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EFFECT OF HOT WATER TREATMENT IN CONTROLLING FUSARIUM WILT OF GLADIOLUS

L. YASMIN¹, M. A. ALI² AND T. K. DEY³

Abstract

The experiment was conducted at the Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Gazipur during 2010-12 following two factors RCB design with four replications. The levels of Factor A (Duration): were i) T₁ = 5 minutes, ii) T₂ = 10 minutes and iii) T₃ = 15 minutes. Factor B (Water temperature): i) A₁ = 50°C, ii) A₂ = 52°C, iii) A₃ = 54°C, iv) A₄ = 56°C and v) A₅ = Control (normal water). The treatment combinations were evaluated against the wilt disease of gladiolus (*Fusarium oxysporum* f. sp. *gladioli*) under naturally infested field condition. Corm treated with hot water at 54°C for 5 minutes was very effective in inhibiting the wilt disease incidence and thereby resulting better corm emergence, flower sticks plot⁻¹, corm plot⁻¹ and comel yield of gladiolus. Corm treated with hot water at 52°C for 10 minutes appeared moderately effective against Fusarium wilt as well as better flower, corm and cormel yield of gladiolus under field condition.

Keywords: Gladiolus, Hot water, *Fusarium oxysporum*, Fusarium wilt.

Introduction

Gladiolus (*Gladiolus* sp) is one of the most popular commercial flower in Bangladesh. It is mainly cultivated for cut-flowers because of its elegant appearance and prolonged vase-life. Gladiolus spikes are most popular in flower arrangements and for preparing attractive bouquets. Its magnificent inflorescence with various colour have made it attractive for use in herbaceous border, bedding, rockeries, pots and for cut-flowers.

The agro-ecological conditions of the country are very conducive for the survival and culture of gladiolus. But there is no authentic report on the statistics of area under cultivation of this crop. The major production belts of this flower are Jessore sadar, Sharsha, Jhikargacha, Kushtia, Chuadanga, Satkhira, Khulna, Chittagong, Mymensingh, Dhaka, Savar and Gazipur. It has great economic value as a cut-flower and its cultivation is relatively easy. Income from gladiolus flower production is six times higher than from that of rice (Momin, 2006).

The major obstacle for cultivation of gladiolus in Subtropical and Mediterranean regions is the various diseases caused by fungi, bacteria and viruses of which Fusarium wilt disease caused by *F. oxysporum* f. sp. *gladioli* is a major problem

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in all over the gladiolus growing areas (Kulkarni, 2006). Fusarium wilt of gladiolus is considered as a serious and highly devastating disease which can cause 60-70% yield loss (Vlasova and Shitan, 1974), the damage may reach upto 100% (Pathania and Misra, 2000). Crop loss of 30% in Germany and 60-80% in Russia was estimated due to *Fusarium* wilt of gladiolus (Bruhn, 1955). It is also a serious problem in India. Fungal infection reduced plant growth and flowering with 15- 28% decrease in the number of florets/spike (Misra *et al.*, 2003). The incidence and severity of the disease vary from locality to locality. Kulkarni (2006) showed that the maximum incidence of leaf blight and corm rot of gladiolus was noticed in Dharwad district (42.81%) followed by the Belgaum district (27.46%) and least in Bangalore district (22.41%) of India.

The pathogen is both seed and soil borne (Cohen and Hass, 1990; Mukhopadhyay, 1995). It causes curving, blending, arching, stunting, yellowing and drying of leaves associated with root and corm rot in the field as well as in the storage. *F. oxysporum* f. sp. *gladioli* causes three types of rot e.g. vascular corm rot, brown rot and basal rot (Partridge, 2003). Vascular rot is also called yellows and is characterized by a brown discoloration in the centre of the corm and extending into the flesh. The leaf symptoms start at the tip of the leaf blade and gradually spread all over the leaf blade. If the plant infected at later stage, it produces weak or small florets. When the plant is infected at early stage and infection is severe, whole plant becomes dry and dies within few days (Misra and Singh, 1998).

The most common method of controlling Fusarium and other fungal diseases infecting gladiolus corms was hot water treatment of the cormels before planting (Vigodsky, 1967; Hsieh, 1985). Cohen and Haas (1990) found that hot-water was effective for dormancy breaking of gladiolus cormels and also for disease management. They found that hot water treatment at 57°C for 30 minutes eradicated Fusarium as well as other pathogens. Others also found that hot water treatment of cormels eliminated the Fusarium wilt pathogen (Anon. 2009). Considering the above situation the present investigation was undertaken to study the effect of hot water treatment to control Fusarium wilt of gladiolus.

Materials and Method

The experiment was conducted at the Floriculture Field of Horticulture Research Centre (HRC) of Bangladesh Agricultural Research Institute (BARI), Gazipur during the period from 2010 to 2012. The experiment was laid out in randomized complete block design with four replications using two factors as Factor A (Duration): i) T₁ = 5 minutes, ii) T₂ = 10 minutes and iii) T₃ = 15 minutes and Factor B (Water temperature): i) A₁ = 50°C, ii) A₂ = 52°C, iii) A₃ = 54°C, iv) A₄ = 56°C and v) A₅ = Control (normal water). Corms were treated with hot water at 50°C, 52°C, 54°C and 56°C for 5, 10 and 15 minutes of each treatment. For control treatment, the corms were soaked in normal water at room temperature for the same time. The corms were dried in shade before planting in the field.

The experiment was conducted in previously *Fusarium oxysporum* infested soil. The recommended dose of fertilizers i.e. cowdung @ 10t/ha, TSP @ 225kg/ha, MoP 190kg/ha were applied to the soil during land preparation and thoroughly mixed with the soil before leveling. Urea @ 200kg/ha was top dressed in two equal splits, one at the four leaf stage and another at spike initiation stage (Woltz, 1976).

The treatments were randomly assigned in each block where unit plot size was 1.25m × 1.6m. Spacing was maintained at 25cm between the rows and 20cm between the plants. Depth of planting of the corms was 6cm. Two adjacent unit plots were separated by 50cm space, and there was 75cm space between the blocks. Data were recorded on Emergence, Days to 50% emergence, Plant height, Pre emergence corm rot, Disease incidence, Percent disease index (PDI), Wilted plant, Days to 50% spike initiation, Spike length, Rachis length, Florets spike⁻¹, Flower sticks plot⁻¹, Flower sticks ha⁻¹, Corms hill⁻¹, Corm yield, Cormels hill⁻¹ and Cormels yield. Assessment of disease incidence, disease index and wilted plant were calculated using following formula (Singh and Arora, 1994):

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants plot}^{-1}}{\text{Number of total plants plot}^{-1}} \times 100$$

$$\text{Percent disease index (\%)} = \frac{\text{Class frequency}}{\text{Total number of sample} \times \text{Maximum grade of scale}} \times 100$$

$$\text{Wilted plant (\%)} = \frac{\text{Number of dead plants plot}^{-1}}{\text{Total number of plants plot}^{-1}} \times 100$$

The effect of different duration and temperature of gladiolus corms played remarkable role on emergence percentage and days to 50% emergence (Table 1). The maximum emergence (99%) was recorded in corm treated at 54°C for 5 minutes and minimum emergence (92%) was observed in normal water. Corm emergence was almost similar in other treatments. The minimum days (13) required to reach 50% corm emergence was in case of 5 minutes treatment at 54°C and maximum days (35) required for 50% emergence was in 15 minutes treatment at 56°C (Table 1). No significant effect was observed in plant height due to hot water treatment.

The interaction effect with various duration and temperature of hot water treatment showed significant influence on *Fusarium* wilt disease of gladiolus (Table 2).

The pre - emergence corm rot, wilted plant and severity of *Fusarium* wilt of gladiolus was low in 54°C for 5 minutes while other treatments were almost statistically similar. The highest wilted plant (19.99%) was recorded in 56°C for 15 minutes. The range of wilted plant was 3.94% to 10.15% in other combinations. Disease severity varied from 22.50 to 29.17% among the treatments.

Table 1. Interaction effect of hot water treatment (duration and temperature) on emergence and vegetative growth of gladiolus

Treatments		Emergence (%)	Days to 50% emergence	Plant height (cm)
Duration (min.)	Temperature (°C)			
5 minutes	50	93 cd	14 cd	36.50
	52	97 b	14 cd	37.84
	54	99 a	13 d	37.95
	56	97 b	14 cd	37.77
	Normal water	93 cd	15 cd	37.62
10 minutes	50	93 cd	14 cd	37.67
	52	97 b	15 cd	37.81
	54	97 b	14 cd	37.55
	56	94 c	21 b	36.78
	Normal water	92 d	15 cd	36.41
15 minutes	50	94 c	16 c	37.67
	52	97 b	20 b	36.67
	54	94 c	20 b	36.51
	56	92 d	35 a	34.50
	Normal water	92 d	15 cd	37.32
CV%		0.71	7.38	9.31

Means followed by the same letters in a column did not differ significantly at the 5% level of probability.

Table 2. Interaction effect of duration and temperature of hot water on disease infection of gladiolus

Treatments		Pre-emergence corm rot (%)	Wilted plant (%)	Disease severity (PDI)
Duration (min.)	Temperature (°C)			
5	50	7.17 a (2.67)	6.76 cd (2.59)	29.17 a (31.50)
	52	3.83 b (1.94)	3.94 e (1.96)	25.00 bcd (28.87)
	54	1.33 c (1.05)	3.85 e (1.94)	22.50 d (27.23)
	56	3.83 b (1.94)	6.53 cd (2.54)	24.17 cd (28.36)
	Normal water	7.17 a (2.67)	9.25 bc (3.03)	29.17 a (31.50)
10	50	7.17 a (2.67)	4.96 de (2.21)	25.00 bcd (28.87)
	52	3.83 b (1.94)	3.94 e (1.96)	22.50 d (27.23)
	54	3.83 b (1.94)	4.82 de (2.17)	25.00 bcd (28.87)
	56	6.33 a (2.51)	6.69 cd (2.58)	26.67 abc (29.99)
	Normal water	8.67 a (2.94)	10.15 b (3.18)	29.17 a (31.50)
15	50	6.33 a (2.51)	4.91 de (2.20)	25.00 bcd (28.87)
	52	3.83 b (1.94)	4.82 de (2.17)	24.17 cd (28.36)
	54	6.33 a (2.51)	6.69 cd (2.58)	25.00 bcd (28.87)
	56	8.67 a (2.94)	19.99 a (4.47)	27.50 ab (30.51)
	Normal water	8.67 a (2.94)	10.15 b (3.18)	29.17 a (31.50)
CV%		13.18	11.61	3.38

Means followed by the same letters in a column did not differ significantly at the 5% level of probability.

A negative correlation between production of flower sticks ha^{-1} and percentage of plant infection was found to be highly significant ($r = -0.819$) (Fig. 1). The negative relationship indicated that flower sticks ha^{-1} decreased with the increasing of plant infection (%). The equation ($y = -723.38x + 124007$) stated that flower sticks ha^{-1} decreases at the rate of 723.38 flower sticks ha^{-1} per unit change of plant infection. The R^2 value indicated that 67.1% reduction of flower sticks ha^{-1} was attributed due to plant infection.

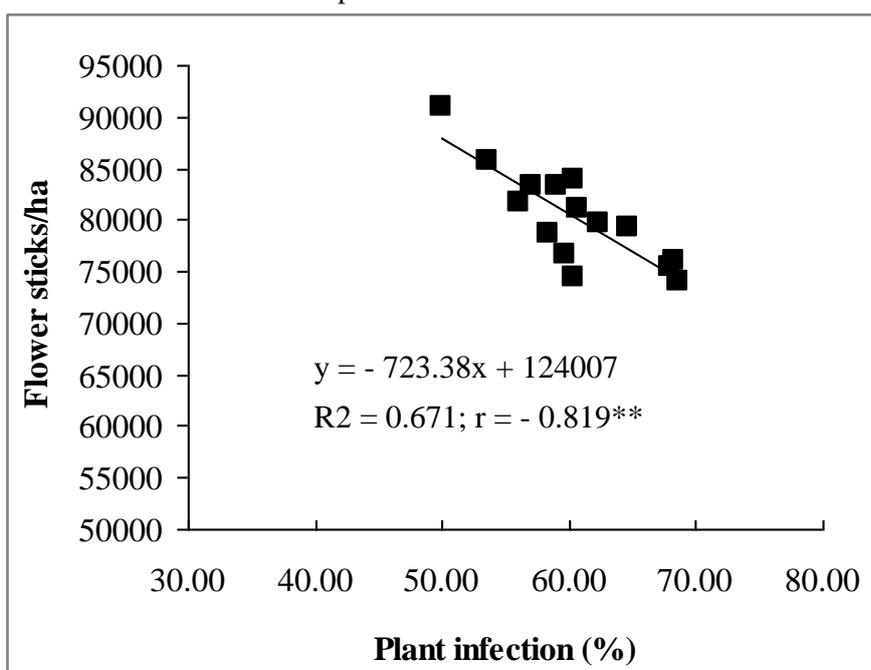


Fig. 1. Relationship between flower sticks ha^{-1} and plant infection (%)

There was significant effect of hot water treatment with various duration and temperature on days to 50% spike initiation and characteristics of gladiolus flower (Table 3). Five minutes treatment at 54°C or 56°C required 85 days for 50% spike initiation. The maximum days (97-98) required in other treatments. The highest number of flower sticks (38.33 plot^{-1}) was obtained from 5 minutes treatment at 54°C and the lowest sticks (31 plot^{-1}) in normal water. Other characters of gladiolus flower did not differ significantly by hot water treatment.

The interaction effect of hot water treatments with various duration and temperature had significant influence on corms plot^{-1} and ha^{-1} but not other characters of corm (Table 4). The number of corms was 54.90 plot^{-1} and 131.00 ha^{-1} in case of 5 minutes treatment at 54°C and minimum number of corms plot^{-1} and ha^{-1} were recorded in 15 minutes treatment at 56°C .

Table 3. Interaction effect of duration and temperature of hot water on flower production of gladiolus

Treatments		Days to 50% spike initiation	Spike length (cm)	Rachis length (cm)	Flower sticks plot ⁻¹
Duration (min.)	Temperature (°C)				
5	50	86 b	67	40	33.33 bcde
	52	86 b	66	42	35.33 abc
	54	85 b	70	43	38.33 a
	56	85 b	66	42	35 abcd
	Normal water	87 b	65	38	32 cde
10	50	86 b	65	41	33.33 bcde
	52	85 b	68	43	36 ab
	54	86 b	65	42	35 abcd
	56	97 a	68	42	32.33 cde
	Normal water	86 b	66	39	31.67 de
15	50	87 b	65	41	34 bcde
	52	97 a	66	41	34.33 bcde
	54	97 a	67	40	33 bcde
	56	98 a	67	40	31.3 e
	Normal water	87 b	65	38	31 e
CV%		1.61	7.28	10.79	6.49

Means followed by the same letters in a column did not differ significantly at the 5% level of probability.

Table 4. Interaction effect of duration and temperature on corm production of gladiolus

Treatments		Corms hill ⁻¹	Corm weight (g)	Corms plot ⁻¹	Corms ha ⁻¹ (000)
Duration (min.)	Temperature (°C)				
5	50	1.33	17.10	46.60 cde	110.67 cd
	52	1.37	17.62	50.97 abc	121.33 abc
	54	1.43	18.83	54.90 a	131.00 a
	56	1.37	17.48	49.60 abcd	118.33 abc
	Normal water	1.30	16.50	44.20 def	105.00 de
10	50	1.37	16.82	48.70 bcde	116.00 bcd
	52	1.40	18.20	52.27 ab	124.67 ab
	54	1.40	17.27	51.73 abc	123.33 abc
	56	1.33	17.43	47.10 bcde	112.00 bcd
	Normal water	1.33	16.83	44.00 ef	104.67 de
15	50	1.37	17.43	49.20 bcde	117.33 bcd
	52	1.37	17.00	50.47 abc	120.33 abc
	54	1.33	16.89	47.07 bcde	112.00 bcd
	56	1.33	16.88	39.07 f	93.33 e
	Normal water	1.33	16.00	44.00 ef	104.67 de
CV%		4.56	9.11	6.86	6.82

Means followed by the same letters in a column did not differ significantly at the 5% level of probability.

The effect of hot water treatment with different duration and temperature showed significant differences on cormel production (Table 5). The highest cormel weight hill^{-1} (28.67g) was recorded in 54°C for 5 minutes and the lowest (19.67g) was recorded in 56°C for 15 minutes. The range of cormel weight was 22.17g to 27.33g hill^{-1} in other treatments. The maximum cormel yield was 1097.0g plot^{-1} and 2.61t ha^{-1} in case of 54°C temperature for 5 minutes. The lowest cormel yield was 576.0g plot^{-1} and 1.37t ha^{-1} recorded in 56°C temperature for 15 minutes. The range of the cormel yield in other treatments was 754.0g to 1021.0g plot^{-1} and 1.79t to 2.43t ha^{-1} .

Table 5. Interaction effect of duration and temperature on cormel production of gladiolus

Treatments		Cormels hill^{-1}	Cormel weight $\text{hill}^{-1}(\text{g})$	Cormel yield $\text{plot}^{-1}(\text{g})$	Cormel yield $\text{ha}^{-1}(\text{t})$
Duration (min.)	Temperature ($^{\circ}\text{C}$)				
5	50	17.67	25.17 de	878.0 c	2.09 c
	52	19.67	26.83 bc	1001.0 b	2.38 b
	54	20.00	28.67 a	1097.0 a	2.61 a
	56	19.67	27.00 b	979.0 b	2.33 b
	Normal water	16.33	23.00 g	782.0 d	1.86 d
10	50	18.67	25.50 de	909.0 c	2.16 c
	52	19.33	27.33 b	1021.0 b	2.43 b
	54	18.00	24.33 ef	903.0 c	2.15 c
	56	14.67	22.17 g	784.0 d	1.87 d
	Normal water	17.33	23.33 fg	770.0 d	1.83 d
15	50	18.00	25.67 cd	924.0 c	2.20 c
	52	18.33	24.33 ef	901.0 c	2.14 c
	54	16.00	22.67 g	800.0 d	1.90 d
	56	14.00	19.67 h	576.0 e	1.37 c
	Normal water	16.67	22.83 g	754.0 d	1.79 d
CV%		10.10	2.97	3.27	3.27

Means followed by the same letters in a column did not differ significantly at the 5% level of probability.

Study on the effect of hot water treatment in controlling Fusarium wilt of gladiolus revealed that corm treatment by hot water was effective. Among the different temperature and duration of hot water treatment, corm treated by hot water at 54°C for 5 minutes was very effective in inhibiting the wilt disease incidence and thereby resulting increased corm emergence (%), flower sticks plot^{-1} , corm plot^{-1} and comel yield of gladiolus under field condition. An investigation was carried out by Cohen and Hass (1990) and found that hot water

had positive effect in disease management and dormancy breaking of gladiolus cormels. They found that hot water treatment of 57°C for 30 minutes eradicated *Fusarium* as well as other pathogens. In an experiment in university of California found it was that hot water treatment of cormels eliminated the *Fusarium* yellows (Anon., 2009). From the present investigation, corm treated by hot water at 52°C for 10 minutes was moderately effective against *Fusarium* wilt. Corm treated by hot water at 56°C for 15 minutes showed maximum plant mortality. In contrast Roistacher *et al.* (1957) reported that this fungus died between 53.3 and 56.7°C whereas cormels died above 56.7°C.

Conclusion

Corm treated with hot water at 54°C for 5 minutes was very effective in inhibiting the disease infestation as well as gave the increased corm emergence (%), flower sticks plot⁻¹, corm plot⁻¹ and comel yield of gladiolus. Besides, corm treated with hot water at 52°C for 10 minutes appeared moderately effective against *Fusarium* wilt as well as better flower, corm and cormel yield of gladiolus.

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**MARKER ASSISTED INTROGRESSION OF BACTERIAL BLIGHT
RESISTANT GENE INTO SUBMERGENCE TOLERANCE RICE
VARIETY BRRI dhan52**

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M. A. K. MIAN⁴ AND N. A. IVY⁵

Abstract

BRRI dhan52 is a unique submergence tolerant rice variety containing prominent genetic background of BR11, a mega rainfed lowland rice (RLR) variety of Bangladesh, but is susceptible to bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The variety is considerably popular in the southern part of Bangladesh due to its high yield in flash flood condition and comparatively medium slender grain. Molecular markers linked to BB resistance genes (*Xa* genes) and submergence QTL (*SUB1*) were utilized in a marker-aided selection program to develop elite breeding lines with broad-spectrum resistance to bacterial blight. Sequence tagged site (STS) and simple sequence repeat (SSR) markers were essentially used to detect the genes for BB and submergence as well. In backcross generation, markers closely linked to *Xa21* and *SUB1* QTL were used to select desirable plants possessing these resistance genes (foreground selection) and microsatellite markers polymorphic between donor and recurrent parent were also used to select plants that have maximum contribution from the recurrent parent genome (background selection). In BC₁F₁ generation, three best plants consequently were selected from previously selected ten double heterozygous (*Xa21* and *SUB1* QTL) plants. The percentage of recipient genome recovery in the best plant 1, 2 and 3 were 78.7%, 75.83% and 75.4%, respectively. Eventually this work illustrates the successful application of marker-assisted breeding for introgression of bacterial blight resistant gene into a rice variety of Bangladesh.

Keywords: Bacterial blight, marker assisted backcrossing, *Xa21* gene, *SUB1* QTL, Rainfed lowland rice.

Introduction

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most widespread and important diseases of rice in most of the rice growing countries due to its high epidemic potential and destructiveness to high yielding cultivars in both temperate and tropical region especially in Asia. It causes

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substantial yield loss ranging from 10% to 30% under field conditions (Reyes *et al.*, 1983) and up to as high as 80% (Singh *et al.*, 1997) and even 100% under very severe conditions (Zhai and Zhu, 1999). Though BB is controlled by various means, host plant resistance is considered to be the most effective, economical and environmentally safe option for management of the BB pathogen (Khush *et al.*, 1989).

The agroecosystems of the country are facing various environmental stresses viz. salinity, submergence, diseases etc. Among the rice growing ecosystems, rainfed lowland is the most challenging one with respect to prevalence of many abiotic and biotic stresses. Among the biotic stresses, bacterial blight disease is one of the important factors in rainfed lowland rice ecosystems. Flash flood submergence is another important hazards to the agriculture of Bangladesh related to climate-change. Submergence stress regularly affects 15 millions hectares or more of RLR areas in South and Southeast Asia. (Neeraja *et al.*, 2007). Recently, BRRI has released two flash flood tolerant rice varieties namely BRRI dhan51 and BRRI dhan52. But unfortunately, BRRI dhan52 has been found susceptible against bacterial blight both under field and controlled screening conditions. The advent of bacterial blight disease becomes more intensified under post-flood submergence condition. For this reason, the incorporation of BB resistant genes in the genetic background of BRRI dhan52 is inevitable. The bacterial blight resistant BRRI dhan52 will hopefully become wider adaptable in the farmers field condition increasing productivity of the flash flood prone areas of the country.

Recently the concept of MAS has provided an advantage of molecular marker-based approaches for crop improvement as compared to selection based solely on phenotype (Tanksley *et al.*, 1989; Paterson *et al.*, 1991). Current approaches and successful research to breeding disease resistant rice cultivars have integrated the use of molecular markers for selection of the desired traits. Gene-based markers for three important BB resistant genes viz. *xa5*, *xa13* and *Xa21* are now available. Recently, MAS has been extensively used to select the lines containing the BB resistant genes viz. *xa5*, *xa13*, and *Xa21* using STS, CAPS and SSR markers (Chen *et al.*, 2000; Chen *et al.*, 2001; Sanchez *et al.*, 2000; Singh *et al.*, 2001 and Joseph *et al.*, 2004).

Undoubtly, marker assisted backcrossing (MABC) is a precise and effective method to introgress a single locus controlling a trait of interest while retaining the essential characteristics of the recurrent parent (Collard and Mackill, 2008). MABC has three main advantages over conventional backcrossing. Firstly, DNA markers can be used for simple and efficient selection of the target locus ('foreground selection'). Secondly, the size of the donor chromosome segment containing the target locus can be minimized ('recombinant selection'). Thirdly, the recovery of the recurrent parent can be accelerated by selecting backcross lines with a higher proportion of recurrent parent genome ('background selection'). This approach has been purposefully used with great success for

‘enhancing’ rice varieties for traits such as bacterial blight resistance gene *XA21*; (Chen *et al.* 2000), the *waxy* locus for grain quality (Zhou *et al.*, 2003) and submergence tolerance *SUB1* (Neeraja *et al.*, 2007; Septiningsih *et al.*, 2009)

Considering the importance of submergence tolerance variety BRR1 dhan52 to the Bangladesh rice economy and the necessity to introduce BB resistance, marker assisted backcross breeding techniques have been successfully used to introgress the *Xa21* resistance gene and *SUB1* QTL into this variety. Microsatellite markers are highly sequence specific and co-dominant. These prominent features make them ideal for use in background selection as they permit the detection of alleles coming from both parents and allow accurate determination of the allelic constitution of the offspring. Hence, a set of microsatellite markers were used that are polymorphic between the donor and recurrent parents in selection for the recurrent genome at backcross generation.

Finally, this work will certainly represent a successful example of the use of molecular markers, in foreground and background selection, for illuminating and pyramiding biotic and abiotic genes of interest into a premium rice variety.

Materials and Method

Plant materials: IRBB60, a near isogenic line in the background of IR24, carrying the four resistant genes *Xa4*, *xa5*, *xa13* and *Xa21* served as the donor for all the crosses attempted. The recipient parents were BRR1 dhan52 for each cross. This variety was derived from the cross BR11/IR40931-33-1-3-2. The variety was released by BRR1 in 2010 as a submergence tolerant variety. Growth duration of this variety is 145 days on normal condition but it requires 155-160 days during flash flood condition. The yield potential of this variety is 5 ton/ha under optimum management and 4.5 ton/ha if affected by 10-15 days flash flood.

