 1980; Rana and Ahuja, 1986).

Stem diameter was affected by seeding rate but row spacing had no effect on stem diameter (Table 1).Increase in seed rate from 5 kg ha^{-1} to 15 kg ha^{-1} resulted in a significant decrease in stem diameter while increased the stem density. This is due to the fact that higher seed rate directly results in higher stem density and a higher stem density resulting in decrease in stem diameter due to the obvious interplant competition (Schmitt & Wulff, 1993; Van Der Werf et al., 1995). Higher plant density produces thin stemmed plants that tend to lodge (Kashiwagi et al., 2008; Venuto & Kindiger, 2008) and high seeding rates should be avoided for sorghum in regions where lodging is a major concern. The effect of interaction between seeding rate and row spacing on stem diameter was significant (Figure 1).No significant effect of increasing the row spacing within a given seed rate was recorded on the stem thickness, however at the highest seed rate (15 kg ha^{-1}), the stem thickness decreased significantly as the row spacing increased to 60cm. The maximum stem diameter (1.88cm) was recorded from the treatment interaction of the lowest seed rate (5 kg ha^{-1}) with the least row spacing (30cm) and the interaction of statistically similar stem thickness (1.88cm) from the interaction of the lowest seed rate (30cm) and wider row spacing (45 &60cm).

Table 1. Effect of seeding rates and row spacing on yield and yield components of sorghum (2-year average)

Treatments	Plant Height (cm)	Stem Diameter (cm)	Stem Density m^{-2}	No. of Heads m^{-2}	No. of Headsplant^{-1}	1000-grain Weight (g)	No. of Grains head^{-1}	Grain Yield (kg ha^{-1})
SR_1(5 kg ha^{-1})	281.67e	1.88a	25.44e	21.22e	0.843 a	17.14 a	1020.6a	2996 a
SR_2 (7.5 kg ha^{-1})	291.22d	1.82b	31.11d	25.00 d	0.803 ab	16.76 b	797.2b	2766 b
SR_3(10 kg ha^{-1})	297.44c	1.78c	40.33c	31.22 c	0.774 bc	16.21 c	648.0c	2557 c
SR_4(12.5 kg ha^{-1})	308.67b	1.73d	51.67b	38.11 b	0.738 cd	15.81 d	536.0d	2357d
SR_5(15 kg ha^{-1})	313.11a	1.60e	59.67 a	42.67 a	0.716 d	15.18 e	489.4e	2329d
LSD (0.05)	*3.374*	*0.021*	*2.099*	*2.112*	*0.0497*	*0.133*	*35.27*	***141.49***
RS_1(30 cm)	309.87a	1.78	43.60a	33.27a	0.781	16.28	757.9 a	2747 a
RS_2(45 cm)	297.40b	1.76	41.00b	30.40 b	0.761	16.21	693.7 b	2622 ab
RS_3(60 cm)	288.00c	1.75	40.33b	31.27b	0.783	16.16	643.1 c	2433 b
LSD (0.05)	*4.670*	*NS*	*1.894*	*1.753*	*NS*	*NS*	*25.62*	***222.86***
RS_1SR_1	296.00	1.88 a	23.33g	19.67g	0.830	17.22	1157.7a	3257
RS_1SR_2	302.00	1.83 bc	30.33 f	24.67 f	0.809	16.88	880.0c	2983
RS_1SR_3	310.00	1.81 cd	43.67 d	34.33 d	0.787	16.37	662.3ef	2690
RS_1SR_4	320.00	1.73 ef	55.67 b	42.33 ab	0.760	15.85	579.7gh	2417
RS_1SR_5	321.33	1.64 f	65.00 a	45.33a	0.697	15.11	510.0i	2390
RS_2SR_1	280.00	1.88 ab	26.67g	22.00g	0.840	17.17	997.0b	2980
RS_2SR_2	290.00	1.82 c	32.00f	25.33 f	0.790	16.72	798.3d	2793
RS_2SR_3	295.00	1.77 de	37.33 e	30.00 e	0.803	16.14	655.0ef	2650
RS_2SR_4	308.33	1.72 f	51.33 c	34.67 d	0.677	15.78	528.3hi	2376
RS_2SR_5	313.67	1.61 f	57.67 b	40.00 bc	0.693	15.25	490.0i	2310
RS_3SR_1	269.00	1.88 a	26.33g	22.00g	0.840	17.04	907.0c	2750
RS_3SR_2	281.67	1.81 cd	31.00 f	25.00 f	0.810	16.67	713.3e	2520
RS_3SR_3	287.33	1.77 de	40.00 de	29.33 e	0.777	16.12	626.7fg	2330
RS_3SR_4	297.67	1.73 ef	48.00c	37.33 cd	0.757	15.80	500.0i	2277
RS_3SR_5	304.33	1.55 g	56.33 b	42.67ab	0.733	15.17	468.3i	2287
LSD (0.05)	*NS*	*0.044*	*3.636*	*3.658*	*NS*	*NS*	*61.10*	*NS*

SR = Seed Rate, RS = Row spacin

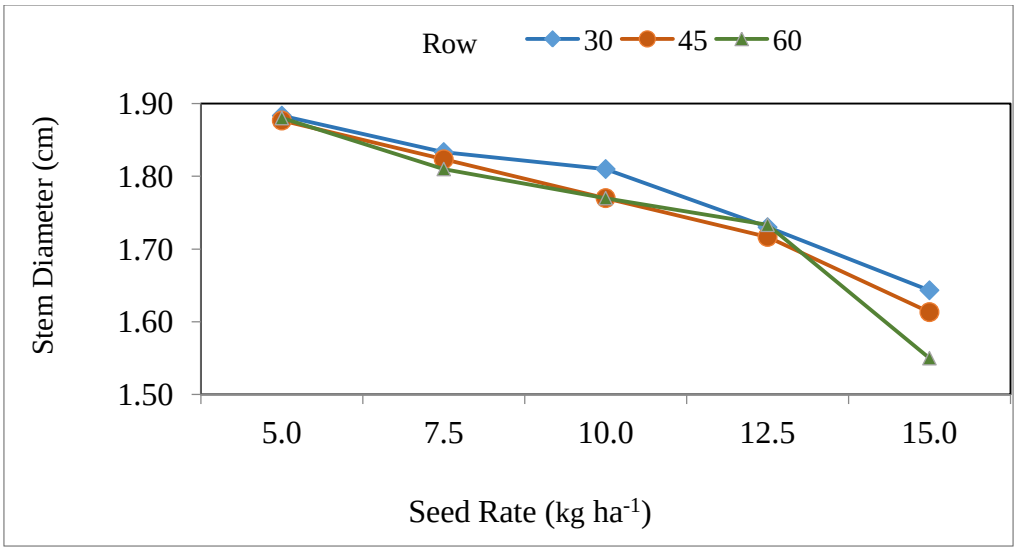


Figure 1. Interactive effect of seed rate and row spacing on stem diameter of sorghum.

Stem density (number of plants m^{-2}) was statistically higher in the narrow row spacing (30cm) than those in wider row spacing of 45cm and 60cm. (Table 1). Increase in seed rate from 5 to 15 kg ha^{-1}resulted in increased stem density from 25.44 to 59.67 stem m^{-2}. Interaction between seeding rate and row spacing was significant for stem density (Figure 2). A trend of differences in the stem density due to interaction between seed rate and row spacing was recorded to be more prominent due to different row spacing interaction with the highest seed rate. Among all the interactions, the highest stem density i.e. 65.00 stems m^{-2} was recorded in the interaction of 30cm row spacing with 15 kg ha^{-1}seed rate followed by statistically lower cluster of stem density in the decreasing trend(57.67,56.33 & 5.67 stems m^{-2}) in the interactions of 45cm row spacing and seed rate of 15 kg ha^{-1}; 60cm row spacing and seed rate of 15 kg ha^{-1}; and 30cm row spacing and seed rate of 12.5 kg ha^{-1}. All the other interactions resulted in further statistically different and lower stem density clusters with the least stem density in three interactions of 60cm, 45cm and 30cm at 5 kg ha^{-1} seed rate having 21.00, 20.67 and 19.67 stem densities in the decreasing trend, respectively. Similar results have been reported by Snider et al. (2012) stating that plant stand establishment may be more favorable under narrow row spacing as compared to wider row spacing i.e. 76cm row spacing, owing to more number of seeds per length of row resulting in increased intra-row competition among plants during the early plant establishment. Such observations have been recorded for other crop species as well (De Bruin & Pedersen, 2008) suggesting decrease in the plant population by increasing row spacing caused by the intra-row competition between plants.

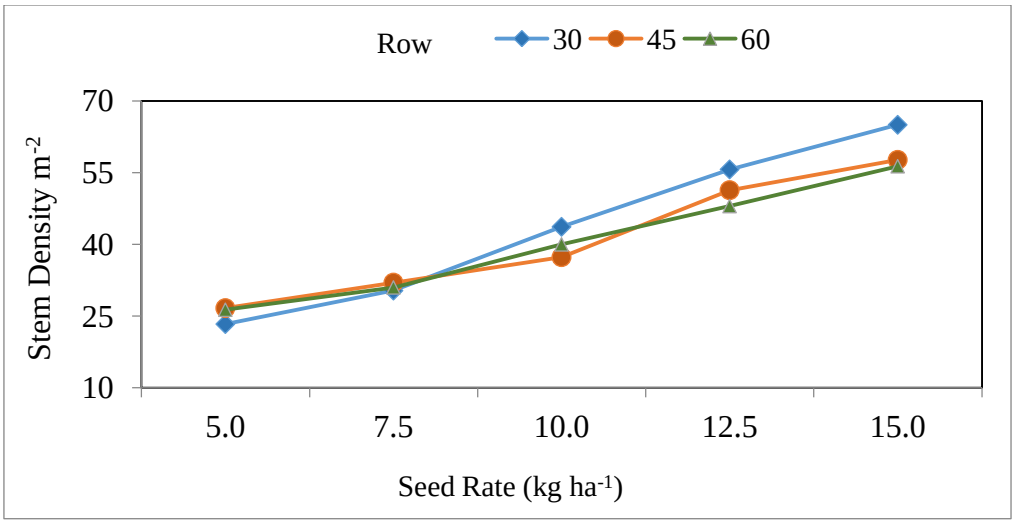


Figure 2. Interactive effect of seed rate and row spacing on density m^{-2} of sorghum

The trend of number of viable heads m^{-2} was similar to that of stem density (Table 1). All treatments of seed rate differed significantly from each other for their effect on the number of viable heads m^{-2}, producing the highest number of viable heads m^{-2}(42.67) in the highest seed rate decreasing with the decrease in the seed rate. This behavior is due to the fact that using the highest seed rate at the narrow row spacing (30cm) would accommodate more number of lines resulting in greater plant to plant distance within the row providing more space to the individual plants for their establishment. Mascagni & Bell (2005) also reported that there had been more tillering for twin rows since the intra-row spacing was greater than the single row for a given seeding rate. The result of number of heads m^{-2} had no effect on sorghum grain yield ha^{-1} and this behavior of sorghum crop is supported by the studies of Mascagni & Bell (2005). The interaction between spacing and seed rate was significant for its effect on the number of viable head m^{-2} (Figure 3). The percentage of head formation on plants was affected significantly with varying the seed rate showing an overall trend of decrease in head formation as the seed rate was increased. The highest percentage of head formation (83.41%) was recorded at 5 kg seed ha^{-1}, having 254,400 plants ha^{-1}, followed by statistically similar 7.5 kg seed ha^{-1}, having 311,100 plants ha^{-1}, producing heads on 80.36% plants. A further decrease of head formation was recorded as the seed rate increased (Table 1). In case of two lower seed rates i.e. 5 kg $ha^{-1}$and 7.5 kg ha^{-1},the intra-row spacing was greater than the higher seed rate and the production of higher percentage was recorded due to the phenomenon explained earlier as for the establishment of plants. Earlier studies have reported that a single row planting, having 124,000-160,000 plant ha^{-1}, produced a greater number of heads $plant^{-1}$ than the twin row planting at any population (Fernandez et al., 2012). They also reported that 76cm row spacing planted 210,000

to 240,000 plants ha^{-1} produced the greatest number of heads per plant than the lower plant population at 76cm row spacing. The results of the current studies are in agreement with the earlier studies reported.

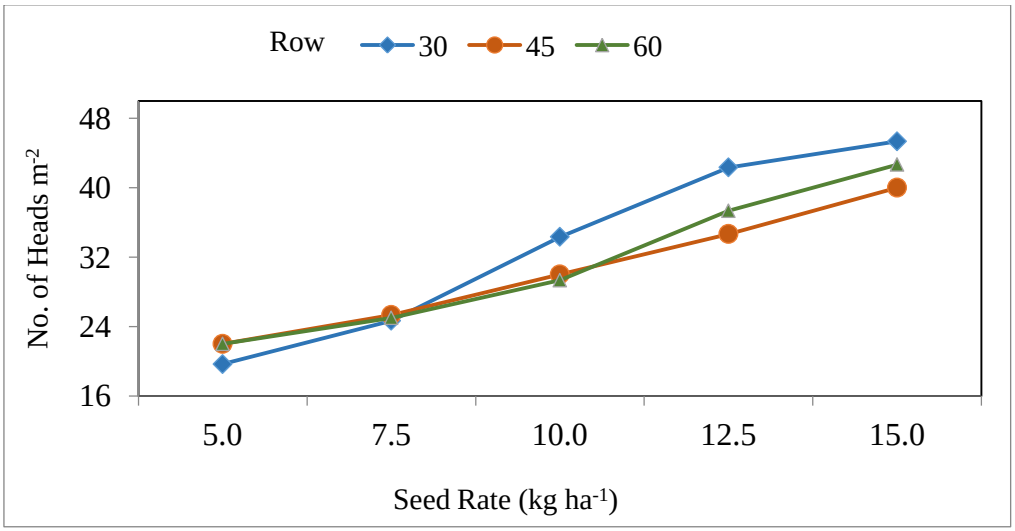


Figure 3. Interactive effect of seed rate and row spacing on number of heads m^{-2}of sorghum.

The effect of row spacing on 1000-grain weight did not differ significantly. On the other hand, seed rates affected 1000-grain weight significantly showing a continuous trend of reduction in the 1000-grain weight as the seed rate was increased. The highest 1000-grain weight of 17.14 g was recorded at 5 kg ha^{-1}seed rate (Table 1). The parameter of increase in 1000-grain weight was reflected in the grain yield increase confirming its contributive factor for grain yield. This study was supported by Fernandez et al. (2012) reporting that the single-row planting at low plant population produced the highest grain weight.

Number of grains per head plays an important role in grain yield of sorghum crop. In the present study, all seed rates were significantly different, producing the highest number of grain per head (1020.6) in the 5 kg seed ha^{-1}. Similarly, closer row spacing (30cm) produced the highest number of grain per head (757.93). The results of the interaction between seed rate and row spacing depicted that the lowest 5 kg seed ha^{-1}with narrow row spacing of 30cm was the best combination, producing 1157.7 grains per head (Table 1). At all three row spacings, number of grains per head decreased with the increase of seed rate (Figure 4). While at all five seed rates, number of grains per head decreased with increase of row spacing (Figure 4). The increase in number of grains per head by using different seed rates, row spacing or the geometry of sowing pattern has been attributed basically due low plant population or more plant to plant distance (Fernandez et al., 2012). The number of seed per head plays an important role in yield determination and has been reported to contribute

70% towards grain yield sorghum as reported by Karchi & Rudich (1966). The current study was in confirmation of number of grains per head playing a major contributive role towards grain yield of sorghum.

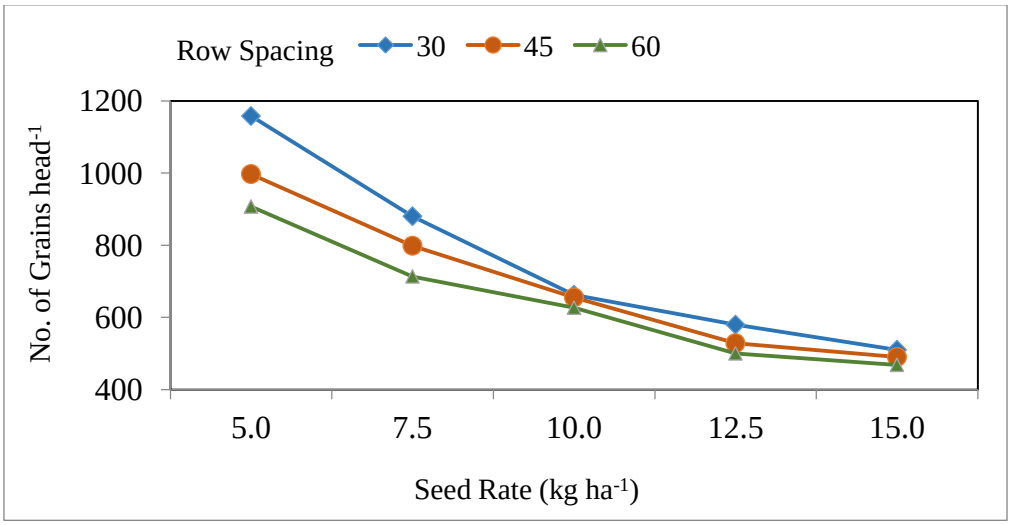


Figure 4. Interactive effect of seed rate and row spacing on number of grains head^{-1} of sorghum

The maximum grain yield (2747 kg ha^{-1}) was obtained with row spacing at 30 cm that was statistically at par with that of 45cm apart row (2622 kg ha^{-1}) but significantly higher than the grain yield (2433 kg ha^{-1}) obtained from row spacing at 60 cm apart (Table 1).Similar response to row spacing has been reported in previous studies where similar row spacing produced higher yields of sorghum grains (Stickler & Laude, 1960; Steiner, 1986), forage (Stickler & Laude, 1960) and other crops such as soybean (De Bruin & Pedersen, 2008). Higher biomass production of sorghum crop with a narrower row spacing of 19cm has been mentioned by Snider et al. (2012), and the phenomenon might be due to the better light interception (Steiner, 1986) and decreased inter-row competition between pants (De Bruin & Pederson, 2008). In contrast, the seed rate studies showed constant decrease in the grain yield with increase in the seed rate (Table 1). Snider et al. (2012) have reported a significant yield reduction with increase in seeding rate from 218,000 to 393,000 ha^{-1}; however the seeding rate 116,000 to 291,000 ha^{-1} did not affect the yields. Higher seed rates have been reported as to increase dry matter production but, in other cases on the contrary, no such effect has been reported to showing inconsistency (Habyarimana et al., 2004; Wortmann et al., 2010). According to the current studies, 30 cm row spacing and 5 kg seed ha^{-1}, requiring the least inputs, were found to be the optimum, among the tested seed rates and row spacing levels, for better above-ground biomass and yield of the cultivar “Sorghum 2011” (Table 1).These findings

suggests a phenomenon that for any given seed rate tested better crop stand can be established by 30cm apart row spacing as compared to other row spacing. This behavior might be attributed to the fact that at wider row spacing of 60cm, each row would receive a higher number of seeds, increasing the number of plants per row, and the intra-row plants would suffer from increased plant-to-plant competition during the early plant establishment stage. Similar results have been depicted from other studies as well in other crops (De Bruin & Pederson, 2008).

These studies indicated consistent increase in plant density m^{-2} with increase in the seed rate in sorghum cultivar "Sorghum 2011". Such increase in plant population by increasing the seed rate has been reported by other workers (Geleta et al., 2002; Habyarimana et al., 2004; Wortmam et al., 2010) and an interplant competition has been reported to have pronounced effect leading to self-thinning at higher seeding rates of some species (Van Der Werf et al., 1995) decreasing the yield.

CONCLUSIONS

Increasing the seed rate from 5 kg ha^{-1} (254,400 plants ha^{-1}) to higher seed rates, the grain yield decreased significantly. Higher grain yield in sorghum cultivar sorghum 2011 was recorded under narrower row spacing of 30-45 cm. Row spacing primarily influences the number of plants m^{-2} (stem densitym^{-2}) and results of the grain yield are considered to be more dependent, or attribute of, plant to plant space using seed rate, row to row distance, different establishing techniques or the planting geometry etc. The present study indicates that the plant characters such as stem diameter and plant height are affected by the plant population, depending upon the seed rate or the plant spacing, where stem diameter was negatively affected, while the plant height was positively affected, with increase in the seed rate. Both the parameters are, in common observation, are affected oppositely as plant height or tiller height increases, the stem diameter decreases. Seed rate and spacing effect was recorded to enhance one of the above parameter and had the contrary effect on the other. Higher seed rate or wider row spacing, within a given seed rate giving more number of plants per row, produces thinner plants that are more vulnerable to lodging. The plant characteristics such as stem diameter, density m^{-2}, number of heads per plant and number of grains per head were inferred to be improved in the treatment combination of lowest seed rate and 30cm row spacing, and the same combination producing better sorghum grain yield. Hence, 5 kg ha^{-1} seed rate of Sorghum with 30cm row spacing is considered to be the optimum for higher grain yield.

REFERENCES

Andrade, F.H., Calvino, P., Cirilo, A., and Baebieri, P. (2002). Yield responses to narrow row depend on increased radiation interception. *Agronomy Journal*, 94 (10), 113-118.

Ayub, M., Tanveer, A., Nadeer, M.A., and Tayyab, M. (2003). Fodder yield and quality of sorghum as influenced by different tillage method and seed rates. *Pakistan Journal of Agronomy*, 2(3), 179-184.

Bryant, H.H., Touchto, J.T., and Moore, D.P. (1986). Narrow row and early planting produce top grain sorghum yields. *Highlights Agriculture Research Alabama Agricultural Experiment Station*, 33, Article 5.

Caliskan, S., Aslan, M., Uremis, I., and Caliskan, M.E. (2007). The effect of row spacing on yield and yield component of full season and double cropped soybean. *Turk Journal of Agriculture*, 31147-31154.

Caravetta, C.J., Cherney, J., and Johnson, H. (1990).Within row spacing influences on diverse sorghum genotypes. 1. Morphology. *Agronomy Journal*, 82(2), 206-210.

Cox, W.J. (1996). Whole plant physiological and yield responses of maize to plant density. *Agronomy Journal*, 88, 489-496.

De Bruin, J.L. and Pederson, P. (2008). Effect of row spacing and seeding rate on soybean yield. *Agronomy Journal*, 100, 204-210.

Fernandez, C.J., Fromme, D.D., and Grichar, W.J. (2012).Grain sorghum response to row spacing and plant populations in the Texas coastal Bend region. *International Journal of Agronomy*, Article ID 238634, P.6.

Geleta, B., Atak, M., Baenziger, P.S., Nelson, L.A., Baltenesperger, D.D., Eskridge, K.M., Shipmam, M.J., and Shelton, D.R. (2002). Seeding rate and genotype effect on agronomic performance and end use quality of winter wheat. *Crop Science*, 42, 827-832.

Habyarimana, E., Bonardi, P., Laureti, D., Di Bari, V., Cosentino, S., and Lorenzoni, C. (2004). Multilocational evaluation of biomass sorghum hybrids under two stand densities and variable water supply in Italy. *Indian Crops Production*, 20, 3-9.

Johnson, WA., Prostko, E.P., and Mullinix, B.G. (2005). Improving the management of dicot weedsin peanut with narrow row spacings and residual herbicides. *Agronomy Journal*, 97(1), 85-88.

Karchi, Z. and Rudich, Y. (1966). Effects of row width and seedling spacing on yield and its components in grain sorghum grown under dryland conditions. *Agronomy Journal*, 58, 602-604.

Kashiwagi, T., Togawa, E., Hirotsu, N., and Ishimaru, K. (2008). Improvement of lodging resistance with QTLs for stem diameter in rice (*Oryza sativa* L.)*Theory and Applied Genetics*, 117, 749-757.

Krishnareddy, S.R., Stewart, B.A., Payne, W.A., Robinson, C.A., and Thomason R.C. (2006). Tillering in dryland grain sorghum clumps as influenced by light, planting density and geometry. *Southern Conservation Systems Conference, Amarillo TX*, June 26-28.

Lemerle, D., Verbeek, B., and Diffy, S. (2006). Influence of field pea (*Pisumsativum*) density on grain yield and competitiveness with annual rye grass (Loliumrigidum) in south-eastern Australia. *Australian Journal of Experimental Agriculture*, 46, 1465-1472.

Lemerle, D., Causens, R.D., Gill, L.S., Peltzer, S.J., Moerkerk, M., Murphy, C.E., Collins, D., and Cullis, B.R. (2004). Reliability of higher seed rates of wheat for increased competitiveness with weeds in low rainfall environment. *Journal of Agriculture Sciences*, 142, 395-409.

Mascagni, H.J., and Bell, B. (2005). Plant patterns for different grain sorghum hybrids, *Louisiana Agriculture Magazine*, 2005.

(http://www.isuageenter.com/en/communications/publications/agmag/archive/2005/winter/plant+patterns+for+different+grain+sorghum+hybrids.htm)

McMurray, L. (2004). Plant density inputs Kaspa field pea's grain yield. *Australian Farm Journal*, 45-46.

McRae, F.J., McCaffery D.W., and Mathews, P.W. (2008). Winter crop variety sowing guide. NSW, *Department of Primary Industries*, 74-85.

Pawlowski, F., Jedruszczak, M., and Bojarezyk, M. (1993). Yield of soybean on loens soil depending on row spacing and sowing rate. *Field Crop Abstracts*, 46(2), 978.

Rana, J.S., and Ahuja, K.N. (1986). Response of mungbean varieties to different row spacings.*Indian Journal of Agronomy*, 31(3), 308-309.

Schmitt, J., and Wulff, R.D. (1993). Light spectral quality, phytochrome and plant competition. *Trends Ecology & Evolution*, 8, 47-51.

Snider, J.L., Raper, R.L., and Schwab, E.B. (2012).The effect of row spacing and seed rate on biomass production and plant stand characteristics of non-irrigated photoperiod-sensitive sorghum (*Sorghum bicolor* L. Moench). *Industrial Crops and Products*, 37, 527-535.

Srivastava, S.N., Namdeo, K.N., Tripathi, A.R. and Pandey, R.P. (1980). Effect of row spacings and levels of phosphorus on blackgram (*Vigna mungo, var. RadiataHepper*).*Madras Agriculture Journal*, 67(12), 771-773.

Staggenborg, S.A. (1999). Grain sorghum response to row spacings and seeding rates in Kansas. *Journal of Production Agriculture*, 12(3), 390-395.

Steiner, J.L. (1986). Dryland grain sorghum water use, light interception and growth response to planting geometry. *Agronomy Journal*, 78, 720-726.

Stickler, F.C., and Laude, H.H. (1960). Effect of row spacing and plant population on performance of corn, grain sorghum and forage sorghum. *Agronomy Journal*, 52, 275-277.

Van Der Werf, H.M.G., Wijlhuizen, M., and de Shutter, J.A.A. (1995). Planting density and self-thinning affect yield and quality of fibre hemp (*Cannabis sativa* L.). *Field Crops Research*, 40,153-164.

Venuto, B., and Kindiger, B. (2008). Forage and biomass feedstock production from hybrid forage sorghum and sorghum sudan grass hybrids. *Grassland Science*, 54, 189-196.

Widdicombe, W.D., and Thelen, K.D. (2002). Row width and plant density effects on corn grain production in the northern Corn Belt. *Agronomy Journal*, 94, 1020-1023.

Walker, R.H., and Buchanan, G.A. (1982).Crop manipulation in integrated weed management systems. *Weed Science*, 30, 17-24.

Wortmann, C.S., Liska, A.I., Ferguson, R.B., Lyon, D.I., Klein, R.N., and Dweikat, I. (2010). Dryland performance of sweet sorghum and grain crops for biofuel in Nebraska, *Agronomy Journal*, 102, 319-326.

SAARC J. Agri., 15(2): 93-102 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35150*

EFFECTS OF ORGANIC AND INORGANIC FERTILIZERS ON LETTUCE (*Lactuca sativa* L.) AND SOIL PROPERTIES

M.B. Hossain[1*] and K.S. Ryu[2]

[1]Soil Science Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2200, Bangladesh
[2]Laboratory of Soil Science, Department of Food and Bioenvironmental Science, College of Life & Environment, Daegu University, Kyongsan, Kyongbuk, Korea-712714, Republic of Korea

ABSTRACT

A Greenhouse experiment was conducted to identify the suitable dose of organic fertilizer for lettuce production. Different doses of organic fertilizer (6.5, 13 and 26 t ha^{-1}) and the recommended dose of chemical fertilizer (RDCF) as standard were selected for this experiment. Application of 13 t ha^{-1} organic fertilizer significantly increased leaf size (length and breadth) of lettuce. This treatment also increased 14, 25, 21, 32, 24, 27, 36 and 168% fresh weight, dry weight, N, P, K, Ca, Mg & Na uptake over RDCF, respectively. Organic matter content was increased of 17.79, 43.82 and 89.89% in 6.5, 13 and 26 t ha^{-1} organic fertilizer treated plots respectively over recommended dose of chemical fertilizers. Data also indicated that organic fertilizer @ 26 t ha^{-1} resulted in significant increase in pH, total nitrogen (24%), organic matter (90%) and Zn (29%) compared to RDCF and decreased electrical conductivity, mineral nitrogen (NH_4^+-N & NO_3^--N) and cadmium and lead (Cd & Pb) in soil. Positive and significant correlation was observed on yield and yield attributes of lettuce and soil nitrogen, organic matter with pH, total nitrogen with mineral nitrogen and negative correlation was found with applied organic fertilizer with cadmium and lead. Based on these results, organic fertilizer @ 13 t ha^{-1} without chemical fertilizer could be recommended to increase lettuce yield as well as mitigate heavy metals in soil.

Keywords: Organic fertilizer doses, lettuce yield, nutrient uptake, soil chemical properties

INTRODUCTION

Lettuce is one of the most important commercial vegetables and major food items in Republic of Korea. It is consumed as salad and a fast food item. As such it assumes importance to carry out research in order to optimize the production of lettuce and also guarantee the environmental sustainability in crop production areas. Crops

[*] Corresponding author e-mail: belalbina@gmail.com

Received: 05.09.2017 *Accepted: 30.09.2017*

success depends on nutrient input during growth. Sole use of chemical fertilizers often declines soil fertility and the resultant crop productivity due to nutrient imbalance in soil, which has been recognized as one of the most important factors that limit crop yield. Therefore, the use of chemical fertilizer may not keep pace with time in maintenance of soil health for sustaining the productivity. Every day increases the concern with food intake that received excessive chemical molecules when consumed outside the prudential limits cause disease in the short and long term (Moreira et al., 2014). Lettuce is actually consumed in natural and as such consumers' concern has been steadily increasing how it is cropped and parallel to this fact is that there is an increasing demand for organic products. The use of mineral fertilizers in lettuce growth is a common agricultural practice that brings satisfactory results in terms of yield, however, consumer's health, production cost and product quality should be considered. Organic farming practices involved on the management of soil organic matter (SOM) and nutrient availability include crop rotation, cover cropping and soil amendment with compost and/or manures (Brito et al., 2012). Organic fertilizers also improve soil physical and chemical properties and reduce the needs for mineral fertilizers. The application of organic fertilizers in lettuce increases the yield and nutrient content in plants. Knowing that leafy vegetables respond well to organic manure results obtained from an experiment with arugula and lettuce, concluded that organic matter mineralization contributed for nutrients to plants supplying their needs during development, because the area was managed with organic practices about five years (Oliveira et al., 2010). Organic compound has a positive effect on fresh weight plant as pointed by an experiment (Villas Boase et al., 2004), which tested three doses (30, 60 and 120 t ha^{-1}). It could be observed that the compound bean straw increased fresh weight of plant and the values of N, K, Ca, Mg, Cu, Fe & Zn in lettuce plants (Vilas et al., 2004). This study aimed at evaluating the effect of compost rates and recommended dose of mineral (RDM) fertilizers on lettuce (*Lactuca sativa* L.) and soil properties.

MATERIALS AND METHODS

A controlled glasshouse experiment was conducted at Daegu University, Kyongsan, Daegu in Republic of Korea to compare the performance of organic fertilizer doses and recommended dose of chemical fertilizers (RDCF) on growth and yield of lettuce, nutrient uptake and their effect on soil chemical properties. Glasshouse controller was maintained to monitor and control temperature (15-25^{O}C), lighting (250 µmol m^{-2} sec^{-1}) and humidity (65-95%). CO_2 monitoring control was used for the experiment. Initial soil samples were collected from the experimental and mixed it after final land preparation. Initial soil and organic fertilizer properties are presented in table 1.

Table 1. Physical and chemical properties of soil and organic fertilizer

Parameters	Soil	Organic fertilizer
Textural class	Silt loam	
Soil reaction (pH)	7.3	
Electrical conductivity (dS m^{-1})	0.3	
Organic matter (%)	9.8	
Total nitrogen (%)	0.19	0.45% (total nitrogen)
Available P_2O_5 (mg kg^{-1})	370	0.14% (total phosphorus)
Exchangeable K ($cmole_c$ kg^{-1})	2.2	0.62% (total potassium)
Exchangeable Ca ($cmole_c$ kg^{-1})	15.4	
Exchangeable Mg ($cmole_c$ kg^{-1})	0.06	
Cadmium (cmolec kg^{-1})	nd	
Cupper ($cmole_c$ kg^{-1})	1.54	
Lead ($cmole_c$ kg^{-1})	0.42	
Zinc ($cmole_c$ kg^{-1})	17.46	

nd=non-detectable.

Four treatments comprised of recommended dose of chemical fertilizers (RDCF) (urea, fused super phosphate and muriate of potash @ 440, 500 and 250 kg ha^{-1}, respectively), 50% recommended dose of organic fertilizer (6.5 t ha^{-1}), 100% recommended dose of organic fertilizer (13 t ha^{-1}) and 200% recommended dose of organic fertilizer (26 t ha^{-1}). Field experiment was laid out in a randomized block design with three replications in a plot size of 2.5m × 4m. Lettuce was the test crop used in the experiment. Row to row and plant to plant distances were 30cm × 25cm. Half dose of nitrogen as urea, full dose of fused super phosphate and muriate of potash were applied as basal and the remaining half dose of urea was applied after 20 days from the date of lettuce plantation. The crop was cultivated following normal cultural practices. Soil samples were collected from the experimental plots after harvest of lettuce. These samples were dried, powdered and sieved through 2 mm sieve for analysis of pH, organic carbon, total nitrogen, EC, Cd, Pb, Cu and Zn. The pH of soil samples was measured in 1:2.5 soils: distilled water suspension with the help of pH meter and organic carbon was measured by chromic acid digestion method (Walkley and Black, 1934). Electrical conductivity (EC), Cadmium (Cd), Cupper (Cu), lead (Pd), and Zinc (Zn) were analyzed by the following standard methods. Harvested plants were washed, dried and ground by using a stainless steel mill and then digested with tri-acid (HNO_3-$HClO_4$-H_2SO_4) mixture. P, K, Ca, Mg,

and Na in soil solution and plant extracts except nitrogen were analyzed by ICP-AES (Inductively coupled plasma-atomic emission spectrometer) using a Varian Liberty Series II, Aus. NH_4^+-N and NO_3^--N were determined by steam distillation of K_2SO_4 extract using MgO and Deverda's alloy, respectively (Bremner, 1965). Data were statistically analyzed and Duncan's Multiple Range was applied to examine significant differences between the treatment means (Gomez and Gomez, 1984).

RESULTS

Data regarding the effect of organic fertilizer rates and the recommended dose of chemical fertilizers (RDCF) on plant height, leaf number, leaf length and breadth are summarized in table 2. Organic fertilizer doses (6.5, 13 and 26 t ha^{-1}) and RDCF did not produce significant results except leaf number of lettuce. Plant height of lettuce was found higher in most of the treatments except 26 t ha^{-1} organic fertilizer treatment but the differences were not significant. It was found that application of organic fertilizer (13 t ha^{-1}) resulted in significantly greater of 9.2% and 8.3% plant height compared with 26 t ha^{-1} organic fertilizer rate and RDCF, respectively. Leaf number is known as primary yield attribute, which also plays an important role in yield formation of lettuce. Leaf number ranged from 21.5 to 23.90 in different treatments. Application of 13 t ha^{-1} organic fertilizer also produced the maximum leaf number and size of lettuce, which followed by the application of 6.5 t ha^{-1} organic fertilizer. All the organic fertilizer doses produced the higher leaf number than RDCF. Leaf size in respect of leaf length and breadth, organic fertilizer @ 13 t ha^{-1} produced the maximum leaf size (22.97cm × 11.69cm), while the treatment organic fertilizer (26 t ha^{-1}) and the RDCF had the smallest leaf size. Organic fertilizer @ 26 t ha^{-1} produced lower leaf length (21.85cm) than RDCF and the opposite results were found in respect of leaf breadth in these treatments. Mean dry weight of lettuce ranged 0.76 and 0.95 t ha^{-1} in RDCF and organic fertilizer (13 t ha^{-1}), respectively. Among the organic fertilizer doses, 13 t ha^{-1} accounted for higher yield with an increase of 25 percent over RDCF. Further increase in organic fertilizer dose resulted in yield reduction of lettuce. Organic fertilizer dose 13 t ha^{-1} also performed better lettuce yield than 6.5 t ha^{-1} treated plots. However, the plot supplied with 26 t ha^{-1} could not sustain the yield until the amount of organic fertilizer was increased from 13 t ha^{-1}. Organic fertilizer applied @ 13 t ha^{-1} also significantly increased fresh weight of lettuce by 7.0 and 22.4 percent over organic fertilizers applied @ 6.5 and 26 t ha^{-1}, respectively. Effects of organic fertilizer doses and RDCF on N, P, K, Ca, Mg & Na uptake of lettuce are summarized in table 2. N, P, Ca, Mg & Na uptake results were statistically significant in different treatments. Maximum N uptake was observed in 13 t ha^{-1} organic fertilizer followed by 6.5 t ha^{-1} organic fertilizer. Nitrogen uptake was increased with increasing of organic fertilizer rates and such increases were found significantly higher up to 13 t ha^{-1} organic fertilizers applied. Similar trend was observed in respect of P and K uptake by lettuce. Potassium uptake was higher than any other nutrients studied in the experiment but the uptake results

were not statistically significant in different treatments. Its uptake ranged from 74.50 to 92.25 kg ha^{-1} in RDCF and 13 t ha^{-1} organic fertilizers treated plots, respectively. Potassium uptake was increased from 9.96, 19.24 and 6.58 percent in 6.5, 13 and 26 t ha^{-1} applied organic fertilizer rates over RDCF. Maximum Ca and Mg uptake were found in 13 t ha^{-1} organic fertilizer treated plots and the second highest calcium (Ca) and magnesium (Mg) uptake was found in 6.5 t ha^{-1} organic fertilizer. On the other hand, organic fertilizer (6.5 t ha^{-1}) produced the highest Na uptake in lettuce leaf and the lowest Na uptake was found in RDCF.

Effects of organic fertilizer rates and RDCF on chemical properties of soil are summarized in table 3. Soil reaction was increased with the increase of organic fertilizer doses. Soil pH varied from 6.83 to 7.93. The lowest pH was recorded in RDCF treated plots and the highest pH was observed in 26 t ha^{-1} organic fertilizer treated plots. Same pH (7.87) was found in 6.5 and 13 t ha^{-1} levels. The use of organic fertilizer (6.5, 13 and 26 t ha^{-1}) decreased the potential acidity from 15.2 to 16.7% compared to RDCF, indicating that organic fertilizer promoted the increase of bases in soil exchange complex. Electrical conductivity (EC) results were not statistically significant in different treatments (Table 3). Maximum EC (0.22 dS m^{-1}) was observed in RDCF treated plots. Recommended doses of RDCF produced higher EC value than different doses of organic fertilizer. Same EC value (0.13 dS m^{-1}) was found in 13 and 26 t ha^{-1} organic fertilizer doses. Total nitrogen ranged from 0.16% in 6.5 t ha^{-1} to 0.21% supplied with organic fertilizer @ 26 t ha^{-1}. Available nitrogen content results were not statistically significant. In general, organic fertilizer 6.5 and 13 t ha^{-1} increased 21 and 26 percent NH_4^+-N and 11 and 19 percent NO_3^--N over RDCF, respectively. On the other hand, RDCF treated plots increased 81 and 50 percent NH_4^+-N and NO_3^--N over the 26 t ha^{-1} organic fertilizer respectively. Available N (NH_4^+-N and NO_3^- -N) content was increased with the increase of organic fertilizer rates except 26 t ha^{-1}. Ammonium-N (NH_4^+-N) content was higher than NO_3^--N except 26 t ha^{-1} organic fertilizer treated plots. Different treatments had significant effect on soil organic matter status in soil. Organic matter in soil was varied from 0.89 to 1.69 per cent. The lowest organic matter was recorded in RDCF and the highest was in 26 t ha^{-1} organic fertilizer treated plots. Organic matter content was also increased of 17.79, 43.82 and 89.89% in 6.5, 13 and 26 t ha^{-1} organic fertilizer treated plots respectively over RDCF. Organic matter was increased with the increase of organic fertilizer doses and such increases were found significantly higher up to 26 t ha^{-1}. Heavy metals (Cd & Pb) were decreased with the increase of organic fertilizer doses. Organic fertilizer (26 t ha^{-1}) helped to reduce heavy metals contents in soil except Zn. Maximum heavy metals concentrations were found in RDCF treated plots. Zinc content was increased with the increase of organic fertilizer level. Correlation coefficients of yield attributes and soil quality traits were worked out (Figure 1a, b, c, d, e, f, g, h & i) in order to evaluate their influence on lettuce yield. Plant height, leaf number, NH_4^+-N and NO_3^- -N content were performed

Table 2. Effect of different levels of organic fertilizer and mineral fertilizer on growth, yield and nutrient uptake of lettuce

Treatment	Plant height (cm)	No. of leaf	Leaf size (cm)		Fresh weight (t ha^{-1})	Dry weight (t ha^{-1})	N	P	K	Ca	Mg	Na
			Length	Breadth			----------(kg ha^{-1})----------					
Chemical fertilizers (RDCF)	26.98	21.53	21.93	11.01	1.85	0.76	28.68	5.14	74.50	14.54	3.01	1.93
Organic fertilizer (6.5 t ha^{-1})	28.68	22.77	22.37	11.40	1.98	0.93	33.73	5.98	82.74	15.93	4.03	5.58
Organic fertilizer (13 t ha^{-1})	29.23	23.90	22.97	11.69	2.10	0.95	34.71	6.81	92.25	18.51	4.09	5.17
Organic fertilizer (26 t ha^{-1})	26.78	23.77	21.85	11.10	1.91	0.86	30.13	6.31	79.75	14.48	3.50	4.36
$LSD_{0.05}$	NS	2.21	NS	NS	0.11	0.07	3.95	1.42	NS	2.52	1.00	3.21

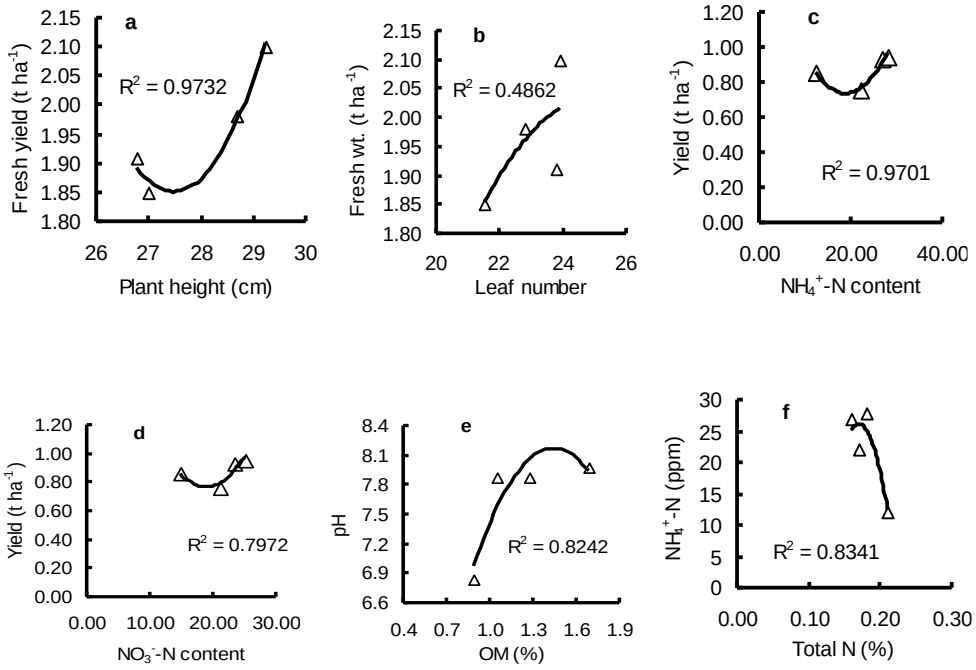
LSD=Least Significant Difference.

Table 3. Effect of different levels of organic fertilizer and mineral fertilizer on chemical properties in post harvest soil

Treatment	pH	EC dS m^{-1}	Total nitrogen (%)	Mineral nitrogen (ppm)		Organic matter (%)	Heavy metals (ppm)		
				NH_4^+ - N	NO_3^- - N		Cd	Pd	Zn
Chemical fertilizers (RDCF)	6.83	0.22	0.17	22.17	21.00	0.89	1.74	0.43	14.12
Organic fertilizer (6.5 t ha^{-1})	7.87	0.12	0.16	26.83	23.33	1.05	1.36	0.41	15.30
Organic fertilizer (13 t ha^{-1})	7.87	0.13	0.18	28.00	25.00	1.28	0.98	0.37	16.39
Organic fertilizer (26 t ha^{-1})	7.97	0.13	0.21	12.25	14.91	1.69	0.43	0.06	18.28
$LSD_{0.05}$	0.30	NS	NS	NS	NS	0.13	0.22	NS	3.71

LSD=Least Significant Difference.

positive and significant correlation with lettuce yield to indicate that lettuce yield was increased with the increase of the above mentioned parameters. Soil pH had positive and significant correlation with organic matter (OM) content in soil suggesting that increased OM helped to increase pH in soil. On the other hand, NH_4^+-N and NO_3^- -N contents were decreased with the increase of total nitrogen in soil. A negative and significant correlation was observed between organic fertilizer doses and heavy metals (Cd & Pb). Heavy metals decreased with the increase of organic fertilizer doses. Plant height (r^2=0.973), leaf number (r^2=0.486), NH_4^+-N content (r^2=0.970), NO_3^- -N content (r^2=0.797) showed significant positive correlation with yield of lettuce. On the other hand, total nitrogen showed significant negative correlation with NH_4^+-N content (r^2=0.834) and NO_3^- -N content (r^2=0.830). From regression studies it was clear that available mineral nitrogen performed significant effect on growth of lettuce. Organic fertilizer (@ 6.5 and 13 t ha^{-1}) plots increased available nitrogen in soil.



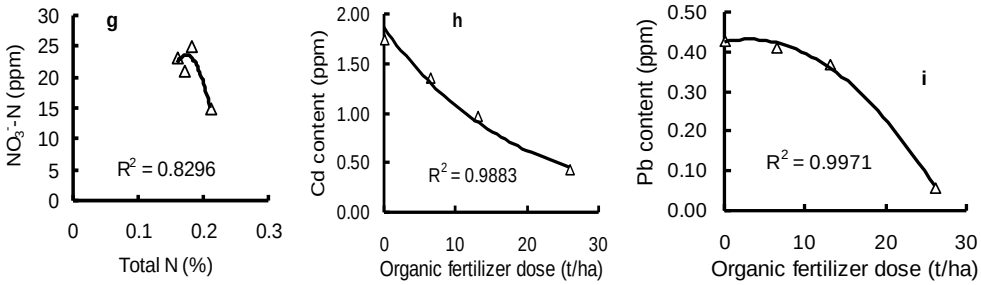


Figure 1. Relationship between plant height and fresh weight of lettuce (a), leaf number and fresh weight of lettuce (b), NH_4^+ -N content and dry weight of lettuce (c), NO_3^- -N content and dry yield of lettuce (d), Organic matter and pH (e), total nitrogen and NH_4^+ -N (f), total nitrogen and NO_3^- -N (g), organic fertilizer level and Cd content (h) and organic fertilizer level and Pd content.

DISCUSSION

Crop success depends on the availability of nutrients especially nitrogen during growth. Among the organic fertilizers doses, @ 26 t ha^{-1} produced the lowest yield of lettuce. Reduced growth (plant height and leaf size) of lettuce in this study was directly related to the insufficient dynamics of N in soil plant-system because the lowest mineral nitrogen content was found in 26 t ha^{-1} organic fertilizer treated soil. Higher dose (26 t ha^{-1}) of organic fertilizer produced the maximum organic matter in soil and this increased of organic matter is needed more mineral nitrogen (NH_4^+-N and NO_3^- -N) for the formation of optimum C:N (10-12:1) ratio in soil, as a result, lettuce growth and development were hampered due to the insufficiency of mineral nitrogen. Researchers reported that N is the main yield factor and considered as the characteristic constituent of functional plasma, an integral part of chlorophyll molecules, proteins, amino acids, nucleic acids (RNA and DNA), nucleotides, phosphotides, alkaloids, enzymes, coenzymes, hormones and vitamins (Castellanos et al., 2000). After application of higher dose of organic fertilizer, nitrogen immobilization was happened in the first crop season followed by mineralization during the second crop (Lynch et al., 2004). Nitrogen content from supplied organic fertilizer in this experiment was 0.45%. Melgarejo et al. (1997) indicated that low nitrogen enriched (>2%) organic materials immobilized mineral nitrogen in soil. On the other hand, organic fertilizer @ 13 t ha^{-1} produced the maximum lettuce yield. It may be attributed to the potential effect of this amendment level to improve water holding capacity, microbial activity, physicochemical and nutritional properties of soils. Improvement of soil physical, chemical and biological properties was reflected in lettuce growth, yield and nutrient uptake. Our present investigated results indicated that the availability of nutrient was increased in soil when nutrients were supplied

through optimum organic fertilizer dose. Similar advantage of organic manures in terms of crop growth and nutrient uptake was reported (Haruna, 2011). This could happen due to the synchronization with mineral nitrogen (NH_4^+-N and NO_3^- -N) and nutritional demands of lettuce crop. Organic fertilizer (26 t ha^{-1}) helped to increase pH and reduce heavy metals contents in soil except Zn because organic residues have the potential of transforming electrochemical properties of acidic soils for improving base retention and increasing the soil pH (Krishna et al., 2004). Heavy metals solubility depends on the degree of pH and dissolves organic matter in soil. Organic matter solubility is relatively low between around pH 4.6 and 6.4 but increase markedly beyond this range to a maximum at pH 7.7. Linear regression analysis showed that pH was positively correlated with dissolved organic carbon concentration from 4.6 to 7.7 pH range. Dissolve organic matter increased with the increase of pH in soil because pH increases the negative charge on such surfaces is increased and repels negatively charged molecules into the soil solution, thereby increasing dissolve organic matter concentration. Cadmium showed the lowest solubility in soil by the high pH and high solubility of dissolve organic carbon. These results indicated that pH showed a strong relationship (negative) with Cd concentrations in soil (Ashworth and Alloway, 2008). But the inconsistent results were found in respect of Pd concentration in soil. These phenomena might have happened due to the strong adsorption capacity of solid organic phase in soil.

