ISSN 0258 - 7122

BJAR

Volume 40 Number 1
March 2015

Bangladesh Journal of Agricultural Research

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Bangladesh
Journal of
AGRICULTURAL
RESEARCH

Volume 40 Number 1
March 2015

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Rate of Subscription
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US $ 10.00 per copy (abroad)

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BANGLADESH JOURNAL OF AGRICULTURAL RESEARCH

Vol. 40	**March 2015**	**No. 1**

C O N T E N T S

ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 1-16, March 2015

THE THIRD GENOTYPIC CLUSTERS OF *Bemisia tabaci* (GENNADIUS) (HEMIPTERA: ALEYRODIDAE) FOUND IN BANGLADESH

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Abstract

The sweetpotato whitefly, *Bemisia tabaci* is a species complex that possessed several biotypes including different genotypic clusters within species, which may differ from each other genetically and physiologically but morphologically alike. This study was performed by molecular analysis for easy identification of whitefly and describes its biotype throughout Bangladesh. Whiteflies have been identified from different places of Bangladesh based on mitochondrial cytochrome oxidase subunit I (mtCOI) gene and 16S ribosomal RNA gene sequences analysis. The mtCOI sequences of BW3 (collected from eastern part of Bangladesh) whitefly were diverged by 14.5% and 15.1% compared with B and Q biotypes from Korea and it also diverged by 15.4% and 13.7% from each other compared to BW1 (collected from southern part of Bangladesh) and BW2 (collected from northern part of Bangladesh), respectively within the country. The 16S rRNA sequences of BW3 whitefly were more deviated by 41.5%, 10.7%, 42.7% and 12.6% compared with the country populations from BW1, BW2, B and Q biotypes, respectively. Moreover, it showed high divergences from indigenous whiteflies of southern and northern part of Bangladesh which clustered in a different clade on both mtCOI and 16S rRNA phylogeny. Therefore, till date three genotypic cluster of indigenous whitefly BW1, BW2 and BW3 are identified from Bangladesh.

Keywords: *Bemisia tabaci*, BW3, mtCOI gene, 16S rRNA gene, indigenous whitefly.

Introduction

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a very complex species consists of at least 24 biotypes in tropical and sub-tropical region around the World (Ahmed *et al*., 2009). This devastating global insect pest caused damage directly by sucking the plant sap from phloem, indirectly by excreting honeydews that produce sooty mould, and by spreading 111 plant virus diseases (Martin *et al*., 2000; Jones, 2003; Mughra *et al*., 2008). *Bemisia tabaci* is a genetically different groups of insect that morphologically indistinguishable (Frohlich *et al*., 1999; De Barro *et al*., 2000; Boykin *et al*., 2007). Significant

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attempt has been committed to the analysis of the biological and genetic variation of *B. tabaci* and has been recognized different biotypes into the species (Perring, 2001). Among them, two predominantly aggressive biotypes, known as B and Q, are distributed everywhere around the World (De Barro, 2005; De Barro *et al*., 2005; Chu *et al*., 2006; Martinez-Carrillo & Brown, 2007) whereas, in Bangladesh yet B and/ or Q biotype are absent but indigenous biotype BW1 and BW2 recorded recently (Maruthi *et al*., 2007; Jahan *et al*., 2011; Jahan, 2012). The *B. tabaci* is not genetically consistent. Based on mitochondrial DNA markers, the *B. tabaci* complex can be placed into five major groups according to their geographical origin: (1) New World (US, Mexico, Puerto Rico), (2) Southeast Asia (Thailand, Malaysia), (3) Mediterranean basin (Southwest Europe, North Africa, Middle East), (4) Indian subcontinent (Bangladesh, India, Myanmar, Nepal and Pakistan), (5) Equatorial Africa (Cameroon, Mozambique, Uganda, and Zambia); an additional more distantly related group contained the B relatives from many world sites and the non-B relatives from the Middle East (Frohlich *et al*., 1999). At least three distinct genotypes, apparently indigenous to India, which are also present in China, Malaysia, Nepal, Pakistan and Thailand. These coexist with the B biotype, which was first reported in India in 1999, and has since spread rapidly to other states in south India (Rekha *et al*., 2005). This may support the conclusion of De Barro *et al*. (2005) that using mtCOI and internal transcribed spacer region 1 (ITS1) markers, some individuals from Asia did not fit into either of the two clusters (called Asia1 and Asia2), and they remained classified as ‘unresolved’ in the Asia group. In a similar study, two indigenous *B. tabaci* genotypes have been reported from neighboring Pakistan, and one from the Punjab area was similar to the Indian populations (Brown, 2001; Simon *et al*., 2003), suggesting their wider occurrence. The mtCOI DNA diversity of Asian *B. tabaci* therefore is comparable to that from African populations, where at least five distinct clades of *B. tabaci* have been recorded on cassava (Legg *et al*., 2002; Berry *et al*., 2004).

In this study, the genetic differences between the *B. tabaci* B and Q biotype populations from Korea and indigenous whitefly from Bangladesh which was collected from various places on different host-plants as bean, eggplant, and okra were investigated for identification.

Materials and Method

Whiteflies collection

Samples of adult *B. tabaci* were collected from bean, eggplant and okra of different places of eastern part of Bangladesh such as, Chittagong and Cox’s Bazar in 2012 (Table 1) and were immediately preserved in 99% ethanol (alcohol) and stored at -20^{0}C. Adults of *B. tabaci* B and Q biotypes were collected from whitefly rearing house in Insect Molecular Physiology Laboratory at Kyungpook National University in South Korea on cucumber and tomato plants in 2011 for morphological and genetic sequences comparison of Bangladesh whiteflies.

DNA extraction

Total genomic DNA was extracted from individual *B. tabaci* according to protocol supplied by Invitrogen Purelink Genomic DNA mini kit. After removing from ethanol the sample had been washed with double-distilled water to remove alcohol, individual whiteflies were homogenized in 180 µl genomic digestion buffer using a 1.5 ml microcentrifuge tube and micropestle (homogenizer). Then added 200 µl genomic lysis/ binding buffer (1% SDS, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 25 mM NaCl, Proteinase K 200 mg/ml) and after that immediately added 200 µl absolute ethanol. Subsequently added wash buffer into the genomic column and finally added 20 µl genomic elusion buffer (Invitrogen Purelink, Carisbad, CA, USA). After 1 min incubation at room temperature, samples were centrifuged at about 12000 rpm for 1 min, and the supernatants/pellets were directly used for PCR detection of the secondary endosymbionts or were stored at -20°C for later use. These procedures were followed by Dellaporta *et al*., 1983 and Jahan *et al*., 2011.

Table 1. List of collection with GenBank accession no. for the sequences of collected *B. tabaci* in Bangladesh.

Name of Specimen	Host plant	Location	Year	Gene	GenBank Accession no.
B. tabaci	Bean	Chittagong	2012	mtCOI	JX417071
B. tabaci	Bean	Chittagong	2012	16S rRNA	JX417075
B. tabaci	Bean	Patuakhali	2011	mtCOI	JN018067
B. tabaci	Bean	Patuakhali	2011	16S rRNA	JQ305697
B. tabaci	Eggplant	Chittagong	2012	mtCOI	JX417072
B. tabaci	Eggplant	Chittagong	2012	16S rRNA	JX417076
B. tabaci	Eggplant	Kurigram	2011	mtCOI	JQ305088
B. tabaci	Eggplant	Kurigram	2011	16S rRNA	JQ305699
B. tabaci	Eggplant	Cox’s Bazar	2012	mtCOI	JX417073
B. tabaci	Eggplant	Cox’s Bazar	2012	16S rRNA	JX417077
B. tabaci	Okra	Cox’s Bazar	2012	mtCOI	JX417074
B. tabaci	Okra	Cox’s Bazar	2012	16S rRNA	JX417078

Primer design and PCR amplification

Bemisia tabaci was determined using the genomic DNA which was collected from Bangladesh with the primers listed in Table 2, using Polymerase Chain

Reaction (PCR). All PCR reaction mixture performed in 20 µl volume that included 1 µl of each primer (Forward and Reverse), 1 µl of DNA template and 17 µl smart buffer which were supplied by the manufacturer (Smart taq pre-mix). All PCR reactions were carried out on the PTC-200 DNA engine thermal cycler (MJ Research PTC-200 DNA Engine Thermal Cycler PCR).

Table 2. Nucleotide sequences of primers listed for *B. tabaci* identification.

Gene	Primer Name	Primer Direction	Primer Sequence (5' to 3')	Size (bp)	Reference	Tm. (°C)
mtCOI	C1-J-2195	Forward	TTGATTTTTTGGTCAT CCAGAAGT	860	Simon *et al.* 1994	52°C
	L2-N-3014	Reverse	TCCAATGCACTAATC TGCCATATTA			
16S rRNA	LR-J-12887	Forward	CCGGTTTGAACTCAG ATCATGT	520	Simon *et al.* 1994	55°C
	LR-N-13398	Reverse	CGCCTGTTTAACAAA AACAT			

PCR condition

The mixtures with the cytochrome oxidase sub-unit1 (CO1) primer C1-J-2195(F) and L2-N-3014(R) were amplified in a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) with a 1 minute initial denaturation at 95°C, 35 cycles (1 min at 94°C, 30 sec at 52°C, 2 min at 72°C), and finally by a 5 min extension at 72°C. For 16S rRNA primers LR-J-12887 (F) and LR-N-13398 (R) were also amplified with a 2 min initial denaturation at 94°C, 35 cycles (30 sec at 94°C, 1 min at 55°C, 1 min at 72°C), and finally by a 10 min extension at 72°C.

Gel-electrophoresis

Electrophoresis of amplified PCR products (5 µl) were done using 1.0% agarose gels with 1% TAE buffer at 100 V for 30 minutes with 100 bp ladder as DNA marker and the gels were then stained by 10 µl Ethidium Bromide for 20 minutes. When fragments with the expected size were visible on the gels, then the rest of 15 µl of PCR products were used for sequencing.

Sequence analysis

DNA sequences were aligned using CLUSTAL W (Thompson *et al*., 1994). The aligned sequences were checked and compared the sequences similarity with the online published sequences using BLAST in the National Centre for Biotechnology Information (NCBI). Sequences were aligned and arranged using the Clustal W2 multiple alignments in BioEdit (version 7.0). The sequences divergences calculated by Molecular Evolutionary Genetics Aanlysis (MEGA) among intraspecific, interspecific species, based on Kimura-2-parameter (K2P) distances (Tamura *et al*., 2007). Phylogenetic relationships were inferred by MEGA Software Version 4.0 (Tamura *et al*., 2007) using neibour-joining method (NJ). Bootstrap values were obtained from 1000 replicates. The sequences are deposited in the GenBank database.

Results

Identification of *B. tabaci* in Bangladesh

The sweetpotato whitefly, *B. tabaci* was identified from the collected specimen in Bangladesh using PCR, and compared the similarity of mtCOI and 16S rRNA gene sequences with related information published in NCBI database. The DNA fragment at 860 bp to C1-J-2195(F) and L2-N-3014(R) and at 520 bp to LR-J-12887 (F) and LR-N-13398 (R) primer sets were shown in PCR amplification (Figure 1). The partial mtCOI and 16S rRNA gene sequences have been provided here that was submitted for registration to the NCBI GenBank database (Table1). Moreover, we provide here the comparison of similarity using both mtCOI and 16S rRNA nucleotides in total length of sequences (Table 3). We found that all sequences showed maximum similarity with *B. tabaci* around the World.

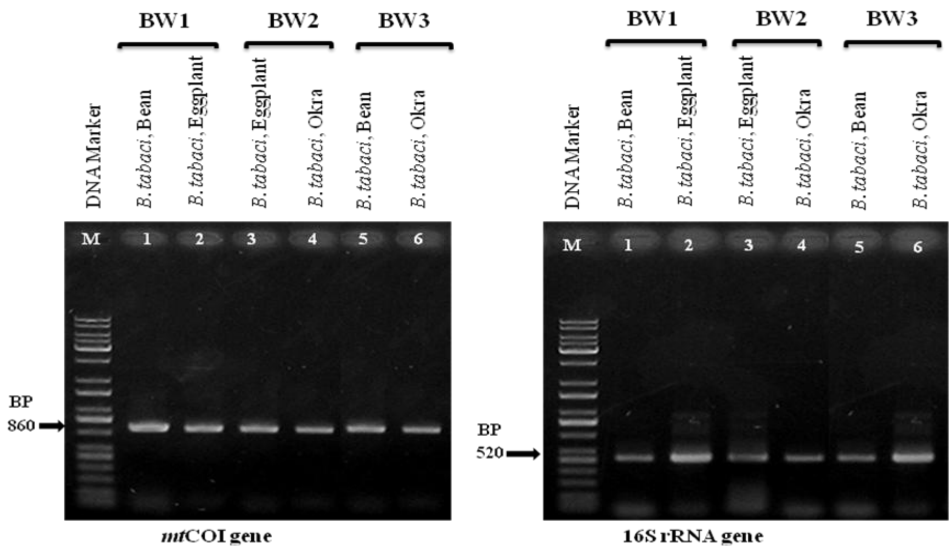

Figure1. PCR Amplification of mtCOI gene region of *B. tabaci* with C1-J-2195 and L2-N-3014 primers and 16S rRNA gene with LR-J-12887 and LR-N-13398 primer set to distinguish *B. tabaci*. M: molecular weight standard-DNA marker (100 bp ladder).

Biotype detection from Bangladesh whiteflies

Biotype of *B. tabaci* collected from Bangladesh has been examined using PCR amplification with 16S rRNA gene sequence digested with restricted enzyme Hinf1 (Figure 1). The result revealed that collected whiteflies from eastern part of Bangladesh are indigenous (native) BW3 biotype (Figure 4 & 5). More interesting things that collected samples from eastern part were shown very dissimilarity from B biotype, Q biotype and previously collected BW1 BW2 whitefly but they all are truly indigenous whitefly (Figure 4 & 5). We revealed another new genotypic cluster of *B. tabaci* (BW3) in Bangladesh. This new genotype of whiteflies showed score of similarity 85.51%, 84.87%, 84.61% and 86.32% with B and Q biotype (Korea), BW1 (southern part of Bangladesh) and BW2 (northern part of Bangladesh), respectively for mtCOI gene (Table 3). The 16S rRNA sequences of BW3 (newly collected from eastern part of Bangladesh) whitefly were shown similarity by 57.32%, 87.38%, 58.54% and 89.29% compared with B biotype, Q biotype, BW1 and BW2, respectively (Table 3).

Table 3. Similarity of both mtCOI and 16S rRNA nucleotide sequences of *B. tabaci* from each other with another biotype.

Sequence of *B. tabaci* (A)	Sequence of *B. tabaci* (B)	Score of Similarity of mtCOI (%)	Score of Similarity of 16S rRNA (%)
B-biotype, Korea	Q-biotype, Korea	94.73	50.0
B-biotype, Korea	BW1, Bangladesh(South)	84.06	91.46
B-biotype, Korea	BW2, Bangladesh(North)	84.85	53.66
B-biotype, Korea	BW3, Bangladesh(East)	85.51	57.32
Q-biotype, Korea	BW1, Bangladesh(South)	83.55	54.15
Q-biotype, Korea	BW2, Bangladesh(North)	84.34	90.24
Q-biotype, Korea	BW3, Bangladesh(East)	84.87	87.38
BW1, Bangladesh(South)	BW2, Bangladesh(North)	90.26	54.88
BW1, Bangladesh(South)	BW3, Bangladesh(East)	84.61	58.54
BW2, Bangladesh(North)	BW3, Bangladesh(East)	86.32	89.29

Sequence analysis

The profile of PCR amplification of 16S rRNA gene with primer, LR-J-12887 (5’- CCGGTTTGAACTCAGATCATGT-3’), LR-N-13398 (5’- CGCCTGTTTAACAAAAACAT-3’) showed consistent and single fragment of DNA in the length of 520 bp. Similarly, the primer set of C1-J-2195(F) and L2-

N-3014(R) demonstrated consistent and single DNA fragment of 860 bp to distinguish the whiteflies including all biotypes (Figure 1). The sequences for all samples were obtained by purification of PCR product using ProMega Gel purified kit and can compare easily among the all sequences by clustalW2 alignment (Figure 2 & 3). Here, it was found dissimilarity score of BW3 are 15.39% and 13.68% for mtCOI gene and 41.46% and 10.71% for 16S rRNA gene compared with BW1 and BW2, respectively (Table 3). Moreover, the sequences of mtCOI and 16S rRNA for BW3 were analyzed based on pairwise distance (character difference) and nucleotide different. It revealed that BW3 was highly divergent from B & Q biotype (Korea), BW1 and BW2, which expressed 110 and 198 nucleotides were different from B biotype, 115 and 51 nucleotides were different from Q biotype, 117 and 204 nucleotides were different from BW1, 103 and 43 nucleotides were different from BW2 in mtCOI and 16S rRNA gene sequences, respectively (Table 4 & 5). These sequences of BW3 had shown the proportions of A+T and G+C in residue compositions of 69.0% and 31.0% for mtCOI as well as 73.7% and 26.3% for 16S rRNA, respectively. The average proportion of T: C: A: G was 42.9: 13.0: 26.1: 18.0 for mtCOI and 30.4: 12.7: 43.3: 13.6 for 16S rRNA, respectively with a narrow standard error around means, but base composition varied substantially in different portions within the sequences of Bangladesh indigenous whiteflies. Among these 760 bp nucleotide, 568 characters were conserved and 192 characters were variable (Table 6 & 7). The sequence divergence in pairwise comparisons revealed that BW3 was very diverse group of whitefly in phylogenetic tree where number of nucleotide changed 33-210 from each other, and lowest distance value was 0.053 and highest was 0.532 among all tested population (Table 4 & 5).

Table 4. Pairwise distance among 5 different biotypes of *B. tabaci* in Bangladesh based on sequences of the fragment of mtCOI gene.

	1	2	3	4	5
[1]		0.099	0.154	0.159	0.165
[2]	75		0.136	0.152	0.157
[3]	117	103		0.145	0.152
[4]	121	115	110		0.053
[5]	125	119	115	40	

Table 5. Pairwise distance among 5 different biotypes of *B. tabaci* in Bangladesh based on sequences of the fragment of 16S rRNA gene.

	1	2	3	4	5
[1]		0.532	0.516	0.084	0.527
[2]	210		0.109	0.514	0.101
[3]	204	43		0.501	0.129
[4]	33	203	198		0.511
[5]	208	40	51	202	

(*Bemisia tabaci* from Southern part of Bangladesh (1), Northern part of Bangladesh (2), Eastern part of Bangladesh (3), B biotype from Korea (4); and Q biotype from Korea (5)). Distance in-between mtCOI gene for 5 different biotypes of *B. tabaci* in Bangladesh (below diagonal: total nucleotide differences, above diagonal: mean character differences) using Kimura 2-parameter (*Bemisia tabaci* from Southern part of Bangladesh (1), Northern part of Bangladesh (2), Eastern part of Bangladesh (3), B biotype from Korea (4); and Q biotype from Korea (5)). Distance in-between 16S rRNA gene for 5 different biotypes of *B. tabaci* in Bangladesh (below diagonal: total nucleotide differences, above diagonal: mean character differences) using Kimura 2-parameter

```
B      AATTTTTCTTTATAAGTTGTT-------CT----TAATCCAAC---ATCGAGGTCGCA-- 44
Q      AATTCTGCTCAATGAGTAAGTTAAATAGCTGCAGTAAATTAACTGTACTAAGGTAGCATA 60
BW1    AATTTTTCTTTATAAGCTATT-------CT----TAATCCAAC---ATCGAGGTCGCA-- 44
BW2    AGCTCTGCTCAATGACAAATTTAAATAGCTGCAGTAAATTAACTGTACTAAGGTAGCATA 60
BW3    AATTCTGCTCAATGATAAACTTAAATAGCTGCAGTAAATTAACTGTACTAAGGTAGCATA 60
       *  * * **  ** *     *       **    ***   ***   *   **** ***

B      ---ATTTATTTTGTAAATGAGAACTTC--TCAAAAATTTTGCGCTGTTATCCCTTAGGTA 99
Q      ATAAGTTGCTTTATAATTAAAAGCTTGAATGAAAGAA---ATAATGTGA------AAGAA 111
BW1    ---ATTTATCTTGTGAATAAGAGCTTC--TCAAAAATTTTGCGCTGTTATCCCTTAGGTA 99
BW2    ATAAATTGCTTTATAATTAAAAGCTTGAATGAAAGAA---ACAATGTGA------AACAA 111
BW3    ATAAATTGCTTTATAATTGAAAGCTTGAATGAAGGAA---ACAATGAAA------AACAA 111
          * **   ** * * * * * ***   * **  *        **  *      *   *

B      ACTTGTTTTATTTCATATTTGGATAATTTAAT-TTGATTTAAATCATTTTT--TTATATC 156
Q      GCTT--TTTAAACTAAAATAGTGAAAATTAATATTTAAGTGAAAAATCTTAAATAGAATC 169
BW1    ACTTGTTTTAATTCTTATTTGGATAGTTTAAC-CTGAGGTAATTCATTTTA--TTATATT 156
BW2    ACTT--TTTAAACTAAAAAAGTAAAAATTAATATCTAAGTGAAAAATCTTAAATTAAATC 169
BW3    ACTT--TTTAATCTAAAAAAGTAAAAATTAGTATTTAAGTGAAAAATCTTAAATTAAATC 169
        ***  ****      *   *   *  ***      *  * *   ** **   *   **

B      TAAAGATTTATTTATTTAAAAATCTCCCCAATCTAACAAATTAACCAAATGAAATTAAGT 216
Q      AAAAGACAAG-------AAGACCCTTTAGAACTTTACAAATTGATTTAATATCATTTAGT 222
BW1    TAAAGATTTATTTATTTAAAAATCTCCCCAACTTGATAAATTAATCTAATGAACATAATT 216
BW2    AAAAGACAAG-------AAGACCCTTTAGAACTTTATAAGTTAAACTATACTCATTAAAT 222
BW3    AAAAGACAAG-------AAGACCCTTTAGAACTTTACAAGCCTCACCATCTTAACTGGAT 222
        *****           ** *  **    **  * * **        *       *   *

B      TAATTTGTAAAGTTCTAAAGGGTCTT-------CTTGTCTTTTGATTTTATTTAAGATTT 269
Q      TAATTTGTTAAATTGGGGAGATTTTTAAATAAATAAATCTTTAGATATAAA--AAAATGA 280
BW1    TAACTTATAAAGTTCTAAAGGGTCTT-------CTTGTCTTTTGATTTAATTTAAGATTT 269
BW2    TGACTTATTAAGTTGGGGAGATTTTTAAATAAATAAATCTTTAAATATAAA--AAAATGA 280
BW3    TGTCTTGTTAAATTGGGGAGATTTTTAAATAAATAAATCTTTAAATATAAT--AAGATGA 280
       *   ** * ** **    **  * **           *****  ** * *   ** **

B      TTCACTTAAATATTAATTTTTACTACTTTAGTTTAAA--AAGCTTGTT------TCACAT 321
Q      TTTAAATCAG-ATTAAATTATCCAAATGTGAAATAAAACAAGTTACCTAAGGGATAACAG 339
BW1    TTCACTTAAATATTAATTTTTACTCTTTTAGTTTAAA--AAGTTTGTT------TCACAT 321
BW2    ATTAACTCAA-GTTAAAACATCCAAATAAGAATAAAAACAAGTTACCTAAGGGATAACAG 339
BW3    TATACTTCAA-ATTAAATTATCCACGTATGAACTAAAACAAGTTACCTAAGGGATAACAG 339
          *  * *   ****    * *   *       ***  *** *   *      * ***

B      TATT---TCTTTCATTCAAGCTTTTAATTATAAAGCAACTTATTATGCTACCTTAGTACA 378
Q      CGCAAAATTTTTGAG--AAGTTCTCATTTACAAAATAAATT-----GCGACCTCGAT--- 389
BW1    TGTT---TCTTTCATTCAAGCTTTTAATTATAAAGCAACTTATTATGCTACCTTAGTACA 378
BW2    CGCAAAACTTTTGAG--AAGTTCTCATTCACAAAATAAATT-----GCGACCTCGAT--- 389
BW3    CGCAAAATTTTTGAG--AAGATCTTATTCACAAAATACATT-----GCGACCTCGAT--- 389
                *** *   *** * * * * * ***  *  **     ** ****   *

B      GTTAATTTACTGCAGCTATTTA-ATTTACTCAT 410
Q      GTTGGATTA--AGAACAACTTATAAAGAAAAAT 420
BW1    GTTAATTTACTGCAGCTATTTA-AATTTGTCAT 410
BW2    GTTGGATTA--AGAATAACTTATAAAGAAAAAT 420
BW3    GTTGGATTA--AGAATAACTTATAGAGAAAAAT 420
       ***   ***    *   * *** *       **
```

Fig. 2. Sequence alignment of Bangladesh whitefly (BW1, BW2 and BW3) and compared with B and Q biotype of Korea using 16S rRNA gene nucleotide sequences by ClustalW2 program.

```
B      TTGTTTCTCATCTAATCAGCAGTGAGGCTGGAAAATTAGAGGTATTTGGAAGGTTGGGTA 60
Q      TTGTTTCTCATTTAATTAGCAGCGAGGCTGGAAAATTAGAGGTATTTGGAAGGTTGGGGA 60
BW1    TTGTTTCTCATTTAATTAGAAGCGAAGCTGGAAAACTTGAAGTATTTGGTAGGTTGGGAA 60
BW2    TTGTTTCTCATTTAATTAGGAGTGAGGCTGGAAAGCTTGAAGTATTTGGCAGATTAGGTA 60
BW3    TCGTTTCTCATTTAATTAGGAGTGAAGCTGGGAAACTTGAGGTATTTGGCAGGTTAGGAA 60
       * ********* **** ** ** ** ***** **   * ** ******** ** ** ** *

B      TAATTTATGCTATATTGACTATTGGTATTCTAGGGTTTATTGTTTGAGGTCATCATATAT 120
Q      TAATTTATGCTATATTGACTATTGGTATCTTAGGGTTTATTGTTTGAGGACATCATATAT 120
BW1    TAATTTATGCTATAGTAACTATTGGAATTCTAGGTTTTATTGTGTGAGGTCATCACATAT 120
BW2    TAATTTATGCTATAGTAACGATTGGCATTCTAGGTTTTATTGTGTGAGGTCATCATATAT 120
BW3    TAATTTATGCTATAATAACTATTGGCATTTTGGGGTTTATTGTTTGAGGTCATCATATAT 120
       ************** * ** ***** **   * ** ******** ***** ***** ****

B      TCACAGTTGGAATAGATGTAGATACTCGAGCTTATTTCACTTCAGCCACTATAATTATTG 180
Q      TTACAGTTGGAATAGATGTAGATACTCGAGCTTATTTCACTTCAGCTACTATGATTATTG 180
BW1    TTACCGTTGGGATAGATGTTGATACTCGGGCTTATTTTACTTCAGCCACTATAATTATCG 180
BW2    TTACTGTTGGAATAGATGTGGACACTCGGGCTTATTTTACTTCAGCTACTATGATTATTG 180
BW3    TTACTGTTGGTATAGATGTTGATACTCGAGCTTATTTTACTTCAGCTACTATGGTTATTG 180
       * ** ***** ******** ** ***** ******** ******** *****   **** *

B      CTGTTCCCACAGGAATTAAAATTTTTAGTTGGCTTGCTACTTTGGGTGGAATAAAGTCTA 240
Q      CCGTTCCTACAGGAATTAAAATTTTTAGTTGGCTTGCTACTTTGGGTGGAATAAAGTCCA 240
BW1    CTGTTCCGACCGGAATTAAAATCTTTAGGTGACTTGCTACTCTAGGTGGAATAAAATCTA 240
BW2    CTGTTCCGACTGGAATTAAAATCTTTAGGTGACTTGCTACTCTAGGTGGAATAAAGTTTA 240
BW3    CTGTTCCAACTGGGATTAAGATTTTCAGGTGGCTTGCTACTTTAGGTGGAATAAAATCCA 240
       * ***** ** ** ***** ** ** ** ** ********* * *********** *   *

B      ATAAATTAAGGCCTCTTGGCCTTTGATTTACAGGATTTTTATTTTTATTTACTATAGGTG 300
Q      ATAAATTCAGGCCCCTTGGCCTTTGATTTACAGGATTTTTATTTTTATTTACTATAGGTG 300
BW1    ACAAGTTTAGTCCTCTTGGACTTTGGTTTACTGGATTTCTTTTTTTATTTACCATGGGTG 300
BW2    ACATATTTAGTCCGCTTGGACTTTGTTTTGCTGGATTTCTTTTCTTATTTACTATGGGTG 300
BW3    ATAAATTAAGGCCGTTAGTTCTTTGATTTACAGGATTTTTATTCTTATTTACCATGGGTG 300
       * *  ** ** **  * *  ***** *** * ****** * ** ******** ** ****

B      GGTTAACTGGAATTATTCTTGGTAATTCTTCTGTAGATGTGTGTCTGCATGACACTTATT 360
Q      GATTAACTGGAATTATTCTTGGTAACTCTTCTGTAGATGTGTGTTTGCATGACACTTATT 360
BW1    GGTTAACTGGGATCATTCTTGGTAATTCCTCTGTTGATGTCTGCTTACATGATACTTACT 360
BW2    GATTAACTGGAATTATTCTGGGTAATTCTTCTGTTGATGTCTGCTTACATGATACTTACT 360
BW3    GACTAACTGGGATTATTCTTGGTAATTCTTCTGTTGATGTTTGTTTGCATGATACTTATT 360
       *  ******* ** ***** ***** ** ***** ***** **  * ***** ***** *

B      TTGTTGTTGCACATTTTCATTATGTTTTATCAATAGGAATTATTTTTGCTATTGTAGGAG 420
Q      TTGTTGTTGCGCATTTTCATTATGTCTTATCAATAGGAATTATTTTTGCTATTGTAGGAG 420
BW1    TTGTTGTTGCTCATTTTCATTATGTTCTATCTATAGGAATTATCTTTGCTATTGTGGGAG 420
BW2    TTGTTGTTGCTCATTTTCATTATGTTTTATCTATAGGAATTATCTTTGCTATCGTGGGAG 420
BW3    TTGTTGTTGCTCATTTTCATTATGTTTTATCCATAGGAATCATTTTTGCTATCATAGGAG 420
       ********** **************    **** ******** ** ********   * ****

B      GAGTTATCTATTGATTTCCACTAATCTTAGGTTTAACCTTAAATAATTATAGATTGGTGT 480
Q      GAGTTATCTATTGATTTCCATTAATCTTGGGCTTAACCTTAAATAATTATAGCTTGGTGT 480
BW1    GTGTTATTTATTGATTTCCATTAATTTTGGGCTTAACACTAAATAGTCATAGTTTGGTAT 480
BW2    GAGTTATTTATTGATTTCCGGTAATCTTGGGATTAACACTAAATAGACATAGTTTGGTAT 480
BW3    GTTTTATTTACTGATTTCCATTAATCTTAGGTCTAACATTAAATAACCATAATTTGGTAT 480
       *  **** ** ********   **** ** **  ****  ******   ***  ***** *

B      CTCAATTTTATATCATGTTTATTGGAGTAAATTTAACTTTTTTTCCTCAGCATTTTCTTG 540
Q      CTCAATTTTATATCATGTTCATTGGAGTAAATTTAACTTTTTTTCCTCAGCATTTTCTTG 540
BW1    CGCAGTTTTACATTATGTTTTTAGGAGTAAATTTAACGTTTTTCCACAACATTTCCTTG 540
BW2    CACAGTTTTACATTATGTTTTTGGGAGTAAATTTAACGTTTTTCCCGCAACATTTTCTTG 540
BW3    CTCAGTTTTATATTATATTTTTGGGCGTTAACCTAACATTTTTTCCACAACACTTTCTTG 540
       * ** ***** ** ** **  * ** ** **  **** ***** ** ** ** ** ****

B      GTTTAGGGGGAATGCCTCGTCGATATTCAGATTATGCTGATTGCTATCTAGTATGAAATA 600
Q      GTTTGGGGGGAATGCCTCGCCGATATTCAGATTATGCTGATTGTTATCTAGTATGGAACA 600
BW1    GGTTGAGGGGAATACCTCGACGGTACTCAGACTATCCCGATTGTTATCTGATATGAAATA 600
BW2    GGCTGAGAGGTATACCTCGTCGCTATTCAGATTATCCTGACTGTTATCTAATATGAAATA 600
BW3    GATTAAGCGGAATACCTCGTCGGTATTCAGATTATCCTGATTGTTATCTCATATGAAATA 600
       *  *  * ** ** ***** ** ** ***** *** * ** ** *****  **** ** *

B      AAATTTCTTCTGCGGGAAGGATTCTGAGTATTATTTCTGTTATTTATTTTTTATTTATTG 660
Q      AAATTTCTTCTGCGGGAAGGATTTTGAGTATCATTTCTGTTATTTATTTTTTATTTATTG 660
BW1    AAATTTCTTCTGCGGGGAGAATTTTAAGTGTTATTTCCGTCATTTATTTTTTATTTATTG 660
BW2    AAATTTCTTCTGCGGGGAGAATTTTGAGCATTATTTCTGTTATTTATTTTTTATTTATTG 660
BW3    AAATTTCTTCTGCGGGGAGTATCTTGAGAATTATTTCTGTTATCTATTTTTTATTTATTG 660
       **************** ** **   * **   * ***** ** ** ****************

B      TTT-AGAATCCTTTCTTCTTCTGCGGTTAGTAAGATTTAAGCTTGGTGTAAGTAGGCATC 719
Q      TTTTAGAATCTTTTCTTCTTTTGCGTTTAGTAAGATTTAAGCTTGGTGTAAGCAGACATC 720
BW1    TTTTGGAATCTTTACTCCTTTTGCGGTTGGTGGGGTTTAAGCTTGGCATAATTAGACATT 720
BW2    TTTTAGAGTCTTTGCTTCTTTTGCGGTTAGTGGGTTTTAAGCTTGGTATAATTAGCCACT 720
BW3    TTTTAGAATCTTTGCTTCTTTTACGGCTAGTAAGATTTAAACTTAGGATAATTAGGCATT 720
       ***   ** ** ** ** *** * **   * **   * ***** *** *   ***   ** **

B      TAGAATGAAAGATTAATAAACCAGCTCTTAATCACAGTTT 759
Q      TAGAATGGAAAATTAATAAACCAGCCCTTAATCACAGTTT 760
BW1    TAGAGTGGAAGATTAATAAGCCGGCTCTTGGGCATAGTTT 760
BW2    TAGAATGAAAAATTAATAAGCCGGCTCTTAGGCATAGTTT 760
BW3    TAGAATGAAAAATCAATAAGCCAGTTCTTAGTCATAGTTT 760
       **** ** ** ** ***** ** *  ***   ** *****
```

Fig. 3. Sequence alignment of Bangladesh whitefly (BW1, BW2 and BW3) and compared with B and Q biotype of Korea using mtCOI region nucleotide sequences by ClustalW2 program

Phylogenetic analysis

The Neibour-joining phylogenetic tree reconstruction based on 21 mitochondrial cytochrome oxidase subunit-I (mtCOI) sequences (B biotype from Israel, Viet Nam, China and Korea; Q biotype from Costa Rica, China, Egypt and Korea; BW1 from southern part of Bangladesh, BW2 from northern part of Bangladesh and newly collected Indigenous *B. tabaci* from eastern part of Bangladesh were compared) is shown in Figure 6. It revealed that mtCOI sequence of *B. tabaci* of eastern part of Bangladesh from Chittagong and Cox's Bazar which collected from bean, eggplant and okra were clustered together individually with high distance from each other (Figure 4). It was clear that third genotypic cluster was present in Bangladesh indigenous whiteflies which was BW3. Here mtCOI sequence of *Aleurodicus dispersus* was used as out group of phylogeny.

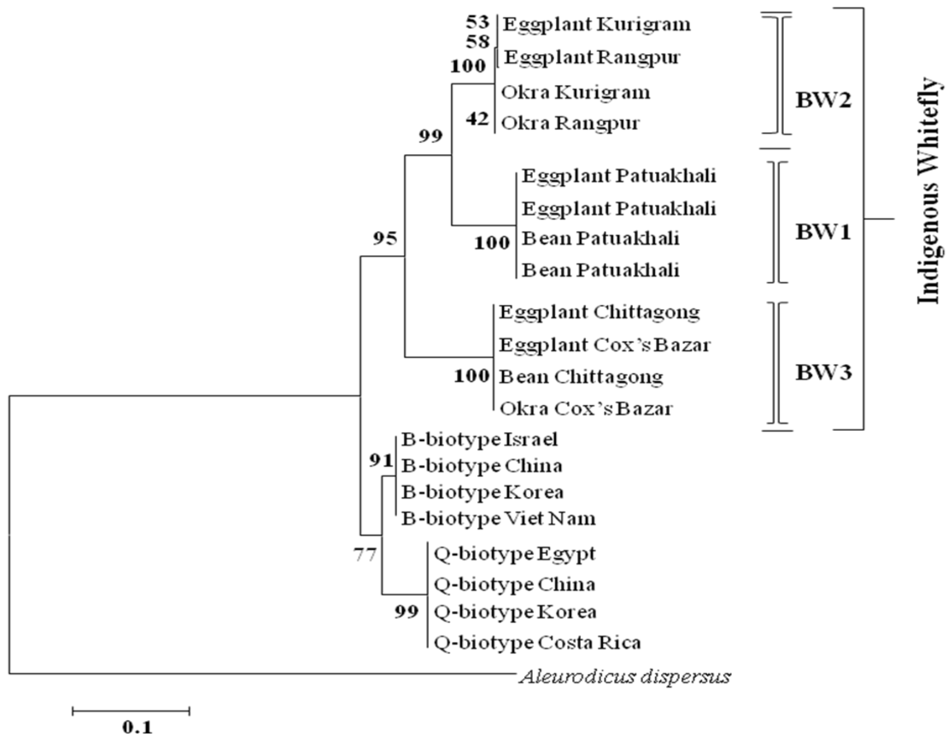

Fig. 4. Phylogenetic relationships of *B. tabaci* populations based on a fragment (~860 bp) of the mitochondrial COI sequences. Neibour-joining phylogenetic tree reconstructed using the whitefly mitochondrial cytochrome oxidase subunit-I (mtCOI) sequences as a molecular marker according to the Bayesian method. The numbers placed at each node indicate the bootstrap support for values > 50. The horizontal branch length is drawn to scale, and the bar indicates the distance of 0.1 nt replacements per site.

Table 6. Percentage of nucleotide frequencies in variable DNA sites of 5 different biotypes of *B. tabaci* in Bangladesh based on sequences of the fragment of mtCOI gene.

B. tabaci biotypes	Nucleotide composition (%)				Conserved sites (%) (568/760)				Variable sites (%) (192/760)				Parsim-info sites (%) (92/760)				Total	
	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G		
BW1	41.3	13.9	24.1	20.7	43.3	12.3	25.2	19.2	35.4	18.8	20.8	25.0	25.0	20.7	26.1	28.3	760	
BW2	42.4	13.0	24.2	20.4	43.3	12.3	25.2	19.2	39.6	15.1	21.4	24.0	25.0	20.7	22.8	31.5	760	
BW3	42.9	13.0	26.1	18.0	43.3	12.3	25.2	19.2	41.7	15.1	28.6	14.6	33.7	10.9	34.8	20.7	760	
Bt-B	42.4	12.5	26.1	19.0	43.2	12.3	25.2	19.2	40.1	13.0	28.6	18.2	30.4	12.0	32.6	25.0	759	
Bt-Q	42.1	13.0	25.5	19.3	43.3	12.3	25.2	19.2	38.5	15.1	26.6	19.8	27.2	15.2	30.4	27.2	760	
Avg.	42.2	13.1	25.2	19.5	43.3	12.3	25.2	19.2	39.1	15.4	25.2	20.3	28.3	15.9	29.3	26.5	759.8	

Table 7. Percentage of nucleotide frequencies in variable DNA sites of 5 different biotypes of *B. tabaci* in Bangladesh based on sequences of the fragment of 16S rRNA gene.

B. tabaci biotypes	Nucleotide composition (%)				Conserved sites (%) (185/427)				Variable sites (%) (242/427)				Singleton sites (%) (55/427)				Total
	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G	
BW1	44.9	14.1	30.6	10.4	44.2	5.5	44.2	6.1	45.4	20.0	21.3	13.3	37.7	20.8	28.3	13.2	427
BW2	28.9	12.0	46.9	12.2	41.8	6.8	44.6	6.8	19.5	15.8	48.5	16.2	25.5	14.5	49.1	10.9	427
BW3	30.4	12.7	43.3	13.6	41.8	6.8	44.6	6.8	22.0	17.0	42.3	18.7	34.5	18.2	30.9	16.4	427
Bt-B	45.4	13.6	31.4	9.6	44.2	5.5	44.2	6.1	46.3	19.2	22.5	12.1	49.1	9.4	34.0	7.5	427
Bt-Q	31.7	9.6	44.4	14.4	42.0	6.8	44.3	6.8	24.1	11.6	44.4	19.9	34.5	5.5	38.2	21.8	427
Avg.	36.2	12.4	39.4	12.1	42.8	6.3	44.4	6.5	31.4	16.7	35.8	16.0	36.2	13.7	36.2	14.0	427.0

Bemisia tabaci from Southern part of Bangladesh (BW1), Northern part of Bangladesh (BW2), Eastern part of Bangladesh (BW3), B biotype from Korea (Bt-B); and Q biotype from Korea (Bt-Q).

The same analysis constructed by Neibour-joining phylogenetic tree based on twenty three 16S ribosomal RNA (16S rRNA) sequences (B biotype from Israel, China and Korea; Q biotype from Spain, China and Korea; Indigenous *B. tabaci* BW1 and BW2 from southern part and northern part of Bangladesh, China, India, Pakistan, Sri Lanka and Japan were compared with newly collected whitefly from eastern part of Bangladesh) is shown in Figure 5. It revealed that ribosomal RNA (16S rRNA) sequences of *B. tabaci* of eastern part of Bangladesh which was collected from bean, eggplant and okra were clustered together individually with high distance from each other (Figure 5). It is undoubtedly revealed that third genotypic cluster of *B. tabaci* in Bangladesh which is BW3.

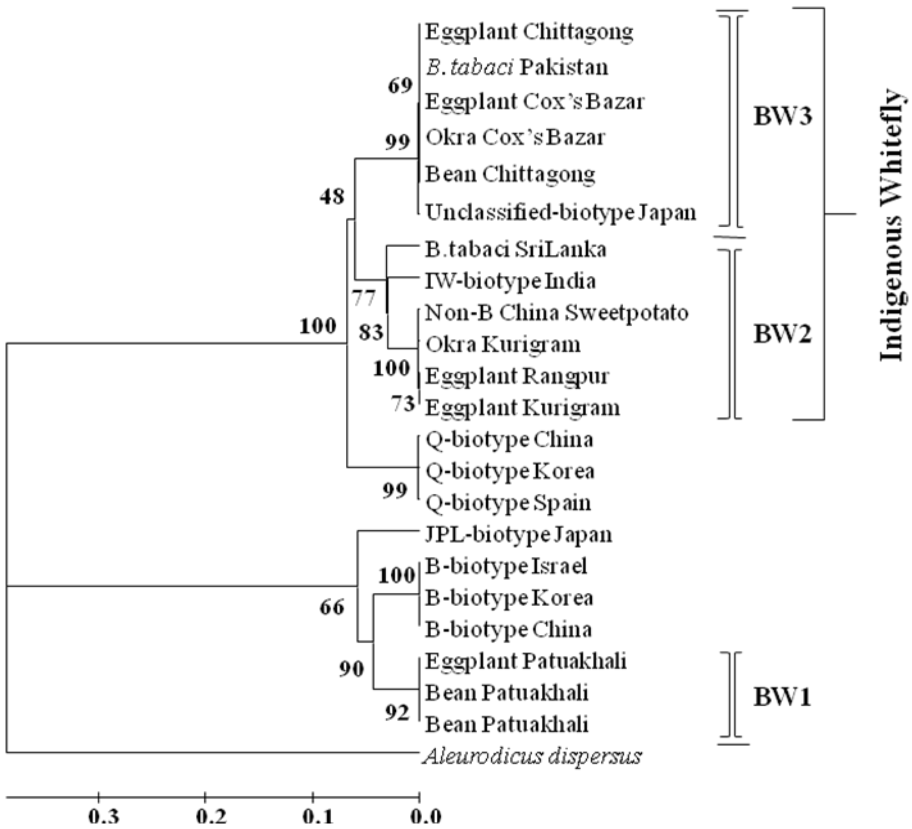

Fig. 5. Phylogenetic relationships of *B. tabaci* populations based on a fragment (~520 bp) of the mitochondrial 16S rRNA sequences. Neibour-joining phylogenetic tree reconstructed using the whitefly 16S ribosomal RNA (16S rRNA) sequences as a molecular marker according to the Bayesian method. The numbers placed at each node indicate the bootstrap support for values > 50. The horizontal branch length is drawn to scale, and the bar indicates 0.0-0.3 nt replacements per site.