Molecular marker analysis

For successful molecular marker analysis salient procedures that have been followed can be depicted below: DNA was extracted from young leaves of 2-week-old plants using a modified protocol as described by Zheng *et al.*, (1995). PCR was performed in 10 µl reactions containing 25 ng of DNA template, 1 µl PCR buffer(10X), 0.2 µl of 10 mM dNTP, 0.50 µl each of 10 µM forward and reverse primers and 0.1 µl of *Taq* DNA polymerase (5 U/µl) using 96-well G-Storm thermal cycler. After initial denaturation for 5 min at 94°C, each cycle comprised 45 sec denaturation at 94°C, 45 sec annealing at 55°C, and 1:30 min extension at 72°C with a final extension for 7 min at 72°C at the end of 35 cycles. The PCR products were mixed with bromophenol blue gel loading dye and were analyzed by electrophoresis on 8% polyacrylamide gel using mini vertical polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). The gels were stained in 0.5 mg/ml ethidium bromide and photos were taken using Alpha Imager 1220 (Alpha Innotech, CA,

USA). Microsatellite or simple sequence repeat (SSR) markers were used for selection (IRGSP, 2005; McCouch *et al.*, 2002; Temnykh *et al.*, 2001).

For foreground selection, gene-based STS marker Xa21 specific to the bacterial blight resistance gene of *Xa21* was used in the confirmation of *Xa21* and STS marker ART5 was used for the confirmation of *SUB1* QTL. ART5 was a gene-based marker specific to the promoter region of putative *Sub1C* gene within *SUB1* QTL (Septiningsih *et al.*, 2009 and IRGSP, 2005).

A total of 4 (Xa21, ART5, RM8300 and Sub1C173) tightly linked and gene-based foreground markers were surveyed over two parents viz. BRRI dhan52 and IRBB60. Two primers were obtained as polymorphic between two parents and two primers were monomorphic. Both the primers were used as gene-based markers initially in the foreground selection because of their clear codominant nature and capability of producing conspicuous bands. However, the individuals that were heterozygous for the foreground markers were selected in this selection step.

Microsatellite markers unlinked to *Xa21* and *SUB1* QTL covering all the chromosomes including the *Xa21* carrier chromosome 11 that were polymorphic between the two parents, were used for background selection to recover the recipient genome. Out of 304 SSR primers surveyed, a total of 61 microsatellite markers of the target QTL were used for background selection initially (Table 1).

Table 1. List of background markers of all 12 chromosomes used in the background selection

Chr 1	Chr 2	Chr 3	Chr 4	Chr 5	Chr 6
RM495	RM110	RM231	RM16301	RM153	RM540
RM583	RM211	RM7	RM119	RM413	RM587
RM581	RM423	RM232		RM548	RM510
RM493	RM341	RM411		RM593	RM314
RM212	RM208	RM293		RM574	RM402
RM104		RM565		RM334	RM527
		RM85			RM340
Chr 7	Chr 8	Chr 9	Chr 10	Chr 11	Chr 12
RM481	RM337	RM296	RM228	RM286	RM4A
RM432	RM407	RM23778	RM590	RM5704	RM511
RM560	RM152	RM23901		RM229	RM235
RM336	RM126	RM23958		RM457	RM12
RM455	RM72	RM245		RM254	
	RM5556				
	RM256				

Data analysis

Data analyses pertaining to obtain desirable results can be mentioned below:

The molecular weights of the different alleles were measured using Alpha Ease Fc 5.0 software. The marker data was analyzed using the software Graphical Genotyper (GGT 2.0) (Van Berloo, 2008). The homozygous recipient allele, homozygous dominant allele and heterozygous allele were scored as 'A', 'B' and 'H'. The percent markers homozygous for recipient parent (%A) and the percent recipient alleles (%R) were calculated.

Results and Discussion

Results and Concomitant relevant discussion can be mentioned below:

F₁ and backcross seed production: DNA samples were collected from 75 F₁ plants and PCR was carried out using Polymorphic SSR marker RM23958. According to PCR bands produced from the 75 F₁ plants, 50 were scored as H, 25 were scored as A. Score 'H' represented heterozygous alleles for donor and recipient parent. Figure 1 shows the partial view of the gel picture of F₁ confirmation using SSR markers RM23958. The heterozygous 50 F₁'s were confirmed as true F₁. A total of 2476 BC₁F₁ seeds were produced from 20 F₁ plants.

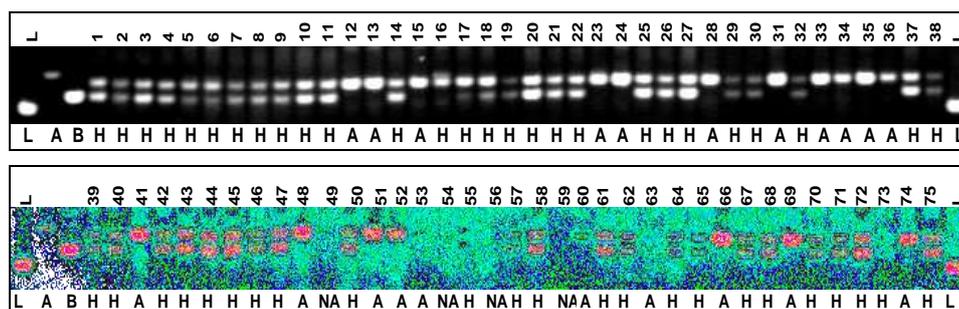


Fig.1 Confirmation of F₁s plants using SSR marker RM23958.

Foreground selection: Foreground selection was initially carried out by the gene-based marker Xa21 for *Xa21* gene and gene-based marker ART5 for *SUB1* QTL and the individual plants that were heterozygous at both the *Xa21* and *SUB1* loci were identified reducing the population size for further selection. Out of 72 plants, 30 plants were found showing the locus for the gene-based marker Xa21 as heterozygous state (Score H), 41 plants were found with the locus fixed for recipient allele (susceptible allele) (Score A). Here, in BC₁F₁ generation, 30 plants showed 'H' score and 41 plants showed 'A' score which indicated that the results fitted to the expected 1:1 ratio of this generation with a non-significant chi square value of 1.718 at a probability level of 0.05. The single plants with B score were produced due to accidental failure of backcrossing.

Out of 30 heterozygous plants for Xa21, 23 plants were used in foreground selection for the detection of *SUB1* QTL by using ART5 marker. 11 plants were found showing the locus for the gene-based marker as heterozygous state (Score H), 12 plants were found with the locus fixed for recipient allele (susceptible allele) (Score A). Figure 2 and 3 showed the foreground selection of BC₁F₁ generation.

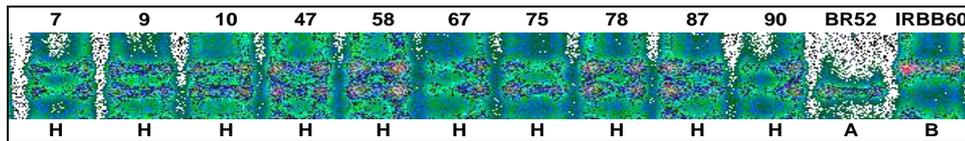


Fig. 2. Complete view of gel picture of the foreground selection with the gene-based marker Xa21 in BC₁F₁ plants.

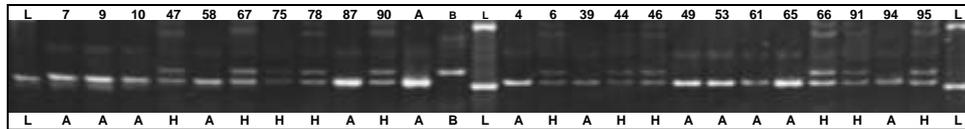


Fig. 3. Gel picture of the foreground selection using marker ART5 in population of BC₁F₁ generation.

Background Selection: A total of 61 microsatellite markers were used for background selection over 10 BC₁F₁ plants resulting from foreground selection. The percent markers homozygous for the recipient parent ranged from 37.70 to 57.37% in those 10 plants (Table 2). In the plant number 78 and 90 (Table 2) of BC₁F₁ generation, 35 markers out of 61 markers (57.37%) were like recipient parent type. But if the alleles of the homologous chromosomes were considered, the percentage of recipient alleles in plant number 78 and 90 was 78.7%. So, these two plants had been considered as the best plants of that population. Figure 4 shows the graphical genotype of the best plant 78.

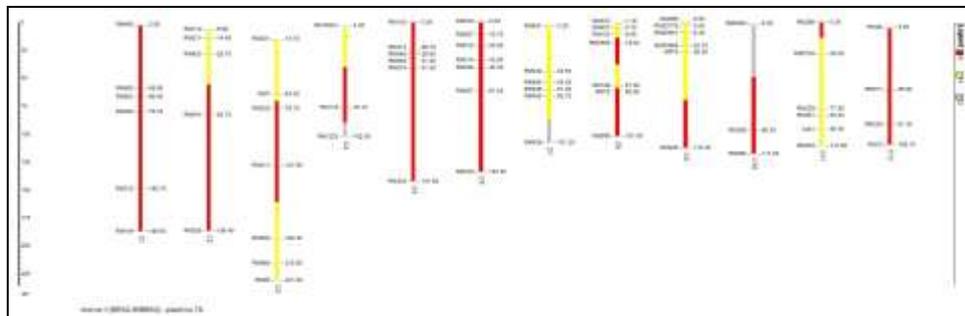


Fig. 4. Graphical genotype of the selected best plant 78 of BC₁F₁ generation. The red coloured regions on the chromosomes indicated homozygous region for the recipient genome while the yellow coloured regions indicated the heterozygous regions and the gray coloured regions indicated unknown region. The distances were represented in cM based on published map of Temnykh *et al.* (2001).

Table 2. Results of background selection, BC₁F₁ generation (No. of markers homozygous for recipient A, donor B, or heterozygous H)

Items	Plant Numbers									
		6	44	46	47	67	75	78	90	91
A	31	31	25	30	23	25	35	35	30	24
H	29	27	35	31	37	36	26	26	29	36
B	0	1	0	0	1	0	0	0	0	0
%A	51.67	53.1	41.66	49.18	37.70	40.98	57.37	57.37	50.8	40.0
%	75.83	76.72	70.83	74.6	69.2	70.5	78.7	78.7	75.4	70.0
Recipient allele										
Rank	2	8	5	4	9	6	1a	1b	3	7

BRR1 dhan52 is a medium slender grain submergence tolerant indica rice variety which can tolerate 10-15 days flash flood condition and yield potential is 4.5 to 5.0 ton/ha. In the present work, we have introgressed BB resistant gene *Xa21* and confirm the presence of *SUB1* QTL in BRR1 dhan52 with the objective of developing BB resistant BRR1 dhan52-*Xa21* line that retain the yield and quality characteristics of BRR1 dhan52.

Although the average percentage of the recurrent parent genome is 75% for the entire BC₁F₁ population, some individuals possess more or less of the recurrent parent genome than others (Collard *et al.*, 2008). Our selected BC₁F₁ plants had a recurrent parent genome than that of expected 75%.

In Foreground as well as and Background selection, selection of BC₁F₁ generation was completed and the best plants identified possessed 79% recipient parent genetic background along with the target genes in the heterozygous state. The recovery of recipient parent genome in the best plant indicated that one more backcrossing and a final self pollination in the backcross-F₂ generation would be required in order to develop BRR1 dhan52-*Xa21* homozygous line.

The current output of marker-assisted backcrossing activities of this work reflected that the approaches could be routinely used in order to pyramid multiple genes or QTLs, conferring tolerances or resistances to multiple biotic and abiotic stresses, into the genetic background of high yielding varieties and also it could be helpful to achieve wider adoption of the newly developed variety in the flash flood prone ecosystem of the country where there are outbreaks of bacterial blight disease.

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**PRODUCTION POTENTIAL AND ECONOMICS OF MUNG BEAN IN
RICE BASED CROPPING PATTERN IN SYLHET
REGION UNDER AEZ 20**

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Abstract

The study was conducted at the farmers field in Sylhet under AEZ 20 during three consecutive years 2012-13, 2013-14 and 2014-15 to determine the productivity and profitability of cropping patterns *viz.*, IP: improved pattern (Mung bean-T. aus-T. aman rice) and FP: farmer's pattern (Fallow-T. aus-T. aman rice) through incorporation of high yielding varieties and improved management practices. The experiment was laid out in randomized complete block design with six dispersed replications. Results showed that the improve pattern with management practices provided 10.85 and 14.32% higher grain yield of T. aus and T. aman rice, respectively; also contributed more T. aman mean rice equivalent yield (11.81 t ha⁻¹) compared to farmer's pattern. Mean sustainable yield index (77.63%), production efficiency (47.88 kg ha⁻¹day⁻¹), and land use efficiency (67.66%) were maximum in Mung bean-T. aus- T. aman rice cropping system. Similarly, the highest mean gross margin (Tk.126762 ha⁻¹) with benefit cost ratio (2.10) was obtained from improved pattern. Three years results revealed that 42% extra cost provides an ample scope of considerable improvement of the productivity of improved pattern with the inclusion of Mungbean before T. aus rice.

Keywords: Agronomic performance, land use efficiency, production potential, sustainable yield index, fallow land utilization

Introduction

In Sylhet region, mainly Fallow - T. aus - T. aman rice cropping pattern is widely followed by farmers under rainfed condition. Transplantation of aus rice is being dependant on rainfall, which sown during early monsoon (early May). This delayed transplantation of T. aus rice that causes late cultivation and harvesting of T. aman rice, which hampered timely cultivation of *rabi* crops; so, winter crops are not possible to be grown. The soils under these cropping pattern areas are generally heavy silty clay loams to clays and the top soil quickly becomes dry and hard after the harvest of T. aman rice. In Eastern Surma Kushiara Floodplain of Sylhet region, a vast area remains fallow for a long time after the harvest of T. aman rice due to moisture stress up to next season for cultivation of T. aus rice following the existing cropping pattern (Fallow-T. aus-T. aman rice). However, the yields of rice are very low compared to other regions of the country. Farmers

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try to improve crop productivity through addition of chemical fertilizers but, chemical fertilizers are expensive and generally cannot afford to buy many poor farmers. Higher crop productivity can also be achieved through the use of organic fertilizers such as compost or farmyard manure and recycling of crop and organic residues in production systems which can improve soil health. Nevertheless, lack of available organic fertilizers and high transport cost are major constraints. Consequence of this scenario, incorporation of legumes into the existing cropping systems seems to be a logical approach. Legumes are known to biologically fix atmospheric nitrogen (N) in symbiosis with Rhizobium bacteria. This can partly reduce the N fertilizer requirement of the main field crop in rotation. Thus it becomes a modest source of N for resource-poor farmers (McDonagh *et al.*, 1995). Inclusion of grain legumes plays an important role for increasing cropping intensity or even sustaining crop productivity along with improving nutritional status of the people and maintaining soil health (Becker *et al.*, 1995; Norman *et al.*, 1984).

Generally, rainfall starts in February and prevails up to November in each year that offers an excellent opportunity for the production of short duration pulse crops before T. aus rice. Nazrul and Shaheb (2012b) reported that pulse crops can be grown well in fallow land of Sylhet where mungbean (var. BARI Mung-6), chickpea (var. BARI Chola-5) and lentil (var. BARI Mosur-6) could be more suitable and produced higher seed yield. To enhance the crop production through utilization of fallow land in Sylhet region, the potato-rice and chickpea-rice based cropping patterns have been developed (Nazrul *et al.*, 2013; Nazrul and Shaheb, 2012a; Shaheb *et al.*, 2011).

A number of reports on different cropping pattern are available in Bangladesh and India that an additional crop could be introduced without much changes or replacing the existing ones for considerable increases of productivity as well as profitability of the farmers (Azad *et al.*, 1982; Malavia *et al.*, 1986; Soni and Kaur, 1984; Khan *et al.*, 2005; Nazrul *et al.*, 2013, Kamrozzaman *et al.*, 2015). But, little effort has been made for on-farm evaluation of the improved technologies of Mungbean-T. aus-T. aman rice cropping pattern in Sylhet area. The present study was therefore, initiated with a view to determine productivity and economic feasibility of an improved package of technologies over the farmer's existing practices.

Materials and Method

The study was carried out during three consecutive years 2012-13, 2013-14 and 2014-15 at farmer's field, Sylhet (24°54' N latitudes and 91°58' E longitude) located in Agro Ecological Zone (AEZ)-20; under Eastern Surma Kushiya Floodplain. This trial was conducted to derive the economic consequences of two cropping patterns viz. IP: improved pattern (Mungbean-T. aus-T. aman) and FP: farmer's pattern (Fallow-T. aus-T. aman) through incorporation of high yielding varieties and improved management practices.

Annual monthly total rainfalls, along with maximum and minimum average temperatures during the study period are presented in Fig. 1. The highest amount of average monthly rainfall occurred in June followed by August, July and May, whereas lowest amount of rainfall occurred in January followed by December and February. Rainfall increases gradually from the month of January to June and then decreases. The crops received 3765, 3938 and 3760 mm total rainfall during crop season of 2012-13, 2013-14 and 2014-15, respectively. The monthly mean maximum air temperature was 30.33, 31.24 and 30.31 °C and minimum 20.83, 20.77 and 20.62 °C during the crop season of 2012-13, 2013-14 and 2014-15, respectively.

The soil was clay loam with low organic matter content (1.63%) and soil pH ranged from 4.10 to 5.63 acidic in nature. The initial status of N (0.07%), P (7.59 µg/soil), K (0.18 meq/100g soil), S (10.80 µg/soil), B (0.34 µg/soil) and Zn (1.27 µg/soil) was very low, low, low, low, medium and medium, respectively. The trial was laid out in randomized complete block design with six dispersed replications. Two plots of 500 m² were selected for each replication. One plot was under the improved pattern and the other farmer's pattern.

In the improved pattern, mung bean var. BARI Mung-6 was introduced against fallow period. The T. aus rice var. BRR1 dhan48 and T. aman rice var. Binadhan-7 was introduced instead of BR-26 and BRR1 dhan33, respectively. The agronomic parameters and cultural operation for crop production under improved and farmer's practices are presented in Table 1. All field operation and management practices of both farmer's and improved pattern were closely monitored and the data were recorded for agro-economic performance. The differences between mean was compared by t-test.

Agronomic performance *viz.*, land use efficiency, production efficiency, rice equivalent yield and sustainable yield index of cropping patterns were calculated. Land use efficiency is worked out by taking total duration of individual crop in a sequence divided by 365 days (Tomer and Tiwari, 1990). It is calculated by following formula:

$$\text{Land use efficiency} = \frac{d_1 + d_2 + d_3}{365} \times 100$$

Where d_1 , d_2 and d_3 the duration of first, second and third crops of the pattern.

Production efficiency: Production efficiency values in terms of Kg ha⁻¹day⁻¹ were calculated by total production in a cropping sequence divided by total duration of crops in that sequence (Tomer and Tiwari, 1990).

$$\text{Production efficiency} = \frac{Y_1 + Y_2 + Y_3}{d_1 + d_2 + d_3} \text{ (Kg ha}^{-1}\text{day}^{-1}\text{)}$$

Where, Y_1 : Yield of first crop; and d_1 : Duration of first crop of the pattern; Y_2 : Yield of second crop and d_2 : Duration of second crop of the pattern; Y_3 : Yield of third crop and d_3 : Duration of third crop of the pattern.

Sustainable Yield index (SYI) was worked out by the following formula suggest by Krishna and Reddy (1997).

$$\text{Sustainable yield index} = \frac{Y_{\text{mean}} - SD}{Y_{\text{max}}} \times 100$$

Where, Y_{mean} : Estimated mean yield of a practice over years; SD: Estimated standard deviation; Y_{max} : Observed maximum yield in the experiment over the years.

Rice equivalent yield:

For comparison between crop sequences, the yield of all crops was converted into rice equivalent yield (REY) on the basis of prevailing market price of individual crop (Verma and Modgal, 1983).

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

The economic indices like gross and net returns and benefit cost ratio were also calculated on the basis of prevailing market price of the produces. For economic evaluation of two different cropping sequences averaged data of two crop cycles were used. The gross cost of cultivation of different crops was calculated on the basis of different operations performed and materials used for raising the crops. Benefit cost ratio (BCR) was also calculated by the following formula:

$$\text{Benefit Cost Ratio (BCR)} = \frac{\text{Gross return (Tk ha}^{-1}\text{)}}{\text{Total (variable) cost of cultivation (Tk ha}^{-1}\text{)}}$$

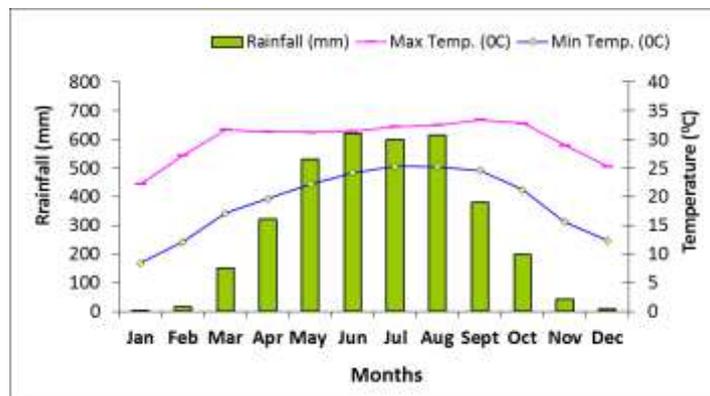


Fig. 1. Average of three years monthly total rainfall (mm), maximum and minimum air temperatures during study period (Source: Metrological Department, Sylhet).

Table 1. Agronomic practices of improved (Mungbean-T. aus-T. aman) and farmer's existing (Fallow-T. aus-T. aman) cropping patterns during 2012-15

Parameters	Cropping pattern index	Farmer's pattern (FP)			Improved pattern (IP)		
		2012-13	2013-14	2014-15	2012-13	2013-14	2014-15
Variety	C ₁	Fallow	Fallow	Fallow	BARI Mung-6	BARI Mung-6	BARI Mung-6
	C ₂	BR-26	BR-26	BR-26	BRR1 dhan48	BRR1 dhan48	BRR1 dhan48
	C ₃	BRR1 dhan33	BRR1 dhan33	BRR1 dhan33	Binadhan-7	Binadhan-7	Binadhan-7
Date of Sowing/	C ₁	Fallow	Fallow	Fallow	2-5 March	2-5 March	2-5 March
Transplant	C ₂	10-15 May	10-15 May	10-15 May	10-15 May	10-15 May	10-15 May
	C ₃	20-25 August	20-25 August	20-28 August	15-20 August	15-18 August	15-20 August
Seed rate (kg ha ⁻¹)	C ₁	Fallow	Fallow	Fallow	40-45	40-45	40-45
	C ₂	35-40	35-40	35-40	25-30	25-30	25-30
	C ₃	30-35	30-35	30-35	25-30	25-30	25-30
Planting method	C ₁	Fallow	Fallow	Fallow	Line	Line	Line
	C ₂	Line	Line	Line	Line	Line	Line
	C ₃	Line	Line	Line	Line	Line	Line
Spacing (cm)	C ₁	Fallow	Fallow	Fallow	30 × 0	30 × 0	30 × 0
(Row × Hill)	C ₂	20 × 10	20 × 10	20 × 10	25 × 15	25 × 15	25 × 15
	C ₃	20 × 15	20 × 15	20 × 15	25 × 15	25 × 15	25 × 15
Fertilizer dose (kg ha ⁻¹)	C ₁	Fallow	Fallow	Fallow	23-20-17-15-1.0	23-20-17-15-1.0	23-20-17-15-1.0
(NPKSZn)	C ₂	83-20-40-5-1	83-20-40-5-1	83-20-40-5-1	75-15-30-6-0.6	75-15-30-6-0.6	75-15-30-6-0.6
	C ₃	92-24-60-8-0.5	92-24-60-8-0.5	92-24-60-8-0.5	90-10-35-8-1.0	90-10-35-8-1.0	90-10-35-8-1.0

Table 1. *Cont'd.*

Parameters	Cropping pattern index			Farmer's pattern (FP)			Improved pattern (IP)		
	2012-13	2013-14	2014-15	2012-13	2013-14	2014-15	2012-13	2013-14	2014-15
Fertilizer application	Fallow	Fallow	Fallow	All fertilizer nutrients-NPKSZn applied as basal during final land preparation.	All fertilizer nutrients-NPKSZn applied as basal during final land preparation.	All fertilizer nutrients-NPKSZn applied as basal during final land preparation.	All fertilizer nutrients-NPKSZn applied as basal during final land preparation.	All fertilizer nutrients-NPKSZn applied as basal during final land preparation.	All fertilizer nutrients-NPKSZn applied as basal during final land preparation.
method	C ₁	C ₂	C ₃	All PKS used as basal during final land preparation. N used in 2 equal splits at 15-20 DAT and another one in 35-40 DAT.	All PKS used as basal during final land preparation. N ₂ used in 2 equal splits at 15-20 DAT and another one in 35-40 DAT.	All PKS used as basal during final land preparation. N ₂ used in 2 equal splits at 15-20 DAT and another one in 35-40 DAT.	Half of nitrogen and all other fertilizers applied as basal during final land preparation. Remaining nitrogen was top dressed at 40-45 DAS under moist soil condition	Half of nitrogen and all other fertilizers applied as basal during final land preparation. Remaining nitrogen was top dressed at 40-45 DAS under moist soil condition	Half of nitrogen and all other fertilizers applied as basal during final land preparation. Remaining nitrogen was top dressed at 40-45 DAS under moist soil condition

Table 1. Cont'd.

Parameters	Cropping pattern index	Farmer's pattern (FP)			Improved pattern (IP)		
		2012-13	2013-14	2014-15	2012-13	2013-14	2014-15
Weeding (no.)	C ₁	Fallow	Fallow	Fallow	1	1	1
	C ₂	2	2	2	2	2	2
	C ₃	1	1	1	2	2	2
Irrigation/Rainfed	C ₁	Fallow	Fallow	Fallow	Rainfed	Rainfed	Rainfed
	C ₂	Rainfed	Rainfed	Rainfed	Rainfed	Rainfed	Rainfed
	C ₃	Rainfed	Rainfed	Rainfed	Rainfed	Rainfed	Rainfed
Insect-pest control	C ₁	Fallow	Fallow	Fallow	IPM	IPM	IPM
	C ₂	Chemical	Chemical	Chemical	IPM	IPM	IPM
	C ₃	Chemical	Chemical	Chemical	IPM	IPM	IPM
Harvest time (date)	C ₁	Fallow	Fallow	Fallow	7-10 May	7-10 May	7-10 May
	C ₂	7-10 Aug	7-10 Aug	7-10 Aug	28-30 July	28-30 July	28-30 July
	C ₃	10-16 Dec	10-16 Dec	10-15 Dec	15-20 Nov	15-20 Nov	15-20 Nov
Field duration (days)	C ₁	Fallow	Fallow	Fallow	60-66	58-65	60-66
	C ₂	105-110	105-110	105-110	100-105	100-105	100-105
	C ₃	112-115	112-115	105-115	90-96	90-95	90-96
Total duration (days)		217-225	217-225	210-225	250-267	248-265	250-267

C₁: Fallow/Mungbean; C₂: T. aus; C₃: T. aman; IPM: integrated pest management.