CONCLUSION

From the above discussion it may be concluded that the growth and yield of lettuce were the highest in recommended dose of organic fertilizer (13 t ha^{-1}). So, organic fertilizer (13 t ha^{-1}) may be recommended to the growers for getting better yield of lettuce.

ACKNOWLEDGEMENTS

Financial assistance received from the Korea Research Foundation, Govt. of Korea is gratefully acknowledged.

REFERENCES

Ashworth, D.J., and Alloway, B.J. (2008). Influence of dissolved organic matter on the solubility of heavy metals in sewage-sludge-amended soils. *Communications in Soil Science and plant analysis,* 39, 538-550.

Villas Boas, R.L., Passos, J.C., Fernandes, D.M., Bull, L.T., Cezar, V.R.S., and Goto, R. (2004). Doses effects and organic compounds types in lettuce crop in two soils under protect environment. *Horticultura Brasileira*, 22, 28-34.

Bremner, J.M. (1965). In Methods of Soil Analysis. Am. Soc. Agron., Madison. P. 1179-1237.

Brito, L.M., Pinto, R., Mourao, I., and Coutinho, J. (2012). Organic lettuce, rye/vetch and Swiss chard growth and nutrient uptake response to lime and horse manure compost. *Organic Agriculture*, 2(3-4), 163-171.

Castellanos, J.Z., Uvalle-Bueno, J.X., and Aguilar-Santelises, Y.A. (2000). Manual de interpretacion de analisis de suelos, aguas agricolas, plantas y ECP. 2nd ed. INIFAP, Chapingo, Mexico.

Gomez, K.A., and Gomez, A.A. (1984). Statistical procedures for agricultural research, 2nd ed., John Wiley and Sons, Singapore. p. 98.

Haruna, I.M. (2011). Growth and yield of sesame (*Sesamum indicum* L.) as affected by poultry manure, nitrogen and phosphorus at Samaru, Nigeria. *The Journal of Animal and Plant Sciences*, 21(4), 653-659.

Krishna, G.M., Arun, K.M., Kuntal, M.H., Kali, K.B., Prabir, K.G., and Manoranjan, M. (2004). Rice residue- management options and effects on soil properties and crop productivity. *Food, Agriculture and Environment,* 2(1), 224-231.

Lynch, D.H., Voroney, R.P., and Warman, P.R. (2004). Nitrogen availability from composts for humid region perennial grass and legume-grass forage production. *Journal of Environmental Quality*, 33, 1509-1520.

Melgarejo, M.R., Ballesteros, M.I., and Bendeck, Y.M. (1997). Evaluacion de algunos parametros fisicoquímicos y nutricionales en humus de lombriz y compost derivados. *Revista Colombiana de Quimica*, 26(2), 1-11.

Moreira, M.A., Santos, C.A.P., Lucas, A.A.T., Bianchini, F.G., de Souza, I.M., and Viegas, P.R.A. (2014). Lettuce production according to different sources of organic matter and soil cover. *Agricultural Sciences*, 5(2), 99-105.

Oliveira, E.Q., Souza, R.J., Cruz, M.C.M., Marques, V.B., and Franca, A.C. (2010). Lettuce and arugula yield in consorted systems under organic and mineral fertilization. *Horticultura Brasileira*, 28, 36-40,

Vilas, B.R.L., Passos, J.C., Fernandes, D.M., Bull, L.T., Cezar, V.R.S., and Goto, R. (2004). Efitos de doses de compostos organicos na producao de alface em dois solos sob ambiente protegido. *Horticultura Brasileira*, 22, 28-34.

Walkley, A., and Black, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 34, 29-38.

SAARC J. Agri., 15(2): 103-112 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35150*

INCREASING CROPPING INTENSITYAND PRODUCTIVITY THROUGH BORO-T.AUS-T.AMAN-MUSTARD CROPPING PATTERN IN BANGLADESH

M.A.H. Khan[1*], M.S. Zaman[1], M.K. Hasan[2] and A.S.M.M.R. Khan[2]

[1]On-Farm Research Division, Bangladesh Agricultural Research Institute, Mymensingh, Bangladesh
[2]On-Farm Research Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh

ABSTRACT

The experiment was conducted at Multiplication Testing Site (MLT) Trishal of on-farm research division, Bangladesh Agricultural Research Institute, Mymensingh during 2015-16 and 2016-17 to study the comparative agro-economic performance of four crops pattern for increasing cropping intensity and productivity as compared with existing farmers' pattern. Four crops pattern (Boro-T.Aus-T.Aman-Mustard) and farmers' existing pattern (Boro-fallow-T.Aman- fallow) as control were tested. On an average, organic matter 3.94 t $ha^{-1}$and 2.60 t ha^{-1} were added to soil in four crops pattern and farmers existing pattern by incorporation of biomass of respective crops. Two years average results showed that the highest rice equivalent yield (20.63 t ha^{-1}) was obtained from four crops pattern. The highest average gross return and gross margin of the four crops pattern were obtained Tk.359570 and Tk. 170162 ha^{-1} which were 80 and 207 % higher over farmers' pattern. Farmers' practice gave the lower gross return (Tk. 199790 ha^{-1}). The mean marginal benefit cost ratio (MBCR) was found 2.23 which indicated the superiority of four crops pattern over the farmers' existing pattern. The marginal benefit cost ratio (MBCR) analysis also showed that inclusion of mustard and T.Aus rice in the existing pattern might be profitable and acceptable to the farmers. Nutrient uptake and balance showed that considerable amounts of N, P, K and S were removed by crops every year. However, the N, K and S balances were found negative in all cases but P balance was found positive. From the above result showed that four crops can be grown successfully one after another in sequence of the tested pattern.

Keywords: Cropping intensity, cropping pattern, land utilization index, rice equivalent yield

[*] Corresponding author e-mail: helim1367@gmail.com

Received: 21.05.2017 *Accepted: 09.09.2017*

INTRODUCTION

Bangladesh is a densely populated (1008 per sq. km.) country of the world with an area of 147570 sq. km with population growth rate 1.89 percent (BBS 2015). At present total cultivable land is 8.5 million hectares which is decreasing at the rate of about 0.87% per year due to construction of houses, roads and industrial infrastructure. There is very little scope of increasing cultivable land but there are some scope of increasing productivity and cropping intensity from existing level of 191 to 300 % or more by improving the present cropping patterns incorporating short duration crops like; mustard, mungbean, potato, T.Aus etc. in the rice based cropping patterns following the modern variety and technologies. Sustainable crop production in Bangladesh through improvement of cropping patterns in rice based cropping system is regarded as an important in national issues such as food security, poverty alleviation and creation of job opportunity. The main challenge of the new millennium is to increase yield of per unit area by at least 50 % through manipulating the limited land resource. In order to produce more food within a limited area, two most important options to be adopted are i) to increase the cropping intensity by producing three or more crops from the same piece of land in a year and ii) to increase the production efficiency of the individual crop by using optimum management practices. It is reported that more than 60 % of the cultivable lands in Mymensingh region are used for cultivating two rice crops i.e. Boro and T.Aman. As a result, the vast area remains fallow for 75 days after T.Aman and 75 days after Boro harvest. So, there is a great scope of increasing cropping intensity as well as crop productivity. The areas of oilseed and pulse in rabi season are decreasing because of increasing cultivation of Boro rice. Recently with the development of short duration T.Aus and T.Aman rice and mustard varieties opportunities have been created to accommodate four crops in same piece of land in a year. Potential adoption of mustard and T.Aus in Boro- fallow-T.Aman cropping system would generate employment and additional income for the rural poor and producing more of these crops utilizing fallow and underutilized lands in the country. Considering the above issues, the present study was undertaken to evaluate the feasibility of increasing cropping intensity and productivity by growing four crops in a year in a same piece of land by incorporating mustard and T.Aus rice in the existing cropping system.

MATERIALS AND METHODS

The trial was conducted to increase cropping intensity and productivity by incorporating mustard and T.Aus rice in the existing cropping system (Boro-fallow-T.Aman-fallow) during 2015-16 and 2016-17. The experimental site belongs to Old Brahmaputra Floodplain Agro-ecological Zone (AEZ-9) of Mymensingh. The geographical position of the area is between $24^{o}45^{/}$ N latitude and $90^{o}24^{/}$ E longitude. The land was medium high and the soil of the study area was sandy loam in texture with well drainage system and almost neutral in reaction having pH range of 6.0 to 6.9. Maximum rainfall was received during the months of April to September. The

meteorological data of the experimental site revealed that the highest temperature (33.9^{o}C) in August and the lowest in December (10.1^{o}C). The relative humidity was the highest (84.5%) in August and the lowest (75.2 %) in March. The crop received (140.5mm) rain showers from October to March. Monthly mean maximum and minimum air temperature (31.9 and 19.3^{o}C), total rainfall (2018 mm) and relative humidity (82.7 %) were prevailing during the study period. The land type was medium high and the soil texture of the experimental plots was clay loam which belongs to Old Brahmaputra Floodplain soil (AEZ-9). Initial and final soil samples were collected and analyzed (Table 1). General soil types predominantly include Dark Grey Floodplain soils. Organic matter content was low, top soils were acidic to neutral and sub-soils were neutral in reaction. In general, fertility level including N, K and B was low.

Table 1. Initial and post harvest soil test values of farmers field at Trishal, Mymensingh

Sample	Land type	Rainfed/ Irrigated	pH	OM (%)	Total N (%)	K (meq/100 g)	P (Bray)	S	Zn	B
							($\mu g\ g^{-1}$)			
Initial	MHL	Irrigated	6.07	1.39	0.073 (VL)	0.070 (VL)	17.30 (Opt.)	24.42 (Opt.)	1.28 (M)	0.08 (VL)
Post harvest	MHL	Irrigated	6.08	1.28	0.068 (VL)	0.067 (VL)	22.67 (H)	22.94 (Opt)	1.25 (M)	0.11 (VL)

The experiment was laid out in a randomized complete block design with six dispersed replications. Two cropping pattern viz. four crops pattern and farmers' existing pattern were the treatments variables of the experiment. The unit plot size was 1000-1200 Sq. m. Boro rice was the first crop of the sequence. Seedlings of rice were grown in adjacent plot and transplanting was done with 35-40 days old seedlings of rice var. BRRI dhan28 at a spacing of 20 cm × 15 cm during 26 to 31 January, 2015 and 2016 in four crops pattern. Fertilizer management and intercultural operations like weeding, mulching, irrigation and pest management were done according to BRRI (2013). Boro rice was harvested during 26 April to 06 May, 2015 and 2016 in two consecutive years. Rice plant was harvested at 30 cm height from soil surface and remaining parts of the plants was incorporated in soil. T.Aus and T.Aman rice was the second and third crop of the sequence. Seedlings of rice were grown in adjacent plot and transplanting was done with 25-30 days old seedlings of T.Aus rice var. BRRI dhan48 were transplanted with 20 cm × 15 cm during 9 to 13 May, 2015 and 2016 and T.Aman rice var. BRRI dhan57 were transplanted with 20 cm × 15 cm during 8 to 15 August, 2015 and 2016 in both years. Fertilizer management and intercultural operations like weeding, mulching, irrigation and pest management were done according to BRRI (2013). T.Aus rice was harvested during

1 to 6 August, 2015 and 2016 and T.Aman rice was harvested during 22 to 31 October, 2015 and 2016 in two consecutive years. T.Aus rice plant was harvested at 30 cm and T.Aman rice plant was harvested at 15 cm from soil surface and remaining parts of the plants was incorporated in soil. Mustard was grown during *rabi* season and it was the fourth crop of the sequence. Fertilizer management and intercultural operations like weeding, mulching, irrigation and pest management were done according to FRG, 2012. Mustard var. BARI sarisha-14 was seeded as broadcast method with seed rate of 7 kg ha^{-1}. The crop was sown during 3 to 10 November, 2015 and 2016 and harvested during 19 to 24 January, 2016 and 2017, respectively. Crop nutrient uptake was estimated following the standard value of FRG, 2012. Agronomic performance like field duration, rice equivalent yield (REY), production efficiency and land utilization index of cropping patterns were calculated.

Rice equivalent yield (REY)

For comparison between crop sequences, the yield of every crop was converted into rice equivalent on the basis of prevailing market price of individual crop (Verma and Modgal, 1983). Rice equivalent yield (REY) was computed as yield of individual crop multiplied by market price of that crop divided by market price of rice.

$$\text{Rice equivalent yield (t ha}^{-1}\text{yr}^{-1}) = \frac{\text{Yield of individual crop} \times \text{market price of that crop}}{\text{market price of rice}}$$

Production efficiency

Production efficiency value in terms of kg $ha^{-1}day^{-1}$ was calculated by total main product in a cropping pattern divided by total duration of crops in that pattern (Tomar and Tiwari, 1990).

$$\text{Production Efficiency (kg ha}^{-1}\text{ day}^{-1}) = \frac{\sum Y_i}{\sum d_i}$$

Where, Y_i= Yield (kg) of i^{th} crop, d_i= Duration (day) of i^{th} crop of the pattern and i= 1, 2, 3, 4

Land utilization index (LUI)

It was worked-out by taking total duration of crops in an individual cropping pattern divided by 365 days (Rahman et al., 1989). It was calculated by the following formula:

$$\text{Land Utilization Index (\%)} = \frac{d_1 + d_2 + d_3 + d_4}{365} \times 100$$

Where d_1, d_2, d_3 and d_4 the duration of 1^{st}, 2^{nd} 3^{rd} and 4^{th} crop of the pattern

Economic analysis was done on the basis of prevailing market price of the commodities. The inputs used included seed, fertilizer, labour and insecticides. The MBCR of the farmer's prevalent pattern and any replacement for it can be computed

as the marginal value product ((MVP) over the marginal value cost (MVC). The Marginal of prevalent pattern (F) and any potential replacement (E) which was computed as (CIMMYT, 1988).

$$\text{Marginal Benefit Cost Ratio (MBCR)} = \frac{\text{Gross return (E) - Gross return (F)}}{\text{TVC (E) - TVC (F)}} = \frac{MVP}{MVC}$$

RESULTS AND DISCUSSION

Soil chemical analysis of four crop pattern revealed that on an average, pH of the soil increased slightly whereas organic matter a little bit decreased (0.11%) in four crops pattern (Table 1). There was no definite trend followed by the elements but total N, K and B maintained below critical level and P, S and Zn maintained above critical level. Organic matter added to soil through incorporation of non-economic plant parts helped to maintain the quality of soil (Table 2).

Table 2. Addition of organic matter from crop residues in soil of four crops pattern and farmers' pattern at Trishal, Mymensingh during 2015-16 and 2016-17

Crops	4-Crops pattern added residues (t ha^{-1})			Farmers' pattern added residues (t ha^{-1})		
	2015-16	2016-17	Mean	2015-16	2016-17	Mean
Boro rice (30cm)	1.73	1.78	1.76	1.95	1.99	1.97
T.Aus rice (30cm)	1.55	1.59	1.57	-	-	-
T.Aman rice (15cm)	0.59	0.63	0.61	0.62	0.64	0.63
Mustard	-	-	-	-	-	-
Total	3.87	4.00	3.94	2.57	2.63	2.60

Grain yield and by-product yield

Results of four crops pattern (Boro-T.Aus-T.Aman-Mustard) and farmer's existing pattern (Boro-fallow-T.Aman-fallow) have been presented in tables 3-5. Grain yield of Boro rice was 5.96 t ha^{-1} in 1st year and 6.24 t ha^{-1} in 2nd year. Mean grain and straw yields of Boro rice were 6.10 and 6.17 t ha^{-1}. Grain yield of T.Aus rice was 4.88 and 5.24 t ha^{-1} in two consecutive years and mean grain and straw yields were 5.06 and 5.40 t ha^{-1} whereas T.Aman rice grain yields were 4.39 and 4.38 t ha^{-1} in 1st and 2nd year and mean grain and straw yields of T.Aman rice were 4.38 and 4.95 tha^{-1}. Seed yield of mustard were 1.52 and 1.46 t ha^{-1} and stover yields were 2.95 and 2.80 t ha^{-1} in two successive years, respectively. Mean seed yield of mustard was 1.49 t ha^{-1}. Mustard seed yield decreased 4% by 2nd year and it might be due to shorter duration (6 days) than 1st year. It was revealed that all the 4-crops under Boro-T.Aus-

T.Aman-Mustard cropping pattern gave higher grain and by-product yield (Table 3). T.Aman rice in 4-crops pattern produced 11% higher grain yield over farmers' practice due to change of variety with improved production technologies. Similar results were also obtained by (Nazrul et al., 2013; Khan et al., 2006). Farmers' pattern gave lower yield due to imbalance use of fertilizers and poor management practices. Four crops pattern produced higher by-product yield (19.40 t ha^{-1}) over farmers' practice (11.76 t ha^{-1}). Mean by-product yield of four crops pattern was 65 % higher over farmer's pattern due to change of variety with improved technologies and inclusion of two crops in the existing pattern.

Table 3. Agronomic parameters of four crops pattern and farmers' existing pattern at Trishal, Mymensingh during 2015-16 and 2016-17

Parameters	Years	Four Crops Pattern				Farmers' Pattern	
		Boro	T.Aus	T.Aman	Mustard	Boro	T.Aman
Variety	2015-16	BRRI dhan28	BRRI dhan48	BRRI dhan57	BARI Sarisha-14	BRRI dhan29	BRRI dhan32
	2016-17	BRRI dhan28	BRRI dhan48	BRRI dhan57	BARI Sarisha-14	BRRI dhan29	BRRI dhan32
Sowing/ planting time	2015-16	27-31 Jan.	09-13 May	08-12 Aug.	03-09 Nov.	09-13Jan.	10-15 Aug.
	2016-17	26-31Jan.	13-16 May	11-15 Aug.	05-10 Nov.	26-29 Jan.	09-13 Aug
Seedling age (days)	2015-16	35-40	25-30	25-30	-	40-45	30-35
	2016-17	35-40	25-30	25-30	-	35-40	30-35
Harvesting time	2015-16	26-28 April	01-06 Aug.	22-25 Oct.	22-24 Jan.	10-15May	15-20 Nov.
	2016-17	02-06 May	01-05 Aug.	28-31 Oct.	19-22 Jan.	26-29May	12-16 Nov.
Grain yield (t ha^{-1})	2015-16	5.96	4.88	4.39	1.52	6.87	3.92
	2016-17	6.24	5.25	4.37	1.46	6.98	3.94
Straw yield (t ha^{-1})	2015-16	5.94	5.15	5.10	2.95	6.99	4.27
	2016-17	6.39	5.64	4.80	2.80	7.49	4.77
Field duration (days)	2015-16	100	78	76	80	121	97
	2016-17	96	79	77	74	121	94
TAT (days)	2015-16	7	10	7	7	92	55
	2016-17	8	12	11	8	88	62

Field duration

Field duration of a cropping pattern comprises on the individual crop duration. Farmers' cropping pattern Boro-fallow-T.Aman-fallow has needed 218 and 215 days

field duration in 1st and 2nd year. The newly introduced two crops in the farmers existing pattern were mustard (var. BARI Sarisha-14) and T.Aus (var. BRRI dhan48). A short duration T.Aman rice var. BRRI dhan57 was also introduced to minimize the field duration of the crop. Total field duration of four crops pattern Boro-T.Aus - T.Aman-Mustard has needed 334 and 326 days (excluding seedling age of rice) to complete the cycle in 1st and 2nd year (Table 3). Thus, long turn around period of 147-150 days in the farmers existing pattern was utilized. Result indicated that mustard and T.Aus rice could be easily fitted in Rice-Rice cropping pattern with an average of 35 days turn around time in a year. Similar trend was also observed by Mondal et al. (2015) who reported that all the tested four crops pattern can be grown successfully one after another in sequence.

Rice equivalent yield

Total productivity of four crops pattern and farmers' pattern were evaluated in terms of rice equivalent yield (REY) and it was calculated from the yield of component crops. Mean rice equivalent yield (20.63 t ha^{-1} yr^{-1}) was found in four crops pattern and farmers' existing pattern produced (10.72 t ha^{-1} yr^{-1}) rice yield (Table 4). Inclusion of mustard in rabi season and T.Aus in kharif-1 season in existing cropping pattern increased total productivity by 92 % compared to farmers' practice. These results are in agreement with Mondal et al. (2015). They reported that total productivity increased by 67 % over farmers' practice.

Table 4. Rice equivalent yield, production efficiency and land utilization index of four crops pattern and farmers' existing pattern at Trishal, Mymensingh during 2015-16 and 2016-17

Items	Four crops pattern			Farmers' pattern		
	2015-16	2016-17	Average	2015-16	2016-17	Average
REY (t $ha^{-1}yr^{-1}$)	20.30	20.95	20.63	10.52	10.92	10.72
PE (kg $ha^{-1}day^{-1}$)	60.78	53.50	57.14	48.89	50.79	49.64
LUI (%)	92	89.32	90.66	58.00	58.90	58.45

REY= Rice Equivalent Yield, PE= Production Efficiency and LUI= Land Utilization Index

Production efficiency

Mean maximum production efficiency (57) in terms of kg $ha^{-1}day^{-1}$ was obtained from four crops pattern and minimum (50 kg ha^{-1} day^{-1}) in farmers' practice (Table 4). The higher production efficiency in four crops pattern might be due to inclusion of high yielding mustard and T.Aus rice varieties and improved management practices. Similar trend were noted by Nazrul et al. (2013) and Khan et al. (2006).

Land utilization index (LUI)

Land utilization index is the effective use of land in a cropping year, which mostly depends on crop duration. Land utilization index (LUI) indicated that four crops pattern used the land for 91% period of the year, whereas farmers' pattern used the land for 58 % period of the year (Table 4). The higher land use efficiency in four crops pattern because this pattern occupied the field for longest duration (326-334 days) whereas the farmers' pattern occupied the field for 215-218 days of a year.

Cost and return analysis

From the economic point of view, Boro-T.Aus -T.Aman- Mustard rice cropping pattern showed its superiority over Boro-T.Aman (farmers' pattern) cropping pattern. Mean gross return of four crops pattern was found Tk. 359570 ha^{-1} and farmers' pattern was Tk.199790 ha^{-1} which was more than 80 % higher over farmers' pattern (Table 5). Two rice crop patterns (Farmers' pattern) gave the lower gross return Tk. 199790 ha^{-1}. Mean variable cost was higher in four crops pattern (Tk. 189408 ha^{-1}) might be due to inclusion of two component crops in the pattern. The mean gross margin was significantly higher in four crops pattern (Tk.170162 ha^{-1}) than farmers' pattern (Tk. 82117 ha^{-1}). Four crops pattern achieved higher gross margin mainly due to higher yield advantages of the component crops. The mean marginal benefit cost ratio (MBCR) was found 2.23 which indicated the superiority of the four crops pattern over the farmers' pattern. The marginal benefit cost ratio (MBCR) also showed that inclusion of mustard and T.Aus in the existing pattern might be profitable and acceptable to the farmers. These results are supported by Mondal et al. (2015). They reported that inclusion of T.Aus, potato, mustard and mungbean in the existing pattern were profitable and acceptable to the farmers and grown successfully one after another in sequence of one year cycle.

Table 5. Cost and return analysis of four crop based cropping pattern and farmers' cropping pattern at Mymensingh during 2015-16 and 2016-17

Parameters	Gross return (Tk. ha^{-1})			Total variable cost (Tk. ha^{-1})			MBCR		
	2015-16	2016-17	Mean	2015-16	2016-17	Mean	2015-16	2016-17	Mean
4-Crops pattern	358820	360320	359570	186381	192435	189408	2.32	2.13	2.23
Farmers' pattern	192460	207120	199790	114771	120575	117673	-	-	-

Price (Tk. kg^{-1}): Mustard-50.0, Boro rice-15.0, T.Aman-16.0, T.Aus rice-14.50, Stover-1.0 and Straw-2.0

Apparent soil nutrient balance

Total N, P, K and S uptake by different crops at the farmer's field are presented in table 6. The partial net balance of N was negative in both pattern and ranged from -74

to -223 kg ha^{-1}. Nitrogen replenishment through chemical fertilizer and organic matter addition either singly or incombination was not enough to balance N removal by crops since much of applied N was lost from the soil. The N balance thus, was negative (Table 6). The P balance was favourable as expected due to individual crop basis fertilization. Excess amount of P accumulated in the soil and positive effect of P was reflected in four crops pattern. In farmers pattern, P balance was also negative but the amount is very low (-2kg ha^{-1}). In case of K, it was evident that this element was removed in excess of the quantity added as fertilizer in both pattern. The partial net balance of K was negative and ranged from -79 to -128 kg ha^{-1}. This may lead to K depletion in the long run. There was negative balance of S in both pattern and it ranged from -3 to -15 kg ha^{-1}. This results are supported by Khan et al. (2006) and Ishaque et al. (1998).

Table 6. Effect of Boro-T.Aus-T.Aman-Mustard cropping pattern on the soil nutrient balance at Trishal, Mymensingh (Average of 2015-16 and 2016-17)

Pattern	Nutrient uptake (kg ha^{-1})				Nutrient added (inorg.+org.) (kg ha^{-1})				Apparent nutrient balance (kg ha^{-1})			
	N	P	K	S	N*	P	K	S	N	P	K	S
4-Crops	380	60	321	58	393	83	193	45	-223	23	-128	-15
FP	178	31	160	17	260	29	81	14	-74	-2	-79	-3

*40% of applied fertilizer/manure N was considered effective

CONCLUSION

Mustard and T.Aus rice could be easily fitted in the existing pattern with higher rice equivalent yield and higher benefit. Besides, cultivation of four crops, Boro (var. BRRI dhan28)-T.Aus (var. BRRI dhan48)-T.Aman (var. BRRI dhan57)-Mustard (var. BARI Sarisha-14) pattern in a year in the same piece of land could be created more employment opportunity as well as increased production of rice and mustard for the farmers at the same time cropping intensity and productivity could be increased.

ACKNOWLEDGEMENT

Authors are grateful to Ministry of Agriculture, Peoples Republic of Bangladesh for a research grant to OFRD, BARI for development of four crop based cropping patterns.

REFERENCES

BBS (Bangladesh Bureau of Statistics). (2015). Statistical Pocket Book Bangladesh, Statistics and Informatics Division, Ministry of Planning, Government People's Republic of Bangladesh, Dhaka, p. 92.

BRRI (Bangladesh Rice Research Institute). (2013). Modern Rice Cultivation, 17th edition, Bangladesh Rice Research Institute, Joydebpur, Gazipur-1701.

FRG (Fertilizer Recommendation Guide). (2012). Nutrient uptake by various crops at definite yield level. Bangladesh Agricultural Research Council. Farmgate, New Airport Road, Dhaka-1215, p.7.

CIMMYT (International Maize and Wheat Improvement Centre). (1988). Agronomic data to farmer recommendation: An economic training manual. International Maize and Wheat Improvement Centre, Mexico, D.F, p.79.

Ishaque, M., Panaullah, G.M., Bhuyian, N.I., and Saha, P.K. (1998). Integrated nutrient management with inorganic fertilizer and green/ organic manures for Boro-T.Amanrice cropping pattern. In proceedings "National Workshop on Integrated Nutrient Management for Crop Production and Soil Fertility" held on 24-25 March, 1998 at BARI, Gazipur, Bangladesh, p. 111-114.

Khan, M.A., Hossain, S.M.A., and Khan, M.A.H.K. (2006). A study on some selected jute based cropping patterns at Kishoregonj. *Bangladesh Journal of Agricultural Research*, 31 (1), 85-95.

Khan, M.A.H., Khurram, M.M.H., Chowdhury, D.A., Quayyum, M.A. and Islam, M.F. (2006). Integrated nutrient management in wheat-Jute-T.Amanrice cropping system. *Bangladesh Journal of Agricultural Research*, 31(2), 249-257.

Mondal, R.I., Begum, F., Aziz, M.A., and Sharif, S.H. (2015). Crop sequences for increasing cropping intensity and productivity. *SAARC Journal of Agriculture*, 13(1), 135-147.

Nazrul, M.I., Shaheb, M.R., Khan, M.A.H., and Khan, A.S.M.M.R. (2013). On-Farm evaluation of production potential and economic returns of potato-rice based improved cropping system. *Bangladesh Agronomy Journal*, 16 (2), 41-50.

Rahman, M.M., Khan, M.H., Mallick, R.N., and Hudgens, R.E. (1989). *Guidelines for Farming Systems Research Methodology.* Bangladesh Agricultural Research Council, Farmgate, Dhaka-1215.

Tomer, S.S., and Tiwari, A.S. (1990). Production potential and economics of different crop sequences. *Indian Journal of Agronomy*, 35 (1&2), 30-35.

Verma, S.P., and Modgal, S.C. (1983). Production potential and economics of fertilizer application as resources constraints in maize, wheat crop sequence. *Himachal Journal of Agricultural Research*, 9 (2), 89-92.

SAARC J. Agri., 15(2): 113-123 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35163*

SHORTAGE OF WATER IN TEESTA RIVER BASIN AND ITS IMPACT ON CROP PRODUCTION IN NORTHERN BANGLADESH

M.L. Raihan*, M.A. Sarker and M.A.M. Miah

Department of Agricultural Extension Education, Bangladesh Agricultural University
Mymensingh, Bangladesh

ABSTRACT

The objectives of the study were to (i) assess the extent of water shortage in the downstream of Teesta River Basin (TRB) areas; (ii) explore the problems associated with crop production due to water shortage in TRB areas and (iii) examine the impact of water shortage on crop production in TRB areas. The study was conducted in four villages under Nilphmari district during April, 2015. By secondary data analysis and farmers' perception it was clear that water flow and discharge of Teesta river was decreasing significantly during the last 15 years. The major impact was the dramatic increase in costs of irrigation of major crops and ultimately rise in the costs of production and less profit from farming. All of the farmers (100%) opined that the irrigation costs of major crops have been increasing due to shortage of water. The cultivation of LWRC due to water shortage was also a major impact of water shortage on crop production. Farmers were concentrating more on cultivating maize, tobacco, wheat, different types of vegetables etc. compared to rice particularly in dry season. Different types of problems like increased amount of heavy metal in crop land due to continuous uplifting of ground water, decrease soil fertility, increase pests and diseases to crops, fallowing of high and medium high land, increase of fertilizer and pesticide costs etc. were affecting farmers severely in crop production.

Keywords: Water shortage impact, crop production, Teesta river basin

INTRODUCTION

Bangladesh is a very small agrarian country with 142.32 million people (BBS, 2011). In the recent time agriculture sector of the country is facing many challenges especially due to consequences of climate change and manmade causes. The Teesta river being a major source of ground water recharge in North- Western part of Bangladesh and was used for irrigation purposes for long time. Teesta Barrage

* Corresponding author e-mail: masarker@bau.edu.bd

Received: 15.10.2107 *Accepted: 25.11.2017*

Project (TBP), which is located at the Teesta Flood Plains (TFP) at Dalia point in Nilphamari district, has been a major source of surface water irrigation in North-Western part of Bangladesh by gravity flow since 1990. Reduction of dry season flow of Teesta has significant consequences on its ecosystem services. It is through this process, the mighty Teesta has been tamed as it lost its might and its flow has reduced to only a few cusecs in dry periods (Haque et al., 2014). For the last several years these areas are facing serious water related difficulties like river bed siltation, low water flow, fresh and water bodies becoming dry etc. (The Daily Star, 16-04-2011). Of the147 billion cubic meters required in the country during dry season, only 90 billion cubic meters is available. This 40% deficit leads to drought in some regions (Mbugua and Snijders, 2011). People face many problems in dry season getting water in tube wells and in irrigation canals. Therefore people rely on deep tube well or shallow tube well. Not all farmers have access to these means. Because of water shortage crop production is decreasing, causing less income for farmers and less availability of food for the local communities and serious impact on annual crop production (Mullick et al., 2011). People are now suffering due to the rising costs of lifting water, thereby increasing cost of irrigation and crop production (Haque et al., 2014). The price for installation of shallow machine is much higher compared to past years. In some high land areas, the groundwater level is so deep that sinking of shallow machine is too costly, especially in some areas of Nilphamari Sadar and Joldhaka upazila (Mbugua and Snijders, 2011). Again, farmers of these area are now changing their cropping pattern (larger area coverage by maize and tobacco) without considering ecological effect and food security and moving to other professions other than farming (Islam and Higano, 2011). Arsenic is a great problem in the study area and this problem worsening continually due to the injudicious uplifting of ground water (Rahman, 2005). In view of the above background and facts, the present study aimed at gathering information regarding the following questions:

- What extent the water shortage is found in Teesta River Basin?
- What extent of problems confronted by the farmers engaged in crop production due to shortage of water?
- What extent the impact of water shortage on crop production?

MATERIALS AND METHODS

This study employed both quantitative and qualitative research approaches in order to get a comprehensive view of the shortage of water and its impact on crop production. The study were conducted in four villages namely Dubachuri Gondeyapara, Hajipara, Nakbakta and Kalikaganj under Ramnagar, Laxmichap, Saulmari and Daoabari unions respectively in Nilphamari Sadar and Joldhaka upazila under Nilphmari district. Nilphamari district was selected purposively as it is one of the most affected districts due to water shortage in Teesta river of Bangladesh (Mbugua and Snijders, 2011). About 1250 farm families live in each village of Ramnagar, Laxmichap,

Daoabari and Soalmari unions of Nilphamari Sadar and Joldhaka upazilas (BBS, 2011). List of farmers were taken from respective Sub-Assistant Agriculture Officers (SAAOs) of each village and farmers were randomly taken as respondents. The target population was 300, which was taken randomly from four unions.

Out of these 300 populations, 25 percent populations were selected from each village as sample. Hence, the sample size was 75. In order to collect relevant data for the study, a structured interview schedule was carefully prepared keeping the objectives in mind. The interview schedule was pre-tested with 20 farmers by the researcher. A sub-sample of 20 farmers was selected for FGD. The dependent variable of the study was the impact of water shortage on crop production and the explanatory variables were eight (8) selected socio-economic characteristics. Causes of water shortage were measured by two dimensions such as change in climate and increase in withdrawal of water from upstream. The impact on crop production was measured on the basis of the extent of changes occurred in two (2) selected dimensions of crop production as a result of water shortage in Teesta river. i) Changes of crop choices due to water shortage and ii) Changes in irrigation cost of major crops due to water shortage. The respondents were asked to mention their common cropping pattern or crop choices pattern they had been practicing over the years in three common seasons, namely, *Rabi* (16 October-15 March), *Kharif-1* (16 March-30 June) and *Kharif-2* (1 July-15 October). The separate information regarding cropping pattern of the respondents were written under "before 2000" and "at present". The respondents were asked to mention their irrigation costs (in '000' taka) for major crops like rice (Boro and Aman), wheat, maize, tobacco and potato per hectare area in "before 2000" and "at present". The changes in average costs were computed and compared using "T-test". Eight characteristics of the farmers were selected as independent variables of the study. A Problem Facing Index (PFI) for each 10 selected problems was computed by using the following formula:

$$PFI = (P_h \times 3) + (P_m \times 2) + (P_l \times 1) + (P_n \times 0)$$

Where,

P_h = Number of responses indicating high problem

P_m = Number of responses indicating medium problem

P_l = Number of responses indicating low problem

P_n = Number of responses indicating no problem

Problem Facing Index (PFI) for any one of the selected problem could range from 0 to 225, where, 0 indicated no problem facing and 225 indicated highest problem facing.

RESULTS AND DISCUSSIONS

Selected characteristics of the farmers

Table 1. Summary statement showing categories and salient features of the selected characteristics of the farmers (N=75)

Selected characteristics	Measuring unit	Categories	Respondents		Mean	SD
			No	%		
Age	Year	Young (18- 35)	18	24	46.89	10.91
		Middle (36-55)	37	49.3		
		Old (> 55)	20	26.7		
Level of education	Years of schooling	Illiterate (0)	20	26.7	4.84	4.35
		Primary (1-5)	27	36		
		Secondary (6-10)	20	26.7		
		Higher secondary (>12)	8	10.6		
Farm size	Hectare	Landless (0.002-0.02)	0	0	1.18	0.62
		Marginal (0.021-0.2)	0	0		
		Small (0.21-0.99)	41	54.7		
		Medium (1.0-3.0)	33	44		
		Large (> 3.0)	1	1.3		
Annual family income	Thousand taka	Low (up to 96)	43	57.3	97.37	23.69
		Medium (97-130)	26	34.7		
		High (> 130)	6	8		
Agricultural training experience	Days	No training (0)	21	28	4.04	3.99
		Short duration (1-7)	34	45.3		
		Medium duration (8-15)	20	26.7		
		Long duration (> 15)	0	0		
Extension media contact	Scale	Low contact (up to 12)	33	44	18.29	9.97
		Medium contact (13-24)	17	22.7		
		High contact (>24)	25	33.3		
Organizational participation	Scale	Low participation (up to 7)	30	40	8.76	3.34

Selected characteristics	Measuring unit	Categories	Respondents		Mean	SD
			No	%		
Knowledge on sustainable use of water resources	Scale score	Medium participation (8-14) (8-14)	41	54.7	10.09	5.61
		High participation (> 14)	4	5.3		
		Low knowledge (up to 7)	37	49.3		
		Medium knowledge (8-14)	14	18.7		
		High knowledge (>14)	24	32		

Note: SD=Standard Deviation

Data in table 1 revealed that majority of the farmers (49.3%) were middle-aged and considerable proportion of the farmers (36%) had primary education. The highest proportion of the farm size was small (54%). Majority of the farmers (57.3%) had low annual family income and short duration training experience (45.3%) while the significant proportion of farmers had low extension media contact (44%), medium organizational participation (54.7%) and highest proportion of farmers had low knowledge on sustainable use of water resources (49.3%).

Water flow in Teesta river during dry season

Water flow of Teesta River has been significantly decreasing during the last 15 years. According to the data provided by BWDB, it is in remarkably declining phase. A graphic representation of water flow in Teesta river during last 15years is given below to providing an understand of the water scarcity scenario.

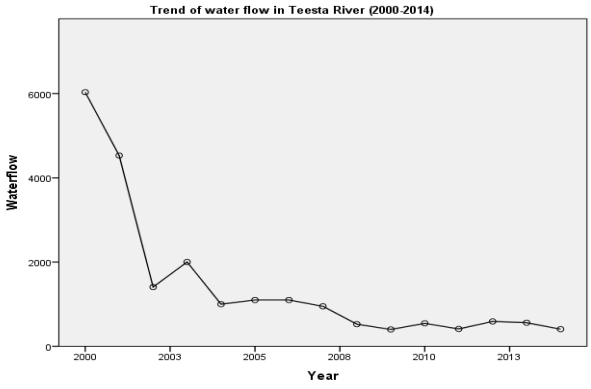


Figure 1. Trend of water flow in Teesta River (2000-2014) (Unreleased data source: BWDB, 2015)

Changes in Coverage of Major Available Sources of Water for Agricultural Production

This was measured to know the real coverage by different water sources for agricultural production i.e., irrigation. From the data presented in table 2 revealed that four main sources of water for agricultural productions were available. There is a remarkable change in coverage by deep tube well. Before 2000, no coverage was done by deep tube well because surface water i.e. water from Teesta river was sufficiently available. But now, the coverage has been significantly increasing up to 85.33 percent. The 't' value was 18.106 which is statistically significant at 1.0 percent level. But, this trend is not good for sustainable agriculture. There are plenty of heavy metals like arsenic, lead etc. are being lifted with ground water and the level is continually falling.

Table 2. Distribution of coverage of major available sources of water for agricultural production

S	Extent of irrigation coverage								't' value	Significance level
	Before 2000				At present (in 2014)					
	H	M	TSE	NAA	H	M	TSE	NAA		
TRW	70 (93.3)	5 (6.6)	0 (0.0)	0 (0.0)	3 (4.0)	15 (20)	38 (50.6)	19 (25.3)	-22.36	.000
STW	16 (21.3)	15 (20)	25 (33.3)	19 (25.3)	12 (16.0)	10 (13.3)	17 (22.6)	36 (48.0)	-6.08	.000
DTW	0 (0.0)	33 (44)	10 (13.3)	32 (42.6)	64 (85.3)	7 (9.3)	4 (5.3)	0 (0.0)	18.01	.000
RW	73 (97.3)	2 (2.6)	0 (0.0)	0 (0.0)	33 (44.0)	37 (49.3)	5 (6.6)	0 (0.0)	-9.12	.000

Notes: i) Figures in the parenthesis showing the percentage of respondents ii) S= Source, H=High, M=Moderate, TSE=To some extent, NAA=Not at all, TRW= Teesta River Water, STW= Shallow Tube Well, DTW= Deep Tube Well, and RW= Rain Water

Changes in Crop Choices due to Water Shortage

This was measured by the changes in cropping pattern and crop choices against existing three cropping seasons namely, *Rabi*, *Kharif-1* and *Kharif-2* due to water scarcity.

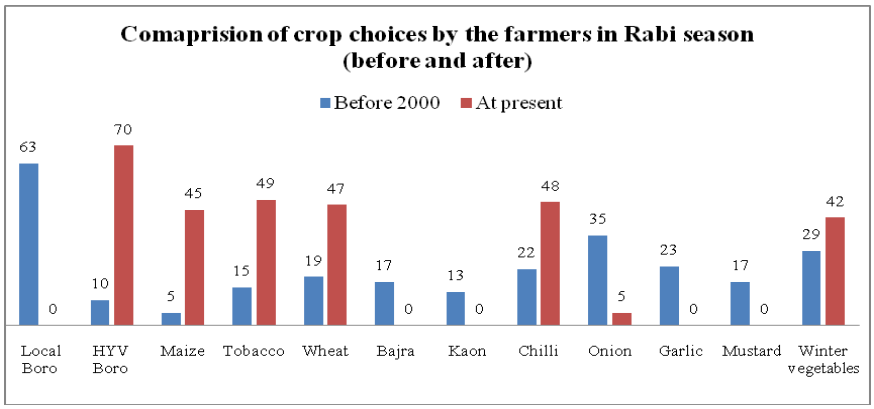


Figure 2. Distribution of crop choices by the farmers in Rabi season (before 2000 and at present)

A great varietal change has been occurred. Before 2000, only China dhan as local Boro was cultivated. In case of HYV Boro, only BR-14 or Gazi dhan was cultivated by farmers but now BRRI dhan-28, BRRI dhan-29, BRRI dhan-33, BRRI dhan-50, Hybrid ACI, Sonar Bangla etc. are being cultivated by farmers. A remarkable change has also been occurred in maize cultivation. Maize is a low water requiring crop and farmers are cultivating maize more than the previous time. The numbers of farmers were only 5 before 2000, whereas it is now 45. There is a problem of continuous maize cultivation on the same land. Because, maize is a nutrient exhaustive crop, it uptakes much more nutrient from soil. Thus, decrease soil fertility if it continues more than 2 years at a stretch. Most of the farmers are not concerned or indifferent about this. A great change also occurred in tobacco cultivation because of water shortage. The farmers are compelled to cultivate this crop because profit is high compared to rice and water requirement is also less compared to rice cultivation. Before 2000, 15 farmers had cultivated tobacco, but now almost all farmers i.e. 49 farmers are cultivating tobacco without considering its ecological impact or health hazards. Wheat cultivation is also significantly increasing because of the same reason mentioned in case of other two crops earlier. Only 19 farmers had cultivated wheat before 2000, but now 47 farmers are cultivating wheat.

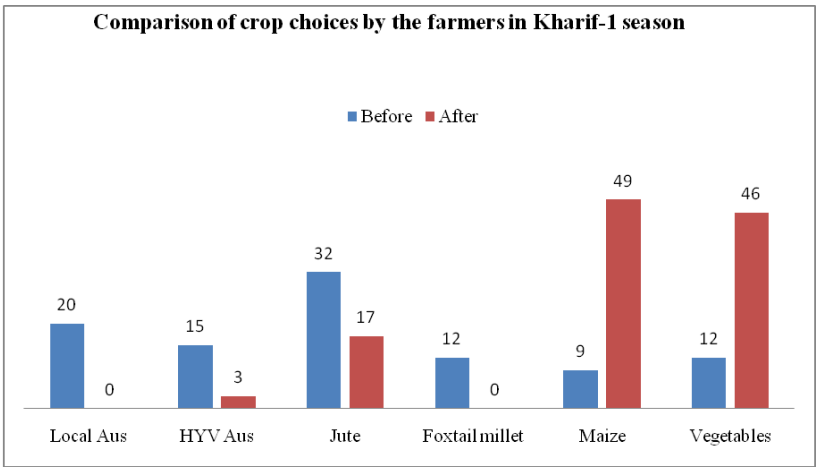


Figure 3. Distribution of crop choices by the farmers in Kharif-1 season (before 2000 and at present)

There is remarkable changes occur in maize and vegetables cultivation. 49 and 46 farmers are cultivating maize and vegetables compared to 9 and 12 farmers before 2000. This is due to water scarcity also remains in Kharif-1 season and farmers are shifting their cultivation pattern. Kachu, spinach etc. vegetables are now generally cultivated in Kharif-1 season.

Changes in Irrigation Costs of Major Crops due to Water Shortage

Table 4 depicts that before 2000, the average cost of irrigation in case of Boro rice was only Tk. 4352.00 per hectare whereas the average cost is now Tk. 25092.66. The 'T' value was 178.408 and it was significant at 1.0 percent level. We know, Boro rice is a high water requiring crop and irrigation cost is vital for the crop production. During FGDs, the farmers opined that they are producing Boro rice with very less coverage considering no profit due to severe increase of cost of irrigation.

Table 3. Changes in irrigation costs of major crops (Source: Field data)

Sl. No.	Crops	Average cost before 2000 (in Taka ha^{-1})	Average cost at present (in Taka ha^{-1})	'T' value	Significance level
1	*Boro* rice	4352.00	25092.66	178.41	.000
2	*Aman* rice	828.00	5873.33	30.73	.000
3	Wheat	1434.00	5553.33	144.75	.000
4	Maize	1568.00	6584.00	254.21	.000
5	Toacco	1568.00	6584.00	254.21	.000
6	Potato	1309.33	4606.00	312.10	.000

It is now much clear that water scarcity is largely contributing in increasing cost of irrigation in almost all crops. So, the crop cultivation is facing many challenges and farmers are getting less profit from farming. With the water shortage and increasing of irrigation costs, some high lands are remain fallow and that is also causing a threat to crop production and sustainable livelihood of farmers.

Problems Faced by the Farmers in Crop Production Due to Water Shortage

The data presented in table 4 show that the highest portions of the respondents (58.7 percent) have faced medium constraints in crop production, while 40.0 percent of the respondents faced high constraints and only 1.3 percent faced low constraints. This means that the large portion (98.7 percent) of the farmers have faced medium to high problems in crop production mainly because of shortage of water for irrigation,

Table 4. Distribution of the farmers according to the problems faced by them

Category	No	Percent	Mean	Standard deviation
Low problems (up to 10)	1	1.3	19.04	4.24
Medium problems (11-20)	44	58.7		
High problems (above 20)	30	40.0		
Total	75	100.0		

For having a better understanding regarding farmers' problems in crop production it was necessary to have an idea about the comparative problem facing in 10 selected problems. The computed PFI of the 10 problems ranged from 98 to 225 (against a possible range from 0 to 225) which are arranged in rank order according to their PFI as shown in table 5.

Table 5. Rank order of problems faced by the farmers in crop production due to water shortage

Sl. No.	Name of the problems	Extent of problems				PFI	Rank order
		High	Medium	Low	NAA		
1	Irrigation cost increase	75	0	0	0	225	1
2	Less profit in farming	72	3	0	0	222	2
3	Heavy metal in crop land increase	13	12	41	9	104	9
4	Problem in land preparation on time	22	29	24	0	148	7
5	Problem in intercultural operation	12	17	39	7	109	8
6	Soil fertility decrease	52	19	4	0	198	4
7	Water level is going down	71	4	0	0	221	3

Sl. No.	Name of the problems	Extent of problems				PFI	Rank order
		High	Medium	Low	NAA		
8	Fallowing of high land and medium high land	7	24	29	15	98	10
9	Insect infestation and disease infection	33	18	24	0	159	6
10	Fertilizer and pesticide cost increase	29	43	3	0	176	5

N.B. NAA = Not at all

In the study area, it was observed that the farmers are facing different types of problems in crop production and its related practices due to water scarcity. They are always struggling against these unexpected events. It is evident from the table 4.6 that increase in cost of irrigation due to water shortage was the major and most prominent problem for farmers in crop production. All the farmers (100%) are strongly facing the problem of irrigation cost increase. Due to water shortage, farmers cannot let their land to remain fallow rather they produce crops through irrigation by groundwater. They require more fuel or diesel for uplifting groundwater which is becoming expensive gradually. Again, the second most important problem was less profit in farming. It was directly associated with the first one. Farmers were compelled to irrigate by groundwater using different machines and that is why cost of production increased and farmers could not sell their agricultural produces particularly rice in reasonable price compared to cost of production. They got very low or no profit from rice cultivation. The third important problem arises due to continuous uplifting of groundwater and the decreasing level of underground water. Soil fertility decrease was another important problem which was number 4 problem according to rank order followed by fertilizer and pesticide cost increase (5), insect infestation and disease infection to crops increase (6), problem in land preparation (7), problem in intercultural operation (8), heavy metal in crop land increase (9), and fallowing of high land (10). Increase in insect pest and disease in crops because of water shortage, climate change and global warming. The cost of fertilizer and pesticides are also increasing due to the above mentioned problems. Biodiversity were greatly hampered. Unavailability of water in due time caused problems in land preparation and intercultural operations. As a result, crop productions were also hampered. Water shortage caused problems in irrigation of high lands creating land fallowing problem.

CONCLUSION

On the basis of findings of the study, their logical interpretation and other relevant facts, we can say that the water flow of Teesta river has been decreasing gradually. Shortage of water was becoming high. Though it was the main source of irrigation water, livelihood, maintaining biodiversity, ecosystem balance. Decrease in the

overall flow of Teesta river was higher. Due to climate change, upstream diversion of water by neighbouring country etc. water becoming scarce day by day. The cost of irrigation for crop production was found to be drastically increasing in the study area due to water shortage in Teesta river. Thus, benefit-cost ratio was also lowering and the farmers faced serious financial problems as farming was getting less profitable. Farmers faced a number of major and minor problems in crop production due to water shortage in Teesta river basin. Cropping pattern of the study area was significantly changing. Cultivation of low water requiring crops was increasing. Thus, if the present situation continues, it can be concluded that farmers might not be able to produce higher water requiring crops, mainly Boro rice. So, food security may face a great challenge. Knowledge of farmers on sustainable use of water resources were not up to the mark level, so it should be increased in a planned way. It can be concluded clearly that urgent proper sharing of Teesta river water with the neighboring country through proper negotiation and treaty should be accomplished by the government with advocacy from NGOs and other learned persons.