Discussion

Bemisia tabaci populations revealed high genetic diversity in southern Asia. Phylogenetic analysis of mtCOI and 16S rRNA gene sequence data can be separated clearly the *B. tabaci* samples into north, south and east indigenous groups of Bangladeshi whitefly (Figure 4 & 5). Different cropping pattern and diverse climatic conditions in the northern, eastern and southern regions of Bangladesh may be responsible for the apparent diversity in *B. tabaci*, which otherwise grouped in geographic location. Tobacco, potato, and vegetables are grown almost as monocultures in the dry arid conditions in Kurigram, northern part of Bangladesh whereas the south is relatively cool and mixed crops of cereals, pulses, and vegetables predominate. However, divisions were not based on the host-plant from which the samples were collected, which was one of the criteria used to identify two *B. tabaci* biotypes (cassava and sweet potato) in south India (Lisha *et al*., 2003). The RAPD-PCR technique has been used before to distinguish indigenous populations of *B. tabaci* from those of the introduced B biotype (Gawel & Bartlett, 1993; Perring *et al*., 1993; De Barro & Driver, 1997; Guirao *et al*., 1997; Moya *et al*., 2001). Based on phylogenetic analyses of mtCOI and 16S rRNA gene sequences, the Asian *B. tabaci* formed three clusters each supported with a high bootstrap score, which indicate the existence of at least two genotypic clusters (BW1 and BW2) of *B. tabaci* indigenous to Bangladesh. This may support the conclusion of De Barro *et al*. (2005) that using mtCOI and internal transcribed spacer region 1 (ITS1) markers, some individuals from Asia did not fit into either of the two clusters (called Asia1 and Asia2), and they remained classified as 'unresolved' in the Asia group.

This research work has been successfully carried out for monitoring *B. tabaci* and detecting its genotypic clusters from Bangladesh. Jahan *et al*. (2011) previously mentioned that all Bangladeshi whiteflies were indigenous and there was apparently absent B and Q biotype of *B. tabaci*. The present findings also supported the previous report of Jahan *et al*., 2011. In the present study for detecting the third genotypic cluster of *B. tabaci*, it was found that whiteflies of eastern part of Bangladesh make a distinct clade in Phylogenetic tree based on mtCOI and 16S rRNA sequences separately (Figure 4 & 5). Although, Jahan (2012) reported two genotypic clusters present in Bangladesh. However, all sequences data currently found in GenBank were compared for preparing the phylogeny of whiteflies using mtCOI sequences of different biotypes including indigenous whiteflies from different countries.

Some Bangladeshi *B. tabaci* shared about 99% mtCOI sequence identity with populations from other Asian countries like India, Pakistan, Myanmar, Japan and Nepal. There may be several explanations for this, including the existence of a cline of distinct *B. tabaci* genotypes across Asian countries that allow gene flow between them. Huge geographical distances and natural barriers, such as the Himalayan mountain range, however, represent significant physical barriers for natural movement and therefore probably restrict gene flow, for example, between the Bangladesh and Myanmar *B. tabaci* populations. The most likely reason for the similarity of these populations may be the movement of *B. tabaci* between the countries as a result of human activities. The most recent example of such introduction has been the arrival of the B biotype in India, Pakistan, and China (Banks *et al*., 2001; Simon *et al*., 2003; Zhang *et al*., 2005), and more recently the Q biotype in China (Zhang *et al*., 2005). The data, which presented here, highlight the real and increasing threat posed by the movement of *B. tabaci* and potentially new viruses to agriculture in Asia.

Phylogenetic analysis of mtCOI and 16S rRNA gene sequences with reference *B. tabaci* sequences from other countries divided them into two genotypic clusters. Each cluster supported with high bootstrap values (55–100%) and the individuals belonging to each cluster shared high nucleotide identities (up to 100%) (Figure 6 & 7). This result supported by Rekha *et al*. (2005) who reported that at least three distinct genotypes, of indigenous whitefly to India, which are also present in China, Malaysia, Nepal, Pakistan, Japan and Thailand.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 17-34, March 2015

GRAIN YIELD, NUTRIENT BALANCE AND ECONOMICS OF T. *AMAN* RICE CULTIVATION AS INFLUENCED BY NUTRIENTS MANAGEMENT

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Abstract

A field experiment was conducted at Regional Wheat Research Centre of the Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh during 2007 and 2008. The objectives were to find out the optimum nutrient management practice for grain yield, nutrient balance and economics of T. *Aman* rice. Twelve nutrient management treatments (with and without CRI) were tested in RCBD with 3 replications. Treatments were T_1=HYG (0-80-16-44-12-2-0), T_2=MYG (0-56-12-32-8-1.5-0), T_3=IPNS (5000-65-13-32-9-2-0), T_4=STB (0-68-15-37-11-2-0), T_5=FP (0-39-7-12-0-0-0), T_6=CON (0-0-0-0-0-0-0), T_7=HYG+CRI(Crop residue incorporation), T_8=MYG+CRI, T_9=IPNS+CRI, T_{10}=STB+CRI, T_{11}=FP+CRI, T_{12}=CON+CRI kg ha^{-1} CDNPKSZnB for T. *Aman* rice. On an average, maximum grain yield of T. *Aman* rice was obtained from STB+CRI (5.24 t ha^{-1}) followed by IPNS+CRI (5.13 t ha^{-1}), STB (5.12 t ha^{-1}), IPNS (5.03 t ha^{-1}), HYG+CRI (4.50t ha^{-1}) and HYG (4.41 t ha^{-1}). Numerically but not statistically higher yield and yield contributing parameters were noticed in CRI plots than without CRI. Except N and K remaining nutrient balance like P S Zn and B were found positive in case of HYG, MYG, IPNS and STB along with or without CRI nutrient managements while FP and CON (Control) showed negative balance. The maximum BCR was observed in STB (3.25) followed by STB+CRI (3.14) and IPNS (2.98) and similar trend was observed in MBCR.

Keywords: T. *Aman* rice, yield, nutrient balance, nutrient management and crop residue incorporation

Introduction

Bangladesh is a country of 0.148 million sq.km and it has to feed about 150 million people (BBS, 2012). In order to produce more food within a limited area, two most important technique is to be adopted, which is to increase the productive efficiency of the individual crop depending on how well it utilizes the basic resources especially, the limiting ones, water and nutrients. Bangladesh is the fourth largest producer and consumer of rice in the world. Rice is the staple

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food in Bangladesh. Rice is extensively grown in Bangladesh which covers 75% of the total cropped area and about 60% labor is engaged in rice production. Rice alone contributes around 10% to the GDP. Rice alone contributes about 95% to the total food grain production(BER,2010).It provides 75% of the calories and 55% of the proteins in the average daily diet of the people (Bhuiyan *et al*., 2002).The national mean yield (2.60 t ha^{-1}) of rice in Bangladesh is lower than the potential national yield (5.40 t ha^{-1}) and world average yield (3.70 t ha^{-1}) (Pingali*et al*., 1997).The lower yield of transplanted *Aman* rice has been attributed to several reasons, one of them being imbalanced nutrients management. Crop residue is a vital natural resource for conserving and sustaining soil productivity. It is the primary substrate for replenishment of soil organic matter. Upon mineralization, crop residue supplies essential plant nutrients (Walters *et al*., 1992). Additionally, residue incorporation can improve physical and biological conditions of the soil and prevent soil degradation (Nyborg *et al*., 1995). Incorporation of crop residues of either rice straw or wheat straw increased the yield and yield components of rice and nutrient uptake and also improved the physico-chemical properties of the soil, which provided better soil environment for crop growth. Increasing levels of NPK application increased the yield-attributing characters and nutrient uptake by both the crops, which ultimately increased the grain and straw yields (Das *et al.,* 2003). Therefore, the present study was undertaken to find out the optimum nutrient management practice for grain yield, apparent nutrient balance in soil and economics of T. *Aman* rice cultivation under AEZ-28.

Materials and Method

The experiment was carried out at the Regional Wheat Research Centre of Bangladesh Agricultural Research Institute Joydebpur, Gazipur. The experimental field of Gazipur belongs to the agro-ecological zone of Modhupur Tract (AEZ-28). The initial soil of the experimental field was analyzed for chemical properties before setting up the experiment. The initial soil status was pH 6.48, OM (%) 1.07, Total N (%) 0.055, available P(µg g^{-1}) 3.76, exchangeable K (meq 100 g^{-1}) 0.15, available S(µgg^{-1}) 9.91, available Zn(µg g^{-1}) 0.24 and available B(µg g^{-1}) 0.16. Morphological characters are Grey Terrace soils, medium high land, not well drained, above flood level and grey soil clour. Physiological characters are silty loam to loam having more or less near neutral soil pH with very low to low soilfertility. T. *Aman* rice variety (BRRIdhan39) was tested in *Kharif*-II season during 2007 and 2008, respectively. Twelve nutrient management treatments were tested in RCBD with 3 replications. Treatments were T_1=HYG (0-80-16-44-12-2-0), T_2=MYG (0-56-12-32-8-1.5-0), T_3=IPNS (5000-65-13-32-9-2-0), T_4=STB (0-68-15-37-11-2-0), T_5=FP (0-39-7-12-0-0-0), T_6=CON (0-0-0-0-0-0-0), T_7=HYG+CRI, T_8=MYG+CRI, T_9=IPNS+CRI, T_{10}=STB+CRI, T_{11}=FP+CRI, T_{12}=CON+CRI kg ha^{-1}

CDNPKSZnB for T. *Aman* rice.(Here, HYG= High Yield Goal, MYG= Moderate Yield Goal, IPNS= Integrated Nutrient Management System, STB= Soil Test Based, FP= Farmers Practice, CON= Control, CD= Cowdung and CRI= Crop Residue Incorporated). The previous crop mungbean was cultivated which was demarked individually plot earlier in the whole experimental area and in case of CRI plots, total biomass (except pod) of mungbean was incorporated as residue before T. *Aman* rice transplanting. In second year, the land was used for any rabi crop after harvest of T. Aman rice. The rates for N, P, K, S, and Zn application were calculated based on the soil test value following the soil test interpretation (FRG, BARC, 2005). The rate for each element was considered as 100%. Accordingly, the full or 100% rate of N, P, K, S, and Zn for each crop was applied. In case of Integrated Plant Nutrient System (IPNS) treatment the amount of nutrients available in cowdung (CD) was deducted from the total amount of chemical fertilizers and adjusted accordingly. The rates for chemical fertilizers were fixed on soil test basis (STB) with a high yield goal (HYG) for specific crop basis as per BARC (FRG, BARC, 2005). The exact fertilizer nutrient for making the recommendation was computed with the following formula:

$$F_r = U_f - \frac{C_i}{C_s} \times (S_t - L_s)$$

Where

F_r = Fertilizer nutrient required for a given soil test value

U_f = Upper limit of the recommended fertilizer nutrient for the respective soil test value interpretation (STVI) class

C_i = Units of class intervals used for fertilizer nutrient recommendation

C_s = Units of class intervals used for STVI class

S_t = Soil test value

L_s = Lower limit of the soil test value within STVI class.

The sources of N, P, K, S, and Zn were urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum, and zincsulphate, respectively. The farmers' practice (FP) for fertilizer rates was determined on the basis of data collected through interviewing thirty (30) farmers from adjacent locality. Total residue was chopped just after harvest and ploughed down to the soil by spade for decomposition in respective CRI plots. Thirty-day old seedlings were transplanted from 1-7 July each year. Three seedlings per hill were used following a spacing of 20cm x 15cm.The whole amount of TSP, MoP, gypsum and zincsulphate were applied at the time of final land preparation as per treatment. Urea was applied into three splits at 15, 30 and 45 days after transplanting. Intercultural operations like weeding, irrigation and pest control measures were taken as and when necessary. T. Aman rice was harvested on 25-31 October each year.

The crops were harvested from 10 m2 at full maturity. A sub-sample of 200 g dry biomass for each of crop was collected for chemical (nutrient uptake) analysis. The sub-samples were dried in an oven for 72 hours at 700C. Apparent nutrient balance (added-uptake) was calculated by using the following formula.

Xa = (Xf + Xr+ Xi+ Xb+Xcri)-Xrem, Where

Xa = Apparent gain (+) or loss (-) of nutrient (kg ha^{-1})

Xf = Nutrient added through inorganic sources (kg ha^{-1})

Xr = Nutrient added through rainfall (kg ha^{-1}). (Not considered)

Xi = Nutrient added through irrigation water (kg ha^{-1}). (Not considered)

Xb= Nutrient added through BNF (kg ha^{-1}). (Not considered)

Xcri= Nutrient added through crop residue incorporation (kg ha^{-1}).

Xrem= Nutrient removed by crops and loss through different systems (kg ha^{-1}).

The data were analyzed statistically by the F-test andthe mean comparisons of the treatments were evaluated by DMRT (Duncan's Multiple Range Test).

Results and Discussion

Plant population

Plant population was not influenced significantly by different nutrient management treatments in both the years (Table 2).

Plant height

The nutrient management treatments without or with CRI influenced the plant height significantly in both theyears. The plant height was statistically identical except control without or with crop residues incorporation (Table 2). In a field study, Basak *et al.* (2008 a) recorded the highest plant height with STB nutrient in T. *Aman* rice of Mustard-*Boro* rice-T. *Aman* rice cropping pattern and Awal *et al.* (2007) also reported similar result in T. *Aman* rice on Wheat-Jute-T. *Aman* rice cropping pattern.

Panicles number

Number of panicles m^{-2} differed significantly due to application of nutrients in soil both the years. The highest number of panicles m^{-2} was 250 under STB with crop residues integration, which was statistically identical with STB (249),

IPNS+CRI (245), IPNS (243), HYG+CRI (240) and HYG (237) in 2007. In 2008, the trend was similar. From the two years results, it was observed that the maximum panicles m^{-2} obtained from HYG, IPNS and STB, without or with CRI nutrient management treatments compared to other treatments. STB was the best among those treatments might be due to proper nutrient was added into the soil resulted maximum number of panicles m^{-2} followed by IPNS and HYG, respectively. The lowest panicles m^{-2} was found in control due to lack of proper nutrient. Increased number of panicles m^{-2} was found in all the treatments along with CRI than without CRI which might be due to the crop residual effect (Maskina *et al*., 1987). Ali *et al*. (2003) stated that STB nutrient gave the highest panicles m^{-2} in T. *Aman* rice in Mustard-*Boro* rice-T. *Aman* and Basak *et al.* (2008) stated similar findings in T. *Aman* rice of Mustard-*Boro* rice-T. *Aman* rice cropping pattern.

Grains panicle^{-1}

The nutrient management treatments HYG, IPNS and STB without or with CRI produced higher number of grains panicle^{-1} which ranged from 78 to 88 in both the years. From the two years results, it was revealed that the nutrient management treatments HYG, IPNS and STB along with or without CRI produced the maximum number of grains panicle^{-1}. Among those treatments, STB gave the best performance that might be due to appropriate nutrient dose applied into the soil, while the nutrient applied was higher in case of HYG. Control produced the minimum grains panicle^{-1} due to no addition of nutrient into the soil. However, the increasing trend was observed in all the treatments along with CRI than without CRI which might be due to the effect of crop residues (Naser *et al*., 2001). However, there was no significant difference between with or without CRI. These findings were similar to the findings of Zaman *et al*., 2007 a & b; Awal *et al*., 2007.

Sterile spikelet panicle^{-1}

The number of sterile spikelets panicle^{-1} differed significantly due to application of different nutrients without or with incorporation of crop residues. The highest number of sterile spikelets panicle^{-1} was 57 under control without CRI followed by with control with CRI (57). In case of other nutrient management treatments the number of sterile spikelets panicle^{-1} ranged from 32 to 42. The lowest sterile spikelets panicle^{-1} (32) was obtained with HYG treatment without CRI. Similar trend was found in 2008 (Table 3).The highest number of sterile spikelets panicle^{-1} in case of control without or with CRI might be due to the absence of proper nutrients in the soil. HYG, IPNS and STB without or with CRI produced the minimum number of sterile spikelets panicle^{-1}.

Table 1. Total addition of extra nutrients into the soil through previous crop (mungbean) residues incorporation in T. *Aman* rice (kg ha^{-1}yr^{-1}) during 2007 and 2008 (assuming nitrogen mineralization rate 40%).

Nutrient management	Kg ha^{-1}		Kg ha^{-1}		Kg ha^{-1}		Kg ha^{-1}		Kg ha^{-1}		Kg ha^{-1}	
	N		P		K		S		Zn		B	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
HYG+CRI	17	18	11	12	41	44	9	9	0.14	0.15	0.10	0.11
MYG+CRI	11	12	8	9	32	35	8	8	0.11	0.08	0.08	0.07
IPNS+CRI	13	15	10	11	40	44	9	9	0.13	0.14	0.09	0.09
STB+CRI	13	14	10	11	37	40	9	10	0.11	0.12	0.09	0.08
FP+CRI	9	10	7	8	24	28	6	7	0.08	0.09	0.07	0.07
CON+CRI	6	6	4	4	17	17	4	4	0.05	0.05	0.04	0.03

Grain weight

Different nutrient management treatments over the years did not influence 1000-grain weight significantly (Table 3).

Grain yield

The grain yield irrespective of treatment was found slightly higher in 2008 than 2007 (Table 3). It was observed that HYG, IPNS and STB nutrient management treatments without or with crop residues incorporation produced the maximum grain yield and those were statistically identical over the years. Among those treatments, STB gave the highest yield, which might be due to the combined effect of higher number of tillers m^{-2}, panicles m^{-2} and grains panicle^{-1}. MYG and FP without or with CRI gave average and low grain yield, respectively might be due to the effect of moderate and low number of tillers m^{-2}, panicles m^{-2} and grains panicle^{-1}. The lowest grain yield was found in control treatment. However, the grain yield in all the treatments with CRI was found superior to without CRI that might be due to the effect of conservation agriculture, more soil microbial activities through crop residual incorporation resulting the yield was increased (Kavimadan *et al*., 1987 and Ladha *et al*., 1987). Moreover, the overall trend was similar in both the years. These results are in agreement with that of Akhteruzzaman *et al*. (2009). On an average, maximum grain yield (5.24 t ha^{-1}) was recorded from STB+CRI followed by IPNS+CRI, STB and IPNS. It is noted that STB, IPNS and incorporation of residues played vital role in increasing grain yield as well as improved of soil health. Timsina *et al*. (2006 a) reported the highest grain yield with STB nutrient in T. *Aman* rice on rice-wheat system. Similar findings were also reported by many scientists (Quayyum *et al*., 2001 and 2002; Chowdhury *et al*., 2002; Basak *et al*., 2008 a; Roy *et al*., 2008; Ali *et al*., 2003).

Straw yield

The significantly highest straw yield was 6.43 t ha^{-1}in HYG+CRI which was identical to IPNS+CRI (6.29 t ha^{-1}), HYG (6.26 t ha^{-1}), IPNS (6.13 t ha^{-1}). MYG treatment yielded similar to STB. FP gave low straw yield without or with crop residue incorporation. The lowest straw yield was 1.44 t ha^{-1} under control without CRI. In 2008, the trend was similar to the previous year. However, the highest straw yield was 6.92 t ha^{-1} under HYG+CRI and the lowest was 1.69 t ha^{-1} in control without CRI (Table 3).Among the treatments, HYG gave the maximum straw yield which was followed by IPNS and STB, which might be contributed through plant height and biomass. The straw yield was higher in all the treatments along with CRI than without CRI, might be due to the residual effect of the crop (Thakur and Singh, 1987; Kavimadan *et al*. 1987 and Ladha *et al*. 1987).

Table 2. Plants m^{-2}, plant height, panicles m^{-2} and of grains $panicle^{-1}$ of T. *Aman* rice as influenced by different nutrient management during 2007 and 2008.

Nutrient management	Plants m^{-2}		Plant height (cm)		Panicles m^{-2}		Grains $panicle^{-1}$	
	2007	2008	2007	2008	2007	2008	2007	2008
HYG	32.0	32.8	114.2 a	119.9 a	237 abc	249 ab	78 ab	82 ab
MYG	32.0	32.8	106.7 a	112.4 a	217 c	230 bc	67 bc	71 bc
IPNS	32.3	33.1	110.9 a	116.7 a	243 a	256 a	82 a	86 a
STB	32.0	33.3	108.9 a	115.7 a	249 a	261 a	83 a	87 a
FP	32.0	32.7	106.3 a	112.1 a	194 d	207 d	51 d	55 d
CON	31.6	32.6	88.1 b	93.8 b	151 e	164 e	25 e	29 e
HYG+CRI	32.2	33.1	114.5 a	120.3 a	240 ab	251 ab	79 a	83 a
MYG+CRI	32.6	33.1	109.9 a	114.7 a	219 bc	232 bc	67 c	71 bc
IPNS+CRI	32.3	33.2	111.3 a	117.1 a	245 a	257 a	84 a	88 a
STB+CRI	32.4	33.5	111.0 a	116.7 a	250 a	263 a	85 a	88 a
FP+CRI	32.3	33.1	107.2 a	112.9 a	196 d	209 d	53 d	570 d
CON+CRI	31.7	32.8	89.7 b	95.4 b	153 e	166 e	26 e	30 e
CV (%)	2.60	4.48	3.93	3.73	6.08	5.85	7.07	6.65
Level of sig	NS	NS	**	**	**	**	**	**

Table 3. Number of sterile spikelets panicle^{-1},1000-grain weight, grain yield and dry straw yield of T. *Aman* rice as influenced by different nutrient management during 2007 and 2008.

Nutrient management	Number of sterile spikelets panicle^{-1}		1000-grain weight (g)		Grain yield (t ha^{-1})		Straw yield (t ha^{-1})	
	2007	2008	2007	2008	2007	2008	2007	2008
HYG	32 d	34 d	21.95	23.40	4.31 ab	4.51 ab	6.26 a	6.75 a
MYG	40 bcd	42 bcd	22.55	23.73	3.50 b	3.70 b	4.22 bc	4.71 bc
IPNS	42 bc	44 bc	21.95	23.53	4.93 a	5.13 a	6.13 a	6.62 a
STB	33 cd	35 cd	22.35	23.35	5.02 a	5.22 a	5.36 ab	5.85 ab
FP	33 cd	35 cd	22.29	23.47	2.41 c	2.61 c	2.88 d	3.37 d
CON	57 a	59 a	22.09	23.13	1.21 d	1.31 d	1.44 e	1.69 e
HYG+CRI	32 d	34 d	22.42	23.60	4.35 ab	4.55 ab	6.43 a	6.92 a
MYG+CRI	40 bcd	42 bcd	22.22	23.27	3.54 b	3.73 b	4.25 bc	4.76 bc
IPNS+CRI	42 b	44 b	22.75	23.93	5.03 a	5.23 a	6.29 a	6.78 a
STB+CRI	33 cd	35 cd	22.69	23.87	5.14 a	5.34 a	5.42 a	5.91 ab
FP+CRI	33 cd	35 cd	22.69	23.87	2.43 c	2.64 c	2.92 d	3.41 d
CON+CRI	57 a	58 a	22.17	23.13	1.26 d	1.36 d	1.46 e	1.75 e
CV (%)	8.71	8.34	3.90	3.71	9.76	9.29	10.92	10.05
Level of sig	**	**	NS	NS	**	**	**	**

In a column, mean values having common letter(s) do not differ significantly whereas mean values with dissimilar letter(s) differ significantly as per DMRT.

T_1=HYG (0-80-16-44-12-2-0), T_2=MYG (0-56-12-32-8-1.5-0), T_3=IPNS (5000-65-13-32-9-2-0), T_4=STB (0-68-15-37-11-2-0), T_5=FP (0-39-7-12-0-0-0), T_6=CON (0-0-0-0-0-0-0), CD, N, P, K, S and Zn (kg ha^{-1}), respectively and CRI= Crop Residue Incorporation.

Apparent nutrient uptake and balance

Nitrogen

From the mean data it was observed that the added of nutrient ranged from 0 to 55 kg $ha^{-1}yr^{-1}$ (40% of applied chemical/cowdung/crop residues nutrient N was considered effective) while uptake ranged from 25 to 116 kg $ha^{-1}yr^{-1}$among different treatments (Fig.1). Maximum uptake was found in IPNS+CRI (116 kg $ha^{-1}yr^{-1}$) followed by STB+CRI (115 kg $ha^{-1}yr^{-1}$). Minimum uptake was estimated in CON (25 kg $ha^{-1}yr^{-1}$). The apparent nutrient balance was found negative in all treatments ranging from -20 to -82 kg $ha^{-1}yr^{-1}$. The highest negative balance was found in STB (-82 kg $ha^{-1}yr^{-1}$) followed by IPNS (-68 kg $ha^{-1}yr^{-1}$). The lowest negative balance was observed in CON+CRI (-20 kg $ha^{-1}yr^{-1}$). Fig. 1, showed that the nitrogen balance was negative as the uptake was higher compared to added nitrogen (40% of applied chemical/cowdung/crop residues nutrient N was considered effective). Nitrogen replenishment through chemical fertilizer, cowdung addition, crop residue incorporation either singly or in combination was not enough to balance N removal by crop; so much of the applied N was lost from the soil through depletion. The N balance thus was negative in all treatments appeared to have been removed in excess of the quantity added in soil. However, the N balance was less negative in those treatments where crop residues were incorporated than without incorporation which might be due to addition of extra N came from previous crop residues (6 to 18 kg $ha^{-1}yr^{-1}$) as shown (Table 1). Present findings are also in agreement with the observation of Timsina *et al*., 2001, 2006 (b) and Rahman *et al.*, 1998.

Phosphorus

The added phosphorus was in the range from 0 to 28 kg $ha^{-1}yr^{-1}$in respective of different treatments. The uptake was ranged from 5 to 26 kg $ha^{-1}yr^{-1}$. The treatment IPNS+CRI showed maximum uptake (26 kg $ha^{-1}yr^{-1}$) followed by STB+CRI (25 kg $ha^{-1}yr^{-1}$). The lowest uptake was found in CON and CON+CRI (5 kg $ha^{-1}yr^{-1}$). Only control plot along with or without CRI treatments showed negative balance ranged from -1 to -5 kg ha^{-1} yr^{-1} and remaining all the treatments showed positive balance ranged from 1 to 7 kg ha^{-1} yr^{-1}(Fig. 2). From the figure 2, it was observed that except control plots along with or without CRI treatments, all treatments showed positive balance due to addition of higher amount of phosphorus while uptake was lower that might be due to total dry matter content as well as the variation of concentration of the nutrient of the crops. In HYG, MYG, IPNS, STB and FP along with or without CRI treatments the balance appeared positive with trace amount due to addition of adequate nutrient into the soil whereas uptake was a little bit lower. However, the positive balance was higher in those treatments where the crop residue were incorporated with soil than without incorporated treatments which might be due to addition of extra nutrient in the range of 4-12 kg $ha^{-1}yr^{-1}$from the mean data (Table 1). Similar results were also found by Saleque *et al*. (2006).

Potassium

The quantity of added nutrient (K) was in the range of 0 to 87 kg ha^{-1}yr^{-1}and uptake by the crop varied from 22 to 116 kg ha^{-1}yr^{-1}. Maximum uptake was found in STB+CRI (116 kg ha^{-1}yr^{-1}) followed by IPNS (104 kg ha^{-1}yr^{-1}). Minimum uptake was observed in CON (22 kg ha^{-1}yr^{-1}). Among the nutrient managements, all treatments showed negative balance in the range of -1 to -66 kg ha^{-1}yr^{-1}. Maximum negative balance was observed in STB (-66 kg ha^{-1}yr^{-1}) and minimum was found in MYG+CRI (-1 kg ha^{-1}yr^{-1}) as shown in Fig. 3. However, the negative balance was shown lower in those treatments where crop residues were incorporated than without incorporated plots. It might happen due to addition of extra nutrient in the range of 17 to 44kg ha^{-1}yr^{-1}from the mean data through crop residues incorporation (Table 1). This result is also agreement with Panaullah *et al*. (2006).

Sulphur

From the mean data it was observed that quantity of added nutrient ranged from 0 to 21 kg ha^{-1}yr^{-1}and the uptake ranged from 4 to 19 kg ha^{-1}yr^{-1} with irrespective treatments. Among the treatments, maximum uptake was observed in STB+CRI (19 kg ha^{-1}yr^{-1}) followed by IPNS+CRI and HYG+CRI (17 kg ha^{-1}yr^{-1}). Minimum uptake was found in CON (4 kg ha^{-1}yr^{-1}). The negative balance was observed in FP and CON with and without CRI treatments was -1 to -8 kg ha^{-1}yr^{-1}. Remaining treatments showed positive balance ranged from 1 to 4 kg ha^{-1}yr^{-1}(Fig.4). Among the treatments, the maximum positive balance was observed in HYG+CRI and IPNS+CRI (4 kg ha^{-1}yr^{-1}) followed by MYG+CRI (2 kg ha^{-1}yr^{-1}). This result is in agreement with Khan *et al.* (2005).

Zinc

The amount of nutrient added in different nutrient treatment was in the range of 0 to 2.15 kg ha^{-1} and uptake was in the range 0.12 to 0.65 kg ha^{-1}yr^{-1}with different treatments shown in Fig 5. Maximum uptake was observed in STB (0.65 kg ha^{-1}yr^{-1}) that was followed by IPNS (0.64 kg ha^{-1}yr^{-1}). Minimum uptake was found in CON (0.12 kg ha^{-1}yr^{-1}). The highest negative balance was noticed in FP (-0.31 kg ha^{-1}yr^{-1}) and the lowest in CON+CRI (-0.08 kg ha^{-1}yr^{-1}). Other treatments showed positive balance ranged from 1.12 to 1.61 kg ha^{-1}yr^{-1}. Among the treatments, maximum positive balance was noticed in HYG+CRI (1.61 kg ha^{-1}yr^{-1}) followed by IPNS+CRI (1.54 kg ha^{-1}yr^{-1}). Minimum positive balance was observed in MYG (1.12 kg ha^{-1}yr^{-1}). From the mean data of two years, it was noticed that farmers' practice and control treatments showed negative balance of zinc. Because there was poor and no nutrient (native nutrient was available only in the soil) was added into the soil whereas a considerable amount of nutrient was removed by the crop through total dry matter weight and nutrient concentration variation in respective treatments consequently the balance became negative. Similar results were reported by Bhuiyan (2004) in wheat-T. *Aus*/Mungbean-T.*Aman* rice cropping pattern and Basak *et al*. (2008) in Groundnut-T.*Aus*-T.*Aman* rice cropping pattern.

Boron

The range of added boron was 0 to 0.11 kg $ha^{-1}yr^{-1}$and uptake ranged from 0.04 to 0.27 kg $ha^{-1}yr^{-1}$. The uptake was the highest in both IPNS and STB (0.27 kg ha^{-1}) and the lowest uptake in CON (0.04 kg $ha^{-1}yr^{-1}$) in Fig. 6. The highest negative balance was found in both IPNS and STB (-0.27 kg $ha^{-1}yr^{-1}$) and the lowest negative balance was observed in CON+CRI (-0.01 kg $ha^{-1}yr^{-1}$). From the above results, it was observed that the balance was negative in all the treatments due to no addition of born nutrient in the soil from external sources (native boron available in the soil only). Although some amount of nutrient was removed by the plants for total dry matter production and variation of nutrient concentration (concentration table was not shown here). Similar results were reported by Bhuiyan (2004) in Wheat-T. *Aus*/ Mungbean- T. *Aman* rice cropping pattern.

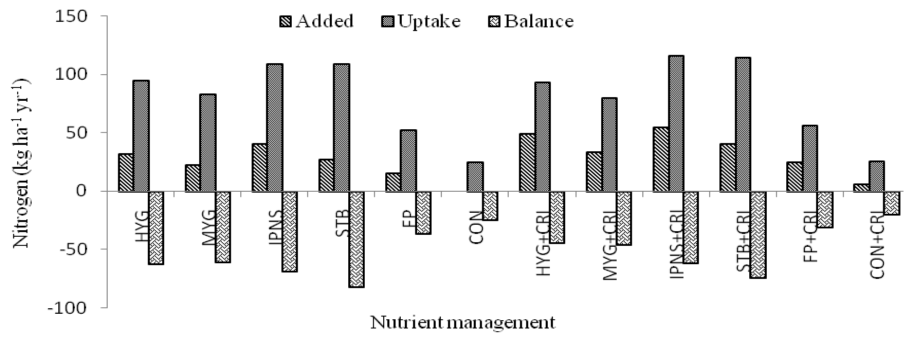

Fig. 1. Apparent N balance of T. *Aman* rice as influenced by different nutrients management (two years mean).

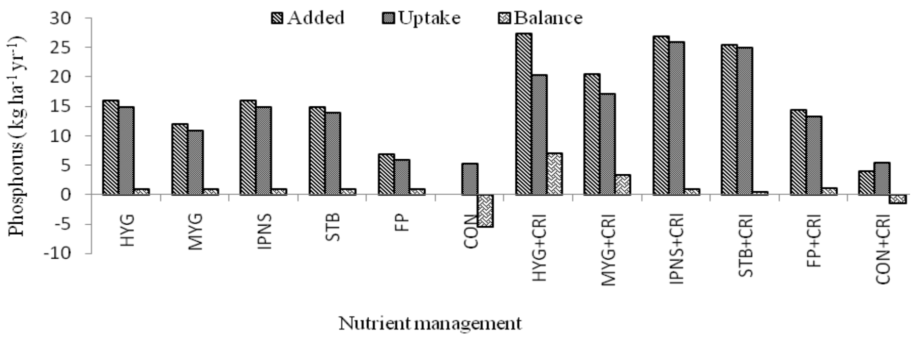

Fig. 2. Apparent P balance of T. *Aman* rice as influenced by different nutrients management (two years mean).

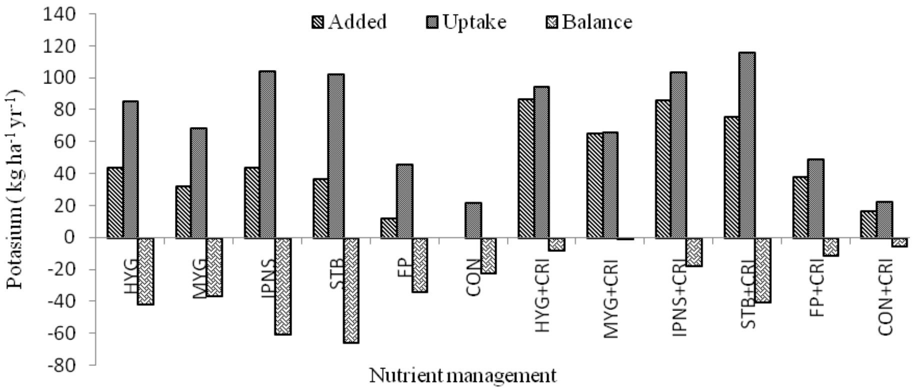

Fig. 3. Apparent K balance of T. *Aman* rice as influenced by different nutrients management (two years mean).

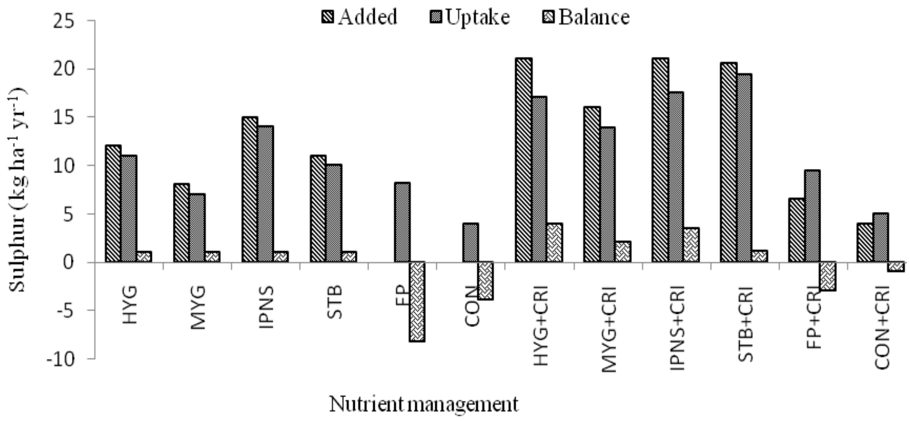

Fig. 4. Apparent S balance of T. *Aman* rice as influenced by different nutrients management (two years mean).

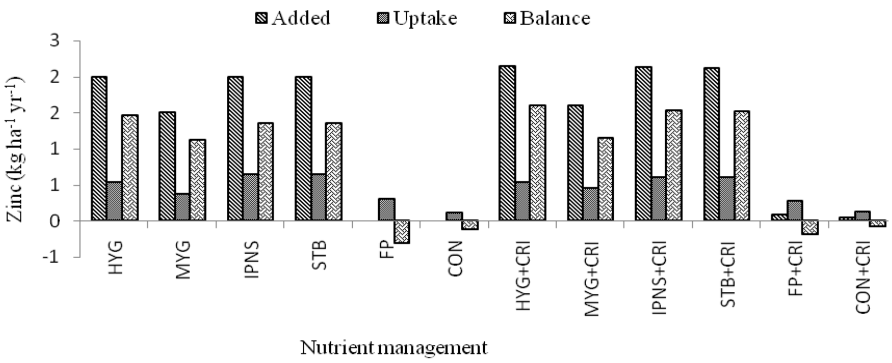

Fig. 5. Apparent Zn balance of T. *Aman* rice as influenced by different nutrients management (two years mean).

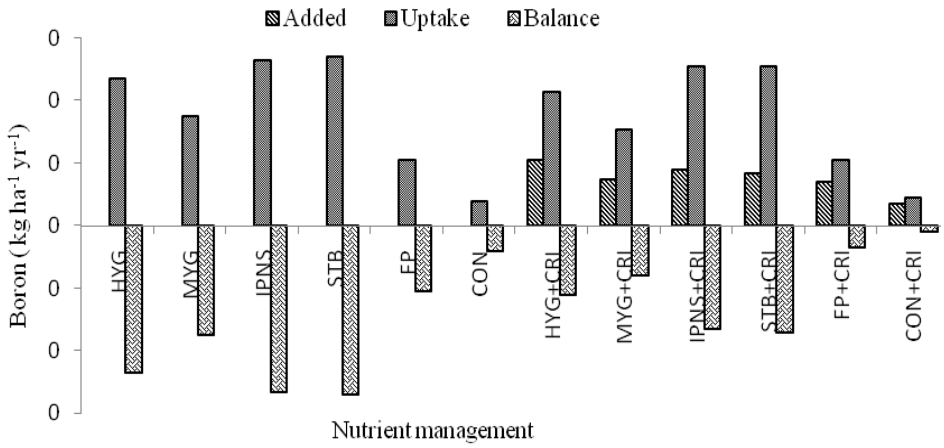

Fig. 6. Apparent B balance of T. *Aman* rice as influenced by different nutrients management (two years mean).

Economics of mungbean cultivation as influenced by different nutrient managements

Average of two years result showed that STB+CRI nutrient management gave the highest gross return (Tk. 55738 ha^{-1}) followed by IPNS+CRI (Tk. 54568 ha^{-1}), STB (Tk.54420 ha^{-1}) and IPNS (Tk. 53520 ha^{-1}) nutrient management treatments due to higher yield. Similar trend was followed in gross margin and net return. Due to higher yield obtained from STB nutrient management, higher BCR (3.25) followed by STB+CRI (3.14), IPNS (2.98) and IPNS+CRI (2.87). Similarly, the highest MBCR was found in STB (9.97) followed by STB+CRI (8.23) due to comparatively lower variable cost. Control plots produced the lowest gross return, gross margin, net return and BCR due to low yield (Table 4). The overall economic performance of the aforesaid of T. *Aman* rice is sustainable, considering applied STB nutrient management. STB+CRI nutrient management also gave higher gross margin, net return and BCR compared to other nutrient managements like STB. Many scientists (Ali *et al*., 2009; Biswas *et al*., 2004, 2007, 2008; Zaman *et al*., 2007 a & b) also reported that conducted similar type of experiments with different cropping patterns without crop residue incorporation into the soil and found more or less similar results. However, STB and IPNS nutrient managements along with and without crop residue incorporation might be suitable for T. *Aman* rice production in economic point of view.

Table 4. Economic performance of T. *Aman* rice as influenced by different nutrient managements (mean of 2007 and 2008).

Nutrient management	Total cost (Taka)	Variable Cost (Taka)	Gross return (Taka)	Gross margin (Taka)	Net return (Taka)	BCR	MBCR (over control)
	1	2	3	4=(3-2)	5=(3-1)	6=(3/1)	7
HYG	17310	4661	46903	42242	29593	2.71	7.19
MYG	16033	3384	38233	34849	22200	2.38	7.35
IPNS	17972	5323	53520	48197	35548	2.98	7.54
STB	16767	4118	54420	50302	37653	3.25	9.97
FP	14412	1763	26663	24900	12251	1.85	7.54
CON	12649	0	13383	13383	734	1.06	CON
HYG+CRI	18330	5681	47333	41652	29003	2.58	5.98
MYG+CRI	16963	4314	38603	34289	21640	2.28	5.85
IPNS+CRI	18987	6338	54568	48230	35581	2.87	6.50
STB+CRI	17797	5148	55738	50590	37941	3.14	8.23
FP+CRI	15172	2523	26933	24410	11761	1.78	5.37
CON+CRI	13179	530	13903	13373	724	1.06	0.98

HYG=0-80-16-44-12-2-0, MYG=0-56-12-32-8-1.5-0, IPNS=5-65-13-32-9-2-0, STB=0-68-15-37-11-2-0, FP=0-39-7-12-0-0-0, CON=0-0-0-0-0-0-0, CD (t ha^{-1}), N, P, K, S, Zn, B (kg ha^{-1}), respectively and CRI= Crop Residue Incorporation.

Input and output prices: Urea-6.50 (Tk. kg^{-1}), TSP-19.00, MP-15.00, Gypsum-4.60, Zincsulphate-65.00, Boric acid-100, Cowdung-0.32 and Crop reside-0.50 (dry basis), (Tk. kg^{-1}) Rice grain-10 and Rice straw-0.50 (dry basis)

Conclusion

Soil test based and integrated plant nutrient system nutrient management along with or without crop residue incorporation could be suitable for getting economically higher grain yield of T. *Aman* rice keeping improvement soil health.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 35-51, March 2015

STUDY ON WATER SORPTION ISOTHERM OF SUMMER ONION

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Abstract

The water sorption characteristics of dehydrated onion and onion solutes composite by vacuum drying (VD) and air drying (AD) were developed at room temperature using vacuum desiccators containing saturated salt solutions at various relative humidity levels (11-93%). From moisture sorption isotherm data, the monolayer moisture content was estimated by Brunauer-Emmett-Teller (BET) and Guggenheim-Anderson-de Boer (GAB) equation using data up to a water activity of 0.52 and 0.93 respectively. Results showed that in case of non treated samples the monolayer moisture content values (W_o) of BET gave slightly higher values than GAB (9.7 vs 8.2) for VD, while GAB gave higher value than BET (11.0 vs 9.8) for AD. It is also seen that the treated and non treated onion slice and onion powder absorbed approximately the same amount of water at water activities below about 0.44 and above 0.44 the treated samples begin to absorb more water than the non treated samples. It was observed that 10-20% added of sugar gave no change in water sorption capacity while the amount of sorbed water increases with increasing amount added salt for mix onion product.