Results and Discussion

Grain/Seed Yield of the Cropping Patterns

Improved pattern took 249-266 days against 214-225 days due to inclusion of mungbean in the pattern. This indicates that mungbean could easily be grown or fitted before T. aus rice. The yield of rice was significantly higher in the improved pattern as compared to farmers existing pattern during individual years and also in mean data (Table 2). Variation in the yield of rice as evident in the improved pattern might be due to change of variety with improved production technologies. Similar results were also obtained by Nazrul *et al.* (2013) and Khan *et al.* (2005) in case of rice based cropping sequences. In all the years, farmers' pattern gave lower grain yield of rice due to imbalance use of fertilizers and traditional management practices. But in year of 2012-13, the yield of T. aus rice was little lower in improved pattern due to partial lodging. Furthermore in year of 2014-15, the yield of T. aman rice was also insignificantly lower in improved pattern compared to farmer's pattern due to attack of rice bug at panicle formation to flowering. Chlorpyrifos insecticide (Dursban 20 EC @ 20 ml in 10 liters of water for 5 decimal areas) was sprayed for controlling this pest. The rice variety, Binadhan-7 in improved pattern performed better than BRRI dhan33 in farmers' practices due to higher yield potential of the variety.

By-product yield of the cropping patterns

The improved cropping pattern produced higher amount of total by-product yield (11.13 t ha⁻¹) than the by-product yield of the crops (9.03 t ha⁻¹) of the farmers' pattern (Table 2). The by-product yield of improved pattern was higher due to introduction and change of variety with improved technologies for the component crops. In all the years, mungbean contributed valuable by-product. On the contrary, farmers are not able to sale by-product (rice straw) in the local market; whereas, the by-product of mungbean can be used as green manure and incorporate in the field for soil health improvement.

Rice equivalent yield

The component crops of Mungbean-T. aus-T. aman rice cropping pattern under improved practices (IP) gave higher T. aman rice equivalent yields against grain yield as well as by-product in all the years. The mean rice equivalent yield under improved cropping pattern also produced higher rice equivalent yield over farmers' traditional cropping pattern (Table 3). On an average, the T. aman rice equivalent yield in improved pattern increased 73% over the crops under farmers' practices. Inclusion of high yielding new crop varieties with improved management practices increased the higher T. aman rice equivalent yield. It was also due to higher price of components crops in the improved pattern. Lower rice equivalent yield was obtained in the farmers' pattern probably due to variety and traditional management practices.

Production efficiency

Maximum production efficiency was obtained from improved pattern during individual years and also means data (Table 3). The higher production efficiency of improved cropping pattern might be due to inclusion of a new or modern varieties and management practices. In conversely, the lowest production efficiency was observed in farmers' pattern where crop remained in the field for shorter time and yields were also lower, leading to lower production per day. Mean production efficiency ($47.88 \text{ kg ha}^{-1}\text{day}^{-1}$) was higher in improved pattern and lower ($36.97 \text{ kg ha}^{-1}\text{day}^{-1}$) in farmers' pattern. Similar trend were noted by Nazrul *et al.* (2013) and Khan *et al.* (2005) in case of improved cropping sequences.

Land use efficiency

Land use efficiency is the effective use of land in a cropping year, which mostly depends on crop duration. The average land-use efficiency indicated that improved pattern used the land for 67.66% period of the year, whereas farmer's pattern used the land for 50.68% period of the year (Table 3). The land use efficiency was higher in improved pattern due to cultivation of mungbean as component crop in fallow period.

Table 2. Productivity of improved (Mungbean-T. aus-T. aman) and farmer's existing (Fallow-T. aus-T. aman) cropping patterns during 2012-15

Years	Cropping patterns	Seed/Grain yield (t ha^{-1})			By product yield (t ha^{-1})		
		Fallow/ Mungbean	T. aus	T. aman	Fallow/ Mungbean	T. aus	T. aman
2012-13	FP	-	3.25	3.75	-	4.36	4.79
	IP	1.12	3.20	4.50	1.68	4.31	4.86
2013-14	FP	-	3.51	3.64	-	4.40	4.51
	IP	1.30	3.85	4.35	1.98	4.65	4.70
2014-15	FP	-	3.47	3.70	-	4.37	4.65
	IP	1.10	4.30	3.85	1.66	4.92	4.49
Mean	FP	-	3.41	3.70	-	4.37	4.66
	IP	1.17	3.78	4.23	1.77	4.62	4.74

Note- FP: Farmer's pattern, IP: Improved patter;

The costs (Tkkg^{-1}): rice seed (32.00), mungbean seed (150.00), and, urea (20.00), TSP (22.00) and MoP (15.00);

Among field operations, the cost of plowing was taken as $\text{Tk } 10 \text{ decimal}^{-1}$, labour cost of $\text{Tk } 300\text{m}^{-1}\text{day}^{-1}$. Gross returns included income from sale of main and by-products (Tk ka^{-1}) of all crops; T. aus rice (19.00), T. aman rice (20.50), mungbean (70.00), stover (1.00).

Sustainable yield index

The sustainable yield index (SYI) of farmer's and improved cropping pattern is presented in Table 3. The values of sustainable yield index as a measure of sustainability of the system which was high in the improved cropping system (66.88-95.72%) over farmer's practices (63.55-76.63%). The results showed that between two different cropping systems Mungbean-T. aus-T. aman rice recorded the highest mean SYI of 77.63% followed by Fallow-T. aus-T. aman rice (70.10%). So, cropping system involving mungbean in fallow period and modern varieties of T. aus and T. aman rice recorded higher SYI compared to fallow-rice based crop sequences. The results are in agreement with the findings of Nazrul *et al.* (2013) and Ram *et al.*, (2012). This indicated that improved pattern is therefore, more stable than farmer's pattern. Mungbean is providing special advantage regarding utilization of fallow land and can improve the soil health.

Table 3. Rice equivalent yield, production efficiency, land use efficiency and sustainable yield index of improved and farmers patterns at farmer's field during 2012-15

Years	Cropping patterns	Rice (T. aman) equivalent yield (tha ⁻¹)	Production efficiency (Kg ha ⁻¹ day ⁻¹)	Land use efficiency (%)	% sustainable yield index (SYI)
2012-13	FP	6.76	35.57	52.05	63.55
	IP	11.36	44.54	69.86	66.88
2013-14	FP	6.89	37.24	50.68	70.13
	IP	12.43	50.12	67.94	80.30
2014-15	FP	6.86	38.11	49.31	76.63
	IP	11.66	48.99	65.20	95.72
Mean	FP	6.83	36.97	50.68	70.10
	IP	11.81	47.88	67.66	77.63

Economic

Between two crop sequences, the improved cropping pattern showed its superiority over farmers' existing pattern during three consecutive years of cropping season. On an average, gross return of the improved pattern was Tk.242105 ha⁻¹ which was more than 73% higher than farmers' pattern of Tk.140015 ha⁻¹ (Table 4). The production cost of the improved pattern (Tk.115343 ha⁻¹) was higher than farmers' pattern (Tk.80973 ha⁻¹) due to introduction of mungbean in fallow period, cost of fertilizer and other inputs. The gross margin was substantially higher in the improved pattern (Tk.126762 ha⁻¹) than farmers' pattern (Tk. 59042 ha⁻¹). Inclusion of mungbean and improved varieties of rice in these cropping systems, increasing the system productivity fetched higher market price; thereby, increasing the gross margin. The 114% additional gross margin was achieved by adding 42% additional cost in the improved pattern. Returns per Taka invested were highest for mungbean-T. aus-T. aman rice (2.10) over the farmers' pattern (1.73).

Table 4. Cost benefit analysis of improved and farmer's existing cropping pattern at farmer's field (average of three years)

Years	Cropping patterns	Gross return (Tk ha ⁻¹)	Cost of cultivation (Tk ha ⁻¹)	Gross margin (Tk ha ⁻¹)	BCR
2012-13	FP	138580	80670	57910	1.72
	IP	232880	115105	117775	2.02
2013-14	FP	141245	80670	60575	1.82
	IP	254815	115105	139710	2.21
2014-15	FP	140630	81580	59050	1.72
	IP	239030	115820	123210	2.06
Mean	FP	140015	80973	59042	1.73
	IP	242105	115343	126762	2.10

The costs (Tk kg⁻¹): rice seed (32.00), mungbean seed (150.00), and, urea (20.00), TSP (22.00) and MoP (15.00).

Among field operations, the cost of plowing was taken as Tk. 10 decimal⁻¹, labour cost of Tk. 300m⁻¹day⁻¹. Gross returns included income from sale of main and by-products (Tk ka⁻¹) of all crops; T. aus rice (19.00), T. aman rice (20.50), mungbean (70.00), stover (1.00).

Conclusion

Three years study revealed that Mungbean-T. aus-T. aman rice cropping pattern is more productive, sustainable and remunerative for medium high land under Eastern Surma Kushiyara Floodplain (AEZ 20). So, farmers of commanding area could follow Mungbean (var. BARI Mung-6)-T. aus (var. BRRI dhan48)-T. aman rice (var. Binadhan-7) cropping pattern for higher productivity and profitability as well as utilization of fallow land sustainable soil health.

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COMBINING ABILITY ESTIMATES IN MAIZE (*ZEA MAYS* L.) THROUGH LINE \times TESTER ANALYSIS

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Abstract

Eighteen advanced S₄ lines of maize extracted from NK46 were evaluated through line \times tester method by using two testers for grain yield and its components. General combining ability (GCA) and specific combining ability (SCA) effects of crosses were determined to evaluate the prospective inbred lines. Highly significant genotypic differences for all of six characters, i.e. days to 50% tasselling, Days to 50% silking, plant height, ear height, 1000 kernel weight, and yield indicated presence of wide range of variability among the genotypes for those traits. Non-additive gene action was predominant. Four lines for days to 50% tasselling, one for days to 50% silking, two for plant height, one for ear height, three for 1000 kernel weight, and four for yield were found with significant GCA effects in desired direction. For days to tasselling, days to silking, plant height and ear height, none of 36 cross combinations confirmed significant SCA effects. Five combinations showed significant positive SCA effects for 1000 kernel weight. For yield two crosses showed significant positive SCA. Considering the results of present study, based on GCA and SCA analysis of observed characters seven lines namely, NK46-2, NK46-4, NK46-10, NK46-13, NK46-18, NK46-43 and NK46-44 were selected for further breeding program

Keywords: *Zea mays* L., Line \times Tester, combining ability.

Introduction

Maize (*Zea mays*. L) is the third most important cereal crop with abundant natural diversity. In Bangladesh, its area and production is increasing. Maize is a highly allogamous crop and it has been successfully exploited for the production of hybrids. Superior inbred lines with good combining ability are prerequisite for development of superior hybrids. Maize breeding methodology generally involves the development of inbred lines from heterogeneous source population through continuous selfing. Selection of segregants at early generations is very important to reduce the number of inbred lines to a manageable size. Combining ability at early and advanced generation in maize is an excellent tool which helps

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to discern the goal and direction in a breeding program (Manonmani and Khan, 2003). Its role is important to decide parents, crosses and appropriate breeding procedure to be followed to select desirable segregants (Salgotra *et al.*, 2009). It has been reported that selection during inbreeding based on the performance of test cross progeny is highly effective in improving the GCA of inbred lines. In this context, L×T analysis (Kempthorne, 1957) has widely been used for evaluation of large number of lines by crossing them with testers to obtain superior inbred lines with desired traits. The present study involving a line × tester analysis was aimed to determine the general combining ability (GCA) and specific combining ability (SCA) of developed crosses for different traits and to explore the superior lines with earliness, low plant and ear height with higher yield for advancing to the next generation.

Materials and Method

Eighteen lines of maize were selected from S₄ lines which were developed from a source population of NK46 through recycling (repeated inbreeding) for four generations. Two inbred lines with diverse genetic base namely BIL28 (Jahan *et al.* 2014) and BIL79 were selected as testers. During rabi, 2012-13 the hybridization was performed following Line × Tester mating design using 18 S₄ lines (listed in Table 1) as female and testers as male parents to obtain 36 cross combinations. The 36 cross combinations and 18 advanced lines and two testers were grown in an Alpha lattice design with two replications with spacing of 75 x 20 cm at Regional Agricultural Research Station, Bangladesh Agricultural Research Institute, Hathazari, Chittagong, Bangladesh during rabi, 2013-14. Seeds were sown in 29 November, 2013. One border row was used at each end of the replication to minimize the border effect. Fertilizers were applied @ 250, 120, 120, 40, 5 and 1 kg/ha of N, P₂O₅, K₂O, S, Zn and B respectively. One third urea and total amount of other fertilizers were applied at final land preparation. The remaining urea was applied in two installments as top dressing. Standard agronomic practices including two weeding and four irrigations and plant protection measures were followed to raise a healthy crop. The data were recorded on 10 randomly selected plants for quantitative characters viz. days to 50% tasselling, days to 50% silking, plant height (cm), ear height (cm), 1000 grain weight (g), and grain yield (g). Combining ability analysis was done as per the method given by Kempthorne (1957).

Results and Discussion

The analysis of variance presented in Table 2 revealed highly significant differences among the genotypes for all the characters, suggesting sufficient genetic variability among the genotypes. Highly significant differences between parents (lines and testers) and interactions of parents and crosses for all the traits indicated wide range of variability present among them. Variability between crosses is highly significant for days to tasselling, 1000 kernel weight and yield

which were in conformity with Shushay *et al.* (2013). Lines differed at highly significant level in favor of days to tasselling, days to silking, ear height, and yield and at significant level for plant height which represented prevalence of substantial variability in them. Testers differed significantly for days to 50% tasselling and days to 50% silking where difference between Line×Tester was highly significant for 1000 kernel weight and yield (Table 2). Jahan *et al.* (2014) also found significant difference for days to tasselling, and silking between testers and for yield between Line×Tester interaction. Similar genotypic difference for grain weight, grain yield and other characters were reported by Narro *et al.* (2003) and Sofi and Rathor (2006).

Table 1. List of 18 selected S₄ lines and two testers used in Line×Tester mating

Sl. No.	Parents	Sl. No.	Parents
	Line		Line
1	NK46-2	10	NK46-18
2	NK46-4	11	NK46-21
3	NK46-6	12	NK46-22
4	NK46-7	13	NK46-23
5	NK46-8	14	NK46-34
6	NK46-10	15	NK46-39
7	NK46-12	16	NK46-40
8	NK46-13	17	NK46-43
9	NK46-14	18	NK46-44
Tester			
1	BIL 28		
2	BIL 79		

The higher estimation of dominance variance (σ^2_{sca}) as compared to additive variance (σ^2_{gca}) which produced the below one ratio of σ^2_{gca} to σ^2_{sca} for all the six characters (Table 2) probably due to predominance of non-additive gene action suggested the scope of improvement of these characters through heterosis breeding. Similar non-additive gene action for all traits under this study was also reported by Talukder *et al.* (2016). Suneetha *et al.* (2000) got non-additive gene action for days to 50% tasselling and days to 50% silking. Amin *et al.* (2014a) found non-additive gene action for plant height, 1000 grain weight and yield. Singh and Singh (1998) reported non-additive gene action for plant height, ear height, 1000 grain weight where Mahto and Ganguly (2001) also reported non additive gene action for grain yield that supported the present study. However, Amin *et al.* (2014b) found similar results for days to silking, and yield but different results i.e. additive gene action for plant height, ear height and grain weight which might be due to use of different genotypes and mating design in their study.

Table 2. ANOVA showing mean squares and estimates of variance for grain yield and other characters in maize

Source	df	Days to 50% tasselling	Days to 50% silking	Plant height (cm)	Ear height (cm)	1000-kernel weight (g)	Yield (t/ha)
Genotypes	55	36.83**	32.83**	901.53**	553.74**	2623.53**	8.86**
Parents (P)	19	27.75**	26.87**	1142.34**	716.35**	1943.51**	4.33**
P vs C	1	860.89**	857.59**	18003.01**	10520.76**	10142.42**	190.96**
Crosses (C)	35	18.22**	12.50	282.19	180.70	2777.86**	6.12**
Lines (L)	17	29.24**	20.89**	401.45*	315.16**	3190.79	8.29*
Testers (T)	1	29.39*	17.01*	5.31	13.52	5341.12	1.01
L × T	17	6.54	3.84	179.23	56.07	2214.15**	4.25**
Error	55	5.92	8.06	183.32	159.99	330.79	1.47
Estimates of component of variances							
σ^2g (Line)	-	5.68	4.26	55.55	64.77	244.16	1.01
σ^2g (Tester)	-	0.63	0.37	4.83	1.18	86.86	0.09
σ^2gca	-	0.22	0.16	1.93	2.33	10.54	0.03
σ^2sca	-	0.31	2.11	2.05	51.96	941.68	1.39
σ^2gca/σ^2sca	-	0.71	0.08	0.94	0.04	0.01	0.03

*P=0.05 and **P=0.01

The contribution of lines, testers and their interactions to total variances were presented in Table 3. The proportional contribution of lines and interactions to total variances were much higher than testers in all the traits. However, the contributions of lines were higher than the interactions to total variances for all the characters. This suggested female parents contributed maximum to total variance in maize, followed by interaction. Testers contributed the lowest to total variance, which is in agreement with Amiruzzaman and Amin (2011a, b), Parvin (2009), Rissi *et al.* (1991) and Talukder and Banik (2012).

Table 3. Proportional contribution of lines, testers and their interactions to total variance in maize

Source	Days to tasselling	Days to silking	Plant height (cm)	Ear height (cm)	1000-kernel weight (g)	Yield (t/ha)
Line	77.96	81.19	69.10	84.71	55.79	65.79
Tester	4.61	3.89	0.05	0.21	5.49	0.47
Line × Tester	17.43	14.92	30.85	15.07	38.71	33.74

General combining ability effects

Selection of parents with good general combining ability is a prime requisite for any successful breeding program especially for heterosis breeding. The general combining ability effects and *per se* performance of parents (line and tester) were presented in Table 4. Negative GCA effects are desired for days to tasselling, days to silking, plant height and ear height; but in case of yield the choice for GCA effects is in positive direction. The line NK46-10 and NK46-40 exhibited highly significant negative GCA effects for days to tasselling where NK46-18 and NK46-44 showed significant negative GCA effects. For days to silking only NK46-40 showed highly significant negative GCA effects. These lines can be utilized for evolving earliness. Hussain *et al.* (2003), Roy *et al.* (1998), and Uddin *et al.* (2006) also observed similar phenomenon in their study. For plant height, line NK46-22 contributed significant negative GCA effects where NK46-4 was with significant negative GCA effect for both plant height and ear height. This indicated that these parents can be utilized for developing dwarf hybrids to reduce yield loss due to root and stem lodging. One line (NK46-39) with highly significant positive GCA effects and two lines (NK46-8 and NK46-23) with significant positive GCA in favor of 1000 kernel weight found in the study may be important for developing bold grain, whereas other five lines showed significant negative GCA for this trait. Similar to the current findings both positive and negative GCA effects for 1000 kernel weight were reported by Koppad (2007) and Wali *et al.* (2010). Parents with good GCA effects for 1000-kernel weight were not found with good GCA for yield. It might occur due to the effects of other yield contributing characters like ear/plant, length of ear, row/ear, kernel/row etc. Highly significant positive GCA effects for yield in NK46-2, NK46-13, NK46-18, NK46-43 and NK46-44 indicated that these parents were good general combiner. These lines were promising to be used for exploiting more positive alleles for yield. Significant GCA effect for yield in maize was also reported by Ivy and Hawlader (2000) and Paul and Duara (1991). The lines with high GCA effects representing additive gene action in inheritance of traits, may be utilized in hybridization program to improve respective traits through transgressive segregation.

Specific combining ability effects (SCA)

Specific combining ability and mean of the crosses for grain yield, its components and other characters were presented in Table 5. In this investigation, none of the crosses exhibited significant negative or positive SCA effects for days to 50% tasselling, 50% silking, plant height and ear height, although some crosses involved significant negative general combining parents. In case of maize, negative value is expected for these traits to develop early and short statured plant. Sixteen crosses revealed negative but non-significant SCA effects for both days to tasselling and silking. Nine crosses were non-significant negative specific combiner for both plant height and ear height. Uddin *et al.* (2006) also

reported failure of some crosses to show significant negative SCA effects for plant height and ear height while parents were found with significant negative GCA effects. Significant positive SCA effects were found in NK46-8×BIL28, NK46-13×BIL79, NK46-22×BIL79, NK46-40×BIL79 and NK46-43×BIL28, for 1000-kernel weight where only the parent NK46-8 was with good GCA effects for this trait. Although 1000-kernel weight is one of the important yield contributing characters the crosses with high SCA effects for this trait failed to give high SCA effects for yield. It might be due to the effects of other yield contributing characters. Positive SCA effect is expected for yield and NK46-4×BIL79 and NK46-10×BIL28 combinations exhibited significant positive SCA effects which were in desired direction. But the parents confirming significant positive GCA effects could not produce cross combinations with significant positive SCA. Das and Islam (1994), Shushay *et al.* (2013) and Uddin *et al.* (2006) found crosses with significant positive SCA effects for yield. The present results were in line with the earlier findings of Ivy and Howlader (2000) where they stated that good general combiner do not always show high SCA effects in their crosses. On the contrary Paul and Duara (1991) reported that parents with high GCA always produce hybrids with high SCA.

Table 4. General combining ability (GCA) effects and mean of parents for grain yield, yield components and other characters in maize

Sl. No.	Parents	Days to 50% tasselling		Days to 50% silking		Plant height (cm)	
		GCA	mean	GCA	mean	GCA	mean
Tester							
1	BIL 28	0.64	101.00	0.49	105.50	-0.27	191.20
2	BIL 79	-0.64	94.50	-0.49	98.50	0.27	195.10
	SE (g_i)	0.41	-	0.47	-	2.26	-
	SE ($g_i g_j$)	0.57	-	0.67	-	3.19	-
Line							
1	NK46-2	-0.89	95.00	-1.13	98.50	-3.67	159.75
2	NK46-4	2.86*	107.50	2.88	110.50	-16.96*	130.00
3	NK46-6	0.36	100.50	0.63	104.50	-9.97	171.00
4	NK46-7	-0.89	103.00	0.13	107.00	-5.72	164.70
5	NK46-8	-1.89	97.50	-0.88	101.00	-7.27	206.70
6	NK46-10	-3.64**	102.00	-2.63	106.50	0.63	146.10
7	NK46-12	3.11*	102.50	3.13*	105.50	-1.02	174.90
8	NK46-13	4.61**	105.00	2.88	107.50	0.38	148.45
9	NK46-14	-0.64	96.00	-1.63	99.50	18.45*	200.70
10	NK46-18	-2.89*	99.00	-2.13	102.50	14.13	191.60

Sl. No.	Parents	Days to 50% tasselling		Days to 50% silking		Plant height (cm)	
		GCA	mean	GCA	mean	GCA	mean
11	NK46-21	2.86*	97.50	1.88	100.50	15.83	171.60
12	NK46-22	3.11	97.50	2.88	101.00	-2.12*	163.50
13	NK46-23	-0.89	99.00	-0.88	103.00	-2.67	177.10
14	NK46-34	1.11	100.50	0.63	103.00	-6.59	158.04
15	NK46-39	-1.64	92.50	-1.88	96.50	-0.66	209.75
16	NK46-40	-4.64**	96.00	-4.13**	98.50	7.73	182.50
17	NK46-43	2.61*	100.00	2.63	103.00	-12.72	212.10
18	NK46-44	-2.64*	102.00	-2.38	105.50	12.28	213.50
	SE (g_i)	1.22	-	1.42	-	6.77	-
	SE ($g_i g_j$)	1.72	-	2.01	-	9.57	-

*P=0.05 and **P=0.01

Table 4. cont'd.