REFERENCES

BBS. (2011). Statistical Year Book of Bangladesh. 2005, Bangladesh Bureau of Statistics. Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka.

Haque, A.K., Aich, D., Subhani, R., Bari, E., and Diyan, M.A. (2014). A tale of tamed river: The value of ecosystem services of Teesta river-an estimate of value of provisional services in agriculture and fisheries. Asian Center for Development, Dhaka.

Islam, M.F., and Higano, Y. (2001). Equitable Sharing of Bilateral International Water: a Policy Measure for Optimal Utilization of the Teesta river. *Studies in Regional Science,* 32 (1), 17-32.

Mullick, M.R.A., Babel1, M.S., and Perret, S.R. (2011). Discharge Based Economic Valuation of Irrigation Water: Evidence from the Teesta River. *Bangladesh Journal of Irrigation and Drainage*, 60, 481-492.

Rahman, M.M. (2005). Bangladesh-From a Country of Flood to a Country of Water Scarcity-Sustainable Perspective for Solution. Seminar on Environment and Development, Hamburg, Germany, Entwicklungs Forum Bangladesh.

SAARC J. Agri., 15(2): 125-135 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35162*

TOXICITY AND PHYSIOLOGICAL EFFECTS OF SOME PLANT EXTRACTS ON FRUIT FLY INFESTING ASH GOURD

M.R. Amin[1*], S.M.A. Shafiullah[1], E. Mondal[1] and T. Ahmed[2]

[1]Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University Gazipur, Bangladesh

[2]Department of Agroforestry and Environment, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

ABSTRACT

The aqueous extracts of neem *Azadirachta indica*, eucalyptus *Eucalyptus globulus*, telakucha *Coccinia indica*, mahogany *Swietenia macrophylla* and joba *Hibiscus rosa-sinensis* leaves were used to evaluate their toxicity and physiological effects on fruit fly *Bactrocera cucurbitae* (Diptera: Tephritidae) reared on ash gourd *Benincasa hispida.* The investigations were done with 1, 2 and 4% extracts and an untreated control. The toxicity of the extracts varied with plant species, concentration and exposure period. Among the treatments, neem revealed the most toxic effect as it showed the lowest LC_{50} and LC_{95} values as well as steepest slope of the concentration curve. The order of toxicity of the plants was neem> eucalyptus> telakucha> mehagony> joba. All the extracts reduced pupation, adult emergence, protein content in larval body and body weight of male and female flies. The highest reduction of protein, body weight of male and female flies, pupation and adult emergence were found with 4% neem extract. Among the tested extracts, 4% neem extract was found as the most effective botanical insecticide against *B. Cucurbitae*

Keywords: Botanical insecticides, *Bactrocera cucurbitae,* mortality, growth, development

INTRODUCTION

Ash gourd *Benincasa hispida* L. is one of the important cucurbitaceous vegetables in Bangladesh and other south-east Asian countries. This vegetable is mostly infested with fruit fly, epilachna beetle and red pumpkin beetle. Among these pests, fruit fly *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) is the most destructive which causes damage to all the cucurbitaceous vegetables and its infestation level ranges from 20 to 100% depending on the cucurbitaceous species, climatic region and

[*] Corresponding author e-mail: mramin.bsmrau@gmail.com

Received: 19.09.2017 *Accepted: 30.11.2017*

cultivation season (Atwal and Dhaliwal, 2000; Ramadan and Messing, 2003; Mannan, 2004; Sapkota et al., 2010). In Bangladesh, *B. Cucurbitae* caused 71.5% and 21.0% fruit infestation on sweet gourd and ridge gourd, respectively (Amin et al., 2011).

The female fruit flies lay eggs inside the young, green and tender fruits or corolla of the flowers or stems by inserting their ovipositor. The maggots feed on the flowers, stems and mostly inside the developing fruits. The fruits attacked in early stages fail to develop properly, and drop or rot on the plant. Rabindranath and Pillai (1986) reported that the fruit fly infestations deteriorate fruit quality and yield of ash gourd. Due to internal feeding behavior of larvae, management of fruit fly is difficult. Bagging of fruits in scaffold is a suitable method in suppressing fruit fly infestation but this method is laborious and expensive (Mukherjee et al., 2007; Amin et al., 2008).

The farmers of Bangladesh are mostly relied on synthetic insecticides to combat this manacle pest. The synthetic insecticides not only kill the pest, but also direct killer of insect pollinators and natural enemies and create resistance to pest (Azad et al., 2011). Their excessive and inadvertent use in food crops and vegetables are disastrous to environment and human health (Kim et al., 2003; Desneux et al., 2007).

Now-a-days, environmentally safe products such as plant extracts, oils, and dusts are being incorporated in integrated pest management program for depletion of the use of chemical insecticides (Yuya et al., 2009). Many researchers in different countries have focused on the use of plant materials and extracts as insecticides against various insect pests (Moulton et al., 2002; Osoria et al., 2008).

The neem based insecticides containing azadirachtin have toxicity, repellence, feeding and oviposition deterrence, insect growth regulator activity, and have been reported to control more than 400 species of insects (Pineda et al., 2009). The active ingredients in neem have the ability to function at hormonal concentrations and produce ecdysone-type effects in susceptible insects (Govindachari et al., 2004). Ulrichs et al. (2008) reported that the third instar larvae of *Spodoptera litura* fed on castor leaves treated with salt grass *Porteresia coarctata* (Roxb.) leaf extract at different concentrations showed significant reduction in protein and DNA content in the fat body and midgut tissues.

Bangladesh possesses a lot of botanical species which have medicinal and insecticidal properties. That is why the botanicals are chief and these are promising sources of pest control materials. The botanical compounds azadirachtin, pyrethrum, nicotine and rotenone are recognized as effective insect-control agents (Isman, 1997). Therefore, in this study the locally available plants namely neem *Azadirachta indica*, eucalyptus *Eucalyptus globules*, joba *Hibiscus rosa-sinensis*, telakucha *Coccinia indica* and mahogany *Swietenia macrophylla* leaf extracts were evaluated against the mortality of fruit fly *B. cucurbitae* larvae reared on ash gourd fruit. The study also estimated the pupation and adult emergence rate, protein content in the adult flies and their body weight.

MATERIALS AND METHODS

Study site and condition

The study was conducted in the laboratory of the Department of Entomology, Bangabandhu Shaikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh during November 2015 to June 2016. The insects were maintained in the laboratory at 12:12 LD, 28 ± 2°C and 75 ± 5% RH providing ash gourd which was cultivated in the experimental field of BSMRAU.

Preparation of extracts

Leaves of neem *Azadirachta indica,* eucalyptus *Eucalyptus globulus*, joba *Hibiscus rosa-sinensis,* telakucha *Coccinia indica* and mahogany *Swietenia macrophylla* were collected from the BSMRAU campus and transported to the entomology laboratory. The collected leaves were washed with tap water and air dried for 7 days in the shade. Furthermore, the leaves were dried in an oven at 50-60˚C for 24h to obtain constant weight. The leaves were powdered mechanically by using an electric blender and passed through 40 mesh screen. The powder of each plant species were separately extracted in water. For each preparation, 10g powder was macerated in a 2.5 L capacity glass bottle using 1L water. Then the sample was shaken for 72h using an electric shaker. The extract was filtered and the filtrate was considered 1% concentration (1g 100 ml^{-1}). Similarly, 2 and 4% extracts were prepared and stored in a refrigerator at 4^{o}C until insect bioassay.

Mortality test

Fresh ash gourd fruits were collected from the field and these were cut into pieces and therefore, treated with the extracts. The fruit pieces were then transferred into 9 cm diameter Petri dishes that contained maggots (first and second instar larvae). Each Petri dish had 10 maggots and fruit pieces were used as their diet. An untreated control observation was done with fresh fruit. Number of insect mortality in each Petri dish was recorded at 24, 48 and 72h after treatment and percent mortality was calculated. The observed mortality was corrected following the formula of Schneider-Orellis (1949).

Observation of pupation and adult insect emergence

The 4% extracts of each plant was poured distinctly into the glass containers which had ash gourd pieces. The extracts were mixed with the fruit pieces and the pieces were placed in different Petri dishes and five pairs of newly emerged maggots were released in each Petri dish. The Petri dishes along with the maggots and ash gourd pieces were kept in the laboratory. A control observation was made with maggots and fresh ash gourd pieces. The Petri dishes were kept in net proof cages providing sands on the bottom for pupation of the larvae. Daily observation was made to know the rate of pupation and adult emergence.

Estimation of protein and body weight

For determination of protein, 6 third instar larvae (One larva from each extract treated and untreated fruits) were selected and oven dried at 60^{o}C and then powdered. Protein was determined by conventional Kjeldahl method following the protocol of Amin et al. (2011) and the percent reduction of protein in the treatments was calculated. In total 60 adult insects (five male and five female from each extract treated and untreated fruits) were used to determine their body weight. The weight was determined using a digital balance (AG 204, Mettler Toledo, Switzerland) and percent body weight reduction of the insects in different treatments were calculated.

Statistical analysis

Probit and Chi statistics were employed to analyze the toxicity and protein reduction, respectively. Pupation, adult emergence and body weight reduction data were analyzed by one way analysis of variance (ANOVA) and the mean differences were evaluated using Tukey's HSD posthoc statistics. All the analyses were performed using IBM SPSS 19.

RESULTS

The plant extracts at 24h after treatment showed toxicity effect on the maggots of *B. cucurbitae* and the calculated LC_{50} and LC_{95} values ranged from 9.3 (6.0-63.7) to 13.9 (9.2-40.2) and 18.7 (11.1-150.5) to 37.2 (20.2-101.4) g /100 ml, respectively (Table 1). Toxicity results differed significantly among the extracts and neem extract was found as the most effective one. Its concentration response curve showed the steepest slope which indicated that small variations in the concentrations induced greater responses in mortality. The order of mortality activity of the different plant extracts at 24h post treatment showed neem> eucalyptus> telakucha> mahogany> joba.

Table 1. Toxicity effect of different plant extracts on the larvae of *Bactrocera cucurbitae* exposed to 24h post treatment

Plant	Slope (± S.E)	LC[a]50 (95% fl)	TR_{50}	LC95[a](95%fl)	TR_{95}	χ^2 (df)	P
Neem	0.17 ± 0.03	9.3 (6.0- 63.7)	1.49	18.7 (11.1-150.5)	1.75	58.6 (13)	< 0.001
Eucalyptus	0.17 ± 0.03	9.4 (5.9 – 198.2)	1.48	19.0 (10.9-473.6)	1.72	66.3 (13)	< 0.001
Telakucha	0.13±0.03	10.0(6.4- 59.3)	1.39	23.2(13.5-158.1)	1.41	34.9 (13)	< 0.01
Mahogany	0.13 ± 0.03	11.6 (7.1- 201.2)	1.20	23.9 (13.4-471.8)	1.37	38.6 (13)	< 0.001
Joba	0.09 ± 0.03	13.9 (9.2-40.2)	-	32.7(20.2-101.4)	-	22.1 (13)	< 0.05

Each datum represents the mean of five replicates, each set up with 10 adults (n = 50). Concentrations are expressed as g ml^{-1} fl stands for fiducial limits. [a]Different concentrations (1, 2 and 4g 100ml^{-1}) .

The toxicity of the plant extracts at 48h after treatment showed LC_{50} and LC_{95} values from 8.4 (5.5-51.5) to 17.7 (10.6-113.9) and 20.0 (12.5-72.2) to 39.9 (22.5-276.1) g $100ml^{-1}$, respectively (Table 2). The neem extract revealed the lowest LC_{50} and the telakucha extract showed the lowest LC_{95} values which were statistically similar to eucalyptus. The concentration response curve of the telakucha and eucalyptus extracts showed identical having the steepest slope. The order of toxicity of the plant extracts at 48h post treatment was neem> eucalyptus> telakucha > mahogany> joba.

Table 2. Toxicity effect of different plant extracts on the larvae of *Bactrocera cucurbitae* exposed to 48h post treatment

Plant	Slope (± S.E)	LC^a50 (95% fl)	TR_{50}	$LC95^a$ (95%fl)	TR_{95}	χ^2 (df)	P
Neem	0.13 ±0.03	8.4 (5.5- 51.5)	2.11	20.7 (12.2-152.9)	1.93	45.3 (13)	< 0.001
Eucalyptus	0.14 ± 0.03	8.7(5.8 – 38.9)	2.03	20.0 (12.0 -105.2)	2.0	44.9 (13)	< 0.001
Telakucha	0.14 ± 0.03	8.7 (5.9 – 27.3)	2.03	20.0 (12.5-72.2)	2.0	36.6 (13)	< 0.001
Mahogany	0.10 ± 0.03	12.3 (7.6 – 113.3)	1.44	28.8 (16.2-296.6)	1.39	24.3 (13)	< 0.05
Joba	0.07 ± 0.03	17.7 (10.6 – 113.9)	-	39.9 (22.5-276.1)	-	11.3 (13)	0.58

Each datum represents the mean of five replicates, each set up with 10 adults (n = 50). Concentrations are expressed as g ml^{-1} fl stands for fiducial limits. [a]Different concentrations (1, 2 and 4g 100 ml^{-1}).

The toxicity effects of different plant extracts at 72h after treatment revealed LC_{50} and LC_{95} values from 6.1 (4.9-9.3) to 10.7 (7.7-21.5) and 13.5 (10.0-22.8) to 26.7 (17.9-57.9) g/100 ml, respectively (Table 3). Among the treatments, neem revealed the most toxic effect as it showed the lowest LC_{50} and LC_{95} values having the steepest slope of the concentration curve. The order of toxicity of the plants was neem> eucalyptus> telakucha> joba> mahogany.

Table 3. Toxicity effect of different plant extracts on the larvae of *Bactrocera cucurbitae* exposed to 72h post treatment

Plant	Slope (± S.E)	LC^a50 (95% fl)	TR_{50}	$LC95^a$(95%fl)	TR_{95}	χ^2 (df)	P
Neem	0.22 ±0.03	6.1 (4.9- 9.3)	1.75	13.5 (10.0-22.8)	1.98	33.5 (13)	< 0.001
Eucalyptus	0.13 ± 0.03	7.6 (5.0 – 51.1)	1.41	20.6 (11.9-192.5)	1.30	46.8 (13)	< 0.001
Telakucha	0.16 ± 0.03	7.8 (5.4 – 27.6)	1.37	17.8 (11.0-74.2)	1.5	52.0 (13)	< 0.001
Mahogany	0.10 ± 0.03	10.7(7.7 – 21.5)	-	26.7 (17.9 -57.9)	-	20.2 (13)	0.09
Jaba	0.11 ± 0.03	10.1 (6.3 – 145.8)	1.06	25.7 (14.3-435.9)	1.04	32.8 (13)	< 0.01

Each datum represents the mean of five replicates, each set up with 10 adults (n = 50). Concentrations are expressed as g ml^{-1} fl stands for fiducial limits. [a]Different concentrations (1, 2 and 4g 100 ml^{-1}) .

The plant extracts significantly ($\chi^2 = 9.2$, df = 4, $p < 0.05$) reduced the protein content in the larval body and the reduction rate varied from 5.6 to 17.6% (Figure 1). Among the treatments, neem and joba revealed the highest and lowest reduction, respectively.

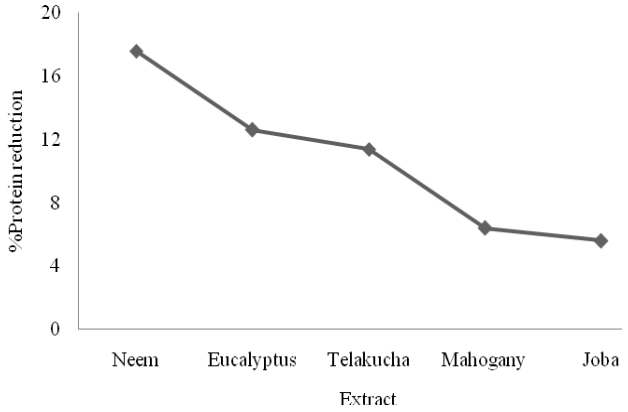


Figure 1. Effect of plant extracts on the protein reduction in *Bactrocera cucurbitae* larvae reared on ash gourd. $\chi 2_{(4)} = 9.2$, $p < 0.05$.

The pupation reduction rate among the treatments ranged from 30.0 ± 6.2 to 53.3 ± 3.3% (Figure 2) and the results differed significantly ($F_{4, 20} = 3.2$, $p < 0.05$). The highest and lowest pupation rates were found in neem and joba, respectively. The plant extracts significantly ($F_{4, 20} = 3.7$, $p< 0.05$) reduced the adult emergence and the results varied from 1.9 ± 0.8 to 5.0 ± 0.8% (Figure 3). Neem and joba depicted the highest and lowest level of reduction, respectively.

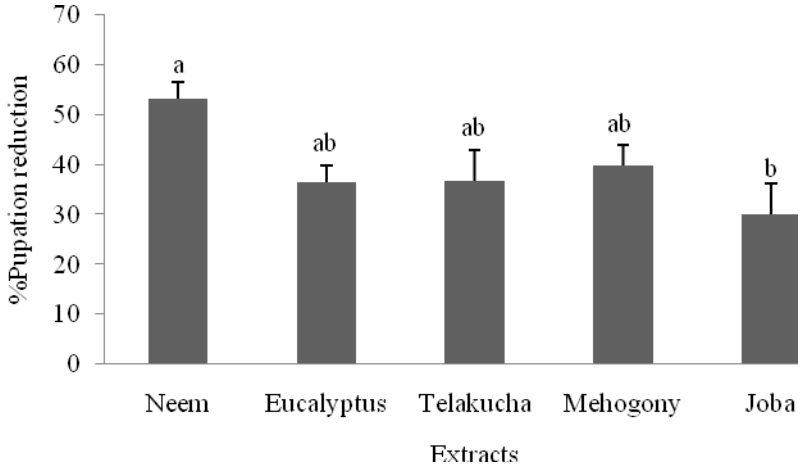


Figure 2. Effect of different plant extracts on the pupation reduction of *Bactrocera cucurbitae* larvae reared on ash gourd. Data expressed as mean ± SE. Bars with no common letter(s) are significantly different by Tukey's HSD posthoc statistic at $p \leq 0.05$.

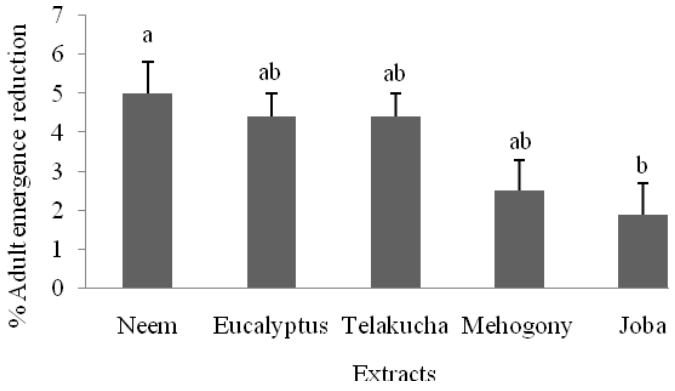


Figure 3. Effect of different plant extracts on the adult emergence reduction of *Bactrocera cucurbitae* larvae reared on ash gourd. Data expressed as mean ± SE. Bars with no common letter(s) are significantly different by Tukey's HSD posthoc statistic at $p \leq 0.05$.

The effect of plant extracts on the body weight reduction of male and female fruit flies are presented in figure 4. The body weight reduction of male and female flies ranged from 11.0 ± 0.9 to 14.6 ± 0.6% and 3.8 ± 0.9 to 8.2 ± 0.5%, respectively. Among the treatments, neem depicted the highest level of reduction and the results differed significantly (Male: $F_{4, 20} = 4.0$, $p < 0.05$; Female: $F_{4, 20} = 3.1$, $p < 0.05$).

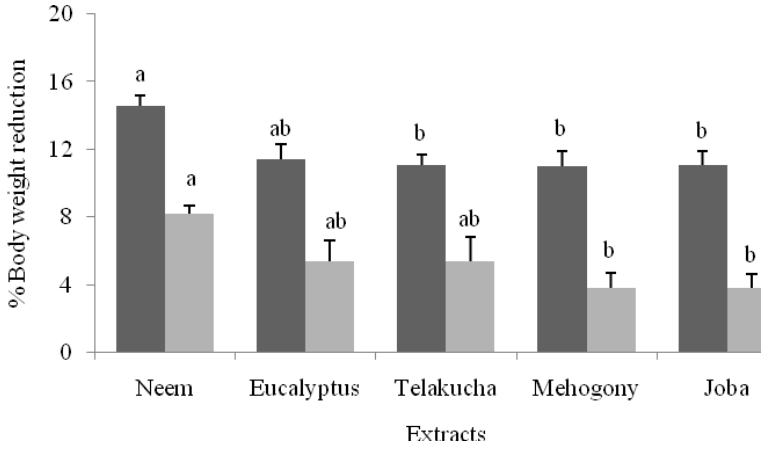


Figure 4. Effect of different plant extracts on the body weight of adult male (■) and female (□) fruit flies reared on ash gourd. Data expressed as mean ± SE. Bars with no common letter(s) are significantly different by Tukey's HSD posthoc statistic at $p \leq 0.05$.

DISCUSSION

Plants are sources of different alkaloids such as azadirachtin, azdirol, cymarin, digitoxin, kulactone, limocinin, salanin, toosendanin, xanthotoxin etc which are soluble in water and toxic to herbivore insects. Many authors reported that the aqueous extract of plants has significant effect on insect mortality, their growth and reproduction (Ciepielewska et al., 2005; Roy et al., 2005; Roy et al., 2010; Roy et al., 2012; Ahad et al., 2015; Ahad et al., 2016). There is report that 4% aqueous extract of neem, akanda and biskatali leaves had significant toxicity effect on lesser grain borer and rice weevil (Amin et al., 2000; Shahjahan and Amin, 2000).

In the present study, all the extracts showed mortality effect on the fruit fly maggot and the results varied with plant species, extract concentrations and exposure periods. This might be the cause that the ingredients of the plants dissolved in water in varied levels and time and those have the ability to dysfunction the nutritional balance of the larvae, thus caused mortality. Fouad et al. (2014) reported that the plant extracts possess broad spectrum toxic substances that interrupt insect's normal physiology and influence on their feeding and mortality.

The tested plant extracts exhibited significant reduction of protein content in larval body, and body weight of adult male and female flies. The extracts might have retarded the maggots from food consumption and efficiency of the conversion of digested food thus reduced the protein content and body weight. Gnanamani and Dhanasekaran (2014) observed 45.4, 13.5, 45.0, 36.9 and 33.1% protein depletion in the haemolymph of the castor hairy caterpillar *Pericallia ricini* larvae when they were treated with *Catharanthus roseus, Datura metel, Delonix regia, Eucalyptus globulus* and *Pongamia glabra* plant extracts, respectively.

The present findings showed that the plant extracts significantly reduced the pupation and adult emergence of fruit fly. The alkaloid substances of the plants dissolved in water might have acted on the neuroendocrine system of the maggots and interrupted their normal processes of molting and metamorphosis, thus they retained as malformed larvae or pupae. Whatever, the larvae turned into pupae and then adults, most of them exhibited deformations of the wings or other parts of the body. This finding is in accordance with Martinez (2011) who reported that the neem compound azadirachtin and its derivatives typically cause growth inhibition and changes in the metamorphosis of insects. Roy et al. (2014) reported that the leaf extracts of common cocklebur *Xanthium strumarium* retarded adult emergence of the pulse beetle *Callosobruchus chinensis.*

The antifeedant, larvicidal and growth inhibition activities of insecticides, controlling the pests at the early stage before they can disperse on the plant would be more preferable in the management of insect pests. In the present study, all the plant extracts retarded growth and development of fruit fly larvae as well as shown their mortality. However, neem and eucalyptus showed potential effect in comparison to other extracts.

The findings are important baseline information for the potential use of neem and eucalyptus leaf extracts as promising and safe insecticidal agent against *B. cucurbitae*. These plant extracts could be incorporated into the integrated management programs of fruit fly in ash gourd field, which could reduce control costs and environmental adverse effects associated with the use of broad spectrum insecticides.

REFERENCES

Ahad, M.A., Nahar, M.K., Amin, M.R., Suh, S.J., and Kwon, Y.J. (2015). Biological activities of five weed extracts against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). *Korean Journal of Applied Entomology,* 54, 83-89.

Ahad, M.A., Nahar, M.K., Amin, M.R., Suh, S.J., and Kwon, Y.J. (2016). Effect of weed extracts against pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) of mung bean. *Bangladesh Journal of Agricultural Research,* 41, 75-84.

Amin, M.R., Ara, R., Mukherjee, S., Bhuyain, M.M.H., and Uddin, M.N. (2008). Economic evaluation of different management practices of cucurbit fruit fly (*Bactocera cucurbitae*) in sweet gourd. *Journal of Science and Technology,* 6,109-113.

Amin, M.R., Sarkar, C., and Chun, I.J. (2011). Comparison of host plants infestation level and life history of fruit fly (*Bactrocera cucurbitae* Coquillett) on cucurbitaceous crops. *Horticulture, Environment, and Biotechnology,* 52, 541-545.

Amin, M.R., Shahjahan, M., Eltaj ,H.F., Iqbal, T.M.T., and Hossain, M.A. (2000). Use of akanda, biskatali and neem as botanical insecticides against lesser grain borer. *Bangladesh Journal of Entomology*, 10, 1-13.

Atwal, A.S., and Dhaliwal, G.S. (2000). Agricultural Pests of South Asia and Their Management. Kalyani Publication. New Delhi-110002, p. 277-278.

Azad, H.M.S., Amin, M.R., Tithi, D.A., and Hossain, S.M.A. (2011). Performances of three cotton varieties cultivated under economic threshold level based insecticide sprayed and non-sprayed conditions. *Our Nature,* 9, 21-25.

Ciepielewska, D., Kordan, B., and Nietupski, M. (2005). Effect of plant extracts on some stored-product insect pests. *Polish Journal of Natural Sciences,* 18, 7-14.

Desneux, N., Decourtye, A., and Delpuech, J.M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology,* 52, 81-106.

Fouad, H.A., Faroni, L.R.D.A., Tavares, W.S., Ribeiro, R.C., Freitas, S.S., and Zanuncio, J. S.(2014). Botanical extracts of plants from the Brazilian cerrado for the integrated management of *Sitotroga cerealella* (Lepidoptera: Gelechiidae) in stored grain. *Journal of Stored Product Research,* 57, 6-11.

Gnanamani, R., and Dhanasekaran, S. (2014). Impact of plant extracts on total free amino acids alterations in the haemolymph of *Pericallia ricini. Journal of Entomology and Zoology Studies,* 2, 62-67.

Govindachari, T.R., Suresh, G., Geetha, Gopalakrishnan., and Wesly, S.D. (2004). Insect antifeedant and growth regulating activity of neem seed oil- the role of major tetranotriterpenoids. *Journal of Applied Entomology*, 124, 287-291.

Kim, S.I., Park, C., Ohh, M.H., Cho, H.C., and Ahn, Y.J. (2003). Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). *Journal of Stored Product Research*, 39, 11-19.

Mannan, M.A. (2004). Study on the relative preference of fruit fly (*Bactrocera cucurbitae)* on different cucurbit. Annual Research Report (2004-05), Division of Entomology, BARI, Gazipur. p. 54.

Martinez, S.S. (2011). O *nim, Azadiractina indica*-natureza, ususmúltiplos, *produção.* IAPAR, Londrina, p. 205.

Moulton, J.K., Pepper, D.A., and Dennehy, T.J. (2000). Beet armyworm (*Spodoptera exigua*) resistance to spinosad. *Pest Management Science*, 56, 842-848.

Mukherjee, S., Tithi, D.A., Bachchu, A.A., Ara, R., and Amin, M.R. (2007). Life history and management of cucurbit fruit fly *Bactrocera cucurbitae* on sweet gourd. *Journal of Science and Technology (Dinajpur)*, 5, 14-24.

Osoria, A., Martinez, A.M., Schneider, M.I., Diaz, O., Corrales, J.L., Aviles, M.C. (2008). Monitoring of beet armyworm resistance to spinosad and methoxyfenozide in Mexico. *Pest Management Science*, 64, 1001-1007.

Pineda, S., Martínez, A.M., Figueroa, J.I., Schneider, M.I., Estal, P.D., Viñuela, E., Gómez, B., Smagghe, G., and Budia, F.(2009). Influence of azadirachtin and Methoxyfenozide on Life Parameters of *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 102, 1490-1496.

Rabindranath, K., and Pillai, K.S. (1986). Control of fruit fly of bitter gourd using synthetic pyrethroids. *Entomology*, 11, 269-272.

Ramada, M., and Messing, R.H. (2003). A survey for potential biocontrol agents of *Bactrocera cucurbitae* (Diptera: Tephritidae) in Thailand. *Proceedings of the Hawaiian Entomological Society*, 36,115-122.

Roy, B., Amin, M.R., Jalal, S., and Sarker, B.C. (2012). Bioefficacy of blood leaf plant extracts again pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Bangladesh Journal of Entomology*, 22, 69-77.

Roy, B., Amin, M.R., Jalal, S., Kwon, Y.J., and Suh, S.J. (2014). Evaluation of common cocklebur *Xanthium strumarium* leaf extract as post-harvest grain protectant of black gram against pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae) and isolation of crude compound. *Entomological Research*, 44, 254-261.

Roy, B., Amin, M.R., Uddin, M.N., Islam, A.T.M.S., Islam, M.J., and Halder, B.C. (2005). Leaf extracts of shiyalmutra (*Blumea lacera*) against lesser grain borer and rice weevil. *Journal of Biological Sciences*, 5, 201-204.

Roy, B., Jalal, S., Sarker, B.C., Amin, M.R., Jeon, Y.H. and Chun, I.J. (2012). Evaluation of insecticidal activities of common cocklebur fruit extract against pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Korean Journal of Applied Entomology*, 51, 325-329.

Roy, B., Sarker, B.C., Amin, M.R., Roy, B.C., and Jalal, S. (2010).Bioefficacy of shiyalmutra leaf extract again rice weevil. *Journal of Science and Technology*, 8, 1-5.

Sapkota, R., Dahal, K.C., and Thapa, R.B. (2010). Damage assessment and management of cucurbit fruit flies in spring-summer squash. *Journal of Entomology and Nematology*, 2, 7-12.

Shahjahan, M., and Amin, M.R. (2000). Evaluation of some plant extracts against rice weevil, *Sitophilus oryzae* L. *Journal of the Asiatic Society of Bangladesh*, 26, 213-222.

Ulrichs, C., Mewis, I., Adhikary, S., Bhattacharyya, A., and Goswami, A. (2008). Antifeedant activity and toxicity of leaf extracts from *Porteresia coarctata* Takeoka and their effects on the physiology of *Spodoptera litura* F.). *Journal of Pest Science*, 81, 79-84.

SAARC J. Agri., 15(2): 137-146 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35160*

AN EXERTION OF ENHANCE MILK YIELD OF NATIVE PABNA CATTLE THROUGH USING DIFFERENT TYPES OF CALCIUM SALT OF FATTY ACID

D. Yeasmin[1], N. Islam[2], N.R. Sarker[1], N. Huda[3*], A. Habib[1], F. Tabassum[1]

[1]Fodder Research and Development Project, Bangladesh Livestock Research Institute
Savar, Dhaka, Bangladesh
[2]Department of Dairy Science, Bangladesh Agricultural University, Mymenshing, Bangladesh
[3]Animal Production Research Division, Bangladesh Livestock Research Institute
Savar, Dhaka, Bangladesh

ABSTRACT

Keeping consideration on increasing quality milk production through addition of conventional fat, an experiment accords CRD design was conducted at Bangladesh Livestock Research Institute, Savar, Dhaka for a period of 30 days including 5 days of digestibility trial. Twenty lactating Pabna cattle of average 250.1 ± 13.64 kg (*Bos indicus*) of initial body weight with average milk yield of 2.88 ± 0.38 kg were selected and divided into four groups randomly having five cows in each. The imposed treatments were T_0, T_1, T_2, and T_3; in where T_o= German grass + Concentrate mixture without Ca salt of fatty acid + 2.5 % DCP, T_1= German grass + Concentrate mixture + 2.5 % soybean based Ca salt of fatty acid, T_2= German grass + Concentrate mixture + 2.5 % mustard based Ca salt of fatty acid and T_3= German grass + Concentrate mixture + 2.5% palm-oil based Ca salt of fatty acid. The result showed that, calcium salt of fatty acid influenced to increase of milk yield significantly among ($P<0.001$) the treatments; in where highest yield gained in T_1 (3.53 kg d^{-1}) followed by T_2, T_0 and T_3 (3.33, 2.44 and 2.24, respectively). Besides this, all other parameters analytically examined in this experiment i.e. intake, apparent digestibility, gained body weight and chemical composition of milk showed non-significant difference among the treatments. From the analytical value and discussion, it may be suggested that, calcium salt of fatty acid formulated from soybean oil or mustard oil could be used for enhancing better quality milk yield.

Keywords: Ca salt of fatty acid, milk yield, chemical composition of milk, nutrient intake, nutrient digestibility, gained weight

INTRODUCTION

For tactful diet formulation with much more energy than conventional fat consults addition of fat as feed in the diet very commonly. The supplemental fat of diet,

* Corresponding author e-mail: hudanazmul1971@gmail.com

Received: 07.09.2017 *Accepted: 10.11.2017*

provide calories and as well as impacts on tissue metabolism by altering genetic expression (Sumida et al., 1993) or on supplying precursors (acetyl Co-A) for synthesis of other fatty acids or steroids (Staples et al., 1998), or competing with other cell components (Thatcher et al., 2004). In addition, metabolizible energy in the form of supplemental fat is utilized more efficiently than that contained in cereals and forage (Palmquist and Jenkins, 1980). And, another beautiful mechanism is, rumen inert fat can enhance energy density of lactation diets without adversely affecting fiber digestion; thus, effectively overcome the shortcomings of conventional fat being introduced in the rumen (Jenkins and Palmquist l984; Grummer, 1988). The development of Ca salts of fatty acids (CaFA), which are considered inert in the rumen, offers a method of increasing production and efficiency without impairing fermentative digestion (Chalupa et al., 1986). In this regard, several experiments had done and they suggested that, inclusion of Ca salts of FA increased overall milk production of dairy cows (Moallem et al., 2000); by means of milk yield or milk fat percentage (Klusmeyer et al., l99l) or both (Rabiee et al., 2012). A major concern is, "Ca salts" are thought to be insoluble in the rumen which resembles an experiment; in where a higher proportion of unsaturated FA in duodenal digesta when a diet containing Ca salts of FA from palm oil was fed (Wu et al., 1991). But, it is more established that, calcium salt of fatty acid have possible protection against bio-hydrogenation depending on the conditions in rumen and if feeding saturated fats or Ca salts of long chain fatty acids are practiced, then it may minimize any detrimental effects on ruminal fermentation and saturated fatty acids are less likely to alter fermentation in the rumen than unsaturated fatty acids, because saturated fatty acids are less soluble and therefore, are less likely to absorb by bacteria (Chalupa et al., 1984). Saturated fatty acids also react more readily with metal ions to form insoluble salts of fatty acids (Palmquist and Jenkins, 1982) and preformed Ca salts of fatty acids (CaS) does not alter fermentation in the rumen because of their insolubility (Chalupa et al., 1984 and Chalupa et. al., 1986). It is exercised and proved that, supplementation of calcium salts of long chain fatty acids (Ca-LCFA) as a rumen inert fat (PF) has no detrimental effect on fermentation and apparent nutrient digestion (Naik et al., 2009). Even ruminal pH, total VFA, molar percentage acetate propionate, and milk yield and fat percentage were not affected by fat supplementation (Grummer, 1988). Moreover, calcium salts of palm oil fatty acids at a 4% level in the concentrate mixture resulted with improved milk production and milk quality in terms of polyunsaturated fatty acids without affecting the digestibility of nutrients (Sajith et al., 2008). For a long time, supplemental fat is increasingly included in the diets of high yielding dairy cows (Kellogg et al., 2001). This allows to modify the fatty acids (FA) pattern of the milk fat (Precht et al., 2001) and to improve the energy supply of the cow. Furthermore, supplemental fat act as nutritional modifier of physiology and metabolism (Voigt et al., 2005). However, unprotected, unsaturated FA can be toxic to the rumen microbes unless saturated by microbial hydrogenation (Harfoot, 1981). There are different commercial sources of

rumen inert fats including hydrogenated fatty acids and calcium salts of fatty acids and these fat sources were originally designed to increase the calorie intake of dairy cows with minimal impact on rumen microbial activity indeed. In aspect of improved milk production and altering composition of milk fat, there has no doubt on influencing supremacy of Calcium salt of fatty acid. But, regarding this context, a few works have done in Bangladesh. So very logically, this experiment was undertaken to find out the effect of feeding different types of calcium salt of fatty acids on feed intake, digestibility, milk yield, milk composition of dairy cow and finally to recommend a perfect one.

MATERIALS AND METHODS

An experiment accords CRD design was conducted at "Pachutia Cattle Research Farm" of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka for a period of 30 days including 5 days of digestibility trial from 10 September, 2013 to 10 October, 2013. Twenty lactating Pabna cattle of average 250.1 ± 13.64 kg (*Bos indicus*) of initial body weight with average milk yield of 2.88 ± 0.38 kg were selected and divided into four groups randomly having five cows in each with maintained similarity of body weight and milk yield of each group as much as possible. These groups were then imposed to treatments of T_0, T_1, T_2, and T_3; in where T_o= German grass + Concentrate mixture without Ca salt of fatty acid + 2.5 % DCP, T_1= German grass + Concentrate mixture + 2.5 % soybean based Ca salt of fatty acid, T_2= German grass + Concentrate mixture + 2.5 % mustard based Ca salt of fatty acid and T_3= German grass + Concentrate mixture + 2.5% palm oil based Ca salt of fatty acid. Due to the abundance of German grass at research farm it was used as basal diet of cow. Three different types of calcium salt of fatty acid were prepared using sodium hydroxide (NaOH) solution and saturated solution of calcium chloride ($CaCl_2$). As for soybean oil, at first five parts of soybean oil were added to four parts of aqueous solution of NaOH (6M) and the hydrolysis of oil triacylglycerols was performed at 95 to 100°C with continuous agitation and bubbling Na. When no more soybean oil was visible, the resulting blend was left to stand at 5°C until Na soaps had solidified. The Na soaps then were dissolved in hot water (95 to 100°C) using a 1:5 ratio of soap to water, and a saturated solution of $CaCl_2$ at a ratio of 2.5 parts and 4.5 parts of soap to water was added for salting out. A filter cloth was used to filter the Ca salts, and tap water was used to remove residual NaOH and excess $CaCL_2$. The Ca salts were finally dried both in air and sun, and kept at about 20°C until use for feeding. For mustard oil and palm oil same manner was followed. Preparation of Ca salt of fatty acid was done on the basis of availability of ingredient by modifying the method of Chouinard et al. (1998). The concentrate mixture was prepared by weighing and mixing of wheat bran (45%), maize crushed (10%), wheat crushed (10%), soybean meal (5%), til oil cake (10%), kheshari (15%), fish meal (2%), salt (0.5%), DCP or Calcium salt of fatty acids (2.5%) manually. The nutrient composition of concentrate mixture was dry matter (90.21%), organic matter

(91.49%), crude protein (16.07%), ADF (21.29%), NDF (55.69%) and ash (8.51%). All the ingredients were mixed properly and then allowed to animals. The amount of supplied and refused feeds was recorded everyday and from there actual feed intake of each animal was found out periodically after every fortnight and then incremental feed was adjusted to the animal diets. The nutrient requirements of animal (varying amount of milk production) were calculated based on the recommendation of NRC, 1984. Live weight of all animals were also measured fortnightly at morning with empty stomach. Animals were housed in individual well equipped stanchion barn and offered concentrate rations as two equal portions at 6:00 and 13:00 h before milking and roughages was offered thrice a day; as one third at morning after milking and remaining two third at noon after milking again. Abundant clean water was made available all the period of experiment and prior to experiment, all the cows were de-wormed for internal parasites using Tetranid Bolus (Techno Drugs, Bangladesh). A conventional digestion trial was performed for 5 days at the end of feeding trial and that time feed intake, refusal of feed and was recorded daily. Composite samples of supplied feed, residue and feces of individual animal were stored at -20 ^{0}C. At the time of chemical analysis, 1 mm screen sieve was used for mixed dried feces except DM and CP. Samples of fresh fodder, concentrates, feed refusals and feces were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). DM contents by oven drying at 105 ^{0}C for overnight, ash at 550 ^{0}C for 8 h, total nitrogen (N) by Kjeldahl procedure and CP calculation from N content (CP = N × 6.25) according to the official methods of AOAC (2005) were determined. NDF and ADF were determined following procedure proposed by Goering and Van Soest (1970). Apparent digestibility coefficient for DM, OM, CP, NDF and ADF was calculated from dietary intake of constituent and amount recovered in feces. Milk samples were collected from each cow at 15 days interval and were analyzed for fat, protein, lactose, SNF and total ash contents by milk analyzer (Lactostar, Funk Gurbar). The data were analyzed using "MSTAT-C" statistical program to compute analysis of variance (ANOVA) for a Complete Randomized Design (CRD) and the mean values with standard error of difference (SED) were recorded. The difference among the treatment means were determined by Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS

In this experiment, all the intake parameters of nutrients showed that, they are non-significant among the treatments. In case of total dry matter intake, little bit highest value observed in T_1 (2.5 % soybean oil based calcium salt of fatty acid) treatment with 7.99 kg d^{-1} followed by T_3 (2.5% palm oil based calcium salt of fatty acid), T_2 (2.5 % mustard oil based calcium salt of fatty acid) and T_0 (without calcium salt of fatty acid) (7.93, 7.92 and 7.84), respectively. Result of dry matter intake from roughage varied as same before but in case of DMI of concentrate it was different as before (Table1). The metabolizeble energy requirement of different treatments were

50.4, 49.4, 47.6 and 50.0 (T_0, T_1, T_2 and T_3), respectively (Table1). And, after the experiment, increased ME intake was observed with all the treatments. Among the treatments, highest MEI value was observed in T_1 with 55.4 MJ d^{-1} followed by T_0, T_3 and T_2 (54.4, 53.6 and 50.8, respectively), shown in table1. Same manner was seen in the context of ME intake from roughage i.e. highest in T_1 with 37.0 MJ d^{-1} followed by T_0, T_3 and T_2 (36.2, 34.8 and 32.6, respectively), shown in table1. But, from concentrate T_3 treatment harvest the highest ME i.e. 18.8 MJ kg^{-1}. In case of crude protein intake, highest total crude protein intake was observed in T_3 treatment with 995.2 gm d^{-1} followed by T_0 & T_1 and T_2 (994.4 and 992.4, respectively), shown in Table1. CP intake from roughage was observed as same manner as intake of total CP. From concentrate, highest crude protein was ingested by T_0 (385.4 gm d^{-1}) followed by T_1, T_3 and T_2 (384.6, 384.4 and 383.6, respectively), shown in table1. The effect of calcium salt of fatty acid on apparent digestibility of different treatments for all the parameters were also found non-significant. Highest dry matter and crude protein digestibility was found in T_0 (71.68 and 72.04, respectively) followed by T_3, T_2 and T_1 (71.24 & 71.39, 71.05 & 71.29 and 69.31 & 69.23, respectively), shown in table1. The effect of different types of calcium salt of fatty acid was clearly visible with the milk yield of different treatments. The average daily milk yield of whole experimental period were 2.44, 3.53, 3.33 and 2.24 kg in treatment groups T_0 (without calcium salt of fatty acid), T_1 (2.5 % soybean oil based calcium salt of fatty acid), T_2 (2.5 % mustard oil based calcium salt of fatty acid) and T_3 (2.5% palm oil based calcium salt of fatty acid), respectively; which differed significantly ($P<0.001$) among treatments with gained highest yield in T_1 (3.53 kg d^{-1}) followed by T_2, T_0 and T_3 (3.33, 2.44 and 2.24, respectively), shown in table 2. Here, T_1 did not differed significantly with T_2 and T_0 also did not differed significantly with T_3 but T_1 & T_2 together differed significantly ($P<0.001$) with other two treatment groups i.e. T_0 & T_3. This result indicates significant improvement in milk yields distinctly due to supplementation of Calcium salt of fatty acid. However, the compositions of milk against fed different diets represented the result differently. The average milk fat content of T_0, T_1, T_2 and T_3 groups were 4.01, 3.35, 3.55 and 3.66 g kg^{-1} of milk, respectively. There was no significant difference ($p>0.05$) in terms of fat, protein, lactose, total minerals (estimated) or solids not fat (g kg^{-1}) among treatments (Table 2). Finally, very logically as all animals of each treatment got the same quality feed ingredient except Ca salts of fatty acid then any significant difference of live weight gain among treatments was not occurred (Table 3).

Table 1. Effect of different types of calcium salt of fatty acid on intake and digestibility of different treatment groups of Pabna lactating cow

Parameter	Mean ±SE of different treatment group				Level of significance
	T_0	T_1	T_2	T_3	
ME (MJ) requirement	50.40±2.03	49.40±3.05	47.60±3.74	50.00±3.78	NS
DMI from roughage (kg)	5.45±.068	5.62±.094	5.53±.14	5.58±.079	NS
DMI from concentrate (kg)	2.37±.046	2.37±.085	2.38±.076	2.35±.022	NS
Total DMI (kg)	7.84±.06	7.99±.12	7.92±.21	7.93±.09	NS
ME from roughage (MJ)	36.20±1.83	37.00±2.47	32.60±2.06	34.80±2.48	NS
ME from conc. (MJ)	18.20±.58	18.40±.51	18.20±.80	18.80±.66	NS
Total ME intake (MJ)	54.40±1.75	55.40±2.25	50.80±2.39	53.60±3.08	NS
CP from roughage (g)	609.00±2.19	609.80±3.01	608.80±2.73	610.80±1.36	NS
CP from conc. (g)	385.40±1.21	384.60±3.19	383.60±.93	384.40±.68	NS
Total CP intake (g)	994.40±1.50	994.40±1.78	992.40±2.23	995.20±1.32	NS
Digestibility of DM	71.68±2.08	69.31±1.65	71.05±1.81	71.24±1.17	NS
Digestibility of OM	69.05±1.18	66.74±1.57	69.05±1.18	69.05±1.18	NS
Digestibility of CP	72.04±2.21	69.23±1.44	71.29±1.14	71.39±1.07	NS
Digestibility of ADF	68.26±1.21	65.89±1.61	68.26±1.21	68.26±1.21	NS
Digestibility of NDF	71.60±.60	72.70±.51	71.40±.76	71.90±.58	NS

Significant level= NS: Non Significant=P>0.05; SE: Standard error; T_0= without calcium salt of fatty acid; T_1 = 2.5 % soybean oil based calcium salt of fatty acid; T_2 = 2.5 % mustard oil based calcium salt of fatty acid; T_3 = 2.5% palm oil based calcium salt of fatty acid

Table 2. Effect of different types of calcium salt of fatty acid on milk yield and composition of different treatment group of Pabna lactating cows

Parameter	Mean ±SE of different treatment group				Level of significance
	T_0	T_1	T_2	T_3	
Initial milk yield	2.86±.21	2.9±.22	2.84±.48	2.94±.29	NS
Final milk yield	2.44^{b}±.15	3.53^{a}±.13	3.33^{a}±.21	2.24^{b}±.28	***
Fat (g kg^{-1})	4.01±.21	3.35±.19	3.55±.19	3.73±.26	NS
Protein (g kg^{-1})	3.65±.02	3.61±.05	3.70±.05	3.71±.04	NS
Lactose (g kg^{-1})	4.95±.30	5.21±.07	5.33±.07	5.34±.06	NS
Solids not fat (g kg^{-1})	9.67±.06	9.60±.14	9.83±.13	9.84±.11	NS
Estimated mineral (g kg^{-1})	0.65±.02	0.62±.06	0.67±.02	0.72±.03	NS

Significant level= (Non Significant=P>0.05; ***=P<0.001, highly significant), ^{abcd} values with different

superscripts in the same row differ significantly; SE: Standard error, T_0= without calcium salt of fatty acid; T_1 = 2.5 % soybean oil based calcium salt of fatty acid; T_2 = 2.5 % mustard oil based calcium salt of fatty acid; T_3 = 2.5% palm oil based calcium salt of fatty acid

Table 3. Effect of different types of calcium salt of fatty acid on live weight changes of different treatment groups of Pabna lactating cow

Parameter	Mean (±SE) of different treatment group				Level of significance
	T_0	T_1	T_2	T_3	
Initial Live weight (kg)	258.2 ± 9.26	265 ± 13	224 ± 19.21	253.2 ± 13.09	NS
Final live weight (kg)	279.2 ± 3.48	285 ± 7.73	244.2 ± 17.16	269.8 ± 10.01	NS
Live weight gain (kg)	21 ± 10.47	20 ± 8.05	20.2 ± 5.77	16.6 ± 5.90	NS
Daily live weight gain (kg d^{-1})	0.70 ± 0.34	0.66 ± 0.26	0.67 ± 0.19	0.55 ± 0.19	NS

Significant level= NS: Non Significant=P>0.05; SE: Standard error, T_0= without calcium salt of fatty acid; T_1 = 2.5 % soybean oil based calcium salt of fatty acid; T_2 = 2.5 % mustard oil based calcium salt of fatty acid; T_3 = 2.5% palm oil based calcium salt of fatty acid

DISCUSSION

The intake of total DM was higher in T_2 group (2.5 % calcium salt of fatty acid) may be due to efficient microbial growth which may not the exact cause. Wanapat and Khampa (2006) described that, the combined use of feeding with roughage and concentrate after 4h with MSPF could improve rumen P^H, microbial protein synthesis but reduced protozoal population in dairy steers. Hristov et al. (2004) viewed long chain unsaturated fatty acid ($C_{18:3}$, $C_{18:2}$, $C_{18:1}$) decreased protozoal numbers. But discrete or overall performances of roughage and concentrate did not differ significantly which was resembles to the findings of Klusmeyer et al., 1991. Feeding vegetable oils rich in linoleic acid (Go mez-Corte et al., 2008) also respond similarly was found. In case of apparent digestibility, Naik et al. (2009) evaluated that, there had no effect on apparent digestibility of DM and organic matter (OM) when calcium salts of long chain fatty acids (Ca-LCFA) as a rumen inert fat (PF) was used as adult buffalo feed. CP digestibility was not significantly differed among treatments as like as the findings of Voigt et al. (2006), in where carbohydrates and crude protein did not differ significantly. And, Schauff and Clark (1989) suggest that Ca salts of fatty acids were inert in the rumen and did not greatly alter fermentation in the rumen, apparent total tract digestibility of DM, organic matter, ADF, NDF, and CP. Significant improvement of milk yield was observed among treatments through incorporation of different types of Ca salts of fatty acid at 2.5% level in the concentrate mixture in this experiment. In the study of Sajith et al. (2008) got improved quality milk production without affecting the digestibility of nutrients through incorporation of polyunsaturated calcium salts of fatty acids at a 4% level in the concentrate mixture. By using calcium salts of fatty acids Rabiee et al. (2012) and Fahey et al. (2002) also obtained the similar result. There was no significant ($p>0.05$)

difference for the parameters of milk protein, lactose, total minerals and SNF. Similar result was also reported by Perfield et al. (2002) except milk fat who conducted an experiment by feeding rumen protected CLA to pregnant cows.