Keywords: BET equation, GAB equation, Monolayer moisture content, Water activity

Introduction

Summer onion (*Allium cepa* L.) is one of the most important spice crop grown all the year round in Bangladesh. Even any curry cannot be think without onion. It is used as salad or cooked in various ways in all curries, fried or baked. It has very good medicinal value. Nutritive value of onion varies from variety to variety. It major value is in its flavour. Onion ranks medium in caloric value and minerals, low in protein and very low in vitamins (Pandey, 2004).

Sorption isotherm is considered as one of the most fundamental elements for understanding dehydration process based on the removal of majority of water content in produce (Dauthy, 1995). Sorption isotherm depicts relationship of equlibrium moisture content of products to water activity (a_w) which is defined as the ratio of vapour pressure of water in a product (food) to the vapour pressure of saturated water at the same temperature and atmospheric pressure. Since water activity of food affects the rate of biological and chemical reactions, the drying

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process when properly designed could inhibit the growth and reproduction of microorganisms that could lead to decay (Herringshaw, 1997). It also prevents biochemical reactions associated with moisture content and results in nutrient loss and deterioration of product quality including organoleptic properties (Haralampu and karel, 1983 and Okos *et al.*, 1992).

Optimal design of drying and storage process is based on knowledge of moisture sorption isotherms and water activity. Moisture sorption isotherms added to the food isosteric heat of sorption are essential for designing efficient dryers and several other food preserving methods. These parameters are useful for predicting stability changes in produce, selecting packing material and ingredients and estimating the energy required for the drying process (Lee and Labuza, 1975).

The moisture sorption isotherms can be obtained by process either increasing or decreasing moisture content are termed as adsorption isotherm and desorption isotherm respectively. Desorption isotherms generally result in higher values of equilibrium moisture content than adsorption isotherm at a given water activity. and this phenomena is termed as hysteresis (Young and Nelson, 1976). This behaviour is especially exihibited by hygroscopic products. At constant moisture content desorption isotherm gives lower water activity than adsorption path. For safety reason it is advised to determined adsorption isotherm particularly for stored products in a changing external environment.

Many researchers have devised a wide variety of ways for describing sorption isotherms, resulting in a large number of theoretical, semitheoretical and emperical equations (Chirife and Iglesias, 1978). Among these theoretical approaches, the BET theory advanced by Brunauer *et al.* 1938 has been the most successful and widely used in the food industry due to its simplicity and more easily interpretable results with minimum data input. The theory, generally known as BET theory, utilizes the assumption that Vander waals forces account for sorption of water molecules on product surfaces.

The monolayer sorption and the heat of sorption are important because these values are measures of the molecular level status of the total system as defined by the BET theory. The BET monolayer value has been said to be optimal water content for stability of low-moisture materials (Roos, 1995).

The GAB model was found to be the most suitable for describing the sorption data. The monolayer moisture content was estimated using the Brunauer-Emmett-Teller (BET) equation. The BET model (Brunauer *et al.* 1938) gave the best fit to the data at a_w of up to 0.5 (Bell and Labuza, 2000 and Roos, 1995). Guggenheim-Anderson-de Boer (GAB) sorption model introduces a third state of sorbed species intermediates to the tightly bound and free states (Anderson, 1946).

Hossain *et al.* (2001) observed that higher sorption isotherms at lower temperature and the modified BET model was found to be the best fit model in the temperature range of 20-25^{0}C and relative humidity range of 11-97%.Therefore, the present study was undertaken to determine relationship between a_w and quality characteristics of different dried onion and onion solute composites .

Materials and Method

Sample

The experiment was conducted in the Department of Food Technology and Rural Industries, Bangladesh Agricultural University (BAU), Mymensingh during the period of January, 2011 to July 2011. Freshly harvested summer onion variety of BARI piaz-2 was procured from Spices Research Center (SRC) of Bangladesh Agricultural Research Institute (BARI) and stored at room temperature. Sucrose and salt were bought from local market. All other chemicals were of reagent grade and collected from the scientific store.

Method

Onions free of mechanical injury and disease were cleaned and washed with tap water and spread on a perforated tray to drain out excess water and subsequently used for preparation of samples. After peeling onions were cut into slices of approximately 5mm thickness by an electric slicer or made into slurry by electric blender before being made into samples. When studying sorption behavior of osmosed samples, onion slices were first osmosed (24 hr, in appropriate solution) and slices were then either i) directly dried in vaccum dryer at 70^{0}C or ii) ground to a slurry before vaccum drying. In some cases the ground slurry was blended with known amount of solutes prior to being vaccum dried.

Determination of Sorption isotherm

Procedures for determining equilibrium moisture content as influenced by water activity in food materials are described in detail by Gal (1983) and Shatadal and Jayas (2000). The principal methods are gravimetric and menometric/ hygrometric. The gravimetric method is divided into static and dynamic methods. As the static gravimetric method is simple and reliable, procedures noted by Islam (1980) with certain modifications were applied in determining sorption isotherm in this study.

The dehydrated 2g onion samples were taken in each crucible. Nine desiccators with nine saturated salt solution (Table-1) were used to determine the equilibrium moisture content for vaccum dried onion at room temperature (about 25^{0}C) over a water activity range 0.11 to 0.93. The sample and the solution was separated by a perforated plate to avoid mixing. The dessicators were evacuated to less than 50 torr. The crusibles were kept in vaccum desiccators over saturated salt solution with two replication. At various intervals, the vacuum was broken with air, the sample weighed and replaced in the desiccators, which was then re-evacuated. After attaining equilibrium the crucible were collected from each desiccators and the equilibrium moisture contents of the dried samples were determined by oven method (AOAC, 2000).

The following salt solutions (Table 1) of known water activity were used for the study.

Table 1. Water activity of saturated salt solution.

Saturated salt solution	Water activity (a_w)[b]
LiCI [a]	0.11
$KC_2H_3O_2$ [a]	0.20
$MgCI_2.6H_2O$ [a]	0.33
K_2CO_3 [a]	0.44
$Mg(NO_3)_2.6H_2O$ [a]	0.52
$CuCI_2$ [a]	0.68
NaCI [a]	0.75
KCI [a]	0.85
KNO_3 [a]	0.93

i) a indicates all chemicals were of reagent grade

ii) b indicates Labuza *et al.* (1976)

The BET theory states that sorption behavior can be represented by the following equation, popularly known as BET equation, derived either kinetically from thermodynamic considerations or from statistical mechanics (Adamson, 1963).

$$\frac{W}{W_0} = \frac{C_a}{(1-a_w)\,[1+(c-1)\,a_w]} \quad (1)$$

This may be rearranged as:

$$\frac{a_w}{(1-a_w)\,W} = \frac{1}{W_0\,C} + \frac{a_w\,(C-1)}{W_0\,C} \quad (2)$$

Where

a_w = water activity

W = moisture content, dry basis (db)

W_0 = monolayer moisture content (db)

C = energy constant

The average heat of sorption, ΔH_s , is given by,

ΔH_s = RT (Inc)---(3)

Where

R = gas constant

T = absolute temperature

Plotting $\frac{a_w}{(1-a_w)W}$ vs. a_w, the monomolecular moisture content and heat of sorption can be determined from the slope and intercept.

Curl *et al.*, (1976) rearranged the classical BET equation give:

$$\frac{a_w}{(1-a_w)\,W} = \frac{1}{W_0} + \frac{1}{CW_0}\,(\frac{1-a_w}{a_w}) ---------------------- \quad (4)$$

From a plot of $\frac{a_w}{W\,(1-a_w)}$ vs. $\frac{1-a_w}{a_w}$, a straight line is obtained which intersects the ordinate at a point equal to $\frac{1}{W_0}$ and the abscissa at a point equal to –C. The use of the rearranged equation enables one to directly estimate the value of C and W_0.

The monolayer sorption and the heat of sorption are important because these values are measures of the molecular level status of the total system as defined by the BET theory. The BET monolayer value has been said to be optimal water content for stability of low-moisture materials (Roos,1995).

The GAB model was found to be the most suitable for describing the sorption data. The monolayer moisture content was estimated using the Brunauer-Emmett-Teller (BET) equation. The BET model (Brunauer *et al.* 1938) gives the best fit to the data at a_w of up to 0.5 (Bell and Labuza, 2000 and Roos 1995). The GAB equation has a similar form as that of BET, but has an extra constant, K (equation-5). BET is actually a special case of GAB.

The GAB equation is usually presented in the following form:

$$W = \frac{W_m K C a_w}{(1-Ka_w)\,(1-Ka_w+CKa_w)} --(5)$$

Where,

W = the equilibrium moisture; fraction (db)

W_m = the monolayer moisture content; fraction (db)

C = the GAB multi-layer constant

K = factor correcting properties of multilayer with respect to the bulk liquid

The following procedure is suggested by Biozt (1983) to fit data on water activities and equilibrium moisture content.

Equation (5) can be transformed as follows:

Equation (5) can be transformed as follows:

$$\frac{a_w}{W} = \alpha a_w^2 + \beta a_w + \gamma \text{--} \quad (6)$$

Where,

$$\alpha = \frac{k}{W_m}\left(\frac{1}{C} - 1\right)$$

$$\beta = \frac{1}{W_m}\left(1 - \frac{2}{C}\right)$$

$$\gamma = \frac{1}{W_m C k}$$

Equation (6) indicates that GAB equation is a three-parameter model. The water activity and equilibrium moisture content data are regressed using equitation (6) and values of three coefficients α, β and γ are obtained. From these coefficients, the values of K, W_m, and C can be calculated.

Results and Discussion

The sorption isotherm is a valuable tool for food researcher for its importance in dehydration process, in packaging and in changing of quality during storage. The sorption isotherm of vaccum oven dried (VD) and air dried (AD) onion, water sorption behaviour as influenced by physical state of onion and onion solute composites, sorption behavior of onion composites as affected by composition and concentration of added solutes and sorption behaviour of onion at different temperature were developed and discussed one by one in the following sections.

Comparison of sorption behaviour of vacuum oven dried and air dried onion slice

Adsorption isotherms for dehydrated onion obtained by VD and AD were developed to determine how the onion product will behave in a confined environment. The experimental moisture sorption data obtained corresponding to a_w values for both VD and AD are presented in Fig. 1.

It is seen that samples absorbed little water particularly at below a_w (<0.52). Thereafter the water absorption was comparatively higher at water activity (a_w) values above 0.52. Water uptake in both the sample was low upto 0.52 a_w, medium in the range of 0.52 to 0.8 a_w, while beyond 0.8 a_w water sorption rapidly increases as a_w approaches 1.0, indicating that the isotherms have three distinct regions as noted by Ouaouich, (2004). Almost similar water sorption behaviour was observed for VD and AD onion slices (Chirife and Iglesias, 1978). However, Kamruzzaman (2005) mentioned that vaccum dried aroids gave higher equilibrium moisture content at each a_w values except 0.75. The differences might be due to differences in chemical constituents' composition (Rao and Rizvi, 1986) and structure of the raw material.

The monolayer moisture content was estimated using BET and GAB equation. The BET equation is an extension of the Langmuir relationship that accounts for multilayer coverage.

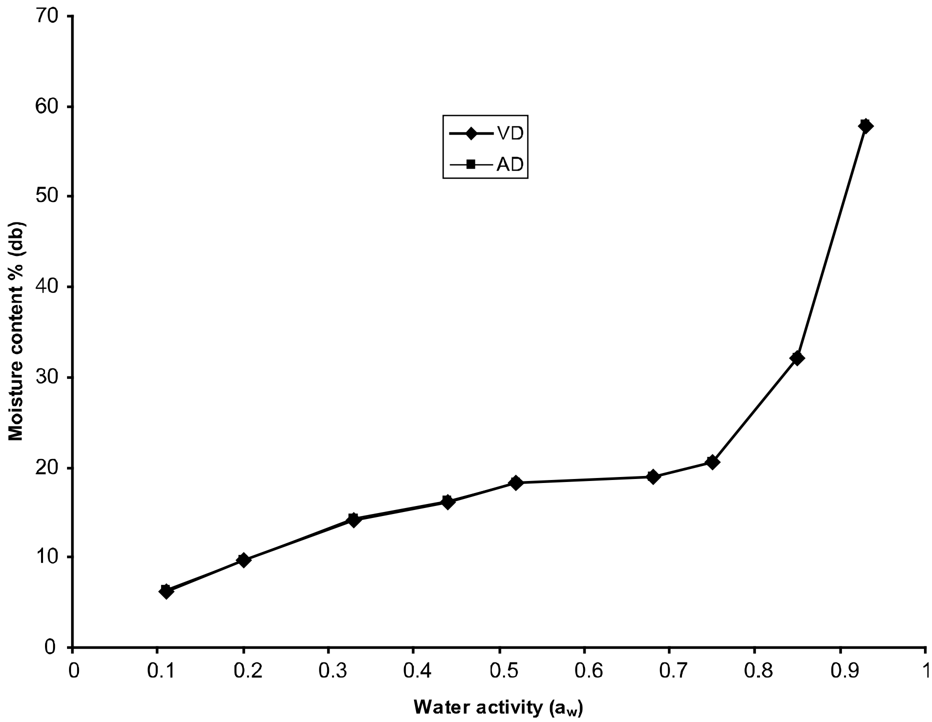

Fig. 1. Graphical presentation of sorption isotherm of vacuum dried and air dried onion

BET equation (1) was used (for data only up to a_w of 0.52) to calculate monolayer moisture content (W_0) and energy constant (C). W_0 represents the optimal moisture for maximum storage stability in the dry state. The following equations were obtained for AD and VD. These equations were then used to calculate W_o and C for both AD and VD as shown in Table 2.

$$\frac{a_w}{(1-a_w)W_0} = 0.0069 + 0.0959\, a_w \text{ (for VD)------------------------------(1)}$$

$$\frac{a_w}{(1-a_w)W_0} = 0.0068 + 0.0954\, a_w \text{ (for AD)-------------------------------(2)}$$

Table 2. Monolayer moisture content and energy constant for vacuum dried (VD) and air dried (AD) sample.

Sample	Energy constant (cal/g-mole)	Monolayer moisture content (g/100 g solid)
VD (BET)	14.90	9.73
AD (BET)	15.03	9.79
VD (GAB)	26.02	8.21
AD (GAB)	22.39	10.99

The monolayer moisture content of onion was found to be 9.73 and 9.79 g water per 100g solid for VD and AD respectively. The calculated monolayer moisture contents are greater than those found by Islam, (1980) who reported 5.5% moisture content (db) for potato slice and 6% moisture content (db) for potato powder as W_o. Kamruzzaman (2005), however, found 9.60 and 7.52% moisture content (db) as W_o for VD and AD of Aroids respectively.

GAB (Guggenheim-Anderson-De Boer) being another important model for describing sorption isotherm behavior. The sorption data of VD and AD onion were analyzed as per equation 6 and the following equation were obtained:

$$\frac{a_w}{W} = -0.0804\, a_w^2 + 0.0926 a_w + 0.0052 \text{ …… For VD…………. (3)}$$

$$\frac{a_w}{W} = -0.0813\, a_w^2 + 0.0942 a_w + 0.0045 \text{ …… For AD…………. (4)}$$

From Fig. 1 it is seen that at VD and AD onion slices gave about 18% and 19% moisture content at 0.6 a_w. It may be mentioned here that the current study was concerned with adsorption isotherm so as to avoid risk due to hysteresis effect.

At same moisture content adsorption path gives higher water activity than desorption path. Thus product dried to safe a_w level according to adsorption isotherm will be even safer when it follows desorption path.

The coefficients found α, β and γ were -0.0804, 0.0926 , 0.0052 and -0.0813, 0.0942, 0.0045 for VD and AD respectively (equation 3 and 4) . The monolayer moisture content were found 8.21 and 10.99 g water per 100 gm dry-solids for VD and AD respectively, when K= 0.9 (Table 2).

Compared to monolayer moisture content of GAB equation BET gave slightly higher value (9.73 vs. 8.21) for VD. On the other side, for AD, GAB gave higher value than BET (10.99 vs. 9.79). However, variations are not very high. Considering experimental limitation and design criteria, both models could be utilized. It should, however, be mentioned that BET model is applicable up to 0.52 a_w, while GAB is valid up to 0.93 to 0.973 a_w (Roos, 1995 and Talla, 2012) giving an added advantage in predicting sorption behaviour over a wider region. Monolayer moisture content value is important for dry foods and this levels of moisture content is the most stable to chemical reactions (Labuza, 1972; Bluestein and Labuza, 1975).

Considering very low differences in monolayer moisture content values between the VD and AD and from similar sorption behaviour shown in the entire a_w range (0.11 to 0.93 a_w) by both VD and AD it may be concluded that both AD and VD samples could be used for analyzing sorption behaviour of onion and thus also used for determining end point of drying.

Water sorption behaviour as influenced by physical state of onion and onion solute composites

Water sorption isotherms were used to demonstrate changes in structure of food materials. Thus the water sorption behavior of onion samples, in the form of slices and powder (prepared from plain and osmosed materials) and onion powder blended with salt were examined to determine if gross onion structure had an influence on water uptake.

The results shown in Fig. 2, indicated that there were no difference in water sorption capacity of slices and powder. At higher water activity, non-treated (plain) slices (the Fig 2 is redrawn from Fig.1 for comparison) begin to absorb more water than powder. No definite deviation as to sorption behaviour was noted for the osmosed samples as well as onion powder blended with salt when compared among themselves.

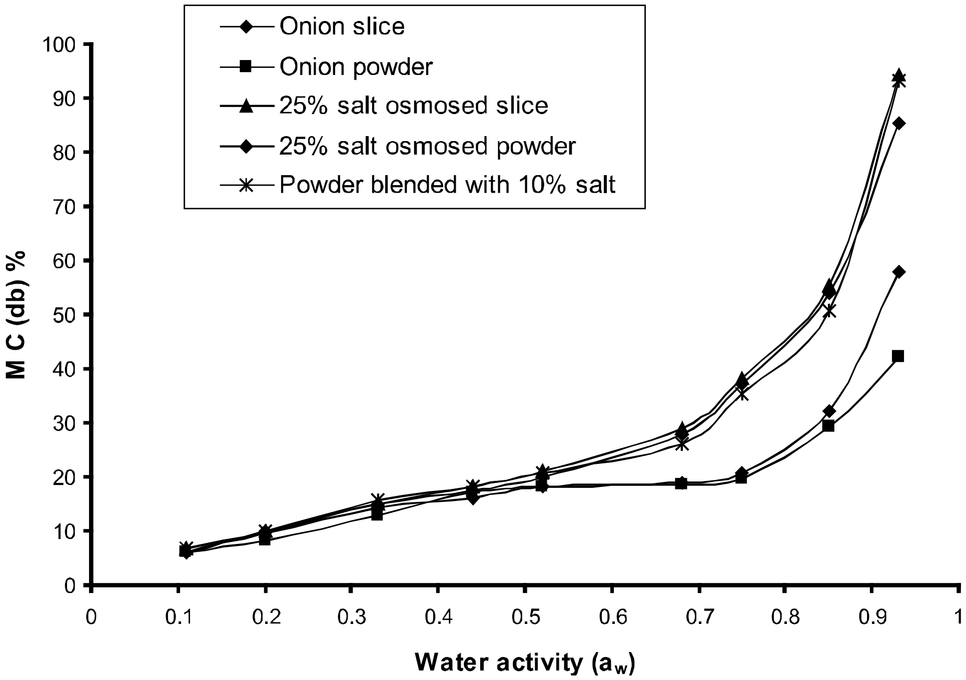

Fig. 2. Water sorption isotherm of onion and onion solute composite

The difference in sorption capacity between slices and powder at higher water activity (at or above 0.75 a_w) is due to differences in the structural integrity of onion slices and powder. When onion powder is made, the cellular structure and thus also capillaries are somewhat destroyed, whereas minimum damage is caused to the structure when slices are prepared. Since at higher water activity, a large amount of the sorbed water is found in capillaries and intercellular spaces (Labuza, 1968; Nickerson and Sinskey, 1972 and Christian, 1968) of slices could be expected to sorb more moisture than powder. However, this difference may not be critical to the goal of this study since to obtain stable dehydrated product from non treated onion, the moisture content of the product should correspond to water activity below 0.75.

Comparison between treated samples-osmosed slices, powder from osmosed slices and a powder-salt blend and nontreated samples (onion slices and onion powder) showed that both groups (treated and nontreated samples) absorbed approximately the same amount of water at water activities, below about 0.44 (Fig. 2). At water activities above 0.44, the treated samples begin to adsorb more water than the nontreated and the difference becomes larger as water activity approaches 1 (Fig. 2). The similarity of water sorption behavior at lower water activity is confirmed by the calculated values of BET and GAB monolayer moisture content (Table 2). The calculation method is the same as noted in the section -1. In this cases (except for onion slices) GAB values are higher compared to BET which is in agreement with literature (Timmermann, 2003).

The treated samples having infused or added salt sorbs very little water at water activities below 0.44 and thus the adsorption is primarily due to the polymeric material of the onion tissue. At higher water activities, considerable adsorption takes place which can be attributed to the presence of infused or added salt. This behavior is in good agreement with the literature (Saravacos and Stinchfield, 1965; Islam and Farouk,1981).

From these results, two important conclusions can be drawn. One was that, at water activities of interest relative to the stability of dehydrated onion product, sorption isotherm studies for osmosed materials could be simulated using a model system made of powder (vaccum dried) and salt. The other conclusion was that by treating with salt, the water sorption capacity of the onion product can be made higher so that at water activity values at which the dehydrated products are shelf-stable, the product can be preserved at higher moisture content. This would possibly reduce the drying cost compared to nontreated products, since less moisture removal will be required. As mentioned earlier (section -1) that for experimental reasons, this study has been concerned with the adsorption isotherm. Since the adsorption isotherm has a lower moisture content at each water activity than the corresponding desorption isotherm (which is closer in concept to drying process), it is expected that the dried product would be safely preserved at moisture contents even higher than those presented here (i. e. still at a safe a_w level). Accordingly, the values used in this study represent conservative estimates of safe moisture contents.

Table 3. Monolayer moisture content of onion and onion solute composites

Product	Monolayer moisture content for BET equation	Monolayer moisture content for GAB equation	Moisture content at a_w 0.65
Onion slice[a]	9.73	8.21	18.843
Onion powder[b]	10.27	14.75	18.465
Osmosed slice[c]	11.38	15.08	27.423
Osmosed powder[d]	11.06	13.51	26.433
Powder, salt blend[e]	11.06	15.13	25.141

[a]Onion sliced and vaccum dried.

[b]Onion sliced and vaccum dried and powdered

[c]Onion slice osmosed (24 hr in 25% salt solution) and vaccum dried.

[d]Onion sliced osmosed (24 hr in 25% salt solution) and vaccum dried.and powdered

[e]Vaccum dried onion powder blended with 10% salt

Sorption behavior of onion composites as affected by composition and concentration of added solutes

Different concentration of salt and sugar singly or in combination were used to examine the influence of composition and concentrations of these two osmosis solutes on water sorption behavior of onion at a_w ranging from 0.52 to 0.93 as below a_w 0.52 added solute (s) does not have additional influence in water uptake (cf. section 2). The results are shown as a series of water sorption isotherms (Fig. 3).

An examination of the sorption isotherms (Fig. 3) showed that 10-20% added sugar gives slight change above 0.75 a_w in water sorption capacity. On the other hand, addition of salt significantly increases the water sorption capacity of the mixed onion product and the amount of sorbed water increases with increasing amount of added salt. When both salt and sugar are added (each at the 10 percent level) to onion, there appears to be no synergistic effect in so far as water sorption behavior is concerned and the increased amount of water uptake at each water activity is only due to the presence of the added salt.

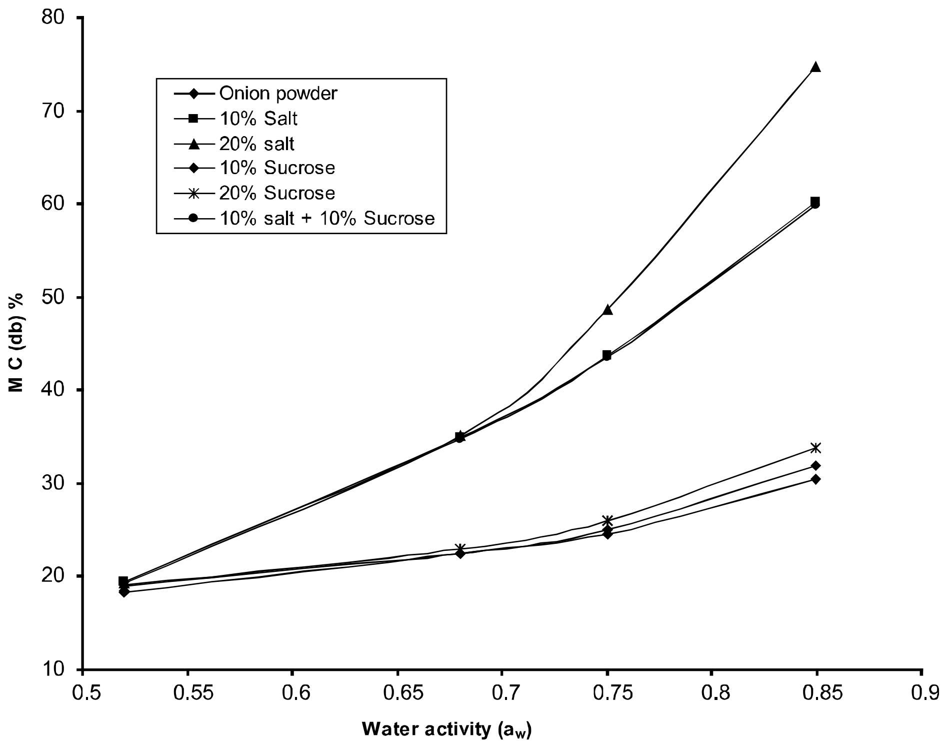

Fig. 3 Effect of composition and concentration of added solutes on water sorption behaviour

It has been shown (Fig. 2) that the sorption isotherm of onion with added salt begins to deviate from the isotherm for plain onion at water activities above 0.44. It can now be seen (Fig. 3) that the isotherm for onion with 10 and 20% added salt deviate from each other at water activities above 0.68, with samples having 20% salt levels giving higher moisture content than that with 10% salt. The steady increase in EMC with increasing salt content, especially at higher water activities, demonstrates the fact that salt is a good water binding agent particularly around its saturation vapor pressure (Islam, 1980). Gal (1971) observed a similar phenomenon, where the equilibrium moisture content of casein increased with increasing salt content, until the protein became saturated with ion pairs. Shibata *et al.* (1976) demonstrated that the water sorption isotherm of dried noodle is strongly influenced by added salt (NaCl) above an a_w of 0.6. Islam and Farouk (1981) also showed that at higher water activities, large proportion of water may be sorbed by added salt. While this statement is true for salt, it seems from the above results that it does not hold for sugar-onion blends over the water activity ranges of interest to the stability of dehydrated products (0.62 to 0.75 a_w) as defined by Nickerson and Sinskey (1977).

This implies that only salt would be useful to develop dehydrated onion products having higher equilibrium moisture contents corresponding to the water activity ranges in which dehydrated products are shelf stable. Thus added salt might reduce the cost of drying as the final moisture content required for stability is increased. However, it should be noted that water activity is not the only factor determining the stability of dehydrated product. Presence of solute and/or preservatives, temperature etc. might have significant influence on product stability.

Sorption behaviour of onion at different temperature

The experimental results of adsorption equilibrium moisture contents of dried onion at temperatures of 10, 30 and 50^{0}C are presented in Fig. 4. Higher equilibrium moisture contents were found at the lower temperature for the same water activity. For most of the food materials the equilibrium moisture content values decrease with an increase in temperature at a constant water activity. The reason may be that with an increase in temperature the water molecules get activated due to an increase in their energy level, causing them to become less stable and to break away from the water binding site of the food material, thus decreasing the monolayer moisture content. Similar results have been reported by many investigators for food materials low in sugar content (Shatadal and Jayas, 2000). Turhan *et al.* (1997) presented similar effect of temperature on adsorption and desorption isotherms for Turkish red chilli, while Talla (2012) found similar temperature effect for sorption behaviour of rough rice and kilishi respectively. According to the classification of isotherm curves proposed by Brunauer *et al.* (1940) all of the isotherm curves were found to follow a pattern of sigmoid in shape (type II).

The implication of effect of temperature on sorption behaviour is very crucial particularly for import/export business as packaged food material with constant moisture content would give higher water activity when transported to a country with higher ambient temperature and the stability of product might be at risk.

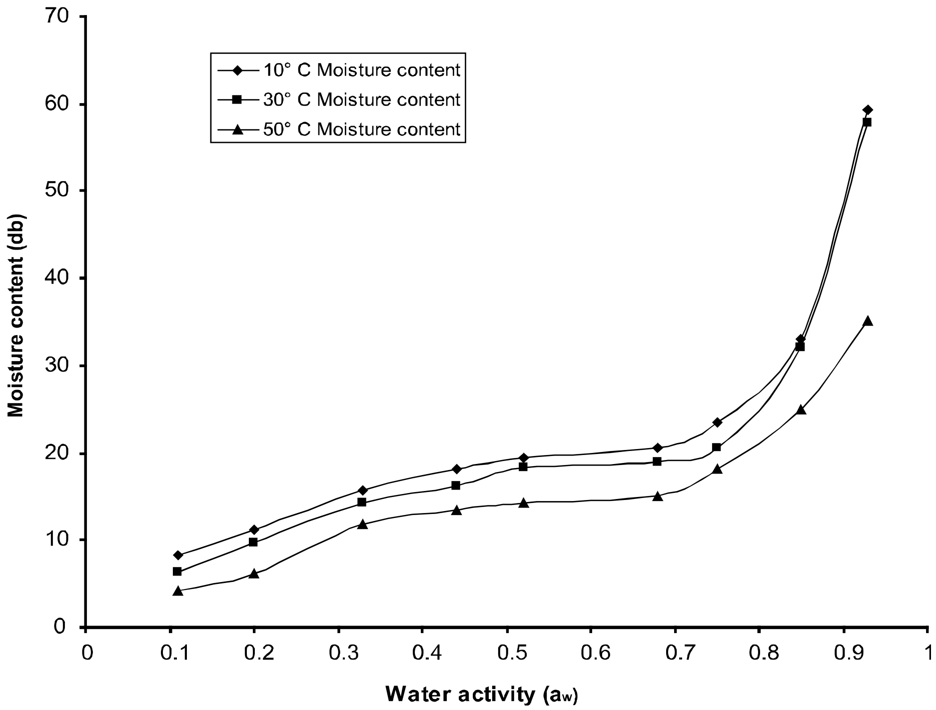

Fig. 4. Sorption isotherm of summer onion at different temperature

Conclusion: AD or VD samples could be used for analyzing sorption behaviour of onion. Except for onion slices, onion sample with infused or added salt gave higher GAB monolayer values compared to BET. Salt is preferred as osmosis solute as it is cheaper and it gives higher EMC compared to sugar particularly at higher $a_{w.}$ The higher the salt content following osmosis the higher is the moisture content at water activities of interest to stability of dehydrated products (0.62 to 0.75 a_w). The effect of temperature or sorption isotherm demands precautionery measures when packaged dried onion would be transported to a country with higher ambient temperature.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 53-60, March 2015

EFFECT OF NITROGEN LEVEL AND LEAF CUTTING FREQUENCY ON FOLIAGE AND SEED YIELDS OF CORIANDER

M. MONIRUZZAMAN[1] AND M. M. RAHMAN[2]

Abstract

A field experiment was conducted at BSMRAU farm, Gazipur to evaluate the effects of four nitrogen levels (0, 40, 80 and 120 kg/ha) and four levels of leaf cutting (no cutting, one cutting at 30 DAS), two cuttings at 30 & 45 DAS and three cuttings at 30, 45 & 60 DAS) on three genotypes of coriander (*Coriandrum sativum* L.) (CS001, CS002 and CS003). The genotype CS003 produced the highest foliage yield (8.92 t/ha) and the genotype CS001 gave the highest seed yield (0.93 t/ha). The maximum foliage and seed yields were obtained from the N application at 80 kg N/ha. The maximum foliage yield (11.21 t/ha) was recorded with the three cuttings, but the highest seed yield was noted with the one cutting (1.06 t/ha). The 80 kg N/ha coupled with three cuttings gave the top most foliage yield while the same rate accompanied with one cutting gave the top most seed yield for all genotypes.

Keywords: Nitrogen, leaf cutting, green leaf, seed yield, coriander, *Coriandrum sativum* L.

Introduction

Coriander (*Coriandrum sativum* L.*)* in Bangladesh is an important spice crop grown in *rabi* season and it is known as 'dhonia'. The young plant of coriander is used as appetizer in preparing fresh chutneys and sauces and leaves are used to flavour food, curries, soups, fish sauce, etc. and seeds are used in cakes, soups, sausage, pickles, curries, etc. (Janardhanan and Thoppil, 2004; Tiwary and Agarwal, 2004). Coriander seeds are also used in preparation of medicines (Sharma and Sharma, 2004). Two types of genotypes are available in respect of seed and foliage production purposes. Some genotypes are exclusively cultivated for seed purposes and some are cultivated for seed as well as foliage (leaf) production. The later is called dual purpose genotype. In Bangladesh, seed purpose varieties as well as dual purpose varieties are imported from foreign countries.

Leaf cutting and nitrogen application have been reported to increase the number of branches and umbels per plant which in turn increased the yield of leaves and seeds in coriander (Thakral *et al.* 1992; Tiwari *et al*., 2002). Application of nitrogen encourages vegetative growth which resulted in the increased yield of

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leaves and seeds of coriander (Datta *et al.* (2008). The present study was undertaken to find out the optimum dose of nitrogen for higher leaf and seed yield and to determine the frequency of leaf cutting in relation to leaf and seed yield of coriander.

Materials and Method

The experiment was conducted at the research farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur (AEZ-28) during November 2009 to May 2010. The experimental field comprised with the Piedmont plain soil having medium loamy to moderately fine texture (sandy clay loam). The soil was poor in organic matter (1.91%) and moderately acidic (pH 5.90). The total N, available P, exchangeable K, available S and B in soil were 0.079%, 30.77 ppm, 0.35 meq/100 g soil, 13.4 ppm and 0.31 ppm, respectively.

The experiment was laid out in a split-split plot design with three replications having three genotypes (CS001, CS002 and CS003) in main plots, four nitrogen levels (0, 40, 80 and 120 kg/ha) in sub-plots and four levels of leaf cutting (No cutting, one cutting at 30 DAS, two cuttings at 30 and 45 DAS and three cuttings at 30, 45 and 60 DAS) in sub-sub plots. The coriander genotypes were collected from Siddique Bazzar, Dhaka. The unit plot size was 3 x 1 m. The first and the second leaf cutting were done 4 cm above the ground leaving the terminal bud during the third cutting the suitable leaves are cut.

The land was fertilized @ 5 t cowdung, 40 kg P, 40 kg K and 20 kg S per hectare. Full amount of cowdung, TSP, MOP and gypsum was added as basal application. One- half of urea was applied during final land preparation and the rest of the urea was applied in three equal instalments after each cutting as top dress. The seeds (fruits) were rubbed for separating the two mericarps (seeds) and were soaked in water for 24 hours to enhance germination. Seed were also treated with Bavistin at 2g per kg of seeds before sowing. The seeds were sown in 20 cm apart lines continuously by hand @ 30 kg/ha. Sowing was done on November 9, 2009. First and second weedings were done after 25 and 60 days after sowing, respectively. Plant thinning was done at 25 DAS maintaining 10 cm distance between plants. Irrigation was done at 30, 45, 60 and 85 days after sowing. Malathion @ 1.5 ml/l was sprayed during fruiting stage to reduce the attack of aphid.

Harvesting of leaves was done at 30, 45 and 60 days after sowing. Seeds were harvested when half of the fruits on the plant changed from green to brown colour (Singhania *et al*. 2006). Seeds were dried in the sun to attain 10% moisture.

Data were recorded umbels/plant, umbelletes/plant, seeds/umbel, 1000-seed weight (g), stover yield and seed yield (t/ha). Plot yield was converted to per hectare yield. The data were analyzed statistically by MSTAT-C Program and mean comparison was done following the Duncan Multiple Range Test (DMRT).

Results and Discussion

Data on effects of genotypes, N levels and leaf cutting frequency have been shown in Tables 1-5.

Effect of genotypes: Umbels/plant (33.1), umbellates/umbel (5.47) and seeds/umbel (20.52) were found highest with CS001 followed by CS002 and then CS003 (Table 1). The CS002 gave the maximum 1000-seed weight (11.7 g) compared to other genotypes. The genotypes CS001 and CS003 gave the identical result in respect of 1000-seed weight. The genotype CS003 produced the highest foliage yield (8.92 t/ha) and the CS001 did the lowest (6.95 t/ha). The highest seed yield was recorded from CS001 (0.93 t/ha) followed by CS002 (0.89 t/ha) and then CS003 (0.79 t/ha). The genotype CS003 produced the higher stover yield (1.95 t/ha) and the CS001 and CS002 gave an identical yield (1.36 t/ha).

Effect of nitrogen level: Application of 80 kg N/ha produced the highest umbellates/umbel (5.75) followed by 120 kg N/ha (Table 1). The maximum seeds/umbel was recorded at both 80 and 120 kg N/ha (21.3) followed by 40 kg N/ha (20.3). The highest 1000-seed weight was obtained from the application of 80 kg N/ha. The yield of foliage increased progressively with the increase of N levels up to 80 kg N/ha beyond which it declined (Table 1). Similar trend was observed with N application at 80 kg N/ha (1.11 t/ha). Higher rate of N application (120 kg/ha) was not beneficial in respect of seed yield as it produced more vegetative growth and less reproductive growth. Thakral *et al.* (1992) reported the highest number of umbels/plant and seed yield (15.98 q/ha) at 60 kg N/ha and the highest green leaf yield at 90 kg N/ha. Datta *et al*. (2006) observed that number of umbels/plant, number of seeds/umbel green leaf yield and seed yield increased significantly up to 60 kg N /ha. The stover yield due to 80 and 120 kg N/ha was statistically similar (Table 1).The N control treatment for all parameters showed the lowest results.

Effect of leaf cutting frequency: Umbels/plant (36.5), umbellates/umbel (5.70) and seeds/umbel (24.3) were recorded as the highest from one cutting (C_1) followed by no cutting (C_0) and their lowest values were obtained from three cuttings (C_3). The 1000-seed weight was not influenced by cutting frequency. The yield of foliage increased with the increasing frequency of cuttings (Table 1). The significantly maximum foliage yield (11.21 t/ha) was obtained from three cuttings (C_3) and the minimum (9.24 t/ha) from one cutting (C_1). This result is in agreement with Thakral *et al.* (1992) and Datta *et al.* (2008) in coriander. The maximum seed yield was obtained from one cutting (C_1) (1.06 t/ha) followed by

Table 1. Effect of genotypes, nitrogen levels and leaf cutting frequency on yield attributes of coriander.

Factor	Umbels / plant (no.)	Umbellates /umbel (no.)	Seeds /umbel (no.)	1000 -seed weight (g)	Foliage yield (t/ha)	Dry seed yield (t/ha)	Stover yield (t/ha)
Genotype							
CS001	33.13 a	5.47 a	20.52 a	10.88 b	6.95 c	0.93 a	1.36 b
CS002	29.52 b	5.20 b	19.96 b	11.74 a	7.35 b	0.89 b	1.36 b
CS003	26.82 c	5.03 c	18.69 c	10.85 b	8.92 a	0.79 c	1.95 a
Nitrogen levels							
N_0	20.63 c	4.64 d	16.09 c	10.87 d	4.99 d	0.49 d	1.02 c
N_1	28.88 b	4.92 c	20.28 b	11.23 b	7.40 c	0.83 c	1.47 b
N_2	34.81 a	5.75 a	21.26 a	11.34 a	10.07 a	1.11 a	1.87 a
N_3	34.97a	5.63 b	21.26 a	11.19 c	8.49 b	1.06 b	1.88 a
Cutting frequency							
C_0	31.46 b	5.48 b	21.69 b	11.16	0.00 d	0.97 b	1.68 b
C_1	36.48 a	5.70 a	24.26 a	11.16	9.24 c	1.06 a	1.94 a
C_2	25.92 c	5.25 c	19.91 c	11.15	10.51 b	0.81 c	1.46 c
C_3	25.23 d	4.51 d	13.03 d	11.18	11.21 a	0.65 d	1.14 d
CV (%)	4.60	3.25	5.41	2.62	3.00	6.87	9.14

Means showing different letters are significantly different at 5% level of probability by DMRT

N_0 = 0 kg/ha, N_1 = 40 kg/ha, N_2 = 80 kg/ha and N_3 = 120 kg/ha

C_0 = No foliage cutting

C_1 = One foliage cutting at 30 DAS

C_2 = Two foliage cutting at 30 DAS (except terminal portion) and 45 DAS

C_3 = Three foliage cutting at 30, 50 (except terminal portion) and 60 (only the suitable leaves) DAS

Table 2. Interaction effect of nitrogen levels and foliage cutting on yield and yield attributes of coriander.

Treat. comb.	Umbels/plant (no.)			Umbellates/umbel (no.)			1000-seed weight (g)		
	CS001	CS002	CS003	CS001	CS002	CS003	CS001	CS002	CS003
N_0C_0	23.20 h	21.50 g	20.80 g	5.03 f	4.80 ef	4.72 gh	10.70 f	11.37f	10.63f
N_0C_1	26.91 fg	24.94 f	24.13 ef	5.40 e	4.99 d	4.91 ef	10.73ef	11.27g	10.60f
N_0C_2	16.02 i	17.63 h	17.05 h	4.83 g	4.66 f	4.56 h	10.60 g	11.30fg	10.53g
N_0C_3	18.55 i	17.20 h	16.64 h	4.16 i	3.84 h	3.76 j	10.80 e	11.33fg	10.70e
N_1C_0	32.01 d	29.70 e	28.70 c	5.50 e	5.09 d	4.98 e	10.93cd	11.93bc	10.80d
N_1C_1	37.13 b	34.41 d	36.19 a	5.72 d	5.29 c	5.19 d	10.90d	11.90bcd	10.83d
N_1C_2	26.24 g	24.33 f	25.58 d	5.11 f	4.94 de	4.76 fg	10.87d	11.93bc	10.93c
N_1C_3	25.61 g	23.74 f	22.96 f	4.40 h	4.08 g	3.98 i	10.93cd	11.87cd	10.97c
N_2C_0	34.80 c	44.50 b	31.20 b	6.29 b	5.81 b	5.69 b	11.00c	11.97b	11.10b
N_2C_1	40.36 a	51.62 a	36.19 a	6.51 a	6.04 a	5.93 a	11.10a	12.05a	11.17a
N_2C_2	28.53 e	36.49 c	25.57 d	5.87 cd	5.64 b	5.52 c	11.07ab	12.04a	10.97c
N_2C_3	27.84 ef	35.61 cd	24.95 de	5.03 f	4.65 f	4.55 h	11.08ab	12.07a	10.73e
N_3C_0	35.17 c	44.60 b	31.30 b	6.30 b	5.82 b	5.70 b	10.90d	11.73e	10.83d
N_3C_1	40.43 a	51.65 a	36.25 a	6.50 a	6.03 a	5.95 a	10.77ef	11.83d	10.93c
N_3C_2	28.50 e	36.55 c	25.60 d	5.90 c	5.69 b	5.50 c	10.93cd	11.73e	10.93c
N_3C_3	29.37 ef	35.66 cd	26.00 d	4.99 fg	5.83 b	4.89 efg	10.90d	11.73e	11.00c
CV (%)		4.60			3.25			2.62	

Means showing different letters are significantly different at 5% level of probability by DMRT

N_0 = 0 kg/ha, N_1 = 40 kg/ha, N_2 = 80 kg/ha and N_3 = 120 kg/ha

C_0 = No foliage cutting, C_1 = One foliage cutting at 30 DAS, C_2 = Two foliage cuttings at 30 DAS (except terminal portion) and 45 DAS

C_3 = Three foliage cutting at 30, 45 (except terminal portion) and 60 (only the suitable leaves) DAS

Table 2. contd.