Sl. No.	Parents	Ear height (cm)		1000 kernel weight (g)		Yield (t/ha)	
		GCA	mean	GCA	mean	GCA	mean
Tester							
1	BIL 28	0.43	94.40	8.61	297.50	-0.12	7.33
2	BIL 79	-0.43	93.50	-8.61	255.50	0.12	4.86
	SE (g_i)	2.11	-	3.03	-	0.20	-
	SE ($g_i g_j$)	2.98	-	4.29	-	0.29	-
Line							
1	NK46-2	-12.12	61.63	10.67	367.50	1.66**	7.25
2	NK46-4	-16.71*	60.25	-28.05**	311.60	-0.74	6.62
3	NK46-6	-2.87	76.70	-24.80*	268.00	-2.55**	5.96
4	NK46-7	-1.46	77.80	-17.30	250.50	0.34	4.38
5	NK46-8	-5.76	107.00	58.43*	279.00	0.48	7.97
6	NK46-10	-6.26	59.30	4.56	331.00	-0.35	6.45
7	NK46-12	-1.96	75.70	3.13	298.00	0.36	5.64
8	NK46-13	-2.61	52.00	11.50	317.50	1.92**	6.77
9	NK46-14	12.24	84.70	-46.37**	322.31	-1.64*	9.97
10	NK46-18	15.54*	90.70	12.95	338.54	2.21**	6.28
11	NK46-21	10.89	80.50	18.20	322.94	0.79	8.92
12	NK46-22	-6.96	61.30	-34.05**	352.50	-2.10**	6.83

Sl. No.	Parents	Ear height (cm)		1000 kernel weight (g)		Yield (t/ha)	
		GCA	mean	GCA	mean	GCA	mean
13	NK46-23	-1.61	70.90	24.95*	331.50	-0.62	5.76
14	NK46-34	7.34	60.88	-9.53	298.50	-1.21	5.16
15	NK46-39	-2.61	110.40	46.62**	339.50	-0.02	7.06
16	NK46-40	4.89	72.60	-37.74**	297.00	-1.44	8.74
17	NK46-43	-2.86	114.10	6.95	293.00	1.86*	4.95
18	NK46-44	12.89	105.30	-0.07	292.00	1.06**	5.26
	SE (g_i)	6.32	-	9.09	-	0.61	-
	SE (g_i-g_j)	8.94	-	12.86	-	0.86	-

*P=0.05 and **P=0.01

Table 5. Specific combining ability (SCA) and mean of the crosses for grain yield, its components and other characters in maize

Sl. No.	Crosses	Days to tassel		Days to silk		Plant height (cm)	
		SCA	mean	SCA	mean	SCA	mean
1.	NK46-2 × BIL28	-0.89	92.50	-0.49	96.00	6.37	207.30
2.	NK46-2 × BIL79	0.89	93.00	0.49	96.00	-6.37	195.10
3.	NK46-4 × BIL28	0.36	97.50	0.01	100.50	3.48	191.13
4.	NK46-4 × BIL79	-0.36	95.50	-0.01	99.50	-3.48	184.70
5.	NK46-6 × BIL28	-0.64	94.00	-0.74	97.50	-1.13	193.50
6.	NK46-6 × BIL79	0.64	94.00	0.74	98.00	1.13	196.30
7.	NK46-7 × BIL28	-0.39	93.00	-0.24	97.50	-4.78	194.10
8.	NK46-7 × BIL79	0.39	92.50	0.24	97.00	4.78	204.20
9.	NK46-8 × BIL28	-0.39	92.00	-0.24	96.50	0.17	197.50
10.	NK46-8 × BIL79	0.39	91.50	0.24	96.00	-0.17	197.70
11.	NK46-10 × BIL28	-0.64	90.00	-1.49	93.50	15.17	220.40
12.	NK46-10 × BIL79	0.64	90.00	1.49	95.50	-15.17	190.60
13.	NK46-12 × BIL28	1.11	98.50	1.26	102.00	-3.38	200.20
14.	NK46-12 × BIL79	-1.11	95.00	-1.26	98.50	3.38	207.50
15.	NK46-13 × BIL28	-1.39	97.50	-0.99	99.50	-10.28	194.70
16.	NK46-13 × BIL79	1.39	99.00	0.99	100.50	10.28	215.80
17.	NK46-14 × BIL28	2.36	96.00	1.51	97.50	0.30	223.35
18.	NK46-14 × BIL79	-2.36	90.00	-1.51	93.50	-0.30	223.30
19.	NK46-18 × BIL28	-0.39	91.00	0.01	95.50	-0.73	218.00
20.	NK46-18 × BIL79	0.39	90.50	-0.01	94.50	0.73	220.00

Sl. No.	Crosses	Days to tassel		Days to silk		Plant height (cm)	
		SCA	mean	SCA	mean	SCA	mean
21.	NK46-21 × BIL28	1.86	99.00	2.01	101.50	-1.53	218.90
22.	NK46-21 × BIL79	-1.86	94.00	-2.01	96.50	1.53	222.50
23.	NK46-22 × BIL28	0.11	97.50	0.01	100.50	-7.18	195.30
24.	NK46-22 × BIL79	-0.11	96.00	-0.01	99.50	7.18	210.20
25.	NK46-23 × BIL28	0.11	93.50	-0.24	96.50	-11.23	190.70
26.	NK46-23 × BIL79	-0.11	92.00	0.24	96.00	11.23	213.70
27.	NK46-34 × BIL28	1.61	97.00	1.26	99.50	5.66	203.68
28.	NK46-34 × BIL79	-1.61	92.50	-1.26	96.00	-5.66	192.90
29.	NK46-39 × BIL28	-2.64	90.00	-1.24	94.50	2.86	206.80
30.	NK46-39 × BIL79	2.64	94.00	1.24	96.00	-2.86	201.63
31.	NK46-40 × BIL28	-1.64	88.00	-0.99	92.50	-0.53	211.80
32.	NK46-40 × BIL79	1.64	90.00	0.99	93.50	0.53	213.40
33.	NK46-43 × BIL28	0.61	97.50	0.26	100.50	10.22	202.10
34.	NK46-43 × BIL79	-0.61	95.00	-0.26	99.00	-10.22	182.20
35.	NK46-44 × BIL28	0.86	92.50	0.26	95.50	-3.48	213.40
36.	NK46-44 × BIL79	-0.86	89.50	-0.26	94.00	3.48	220.90
	SE (<i>Sij</i>)	1.72	-	2.01	-	9.57	-
	SE (<i>Sij-Skl</i>)	2.43	-	2.84	-	13.54	-

*P=0.05 and **P=0.01

Table 5. cont'd.

Sl. No.	Crosses	Ear height (cm)		1000 kernel weight (g)		Yield (t/ha)	
		SCA	mean	SCA	mean	SCA	mean
1.	NK46-2 × BIL28	1.65	90.68	-25.69	321.65	-1.41	9.46
2.	NK46-2 × BIL79	-1.65	86.50	25.69	355.80	1.41	12.52
3.	NK46-4 × BIL28	-6.03	78.40	23.89	332.50	-2.11*	6.37
4.	NK46-4 × BIL79	6.03	89.60	-23.89	267.50	2.11*	10.82
5.	NK46-6 × BIL28	0.53	98.80	-7.86	304.00	1.15	7.81
6.	NK46-6 × BIL79	-0.53	96.88	7.86	302.50	-1.15	5.74
7.	NK46-7 × BIL28	-4.98	94.70	14.64	334.00	0.53	10.09
8.	NK46-7 × BIL79	4.98	103.80	-14.64	287.50	-0.53	9.26
9.	NK46-8 × BIL28	-3.08	92.30	33.90*	429.00	0.77	10.46
10.	NK46-8 × BIL79	3.08	97.60	-33.90*	343.97	-0.77	9.16
11.	NK46-10 × BIL28	10.42	105.30	19.00	360.23	2.13*	10.99

Sl. No.	Crosses	Ear height (cm)		1000 kernel weight (g)		Yield (t/ha)	
		SCA	mean	SCA	mean	SCA	mean
12.	NK46-10 × BIL79	-10.42	83.60	-19.00	305.00	-2.13*	6.97
13.	NK46-12 × BIL28	-2.58	96.60	-24.80	315.00	0.42	9.99
14.	NK46-12 × BIL79	2.58	100.90	24.80	347.36	-0.42	9.39
15.	NK46-13 × BIL28	0.77	99.30	-39.06**	309.10	-0.66	10.47
16.	NK46-13 × BIL79	-0.77	96.90	39.06**	370.00	0.66	12.03
17.	NK46-14 × BIL28	1.12	114.50	-17.12	273.17	-0.27	7.30
18.	NK46-14 × BIL79	-1.12	111.40	17.12	290.19	0.27	8.08
19.	NK46-18 × BIL28	2.62	119.30	5.89	355.50	-1.10	10.32
20.	NK46-18 × BIL79	-2.62	113.20	-5.89	326.50	1.10	12.76
21.	NK46-21 × BIL28	0.57	112.60	0.14	355.00	0.70	10.71
22.	NK46-21 × BIL79	-0.57	110.60	-0.14	337.50	-0.70	9.54
23.	NK46-22 × BIL28	-3.98	90.20	-27.61*	275.00	-0.15	6.96
24.	NK46-22 × BIL79	3.98	97.30	27.61*	313.00	0.15	7.50
25.	NK46-23 × BIL28	-0.83	98.70	16.89	378.50	0.35	8.94
26.	NK46-23 × BIL79	0.83	99.50	-16.89	327.50	-0.35	8.49
27.	NK46-34 × BIL28	-0.88	107.60	-0.59	326.54	-0.97	7.03
28.	NK46-34 × BIL79	0.88	108.50	0.59	310.50	0.97	9.21
29.	NK46-39 × BIL28	4.17	102.70	9.56	392.85	0.14	9.34
30.	NK46-39 × BIL79	-4.17	93.50	-9.56	356.50	-0.14	9.30
31.	NK46-40 × BIL28	1.97	108.00	-33.93*	265.00	0.53	8.30
32.	NK46-40 × BIL79	-1.97	103.20	33.93*	315.63	-0.53	7.48
33.	NK46-43 × BIL28	-0.08	98.20	37.39**	381.00	0.72	11.80
34.	NK46-43 × BIL79	0.08	97.50	-37.39**	289.00	-0.72	10.59
35.	NK46-44 × BIL28	-1.33	112.70	15.38	351.98	-0.76	9.51
36.	NK46-44 × BIL79	1.33	114.50	-15.38	304.00	0.76	11.27
	SE _(Sij)	8.94	-	12.86	-	0.86	-
	SE _(Sij-Skl)	12.65	-	18.19	-	1.21	-

*P=0.05 and **P=0.01

Considering GCA and SCA effects in preferential direction some lines can be selected for further breeding. NK46-10 was observed with good GCA for days to tasselling and good SCA for yield. NK46-18 was good general combiner for days to tasselling as well as yield. Thus NK46-10 and NK46-18 were selected for earliness and yield. The line NK46-4 was selected for desired GCA effects regarding plant height and ear height and good SCA effect for yield (Table 4 and

Table 5). On the other hand NK46-2, NK46-13, NK46-43 and NK46-44 were selected for good GCA effects for yield only.

Conclusion

Seven lines namely, NK46-4 for short plant and ear height and yield, NK46-10 and NK46-18 for earliness as well as yield, NK46-2, NK46-13, NK46-43 and NK46-44 for yield only were selected. These lines may be utilized for advancing to next generation.

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**SEED PRODUCTION POTENTIALITY IN YIELD AND QUALITY OF
EGGPLANT (*Solanum melongena* L.) GROWN UNDER SUMMER AND
WINTER SEASONS**

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N. A. IVY⁴ AND S. AHMAD⁵

Abstract

Two separate field experiments were conducted at the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during April to October 2012 (summer season) and October 2012 to March 2013 (winter season) to evaluate and compare the seasonal effect on fruit set, seed yield and seed quality of eggplant. Six eggplant varieties (BARI Begun-1, BARI Begun-5, BARI Begun-6, BARI Begun-8, BARI Begun-9 and Khotkhotia) were grown separately with proper isolation in both the seasons. Significant variation in fruit set per plant, seed yield per fruit and seed quality of eggplant were observed due to execution of growing seasons. The highest seed yield per fruit was obtained from BARI Begun-6 in both the seasons. Number of fruits per plant, seeds per fruit and 1000-seed weight showed the highest in winter season (October to March) as a result the highest seed yield was obtained from the same season. Winter season also showed the best seed quality attributes like germination (%), co-efficient of germination and vigour index irrespective of variety used. Based on seed yield and seed quality, winter season (October to March) found to be more favourable for quality seed production of eggplant in Bangladesh condition.

Keywords: *Solanum melongena* L, growing season, seed yield and quality.

Introduction

Eggplant (*Solanum melongena* L.) is one of the most popular, year round and economically important vegetable among small scale farmers and low income consumers of the entire universe (FAO, 2000). It is a perennial but grown commercially as an annual crop and extensively cultivated in all parts of Bangladesh (Chada, 1993) and covers about 15% of the total vegetable area of the country (Rahman, 2005).

Eggplant is originated from Indian subcontinent and is now generally grown as a vegetable throughout the tropical, sub-tropical and warm temperate areas of the world (Chen, 2001). Environmental factors are important for the production of eggplant seed. The crop requires a long and warm season during growth and fruit maturation. The same climatic factors that influence the cultivation of eggplant as a market vegetable also act as seed production. Eggplant is more susceptible to lower temperatures than tomato and pepper (Chen, 2001). Its production benefit

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from day time temperatures of 27°C to 32°C and warm night temperatures of 21°C to 27°C (Hartman *et al.*, 1988). The optimum growing temperature is 22-30°C and growth ceases at temperatures below 15°C. Pollen deformity increases at temperature of 15-16°C (Malarkodi *et al.*, 2006). A daily mean temperature of 18 to 21°C is most favourable for better growth and yield. Brinjal seeds germinate well at 25°C (Bose *et al.*, 1993) whereas, Sanwal *et al.* (1997) reported that plant grown in the field with maximum temperatures in the range 36.7°C-39.8°C reduced fruit set and fruit yield. As a result increased anther tip burning, reduced pollen germination and pollen tube lengths which affect the total seed yield. Eggplant requires 3000-8000 foot candles of light, minimum 10°C, maximum 35°C and optimum of 20-25°C temperature for crop growth (Hazra and Som, 1999). Therefore, considering above facts, the present investigation was undertaken to evaluate the seasonal effect on fruit set, seed yield and seed quality of eggplant.

Materials and Method

Two separate field experiments were conducted at the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during April to October 2012 (summer season) and October 2012 to March 2013 (winter season). The soil was silty clay loam with a pH 5.90 and belongs to the 'Shallow Red-Brown Trace' soil of Salna series under Madhupur tract (24.09° N latitude and 90.26° E longitude) at 8.5 m above the sea level. The total rainfall during the growing season was 1130.80 mm in summer season (April 2012 to October 2012) and 182.80 mm in winter season (October 2012 to March 2013). Monthly maximum and minimum air temperatures (°C) and relative humidity (%) during the crop growth period are presented in Figure 1.

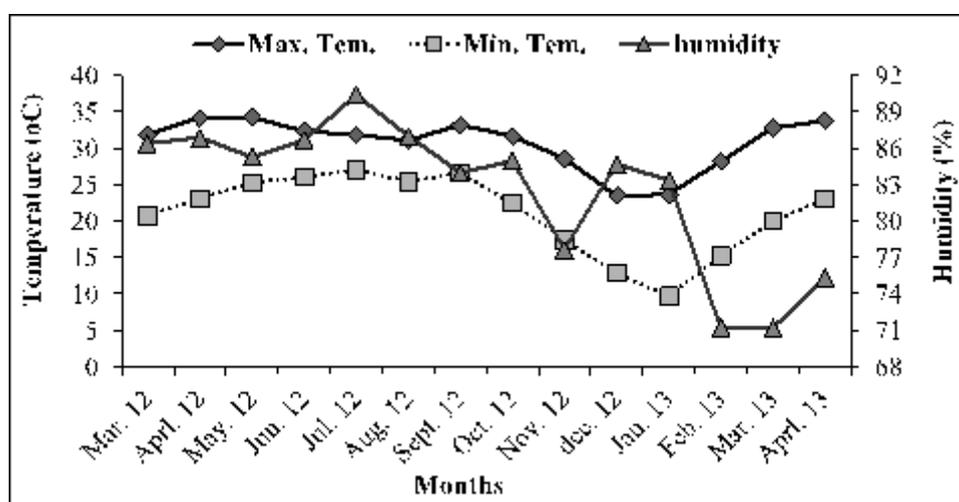


Figure 1. Average max. and min. temperature (°C) and relative humidity (%) during experimental period.

Six varieties of eggplant viz. BARI Begun-1, BARI Begun-5, BARI Begun-6, BARI Begun-8, BARI Begun-9 and Khotkhotia were used in the study. These varieties were grown separately with proper isolation in both the seasons. Healthy, uniform and disease free eggplant seedlings of 35 days old were transplanted on 25 May 2012 (summer season) and on 2 November 2012 (winter season). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The unit plot size was 0.80 m x 10.5 m and fifteen seedlings were planted in a plot with a plant spacing of 70 cm apart in single row maintaining 50 cm drain between the plots. The crop was fertilized with 210-100-120-30 kg N P K S ha⁻¹ in the form of urea, triple super phosphate (TSP), muriate of potash (MoP) and gypsum, respectively. In addition, cowdung @ 10 t ha⁻¹ was also applied. Total amount of TSP, gypsum, well decomposed cowdung and half of MoP were applied at the time of final land preparation while, the entire urea and rest of MoP was applied at three equal installment. First one-third of urea and one-third of MoP was applied at 15 days after transplanting (DAT) and rest amount of urea and MP were applied in two equal split at 30 and 50 DAT followed by irrigation. Flood irrigation was provided into the drain between two plots immediately after transplanting. Irrigation was applied time to time considering the moisture content status of the field. Weeding was done timely to minimize crop weed competition and mulching was done followed by top-dressing and irrigation at 15 days interval. To protect white flies and brinjal shoot and fruit borer, Admire @ 1.0 ml per litre water and Mackjoint 5 SG was sprayed at 7 days interval alternatively. Furadon 5G @ 5 kg ha⁻¹ was also applied in the pit before transplanting.

Full ripen fruits of eggplant (60 DAA) were harvested from the experimental field. Standard size fruits (not the biggest one or the smallest) were selected for seed extraction purpose. The selected fruit were kept in ambient condition for five days post-harvest ripening. These allow the seeds to mature fully and the fruits become soften. The seeds were separated by washing under running tap water and dried to about 8% moisture content. Data on fruit per plant, seed per fruit, 1000-seed weight, seed yield per fruit, germination percentage, co-efficient of germination, seedling length, dry weight of seedling, electrical conductivity test and seedling vigour index were determined.

In the laboratory, germination test were done. Germination percentage was calculated by the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds tested}} \times 100$$

For electrical conductivity test, 2.00 g seeds of each sample were taken in a conical flask containing 50 ml de-ionized water and incubated at 20±1°C for 20 hours. After 20 hours, water of the beaker containing seeds was decanted in order to separate the seeds. The electrical conductivity meter (Model: Lutron CD-4301)

was used. Three replications of measurements were made for each sample of seed and expressed on $\mu\text{S cm}^{-1}\text{g}^{-1}$.

Co-efficient of germination and vigour index were calculated using the following formula (Copeland, 1976).

$$\text{Co-efficient of germination} = \frac{100(A_1 + A_2 + \dots + A_x)}{A_1T_1 + A_2T_2 + \dots + A_xT_x}$$

Where, A= number of seed germinated, T= time corresponding to A and X= Number of days to final count.

$$\text{Vigour index} = \frac{\text{Number of normal seedlings}}{\text{days of first count}} + \dots + \frac{\text{Number of normal seedlings}}{\text{days of final count}}$$

The recorded data on different parameters were subjected to analysis of variance (ANOVA). Microsoft EXCEL and CROPSTAT software programs were used wherever appropriate and the means were compared by least significant difference (LSD) test (Gomez and Gomez, 1984).

Results and Discussion

Seasonal effects on quantity and quality of seed and seedling growth of eggplant have been presented and discussed character wise through different graphs.

Number of fruits per plant

Number of fruits per plant was influenced significantly by growing season within and among the variety. The maximum number of fruits per plant was obtained from BARI Begun-8 followed by BARI Begun-1 irrespective of season (Fig. 2). Variation of fruits per plant among the varieties was possibly due to difference in genetic makeup of the crops. Higher numbers of fruit per plant were observed from winter season in all variety. The result indicates that winter season is more favourable to fruit set of eggplant than summer. This might be due to clear sunshine, cool and gentle weather, high movement of pollinators. Bose *et al.* (1993) also reported that in the hills of Kulu valley in Himachal Pradesh, India the brinjal seeds should be sown in the nursery bed during the months of March and April for growing seed crop. But, Desai *et al.* (1997) reported from India that brinjal seeds may be sown in November for fruit yield and April – May for growing seed crop.

Number of seeds per fruit

Number of seeds per fruit differed significantly among the varieties (Fig. 2). The variety BARI Begun-6 produced the highest number of seeds per fruit, which was statistically superior to rest of the varieties. The lowest number of seeds per fruit was from BARI Begun-1. Number of seeds per fruit was unaffected by growing season in most of the variety except BARI Begun-6 (Fig. 2). There was

a significant different in number of seeds per fruit in BARI begun-6 by growing season. However, numerically higher number of seeds per fruit was found from winter season in all variety. This result indicated that eggplant planted in normal planting time (October to November) gave higher seed yield. Agrawal (2003) also suggested that the seed crops should invariably be sown at their normal planting time.

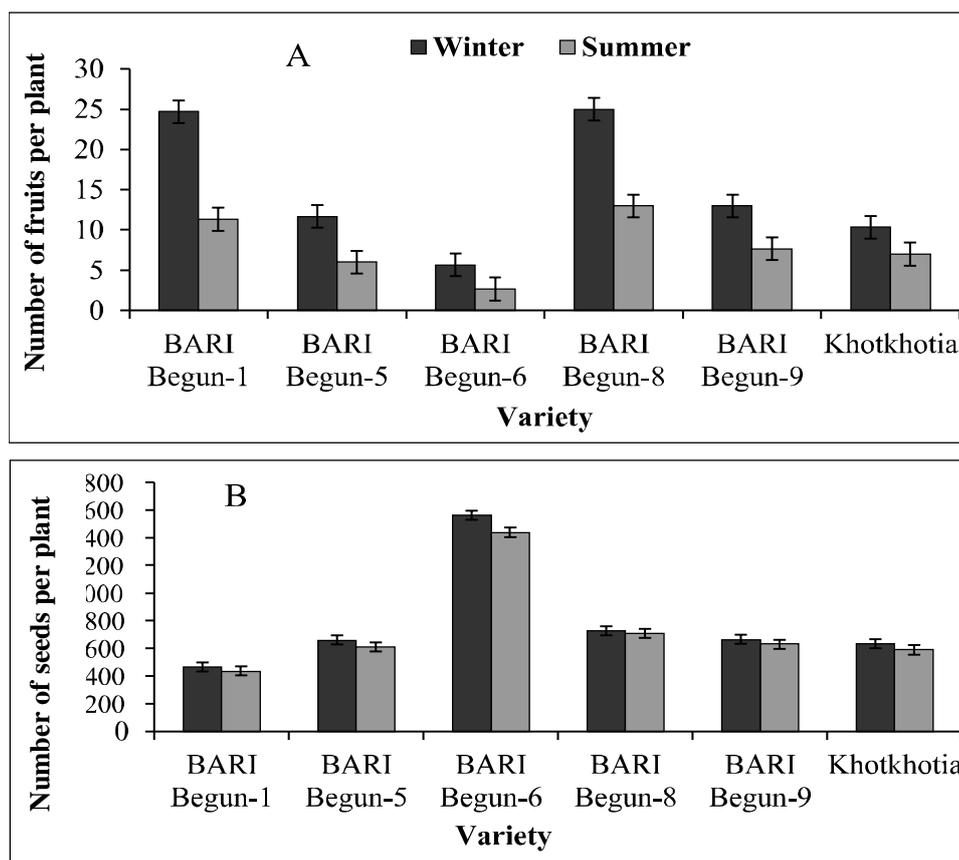


Figure 2. Number of fruits per plant (A) and number of seeds per fruit (B) as influenced by crop growing season.

1000-seed weight

Seed size is an important parameter of seed quality, because bigger seed encourages better seedling establishment in the field. Significant difference in 1000-seed weight was observed in six varieties of eggplant (Fig. 3). The maximum weight of 1000-seed was observed in the variety BARI Begun-5 followed by BARI Begun-6. However, 1000-seed weight was not influenced significantly by growing season of eggplant. All variety showed the same trend of seed weight when grown in different season. But the maximum 1000-seed

weight was found in the variety BARI Begun-5 followed by BARI Begun-6 irrespective of growing seasons. This result showed that higher seed weight was recorded from winter crops than summer.

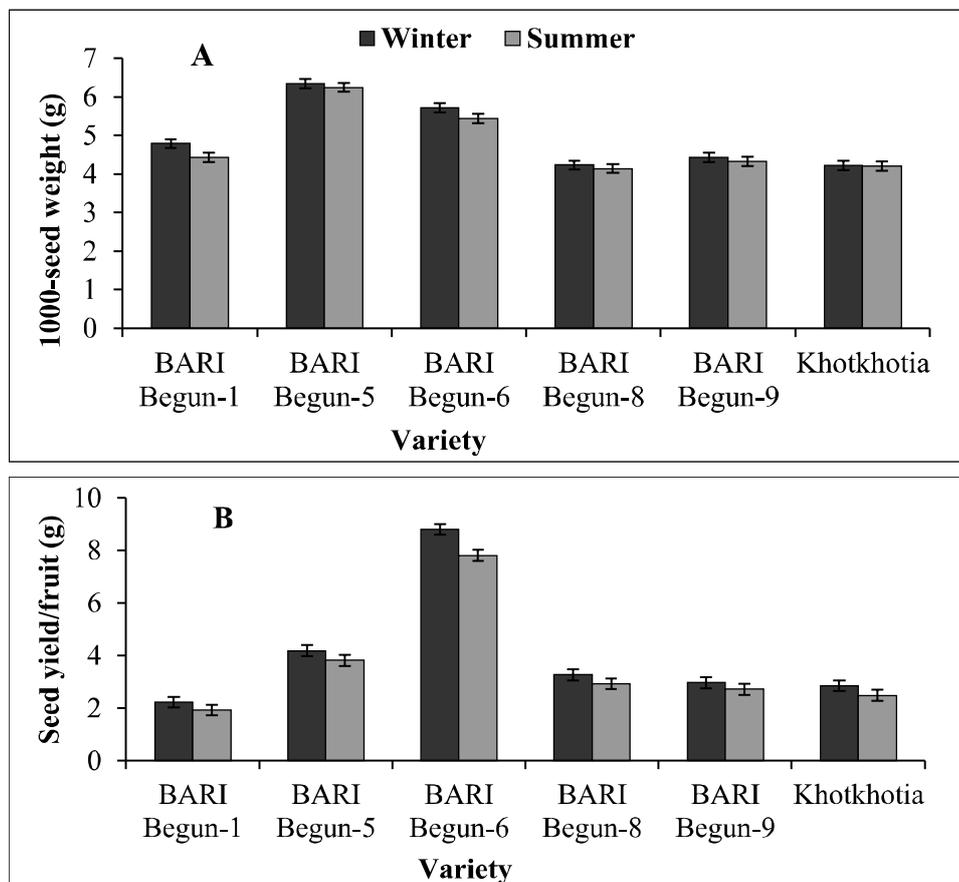


Figure 3. 1000-seed weight (A) and seed yield per fruit (B) of eggplant as influenced by crop growing season.

Seed yield per fruit

Seed yield per plant is of great importance as it constitute total seed yield when uniform stand establishment is achieved. In this study seed yield per fruit varies significantly among six varieties of eggplant (Fig. 3). The highest seed yield per fruit was found in the variety BARI Begun-6 and the lowest from BARI Begun-1. However, the amount of seed per fruit of eggplant remained unaffected by growing season. Only BARI Begun-6 produced significantly higher amount of seed per fruit when grown in winter season. The variety BARI Begun-1 performed lowest seed yield in both seasons. However, numerically higher amount of seed per fruit was recorded from winter season. As numbers of fruit

per plant, seeds per fruit and seed weight determine final seed yield of eggplant, higher number of fruit and higher seed weight increased seed yield of eggplant when grown in winter season.

Seed quality attributes

Seed quality was evaluated in terms of percent germination, electrical conductivity, vigour index and coefficient of germination which have been described in the following headings.

Seed leachate conductivity

Electrical conductivity test provides a quick decision about the seed quality. This test is related to deterioration sequence of seeds. Seed leachate conductivity was not influenced significantly by growing season within and among the variety (Fig. 4). The maximum electrical conductivity was found in the variety Khotkhotia in both the seasons. However, lower electrical conductivity was recorded in all variety from the seeds of winter crops. The result indicated that winter crops produced seeds with better cell membrane integrity and hence these seeds are vigorous.