CONCLUSION

Based on the above finding it may be concluded that, calcium salt of fatty acid formulated from soybean oil or mustard oil could be used for enhancing better quality milk yield and it may recognized as an effective technique.

REFERENCES

AOAC. (2005). Official Methods of Analysis, Centennial Edition. Association of Official Analytical Chemist, Washington DC.

Chalupa, W., Vecchiarelli, B., Elser, A.E., Kronfeld, D.S., Sklan, D., and Palmquist, D.L. (1986). Rumen fermentation in vitro as influenced by long chain fatty acids. *Journal of Dairy Science*, 69(5), 1293-1301.

Chalupa, W., Rickbaugh, B., Kronfeld, D.S. and Sklan, D. (1984). Rumen fermentation in vitro as influenced by long chain fatty acids. *Journal of Dairy Science*, 67, 1439-1450.

Chouinard, P.Y., Girard., and Brisson, G.J. (1988). Fatty acid profile and physical properties of milk fat from cows fed calcium salt of fatty acids with varying unsaturation. *Journal of Dairy Science*, 81, 471-481.

Fahey, J., Mee, J.F., Murphy, J.J., and O'Callaghan, D.(2002). Effects of calcium salts of fatty acids and calcium salt of methionine hydroxy analogue on plasma prostaglandin F_2 alpha metabolite and milk fatty acid profiles in late lactation Holstein-Friesian cows. National Center for Biotechnology Information, U.S. National Library of Medicine. *Theriogenology*, 58(8), 1471-1482.

Go Mez-Corte, P., Frutos, S.P., Mantecon, A.R., Juarez, M., De la Fuente, M.A., and Hervast, G. (2008). Milk production, conjugated linoleic acids content, and in vitro ruminal fermentation in response to high levels of soybean oil in dairy cattle diet. *Journal of Dairy Science*, 91, 1560-1569.

Goering, H.K., and Van., Soest, P.J.(1970). Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Application). Agricultural Handbook No. 379, Agricultural Research Service, U.S. Department of Agriculture.

Grummer, R.R. (1988). Influence of prilled fat and calcium salt of palm oil acids on ruminal fermentation and nutrient digestibility. *Journal of Dairy Science*, 71, 117-123.

Harfoot, C.G. (1981). Lipid metabolism in the rumen. In: *Lipid metabolism in ruminants animals*. Edn. Pergamon Press, Oxford, UK.

Hristov, A.N., Ivan, M., and McAllister, T.A. (2004). In Vitro effects of individual fatty acids on protozoal numbers and on fermentation products in ruminal fluid from cattle fed a high- concentrate, barley-based diet. *Journal of Animal Science*, 82, 2693-2704.

Jenkins, T.C., and Palmquist, D.L. (1984). Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy cows. *Journal of Dairy Science*, 67(5), 978-986.

Kellogg, D.W., Pennington, Johnson, J.A., and Panivivat, Z.B. (2001). Survey of management practices used for the highest producing DHI herds in the United States. *Journal of Dairy Science*, 84, 120-127.

Klusmeyer, T.H., Lynch, G.L., Clark, J.H., and Nelson, D.R. (1991). Effects of calcium salts of fatty acids and protein source on ruminal fermentation and nutrient flow to duodenum of cows. *Journal of Dairy Science*, 74, 2206-2219.

Moallem, U., Folman, Y., and Sklan, D. (2000). Effects of somatotropin and dietary calcium soaps of fatty acids in early lactation on milk production, dry matter intake, and energy balance of high-yielding dairy cows. *Journal of Dairy Science*, 83, 2085-2094.

Naik, P.K., Saijpaul, S., and Rani, N. (2009). Effects of ruminally protected fat on in vitro fermentation and apparent nutrient digestibility in buffaloes. *Journal of Animal Feed Science Technology*, 153, 68-76.

Palmquist, D.L., and Jenkins, T.C. (1980). Fat in lactation rations: review. *Journal of Dairy Science*, 63(1), l-14.

Palmquist, D.L., and Jenkins, T.C. (1982). Effects of added fat and calcium on in vitro formation of insoluble fatty acids soap and cell wall digestibility. *Journal of Animal Science*, 66, 957-970.

Perfield, I.J.W., Bernal-santos, G., Overton, T.R., and Bauman, D.E.(2002). Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. *Journal of Dairy Science*, 85, 1609-1617.

Precht, D., Voigt, J., Hagemeister, H., and Kanitz, W. (2001). The influence of dietary rumen protected linoleic acid on milk fat composition, spreadability of butter and energy balance in dairy cows. *European Journal of Lipid Science and Technology*, 103, 783-792.

Rabiee, A.R., Breinhild, K., Scott, W., Golder, H.M., Block, E., and Lean, I.J.(2012). Effect of fat additions to diets of dairy cattle on milk production and components: a meta-analysis and meta-regression. *Journal of Dairy Science*, 95(6), 3225-3247.

Sajith, P., Anil, K., and Tiwari, D.P. (2008). Effect of Feeding Calcium Salts of Palm Oil Fatty Acids on Performance of Lactating Crossbred Cows. *Asian-Australasian Journal of Animal Science*, 21(3), 376-385.

Schauff, D.J., and Clark, J.H. (1989). Effects of prilled fatty acids and calcium salts of fatty acids on rumen fermentation, nutrient digestibilities, milk production, and milk composition. *Journal of Dairy Science*, 72(4), 917-927.

Staples, C.R., Burke, J.M., and Thatcher, W.W. (1998). Influence of supplemental fats on reproductive tissues and performance of lactating cows. *Journal of Dairy Science*, 81, 856-871

Sumida, C.R., Graber., and Nunez, E.(1993). Role of fatty acids in signal transduction: modulators and messengers. *Prostaglandins Leukot Essent Fatty Acids*, 48, 117-122.

Thatcher, W.W., Bilby, T., Staples, C.R., MacLaren, L., and Santos, J. (2004). Effects of polyunsaturated fatty acids on reproductive processes in dairy cattle. In Proc. Southwest Nutrition & Management Conference, Bioproducts, Inc. Pre-Conference Symposium. Phoenix, AZ, February 26, 2004.

Voigt, J., Kanitz, K., Precht, W., Becker, D., Schneider, F., Spitschak, F., Schonhusen, M., Junghans, U., Aschenbach, P., and Gdbel, J.R. (2005). Verwertung von glucose and langkettigenfetts duren durch die laktierende milch kuhbeifutter ungeinerfettan gereichertendidt. In: Deutsch. Tierdrztl. Wochenschr. 112, S423-425.

Voigt, J., Kuhla, S., Gaafar, K., Demo, M., and Hagemeister, H. (2006). Digestibility of rumen protected fat in cattle. *Slovak Journal of Animal Science*, 39(1-2), 16-19.

Wu, Z., Ohajuruka, O.A., and Palmquist, D.L. (1991). Ruminal synthesis, biohydrogenation, and digestibility of fatty acids by dairy cows. *Journal of Dairy Science*, 74, 3025-3034.

Steel, R.G.R., and Torrie, J.H. (1980). Principles and Procedures of Statistics: A Biometrical Approach. 2nd Ed. McGraw-Hill Book Company, New York.

SAARC J. Agri., 15(2): 147-161 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35159*

INTEGRATED USE OF MANURE AND FERTILIZERS INCREASES RICE YIELD, NUTRIENT UPTAKE AND SOIL FERTILITY IN THE BORO-FALLOW-T.AMAN RICE CROPPING PATTERN

S. Bilkis[1*], M.R. Islam[2], M. Jahiruddin[2], M.M. Rahaman[2]

[1]Sylhet Agricultural University, Sylhet, Bangladesh
[2]Department of Soil Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

ABSTRACT

The effect of integrated use of manure and fertilizers on crop yield, nutrient uptake and soil fertility was studied in the Boro-fallow-T. Aman cropping pattern over two years. The experiment was set up at Bangladesh Agricultural University (BAU) farm, Mymensingh under the AEZ 9 (Old Brahmaputra Floodplain). The field trial consisted of eight treatments and control (no fertilizer or manure), 100% chemical fertilizers (CF), and IPNS based six treatments with six types of manure. Cowdung (CD), CD slurry, Trichocompost (TC) and vermicompost (VC) were added to soil at 5 t ha^{-1} and poultry manure (PM) and PM slurry applied at 3 t ha^{-1}. For all IPNS treatments, nutrient supply from manure was adjusted with that from chemical fertilizers. In each crop cycle, manure was applied to the first crop (Boro rice) and the residual effect was evaluated on the succeeding crop (T.Aman rice). The IPNS based treatments significantly increased the grain and straw yields of Boro rice and it had also positive residual effect on T.Aman rice. Trichocompost and vermicompost, among the six IPNS treatments, demonstrated higher crop yield and that was followed by poultry manure slurry and cowdung slurry. Integrated use of manure with fertilizers gave on an average 8.3-33.8% and 2.9-18.3% higher grain yield in Boro and T. Aman rice, respectively over sole fertilizers treatment. Higher nutrient uptake by crops (N, P, K & S) was also observed in IPNS treated plots. The IPNS treatments improved soil fertility in terms of increasing organic matter, N, P and S contents of soil after two crop cycles. The study suggests that manure and fertilizers should be used in an integrated manner to achieve sustainable crop yield, without incurring loss to soil fertility.

[*] Corresponding author e-mail: sbilkis26@gmail.com

Received: 05.09.2017 *Accepted: 04.10.2017*

Keywords: Cowdung, poultry manure, trichocompost, vermicompost, rice yield, soil fertility

INTRODUCTION

In Bangladesh, with advancement of time, nutrient mining increases due to increasing cropping intensity (191%, BBS 2017), use of modern varieties, nutrient leaching, gaseous loss, soil erosion and imbalanced application of fertilizers with no or little addition of organic manure. Higher is the crop yield, higher is the nutrient removal from soil. Nutrient deficiency in this country's soils has arisen chronologically N, P, K, S, Zn and B (Islam, 2008; Jahiruddin and Satter, 2010). About 45% of net cultivable areas of Bangladesh contain less than 1% OM (FRG, 2012). Organic manure is a good source of nutrients, especially N, P & S and it's a good means of soil rejuvenation (Jeptoo et al., 2013). So, use of OM could be an inevitable practice in the coming years for ensuring sustainable crop productivity without affecting soil fertility (Heikamp et al., 2011; Premsekhar and Rajashree, 2009).

Bio-slurry, by-product of biogas plant, can be a potential source of manure for crop production (Yu et al., 2010; Abubaker, 2012). Bio-slurry is the residual manure generated through anaerobic decomposition of various organic materials, chiefly cowdung and poultry manure, in presence of anaerobic microbes in the biogas digester. About 25-30% of organic matter is converted into biogas during the anaerobic fermentation process, while the rest becomes available as manure (bio-slurry).

As stated by Rahman et al. (2008), unbalanced use of chemical fertilizers has affected soil health, causing a substantial decrease in soil organic carbon. As a general rule, use of organic fertilizers especially in composted form produces positive effect on soil health and fertility, which consequents increased crop yield on a long-term basis (Mehdizadeh et al., 2013).

Manure or fertilizer alone cannot sustain soil fertility and crop yield over time, their combination is essential (FRG, 2012). Nambiar (1997) viewed that integrated use of organic manure and chemical fertilizers would be quite promising not only in providing greater stability in production, but also in maintaining soil fertility status. Limited data are available on the growth, yield and nutrient content of agronomic crops treated with bioslurry, vermicompost and trichocompost. It is essential to develop a strong workable and compatible package of nutrient management through organic and inorganic sources for various crops based on scientific facts, local conditions and economic viability (Kannaiyan, 2000).

The present study was, therefore, undertaken to develop an efficient nutrient management practice following the concept of IPNS approach in the rice-rice cropping pattern.

MATERIALS AND METHODS

The experiment was conducted at the soil science field laboratory of Bangladesh Agricultural University (BAU), Mymensingh for two consecutive years, 2012 and 2013. The experimental site was situated at 24.75^0 N latitude and 90.5^0 E longitude. The soil belongs to Sonatala series under agro-ecological zone (AEZ) 9, known as Old Brahmaputra Floodplain (FAO/UNDP, 1988). The soil was silt loam in texture having 6.29 pH, 1.85% OM, 0.124% total N, 3.96 mg kg^{-1} available P, 0.11 cmol kg^{-1} exchangeable K and 11.9 mg kg^{-1} available S.

Variety BRRI dhan29 and BINA dhan 7 was used for Boro and T. Aman rice, respectively. The experiment was laid out in a randomized complete block design (RCBD), with three replications. There were eight treatments. as shown in table 1. Treatments T_3-T_8 received a definite amount of nutrients from different types of manure and the rest amount of nutrients came from chemical fertilizers so that the rate of every nutrient application was the same over the fertilizer and manure + fertilizer treatments. The fertilizer doses were rationalized for the second crop, as outlined in the Fertilizer Recommendation Guide (FRG, 2012). The rate of manure application was 5 t ha^{-1} for cowdung, its slurry, and tricho- and vermi compost, and 3 t ha^{-1} for poultry manure and its slurry. Full amount of P, K and S fertilizers was applied as basal during final land preparation and urea was applied in 3 equal splits - the one-third during final land preparation and the two-thirds during tiller and panicle initiation stages of crop growth. All types of manure were applied seven days before transplanting. Thirty five days' old seedlings were used for transplanting of both Boro and T.Aman rice. Boro rice was transplanted on 24 January in 2012 and 28 January in 2013 and was harvested correspondingly on 20 May 2012 and 22 May 2013. T.Aman rice was transplanted on 6 August 2012 and 28 July 2013 and harvested respectively on 8 November 2012 and 9 November 2013. Weeding and irrigation were done whenever required.

At maturity, the crop was harvested and agronomic data viz. plant height, tillers $hill^{-1}$, panicle length (cm), grains $panicle^{-1}$, 1000-grain weight (g) and grain and straw yields were recorded. Grain yield was expressed at 14% moisture basis and straw yield at sun dry basis, drying was done for a period until a constant weight was obtained. The grain and straw samples were collected, dried and ground for analysis of N, P, K & S contents following standard methods (H_2SO_4 digestion for N and HNO_3-$HClO_4$ digestion for P, K & S). Nutrient uptake by the grain and straw was calculated by multiplying their percent concentration with the corresponding yield. Composite soil samples were collected from every plot and prepared for chemical analysis. Soil pH was measured by glass electrode pH meter with soil-water ratio 1:2.5 (McLean, 1982), organic matter by wet oxidation method (Nelson and Sommers, 1982), N by Micro-Kjeldahl method (Bremner and Mulvaney, 1982), P by 0.5M $NaHCO_3$, pH 8.5 extraction method (Olsen and Sommers, 1982), K by NH4OAc, pH 7.0 extraction method (Barker and Surh, 1982), S by 0.15M $CaCl_2$

extraction method (Page et al., 1982), Zn by 0.005M DTPA, pH 7.3 extraction method (Lindsay and Norvell, 1978) and hot water-0.02M $CaCl_2$ method (Page et al., 1982). All data were analyzed statistically by MSTATc computer programme following the F-test and the mean comparisons of the treatments were done by Duncan's Multiple Range Test (DMRT) at 5% level.

Table 1. Treatment combinations of organic manure and chemical fertilizers

Treatment code	Treatment combinations
T_1	Control (no manure or fertilizer)
T_2	HYG based 100% chemical fertilizer (CF) [FRG-2012]
T_3	CD + CF (IPNS basis)
T_4	CD slurry + CF (IPNS basis)
T_5	PM + CF (IPNS basis)
T_6	PM slurry + CF (IPNS basis)
T_7	TC + CF (IPNS basis)
T_8	VC + CF (IPNS basis)

HYG = High Yield Goal, CF = Chemical Fertilizer, IPNS = Integrated Plant Nutrition System, CD = Cowdung, CD slurry = Cowdung slurry, PM = Poultry manure, PM slurry = Poultry manure slurry, TC =Trichocompost, VC = Vermicompost

RESULTS

Effects of integrated use of manure and fertilizers were investigated directly on Boro rice and their residual effects on T.Aman rice. The results are described below.

Yield and yield attributes of Boro rice

Significant variation was recorded for the plant height of Boro rice (BRRI dhan29) due to application of different manure and chemical fertilizers (Table 1). The tallest plant (101.5 cm) was observed in T_7 treatment (trichocompost) and the shortest (78.6 cm) was found in control (T_1) plot. In the second year (2013), the T_4 treatment recorded the highest plant height (95.7 cm), which was statistically similar with other treatments except control (T_1), the number of tillers $hill^{-1}$ showed a significant variation due to imposed treatments (Table 2). The highest number of tillers $hill^{-1}$ (14.8 in the first year and 12.3 in the second year) was found in T_7 treatment. The lowest number of tillers $hill^{-1}$ was produced in T_1 (control) treatment in both the years. Panicle length was not significantly influenced by the treatments (Table 2). In both years, the maximum number of grains $panicle^{-1}$ (144.7 and 147.5) was recorded in T_7 treatment. Manure treated plots produced higher number of grains $panicle^{-1}$ as compared to absolute fertilizer treated plots. The highest 1000-grain weight was

recorded in T_7, which was statistically similar with all other treatments except control over the years.

The grain yield of Boro rice was significantly influenced by different treatments (Table 3). In 2012, the highest grain yield (6.44 t ha^{-1}) was obtained from T_7 treatment (TC + CF), which was statistically similar with T_5 (6.20 t ha^{-1}), T_6 (6.30 t ha^{-1}) and T_8 (6.34 t ha^{-1}). In this year all the manure treated plots produced higher yield compared to absolute chemical fertilizer (T_2) and control plots. Similar trend of grain yield was observed in 2013. In both years, the control treatment (T_1) produced the lowest grain yield. Like grain yield, the straw yield of Boro rice was markedly influenced by different treatments (Table 3). In 2012, the highest straw yield (6.79 t ha^{-1}) was obtained from T_7 treatment, which was statistically similar with T_4, T_5, T_6 and T_8 treatments. In 2013, T_7 recorded the highest straw yield (7.48 t ha^{-1}), which was identical with all other treatments except T_2 and T_1. The lowest straw yields of 2.93 t ha^{-1} and 3.11 t ha^{-1} were recorded in control (T_1) treatment.

Table 2. Effects of integrated use of manure and fertilizers on plant height, tillers and panicle length of Boro rice (BRRI dhan29)

Treatments	Plant height (cm)		Tillers $hill^{-1}$		Panicle length (cm)	
	2012	2013	2012	2013	2012	2013
T_1	78.6d	75.4b	8.8c	6.2d	20.6b	22.2c
T_2	94.7c	92.6a	13.0b	9.8c	23.7a	24.8b
T_3	94.1c	92.3a	13.4ab	9.7c	23.7a	25.2ab
T_4	96.1bc	95.7a	14.3ab	10.7bc	24.3a	25.8ab
T_5	100.1ab	95.5a	14.8a	11.2ab	23.1a	26.1ab
T_6	96.9abc	94.1a	14.7a	10.1bc	23.8a	26.6ab
T_7	101.5a	95.2a	14.8a	12.3a	24.3a	26.9a
T_8	98.6abc	95.1a	14.8a	11.1b	24.1a	26.8a
CV (%)	2.80	2.40	6.23	6.29	3.36	3.70
Significant level	**	**	**	**	**	**
SE (±)	1.536	1.274	0.519	0.368	0.454	0.547

Means followed by same letter in a column are not significantly different at 5 % level by DMRT.

SE (±) = standard error of means, CV = Coefficient of variation,** = Significant at 1% level

Table 3. Effects of integrated use of manure and fertilizers on grains panicle^{-1} and 1000-grain weight and grain and straw yields of Boro rice (BRRI dhan29)

Treatments	Grains panicle^{-1}		1000-grain weight (g)		Grain yield (t ha^{-1})		Straw yield (t ha^{-1})	
	2012	2013	2012	2013	2012	2013	2012	2013
T1	87.83c	93.83c	19.15b	20.19b	2.61c	2.71c	2.93c	3.11c
T2	129.5b	135.4b	20.72a	22.27a	5.44b	5.96b	6.09ab	6.17b
T3	135.3ab	138.8ab	20.70a	22.37a	5.49b	6.17b	6.04b	6.54ab
T4	137.9ab	141.2ab	21.08a	22.38a	6.10ab	6.54ab	6.21ab	6.69ab
T5	137.5ab	142.8ab	21.08a	22.40a	6.20a	6.77ab	6.71ab	7.22a
T6	139.2ab	144.5ab	21.03a	22.50a	6.30a	6.81ab	6.43ab	6.96ab
T7	144.7a	147.5a	21.17a	22.66a	6.44a	7.37a	6.79a	7.48a
T8	141.0ab	146.3a	21.07a	22.53a	6.34a	7.31a	6.63ab	7.39a
CV (%)	5.25	3.83	2.36	2.10	6.78	6.57	9.0	7.57
Sig. level	**	**	**	**	**	**	**	**
SE (±)	3.991	3.013	0.157	0.245	0.220	0.227	0.323	0.282

Means followed by same letter in a column are not significantly different at 5 % level by DMRT.

SE (±) = standard error of means, CV = Coefficient of variation,** = Significant at 1% level

Nutrient uptake by Boro rice

The N uptake by Boro rice was significantly affected by the treatments (Table 4). The highest total N uptake (145.8 kg ha^{-1},146.3 kg ha^{-1}) was recorded in T_7 treatment in both years. Cowdung, CD slurry, PM, PM slurry, trichocompost and vermicompost receiving treatments also showed higher N uptake in comparison to absolute chemical fertilizer (T_2) and control (T_1) treatments. The highest K uptake (121.8 kg ha^{-1}) in 2012 was recorded in T_7 treatment, which was statistically similar with T_8 (120.7 kg ha^{-1}) and T_5 (116.8 kg ha^{-1}). In 2013, the highest K uptake (153.7 kg ha^{-1}) was found in T_7 and it was statistically similar with T_4 (136.0 kg ha^{-1}), T_5 (141.3 kg ha^{-1}), T_6 (137.2 kg ha^{-1}) and T_8 (144.3 kg ha^{-1}). To the lowest K uptake was always noted in control (T_1). For both years, the T_7 showed the highest P uptake (18.66 kg ha^{-1}, 23.8 kg ha^{-1}), which was statistically similar with T_5, T_6 and T_8 treatments (Table 4). All other manure treated plots except CD showed higher P uptake as compared to T_2 treatment. Application of manure and fertilizer significantly influenced S uptake by Boro rice (Table 4). The highest S uptake was recorded from T_7 (12.08 kg ha^{-1}, 11.73 kg ha^{-1}) and the lowest S uptake in T_1 compared to T_2 and other treatments.

Table 4. Effects of integrated use of manure and fertilizers on the N, K, P and S uptake by Boro rice (BRRI dhan29)

Treatments	N uptake (kg ha^{-1})		K uptake (kg ha^{-1})		P uptake (kg ha^{-1})		S uptake (kg ha^{-1})	
	2012	2013	2012	2013	2012	2013	2012	2013
T1	43.26d	43.32e	42.28d	53.48d	4.96d	6.94d	2.77e	3.25e
T2	103.2c	104.4d	105.2bc	110.5c	13.99b	17.04c	9.64cd	8.94cd
T3	99.04c	109.5cd	103.9bc	129.5b	11.93c	18.04c	9.51d	8.24d
T4	121.7b	118.7bc	100.4c	136.0ab	13.74bc	18.00c	9.56d	8.99cd
T5	129.3b	118.9bc	116.8a	141.3ab	18.39a	21.67ab	11.12ab	10.11bc
T6	129.3b	125.9b	114.0ab	137.2ab	16.72a	21.38b	10.12bcd	9.95bc
T7	145.8a	146.3a	121.8a	153.7a	18.66a	23.80a	12.08a	11.73a
T8	130.6b	132.5b	120.7a	144.3ab	17.15a	22.47ab	11.03abc	10.35b
CV (%)	5.59	6.75	6.08	7.44	7.65	6.62	8.04	7.20
Sig. level	**	**	**	**	**	**	**	**
SE (±)	3.638	4.384	3.618	5.401	0.638	0.713	0.440	0.372

Means followed by same letter in a column are not significantly different at 5 % level by DMRT.SE (±) = Standard error of means, CV = Coefficient of variation, ** = Significant at 1% level

Residual effects on T.Aman rice

T.Aman rice received N, P, K & S from chemical fertilizer source only. Plant height varied significantly due to residual effect of different types of manure used (Table 5). In the first year, T_4 produced the tallest plant (91.7 cm) which was statistically similar with T_5, T_6, T_7 and T_8 treatments. In the second year, the tallest plants were observed with T_8 treatment, which however was statistically identical with all other treatments except T_1 which exhibited the shortest plants in both years. In 2012, T_7 produced the maximum number of tillers hill^{-1} (Table 5) while in 2013 the highest number of tillers was found in T_8 treatment. The minimum number of tillers hill^{-1} was always noted in the control (T_1).

In 2012, the highest panicle length (26.8 cm) was recorded in T_7 and it was statistically similar with all other treatments except T_1 and T_2. In 2013, the treatment effects were mostly similar; both T_7 and T_8 produced the biggest size panicle (25.6 cm). In 2012, the maximum number of grains panicle^{-1} was obtained from T_7 treatment, which was statistically similar with T_4, T_5, T_6 and T_8 treatments (Table 6). Almost similar trends were observed in 2013. The 1000-grain weight ranged from 20.8 - 22.4 g in 2012 and 20.8 - 23.1 g in 2013, the highest 1000-grain weight being

noted in T_8 and T_7, respectively (Table 6). The control treatment had the lowest 1000-grain weight. However, in the first year, variation in 1000-grain weight due to different treatments was non-significant, and in the second year, all the treatments were similar except T_3 and T_1 in terms of 1000-grain weight.

Table 5. Residual effects of integrated use of manure and fertilizers on plant height, tiller production and panicle length of T.Aman rice (BINA dhan7)

Treatments	Plant height (cm)		Tillers hill^{-1}		Panicle length (cm)	
	2012	2013	2012	2013	2012	2013
T1	79.7d	80.5b	9.1d	8.4c	21.96c	22.0b
T2	88.9c	90.7a	11.7c	11.5b	25.68b	25.0a
T3	89.5bc	91.8a	11.8bc	11.7ab	25.73ab	25.1a
T4	91.7a	90.4a	11.8bc	12.6ab	26.03ab	25.2a
T5	89.9abc	89.5a	13.1abc	12.7ab	26.17ab	25.3a
T6	90.9ab	90.9a	12.5abc	13.4ab	26.11ab	25.2a
T7	91.6a	92.1a	13.9a	13.9a	26.82a	25.6a
T8	89.9abc	92.5a	13.2ab	14.0a	26.77a	25.6a
CV (%)	2.17	2.86	6.39	9.79	2.47	5.21
Significant level	**	**	**	**	**	**
SE (±)	0.576	1.106	0.447	0.694	0.323	0.675

Means followed by same letter in a column are not significantly different at 5 % level by DMRT.SE (±) = Standard error of means, CV = Coefficient of variation, ** = Significant at 1% level

The grain yield of T.Aman rice responded significantly to the residual effect of different treatments (Table 6). In 2012, the highest grain yield (4.70 t ha^{-1}) was obtained in T_7, and the next highest yield was due to T_4, T_6 and T_8 treatments, their yields were statistically similar. The lowest grain yield (2.47 t ha^{-1}) was observed with the control (T_1). In 2013, T_7 also produced the highest grain yield (5.15 t ha^{-1}), which was statistically similar with T_8 (4.78 t ha^{-1}). Combined application of manure with fertilizers gave better grain yield compared to sole chemical fertilizers. In 2012, the highest straw yield (4.85 t ha^{-1}) was obtained from T_7, which was identical with T_8 (Table 6). In 2013, T_7 gave the maximum straw yield (5.03 t ha^{-1}), which was significantly higher than all other treatments. Considering mean yield of two years, T_7 produced the highest straw yield (4.94 t ha^{-1}). Other manure treatments exhibited comparable yields with T_2. The lowest straw yield was recorded with the control (T_1).

Nutrient uptake

In 2012, the highest N uptake (98.67 kg ha^{-1}) was found in T_7, which was similar to T_8 (Table 7). Statistically similar N uptake was observed in T_4 and T_6 treatments, which was significantly higher than absolute fertilizer treatment (T_2). The lowest N

uptake by rice (37.72 kg ha^{-1}) was noted in control (T_1). Similarly in 2013, the higher N uptake (99.47 kg ha^{-1}) was recorded in T_7 that was statistically similar with T_8 (91.61 kg ha^{-1}).

Table 6. Effects of integrated use of manure and fertilizers on grains $panicle^{-1}$ and 1000-grain weight, grain and straw yields of T. Aman rice (BINA dhan7)

Treatments	Grains $panicle^{-1}$		1000-grain weight (g)		Grain yield (t ha^{-1})		Straw yield (t ha^{-1})	
	2012	2013	2012	2013	2012	2013	2012	2013
T1	78.5c	72.5c	20.81	20.82c	2.47d	2.60e	2.83e	2.66d
T2	100.5b	94.43b	21.93	22.49ab	3.69c	4.08d	3.81d	4.13c
T3	101.2b	94.30b	21.74	22.44b	3.79c	4.31cd	4.07cd	4.35bc
T4	109.2a	94.30b	22.11	22.63ab	4.15b	4.38bcd	4.34bc	4.45b
T5	110.8a	100.0ab	22.09	22.79ab	4.32b	4.41bcd	4.35bc	4.62b
T6	112.3a	103.8ab	22.04	22.78ab	4.35b	4.58bc	4.39bc	4.54b
T7	113.5a	106.1a	22.35	23.06a	4.70a	5.15a	4.85a	5.23a
T8	112.8a	103.9ab	22.37	22.81ab	4.35b	4.78ab	4.64ab	4.92b
CV (%)	3.67	5.46	2.99	3.55	4.65	5.88	5.96	3.56
Sig. level	**	**	NS	**	**	**	**	**
SE (±)	2.22	3.032	0.378	0.3314	0.106	0.145	0.149	0.876

Means followed by same letter in a column are not significantly different at 5 % level by DMRT.SE (±) = Standard error of means, CV = Coefficient of variation, ** = Significant at 1% level

There was a positive residual effect of the treatments on the K uptake by the crop. In 2012, the highest K uptake (109.9 kg ha^{-1}) was recorded with T_7, which was statistically similar with T_4, T_5 and T_8 treatments (97.96, 100.7 and 100.1 kg ha^{-1}, respectively). The lowest K uptake by rice (44.81kg ha^{-1}) was observed in control (T_1). In 2013, the highest K uptake (110.7 kg ha^{-1}) was found in T_7, which was statistically identical with T_8. In 2012, the highest P uptake (14.12 kg ha^{-1}) was recorded with T_7 and it was statistically similar with T_5, T_6 and T_8 treatments, showing 13.18, 13.70 and 13.43 kg ha^{-1}, respectively. In 2013, the T_7 also showed the highest P uptake (15.84 kg ha^{-1}), followed by T_8 (15.13 kg ha^{-1}). The T_5 (14.03 kg ha^{-1}) and T_6 (14.38 kg ha^{-1}) had identical P uptake followed by the T_3 and T_4 treatments. The lowest P uptake by the crop (6.09 kg ha^{-1} in 2012 and 6.61 kg ha^{-1} in 2013) was observed in control (T_1) plot. In the first year, T_7 treatment had the highest (8.66 kg ha^{-1}) S uptake, which was significantly different from all other treatments (Table 10). The lowest S uptake by the crop (3.01 kg ha^{-1}) was found in control (T_1) treatment. In the second year, the highest S uptake by T.Aman rice (9.19 kg ha^{-1}) was observed in T_7 and the next highest S uptake (8.37 kg ha^{-1}) was noted in T_8 treatment (Table 7).

Table 7. Effects of integrated use of manure and fertilizers on N, K, P and S uptake by T.Aman rice (BINA dhan7)

Treatments	N uptake (kg ha^{-1})		K uptake (kg ha^{-1})		P uptake (kg ha^{-1})		S uptake (kg ha^{-1})	
	2012	2013	2012	2013	2012	2013	2012	2013
T1	37.72d	41.63e	44.81d	44.52e	6.09d	6.61e	3.01e	3.88d
T2	62.10cd	73.47d	79.80c	89.04d	10.74c	12.22d	5.31d	6.71c
T3	67.89bcd	79.88cd	83.65c	91.02cd	11.13c	12.84cd	5.59d	6.99c
T4	78.98bc	84.58bc	97.96ab	93.22bcd	12.51b	12.98cd	6.52c	7.11c
T5	72.16bcd	84.71bc	100.7ab	97.31bc	13.18ab	14.03bc	5.89d	7.29c
T6	74.64bc	87.24bc	89.06bc	99.27b	13.70ab	14.38bc	7.72b	7.30c
T7	98.67a	99.47a	109.9a	110.7a	14.12a	15.84a	8.66a	9.19a
T8	84.49ab	91.61ab	100.1ab	106.6a	13.43ab	15.13ab	7.86b	8.37b
CV (%)	3.15	6.13	7.67	4.48	5.36	6.01	5.51	4.46
Sig. level	**	**	**	**	**	**	**	**
SE (±)	1.375	2.845	3.907	2.368	0.367	0.450	0.201	0.183

Means followed by same letter in a column are not significantly different at 5 % level by DMRT.SE (±) = Standard error of means, CV = Coefficient of variation, ** = Significant at 1% level

Change in soil properties

Soil samples from every plot after two crop cycles were analyzed for pH, organic matter, N, P, K, S, Zn and B contents (Table 8). There was a little change in soil pH and OM over the treatments. Total N content of soil was higher in plots when organic manure was added to soil and it ranged from 0.138 - 0.160% (initial level 0.132%). There was a decreasing trend of K content in all the treatments compared to initial soil status. Available P content varied from 3.92 - 9.52 mg kg^{-1} (initial status 3.61 mg kg^{-1}). Addition of organic manure considerably increased the soil S level. The soil Zn content had remarkably increased, much increase being observed in the TC and PM slurry amended plots. Like Zn content, all the treatments except control increased the B content of soil.

Table 8. Changes in soil properties as influenced by manure and fertilizer treatments in the Boro-fallow- T.Aman cropping pattern

Treatments	pH	OM (%)	Total N (%)	K (cmol kg^{-1})	P	S	Zn	B
					(mg kg^{-1})			
Initial soil	6.14	2.35	0.132	0.12	3.61	12.6	0.65	0.250
Post-harvest soil (after 2-crop cycle)								
T1	5.99	2.34	0.138	0.087	3.92	11.17	0.63	0.231
T2	6.02	2.32	0.143	0.093	4.3	14.43	0.89	0.265
T3	6.16	2.55	0.154	0.106	4.77	14.63	0.87	0.461
T4	6.18	2.52	0.155	0.109	4.87	17.08	0.88	0.323
T5	6.18	2.54	0.157	0.113	7.60	18.91	0.91	0.431
T6	6.13	2.57	0.155	0.103	9.52	17.76	0.93	0.375
T7	6.31	2.48	0.159	0.118	7.38	18.53	0.95	0.415
T8	6.23	2.45	0.143	0.103	5.72	17.31	1.08	0.461

DISCUSSION

Integrated use of inorganic and organic sources of nutrients (called IPNS) produced significantly higher grain and straw yields of both Boro and T.Aman rice compared to sole inorganic source (fertilizer) use. Treatment containing Trichocompost recorded the highest crop yield, followed by vermicompost, PM slurry and PM treatments. The trichocompost treated plots demonstrated 21.1% and 27.7% higher grain yield of Boro and T.Aman rice, respectively over 100% fertilizer treatment.

Use of Trichoderma compost technology is at initial level in our country. Trichoderma fungi decomposes organic materials in which they grow, colonize plant root system and attack other fungi in the plant's root system and releases compounds that activate plant defense mechanism (Rabbani, 2013). The potential of organic amendments over chemical fertilizers in suppression of disease incidence has long been recognized (Hadar, 2011; Pane et al., 2011; Bonanomi et al., 2007).

Zaman (2002) reported a comparable crop yield in the rice-rice pattern due to application of 70% NPKS fertilizers plus 3 t ha^{-1} poultry manure with 100% NPKS sole fertilizers. Rahman (2013) observed a 15-20% yield increase due to combined use of fertilizers with poultry manure or household compost over 100% sole fertilizer treatment. Poultry manure can supply organic C to soil in one way and some growth hormones and concentrates feed to poultry birds can influence the plant growth on the other way.

Between slurry and original manure, the slurry treatment produced statistically higher yield of crops. As reported by Haque (2014), bio-slurry and original manure can give similar crop yield, with an advantage that cowdung slurry at 5 t ha^{-1} (15% moisture)

application can substitute 18- 32% N, 40-100% P and 13-34% K, and poultry manure slurry at 3 t ha^{-1} application can substitute 15-21% N, 50-100% P and 8 - 18% K for the chemical fertilizers in the rice-based cropping systems.

Tricochompost, vermicompost, PM and PM slurry showed also higher and comparable N, P, K and S uptake. Organic manure releases nutrients slowly and it is reflected on the nutrient concentration as well as nutrient uptake, as reported by Saidu et al. (2012), Ayoola and Makinde (2007). The increased uptake of nutrients due to NPKS fertilization and organic manure application was due to addition of nutrients and proliferous root system developed under balanced nutrient application resulting in better absorption of water and nutrients along with improved physical environment (Laxminarayana, 2006; Kler and Walia, 2006).

Organic manure showed a positive effect on soil properties, as determined after two crop cycles. Soil organic matter and N contents, and P, S, Zn & B availability in soil showed an increasing trend in IPNS treated plots. On the contrary, the exchangeable K content decreased across the treatments showing a K mining. Soil organic matter undergoes mineralization and releases substantial quantities of nitrogen, phosphorus, sulphur and smaller amount of micronutrients (Rahman et al., 2013). Vermicompost contains most nutrients in plant available form such as nitrate, phosphate and exchangeable calcium and soluble potassium (Edwards, 1998; Orozco et al., 1996). Organic fertilizer application, therefore exhibited potential in improving crop yield, N use efficiency and soil health in acid lateritic soil of the subtropical climate (Murmu et al., 2013).

Thus, sustainable production of crops cannot be achieved by using chemical fertilizers alone because of deterioration in soil physical and biological environments (Khan et al., 2008). Integrated use of both organic manure and chemical fertilizers appears as the best approach in providing greater stability in production and improving soil fertility status, as evidenced in the past (Islam et al., 2011; Sood, 2007; Singh and Lal, 2006).

CONCLUSION

Among the IPNS treatments, trichocompost and vermicompost containing treatments showed higher crop yield, followed by poultry manure slurry and cowdung slurry. The IPNS based treatments gave on an average 8.3-33.8% and 2.9-18.3% higher grain yield in Boro and T.Aman rice, respectively over sole chemical fertilizer treatment. Higher nutrient uptake by crops (N, K, P & S) was also observed in IPNS treated plots. The IPNS treatments improved soil fertility in terms of increasing organic matter, N, P and S contents of soil after two crop cycles. It is concluded that manure and fertilizers should be used in an integrated manner to achieve sustainable crop yield, with sustained soil fertility.

ACKNOWLEDGEMENT

The work was supported by the Higher Education Quality Enhancement Project (HEQEP) which had provided full research cost and offered Ph.D. fellowship to the first author.

REFERENCES

Abubaker, J. (2012). Effects of fertilization with biogas residues on crop yield, soil microbiology and greenhouse gas emissions. *Acta Universitatis Agriculturae Sueciae*, Ph.D. Thesis, Dept. of Microbiology, *Swedish University of Agricultural Sciences*, 46, 1-79.

Ayoola, O.T., and Makinde, E.A. (2007). Fertilizer treatment effects on performance of cassava under two planting patterns in a cassava- based cropping system in South-West Nigeria. *Research Journal of Agriculture and Biological Sciences,* 3(1), 13-*20.*

Barker, D.E., and Suhr, N.H. (1982). Potassium. Atomic absorption and flame emission spectroscopy. In: *Methods of soil analysis, Part 2, Chemical and microbiological properties,* A.L. Page, R.H. Miller and D.R. Keeney (eds.), p. 13-26. American Society of Agronomy Inc., Madison, WI, USA.

Bonanomi, G, Antignani, V., Pane, C., Scala, F. (2007). Suppression of soil borne fungal diseases with organic amendments. *Journal of Plant Pathology*, 89, 311-324.

Bremner, J.M., and Mulvaney, C.S. 1982. Total nitrogen. In: *Methods of soil analysis, Part 2, Chemical and microbiological properties,* A.L. Page, R.H. Miller and D. R. Keeney (eds.), p. 595-624. American Society of Agronomy and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

BBS, (2017). Bangladesh Bureau of Statistics. The Year Book of Agricultural Statistics of Bangladesh. Stat. Div. Minis. Planning, Govt. Peoples Repub. Bangladesh, Dhaka.

Edwards, C.A. (1998). The use of earthworm in the breakdown and management of organic wastes. In *Earthworm Ecology,* C.A. Edwards (Ed) pp. 327-254, CRC Press Boco Roton, FL.

FAO/UNDP. (1988). Land Resources Appraisal of Bangladesh for Agricultural Development, Report-2, Agro-ecological Regions of Bangladesh, BGD/81/035.

FRG. (2012). Fertilizer Recommendation Guide. Bangladesh Agricultural Research Council (BARC), Dhaka, Bangladesh.

Hadar, Y. (2011). Suppressive compost: when plant pathology met microbial ecology, *Phytoparasitica*, 39, 311-314.

Haque, M.A. (2014). Mineralization of bioslurry and its integrated use with fertilizers in the rice based cropping systems. Ph. D Thesis, Department of Soil Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Heitkamp, F., Raupp, J., and Ludwig, B. (2011). Soil organic matter pools and crop yields as affected by the rate of farmyard manure and use of biodynamic preparations in a sandy soil. *Organic Agriculture.* l1, 111-124.

Islam, M.S. (2008). Soil fertility history, present status and future scenario in Bangladesh. *Bangladesh Journal of Agriculture and Environment,* 4,129 -152.

Islam, M.M., Karim, A.J.M.S., Jahiruddin, M., Majid, N.M., Miah, M.G., Ahmed, M.M., and Hakim, M.A. (2011). Effects of organic manure and chemical fertilizers on crops in the radish-stem amaranth-Indian spinach cropping pattern in homestead area. *Australian Journal of Crop Science*, 5, 1370-1378.

Jahiruddin, M., and Satter, M.A. (2010). Research priority in agriculture and development of vision document-2030 and beyond. Land and soil resource management. Bangladesh gricultural Research Council. Dhaka.

Kannaiyan. (2000). Bio-fertilizers – key factor in organic farming. The Hindu survey of Indian Agriculture, p. 165-173.

Khan, Z., Paigham, S., Muhammad, A., Zagda, K., Shah, P., and Arfi, M.(2000). Management of organic farming: effectiveness of farmyard manure and nitrogen for maize productivity. *Sarhad Journal of Agriculture,* 16(5)**,** 461-465.

Jeptoo, A., Aguyoh, J. N., and Saidi, M. (2013). Improving Carrot Yield and Quality through the Use of Bio-Slurry Manure. *Sustainable Agricultural Research,* 2(1), 164-172.

Kler, D.S., and Walia, S.S. (2006). Organic, integrated and chemical farming in wheat under maize-wheat cropping system. *Indian Journal of Agronomy*, 51, 6-9.

Laxminarayana, K. (2006). Effect of integrated use of inorganic and organic manures on soil properties, yields and nutrient uptake of rice in Ultisols of Mizoram. *Journal of the Indian Society of Soil Science,* 54, 120-123.

Lindsay, W.L., and Norvell, W.A. (1978). Development of a DTPA soil test for Zn, Fe, Mn and Cu. *Soil Science Society of America Journal*, 42, 421-428.

McLean, E.O. (1982). Soil pH and lime requirement. In: *Methods of soil analysis, Part 2, Chemical and microbiological properties,* A.L. Page, R.H. Miller and D.R. Keeney (eds.), p. 199-224. American Society of Agronomy Inc., Madison, WI, USA.

Mehdizadeh, M., Dorbandi , E.I., Naseri-Rad, H., and Tobeh, A. (2013). Growth and yield of tomato (*Lycopersicon esculentum* Mill.) as influenced by different organic fertilizers. *International Journal of Agronomy and Plant Production*, 4(4), 734-738.

Murmu, K., Swain, D.K., and Ghosh, B.C. (2013). Comparative assessment of conventional and organic nutrient management on crop growth and yield and soil fertility in tomato-sweet corn production system. *Australian Journal of Crop Science*, 7(11), 1617-1626.

Nambiar, K.K.M. (1997). Nutrient management for soil fertility restoration and sustaining cropping system productivity. In *nutrient management practices in crops and cropping system*. C.P. Ghonsikar and V.S. Shinde (eds.) Scientific Publishers. India. P. 213-250.

Nelson, D.W., and Sommers, L.E. (1982). Total carbon, organic carbon, and organic matter. In: *Methods of soil analysis, Part 2, Chemical and microbiological properties,* A.L. Page, R..H. Miller and D.R. Keeney (eds.), p.539-579. American Society of Agronomy Inc., Madison, WI, USA.

Olsen, S.R., and Sommers, L.E. (1982). Phosphorus. In: *Methods of soil analysis, Part 2, Chemical and microbiological properties,* A.L. Page, R.H. Miller and D.R. Keeney (eds.), p. 403-430. American Society of Agronomy Inc., Madison, WI, USA.

Orozco, F.H., Cegarra, J., Trujillo, L.M., and Roig, A. (1996). Vermicomposting of coffee pulp using the earthworm *Eisenia Foetida*: effects on C and N contents and the availability of nutrients. *Biology and Fertility of Soil,* 22, 162-166.

Page, A.L., Miller, R.H., and Keeney, D.R. (1982). Methods of soil analysis part 21th edn. Amer. Soc. Agron. Inc. Madi Wisconsis, USA.

Pane, C., Spaccini, R., Piccolo, A., Scala, F., and Bonanomi, G. (2011). Compost amendments enhance peat suppressiveness to *Pythium ultimum, Rhizoctonia solani and Sclerotinia minor*. *Biological Control,* 56, 115-124.

Premsekhar, M., and Rajashree, V. (2009). Influence Organic Manures on Growth, Yield and Quality of Okra. . *American-Eurasian Journal of Sustainable Agriculture,* 3(1), 6-8.

Rabbani, M.G., Jahiruddin, M., Islam, M.S., and Haque, M.M.(2013). Assessment of the Fertilizer Market with Focus on Micronutrient and Organic Compost. Study Report, KATALYST, Dhaka.

Rahman, M.A. (2013). Integrated use of fertilizer with manure on mustard, potato and wheat and their residual effects on succeeding crops. Ph. D. Thesis. Department of Soil Science, Bangladesh Agricultural University, Mymensingh.

Rahman, M.H., Islam, M.R., Jahiruddin, M., Rafii, M.Y., Hanafi, M.M., and Malek, M.A. (2013). Integrated nutrient management in maize-legume-rice cropping pattern and its impact on soil fertility. *Journal of Food, Agriculture & Environment*, 11(1), 648-652.

Rahman, S.M.E., Islam, M.A., Rahman, M.M., and Oh, D.H. (2008). Effect of cattle slurry on growth, biomass yield and chemical composition of maize fodder. *Asian-Australian Journal of Animal Science,* 2 (1), 1592-1598.

Saidu, A., Abayomi, Yakeen, A., Aduloju., and Morolake, O.(2012). Evaluation of complementry use of organic and inorganic fertilizers on the performance of upland rice (*Oryza sativa* L.). *International Journal of Advanced Biological Research*, 2(3), 487-491.

Singh, S.K., and Lal, S.S. (2006). Effect of organic sources of nutrients on potato production in South Bihar. Paper presented at national symposium on conservation agriculture and environment, BHU, Varanasi, India, 26-28 October 2006, 284.

Sood, M.C. (2007). Integrated nutrient supply and management for potato production in Mid Hills of Shimla. *Potato Journal*, 34, 101-102.

Yu, F.B., Luo, X.P., Song, C.F., Zhang, M.X., and Shan, S.D. (2010). Concentrated biogas slurry enhanced soil fertility and tomato quality. *Plant Soil Science,* 60(3), 262-268.

Zaman, S.K. (2002). Integration of fertilizer and manure for susainable soil fertility and productivity in rice-rice cropping pattern. Ph.D. Thesis. Dept. Soil Sci., BAU, Mymensingh.