Treatment combination	Foliage yield (t/ha)			Seed yield (t/ha)		
	CS001	CS002	CS003	CS001	CS002	CS003
N_0C_0	0.00 l	0.00 k	0.00 l	0.60 i	0.54 i	0.45 j
N_0C_1	5.70 k	5.76 j	6.31 k	0.55 i	0.53 i	0.52 i
N_0C_2	6.06 j	6.16 i	7.77 j	0.49 j	0.48 i	0.43 j
N_0C_3	6.70 i	6.77 h	8.69 i	0.41 k	0.52 i	0.42 j
N_1C_0	0.00 l	0.00 k	0.00 l	0.89 f	0.91 ef	0.81 e
N_1C_1	7.41 h	9.31 g	9.76 h	1.03 e	1.03 d	0.90 d
N_1C_2	8.61 f	10.43 e	11.27 g	0.87 f	0.81 g	0.65 g
N_1C_3	8.71 f	10.90 d	12.42 e	0.77 g	0.53 i	0.72f
N_2C_0	0.00 l	0.00 k	0.00 l	1.37 b	1.32 b	1.19 b
N_2C_1	11.77 c	11.01 d	13.75 d	1.45 a	1.42 a	1.30 a
N_2C_2	13.19 b	12.05 b	15.68 b	1.11 d	1.22 c	0.95 d
N_2C_3	14.24 a	12.55 a	16.64 a	0.70 h	0.65 h	0.70 fg
N_3C_0	0.00 l	0.00 l	0.00 l	1.27 c	1.25 c	1.11 c
N_3C_1	8.02 g	10.01 f	12.02 f	1.42 ab	1.32 b	1.22 b
N_3C_2	9.91 e	11.08 d	13.85 d	1.05 de	0.95 e	0.70 fg
N_3C_3	10.85 d	11.64 c	14.44 c	0.91 f	0.89 f	0.58 h
CV (%)		3.00			6.87	

Means showing different letters are significantly different at 5% level of probability by DMRT

N_0 = 0 kg/ha, N_1 = 40 kg/ha, N_2 = 80 kg/ha and N_3 = 120 kg/ha

C_0 = No foliage cutting

C_1 = One foliage cutting at 30 days after sowing (DAS)

C_2 = Two foliage cuttings at 30 DAS (except terminal portion) and 45 DAS (except terminal portion)

C_3 = Thrice foliage cuttings at 30, 45 DAS and 60 DAS (only suitable leaves) no cutting (0.97 t/ha) and the lowest seed yield was recorded from three cuttings at 30, 45 and 60 DAS (C_3). The single cutting (30 DAS) did not affect the vegetative and reproductive growth that resulted in the highest seed yield. The plants of no cutting had a lot of leaves at the basal portion of the stems; these older leaves actually acted as sink that reduced the seed yield. This finding also corroborates with the findings of Thakral *et al.* (1992). On the contrary, Datta *et al*. (2008) obtained significantly higher seed yield from no cutting compared to one cutting. Tiwari *et al*. (2002) observed the highest number umbels per plant and the highest seed yield with two leaf cuttings. Rema *et al*. (1997) reported that once leaf plucking at 50 DAS did not significantly reduce seed yield compared to the control treatment. In case of stover yield similar trend was also observed with cuttings where one cutting (C_1) produced the maximum stover yield (1.94 t/ha) significantly compared to other cuttings.

Interaction effect of nitrogen levels and foliage cutting: For all genotypes, the maximum umbels/plant was recorded from N_3C_1 closely followed by N_2C_1 and N_1C_1 in CS003. The second highest umbels/plant was obtained from N_3C_0 closely followed by N_2C_0. The maximum number of umbellates/umbel was obtained from N_2C_1 closely followed by N_3C_1 in each genotype. The N_3C_1 and N_2C_1 gave the identical second maximum umbellates/umbel. The maximum 1000-seed weight was obtained from N_2C_1 closely followed by N_2C_2 and N_2C_3 in CS001 and CS002 except CS003. The N_3 treatment failed to give the maximum 1000-seed weight irrespective of cutting treatments in all genotypes. The maximum foliage yield was obtained from N_2C_3 in CS001 (14.2 t/ha), CS002 (12.6 t/ha) and CS003 (16.6 t/ha). The N_3C_3 was unable to give the highest foliage yield probably due to non utilization of the whole nitrogen. The second highest foliage yield was recorded from N_2C_2 in all genotypes followed by N_3C_3 in CS002 and CS03 and by N_2C_1 in CS001. The result was in close conformity with Thakral *et al.* (1992). But the result differed from Datta *et al*. (2008) who obtained the maximum leaf yield from the interaction of 90 kg N/ha and two leaf cuttings. The combination N_2C_1 produced the maximum seed yield in all genotypes, highest in CS001. In CS001, N_2C_1 and N_3C_1 gave the identical seed yield. The second highest seed yield was recorded from N_2C_1. The lowest yield was noticed in N_0C_3. Although the combinations of N_3C_1 and N_2C_1 showed identical results with regard umbels/plant and umbellates/umbel, N_3C_1 failed to give the maximum 1000-seed weight compared to N_2C_1. For this reason the N_3C_1 combination gave lower yield (1.32 t/ha. Thakral *et al.* (1992) reported that significantly the highest yield of seeds was obtained from the application 60 kg N/ha with one leaf cutting in coriander.

From the investigation, it appears that the response of coriander crop to nitrogen levels and leaf cuttings was fairly well in terms of the production of foliage (green leaves) as well as seed in Salna areas (AEZ-28). It was evident that the application of 80 kg N/ha with two cuttings of foliage at 30 and 45 DAS was the most suitable for coriander cultivation. Application of 80 kg N/ha with one cutting of foliage at 30 DAS might also be advocated for coriander production for foliage as well as seed.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 61-69, March 2015

GREEN COB AND FODDER YIELD OF SWEET CORN AS INFLUENCED BY SOWING TIME IN THE HILLY REGION

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Abstract

A field experiment was conducted at the farm of Hill Tract Agricultural Research Station, Ramgarh, Khagrachari Hill District during *Rabi* season of 2010-11 and 2011-12 to determine the optimum sowing time for better yield of green cob as well as fodder of sweet corn (var. BARI Sweet corn-1) in the hilly region. Five sowing dates (November 20, November 30, December 10, December 20 and December 30) were included in the study. During 2010-11, the highest green cob yield was obtained from 20 November sowing (8.43 t/ha) followed by 30 November sowing (7.81 t/ha) and the lowest yield (5.00 ton/ha) from 20 December sowing. During 2011-12, the maximum green cob yield (8.60 t/ha) was also obtained from 20 November, which was statistically identical with that of 30 November (8.03 t/ha), 10 December (7.67 t/ha) and 20 December (8.11 ton/ha) sowing. Average of two years result showed that, the maximum fodder yield (39.99 t/ha) was obtained from 30 November sowing which was at par with that of 20 November sowing. Maximum TSS (Total soluble sugar) value of Sweet corn was obtained from 20 November sowing during 2010-11 and 30 November sowing during 2011-12. Across over two years, 20 November to 30 November sowing was found suitable for sweet corn production in the hilly areas in terms of green cob and fodder yield and also TSS value.

Keyword: Sweet corn, green cob, fodder, hill region

Introduction

Sweet corn is mainly produced for human consumption either as a fresh cob or processed product. The kernel of sweet corn is translucent in fresh condition. The green cob is suitable for fresh consumption due to its sweet and delicious taste. Tribal people of hilly areas normally take any type of corn as boiled or roasted condition. Generally the people of this area cultivate the corn as jhum along with other crops. Sweet corn may be a good option for them. BARI already released a sweet corn variety i.e. BARI Sweet corn-1. As sweet corn is harvested early (i.e.20-25 days after silking) then the plant and husk could be used as fodder.

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Potential yield of sweet corn can be achieved through optimum use of inputs and agronomic practices. Planting date and variety selection are the major factors affecting maize production in addition to soil fertility, temperature regimes and irrigation (Ramankutty *et al.,* 2002). For optimization of yield, planting at the appropriate time is very critical (Anapalli *et al.,* 2005). Rogers *et al.* (2000) reported that The effects of sowing time on sweet corn yield and quality were examined by sowing three sweet corn hybrids (Sheba, Challenger and XP1029) of varying maturity at approximately fortnightly intervals from 21 September 1999 to 20 January 2000 in New Zealand. Delayed sowing reduced total crop biomass by 0.86 t/ha per 10-days delay. Harvestable ear yield declined by 1.6 t/ha per 10-days delay in sowing, mainly through reduced mass of harvestable secondary ears. Photoperiod and temperature influence the time from sowing to tassel initiation with appreciable genetic differences in relative sensitivity to these factors (Ellis *et al.,* 1992). Yield can be increased to a greater extent provided high yielding varieties are identified and planted at proper time (Khan *et al.,* 2009; and Arif *et al.,* 2001). The influence of sowing date on crop development and yield of maize was studied during 1983-85 at Arlington, Wisconsin. Sowing dates were April 26 to May 6 (early), May 14 to 19 (middle), and May 27 to June 6 (late). The highest grain yields were generally obtained when planting was completed by early May, with yield declining as planting was delayed (Imholte and Carter, 1987). Herbek *et al.* (1986) emphasised that a yield increase trend was observed as planting date progressed from the first planting date (late April) to the second (mid-May). Yield increased with delayed planting dates. Erbay and Koycu (1986) reported that the highest yield was obtained from the Akponar variety sown on the May 3 and that yield decreased as planting time was delayed. Yield and tasseling period decreased with the delay in sowing dates. Therefore, the present study was conducted to determine the optimum sowing time for better marketable yield of green cob as well as fodder of sweet corn in the hilly areas.

Materials and Method

A field experiment was conducted at the farm of Hill Tract Agricultural Research Station, Ramgarh, Khagrachari Hill District during *Rabi* season of 2010-11 and 2011-12, in the hilly region. The soil of this hill area having acidic soil where p^H mostly ranges from 4.5-5.0 belongs to AEZ 29. Five different dates of sowing viz. November 20, November 30, December 10, December 20 and December 30 at 10-days interval were included as treatment variables in the experiment. The trial was laid out in a randomized complete block design with three replications. The unit plot size was 4.5m×3.5m. Seeds of sweet corn (var. BARI Sweet corn-1) were sown according to treatment with the spacing of 45 cm×25 cm. The experimental plots were prepared and labeled properly. Lime was also applied due to minimize the soil acidity in hilly areas. Fertilizers at the rate of 150-60-90-

20 kg/ha of NPKS were applied in the form of Urea, Triple Super Phosphate (TSP), Muriate of Potash (MoP) and Gypsum, respectively. The full amount of TSP, MoP, Gypsum and 1/3 of urea were applied as basal dose during final land preparation. The remaining 2/3 of urea were applied as side dressing at 30 and 55 days after emergence. Weeding, irrigation and insecticides spraying were done as when required to maintain an optimum growth condition for the crop. The green cob was harvested at 20-24 days after silking when the corn silks were turned to dry as brown color. The collected data were analyzed statistically and means were separated using LSD test at 5% level of significance.

Results and Discussion

Days to emergence showed considerable variation among the sowing time (Fig. 1) and this might be occurred due to variation of prevailing temperature of growing period (Table 3 and 4). Days to tasseling of sweet corn was also markedly differed by sowing time (Fig.1). This result is in agreement with the findings of Khan *et al.* (2009). They reported that days to tasseling were significantly affected by date of sowing. Days to tasseling decreased with delaying of sowing time. This might be due to differences in photoperiod and temperature among sowing times. Photoperiod and temperature can influence the timing of development events in maize (Aitken, 1977 and Allison and Daynard, 1979). These results are also in conformity with that of Khan *et al.* (2009), Khan *et al.* (2004) and Shaw (1988) who reported the dependence of tasseling duration on temperature and variety. Days to silking was influenced by sowing time (Fig.1). The minimum days to silking were recorded from 30 November sowing and the maximum days to silking were recorded from 20 December sowing. Significant effects of sowing date on days to silking in corn are reported by Khan *et al. (*2009) and Shafi *et al.* (2006).

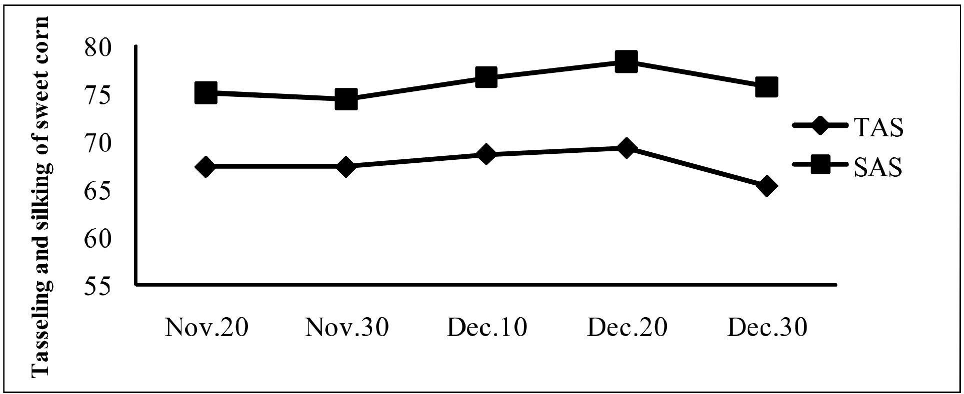

Fig.1: Effect of sowing time on tasseling and silking of var. BARI Sweet corn-1.

Table 1. Effect of sowing time on the yield contributing characters of var. BARI sweet corn-1.

Sowing time	Plant height (m)		Cob length (cm)		Cob diameter (cm)		Weight of individual cob (g)	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Nov 20	2.40	2.46	15.85	17.46	4.09	4.82	107.4	156.77
Nov 30	2.61	2.63	16.03	16.80	4.21	3.82	143.2	131.50
Dec 10	2.68	2.57	16.66	17.00	4.18	4.44	139.43	159.43
Dec 20	2.79	2.52	15.87	17.87	4.44	4.5	169.23	192.37
Dec 30	2.81	2.70	17.37	18.07	4.18	4.5	149.4	196.00
$LSD_{(0.05)}$	NS	NS	NS	0.42	0.16	0.35	6.3	8.861
CV (%)	6.37	5.76	6.37	4.12	1.93	4.28	9.55	2.81

Table 2. Effect of sowing time on the yield of green cob, fodder and TSS of var. BARI Sweet corn-1.

Sowing time	Yield of green cob (t/ha)			Fodder yield (t/ha)			TSS (%)		
	2010-11	2011-12	Mean	2010-11	2011-12	Mean	2010-11	2011-12	Mean
Nov 20	8.43	8.60	8.51	38.76	38.94	38.85	13.78	12.96	13.37
Nov 30	7.81	8.03	7.92	38.32	39.99	39.15	13.57	14.46	14.01
Dec 10	5.59	7.67	6.63	40.61	34.39	37.50	13.00	13.91	13.46
Dec 20	5.00	8.11	6.55	38.22	37. 08	37.65	13.27	13.31	13.29
Dec 30	6.46	6.15	6.30	37.47	33.94	35.70	12.54	13.62	13.08
$LSD_{(0.05)}$	1.35	2.26	-	NS	NS	-	1.10	0.59	-
CV (%)	5.78	9.91	-	9.05	6.73	-	4.42	2.52	-

Sowing time significantly influenced the cob length, cob diameter, weight of individual cob, yield of green cob, yield of fodder and TSS value of BARI sweet corn-1 (Table 1and 2) but no significant variation was observed in plant height. The maximum cob length was recorded from 30 December sowing in 2011-12 but at par to Dec 20 sowing but significant difference was not found during 2010-11. During 2010-11, maximum cob diameter (4.44 cm) was recorded from 20 December sowing whereas minimum from 20 November (4.09 cm) sowing. But during 2011-12, maximum cob diameter (4.82 cm) was recorded from 20 November sowing whereas minimum from 30 November (3.82 cm) sowing. The highest individual cob weight (169.23 g) was obtained from 20 December sowing and the lowest weight of individual cob (107.40 g) was obtained from 20 November sowing. But during 2010-11, individual cob weight (196.00 g) was obtained from 30 December sowing and 20 December (192.37 g) were statistically at par and the lowest weight (131.50 g) from 30 November sowing. Individual cob weight was found higher in late sowing. Temperature was lower at late sown condition (Table 3 and 4). Sencar *et al.* (1997) stated that weight of individual cob increased with delayed sowing time. The maximum green cob yield (8.43 t/ha) was recorded from 20 November sowing followed by nearly 30 sowing in 2010-11 but at par all sowing time except Dec. 30 in 2011-12. It was sorted that green cob increased up to Dec.20 in 2011-12 but dramatically reduced after Nov.30 sowing during 2010-11. In both the years, lowest yield was recorded from 30 December (6.11 t/ha) in 2011-12 but lower yield from Dce.10 to 30 in 2010-11. Average over the years, maximum yield was recorded 20 November sowing (8.51 t/ha) followed by 30 November sowing (7.92 t/ha). There was trend to decrease yield with the advancement of sowing time. Lower yield was recorded from 10 December sowing onward. This might be due to differences of environmental condition during the growing period due to different sowing time. Environmental conditions may have reduced photoassimilate production during the lag phase of late sown maize, because both temperature and incident solarradiation were low at that time which affected biomass production and perhaps sink activity (Ou-Lee and Setter, 1985). Some researchers also stated that delaying the sowing date resulted in decreased yields (Ishimura *et al.* (1984), Tomorga *et al.* (1985), Imholte and Carter (1987). Fodder yield was statistically identical in both the years but maximum fodder yield (40.61 t/ha) was found from 10 December sowing but the maximum fodder was recorded (39.99 t/ha) from 30 December sowing during 2011-12. But higher mean yield of fodder was recorded from 30 November (39.15 t/ha) followed by 20 November sowing (38.85 t/ha). The minimum green fodder yield was recorded from late sowing. Sweetness of the green cob i.e., TSS (% brix) value varied significantly due to different sowing dates. The TSS value was recorded statistically identical in different sowing time except Dec. 30 sowing during 2010-11 but Nov. 30 and Dec.10 sowing was identical in 2011-12. The highest mean TSS value was recorded at 30 November sowing and reduced TSS statistically with the advancement of sowing time.

Table 3. Weather data during 2010-2011 (10-days Interval).

Date	Temperature (0^C)		Mean	Humidity (%)	Rainfall (cm)
	Maximum	Minimum			
1-10 November,2010	24.0	20.0	22.00	77.45	0
11-20 November,2010	26.0	19.0	22.50	77.55	0
21-30 November,2010	24.5	19.0	21.75	75.00	0
1-10 December,2010	24.00	18.00	21.60	78.70	0
11-20 December,2010	19.80	16.0	18.65	80.45	0
21-31 December,2010	20.00	17.00	19.17	81.63	0
1-10 January,2011	24.00	13.70	18.85	77.95	0
11-20 January,2011	20.50	12.50	16.50	78.60	0
21-30 Janury,2011	24.00	13.36	18.68	76.04	0
1-10 February,2011	25.40	14.30	19.85	58.40	0
11-20 February,2011	26.70	15.80	21.25	58.37	0
21-28 February,2011	27.75	18.37	23.06	56.86	0
1-10 March,2011	29.80	18.20	24.00	73.25	0
11-20 March,2011	30.70	20.70	25.70	73.10	0
21-31 March,2011	31.45	23.81	27.63	74.40	0
1-10 April,2011	32.70	23.10	27.90	74.50	-
11-20 April,2011	33.70	26.70	30.20	73.80	-
21-30April,2011	33.40	24.70	29.05	74.35	2.80
1-10 May,2011	33.20	24.70	28.95	73.35	-
11-20 May,2011	34.20	28.00	31.10	76.10	-
21-31May,2011	31.00	27.60	29.30	77.95	28.48

Source: Daily Weather Data, Khagrachari, 2011-12

Table 4. Weather data during 2011-2012 (10-days Interval)

Date	Temperature (0^C)		Mean	Humidity (%)	Rainfall (cm)
	Maximum	Minimum			
1-10 November,2011	24.7	20.6	22.65	78.45	0
11-20 November,2011	26.5	19.4	22.95	79.55	0
21-30 November,2011	24.8	19.2	22.00	78.00	0
1-10 December,2011	24.40	18.80	21.60	80.70	0
11-20 December,2011	20.80	16.5	18.65	84.45	0
21-31 December,2011	20.81	17.54	19.17	81.63	0
1-10 January,2012	21.50	17.60	19.55	80.65	0
11-20 January,2012	18.50	14.5	16.50	78.30	0
21-30 Janury,2012	18.36	13.36	15.86	75.90	0
1-10 February,2012	24.20	14.20	19.20	72.30	0
11-20 February,2012	26.20	15.80	21.00	69.73	0
21-29 February,2012	27.66	17.11	22.38	61.90	0
1-10 March,2012	28.60	22.40	25.50	72.80	0
11-20 March,2012	28.10	20.20	24.15	68.75	0
21-31 March,2012	31.81	25.54	28.67	70.30	0
1-10 April,2012	29.70	23.70	26.70	69.60	-
11-20 April,2012	30.20	25.10	27.65	68.45	-
21-30April,2012	30.80	27.30	29.05	71.70	19.56
1-10 May,2012	29.50	25.30	27.40	71.70	-
11-20 May,2012	33.60	27.70	30.65	72.15	-
21-31May,2012	32.90	29.90	31.40	75.50	11.94

Source: Daily weather Data, Khagrachari, 2011-12

Conclusion

The result revealed that 20 November to 30 November sowing could be suitable for sweet corn cultivation in hilly region in respect of green cob yield as well as fodder yield and TSS value.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 71-76, March 2015

YIELD RESPONSE OF SUMMER COUNTRY BEAN TO BORON AND MOLYBDENUM FERTILIZER

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Abstract

Field experiments were conducted in the Grey Terrace Soil (*Aeric Albaquept*) under AEZ-28 at the Bangladesh Agricultural Research Institute (BARI) farm, Gazipur during summer seasons of 2010 and 2011 to determine the optimum rate of boron and molybdenum combination for maximizing the yield of summer country bean (var. BARI Sheem-3). Four levels each of B (0, 1, 2 and 3 kg ha^{-1}) and Mo (0, 0.5, 1.0 and 1.5 kg ha^{-1}) were used as treatment variables. The results indicated that application of B and Mo fertilizer combination exerted significant influence on the number of pods $plant^{-1}$, individual pod weight and pod yield ha^{-1} in both the years. The highest pod yield (9.58 t ha^{-1} in 2010 and 9.42 t ha^{-1} in 2011) was produced by the combination of 2 kg B and 1.5 kg Mo ha^{-1} and it was statistically identical with 2 kg B and 1.0 kg Mo ha^{-1} combination. Addition of B beyond 2 kg ha^{-1} along with higher doses of Mo created a detrimental effect to reduce yield irrespective of years. The results revealed that application of 2 kg B and 1 kg Mo ha^{-1} combination with a blanket dose of 50 kg N, 40 kg P, 60 kg K and 20 kg S ha^{-1} plus cowdung 5 t ha^{-1} might be optimum for summer country bean cultivation in Grey Terrace Soil of Gazipur.

Introduction

Country bean (*Dolichos lablab*) is one of the major vegetables from the Leguminosae family. In addition to the protein contributions of the legume, it is rich in other nutrients such as starch, dietary fiber, protective phytochemicals, vitamins and elements (Saikia *et al*., 1999). Country bean is grown in Bangladesh during summer and winter seasons. Summer country bean is a newly introduced vegetable crop in this country. The yield of summer country bean is low in Bangladesh as compared to the other countries of the world. The reasons of lower yield can be attributed to imbalanced use of fertilizer and lack of high yielding varieties. The farmers of Bangladesh usually use N, P and K for crop production. Use of micro nutrients is limited. However, boron influenced the absorption of N, P, K and its deficiency changed the equilibrium of optimum of those three macronutrients (Raj, 1985). It influences yield and yield attributes of French bean (Singh *et al*., 1989; Singh and Singh, 1990). Patra (1989) found higher yield in soybean due to application of B at the rate of 2 kg ha^{-1}. Molybdenum is an important element to help in protein synthesis and fixation of atmospheric

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nitrogen in the root of legume by nodule bacteria. Singh *et al.* (2008) reported that application of Mo alone or combination with *S. rhizobium* significantly increased the grain of blackgram as compared to control. Ruschel *et al*. (1970) reported that application of molybdenum fertilizer in combination with boron significantly increased bean yield. The influence of Mo and B on different grain legumes have been reported by Bhuiyan *et al*. (1997) and Chowdhury *et al*. (1998). Information on the use of B and Mo in combination with NPKS and organic manure for summer country bean cultivation in Bangladesh is scanty. In view of this, field experiments were conducted to determine the optimum rate of B and Mo combination in presence of NPKS and cowdung for maximizing the yield of summer country bean in Grey Terrace Soil of Gazipur.

Materials and Method

Field experiments were carried out at the Bangladesh Agricultural Research Institute (BARI) farm, Joydebpur, Gazipur during summer (Kharif) seasons of 2010 and 2011. The soil of the experimental field was loam in texture. It belongs to the Chhiata series of the Grey Terrace Soil (*Aeric Albaquept*) under the AEZ-28 (Modhupur Tract). Results of initial soil analysis are presented in Table 1. The soil test values showed that the experimental soil was deficient in N, P, K, S and B. The soil was slightly acidic. Organic matter content of the soil was very low.

Table 1. Chemical properties of the initial soil sample (average data of years 2010 and 2011).

pH	OM (%)	Ca	Mg	K	Total N (%)	P	S	B	Fe	Zn
		Meq100^{-1}g soil				mg kg^{-1}				
6.5	0.80	0.6	0.5	0.12	0.040	8	9	0.10	110	1.0
Critical level		2.0	0.8	0.20	0.12	14	14	0.2	10	0.6

The treatment consisted of four levels each of boron (0, 1, 2 and 3 kg B ha^{-1}) and molybdenum (0, 0.5, 1.0 and 1.5 kg ha^{-1}). The treatments were factorial combination of the two factors and the experiment was conducted using randomized complete block design with three replications. The unit plot size was 4 m x 4 m. Thirty day old seedlings of BARI Sheem-3 were transplanted on 28 April, 2010 and 11 May, 2011 at a spacing of 1.5 m x 1.5 m. Boron and molybdenum were applied as boric acid and ammonium molybdate, respectively in the pits. Blanket dose of 50 kg N, 40 kg P, 60 kg K and 20 kg S ha^{-1} in the form of urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum and cowdung at 5 t ha^{-1} were added to every plot. TSP, MoP, gypsum, cowdung and half of urea were applied in the pits as basal and remaining half of urea was applied after third week of transplanting. Intercultural operations such as weeding, irrigation and insecticide spray were done as and when required. Pods of country bean were harvested from each plot during July-August, 2010 and

July- September, 2011 and recorded. Twenty pods were randomly selected from each plot to record the data on yield components. Weight of total pod plot^{-1} was recorded to compare per hectare yield. All the data were analyzed by computer using MSTAT-C package and mean values were compared by Duncan's New Multiple Range Test (Gomez and Gomez, 1984).

Results and Discussion

Results obtained from interaction of B and Mo levels were significant. Hence, only interaction effects on different plant characters arediscussed here. Interaction effects of B and Mo levels were significant in respect of number of pods plant^{-1} and weight of individual pod of summer country bean in both the years (Figs. 1 and 2). Application of B and Mo fertilizer significantly increased the number of pods plant^{-1} with increasing rate for B up to 2 kg ha^{-1} and for Mo up to 1.5 kg ha^{-1} combination and it was statistically identical with 2 kg B ha^{-1} and 1.0 kg Mo ha^{-1} combination in both the years. These two treatments, however, were significantly different from all other treatment combinations. Number of pods plant^{-1} varied from 161 to 392 in 2010 and 157 to 388 in 2011 due to different combination of B and Mo. The higher levels of B and Mo fertilizers in combination significantly reduced the number of pods plant^{-1} irrespective of years. The lowest value was recorded from B_0Mo_0 treatment. Almost similar trend of variations in respect of weight of individual pod was noticed due to interaction of B and Mo fertilization. The results thus indicate that B and Mo fertilizers along with NPKS and cowdung played key role to enhance pods plant^{-1} and weight of unit pod to a considerable extent.Boron and molybdenum levels also interacted significantly with respect to pod yield ha^{-1} (Fig. 3). Pod yield differed from 4.14 to 9.58 t ha^{-1} in 2010 and 3.97 to 9.42 t ha^{-1} in 2011 due to different levels of B and Mo combination. The highest pod yield (9.58 t ha^{-1} in 2010 and 9.42 t ha^{-1} in 2011) of summer country bean was achieved with the combined application of 2 kg B and 1.5 kg Mo ha^{-1} in presence of 50 kg N, 40 kg P, 60 kg K, 20 kg S ha^{-1} and cowdung at 5 t ha^{-1} and it was statistically identical with 2 kg B and 1.0 kg Mo ha^{-1} combination in both the years. These two treatment combinations were significantly different from all other treatment combinations. The soil test value showed that experimental field was highly deficient in different nutrients. Application of B and Mo along with other essential nutrients to the soil helped in maintaining soil fertility and offered favorable response, which was reflected by higher yield. Moreover, supply of 2 kg B and 1.5 kg Mo ha^{-1} combination in presence of NPKS and cowdung for better growth and plant development which resulted in higher yield due to higher pods plant^{-1} and pod weight.Similar finding was obtained by Shil *et al.* (2007).

They reported that combined application of B and Mo provided a beneficial effect on seed yield of chickpea. The beneficial effect of the combined application of B and Mo including Zn has been reported with chickpea grown in calcareous soil (Jahiruddin, 2008) and also with French bean (Kushwaha, 1999). Addition of B level beyond 2 kg ha^{-1} along with higher dose of Mo created a detrimental effect to reduce yield irrespective of years. The negative response of higher dose of B and Mo in the experiment might be due to imbalance caused by increasing level B and Mo without concomitant increase in other fertilizer. The results showed that yield of summer country bean increased with the increase of B and Mo application upto a certain limit. Plants grown without B and Mo (B_0Mo_0) gave the lowest pod yield of 4.14 t ha^{-1} in 2010 and 3.97 t ha^{-1} in 2011.

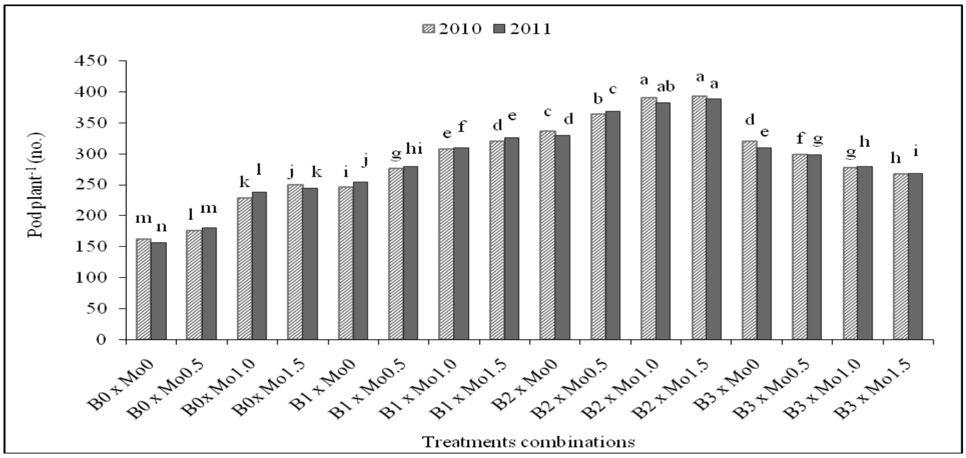

Fig. 1. Interaction effect of boron and molybdenum on the pods plant^{-1}of summer country bean.

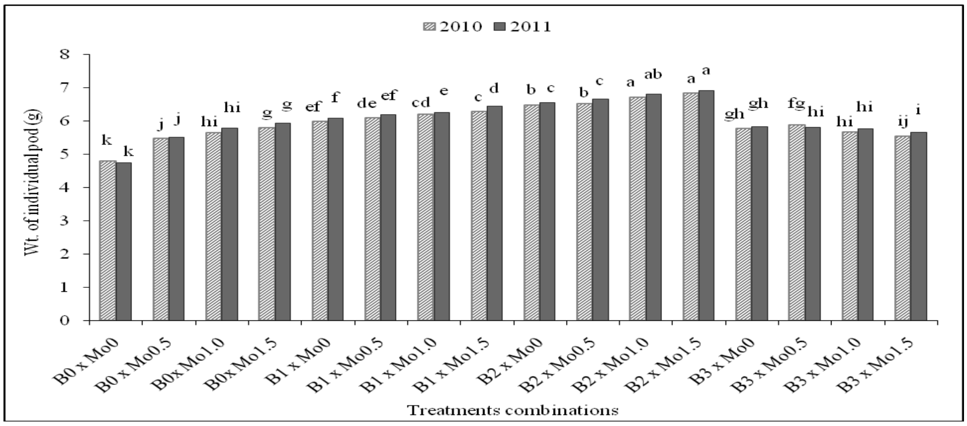

Fig. 2. Interaction effect of boron and molybdenum on the weight of individual pod of summer country bean

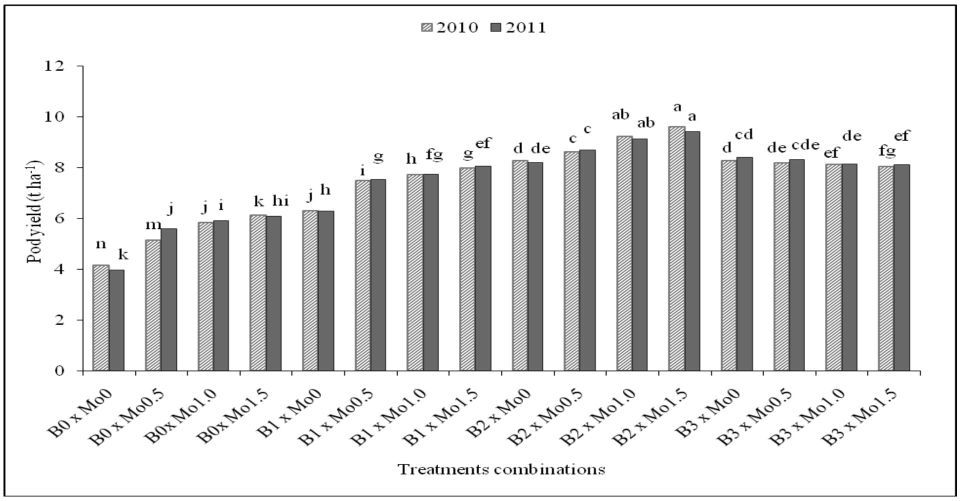

Fig. 3. Interaction effect of boron and molybdenum on the pod yield ha^{-1} of summer country bean

Conclusion

Results of two years reveal that there exists a scope for yield improvement of summer country bean by application of B and Mo. Application of 2 kg B and 1 kg Mo ha^{-1} combination in presence of 50 kg N, 40 kg P, 60 kg K, 20 kg S ha^{-1} and cowdung at 5 t ha^{-1} for BARI Sheem-3 might be optimum for obtaining maximum pod yield in Grey Terrace Soil (AEZ-28) of Gazipur.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 77-93, March 2015

SEED YIELD, NUTRIENT BALANCE AND ECONOMICS OF MUNGBEAN CULTIVATION AS INFLUENCED BY DIFFERENT NUTRIENTS MANAGEMENT UNDER AEZ-28

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Abstract

A field experiment was conducted at Regional Wheat Research Centre of the Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh for 2 consecutive years during 2007 and 2008 to find out the optimum nutrient management practice for seed yield, nutrient balance and economics of mungbean. Twelve nutrient management treatments were tested in RCBD with 3 replications. Treatments were without CRI T_1=HYG (0-24-40-48-24-3-1.2), T_2=MYG (0-20-36-40-20-2-1), T_3=IPNS (5000-9-37-36-21-3-1.2), T_4=STB (0-20-36-40-22-2-1), T_5=FP (0-6-5-4-0-0-0), T_6=CON (0-0-0-0-0-0-0) and with CRI T_7=HYG+CRI, T_8=MYG+CRI, T_9=IPNS+CRI, T_{10}=STB+CRI, T_{11}=FP+CRI, T_{12}=CON+CRI kg ha^{-1} CDNPKSZnB for mungbean. The maximum seed yield of mungbean was obtained from STB+CRI (1.57 t ha^{-1}) followed by IPNS+CRI (1.54 t ha^{-1}), STB (1.54 t ha^{-1}), IPNS (1.52 t ha^{-1}), HYG+CRI (1.44 t ha^{-1}) and HYG (1.41 t ha^{-1}) in 2007. Similar trend was found in 2008. Numerically higher yield and yield contributing parameters were noticed in CRI plots than without CRI. N and K balance were found negative in all the treatments. P, S, Zn and B balance were found positive in case of HYG, MYG, IPNS and STB along with or without CRI nutrient managements. While in case of FP and CON, the balance was shown almost negative. The maximum gross return and margin was obtained from STB+CRI followed by STB. Slightly higher BCR (3.00) was recorded from STB followed by STB+CRI (2.91).

Keywords: Mungbean, Seed yield, Economics, Nutrient balance and Crop Residue Incorporation.

Introduction

Bangladesh is a small country of 0.148 million sq.km and it has to feed about 145 million people of this country (BBS, 2012). In order to produce more food within a limited area, two most important techniques are to be adopted, which is to increase the productive efficiency of the individual crop depending on how well

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it utilizes the basic resources especially, water and nutrients. Bangladesh grows different types of pulse crops. Among the mmungbean is one of the important one for its short duration character (Ahmed *et al.*, 1978). In our country mungbean ranks third in respect of acreage and production while it ranks first in respect of market price. Mungbean grain contains 51% carbohydrate, 26% protein, 10% moisture, 4% mineral and 3% vitamins (Kaul, 1982). Moreover, its residues have capacity to improve the soil fertility thus increase the productivity of land. The average yield of mungbean is 0.69 t ha^{-1} which is very low compared to other mungbean growing countries. There are many reasons of low yield of mungbean of which optimum fertilizer from both organic and inorganic sources along with crop residue management are important which affect the yield contributing characters and seed yield of mungbean. Therefore, the present study was undertaken with the objectives to find out the optimum nutrient management practice for seed yield, apparent nutrient balance in soil and economics of mungbean cultivation under AEZ-28.

Materials and method

The experiment was carried out at the Regional Wheat Research Centre of Bangladesh Agricultural Research Institute Joydebpur, Gazipur. The experimental field of Gazipur belongs to the agro-ecological zone of Modhupur Tract (AEZ-28). Mungbean was grown in the same location. The initial soil of the experimental field was analyzed for chemical properties before setting up the experiment. The initial soil status was pH 6.48, OM (%) 1.07, Total N (%) 0.055, available P (μg g^{-1}) 3.76, exchangeable K (meq 100 g^{-1}) 0.15, available S (μg g^{-1}) 9.91, available Zn (μg g^{-1}) 0.24 and available B (μg g^{-1}) 0.16. Morphological characters are Grey Terrace soils, medium high land, not well drained, above flood level and grey soil clour. From the initial status of the soil, it was observed that physical characters are silty loam to loam having more or less near neutral soil pH with very low to low soil fertility level. Mungbean (BARI Mug 6), variety was tested in *Kharif*-I season during 2007 and 2008. Twelve nutrient management treatments were tested in RCBD with 3 replications. Treatments were T_1=HYG (0-24-40-48-24-3-1.2), T_2=MYG (0-20-36-40-20-2-1), T_3=IPNS (5000-9-37-36-21-3-1.2), T_4=STB (0-20-36-40-22-2-1), T_5=FP (0-6-5-4-0-0-0), T_6=CON (0-0-0-0-0-0-0), T_7=HYG+CRI, T_8=MYG+CRI, T_9=IPNS+CRI, T_{10}=STB+CRI, T_{11}=FP+CRI, T_{12}=CON+CRI kg ha^{-1} CDNPKSZnB for mungbean. The previous crop was potato and total foliage (haulm) as residue of potato was incorporated before mungbean sowing. The rates for N, P, K, S, Zn and B application were calculated based on the soil test value following the soil test interpretation (BARC, 2005). Accordingly, the full or 100% rate of N, P, K, S, Zn and B for each crop was applied. In case of Integrated Plant Nutrient System (IPNS) treatment the amount of nutrients available in cowdung (CD) was deducted from the total amount of chemical fertilizers and adjusted accordingly.

The rates for chemical fertilizers were fixed on soil test basis (STB) with a high yield goal (HYG) for specific crop basis as per BARC, 2005. The exact fertilizer nutrient for making the recommendation was computed with the following formula:

$$F_r = U_f - \frac{C_i}{C_s} \times (S_t - L_s)$$

Where

- F_r = Fertilizer nutrient required for a given soil test value
- U_f = Upper limit of the recommended fertilizer nutrient for the respective soil test value interpretation (STVI) class
- C_i = Units of class intervals used for fertilizer nutrient recommendation
- C_s = Units of class intervals used for STVI class
- S_t = Soil test value
- L_s = Lower limit of the soil test value within STVI class.

For HYG, MYG and IPNS management recommended cowdung was adjusted into chemical nutrient and added according to the thumb rule i.e. 1 ton decomposed cowdung contains 3 kg N, 0.6 kg P, 2.4 kg K and 0.6 kg S (BARC, 2005). The sources of N, P, K, S, Zn and B were urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum, zincsulphate and boric acid, respectively. The farmers' practice (FP) for fertilizer rates was determined on the basis of data collected through interviewing thirty (30) farmers from adjacent locality. It appeared that the farmers generally did not use S, Zn and B fertilizers. Residue of previous potato crop was in-situ incorporated to the soil for crop residue incorporation plots only. Total fresh residue was chopped just after harvest and ploughed down to the soil by spade for decomposition in respective CRI plots with available moisture consequently that was decomposed. Moreover a light irrigation was applied for germination of mungbean seed which enhanced the decomposition of residue. Turn Around Time (TAT) was about 10-15 days potato harvest and mungbean sowing. Seeds were sown on 5-7 March each year in 30 cm of continuous solid lines @ 40 kg ha^{-1} and seeds were covered with soil. The whole amount of urea TSP, MoP, gypsum, zincsulphate and boric acid were applied at the time of final land preparation as per treatment. The mungbean field was then irrigated at 15 and 30 days after germination. Weeding and plant protection measures were followed as and when necessary. Mungbean was harvested on 25-31 May each year. The crops were harvested at full maturity. An area of 10 m^2 in each plot was harvested to record seed and biomass yields. A sub-sample of 200 g dry biomass for each of crop was collected for chemical (nutrient uptake) analysis. The data on the yield contributing characters were recorded from 10 randomly selected plants for mungbean. For dry biomass yield, total fresh biomass yield was measured from the harvested area. Then a sub-sample from each plot was collected and weighed to record fresh weight. The sub-samples were dried in an oven for 72 hours at 70^0C. Oven dry of each sample was recorded and expressed as dry biomass yield (t ha^{-1}). Apparent nutrient balance (added-uptake) was calculated using the following formula.

$X_a = (X_f + X_r + X_i + X_b + X_{cri}) - X_{rem}$

Where

Xa = Apparent gain (+) or loss (-) of nutrient (kg ha^{-1})

Xf = Nutrient added through inorganic sources (kg ha^{-1})

Xr = Nutrient added through rainfall (kg ha^{-1}). (Not considered)

Xi = Nutrient added through irrigation water (kg ha^{-1}). (Not considered)

Xb= Nutrient added through BNF (kg ha^{-1}). (Not considered)

Xcri= Nutrient added through crop residue incorporation (kg ha^{-1}).

Xrem= Nutrient removed by crops and loss through different systems (kg ha^{-1}).

Data were analyzed statistically by the F-test to examine whether the treatment effects were significant. The mean comparisons of the treatments were evaluated by DMRT (Duncan's Multiple Range Test).