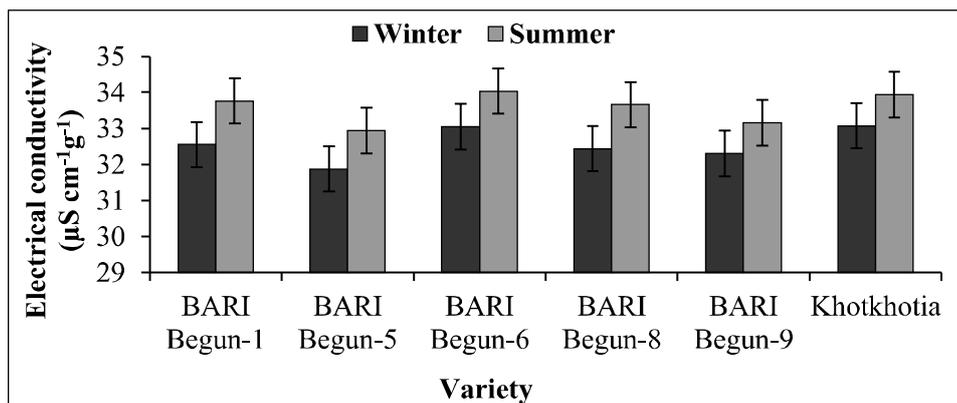


Figure 4. Electrical conductivity of eggplant seed as influenced by crop growing season.

Germination percentage

Germination percentage of seed is strongly related to individual seed weight as germination is energy requiring process. In this study, seed germination percentage was influenced significantly by growing season within the variety. The maximum germination percentage was found in the variety Khotkhotia which was statistically similar to the variety BARI Begun-6 and BARI Begun-8 irrespective of growing seasons. Always higher germination was found from the seeds of winter (October sowing) crops in all variety (Fig. 5). The seeds harvested from winter crop can accumulate higher dry matter due to favourable

environment for crop growth and this may enhance germination capacity of eggplant seed. The result indicates that winter crops may produce high quality seed than summer (April sowing) crops of eggplant.

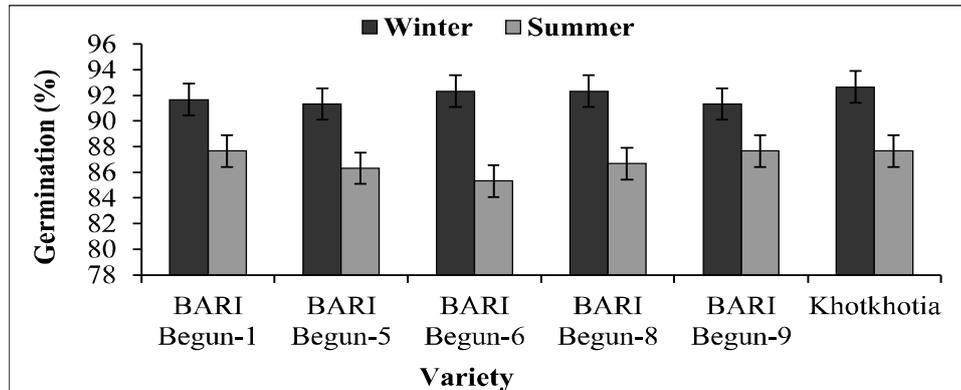


Figure 5. Germination percentage of eggplant seed as influenced by crop growing season.

Co-efficient of germination

Significant variation was observed in co-efficient germination among the six eggplant varieties. The variety Khotkhotia exhibited maximum value followed by BARI Begun-1, BARI Begun-5, BARI Begun-8 and BARI Begun-9. Co-efficient of germination of eggplant also varied greatly by crop growing season. Higher co-efficient of germination was found in the seeds harvested from winter crops of eggplant (Fig. 6). The result indicated that seeds of winter crops was more vigorous than seeds of summer crops as higher the co-efficient value indicates higher speed of germination and higher seed vigour. The seeds having lower vigour values can't germinate well under field condition.

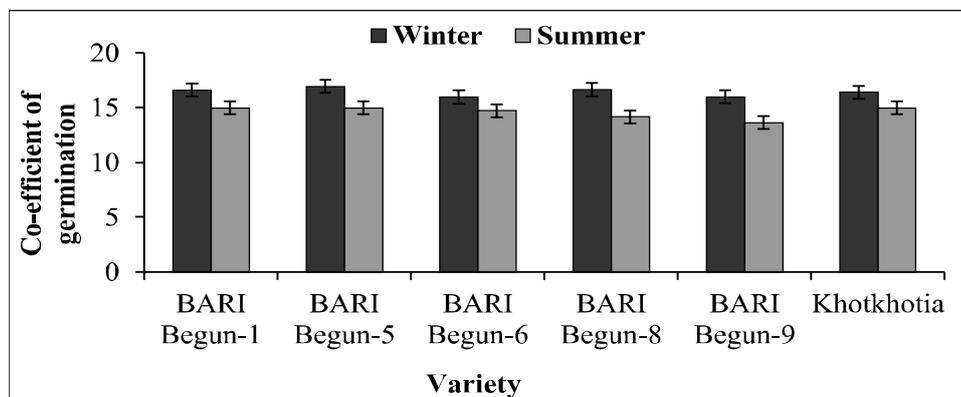


Figure 6. Co-efficient of germination of eggplant seed as influenced by crop growing season.

Vigour index

Significant variation was observed in vigour index among the six eggplant varieties (Fig.7). The variety BARI Begun-1 showed maximum vigour index followed by BARI Begun-5, BARI Begun-6, BARI Begun-8, BARI Begun-9 and Khotkhotia showed the least. Marked variation was also noticed in vigour index by growing season. Higher seedling vigour index was found in the seeds harvested from winter crops of eggplant (Fig. 7). The result indicated that seeds of winter crops were more vigourous than seeds of summer crops of eggplant.

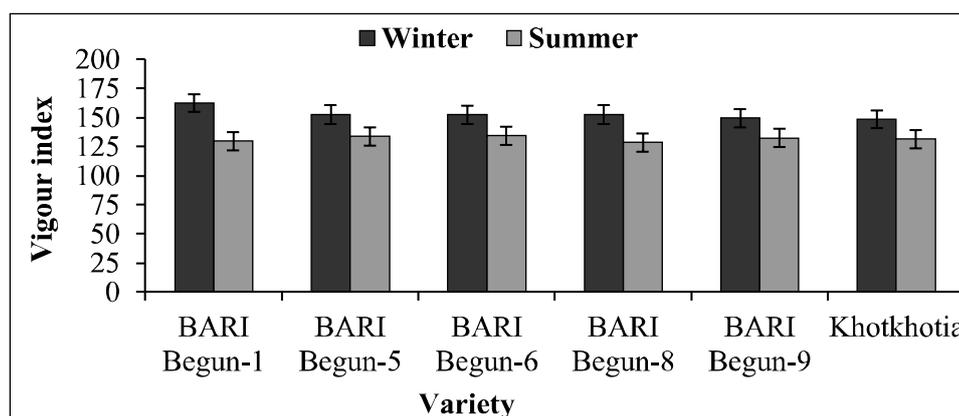


Figure 7. Vigour index of eggplant seedling as influenced by crop growing season.

Conclusion

The result of the study indicated that winter season (October to March) is more favourable to produce higher amount of better quality eggplant seed compared to summer season (April to October) in Bangladesh condition.

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EFFECT OF COMPOST TEA, STREPTOMYCIN AND CUPRAVIT IN CONTROLLING BACTERIAL LEAF BLIGHT OF RICE

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Abstract

Compost tea, streptomycin and cupravit were evaluated against the Bacterial Leaf Blight diseases of rice cv. BR11 in two management approaches (preventive and curative) under field condition. The selected cultivar BR11 was cultivated in the field laboratory of the Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh during the period from July to December 2012. The field experiment was carried out with seven treatments in Randomized Complete Block Design (RCBD) and each was replicated thrice. The treatments were T₀ = Control, T₁ = Compost tea as foliar spray 2 times (preventive) @ 1:5 w/v, T₂ = Compost tea as foliar spray 2 times (Curative) @ 1:5 w/v, T₃ = Streptomycin as foliar spray 2 times (preventive) @ 1gm/10 L, T₄ = Streptomycin as foliar spray 2 times (Curative) @ 1gm/10 L, T₅ = Cupravit as foliar spray 2 times (preventive) @ 0.2%, T₆ = Cupravit as foliar spray 2 times (Curative) @ 0.2%. Cupravit as foliar spray 2 times (Curative) @ 0.2% showed marked effect in reducing Bacterial Leaf Blight diseases of BR11 rice as well as enhancing yield and yield contributing characters. The effect of compost tea also produced similar effects on disease and yield of rice over control. Among the application options of different treatment, curative application provided better results than preventive under field condition.

Keywords: Compost tea, streptomycin, cupravit, bacterial leaf blight, rice

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop in Bangladesh covering 11533198.38 hectares of land (BBS, 2012). The average world yield of rice is 4.48 metric tons/hectare, but the average yield in Bangladesh is measurably poor, only 2.94 metric tons/hectare (FAO, 2013; BBS, 2012). This indicates that average per hectare yield of rice in Bangladesh is extremely low compared to other rice growing countries of the world.

Among the many reasons of low yield of rice in Bangladesh, diseases and insect pests play a major role, sometimes leading to disastrous consequences (Fakir, 1982). Out of 32 diseases of rice, ten are considered major, among them bacterial

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leaf blight (BLB) has been considered as most important one because of their widespread outbreak and significant yield loss potential (Khan *et al.*, 2010).

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is the most devastating bacterial disease of rice worldwide (Thein and Prathuangwong, 2010). The yield loss caused by BLB in Bangladesh has been estimated as 5-29% depending on the crop stage, variety and season (BRRI, 2004). This disease is being controlled especially by spraying chemical or bactericides in the field as well as by treating seeds with the seed treating chemicals and using disease resistant cultivars. Especially in Bangladesh, there is no recommended bactericides that can control BLB effectively under field condition (Khan *et al.*, 2010).

Farmers generally use chemicals for the control of this disease but indiscriminate use of chemicals resulted environmental pollution and health hazards. As an alternate means of avoiding these problems, biological agents are being used for combating the diseases with the aim of increasing crop production. The biological control of pathogen offers environmentally safe, durable and cost effective alternatives to chemical compounds (Perveen *et al.*, 1994). Living microorganisms such as bacteria, viruses or fungi are employed in biological control either as antagonists, parasites or competitor (Kwok *et al.*, 1987).

Compost tea has been defined simply as liquid extract from compost material that may contain organic and inorganic soluble nutrients, and a large number of organisms including bacteria, fungi, protozoa and nematodes (Rou, 2003). It is emerging as a crop protection tool and has been using for organic agriculture for a number of reason viz. it contains microorganisms which can reduce the incidence of foliar and/or soil-borne diseases, a readily available nutrients which rapidly benefit plant growth through direct contribution to plant nutrition and is easily integrated into existing plant fertility and disease control programs due to its easy application via existing irrigation or spray equipment, or as a soil drench. Assuming all the above mentioned facts, the present study was undertaken to find out the effect of compost tea on the incidence and severity of Bacterial Leaf Blight disease and impact on yield of BR11 rice.

Materials and Method

Experimental Site and Seed Sowing

An experiment was conducted at the Field Laboratory of Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during the period from July 2012 to December 2012. A high yielding cultivar BR11 (Mukta) was selected for this study. Seeds of BR11 (Mukta) were collected from seed store of Bangladesh Agricultural Development Corporation (BADC), seed building, Monakhola, Mymensingh.

A small piece of medium low land of the experimental farm of the Department of Plant pathology, Bangladesh Agricultural University, Mymensingh was puddled with the power tiller. Clean and mature seeds were soaked in tap water for 24 hours and incubated 48 hours for germination before sowing in the seed bed. The germinated seeds were sown in the seed bed on 5 July 2012.

Field Experiment Setting

The experiment was laid out in a Randomized Complete Block Design (RCBD) with 3 replications maintaining distance between the blocks and the plots as 50 cm and 25cm, respectively. A total of 7 treatments were used henceforth designated as T₀ (Control), T₁ (Compost tea as foliar spray 2 times (preventive) @ 1:5 w/v, T₂ (Compost tea as foliar spray 2 times (Curative) @ 1:5 w/v, T₃ (Streptomycin as foliar spray 2 times (preventive) @ 1gm/10 L, T₄ (Streptomycin as foliar spray 2 times (Curative) @ 1gm/10 L, T₅ (Cupravit as foliar spray 2 times (preventive) @ 0.2%) and T₆ (Cupravit as foliar spray 2 times (Curative) @ 0.2%.

The main land was prepared by power tiller on 8th August 2012. Later on the individual plots were cleaned from weeds and stubbles. Organic amendments and Chemical fertilizers were applied in the field as recommended by Bangladesh Agricultural Research Council (Anonymous, 2005). The border of each unit plot was raised to prevent the fertilizer and nutrients movement from one plot to others.

Transplanting of Seedlings of BR 11 (Mukta) and Preparation of Compost Tea

After preparing the land, 32 days of old seedlings of BR 11 (Mukta) were uprooted carefully to avoid root injury. The seedlings were transplanted putting three seedlings/hill where plant to plant and row to row spacing were 15 cm and 20 cm, respectively. Weeding and other cultural practice was performed in time.

Compost tea was obtained by mixing compost with tap water at a ratio of 1:5(w/v) followed by fermentation for one week. Then it was stirred once every day and allowed to ferment in the Net house of Seed Pathology Centre, BAU, Mymensingh at 25°C. After 7 days, the solution was filtered through cheese cloth to obtain the compost tea.

Inoculum preparation and Inoculation of test entries

BXO9, a virulent and reference isolate of major races of *Xanthomonas oryzae* pv. *oryzae* (Khan *et al.*, 2010) was obtained from the Plant pathology Division, BRRI for inoculation. The isolate was cultured on Peptone Sucrose Agar (PSA) slants for 72 hours at 30°C. Inoculum was prepared by mixing the cultured bacteria with 10 ml sterile distilled water in a slant. Before inoculation the concentration of the bacterial suspension was adjusted to 10⁸ to 10¹⁰ CFU/ml

using sterile water. The leaf clipping inoculation method was adopted in this experiment. The scissors were dipped into bacterial suspension, and then the tip of fully expanded leaves were clipped as described by Kauffman *et al.* (1973).

Identification of the symptom of Bacterial Leaf Blight of rice

The rice plants were examined periodically and bacterial leaf blight infection was identified on the basis of symptoms developed on the plants as described by Tabei and Mukoo (1960). At first a tiny water soaked lesion appeared from the leaf margin, then turned yellow while enlarging both the length and width to develop an elongated irregular lesion. The border of the lesion adjoining the healthy part showed a wavy margin lesions might start on one or both margins of the leaf.

Assessment of incidence and severity of disease

Data on lesion length, relative lesion length and leaf area damaged were recorded at 7 and 14 days after inoculation (DAI). Lesion length was measured by measuring scale. Total number of inoculated leaves per hill and number of infected leaves per hill was recorded. Both at natural and inoculated conditions, 5 hills and 5 leaves were randomly selected from each plot to collect data on total number of leaves per hill, number of infected leaves per hill, total leaf length (cm) and leaf lesion length (cm).

The disease incidence was calculated by the following formula:

$$\text{Disease incidence (I)\%} = \frac{\text{Number of infectedleaves}}{\text{Totalnumber of leaves}} \times 100$$

Percent leaf area diseased/severity was assessed by the following formula:

$$\text{Severity \%} = \frac{\text{Leaf area diseased}}{\text{Totalleaf area}} \times 100$$

Harvesting and recording of data

Randomly selected 15 tillers from each plot (inoculated and non-inoculated) were used for recording the data on Plant height (cm), No of tiller/plant, Panicle length (cm), Total number of grains/panicle, Chaffy grain number/ panicle and Grain weight (gm)/plot.

Analysis of data

The collected data were analyzed using the Analysis of variance (ANOVA) technique, and the mean differences were adjudged by Duncan's Multiple Range Test (Gomez and Gomez, 1984) using the statistical computer package.

Results and Discussion

Effect of compost tea, streptomycin and cupravit on against BLB of rice in the field

Effect of different treatments on incidence of BLB at 7 DAI was variable and ranged from 52.82 % to 86.26 % (Fig. 1). Highest BLB incidence (86.26%) was observed in T₀ (control) which was statistically similar to T₃ (streptomycin preventive), the lowest disease incidence (52.82%) was in T₆ (cupravit curative).

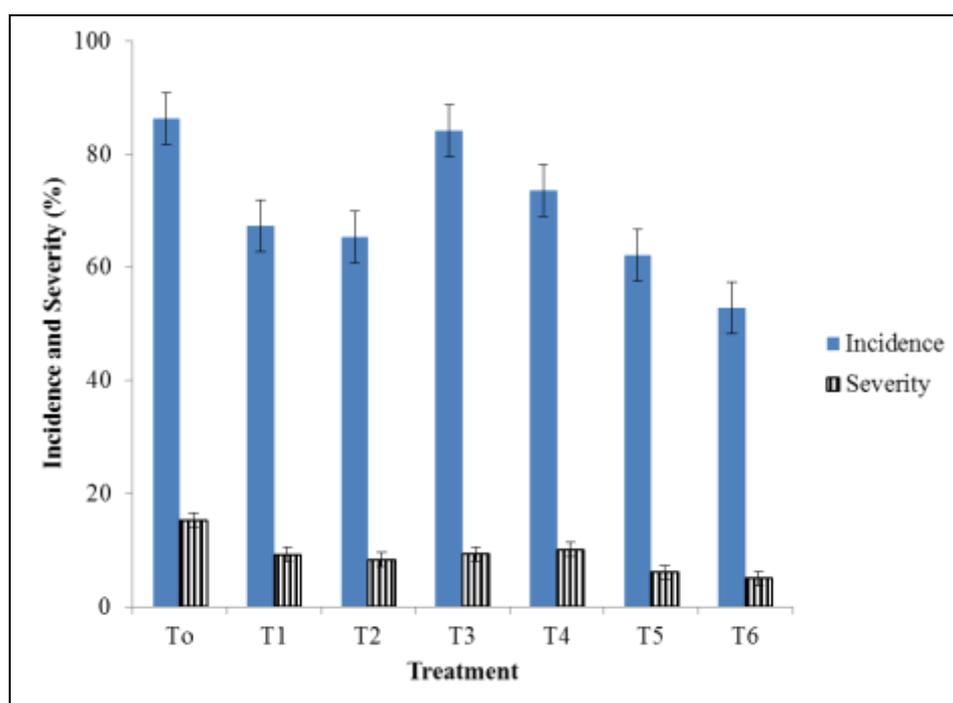


Fig.1. Effects of different treatments on the incidence and severity of BLB of cv.BR11, at 7 DAI.

[Where, T₀=Control, T₁=Compost tea (preventive), T₂=Compost tea (Curative), T₃=Streptomycin (preventive), T₄=Streptomycin (Curative), T₅=Cupravit (preventive) and T₆=Cupravit (Curative)]

Besides, disease severity at 7 DAI ranged from 5.01% to 15.33% (Fig.1). Highest disease severity (15.33%) was observed in T₀ (control) which is statistically similar to T₄ (streptomycin curative) whereas the lowest disease severity (5.01%) was found in T₆ (cupravit curative) which is statistically similar to T₅ (cupravit preventive).

Incidence of BLB varied from 83.09% to 94.04% at 14 DAI in field (Fig.2). Highest BLB incidence (94.04%) was observed in T₀ (Control) which was statistically similar to T₄ (streptomycin curative), T₃ (streptomycin preventive),

while the lowest BLB incidence (83.09%) was observed in T₆ (cupravit curative), which was statistically similar to T₅ (cupravit preventive) and T₂ (compost tea curative).

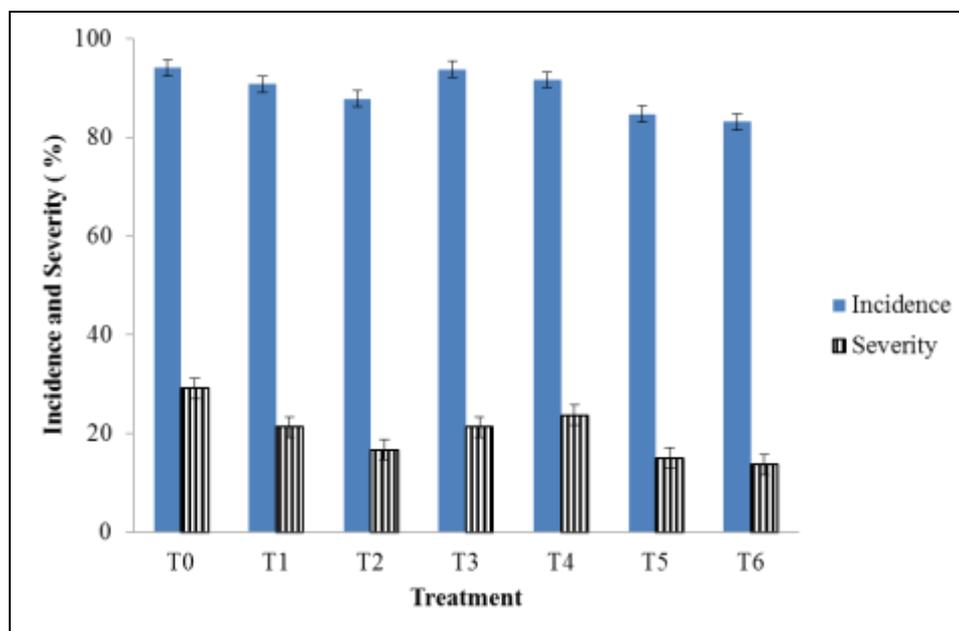


Fig.2. Effects of different treatments on the incidence and severity of BLB of rice cv. BR11 at 14 DAI.

[Where, T₀=Control, T₁=Compost tea (preventive), T₂=Compost tea (Curative), T₃=Streptomycin (preventive), T₄=Streptomycin (Curative), T₅=Cupravit (preventive) and T₆=Cupravit (Curative)]

The disease severity of rice under different treatments at 14 DAI was also varied (Fig. 2) and the highest severity (29.21%) was found in control treatment (T₀), while the lowest severity (13.69 %) was recorded in T₆ (cupravit curative), which was statistically similar to T₂ (compost tea curative) and to T₅ (cupravit preventive).

There was a significant variation among the treatments regarding the incidence of bacterial leaf blight of rice that varied from 52% to 94%. BLB incidence was significantly lower in compost tea treatment which was statistically similar to cupravit whereas application of streptomycin did not show any significant effect on BLB of rice. Similarly cupravit showed marked effect on severity of BLB which is statistically similar to compost tea application. This result was fully in agreement with the findings (Kumar *et al.*, 2009) who found that among the chemicals copper oxychloride (0.25%) was most effective over other chemicals. Likewise, compost tea foliar sprays also gave good response to BLB management as compared to Streptomycin and control treatments. These results were fully agreement with the findings of Wickramaarachchi *et al.* (2003). Ryan

et al. (2005) also explored the use of aerated water extracts or teas from compost to control foliar diseases. In this experiment streptomycin (antibiotics) had little effect on BLB of rice as compared to cupravit and compost tea. Similar findings were reported by Padmanabhan and Jain (1966).

Effect of Compost tea, streptomycin and cupravit on yield and yield components of rice cv.BR11

Table 1. Effect of different treatment on the growth and yield of rice CV. BR11

Treatments	Plant height (cm)	No. of tiller/plant	Panicle length (cm)	Total grain number/panicle	Chaffy grain number/panicle	Grain weight (g)/plot
T ₀	104.80ab	14.27ab	22.39b	132.69d	45.78b	726.67e
T ₁	100.60bc	16.00ab	24.50b	144.78c	42.22bc	793.33c
T ₂	107.10a	16.27ab	22.29b	159.22b	45.22bc	826.67b
T ₃	98.17c	13.93b	22.16b	147.01c	51.33a	760.00d
T ₄	95.97d	14.33ab	23.44b	146.89c	43.89bc	726.67e
T ₅	98.78bc	17.20a	23.58b	158.55b	41.44bc	766.67d
T ₆	100.20bc	17.00a	27.41a	180.33a	41.00c	873.33a
LSD	6.48	1.95	2.44	11.95	3.51	17.01
CV (%)	3.61	7.05	5.80	4.40	4.45	1.22
LS	*	**	**	**	**	**

LSD= Least Significance Difference

CV= Coefficient of Variation

LS = Level of significance

** and * = Significant at 1% and 5% level respectively

Within the table different letter (s) in same column indicate significance difference by DMRT

[Where, T₀=Control, T₁=Compost tea (preventive), T₂=Compost tea (Curative), T₃=Streptomycin (preventive), T₄=Streptomycin (Curative), T₅=Cupravit (preventive) and T₆=Cupravit (Curative)]

Plant height (cm)

Effect of different treatments on plant height was variable that ranged from 95.97 cm to 107.10 cm (Table 1). Highest plant height (107.10 cm) was observed in T₂ (compost tea curative) which was statistically similar to T₀ (control) and the lowest plant height (95.97 cm) was in T₄ (streptomycin curative).

Number of tillers/ plant

Significant effect of different treatments on the no of tiller/plant was observed (Table 1). Highest tiller number (17.20) was observed in T₅ (cupravit preventive)

which was statistically similar to T₆ (cupravit curative), T₂ (compost tea curative) and T₁ (compost tea preventive) whereas the lowest tiller number (13.93) was in T₃ (streptomycin preventive) which was statistically similar to T₄ (streptomycin curative) and T₀ (control).

Panicle length (cm)

Mean panicle lengths ranged from 22.16 cm to 27.41 cm among the treatments (Table 1). Maximum panicle length was recorded from T₆ (cupravit curative), while the minimum panicle length was in T₃ (streptomycin preventive), T₂ (compost tea curative) and T₀ (control).

Total number of grains/panicle

Effect of different treatment on grain number /panicle varied significantly (Table 1). Mean number of grain /panicle ranged from 132.69 to 180.33. The highest number (180.33) of grain /panicle was recorded in T₆ treatments plant and the lowest number (132.69) of Grain/ panicle was recorded in T₀ control plants.