SAARC J. Agri., 15(2): 163-175 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35146*

EFFECT OF DROUGHT STRESS ON WATER RELATION TRAITS OF FOUR SOYBEAN GENOTYPES

J.A. Chowdhury[1], M.A. Karim[2], Q.A. Khaliq[2], A.U. Ahmed[3*] and A.T.M.A.I. Mondol[4]

[1]Agronomy Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[2]Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University Gazipur-1706, Bangladesh
[3]Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[4]Soil Science Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

ABSTRACT

An experiment was conducted in a venyl house at the environmental stress site of Bangabandhu Sheikh Mujibur Rahman Agricultural University during September to December 2012 to know the internal water status under drought stress in soybean genotypes, viz. Shohag, BARI Soybean-6, BD2331 (relatively stress tolerant) and BGM2026 (susceptible). Drought (water) stress reduced the leaf water potential in all the genotypes though was more negative in tolerant genotypes than in susceptible ones. The lowest leaf water potential was obtained from BARI Soybean-6 (-1.58 MPa) and the highest in BGM2026 (-1.2 MPa). Relative water content (RWC) decreased remarkably in all the genotypes and reduction was more in susceptible than tolerant genotypes. At 8.00 am, RWC of stressed plants decreased by 9.58, 9.02, 8.90 and 13.90% in the genotype Shohag,, BARI Soybean-6, BD2331 and BGM2026 at vegetative stage, respectively. Drought stress decreased the exudation rate in all the genotypes of soybean and it was 24, 27, 22 and 12 mg h^{-1} in the genotype Shohag, BARI Soybean-6, BD2331 and BGM2026 at vegetative stage, respectively. Leaf temperatures in drought stressed plant were higher than in well-watered plants. Shohag, BARI Soybean-6, BD2331 and BGM2026 showed 4.7, 4.5 5.2 and 11.07% increase in leaf temperature due to water stress. At drought stressed treatment reduction in leaf water potential, relative water content, exudation rate and water retention capacity were noticed at the three growth stages in all the genotypes with a concurrent increase in leaf temperature. Genotypes BARI Soybean-6, Shohag and BD2331 showed considerably less reduction in relative water content, exudation rate and water retention capacity, high reduction in leaf water potential and less increase in leaf temperature during drought were considered as drought tolerant. However genotype BGM2026 showed considerably high reduction in

[*] Corresponding author e-mail**:** kajalashraf@gmail.com

Received: 19.09.2016 *Accepted: 20.08.2017*

relative water content, exudation rate and water retention capacity, low reduction in leaf water potential and high increase in leaf temperature was considered as drought susceptible.

Keywords: Drought, stress, soybean, genotypes

INTRODUCTION

Water is absolutely necessary for the functioning of protoplasm of cell. Water deficit stress affects water status in plant. Thus adequacy and inadequacy of water are the limiting factors for life both in land and water environment (Onwugbuta-Enyi, 2004). Several methods are used to characterize plant water status under water stress conditions. Determination of water relation components in whole plant or cellular level is important for determination of tolerance genotypes to environmental stresses especially to water deficit stress. It may be possible to improve the water stress tolerance of soybean by understanding the water relation parameters that are associated with high productivity. Among the several methods used to characterize internal plant water status under water stress conditions, relative water content and leaf water potential are used as the indicators of degree of water stress. The adequacy and inadequacy of water reflects on the status of plant water relations viz. leaf water potential, relative water content, exudation rate etc. of a plant and decrease them (Omae et al., 2007). Leaf water potential and relative water content are useful means for determining the physiological water status of plants (Gonzales & Gonzales-Vilar, 2001). Thus an understanding of the influence of drought on leaf water relations is crucial for classifying the mechanism of drought tolerance of a plant (Omae et al., 2007).

Leaf water potential is considered to be a reliable parameter for quantifying plant water stress response. Nayyer et al. (2005) has suggested that the leaf water potential is a prominent character that can be selected for improving drought tolerance of different crops. Jones in 1990 revealed that a majority of workers used leaf water potential to measure plant water status. Leaf water potential expresses the totality of turgor and osmotic potentials and under drought stress, adjustment in osmotic potential or maintenance of turgor could result in the maintenance of leaf water potential (Ocampo and Robles, 2000). The potential varies greatly, depending on the type of plant and environmental conditions. Water stressed plants showed a marked reduction in xylem exudation rate compared to well-watered condition (Aziz, 2003). Leaf temperature is also related to water stress. Ehrler et al. (1978) reported that canopy temperature provides a good indication of plant water potential. This study was initiated to determine and compare the variations in the internal water status of four soybean genotypes due to drought.

MATERIALS AND METHODS

A pot experiment in a vinyl house was conducted at the Bangabandhu Sheikh Mujibur Rahman Agricultural University during September to December 2012. Three relatively water stress tolerant (Shohag, BARI Soybean-6 and BD2331) and one susceptible (BGM-2026) genotypes, selected from the previous experiment, were used in this study to know the internal water status under drought stress in soybean. Seeds of tolerant and susceptible genotypes were sown in plastic pots. The soil of the pot was filled with mixture of soil and cow dung at a ratio of 4:1. Pot contained 12.0 kg of soil which was equivalent to 9 kg oven dry soil and holds about 28% moisture at field capacity (FC). Soil used in the pot was sandy loam. The soil of the pot was fertilized uniformly with 0.15, 0.18, 0.36 and 0.1 g urea, triple super phosphate, muriate of potash and gypsum corresponding to 24-30-60-15 kg NPKS per hectare, respectively. Six seeds pot^{-1} were sown on 3 September, 2012. After seedling establishment two uniform and healthy plants pot^{-1} were allowed to grow. Two watering treatments of the plants viz. drought stress (water stress) (50% water of the FC) and non-stress (control) (80% water of FC) were applied at 21 days after emergence (DAE) and maintained throughout the growing season. The pots were arranged in a completely randomized design (factorial) with four replications (two plants pot^{-1} considered as one replication). There were eight treatment combinations, including four genotypes and two water regime treatments (hereafter referred to as non-stress and water stress treatments). Normal management practices (Khan, 2013) were applied for all the treatments.

Data were collected on the following parameters

Relative water content (RWC) in leaf

Relative water content (RWC) of leaves was measured at vegetative, flowering and pod development stages of each genotype at 8:00 am and 1:00 pm. Fully developed 3rd leaf from the top was used for RWC measurement. Immediately after cutting, leaves were sealed within plastic bags and kept in ice box and quickly transferred to the laboratory. The fresh weight of leaves from each treatment was recorded just after removal. Turgid weight (TW) was obtained after soaking leaves in distilled water in beakers for 24 hours at room temperature (about 20ºC) and under low light condition of the laboratory. After soaking, leaves were quickly and carefully blotted dried with tissue paper in preparation for determining turgid weight. Dry weight (DW) of the leaf was obtained after oven drying the leaf samples for 72 hour at 70ºC. RWC was calculated using the formula of Schonfeld et al. (1988):

RWC (%) = (FW – DW) / (TW – DW) x 100

Where, FW = Fresh weight

DW = Dry weight

TW = Turgid weight

Water retention capacity (WRC) were calculated as follows (Sangakkara et al., 1996).

$$\text{Water retention capacity (WRC)} = \frac{\text{Turgid weight}}{\text{Dry weight}}$$

Leaf water potential

Leaf water potential was measured at 6:30 am with the help of Scholander Pressure Bomb apparatus. The third uppermost fully expanded leaf was cut carefully with sharp blade from 4 replicated plants of each treatment. The petiole of cut leaf was set in the apparatus and pressure was applied to the leaf from a cylinder of compressed gas until xylem sap appeared at the cut surface of the leaf (detected by using a magnifying glass). The gas flow was immediately stopped and the pressure was noted in the gauge.

Xylem exudation rate (XER)

Xylem exudation rates at vegetative, flowering and pod development stages were measured at 9:00 am at 5 cm above from stem base. At first, dry cotton was weighed. A slanting cut on stem was made with a sharp knife. Then the weighed cotton was placed on the cut surface. The exudation of sap was collected from the stem for 1 hour at normal temperature. The final weight of the cotton with sap was taken. The exudation rate was calculated by deducting cotton weight from the sap containing cotton weight and expressed per hour basis as follows;

$$\text{Xylem exudation rate} = \frac{\text{(Weight of cotton + sap) – (Weight of cotton)}}{\text{Time}} \text{ mg } h^{-1}$$

Statistical analysis

The data were analyzed by MSTAT-C statistical program. The difference between the treatments means were compared by Least Significant Difference (LSD) test (Gomez and Gomez, 1983).

RESULTS AND DISCUSSION

Leaf water potential

Water stress decreased the leaf water potential (LWP) at pod development stages studied in all the four soybean genotypes (Figure 1). Water stress significantly reduced leaf water potential of soybean plant and the potentials fell from -0.88 MPa in unstressed leaves to -1.18 MPa in drought stressed leaves (Makbul et al., 2011). Other researchers also reported that leaf water potential decreased under drought stress conditions (Siddique et al., 2000). Such observation also observed in snap bean by Omae et al. (2007) and in soybean by Ohashi et al. (2000). Leaf water potential in all the genotypes was higher under control condition than that in stress condition.

Under stress condition the LWP of BARI Soybean-6 was more negative which was followed by Shohag and BD2331 and the minimum in BGM2026. The leaf water potential recorded at pod development stage varied from -1.00 to -1.2 MPa and from -1.2 to -1.58 MPa under non-stress and water stress condition, respectively. Under water stress condition the lowest leaf water potential was obtained from BARI Soybean-6 (-1.58 MPa) and the highest in BGM2026 (-1.2 MPa). The highest reduction in leaf water potential 31.66% was recorded in BARI Soybean-6. The changes in water potential might be due to change in osmotic pressure, the osmotic components of water. Gonzalez et al. (2008) recorded a significant decrease in leaf water potential under drought stress in barley.

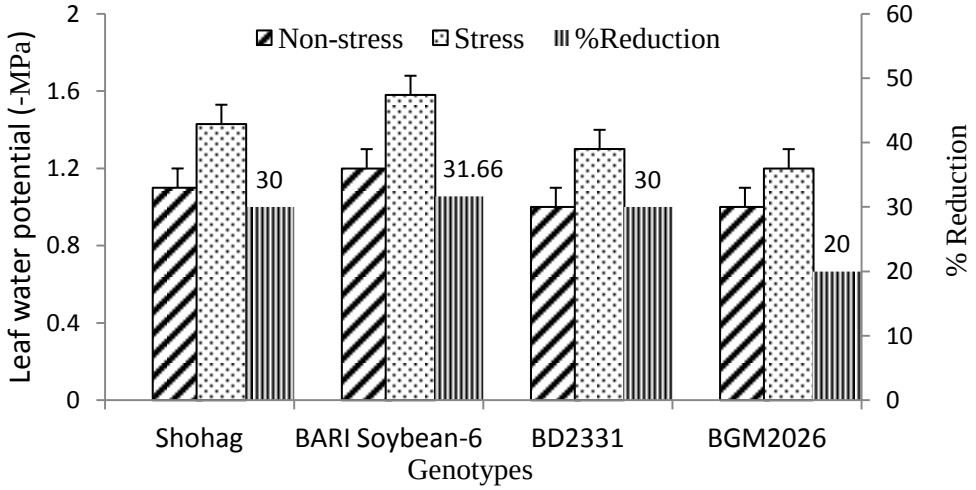


Figure 1. Leaf water potential in soybean at pod development stage as affected by two water regimes [Vertical bar represent LSD value at 5% level of significant]

Relative leaf water content

Relative water content (RWC) is an important determinant of metabolic activity and survival of leaf. RWC values of four genotypes at three different stages are shown in figures 2, 3 and 4. Water stress significantly reduced RWC at two sampling times (8:00am and 1:00 pm) across the genotypes at different growth stages in all the four soybean genotypes studied. The reduction in RWC due to water stress was also reported by Omae et al. (2005) and Omae et al. (2007) in snap bean. Plants grown under water stress conditions showed a lower RWC than those grown under non stress conditions. Relative water content was higher in the morning, while decreased at noon. Several researchers reported that RWC of different crops was the highest in the morning and gradually decreased thereafter (Omae et al., 2005). Schonfeld et al. (1988) reported that the cultivars that were resistant to drought had more RWC. BARI Soybean-6 had higher RWC than the rest of genotypes and genotype

BGM2026 had the lowest RWC at all the three growth stages under both non-stress and stress condition. Upreti et al. (2000) reported that sensitive pea genotypes were more affected by a decline in relative water content than tolerant ones under drought stress condition. The RWC of all the genotypes fell at noon, possibly due to higher evaporation resulting from increased temperature and light intensity.

Water stress significantly reduced RWC at two sampling times (8:00am and 1:00 pm) across the genotypes at different growth stages in all the four soybean genotypes studied. At 8.00 am, RWC of water stressed plants of Shohag decreased by 9.58, 10.32 and 10.94%, BARI Soybean-6 decreased 9.02, 9.84 and 10.65%, BD2331 decreased 8.90, 11.68 and 12.94%, and BGM2026 decreased 13.90, 15.31 and 16.21% compared to control plants at vegetative, flowering and pod development stages, respectively. At 1.00 pm, RWC of water stressed plants decreased by 11.21, 12.55 and 13.40% in Shohag, decreased 10.79, 11.60 and 13.10% in BARI Soybean-6, 12.48, 14.27 and 18.74 % in BD 2331and 19.22, 21.51 and 25.45% in BGM2026 at three growth stages, respectively. The higher reduction was found in BGM2026 at both the day time. Similar results were observed by Rosales-Serna et al. (2004) in tolerant cultivar Pinto Villa compared to susceptible cultivar Bayo Madero; which was explained as to be related to the lower stomatal index in the adaxial surface in Pinto Villa in comparison with Bayo Madero (Aguirre et al., 1999), or to a higher capability for soil extraction under drought stress. Parsons and Howe (1984) opined that among several methods used to characterize internal plant water status under drought conditions, RWC is an integrative indicator.

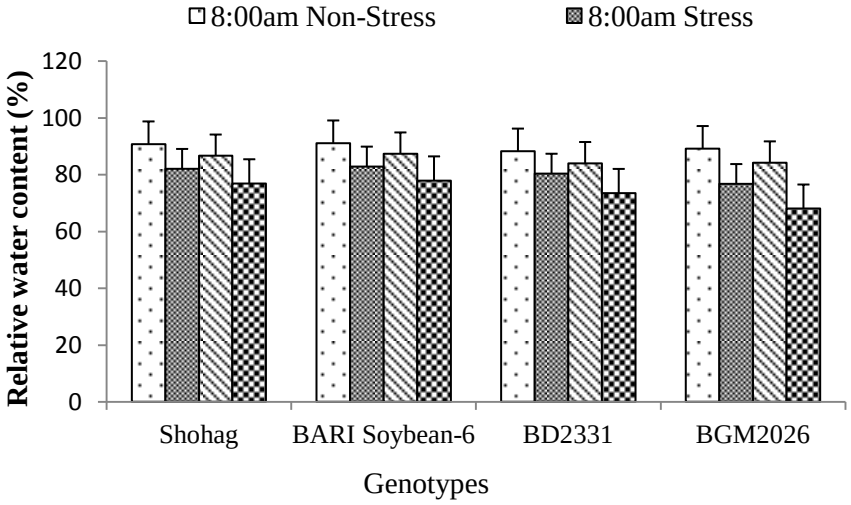


Figure 2. Relative water content (RWC) in soybean genotypes under non-stress and water stress conditions at vegetative stage [Vertical bar represent LSD value at 5% level of significant]

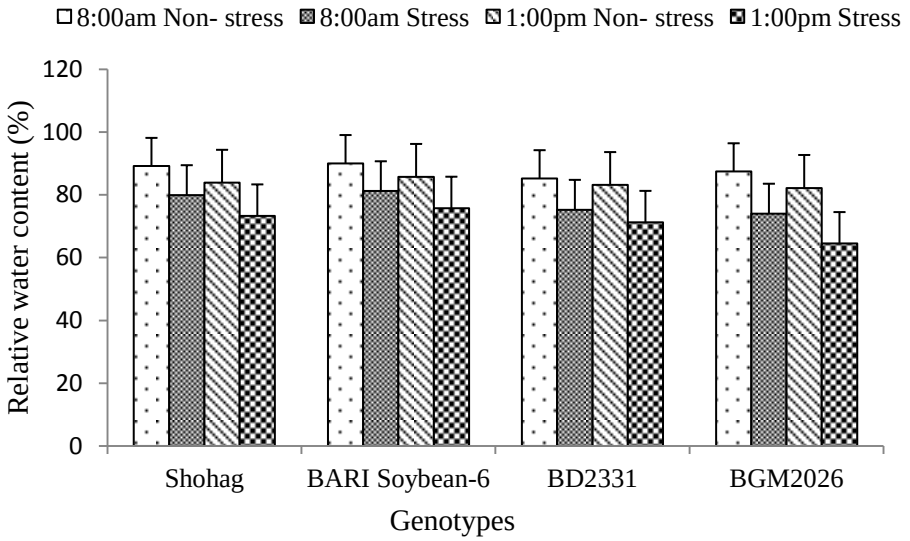


Figure 3. Relative water content (RWC) in soybean genotypes under non-stress and water stress conditions at flowering stage [Vertical bar represent LSD value at 5% level of significant]

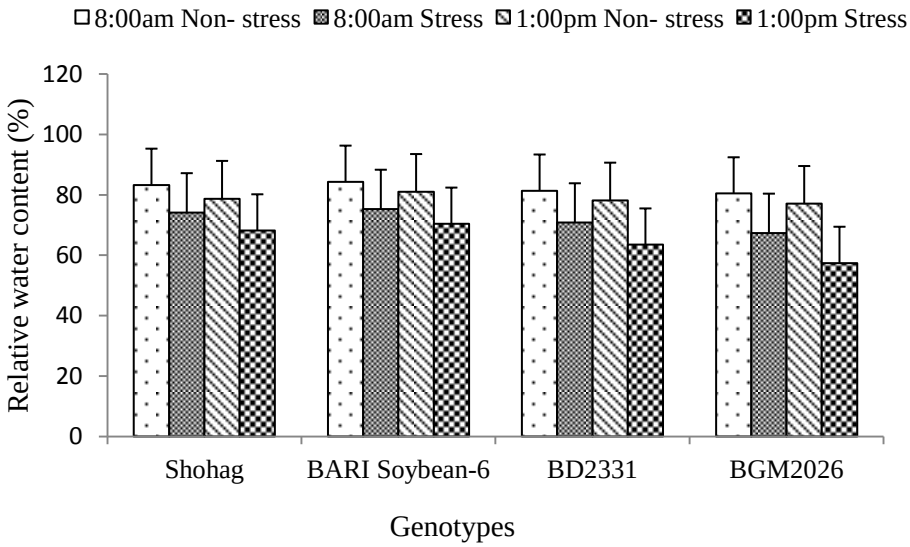


Figure 4. Relative water content (RWC) in soybean genotypes under non-stress and water stress conditions at pod development stage [Vertical bar represent LSD value at 5% level of significant]

Water retention capacity

The turgid weight/dry weight (TW/DW) ratio illustrates the water retention capacity (WRC) of plants that are determined by the cell structures. Plants grown under a high moisture regime maintains a higher ratio and that might be due to the lower destruction of plant tissues by moisture deficit (Sangakkara et al., 1996). Water stress decreased the WRC significantly which was affected more at noon compared to that at morning (Figure 5). Among the genotypes, the WRC ranged from 6.6 to 7.2 and 6.0 to 7.0 at morning and noon respectively under non-stress and from 6.0 to 6.3 and 5.1 to 5.5 at morning and noon, respectively under water stress condition. Genotype BGM2026 presented the highest WRC value under non-stress condition but the lowest under water stress condition and decreased considerably at morning (16.66%) and noon (27.14%) while Shohag and BARI Soybean-6 presented the lowest TW/DW values under non-stress condition. The reduction rate of WRC was minimal which are 7.57% for Shohag, 13.33% for BARI Soybean-6, and 7.57% for Shohag, and 13.11% for BARI Soybean-6 at morning and noon, respectively (Figure 6). The higher reduction in WRC for BGM2026 indicated a greater damage in cell structure due to water stress than Shohag and BARI Soybean-6. Sanagakkara et al. (1996) and Martinez et al. (2007) observed similar results in *Phaseolus vulgaris*. This reduction was also observed in Mediteranean shrub *Artiplexhalimus* (Martinez et al., 2004). The reduction in the leaf TW/DW could be result of hemi-cellulose and cellulose accumulation in the cell well as reported by Wakabayashi et al. (1997). Martinez et al. (2007) pointed out that there is a negative relationship between TW/DW and drought resistance index (DRI) under water stress. Martinez et al. (2004) also observed that a decrease in the leaf TW/DW indicated a decrease in cell size. A reduction in cell size is one of the most common anatomical changes observed in water stressed leaves (Tardieu et al., 2000). In the present study, Shohag and BARI Soybean-6 showed the lowest reduction in WRC, and thus an indication of their tolerance to water stress.

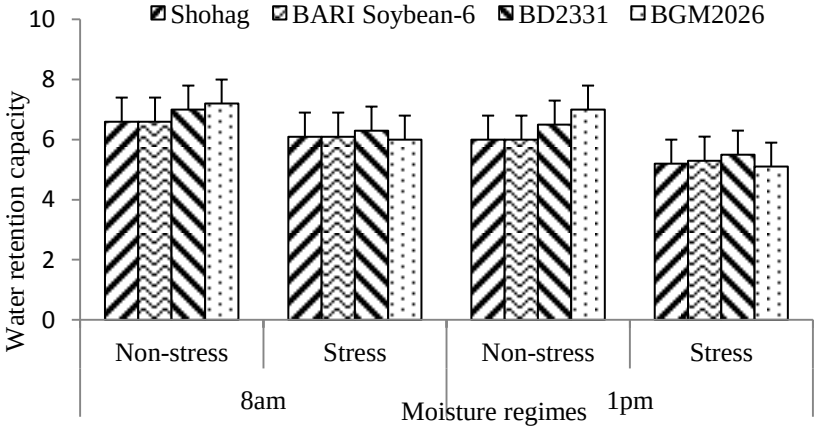


Figure 5. Water retention capacity in four soybean genotypes grown under non-stress and water stress conditions at pod development stage [Vertical bar represent LSD value at 5% level of significant]

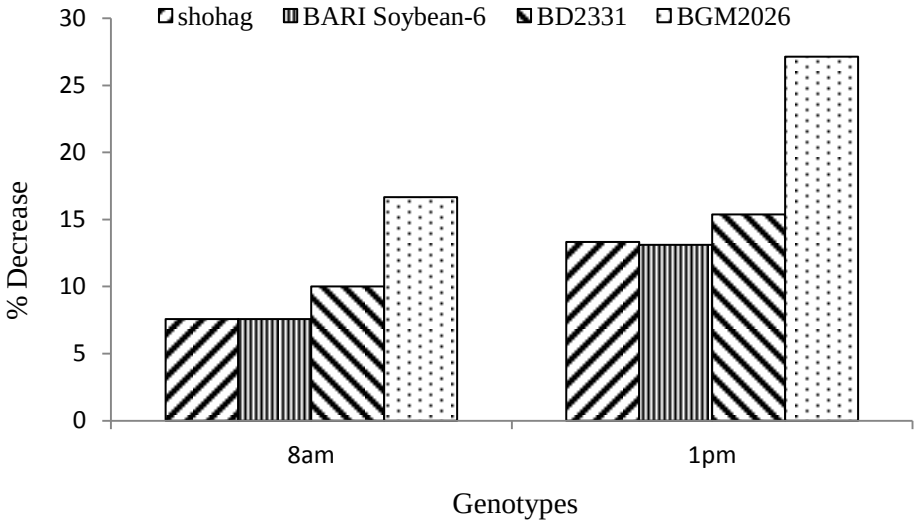


Figure 6. Reduction percent of water retention capacity in four soybean genotypes grown under non-stress and water stress conditions at pod development stage

Xylem exudation rate

Xylem exudation rate is known as the flow of sap from cut end of stem against the gravitational force. Under normal condition, exudation rate is higher than that under any kind of stress conditions. Thus, the exudation rate can be used as an indicator to measure the severity of water stress. Water stress drastically reduced the exudation rate in all the genotypes at all the growth stages studied. Exudation rate is directly associated with the flow of transpiration. Decreased exudation rate means lower water uptake by the plant. In this experiment it was found that drought stress substantially decreased the exudation rate in all the genotypes of soybean at all the three growth stages studied (Figure 7). The exudation rate in genotype BGM2026 was much lower than that of other genotypes in stressed condition. The exudation rate was not significant under non-stress condition, while marked variation was observed due to water stress irrespective of genotypes. The exudation rate varied from 79 to 82, 99 to 107 and 92 to 100 mg h^{-1}at vegetative, flowering and pod development stages, respectively under non-stress condition and 12 to 27, 8.3 to 20.2 and 4.5 to 16.3 mg h^{-1} at vegetative, flowering and pod development stage respectively under water stress condition. At all the growth stages, the highest exudation rate was recorded in BARI Soybean-6 which was followed by Shohag and BD2331, while the genotype BGM2026 was affected more and had the lowest exudation rate under water stress condition which indicated that the former three genotypes absorbed more water than that of BGM2026 under water stress condition.

The reduction percent of exudation rate was the highest in BGM2026 (85.36, 92.24 and 95.5% at vegetative, flowering and pod development stages respectively) due to the effect of water stress (Figure 8). The results obtained in this study were in agreement with those obtained by Baque (2006) who reported that exudation rate was higher in control and lower in moister stress in wheat. Reduction in water uptake by other plants due to water stress was also reported by Choudhury (2009) in french bean.

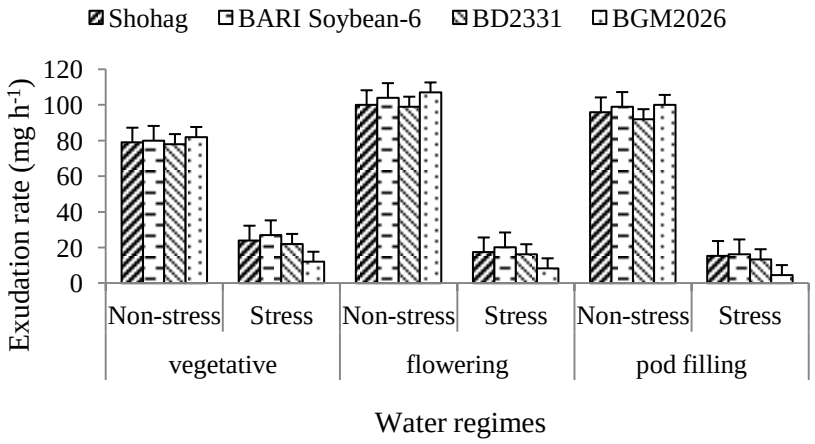


Figure 7. Xylem exudation rate in four soybean genotypes grown under non-stress and water stress conditions at different growth stages [Vertical bar represent LSD value at 5% level of significant]

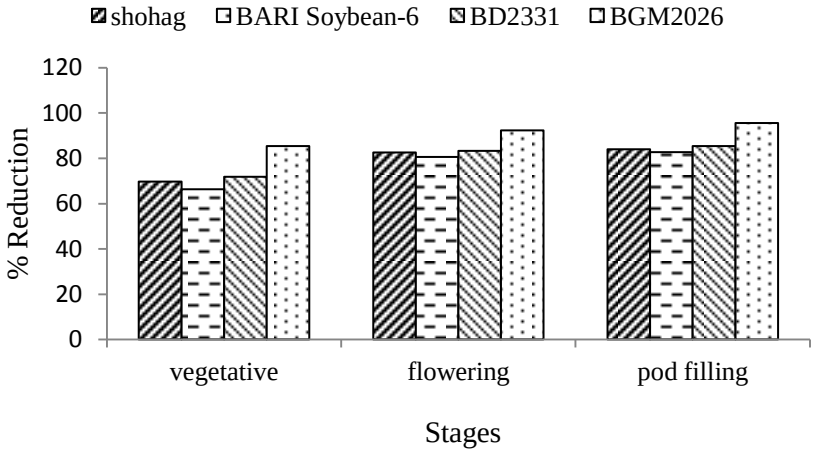


Figure 8. Reduction percent of Xylem exudation rate in four soybean genotypes grown under non-stress and water stress conditions at different growth stage

Leaf temperature

Water stress increased the leaf temperature of all the genotypes under study (Figure 9). Leaf temperatures in drought stressed plant were higher than in well-watered plants. Leaf temperature ranged from 34.98 to 39.18^{0}C and 36.57 to 41.41^{0}C under non-stress and water stress conditions, respectively. Under non-stress environment genotype BD2331 showed the highest leaf temperature, while BGM2026 showed the highest under water stress environment. Shohag, BARI Soybean-6 and BD2331 showed only 4.7, 4.5 and 5.2% increase in leaf temperature due to water stress while genotype BGM2026 showed 11.07% increase in leaf temperature. Increase in leaf temperature due to water stress might be attributed to low transpiration under drought. Winter et al. (1988) also found significant difference in leaf temperature between droughts stressed and irrigated plants.

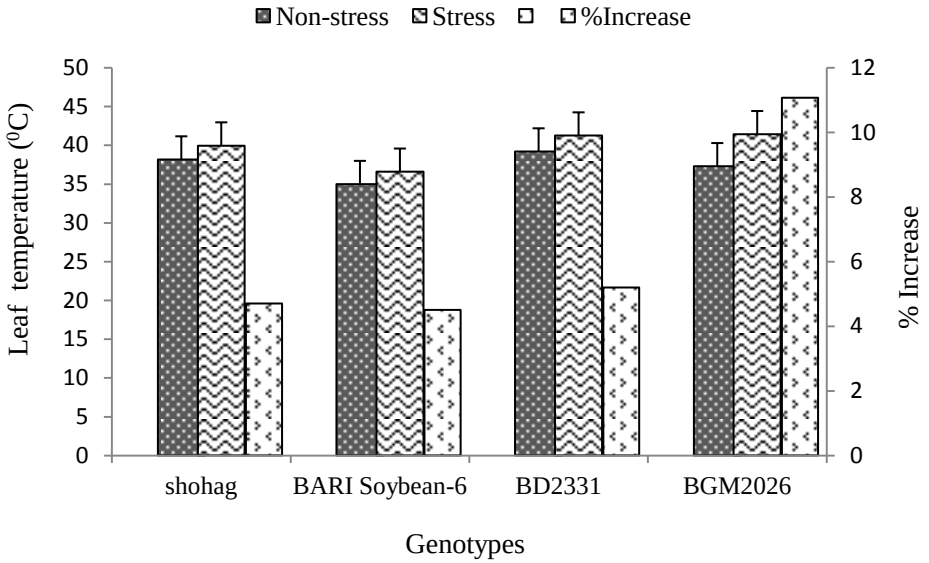


Figure 9. Leaf temperature in four soybean genotypes grown under non-stress and water stress conditions at pod development stage [Vertical bar represent LSD value at 5% level of significant]

CONCLUSION

Based on findings of the present study it may concluded that high water stress tolerance of Shohag, BARI Soybean-6, BD2331 is associated with maintaining better plant water relations which is reflected by higher relative water content, water retention capacity, exudation rate, lower leaf water potential and leaf temperature than in case of BGM2026.

REFERENCES

Aguirre, J.F., RuizL, P.J., Kohashi-Shibata, J., Trejo-Lopez, C., and Acosta-Gallegos, J.A. (1999). Morphological observations in the leaf surface of *Phaseolus vulgaris* L. and their possible relationship to stomotal response. *Annual Report of the Bean Improvement Cooperative*, 42, 75-76.

Aziz, M.A. (2003). Growth, yield and some physiological mechanisms of salinity tolerance in mungbean. A Ph.D. Dissertation. Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, p. 127.

Choudhury, A.K. (2009). Water stress tolerance of french bean (*Phaseolus vulgaris* L.). Ph. D. Dissertation. Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur-1706. Bangladesh.

Ehrler, W.L., Idso, S.B., Jackson, R.D., and Reginato, R.J. (1978). Wheat canopy temperature: Relation to plant water potential. *Agronomy Journal*, 70, 251-256.

Gonzales, L., and Gonzales-Vilar, M. (2001). Determination of relative water content. In: Reigosa M.J. (ed.). *Handbook of plant Ecophysiology Techniques*, Dordrecht: Kluwer Academic Publishers, p. 207-212.

González, A., Martín, I. and Ayerbe, L. (2008). Yield and osmotic adjustment capacity of barley under terminal water-stress conditions. *Journal of Agronomy and Crop Science*, 194, 81-91.

Khan, M.S.A. (2013). Evaluation of soybean genotypes in relation to yield perfopmance, salinity and drought tolerance. A Ph.D. Dissertation. Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, p. 7

Makbul, S., Guler, N.S., Durmus, N., and Guven, S. (2011). Changes in anatomical and physiological parameters of soybean under drought stress. *Turkey Journal of Botany*, 35, 369-377.

Nayyer, H., Kaur, S., Singh, K.J., Dhir, K.K., and Bains, T. (2005). Water stress induced injury to reproductive phase in chickpea: evaluation of stress sensitivity in wild and cultivated species in relation to abscisic acid and poly amines. *Journal of Agronomy and Crop Science*, 191, 450-457.

Ocampo, E.T.M., and Robles, R.P. (2000). Drought tolerance in Mungbean II. Stomatal Movement, Photosynthesis and leaf water potential. *Philippine Journal of Crop Science*, 25, 7-15.

Ohashi, Y., Saneoka, H., and Fujita, K. (2000). Effect of water stress on growth, photosynthesis and photoassimilate translocation in soybean and tropical pasture legume siratro. *Soil Science and Plant Nutrition*, 46, 417- 425.

Omae, H., Kumar, A., Egawa, Y., Kashiwaba, K., and Shono, M. (2005). Midday drop of leaf water content to drought tolerance in snap bean (*Phaseolus vulgarid* L.). *Plant Production Science*, 8, 465-467.

Omae, H., Kumar, A., Kashiwaba, K., and Shono, M. (2007). Assessing drought tolerance of snap bean (*Phaseolus vulgaris*) from genotypic differences in leaf water relations, shoot growth and photosynthetic parameters. *Plant Production Science*, 10, 28-35.

Onwugbuta-Enyi, J. (2004). Water balance and proximate composition in cowpea (Vignaunguiculata (I) Walps) seedlings exposed to drought and flooding stress. *Journal of Applied Science and Environmental Management*, 8, 55-57.

Parsons, L.R., and Howe, T.K. (1984). Effects of water stress on the water relatins of *Phaseolus vulgaris* and the drought resistant *Phaseolus acutifolius*. *Physiology of Plant*, 60,197-202.

Rosales-Serna, R., Kohashi-Shibata, J., Acosta-Gallegos, J. A., Trejo-Lopez, C., Ortiz-Cerceres, J., and. Kelly, J.D. (2004). Biomas distribution, maturity acceleration and yield in drought-stressed common bean cultivars. *Field Crops Research*, 85, 203-2011.

Schonfeld, M.A., Johnson, R.C., Carver, B.F., and Mornhinweg, D.W. (1988). Water relations in winter wheat as drought resistance indicators. *Crop Science*, 28, 526-531.

Siddique, M.R.B., Hamid, A., and Islam, M.S. (2000). Drought stress effects on water relations of wheat. *Botanical Bulletin of Academia Sinica*, 41, 35-39.

Upreti, K. K., Murti, G.S.R and Bhatt, R.M. (2000). Response of pea cultivars to water stress: changes in morpho-physiological characters, endogenous hormones and yield. *Vegetable Science*, 27, 57-61.

Winter, S.R., Musick, J.T., and Porter, K.B. (1988). Evaluations of screening techniques for breeding drought-resistant winter wheat. *Crop Science*, 28, 512-516.

SAARC J. Agri., 15(2): 177-192 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35147*

SELECTION OF DROUGHT TOLERANT WHEAT GENOTYPES BY OSMOTIC STRESS IMPOSED AT GERMINATION AND EARLY SEEDLING STAGE

R.R. Saha[1*], A. Hannan[2], A. Nessa[2], M.A. Malek[3] and M.R. Islam[4]

[1]Plant Physiology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[2]Seed Technology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[3]Plant Genetic Resources Center, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[4]Regional Agricultural Research Station, BARI, Ishurdi, Pabna, Bangladesh

ABSTRACT

An experiment on hundred wheat genotypes under different levels of osmotic stress was carried out during 2014 to select the genotype(s) tolerant to drought at germination and early seedling stage. Different levels of osmotic stress were imposed by using polyethylene glycol (PEG). Three osmotic stress levels viz. control (distilled water), 15% PEG solution and 25% PEG solution were used. Among the 100 genotypes the rate of germination percentage, final germination (%), root and shoot dry weight, amount of respiration and vigour index under PEG treatment was found significantly lower than that of control condition. Compared to control condition relative decrease in rate of germination, final germination, amount of respiration and vigour index among the wheat genotypes were found more at 25% PEG than that of 15% PEG treatment. However, the seed metabolic efficiency was significantly higher in wheat genotypes under both 15% PEG and 25% PEG treatment compared to the control condition. A significant positive correlation exists between the important growth parameters like rate of germination (%), final germination (%), shoot dry weight, root dry weight and vigour index. On the basis of these physiological traits against osmotic stress, nine genotypes of wheat such as BD-480, BD-498, BD-501, BD-513, BD-514, BD-519, BD-592, BD-618 and BD- 633 were selected as drought tolerant.

Keywords: Drought tolerant, osmotic stress, wheat genotypes

INTRODUCTION

Wheat is one of the most important cereal crops of the world. In most areas of the world, wheat is a principal food. In Bangladesh, it is the second most important grain crop after rice and grown in winter season which prevailing drought condition due to

[*] Corresponding author e-mail: saharinarani@yahoo.com

Received: 17.04.2017 *Accepted: 04.08.2017*

lack of rains. Drought is a severe limitation of plant growth, development and productivity, particularly in arid and semi-arid regions (Galle et al., 2007). Seed germination and early seedling growth are potentially the most critical stages for water stress (Ahmad et al., 2009). Besides the reduction in total germination, comparatively low soil moisture availability results in delayed emergence, a criterion of particular importance in the vigor and subsequent yielding ability of many crops (Azam and Allen, 1976). The rate and degree of seedling establishment are extremely important factors in determining both yield and time of maturity (Brigg and Aylenfisu, 1979).

Abiotic stresses like drought can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth, and development. Among these, drought is a worldwide problem, constraining global crop production seriously and recent global climate change has made this situation more serious (Pan et al., 2002). Plants respond to drought with physiological and biochemical changes. The impacts of drought condition depend on their severity and the stage of plant growth during which they occur. Seedling emergence is one of the most sensitive growth stages that are susceptible to water deficit. Therefore, seed germination, seedling vigour and coleoptiles's length are prerequisites for successful stand establishment of wheat under drought conditions. Simulation of drought stress by polyethylene glycol (PEG) induces drought stress on the plants (Jiang et al., 1995). PEG-6000 has long been utilized as a reliable marker under laboratory conditions for testing the drought tolerant genotypes which could be a cost effective and still potential approach. With this view the present study was conducted to select wheat genotype(s) for their drought tolerance at the germination and early seedling stage by using PEG induced water stress.

MATERIALS AND METHODS

The experiment was conducted in both the laboratories of Plant Physiology Division and Seed Technology Division, BARI, Gazipur during 2014. The experiment was laid out in a completely randomized design with two replications. Hundred wheat genotypes were tested under osmotic stress imposed by using polyethylene glycol (PEG6000). Solutions were prepared according to Baloch et al. (2012). There were three osmotic stress levels i.e. T_1-control (with distilled water), T_2-15% PEG solution and T_3-25% PEG solution. Thirty seeds were placed in Whatman number 1 filter paper in petridishes. After placing the seeds in petridishes, measured volume of 10 ml PEG solution or distilled water was given in the petridishes. Three days after placement of seeds in the petridishes, 10 ml distilled water was added in each petridishes to minimize the evapo-transpiration losses. Germination was counted at 24-hour interval and continued up to the 7 day. The seeds were considered germinated when plumule and radicle came out and longer than 2 mm. After 10 days of treatment, other parameters such as number of seedling, dry weight of shoot, root and remaining seed was taken on all seedlings of each treatment which having at

least 3 cm long in both root and shoot. The rate of germination was calculated using the formula according to Krishnasamy and Seshu (1990). Seed metabolic efficiency (SME) and amount of seed material respired (SMR) were calculated using the formula according to Rao and Sinha (1993). All percentage data were transformed into arcsine value and recorded data were analyzed statistically (ANOVA and correlation). Least Significant Difference (LSD) was used to compare the mean differences among the treatments.

RESULTS & DISCUSSION

Different seedling traits

Varying response of wheat genotypes to PEG treatment is very important for screening drought tolerant genotypes at early seedling stage before conducting extensive and expensive field tests. In the present study, rate of germination (%), final germination (%), root and shoot dry weight, amount of respiration of 100 wheat genotypes were significantly decreased due to osmotic stress i.e. PEG treatment compared to that of control treatment (Table 1 & 2). Roza et al. (2010) noted that significant decrease in shoot and root dry matter of wheat at PEG treatment. On the other hand, dry weight of remaining seed, root-shoot ratio and seed metabolic efficiency were significantly increased at PEG treatment than that of control condition (Table 2). It was observed that the range between maximum and minimum values of rate of germination (%) and final germination (%) among the 100 wheat genotypes under PEG treatment was higher than that of control treatment. It might be due to variable responses of wheat genotypes to osmotic stresses. It was also found that the rate of germination and final germination relatively decreased more in 25% PEG than that of 15% PEG treatment. This was probably due to depression in traits due to PEG desiccation. The remaining seed dry weight was higher in both 15% PEG and 25% PEG treatment than that of control treatment. The result indicated that PEG induced stress condition might be affected the transformation of seed reserve to available form which was essential for producing seedling organ i,e, root and shoot. It is also observed that dry matter was more or less equally distributed in root, shoot and remaining seed but slightly higher dry matter utilized for respiration under control condition. Whereas, in case of PEG treatment, most of the dry matter remained in seeds which might be due to inhibitory effect of osmotic stress on breakdown of seed reserves resulting lower accumulation of root and shoot dry matter as well as utilizing smaller amount of dry matter for respiration. For this reason, decreasing trend was presumably found in seed germination as well as seedling growth.

Root and shoot ratio significantly varied among the wheat genotypes under different level of stress (Table 2). Higher root-shoot ratio was observed in all the genotypes under drought stress condition i.e. PEG treatments compared to control condition. This might be due to the tendency of enhanced root growth for surviving under stress

condition. The development of root system under water deficit conditions appears to be a very viable criterion to select water stress tolerant genotypes of a crop because the roots take the moisture from lower layers of soil. Dhanda et al. (2004) reported that continued growth of roots in dry soil is particularly important to avoid drought stress. On the other hand, significantly decreasing amount of respiration was found in all the genotypes under PEG treatment than that of control condition which indicated a lower enzymatic activity under stressful conditions. However, the seed metabolic efficiency was significantly higher in wheat genotypes under both 15% PEG and 25% PEG treatment compared to the control. Under stress condition, the higher value of seed metabolic efficiency indicated the seeds may have higher efficiency to utilize seed reserves for producing root and shoot rather than respiration and/or unable to breakdown the seed reserve resulting higher remaining seed dry weight.

Vigour index

Vigour index differed significantly among the wheat genotypes under variable drought condition (Table 3). Due to PEG treatment considerable reduction was found in seed vigour index in all the genotypes compared to control condition. Relatively more reduction of vigour index was observed among the wheat genotypes at 25% PEG than that of 15% PEG treatment. Similar to present findings, Bayoumi *et al.* (2008) and Rauf et al. (2007) also noted significant reductions in all seedling traits by osmotic stress in wheat. However, among the 100 genotypes, nine genotypes such as BD-480, BD-498, BD-501, BD-513, BD-514, BD-519, BD-592, BD-618 and BD-633 showed relatively less reduction in vigour index at both 15% PEG and 25% PEG treatment and these genotypes were regarded as drought tolerant.

Correlations among seedling traits

Correlations are important statistical parameters for selection and crop improvement program (Baloch et al. (2012). Rate of germination showed significantly positive correlation with shoot dry weight (r = 0.64), amount of respiration (r = 0.46) and vigour index (r = 0.6) but negative correlation with remaining seed dry weight (r = - 0.57) and root-shoot ratio (r = - 0.39) (Table 4). Final germination (%) and root dry weight expressed significantly positive correlation with vigour index (r = 0.78 and 0.7, respectively). Shoot dry weight showed significantly positive correlation with amount of respiration (r = 0.54) and vigour index (r = 0.81) but significantly negative correlation with remaining seed dry weight, total seedling dry weight and root-shoot ratio. In case of remaining seed dry weight, significant positive correlation was found with total seedling dry weight (r = 0.88) but significantly negative correlation with amount of respiration (r = -0.64) and vigour index (r = - 0.65). Total dry weight of seedling positively correlated with seed metabolic efficiency (r = 0.48) and negatively correlated with amount of respiration (r = - 0.56). Similarly amount of respiration showed significantly negative correlation with seed metabolic efficiency(r = -0.8). Moreover, rate of germination (%), final germination (%), root and shoot dry weight and vigour index exhibited significant positive correlations with each other,

which suggesting that increase in any one of those traits correspondingly increase the other traits. It means that if one reliable trait is picked in osmotic stress and used as selection criterion that will lead to improve other seedling traits for drought conditions (Baloch et al., 2012). Several other workers (Bayoumi et al., 2008, Dhanda et al., 2004, Rauf et al., 2007 and Baloch et al., 2012) also noted positive correlations among wheat seedling traits under osmotic or water stress condition.

Table 4. Correlation coefficient (r) among different seedling traits of wheat genotypes as affected by polyethylene glycol (PEG-6000) treatment

	RG	FG	RDW	SDW	RSDW	TDW	R:S	ASR	SME	VI
		0.45	0.3	0.64	-0.57	-0.37	-0.39	0.46	-0.22	0.6
RG	1	NS	NS	**	**	NS	**	**	NS	**
			0.39	0.44	-.44	-0.25	-0.2	0.22	-0.08	0.78
FG		1	NS	NS	NS	NS	NS	NS	NS	**
				0.4	-0.29	0.1	0.37	0.12	0.22	0.7
RDW			1	NS	NS	NS	NS	NS	NS	**
					-0.73	-0.38	-0.63	0.54	-0.18	0.81
SDW				1	**	**	**	**	NS	**
						0.88	0.43	-0.64	0.39	-0.65
RSDW					1	**	NS	**	NS	**
							0.39	-0.56	0.48	-0.27
TDW						1	NS	**	**	NS
								-0.38	0.29	-0.25
R:S							1	NS	NS	NS
									-0.8	0.42
ASR								1	**	NS
										-0.06
SME									1	NS
VI										1

**Significant at 1% probability level, NS=Non-significant

RG-Rate of germination, FG-Final germination, RDW- Root dry weight, SDW-Shoot dry weight, RSDW- remaining seed dry weight, TDW-Total dry weight, R:S-Root –shoot ratio, SMR - amount of seed material respired, SME-Seed metabolic efficiency, VI-Vigour index

CONCLUSION

Significant positive correlation exists among some important growth parameters like rate of germination (%), final germination (%), shoot and root dry weight with vigour index. On the basis of these physiological traits against osmotic stress, nine wheat genotypes such as BD-480, BD-498, BD-501, BD-513, BD-514, BD-519, BD-592, BD-618 and BD- 633 were selected as drought tolerant.

REFERENCES

Ahmad, S., Ahmad, R., Ashraf, M.Y., Ashraf, M., and Waraich, E.A. (2009). Sunflower (*Helianthus Annuus* L.) response to drought stress at germination and seedling growth stages. *Pakistan Journal of Botany*, 41, 647-654.

Azam, G., and Allen, R.E. (1976). Interrelationship of seedling vigor criterion of wheat under different field situations and soil water potentials. *Crop Science*, 16, 615-618.

Baloch, M.J., Dunwell, J., Khakwani, A.A., Dennet, M., Jatoi, W.A., and Channa, S.A. (2012). Assessment of wheat cultivars for drought tolerance via osmotic stress imposed at early seedling growth stages. *Journal of Agricultural Research,* 50(3), 299-310.

Bayoumi, T.Y., Manal, H.E., and Metwali, E.M. (2008). Application of physiological and biological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *African Journal of Biotechnology*, 7(14), 2341-2352.

Brigg, K.G. and Aytenfisu, A. (1979). The effect of seedling rate, seeding date and location on grain yield, maturity, protein percentage and protein yield of some spring wheats in central Alberia. *Canadian Journal* of *Plant Science*, 59, 1129-1146.

Dhanda, S.S., Sethi G.S., and Behl, R.K. (2004). Indices of drought tolerance in wheat genotypes at early stages of plant growth. *Journal of Agronomy and Crop Science*, 190, 6-12.

Galle, A., Haldimann, P., and Feller, U. (2007). Photosynthetic performance and water relations in young pubescent oak (Quercus pubescens) trees during drought stress and recovery. *New Phytologist*, 174,799–810.

Jiang, Y., Macdonald, S.E., and Zwiazak, J.J. (1995). Effects of cold storage and water stress on water relations and gas exchange of white spruce (*Picea glauca*) seedlings. *Tree Physiology*, 15(4), 267-273.

Krishnasamy, V., and Seshu, D.V. (1990). Germination after accelerated aging and associated characters in rice varieties. *Seed Science and Technology*, 18, 353-359.

Pan, X.Y., Wang, Y.F., Wang, G.X., Cao, Q.D., and Wang, J. (2002). Relationship between growth redundancy and size inequality in spring wheat population mulched with clear plastic film. *Chinese Journal of Plant Ecology*, 26(2), 177-184.

Rao, D.G., and Sinha, S.K. (1993). Efficiency of mobilization of seed reserves in sorghum hybrids and their parents as influnced by temperature. *Seed Research*, 21(2), 97-100.

Rauf, M., Munir, M., Ul-Hassan, M., Ahmed, M., and Afzal, M. (2007). Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. *African Journal of Biotechnology*, 6(8), 971-975.

Roza, G. M., ShahzadJamaati-e-Somarin, K., and Zabihi-e-Mahmoodabad, R. (2010). Effects of polyethylene glycol and NaCI stress on two cultivars of wheat (*Triticum durum*) at germination and early seedling stages. *American-Eurasian Journal of Agricultural & Environmental Science*, 9, 86-90.

Table 1. Effect of drought stress through polyethylene glycol (PEG-6000) solution on rate of germination, final germination, dry weight of root and shoot wheat genotypes.