Table 1.Total addition of extra nutrients into the soil through crop residues incorporation in mungbean (kg $ha^{-1}yr^{-1}$) during 2007 & 2008 (Assuming nitrogen mineralization rate 40%).

Nutrient management	kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}		kg ha^{-1}	
	N		P		K		S		Zn		B	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
HYG+CRI	17	18	11	12	41	44	9	9	0.14	0.15	0.10	0.11
MYG+CRI	11	12	8	9	32	35	8	8	0.11	0.08	0.08	0.07
IPNS+CRI	13	15	10	11	40	44	9	9	0.13	0.14	0.09	0.09
STB+CRI	13	14	10	11	37	40	9	10	0.11	0.12	0.09	0.08
FP+CRI	9	10	7	8	24	28	6	7	0.08	0.09	0.07	0.07
CON+CRI	6	6	4	4	17	17	4	4	0.05	0.05	0.04	0.03

On an average of two years, major plant nutrients like N (6 to 18 kg ha^{-1} yr^{-1}), P (4 to 12 kg ha^{-1} yr^{-1}), K (17 to 44 kg ha^{-1} yr^{-1}), S (4 to 10 kg ha^{-1} yr^{-1}) and minor nutrients like Zn (0.05 to 0.15 kg ha^{-1} yr^{-1}) and B (0.03 to 0.11 kg ha^{-1} yr^{-1}) were added into the soil through crop residues incorporation. The amount was varied depend on irrespective treatment as well as the crop growth. That was an approach for conservation agriculture which might have played a vital role for sustaining soil health like fertility and productivity. This approach might be helpful for a long run.

Results and discussion

Plants population

The number of plants m^{-2} did not vary significantly by different nutrient management treatments in both the years (Table 2).

Plant height

The nutrient management treatments without or with crop residues incorporation significantly influenced the plant height in both the years. The significantly taller plant was recorded under HYG with crop residues incorporation (37.2 cm) followed by remaining nutrient management treatments except, FP without CRI, control without and with CRI (28.4 cm). The treatment HYG+CRI produced the tallest plant (42.1 cm) which was statistically identical to HYG (41.6 cm), IPNS+CRI (41.1 cm) and STB+CRI (40.6 cm). The plant height under different nutrient management ranged from 30.3 cm to 36.1 cm whereas lowest (28.1 cm) in control in Table 2. However, the trend of plant height was observed more or less similar over the years that might be due to similar nutrient management but tendency is taller plant in CRI incorporation treatment might be due to crop residue effect (Naser *et al*., 2001).

Pods plant^{-1}

Number of pods plant^{-1} differed significantly due to application of nutrients without or with crop residues incorporation in 2007 (Table 2). The significant more number of pods plant^{-1} (12.13) was obtained from STB+CRI followed by STB (12.10), IPNS+CRI (12.01), IPNS (11.98), HYG+CRI (10.83) and HYG (10.80). The lowest number was found in control (4.80) treatment. In 2008, the trend of number of pods plant^{-1}due to nutrient management treatments was similar previous year.

The highest number of pods palnt^{-1} was obtained from the treatment STB without or with crop residues incorporation that might be due to proper nutrient management followed by IPNS and HYG without or with CRI. Similar findings were reported by Singh and Pareek (2003) where the highest number of pods plant^{-1} was recorded with nutrient applied @ P_2O_{545} kg ha^{-1} in mungbean. In another field experiment, Quayyum *et al*. (2002) also reported that STB nutrient N_{20} P_{20} K_{20} gave the highest number of pods plant^{-1} in wheat-mungbean- T. *Aman* rice cropping pattern.

Pod length

The nutrient management packages had significant effect on the length of pod in 2007. However, the nutrient management treatments HYG, MYG, IPNS, and STB without and with CRI showed statistically identical length of pod with exception of MYG without CRI. While in 2008, the maximum length of pod (9.65 cm) was obtained under STB+CRI which were statistically identical to all treatments except control. The minimum length of pod was recorded under control without CRI in both the years (Table 2).

However, the length of pod was more or less similar over the years might be due to the effect of similar nutrient management.

Seeds pod^{-1}

Different nutrient management treatments showed significant effect on seeds pod^{-1} (Table 2). Among the treatments, STB+CRI gave the maximum seeds pod^{-1} (9.81) followed by HYG and IPNS without or with CRI, and were statistically identical. Number of seeds pod^{-1} was the lowest (5.37) in control in 2007. During 2008, most of the nutrient management treatments except, MYG, FP and control without or with CRI were statistically identical and varied from 9.53 to 10.43. The maximum number of seeds pod^{-1} (10.43) was obtained under STB+CRI followed by STB (10.42). FP showed lower seeds pod^{-1} in both the years.

The higher number of seeds pod^{-1} in case of CRI treatment might be due to the effect of the crop residues incorporation. Similar trend was observed over the years because the nutrient management treatments were same in both the years. Similar findings were reported by Siag and Yadav (2003) where the maximum number of seeds pod^{-1} was obtained when nutrient was applied @ S_{40} kg ha^{-1} in mungbean.

Seed weight

The nutrient management treatments HYG, IPNS and STB without and with CRI produced significantly higher 1000-seed weight that ranged from 45.77 to 47.57 g. Among the treatments, STB+CRI gave the maximum 1000-seed weight (47.57 g). The lowest 1000-seed weight (35.17 g) was recorded under the control treatment. Similar trend was found in 2008 but the values were higher than the previous year that might be due to crop residue incorporation (Table 2).

This observation might be due to residual effect of the crop residues although it was statistically identical in other crop in rice Naser *et al*., 2001. In another field study, Singh and Pareek (2003) reported the maximum 1000-seed weight by using nutrient @ P_2O_{545} kg ha^{-1} on mungbean.

Seed yield

Most of the nutrient management treatments except MYG, FP and control without or with crop residue incorporation were statistically identical for seed yield in 2007. All those treatments yielded 1.41 to 1.57 t ha^{-1} and were superior to MYG, FP and control with or without crop residue incorporation. The treatment STB+CRI produced the highest seed yield (1.57 t ha^{-1}). On the other hand, MYG without or with CRI produced 1.24 and 1.26 t ha^{-1}, respectively. FP produced lower yield in the range of 0.63 to 0.64 t ha^{-1}. The lowest yield was 0.33 t ha^{-1} in control plot. In 2008, the trend of seed yield due to nutrient management treatments was similar to the findings of previous year.

Moreover, it was observed that the nutrient management treatments like, HYG, IPNS and STB without or with crop residues incorporation gave higher seed yield. These results might be due to the cumulative effect of higher number of pods $plant^{-1}$, seeds pod^{-1} and 1000-seed weight. Among the treatments, STB produced the highest yield while lowest seed yield in control. However, the seed yield was numerically higher, though not statistically significant in all the treatments along with crop residues incorporation than without CRI might be due to effect of crop residue incorporation as well as more soil microbial activities for having conservation agriculture. Similar results were reported by Akhterruzzaman *et al.* (2009) where they observed the highest seed yield of mungbean with STB nutrient (N_{22} P_9 K_{11} S_2 kg ha^{-1}) in wheat-mungbean- T. *Aman* rice cropping pattern in high Ganges river floodplain soil. Timsina *et al.* (2006 a) reported the maximum seed yield from STB nutrient @ N_{20} P_{20} K_{20} in wheat-mungbean-T. *Aman* rice cropping pattern in northern part of Bangladesh. In addition, Chowdhury *et al*. (2002) revealed that STB nutrient N_{15} P_{15} K_0 S_{10} kg ha^{-1} gave the highest mungbean seed yield in wheat-mungbean-T. *Aman* rice cropping pattern. Moreover, these results were also in agreement with other authors (Saha *et al*., 2000; Quayyum *et al.*, 2001 and 2002; Singh *et al*., 2003).

Dry biomass yield

The maximum dry biomass yield (2.36 t ha^{-1}) was obtained from HYG+CRI, which was statistically identical to HYG (2.32 t ha^{-1}), IPNS+CRI (2.29 t ha^{-1}), IPNS (2.27 t ha^{-1}), STB+CRI (2.14 t ha^{-1}) and STB (2.10 t ha^{-1}). The trend was similar to the previous year with some exception in 2008. HYG+CRI produced the highest biomass yield (2.51 t ha^{-1}), which was statistically identical to a number of treatments. The lowest biomass yield of 097 and 1.01 t ha^{-1} was recorded in control treatment in 2007 and 2008 (Table 2).

Table 1. Crop characters, yield components and yield of mungbean as influenced by different nutrient management during 2007 and 2008.

Nutrient manage	Number of plants m^{-2}		Plant height (cm)		Number of pods $plant^{-1}$		Length of pod (cm)		Number of seeds pod^{-1}		1000-seed weight (g)		Seed yield ($t\ ha^{-1}$)		Biomass yield ($t\ ha^{-1}$)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
HYG	32.0	32.3	36.5 ab	41.6 a	10.80 ab	11.30 ab	7.60 ab	8.12 a	9.49 ab	9.53 ab	45.77 ab	50.15 ab	1.41 ab	1.48 ab	2.32 a	2.47 ab
MYG	32.0	32.2	33.2 abc	32.4 bc	9.07 b	9.51 b	6.83 bc	8.09 a	8.73 b	7.77 bc	39.37 bc	43.42 bc	1.24 b	1.30 b	1.85 c	2.00 bc
IPNS	32.5	32.3	36.1 ab	36.1 b	11.98 a	12.48 a	8.65 ab	9.15 a	9.69 a	10.31 a	47.48 a	52.03 a	1.52 a	1.69 a	2.27 a	2.42 ab
STB	32.6	32.8	34.8 ab	35.3 b	12.10 a	12.61 a	8.87 a	9.61 a	9.78 a	10.42 a	47.51 a	52.25 a	1.54 a	1.71 a	2.10 ab	2.25 ab
FP	32.0	32.2	31.9 bc	30.3 cd	5.90 c	6.18 c	7.23 ab	7.83 a	6.87 c	7.33 c	39.17 bc	43.52 bc	0.63 c	0.71 c	1.42 d	1.51 d
CON	31.7	32.0	28.4 c	28.1 d	4.80 c	5.01 c	5.13 c	5.35 b	5.37 d	5.57 c	35.17 c	38.31 c	0.33 d	0.38 d	0.97 e	1.01 e
HYG+CRI	32.1	32.7	37.2 a	42.1 a	10.83 ab	11.34 ab	7.77 ab	8.42 a	9.51 ab	9.61 ab	45.81 ab	50.23 ab	1.44 ab	1.51 ab	2.36 a	2.51 a
MYG+CRI	32.3	32.3	34.3 ab	34.5 bc	9.10 b	9.54 b	7.30 ab	8.31 a	8.76 b	7.81 bc	39.43 bc	43.52 bc	1.26 b	1.32 b	1.88 bc	2.02 abc
IPNS+CRI	31.9	32.7	37.0 ab	41.1 a	12.01 a	12.60 a	8.70 ab	9.17 a	9.70 a	10.32 a	47.52 a	52.07 a	1.54 a	1.70 a	2.29 a	2.49 ab
STB+CRI	32.3	33.0	36.1 ab	40.6 a	12.13 a	12.73 a	9.13 a	9.65 a	9.81 a	10.43 a	47.57 a	52.37 a	1.57 a	1.73 a	2.14 a	2.30 ab
FP+CRI	32.0	32.2	32.3 abc	32.9 bc	6.00 c	6.21 c	7.27 ab	7.95 a	6.91 c	7.36 c	39.20 bc	43.60 bc	0.64 c	0.72 c	1.45 d	1.65 cd
CON+CRI	31.9	32.2	28.8 c	28.1 d	4.83 c	5.03 c	5.17 c	5.39 b	5.43 d	5.59 c	35.50 c	38.51 c	0.36 d	0.41 d	1.02 e	1.03 e
CV (%)	5.25	8.43	5.91	4.90	8.84	9.19	9.93	9.80	4.11	9.89	6.46	5.85	7.51	10.27	6.96	9.63
Lev. of sig	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**

In a column, mean values having common letter(s) do not differ significantly whereas mean values with dissimilar letter(s) differ significantly as per DMRT.

HYG=0-24-40-48-24-3-1.2, MYG=0-20-36-40-20-2-1, IPNS=5000-9-37-36-21-3-1.2, STB=0-20-36-40-22-2-1, FP=0-6-5-4-0-0-0, CON=0-0-0-0-0-0-0, CD, N, P, K, S, Zn, B ($kg\ ha^{-1}$), respectively and CRI= Crop Residue Incorporation.

Here, HYG= High Yield Goal, MYG= Moderate Yield Goal, IPNS= Integrated Nutrient Management System, STB= Soil Test Based, FP= Farmers Practice, CON= Control, CD= Cowdung and CRI= Crop Residue Incorporated.

From the results of two years, it was found that HYG, IPNS and STB nutrient management treatments without or with CRI gave higher dry biomass yield. These results might be due to the effect of proper nutrient management, while MYG and FP gave and low biomass yield might be due to the effect of respective nutrient management. The lowest biomass yield was noticed in control as influenced no nutrient added into the soil i.e. native nutrient effect. However, over all the trend was more or less similar over the years might be due to applying the same nutrient management treatments. Chowdhury *et al*. (2002) reported that STB nutrient N_{15} P_{15} K_0 S_{10} kg ha^{-1} produced the highest dry biomass yield in wheat-mungbean-T. *Aman* rice cropping pattern.

Apparent nutrient uptake and balance

Total nutrient uptake (seed+ biomass) of N P K S Zn and B was significantly influenced by different nutrient management treatments in 2007 and 2008 for mungbean (statistically analyzed but not shown here) and balance (two years mean) (not statistically analyzed) was calculated normally and discussed below (Fig. 1-6).

Nitrogen

From the mean data it was observed that the added of nutrient ranged from 0 to 41 kg ha^{-1}yr^{-1} (40% of applied chemical/cowdung/crop residues nutrient N was considered effective) while uptake ranged from 26 to 93 kg ha^{-1}yr^{-1}among different treatments (Fig.1). Maximum uptake was found in STB (93 kg ha^{-1}yr^{-1}) followed by STB+CRI (91 kg ha^{-1}yr^{-1}). Minimum uptake was estimated in CON (26 kg ha^{-1}yr^{-1}). The apparent nutrient balance was found negative in all treatments ranging from -26 to -73 kg ha^{-1}yr^{-1}. The highest negative balance was found in STB (-73 kg ha^{-1}yr^{-1}) followed by HYG and IPNS (-65 kg ha^{-1}yr^{-1}). The lowest negative balance was observed in CON+CRI (-21 kg ha^{-1}yr^{-1}).

Fig. 1, shows that the nitrogen balance was negative as the uptake was higher compared to added nitrogen (40% of applied chemical/cowdung/crop residues nutrient N was considered effective). The N balance thus was negative in all treatments appeared to have been removed in excess of the quantity added in soil. However, the N balance was less negative in those treatments where crop residues were incorporated than without incorporation which might be due to addition of extra N came from crop residues (6 to 18 kg ha^{-1}yr^{-1}) as shown (Table 2). Present findings are also in agreement with some other researchers (Timsina *et al*., 2001 and 2006 (b)).

Phosphorus

The added phosphorus was in the range from 0 to 52 kg ha^{-1}yr^{-1}in respective of different treatments. The uptake was ranged from 5 to 19 kg ha^{-1}yr^{-1}. The treatments STB +CRI showed the highest uptake (19 kg ha^{-1}yr^{-1}) followed by IPNS+CRI (18 kg ha^{-1}yr^{-1}). The lowest uptake was found in CON and CON+CRI (5 kg ha^{-1}yr^{-1}). The negative balance was found in CON+CRI and CON -1 and -5 kg ha^{-1}yr^{-1}, respectively and FP (-5 kg ha^{-1}yr^{-1}) nutrient management treatments from mean data shown in Fig. 2. On the other hand, remaining treatments showed positive balance in the range of 3-35 kg ha^{-1}yr^{-1}. Maximum positive balance was found in HYG+CRI (35 kg ha^{-1}yr^{-1}) followed by IPNS+CRI (33 kg ha^{-1}yr^{-1}). Minimum positive balance was noticed in FP+CRI (3 kg ha^{-1}yr^{-1}).

From the figure 2, it was observed that the except FP and CON without or with crop residue incorporation treatments showed positive balance due to addition of higher amount of phosphorus nutrient with lower nutrient uptake that might be due to total dry matter content as well as the variation of concentration of the nutrient of the crops. Similar results were also found by Saleque *et al*. (2006).

Potassium

The quantity of added nutrient (K) was in the range of 0 to 90 kg ha^{-1}yr^{-1}and uptake by the crop varied from 21 to 65 kg ha^{-1}yr^{-1}. Maximum uptake was found in IPNS+CRI and STB+CRI (65 kg ha^{-1}yr^{-1}) followed by IPNS and STB (64 kg ha^{-1}yr^{-1}). Minimum uptake was observed in CON (21 kg ha^{-1}yr^{-1}). Among the nutrient managements, all treatments showed negative balance in the range of -4 to -29 kg ha^{-1}yr^{-1} except HYG+CRI, MYG+CRI, IPNS+CRI and STB+CRI. The positive balance ranged from 14 to 31 kg ha^{-1}yr^{-1}. Maximum positive balance was observed in HYG+CRI (31 kg ha^{-1}yr^{-1}) and minimum was found in both STB+CRI (14 kg ha^{-1}yr^{-1}) as shown in Fig. 3. This result is also agreement with Panaullah *et al*. (2006) but differed from Rahman *et al*., 2002.

Sulphur

From the mean data it was observed that quantity of added nutrient ranged from 0 to 33 kg ha^{-1}yr^{-1}and the uptake ranged from 4 to 15 kg ha^{-1}yr^{-1}with irrespective treatments. Among the treatments, maximum uptake was observed in STB+CRI (15 kg ha^{-1}yr^{-1}) followed by IPNS+CRI (13 kg ha^{-1}yr^{-1}). Minimum uptake was found in CON and CON+CRI (4 kg ha^{-1}yr^{-1}). The negative balance as observed in FP and CON with and without CRI treatments was -1 to -8 kg ha^{-1}yr^{-1}. Remaining treatments showed positive balance ranged from 8 to 22 kg ha^{-1}yr^{-1}shown in Fig. 4. Among the treatments, the maximum positive balance was observed in HYG+CRI (22 kg ha^{-1}yr^{-1}) that was followed by IPNS+CRI (20 kg ha^{-1}yr^{-1}). The minimum positive balance was found in MYG (8 kg ha^{-1}yr^{-1}).

From the above results it was observed that only two treatments such as FP and CON with and without CRI showed negative balance (two years mean) due to addition of low or no nutrient in the soil as uptake was high which might be due to total dry matter accumulation and variation of nutrient concentration of respective treatments. Rest of the treatments showed positive balance that might be due to addition of high amount of nutrient. The result is in agreement with Khan *et al.* (2005).

Zinc

The amount of nutrient added in different nutrient treatment was in the range of 0 to 3 kg ha^{-1} and uptake was in the range 0.06 to 0.20 kg $ha^{-1}yr^{-1}$with different treatments shown in Fig 5. Maximum uptake was observed in IPNS (0.21 kg $ha^{-1}yr^{-1}$) that was followed by a number of treatments like STB, STB+CRI and IPNS+CRI (0.20 kg $ha^{-1}yr^{-1}$). Minimum uptake was found in CON and CON+CRI (0.06 kg $ha^{-1}yr^{-1}$). The highest negative balance was noticed in FP (-0.10 kg $ha^{-1}yr^{-1}$) and the lowest in CON+CRI (-0.01 kg $ha^{-1}yr^{-1}$). Other treatments showed positive balance ranged from 1.80 to 2.96 kg $ha^{-1}yr^{-1}$. Among the treatments, maximum positive balance was noticed in HYG+CRI (2.96 kg $ha^{-1}yr^{-1}$) which was followed by IPNS+CRI (2.93 kg $ha^{-1}yr^{-1}$). Minimum positive balance was observed in STB (1.80 kg $ha^{-1}yr^{-1}$).

From the mean data of two years, it was noticed that farmers' practice and control treatments showed negative balance of zinc. Because there was poor and no nutrient (native nutrient was available only in the soil) was added into the soil whereas a considerable amount of nutrient was removed by the crop through total dry matter production and nutrient concentration variation in respective treatments consequently the balance became negative. On the other hand, rest of the treatments had positive balance. Similar results were reported by Basak *et al*. (2008) in Groundnut-T.*Aus*-T.*Aman* rice cropping pattern.

Boron

The range of added boron was 0-1.20 kg $ha^{-1}yr^{-1}$and uptake ranged from 0.04 to 0.15 kg $ha^{-1}yr^{-1}$. The uptake was the highest in STB (0.15 kg ha^{-1}). The CON and CON+CRI (0.04 kg $ha^{-1}yr^{-1}$) had the lowest uptake. The minimum negative balance was found in FP+CRI and CON+CRI (-0.01 kg $ha^{-1}yr^{-1}$) and the maximum was in FP (-0.07 kg $ha^{-1}yr^{-1}$) in Fig. 6. Other treatments showed positive balance ranged from 0.86 to 1.17 kg ha^{-1}. The highest positive balance was found in HYG+CRI (1.17 kg $ha^{-1}yr^{-1}$). The lowest positive balance was observed in STB (0.86 kg $ha^{-1}yr^{-1}$). Similar results were reported by Bhuiyan (2004) in Wheat-T. *Aus*/ Mungbean- T. *Aman* rice cropping pattern.

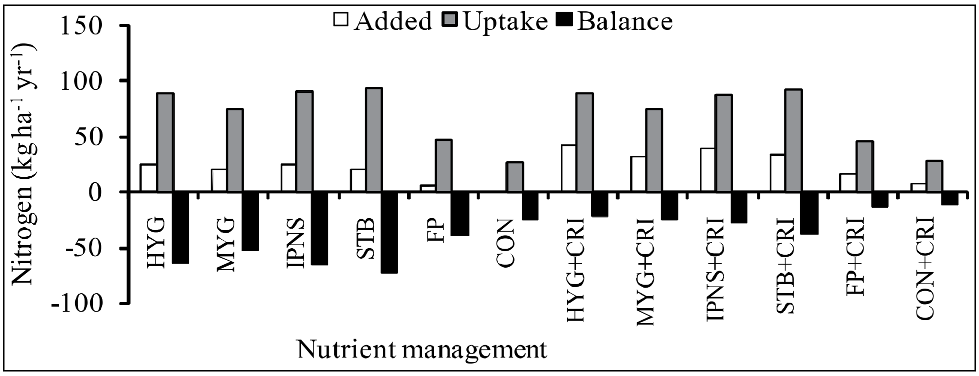

Fig. 1. Apparent N balance of mungbean as influenced by different nutrient management at (two years mean)

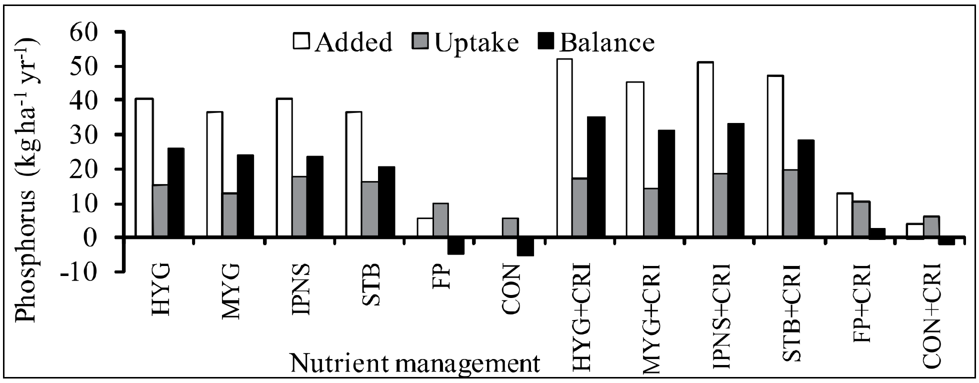

Fig. 2. Apparent P balance of mungbean as influenced by different nutrient management (two years mean)

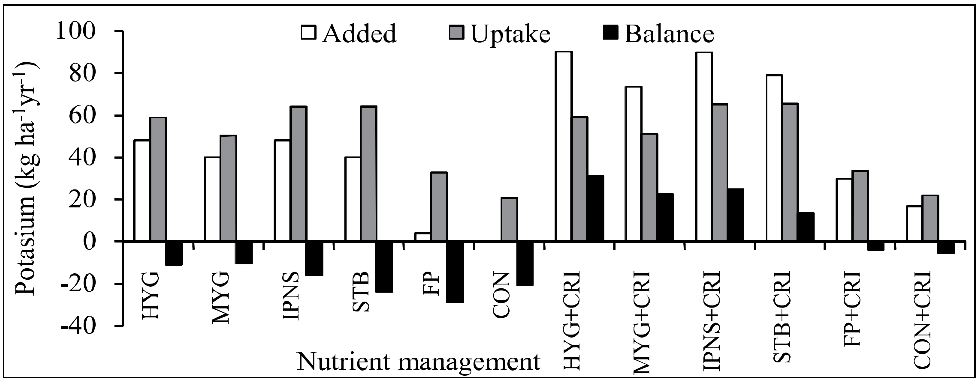

Fig. 3. Apparent K balance of mungbean as influenced by different nutrient management two years mean)

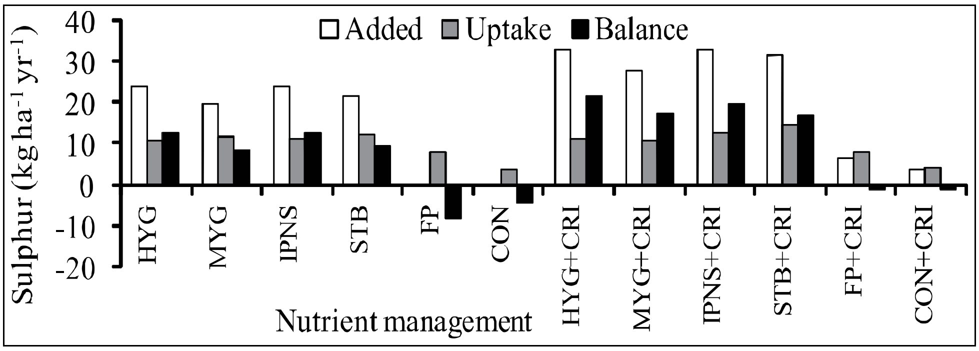

Fig. 4. Apparent S balance of mungbean as influenced by different nutrient management (two years mean)

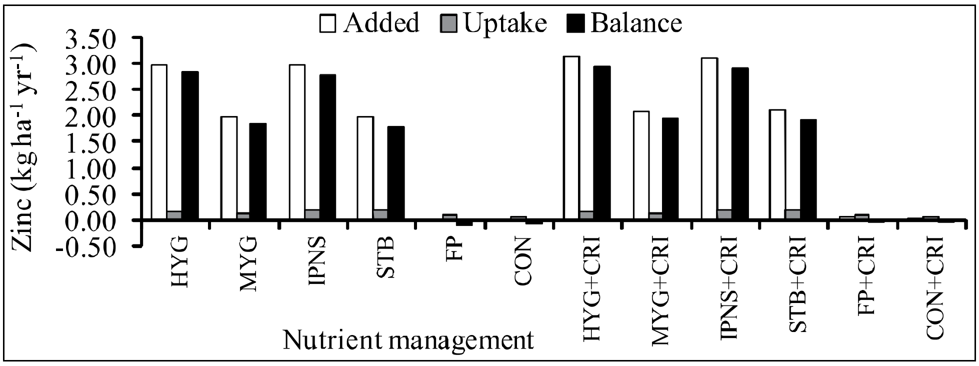

Fig. 5. Apparent Zn balance of mungbean as influenced by different nutrient mamagement (two years mean).

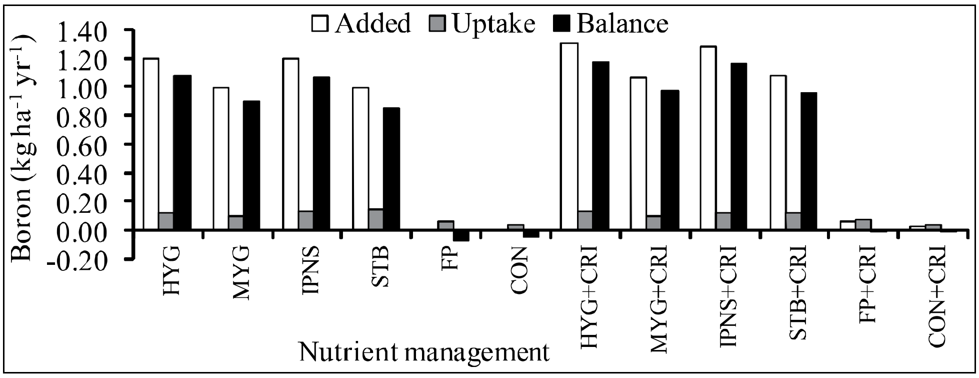

Fig.6. Apparent B balance of mungbean as influenced by different nutrient management (two years mean).

Economics of mungbean cultivations influenced by different nutrient management

Average of two years result showed that STB+CRI nutrient management gave the highest gross return (Tk. 41859 ha^{-1}) followed by STB (Tk. 41224 ha^{-1}), IPNS+CRI (Tk. 40981 ha^{-1}), and IPNS (Tk. 40844 ha^{-1}) nutrient management treatments due to higher yield. Table 3 revealed that STB+CRI nutrient management produced the highest net return (Tk. 27470 ha^{-1}) followed by STB (Tk. 27463 ha^{-1}), IPNS (Tk. 25394 ha^{-1}), and IPNS+CRI (Tk. 24908 ha^{-1}) due to lower total costs. In STB nutrient management, the cost of crop residue was not considered in the calculation that resulted in low total cost. Due to higher yield obtained STB nutrient management was much profitable (BCR, 3.00) compared to other nutrient managements like STB+CRI (2.91), IPNS (2.64), and IPNS+CRI (2.55). Control plots produced the lowest gross return, net return and BCR due to low yield. The overall economic performance of the aforesaid mungbean is sustainable and feasible considering applied STB nutrient management. The other best alternative nutrient managements were STB+CRI, IPNS, and IPNS+CRI. Nevertheless, STB+CRI nutrient management also gave higher net return compared to other nutrient managements like STB. Many scientists (Ali *et al*., 2009 a and b; Biswas *et al*., 2004, 2007, 2008; Zaman *et al*., 2007 a and b) also conducted similar type of experiments with different cropping patterns without crop residue incorporation into the soil and found more or less similar results. However, STB and IPNS nutrient managements along with and without crop residue incorporation might be suitable for mungbean production as well as economic point of view.

Table 3. Economic performance of potato as influenced by different nutrient management (mean of 2006-07 and 2007-08).

Nutrient management	Total cost (Taka	Gross return (Taka	Net return (Taka)	BCR
	1	2	3=(2-1)	4=(2/1)
HYG	14788	37044	22256	2.51
MYG	13705	32232	18527	2.36
IPNS	15450	40844	25394	2.64
STB	13761	41224	27463	3.00
FP	8115	17117	9002	2.11
CON	7287	9123	1836	1.26
HYG+CRI	15355	37052	21696	2.41
MYG+CRI	14210	32363	18153	2.28
IPNS+CRI	16072	40981	24908	2.55
STB+CRI	14389	41859	27470	2.91
FP+CRI	8527	17387	8859	2.04
CON+CRI	7542	9880	2338	1.31

Here, HYG=0-24-40-48-24-3-1.2, MYG=0-20-36-40-20-2-1, IPNS=5-9-37-36-21-3-1.2, STB=0-20-36-40-22-2-1, FP=0-6-5-4-0-0-0, CON=0-0-0-0-0-0-0, CD (t ha-1), N, P, K, S, Zn, B (kg ha^{-1}), respectively and CRI= Crop Residue Incorporation. BCR=Benefit-cost ratio ; MBCR=Marginal benefit-cost ration

Input and output prices: Urea-6.50 (Tk. kg^{-1}), TSP-19.00, MP-15.00, Gypsum-4.60, Zincsulphate-65.00, Boric acid-100, Cowdung-0.32 and Crop reside-0.25 (Tk. kg^{-1}) (dry basis) .

Mungbean seed -25 (Tk. kg^{-1}) and Biomass-0.25 (Tk. kg^{-1}) (dry basis)

Conclusion

Soil test based (0-20-36-40-22-2-1 CDNPKSZnB kg ha^{-1}) and integrated plant nutrient system (5-9-37-36-21-3-1.2 CDNPKSZnB kg ha^{-1}) along with or without crop residue incorporation could be suitable for getting higher seed yield with profitable cultivation of mungbean keeping sustainable good soil health.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 95-108, March 2015

CONSTRAINTS AND SUGGESTIONS FOR MODERN VARIETY POTATO PRODUCTION TECHNOLOGY

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Abstract

A study was carried out to determine the production constraints of modern varieties of potatoes and also find out the probable suggestions to overcome the constraints as verified the views between the farmers and extension officials. Data were collected from 232 farmers sampled randomly from 1547 potato growers and 51 extension officials from 153 population available from the study area of three upazila namely; Durgapur, Mohanpur and Bagmara under Rajshahi district of northwest area of Bangladesh. The study area was selected through multistage sampling procedure with continuous field observation and consultations with teachers, extension personnel, experts and contact farmers along with literatures reviewed. Thus, twenty important constraints regarding potato production were identified and possible suggestions for overcoming the constraints were suggested by using the same procedure. The importance of the constraints and the suggestions were measured by using 'Important Constraints Score Index (IPSI)' and 'Important Suggestion Index (ISSI)'. Out of 20 constraints and suggestions views of farmers and officials differed significantly for 15 items and 13 items.

Keywords: Constraints, suggestions, potato production.

Introduction

The potato refers to starchy tubers produced under soil from the crop plant *Solanum tuberosum* L. of the botanical family Solanaceae ranks among the four most important food crops in the world including wheat, rice and corn (Van-Diepan, 2003). Potato is the third most important crop of Bangladesh followed by rice and wheat. It is commonly used as vegetables and contributes 63 percent of the total annual vegetable production in Bangladesh (BBS, 2008). Bangladesh has made a reasonable progress in potato production during 1950-51 to 2011-12. Area and production under potato has increased to double during the same period due to favorable soil and climate. In general, potato takes 90 to 115 days for maturity but early variety potato could be harvested after 75 days of plantation. Bangladesh has good potential to produce potato yields more than 30 ton per

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hectare but actual yield is around 19.07 tons per hectare in 2012 (The Financial Express, 2013). Potato is ideally suited to places where land is limited and labour is abundant. Moreover, the potato is a highly productive crop which produces more food per unit area and per unit time than cereal crops. The factors that influence potato yield and quality includes cultivar, soil type, weather conditions, water management, fertilization, plant population, seed size, pests and diseases. The potato gives a high return compared to other crops in Bangladesh and it is cultivated during the Rabi season from November to February. The selection of high yielding varieties along with other production factors plays an important role to increase the yield per unit area (Khan, 2009). With access to production inputs, continued expansion in post harvest support in the form of roads and cold storages in the country, farmers continue to find potatoes an extremely attractive crop to grow. To accelerate potato production, the present study was undertaken with the objectives:

I. Determine the chronology of important potato production constraints and depict the priority of important suggestions and

II. Compare the significant relationship of views between farmers and extension officials.

Materials and Method

Modern variety potato farming is done in almost all agricultural zones of Bangladesh either in large or in a small scale. The researcher considered mainly two varieties namely Cardinal and Diamont as modern potato varieties for the study area due to their seed availability, production nature and market demand than others like Hira, Dhira, Chamak, Kufri Sindhuri, Granola etc. But as a potential area in northwest Bangladesh the study was conducted with randomly selected twenty villages in four unions of three upazilas (Durgapur, Mohanpur and Bagmara) under Rajshahi district. Out of available 1547 potato farmers a sample of 15% of them (232) was selected by using a Table of Random numbers (Kerlinger, 1973) and another second group of 15% extension personnel (51) were selected from the study area and 51 extension officials were interviewed for the constraints of potato production with possible suggestions for overcoming the constraints by the same questions as prepared in interview schedule for the farmers. A four point of awareness scale was prepared in this study for measuring potato production constraints of the farmers. Initially 24 statements were prepared and carefully examined by extension experts and their existence in the study area was also checked by farmers outside the study respondents during pre-testing of interview schedule. Out of 24 statements, 20 were selected as their existence more or less prevailing in the study area. To determine the degree of consideration a four point of awareness scale is set up against each selected constraint. Data were collected during the month of July 2010 to February 2011.

Necessary secondary data were also collected from different sources in addition to primary data.

Measurement of the importance of constraints in potato production

Extent or importance of each constraint in potato production as opined either by the farmers or by the agricultural extension officials was computed. The respondents of the study area pointed out the extent of different constraints by putting a tick mark in any one of four columns such as very important (extreme), important (high), moderate (medium) and less important (low). These responses were quantified by assigning weights very important, important, moderately important and less important as 4, 3, 2 and 1.

The important score was computed for each constraint by summing the weights for responses of the respondents against that constraint. For easy understanding of the constraint, the importance score (IS) of each of the constraint was computed and was expressed in percentage by using the following formula: IS = $P_{vh} \times 4 + P_h \times 3 + P_m \times 2 + P_l \times 1$ and

$$\text{Important Constraint Score Index (ICSI)} = \frac{\text{Observed Constraint Score}}{\text{Possible Constraint Score}} \times 100$$

The possible important constraint score index (ICSI) of a constraint could range from 232 to 928, where 232 indicated that the statement is less important as a constraint in potato production, while 928 indicated very high constraint faced by the farmers in the cultivation of potato.

Measurement of the importance of suggestions in potato production

In respect of each suggestion, the respondents indicated importance of solutions/suggestions by putting a tick mark in any one of four responses such as very important (extreme), important (high), moderate (medium) and less important (low). These responses were quantified by assigning weights very important, important, moderately important and less important as 4, 3, 2 and 1.

The important score (IS) of solutions/suggestions were computed by summing the weights for responses of the respondents. Therefore, the importance score of solution/suggestion could range from 232 to 928 for farmers while it could range from 51 to 204 for officers. For easy understanding, the importance score (IS) of each of the solution/suggestion was computed and expressed in percentage by using the following formula:

$$\text{Important Solution Score Index (ISSI)} = \frac{\text{Observed Solution Score}}{\text{Possible Solution Score}} \times 100$$

Where zero indicated no important suggestion for encouraging the production of potato and 100 indicated very high encouraging suggestion for practicing technologies in potato cultivation by the farmers i.e. higher the ISSI, the higher was the solution important for practicing improved technologies in potato cultivation.

Comparing the means of constraints of potato production by two independent groups of farmers and extension officials, *t*-test was used.

$$\text{The ratio} = \frac{\text{difference}}{\text{standard error of difference}}$$

When, the standard error of difference is estimated from the sample is denoted by t. *t*-test is used for small samples, as *t*-distribution tends to be normal distribution when *df* is greater than 30 (in this case 50 and 231).

t is calculated by the formula,

$$t = \frac{\overline{x_1} - \overline{x_2}}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \text{ at } n_1 + n_2\text{-}2\text{df}$$

$$S = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}}$$

Where, $\bar{x}_1$ = mean of first sample (farmers); $\bar{x}_2$ = mean of second sample (officials); n_1= number of observation in first sample; n_2= number of observation in second sample

Results and Discussion

Constraints in potato production as perceived by the farmers and extension officials

It was assumed that the views of farmers may differ with that of agricultural personnel regarding importance of constraints in potato production. As such cross check the matter between end user of potato technologies and extension service provider concerned with the technologies were recorded. The constraints with rank order as opined by the farmers are presented in Table 1.

Comparative analysis of constraints

From the rank orders of farmers and officials it has been seen that the views are not same and the degree of importance given by scoring also differed from one another. Important Constraints Index Score (ICIS) as obtained from farmers ranged from 42.24 to 90.30 and ICIS from officials ranged from 51.47 to 69.11 percent. The constraints range of farmers is much more than that of officers and the second category of opinion (Important, weight as assigned 3) ranked the top in total score from both type of respondents. Farmers opined low or less important category as last grade while officials opined very important category as last grade. Mostly farmers of Bangladesh are subsistence and they always try to ensure their food production only for survival. But they cultivate some cash crops for their economic gain as to meet cost involvement in agriculture and to ensure food security by increasing own capital. Potato is one of the leading cash crop, requires initial high investment for its production. The market price is observed low during harvesting season but it is unwise to store at home due to rapid decomposition. On the other hand, potato is not procured by the Government like other crops (rice and wheat) which create the frustration to the growers. This scenario ultimately shows a fall of law of diminishing returns of economic analysis. To protect the crop, in case of seed, good care is required which lead to need enough money. The most important constraints for farmers' ranked as 1, 2, 3 and 4, respectively i.e. initial investment high, lack of Govt. procurement, storing cost at cold storages and low market price of potato. The concerned officials opined that lack of Govt. procurement, storing cost at cold storages, lack of information and high price of pesticides is ranked 1, 2, 3 and 4 accordingly. From the both group of respondents it has been found that each group had given prime concern of cost involvement as the leading constraint of potato production. Other constraints have also ranked by them with their views but the degree of importance and ranking differs distinctly. From the above observations it has been thought to proceed for looking into the consistency or inconsistency for evaluating the difference statistically by *t*-test. Out of 20 constraints it was found that views of farmers and officers differed insignificantly only for 5 items and the rest 15 differed significantly. Insignificant difference of five constraints are storing cost at cold storage, low market price of potato, lack of timely quality seed supply, lack of seed cold storage and high price of pesticides. Most of cold storage are private enterprise and their regulation or operation are not controlled by extension departments and all the potato growers are supposed to admit either high price rate/bag at cold storage or to sell their produce at low price immediately after harvesting. No alternative option for farmers or no liability of the extension department to procure potato like rice or wheat as done by food department. So neither farmer nor extension officials might claim each other regarding the remarking situation which creates same perception of these two constraints. Not more than 10% of total seed demand is met by the Government agency BADC whole to fulfill the rest amount farmers have to run towards different companies or persons to get the quality seed. Though their field is ready in time to plant tuber but deviation due to seed supply situation. As this reality

Table 1. Rank order of different constraints in potato production as opined by farmers.

Sl. No.	Constraints	Very important (4)	Important (3)	Moderately important (2)	Less important (1)	IS	ICSI	Rank order
1	High initial investment	155	65	11	01	838	90.30	01
2	Lack of govt. procurement	125	69	14	24	759	81.78	02
3	Storing cost at cold storages	107	83	34	08	753	81.14	03
4	Low market price of potato	104	84	21	23	733	78.98	04
5	Lack of timely quality seed supply	58	136	22	16	700	75.43	05
6	Lack of cowdung manure	78	86	53	15	692	74.46	06
7	Middle man in marketing	98	56	46	32	684	73.70	07
8	Labor crisis	82	83	38	29	682	73.49	08
9	Indigenous storing	43	111	64	14	646	69.61	09
10	Mismanagement in cold storage	49	76	58	49	638	68.75	10
11	Short of capital	40	73	108	11	606	65.30	11
12	Improper loan support	39	83	81	29	596	64.22	12
13	Lack of seed cold store	33	73	96	30	573	61.74	13
14	Lack of balanced plant nutrients & pesticides	25	87	62	58	543	58.51	14
15	Lack of information	16	55	115	46	505	54.41	15
16	Lack of training	10	39	145	38	485	52.26	16
17	Virus attack	20	66	46	100	470	50.64	17
18	Lack of irrigation support	27	42	38	125	435	46.87	18
19	High price of pesticides	10	41	86	95	430	46.33	19
20	Plowing tillers	10	23	84	115	393	42.24	20
	Total	1129	1431	1222	858			

IS = Importance Score, ICSI = Important Constraint Score Index.

Table 2. Rank order of different constraints in potato production as opined by the extension official.