Chaffy grain number/ panicle

The number of chaffy grain number/panicle was also influenced by the different treatments (Table 1). The highest number (51.33) of chaffy grain /panicle was recorded in T₃ (streptomycin preventive) and the lowest number (41.00) of chaffy grain number/panicle was recorded in T₆ (cupravit curative) which was statistically similar to T₂ (compost tea curative), T₄ (streptomycin curative) and T₅ (cupravit preventive).

Grain weight (g/plot)

The average grain weight (g)/plot were also varied statistically (Table 1). Maximum weight (873.33g) of grain was recorded in T₆ (cupravit curative) and the lowest weight (726.67 gm) of grain were also recorded in plants with T₀ (control) which was statistically similar to T₄ (streptomycin curative).

Foliar application of compost tea did not show any significant effect on most of the yield contributing components except grain weight /plot. Grain weight under different treatments ranged from 726 to 873 g. In case of spraying compost tea (preventive) the per plot yield was 826.67gm where compost tea (curative) 793.33g. This result indicated that the compost tea enhanced the grain weight which was supported by Ngakou *et al.* (2012).

Among the application options preventive and curative, curative application provided better results than preventive under field condition. This finding was supported by Mary *et al.* (2001). They used prophylactic and curative spraying of streptocycline (500 ppm), streptomycin + oxytetracycline (1:9, 250 and 500 ppm), bacitracin-100 (500 ppm) and cowdung extract (20 g/litre) on rice cultivars

TNI and Jyothy and observed a reduction in bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) incidence and an increase in straw and grain yields in all treatments except bacrinol-100. They also reported that prophylactic and curative spraying of streptomycin + oxytetracycline and cowdung extract produced the highest grain and straw yields for TNI and Jyothy, respectively. The percentage of disease index was lower for curative (40.16) than for prophylactic (42.22) spraying.

Conclusion

The compost tea was highly effective to control bacterial leaf blight (BLB) in rice which could replace the chemical fungicides without any risk to human, animal and environment. So, growers could be motivated to apply the cost effective compost tea for efficient management of BLB of rice for better yield.

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GENETIC ANALYSIS OF YIELD AND YIELD CONTRIBUTING TRAITS IN BORO RICE (*Oryza sativa* L.) OVER ENVIRONMENTS

K. PRIYANKA¹ AND H. K. JAISWAL²

Abstract

Hayman's component analysis was employed to estimate genetic components of variation for yield and yield related traits in *boro* rice. Nine diverse lines of *boro* rice were crossed in diallel mating design excluding reciprocals and all the parental lines along with their 36 crosses were evaluated in randomized block design over 3 seasons in 3 replication. Significant differences among genotypes were observed for all the traits over seasons. Component analysis indicated importance of both additive and dominance components. However, greater magnitude of dominance component than its corresponding additive component of variance exhibited greater role of dominance in the inheritance of these traits. The average degree of dominance was more than unity indicating over-dominance for all the traits. Most of the traits exhibited low to moderate narrow sense heritability.

Keywords: *Boro* rice, component analysis, diallel, gene action, heritability

Introduction

Rice is a major food crop for more than half of the world population. About 90 percent of all the rice grown in the world is produced and consumed in Asia. The largest rice producing countries include China, India, Indonesia, Vietnam, Thailand and Bangladesh. In Bangladesh, rice is cultivated in all the three seasons i.e. aus, aman and *boro*, accounting for around 34.5 million metric tons of rice. *Boro* rice is famous for high productivity. India is the world's second largest producer of rice after China, accounting for about 20% of the world rice production. To meet the food demand of the growing population and to achieve food security in the country, the present level of production need to be increased by 2 million tones every year. Amelioration of grain yield is primary objective in plant breeding experiments. It is a complex trait governed by polygenes and highly influenced by environment. Grain yield depends on a number of growth and yield component traits directly or indirectly. Therefore, selection of yield components would be more beneficial than yield per se.

Formulation of an effective breeding strategy requires the sound knowledge of nature and magnitude of gene action. Biometrically, Hayman's component analysis is an important tool which clearly provides information about various components of variance (Hayman, 1954) and in this way, helps to find the nature of various gene effects. Thus, the present study was conducted over three

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seasons to have a reliable information of the various components of genetic variation for yield and yield related traits in *boro* rice.

Materials and Method

Seeds of nine diverse genotypes of *boro* rice (IR 8, IR 36, IR 64, HUR 36, HUR 105, MTU 1010, Jaya, Krishna Hamsa and Gautam) were sown in nursery at three different dates with 15 days interval between successive sowings to ensure synchronization in flowering for the purpose of hybridization. In *kharif*, raised nursery beds were made. Twenty one days old seedlings were transplanted in the crossing block. Raising a good nursery in *kharif* season was not a big deal in case of rice but in *boro* season special care was needed due to low temperature at vegetative stage. Firstly, seeds were soaked in water overnight and kept in moistened gunny bags for two days for sprouting. Then these sprouted seeds were spread uniformly in prepared seed beds (wet bed nursery) in last week of November. Nursery beds were covered with polythene sheets during night hours to avoid cold injury. Sixty days old seedlings were transplanted in the crossing block. A standard spacing of 20 x 15 cm was adopted for the planting. Row length was maintained at 2 m. Single seedling per hill was transplanted. Recommended agronomic package of practices for each season was followed to raise a good crop. At the time of flowering, crosses were made in 9 x 9 diallel mating design without reciprocals during *kharif*-2014, *boro*-2014 and *kharif*-2015 to generate 36 crosses. All the 36 crosses along with 9 parents were evaluated in randomized block design in three replications for three seasons i.e. *boro*-2014, *kharif*-2015 and *boro*-2015 at Agricultural Research Farm of Banaras Hindu University. All the genotypes were evaluated for days to 50% flowering, days to maturity, plant height, effective tillers per plant, main panicle length, flag leaf length, flag leaf width, grains per panicle, 100 seed weight and yield/plant. Data was recorded from ten randomly selected plants from each entry in each replication. Mean data over replications were used for statistical analysis.

The analysis of variance was performed as per method suggested by Panse and Sukhatme (1967) for randomized block design. Diallel component analysis was performed with the help of statistical software Windostat v.9.2 (Windostat Services, Hyderabad, A.P., India).

Results and Discussion

Analysis of variance (ANOVA) showed significant variations for all the traits among seasons, genotypes and season x genotype interaction (Table 1). Significant season x genotype interaction indicated the differential response of genotypes over the seasons, so a separate analysis for each environment was carried out in order to assess the reliable estimates of various genetic components. Separate analysis for individual environment could also help in selection of traits which exhibited consistency in gene action, thus those traits could be reliably utilized in breeding programme irrespective of the environments. Analysis of variance indicated significant differences among the

treatments i.e. 9 parents and 36 crosses over the three seasons (Table 2). Significant variation for the yield traits in rice were reported by Hosseini *et al.* (2005), Sharma (2006), Dey *et al.* (2013) and Allahgholipour *et al.* (2015).

The estimates of components of genetic variation (\hat{D} , \hat{H}_1 , \hat{H}_2 , \hat{h}^2 , \hat{F} and \hat{E}) are presented in Table (3). The estimates of additive component of genetic variation were significant for most of the traits in all the growing seasons except days to 50% flowering (*boro-2015*), days to maturity (*boro-2015*), effective tillers/plant (in all the seasons), panicle length (in all the seasons), flag leaf length (*boro-2015*), flag leaf width (*boro-2015*), grains/panicle (*boro-2014*) and yield/plant (in all the seasons). The estimates of dominance components, \hat{H}_1 and \hat{H}_2 were highly significant for all the traits over all the three seasons (*boro-2014*, *boro-2015* and *kharif-2015*). In general, for yield traits estimates of additive component (\hat{D}) were smaller than those of dominance components (\hat{H}_1 and \hat{H}_2) for all the three seasons.

The estimates of net dominance effect, i.e. \hat{h}^2 , were positive and significant for days to 50% flowering (*boro-2014*), days to maturity (*boro-2014* and *boro-2015*), plant height (*boro-2015* and *kharif-2015*), effective tillers/plant (*kharif-2015*), panicle length (*kharif-2015*), flag leaf length (*boro-2014*), flag leaf width (*kharif-2015*), grains/panicle (*boro-2015* and *kharif-2015*) and 100 seed weight (in all the seasons). Positive and significant \hat{h}^2 for most of the traits was reported by Verma and Srivastava (2004).

The estimates of component \hat{F} were significant and positive for plant height (*boro-2015*), flag leaf length (*kharif-2015*), flag leaf width (*boro-2014*), grains/panicle (*boro-2015* and *kharif-2015*) and 100 seed weight (*kharif-2015*) suggesting an excess of positive genes controlling these traits. Raju *et al.* (2011) also reported an overall excess of dominant genes for most of the yield and quality traits except plant height, panicle length and kernel breadth.

Component \hat{E} was non-significant for most of the traits except flag leaf width (*boro-2014*). Verma and Srivastava (2004) and Kumar (2011) also reported non significant estimates of \hat{E} for all the yield traits under study which support the findings of the present investigation.

Most of the earlier studies reported significance of both additive and dominance genetic variance for most of the yield traits. Li and Chang (1970), Murai and Kinoshita (1986), Xu and Shen (1991), Mahmood *et al.* (2004), Verma and Srivastava (2004) and Kumar (2011) reported importance of both additive and dominant genes for all the traits under study. Raju *et al.* (2011) reported significant additive and non additive component of variance for the traits, plant height, panicle length, 1000-grain weight and kernel length/breadth ratio. The dominant component of variance was significant for days to 50% flowering, ear

bearing tillers, panicle weight and grain yield/plant and additive component was significant for kernel length and breadth. The non-additive component of variance dominated the additive one for most of the yield traits except 1000-grain weight. Verma and Srivastava (2004), Kumar *et al.* (2008) and Habib *et al.* (2014) reported involvement of both additive and dominance component of variance in the inheritance of the yield traits under study but the magnitude of dominance component was greater than the additive component of variance which indicated greater role of dominance in genetic control of these traits, which supports the present findings.

The proportions of components of genetic variation are presented in Table 4. The estimates of degree of dominance $(\hat{H}_1/\hat{D})^{1/2}$ were higher than the unity for most of the traits in all the three seasons, suggesting the presence of over-dominance type of intra-allelic interactions. The estimates were close to unity for days to 50% flowering (1.400 in *kharif-2015*), days to maturity (1.335 in *kharif-2015*), plant height (1.384 in *boro-2014* and 1.337 in *boro-2015*) and grains/panicle (1.237 in *kharif-2015*) suggesting complete to over-dominance for the trait. Raju *et al.* (2011) reported over-dominance for days to flowering, ear bearing tillers per plant, panicle weight, grain yield/plant and kernel length and kernel length/breadth ratio and partial dominance for plant height, 1000 grain weight and kernel breadth. The average degree of dominance more than unity for most of the traits was also reported by Verma and Srivastava (2004), Kumar (2011) and Habib *et al.* (2014) which is in agreement with present findings. Mahmood *et al.* (2004) reported less than unity average degree of dominance for the trait plant height, number of productive tillers per plant and number of primary branches per panicle, suggesting partial dominance for these traits. Akram *et al.* (2007) reported over-dominance for panicle length, grain yield/plant and 100 seed weight, whereas partial to complete dominance for tillers per plant.

The ratio $\hat{H}_2/4\hat{H}_1$ was found close to the expected value of 0.25 for most of the traits, suggesting nearly symmetrical distribution of positive and negative alleles at loci showing dominance for the traits. An asymmetrical distribution of positive and negative alleles was observed in case of panicle length (0.178 in *boro-2014*), flag leaf length (0.155 in *kharif-2015*), flag leaf width (0.181 in *boro-2015*), grains/panicle (0.179 in *boro-2014*, 0.148 in *boro-2015* and 0.140 in *kharif-2015*) and yield/plant (0.198 in *boro-2014* and 0.181 in *boro-2015*). Various researchers have reported closeness as well as deviations from the value 0.25 for most of the yield and quality traits in their materials (Murai and Kinoshita, 1986; Xu and Shen, 1991; Verma and Srivastava, 2004; Akram *et al.*, 2007; Kumar, 2011 and Raju *et al.*, 2011) which is in agreement with the present findings. However, Habib *et al.* (2014) reported less than 0.25 for all the traits suggesting asymmetrical distribution of genes in the parents which may be due to differences among the materials under study.

Table 3. Components of genetic variation for yield and yield contributing traits for 9x9 diallel in *Boro* rice in three seasons

Component	Season	DTF	DTM	PH	ET/P	PL	FL	FW	G/P	100SW	Y/P
\hat{D}	<i>Boro</i> -2014	68.399*	36.061*	114.686*	1.738	2.891	65.288*	0.021*	891.203	0.164*	4.041
	<i>Boro</i> -2015	64.168	65.962	172.458*	1.219	1.161	37.875	0.006	794.003*	0.174*	2.680
	<i>Kharif</i> -2015	94.561*	93.392*	74.215*	1.095	4.568	93.667*	0.027*	1401.425*	0.162*	0.874
\hat{H}_1	<i>Boro</i> -2014	273.891*	270.448*	219.824*	14.759*	26.470*	288.402*	0.092*	4974.33*	0.507*	134.155*
	<i>Boro</i> -2015	356.004*	383.304*	308.144*	8.368*	24.934*	263.696*	0.087*	3081.517*	0.693*	211.363*
	<i>Kharif</i> -2015	185.261*	166.534*	232.242*	14.858*	30.738*	246.164*	0.124*	2143.826*	0.373*	55.574*
\hat{H}_2	<i>Boro</i> -2014	230.276*	236.944*	185.018*	14.213*	18.807*	233.170*	0.076*	3568.331*	0.420*	106.026*
	<i>Boro</i> -2015	314.359*	336.402*	258.698*	7.936*	20.781*	221.792*	0.063*	1827.368*	0.591*	153.380*
	<i>Kharif</i> -2015	162.649*	149.516*	191.881*	14.551*	24.360*	153.070*	0.107*	1199.832*	0.305*	48.465*
\hat{h}_2	<i>Boro</i> -2014	52.481*	69.741*	2.962	0.065	0.125	186.436*	0.0001	1068.599	0.177*	8.462
	<i>Boro</i> -2015	88.777	148.969*	127.377*	0.365	-0.206	8.730	0.010	1420.957*	0.32*	9.397
\hat{F}	<i>Kharif</i> -2015	11.519	20.586	62.36*	8.858*	12.045*	9.758	0.048*	2063.406*	0.257*	10.976
	<i>Boro</i> -2014	31.133	24.745	70.022	1.073	6.800	67.875	0.028*	2110.841	0.212	-9.881
	<i>Boro</i> -2015	26.402	34.786	149.561*	0.868	2.769	42.161	0.01	1903.741*	0.211	1.220
\hat{E}	<i>Kharif</i> -2015	17.430	20.934	38.553	-0.266	7.831	151.353*	0.037	2199.099*	0.180*	-4.095
	<i>Boro</i> -2014	0.546	0.467	0.253	0.525	0.588	0.922	0.005*	16.686	0.00014	1.979
	<i>Boro</i> -2015	0.474	0.538	0.438	0.440	0.548	0.495	0.004	12.556	0.00027	1.974
<i>Kharif</i> -2015	0.516	0.519	0.241	0.422	0.510	0.768	0.004	13.059	0.00009	1.350	

*p<0.05; **p<0.01; ***p<0.001; SV-Source of variation; DF – Degrees of freedom; DTF – Days to 50% flowering; DTM – Days to maturity; PH- Plant height; ET/P – Effective tillers/plant; PL- Panicle length; FL – Flag leaf length; FW- Flag leaf width; G/P-Grains/panicle; 100SW- 100 seed weight; Y/P- Yield/plant; \hat{D} - variance component due to additive gene effects; \hat{H}_1 - variance component due to dominance deviations; \hat{H}_2 - estimate of dominance genetic variance due to proportion of positive and negative genes; \hat{h}^2 - net dominance effect; \hat{F} - mean of covariance of additive and dominance effects over all arrays; \hat{E} - environmental component of variation.

Table 4. Proportion of genetic components of variation for yield and yield contributing traits in *boro* rice over seasons

Component	Season	DTF	DTM	PH	ET/P	PL	FL	FW	G/P	100SW	Y/P
$(\hat{H}_1 / \hat{D})^{1/2}$	<i>Boro</i> -2014	2.001	2.071	1.384	2.914	3.026	2.102	2.117	2.363	1.759	5.762
	<i>Boro</i> -2015	2.355	2.411	1.337	2.620	4.634	2.639	3.697	1.970	1.996	8.880
	<i>Kharij</i> -2015	1.400	1.335	1.769	3.684	2.594	1.621	2.128	1.237	1.517	7.974
$\hat{H}_2 / 4 \hat{H}_1$	<i>Boro</i> -2014	0.210	0.219	0.210	0.241	0.178	0.202	0.206	0.179	0.207	0.198
	<i>Boro</i> -2015	0.221	0.219	0.210	0.237	0.208	0.210	0.181	0.148	0.213	0.181
	<i>Kharij</i> -2015	0.219	0.224	0.207	0.245	0.200	0.155	0.215	0.140	0.204	0.218
$\hat{K}\hat{D}/\hat{K}\hat{R}$	<i>Boro</i> -2014	1.257	1.209	1.566	1.237	2.271	1.657	1.960	3.010	2.165	0.650
	<i>Boro</i> -2015	1.191	1.246	1.960	1.314	1.693	1.535	1.506	4.109	1.872	1.053
	<i>Kharij</i> -2015	1.141	1.183	1.344	0.936	1.987	2.987	1.910	4.470	2.155	0.546
\hat{h}_2 / \hat{H}_2	<i>Boro</i> -2014	0.228	0.294	0.016	0.005	0.007	0.800	0.001	0.299	0.423	0.080
	<i>Boro</i> -2015	0.282	0.443	0.492	0.046	0.010	0.039	0.166	0.778	0.542	0.061
	<i>Kharij</i> -2015	0.071	0.138	0.325	0.609	0.489	0.064	0.447	1.720	0.843	0.226
\hat{h}_2 (ns)%	<i>Boro</i> -2014	41.0	37.6	46.1	12.9	26.2	30.8	14.8	9.30	15.7	42.5
	<i>Boro</i> -2015	33.4	31.6	35.7	13.9	18.1	25.2	34.2	13.3	18.0	42.4
	<i>Kharij</i> -2015	54.8	54.1	44.1	17.0	17.6	31.2	11.7	18.9	24.8	31.0

DTF – Days to 50% flowering; DTM – Days to maturity; PH- Plant height; ET/P – Effective tillers/plant; PL- Panicle length; FL – Flag leaf length; FW- Flag leaf width; G/P-Grains/panicle; 100SW- 100 seed weight; Y/P- Yield/plant; $(\hat{H}_1 / \hat{D})^{1/2}$ – average degree of dominance; $\hat{H}_2 / 4 \hat{H}_1$ – proportion of dominant genes with positive and negative effects; $\hat{K}\hat{D}/\hat{K}\hat{R}$ – proportion of dominant and recessive genes; \hat{h}_2 / \hat{H}_2 – proportion of gene(s)/gene(s) blocks exhibiting dominance; \hat{h}_2 (ns)% – narrow sense heritability estimate

The ratio of dominance to recessive genes, i.e. $\overline{KD}/\overline{KR}$, was greater than unity for all the yield traits except effective tillers per plant (0.936 in *kharif*-2015) and yield/plant (0.650 in *boro*-2014 and 0.546 in *Kharif*-2015) in all the three seasons suggesting presence of an excess of dominant genes in parental lines. For effective tillers/plant (*kharif*-2015) it was close to unity indicating an equal proportion of dominant and recessive gene among parents. However, an excess of recessive genes were observed for yield/plant (*boro*-2014 and *kharif*-2015). Akram *et al.* (2007) also reported presence of dominant alleles in parental alleles for most of the traits as the ratio was more than unity. Verma and Srivastava (2004) reported occurrence of more recessive genes for the trait 50% flowering, panicle length, flag-leaf area and grain yield and occurrence of dominant genes in other yield traits. Raju *et al.* (2011) reported excess of dominant alleles for days to flowering, ear bearing tillers per plant, panicle weight, 1000 grain weight, grain yield/plant, kernel length and kernel length/breadth (L/B). Kumar (2011) reported distribution of more dominant alleles for the traits day to first flowering, plant height, grain yield/plant, 1000 grain weight, grain length and grain breadth and distribution of more recessive alleles for number of productive tillers, panicle length, number of grains per panicle and grain length/breadth (L/B). Habib *et al.* (2014) found less than unity value for plant height, number of tillers/plant, panicle length, total spikelets/plant, number of seeds/panicle, days to 50% flowering, days to maturity, panicle number/plant, grain weight/panicle, grain yield/plot and 1000 seed weight indicating excess of recessive alleles.

The ratio of \hat{h}^2/\hat{H}_2 denotes an approximate number of genes/group of genes controlling the trait exhibiting dominance. The estimate ranged from 0.001 (flag leaf width in *boro*-2014) to 1.720 (grains/panicle in *kharif*-2015) in various seasons, which indicated that at least one to two genes or group of genes showing dominance were present for different traits. In *boro*-2014, all the traits were controlled by single gene or gene group. In *boro*-2015, all the traits were controlled by single gene or gene group exhibiting dominance. In *kharif*-2015, all the traits were controlled by single gene or gene group except grains/panicle which was controlled by two gene or gene groups exhibiting dominance. Li and Chang (1970) reported one to five gene or gene groups exhibiting dominance for seeding to heading period, plant height, number of panicles per plant, panicle length, panicle weight and number of spikelets per panicle in rice. Murai and Kinoshita (1986) reported one to twelve gene or gene groups exhibiting dominance for different yield traits. Verma and Srivastava (2004) reported one major gene group involved in expression of panicle length and plant height in F_1 while the remaining traits showed the presence of at least one major group of genes controlling their inheritance. Kumar (2011) found the ratio less than one for all the traits under study and reported that all the traits were under the control of at least single group of genes. Xu and Shen (1991) and Akram *et al.* (2007) reported one to three gene or gene groups and Habib *et al.* (2014) reported three to five genes or gene groups showing dominance for traits under study.

The estimates of narrow sense heritability were low to moderate for most of the yield traits in all the three seasons. Days to 50% flowering showed highest heritability (33.4 to 54.8%) over the seasons followed by plant height (35.7% to 46.1%) and yield/plant (31.0% to 42.5%). Raju *et al.* (2011) also reported low to moderate narrow sense heritability for yield components such as days to 50% flowering, ear bearing tillers, panicle weight besides grain yield/plant indicating prevalence of non additive gene action i.e. dominant and epistasis in these traits. However, high heritability was observed by Raju *et al.* (2011) for 1000-grain weight indicating prevalence of additive gene action and direct selection for this trait can be done for genetic improvement. Direct selection is effective for the traits showing high heritability and desirable mean performance.

Conclusion

The present investigation indicated the importance of dominance type of genetic effects in inheritance of yield and yield related traits in rice over three seasons. Certainly, heterosis breeding would be more beneficial to harvest the dominance gene effects of these traits. The low to moderate narrow sense heritability for most of traits suggested that direct selection for these traits may not be effective and reliable.

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EFFECT OF BIOSLURRY ON TRANSPLANTED AUS RICE UNDER RAINFED CONDITION IN SYLHET

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Abstract

An experiment was conducted in farmer's field with the aim to evaluate the effect of integrated use of bioslurry and inorganic fertilizer on the agro-economic performance of transplanted aus rice (t. aus) (cv. BRRIdhan42) in AEZ 20. The trial was laid out in a randomized complete block design with three replications. There were three treatments *viz.*, T₁: soil test based inorganic fertilizer dose for high yield goal (HYG), T₂: cowdung (CD) slurry @ 5 t ha⁻¹ with integrated plant nutrition system (IPNS) basis inorganic fertilizer dose for HYG and T₃: Farmer's practice (average of 20 farmers fertilizer dose). The highest mean grain yield (3.93 t ha⁻¹) of t. aus rice was obtained from the treatment T₂ followed by T₁ (3.46 t ha⁻¹). Gross return, gross margin and benefit cost ratio were also observed higher in T₂. Therefore, fertilizer package NPKSZn @ 56-4-20-9-1 kg ha⁻¹ + cow dung slurry @ 5 t ha⁻¹ in IPNS approach might be helpful for getting higher grain yield of t. aus rice and maintaining sustainable soil health as well.

Keywords: Bioslurry, IPNS, transplanted aus rice, benefit cost ratio, soil health.

Introduction

The fertility status of Bangladesh soils is gradually declining and becoming a critical issue for sustainable crop production. Most of the soils are depleted and decline in crop yields is observed everywhere if proper fertility management is not made (Islam, 2006). A good soil should have at least 2.0% organic matter (OM), but in Bangladesh most of the soils have less than 1.5% OM and some soils have even less than 1% OM (FRG, 2012). Proper soil fertility management is, therefore, one of the prime importances in an endeavor to increase crop productivity and food security.

Bio-slurry produced from biogas plant is rich in nutrients and can be used successfully for economic crop production. It is an important type of organic manure that is applied in the form of semi-liquid or compost. It contains considerable amount of plant nutrients compared to cowdung, poultry manure or even compost that improves the soil fertility. It has no toxic or harmful effects on plants. The nutrient quality of bioslurry is higher than that of compost manure and chemical fertilizer. Because of the minimal loss of nitrogen in slurry, it is more effective as fertilizer than composted cattle dung (Islam, 2006; BCAS, 2009). Joshi *et al.* (1994) reported that use of bio-slurry along with inorganic fertilizers may be a good option for increasing soil fertility. The preliminary

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experimental results also indicated that the yield of crops and vegetables could be increased from 10 to 30 percent through the application of slurry (CMS/FAO, 1996). Bonten *et al.* (2014) asserted that nutrients in bio-slurry, especially nitrogen, are more readily available than in manure, leading to a larger short term fertilization effect. Batsai *et al.* (1979) and Shaheb and Nazrul (2011) observed that chemical fertilizer along with bioslurry produced the highest cabbage yield. Although animal waste causes environmental pollution (Paik, 1999) when applied to land without appropriate controls and management (Balsari *et al.*, 2005), the agronomic utilization of slurry represents the best solution for their reuse specially as a potential source of soil nutrients for smallholder rural farmers. However, the utilization of bio-slurry along with inorganic fertilizers may be a good option for increasing crop productivity and soil fertility. The maintenance of organic matter, soil fertility and sustainable crop production are some alarming issues for the farmers and agricultural scientists. Research work on the bioslurry is lacking in acidic soil of Sylhet, Bangladesh. Therefore, the present study was undertaken to evaluate the efficiency of bio-slurry on the performance of t. aus rice under rainfed condition in AEZ 20.