Genotypes	*Rate of Germination (%)			*Final Germination (%)			Root dry weight (mg)			Shoot dry weight(mg)		
	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%
BD-466	49	22	10	82	71	24	3.7	3.6	3.3	6.7	4.6	4.3
BD-470	60	38	5	85	67	36	5.0	5.1	5.6	9.8	5.1	4.1
BD-473	82	42	7	92	74	63	3.6	4.5	3.7	7.9	5.0	3.9
BD-476	64	36	11	90	69	42	4.5	4.1	2.6	8.4	5.5	4.5
BD-479	75	35	7	93	72	41	3.9	4.3	3.3	8.1	4.9	4.2
BD-480	79	43	10	95	84	76	4.2	4.5	3.5	8.4	5.1	4.2
BD-481	68	44	10	88	69	59	3.4	4.2	3.7	7.2	5.5	4.7
BD-483	83	54	40	78	58	54	4.1	3.7	4.2	7.9	7.0	5.3
BD-487	56	29	10	88	77	38	4.0	4.6	3.0	8.2	5.5	4.0
BD-488	64	46	19	75	56	38	4.4	3.7	2.8	9.3	6.2	4.6
BD-489	76	43	11	95	75	62	4.1	5.3	4.0	8.4	5.5	3.9
BD-491	72	45	20	88	71	60	4.0	4.4	4.7	9.1	6.3	5.8
BD-493	94	83	20	87	65	63	5.1	6.4	0.5	9.9	7.8	8.3
BD-496	97	92	17	100	82	69	7.1	6.0	7.0	10.6	8.6	8.4
BD-497	98	91	8	85	72	64	5.7	6.6	7.1	11.6	7.0	8.6
BD-498	93	87	13	90	81	68	5.1	4.8	5.6	7.9	6.9	6.7
BD-499	98	88	16	98	83	70	5.5	6.6	8.2	10.6	9.5	9.5
BD-500	94	90	2	88	74	66	6.3	5.1	7.2	9.7	5.8	7.8
BD-501	93	91	15	95	81	70	6.5	7.1	7.2	9.2	9.5	7.8
BD-505	94	90	22	88	75	69	6.2	6.7	6.9	10.0	8.0	7.4
BD-507	96	76	11	88	72	70	5.9	5.3	6.9	9.9	5.8	8.8
BD-508	93	95	17	98	83	69	6.8	5.6	6.4	8.8	8.2	7.6
BD-509	86	77	4	98	57	56	5.6	5.6	6.1	9.5	7.8	7.6
BD-510	98	91	13	100	87	72	5.5	6.0	6.5	11.9	6.5	8.4
BD-511	73	70	19	87	72	68	7.1	5.7	6.9	9.1	5.7	6.2
BD-512	94	88	8	83	69	65	5.8	5.7	6.9	9.5	5.0	5.5
BD-513	93	91	16	75	63	65	6.1	7.8	6.7	7.0	6.2	5.7
BD-514	98	97	39	97	78	63	3.9	5.2	6.5	5.1	7.0	5.7
BD-516	97	90	20	98	81	71	5.2	5.1	7.0	9.9	5.5	5.7
BD-517	98	97	2	80	80	62	4.1	4.8	6.8	9.1	5.0	5.8
BD-519	98	95	19	95	81	67	3.0	5.1	7.1	7.0	5.0	5.4
BD-522	69	68	67	85	74	61	4.4	5.5	7.9	10.0	6.0	6.9
BD-524	97	95	18	95	84	68	4.7	4.4	7.0	7.7	4.8	6.8
BD-525	86	73	5	95	71	66	2.4	4.3	4.8	5.0	4.2	4.9
BD-527	98	81	2	92	75	62	2.8	3.8	4.2	6.1	4.1	4.2
BD-529	85	78	7	98	81	71	6.2	3.8	6.3	7.1	3.6	4.9

Genotypes	*Rate of Germination (%)			*Final Germination (%)			Root dry weight (mg)			Shoot dry weight(mg)		
	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%
BD-531	98	97	6	97	81	71	6.7	6.8	3.8	8.7	5.4	3.3
BD-533	98	87	22	98	81	63	3.8	6.5	4.9	8.7	4.7	4.8
BD-534	98	51	40	98	78	57	6.8	6.0	4.3	7.9	5.0	4.6
BD-535	93	48	33	87	69	41	6.3	5.2	5.5	9.4	4.1	4.3
BD-536	98	54	22	98	87	69	9.0	4.3	6.5	8.8	5.7	4.6
BD-537	90	34	25	95	83	54	7.8	5.1	5.9	8.0	3.2	4.5
BD-539	90	25	30	88	78	36	8.8	5.0	3.2	10.0	4.2	3.1
BD-540	92	31	20	88	71	21	6.8	5.2	6.8	9.2	5.2	8.6
BD-541	98	27	33	98	81	51	7.6	5.3	3.9	9.5	4.5	4.9
BD-542	96	57	20	83	69	60	5.3	6.2	2.3	7.4	4.6	3.3
BD-545	73	32	18	93	81	60	8.7	5.3	4.9	9.4	3.6	4.5
BD-546	63	19	10	47	27	8	6.3	3.0	1.9	9.2	2.7	2.3
BD-547	97	43	11	98	86	68	7.1	5.0	4.5	8.7	4.3	5.3
BD-551	35	24	20	85	39	3	4.2	2.9	5.0	6.8	3.5	1.8
BD-552	82	28	9	90	68	66	5.7	6.2	4.1	8.3	6.1	4.0
BD-560	76	33	13	95	83	56	6.3	5.6	4.0	7.8	5.3	4.2
BD-562	95	54	21	100	84	72	7.1	6.7	5.0	9.5	5.7	4.8
BD-563	88	48	11	98	86	71	7.6	7.1	5.5	10.1	6.1	4.1
BD-567	84	54	22	98	86	74	6.7	5.3	3.9	6.9	5.4	4.6
BD-568	98	27	14	100	74	66	7.1	5.7	4.4	8.3	4.1	4.5
BD-569	85	20	16	100	89	59	7.1	6.0	4.8	9.0	5.6	4.5
BD-570	83	41	10	90	84	32	6.0	5.0	4.1	8.7	4.5	3.4
BD-574	85	40	3	97	80	32	5.6	6.6	4.1	7.7	5.4	4.5
BD-576	96	46	25	97	86	60	5.6	6.0	3.9	8.5	6.2	4.6
BD-579	95	39	8	98	86	51	8.1	6.7	3.3	9.2	6.4	4.4
BD-581	89	47	10	90	78	59	5.7	5.7	4.9	9.2	4.9	3.5
BD-582	95	58	32	93	77	68	8.0	8.0	6.5	9.5	6.2	4.8
BD-583	95	47	22	98	83	68	8.1	6.1	5.1	8.5	6.5	4.8
BD-584	97	42	27	97	78	69	6.1	6.0	3.6	9.1	6.8	4.0
BD-590	93	12	28	97	80	70	5.7	3.7	3.6	7.7	4.1	3.4
BD-592	94	98	19	85	84	74	6.5	8.2	7.1	9.4	7.7	6.1
BD-593	97	63	8	98	81	60	4.7	5.8	5.6	7.5	6.1	5.5
BD-598	97	93	7	100	87	60	5.8	8.0	6.5	9.8	6.0	4.4
BD-599	97	95	7	97	84	66	7.2	8.7	5.9	8.6	7.3	5.5
BD-600	97	88	7	97	87	72	7.4	8.5	5.7	10.5	7.8	6.2
BD-601	98	93	7	95	84	56	6.6	8.3	6.0	10.2	7.0	5.3
BD-602	96	98	20	95	80	72	5.9	6.9	5.2	9.3	8.2	6.6
BD-603	98	98	18	95	81	67	7.2	8.0	6.2	9.5	8.2	7.1
BD-604	98	93	27	93	82	71	4.4	6.3	4.7	10.0	7.3	6.1

Genotypes	*Rate of Germination (%)			*Final Germination (%)			Root dry weight (mg)			Shoot dry weight(mg)		
	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%
BD-605	95	98	10	98	87	74	6.3	7.0	5.3	10.3	6.9	5.6
BD-607	92	87	22	85	75	60	5.5	7.1	4.7	10.0	6.7	5.5
BD-608	97	88	75	93	77	62	6.4	7.8	5.8	9.8	7.8	5.8
BD-610	40	10	10	85	33	11	4.6	4.5	3.6	9.0	7.0	4.8
BD-611	95	87	5	92	82	65	4.9	7.0	4.1	9.6	6.5	4.9
BD-612	97	90	65	93	83	67	5.9	6.8	8.1	9.5	6.7	5.7
BD-613	96	95	47	92	79	65	6.8	6.8	6.4	10.2	6.5	5.3
BD-614	96	88	25	92	81	60	5.9	7.0	4.1	8.4	8.2	7.1
BD-616	97	91	40	95	84	66	5.7	5.7	4.8	7.8	5.8	4.6
BD-617	95	90	55	95	75	59	5.8	7.5	5.9	9.3	7.6	4.8
BD-618	93	87	62	80	66	56	3.6	7.6	6.7	8.4	7.3	6.4
BD-622	97	93	64	98	86	77	4.6	5.7	2.2	8.7	7.3	5.6
BD-628	94	92	43	90	72	51	5.2	5.3	4.9	8.0	6.2	4.2
BD-629	98	86	16	87	71	51	3.6	7.0	6.2	9.0	5.8	4.4
BD-632	93	89	49	97	83	66	4.9	7.0	5.9	8.6	5.5	4.2
BD-633	95	91	44	90	74	68	5.3	7.3	6.2	8.3	9.0	6.1
BD-634	98	93	41	92	77	66	5.5	7.1	7.3	10.2	7.6	5.6
BD-635	93	93	31	100	77	69	5.5	5.8	5.1	10.5	7.3	5.0
BD-636	96	90	52	92	80	71	5.2	5.0	5.7	8.4	5.8	4.6
BD-638	95	91	11	93	83	48	5.6	4.0	5.3	10.8	7.7	4.9
BD-641	92	82	28	80	71	59	4.1	5.1	3.4	8.0	5.1	4.0
BD-642	98	93	64	77	71	59	4.1	5.7	3.4	8.2	5.5	5.5
BD-643	93	91	62	100	81	54	5.8	6.0	4.4	9.5	6.2	5.6
BD-644	98	95	65	85	71	51	4.2	5.0	4.3	9.0	6.1	4.3
BD-645	95	93	69	87	74	66	5.1	5.7	4.8	9.0	6.1	5.3
LSD (0.05)	14.36			9.93			1.47			1.46		
CV (%)	14.03			8.29			13.49			10.99		

* Percentage data were transformed into arcsine value for analysis

Table 2. Effect of drought stress through polyethylene glycol (PEG-6000) solution on remaining seed dry weight , Root - Shoot ratio, amount of respiration and seed metabolic efficiency wheat genotypes.

Genotypes	Remaining seed dry weight (mg)			Root:Shoot			Amount of respiration			Seed metabolic efficiency (g/g)		
	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%
BD-466	4.4	10.0	12.2	0.6	0.8	0.8	6.0	2.7	3.0	1.7	3.0	2.5
BD-470	9.3	19.9	27.7	0.5	1.0	1.4	14.0	8.0	2.8	1.1	1.3	3.5
BD-473	7.2	15.1	18.3	0.5	0.9	0.9	13.1	7.1	5.8	0.9	1.3	1.3
BD-476	7.3	15.9	23.4	0.5	0.7	0.6	15.8	10.6	5.5	0.8	0.9	1.3
BD-479	7.2	17.5	21.5	0.5	0.9	0.8	17.6	10.1	7.9	0.7	0.9	0.9
BD-480	5.7	13.3	18.5	0.5	0.9	0.8	11.5	6.9	3.7	1.1	1.4	2.1
BD-481	6.9	13.3	17.4	0.5	0.8	0.8	15.1	9.5	6.7	0.7	1.0	1.3
BD-483	6.4	16.3	18.8	0.5	0.5	0.8	16.6	7.9	6.6	0.7	1.4	1.4
BD-487	5.7	14.0	18.4	0.5	0.8	0.8	12.2	6.1	4.9	1.0	1.6	1.4
BD-488	8.5	22.1	22.8	0.5	0.6	0.6	18.0	8.2	10.0	0.8	1.2	0.7
BD-489	5.1	15.3	21.2	0.5	1.0	1.0	15.0	6.5	3.6	0.8	1.6	2.2
BD-491	6.9	16.2	19.0	0.4	0.7	0.8	13.0	6.2	3.6	1.0	1.7	2.9
BD-493	4.0	7.7	6.2	0.5	0.8	0.1	12.2	9.3	6.2	1.2	1.5	1.4
BD-496	3.2	9.3	8.0	0.7	0.7	0.8	9.4	6.4	6.8	1.9	2.3	2.3
BD-497	4.1	9.7	7.8	0.5	0.9	0.8	9.1	7.2	6.9	1.9	1.9	2.3
BD-498	6.3	7.2	6.6	0.7	0.7	0.8	6.4	6.8	6.7	2.0	1.7	1.8
BD-499	8.9	11.5	10.7	0.5	0.7	0.9	14.2	11.7	10.8	1.1	1.4	1.6
BD-500	6.1	14.5	12.0	0.7	0.9	0.9	6.8	3.5	3.9	2.4	3.1	3.8
BD-501	6.8	9.4	10.2	0.7	0.8	0.9	14.5	10.9	11.8	1.1	1.5	1.3
BD-505	6.2	9.2	7.8	0.6	0.8	0.9	12.1	10.7	12.6	1.3	1.4	1.1
BD-507	6.1	13.0	10.4	0.6	0.9	0.8	11.1	8.9	6.9	1.4	1.3	2.3
BD-508	7.5	13.4	10.9	0.8	0.7	0.9	13.5	9.3	11.6	1.2	1.5	1.2
BD-509	8.2	12.5	12.5	0.6	0.7	0.8	10.8	8.1	7.8	1.4	1.7	1.8
BD-510	7.8	14.5	12.1	0.5	0.9	0.8	9.3	7.5	7.6	1.9	1.7	2.0
BD-511	6.8	17.4	16.3	0.8	1.0	1.1	8.7	2.9	3.5	1.9	3.9	3.7
BD-512	9.2	21.1	15.5	0.6	1.1	1.3	12.7	5.4	9.3	1.2	2.0	1.3
BD-513	14.9	17.4	16.9	0.4	1.3	1.2	9.4	4.1	5.2	1.1	3.4	2.4
BD-514	10.4	18.2	14.6	0.6	0.7	1.2	10.5	4.5	7.1	0.9	2.7	1.7
BD-516	10.8	23.7	16.2	0.5	0.9	1.2	11.2	2.8	8.2	1.4	3.8	1.6
BD-517	14.2	23.5	20.6	0.5	1.0	1.2	9.5	3.6	3.7	1.4	2.7	3.4
BD-519	13.1	17.0	13.9	0.4	1.0	1.3	6.4	4.3	4.0	1.6	2.3	3.1
BD-522	20.3	29.2	21.8	0.4	0.9	1.2	8.3	3.3	6.4	1.7	3.4	2.3
BD-524	8.6	16.6	16.3	0.6	0.9	1.0	12.4	7.6	4.2	1.0	1.2	3.3
BD-525	10.6	12.8	10.0	0.5	1.0	1.0	5.6	2.3	4.0	1.3	3.8	2.4
BD-527	13.5	19.8	18.6	0.5	0.9	1.0	10.6	5.4	6.1	0.9	1.5	1.4
BD-529	9.7	16.9	13.4	0.9	1.1	1.3	11.1	9.7	9.5	1.2	0.8	1.2

Genotypes	Remaining seed dry weight (mg)			Root:Shoot			Amount of respiration			Seed metabolic efficiency (g/g)		
	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%
BD-531	4.2	12.1	19.5	0.8	1.3	1.1	14.6	10.0	7.7	1.1	1.2	0.9
BD-533	5.3	12.7	16.4	0.4	1.4	1.0	13.6	7.6	5.4	0.9	1.5	1.8
BD-534	5.6	14.0	18.6	0.9	1.2	0.9	11.9	7.2	4.6	1.2	1.5	1.9
BD-535	3.9	12.9	17.0	0.7	1.1	1.1	15.2	12.4	7.9	1.0	0.8	2.5
BD-536	4.0	11.8	15.5	1.0	0.8	1.4	14.9	14.9	10.1	1.2	0.7	1.1
BD-537	4.7	12.4	19.1	1.0	1.6	1.3	17.6	17.3	8.5	0.9	0.5	1.2
BD-539	5.1	16.6	25.0	0.9	1.2	1.0	14.6	12.7	7.2	1.3	0.7	0.9
BD-540	5.9	15.1	15.3	0.7	1.0	1.2	16.8	13.2	8.1	1.0	0.8	3.2
BD-541	5.1	15.3	21.4	0.8	1.2	0.8	13.8	10.7	5.7	1.2	0.9	1.6
BD-542	4.0	14.7	19.4	0.7	1.4	0.7	14.8	6.0	6.6	0.9	1.8	0.9
BD-545	4.3	15.0	22.0	0.9	1.5	1.1	15.7	14.1	6.6	1.2	0.6	1.4
BD-546	8.0	21.7	16.5	0.7	1.1	0.8	16.6	12.6	19.3	0.9	0.5	0.2
BD-547	5.1	17.1	18.6	0.8	1.2	0.8	14.0	8.5	6.6	1.1	1.1	1.5
BD-551	7.3	10.1	10.8	0.6	0.8	2.7	7.8	9.7	9.1	1.4	0.7	0.8
BD-552	4.6	13.9	17.7	0.7	1.0	1.0	14.4	6.7	7.2	1.0	1.8	1.1
BD-560	5.2	14.8	20.5	0.8	1.0	0.9	13.0	6.5	3.6	1.1	1.7	2.3
BD-562	5.0	15.4	19.4	0.8	1.2	1.1	14.7	8.5	7.1	1.1	1.5	1.4
BD-563	7.4	22.0	29.2	0.8	1.2	1.3	16.8	8.7	5.2	1.1	1.5	1.8
BD-567	5.1	17.1	20.9	1.0	1.0	0.9	14.8	5.6	4.0	0.9	1.9	2.1
BD-568	3.5	15.6	17.6	0.9	1.4	1.0	11.8	5.4	4.2	1.3	1.8	2.1
BD-569	5.0	19.1	24.3	0.8	1.1	1.1	17.2	7.6	4.6	0.9	1.5	2.0
BD-570	4.1	15.9	18.8	0.7	1.1	1.2	10.3	3.7	2.9	1.4	2.6	2.5
BD-574	4.0	12.9	17.0	0.7	1.2	0.9	13.4	5.8	5.1	1.0	2.1	1.7
BD-576	4.6	13.3	18.2	0.7	1.0	0.8	11.8	5.0	3.7	1.2	2.5	2.3
BD-579	5.4	15.3	24.4	0.9	1.0	1.0	15.7	10.0	6.3	1.1	1.3	2.8
BD-581	4.9	14.9	20.5	0.6	1.2	1.4	14.1	8.4	5.0	1.1	1.3	1.7
BD-582	4.2	15.3	21.3	0.8	1.3	1.4	16.0	8.3	5.0	1.1	1.7	2.3
BD-583	6.1	16.7	25.1	1.0	0.9	1.1	15.6	9.1	3.2	1.1	1.4	3.1
BD-584	4.8	13.6	20.0	0.7	0.9	0.9	15.2	8.7	7.5	1.0	1.5	1.0
BD-590	3.3	15.1	16.2	0.7	0.9	1.1	12.2	6.1	5.7	1.1	1.3	1.3
BD-592	5.4	8.3	13.3	0.7	1.1	1.2	8.2	5.4	4.1	1.9	3.0	3.3
BD-593	6.6	8.3	13.8	0.6	0.9	1.0	8.5	7.2	3.3	1.4	1.7	3.4
BD-598	5.4	12.1	16.0	0.6	1.3	1.5	9.7	4.5	3.8	1.6	3.1	2.9
BD-599	6.5	12.9	19.9	0.9	1.2	1.1	12.1	5.5	3.1	1.3	2.9	3.7
BD-600	5.2	14.2	19.5	0.7	1.1	0.9	15.1	7.7	6.8	1.2	2.1	1.8
BD-601	5.7	16.2	20.6	0.7	1.2	1.1	11.4	4.4	4.0	1.5	3.5	2.9
BD-602	5.1	8.9	17.7	0.6	0.8	0.8	12.7	9.0	3.6	1.2	1.7	3.3
BD-603	7.1	13.6	16.7	0.8	1.0	0.9	10.7	4.5	4.4	1.6	3.6	3.1
BD-604	5.5	12.3	15.6	0.4	0.9	0.8	8.5	4.4	3.0	1.7	3.1	3.6

Genotypes	Remaining seed dry weight (mg)			Root:Shoot			Amount of respiration			Seed metabolic efficiency (g/g)		
	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%	Control	PEG 15%	PEG 25%
BD-605	7.4	16.0	19.8	0.6	1.0	1.0	7.8	3.9	4.1	2.1	3.5	2.7
BD-607	5.4	13.3	17.3	0.6	1.1	0.9	9.0	3.7	3.3	1.7	3.8	3.1
BD-608	7.8	14.3	20.9	0.7	1.0	1.0	15.0	9.0	6.5	1.1	1.7	1.8
BD-610	10.6	23.7	25.2	0.5	0.7	0.7	11.9	3.9	2.5	1.1	2.9	3.5
BD-611	6.2	12.5	16.9	0.5	1.1	0.8	9.3	4.1	4.1	1.6	3.3	2.2
BD-612	5.0	12.7	17.9	0.6	1.0	1.4	16.0	10.2	4.6	1.0	1.3	3.0
BD-613	4.5	11.8	17.1	0.7	1.0	1.2	11.7	8.2	4.5	1.5	1.6	2.6
BD-614	6.4	9.1	22.3	0.7	0.9	0.6	13.5	10.0	3.7	1.1	1.5	3.0
BD-616	8.0	10.4	15.4	0.7	1.0	1.1	5.7	5.4	2.4	2.4	2.1	4.0
BD-617	5.1	9.9	18.2	0.6	1.0	1.2	16.4	11.5	7.7	0.9	1.3	1.4
BD-618	7.6	11.6	17.3	0.4	1.0	1.1	16.4	9.5	5.6	0.7	1.6	2.3
BD-622	5.4	12.5	24.0	0.5	0.8	0.4	15.2	8.3	2.0	0.9	1.6	3.9
BD-628	6.6	9.1	15.5	0.7	0.9	1.2	16.6	15.9	12.0	0.8	0.7	0.8
BD-629	4.4	11.6	17.0	0.4	1.2	1.4	12.6	5.4	3.1	1.0	2.4	3.4
BD-632	4.2	11.3	15.7	0.6	1.3	1.4	9.1	3.3	3.2	1.5	3.8	3.2
BD-633	6.5	14.9	20.2	0.6	0.8	1.0	18.6	7.6	6.3	0.7	2.1	2.0
BD-634	5.2	12.6	16.6	0.5	0.9	1.3	13.4	6.9	4.8	1.2	2.1	2.7
BD-635	5.2	14.6	18.1	0.5	0.8	1.0	17.0	10.5	9.8	0.9	1.2	1.0
BD-636	4.1	6.5	14.5	0.6	0.9	1.2	13.9	14.4	6.9	1.0	0.8	1.5
BD-638	5.3	18.5	24.8	0.5	0.5	1.1	15.3	6.7	3.9	1.1	1.7	2.6
BD-641	5.5	13.9	18.4	0.5	1.0	0.9	10.8	4.4	2.6	1.1	2.3	2.8
BD-642	13.0	18.4	23.5	0.5	1.1	0.6	16.2	11.9	9.1	0.8	0.9	1.0
BD-643	7.8	18.2	24.3	0.6	1.0	0.8	16.9	9.6	5.8	0.9	1.3	1.7
BD-644	8.2	16.7	16.9	0.5	0.8	1.0	13.9	7.5	6.8	1.0	1.5	1.3
BD-645	5.2	14.8	20.5	0.8	1.0	0.9	17.0	11.5	8.4	0.8	1.0	1.2
LSD (0.05)		2.59			0.34			0.45			0.53	
CV (%)		10.14			19.45			2.58			15.66	

Table 3. Effect of drought stress through polyethylene glycol (PEG-6000) solution on vigour index and relative vigour index of wheat genotypes.

	Vigour index			Relative vigour index	
Genotypes	Control	15%PEG	25% PEG	15%PEG	25% PEG
BD-466	848	579	182	-32	-79
BD-470	1265	686	350	-46	-72
BD-473	1048	701	479	-33	-54
BD-476	1163	662	300	-43	-74
BD-479	1118	661	302	-41	-73
BD-480	1195	1006	734	-16	-28
BD-481	932	670	494	-28	-47
BD-483	943	626	513	-34	-46
BD-487	1083	770	261	-29	-76
BD-488	1028	551	278	-46	-73
BD-489	1188	805	485	-32	-59
BD-491	1158	755	629	-35	-46
BD-493	1302	916	554	-30	-57
BD-496	1769	1191	926	-33	-48
BD-497	1472	984	686	-33	-53
BD-498	1169	944	838	-19	-28
BD-499	1584	1340	1064	-15	-33
BD-500	1415	808	840	-43	-41
BD-501	1493	1352	1053	-9	-29
BD-505	1434	1102	982	-23	-32
BD-507	1399	802	945	-43	-32
BD-508	1525	1139	966	-25	-37
BD-509	1490	766	762	-49	-49
BD-510	1737	1084	920	-38	-47
BD-511	1406	824	889	-41	-37
BD-512	1276	742	796	-42	-38
BD-513	984	878	795	-11	-19
BD-514	952	869	770	-9	-19
BD-516	1485	859	897	-42	-40
BD-517	1062	777	780	-27	-27
BD-519	944	817	832	-13	-12
BD-522	1217	895	843	-26	-31
BD-524	1178	801	767	-32	-35
BD-525	704	604	444	-14	-37
BD-527	822	588	437	-28	-47

	Vigour index			Relative vigour index	
Genotypes	Control	15%PEG	25% PEG	15%PEG	25% PEG
BD-529	1306	605	792	-54	-39
BD-531	1494	990	500	-34	-67
BD-533	1231	906	611	-26	-50
BD-534	1443	855	508	-41	-65
BD-535	1355	646	397	-52	-71
BD-536	1743	868	761	-50	-56
BD-537	1500	686	563	-54	-62
BD-539	1663	720	227	-57	-86
BD-540	1411	733	323	-48	-77
BD-541	1679	800	451	-52	-73
BD-542	1062	746	334	-30	-69
BD-545	1682	729	563	-57	-66
BD-546	722	153	32	-79	-96
BD-547	1556	792	660	-49	-58
BD-551	937	249	20	-73	-98
BD-552	1255	830	530	-34	-58
BD-560	1337	899	452	-33	-66
BD-562	1655	1041	703	-37	-58
BD-563	1747	1128	672	-35	-62
BD-567	1337	912	624	-32	-53
BD-568	1538	716	587	-53	-62
BD-569	1608	1029	543	-36	-66
BD-570	1323	803	234	-39	-82
BD-574	1278	949	269	-26	-79
BD-576	1360	1041	512	-23	-62
BD-579	1693	1118	388	-34	-77
BD-581	1338	827	495	-38	-63
BD-582	1632	1084	768	-34	-53
BD-583	1627	1037	671	-36	-59
BD-584	1462	1002	527	-31	-64
BD-590	1295	618	496	-52	-62
BD-592	1353	1232	970	-9	-28
BD-593	1196	938	670	-22	-44
BD-598	1554	1221	654	-21	-58
BD-599	1527	1345	751	-12	-51
BD-600	1725	1414	856	-18	-50
BD-601	1593	1201	629	-25	-61

Genotypes	Vigour index			Relative vigour index	
	Control	15%PEG	25% PEG	15%PEG	25% PEG
BD-602	1439	1196	843	-17	-41
BD-603	1588	1318	889	-17	-44
BD-604	1343	1116	760	-17	-43
BD-605	1633	1208	811	-26	-50
BD-607	1317	1037	610	-21	-54
BD-608	1507	1190	719	-21	-52
BD-610	1154	379	89	-67	-92
BD-611	1332	1098	587	-18	-56
BD-612	1433	1115	922	-22	-36
BD-613	1562	1047	761	-33	-51
BD-614	1313	1227	674	-7	-49
BD-616	1283	963	621	-25	-52
BD-617	1430	1137	624	-20	-56
BD-618	1060	981	725	-7	-30
BD-622	1302	1109	596	-15	-54
BD-628	1190	825	461	-31	-61
BD-629	1095	899	540	-18	-51
BD-632	1308	1031	662	-21	-49
BD-633	1230	1192	831	-3	-29
BD-634	1433	1125	851	-22	-41
BD-635	1593	998	701	-37	-56
BD-636	1252	855	726	-32	-42
BD-638	1532	965	491	-37	-68
BD-641	972	719	435	-26	-55
BD-642	940	788	521	-16	-45
BD-643	1532	990	540	-35	-65
BD-644	1118	781	437	-30	-61
BD-645	1217	864	661	-29	-46
LSD (0.05)		143.50		-	-
CV (%)		7.65		-	-

SAARC J. Agri., 15(2): 193-206 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35151*

PROFITABILITY AND RESOURCE USE EFFICIENCY OF POTATO CULTIVATION IN MUNSHIGANJ DISTRICT OF BANGLADESH

H.K. Sujan[1*], F. Islam[1], M.H. Kazal[2] and R.K. Mondal[3]

[1]Faculty of Agribusiness Management, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh
[2]Department of Development and Poverty Studies, Sher-e-Bangla Agricultural University Dhaka 1207, Bangladesh
[3]Department of Agricultural Economics, Sher-e-Bangla Agricultural University Dhaka 1207, Bangladesh

ABSTRACT

Potato is the third largest food crop in Bangladesh by tonnage of production. Its acreage and production are also increasing in day after day. This study was accomplished to examine the profitability and resource use efficiency of potato cultivation in five upazilas of Munshiganj district of Bangladesh. A total of 52 farmers were selected randomly from the study area. Data were collected through farm survey by using a suitable pre-tested questionnaire in February-March, 2016. Profitability analysis, Cobb-Douglas production function, MVP, MFC and Farm Budgeting model were used to analyze the objectives. Average gross return, gross margin and net return were found Tk. 3,47,200, Tk. 1,47,125 and Tk. 1,17,300, respectively. Benefit-cost ratio was found 1.51 and 1.74 on full cost and variable cost basis, respectively. The key production factors, i.e. human labour, land preparation, seed, fertilizer, insecticides and irrigations had significant effect on gross return of potato. Resource use efficiency analysis revealed that farmers were not efficient in using resources in potato cultivation. Human labor, land preparation, insecticide and irrigation were under-utilized and therefore increasing use of those resources could maximize the profitability. Seed and fertilizer constituted major parts of the cost of production hence optimum use of those resources could also enhance the profitability and resource use efficiency of potato cultivation in Munshiganj district.

Keywords: Potato, profitability, resource use efficiency.

INTRODUCTION

Potato is an important and leading staple crop of the world and occupied topmost position after rice and wheat in respect of production consumption (Akhter et al.,

[*] Corresponding author e-mail: mhksujan@gmail.com

Received: 25.05.2017 *Accepted: 20.10.2017*

1998). Bangladesh experienced much progress in its potato production in the past decades as it has increased by 5 percent per annum (Islam et al., 2000). The country has ranked seventh position in the world in terms of potato production in 2015 (FAO, 2015). In 2014-15, around 92,54,000 metric tons of potato have been produced from 4,71,000 hectares (3.09% of total cultivated area) of land in Bangladesh (BBS, 2015). Among all crops, potato *(Solanum tuberosum* L.) is one of the most important vegetables as well as cash crops in Bangladesh (Haque et al., 2012). In fact, short cycle of potato frees the land for cultivating other crops (Walker et al., 1999). Per unit of land and time potato was more productive than any other food crops (Azimuddin et al., 2009). The annual growth rates of area, production and yield of potato were estimated at 7.14%, 9.90% and 2.76% during 1989-90 to 2008-09, respectively (Miah et al., 2011). Potato production is highly profitable and it could provide cash money to farmer. In terms of profitability, potato production was more attractive than any other winter vegetables. Per unit yield and gross return of potato were found higher than other competitive crops (Akhter et al., 2001). The farmers who used quality seed obtained higher yield and profit. But scarcity of quality seed compelled some farmers to use the inferior seed (Huq, 1998). As a result, the Tuber Crop Research Centre (TCRC) of BARI released 40 HYV potato varieties which have good yield potential and tolerant to insect pests and diseases (Haque et al., 2012). BADC also produces quality potato seeds under contract farming and distribute them to the producers, yet evidence is lacking (Moniruzzaman et al., 2015). Diamant, Cardinal and Granola are the most popular varieties among all the released varieties of BARI and are largely grown in Munshiganj, Rangpur and Bogra district respectively (Khalil et al., 2013*).* These varieties have been distributed to the farmers through different GOs, NGOs and private firms. Potato, a high biomass yielder, utilizes huge quantities of nutrient particularly nitrogen, phosphorus and potassium (Elias et al., 1992). Efficiency and input use pattern varies with the socio-economic characteristics of farmer or manager (Islam et al., 2016). Technical and managerial skills on cultivation practices and provision of technical knowledge to control diseases as well as proper allocation of inputs and available resources would help to increase profitability and productivity of potato (Bajracharya and Sapkota, 2017). Several studies in other countries have shown that there is significant potential for raising agricultural output or profitability by improving productive (technical and allocative) efficiency using existing resources (Rahman, 2002). Yadav et al. (2015) worked on productivity, profitability and resource use efficiency of potato in India and found that potato based various cropping sequence differ significantly for most of the desirable parameters which influence the utilization of natural resources. Bajracharya and Sapkota (2017) conducted a research on profitability and productivity of potato (*Solanum tuberosum)* in Baglung district of Nepal and found the average productivity was 9.89 ton per hectare with per hectare total cost and total income of NRs. (Nepali rupees) 1,97,186 and NRs. 2,68,047, respectively. Islam et al. (2000) carried out a research on the title "Potato production system in Bangladesh:

Resource use, productivity, efficiency and comparative profitability of true potato seed technology over traditional tuber technology" and found from efficiency analysis that the potato growers using TPS technology allocated their resources in rational stage of production. Agricultural production policy decisions in Bangladesh are constrained by lack of information on profitability of growing different agricultural crops (Sarkar et al., 2014). Some economic investigations on potato cultivation in Bangladesh were undertaken by different agencies but which were not adequate. Nevertheless, sufficient number of research work were not undertaken for analyzing the profitability and resource use efficiency of potato production in a major potato producing area like Munshiganj district. So, the specific objective of the present study is to analyze the profitability, resource use efficiency and the factors affecting the production of potato in the selected study area.

MATERIALS AND METHODS

A micro-level study based on primary cross-section data was designed to attain the objectives of this study. The methodology of the study is mainly about the sampling procedure, collection of data and analytical framework.

Sampling technique

The study was conducted in five upazilas of Munshiganj district which were: Sreenagar, Sirajdikhan, Tongibari, Munshiganj Sadar and Gazaria. A total of 52 potato farmers taking at least 10 farmers from each upazila were selected by random sampling technique. Since the study focuses on resources use efficiency in a predominantly potato growing area an attempt was made to choose respondents from those areas which had an average level of agricultural performance in their respective sub-regions.

Method of data collection

Following the conventional survey techniques, primary data on resource availability and their use, input-output levels, prices of farm production and inputs as well as some other information were collected by interviewing the farmers personally using a designed and pre-tested questionnaire in February-March, 2016.

Analytical Framework

Both fixed cost and variable cost were taken into account in calculating cost of potato cultivation. Land use cost was calculated on the basis of per year existing lease value of land. Irrespective of potato varieties, the profitability of potato production was examined on the basis of gross return, gross margin, net return and benefit cost ratio analysis. The collected data were edited, summarized, tabulated and analyzed to fulfill the objectives of the study.

Empirical model

Different parameters of costs and return were analyzed to measure the profitability of potato cultivation on the study area. The following algebraic equation was developed to assess the costs and returns of potato production (Sujan et al., 2017).

$$GR_i = \sum_{i=1}^{n} Q_{mi} P_{mi} + \sum_{i=1}^{n} Q_{bi} P_{bi}$$

Where,

GR_i = Gross return from i^{th} product (Tk. ha^{-1})

Q_{mi} = Quantity of the i^{th} main product (Tk. ha^{-1})

P_{mi} = Average price of the i^{th} main product (Tk. ha^{-1})

Q_{bi} = Quantity of the i^{th} by product (kg ha^{-1})

P_{bi} = Average price of the i^{th} by product (Tk. ha^{-1})

i = 1,2,3,……n

Net return was calculated by deducting all costs (variable and fixed) from gross return. To determine the net return of potato production the following equation was used in the present study:

$$\pi = P_y Y - \sum_{i=0}^{n} P_{xi} X_i - TFC$$

Where,

π = Net return (Tk. ha^{-1})

P_y = Per unit price of the product (Tk. kg^{-1})

Y = Quantity of the product per (kg ha^{-1})

P_{xi} = Per unit price of i^{th} inputs (Tk.)

X_i = Quantity of the i^{th} inputs (kg ha^{-1})

TFC = Total fixed cost (Tk. ha^{-1})

i = 1,2,3,……., n (number of inputs).

The Cobb-Douglas production function is used for functional analysis of the data. It is the most widely used model for fitting agricultural production data, because of its mathematical properties, ease of interpretation and computational simplicity (Heady and Dillon, 1969). It is a homogeneous function that provides a scale factor enabling one to measure the return to scale and to interpret the elasticity coefficients with relative ease. It is also relatively easy to estimate because in logarithmic form it is linear and parsimonious (Beattie and Taylor, 1985). Thus, Cobb-Douglas specification provides an adequate representation of the agricultural production

technology. The production of potato is likely to be influenced by different factors, such as human labour, land preparation, seed, manure, chemical fertilizer, insecticide, irrigation, etc. The functional form of the Cobb- Douglas regression equation was as follows:

$$Y = AX_1^{\beta 1} X_2^{\beta 2} \text{ ---------- } X_n^{\beta n} e^{ui}$$

The production function was converted to logarithmic form so that it could be solved by Ordinary Least Square (OLS) method i.e.

$$\text{Ln } Y = \alpha + \beta_1 \ln X_1 + \beta_2 \ln X_2 + \ldots\ldots\ldots\ldots + \beta_n \ln X_n + U_i$$

The empirical production function was the following:

$$\ln Y = \alpha + \beta_1 \ln X_1 + \beta_2 \ln X_2 + \beta_3 \ln X_3 + \beta_4 \ln X_4 + \beta_5 \ln X_5 + \beta_6 \ln X_6 + \beta_7 \ln X_7 + U_i$$

Where, Y = Return (Tk. ha^{-1});

X_1 = Human Labor (Tk. ha^{-1});

X_2 = Land preparation cost (Tk. ha^{-1});

X_3 = Seed (Tk. ha^{-1});

X_4 = Manure (Tk. ha^{-1});

X_5 = Chemical fertilizer (Tk. ha^{-1});

X_6 = Insecticide cost (Tk. ha^{-1});

X_7 = Irrigation cost (Tk. ha^{-1});

α = Intercept;

β_1, β_2 ---- β_7 = Coefficients of the respective variables; and

U_i = Error term.

In order to test the resource use efficiency, the ratio of marginal value product (MVP) to the marginal factor cost (MFC) for each input is computed and tested for its equality to 1 (Sujan et al., 2017).

$$\text{i.e. } \frac{\text{MVP}}{\text{MFC}} = r$$

Where, r = Efficiency ratio;

MVP = value of change in output resulting from a unit change in variable input (Tk.);

MFC = price paid for the unit of variable input (Tk.);

Under this method, the decision rules are that, when;

r >1, the level of resource use is below the optimum level, implying under utilization of resources. Increasing the rate of use of that resource will help increase productivity.

r <1, the level of resources use is above the optimum level, implying over utilization of resources. Reducing the rate of use of that resource will help improve productivity.

r = 1, the level of resource use is at optimum implying efficient resource utilization.

The marginal productivity of a particular resource represents the additional to gross returns in value term caused by an additional one unit of that resource, while other inputs are held constant. When the marginal physical product (MPP) is multiplied by the product price per unit, the MVP is obtained. The most reliable, perhaps the most useful estimate of MVP is obtained by taking resources (X_i) as well as gross return (Y) at their geometric means (Dhawan and Bansal, 1977). Since all the variables of the regression model were measured in monetary value, the slope co-efficient of those explanatory variables in the function represented the MVPs, which are calculated by multiplying the production co-efficient of given resources with the ratio of geometric mean (GM) of gross return to the geometric mean (GM) of the given resources, i.e.

$$\text{In } Y = \text{In } \alpha + \beta_i \text{ In } X_i$$

$$\frac{dY}{dX_i} = \beta_i \frac{Y}{X_i}$$

$$\text{Therefore, MVP } (X_i) = b_i \frac{\overline{Y}(GM)}{\overline{X}i(GM)}$$

Where, $\overline{Y}$ = Mean value (GM) of gross return in Tk.

$\overline{X}_i$ = Mean value (GM) of different variable input in Tk.

i = 1, 2,……..

MFC is the price of per unit of input. If the MFCs of all the inputs expressed in terms of an additional Tk. in calculating the ratio of MVP to MFC, the denominator will always be one, and therefore, the ratio will be equal to their respective MVP.

RESULTS AND DISCUSSION

Input use pattern

The human labour used for producing potato was found to be 226 man days per hectare of which 31.86% were family supplied. The rest 68.14% labours were used on hire basis. The cost on human labor was calculated by considering different charge for male and female labour. Average wage rate of labour was about Tk. 252. Result shows that there are higher scopes of employment generation in potato cultivation. The average cost of land preparation was Tk. 10,562 per hectare. The average amount of seed and manure used on cultivation were 2,419 and 4,787 kg per hectare, respectively. The seed rate used by the farmers was 61% higher than the recommended seed rate of 1.5 t ha^{-1} (Satter et al., 2005). The chemical fertilizers like

urea, TSP, MoP, gypsum, zink sulphate, and boric acid were used at a rate of 348, 426, 383, 15, 20, and 4 kg per hectare. They used higher doses of urea, TSP, MoP and zink sulphate than the recommended doses (220-250, 120-150, 220-250 and 8-10 kg ha^{-1}) (BARI, 2005). The data tabulated on table 1 further shows that farmers used higher amount of fertilizers compared to other areas might be due to less confidence on the recommended doses of fertilizers. Earlier study on potato also found that Munshiganj's farmers used higher dose of fertilizers (Haq et al., 1995 and Haque et al., 2012).

Table 1. Input use pattern of potato cultivation in the study area

Sl. No.	Items	Amount	Percentage
01	Human labour (man-days/ha):	226	100.00
	Hired labour	154	68.14
	Family labour	72	31.86
02	Land preparation cost (Tk. ha^{-1}):	10,562	-
03	Seed (kg ha^{-1})	2,419	-
04	Manures (kg ha^{-1}))	4,787	-
05	Fertilizers (kg ha^{-1})):		
	Urea	348	-
	TSP	426	-
	MoP	383	-
	Gypsum	15	-
	Zinc Sulphate	20	-
	Boric Acid	4	-

Data Sources: Author's Calculation based on field Survey, 2016.

Cost of cultivation

For determining the cost of cultivation of potato, all variable costs like human labour, land preparation, seed, manures, fertilizers, insecticides and irrigation were calculated per hectare basis. The fixed cost of potato cultivation included cost of land use and interest on operating capital. Average bank interest rate was around 10 percent. One third parts of the interest cost included as interest on operating capital for potato production due to its use for around four month only. The cost of land use was calculated on the basis of per hectare lease value of land. Per hectare lease value of land was around Tk. 45,000 per year of which a half parts were included as cost of potato production due to potato's shorter life. The total cost included fixed cost and variable cost. The cost of potato cultivation was estimated to be Tk. 2,29,900 and Tk. 2,00,075 per hectare on total cost and variable cost basis, respectively. Detail result

tabulated on table 2. The major share in total cost included seed cost (36.82%), followed by human labour cost (24.82%) and chemical fertilizers cost (11.08%).

Haque et al. (2012) found the cost of potato cultivation in Munshigonj (Tk. 2,32,283 ha^{-1}) was found higher than that of Bogra (Tk. 2,05,971 ha^{-1}) and Comilla (Tk. 1,93,636 ha^{-1}) due to the higher cost of human labour, fertilizer and insecticides. They also found the seed cost as constituting the major share (42%) of total cost followed by chemical fertilizers cost (21%) and human labour cost (14%) for potato production in some selected area of Bangladesh. However, cost of tuber seed is an important constraint in potato production. Islam et al. (2000) also found the tuber seed cost as 35 to 40 percent of total cost of production. Scarcity of quality seed in sowing time is the major causes for higher seed cost and government intervention on fertilizer market by providing subsidy on fertilizer is the major cause for lower fertilizer cost of potato production. On the other hand Bajracharya and Sapkota (2017) found FYM constituted highest (45.32%) portion of the cost of production followed by seed and human labor in Baglung district of Nepal.

Table 2. Cost of production of potato in the study area

Sl. No.	Items	Amount (Tk. ha^{-1})	Percentage of total cost
A.	Variable Cost:	2,00,075	87.03
	Land preparation cost	10,562	4.60
	Human labour	57,068	24.82
	Hired labour	38,887	-
	Family labour	18,181	-
	Seed	84,650	36.82
	Organic manure	4,787	2.08
	Chemical fertilizers:	25,474	11.08
	Urea	5,571	-
	TSP	9,371	-
	MoP	5,744	-
	Gypsum	438	-
	Zinc sulphate	4,067	-
	Boric Acid	282	-
	Insecticides	14,148	6.16
	Irrigation	3,386	1.47
B.	Fixed cost:	29,825	12.97
	Land use	23,156	10.07
	Interest on operating capital	6,669	2.90
	Total Cost (A+B)	2,29,900	100.00

Data Sources: Author's Calculation based on field Survey, 2016

Profitability of potato cultivation

The yield of potato was 29.5 tons per hectare which was higher than the national average yield (19.13 t ha^{-1}) (BBS, 2015). Estimated average farm gate price was Tk. 11.75 per kg. The gross return and gross margin of potato cultivation were Tk. 3,47,200 and Tk. 1,47,125 per hectare, respectively. The net return of potato cultivation was Tk. 1,17,300 per hectare. Although extra amount of variable inputs were used by farmer the average benefit-cost ratios (BCR) were 1.51 and 1.74 on full cost and variable cost basis. Estimated BCR implicate that the cultivation of potato was still remunerative to the farmers.

Gross margin was found to be the highest (Tk. 1,91,345) in Munshigonj district than any other potato producing area of Bangladesh by Haque et al. (2012). They also found the net return of potato cultivation was Tk. 1,62,873with the BCR of 1.70 and 1.94 on full cost and variable cost basis, respectively for the same district. Ahmed et al., (2009) also observed around Tk. 2,62,625, Tk. 1,20,221 and Tk. 1,42,403 as gross return, gross cost and net return, respectively with undiscounted benefit-cost ratio 2.18 for potato production in some selected areas of Mymensingh district. On the other hand Bajracharya and Sapkota (2017) found the productivity 9.89 ton per hectare with per hectare profit of NRs.70,861 with BCR of 1.44 from potato production in Baglung district of Nepal. Moreover, Islam et al. (2000) carried out a research on two production systems of potato TPS and traditional tuber technology and found variability in costs and returns for those two. The TPS technology was found to have a higher benefit-cost ratio than the traditional technology.

Table 3. Profitability of potato cultivation in the study area

Sl. No.	Items	Formula	Unit	Amounts
01	Yield	*Y*	kg ha-1	29,549
02	Farm gate Price	*P*	Tk. kg^{-1}	11.75
03	Gross return *(GR)*	*Y*P*	Tk. ha^{-1}	3,47,200
04	Total variable cost	*TVC*	Tk. ha^{-1}	2,00,075
05	Total cost	*TC*	Tk. ha^{-1}	2,29,900
06	Gross margin	*GR-TVC*	Tk. ha^{-1}	1,47,125
07	Net return	*GR-TC*	Tk. ha^{-1}	1,17,300
08	Benefit cost ratio			
	Full cost basis	*GR/TC*		1.51
	Variable cost basis	*GR/TVC*		1.74

Data Sources: Author's Calculation based on field Survey, 2016

Factors affecting gross return of potato

In order to assess the contribution of inputs like human labour, land preparation, seed, manure, chemical fertilizers, insecticide and irrigation for potato production, Cobb-Douglas production function model was used. The estimated values of co-efficient and related statistics of Cobb-Douglas production function have been presented in table-4. All the co-efficients of human labour, fertilizer and irrigation were positive and significant at 1% level. The co-efficient of seed and insecticide application were positive and significant at 5% level. The co-efficient of land preparation was positive and significant at 10% level. Manure application had positive impact on the yield of potato but the effects were not significant at desired level of significance. The study revealed that an increase in 10% cost of human labor, land Preparation, seed, fertilizer, insecticide and irrigation, remaining other factors constant would increase the gross return of potato by 1.84, 0.37, 0.79, 1.75, 0.72 and 0.27%, respectively.

The value of the co-efficient of multiple determination (R^2) of the model was 0.77 indicating about 77 percent of the variation in gross return of potato production were explained by the explanatory variables included in the model.

Table 4. Estimated coefficients and their related statistics of production function for potato

Explanatory Variable	Parameters	Co-efficient	Sd. Error	t-values	P-values
Intercept	β_0	6.733***	0.777	8.664	0.000
Human labor (X_1)	β_1	0.184***	0.061	3.015	0.004
Land Preparation (X_2)	β_2	0.037*	0.020	1.840	0.072
Seed (X_3)	β_3	0.079**	0.032	2.500	0.016
Manure (X_4)	β_4	0.011^{NS}	0.023	0.494	0.624
Fertilizer (X_5)	β_5	0.175***	0.059	2.966	0.005
Insecticide (X_6)	β_6	0.072**	0.031	2.317	0.025
Irrigation (X_7)	β_7	0.027***	0.010	2.773	0.008
R^2		0.77			
F-value		26.08***			
Return to scale		0.59			

Note: ***, ** and * indicate significant at 1%, 5% and 10% level, respectively.

Data Sources: Author's Calculation based on field Survey, 2016

The measure of the overall fit of the estimated regression, F-value (26.08) was significant at 1 percent level of significance, implying that all the explanatory variables included in the model were important for explaining the variations in gross returns of potato production.

Haque et al. (2012) also found all the co-efficients of human labour, land preparation, seed and NPK fertilizer were positive and significant at 1% level of significance indicating 10% increase in the cost of human labor, land preparation, seed and NPK fertilizer, remaining other factors constant would increase the yield of potato by 2.25, 0.76, 3.13 and 3.86%, respectively. Ahmed et al. (2009) also carried out a research on the same and the research revealed that seed and pesticides had positive but TSP and MP had negative significant effect on gross return of potato production in Mymensing district of Bangladesh. Bajracharya and Sapkota (2017) also found human labor, seed, FYM, cost of bullock labor and intercultural operation having significant effect on total income of potato in Baglung district of Nepal where an increase in 1% cost of human labor, seed and FYM would increase the total income of potato by 0.075%, 0.639% and 0.190%, whereas 1% increase in the cost of bullock labor and intercultural operation would decrease income by 0.015% and 0.047%, respectively.

Return to scale

The summation of all the production co-efficient (β_i) indicates the return to scale. The return to scale of potato cultivation in Munshiganj district was found around 0.59 which indicate the diminishing return to scale. It means potato farmers allocated their resources in the rational stage of production (Stage-II) where lower amount of return would be added to the gross return by using each additional units of input to the potato cultivation.

The return to scale of potato cultivation in Munshiganj, Bogra and Comilla district of Bangladesh was found 0.965 by Haque et al. (2012) which was 0.218 for Mymensing district of Bangladesh found by Ahmed et al. (2009). Islam et al. (2000) carried out a research on the same topic in Bangladesh and found from efficiency analysis that the potato growers using TPS technology allocated their resources in rational stage of production. Later, Bajracharya and Sapkota (2017) also found the return to scale of potato cultivation 0.842 in Baglung district of Nepal.