Sl. No.	Constraints	Very important (4)	Important (3)	Moderately important (2)	Less important (1)	IS	ICSI	Rank order
1	Lack of govt. procurement	24	07	04	16	141	69.11	01
2	Storing cost at cold storages	19	11	07	14	137	67.15	02
3	Lack of information	07	17	18	09	136	66.66	03
4	Lack of cow dung manure	17	13	08	13	136	66.66	03
5	High price of pesticides	09	21	15	06	135	66.17	04
6	Middle man in marketing	16	15	06	14	135	66.17	04
7	Improper loan support	16	15	04	16	133	65.19	05
8	Lack of irrigation sup	08	21	15	07	132	64.70	06
9	Low market price of potato	19	08	05	19	129	63.23	07
10	Labor crisis	08	20	14	09	129	63.23	07
11	Lack of seed cold storages	09	18	12	12	126	61.76	08
12	Mismanagement in cold storage	11	17	07	16	125	61.27	09
13	Virus attack	09	17	13	12	125	61.27	09
14	Lack of balanced plant nutrients & pesticides	08	12	24	07	123	60.21	10
15	Lack of training	06	19	15	11	122	59.80	11
16	Lack of timely quality seed supply	08	16	14	13	121	59.31	12
17	Short of capital	10	13	11	17	118	57.84	13
18	Initial investment high	08	12	16	15	115	56.37	14
19	Plowing tillers crisis	06	19	04	22	111	54.41	15
20	Indigenous storing	00	18	18	15	105	51.47	16
	Total	218	309	230	213			

Table 3. Showing significance difference of *t*-value for constraints.

S.l. No.	For the first Group (Farmers)				For the second Group (Officers)				t- value
	$\bar{x}_1$	$\sum x_1^2$	$\left(\sum x_1\right)^2$	IS	$\bar{x}_2$	$\sum x_2^2$	$\left(\sum x_2\right)^2$	IS	
1	3.017	2256	490000	700	2.372	341	14641	121	5.96**
2	2.34	1489	294849	543	2.411	339	15129	123	3.79**
3	2.09	1129	235225	435	2.392	338	14884	122	0.26^{ns}
4	2.568	1724	355216	596	2.607	423	17689	133	0.25^{ns}
5	2.176	1257	255025	505	2.666	346	18496	136	0.03^{ns}
6	1.853	968	184900	430	2.647	399	18225	135	5.90**
7	1.875	1087	189225	435	2.588	384	17424	132	4.36**
8	3.159	2527	537289	733	2.529	396	16641	129	4.07**
9	2.025	1198	220900	470	2.450	361	15625	125	2.65**
10	3.612	3110	702244	838	2.254	315	13225	115	12.50**
11	2.612	1740	367236	606	2.313	338	13924	118	2.17*
12	3.245	2603	567009	753	2.686	445	18769	137	4.30**
13	2.496	1599	328329	573	2.470	366	15876	126	0.007^{ns}
14	2.939	2240	465724	682	2.529	373	16641	129	2.66**
15	2.982	2249	478864	692	2.666	434	18496	136	2.14*
16	1.689	818	153664	392	2.176	305	12321	111	4.04**
17	2.784	1957	417316	646	2.058	249	11025	105	5.69**
18	2.75	1749	407044	638	2.450	373	15625	125	4.16**
19	2.948	2288	467856	684	2.647	429	18225	135	1.76^{ns}
20	3.271	2701	576081	759	2.764	479	19881	141	3.13**

*Significant difference at 5% level **Significant difference at 1% level, NS=Not significant.

might not be overcome so no scope of difference in opinion. Numbers of cold storage are available to preserve potato for table purpose but specialized cold storages only for storing seed is very hardly found. This is why, both farmers and officials are of the same opinion. Due to situational demand like foggy weather quickly support predisposition for late blight of potato. To prevent such attack, fungicides demand increases very rapidly, then the chance of higher price is a country picture of general experience display the same situational opinion by the farmers and extension officials.

Suggestions for overcoming the constraints in potato production

Transfer of technology is meaningless until the technology is adopted by the potential farmers. Attributes of production technologies are considered very important to play a crucial (positive or negative) role for perceiveness towards a technology. An attempt was made to find out the relevant suggestions/solutions for overcoming the constraints in using technologies of potato cultivation. The means of suggestions to overcome the constraints of potato production of two independent groups (farmers and officers) are calculated as followed earlier (for comparing the means of solutions as given by the respondent potato farmers and officers of agricultural extension by *t*-test). The solution/suggestion with rank order as opined by the respondent farmers and officers are presented in Table 4 and 5. If there is permanent procurement system by the Government, farmers are not loosed from the potato production. This is why the suggestion of initiative of Government procurement was come from the farmers as first rank basis. Also production is mainly depended on timely input supply. In potato season, farmers have to move here and there to buy or collect the inputs. Sometimes they are misguided or not get quality input. Day by day the storing cost is increasing. In harvesting time, the price is very low. It is common culture in Bangladesh and potato growers are suffering. Also due to shortage of cold storage they have to sell potato with low price. Farmers feel to need advance training on potato cultivation. With consider to the above field situation, the rank wise (2, 3, 4 and 4) important suggestions by the farmers were input supply, reducing storing cost, price fixation at harvesting period and arranging training. On the other hand, the five important suggestions by the officers to overcome the constraints are in the following order: (i) easy loan support, (ii) cattle farming for manure, (iii) reducing storing cost, (iv) co-operative marketing and (v) reducing price of pesticides. From the rank orders of farmers and officials it has been seen that the views are not same and the degree of importance of suggestions given by scoring also differed from one another. Calculated values are placed in Table 6 for showing significance difference of *t*-value. Out of twenty items of suggestions, seven items have been found to differ insignificantly between two independent groups of respondents but the rest thirteen differed significantly. It might be due to unconsciousness or concealing tendency towards field level limitations

Table 4. Rank order of different solutions as opined by the farmers.

Sl. No.	Solution/ suggestions	Very important (4)	Important (3)	Moderate important (2)	Less imp. (1)	IS	ISSI	Rank order
1	Initiatives of govt. procurement	127	70	16	19	769	82.86	01
2	Input supply	126	69	15	22	763	82.21	02
3	Reducing storing cost	107	84	33	08	754	81.25	03
4	Price fixation at harvesting period	107	80	33	12	746	80.38	04
5	Arrangement of training	68	131	26	07	724	78.01	05
6	Timely quality seed supply	67	130	28	07	721	77.69	06
7	Farm mechanization	59	133	23	17	698	75.21	07
8	Cattle farming for manure	60	130	23	19	695	74.89	08
9	Co-operating marketing	59	131	22	20	693	74.67	09
10	Easy loan support	40	73	107	12	605	65.19	10
11	Financial support	49	75	58	50	587	63.25	11
12	Specialized cold store establishment	33	72	97	30	572	61.63	12
13	Avail ability of agrochemicals	28	90	62	52	558	60.01	13
14	Increasing extension contact	25	88	56	63	539	58.08	14
15	Prevention of virus	10	39	144	39	484	52.15	15
16	Deep tube well irrigation support	27	43	40	122	439	47.30	16
17	Reducing price of pesticides	10	42	86	94	432	46.55	17
18	Providing power tillers by installment	10	41	85	96	429	46.22	18
19	Improvement of ITK	10	41	84	97	428	46.12	19
20	Govt. supervision in cold storages	09	40	86	97	425	45.79	20
	Total	1031	1602	1124	883			

IS = Importance Score, ICSI = Important Solution Score Index.

Table 5. Rank order of different solutions in potato production as opined by the officers to overcome the constraints.

Sl. No.	Solution/ suggestions	Very important (4)	Important (3)	Moderately important (2)	Less imp. (1)	IS	ISSI	Rank order
1	Easy loan support	15	16	08	12	136	66.66	01
2	Cattle farming for manure	17	13	08	13	136	66.66	01
3	Reducing storing cost	18	11	08	14	135	66.17	02
4	Co-operating marketing	16	15	06	14	135	66.17	02
5	Reducing price of pesticides	09	20	16	06	134	65.68	03
6	Deep tube well irrigation support	08	21	15	07	132	64.70	04
7	Farm mechanization	08	20	14	09	129	63.23	05
8	Price fixation at harvesting period	18	08	06	19	127	62.25	06
9	Increasing extension contact	08	17	17	09	126	61.76	07
10	Specialized cold store establishment	09	18	12	12	126	61.76	07
11	Prevention of virus	09	16	15	11	125	61.27	08
12	Govt. supervision in cold storages	11	17	07	16	125	61.27	08
13	Avail ability of agrochemicals	08	13	23	07	124	60.78	09
14	Timely quality seed supply	08	16	14	13	121	59.31	10
15	Arrangement of training	06	18	16	11	121	59.31	10
16	Financial support	12	12	10	17	121	59.31	10
17	Input supply	08	11	17	15	114	55.88	11
18	Initiatives of govt. procurement	04	11	24	12	109	53.43	12
19	Providing power tillers by installment	06	15	08	22	107	52.45	13
20	Improvement of ITK	00	18	18	15	105	51.47	14
	Total	198	306	262	254	1020		

Table 6. Showing significance difference of t-value for suggestions.

S.L. No.	For the first Group (Farmers)				For the second Group (Officers)				t- value
	$\bar{x}_1$	$\sum x_1^2$	$\left(\sum x_1\right)^2$	IS	$\bar{x}_2$	$\sum x_2^2$	$\left(\sum x_2\right)^2$	IS	
1	3.10	2361	519841	721	2.37	341	14641	121	5.96**
2	2.40	1558	311364	558	2.43	344	15376	124	0.20 ns
3	3.12	2378	524176	724	2.37	301	14641	121	7.06**
4	2.69	1737	366025	605	2.74	432	19600	140	0.37 ns
5	2.32	1479	290521	539	2.47	358	15876	126	0.98 ns
6	1.86	976	186624	432	2.62	394	17956	134	5.94**
7	1.89	1101	192721	439	2.58	384	17424	132	4.01**
8	3.21	2576	556516	746	2.49	403	16129	127	4.80**
9	2.08	1126	234256	484	2.45	359	15625	125	3.98**
10	3.28	2719	582169	763	2.23	310	12996	114	6.99**
11	2.53	1741	344569	587	2.37	357	14641	121	0.96 ns
12	3.25	2608	568576	754	2.64	433	18225	135	4.37**
13	2.46	1594	327184	572	2.47	366	15876	126	0.07 ns
14	3.00	2250	487204	698	2.52	373	16641	129	4.43**
15	2.99	2241	483025	695	2.66	434	18496	136	2.35*
16	1.84	965	184041	429	2.09	285	11449	107	1.77 ns
17	1.84	962	183184	428	2.05	249	11025	105	1.58 ns
18	1.83	945	180625	425	2.45	373	15625	125	3.99**
19	2.98	2231	480249	693	2.64	429	18225	135	2.41*
20	3.31	2745	591361	769	2.13	271	11881	109	8.36**

* Significant difference at 5% level ** Significant difference at 1% level, NS=Not significant.

followed by giving as usual progress report to the higher authorities to get recognition of better performance. On the other hand, field level suggestions by the incumbents or end users (farmers) don't consider the limitation of resources but expect subsidy or free support from the Government departments. The situational position of the respondents may draw different degree of importance but do not show unimportance. So combined evaluation of responses is being considered urgent in this regard to increase production of potato.

Conclusion

The most important constraints reported by the farmers and officials ranked in the following order: (i) high initial investment, (ii) lack of Government procurement, (iii) storing cost high at cold storage, (iv) low market price of potato, (v) lack of timely quality seed supply, (vi) lack of cow dung manure and (vii) high price of pesticides. Similarly top ranking of suggestions by farmers and officials (i) initiatives of Govt. procurement, (ii) timely input supply, (iii) reducing storing cost, (iv) price fixation at harvesting level, (v) arrangement of modern method of production training, (vi) easy loan support, (vii) co-operative marketing and (viii) reducing price of pesticides. Out of 20 constraints, it was found that views of farmers and officials differed insignificantly only for 5 items. In case of suggestions, seven items have been found to differ insignificantly between two independent groups of respondents but the rest thirteen differed significantly. It might be due to unconsciousness or concealing tendency towards field level limitations followed by giving as usual progress report to the higher authorities to get recognition of better performance. On the other hand, field level suggestions by the incumbents or end users (farmers) don't consider the limitation of resources but expect subsidy or free support from the Government departments. The situational position of the respondents may draw different degree of importance but do not show unimportance. Modern variety potato production technology is a complex matter and it is considered with different aspects. If a farmer follows all aspect in his field he can get more production. So combined evaluation of responses is being considered urgent in this regard to increase production of potato. To improve and ensure the maximum production, the attitude of growers and service provider should possess same views in spite of significant difference.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 109-119, March 2015

EFFECT OF SOWING DATES AND GENOTYPES ON THE YIELD OF CORIANDER (*Coriandrum sativum* L.)

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Abstract

A field experiment on coriander (*Coriandrum sativum* L.) taking five sowing dates viz. November 01, November 16, December 01, December 16 and December 31 and four selected genotypes viz. CS001, CS007, CS008 and CS011 was conducted during the Winter season of 2009-10 at Bangabandhu Sheikh Mujibur Rahman Agricultural University to study heat efficiency for the crop. The crop sown on November 16 and the genotype CS011 showed the highest heat use efficiency for dry matter, seed and stover yield. Heat use efficiency for dry matter as well as seed yield increased from November 01 to November 16 and then decreased with delayed sowing. November 16 sowing coupled with CS011 gave the maximum heat use efficiency for seed yield. Growing Degree Days (GDD) showed a positive linear response with dry matter accumulation and coefficient of regression was high in November 16 sowing as well as in CS011. Heat use efficiency showed a negative linear response with maximum (y = 2.058 – 0.054, R^2 = 0.682*), minimum (y = 2.123 – 0.070x, R^2 = 0.687*) and mean (y = 2.13 – o.063x, R^2 =0.709*) temperature but positive linear response with relative humidity (y = 0.074x – 5.593, R^2 = 0.702*).

Keywords: Heat use efficiency (HUE), Growing Degree Days, Coriander Genotypes, Dry matter and Sowing Dates

Introduction

Coriander (*Coriadrum sativum* L.) belonging to the family Apiaceae is a spice crop cultivated during winter season in Bangladesh. The coriander is cultivated for both the fresh green herb and the spice seed. Its fresh twigs, leaves and seeds are well known to need any introduction or discipline particularly to the housewife. Particularly all parts of the plant are used as spices and condiments which have pleasant aroma (Shanmugavelu *et al*., 2002).

Crop production mainly depends upon the climatic requirement of a particular crop. Temperature is a very important climatic factor which affects plant growth, development and yield. Temperature also plays a key role in determining sowing

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time (Tiwari and Singh, 1993). Winter crops are vulnerable to high temperature during reproductive stages (Kalra, 2008). Each phenophase of a particular crop has an optimum temperature for its initiation and development and changes in this optimum temperature affects the yield of a crop mainly through changes in phenological processes by influencing plant physiological processes including photosynthesis and respiration (Sharma *et al*., 2003). Coriander is a temperature sensitive crop. Its optimum temperature for germination and early growth is 20-25^{o}C (Singhania *et al*., 2006). Relatively cool weather during vegetative period and warm weather during reproductive stage are ideal for coriander crop (Tiwari *et al*., 2002). The optimum sowing time of coriander in Bangladesh is November 15. The life cycle of the coriander plants become shorter as the sowing is delayed that adversely affects yield of coriander (Ahmed and Haque, 1985). Plants in delayed sowing face adverse climate during vegetative and reproductive stages. In delayed sowing plants do not get optimum temperature during vegetative and reproductive growth in coriander and ultimately seed yield decreases. Heat unit requirement or growing degree day (GDD) has been used for characterizing the thermal response in different winter crops (Rajput *et al.*, 1987; Shanker *et al*., 1996). The quantification of heat use efficiency (HUE) is useful for the assessment of yield potential of a crop in different growing environments (Pal and Murty, 2010). Higher the HUE, higher the yield of wheat (Hussain *et al*., 2010) ; Pal and Murty, 2010) and rice (Islam and Sikder, 2011). The experiment was, therefore, undertaken to observe the heat use efficiency of coriander genotypes under different sowing dates.

Materials and Method

The experiment was conducted to determine the effect of sowing dates and genotypes on the yield of coriander during the winter season of 2009-10 at the research farm of the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. The experiment was laid out in split-plot design having five sowing dates viz. November 01, November 16, December 01, December 16 and December 31 in the main plots and four genotypes viz. CS001, CS007, CS008 and CS011 in the subplots. The unit plot size was 4.8 m^2 (4 m ×1.2 m.). The crop was fertilized @ cowdung 5 t/ha and 80-40-20-20 kg/ha of N-P-K-S. The entire amount of cowdung, phosphorus from TSP, potassium from MP, sulphur from gypsum with one- half of nitrogen as urea were applied during final land preparation. The rest of the nitrogen was topdressed in two equal splits at 30 and 60 days after sowing. The sowing of each genotype was done according to selected sowing dates. Slight watering was done just to supply sufficient moisture needed for quick germination. A recommended agronomic production package was followed for the crop (Razzak *et al*., 2004). Seeds were harvested when half of the green seeds on the plant changed to brown colour as suggested by Singhania *et al.* (2006).

Yield data were collected from the inner rows of each plot to avoid the border effect. In each unit plot 10 plants were selected randomly for recording data on yield components and yield. The data were recorded on seed yield/plant, stover yield (kg), dry matter/m^2 and harvest index (%). For recording drymatter (DM) accumulation over time, 10 plants were harvested at random from 25 days after sowing to maturity (119 days) at 15- day intervals. The crops which recorded less than 119 days for harvest are kept in the field up to 119 days. The plant samples were oven dried at 72°C for 72 hours and weight was taken by electric balance. Dry weights were converted to per m^2 for calculating DM.

Heat Unit/Growing Degree Day (GDD)

Growing degree day (GDD) or heat unit were calculated as per the equation suggested by Iwata (1984) :

$$GDD = \sum\{(T_{max} + T_{min}) - Tb\}$$

Where T_{max} is the maximum temperature (°C),

T_{min} is minimum temperatur (°C),

T_b is the base temperature taken as 4.8°C (Hrnander Devi *et al*., 2002).

The daily maximum and minimum temperature were recorded in Weather Station, Dept. Agricultural Engineering, BSMRAU, Salna, Gazipur situated 100 m away from the field. The heat use efficiency (HUE) for dry matter was calculated by the method suggested by (Sharma *et al.*, 2003) and for seed yield by (Pal and Murty, 2010);

$$HUE = \frac{DM}{\sum HU} \text{ g/m}^2\text{/deg. day)}$$

$$HUE = \frac{Seed\ yield\ (kg/ha)}{\sum HU} \text{ kg seed/ha/deg. day)}$$

Where, DM is dry matter (g/m^2), $\sum$ HU is the cumulative heat unit (°C day).

Correlation and regression analyses were done to study the relationship between GDD (HU) and dry matter, and HUE and weather elements. The data were compiled properly and analyzed statistically by MSTAT Program and mean comparison was done following the Duncan's Multiple Range Test at 5% level of probability.

Table 1. Mean air temperature (°C) during sowing to emergence and 50% flowering to seed- filling stage.

Sowing time	Sowing to emergence			50 % flowering to seed filling stage		
	Max.	Min.	Mean	Max.	Min.	Mean
Nov. 01	28.13	24.77	26.45	21.36	16.82	19.09
Nov. 16	25.45	17.85	21.65	22.19	17.87	20.03
Dec. 01	25.15	17.38	21.27	28.17	23.82	26.00
Dec. 16	20.40	15.60	18.00	26.50	21.75	24.13
Dec. 31	20.70	16.11	18.41	28.05	24.13	26.31

Results and Discussion

The heat use efficiency (HUE) increased with growing period and attained the maximum at seed filling stage in all treatments (Table 2). The maximum HUE was recorded from the crops sown on November 16 followed by December 01 in all phenological stages and its minimal value was recorded from December 31 sown crops. HUE at each phenophase increased from November 01 to November 16 and then decreased with delayed sowing. Mean value of HUE was also the maximum in November 16 sown crop and then decreased with delay in sowing of crop. Similar results were reported by Sharma *et al*. (2003) in wheat crop and Kumar *et al*. (2008) in soybean crop. The genotype CS011 gave the highest HUE in all phenological stages followed by CS001 and CS007 (Table 2). Mean value also revealed that CS011 had the highest HUE followed by CS001, CS007 and CS008.

Table 2. Heat use efficiency (HUE) (g/m^2/deg day) of coriander as influenced by different sowing dates and genotypes at different phenological stages.

Treatment	Phenological stages				
	Branching	50% flowering	Seed filling	Maturity	Mean
Sowing date					
November 01	0.031	0.46 c	0.68 c	0.64 c	0.452 c
November 16	0.036	0.54 a	0.76 a	0.71 a	0.511 a
December 01	0.034	0.50 b	0.72 b	0.67 b	0.481 b
December 16	0.032	0.49 b	0.71 b	0.67 b	0.475 b
December 31	0.029	0.44 d	0.62d	0.59d	0.420 d
Genotype					
CS001	0.032	0.50 b	0.70 b	0.69 b	0.48 b
CS007	0.033	0.48 b	0.65 c	0.62 c	0.45 c
CS008	0.030	0.44 c	0.60 d	0.58 d	0.41 d
CS011	0.039	0.55 a	0.76 a	0.73 a	0.52 a
CV (%)	Ns	3.67	3.62	4.21	3.84

Means showing similar letter (s) in a column are not significant at 5% level by DMRT.

Growing Degree day (GDD) values at harvest were little influenced by sowing dates (Table 3). Higher GDD at harvest was recorded with November 01 sowing (1938 ^{o}C day) followed by December 31 sowing (1922 ^{o}C day) and November 16 sowing (1902 ^{o}C day) while GDD was the lowest in December 16 sowing (1816 ^{o}C day). Around 2000 ^{o}C GDD is optimum for coriander seed production suggested by Carrubba *et al.* (2006).

Higher GDD in November 01 sowing was probably due to the crops subjected to low temperature resulted in long maturity duration (119 days). Although the crops of November 16 sowing matured at 118 days after sowing, it had 36 GDDs less than November 01 sowing because at early growth stage the crops faced somewhat lower temperature which was optimum for early growth. The crop sown on December 31 required 1922 GDDs, which might be due to higher temperature during reproductive stage (Table 1) although maturity duration was only 104 days. The lowest GDDs in December 16 were probably due to medium low temperature during reproductive stage and 107 days of maturity. It was clear that GDD decreased with the advancement of sowings except December 31 sowing. This is in close conformity with the report of Pal and Murty (2010) in wheat. The genotype CS001 had the highest GDDs (2023) followed by CS011 (1873), CS007 (1846) and CS008 (1834) (Table 3). There was no significant difference among CS011, CS007 and CS008 with regard to GDD. The variation in GDDs in different genotypes might be due to variation in maturity days of the genotypes. Heat use efficiency was low in CS001 for higher GDD and less seed yield.

Table 3. Growing Degree days (GDDs), seed yield, stover yield and heat use efficiency (HUE) as influenced by sowing dates and genotypes.

Treatment	GDD at harvest (^{o}C day)	Seed yield (t/ha)	HUE (kg seed /deg. day)	Stover yield (t/ha)	HUE (kg stover /deg. day)
Sowing date					
Nov. 01	1938 a	1.62 c	0.84 b	2.00 ab	1.03 b
Nov. 16	1902 a	1.81 a	0.95 a	2.07 a	1.09 a
Dec. 01	1893 ab	1.71 b	0.90 a	1.96 bc	1.03 b
Dec. 16	1816 b	1.57 d	0.85 b	1.91 cd	1.05 b
Dec. 31	1922 a	1.44 e	0.75 c	1.85 d	0.96 c
Genotype					
CS001	2023 a	1.55 c	0.77 c	2.42 a	1.20 a
CS007	1846 b	1.64 b	0.89 b	1.75 c	0.95 c
CS008	1834 b	1.42 d	0.77 c	1.61 d	0.88 d
CS011	1873 b	1.91 a	1.02 a	2.05 b	1.10 b
CV (%)	4.4	4.12	4.67	3.94	4.01

Means showing similar letter (s) in a column are not significant at 5% level by DMRT.

The highest seed yield was obtained from November 16 sowing (1.81 t/ha) which was followed by December 01 sowing (1.71 t/ha) while the lowest in December 31 (Table 3). This is in agreement with the findings of Ahmed and Haque (1985) and Toncer *et al.* (1998). Seed yield was consistently the highest (2.02, 1.19 and 1.65 t/ha in the 3 years, respectively) after sowing on 25 October in Sudan where average air temperature was close to 25°C in last week of October reported by Mohamed (1992). The temperature of 25°C was optimum for coriander growth that was close to November 16 sowing (25.45°C, Table 1). The November 16 sown gave the highest yield because the crops enjoyed more or less suitable temperature during both vegetative stage and reproductive stage (Table 1). The lower yield was recorded from December 16 sowing followed by December 31 sowing. This happened probably due to very low temperature during early vegetative growth and high temperature above optimum during flowering and fruit setting stage (Table 1) and shorter growing seasons. The lower yield in November 01 sowing compared to November 16 and December 01 sowing could be due to the fact that high temperature as well as low temperature was during vegetative growth and flowering and fruit setting phase, respectively (Table 1). The yield of crop increased from November 01 sowing to November 16 sowing and then decreased gradually. Ahmed and Haque (1985) also reported delayed sowings decreased the seed yield of coriander.

The November 16 sown crop exhibited the maximum HUE of 0.95 kg seed/ha/deg. day closely followed by December 01 sown crop (0.90 kg seed/ha/deg. day) while the December 31(very late) sowing had the lowest HUE (Table 3). From November 16 sowing HUE was found to be decreased with late sowing. Similar results were reported by Pal and Murty (2010) in wheat. The genotype CS011 showed the highest HUE (1.02 kg seed/ha/deg. day) which was followed by CS007 while the genotypes CS001 and CS008 showed the lowest HUE. Heat use efficiency of 0.98-1.51 kg grain/ha/deg. day in fine rice was reported by Islam and Sikder (2011). The maximum HUE of 2.93 kg grain/ha deg. days in wheat was also reported by Pal and Murty (2010).

Dry matter was influenced by cumulative heat unit i.e., growing degree day (GDD) in all the sowings as shown in (Table 4). It was observed that dry matter was directly and linearly associated with cumulative heat units. The correlation between GDD and genotypes were highly significant. The R^2 values ranged from 0.75 to 0.95 which were significant at $p \leq 0.01$. The b values i.e., coefficient of regression ranged from 0.532 to 0.836 which were highly significant at $p \leq 0.01$. The value of slope of regression (b) was the maximum in November 16 sowing. The slope of regression (b) was significantly higher in November 16 sowing as compared to December 16 and December 31 sowings and the slopes of November 16, November 01 and December 16 sowings was at statistically similar level. This shows that the coriander crop sown on November 16 is more efficient in heat utilization as compared to November 01, December 16 and December 31 sowing. Dry matter for genotype was also positively related with cumulative heat unit i.e., growing degree day (GDD) in all genotypes as shown in (Table 4.). Dry matter was directly and linearly associated with cumulative heat units. The correlation between GDD and genotypes were highly significant. The R^2 values ranged from 0.78 to 0.79 which were significant at $p \leq 0.01$.The coefficient of regression ranged from 0.464 to 0.616 which was significant at $p \leq 0.01$. The value of coefficient of regression was maximum in CS011 (0.616) as compared to other genotypes. The slope of regression (b) was significantly higher in CS011 as compared to CS001, CS007 and CS008. The slope of regression line developed for each genotype showed that CS011 was more efficient in heat utilization in comparison to other genotypes.

Table 4. Response equations to the fitted GDD and dry matter produced at different sowing date and by genotypes

Treatment	Regression equation	Coefficient of regression (b value)	R^2 value	Coefficient of correlation (r)
Sowing date				
November 01	y = - 602.58 + 0.833x	0.833** ± 0.135	0.88**	0.94**
November 15	y = - 608.42 + 0.836x	0.836** ± 0.085	0.75**	0.87**
December 01	y = - 449.26 + 0.766x	0.766 **± 0.093	0.93**	0.96**
December 16	y = - 359.78 + 0.648x	0.648** ± 0.078	0.93**	0.96**
December 31	y = - 328.47 + 0.532x	0.532** ± 0.050	0.95**	0.97**
Genotype				
CS001	y = - 216.66 + 0.589x	0.589** ± 0.05	0.78**	0.88**
CS007	y = - 196.03 + 0.544x	0.544** ± 0.07	0.79**	0.89**
CS008	y = - 160.58 + 0.464x	0.464** ± 0.065	0.79**	0.89**
CS011	y = - 216.66 + 0.616x	0.616** ± 0.08	0.79**	0.89**

** indicates significant at the 1% level, ± SE (standard error).

The highest stover yield was recorded from November 16 sowing closely followed by November 01 sowing and the lowest value, from December 31 sowing (Table 3). The genotype CS001 produced the maximum stover yield (2.42 t/ha) followed by CS011 (2.05 t/ha) and the lowest from CS008. The maximum HUE for stover was noticed in November 16 sowing followed by December 01, November 01 and December 16 sowing and the lowest HUE from December 31 sowing (Table 3). The maximum HUE for stover was recorded from CS001 followed by CS011. The highest HUE for stover was recorded in soybean (Kumar *et al.*, 2006).

The highest yielding genotype was CS011 when sown on November 16 (2.05 t/ha) or December 01 (2.02 t/ha) (Table 5). The lowest yielding genotype was CS008 at December 16 and December 31 sowings. The genotype CS011 produced consistently higher seed yield than CS007 irrespective of sowing dates. The genotype CS007 also produced consistently higher seed yield than CS001 across sowing dates, although there was no difference in seed yield between the two genotypes at December 01 sowing. Lower seed yields were recorded in all genotypes at late sowings (December 16 and December 31) as compared to other sowings. Reduction in yield components and thereby seed yield at late sowings were due to short growing period, lower temperature in vegetative growth stage and higher temperature at reproductive phase (flowering, fruit setting and seed filling stage) (Table 1**).**

Table 5. Seed yield (t/ha) of coriander as influenced by interaction of genotype and sowing date

Genotype / Sowing date	CS001	CS007	CS008	CS011
November 01	1.52 g	1.63 f	1.41 hi	1.91 b
November 16	1.76 de	1.81 cd	1.62 f	2.05 a
December 01	1.63 f	1.68 ef	1.51 g	2.02 a
December 16	1.47 gh	1.63 f	1.33 ij	1.86 bc
December 31	1.35 i	1.46 gh	1.25 j	1.70 ef
CV (%)		4.12%		

Means showing similar letter (s) are not significant at 5% level by DMRT.

Table 6. Heat use efficiency of coriander genotypes for seed yield under different sowing dates (kg seed/ha/deg day),

Genotype / Sowing date	CS001	CS007	CS008	CS011
November 01	0.77 ef	0.86 cd	0.75 f	1.00 b
November 16	0.90 c	0.97 b	0.87 cd	1.09 a
December 01	0.83 d	0.90 c	0.81 de	1.07 a
December 16	0.77 ef	0.89 c	0.73 fg	1.00 b
December 31	0.68 gh	0.77 ef	0.67 h	0.90 c
CV(%)		4.67		

Means showing similar letter (s) are not significant at 5% level by DMRT.

Among the five sowing dates, the crops sown on November 16 gave the highest HUE irrespective of genotypes (Table 6). The November 16 sown crops exhibited the maximum HUE (1.09 kg seed/ha/deg. days) for the genotype CS11 closely followed by December 01 sowing (1.07 kg seed/ha/deg. days) for the same genotype (Table 6).There was no significant difference between December 01 sowing and November 01 sowing irrespective of genotypes with regard to heat use efficiency. The December 31 sown crop gave the minimal HUE irrespective of genotypes.

Heat use efficiency of coriander showed a negative linear response with the maximum, minimum and mean temperature, while it showed a positive linear response with relative humidity (Fig.1). R^2 values for weather parameters ranged from 0.682 to 0.709 which were significant at $p \leq 0.05$. In wheat crop, similar results for temperature were obtained by Sharma *et al.* (2003) but they reported a parabolic response with relative humidity. The response equations obtained between heat use efficiency and weather elements can be used for simulating the response of heat use efficiency of coriander crops with parameters.

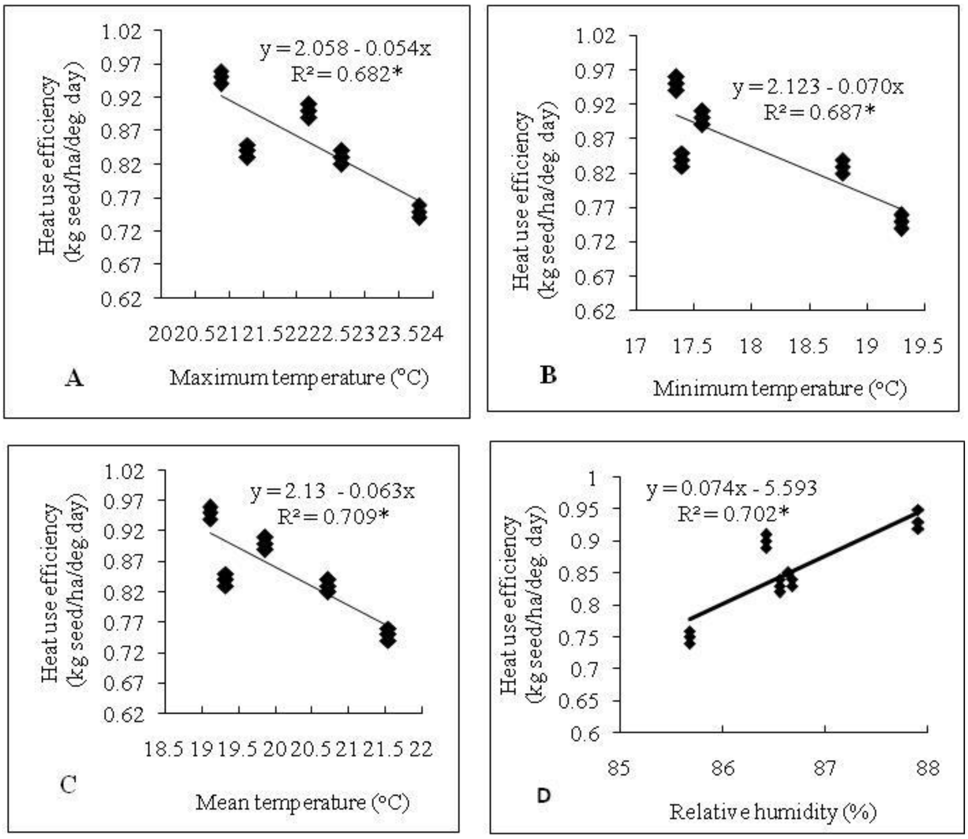

Fig. 1. Relationship between heat use efficiency and weather parameter in coriander crop.

Conclusion

It is, therefore, concluded that sowing of coriander crop on November 16 exhibited higher heat use efficiency for dry matter, seed yield and stover yield. November 16 sowing found the maximum seed yield. Coriander crop sown on November 16 and the genotype CS011 are more efficient in heat utilization. The given agro-climatic conditions of Salna areas (AEZ-28) was found to be beneficial for the coriander crop sown for good harvest. The crop would become suitable for harvest after receiving a thermal sum of 1902 GDD (°C).

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 121-135, March 2015

MOLECULAR DIVERGENCE OF SECONDARY ENDOSYMBIONT, *CARDINIUM* IN *BEMISIA TABACI* (GENNADIUS) AND ASSOCIATES

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Abstract

Whitefly, *Bemisia tabaci* (Hemiptera : Aleyrodidae) harbors numerous secondary endosymbionts, which are transmitted from mother to offspring by both horizontally and vertically, that have crucial role on host selection, biology, and evolution. Bacteria, *Cardinium* was identified in *B. tabaci* as well as in other whitefly population from many different countries by comparing 16S rDNA sequences. *Cardinium* were detected in all tested indigenous *B. tabaci* populations of Bangladesh, Myanmar, Nepal, and the Philippines as well as Q1 biotype of Korea. It was absent in B biotype of Korea and Q biotype of China. *Cardinium* was also detected in three out of five tested *Aleurodicus dispersus* population as well as in five out of seven *Trialeurodes vaporariorum*, whereas they were not detected in *Tetraleurodes acaciae* populatuion. In adition, *Cardinium* was detected in parasitoid *Encarsia formosa* attacking *B. tabaci*. Among the 19 whitefly populations from different countries, present studies identified four phylogenetic groups of *Cardinium*, thereby demonstrating the high diversity of this genus. *Cardinium* phylogeny suggests a correlation of geographical range with ecological variation at the species level.

Keywords: Molecular divergence, Intra-specific variation, *Aleurodicus dispersus*, Endosymbiont, *Bemisia tabaci*, *Cardinium*

Introduction

Endosymbiotic bacteria are harbored within the cells or tissues of many arthropods, including insects (Buchner, 1965; Brown *et al*., 1995; Frohlich *et al*., 1999). The symbiotic relationships between insects and endosymbionts drive evolutionary interactions, resulting in broad ranging activities from neutralism to ammensalism (Moran, 2007; Moya *et al*., 2008).

Endosymbionts can be categorized as either primary or secondary endosymbionts according to their physiological roles. The primary endosymbiont *Portiera aleyrodidarum* is harbored within bacteriocytes and supplements *B. tabaci* with

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essential amino acids for growth and development (Moran and Telang, 1998; Thao *et al*., 2000; Baumann, 2005). Although secondary endosymbionts are not necessary for host survival, they may play important roles in their host's physiology, ecology, and evolution (Zchori-Fein and Brown, 2002; Chiel *et al*., 2007). Until now, seven secondary endosymbionts have been identified in *B. tabaci*, namely *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Orientia*, *Rickettsia*, and *Wolbachia* (Baumann, 2005; Gottlieb *et al*., 2006; Chiel *et al*., 2007; Bing *et al*., 2012).

Cardinium bacteria represent a clade within the Flexibacteraceae, which belongs to the class Sphingobacteria under the phylum Cytophaga-Flavobacterium-Bacteroides (CFB), a phylum that is unrelated to the α-Proteobacteria to which *Wolbachia* belongs. It was first seen in cell cultures established from the tick *Ixodes scapularis* (Kurtti *et al*., 1996). These unusual bacteria were observed in electron micrographs and in later studies their presence was confirmed by PCR and phylogenetic analysis. *Cardinium* are transovarially transmitted Gram-negative pleiomorphic rod-like bacteria about 1-2 micrometer long and approximately 0.5 micrometer wide. They have a distinctive parallel array of hollow filaments resembling microtubules that extend from the inner membrane into the cytoplasm (Bigliardi *et al*., 2006; Nakamura *et al*., 2009). *Cardinium* was originally named *Encarsia* bacterium (EB), (Zchori-Fein *et al*., 2001), and then, Cytophaga-like organisms (CLO), (Hunter *et al*., 2003), Cytophaga-Flavobacterium-Bacteroides (CFB) (Weeks and Breeuwer, 2003) and, finally, *Cardinium hertigii* (Zchori-Fein and Perlman, 2004; Zchori-Fein *et al*., 2004).

All bacteria possess the ability to manipulate the physiological characteristics of their hosts. Specifically, *Wolbachia*, *Arsenophonus*, *Cardinium*, and *Rickettsia* can manipulate host reproduction (Duron *et al*., 2008; Werren *et al*., 2008), *Hamiltonella* can induce virus resistance in pea aphid (Oliver *et al*., 2002). *Rickettsia* increases thermotolerance in *B. tabaci* (Brumin *et al*., 2011). However, in most cases, the presence of endosymbionts is indicated exclusively by polymerase chain reaction (PCR) using DNA template consequent from whole arthropods. Although no direct confirmation of symbiosis can be attained, a positive result strongly represents the symbiotic partners in question. The objectives of this study were to determine the geographic distribution of *Cardinium* infection in *B. tabaci* and other whiteflies as well as the relationship between *Cardinium* infection and evolutionary linkage in whiteflies.

Materials and Method

Collection of whitefly samples

Adult individuals of *B. tabaci* and other whiteflies were collected from various host plants, such as tomato, pepper, ridge gourd, bean, okra, eggplant, and guava grown in Bangladesh, Myanmar, Nepal, China, Philippines and Korea (Tables 2 and 3). In Bangladesh, we collected whiteflies from bean, okra, and eggplant as of the southern, northern and eastern parts of the country during 2011-2012, immediately preserved samples in 99% ethanol, and stored them at -20^0C for further molecular analysis. Adults of *B. tabaci* B and Q biotypes were collected from cucumber, sweet melon, and tomato plants which grown throughout Republic of Korea in 2010 in order to compare the morphologies and genetic sequences of foreign whiteflies. This research work has been performed from January 2011 to June 2012 at the insect molecular physiology lab in the Republic of Korea.

Identification of whiteflies, endosymbionts, and TYLCV

Different genotypic group of *B. tabaci* was determined by amplification the gene of mtCOI region causing specific fragments from extracted genomic DNA samples (Khasdan *et al*., 2005). The presence of *Cardinium* in whiteflies was determined using specific primer sets for *Cardinium* by amplification of 16S rDNA gene fragments as described by Chiel *et al*., 2007. The occurrence of *Tomato yellow leaf curl virus* (TYLCV) on farmhouses of various horticultural crops was surveyed in different geographical regions. Acquisition of this virus by *B. tabaci* was determined using a TYLCV-specific primer set that can amplify conserved intergenic sequences (*Lee et al*., 2010). Specific primer sets for biotypes, endosymbionts, and TYLCV are listed in Table 1. Polymerase chain reaction (PCR) reactions for *Cardinium* were performed in a 20 μl mixture containing 5× SuperTaq PCR buffer (10 mM Tris-HCL, 40 mM KCl, 1.5 mM $MgCl_2$, pH 9.0), 2.5 mM dNTPs, 0.5 μM of each primer, 1 unit of SuperTaq DNA polymerase (SuperBio Co, Korea), and 1 μg DNA of whitefly as a template. The mixtures were amplified using a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) with 5 min of initial denaturation at 95°C, 35 cycles of annealing (1 min at 94°C, 1 min at 58°C, 1 min at 72°C), and 10 min of extension at 72°C. The PCR products were visualized on a 1.0% agarose gel containing ethidium bromide. Expected PCR products were excised from the gel and purified using the Wizard PCR preps DNA purification system (Promega, Madison, WI, USA) and sequenced either directly or by cloning into pGEM-T easy plasmid vector (Promega, Madison, WI, USA).

Table 1. Nucleotide sequences of primer sets for identification of *B. tabaci* and its biotypes as well as detection of *Cardinium* and TYLCV.

Obs.	Targeted gene	Primer Sequence (5' to 3')	Size (bp)	References	Anneal. Temp.
B. tabaci	mtCOI	F-TTGATTTTTTGGTCATCCAGAAGT R-TCCAATGCACTAATCTGCCATATTA	~860	Simon *et al*., 1994	52°C
Biotype	16S rRNA	F-CCGGTTTGAACTCAGATCATGT R-CGCCTGTTTAACAAAAACAT	~520	Simon *et al*., 1994	55°C
Cardinium	16S rDNA	F-GCGGTGTAAAATGAGCGTG R-ACCTMTTCTTAACTCAAGCC	~400	Weeks *et al*., 2003	58°C
TYLCV	Coat Protein	F-TGGGGATTCACAAATGTTTTCT R-CTGAACTTCGACAGCCCAT	~1000	Shatters *et al*., 2009	50°C

DNA sequence analysis

Sequences of the PCR products were determined using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and analyzed using a 3730XL DNA Sequencer (Applied Biosystems, Foster City, USA). Databases were searched using the BLAST algorithm (Altschul *et al*., 1997; Schäffer *et al*., 2001) in NCBI, and sequences were aligned using the MUltiple Sequence Comparison by Log-Expectation (MUSCLE) program (Edgar, 2004). The16S ribosomal DNA sequences of *Cardinium* were analyzed using Bayesian MrBayes 3.0 software. Four Metropolises-coupled Markov Chain Monte Carlo (MCMC) chains were run until standard divergence of the split frequencies become lower than 0.01 (Ronquist and Huelsenbeck, 2003). All sequences were analyzed over 10 million generations, and four sequences were sampled every 100 generations. The first 25% of burn-in (SUMP and SUMT) cycles were discarded prior to the construction of consensus tree, which were visualized by MEGA 4.0 (Tamura *et al.*, 2007).