Materials and Method

The experiment was conducted at farmer's field in multilocation testing site, Moulavibazar (AEZ 20) during the two consecutive years of 2011 and 2012 under rainfed condition in AEZ 20. The experiment was laid out in a randomized complete block design with three replications. Three fertilizer treatments *viz.*, T₁: soil test based inorganic fertilizer dose for high yield goal (HYG), T₂: cowdung (CD) slurry @ 5 t ha⁻¹ with IPNS basis inorganic fertilizer dose for HYG and T₃: Farmer's practice (average of 20 farmers fertilizer dose applied in transplanted aus rice) were used in the trial. The initial soil nutrient status of the experimental soil is presented in Table-1. Treatment-wise nutrient elements were calculated based on the initial soil nutrient status with the help of FRG (2005) and described in Table 2. The unit plot size was 6m × 5m. Rice variety BRRIdhan42 of transplanted aus (t. aus) was used in the trial. Entire amounts of cow dung slurry as per treatment were applied 4 days before final land preparation. The whole amount of phosphorus (P), potassium (K), sulphur (S), boron (B) and 1/3 of nitrogen (N) were applied during final land preparation. The rest of N was top dressed at 21 and 45 days after transplanting of aus rice. Twenty seven day old aus rice seedlings were transplanted on 08-10 June 2011. Plant protection measures and other intercultural operations were done as and when necessary. The crop was harvested on 15 and 17 August in 2011 and 2012, respectively. Five years average monthly maximum and minimum temperatures and monthly total rainfall data have been presented in Fig. 1. Data on yield attributes *viz.*, plant height, plants per m², effective tillers per plant and 1000 grain weight were recorded in time. Individual plot-wise data on grain and straw yields were

recorded and converted into t ha⁻¹. Harvest index and biological yield were calculated by the following formula (Equ. 1 and 2). The collected data were analyzed statistically following the ANOVA technique with the help of MSTAT-C software. The mean differences among the treatment means were adjudged by Least Significant Difference (LSD) test (Gomez and Gomez, 1984).

$$\text{Harvest Index (\%)} = \frac{\text{Grain Yield}}{\text{Biological Yield}} \times 100 \dots\dots\dots(1)$$

$$\text{Biological Yield} = \text{Grain Yield} + \text{Straw Yield} \dots\dots\dots(2)$$

Table 1. Initial soil (0-15cm depth) nutrient status of the experimental field

Nutrients	N (%)	P (µg/g soil)	K (m.eq /100g soil)	S (µg/g soil)	Zn (µg/g soil)	B (µg/g soil)	OM (%)	p ^H
Value	0.08	9.55	0.12	7.53	1.04	0.48	1.85	5.10
Critical limit	0.12	7.0	0.12	10.0	0.6	0.2	-	-
Interpretation	Very low	Low	Low	Low	Medium	Optimum	Medium	Acidic

Table 2. Treatment-wise nutrient elements for transplanted aus rice

Treatments	N-P-K-S-Zn (kg ha ⁻¹) + Cowdung Slurry (CDS)
T ₁ : soil test based inorganic fertilizer dose for high yield goal (HYG)	78-11-45-9-1 + 0
T ₂ : cowdung slurry @ 5 t ha ⁻¹ + IPNS basis inorganic fertilizer dose for HYG	56-4-20-9-1 + CDS @5 t ha ⁻¹
T ₃ : Farmer's practice (average of 20 farmers fertilizer dose)	80-20-25-0-0 + 0

Results and Discussion

Five years average monthly maximum and minimum temperatures and monthly total rainfall data have been presented in Fig. 1. It was observed that the highest amount of average monthly rainfall in Sylhet was recorded in June followed by August, July and May, whereas the lowest amount of rainfall occurred in January followed by December and February. Rainfall increased gradually from the month of January to June and then decreased. However, the annual monthly total rainfall was 3720 mm during the period 2007-12. Average maximum and minimum temperatures were 31.32 and 14.71°C, respectively. Two years research results on the yield attributes and yield of t. aus rice (cv. BRRI dhan42) are presented in Tables 3-5. Results revealed that yield attributes and yield of t. aus rice were significantly influenced by different nutrient packages except plants per m² in 2011. The details discussion is furnished below.

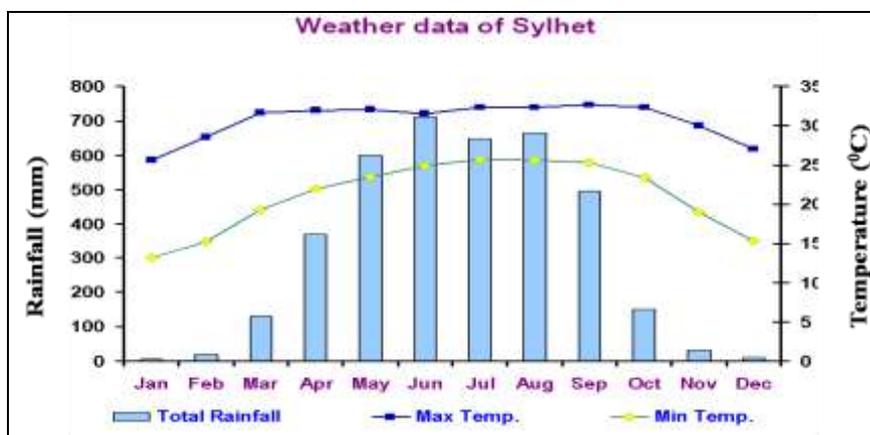


Fig. 1. Monthly average total rainfall, maximum and minimum air temperatures in Sylhet during the period 2007-2013.

Yield attributes of transplanted aus rice

Treatment having CD slurry @5 t ha⁻¹ along with IPNS based inorganic fertilizer gave the longest plant heights of t. aus rice (87.17 and 89cm) compared to that of only inorganic fertilizers in both the years of 2011 and 2012 (Table 3). The shortest plants (83.93 and 86.27 cm) were obtained from farmer's practice (T₃) treatment in both the years, respectively. Like plant height, the highest number of plants per m² (243.40) was obtained from T₂ treatment (CD slurry @ 5 t ha⁻¹ with IPNS based inorganic fertilizer) which was close to T₁ treatment in 2011. The highest number of effective tillers per plant (14.10 and 13.30) was obtained from T₂ treatment (CD slurry @ 5 t ha⁻¹ with IPNS based inorganic fertilizer) but the lowest tillers per plant (10.93 and 10.67) was obtained in T₃ treatment in both the years, respectively. On the contrary, the highest 1000 grain weights (25.15 and 24.88g) were recorded in CD slurry @ 5 t ha⁻¹ with IPNS based inorganic fertilizer and the lowest 1000 grain weights (23.32 and 23.78g) were recorded in farmer's practice (T₃) in 2011 and 2012, respectively (Table 3).

Table 3. Yield attributes and yield of transplanted aus rice in AEZ 20 during 2011 and 2012

Treatments*	Plant height (cm)		Plants m ⁻²		Effective tillers plant ⁻¹ (nos.)		1000 grain weight (g)	
	2011	2012	2011	2012	2011	2012	2011	2012
T ₁	84.77	86.33	239.20	239.20	11.85	11.08	23.77	23.98
T ₂	87.17	89.00	245.60	243.40	14.10	13.30	25.15	24.88
T ₃	83.93	86.27	159.80	236.50	10.93	10.67	23.32	23.78
LSD(0.05)	1.77	2.23	NS	6.28	1.54	1.08	1.55	0.61
CV(%)	0.92	1.13	35.45	1.16	5.54	4.08	2.84	1.12

*T₁: soil test based inorganic fertilizer dose for high yield goal (HYG), T₂: cowdung slurry @5 t ha⁻¹ + IPNS based inorganic fertilizer dose for HYG and T₃: Farmer's practice (average of 20 farmers fertilizer dose).

Table 3. Continued.

Treatments*	Grain yield (t ha ⁻¹)			Straw yield (t ha ⁻¹)			Biological yield (t ha ⁻¹)	Harvest Index (%)
	2011	2012	Mean	2011	2012	Mean		
T ₁	3.57	3.35b	3.46	4.87	4.08	4.48	7.94	43.60
T ₂	4.14	3.71a	3.93	4.43	3.89	4.16	8.09	48.55
T ₃	3.39	3.16b	3.28	4.17	4.35	4.26	7.54	43.46
LSD(0.05)	0.28	0.28	0.28	0.37	0.29	0.33	-	-
CV(%)	3.36	3.61	3.49	3.32	3.18	3.25	-	-

*T₁: soil test based inorganic fertilizer dose for high yield goal (HYG), T₂: cowdung slurry @5 t ha⁻¹ + IPNS based inorganic fertilizer dose for HYG and T₃: Farmer's practice (average of 20 farmers fertilizer dose).

Biological yield and harvest index of transplanted aus rice

Grain and straw yields of t. aus rice (cv. BRR1 dhan42) was influenced significantly by different fertilizer treatments (Table 4). In 2011, the highest grain yield (4.14 t ha⁻¹) was recorded in CD slurry @5 t ha⁻¹ along with IPNS based inorganic fertilizer, while the lowest grain yield (3.39 t ha⁻¹) was obtained from farmer's practice. In 2012, the highest grain yield (3.71 t ha⁻¹) was recorded in T₂ (CD slurry @5 t ha⁻¹ along with IPNS based inorganic fertilizer), which was followed by T₁ (inorganic fertilizers for HYG) (3.35 t ha⁻¹). The lowest grain yield was recorded in farmer's practice (3.16 t ha⁻¹). From the result, it was evident that cowdung bio-slurry has a great potentiality in increasing t. aus rice production. The highest straw yields (4.87 and 4.35 t ha⁻¹) were recorded in T₁ and T₃ treatments, respectively in both the years. It might be due to the accumulation of more dry matter in T₃. From the mean grain yield of two years, it was also found that treatment T₂ provided the highest grain yield (3.93 t ha⁻¹). The highest mean biological yield of t. aus rice (8.09 t ha⁻¹) was achieved from the same treatment (T₂). However, yield increase due to application of CD slurry @5 t ha⁻¹ along with IPNS based inorganic fertilizer (T₂) was 113 and 120% compared to soil test based inorganic fertilizer (T₁) and farmer's practice (T₃), respectively. Similarly, the highest harvest index (48.55) of t. aus rice was recorded in T₃ treatment. Gnanamani and Kasturi Bai (1992) reported that the yield of rice grain showed a 23 percent increase when soil was amended with biodigest slurry compared to SF (containing N, K and P). Similar results were also observed by Bharde (2003). A combination of biogas slurry @ 12.5 t ha⁻¹ and 100% NPK had pronounced effect on enhanced growth and yield attributes and yield of rice (Gurung, 1997). The result is also in conformity with the findings of Shaheb and Nazrul (2011) who reported that inorganic fertilizer along with cowdung slurry 5 t ha⁻¹ in IPNS approach produced the highest yield of cabbage. Higher crop yield due to application of bio-slurry were also reported by Manna and Hazra (1996) and Galli and Lalitpur (2001). Biogas slurry increases agricultural production because of its high content of plant nutrients, growth hormones and enzymes (CMS/FAO, 1996). Garfi *et al.* (2011) asserted that the

potato yield was increased by 27.5 percent with bioslurry compared to the control. Warnars (2012) alluded that bio-slurry increased crop revenues with an average of 25 percent. Thus, it might be summed up that bioslurry has profound effect to increase the production of t. aus rice.

Table 4. Cost and benefit analysis of t. aus rice as influenced by bio-slurry in AEZ 20 during 2011 and 2012

Treatments*	Gross return (Tk. ha ⁻¹)	Total variable cost (Tk. ha ⁻¹)	Gross margin (Tk. ha ⁻¹)	BCR
1	2	3	4 (2-3)	5 (2÷3)
T ₁	78150.00	39716.00	38434.00	1.97
T ₂	86820.00	42882.00	43938.00	2.02
T ₃	74020.00	38676.00	35344.00	1.91

*T₁: soil test based inorganic fertilizer dose for high yield goal (HYG), T₂: cowdung slurry @5 t ha⁻¹ + IPNS based inorganic fertilizer dose for HYG and T₃: Farmer's practice (average of 20 farmers fertilizer dose).

N. B. Price of inputs and outputs (Tk. kg⁻¹):

Price of input (Tk. kg ⁻¹)	Price of output (Tk. kg ⁻¹)
Rice seed-35.00	Rice grain-20.00
Cowdung slurry-1.00	Rice straw-2.00
Urea-12.00	
TSP-22.00	
MOP-15.00	
Gypsum-10.00	
Zinc Sulphate-130.00	

Economic analysis

The cost and return analysis of different fertilizer treatments are presented in Table 5. The highest gross return (Tk.86820 ha⁻¹), net return (Tk.43938 ha⁻¹) and benefit cost ratio (2.02) were obtained in IPNS basis inorganic fertilizer application with 5 t ha⁻¹ cow dung slurry for HYG. Gross return, gross margin and benefit cost ratio were the lowest with farmer's practice. Profitable production of cabbage and cauliflower was found with poultry manure slurry @ 3 t ha⁻¹ or CD slurry @ 5 t ha⁻¹ with IPNS basis inorganic fertilization in Grey Terrace Soil (AEZ-28) of Joydebpur (BARI, 2008). The results of this study are also in conformity with the findings of Shaheb and Nazrul (2011) who reported that inorganic fertilizer applied with bioslurry in an IPNS approach gave higher yield as well as economic return of cabbage. Gnanamani and Kasturi Bai (1992) asserted that the combined application of mineral fertilizer with slurry performed better than the separate application of either mineral fertilizer or slurry in their experimentation.

Table 5. Post harvest (0-15cm depth) nutrient status of the bioslurry applied experimental soil (mean of two years).

Nutrients	N (%)	P ($\mu\text{g/g}$ soil)	K (m.eq /100g soil)	S ($\mu\text{g/g}$ soil)	Zn ($\mu\text{g/g}$ soil)	B ($\mu\text{g/g}$ soil)	OM (%)	p ^H
Value	0.11	10.50	0.10	9.19	1.20	0.47	2.1	5.30
Critical limit	0.12	7.0	0.12	10.0	0.6	0.2	-	-
Interpretation	Low	Low	Low	Low	Medium	Optimum	Medium	Acidic

Post harvest nutrient status of the soil

Post harvest nutrient status of the experimental field at 0-15cm depth of the soil was described in Table 6. Results revealed that soil nutrient status in bioslurry treated plots were increased compared to initial nutrient status of the soil. Soil pH was also slightly increased in the bioslurry treated plots compared to others. It might be due to application of bioslurry. The results are in agreement with the findings of Joshi *et al.* (1994) who reported that use of bio-slurry along with inorganic fertilizers may increase fertility of the soil. Islam (2006) also asserted that integrated plant nutrition system (IPNS) that combines the use of organic (bioslurry) and chemical fertilizers play a vital role in restoring fertility as well as organic matter status of the soils.

Conclusion

From the results, it can be concluded that application of CD slurry @ 5 t ha⁻¹ with IPNS based inorganic fertilizer is better than other two treatments to obtain higher yield and economic return of transplanted aus rice. Cost benefit analysis also confirmed that the same treatment provided the highest gross return, net return and BCR. Soil nutrient status after harvest of t. aus rice also slightly increased due to application of bioslurry with inorganic fertilizers in the soil. Thus, it is recommended that farmers of AEZ 20 should apply cowdung slurry with IPNS basis inorganic fertilizer dose in their field for getting higher economic return and better soil health.

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EFFECT OF DROUGHT STRESS ON BIO-CHEMICAL CHANGE AND CELL MEMBRANE STABILITY OF SOYBEAN GENOTYPES

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AND A. U. AHMED⁴

Abstract

An experiment was conducted in a venylhouse at the environmental stress site of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during September to December 2012 to study the effect of drought stress on proline content, soluble sugar content, chlorophyll content and cell membrane stability of soybean genotypes. Four studied genotypes *viz.*, Shohag, BARI Soybean-6 and BD2331 (relatively stress tolerant) and BGM2026 (susceptible) were tested against two water regimes such as water stress and non-stress. Results indicated that due to drought stress there was an increase in proline content and soluble sugar content and decrease in chlorophyll a content, chlorophyll b content, total chlorophyll content, chlorophyll a/b ratio and cell membrane stability. Proline and soluble sugar showed more content in tolerant genotype than in susceptible ones. Chlorophyll reduction was most significant and cell membrane stability was found minimal in susceptible genotypes. From the result, genotype BGM2026 which recorded the lowest proline, soluble sugar content and highest chlorophyll reduction and cell membrane injury was considered as drought susceptible. The variety/genotype of soybean such as BARI Soybean-6, Shohag and BD2331 were more drought stress tolerant and better mechanisms of drought tolerance.

Introduction

Plant growth is accomplished through cell division, cell enlargement and differentiation involving genetical, physiological, ecological morphological and biochemical events and their complex interaction. The quality and quantity of plant growth depends on these events, which are affected by water deficit (Farooq *et al.*, 2009). The biochemical changes in plant due to water stress led to acclimate to the situation followed by severe functional damage and the loss of plant parts (Chaves *et al.*, 2002). Plants are known to have different mechanisms to adjust water stress conditions. Mechanism of drought tolerance, especially at low plant water status, involve processes at the cellular level, the most important being osmotic adjustment and protection of the membrane system (Mullet and Whitsitt, 1996). An important adjustment under drought stress is to maintain cell turgidity (Ku *et al.*, 2013). To maintain cell turgidity under stress, osmotic adjustment is a common mechanism which involves active accumulation of

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solutes in cells (Ku *et al.*, 2013) and of these solutes, proline is widely distributed in plants and it accumulates in larger amounts than other amino acids in drought-stressed plants (Ashraf, 2004). Proline accumulation is believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans, 2008). Soluble sugars are also considered to play an important role in osmotic adjustment in plants and are widely regarded as adaptive response to water stress conditions (Kameli and Loesel, 1993). Structural integrity of cellular membranes is also important for survival under severe dry periods, or in situations where random droughts occur (Martinez *et al.*, 2004). It is generally accepted that the maintenance of integrity and stability of membranes under water stress is a major component of drought tolerance in plants (Bajji *et al.*, 2002). The degree of cell membrane stability is considered to be one of the best physiological indicators of drought stress tolerance and can be used to screening drought-tolerant genotypes (Kocheva *et al.*, 2004). One of the most important changes under stress is the decrease in the total chlorophyll content (Sarker *et al.*, 1999). Ommen *et al.* (1999) reported that leaf chlorophyll content decreased as a result of drought stress. Plant water stress can affect the ability of the plant to produce chlorophylls, thus affecting leaf greenness (Sandoval-Villa *et al.*, 2002). Hence, the present experiment was undertaken to analyze the drought induced change in bio-chemicals like proline, soluble sugar, chlorophyll content and cell membrane stability of some selected soybean genotypes.

Materials and Method

A pot experiment in a vinyl house was conducted at the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur during September to December 2012. Three relatively water stress tolerant variety/line (Shohag, BARI Soybean-6 and BD2331) and one susceptible (BGM-2026) genotypes (selected from the previous experiment) were tested against two water regimes (water stress and non-stress) at vegetative and pod development stages. There were eight treatment combinations. The pots were arranged in a completely randomized design under factorial arrangement with four replications. Seeds of tolerant genotypes and susceptible genotypes were sown in plastic pots (24 cm internal diameter and 30 cm height). The soil of the pot was filled with mixture of soil and cow dung at a ratio of 4:1. Pot contained 12.0 kg of soil which was equivalent to 9 kg oven dry soil and holds about 28% moisture at field capacity (FC). Soil used in the pot was sandy loam. Fertilizer rates of 70 mg N, 35 mg P, 180 mg K and 20 mg S pot^{-1} in the form of urea, triple super phosphate, muriate of potash and gypsum was added and well mixed with the soil before pouring into the pots. Six seeds pot^{-1} were sown on 3 September, 2012. After seedling establishment two uniform and healthy plants pot^{-1} were allowed to grow. Two watering treatments of the plants viz. drought stress i.e. water stress (50% water of the FC) and non-stress i.e. control (80% water of FC) were applied at 21 days after emergence (DAE) and maintained throughout the growing season. Weeding and spraying were done as normal management practices for all the treatments.

To estimate proline accumulation, samples were collected from top third fully expanded young trifoliolate leaves at pod development stage of soybean genotypes. The collected leaf samples were immediately kept in an ice-bag and brought to the laboratory. Proline was determined by Ninhydrin method (Troll and Lindsley, 1955). The soluble sugar content of soybean leaves was also estimated at pod development stage. Leaf samples were collected from different soybean plants of the respective treatments. Dried and grounded samples were used to estimate soluble sugar by following the method of Yoshida *et al.* (1976).

Chlorophylls were estimated at vegetative, flowering and pod development stages on fresh weight basis extracting the leaf samples with 80% acetone by using Double Beam Spectrophotometer (Model 200-20, Hitachi, Japan). Different chlorophylls were estimated using the following equations (Witham *et al.*, 1986).

$$\text{Chlorophyll a (mg/g tissue)} = [12.7(D663) - 2.69(D645)] \times V / (1000 \times W)$$

$$\text{Chlorophyll b (mg/g tissue)} = [22.9(D645) - 4.68(D663)] \times V / (1000 \times W)$$

$$\text{Total Chlorophyll (mg/g tissue)} = [20.2(D645) + 8.02(D663)] \times V / (1000 \times W)$$

Where, D = optical density reading of the chlorophyll extract at the specific wavelength

V = final volume of the 80% acetone-chlorophyll extract

W = fresh weight in gram of the tissue extracted

For cell membrane stability measurement leaf samples were collected from plants at pod development stage. Cell membrane stability of leaf tissues was calculated as the percentage injury using the following equation (Blum and Ebercon, 1981):

$$\text{Percent injury} = 1 - \frac{\left[\left(1 - \frac{T_1}{T_2} \right) \right]}{\left[\left(1 - \frac{C_1}{C_2} \right) \right]} \times 100$$

Where,

T₁ = first conductivity measurement of desiccation treatment,

T₂ = second conductivity measurement of desiccation treatment,

C₁ = first conductivity measurement of control, and

C₂ = second conductivity measurement of control.

The data were analyzed with MSTATC statistical package program. The difference between the treatments means were compared by Least Significant Difference (LSD) test (Gomez and Gomez, 1984). Functional relationships between proline content and injury index of soybean genotypes as affected by water stress were established through correlation and regression analyses by using Excel program.

Results and discussion

Proline accumulation

In water stress condition free proline increased markedly in all the genotypes under studied (Fig 1). Similar results was also observed by Ashraf and Iram (2005) in *Phaseolus vulgaris*. Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration usually higher in stress tolerant than in stress sensitive plants (Silvente *et al.*, 2012). Significant differences in proline content were observed among genotypes under water stress (Fig 1). The highest accumulation of proline due to water stress was observed in genotype BARI Soybean-6 (131.5%) followed by Shohag (124.8%) and the lowest accumulation in BGM2026 (54.3%). There were no significant differences among the genotypes under non-stress conditions. A similar finding was observed by Stoyanov (2005) who reported that tolerant cultivar showed the highest accumulation of proline in bean plants. Proline accumulation is a common physiological response to many plants in response to drought stress (Mafakheri *et al.*, 2010). Irigoyen *et al.* (1992) reported a relationship between turgor and proline accumulation which could be of useful as a possible drought-injury sensor so selection of new drought tolerant genotypes based on high proline accumulation can be used as a parameter for selection of stress tolerance (Jaleel *et al.*, 2007). Proline is one of the most water soluble amino acids and it is supposed to play a significant role in osmotic adjustment with regard to reduction of osmotic potential due to a net accumulation of solutes (Molaei *et al.*, 2012). Vendruscolo *et al.* (2007) found that proline is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plant to avoid detrimental effects of water stress.

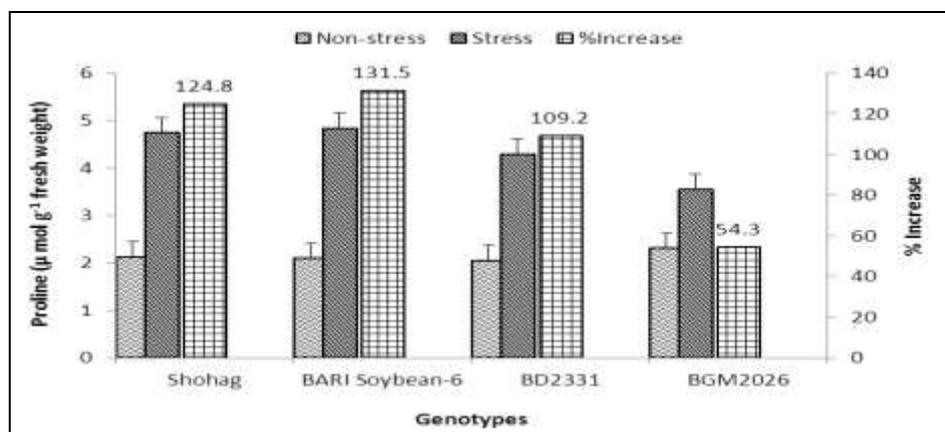


Fig. 1. Proline accumulation in soybean leaf at pod development stage under non-stress and water stress conditions. (Vertical bar represent LSD value at 5% level of significance).

Soluble sugar accumulation

Soluble carbohydrates have a role in osmotic regulations and conservation mechanism (Martin *et al.*, 1993). Total soluble sugar contents in all the genotypes were significantly higher under water stress condition than that under non-stress (Table 1). Sarker *et al.* (1999) also obtained similar results. The highest soluble sugar accumulation was observed in BARI Soybean-6 followed by Shohag, and the lowest in BGM2026 under water stress condition. The total soluble sugar increased due to water stress were 21.8, 25.2, 13.9 and 8.4 mg g⁻¹ dry matter in Shohag, BARI Soybean-6, BD2331 and BGM2026, respectively which was 44, 47, 28 and 17% higher respectively over non-stress. Kundu and Paul (1997) reported that total soluble sugar content was significantly higher at 46 and 67% in the non-irrigated plants of *Brassica campestris* at flowering and pod-filling stages, respectively. As an osmotic agent, the increased sugar, induced by water stress, was significantly correlated to osmotic adjustment and turgor maintenance (Sa'nchez *et al.*, 1998). Another possible function of soluble sugar accumulation under water stress (unrelated to their osmotic contribution) is to form reserve assimilates for seed filling (Sa'nchez *et al.*, 1998).