Resource use efficiency

Resource use efficiency means how efficiently the farmer can use his resources in the production process. It is very important because resource is scarce. For calculating resource use efficiency, the study considered seven input factors namely human labor, land preparation, seed, manure, fertilizer, insecticide and irrigation.

Table 5. Resource use efficiency of different inputs under potato cultivation

Variable	Geometric mean (GM)	$\overline{Y}$(GM)/ $\overline{X}_i$(GM)	Co-efficient	MVP(X_i)	r=MVP/ MFC	Decision rule
Yield *(Y)*	346751.9					
Human labor *(X_1)*	56918.64	6.092	0.184	1.121	1.121	Under-utilization
Land Preparation *(X_2)*	10364.93	33.454	0.037	1.238	1.238	Under-utilization
Seed *(X_3)*	84014.31	4.127	0.079	0.326	0.326	Over-utilization
Manure *(X_4)*	4731.044	73.293	0.011	0.806	0.806	Over-utilization
Fertilizer *(X_5)*	25390.58	13.657	0.175	2.390	2.390	Under-utilization
Insecticide *(X_6)*	14034.89	24.706	0.072	1.779	1.779	Under-utilization
Irrigation *(X_7)*	3053.159	113.572	0.027	3.066	3.066	Under-utilization

Note: *MFC=1TK. Data Sources: Author's Calculation based on field Survey, 2016*

From table-5, it is observed that the ratio of marginal value products (MVP) and marginal factor cost (MFC) for human labour, land preparation, fertilizers, insecticide and irrigation were greater than unity indicating the under-utilization of those resources. Which means increasing the rate of use of those resources would help to increase the productivity. Same ratio for seed and manure were less than unity indicating over-utilization of those variables. Which means reduction of the rate of use of those resources would help to improve the productivity. Islam et al. (2000) conducted a research on potato production system in Bangladesh and found inefficiency in the uses of human labour, seed, manure and fertilizers in TPS technology, which had a potentiality to increase potato output by 20 percent with efficient organization of those resources.

CONCLUSION

It is evidenced from the study that cultivation of potato in Munshiganj district of Bangladesh is a profitable venture. Each taka invested in potato cultivation would return 1.51 taka to its investor. Seed cost was found as the major cost (36.82) constituents of total cost. Besides, over doses chemical fertilizer applied for potato cultivation but their effects on yield were still remunerative. Nevertheless, farmers allocated their resources in the rational stage (Stage-II) of production where diminishing returns to scale (0.59) existed. Sufficient supply of quality seed at fair price in sowing time can help to reduce the cost of production. Furthermore, optimum use of fertilizer can also increase the productivity and profitability of potato cultivation.

REFERENCES

Akhter, S.M.M., Anwar and Asaduzzaman, M. (2001). Potato production in some selected areas of Bangladesh. TCRC. BARI, Joydebpur, Gazipur, Bangladesh.

Akhter, M.E.M.S., Ali, Z.N., Huq, A.K.M.A., Habib, M.A., and Hossain, A.B.S. (1998). Granula, Cleopatra and Binella New Potato Varieties in Bangladesh, *Progressive Science*, 24(1), 133-143.

Ahmed, S., Rashid, M.H.A., and Chowdhury, N. (2009). Comparative profitability of boro rice and potato production in some selected areas of Mymensingh district. *Progressive Agriculture*, 20(1 &2), 253-258. doi: http://dx.doi.org/10.3329/pa.v20i1-2.16880.

Azimuddin, M., Alam, Q.M., and Baset, M.A. (2009). Potato for food security in Bangladesh. *International Journal of Sustainable Crop Production*, 4(1), 94-99.

Bajracharya, M., and Sapkota, M. (2017). Profitability and productivity of potato (*Solanum tuberosum*) in Baglung district, Nepal. *Agriculture & Food Security*, 6, 47. doi: https://doi.org/10.1186/s40066-017-0125-5

BARC, (Bangladesh Agricultural Research Council). (2005). Fertilizer Recommendation Guide, Published by the Bangladesh Agricultural Research Council, Farmgate, Dhaka-1215.

BARI, (Bangladesh Agricultural Research Institute). (2005). Handbook of Agricultural Technology 3rd edition, Gazipur.

BBS, (Bangladesh Bureau of Statistics). (2015). Year book of Agricultural Statistics of Bangladesh, Statistics Division, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh.

Beattie, B.R., and Taylor, C.R. (1985). The Economics of Production. *Montana State University, John Wiley and Sons*, New York, USA.

Dhawan K.C., and Bansal, P.K. (1977). Rationality of the Use of Various Factors of Production on Different Sizes of Farm in the Punjab, *Indian Journal of Agricultural Economics*, 32(3), 121-130.

Elias, S.M., Miah, M.R.U., Hossain, M.J., Torofder, G.S., and Islam, M.S. (1992). Effect of potassium on the growth and yield of potato. *Bangladesh Journal of Agricultural Research*, 17(7), 91-93.

FAOSTAT. (2015). (United Nations Food and Agriculture Organization) (Production/ Core Production Data).

Haque, M.A., Miah, M.A.M., Hossain, S., and Rahman, M.M. (2012). Profitability of BARI released potato (*Solanum tuberosum* L.) varieties in some selected locations of Bangladesh. *Bangladesh Journal of Agricultural Research*, 37(1), 149-158.

Heady, E.O., and Dillon, J.L. (1969). Agricultural Production Functions. *Iowa State University Press*, Ames.

Hoq, A.S.M.A. (1998). Comparative profitability of potato under different situation in Dinajpur. *Bangladesh Journal of Agricultural Research*, 23(1).

Huq, A.S.M.A., Hussain, M.S., and Rashid, M.A. (1995). Potato production in CDP demonstration area: Yield gap analysis. Annual report, Agricultural Ecinomics Division, BARI, Gazipur.

Islam, S.M.F., Anwar, M.M., and Manos, B. (2000). Potato production system in Bangladesh: Resource use, productivity, efficiency and comparative profitability of true potato seed technology over traditional tuber technology. *Acta Hortic*, 536, 261-268 doi: 10.17660/ActaHortic.2000.536.30.

Islam F., and Sujan M.H.K. 2016. Rationality of hog plum cultivation in Jhalokathi district, an empirical analysis in the socio-economic context of Bangladesh. *Journal of Advances in Social Science and Humanities,* 2(1), 8-18. doi: http://dx.doi.org/10.15520/2126.

Khalil, M.I., Haque, M.E., and Hoque, M.Z. (2013). Adoption of BARI recommended potato (*Solanum tuberosum*) varieties by the potato farmers of Bangladesh. *The Agriculturists*, 11(2), 79-86, doi: http://dx.doi.org/10.3329/agric.v11i2.17492.

Miah, M.A.M., Hossain, S.T.M.B., and Rahman, S. (2011). Assessment of potato farmers' perceptions on abiotic stresses and implications for potato improvement research in Bangladesh: A baseline survey. Research report submitted to International Potato Centre (CIP), Lima, Peru.

Moniruzzaman., Hoq, M.S., Islam, M.K., Jasmin, J., and Khanam, M.M. (2015). Agro-economic performance of seed potato production under contract farming in Bangladesh. *Eco-friendly Agricultural Journal*, 8(3), 30-36.

Rahman, K.M.M. (2002). Measuring efficiency of producing rice in Bangladesh: A stochastic frontier analysis. Unpublished [Ph.D Thesis], Institute of Agricultural Policy and Marketing Research, Justus-Liebig University, Giessen, Germany.

Sarkar, M.M.A., and Yesmin, F. (2014). Profitability of potato cultivation in some selected areas of Rangpur district in Bangladesh. *International Journal of Sustainable Crop Production*, 9(1), 11-15.

Satter, M.A., Rahman, M.M., Rashid, M.H., Ali, M.S., and Alam, M.S. (2005). *Krishi Projukti Hatboi* (Hand book on agro technology), 3rd ed, BARI, Gazipur.

Sujan, M.H.K., Islam F., Azad M.J., and Rayhan, S.J. (2017). Financial profitability and resource use efficiency of boro rice cultivation in some selected area of Bangladesh. *African Journal of Agricultural. Research.* 12(29), 2404-2411. doi: 10.5897/AJAR2017.12443.

Walker, T.S., Schmiediche., P.E., and Hijmans, R.J. (1999). World trends and patterns in the potato crop: An economic and geographic survey. *Potato Research*, 42(2), 241-264.

Yadav, S.K., Srivastava, A.K., Bag, T.K., Kumar, Y., and Kumar, K. (2015). Productivity, profitability and resource use efficiency of potato (*Solanum tuberosum*) based cropping systems in eastern Himalayan region. *Indian Journal of Agricultural Sciences*, 85 (7), 921-5.

SAARC J. Agri., 15(2): 207-214 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35165*

Short Communication

REPRODUCTIVE PERFORMANCE OF SPIRALLING WHITEFLY ON GUAVA AND IMPACT OF WEATHER PARAMETERS ON ITS IMMATURE STAGES

I. Hossain, M.M.H. Khan* and S.M.H. Jahan

Department of Entomology, Patuakhali Science and Technology University
Dumki, Patuakhali-8602, Bangladesh

ABSTRACT

The study was conducted to know the reproductive performance of spiralling whitefly on guava. Number of colony, eggs, 1st instar, 2nd instar, 3rd instar and 4th instar nymphs per five leaves ranged from 6 to 15, 8 to 32, 0 to 44, 0 to 22, 0 to 45 and 0 to 28, respectively. Maximum number of adults and nymphs were found in the month of January. Highest longevity of *Aleurodicus dispersus* (21.5 days) was recorded in adult while the lowest was in 2nd instar nymph (6.4 days). The number of colony/leaf and number of 3rd and 4th instar nymphs of *A. dispersus* had significant positive correlation with minimum and maximum temperature while non-significant positive correlation is observed between the number of egg/colony, the number 1st and 2nd instar nymphs with minimum and maximum temperature. *A. dispersus* showed non-significant positive correlation with minimum and maximum relative humidity regarding number of colony/leaf, 2nd instar nymph while non-significant negative correlation with 4th instar nymph.

Key words: *Aleurodicus dispersus,* reproductive performance, life span, weather parameters

INTRODUCTION

Guava is grown in all the districts of Bangladesh and in many other Asian countries. The major areas of guava cultivation are the Gazipur, Barisal and Jessore districts of Bangladesh where successful production is achieved. Spiralling whitefly, *Aleurodicus dispersus* Russell is a highly polyphagous insect species of tropical origin (Martin, 1987) and is reported to infest 99 host plants including fruit trees, vegetable crops, ornamentals, shade and forest trees (Aiswariaya et al., 2007). This insect has introduced in Bangladesh due to poor quarantine system and has become a

* Corresponding author e-mail: mohasin1965@pstu.ac.bd

Received: 05.09.2017 *Accepted: 25.09.2017*

great threat for guava cultivation (Amjad et al., 2009). The eggs are laid .by this species of whitefly, along with deposits of waxy secretions, in a spiraling pattern on the underside of leaves. The first larval stage ('crawler') is the only mobile immature stage (0.32 mm long). During the second larval stage (0.5 mm long), a row of mid-back waxy tufts form on the anterior of the body. During the third larval stage (0.65 mm long), short, evenly-spaced, glass-like, waxy rods emanate from distinctive compound pores along the side of the body (Waterhouse and Norris, 1989). During the early pupal stage (fourth larval stage), sedentary feeding continues (Waterhouse and Norris, 1989). Copious amounts of white, cottony flocculent wax, extending from the dorsum, are then secreted by the pupae; more so than for the larval stages. Young pupae are nearly flat dorsally and flat ventrally. Mature pupae (1.06 mm long) have a swollen ventral surface and are surrounded by a band of wax (Waterhouse and Norris, 1989). Whiteflies cause direct damage by feeding phloem on the undersides of leaves and indirectly through excretion of honeydew on which a black sooty appearance develop from mould fungus growing (Oliveira et al., 2001). Whiteflies develop rapidly in warm weather, and populations can build up quickly in situations where natural enemies are destroyed and weather is favourable. Rainfall and temperature are the major weather parameters affecting the population of *A. dispersus* as they influence the development of each of its six life stages (Banjo and Banjo, 2003) irrespective of the host type associated with the insect. Rainfall, temperature and other weather factors cause seasonal fluctuation and distribution of spiralling whitefly (Delinger, 1986) but it is mostly their combined effect of evapo-transpiration that is more important (Asiwe et al., 2002). When it rains heavily, many small insects get dislodged from plant surfaces by the combined effect of wetness and the kinetic energy of the rain drops as well as strong winds. The density of the spiralling whitefly was positively correlated with maximum temperature and negatively correlated with relative humidity (Krishnamoorthy and Venugopalan, 2010). Aishwariya et al. (2007) reported that the egg density of spiralling whitefly peaked during April, May, August and November months. They also observed that the incidence of all the three stages of *Aleurodicus dispersus* had significant positive correlation with maximum temperature and non-significant positive correlation with minimum temperature, non-significant negative correlation with morning and afternoon relative humidities. Considering above facts the present study was undertaken to know the reproductive performance of spiralling whitefly on guava and to know the impact of weather parameters on immature stages of whiteflies.

MATERIALS AND METHODS

The study was conducted in the homestead area of the Patuakhali Science and Technology University (PSTU) campus and in the entomology laboratory during November 2013 to April 2014. Geographically, the area is covered Gangetic Tidal Floodplain and falls under Agroecological Zone (AEZ-13). The area lies at 0.9 to 2.1 meter above mean sea level (Iftekhar and Islam, 2004). This region occupies a vast

area of tidal floodplain land in the south-west part of Patuakhali district having warm and humid climatic condition. The observation of whitefly was recorded at weekly intervals during 6:30 to 8:30 am. The population of whiteflies (nymphs and adults) were recorded from three leaves one each from the upper, middle and lower position on five randomly selected plants. The population was counted only on five leaves and the whitefly population was expressed on per plant basis. The weekly meteorological data on temperature, relative humidity and rainfall were recorded during the experimental period.

Mass culture of the insect and observation of fecundity and longevity

The laboratory experiment was carried out under normal room temperature (32 ± ^{0}C) and relative humidity (85 ± 5%) with a 14 ± 2:10±2 light and dark cycle (L: D) following completely randomized design (CRD). Twenty pairs of *A. dispersus* adults were released into petridishes with wet cotton of the leaves of each host plant. Adults were allowed to lay eggs for 12 h before being removed. A small pen mark was used to place identifying marks next to 50 whitefly eggs on each of six leaves per species. The infested plants were placed in 60 × 60 × 60 cm^3 cages and development and survival of each stages of whitefly were recorded daily until all the whiteflies emerged. With the exception of the crawlers, which were capable of small distance movement immediately after hatching from egg, all the other immature stages were sessile and cannot move. All stages of whitefly were identified according to their characteristics described by Russell (1965) and Waterhouse and Norris (1989) Therefore, leaves with "pupae" were covered with leaf clip cages to trap emerging adult whiteflies. Emerged adult whiteflies were counted and sexed as described by Gill (1993) and used for daily longevity and fecundity studies.

RESULTS AND DISCUSSION

The number of colony per six leaves ranged from 6 to 15 with mean 10.2 and standard error 1.06 while the number of eggs per colony ranged from 8 to 32 with mean 20.2 and standard error 2.22. The number of 1st instar nymphs/6 leaves ranged from 0 to 44 with mean 8.8 and standard error 4.17. Likewise, the number 2nd instar nymph ranged from 0 to 22 with mean 5.40 and standard error 2.37. The number of 3rd and 4th instars nymphs ranged from 0 to 45 and 0 to 28, respectively with mean 18.1 and 4.60, respectively while standard errors were 5.30 and 2.54, respectively. Among four instars, the number of 3rd instar nymphs was the highest (18.1 nymphs) compared to other instars (Table 1). The relationship between selection of oviposition sites and thereafter growth, survival and reproduction of the offspring are central element in the evolution of host association between herbivorous insects and plant (Thompson, 1988).

Table 1. Fecundity of *Aleurodicus disperses* recorded on six guava leaves during April 2014

Sample No.	No. of colony/6 leaves	No. of egg/ colony	No. of 1st instar nymph/ colony	No. of 2nd instar nymph/ colony	No. of 3rd instar nymph/ colony	No. of 4th instar nymph/ colony
1	6	27	6	0	0	0
2	15	15	20	2	15	6
3	12	22	0	0	0	0
4	6	32	6	5	7	3
5	6	21	0	3	33	3
6	9	13	44	22	0	0
7	9	8	10	0	21	3
8	15	27	2	2	15	28
9	12	15	0	18	45	0
10	12	22	0	2	45	3
Range	6-15	8-32	0-44	0-22	0-45	0-28
Mean ± SE	10.2 ± 1.06	20.2 ±2.22	8.8 ± 4.17	5.40 ± 2.37	18.1 ± 5.30	4.60 ± 2.54

Figure 1 revealed that there was a major peak of different stages of whitefly population in January followed by February. Maximum number of adults, nymphs and pupae were found in the month of January along with maximum number of colony/leaf and number of eggs/colony. After January, population gradually decreased and the lowest number was recorded in April. It was observed that the population of nymph /colony was highest followed by pupae and adult.

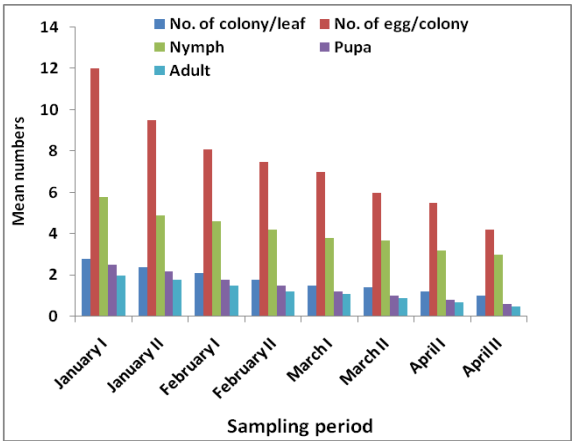


Figure 1. Mean fecundity of *Aleurodicus dispersus* at fortnightly interval on guava during January to April 2014

Abiotic factors such as temperature, rainfall, humidity, fog and sunshine might have tremendous effect on whitefly. *A. dispersus* population as most tropical insects is affected by the climatic conditions which dictate the season (Banjo et al., 2003; Asiwe et al., 2002). A period of moderate rainfall combined with high day temperature which usually occur between April and May, following the onset of rain after the very dry months (December and January) in Nigeria and other tropical regions favours high population of the spiraling whiteflies (Banjo and Banjo, 2003). However the population is at optimum at the drier months of November, December and January (Banjo et al., 2003). During monsoon (June and July) conversely, the population of spiralling whiteflies declines gradually as all the stages of life especially eggs of the insect are washed away by heavy rain couple with wind that is normally associated with such rain (Banjo et al., 2003; Asiwe et al., 2002).

The longevity of different stages of *A. dispersus* is shown in figure 2. Highest longevity of *A. dispersus* (21.5 days) was recorded in adult followed by egg (9.5 days) and pupal (9 days) stages while the lowest was in 2nd instar nymph (6.4 days) followed by 1st instar (7 days) and 3rd instar nymph (8.5 days) (Figure 2).

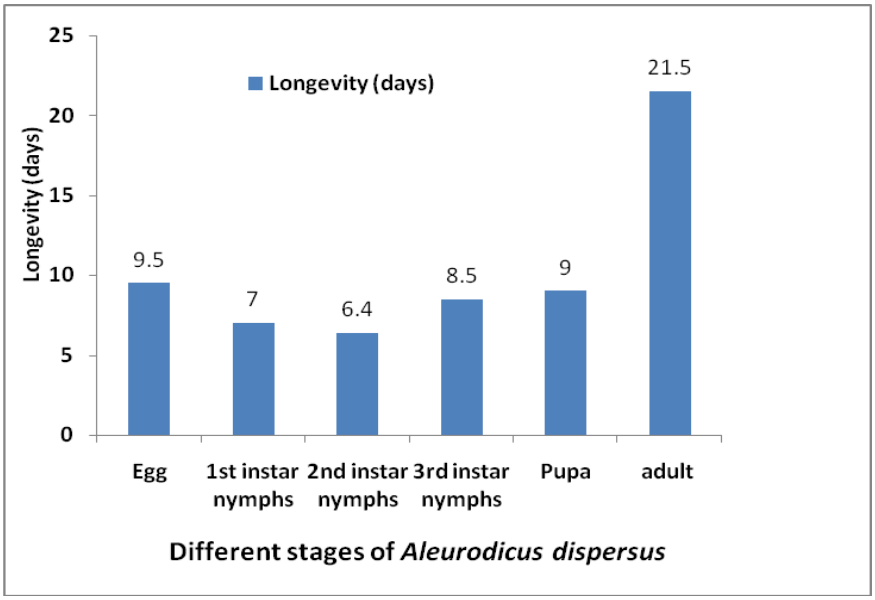


Figure 2. Longevity of different stages of *Aleurodicus dispersus*

From table 2, it is seen that the number of colony/leaf and number of 3rd and 4th instar nymphs of *A. dispersus* had significant positive correlation with minimum and maximum temperature while non-significant positive correlation is observed between the number of egg/colony, the number 1st and 2nd instar nymphs with minimum and maximum temperature. *Aleurodicus dispersus* showed non-significant positive

correlation with minimum and maximum relative humidity regarding number of colony/leaf, 2nd instar nymph while non-significant negatine correlation with 4th instar nymph. The number of egg/colony and 1st instar nymph had non-significant negative correlation with minimum temperature while these stage showed non-significant positive correlation with maximum temperature (Table 2).

Table 2. Correlation coefficient (r) between weather parameters and different stages of spiralling whitefly (*Aleurodicus dispersus*) population on guava

Weather parameters	No. of colony/ leaf	No. of egg/ colony	No. of 1st instar nymph	No. of 2nd instar nymph	No. of 3rd instar nymph	No. of 4th instar nymph
Temperature ^{0}C (Minimum)	0.829*	0.161	0.259	0.270	0.627*	0.524*
Temperature ^{0}C (Maximum)	0.758*	0.466	0.239	0.190	0.615*	0.572*
Relative humidity (%) (Minimum)	0.184	-0.307	-0.434	0.419	0.000	-0.297
Relative humidity (%) (Maximum)	0.263	0.318	0.313	0.427	-0.000	-0.170

*= Significant at 5% level of probability

Krishnamoorthy and Venugopalan (2010) reported that density of the spiralling whitefly was positively correlated with maximum temperature and negatively correlated with relative humidity and support the present findings of incidence in whiefly population with increase in temperature, minimum and maximum relative humidity. Rainfall, temperature and other weather factors cause seasonal fluctuation and are important regulating factors of many tropical insects (Delinger, 1986) but it is mostly their combined effect of evapo-transpiration that is more important (Asiwe et al., 2002). Heavy rainfall and strong wind flow are not favourable for whitefly population due to dislodging from plant surfaces (Banjo, 2010). When it rains heavily, many small insects get dislodged from plant surfaces by the combined effect of wetness and the kinetic energy of the rain drops as well as strong winds. The orientation of the leaves on the plant and consequently the position of the insect on the plant would be critical (Asiwe et al., 2002). *A. dispersus* population as most tropical insects is affected by the climatic conditions which dictate the season (Banjo et al., 2003; Asiwe et al., 2002). Wen et al. (1994) reported that a curvilinear relationship was found between temperature and development rate in *Aleurodicus dispersus* in the laboratory in the range 10-32°C, and a linear regression at 15-25°C. Adults were active at 12.3-32.3°C and adult lifespan decreased as rearing temperature increased from 15 to 30°C. The mean fecundity was highest (28 ± 14.5 eggs/female) at 25°C. A period of moderate rainfall combined with high day temperature which usually occur between April and May, following the onset of rain after the very dry

months (December and January) in Nigeria and other tropical regions favours high population of the spiraling whiteflies (Banjo and Banjo, 2003). Aishwariya et al. (2007) reported that spiralling whitefly was found throughout the year on guava and the incidence of this whitefly was found to build up during April-May. The egg density peaked during April, May, August and November months. The nymphal population was relatively low during June and first fortnight of July and it was found to fluctuate before attaining peak in first fortnight of November and then slowly declined. They also observed that the incidence of all the three stages of *A. dispersus* had significant positive correlation with maximum temperature and non-significant positive correlation with minimum temperature, non-significant negative correlation with morning and afternoon relative humidity. Rainfall was found to show non-significant negative correlation with all stages of *A. dispersus*.

CONCLUSION

Among different developmental stages, highest longevity of *A. dispersus* was recorded in adult compared to nymphs. Highest number of adults, nymphs and pupae were found in the month of January. *A. dispersus* had significant positive correlation with minimum and maximum temperature while non-significant positive correlation with minimum and maximum relative humidity.

REFERENCES

Aishwariya, K.K., Manjunatha, M., and Naik, M.I. (2007). Seasonal incidence of spiralling whitefly *Aleurodicus dispersus* Russell and its natural enemies in relation to weather in Shimoga. *Karnataka Journal of Agricultural Science,* 20(1), 146-148.

Amjad, M., Bashir, M.H., Afzal, M., and Khan, M.A. (2009). Efficacy of some insecticides against whitefly (*Bemisia tabaci* Genn.) infesting cotton under field condition. *Journal of Pakistan Life Science,* 7, 140-143.

Asiwe, J.A.N., Dixon, A.G.O., Jackal, L.E.N., and Nukenine, E.N. (2002). Investigation on the the spread of the spiralling whitefly (*A. dispersus*, Russell) and field evaluation of elite cassava population for genetic resistance. *A Research Article in AJRTC*, 5 (1), 12-17.

Banjo, A.D., and Banjo, F.M. (2003). Life history and the influences of agroclimatological factors on the spiralling whitefly (*A. disperses* Russel) (homoptera: aleyrodidae) on some host plants of economic importance in south-western Nigeria. *Journal of Crop Research,* 26(1), 140-144.

Banjo, A.D., Hassan, A.T., Jackal, L.E.N., Dixon, A.G.O., and Ekanayake, I.J.(2003). Developmental and Behavioural study of spiralling whitefly (*A. dispersus*) on three cassava (*Manihot esulanta* crantz) genotypes. *Journal of Crop Research,* 26(1), 145-149.

Banjo, A.D.(2010). A review on *Aleurodicus dispersus* Russell (Spiraling whitefly) (Hemiptera: Aleyrodidae) in Nigeria. *Journal of Entomology and Nematology*, 2, 1-6.

Delinger, D.L. (1986). Dormancy in Tropical Insects, *Annual Review of Entomology*, 31, 239-264.

Gill, R.J. (1993). The morphology of whiteflies. 13-46. In *Whiteflies: their bionomics, pest status and management* (eds), D. Gerling., Intercept Ltd.

Iftekhar, M.S., and Islam, M.R. (2004). Managing mangroves in Bangladesh: A strategy analysis. *Journal of Coastal Conservation*, 10,139-146.

Krishnamoorthy, A., and Venugopalan, R. (2010). Role of the aphelinid parasitoid *Encarsia guadeloupae* in the suppression of the exotic spiralling whitefly *Aleurodicus dispersus* on banana in India. *Biocontrol Science and Technology*, 14(6), 619-622.

Martin, J.H. (1987). An identification guide to common whitefly species of the world. *Tropical Pest Management*, 33(4), 298-322.

Oliveira, M.R.V., Henneberry, T.J., and Anderson, P. (2001). History, current status and collaborative research projects for *Bemisia tabaci*. *Crop Protection*, 20, 709-723.

Russel, L.M. (1965).A new species of Aleurodicus Douglas and two close relatives (Homoptera: Aleyrodidae). *Florida Entomology*, 48(1), 47-55.

Thompson, J.N. (1988).Evolutionary ecology of the relationship between ovipositon preference and performance of offspring in phytophagous insects. *Entomology Experimental Applied*, 4, 13-14.

Waterhouse, D.F., and Norris. K.R. (1989). Biological control; Pacific prospects; supplement. ACIAR Monograph, 12, p.123.

SAARC J. Agri., 15(2): 215-218 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35166*

Short Communication

THE EFFECT OF FREEZING ON CRYOSURVIVAL OF YAK SPERM

S. Deori*

ICAR-National Research Centre on Yak, Dirang-790101, Arunachal Pradesh, India

ABSTRACT

A study was carried out to study the effect of freezing on cryosurvival of yak semen. Artificial insemination in yak is still in infancy. Semen cryopreservation and use of artificial insemination can be applied in yak husbandry for conservation and rapid multiplication of superior germplasm. Semen was collected from four adult yak bulls using artificial vagina method managed under uniform conditions. A total of 40 ejaculates comprising of 10 ejaculates each bull were collected following twice a week schedule and evaluated for fresh semen characteristics. The fresh yak semen characteristics viz. ejaculate volume (ml), mass activity (0-4), initial sperm motility (%), sperm concentration (x 10^6/ml), live sperm (%), sperm abnormality (%) and intact acrosome (%) were 3.10 ± 0.18, 3.53 ± 0.96, 83.89 ± 2.87, 1180.22 ± 42.32, 77.63 ± 4.23, 8.45 ± 3.33 and 93.61 ± 3.78 respectively. The ejaculates were diluted (1:10) with Tris extender consisting of 6.4 ml glycerol and 20 ml of fresh egg yolk. Straws were equilibrated at 5°C for 4 hours followed by exposure to liquid nitrogen vapour for 10 minutes and finally transferred to liquid nitrogen container for storage. The cryosurvival rate was studied after 7 days of storage in liquid nitrogen. The frozen semen was thawed in warm water (37°C) for 30 seconds for evaluation. Mean values of post-thaw sperm motility (%), live sperm (%) and intact acrosome (%) in yaks were 55.67 ± 4.67, 65.62 ± 3.23 and 89.26 ± 3.67 respectively. In conclusion, yak semen has a better cryosurvival while freezing in tris extender with 6.4 per cent glycerol and 20 per cent egg yolk following an equilibration period of 4h.

Keywords: Cryosurvival, freezing, semen, yak

INTRODUCTION

Yaks are reared on high altitude free–ranges/pastures in trans-Himalayan and Himalayan regions of India and its neighboring countries. It is a unique bovine

* Corresponding author e-mail: sourabhd1@rediffmail.com

Received: 06.09.2017 *Accepted: 20.10.2017*

species of economical importance surviving in high hills snow bound areas under hypoxic and extreme cold conditions (even at-30 to-40°C) above 3000 metre from sea level, where major agriculture is not rewarding due to non-availability of arable lands. Unlike other bovines, yak has been considered as multipurpose animal as they provide milk, meat, fibre/wool, hide, fuel and the much needed transportation to the highlanders. The yak husbandry in India is facing a lot of challenges that leads to decline in yak population. Constraints of transhumance system of yak rearing, unscientific management practices, reproductive problems, nutritional scarcity, degradation of natural pastures and weak marketing linkage are some of the major challenges that makes yak husbandry unpopular. Declining yak population has become a cause of concern to the development authorities as these animals largely cater the needs of the highlanders. Decreasing population accompanied by geographical isolation of yak herds increased the risk of inbreeding that have adversely affected the genetic potential of the animals resulting in reduction in milk production and body size (Roychoudhury and Pathak, 2016).

Cryopreservation of semen and use of Artificial Insemination (AI) can be one of the effective tools for overcoming the inbreeding problem in yaks (Deori et al., 2016). Sperm cryopreservation not essentially only preserve the genetic resources, but also supports transportation of species between remote locations. Transportation of frozen semen is easier and economical in comparison to moving the bulls for natural service. Therefore, this technique may be used successfully in yaks located in inaccessible hilly terrains. Keeping in view the above facts, the present study was designed to see the effect of freezing on the cryosurvival of yak semen and to establish a species-specific standard freezing protocol.

MATERIALS AND METHODS

The study was carried out in the yak farm of the institute located at 9022 feet above mean sea level in the Nyukmadung area of the West Kameng District of Arunachal Pradesh in India. Four yak bulls aged between 3.5 to 4 years were trained for semen donation by artificial vagina method. The bulls were maintained under uniform intensive system of management and fed with seasonal grass *ad libitum* and concentrates of maize, groundnut cake, wheat bran with additional salt and minerals. They were thoroughly examined for sexual and general health before selection. A total of 40 ejaculates comprising of 10 ejaculates from each bull were collected following twice a week schedule and evaluated for fresh semen characteristics.

Ejaculate volume was recorded directly from the glass graduated semen collection tube and expressed in millilitre. Mass activity was estimated immediately after collection of semen. A drop of semen was placed on a pre warmed (37°C) glass slide and examined under low power objective at a magnification of 100X without cover slip. The scoring was done on the basis of wave pattern described by Zemjanis (1970). Initial sperm motility was estimated by taking a fine drop of semen diluted with 4-5 drops of pre warmed (37°C) tris buffer that consisted of 2.422 g tris, 1.36 g

citric acid, 1g fructose and 100 ml triple glass distilled water. A drop of diluted semen was placed on a pre warmed glass slide (37°C) with a cover slip on it and examined under a phase contrast microscope at a magnification of 400X. It was recorded as percentage of progressively motile sperm. Sperm concentration was determined with the help of a Neubauer counting chamber after a dilution of 1:200 with a diluting fluid and expressed in million per millilitre of semen. The percentage of live spermatozoa was determined using Eosin-Nigrosin staining technique described by Blom (1977). Sperm tail and mid piece abnormality were studied by differential interference phase contrast microscopy of wet-mount semen fixed in isotonic formal saline under high power objectives. The sperm head morphology was studied by staining the spermatozoa with Williams stain and examined under microscope of 1000X under oil immersion objectives. The morphological changes of acrosome were studied in stained semen smear using Giemsa staining technique of Watson (1975). Two hundred spermatozoa were examined in each smear at a magnification of 1000X of a compound microscope fitted with artificial illumination and the percentage of intact acrosome was determined.

The ejaculates were diluted (1:10) with tris extender consisting of 2.42 g tris, 1.36 g citric acid, 1.0 g fructose and 73.6 ml of triple glass distilled water. To it 6.4 ml glycerol and fresh egg yolk 20 ml was added. Straw filling and sealing was done by filling and sealing machine at room temperature and the straws were transferred to the cold handling cabinet maintained at 5°C. After the straws reached to 5°C they were equilibrated at 5°C for 4 hours followed by exposure to liquid nitrogen vapour for 10 minutes and storage in liquid nitrogen.

After 7 days of storage in liquid nitrogen, the frozen semen was thawed in warm water (37°C) for 30 seconds for evaluation. Each semen sample was evaluated for sperm motility, live sperm, and intact acrosome after freezing as per the methods discussed previously.

RESULTS AND DISCUSSION

In the present study the fresh yak semen characteristics viz. ejaculate volume (ml), mass activity (0-4), initial sperm motility (%), sperm concentration (x 10^6/ml), live sperm (%), sperm abnormality (%) and intact acrosome (%) were 3.10 ± 0.18, 3.53 ± 0.96, 83.89 ± 2.87, 1180.22 ± 42.32, 77.63 ± 4.23, 8.45 ± 3.33and93.61 ± 3.78 respectively. Mean values of post-thaw sperm motility (%), live sperm (%) and intact acrosome (%) in yaks were 55.67 ± 4.67, 65.62 ± 3.23 and 89.26 ± 3.67 respectively.

In accordance with the present findings, Hazarika et al. (2012) reported ejaculate volume, initial sperm motility, sperm concentration and live sperm count of fresh yak semen irrespective of animals to be 2.83 ± 0.08 ml, 71.5 ± 0.57 %, 1182.5 ± 3.23 million/ml and 80.05 ± 0.74 % respectively. Borah et al. (2015a) reported mean sperm motility and percent live sperm in fresh semen of yak bulls immediately after collection to be 73.00 ± 0.84 and 80.00 ± 0.78 respectively. They further recorded that following equilibration period of 3h, 4h and 5h the post thaw sperm motility

were 38.75 ± 1.62, 47.00 ± 1.64 and 45.00 ± 1.49 percent respectively with an overall mean of 43.58 ± 1.01. In the present study, following freezing, the sperm motility was recorded 55.57 ± 4.67 percent following 4h of equilibration, which is higher than the reported value of Borah et al. (2015a). These may be attributed to differences in season of collection and freezing protocol used.

In an another study, Borah et al. (2015b) found post thaw live sperm in yaks ranged from 69.87 ± 0.41 to 70.40 ± 0.78 percent and percentage of total acrosomal changes ranged from 11.00 ± 0.62 to 13.27 ± 0.53 while employing different thawing methods. These values are in accordance with the present findings.

CONCLUSION

It could be concluded that yak semen has a better cryosurvival while freezing in tris extender with 6.4 percent glycerol and 20 percent egg yolk following an equilibration period of 4h. Frozen yak semen can be suitably used for artificial insemination to curb inbreeding and rapid multiplication of superior germplasm.

REFERENCES

Blom, E. (1977). Sperm morphology with reference to bull fertility. p. 61-81. In Proc. *First All India Symposium on Animal Reproduction.* Ludhiana.

Borah, B.K.D., Deka, B.C., Biswas, R.K., Chakravarty, P., Deori, S., Sinha, S., and Ahmed, K. (2015b). Effect of thawing methods on frozen semen quality of yak (*Poephagus grunniens* L.) bulls, *Veterinary World,* 8(7), 831-834.

Borah, B.K.D., Deka, B.C., Biswas, R.K., Chakravarty, P., Deori, S., Sinha, S Ahmed, K., Dutta, D. J., and Bhattacharyya, D.K. (2015a). Effect of equilibration period on post thaw semen quality of Indian yak. *Indian Journal of Animal Sciences,* 85 (6), 607-609.

Deori, S., Das, P.J., Paul, V., Hussain, M., Medhi, D., Bam, J., Bhattacharya, D., and Deb, S.M. (2016). Cryopreservation of semen for *ex-situ* conservation of Yak (*Poephagus grunniens* L.), p. 109. In Proc. *International Conference Global Biodiversity, Climate Change and Sustainable Development*, 15-18 Oct., Itanagar, India.

Hazarika, D., Sarma, S., Chakravarty, P., Kalita, D.J., Goswami, J., and Deka, B.C. (2012). Testosterone in yak (*Poephagus grunniens* L.) bull seminal plasma and its correlation with physical characteristics of semen. *Indian Journal of Animal Sciences,* 82 (7), 713-714.

Roychoudhury, R., and Pathak, P.K. (2016). Scientific interventions in traditional yak management system for improving profitability. p. 30-36. In Proc. *Fourth Interface Meeting on Holistic approaches to sustain livelihood of yak rearers through scientific interventions in India*, 3-5 March, ICAR-NRC on Yak, Dirang, India.

Watson, P.F. (1975). Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Veterinary Record,* 97, 12-15.

Zemjanis, R. (1970). Collection and evaluation of semen. p.139-155. In *Diagnostic and Therapeutic Techniques in Animal Reproduction*, 2nd ed., Williams and Wilkins Co., Baltimore.

SAARC J. Agri., 15(2): 219-226 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35164*

Short Communication

PHYSIOLOGICAL CHANGES AND SHELF LIFE OF MANGO (*Mangifera indica* L.) INFLUENCED BY POST HARVEST TREATMENTS

M.I. Hoque[1*], S. Chowhan[1], and M. Kamruzzaman[2]

[1]Adaptive Research and Extension Division, Bangladesh Institute of Nuclear Agriculture Mymensingh-2202, Bangladesh

[2]Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202, Bangladesh

ABSTRACT

The experiment examined the efficacy of various post harvest treatments namely control, hot water treatment, thin plastic film, chlorinated water, neem extract and garlic extract on shelf life and quality of mango. Parameters studied were colour, firmness, disease severity, disease incidence, total soluble solids, total weight loss, moisture content, dry matter and shelf life of mango. The longest shelf life of 15.41 days was found in mango fruits wrapped with thin plastic film.

Keywords: Self life, postharvest, physiological change, mango, treatment

INTRODUCTION

Mango is one of the most extensively exploited fruits for food, juice, flavor, fragrance and color, making it a common ingredient in new functional foods often called super fruits (Cole, 1984; Bayarri et al., 2001). The fruit is very popular with the masses due to its wide range of adaptability, high nutritive value, and richness in variety, delicious taste and excellent flavour. A climacteric fruit, the mango ripens quickly after harvest (between 3 and 9 days) (Mitra and Baldwin, 1997). This short period seriously restricts long distance marketing. Apart from that sensitivity to disease and high temperature, and perishability due to faster ripening or softening of the fruit, limit its potential in terms of storage, packaging and transport (Mitra and Baldwin, 1997). Proper postharvest treatments and packaging are required for maintaining better quality, extended shelf life and having access to international markets (Anwar and Malik, 2007). Shelf life of fruits could be extended by precooling, chemical treatments, low temperature, different botanical extracts, and so on. However, ventilated low-density polyethylene (LDPE) have also been found to be beneficial, as this material maintains humidity, which results in less shrinkage during

[*] Corresponding author e-mail: apu.pstu@gmail.comm

Received: 05.09.2017 *Accepted: 10.11.2017*

storage (Tharanathan et al., 2006). Different botanical extracts viz. neem and garlic, and coating like sesame oil influence the shelf life and maintain quality of mango (Rodov et al., 1997). The combination of modified atmospheric packaging (MAP) with effective decay control measures can extend the postharvest life of mango fruit (Rodov et al., 1997). Now-a-days very few fruitful techniques are available in Bangladesh to prolong shelf life of mango. Hence, it is necessary to develop more effective techniques to reduce the postharvest losses for prolonging economic storage life of mango. However, reports on postharvest management practices, especially with regards to mango are very inadequate in scientific literature. At the same time very, little systematic study so has been conducted in Bangladesh to reduce the postharvest losses and extension of shelf life of mango.

MATERIALS AND METHODS

The present experiment was conducted at the laboratory of the Bangladesh Institute of Nuclear Agriculture, Mymensingh, in July 2015. The temperature and relative humidity of the storage room were recorded daily during the study period with a digital thermo hygrometer (THERMO, TFA, and Germany). The minimum and maximum temperatures during the study period of the storage room were 25.2 to 31.3^{o}C, respectively. The materials used for the experiment were the freshly harvested mango fruits of cv. Fazli. The fruits were collected from local grower of Chapinawabganj. The fruits at the commercial maturity with uniform size, shape, and free of any visible defects, disease symptoms and insect infestations were harvested and transported to the laboratory of the Bangladesh Institute of Nuclear Agriculture, Mymensingh, with careful handling to avoid damage and injury. The experiment consisted of six treatments such as: T_0: Control (mango fruit were not subjected to treatments), T_1: hot water treatment (50^{0}C for 5 min), T_2: Thin (20 microns) plastic film, T_3: chlorinated water (two pieces of chlorine tablets were mixed with 1 litre of distilled water), T_4: neem extract (500 g neem leaves/500 L water), T_5: Garlic extract (500 g garlic cloves /500 l water). The experiment was laid out in a completely randomized design (CRD) with three replications of six fruits in each replication.

RESULTS AND DISCUSSION

It was observed that color changes of mango depend on various postharvest treatments. The postharvest treatments showed significant variation in respect of peel color change of mangoes. The changes in colour of mango were determined using a numerical rating scale of 1-7, where 1 = green, 2 = breaker, 3 = up to 25% yellow, 4 = 25-<50% yellow, 5 = 50- <75% yellow, 6 = 75-100% yellow and 7= blackened. Similar method was followed by Hassan (2006). At the 11th day of storage, the scores of peels color was the order of 6.92 (Control)> 6.83 (chlorinated water) > 5.61 (hot water treatment) > 4.67 (garlic extract)> 4.08 (neem extract)>3.83 (thin plastic film). Later at 15 days after storage, peel color of 5.75 was obtained only at thin plastic film. The peel color scores increased as the duration of storage progressed at ambient

temperature. The increase in color score during storage might be due to series of physico-chemical changes like the breakdown of chlorophyll and increase in carotenoid pigments of the pulp caused by enzymatic oxidation and photo degradation.

The faster rate of color change of mango under control treatment may be due to the rapid activity of some enzymes that are responsible for the color changes of mango Robinson (1996). The delay in ripening and senescence of mango fruits in the thin plastic film may be attributed to the inhibition of different chemical changes like chlorophyll breakdown. The result of the present study is also supported by the findings of Robinson (1996). He stated that during color changes, the pulp of the fruit became softer and sweeter as the ratio of sugars to starch increased and the characteristics aroma was produced. These findings were at per with Doreyappa-Gowda and Huddar (2001) who reported that the green peel color of mature Alphonso and other varieties of mango turned from light green or green or dark green to light yellow or yellow or orange yellow due to the breakdown of chlorophyll due to a series of physico-chemical changes during ripening, leading to disappearance of green color.

Firmness of mango was determined by hand feeling using a numerical rating scale of 1-6 where, 1=mature hard, 2=sprung, 3=between sprung and eating ripe, 4=eating ripe, 5=over ripened 6= totally unfit for consumption. Similar method was mentioned by Hassan (2006). Firmness changes showed significant ($P<0.01$) dissimilarity due to the effect of different postharvest treatments. It was observed that the firmness changes occurred at faster rate in control, whereas the rates were slower in those fruits held at thin plastic film. At 11 days after storage, mango reached firmness score of 5.92 in control treatments. The firmness score was significantly less (3.42) in those fruits held at thin plastic film.

The firmness of mango changes due to conversion of starch into sugars. The results of the experiment are supported by the findings of Pesis et al. (2005). They described that creation of an atmosphere with 11% CO_2 and 12% O_2 was the most effective treatment for delaying ripening of bananas. The bananas remained firmer with nice peel color after 1 week at 20^{o}C, but the humidity inside the bags caused some decay development on the crown cut. Storing bananas in air-evacuated bags for 24 to 48 hours reduced O_2 levels (1%) and increased the production of CO_2 up to (30%) but perforating the bags dramatically reduced the CO_2 level to around (9%) and increased the O_2 level (12%).

In general, the moisture content decreased with the increase in storage time under different postharvest treatments. It was observed that the significant variations were found in moisture content at all the days after storage due to different postharvest treatments. Moisture content in the pulp of mango was the highest (87.38%) at 3 days after storage (DAS) due control. On the other hand, the lowest moisture content (84.50%) was found in those fruit treated with 1:1 neem extract.

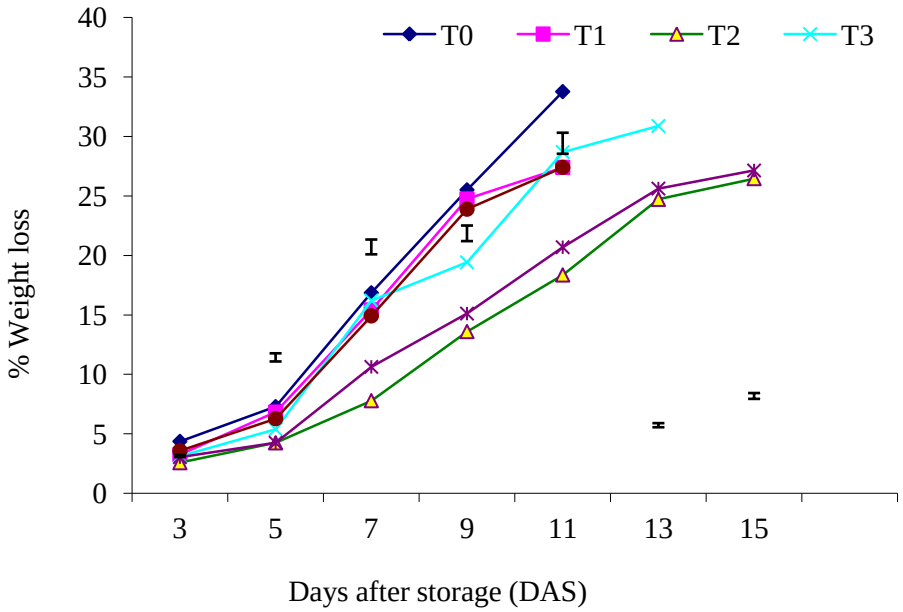


Figure 1. Effect of different postharvest treatments on weight loss at different days after storage. At each day the vertical bars represent LSD at the 5% level of probability

T_0: Control; T_1: Hot water treatment; T_2: Thin plastic film; T_3: Chlorinated water, T_4: Neem extract; T_5: Garlic extract; * significant at the 5% level; ** significant at the 1% level; NS: non-significant; ND: Statistical analysis not done.

Dry matter content varied significantly ($P<0.01$) due to the effect of different postharvest treatments. It was observed that the percent dry matter content increased with the increase in storage duration. At the 15 days after storage, neem extract treatment showed the highest (26.00%) dry matter content. Similar results were reported by Alam (1990). At the same days after storage, the significantly lowest (21.40%) dry matter was recorded in Control.

Highly significant effects, on percent weight loss of mango fruit were observed due to the application of different postharvest treatments. Significantly, the maximum (30.77%) weight loss occurred in control at 11 days after storage. By contrast, minimum weight loss (18.36%) at 11 DAS was observed in fruits held at thin plastic film. Significantly, the minimum (2.58%) weight loss was recorded at initial day after storage also in the fruits held at thin plastic film. The use of neem extract and Garlic extract also contributed to the reduced rate of weight loss (Figure 1).

These results are in agreement with those of Carrillo et al. (2000) who observed that coated or uncoated haden mango in Mexico had an increasing trend of weight loss

with the passage of storage time. However, weight loss was lower in coated fruit (4.0 to 6.5%) as compared to control having higher percent weight loss (0.00 to 9.0%).

These results are further in line with Doreyappa-Gowda and Huddar (2001) who observed that mature green Alphanso and other 7 varieties of mango fruit were influenced by size of fruit, storage temperature, variety and the reduction in length and thickness of fruit during ripening process were attributed to shriveling of fruits due to higher percent loss of water (12.8%) from fruits when stored at high temperature (18-34^{o}C). Perez et al. (2004) also observed that weight loss in Avocado fruit was linear with the storage temperature

Haque (1985) also reported the similar results. He reported that the weight loss of bananas occurred due to the loss of water from the fruits, microbial decay and storage environment like temperature and humidity. High temperature enhanced weight loss but low temperature reduced weight loss during ripening and storage.

Postharvest treatments had highly significant effect on total soluble solids contents of mango. The TSS of mango fruits packaged in control condition ranged from 25.00-30.00% with a mean value of 27.50%. The increasing trend of percent total soluble solids contents of fruit during storage could be attributed mainly to the breakdown of starch into simple sugars during ripening along with a proportional increase in TSS and further hydrolysis decreased the TSS during storage.

The maximum percent total soluble solid contents of mango were observed in control (30.00%) followed by hot water + thin plastic film (28.50%) >neem extract (27.60%) >garlic extract (27.30%) >hot water treatment (27.00%) at 12 days after storage. It was observed that the total soluble solids contents increased from 18.00 to 28.50% up to12 days after storage. The TSS changes were minimal in fruits held at thin plastic film. Garlic extract also exhibited low TSS.