Results

Detection and identification of *Cardinium* in *B. tabaci* populations

To launch the intimate association between *B. tabaci* and infection of its harbored secondary endosymbionts in different biotypes, the presence of *Cardinium* in indigenous whitefly population from Bangladesh, Myanmar, Nepal, and Q biotype of China were examined by PCR amplification of 16S rDNA gene as well as sequences analysis (Table 2). *Cardinium* was detected in all tested whitefly populations regardless of location, but not in B biotype of Korea and Q biotype of China (Table 2).

Cardinium detection in other whiteflies

We collected many other genera of whiteflies from Korea, Bangladesh, Myanmar, Nepal, New Zealand, Japan, and the Philippines. We found that infection of *Cardinium* in spiraling whitefly (*Aleurodicus dispersus*) and greenhouse whitefly (*Trialeurodes vaporariorum*) was very rare (Table 3). On the other hand, *Cardinium* infection was apparently absent in examined acacia whitefly (*Tetraleurodes acaciae*) population (Table 3).

Table 2. Profile of secondary endosymbiotic bacteria in different biotypes of *B. tabaci* on different host plants.

Biotype	Host Plants	Locations	TYLCV	*Cardinium*
B	Cucumber	Goyang, Korea	-	-
Q1	Various	Various places, Korea	+/-	+
Q	Tomato	Qingdao, China	-	-
BW1	Bean	Patuakhali, Bangladesh	-	+
BW1	Bean	Patuakhali, Bangladesh	+	+
BW1	Eggplant	Patuakhali, Bangladesh	-	+
BW1	Eggplant	Patuakhali, Bangladesh	-	+
BW2	Eggplant	Kurigram, Bangladesh	+	+
BW2	Eggplant	Kurigram, Bangladesh	-	+
BW2	Okra	Kurigram, Bangladesh	-	+
Indigenous	Eggplant	Kyuktan, Myanmar	+	+
Indigenous	Ridge gourd	Kyuktan, Myanmar	+	+
Indigenous	Ridge gourd	Magway, Myanmar	-	+
Indigenous	Eggplant	Yangon, Myanmar	-	+
Indigenous	Marigold	Kathmandu, Nepal	-	+
Indigenous	Chili (Pepper)	Kathmandu, Nepal	-	+
Indigenous	Cucumber	Kathmandu, Nepal	-	+
Indigenous	Brinjal	Kathmandu, Nepal	+	+
Indigenous	Tomato	Kathmandu, Nepal	+	+

Present (+), Absent (-).

Table 3. Profile of secondary endosymbiotic bacteria in different foreign whiteflies on various host plants from different countries.

Species	Host plants	Locations	*Cardinium*
Trialeurodes vaporariorum	Tomato	Euiseong, Korea	+
T. vaporariorum	Tomato	Gimcheon, Korea	+
T. vaporariorum	Cucumber	Sangju, Korea	+
T. vaporariorum	Tomato	Kathmandu, Nepal	+
T. vaporariorum	Brinjal	Kathmandu, Nepal	+
T. vaporariorum	Unknown	Japan	-
T. vaporariorum	Unknown	New Zealand	-
Tetraleurodes acaciae	Unknown	Calamba, Philippines	-
Aleurodicus dispersus	Eggplant	Patuakhali, Bangladesh	-
A. dispersus	Guava	Dumki, Bangladesh	-
A. dispersus	Guava	Magway, Myanmar	+
A. dispersus	Unknown	Calamba, Philippines	+
A. dispersus	Unknown	Calamba, Philippines	+

Present (+), Absent (-)

Phylogenetic analysis of *Cardinium*

Neighbour-joining phylogenetic tree reconstructed based on nineteen 16S rDNA sequences of *Cardinium*, which was detected in *B. tabaci*, *T. vaporariorum*, and *A. dispersus* from various countries, is shown in Table 2 and 3. The results showed that the distribution of *Cardinium* endosymbiont was highly diverse due to host and geographical variations. We observed high genetic variance among the 16S rDNA sequences of *Cardinium* from different countries (Fig. 1) and established four distinct clades, namely C1, C2, C3 and C4. Diversified endosymbiont identification for *Cardinium* was performed by sequencing the PCR products of individuals originating from Bangladesh, Myanmar, Nepal, the Philippines, and Republic of Korea (Fig. 1).

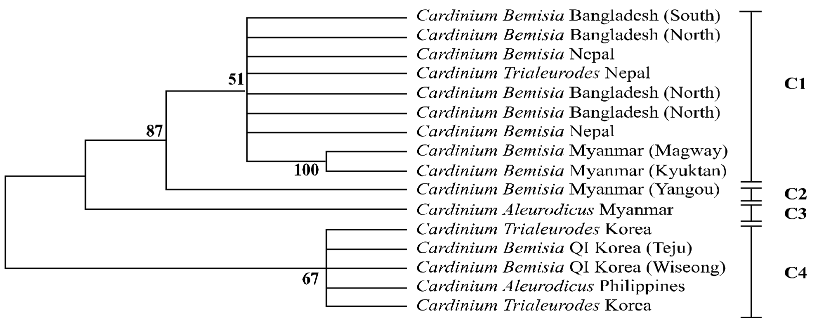

Figure 1. Phylogenetic relationship of 16S rDNA sequences of *Cardinium* in *B. tabaci* were compared with other *Cardinium* in different whitefly. According to the Bayesian method, the neighbor-joining (NJ) tree based on a fragment (~400 bp) using Kimura 2-parameter distances with complete deletion of gap/missing data, by partial 16S rDNA sequences. The number on each branch is the bootstrap support (1,000 replicates).

Analysis of 16S rDNA gene sequences of *Cardinium*

Sixteen sequences of 16S rDNA gene of *Cardinium* in different whiteflies from different countries were analyzed. These sequences had shown the proportions of A+T and G+C in residues composition 48.4% and 51.6% respectively. The average proportion of T: C: A: G was 22.4: 21.4: 25.9: 30.2 with a narrow standard error around means, but base composition varied substantially in different portions within the sequences of species. Among these 311 bp nucleotide, 249 characters were conserved, 62 characters were variable and 30 characters were singleton for parsimony analysis (Table 5). The sequence divergence in pairwise comparisons revealed that *Cardinium* is also very diverse group of endosymbiont like *Arsenophonus* ie. much divergence make 4 clades (C1 – C4) in phylogenetic tree where number of nucleotide changed 1-50 from each other, and distance of value was lowest 0.003 and highest 0.182 among all examined *Cardinium* sequences. *Cardinium* in *B. tabaci* from Bangladesh (BW1 and BW2), Myanmar (Magway and Kyuktan), Nepal and also from *T. vaporariorum* from Nepal make a single clade C1 in the tree (Figs. 1 and 2), *Cardinium* in *B. tabaci* from Myanmar (Yangon) makes another clade C2, *A. dispersus* from Myanmar (Magway) makes C3, and the 4th clade C4 makes by *Cardinium* in Q1 biotype of *B. tabaci* from Korea, *T. vaporariorum* from Korea and *A. dispersus* from the Philippines (Fig. 1 and Table 4). The genetic relationship among *Cardinium* sequences were extracted from neighbor-joining method (NJ). Analysis ran with Kimura's 2-parameter distance model using the Mega 4 program. The inferred phylogenetic topology based on NJ tree was diversified to each other. Result showed that *Cardinium* is divergent due to geographical barrier not depends on host. *Cardinium* makes same clade even though harbored in different species (different hosts).