This increase in TSS is due to the conversion of complex carbohydrates into simple sugars. This is correlated with hydrolytic changes in starch and conversion of starch to sugar being an important index of ripening process in mango and other climacteric fruits and further hydrolysis decreased the TSS during storage (Kays, 1991; Kittur et al., 2001). Similar pattern of TSS was observed in green mature Alphanso and other 7 hybrids or varieties of mango fruit that undergone a series of physico-chemical changes and the major changes were increase in TSS content from 8.55 to 19.0% Brix during ripening and storage at 18-34^{0}C (Doreyappa-Gowda and Huddar, 2001). Similar views were expressed by Manzano et al. (1997) who observed that temperature of storage also affect TSS contents, and TSS contents were high (14.15%) at high temperature (25^{0}C) as compared to lower TSS contents (16.6%) at low temperature (12^{0}C) during 20 days of storage experiment.

It was observed that disease incidence was the highest (100%) in all fruits except in those held at thin plastic film up to 15 days after storage. Generally, the levels of disease incidence were found to gradually increase as the duration of storage

progressed. The disease levels were maintained lower in those fruit held at thin plastic film, and those treated with neem extract, especially at the early part of storage. This result of less disease is possible due to the delayed pathogenic growth and development in fruits held at perforated low-density polyethylene bag. Similar results were reported by Perez et al. (2002).

The levels of disease severity were significantly influenced by postharvest treatments at the different storage time. It was observed that disease severity status of mango was recorded at 3 days interval from day 1 up to 15 days. At day 15, all fruits were spoiled. Disease severity level trended to increase with duration of storage. The lowest disease severity level (0%) was found in mango held in thin plastic film. Disease severity levels were higher especially at the later part of storage in those fruits kept in control. Garlic extracts showed good results in relation to the lower level of disease severity. These results would be attributed to the suppression of fungal growth due to the antifungal properties of garlic. Similar results were reported by Amin (2006).

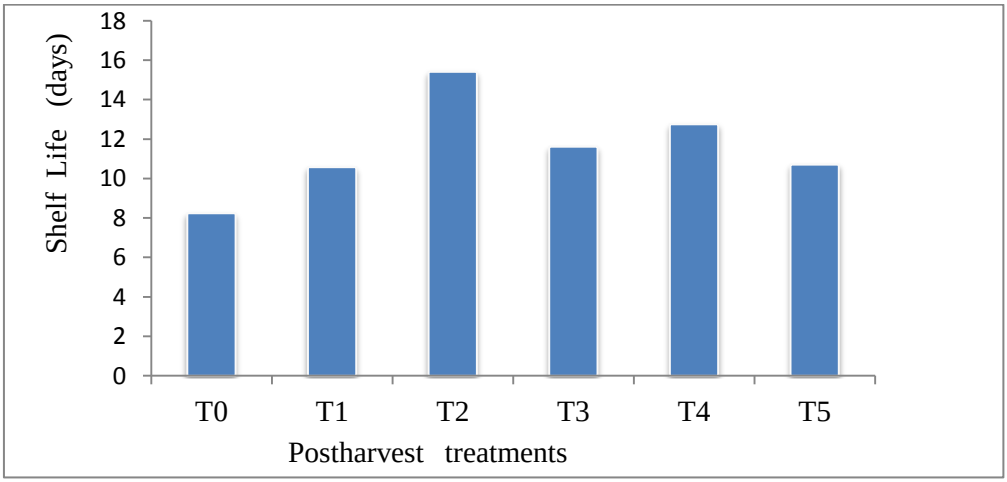


Figure 2. Effect of different postharvest treatments on shelf life at different days after storage

T_0: Control; T_1: Hot water treatment; T_2: Thin plastic film; T_3: Chlorinated water, T_4: Neem extract; T_5: Garlic extract; * significant at the 5% level; ** significant at the 1% level; NS: non-significant; ND: Statistical analysis not done.

Shelf life of mango fruits was significantly affected by different postharvest treatments. It was observed that the extension of shelf life of fruits has been one of the most important concerns of the researchers. Results revealed that the longest shelf life (15.41 days) of mango fruits was recorded in those fruit held at thin plastic Film. The shortest shelf life of 8.25 days was observed from the control fruits (Figure 2).

The longest shelf life as obtained for thin plastic film storage was possibly due to the reduced rate of physico-chemical changes, reduced weight loss and minimal disease severity. Thin plastic film Alphonso mango fruits increased the shelf life of the mango. Pre-cooling has been found to reduce the occurrence and intensity of spongy tissue (Anonymous, 1990).

In the present study, significant extension of shelf life was also recorded in fruit treated neem extract (12.75 days) and garlic extract (10.72 days) (Figure 2). Shelf life extension due to the treatment with garlic extract was possibly due to the suppression of microbial growth. On the other hand, the effects of thin plastic film in shelf life extending would be due to the elevation of CO_2 and reduction of O_2 inside the bags.

CONCLUSION

Postharvest treatments had significant effects on color, firmness, total weight loss, moisture content, total soluble solids (TSS), dry matter content, disease severity, disease incidence and shelf life of mango. The best visual appearance was observed the fruits subjected to thin plastic film. Mango remained fresh until day 15 if held in thin plastic film. Although neem extract showed better result in storage until the 12^{th} day of storage. At the 15^{th} day of storage, mango stored at thin plastic film had firmness score less than other treatments. Mango stored in thin plastic film had reduced weight loss until the 15^{th} day of storage. The highest moisture content was recorded in treatment control (87.38%) at 3 DAS. The neem extract treatment gained the highest dry matter (26%). The longest shelf life of 15.41 days was found in fruits kept in thin plastic film. The fruits subjected to control had the showed shortest shelf life (8.25 days).

REFERENCES

Alam, M.M. (1990). Neem in nematode control. In nematode bio-control (aspect prospects) Delhi, India.CBS. Published distributors Pvt. Ltd, p. 51-55. [Helminth Abst. 46:72].

Amin, A.B.M.R. (2006). Garlic tablet for controlling seed-borne fungal disease of cucumber. An MS thesis submitted of the Department of Plant Pathology, BAU, Mymensingh, p. 1-20.

Anonymous.(1990). Official Methods of Analysis. Association of Analytical Chemists,15^{th} ed. Virginia, Arlington, USA. Hot water treatment affects ripening quality storage life of mango. *Pakistan Journal of Agricultural Sciences*, 44, 23-30.

Anwar, R., and Malik, A.U.(2007). Hot water treatment affects ripening quality and storage life of mango. *Pakistan Journal of Agricultural Sciences*, 44, 23-30.

Bayarri, S., Calvo, C., Costell, E., and Durán, L. (2001). Influence of color perception of sweetness fruit flavor of fruit drinks. *Food Science Technology International*, **7**(1), 399-404.

Carrillo, L.A., Ramirez-Bustamante, F., Valdez-Torres, J.B., Rojas-Villegas, R., and Yahia, E.M. (2000). Ripening quality changes in mango fruit as affected by coating with an edible film. *Journal of Food Quality,* 23, 479-486.

Cole, A.D. (1984). Origin of Cultivated Plants. Vegal Paul Trench Co. London. p. 1-67.

Doreyappy-Gowda, I.N.D., and Huddar, A.G. (2001). Studies on ripening changes in mango (*Mangifera indica L.)* fruits. *Journal of Food Science and Technology*, 38, 135-137.

Haque, M.A. (1985). Ripening time weight loss during ripening of "Amritasagar" banana. *Bangladesh Journal of Agricultural Research,* 10(2), 156-158.

Hassan, M.K. (2006). Constitutive alkenyl resorcinol's and resistance to postharvest diseases in Mango. Ph.D. Thesis, school of agronomy and horticulture, *The University of Quensland Australia,* p. 286.

Kays, S.J. (1991). Postharvest physiology of perishable plant products. Vas Nostr Rein Hold Book, AVI Publishing Co, p. 149-316.

Kittur, F.S., Saroja, N., Habibunnisa, M., and Tharanathan, R.N. (2001). Polysaccharide-based composite coating formulations for shelf-life extension of fresh banana mango. *European Food Research Technology,* 213, 306-311.

Manzano, J.E., Perez, Y., and Rojas, E.(1997). Coating waxes on Haden Mango fruits (*Mangifera indica L*) variety for export. *Acta Horticulturae,* 455, 738-746.

Mitra, S.K., and Baldwin, E.A.(1997).Postharvest physiology storage of tropical subtropical fruits, CABI International, New York, NY, p. 85-122.0rganization, Australia. p. 1-28

Perez, M.B., Rojas, C., and Del-Rio, M.A.(2002). Effect of lipid type amount of edible hydroxyl propyl methylecellulose lipid composite coatings used to protect postharvest quality of marins cv. Forture. *Journal of Food Science,* 67(8), 2903-2910.

Pesis, E., Faure, M., and Marinansky-Ben Arie, R. (1997). Induction of chilling tolerance in mango by temperature conditioning, heat, low O2 ethanol vapours. *Acta Horticulturae*, 455, 629-634.

Robinson, J.C. (1996). A field comparison of ten Cavendish Sub group banana cultivars selected (Musa AAA) over four crop cycles in the subtropics. *Journal of Horticultural Scienc*, 68(4), 511-521.

Rodov, V., Fishman, S., Asuncion, R.D., Peretz, J., and Yehoshua, S.B. (1997). Modified atmosphere packaging (MAP) of Tommy Atkins mango in perforated film. *Acta Horticulturae,* 455, 654-661.

Tharanathan, R.N., Yashoda, H.M., and Prabha, T.N. (2006). Mango (*Mangifera indica L.)* The King of Fruits-An Overview. *Food Reviews International,* 22, 95-123.

SAARC J. Agri., 15(2): 227-239 (2017) *DOI: http://dx.doi.org/10.3329/sja.v15i2.35167*

Status Paper

BUFFALOES FOR DAIRYING IN SOUTH ASIA: POTENTIAL, CHALLENGES AND WAY FORWARD

M.N.A. Siddiky[1*] and M.O. Faruque[2]

[1]SAARC Agriculture Centre, BARC Complex, Farmgate, Dhaka-1215, Bangladesh
[2]Department of Animal Breeding and Genetics, Bangladesh Agricultural University Mymensingh-2202, Bangladesh

ABSTRACT

Buffalo is considered the dairy animal for 21st century due to its higher adaptability and productivity in the changing climatic conditions. There is a large diversity in the buffalo genetic resources and South Asia is home of high yielding source promising buffalo breed of Murrah and Nail Ravi. South Asia is inhabitant of 151.49 million buffalo populations out of 194.29 million of global populations. Besides, about 79.74 % of Asia and 77.9 % of world buffalo populations are inhabitant in South Asian countries. During the last decade, the world buffalo population has been increased by approximately 1.49% annually. South Asia is currently producing 100.74 million metric tons of buffalo milk which is about 96.05 % of Asia and 93.19 % of world buffalo milk production. The share of buffalo milk is around 51.07% of the total milk production of the South Asia. The contribution of buffalo milk in India, Nepal and Pakistan are 51.2%, 66.6% and 59.5% respectively in total milk production. Among the South Asian countries, maximum milk is produced by India followed by Pakistan, Nepal, Bangladesh, Sri Lanka and Bhutan. The productivity of buffalo has been recorded 410 $kg^{-1}buffalo^{-1}year$, 1880 $kg^{-1}buffalo^{-1}year$, 1934 $kg^{-1}buffalo^{-1}year$ and 867 $kg^{-1}buffalo^{-1}year$, 508 $kg^{-1}buffalo^{-1}year$ in Bangladesh, India, Pakistan, Nepal and Sri Lanka respectively. Although most of the buffaloes are non-descript indigenous types and their production potential is not satisfactory. There are different production systems are prevailing such as zero input-low output, low input-medium output and high input-high output. Moreover, selective breeding for buffalo with the same breed under low input-medium output production system and grading up of non-descript buffaloes using improver breed/s under zero input-low output production system has been commonly practiced. The demand of milk has been increasing due to economic solvency and rapid pace of urbanization but most of the countries are deficit in production even it is challenging to meet the

[*] Corresponding author e-mail: nasiddiky.saarc@gmail.com

Received: 10.11.2017 *Accepted: 30.11.2017*

projected demand to achieve the SDG by 2030. To increase the productivity through genetic improvement of buffaloes could be important thrust areas to obtain projected demand of milk. Productive and reproductive efficiency can only be improved by adopting suitable breeding policies and good management practices.

Keywords: Buffalo, dairying, milk, South Asia

INTRODUCTION

Buffalo is called the black gold of South Asia. Buffalo produces high quality, rich, creamier milk as well as lean meat with very low fat and cholesterol content. Its milk is suitable for specially dairy products owning to higher fat, lactose and solid not fat (SNF) contents. Buffaloes are resistant to several tropical diseases prevalent in South Asia, while enduring diverse harsh climates making it a preferred climate resilient livestock species. It has ensured survival for landless and small farmers in many parts of South Asia, while ushering fortunes for the elite buffalo breeders and entrepreneurs. Buffalo holds great hope for food security and poverty alleviation in South Asia, because of the largest population comprising diverse and the best buffalo germplasm of the world. Of the 194.29 million world buffalo population, 97% is concentrated in Asia and 57% in India alone (FAO, 2014). The increasing importance and popularity of buffalo species in the world is evident from its increasing population trends over the last decades with about 1.13% in Asia, 0.92% in Africa, 1.74% in Americas and overwhelming 5.22% in Europe (FAO, 2014). About 150 million households around the world are directly engaged in milk production involving primarily small and marginal households whereas almost the same number is indirectly involved in associated activities related to milk and meat which is the primary source of animal protein for human consumption. This region has a great biodiversity of buffalo germplasm, including the world famous buffaloes Murrah and Nili-Ravi - renowned for high milk production potential. The India and Pakistan share 69.32% and 23.19%, of world buffalo milk production respectively (FAO, 2014). About 71.4% of world buffalo meat is produced in South Asian countries (FAO, 2014). Although buffalo is an essential part of livestock in South Asian countries but it has been neglected in some SAARC countries like Bangladesh, Bhutan and Sri Lanka.

Buffalo population dynamics in South Asia

According to FAO (2014) estimate, about 79.74 % of Asia and 77.9 % of world buffalo population are inhabitant in South Asia. The total buffalo population is around 34.80% of the total cattle and buffalo population in the region. In the region, buffalo population is highest in India followed by Pakistan, Nepal, Bangladesh, Sri Lanka and Bhutan. Buffalo population in Maldives and Afghanistan is not documented yet. Asian buffaloes dominate the world population, representing 92.52% of the worldwide population of 194.29 million (FAO, 2014).

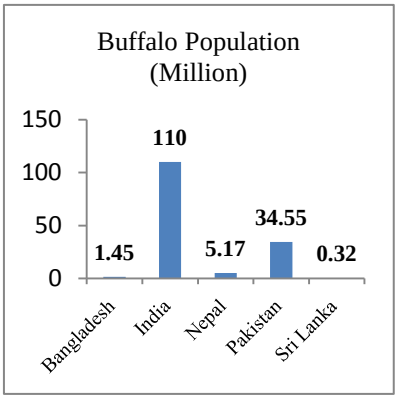


Figure 1. Buffalo population in South Asia

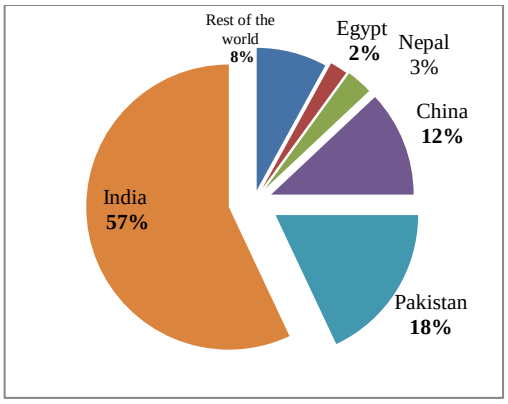


Figure 2. Buffalo population diversity in the world

Table 1. Buffalo populations in the world, in Asia and in South Asia

Region/Country	Total population (million)	Share in world population (%)
World	194.29	100%
Asia	179.75	92.52%
India	110.00	56.61%
Pakistan	34.55	17.78%
Nepal	5.17	2.66%
Bangladesh	1.45	0.746%
Sri Lanka	0.32	0.16%
Bhutan	0.001	-

Source: FAO, 2014; Chakravarty, 2013

Within the Asian region, about 79.74% of buffaloes are in South Asia and the rest 20.26% in other countries (Chakravarty, 2013). The buffalo populations in the world, in Asia and in South Asia are presented in table 1. During last decade, world buffalo population has increased by 20.0 million head and 89.41% of that increases occurred in Asia, in fact, that population growth has been largely contributed by India and Pakistan (FAO, 2014). According to latest livestock census, the buffalo populations are 1.45, 0.001, 110, 5.17, 34.55 and 0.32 millions in Bangladesh, Bhutan, India, Nepal, Pakistan and Sri Lanka respectively (Figure 1). The growth rate of buffalo population in the region was always in positive trend. In the region, total buffalo population was 121.18 million in 2000 and after the passage of time it became 151.49 million in 2014 (Table 2).

Table 2. Growth rate of buffalo population in South Asia (2000-2014) In Million

Year	Bangladesh	India	Nepal	Pakistan	Sri Lanka	Total
2000	0.89	93.83	3.5	22.66	0.30	121.18
2002	0.97	96.53	3.7	24.03	0.28	125.51
2004	1.06	99.72	3.9	25.50	0.30	130.48
2006	1.16	103.42	4.2	27.33	0.31	136.42
2008	1.26	106.01	4.4	29.00	0.31	140.98
2010	1.34	107.37	4.8	29.41	0.42	143.34
2012	1.44	108.70	5.13	32.68	0.41	148.36
2014	1.45	110.00	5.17	34.55	0.32	151.49

(Source: FAOSTAT)

Buffalo genetic resources in South Asia

Regional buffaloes are mostly riverine type (2n=50), however, some swamp type (2n=48) are also available in some agro climatic zones viz. North eastern part of India and Bangladesh. This regional buffaloes comprises of indigenous non-descript, crossbred and pure breed. The genotypic and phenotypic analysis of buffalo genomics has not done properly except India. National Bureau of Animal Genetic Resources (NBAGR) of India has successfully completed phylogenetic analysis of 13 buffalo breeds. The status of buffalo genetic resources in the region are presented in table 3 (Siddiky et al., 2014).

Table 3. Documented buffalo genetic resources in South Asia

Country	Documented buffalo genetic resources
Bangladesh	Indigenous local called Deshi, Nili Ravi, Murrah, crossbred and swamp buffalo
Bhutan	Non descript, Hyakule, Kagye, Murrah, crossbred and wild
India (13 Breeds)	Non descript, Murrah, Nili-Ravi, Jaffarabadi, Banni, Mehsana Marathwada Nagpuri, Pandharpuri, Bhadawari, Surti, Toda, Chilika, Kalahandi, cross breed, swamp and wild
Nepal	Non descript, cross breed, Gaddi, Parkote and Lime and wild
Pakistan	Non descript, Nili-Ravi, Nili, Ravi, Kundhi and Azakheli
Sri Lanka	Non descript, crossbred, Lankan buffalo, wild buffalo and Murrah

(Source: Farm Animal Genetic Resources in SAARC Countries, 2014)

Buffalo milk production in South Asia

South Asia is currently producing 100.74 million metric tons of buffalo milk which is about 96.05 % of Asia and 93.19 % of world buffalo milk production (FAO, 2014).

The share of buffalo milk is around 51.07% of the total milk production of the South Asia. The contribution of buffalo milk to the total milk production in the world is about 13%. The contribution of buffalo milk in India, Nepal and Pakistan are 51.2%, 66.6% and 59.5% respectively in total milk production (FAO, 2014). Among the South Asian countries, maximum milk is produced by India followed by Pakistan, Nepal, Bangladesh, Sri Lanka, and Bhutan. The total milk production scenarios in South Asia are presented in table 4.

Productivity of buffalo in South Asia

River buffalo produce more milk than the Swamp buffalo. Average lactation yield in the best known dairy breeds, Murrah and Nili-Ravi, is around 2000 litre (Agarwal & Tomar, 1998) although elite buffalo with up to 6000 litre also exist in India, Italy and Pakistan, which indicates its great potential for milk production. The productivity of buffalo has been recorded 410 $kg^{-1}buffalo^{-1}year$, 1880 $kg^{-1}buffalo^{-1}year$, 1934 $kg^{-1}buffalo^{-1}year$ and 867 $kg^{-1}buffalo^{-1}year$, 508 $kg^{-1}buffalo^{-1}year$ in Bangladesh, India, Pakistan, Nepal and Sri Lanka respectively (FAO, 2014). Highest productivity recorded in Pakistan and lowest in Bangladesh. The production potentiality of buffaloes in different countries is presented in table 4. According to production potentiality, it is observed that Pakistan and India has the highest milk producing buffaloes than others. Pakistani buffalo Nili-Ravi is the better performing buffalo in the world for milk production. The highest production recorded in elite herd is 2500 litre per 305 days lactation while average production at commercial herd is 1800-2500 litre per 322 days lactation (Pak Dairy Info, 2017). Average milk production of Pakistani buffaloes is 5-7 litre day-1. India is home to great biodiversity of buffalo germplasm, including the world famous Murrah buffaloes-renowned for high milk production potential. The average milk production of Indian buffaloes is 5-6 litre/day. The productivity of buffaloes in different countries are given in table 5.

Table 4. Total milk production in South Asia

Country	Total Milk Production (MMT)	Buffalo Milk Production (MMT)	Percentage of buffalo milk (MMT)
Bangladesh	7.2	0.1	1.4
India	146	74.7	51.2
Nepal	1.8	1.2	66.6
Pakistan	42	25	59.5
Sri Lanka	0.25	0.045	18.0
Total	197.25	100.74	

Source: FAO, 2014 & Hamid et al., 2016

Table 5. Average buffalo milk production in different countries

Country	Average milk yield (kg/animal/year)
Pakistan	1934
India	1880
Vietnam	1000
Nepal	867
Sri Lanka	508
China	563
Bangladesh	410
Italy	815.9
Asia	1389

Buffalo milk quality

Comparative milk composition in buffalo, cow and goat is given in table 6. Buffalo milk is healthy as it is richer in saturated fatty acids. It's much higher total solids (18–23% vs. 13–16%) is useful for making cheese, butter fat, several kinds of traditional sweets and ice creams. Swamp buffalo milk has even higher fat (9–15%), protein (7.1%), lactose (4.90%) and ash (0.89%) (Thac, 1979). Buffalo milk is especially important, and priced higher in Italy, for making Mozzarella cheese (Nanda and Nakao, 2003).

Table 6. Composition milk in buffalo, cow and goat

Constituents	Buffalo	Cow	Goat	Skimmed Milk
Moisture (gm)	81.00	87.50	86.80	92.10
Protein (gm)	4.30	3.20	3.30	2.50
Fat (gm)	6.50	4.10	4.50	0.10
Minerals (gm)	0.80	0.80	0.80	0.70
Carbohydrate (gm)	5.00	4.40	4.60	4.60
Energy calories (kCal)	117.00	67.00	72.00	29.00
Calcium (mg)	210.00	120.00	170.00	120.00
Phosphorus (mg)	130.00	90.00	120.00	90.00
Iron (mg)	0.20	0.20	0.30	0.20

Source: Gupta, 2001

Buffalo genetic improvement

Genetic improvement of large number of buffaloes under different stakeholders in South Asia demands on quality buffalo male germplasm. The easiest way of multiplication of superior germplasm and genetic improvement of buffalo could be

done through artificial insemination (AI). The production of quality male germplasm should be through progeny testing programme. Lack of infrastructure especially lack of animal identification and recording system is the most limiting factor in this regard. In South Asia the animal breeding technology like AI are not even widely adopted because of non-availability of quality male buffalo germplasm at the door step of farmers, lack of breeding infrastructure, non-availability of quality fodders, lack of awareness of rural households and many other problems directly and indirectly associated with the genetic improvement of buffaloes. As a result, the productivity of buffalo is low in South Asia. The adoption of AI reduces the indiscriminate use of breeding bulls, inbreeding among animals and also the chance of reproductive problems of buffaloes due to various sexually transmitted diseases. The breeding policy would be

A. For organized dairy farm

1. Selective breeding of high producing buffalo using most adapted breeds in the institute/ commercial herds under high input-high output production system of the respective country.

2. Cross breeding improver breeds/s and inter se mating using crossbred buffaloes using in organized dairy farm under low input- medium output production system in each country.

B. Un-organized dairy farm

1. Selective breeding for buffalo with the same breed under low input- medium output production system of the respective country.

Nutrition and health care

To enhance productivity, farmers have to be given adequate awareness on balanced feeding of animals. The farmers will be supported with the supply of good quality seeds and seedlings to develop improved pasture where feasible. Farmers will be encouraged to utilize agricultural by- products to increase efficiency and reduce production cost. Awareness to farmers on fodder conservation crop residues enrichments technologies, contract fodder production to make fodder available for feeding the animals round the year will be pursued rigorously. Proactive animal health action plans to prevent infection and parasitic diseases as well as zoonosis and effective laboratory diagnostic services, timely monitoring shall be instituted within the existing regional and national laboratories.

Advantages of buffalo husbandry over cattle

Work/Animal traction

The water buffalo is an important beast of burden in Asian farming. It is widely used to plough, level land, plant crops, puddle rice fields, cultivate field crops, pump water, haul carts, sleds and shallow-draft boats. It is also used to carry people, thresh grain, press sugar cane, haul logs, and more. Buffalo have an advantage over other

draught animals in wet or muddy areas, with their large hooves. Their legs can withstand wet conditions better than cattle. However they are not as fast as cattle, horses or mules. This puts them at a disadvantage in dryer areas. Therefore, the additional income every year through the sale of surplus milk is vital to their well-being and economic security (Agarwal & Tomar, 1998). In India and Pakistan, male Riverine buffalo contribute to 6–12% and 1–2% of farm power, respectively, but provide much of the road haulage. Together with cattle, buffalo plough 30 million hectares of land in India (Nanda et al., 2002).

Utilization of feeds: conversion of meat and milk

The most important and desirable quality of the water buffalo is its extraordinary capacity of utilization less digestible feeds (straw, sugarcane wastes etc.) than cattle. So it requires less concentrate feeds than cattle. This mean that it can produce excellent quality food meat and milk using only crop residues, pasture, and mineral salts, without the addition of supplement concentrates (Hamid et al., 2016). The nutrient requirements of buffalo steer include 0.24 kg DCP, 1.8 kg TDN, 6.6 MCal ME, 14 g Ca, and 11 g P. On *adlibitum* and high concentrate (75:25) based rations the growth rate is 610 g/day with feed efficiency of 7:1. On all roughage rations (Green berseem/ berseem hay) the growth rate is 370 g/day with feed efficiency of 10:1 (Ranjan and Pathak, 1979).

Resistances to diseases

Buffalo is high adaptable and healthily animal that can resist infectious and contagious diseases. However, they must receive the same vaccines, hygienic care and dedicated attention, as like as cattle. Buffaloes have higher degree of resistance and tolerance than cattle against many diseases (Deb et al., 2016).

Soil fertility

Buffalo enrich soil structure and fertility while tracking paddy field. Each year, an adult buffalo produce 4-6 tones of wet manure plus additional urine as bio-fertilizer. This reduces the requirement of chemical fertilizers as well provide soil humus that chemical cannot provide.

Resistance to climatic hazard

Buffalo can survive against tidal wave better than any other livestock species. This is evidence from the cycles that occurs frequently in the coastal area of Bangladesh. Buffalo is the species that can easily survive in coastal ecosystem. Buffalo can be considered one of the suitable options for livelihood improvement in coastal areas (Faruque, 2016).

Housing

Buffalo do not require expensive houses as like cattle. It can even live in open air throughout the year. For 99% of buffaloes in Bangladesh, there is no house at all. This is true in many parts of world including South Asia (Faruque, 2016).

Inherent qualities as meat producer

Buffaloes have a unique ability to utilise coarse feeds, straws and crop residues converting them into protein rich lean meat. Hence buffaloes fit well in poor countries having poor feed resources. Buffalo properly managed and fed as a meat producing animal and slaughtered at 16 to 20 months of age yields a highly satisfactory top quality meat at a much lower cost than the cattle (Ranjan and Pathak, 1979). Since buffaloes have been used as draught animals for centuries, they have evolved with exceptional muscular development. Until recently, little thought was given to use them exclusively for meat production. Buffaloes are lean animals. The sub-cutaneous fat layer of the carcass is usually thinner than that on comparably fed cattle. Fat is low even under feed lot conditions. More lean and less fat compared to cattle, has created a demand for it among health conscious consumers (Kondaiah, 2002).

Increasing popularity of buffalo

The popularity of buffalo dairy farming is increasing day by day in the Indo-Pak subcontinent due to following reasons (Singh, 2013)

- Quick acclimatization to varied climates
- Efficiently thrives on poor forage/ roughage
- Less prone to prevalent diseases than crossbred cows
- Better marketability of surplus males and females
- Better productivity than most indigenous cow breeds
- Higher consistency of milk production
- Better taste and healthy milk-fetches higher price
- Higher fat & SNF - more suitable for sweet meat, yogurt, and cheese making.

Constraints of buffalo development in South Asia

Singh et al. (2013 and Hamid et al. (2016) pointed out the following constrains for development of buffaloes in SAARC region:

- Lack of high yielding buffalo breed in SAARC region except India and Pakistan. The indigenous buffaloes are low producers
- Lack of sufficient number of proven bulls
- Lack of breeding infrastructure
- lack of animal identification and recording system
- Slow adoption of AI and other reproductive biotechnology
- Lack of long-term breed development policy and research programme
- Scarcity of quality feeds, fodder and pasture land

- Higher calf mortality
- Seasonality of reproduction
- Impact of global warming
- Lack of knowledge about the quality of buffalo milk and meat
- Lack of public awareness about buffalo husbandry
- Lack of technical skills about buffalo production of farm holders
- Lack of coordination within research and government organizations, universities, NGOs and beneficiaries

Recommendations of buffalo development in South Asia:

Support product processing and marketing facilities

More dairy groups will be formed and encouraged to take up buffalo farming and they will be supported with required product processing, diversification and marketing facilities. The dairy groups/cooperatives will be supported for establishment of vital infrastructures such as milk collection centers for collection and marketing of fresh milk, milk processing units for collection and processing of local dairy products and dairy sales counter in every town for marketing of products. Through periodic market information and research, preferred dairy products from buffalo milk will be explored. There is an opportunity for the production of mozzarella cheese from buffalo milk. There are feasible small to medium scale processing plants will be encouraged to diversify such products.

Capacity building

The farmers and extension workers lack skills and knowledge in improved buffalo breeding and husbandry practices. Need assessment will be done and training will be imparted to national and field including farmers on buffalo breeding and improved buffalo husbandry practices. Routine disease screening and preventive health care will be emphasized through timely up-dation of skill and knowledge of field staff. National Buffalo Information and Recording System (NBIS) shall be instituted which will ensure traceability for performance recording, disease outbreaks and will help in instituting progeny testing program in the long run. Where possible, awareness and sensitization will be given and farmers will be encouraged to form breeders groups and association to produce quality animals which will form the basis for sustainability of buffalo farming in the country.

Knowledge and skill of national professional will be enhanced to take up research and development works and a provision within national buffalo breeding program shall be created for communication of research findings to the farmers to enhance production. To strengthen networking mechanism and institutional linkages among the buffalo research and production centers within the region, with Asian and world buffalo federations for exchange of gemplasm, scientist visits and information

exchange on latest research and development works can improve and sustain buffalo farming in the region.

Long term intervention areas for buffalo breed improvement program

The combination of AI with Multiple Ovulation and Embryo Transfer (MOET) as advanced reproductive bio-technology approach shall be initiated in a long run which can immensely enhance the rate of genetic gain and productivity in buffalo population. The application of other bio-technologies such as embryo manipulation (splitting, sexing and cloning) for faster multiplication of superior germplasm from highly selected elite donors and facilitate to achieve the target of producing large number of superior bull calves/bulls and their adequate number of quality semen doses are other promising breeding options available for application. Molecular genetic studies for identification of genes of interest (milk quality and quantity traits and Marker Assisted Selection (MAS) are other breeding tools available for identification of superior animals and commercial exploitation of genetic potential of indigenous breeds. Besides, MOET, there may be a limited application of other advanced breeding tools for buffalo breeding in the region.

Considering the constrains and present socio-economic trends in buffalo production and marketing, the following suggestions are being considered:

- Develop/strengthen breeding infrastructure in the region.
- In intensive production system, continuous up gradation of native buffaloes in the plain land with imported semen of Murrah, Nili-Ravi or Mediterranean breed.
- In semi-intensive production system, crossbreeding of native buffaloes with Murrah or Nili Ravi and fixed 50% exotic bloods followed by inter se mating.
- In low input production system, selective breeding should be followed.
- Feeding, disease preventive measures and other husbandry practices should be addressed properly.
- Quality fodder seed production farms should be established. Increase the land for fodder production should be ensured.
- Reproductive biotechnology should be utilized.
- Public awareness for benefit of buffalo production and buffalo milk and meat should be created by different media; Radio like TV, newspaper etc.
- Buffalo milk and meat market and infrastructure should be developed.
- Development of skill manpower by technical training should be ascertained.
- National research and international collaboration should be strengthened.
- Private investment is to be explored and encouraged to invest in buffalo development infrastructure including marketing of milk and meat products in the country.

CONCLUSION

The buffalo dairying in the region is characterized by small-scale, scattered and unorganized buffalo holders; low productivity; inadequate and inappropriate animal feeding and health care; lack of assured year-round remunerative price for milk production; inadequate basic infrastructure for provision of production inputs and services; inadequate basic infrastructure for procurement, transportation, processing and marketing of milk; and lack of professional management. Low productivity of dairy buffaloes is a serious constraint to dairy development in the region. The productivity of dairy buffaloes could be increased by adoptive appropriate breeding strategies. The breeding policy should not only focus on milk yield but should also provide for the production of good quality breeding bulls. Selective breeding with high yielding purebreds, such as Murrah, Nili Ravi, should be given high priority in their home tract and same may be practices for buffaloes maintained under low input system.

REFERENCES

Agarwal, S.K., and Tomar, O.S. (1998). Reproductive Technologies in Buffaloes. Indian Veterinary Research Institute, Izatnagar, India.

Chakravarty, A.K. (2013). Strategies for genetic improvement of buffaloes through production of quality male germplasm in SAARC countries. Paper presented in the seminar on "High Yielding Dairy Buffalo Breed" at SAARC Agriculture Centre, Dhaka on December, 2013.

Deb, G.K., Nahar, T.N., Duran, P.G., and Presicce, G.A. (2016). Safe and sustainable traditional production: The water buffalo in Asia. *Frontiers in Environmental Science*, 4, p.1-7.Doi: 10.3389/fenvs.2016.00038.

FAO. (2014). FAOSTAT Database. Food and Agriculture Organization of the United Nations, Rome, Italy. Available at: http://faostat.fao.org/.

Faruque, M.O. (2016). Buffalo production in Bangladesh. *Asian Buffalo Magazine*, Jan-Dec. 1-7.

Gupta, S. (2001). Handbook of dairy formulations processes and milk processing industries. Engineers India Research Institute, New Delhi

Hamid, M.A., Siddiky, M.N.A., Rahman, M.A., and Hossain, K.M.(2016).Scopes and opportunities of buffalo farming in Bangladesh: A Review. *SAARC Journal of Agriculture*, 14(2), 63-77.

Kondaiah, N. (2002). Meat and by products. In: Handbook of Animal Husbandry. 3rd ed. (Ed. P.S, Dharma). New Delhi, India: DIPA, ICAR, p. 950-975.

Nanda, A.S., Brar, P.S., and Jain, A.K. (2002). Management of buffalo reproduction in India. Proceedings of the First Buffalo Congress of Americas, 16–17 May, Belem Para, Brazil.

Nanda, A.S., and Nakao, T. (2003). Role of buffalo in the socioeconomic development of rural Asia: Current status and future prospectus. *Animal Science Journal*, 74, 443–455.

Pak Dairy Info, 2017. Pakistan first online dairy farming guide. Retrieved on 24 November 2017 from http://www.pakdairyinfo.com/niliravi.htm

Ranjan, S.K., and Pathak, N.N. (1979). Management and feeding of buffaloes. Vikas Publishing House, New Delhi, India.

Siddiky, M.N.A., and Tareque, A.M.M. (eds). (2014). Farm Animal Genetic Resources in SAARC Countries: Diversity, Conservation and Management. SAARC Agriculture Centre, Dhaka, Bangladesh, p. 1-431.

Siddiky, M.N.A. (ed). (2015). SAARC Dairy Outlook. SAARC Agriculture Centre, Dhaka, Bangladesh, p. 1-160.

Singh, I. (2013). Buffalo production in India. Paper presented in the seminar on "High Yielding Dairy Buffalo Breed" at SAARC Agriculture Centre, Dhaka on December, 2013.

Thac, N.D. (1979). Results of studies on F1 crossbreed of Murrah and Vietnamese local buffaloes. Agricultural Publishing House, Hanoi, Vietnam. Results of Research on Science and Technology (1969–1979), p. 154–163.

GUIDELINES OF THE JOURNAL

CALL FOR PAPER

SAARC Agriculture Centre invites research and review papers to be published in the SAARC Journal of Agriculture (SJA). It is half yearly journal, i.e. two issues in a year, first issue at June and second at December of each year.

AIMS AND SCOPE OF THE JOURNAL

SAARC Journal of Agriculture is a half yearly peer-reviewed an open access online journal that publishes original research articles, review papers and short communications in the fields of agriculture, animal sciences, fisheries, natural resources management and allied disciplines. It is an official journal of SAARC Agriculture Centre (SAC). The scientists and researchers from SAARC countries are encouraged to submit their research and review papers for publishing in this journal.

MODE OF SUBMISSION

Authors are requested to submit their manuscript to managing editor through electronic submission process via email- saarcjournal@yahoo.com. Author guidelines and other relevant information are available in the web link of http://www.banglajol.info/index.php/SJA/index.

AUTHOR GUIDELINES

Terms of Submission

The journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers must be submitted on the understanding that they have not been published elsewhere and are not currently under consideration by another journal published by any other publisher. The submitting author is responsible for ensuring that the article's publication has been approved by all the other co-authors. It is also the authors' responsibility to ensure that the articles emanating from a particular institution are submitted with the approval of the necessary institution. Only an acknowledgment from the editorial office confirms the date of receipt. Further correspondence and proofs will be sent to the author(s) before publication unless otherwise indicated. It is a condition of submission of a paper that the authors permit editing of the paper for readability. All enquiries concerning the publication of accepted papers should be addressed to saarcjournal@yahoo.com.

Manuscript Preparation

Manuscripts text should be within 4000 words including references. Manuscript should be written in English in Microsoft Word using font “Times New Roman” and font size 12. The manuscript must be double-spaced. The double-spacing requirement

applies to all written material, including footnotes, references, tables, and figure captions. Authors are requested for limited use of italics, bold, and superscripts and subscripts, however, scientific names should be typed in italics. Word processing features such as automatic footnoting and outlining must be avoided and if numbered list is required to place in the manuscript enter the numbers and use appropriate tabs and indents by hand instead of using automatic outlining.

Manuscripts should be arranged in the following order:

1. Title, Running title and byline.

2. Author–paper documentation (addresses/affiliations, email address of the corresponding author, etc.).

3. Abstract.

4. Introduction.

5. Materials and Methods.

6. Results (sometimes combined with the discussion).

7. Discussion

8. Conclusion

9. Acknowledgments (optional).

10. References.

11. Figure captions, then tables, then the figures themselves.

Title

The title should represent the content of the article. The terms in the title should be limited to those words that give significant information about the content of the article. It is discouraged to start title with 'Study on', 'Effect of', and 'Influence of.' An ideal title briefly identifies the subject, indicates the purpose of the study, and introduces key concept. Scientific names may be given where necessary.

Authorship

There should be a cover page which includes title and all the names of authors. It is encouraged to use full names of authors in bylines. The first person listed in the title is considered as the senior author. An asterisk (*) follows the name of an author denotes the corresponding author and is matched to the words "*Corresponding author" at the bottom of the cover page. In this place addresses for all authors, email address and phone number for the corresponding author are included.

Peer Review Policy

Manuscripts will be sent to reviewers via e-mail for review the manuscripts electronically using track change mode. All manuscripts are subject to peer review and are expected to meet standards of academic excellence. All articles are initially

screened by the managing editor/ associate editor solely or with the help of members of editorial board. If the article is found to be in order as per the 'Guidelines' and there is adequate original information, it is sent to an expert for peer review. On the contrary, the articles that contain inadequate information or are not conform to 'Guidelines' are rejected as such or returned to the author for revision. After the article has been examined by the reviewer or followed by over viewer, it is also suitably edited by a relevant member of Editorial Board. Both reviewer/ over viewer and editorial board comments along with the manuscript are passed on to an author, who resubmits it after incorporating suggestions and comments. The thoroughly revised article will be checked by the editorial board and managing editor in the light of review/ over viewer comments whether it would be worth for publishing or not. The articles accepted for publication by editorial team are checked by a professional editor for English language, uniformity and any other ambiguity before sending to the press for composing. Proofs are mailed to the corresponding authors if needed and are also gone through by the managing editor/ associate editor/ member of editorial board.

Article Processing Charges

Articles are published in the SAARC Journal of Agriculture (SJA) free of charge.

Title and Authorship Information

The following information should be included

- Paper title
- Full author names
- Full mailing addresses
- Email addresses

Abstract

The manuscript should contain an abstract. The abstract should be self-contained and citation-free, single paragraph and should not exceed 200/250 words. An ideal abstract may contain background, rationale, objectives, materials & methods, results and conclusions.

Keywords

Up to 10, in alphabetical order and separated by comma.

Introduction

This section should be succinct, with no subheadings.

Materials and Methods

This part should contain sufficient details so that all procedures can be reflected. It can be divided into subsections if several methods are described. It should include year and place of study.

Results

The content of this section should permit full comprehension of the data reported in figures and tables.

Discussion

A comprehensive discussion section is required to justify the results. Normally a comparison between your results and results from previous works should be given in the Discussion. The Results and Discussion could be merged in a single section if necessary.

Conclusions

This should clearly explain the main findings of the work highlighting its importance and relevance.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full. Please check this section carefully before publication, as amendments or corrections are not allowed after publication

SHORT COMMUNICATIONS

Short Communications typically describe research techniques, apparatus, and observations which were not confirmed normally by repetition (preliminary findings based on a single experiment). These articles are usually shorter than research papers and there are no individual abstract, introduction, materials and methods, results and discussion. Instead, they are written in continuous form without any sub headings.

References

1. In general, not more than 10-15 references would be required. Recent and relevant not more than 20 years old references are encouraged.
2. There is no need to give references for standard procedures of soil and plant analysis, as well as for routine statistical analysis; only the methodology may be indicated.
3. All references quoted in the text must appear at the end of the article and vice-versa. The spellings author's names and dates or years at the two places should be carefully checked.
4. The references should include names of all authors, years (not within brackets), full title of the article, full name of the journal (in italics) (no abbreviations), volume number, issue number, and pages. For book or monograph, the name of the publisher should also be given as well as its volume, edition and relevant pages.
5. The references cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically on author's names, and

chronologically per author.

6. References from standard scientific journals should be preferred, while those concerning unpublished data are generally to be avoided or mentioned as 'Personal communications' in the text. These need not be given in the reference list. A few examples for correct citation of references in the list are given below:

Journal Article

Buhler, D.D. and Mester, T.C.1991. Effect of tillage systems on the emergence depth of giant and green foxtail. *Weed Science*, 39: 200-203

Mowla, G.M., Mondal, M.K., Islam, M.N. and Islam, M.T. 1992. Farm level water utilization in an irrigation project. *Bangladesh Rice Journal*, 3 (1&2): 51-56

Rahman, M.M. 1990. Infestation and yield loss in chickpea due to pod borer in Bangladesh. *Bangladesh Journal of Agricultural Research*, 15(2): 16-23

Book/Bulletin/Reports/Series

Bhuiyan, S.I. 1982. Irrigation system management research and selected methodological issues. *IRRI research paper series no 81*. Los Banos, Manila

De Datta, S. K. 1981. Principles and practices of rice production. Los Banos, Manila

International Rice Research Institute. 2000. International rice trade: a review of 1999 and prospects for 2000. *International Rice Commission Newsletter*, IRRI, Manila

Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics: A biometrical approach. 2nd ed. McGraw-Hill, New York

Westerman, R.L. (ed.) 1990. Soil testing and plant analysis. 3rd ed. SSSA Book Ser. 3. SSSA, Madison, WI

Chapter in a Book

David, H. and Easwaramoorthy. 1988. Physical resistance mechanisms in insect plant interactions. p. 45-70. In T.N. Ananthakrishnan and A. Rahman (ed.), *Dynamics of insect plant interactions: Recent advances and future trends*. Oxford and IBH Publication, New Delhi

Johnson, D. W. and D. E. Todd. 1998. Effects of harvesting intensity on forest productivity and soil carbon storage. p. 351–363. In R. Lal et al. (ed.) *Management of carbon sequestration in soils*. Advances in Soil Science. CRC Press, Boca Raton, FL

Conference/Symposium/ Proceedings

Joshi, B.K. 2004. Crossing frequency and ancestors used in developing Nepalese mid and high hill rice cultivars: Possible criteria for yield improvement and rice genes conservation. p. 502-523. In Proc. *National Conference on Science and Technology*, 4th, Vol. 1. 23-26 Mar., 2004. NAST, Kathmandu, Nepal.

Ramanujam, S. (ed.) 1979. *Proceedings of International Wheat Genet Symposia*, 5th, New Delhi, India. 23–28 Feb. 1978. Indian Soc. Genet. Plant Breeding, Indian Agric. Res. Inst., New Delhi.

Dissertation

Singh, A.A. 2005. Weed management approaches and modeling crop weed interaction in soybean. M. Sc. (Ag.) thesis. Tamil Nadu Agricultural Univ., Coimbatore.

Software and Software Documentation

Minitab. 1998. MINITAB 12. Minitab, State College, PA.

Online publication

Venugopal, D. 2000. Nilgiri tea in crisis: Causes consequences and possible solutions. Retrieved October 11, 2000 from http://www.badaga.org.

Online journal article

Doerge, T.A. 2002. Variable-rate nitrogen management creates opportunities and challenges for corn producers. Crop Manage. doi:10.1094/cm-2002-0905-01-RS.

Tables

1. Each table must be typed on a separate sheet (not to be included in the text) and numbered consecutively in the same order as they mentioned in text.
2. The title should fully describe the contents of the table and explain any symbol or abbreviation used in it as a footnote, using asterisks or small letters viz. a, b, etc.
3. Tables should be self-explanatory, not very large (< 10 columns in portrait and <14 columns in landscape formats respectively) and may cover space up to 20-25% of the text.
4. Maximum size of table acceptable is that can be conveniently composed within one full printed page of the journal. The large sized tables should be suitably split into two or more small tables.
5. Standard abbreviations of units of different parameters should be added between parentheses.
6. The data in the tables should be corrected to minimum place of decimal so as to make it more meaningful.
7. Vertical lines should not be used to separate columns. Similarly, horizontal lines should be used only where these are necessary, not in the body of the article.
8. All tables should be tagged with the main body of the text i.e. after references.

Figures

1. Figures may be given in place of tables where a large number of values are presented that can be interpreted through figures. In no case the same data should be presented in both tables and figures.

2. Originals of the figures should be no larger than twice the final size, of good quality and printed clearly in black on plain white paper or in color. The figures may be sized to fit within the columns of the journal (8 cm width for single column or 17 cm for columns i.e. full page).
3. Lines should be bold enough to allow the figure to be reduced to either single or double column width in the journal.
4. Black and white photographs are also accepted if these are necessary to improve the presentation and quality of the article.

Some useful hints

1. All scientific or technical names as well as all data and facts must be rechecked carefully before submitting the manuscript.
2. Dates and years may be mentioned as 28 May 2007, 28 May to 7 June, and 28-30 May instead of May 28, 2007, 28 May-7 June, and 28 to 30 May, respectively.
3. Avoid numerals and abbreviations at the beginning of a sentence; spell out or change the word order if necessary.
4. A comma may be used for data in thousands or more such as 10,000 or 2,30,000 etc. Alternatively, these data can also be presented as 10.0 or 230.0 if a common expression such as ' $x10^3$' is used in tables or figures. Avoid expressing data in 'lakhs', instead use 'thousand' or 'million'.
5. Only standard abbreviations should be used and these should invariably be explained at first mention. Avoid use of self-made abbreviations such as Rhizo., Azo., buta, isop. etc. for *Rhizobium, Azotobacter*, butachlor, isoproturon, respectively.
6. For names of plant protection chemicals, the first letter of the name need not be capitalized for scientific names but should be capitalized for trade names. All the names should be checked very carefully.
7. Use of unnecessary abbreviations and treatment symbols such as T1, T2 etc. under Materials and Methods or tables without actually using these under Results and Discussion should be avoided.
8. All weights and measurements must be in SI or metric units. Use kg ha^{-1}, or t ha^{-1} but not q ha^{-1}. Do not follow the style g/ha, mg/kg, mg/l, mg/g, ml/l or g per ha, etc
9. Use % after numbers, not per-cent, e.g. 7%. In a series or range of measurements, mention the units only at end, e.g. use 30, 100, 170 and 300C; 20 or 30% more instead of 30C, 100C, 170C and 300 °C; 20% or 30% more.
10. Numeral should be used whenever it is followed by a unit measure or its abbreviations e.g. 1 g, 3 m, 5 h, 6 months etc. Otherwise, words should be used

for numbers one to nine and numerals for larger ones except in a series of numbers when numerals should be used for all in the series.

11. For the composition of fertilizers, manures, crops or soil, the elemental forms (K, P, Mg etc.) should be used and not the oxides.
12. Statistical analysis of data in the standard experimental design should be sound and complete in itself with both ±SE (Standard Error of means) and CD (Critical Difference) or LSD (Least Significant Difference) (P=0.05) values given for comparison of treatment means in tables and figures.
13. Use multiplication sign (×) not a alphabet x for indicating multiplication, crossing, etc

SAARC Agriculture Centre (SAC) is the first SAARC Regional Centre established in 1988 at Dhaka, Bangladesh with an overall objective of promotion of agricultural research and development as well as technology dissemination initiatives for sustainable agricultural development and poverty alleviation in the region.

SAARC Journal of Agriculture (SJA), a half yearly publication from the Centre, is envisaged to serve as a platform for exchange of latest knowledge on breakthrough topics that are of current concern for researchers, extensionists, policy makers and students. It aims to capture the first-hand knowledge on research achievements in the field of agriculture, fisheries, livestock, forestry and allied subjects from the SAARC member countries. SAARC Agriculture Centre welcomes your feedback and suggestions for improving the quality of the journal.