(*Cardinium* in *B. tabaci* from Nepal (1 and 6), Korea (4 and 8), Myanmar (5, 7 and 16), Bangladesh (11, 13, 14 and 15); in *Aleurodicus dispersus* from Myanmar (2), the Philippines (3); in *Trialeurodes vaporariorum* from Korea (9 and 12), Nepal (10)). Distance between 16S rDNA gene for *Cardinium* (below diagonal: total nucleotide differences, above diagonal: mean character differences) using Kimura 2-parameter.

```
Indigenous Bangla     TGGAAGGTCCCCCACACTGGCACTGAGATACGGGCCAGACTCCTACGGGAGGCAGCAGTA   60
Indigenous Myanmar    TGGAAGGTCCCCCACACTGGCACTGAGATACGGGCCAGACTCCTACGGGAGGCAGCAGTA   60
Indigenous Nepal      TGGAAGGTCCCCCACACTGGCACTGAGATACGGGCCAGACTCCTACGGGAGGCAGCAGTA   60
Q-biotype  Korea      TGGAAGATCCCCCACACTGGCACTGAGATACGGGCCAGACTCCTACGGGAGGCAGCAGTA   60
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Indigenous Bangla     GGGAATATTGGTCAATGGGCGCAAGCCTGAACCAGCCATGCCGCGTGCAGGATGAAGGCT   120
Indigenous Myanmar    GGGAATATTGGTCAATGGGCGCAAGCCTGAACCAGCCATGCCGCGTGCAGGATGAAGGCT   120
Indigenous Nepal      GGGAATATTGGTCAATGGGCGCAAGCCTGAACCAGCCATGCCGCGTGCAGGATGAAGGCT   120
Q-biotype  Korea      GGGAATATTGGTCAATGGGCGAAAGCCTGAACCAGCCATGCCGCGTGCAGGATGACAGCT   120
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Indigenous Bangla     CTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAAAAATCCCTGCGGGGGTTCTTGAGA   180
Indigenous Myanmar    CTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAAAAATCCCTGCGGGGGTTCTTGAGA   180
Indigenous Nepal      CTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAAAAATCCCTGCGGGGGTTCTTGAGA   180
Q-biotype  Korea      CTCTGAGTTGTAAACTGCTTTTGTACAGGAGCAAAACAATCCCTGCGGGGGTTCTTGAGA   180
                      ************************************ ***********************

Indigenous Bangla     GTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGGAGGTG   240
Indigenous Myanmar    GTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGGAGGTG   240
Indigenous Nepal      GTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGGAGGTG   240
Q-biotype  Korea      GTACTGTAAGAATAAGCACCGGCTAATTCCGTGCCAGCAGCCGCGGTAATACGGAAGGTG   240
                      ****************************************************** *****

Indigenous Bangla     CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTG   300
Indigenous Myanmar    CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTG   300
Indigenous Nepal      CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTG   300
Q-biotype  Korea      CAAGCGTTATCCGGTTTTATTGGGTTTAAAGGGTGCGTAGGCGGCTTATTAAGTCAGTTG   300
                      ************************************************************

Indigenous Bangla     TGAAATCCTAGTGCTTAACGCTAGAACT   328
Indigenous Myanmar    TGAAATCCTAGTGCTTAACGCTAGAACT   328
Indigenous Nepal      TGAAATCCTAGTGCTTAACGCTAGAACT   328
Q-biotype  Korea      TGAAATCCTAGTGCTTAACGCTAGAACT   328
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Fig. 2. Sequence alignments of *Cardinium* in different biotypes of *B. tabaci* from different countries using partial 16S rDNA gene sequences (5'-3') by the ClustalW2 program.

Table 4. Pairwise distance among 16 *Cardinium* endosymbiont in various whiteflies from different countries based on sequences of the fragment of 16S rDNA gene.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16]
[1]		0.020	0.108	0.020	0.085	0.000	0.085	0.043	0.023	0.000	0.000	0.020	0.000	0.000	0.003	0.010
[2]	6		0.093	0.020	0.104	0.020	0.104	0.043	0.020	0.020	0.020	0.020	0.020	0.020	0.023	0.023
[3]	31	27		0.086	0.182	0.108	0.182	0.108	0.086	0.108	0.108	0.086	0.108	0.108	0.111	0.111
[4]	6	6	25		0.100	0.020	0.100	0.023	0.003	0.020	0.020	0.000	0.020	0.020	0.023	0.023
[5]	25	30	50	29		0.085	0.000	0.126	0.100	0.085	0.085	0.100	0.085	0.085	0.089	0.096
[6]	0	6	31	6	25		0.085	0.043	0.023	0.000	0.000	0.020	0.000	0.000	0.003	0.010
[7]	25	30	50	29	0	25		0.126	0.100	0.085	0.085	0.100	0.085	0.085	0.089	0.096
[8]	13	13	31	7	36	13	36		0.026	0.043	0.043	0.023	0.043	0.043	0.047	0.047
[9]	7	6	25	1	29	7	29	8		0.023	0.023	0.023	0.023	0.023	0.026	0.026
[10]	0	6	31	6	25	0	25	13	7		0.000	0.020	0.000	0.000	0.003	0.010
[11]	0	6	31	6	25	0	25	13	7	0		0.020	0.000	0.000	0.003	0.010
[12]	6	6	25	0	29	6	29	7	1	6	6		0.020	0.020	0.023	0.023
[13]	0	6	31	6	25	0	25	13	7	0	0	6		0.000	0.003	0.010
[14]	0	6	31	6	25	0	25	13	7	0	0	6	0		0.003	0.010
[15]	1	7	32	7	26	1	26	14	8	1	1	7	1	1		0.013
[16]	3	7	32	7	28	3	28	14	8	3	3	7	3	3	4	

Table 5. Percentage of nucleotide frequencies in variable DNA sites of *Cardinium* endosymbiont in various whiteflies from different countries based on sequences of the fragment of 16S rDNA gene.

Cardinium	Nucleotide composition (%)				Conserved sites (%) (249/311)				Variable sites (%) (62/311)				Singleton sites (%) (30/311)				Total
	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G	
Bt-N2	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Ad-M	22.5	21.2	26.4	29.9	21.7	19.7	27.7	30.9	25.8	27.4	21.0	25.8	33.3	26.7	26.7	13.3	311
Ad-P	23.9	21.9	25.5	28.7	21.7	19.7	27.7	30.9	32.8	31.1	16.4	19.7	43.3	33.3	13.3	10.0	310
Bt-K1	22.5	21.9	25.7	29.9	21.7	19.7	27.7	30.9	25.8	30.6	17.7	25.8	33.3	30.0	23.3	13.3	311
Bt-M16	21.2	19.9	29.6	29.3	21.7	19.7	27.7	30.9	19.4	21.0	37.1	22.6	33.3	30.0	23.3	13.3	311
Bt-N7	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Bt-M17	21.2	19.9	29.6	29.3	21.7	19.7	27.7	30.9	19.4	21.0	37.1	22.6	33.3	30.0	23.3	13.3	311
Bt-K2	22.5	21.9	25.7	29.9	21.7	19.7	27.7	30.9	25.8	30.6	17.7	25.8	33.3	30.0	23.3	13.3	311
Tv-K1	22.8	21.5	25.7	29.9	21.7	19.7	27.7	30.9	27.4	29.0	17.7	25.8	33.3	30.0	23.3	13.3	311
Tv-N	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Bt-BW1	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Tv-K2	22.5	21.9	25.7	29.9	21.7	19.7	27.7	30.9	25.8	30.6	17.7	25.8	33.3	30.0	23.3	13.3	311
Bt-BW2	22.5	21.5	25.1	30.9	21.7	19.7	27.7	30.9	25.8	29.0	14.5	30.6	33.3	30.0	23.3	13.3	311
Bt-M18	21.9	21.9	25.4	30.9	21.7	19.7	27.7	30.9	22.6	30.6	16.1	30.6	26.7	33.3	23.3	16.7	311
Avg.	22.4	21.4	25.9	30.2	21.7	19.7	27.7	30.9	25.4	28.4	18.8	27.4	33.8	30.0	22.9	13.3	310.9

(*Cardinium* in *B. tabaci* from Nepal (Bt-N2 and Bt-N7), Korea (Bt-K1 and Bt-K2), Myanmar (Bt-M16, Bt-M17 and Bt-M18), Bangladesh (Bt-BW1 and Bt-BW2); in *Aleurodicus dispersus* from Myanmar (Ad-M), the Philippines (Ad-P); in *Trialeurodes vaporariorum* from Korea (Tv-K1 and Tv-K2), Nepal (Tv-N))

Discussion

This study was attempted to identify and analyze *Cardinium* infection in different hosts from Bangladesh, Myanmar, Nepal, Republic of Korea and the Philippines. The presence of *Cardinium* was very consistent among indigenous genetic groups of *B. tabaci*, Q1 biotype of *B. tabaci* and *T. acaciae*, whereas it was rarely present in *T. vaporariorum* from Korea and Nepal and *A. dispersus* from Myanmar and Philippines but not in Bangladesh. In support of our results, high rates of *Cardinium* infection were reported in invasive and indigenous biotypes as well as Q1 biotype of *B. tabaci* from Korea (Jahan *et al*., 2011; Park *et al*., 2012).

The presence of *Cardinium* was not consistent among *B. tabaci* populations. *Cardinium* was absent in *B. tabaci* both B and Q biotype from Israel, whereas it was present in MS and Q1 biotype (Chiel *et al*., 2007). Gueguen *et al*. (2010) also showed that *Cardinium* was present in Q1 and MS biotypes, but not in B, Q2 or Q3 biotype. Our results further show that *Cardinium* infection was very common in all collected indigenous biotype populations from different countries, except for B biotype from Korea.

Our sequence analysis shows that *Cardinium* deviated into four genotypic groups which clustered in individual clades, each having a different host. Recently, a high level of genetic diversity was reported for *Wolbachia*, with 36 unique strains detected by Ros *et al*. (2012) and using sequences analysis Jahan and Lee, (2012) reported that 4 monophyletic clades of endosymbiont *Wolbachia* found in examined whitefly population. Similarly, 19 allelic profiles and six phylogenetic groups were obtained for the endosymbiont *Arsenophonus* among 152 individuals, demonstrating this bacterium's high diversity (Mouton *et al*., 2012). *Wolbachia* and *Cardinium* have been found to co-infect the same host species (Duron *et al*., 2008).

Conclusion

The present study shows that all of the *B. tabaci* population collected from different host plants in Bangladesh, Myanmar, and Nepal were infected by *Cardinium,* whereas the B biotype from Korea and Q biotype population from China was not infected. According to the 16S rDNA sequence analysis, we identified four phylogenetic clades, illustrating the divergence of *Cardinium* endosymbiont.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 137-151, March 2015

FIELD PERFORMANCE AND FRUIT QUALITY OF STRAWBERRY GENOTYPES UNDER SUBTROPICAL CLIMATE

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Abstract

Thirteen strawberry genotypes collected from different sources were evaluated at the Fruit Research Field of Pomology Division, HRC, BARI, Gazipur, Bangladesh during the winter season of 2009-2010 and 2010-2011 for yield, yield contributing characters and nutrient components of fruit. Among the 13 genotypes studied, the plants of FA 005 produced the maximum number of fruits (43.50 plant^{-1}) followed by FA 006 and FA 007 (37.50 plant^{-1}), while FA 009 and FA 013 produced the minimum number of fruits (9.00 plant^{-1}). The heaviest fruits were produced by FA 006 (18.73 g) followed by FA 007 (17.40 g) and FA 005 (16.96 g) which were statistically similar, while the lightest fruit was found in FA 014 (5.11 g). The fruit yield plant^{-1} of different genotypes varied from 52.00 to 737.70 g and plants of FA 005 produced the maximum yield followed by FA 006 (702.30 g plant^{-1}) and these were significantly higher than those of others. The minimum yield plant^{-1} was recorded in FA 013 and FA 014. The TSS content was highest in FA 007 (8.50 %) followed by FA 017 (8.17 %), whereas the lowest was in FA 009 (6.33 %). The TSS to acid ration was maximum in FA 006 (11.32) followed by FA 017 (11.24), FA 007 (10.80) and FA 005 (10.62), while the lowest was in FA 011 (6.95). The sugar to acid ratio significantly ranging from 3.60 to 5.98, and it was maximum in FA 006 (5.98). Plants of FA 005 contained the maximum amount of ascorbic acid (77.33 mg 100g^{-1}) followed by FA 006 (76.00 mg 100g^{-1}), while the minimum in FA 010 (53.00 mg 100g^{-1}).

Keyword: Strawberry, field performance, yield, fruit quality

Introduction

Strawberries (*Fragaria x ananassa* Duch) is a highly appreciated fruit for its excellent flavour, wonderful taste, attractive colour, high nutrient profile and cosmic medicinal value. Strawberry is now grown successfully in Bangladesh. It was demonstrated that genotype is the main source of variation and single most important factor that influenced the growth, yield and quality of fruit. Today's strawberry

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comprises about 500 commercial cultivars grown worldwide (Galletta and Maas, 1990; Hancock, 1999). Hence, a germplasm pool with good variability for the desirable characters is the basic requirement of any crop improvement program (Singhania *et al*., 2006). On the other hand, crop improvement is primarily dependent on extensive evaluation of available genotypes. Therefore, the choice of a cultivar is of paramount importance for successful strawberry cultivation (Asrey and Singh, 2004). As a new crop it is necessary to evaluate the yield, yield contributing and quality characters of fruits under Bangladesh condition. Yield and yield contributing characters is the ultimate goal of any crop production and the fruit quality and nutrient components is the basic requirement of a crop. Hence, studies about these important traits are necessary for successful cultivation of this crop in a new area like Bangladesh.

Bangladesh Agricultural Research Institute (BARI) has released one variety of strawberry namely BARI Strawberry-1, which is not enough for increasing demand of strawberry cultivation. So it is necessary to develop more variety of this promising crop with higher yield, fruit quality and nutrient profile. There are very little studies done on the performance regarding yield, yield contributing, fruit quality and nutrient components of strawberry in Bangladesh. However, a good number of investigations were done in USA, India, UK, Thailand, Turkey and elsewhere in the world. Therefore, the present study was undertaken to evaluate the collected strawberry genotypes for fruit, yield, yield contributing characters, quality parameters and nutritional components of fruits and to select superior ones.

Materials and Method

The present study was carried out at the Fruit Research Farm of Horticulture Research Centre, Bangladesh Agricultural Research Institute (Latitude $23^{0}59'$ N, Longitude $90^{0}24'$ E, Altitude 14.33 m), Gazipur, Bangladesh during winter season of 2009-2010 and 2010-2011. This region falls in sub-tropical zone having hot summers (May–August) and mild winter (December–February). Cumulative rainfall of about 108 mm during cultivation period with average 79.4 % relative humidity. The mean maximum and minimum temperature during cropping period were 25.89 and 17.05^{0}C, respectively. Soil of the experimental farm was clay loam, having pH 6.2 (slightly acidic), which was low in organic carbon (0.95 %), very low in available phosphorus (9 ppm) and low in potash (0.17 meq/100 g soil).

Healthy and disease free propagules of ‘thirteen strawberry genotypes collected from local and exotic source were considered as treatment and planted in the experiment field the only released strawberry variety namely BARI Strawberry-1 used as check.

The experiment was laid out in randomized complete block (RCB) design with three replications. The unit plot size was 100 x 280 cm and the plants were spaced 50 x 40 cm on open beds. Beds were raised 30 cm above main field with 50 cm drain in-between 2 beds. Each plot contains double row accommodating 14 plants. Daughter plants of strawberry genotypes were planted on 15 October, 2009 and 2010. Data were collected from inner plants from each row to avoid border effect. In each unit plot ten inner plants were selected for recording data.

Runners were removed at every 3 to 4 days intervals in order to make the crown capable to initiate flowers. Straw mulch was applied around the plants as a normal practice in order to conserve soil moisture, decreasing weed and to provide healthy condition for the fruits. Weeds were removed at 15 days interval up to final harvest to keep the crop weed free. Irrigation was given at 7 days interval and whenever necessary to keep the soil moisture available in the field for better plant growth. All other necessary cultural practices and plant protection measures were followed uniformly for all the plots and treatments during the entire period of experimentation. Fruits were usually harvested by hand picking during early in the day when environment was cool, at an interval of 3 to 4 days and handled very carefully. The fruits were harvested at commercial maturity when >80% of the fruit surface showing red colour. Immediately after harvest, strawberries were sorted to eliminate damaged fruit, and selected for uniform size and colour for collecting data.

The following qualitative characters of fruit viz. shape, uniformity, colour, firmness and flavour were recorded by close observation according to the descriptors for strawberry (IBPGR, 1986). On the other hand, number of fruits plant^{-1}, fruit weight (g), fruit length (cm), fruit diameter (cm), yield plant^{-1} (g), days to harvest, harvest duration, deform fruits (%) and number of achenes fruit^{-1} were recorded for quantitative fruit characters. Following chemical and nutrient components were recorded viz. reducing sugar, non-reducing sugar, total sugar, total soluble solid (TSS), pH, titratable acidity, TSS to acid ratio, sugar to acid ratio and ascorbic acid contents of fruits**.**

Two year’s data of different quantitative parameters were pooled and analyzed, following RCB design using MSTAT-C program. The mean comparison was done following the Duncan’s Multiple Range Test (DMRT).

Results and Discussion

Qualitative characters of fruits

Qualitative characters of different strawberry genotypes was studied and shown in Table 1. Fruit shape of different strawberry genotypes was classified into 6 shapes.

Plants of FA 001, BARI Strawberry-1, FA 008 and FA 011 produced conical shaped fruits. While fruits of FA 007 and FA 017 were long conical shaped. On the other hand, FA 006 and FA 016 produced wedged shaped fruits. Fruits of FA 009 and FA 013 was oblate shaped, and that of FA 010 and FA 014 was round shaped and FA 005 was cordate shaped. This findings are similar to that of Jamieson (2003) and Rahman and Ahmad (2009). Uniformity of fruits of different strawberry genotypes was studied and categorized into high, intermediate and low grade. Genotypes of FA 005, FA 006, FA 007, FA 016 and FA 017 produced highly uniform fruits. While fruits of BARI Strawberry-1, FA 008, FA 011 and FA 013 were intermediate in fruit uniformity. Rest of the genotypes produced fruits having low uniformity. Colour of fruits of different strawberry genotypes was studied and classified into light, intermediate, dark and very dark category. Fruits of FA 009, FA 010, FA 013 and FA 014 had light colour. While those of FA 001, FA 008 and FA 011 were intermediate in colour, BARI Strawberry-1, FA 006 and FA 016 had dark, FA 005, FA 007 and FA 017 had very dark coloured fruis.

Table 1. Qualitative characters of fruits in strawberry genotypes.

Genotypes	Shape of fruits	Uniformity of fruits	Colour of fruits	Fruit firmness	Flavour of fruits
FA 001	Conical	Low	Intermediate	Very soft	Slight
FA 005	Cordate	High	Very Dark	Intermediate	Excellent
FA 006	Wedged	High	Dark	Firm	Excellent
FA 007	Long Conic	High	Very dark	Very Firm	Excellent
FA 008	Conical	Intermediate	Intermediate	Very soft	Excellent
FA 009	Oblate	Low	Light	Soft	Poor
FA 010	Round	Low	Light	Soft	Good
FA 011	Conical	Intermediate	Intermediate	Very Soft	Good
FA 013	Oblate	Intermediate	Light	Very Soft	Poor
FA 014	Round	Low	Light	Intermediate	Slight
FA 016	Wedged	High	Dark	Firm	Excellent
FA 017	Long Conic	High	Very dark	Very Firm	Excellent
BARI Strawberry-1	Conical	Intermediate	Dark	Soft	Good

Fruit firmness of different strawberry genotypes was classified into 5 categories viz. very soft, soft intermediate, firm and very firm. Among the genotypes, FA 001, FA 008, FA 011 and FA 013 produced very soft fruits. Whereas fruits of BARI Strawberry-1, FA 009 and FA 010 were soft textured. On the other hand, fruits of FA 005 and FA 014 were intermediate textured. Fruits of FA 006 and FA 016 were firm and those of FA 007 and FA 017 were very firm in texture. Zhang *et al.* (2010) found that fruits of 'Shu Xiang' were very firm in texture. Fruit flavour was classified into 4 group viz. absent, poor, slight, good and excellent. Among the genotypes FA 009 and FA 013 produced fruits with poor in flavour, FA 001 and FA 014 were of slight in flavour. While fruits of BARI Strawberry-1, FA 010 and FA 011 were good in flavour, and FA 005, FA 006, FA 007, FA 008, FA 016 and FA 017 produced fruits with excellent flavour. Rahman and Ahmad (2009) stated that fruit flavour of different strawberry lines distinguishably differed and varied from poor to excellent. Similar fruit flavour was observed in the present investigation.

Yield and yield contributing characters

Average number of fruits plant^{-1} exhibited wide range of variation among the genotypes (Table. 2). The highest number of fruits (43.50) was harvested from FA 005 distantly followed by BARI Strawberry-1 (39.00), FA 017 (38.51), FA 006 (37.50), FA 016 (37.50), FA 008 (37.50) and FA 007 (36.00). The lowest number of fruits (9.00) was produced by the genotype FA 009 and FA 013. Asrey and Singh (2004) found a significant variation in fruits plant^{-1} among the cultivars ranging from 25.33 to 40.66. This result is in conformity with the present finding. Lutchoomun and Cangy (1997) found that strawberry cv. 'Marquise' produced the highest number of fruits plant^{-1} (69) followed by 'Mara des bois' (62), while single plant of cv. 'Selva' produced only 31 fruits which was higher than present observation. The variation in fruits plant^{-1} is due to inherent capability of the genotypes, and also influences of growing environment. The genotype significantly influenced the fruit weight (Table.2). The heaviest fruits were produced by FA 006 (18.73 g) closely followed by FA 007 (17.40 g) and FA 005 (16.96 g), while the lightest fruit was found in FA 014 (5.11 g). Fruit weight recorded in the present experiment was strongly similar to the findings of Crespo (2010), who stated that cv. Asia produced heaviest fruit (26.4 g) and cv. Antea produced the lightest fruits (14.8 g). Present findings are also confirmed with the previous reports of Cordenunsi *et al*. (2002) and Asrey and Singh (2004). They found that single fruit weight of strawberry varied significantly among the studied genotypes. In respect of length and diameter all the genotypes varied significantly probably due to the inherent characters of genotype (Table 2). The longest fruits were produced by FA 007 (5.10 cm) followed by FA 005 (4.50 cm), FA 017 (4.50 cm) and FA 006 (4.40 cm) and the shortest fruits were produced by FA-014 (1.30 cm). The thickest fruit was found in FA 006 (4.20 cm) followed by FA 005 (4.10 cm) and FA 007 (3.80 cm). The thinnest fruit was produced by FA 013 (1.30 cm). This result fully agrees with the previous findings of Asrey and Singh (2004) and Cordenunsi *et al*. (2002).

Asrey and Singh (2004) observed that the size of strawberry fruits among the genotypes varied significantly and fruit length of different strawberry cultivar ranged from 3.49 to 4.21 cm and fruit width ranged from 2.91 to 3.40 cm, respectively.

Table 2. Yield and yield contributing characters in strawberry genotypes

Genotypes	Fruits plant^{-1} (No.)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)
FA 001	25.50 c	9.95 ef	3.40 d	2.50 de
FA 005	43.50 a	16.96 ab	4.50 ab	4.10 ab
FA 006	37.50 b	18.73 a	4.40 ab	4.20 a
FA 007	36.00 b	17.40 ab	5.10 a	3.80 a-c
FA 008	37.50 b	12.07 de	3.90 b-d	3.30 bc
FA 009	9.00 f	9.04 fg	2.20 e	2.20 e
FA 010	16.50 e	7.35 gh	2.00 e	2.20 e
FA 011	21.00 d	7.14 gh	2.10 e	2.10 e
FA 013	9.00 f	5.78 h	1.50 ef	1.30 f
FA 014	13.50 ef	5.11 h	1.30 f	1.70 ef
FA 016	37.50 b	15.33 bc	4.20 bc	3.77 a-c
FA 017	38.51 b	14.47 c	4.50 ab	3.63 a-c
BARI Strawberry-1	39.00 b	13.35 cd	3.63 cd	3.20 cd
CV (%)	6.78	8.61	8.92	11.28

Figures having the same letter(s) in a column do not differ significantly by DMRT at 1 % level of significant.

The analysis of variance indicated a high degree of variation among the genotypes in yield plant^{-1} (Fig. 1). The highest yield plant^{-1} was recorded from the genotype FA 005 (737.70 g) followed by FA 006 (702.30 g) and was significantly higher than others. The lowest yield plant^{-1} was recorded from FA 013 (52.00 g). The variation in yield plant^{-1} was might be due to the inherent character of the genotypes. This was in agreement with the findings of following investigations. From different experiments Lutchoomun and Cangy (1997) and Crespo (2010) stated that fruit yield plant^{-1} in strawberry varied significantly among the cultivars studied ranging from 179.00 to 312.40 g and 386.00 to 624.00 g plant^{-1}, respectively.

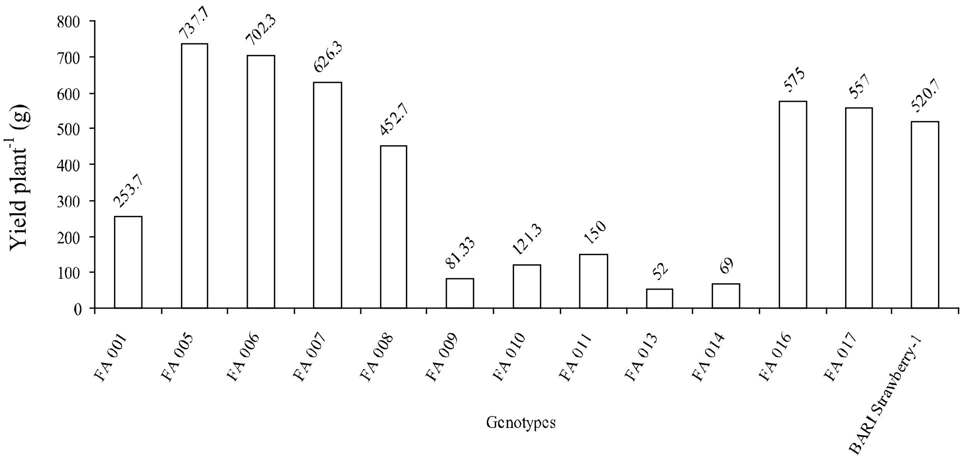

Fig. 1. Yield plant^{-1} in different strawberry genotypes.

The analysis of variance indicates a high degree of variation among the genotypes in days to harvest (Table 3). The genotypes, FA 011 required the minimum days (25.37) for attaining full maturity to harvest from flowering preceded by FA 013 (27.33 days), FA 010 (27.33 days), FA 009 (27.67 days) and FA 014 (28.67). While genotypes FA 007 required maximum days (35.67) from flowering to harvesting. This is in line with the findings of Klein and Perry (1982), who stated that strawberry usually, takes more or less 30 days to achieve full size and maturity of fruits. Anon. (2010) noted that fruits of strawberry matured rapidly, ripening occurs in 20 to 50 days after pollination. Harvest duration is an important character and it indicates a period during which a genotype is able to produce fruits. The longest harvest duration was observed in the genotype FA 007 (94 days) followed by FA 006 (93.67 days) and FA 005 (92.00 days). While, FA 014 had the shortest harvest duration (70.67 days) (Table 3). As a consequence the source-sink relation varies during the harvest and may affect the fruits composition. A significant variation among the strawberry cultivars in harvest duration under conventional system was observed by Macit *et al*. (2007), which is similar with present result.

The analysis of variance indicates a high degree of variation among the genotypes in yield plant^{-1} (Table 3). Among the genotypes, FA 001 produced minimum (10.33 %) deformed fruits preceded by FA 008 and FA 010 (12.33 %), while FA 014 produced maximum (26.00 %) deformed fruits. There was a remarkable variation among the genotypes of strawberry in number of achenes fruit^{-1} (Table 3) and it ranged from 186.70 to 473.30. The highest number of achenes fruit^{-1} was observed in FA 007 (473.30) closely followed by FA 006 (471.70), FA 017 (460.70), FA 016 (460.30) and FA 005 (441.70). The lowest number of achenes fruit^{-1} was found in

FA 013 (186.70). Hansen (1989) stated that strawberry fruits differed in fruit weight and achenes number fruit^{-1}.

Table 3. Days to harvest, harvest duration, percentage of deform fruits and number of achenes fruit^{-1} in strawberry genotypes.

Genotypes	Days to harvesting	Harvest duration	Percentage of deform fruits	Achenes fruit^{-1}
FA 001	29.67 de	88.67 ab	10.33 e	272.30 bc
FA 005	33.33 a-c	92.00 a	21.00 bc	441.70 a
FA 006	34.33 ab	93.67 a	23.33 ab	471.70 a
FA 007	35.67 a	94.00 a	24.33 ab	473.30 a
FA 008	29.67 de	90.00 ab	12.33 e	315.00 b
FA 009	27.67 ef	89.00 ab	16.33 d	276.70 bc
FA 010	27.33 ef	90.00 ab	12.33 e	221.70 cd
FA 011	25.67 f	85.00 b	19.33 cd	210.30 cd
FA 013	27.33 ef	76.00 c	24.33 ab	186.70 d
FA 014	28.67 ef	70.67 c	26.00 a	191.70 d
FA 016	30.33 c-e	90.67 ab	23.00 ab	460.30 a
FA 017	32.00 b-d	89.00 ab	23.67 ab	460.70 a
BARI Strawberry-1	29.33 de	88.67 ab	16.00 d	334.70 b
CV (%)	6.34	3.05	7.51	8.42

Figures having the same letter(s) in a column do not differ significantly by DMRT at 1 % level of significant.

Relationship between number of achenes fruit^{-1} and fruit weight

A positive linear relationship was observed between number of achenes fruit^{-1} and fruit weight (g). The equation was y = 0.0393x - 1.3015 with highly significant value of coefficient of determination (R^2 = 0.9396**) shown in Fig. 2. This regression line coupled with a significant regression coefficient indicated that fruit weight would be increased with a significant manner with the increase in number of achenes fruit^{-1}. So, there is a clear indication that the genotypes having more achenes produced larger fruits. This result corroborates the statement of Darnell (2003) who stated that number of achenes fruit^{-1} positively increased the size and weight of fruit. Annon. (2010) stated that achenes number positively correlated with fruit size and differed significantly among the cultivars, which agreed with the present investigation. Fertile achenes produce auxins that trigger the growth of receptacles during fruit ripening (Nitsch, 1950; Kronenberg, 1959). When achenes are unfertilized, the development of the area on the fruit around the achenes is inhibited, resulting in malformation of the whole fruit (Ledesma *et al*., 2008).

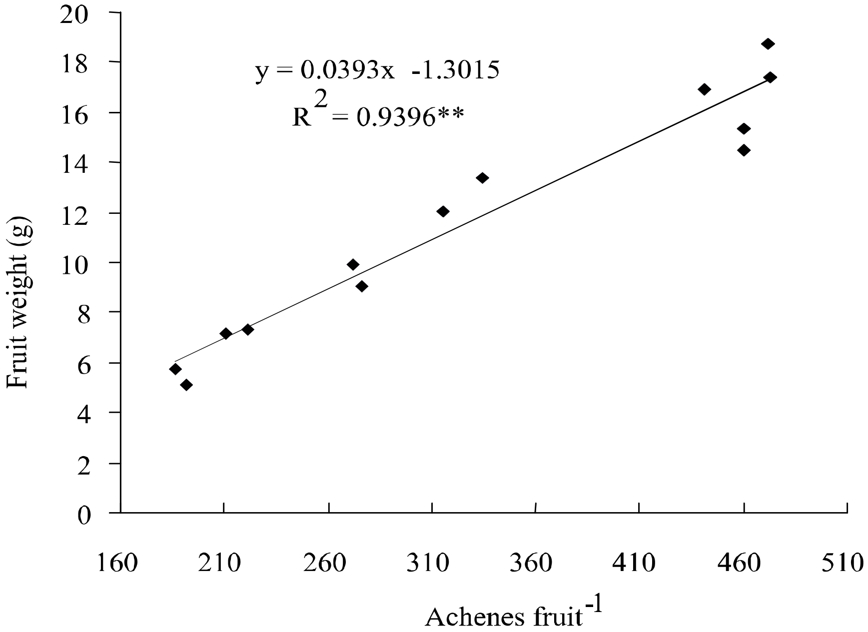

Fig. 2. Relationship between achenes fruit^{-1} and single fruit weight in strawberry.

Chemical and nutrient components

Highly significant variation was observed in reducing sugar content in fruits of different genotypes (Table 4). The highest quantity of reducing sugar was obtained from FA 006 (2.98 %) closely followed by FA 005 (2.95 %), FA 007 (2.94 %), FA 001 (2.91 %), FA 017 (2.91 %), BARI Strawberry-1 (2.89 %). Result of the present investigation is in accord with those of Asrey and Singh (2004), who revealed a significant variation in reducing sugar content of strawberry. Kader (1991) found a large genotypic variation in reducing sugar content which ranged from 3.7 to 5.2 %. This result is higher than that recorded in the present studies probably due to genotypic and environmental variations.

The quantity of non-reducing sugar of strawberry fruit ranging from 1.10 and 1.39 % were statistically non significant.

The component, which affects the taste of the fruit, is the sugar content. The result exhibited highly significant difference in the content of total sugar among the genotypes which ranged from 4.28 % to 3.43 % (Table 4). The highest quantity of total sugar was obtained from FA 005 closely followed by FA 006 (4.23 %), FA

007 (4.20 %), FA 016 (4.13 %), FA 001 (4.10 %), FA 017 (4.10 %) and BARI Strawberry-1 (4.02 %), and the lowest total sugar content was obtained in FA 009 (3.43 %). Total sugar content of strawberry fruits is a heritable trait, and is highly influenced by variability of genotypes. Kader (1991) reported that there is a large genotypic variation in total sugar content of strawberries ranging from 4.10 to 6.60 %. Crespo (2010) stated that total sugar content of strawberry fruits varied among the cultivars and ranged from 40.9 mg g^{-1} (cv. Matis) to 51.8 mg g^{-1} (cv. Asia) which supported the result of present investigation.

Total soluble solids (TSS) of fruits of strawberry genotypes varied significantly and ranging from 8.50 to 6.33 % (Table 4). Among the genotypes, FA 007 contained the highest amount of TSS (8.50 %) closely followed by FA 017 (8.17 %), FA 017 (8.17 %), FA 006 (8.00 %), FA 005 (7.83 %) and FA 016 (7.67 %). The lowest TSS was exhibited by FA 009 (6.33 %). The variation in TSS among the genotypes might be due to the variation of genotypes. Capocasa *et al*. (2008) found that TSS of strawberry fruits varied significantly and rangedfrom 5.8 % to 9.7 % among the cultivars which is in consonance with the present findings. According to Kader (1991) the TSS content of strawberry fruits harvested at commercial ripening stage ranged from 5 to 12 %, depending on cultivar and pre-harvest factors. This is within the range of the present observation. Resende *et al*. (2008) recorded the TSS content of different strawberry cultivars ranging from 7.20 to 8.10 %, which is similar with the present findings. Asrey and Singh (2004) revealed found a strong cultivar variation in TSS which ranged from 4.90 to 7.50 % among the cultivars, while Shaw (1988) reported that this variation was mostly affected by environmental factors.

The pH value of strawberry fruit were statistically non significant and ranged from 3.71 to 3.36. The highest pH value was obtained from in FA 001 while, the lowest in FA 006, and FA 017 (Table 4). Kafkas *et al*. (2007) found that the pH ranging from 3.33–3.43 in the ripe stage of the strawberries but did not change significantly, which is strongly in concur with the present findings.

Titratable acidity (TA) varied significantly among the genotypes (Table 5.). The highest titratable acidity was found in FA 011 (0.960 %) followed by FA 008 (0.923 %), FA 013 (0.920 %) and FA 001 (0.883 %) which were statistically different from others (Table 4). The lowest was found in FA 006 (0.707 %). The titratable acidity and the organic acid content are genetically determined and varied significantly among the genotypes, while less influenced by environment (Shaw, 1988). The result about titratable acidity of present studies agrees with those of

Asrey and Singh (2004), and Resende *et al*. (2008). They reported that the titratable acidity of strawberry fruits varied significantly and ranged from 0.80 to 0.91 % and 0.60 to 0.88 %, respectively among the studied cultivars. Macit *et al*. (2007) found TA of strawberry genotypes varied from 0.34 to 0.41 %, which was lower than present findings and this might be due to differences either in cultivars or in the growing environments.

Table 4. Chemical component and nutrient content of fruits in strawberry.

Genotypes	Reducing sugar (%)	Non reducing sugar (%)	Total sugar (%)	TSS (%)	pH	Titratable acidity (%)
FA 001	2.91 a	1.19	4.10 a-c	6.83 de	3.71	0.883 ab
FA 005	2.95 a	1.13	4.28 a	7.83 a-c	3.63	0.737 de
FA 006	2.98 a	1.34	4.23 a	8.00 ab	3.44	0.707 e
FA 007	2.94 a	1.25	4.20 a	8.50 a	3.36	0.787 c-e
FA 008	2.80 ab	1.26	4.08 a-c	7.17 b-e	3.37	0.923 a
FA 009	2.33 bc	1.29	3.43 d	6.33 e	3.42	0.833 bc
FA 010	2.32 bc	1.10	3.54 cd	6.50 e	3.62	0.777 c-e
FA 011	2.22 c	1.22	3.46 d	6.67 de	3.37	0.960 a
FA 013	2.21 c	1.24	3.60 b-d	6.83 c-e	3.68	0.920 a
FA 014	2.30 bc	1.39	3.44 d	6.67 de	3.41	0.820 bc
FA 016	2.89 a	1.14	4.13 ab	7.67 a-d	3.38	0.780 c-e
FA 017	2.91 a	1.24	4.10 a-c	8.17 a	3.37	0.727 de
BARI Strawberry-1	2.89 a	1.19	4.02 a-c	7.00 c-e	3.36	0.790 cd
CV (%)	7.80	5.55	5.81	5.49	7.35	2.99

Figures having the same letter(s) in a column do not differ significantly by DMRT at 1 % level of significant

Ascorbic acid content of genotypes varied significantly (Fig. 3), and it was found that ascorbic acid content 100 g^{-1} of pulp was the highest in FA 005 (77.33 mg) followed by FA 006 (76.00 mg), while the lowest was found in FA 010 (53.00 mg). Olsson *et al*. (2004) stated that the ascorbic acid content depends on the species and cultivation conditions, which was corroborated with the present investigation. Proteggente *et al*. (2002) recorded 61 mg 100 g^{-1} of ascorbic acid from fresh strawberry, which is within the range of the present observation. This variation in ascorbic acid content might be due to differences in cultivar and climatic condition. According to Lee and Kader (2000), cultivar type can be defined as an important factor affecting ascorbic content of strawberry.

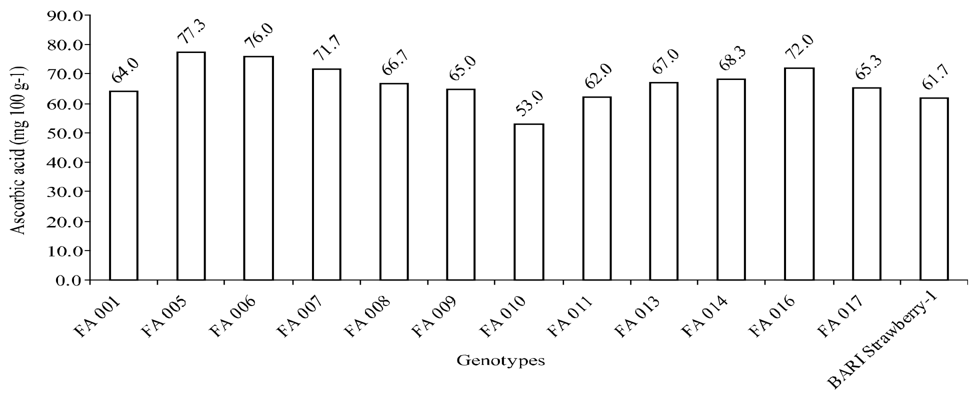

Fig. 3. Ascorbic acid content in fruits of different strawberry genotypes.

The ratio of total soluble solids to acid and sugar to acid is most important for evaluating the taste and determining the maturity of strawberry. According to Kader (1991) high sugar and relatively high acid are required for good flavour. High acid and low sugars produced a tart strawberry, and low acid and high sugar result in a bland taste, while the low sugars and acids, results a tasteless strawberry. The TSS to acid and sugar to acid ratio of fruits of different strawberry genotypes were evaluated and found to be significant (Fig. 4), due to highly significant value of TSS, total sugar and titratable acidity (Table 4). The TSS to acid ratio was found to be highest in FA 006 (11.3) followed by FA 017 (11.2), FA 007 (10.8) and FA 005 (10.6), while the lowest in FA 011 (6.9). The sugar to acid ratio of different genotypes also varied significantly and ranged from 3.6 to 6.0 and followed more or less similar trend of TSS to acid ratio. Among the genotypes, FA 006 exhibited the highest sugar to acid ratio (6.0) followed by FA 005 (5.8) and FA 017 (5.6), while it was lowest in FA 011 (3.6). In an earlier publication, Kafkas *et al.* (2007) reported that the TSS to acid ratio and the sugar to acid ratio in strawberry varied significantly.

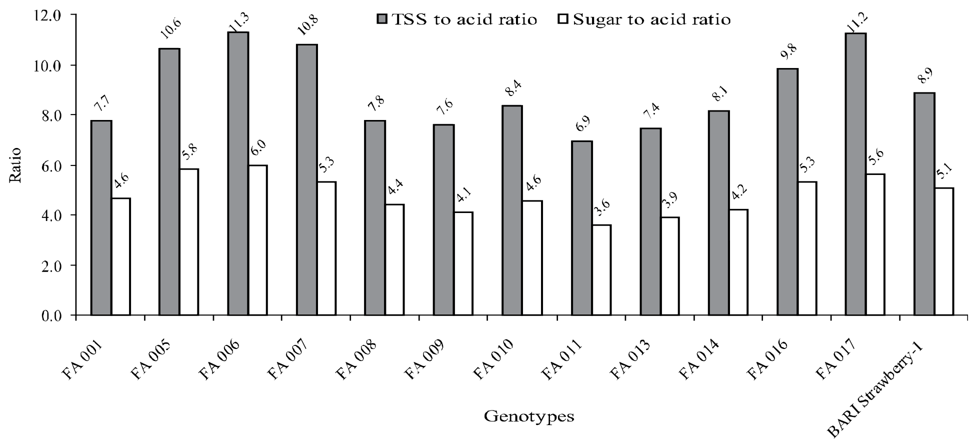

Fig. 4. TSS to acid and sugar to acid ratio in fruits of different strawberry genotypes

Conclusion

The genotypes of strawberries showed remarkable variation in nutrient as well as chemical components of fruit. On the basis of physico-morphological characters, it was concluded that genotype FA 006 and FA 007 were identical with FA 016 and FA 017, respectively. Considering total soluble solids (TSS), sugars, ascorbic acid, TSS to acid and sugar to acid ratio of fruits, the genotypes FA 005, FA 006 and FA 007 were superior to others and found to be promising under Bangladesh condition.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 153-161, March 2015

CORRELATION AND PATH COEFFICIENTS ANALYSES IN BASMATI RICE

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Abstract

Correlation and path coefficients analyses among fourteen morphological characters were studied in six advanced lines of Basmati rice and one commercial check namely BRRI Dhan 29. In general, genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients suggesting that the environmental influence reduces the relationship between yield and yield contributing characters of rice. Correlation coefficient analysis showed significant positive correlation between plant height and panicle length at genotypic level. Number of filled spikelets/panicle showed significant positive correlation with yield at both genotypic and phenotypic levels but significant negative correlation was observed between plant height and yield. Number of effective tillers/plant had negative significant correlation with panicle length and with number of unfilled spikelets/panicle at genotypic level. Number of ineffective tillers/plant had significant negative correlation with 1000-seed weight at both genotypic and phenotypic levels. Path coefficient analysis revealed highest positive direct effect of number of filled spikelets/panicle on grain yield but plant height and number of unfilled spikelets/panicle had negative direct effect on grain.

Keywords: Correlation coefficient, genotypic level, path coefficient, rice, *Oryza sativa.*

Introduction

Rice (*Oryza sativa* L.) belongs to the family Gramineae. It is the staple food for at least 63% of planet inhabitants and contributes on an average 20% of apparent caloric intake of the world population and 30% of population in Asia (Calpe and Prakash, 2007). Over 90 percent of the world's rice is produced and consumed in the Asian Region by six countries (China, India, Indonesia, Bangladesh, Vietnam and Japan) comprising 80% of the world's production and consumption (Abdullah *et al.*, 2006).

Cooked aromatic rice spread fragrance. Aromatic rice is a special group of rice, which is considered best in quality. Among the varieties of aromatic (fine) rice, Basmati rice are the most preferred in the international market and the trade is

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exclusively shared between India and Pakistan. Basmati rices are characterized by superfine grain, pleasant aroma, soft texture and extreme grain elongation with least breadth-wise swelling on cooking (Singh *et al.*, 2000). 'Bas' in Hindi language means "aroma" and 'Mati' means "full of" hence the word Basmati i.e. full of aroma. This rice is different from other rice mainly due to the aroma and elongation post cooking. It has its own unique aroma. This aroma is due to the presence of a chemical called 2-acetyl-1-pyrroline. That is about 12 times more than that of other types of rice. The cooked rice has a soft and flaky texture (Khus and Cruz, 2002). The price of Basmati rice is about 2-3 times higher than the coarse rice (Biswas *et al*., 1992). In spite of low yield potentiality, Basmati rice holds the unique position for its higher demand in the international market. The demand of basmati rice has been increasing in Bangladesh due to increase in per capita income and approaching self-sufficiency in rice production (BRRI, 2004). The climatic conditions of Bangladesh are also suitable to produce quality Basmati rice.

Economic product of rice is the grain yield, which exhibits complex genetics as it is influenced by various yield contributing characters and the environment. In general, increased number of fertile panicles is the single most important yield component associated with rice yield, number of spikelets/panicle; percent filled grains/panicle are also of secondary and tertiary importance (Jones and Synder, 1987). Another trait directly related to panicle is panicle density which chiefly affects the yield potential. These yield contributing components are interrelated with each other showing a complex chain of relationship and also highly influenced by the environmental conditions (Prasad *et al*., 2001). Breeding strategy in rice mainly depends upon the degree of associated characters as well as its magnitude and nature of variation (Zahid *et al*., 2006 and Prasad *et al*., 2001).Therefore, information about the yield contributing traits is of immense importance to the plant breeders for the development of improved varieties/ lines of rice with increased yield potential.

For rational approach towards the improvement of yield and its components, the association of characters with yield and among themselves and the extent of environmental influence on the characters are very much essential. Therefore, the knowledge of association of component characters with yield has great importance to plant breeders, as it helps in their selection with more precision and accuracy. The degree of relationship and association of these components with yield can be measured by correlation coefficients. But selection based on correlation without taking into consideration the interactions between the component characters may sometimes proven misleading (Codawat, 1980). Moreover, it does not give an exact position of the relative importance of direct and indirect effect of the various characters on yield. In path analysis, the correlation coefficient between two traits is separated into the components which measure the direct and indirect effects (Ahmadizadeh *et al*., 2011). Path coefficient analysis provides an exact picture of the relative importance of direct and indirect effects of each of the component character towards yield. With a view to having effective improvement of rice, its character associations as well as the nature and the extent of direct and indirect effects on yield were investigated.

Materials and Method

The study was conducted in the experimental field, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The experimental field belongs to the Agro-ecological zone of "The Modhupur Tract", AEZ-28. Soil pH ranged from 6.0 - 6.6 and had organic matter of 0.84%. Six different advanced lines of Basmati rice namely S1, S2, S5, 42(i), 42(ii), 44(i) collected from Department of Genetics and Plant Breeding from the same University were studied. BRRI Dhan-29 collected from Bangladesh Rice Research Institute was used as check. The experiment was laid out in Randomized Complete Block Design with three replications and with a plot size of 1.5 m × 3.5 m. Recommended cultural practices were followed through the growth period to raise the better crop. The fertilizers N, P, K, S and B in the form of urea, TSP, MOP, Gypsum and Borax, respectively were applied. The entire amount of TSP, MOP, Gypsum and Borax were applied during the final preparation of land. Urea was applied in two equal installments at tillering and panicle initiation stage. Rice seedlings were transplanted in the spacing of 30 cm X 25 cm on January, 2009. Data were recorded on plant height (cm), days to 50% flowering, days to maturity, number of effective tillers/plant, number of ineffective tillers/plant, number of total tillers/plant, panicle length (cm), number of filled spikelet/panicle, number of unfilled spikelets/panicle, number of total spikelets/panicle, weight of 1000-seeds (g), grain yield/plant (g), grain yield/plot (kg). Estimation of phenotypic (r_p) and genotypic (r_g) correlation coefficients were estimated by the formula suggested by Miller *et al*., (1958). The path coefficients were calculated as per the formula given by Dewey and Lu (1959).

Results and Discussion

Correlation studies

Genotypic and phenotypic correlation coefficients between grain yield and other seven characters are presented in Table 1. In general, genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients indicating that there is a strong inherent association between the characters studied. Plant height was correlated negatively and significantly with number of effective tillers/plant, number of ineffective tillers/plant, 1000-seed weight and grain yield at genotypic level. Amirthadevarathinam (1983) also found that grain yield was negatively correlated with plant height. But Bhadru *et al.,* (2011) reported that plant height had a significant positive association with yield which was contradictory with this study. Plant height was significantly and positively correlated with panicle length at both genotypic and phenotypic level. Number of effective tillers/plant was correlated negatively and significantly with panicle length and with number of unfilled spikelets/panicle but positively and significantly correlated with grain yield at genotypic level. Eidi kohnaki *et al.*, (2013), Kiani and Nematzadeh (2012), Akinwale *et al.* (2011), Basavaraja *et al.*, (2011) and Shanthi *et al.*, (2011) reported that productive tillers/plant showed significant positive correlation with grain yield.

Number of ineffective tillers/plant was correlated positively and significantly with 1000-seed weight but negatively and significantly correlated with panicle length at both genotypic and phenotypic levels. Panicle length was correlated negatively and significantly with 1000-seed weight and with grain yield at genotypic level. Similarly negative genotypic correlation of yield/plant was reported with panicle length by Saini and Gagneja (1975). Eidi kohnaki *et al.*, (2013) and Kole *et al.*, (2008) found a significant positive correlation between grain yield with panicle length. Number of filled spikelets/panicle was positively and significantly associated with grain yield at both genotypic and phenotypic levels. Eidi kohnaki *et al.*, (2013), Haider *et al.*, (2012), Kiani and Nematzadeh (2012), Seyoum *et al*., (2012), Akinwale *et al*. (2011), Akhtar *et al*., (2011) and Shanthi *et al.* (2011) reported the positive association of grain yield with filled grains/panicle.

Path coefficient analysis

Path coefficient analysis shown in table 2 revealed the results of direct and indirect effects of various grain components on grain yield (t/ha). It was observed that the highest positive direct effect of number of filled spikelets/panicle was on grain yield followed by 1000-seed weight and days to 50% flowering. The direct positive effect of number of filled spikelets/ panicle on grain yield and positive significant genotypic correlation between these two traits indicates that direct selection through this trait would be much effective for the improvement of grain yield. High direct effect of filled spikelets/panicle on single plant yield was reported by Eidi kohnaki *et al.*, (2013), Kiani and Nematzadeh (2012), Seyoum *et al*., (2012), Bagheri *et al*., (2011), Bhadru *et al*., (2011) and Chandra *et al*., (2009). All these reports support the present findings. Days to 50% flowering, number of effective tillers/plant and number of ineffective tillers/plant also had direct positive effect and positive genotypic correlation with grain yield. So, direct selection through this trait would also be effective for improvement of grain yield. Bhadru *et al*., (2011) and Chandra *et al*., (2009) reported positive direct effect of days to 50% flowering and Eidi kohnaki *et al.*, (2013) and Kiani and Nematzadeh (2012) found the positive direct effect and significant positive

Table 1. Correlation coefficient among yield and yield contributing characters in rice.

		Days to 50 % flowering	Number of effective tillers/plant	Number of ineffective tillers/plant	Panicle length (cm)	Number of filled spikelets /panicle	Number of unfilled spikelets /panicle	1000-seed wt. (g)	Yield (t/ha)
Plant height (cm)	r_g	0.491	-0.878**	-0.998**	0.981**	-0.082	0.390	-0.747*	-0.783*
	r_p	0.009	-0.208	-0.500	0.764*	-0.120	0.100	-0.436	-0.473
Days to 50 % flowering	r_g		-0.341	-0.189	-0.024	0.740*	0.275	-0.572	0.234
	r_p		-0.263	-0.169	-0.059	0.542	0.320	-0.442	0.101
Number of effective tillers/ plant	r_g			0.281	-0.654*	0.056	-0.752*	0.162	0.652*
	r_p			0.272	-0.285	-0.091	-0.405	0.151	0.538
Number of ineffective tillers/plant	r_g				-0.994**	-0.171	0.239	0.957**	0.367
	r_p				-0.674*	-0.165	0.154	0.597*	0.197
Panicle length (cm)	r_g					-0.180	0.011	-0.693*	-0.732*
	r_p					-0.155	-0.051	-0.457	-0.436
Number of filled spikelets/ panicle	r_g						-0.285	-0.640*	0.756*
	r_p						-0.313	-0.498	0.666*
Number of unfilled spikelet/ panicle	r_g							0.549	-0.554
	r_p							0.358	-0.495
1000-seed wt. (g)	r_g								-0.258
	r_p								0.035

* Significant at 5% level, ** Significant at 1% level.

Table 2. Path coefficients of different yield contributing characters on grain yield of rice.

	Plant height (cm)	Days to 50 % flowering	Number of effective tillers /plant	Number of ineffective tillers /plant	Panicle length (cm)	Number of filled spikelets /panicle	Number of unfilled spikelets /panicle	1000-seed wt. (g)	Genotypic correlation with yield
Plant height (cm)	**-0.187**	0.158	-0.050	-0.259	0.168	-0.054	-0.247	-0.313	-0.783*
Days to 50 % flowering	-0.092	**0.323**	-0.019	-0.049	-0.004	0.490	-0.174	-0.240	0.234
Number of effective tillers /plant	0.164	-0.110	**0.057**	0.073	-0.112	0.037	0.476	0.068	0.652*
Number of ineffective tillers /plant	0.187	-0.061	0.016	**0.259**	-0.170	-0.113	-0.151	0.401	0.366
Panicle length (cm)	-0.184	-0.008	-0.037	-0.259	**0.172**	-0.119	-0.007	-0.290	-0.732*
Number of filled spikelets/panicle	0.015	0.239	0.003	-0.044	-0.031	**0.662**	0.180	-0.268	0.756*
Number of unfilled spikelets /panicle	-0.073	0.089	-0.042	0.062	0.002	-0.189	**-0.633**	0.230	-0.554
1000-seed wt. (g)	0.140	-0.185	0.009	0.248	-0.119	-0.423	-0.347	**0.419**	-0.258

Bold figures indicate the direct effects. * Significant at 5% level, ** Significant at 1% level.

Residual effect (R) = ± 0.170.

correlation coefficient between productive tillers/plant and grain yield/plant which also supported the present finding. Panicle length and 1000-seed weight had direct positive effect on grain yield but showed negative genotypic correlation with yield. Akhtar *et al*., (2011) found positive direct effect of 1000-grain weight on yield. However, the results are in contrary with Eidi kohnaki *et al.*, (2013) who reported that the1000-grains weight had negative direct effect on grain yield. Negative direct effect of plant height and number of unfilled spikelets/panicle on grain yield was observed. These two traits also showed negative genotypic correlation with grain yield. The direct effect and correlation coefficient of these two characters were negative, so the direct selection for these traits to improve the yield will not be desirable. Akhtar *et al*., (2011) also reported the negative direct effect of plant height on yield. But Eidi kohnaki *et al*., (2013), Bhadru *et al*., (2011) Nayak *et al.* (2001) and Shanthi and Singh (2001) reported a significant positive association of plant height with yield and also had a positive direct effect on yield both at the phenotypic and genotypic levels which is contradictory with the present study.

The estimated residual effect was 0.170 indicating that about 83% of the variability in grain yield was contributed by the characters studied in path analysis. This residual effect towards yield in the present study might be due to many reasons, such as other characters, which are not included in the investigation, environmental factor and sampling errors. Within the scope of path analysis carried out in the present study, it is therefore, suggested that number of filled spikelets/panicle and number of effective tillers/ plant, the main components of grain yield should be given high priority in the selection programme.

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ISSN 0258-7122
Bangladesh J. Agril. Res. 40(1): 163-176, March 2015

DETERMINATION OF MATURITY INDICES OF BER (*Zizyphus Mauritiana* Lam.) VAR. BARI KUL-2

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Abstract

A study was conducted at Fruit Research Farm and Postharvest Technology Laboratory of Horticulture Research Centre, Bangladesh Agricultural Research Institute during the period from October 2009 to February 2010 to determine the maturity indices of ber. The ber variety BARI Kul-2 was selected for conducting the study. Ber fruits were tagged at fruit setting stage and harvested at 90, 100, 110, 120 days after fruit set (treatments). The physicochemical characters like fruit weight and size, specific gravity, TSS (%), sugar (%), acidity (%), pulp-stone ratio, TSS-acid ratio, sugar-acid ratio as well as subjective sensory attributes like fruit colour and texture, and storage traits like storage life, physiological weight loss (%), ripening status and decay, browning and shriveling (%) of harvested fruits were evaluated for determining the proper stage of commercial maturity. The fruit weight, TSS (%), pulp-stone ratio, TSS/acid ratio, sugar-acid ratio and specific gravity of BARI Kul-2 were found 24.33g, 15.60, 15.66, 39.72, 16.14 and 0.98, respectively, at 110 days after fruit set. Considering all the physical and chemical characters matching with subjective parameters, fruits of BARI Kul-2 was found commercially mature after 110 days of fruit set when the fruits turned into light greenish yellow to greenish yellow colour and specific gravity less than 1.00.

Introduction

Ber (*Zizyphus mauritiana* Lam.) or Indian jujube belongs to the genus *Zizyphus* of the family *Rhamnaceae* or buckthorn family which has about 50 genera and more than 600 species (Pareek, 1983). It is an important fruit in Bangladesh. This fruit is very popular among the people of all social strata for its nourishing value and good taste and lower price. Ber is one of the most nutritious fruits with medicinal value. It is one of the richest sources of Vitamin C, next to aonla and guava but better than citrus fruits and apple (Bal and Uppal, 1992).

The word ‘Maturity’ of a crop refers to an assessment of physiological development which is of two types; physiological maturity and commercial

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maturity (Shewfelt and Prussia, 1993). Physiological maturity is described as "the stage of development when a plant or plant part will continue ontogeny even if detached" whereas commercial maturity is defined as "the stage of development when a plant or plant part possess the prerequisites for utilization by the consumer for a particular purposes" (Watada *et al*., 1984). Maturity is an integral component of quality, especially in the context of commercial maturity (Will *et al*., 1998). On the other hand 'Index' is the sign or indication of the readiness of fruits for harvest according to consumer's choice (Bautista, 1990). Index is of two types-subjective and objective. The former includes colour, size, changes in appearance, feel, sound, smell etc., whereas, the objective indices include chemical constituents, dry matter content, age, weight, length, breadth or diameter etc. Horticultural maturity is equals to commercial maturity. In case of fruit, it is usually refers to the stage when it possesses the necessary characters preferred by the consumers.

Harvesting of fruits at proper stage of maturity is very much important both for maintaining quality and marketing. Ripening of fruit may take place either before or after harvest, but it is generally accepted that postharvest ripening of ber only occurs if the fruit is sufficiently mature when picked. Immature fruits do not have satisfactory sweetness and taste. Overmature fruits, on the other hand, lose their attractiveness and crispiness and became slimy in texture within a very short time (Pareek, 2001). Abbas (1997) stated that fruit colour, percentage of titrable acids and total soluble solids are the most important maturity indices shown for ber fruits grown in Basrah region, but research in India indicates that the specific gravity of the fruit and fruit colour are more suitable indices (Bal, 1980; Bal and Singh, 1978; Bhatia and Gupta, 1985; Bal and Uppal, 1992).

Bangladesh Agricultural Research Institute released a ber variety named BARI Kul-2 in 2002. It is a heavy bearer good quality variety having potentials to commercial cultivation. In view to providing information to growers to harvest at proper stage for better marketing, maturity indices of the variety should be standardized. Therefore, the present study was undertaken to determine the proper stage of maturity in view to find out proper time of harvesting of BARI Kul-2.

Materials and Method

The experiment was conducted on existing ber orchard at Fruit Research Farm and Postharvest Technology laboratory of Horticulture Research Centre, Bangladesh Agricultural Research Institute (BARI) during the period from October 2009 to February 2010. Three trees were selected, each of which was considered as a replication. The selected trees were of 6 years old with approximately uniform growth and size, under same management practices. The trees were fertilized with 25 kg well decomposed cowdung, 750g Urea, 600g

TSP and 600g MP in two split of doses at the end of May and beginning of November with light irrigation (Islam, 2005; Hossain *et al*., 2009). The experiment was laid out in Randomized Completely Block Design (RCBD) with three replications. Fruits were tagged at fruit setting stage and harvested at 90, 100, 110, 120 and 130 days after fruit set (treatments). Two hundred fruits from each tree were plucked randomly for studying different physical, biochemical and quality characteristics. Then these were carried to the laboratory of Postharvest Technology Section, and analyzed for physical and chemical characters on the day of harvesting. One hundred fruits were kept at ambient condition for storage studies. Sensory evaluation was done by panel of judges consisting of five members both for fresh harvested fruits as well as stored ones.

Physical Characters: Weights of fruit and stone were measured by electrical balance. Length, breadth and circumference of fruit were recorded by slide caliper and scale. Pulp-stone ratio was calculated from the following formula:

Pulp-stone ratio = (Fruit weight-stone weight)/Stone weight

Chemical analysis: The biochemical and nutritional parameters were determined by the methods described in the Manual of Analysis of Fruit and Vegetable products (Rangana, 1986). Acidity, vitamin-C and TSS (%) were determined at the day of fruit harvesting. Acidity by treating against standard NaOH solution, ascorbic acid by 2, 6- Diclorophenol-Indophenol Visual Titration Method and TSS (%) by brix meter were determined. These methods were conducted according to Rangana (1986).

Storage studies: Physiological loss in weight (PLW), storage life and physical appearance of harvested fruits were evaluated for storage potentiality. The weight loss (%) and storage life were evaluated as follows:

Physiological loss in weight (%): It was determined by periodic weighing of ber fruits and expressed as percentage of its initial weight by following formula:

$$\%PLW = \frac{W_0 - W_1}{W_0} X100$$

Where, PLW = Physiological loss in weight

W_0 = Initial weight of ber fruits kept for storage (on the day of harvesting)

W_1 = Final weight of ber fruits on selected days after harvesting

Storage life (Day): The storage life of fruits was determined by judging the non-marketability parameters like decay or damaging, shriveling, skin browning, ripening status, odd-flavour etc. at ambient condition.

Sensory evaluation: Sensory evaluation was done both for fresh as well as stored fruits after 3 and 6 days of storage. The physico-morphological qualities like skin colour, pulp texture and taste were evaluated by a panel of judges consisting of 5 members of scientific personnel. They were asked to evaluate the traits by a scoring rate on a 9- point hedonic scale, i.e. 9=Like extremely, 8=Like very much, 7=Like moderately, 6=Like slightly, 5=Neither like nor dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much and 1=Dislike extremely. The different preferences as indicated by scores were evaluated by statistical methods.

Statistical analysis: A two way analysis of variances (ANOVA) was done for different parameters by using statistical method (MSTAT C). The mean separation was done by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Significant variation was found both on physical and chemical characters of fruits as well as storage life and quality attributes.

Physical characters

Weight, length, breadth, and circumference of fruit, stone weight, pulp-stone ratio and specific gravity, all the characters differed significantly (Tables 1 & 2). Fruit weight was found maximum (28.60g) after 130 days of fruit set followed by 120 (26.53g) and 110 days (24.33g). On the contrary, the fruits harvested 90 days after fruit set had the minimum weight (14.41g) among the selected five days of harvesting. Fruit length (4.18 cm), breadth (3.61 cm), and circumference (11.57 cm) were found maximum at 130 days but these were statistically similar with the fruits harvested at110 and 120 days (4.04 and 4.16 for length, 3.44 and 3.55 cm for breadth and 11.25 and 11.43 cm for circumference, respectively). The lowest length, breadth and circumference of fruits were recorded from the fruits harvested 90 days after fruit set. Pulp-stone ratio was computed high (17.75) from the fruits harvested 130 days after fruit set while it was recorded minimum (11.21) at 90 days. Moderate pulp-stone ratio was obtained from the fruits harvested at 110 and 120 days after fruit set (15.66 and 16.81, respectively). Fruits harvested at 110,120 and 130 days after fruit set had the specific gravity <1 (0.98, 0.95 and 0.93, respectively) whereas it was calculated >1 from the fruits harvested at 90 and 100 days after fruit set (1.04 to 1.01, respectively). Bal and Uppal (1992) reported that the best indicator for judging the maturity of ber fruit is that the specific gravity should be less than one. Specific gravity at harvest maturity in Kaithli, Gola and Umran variety were observed as 0.88, 0.93 and 0.81 respectively, in India (Bhatia and Gupta, 1985).

Table 1. Physical characteristics of ber fruits var. BARI Kul-2 influenced by days to maturity.

Days after fruit set (Maturity)	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Fruit circum-ference (cm)	Stone weight (g)	Pulp-Stone ratio	Specific gravity
90	14.41 e	3.25 c	2.83 c	9.13 c	1.18 d	11.21d	1.04 a
100	19.82 d	3.69 b	3.15 b	10.74 b	1.33 c	13.90 c	1.01 ab
110	24.33 c	4.04 a	3.44 a	11.25 a	1.46 b	15.66 b	0.98 ab
120	26.53 b	4.16 a	3.55 a	11.43 a	1.49 ab	16.81 ab	0.95 ab
130	28.60 a	4.18 a	3.61a	11.57 a	1.52 a	17.75 a	0.93 b
CV (%)	2.74	1.82	2.78	1.13	1.76	2.98	1.77

In a column, the means with same letter are not significantly different at 1% level by DMRT

Table 2. Chemical characteristics of ber fruits var. BARI Kul-2 influenced by days to maturity.

Days after fruit set (Maturity)	TSS	Total sugar	Acidity	TSS/Acid ratio	Sugar/Acid ratio	Vitamin C (mg/100g pulp)
90	10.40 c	4.83 c	0.49 a	21.29 d	9.96 c	44.67 d
100	13.07 b	5.83 b	0.42 ab	31.18 cd	14.06 c	64.13 c
110	15.60 a	6.40 b	0.40 abc	39.72 bc	16.14 bc	73.22 bc
120	16.17 a	7.80 a	0.35 bc	46.91 b	22.65 ab	110.40 a
130	16.73 a	8.70 a	0.27 c	61.63 a	29.14 a	83.40 b
CV (%)	4.54	5.13	11.37	11.19	14.58	8.79

In a column, the means with same letter are not significantly different at 1% level by DMRT

Chemical characters

Significant variation was found among the fruits harvested at different days for chemical traits like total soluble solids, total sugar, acidity, TSS-acid ratio, sugar-acid ratio and Vitamin C (Table 2). The highest TSS (16.73%) was recorded from the fruits harvested at 130 days after fruit set which was statistically similar to those of 110 (15.60%) and 120 days (16.17%). Though the total sugar increased with days to maturity, it was statistically similar at 120 and 130 days (7.80 and 8.70%, respectively). Acidity decreased gradually from 90 days to 130 days. It was computed maximum (0.49%) from the fruits harvested at 90 days after fruit set while it was found minimum (0.27%) in 130 days. The highest TSS-acid ratio (61.63) was obtained from the fruits harvested at 130 days followed by 120 days (46.91). Sugar/acid ratio was calculated maximum (29.14) at 130 days. The highest vitamin C (110.4 mg/100g) was obtained from the fruits harvested 120 days followed by 130 days (83.40 mg/100g). Fruits harvested at 90 days after fruit set had the lowest amount of vitamin C (44.67 mg/100g pulp). Vitamin C content was found to be reported high at the early ripening stage in Chinese jujube by Kuliev and Akhundov (1975) and Bi *et al*. (1990).

Sensory evaluation

The acceptability scores for colour and taste were obtained high both by the fruits harvested at 110 and 120 days after fruit set (Table 3). These were 8.83 and 8.23 for colour and taste for the fruits harvested at 120 days after fruit set, however, statistically similar to those (8.67 and 8.17, respectively) of the fruits harvested at 110 days after fruit set. The preference score for texture was obtained high (8.77) by the fruits harvested at 120 days after fruit set followed by 130 and 110 days after fruit set (7.93 and 7.60, respectively). The lowest scores for colour (5.77), texture (4.83) and taste (5.33) were obtained by the fruits harvested at 90 days after fruit set. The preference scores for colour, texture, and taste were found to be decreased with increasing of storage time (Table 3). On 3rd day of storage, the highest score (7.00) for colour was got by the fruits harvested at 110 days after fruit set. On the same day, the scores for texture and taste were found not to be vary significantly among the fruits harvested at 110, 120 and 130 days after fruit set. The acceptability scores of colour, texture and taste declined in all cases on 6th days of storage (Table 3). On the same day, the fruits harvested at 130 days after fruit set had the lower scores for colour (3.10) and taste 4.00), though it got statistically similar score (4.60) for texture of the fruits of 100, 110 and 120 days (4.44, 5.10 and 4.87, respectively) after fruit set. On 6th day of storage, fruits harvested 90 days after fruit set lost their scores drastically as 3.10, 1.63 and 3.23 for colour, texture and taste, respectively (Table 3). The lower scores both for fresh and storage ones of the fruits harvested at 90 and 110 days after set indicate its non-acceptability or less acceptability of proper maturity. On the other hand, it was got lower by the fruits harvested at130 days after fruit set, both at fresh and succeeding storage time, probably because of over maturity.

Table 3. Sensory evaluation of ber fruits var. BARI Kul-2 influenced by days to maturity.

(Preference score: 1-9)

Days after fruit set (Maturity)	Colour			Texture			Taste		
	Storage periods (Days)			Storage periods (Days)			Storage periods (Days)		
	0	3	6	0	3	6	0	3	6
90	5.77 c	3.53 c	3.10 c	4.83 c	2.43 c	1.63 b	5.33 c	4.00 b	3.23 c
100	7.27 b	5.60 b	4.77 a	5.43 c	5.06 b	4.44 a	7.40 b	6.50 a	4.93 ab
110	8.67 a	7.00 a	4.00 b	7.60 b	6.60 a	5.10 a	8.17 a	6.60 a	5.43 a
120	8.83 a	6.60 ab	4.93 a	8.77 a	7.07 a	4.87 a	8.23 a	7.40 a	4.05 bc
130	7.47 b	5.60 b	3.10 c	7.93 ab	6.40 a	4.60 a	7.60 ab	6.47 a	4.00 bc
CV (%)	5.36	8.38	6.26	4.03	5.23	6.25	3.52	8.78	8.46

In a column, the means with same letter are not significantly different at 1% level by DMRT.

Storage attributes

Storage life: Fruits harvested at 110 days after fruit set had the highest storage life (4.50 days) followed by that of 120 days (3.83 days) while it was the lowest (2.00 days) when the fruits were harvested at 90 days after fruit set (Table 4). From Table 4 and 5, it revealed that the best storage life was recorded from the fruits harvested at onset of ripening followed by prior to onset of ripening i.e. colour turning stage. On the contrary, fruits harvested at green or fully ripe stage had short storage life. Several authors also mentioned similar opinion (Abbas, 1997; Al-Niami *et al*. 1989). Though jujube is usually stated as non-climacteric fruit (Kader, 1992), Chinese jujube (*Zizyphus jujuba* Mill. non Lam.) were found to be lie in those group experimentally (Kader *et al.*, 1982), however, some varieties of ber or Indian jujube (*Zizyphus mauritiana* Lam.) was found to shown climacteric behaviour as they produced more CO_2 and ethylene during ripening compare to those of mature green stage (Abbas and Saggar, 1989; Abbas, 1997), and hence the storage life of ber was found to be short likewise to many other tropical fruits (Abbas, 1997). Depending of cultivar and storage conditions, it may vary from 4 to 15 days (Pareek, 2001). Jain *et al*. (1979) had reported lower storage life in Umran fruits, i.e for 3 days in open baskets and 6 days in earthen pots. Similarly, Siddiqui and Gupta (1990) could store Umran fruits for only 3 days in wooden boxes. A longer storage life of 10 days, however reported for Umran variety by Jawanda *et al*. (1980a, b). Owing to low respiration rate, fruits of Umran variety were found to suitable for prolonged storage when harvested at mature-golden yellow stage (Singh *et al*., 1981). In Bangladesh, Islam (2007) observed 4 to 5 days of storage life for different cultivars of ber grown in Rajshahi region. In fact, the short storage life of BARI Kul-2 was considered in this study due the fact mostly of its rapid browning tendency of fruit skin, i.e. pericarp browning within 2 to 5 days of storage at ambient condition (Table 4).

Physiological loss in weight (%): No significant variation was found in physiological loss in weight of stored ber till 3 days of storage (Table 4). However, it was recorded higher (12.53%) on 3rd day from the fruits harvested at 90 days after fruit set. On 6th day of storage, the maximum weight loss (40.50%) was recorded from the fruits harvested at 90 days after fruit set. It was recorded lesser on that day both from the fruits harvested at110 and 120 days after fruit set (18.83% and 16.87%, respectively). In comparison to others, the fruits harvested at 130 days after fruit set had less weight loss both on 3rd and 6th day of storage though it reached to14.73% at latter (Table 4).

Skin blemish: The pericarp of ber was found to becoming brown and shrivel with storage time. The skin of the fruits got blemish through browning, shriveling and undesirable changes. It lowered the preference of the fruits as fresh consumption. The nature and extent of skin blemish in storage were found to be different with maturity differences. At 3 days of storage, the maximum (55.30%) skin blemish was recorded from the fruits harvested at 90 days after fruit set. On the contrary, it was found less (37.97) on the fruits harvested at 110 days after fruit set which was statistically similar (38.63%) to those of 120 days. At 6 days of storage, the skin blemish was found to be increased in all cases. However, it was comparatively less (56.30%) on the fruits harvested at 110 days after fruit set which was statistically not different (59.30%) with those of the fruits harvested at 120 days (Table 4). The skin blemish was recorded high (94.07) from the fruits harvested at 90 days after fruit set. It was statistically similar (90.50%) to those of the fruits of 100 days. The blemish of fruit skin of the fruits of 130 days was also more on 3 (48.10%) and 6 days (77.73%) of storage (Table 4). It could be mentioned that shriveling was found to be appeared earlier mostly on the fruits harvested by 90 days after fruit set whereas browning was exhibited more by the fruits harvested at 130 days. The quick browning of the fruits of latter might be indicating of possible accumulation of degraded polyphenol or increase of tannin in pericarp area of fruit. Decrease of total phenolics with fruit maturity in jujube was reported by Pandey *et al*. (1990) whereas tannin content was found to be increased during ripening by Calderia (1967).

Table 4. Storage life, physiological loss in weight (PLW) and skin blemish of ber fruits var. BARI Kul-2 influenced by storage periods at ambient condition.

Days after fruit set (Maturity)	Storage life	PLW (%)			Skin blemish (%)		
		Storage periods (Days)			Storage periods (Days)		
		0	3	6	0	3	6
90	2.00 c	-	12.53	40.50 a	-	55.30 a	94.07 a
100	2. 67 bc	-	9.40	27.63 b	-	53.93 a	90.50 a
110	4.50 a	-	8.83	18.83 bc	-	37.97 c	56.30 b
120	3.83 ab	-	8.27	16.87 bc	-	38.63 c	59.30 b
130	3.17 a-c	-	7.97	14.73 c	-	48.10 b	77.73 ab
CV (%)	16.82		19.00	15.99		16.11	20.25

In a column, the means with same letter are not significantly different at 1% level by DMRT

Subjective maturity parameters and quality attributes

Fruits were found attractive and properly ripe having light greenish yellow to greenish yellow skin with good taste and flavour when harvested 110 and 120 days after fruit set (Table 5). It retained acceptable appearance on 3 days of storage (Table 6). Fruits harvested at 90 days did not ripen properly. Though the fruits harvested at 130 days were fully ripe and attractive, it became overripe and skin of the fruit turned into brown which was unattractive at 3 days of storage (Table 5 and 6). At 6 days of storage, all the harvested fruits of 130 days after fruit set lost their acceptance of quality severely as fresh consumption. However, fruits harvested at 110 and 120 days after fruit set retained some acceptance of quality till the aforesaid time (Table 6). To obtain good organoleptic quality, Siddiqui and Gupta (1990) recommended harvesting fruits at green-yellow for Kaithli, green-mature or green-yellow or yellow for Gola variety of India. Singh *et al*. (1981) observed that fruits of cultivar Umran developed good organoleptic quality at mature-golden yellow stage. However, Pareek (2001) reported that ber fruits could be harvested at mature-green and mature-golden yellow stages depending on cultivar, distance from market, and expected postharvest uses. At the fully ripe stage, the fruits develop poor organoleptic quality but might be suitable for dehydration.

Table 5. Subjective maturity parameters and quality attributes of ber fruits var. BARI Kul-2 on the day of harvesting as harvested at different days after fruit set.

	Maturity parameters		Quality attributes		
Days after fruit set (Maturity)	Skin colour	Pulp texture	Taste	Attractiveness of fruits on the day of harvesting	Ripening status
90	Green	Less crispy	Not good; astringent	Not attractive	Unripe
100	Light green	Less crispy	Acceptable; slightly astringent	Not so Attractive	Prior to onset of ripening
110	Light Greenish yellow	Crispy	Very good; less astringent	Very much attractive	Onset of ripening
120	Greenish yellow	Crispy	Very good; less astringent	Very much attractive	Fully ripe
130	Full yellow to fully yellow with partially Brownish	Crispy	Good; astringency reduced, taste also reduced	Less attractive	Fully ripe to little bit over ripe

Table 6. Quality attributes of ber fruits var. BARI Kul-2 during storage pertaining to harvested at different days after fruit set.

Days after fruit set (Maturity)	Storage periods							
	At 3 days				At 6 days			
	Physical appearance	Pulp texture	Taste	Ripening status	Physical appearance	Pulp texture	Taste	Ripening status
90	Green, severely Shriveled	Insipid	Unpleasant	Unripe	Green, severely Shriveled	Insipid	Not good	Unripe
100	Dull Green, moderately Shriveled	Insipid	Unpleasant	Not ripe properly	Pale green, severely shriveled	Insipid	Not good	Not ripe properly
110	Skin not so shriveled and colour turns into dull yellow	Less crispy	Acceptable	Ripe properly and flavour still acceptable	Skin shriveled and colour turns into brownish	Crispy-ness fall down	Moderately acceptable	Becoming Over ripe
120	Skin not so shriveled but colour turns into brownish	Medium crispy	Acceptable	Fully ripe and flavour still acceptable	Skin shriveled and colour turns into brownish	Crispy-ness fall down	Moderately acceptable	Becoming Over ripe
130	Skin becoming shriveled and colour turns into brown	Less crispy	Moderately acceptable	Over ripe and flavour becoming unacceptable	Skin severely shriveled and colour turns into deep brown or light tan	More fall down of crispyness	Not acceptable	Over ripe and presence of odd-flavour

Conclusion

Considering physical and chemical characters, sensory attributes and storage behaviour of fruits, it might be concluded that BARI Kul-2 was found horticulturally mature between 110 to 120 days after fruit set. The fruits of BARI Kul-2 were found as greenish yellow and crispy in texture having TSS 15.60-16.17%; pulp-stone ratio 15.66-16.81, TSS-acid ratio 39.72-46.91, sugar-acid ratio 16.14-22.65 and specific gravity 0.98-0.95 while 24.33-26.53g in weight as properly matured. The higher storage life (4.50 days) was recorded from BARI Kul-2 when harvested at 110 days having less decay, browning and shriveling of fruits during storage.

Recommendation

It could be mentioned here that ber fruits could not be mature at a time and several picking may need for total harvesting. Therefore, it would be wise to harvest fruit at colour turning stage rather than mature green or over ripe stage for better storage life with good quality. Regarding this, the selected days to harvest for BARI Kul-2 were found to lie between 110 to 120 days after fruit set i.e. 5 to 15 February at the study area of Bangladesh.

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