Table 1. Soluble sugar accumulation in soybean genotypes under non-stress and water stress conditions at pod development stage

Genotypes	Soluble sugar accumulation (mg g ⁻¹ dry matter)		Percentage increase in soluble sugar over non-stress
	Non-stress	Water stress	
Shohag	49.3	71.1	44
BARI Soybean-6	53.4	78.6	47
BD2331	48.8	62.7	28
BGM2026	50.2	58.6	17
LSD _(0.05) S		3.41	
G		8.09	
SxG		11.45	
CV(%)		11.07	

S=Stress, G=Genotype

Leaf chlorophylls

Water stress significantly affected biosynthesis of leaf chlorophylls as well as chlorophyll a/b ratio in all the genotypes at different growth stages (Tables 2, 3, and 4). Total chlorophyll was significantly decreased by water stress at three growth stages. The chlorophyll a/b ratio was not changed at the vegetative stage though significantly decreased at flowering and pod development stages due to

water stress (Tables 2, 3, and 4). Total chlorophyll content of all the genotypes was higher under non-stress environment at the three stages studied, while water stress caused a reduction in total chlorophyll contents and chlorophyll a/b ratio. Similar results were reported by Makbul *et al.* (2011) in soybean under drought stress conditions. A reduction of chlorophyll formation due to water stress was also reported by Sarker *et al.* (1999). Chlorophyll a, chlorophyll b and a/b ratio were higher in BARI Soybean-6 at all three growth stages under stress and non-stress conditions. Lowest Chlorophyll a and b content and a/b ratio was obtained in BGM2026 under water stress condition. Reduction percent of chlorophyll a and b was the lowest in BARI Soybean-6 and the highest in BGM2026 irrespective of growth stages. The ratio of chlorophyll a/b decreased due to water stress. Decreased in chlorophyll a/b ratio indicated that chlorophyll b is not more sensitive to drought than chlorophyll a (Mafakheri *et al.*, 2010). Water stress decreased chlorophyll a (chl a) more than chlorophyll b (chl b) and thus decreased the ratio. The genotypes BGM2026 had the most affected chlorophyll a/b ratio compared to other genotypes. Sairam and Siravastava (2002) reported that chlorophyll content of resistant and sensitive cultivars to drought stress reduced but resistant cultivar had high chlorophyll content. The decreased in total chlorophyll content may have resulted from a decrease in leaf water status in the soybean (Makbul *et al.*, 2011).

Table 2. Chlorophyll a, chlorophyll b and chlorophyll a/b ratio in soybean leaves at vegetative stage under two water regimes

Genotypes	Chlorophyll a (mg g ⁻¹ leaf tissue)			Chlorophyll b (mg g ⁻¹ leaf tissue)			Chlorophyll a/b ratio	
	Non-stress	Water stress	% reduction	Non-stress	Water stress	% reduction	Non-stress	Water stress
Shohag	2.19	1.95	11	0.93	0.83	10	2.35	2.34
BARI Soybean 6	2.46	2.21	10	0.97	0.89	8	2.53	2.48
BD2331	2.14	1.86	13	0.93	0.82	11	2.30	2.26
BGM2026	2.30	1.79	22	0.96	0.80	17	2.39	2.23
LSD _(0.05) S	0.15			0.04			NS	
G	0.16			0.03			NS	
SxG	NS			NS			NS	
CV(%)	6.11			3.12			4.17	

S=Stress, G=Genotypes, NS=Not significant.

Table 3. Chlorophyll a, chlorophyll b and chlorophyll a/b ratio in soybean leaves at flowering stage under two water regimes

Genotypes	Chlorophyll a (mg g ⁻¹ leaf tissue)			Chlorophyll b (mg g ⁻¹ leaf tissue)			Chlorophyll a/b ratio	
	Non- stress	Water stress	% reduction	Non- stress	Water stress	% reduction	Non- stress	Water stress
Shohag	2.45	2.08	16	1.19	1.02	14	2.05	2.00
BARI Soybean-6	2.67	2.26	15	1.27	1.10	13	2.10	2.05
BD2331	2.24	1.43	18	1.11	0.93	16	2.01	1.96
BGM2026	2.50	1.63	34	1.25	0.86	31	2.00	1.90
LSD _(0.05) S	0.31			0.01			0.04	
G	0.32			0.12			0.039	
SxG	NS			NS			NS	
CV(%)	12.02			8.83			3.88	

S=Stress, G=Genotypes, NS=not significant.

Table 4. Chlorophyll a, chlorophyll b and chlorophyll a/b ratio in soybean leaves at pod development stage under two water regimes

Genotypes	Chlorophyll a (mg g ⁻¹ leaf tissue)			Chlorophyll b (mg g ⁻¹ leaf tissue)			Chlorophyll a/b ratio	
	Non- stress	Water stress	% reduction	Non- stress	Water stress	% reduction	Non- stress	Water stress
Shohag	2.22	1.73	22	1.11	0.92	17	1.00	1.88
BARI Soybean 6	2.50	2.00	20	1.16	0.98	15	2.14	2.04
BD2331	2.06	1.56	24	1.08	0.88	18	1.90	1.77
BGM2026	2.42	1.30	46	1.15	0.76	34	2.10	1.70
LSD _(0.05) S	0.16			0.11			0.10	
G	0.14			0.07			0.07	
Sx 3	0.20			0.11			0.11	
CV(%)	6.06			5.95			3.38	

S=Stress, G=Genotypes

Membrane thermo-stability

Membrane thermo-stability was determined to estimate the percentage of injury of soybean genotypes under water stress condition. According to Blum and Ebercon (1981), injury index signifies the degree of membrane damage due to

stress. Cell membrane thermo-stability as evaluated by the relative electrolyte leakage at 44°C temperature differed among the soybean genotypes under both non-stress and water stress conditions (Table 5). Under non-stress condition all the four genotypes showed identical injury index but under stress condition the genotypes differed significantly for injury index. The genotype BGM2026 presented the greatest membrane damage and the genotype BARI Soybean-6 had the least damage followed by Shohag and BD2331. The injury index was 31.4% higher in BARI Soybean-6 whereas, it was 55.65% higher in BGM2026 in water stress condition than non-stress. Increased injury index was also observed due to water stress in soybean (Sarkar, 1993). The results indicated that the degree of membrane damage was higher due to water stress. The genotype Shohag and BARI Soybean-6 exhibited the lower rate of injury than BGM2026 to cell membranes that means genotype BGM2026 was found to be more injurious than Shohag and BARI Soybean-6. Variety Shohag and BARI Soybean-6 appeared to be relatively tolerant to water stress in terms of membrane stability.

Table 5. Percentage injury of soybean genotypes leaf at pod development stage under non-stress and water stress conditions

Genotypes	Percentage injury of leaf		Increase in injury % in stressed leaf at pod development stage
	Non-stress	Water stress	
Shohag	28.08	37.54	33.68
BARI Soybean-6	27.79	36.51	31.4
BD2331	28.13	38.39	36.47
BGM2026	27.83	43.32	55.65
LSD _(0.05) S		4.38	
G		3.49	
G x S		4.94	
CV(%)		8.73	

S=Stress, G=Genotypes

A negative relationship existed between injury percent and proline content of soybean genotypes in both the water regimes where R^2 were 95 and 0.29 for water stress and non-stress conditions, respectively (Fig. 2). The negative relationship indicated that the increase in proline content decreased the injury percent. Higher R^2 value as found in stress condition than non-stress condition further indicated a stronger relationship between injury index and proline content in the stress condition than control condition. There was a significant and negative correlation between proline accumulation and cell membrane injury (for non-stress $r = -0.54$ and for stress $r = -0.97$). Synthesis of proline and proteins, which have been implicated to have a role in protecting cellular structures during

dehydration, enables plant to survive under a condition of cellular water deficits (Molaei *et al.*, 2012).

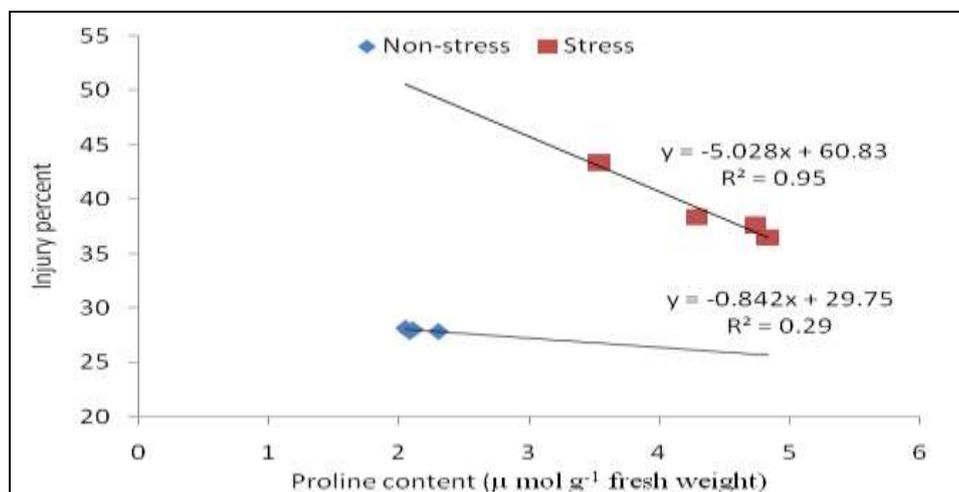


Fig. 2. Relationship between proline content and injury index of soybean genotypes leaf at pod development stage

Conclusion

All biochemical parameters and cell membrane stability of drought tolerant and drought sensitive genotypes showed similar pattern to drought stress. The tolerant genotypes accumulate more proline and soluble sugar than sensitive one. Drought stress decreased chlorophyll a, b and a/b concentration. Cell membrane stability was also higher in tolerant genotype. The variety/genotype Shohag, BARI Soybean-6 and BD2331 is considered as drought tolerant because of their higher proline, soluble sugar accumulation, chlorophyll content and cell membrane stability.

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**EFFECT OF SALT AND WATER STRESS ON GAS EXCHANGE,
DRY MATTER PRODUCTION AND K⁺/Na⁺ IONS
SELECTIVITY IN SOYBEAN**

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A. J. M. S. KARIM⁴ AND M. A. K. MIAN⁵

Abstract

The experiment was conducted in a vinylhouse at the Banghabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh during January to May, 2012 to evaluate the effect of salt and water stress on gas exchange characters, dry matter production and K⁺/Na⁺ ions selectivity in three selected soybean genotypes, namely Galarsum, BD 2331 and BARI Soybean-6. The genotypes were exposed to six treatments viz. (i) control (tap water), (ii) water shortage (irrigation with 70% depletion of available soil water when leaf began to wilt at 10:00 am), (iii) 50 mM NaCl irrigation, (iv) 50 mM NaCl irrigation + water shortage, (v) 75 mM NaCl irrigation, and (vi) 75 mM NaCl irrigation + water shortage conditions. The results revealed that 75 mM NaCl salt + water stress treatment drastically reduced stomatal conductance, photosynthesis and transpiration rate irrespective of soybean genotypes. However, the genotype Galarsum showed minimum transpirational water loss (1.45 mmol H₂O m⁻² s⁻¹) and maximum photosynthesis (20.45 μmol CO₂ m⁻² s⁻¹) as compared to BD 2331 and BARI Soybean-6 under 75 mM NaCl salt + water stress condition. Combined salt and water stress caused greater inhibition of shoot growth than either of the two in soybean. The shoot dry weights were decreased to 24.58, 23.00 and 21.57% of the control in Galarsum, BD 2331 and BARI Soybean-6, respectively at 75 mM NaCl salt + water stress. The genotype Galarsum accumulated higher amount of K⁺ (1.19%) and lower amount of Na⁺ (0.11%) in leaf tissue under 75 mM NaCl salt + water stress. Results indicated that the genotype Galarsum was more capable to cope with the high levels of salt under water stress condition than the other two genotypes.

Keywords: Soybean; Salt and water stress; Photosynthesis; Respiration; Shoot dry matter; Ion uptake

Introduction

Soybean (*Glycine max* L.) is a minor crop in Bangladesh though it is considered as one of the most nutritious crops in the world (Yaklich *et al.*, 2002). Currently, it is gaining popularity in the country for its increasing demand as an ingredient of animal feed as well as for the consciousness of its high nutrition value as human food (Karim *et al.*, 2012).

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Consequently its area is increasing especially in the marginal lands called *charland* (land formed due to accretion of silt on riverbed) and coastal land of southern part of Bangladesh (Islam and Rahman, 2011). Since 1973, the salinity of coastal land has been increased to 27% and the area exceeded one million ha at various levels. More areas are under threat of salinization due to the combined effects of sea level rise, increased tidal effect, introduction of brackish water for shrimp cultivation, continuous reduction of river flow particularly during dry period, capillary upward movement of soluble salts due to presence of high saline ground water table at shallower depth and faulty management of sluice gates in the south and south-western part of Bangladesh (Karim *et al.*, 2014). This causes lower cropping intensity and it is imperative to increase cropping intensity and productivity of the saline belt of Bangladesh. Since soybean is considered as a moderately salt tolerant crop (Mannan *et al.*, 2012), it may be a potential candidate crop for exploiting the relatively low saline areas after rainy season rice (*aman*) harvest in the southern Bangladesh. Besides, salt and water stress prevails at the same time in dry seasons, which very often adds extra harm on plant growth (Karim *et al.*, 1993). The adverse effects of both salt and water stress are primarily due to the restriction of water uptake by the roots (Karim *et al.*, 1993), which decreased relative water content (Orcutt and Nilsen, 2000). Therefore, plants are unable to maintain metabolic activities or turgidity for normal growth because of the low osmotic potential in soil. At the same time, plants absorb damaging amounts of Na^+ and Cl^- (Blum, 1988; Greenway and Munns, 1980; Karim *et al.*, 1992). Na^+ is the primary cause of ion specific damage, resulting due to a range of disorders in enzyme activation and protein synthesis (Tester and Davenport, 2003). In addition, Na^+ acts as a competitor of K^+ uptake in plant (Watad *et al.*, 1991; Schroeder *et al.*, 1994). In salt-adapted cells, no inhibition of K^+ uptake by NaCl was observed indicating a higher K^+/Na^+ selectivity at the plasma membrane (Watad *et al.*, 1991). Therefore, exclusion of Na^+ at root level and maintenance of high K^+ at shoot level are vital for the plants to grow under saline conditions (Munns *et al.*, 2000; Tester and Davenport, 2003). Leaf photosynthetic capacity is suggested to be a key parameter determining crop yield (Jiang *et al.*, 2002; Zhang *et al.*, 2007). The rate of photosynthetic CO_2 assimilation is generally reduced by salinity and drought (Lee *et al.*, 2004; Purwanto, 2003). Salt and water stress have various effects on physiological processes in plants. Extent of physiological changes in plants growing under salt and water deficit conditions is considered as an effective tool for identifying stress tolerant soybean for saline belt of Bangladesh. Therefore, this experiment was undertaken to analyze the physiological response in relation to gas exchange, dry matter distribution and mineral ions accumulation pattern in three high yielding soybean genotypes exposed to salt and water stress conditions.

Materials and Method

A pot experiment was carried out in a vinylhouse of the Department of Agronomy at Banghabandhu Sheikh Mujibur Rahman Agricultural University

(BSMRAU), Gazipur, Bangladesh during January to May, 2012. The location of the experimental site is situated at about 24° 23' north latitude, 90° 08' east longitude and an altitude of 8.4 m. Three genotypes of soybean (Galarsum, BD 2331 and BARI Soybean-6) were grown in six environmental conditions of salinity and water stress. The environmental conditions were (i) control, (ii) water shortage (irrigation with 70% depletion of available soil water when leaf began to wilt at 10:00 am), (iii) 50 mM NaCl irrigation, (iv) 50 mM NaCl irrigation + Water shortage, (v) 75 mM NaCl irrigation, and (vi) 75 mM NaCl irrigation+ Water shortage. The experiment was arranged in a two factor completely randomized design (CRD) with four replications. The genotypes were selected based on their better performance in previous study (Khan *et al.*, 2014). Treatments were imposed after three weeks of seedling emergence. In salt water irrigation and water shortage treatments, initially all pots were irrigated with salt water for a week then water shortage, and thereafter salt water irrigations were applied. The control plants were irrigated with tap water only at field capacity of the soil. Treatments were applied up to harvest. The soils of each pot contained 12 kg air dried sandy loam soil fertilized uniformly with 0.30 g of urea, 0.90 g of triple super phosphate, 0.60 g of muriate of potash and 0.60 g of gypsum before sowing. Five seeds were sown in the soil medium on 20 January, 2012. After the emergence and establishment, two uniform healthy seedlings per pot were allowed to grow for three weeks in the same environment. Admire 200SL @ 1 ml/liter of water was sprayed at 10 and 25 days after emergence to control Jassids and white flies. Ripcord 10 EC @ 1 ml/liter of water was sprayed at 45 and 55 days after emergence to control leaf roller and pod borer. Gas exchange measurements like photosynthetic rate (Pn), stomatal conductance, total conductance to CO₂ and transpiration (E) were measured at 49 days after emergence (after 4 weeks of treatments imposition) by Li-COR 6400 portable photosynthetic system (Li-COR, Lincoln, NE, United States). All the measurements were taken in a bright day between 11:00 and 13:00 h when photosynthetically active radiation (PAR) was between 1100 and 1200 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Photosynthetic water use efficiency (PWUE) was calculated as the ratio between Pn and E. Plants were harvested at 63 days after emergence and different parts of the plant were separated and then oven dried at 70 °C for 4 days to measure the dry weight of the shoot. Dried leaf samples were used to measure the Na⁺ and K⁺ ions concentrations of the respective treatments. Collected data were analyzed using STAR (Statistical Tool for Agricultural Research) program and the treatments means were compared by using Tukeys's Honestly Significant Difference (HSD) Test at $P \leq 0.05$.

Results and Discussion

Stomatal conductance to H₂O: Stomatal conductance to H₂O of soybean genotypes was significantly affected by salinity and water stress treatments (Fig .1). Under water stress treatment, BARI Soybean-6 gave significantly the highest conductance (0.32 mol H₂O m⁻²s⁻¹) to water and BD 2331 (0.14 mol H₂O m⁻²s⁻¹)

showed the least, which was identical to the response of Galarsum. The results however, varied under salt stress and, combined salt and water stress conditions. As compared to the salt stress stomatal conductance to H₂O was highly affected by the combined salt + water stress at both the salinity levels (50 and 75 mM NaCl). However, significantly the highest conductance of 0.21 and 0.16 mol H₂O m⁻²s⁻¹ was recorded in Galarsum at 50 and 75 mM NaCl salt stress, respectively. BD 2331 had the identical response to the conductance at the same treatments. The lowest conductance of 0.13 and 0.12 mol H₂O m⁻²s⁻¹ was obtained by BARI Soybean-6 at 50 and 75 mM NaCl salt stress, respectively. Under 75 mM NaCl salt + water stress condition Galarsum showed significantly the highest conductance (0.04 mol H₂O m⁻²s⁻¹), while both BD 2331 and BARI Soybean-6 genotypes had the least (0.03 mol H₂O m⁻²s⁻¹). Genotypic variation of soybean was observed in respect of stomatal conductance to water. These findings are in conformity with that of Kusvuran (2012) who reported that stomatal conductance of the melon genotypes decreased under salt and drought stress and it was slower in the resistant genotypes than sensitive ones.

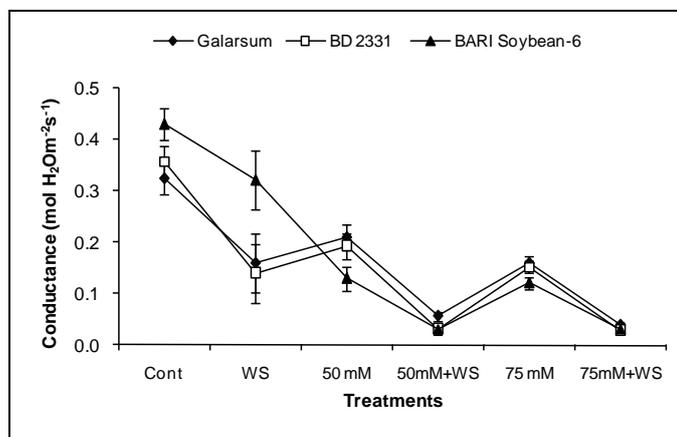


Fig. 1. Stomatal conductance to water as affected by salinity and water stress treatment. Bar represents mean \pm S.E. of the genotypes at the same level of treatment.

Here, Cont = Control, WS = Water stress and mM = NaCl concentration in mM.

Total conductance to CO₂: Total conductance to CO₂ of soybean genotypes was distinctly reduced under water stress, salt stress and, combined salt + water stress conditions as compared to control (Fig. 2). Conductance to CO₂ was significantly high in BARI Soybean-6 (0.29 mol CO₂ m⁻²s⁻¹) under only water stress condition, though drastically reduced in Galarsum and BD 2331. The genotype BD 2331 showed the least conductance (0.14 mol CO₂ m⁻²s⁻¹) under water stress. The total conductance reduced lesser extent in the salt stress conditions as compared to the combined salt + water stress conditions at both the salinity levels (50 and 75 mM NaCl). At the lower level of salt stress conditions Galarsum showed significantly the highest conductance (0.20 mol CO₂ m⁻²s⁻¹), which was identical with that of

BD 2331. BARI Soybean-6 showed the least conductance ($0.12 \text{ mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$). At the highest level of salt stress (75 mM NaCl), Galarsum and BD 2331 showed the highest conductance ($0.12 \text{ mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) and BARI Soybean-6 the least ($0.10 \text{ mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$).

Under the low level of salinity + water stress condition genotype Galarsum showed the highest conductance ($0.05 \text{ mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), which was identical with that of BD 2331 and BARI Soybean-6. Under the high level of salinity + water stress conditions all genotypes had similar conductance to CO_2 . Total conductance to CO_2 through stomata and mesophyll decreased due to low water availability under salt and water stress. Turner *et al.* (1998) reported that stomatal conductance decreased as the osmotic potential decreased in sorghum and sunflower. Similar results were also found by Dadkhah (2010) in sugar beet.

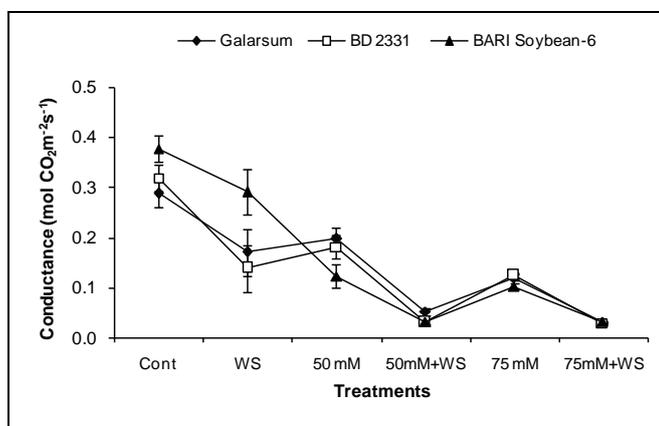


Fig. 2. Total conductance to CO_2 as affected by salinity and water stress treatment. Bar represents mean \pm S.E. of the genotypes at the same level of treatment.

Here, Cont = Control, WS = Water stress and mM = NaCl concentration in mM.

Photosynthetic rate: Photosynthetic rate (P_n) of soybean genotypes significantly declined when plants were exposed to water stress, salt stress and, combined salt + water stress conditions (Fig. 3). Under only water stress condition, BARI Soybean-6 depicted significantly the highest photosynthetic rate ($35.44 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), while it drastically reduced in Galarsum ($25.24 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) and BD 2331 ($21.34 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) genotypes. As compared to only the salt water stress and, the combined salt + water stress conditions, P_n rate reduced lesser extent in the salt stress condition. However, the reduction was higher at higher salt stress both in only salt stress and, the combined salt + water stress conditions due to more restriction of water uptake by the roots. The results are in agreement with that of Netondo *et al.* (2004) who reported that photosynthetic activity decreased when plants were grown under saline conditions. However, significantly the highest photosynthetic rates of 34.49 and $29.47 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ were recorded in Galarsum at 50 and 75 mM NaCl salt stress, respectively. BD 2331 had identical rate at the same treatments. The lowest photosynthetic rate of

29.70 and 27.30 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ were recorded in BARI Soybean-6 at 50 and 75 mM NaCl salt stress, respectively. Under 50 mM NaCl + water stress condition, Galarsum (23.62 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) showed significantly the highest Pn rate followed by BARI Soybean-6 (21.55 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$). BD 2331 (20.76 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) showed the lowest rate. Galarsum (20.45 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) also showed significantly the highest Pn rate under 75 mM NaCl + water stress condition. BD 2331 showed the least rate (20.36 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), which was identical with the Pn rate of BARI Soybean-6 (20.38 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) in the same treatment. The rate of photosynthetic CO_2 assimilation is generally reduced by salinity and drought. This reduction is partly due to a reduced stomatal conductance and consequent restriction of the availability of CO_2 for carboxylation (Brugnoli and Lauteri, 1991). Wang *et al.* (2011) reported that photosynthetic rate decreased under salt and water severity in tamarisk seedlings. Eisa *et al.* (2012) also reported that the net photosynthesis rates were greatly decreased by high salinity and salt-induced photosynthesis inhibition was accompanied with a decrease in transpiration rates but also with improved water use efficiency.

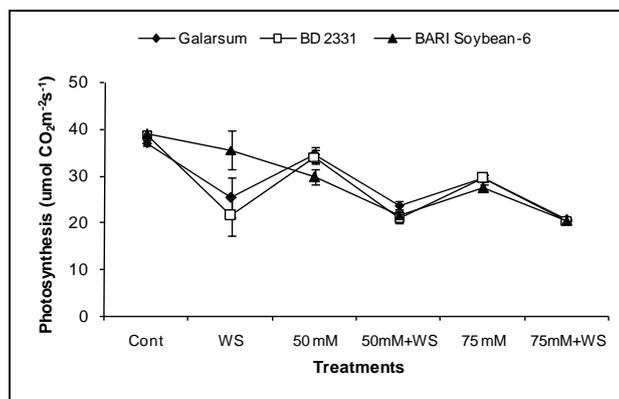


Fig. 3. Leaf photosynthesis as affected by salinity and water stress treatment. Bar represents mean \pm S.E. of the genotype at the same level of treatment.

Here, Cont = Control, WS = Water stress and mM = NaCl concentration in mM.

Transpiration rate: Water stress, salt stress and, combined salt + water stress led to a remarkable reduction in transpiration rate (E) of soybean genotypes as compared to control (Fig. 4). The reduction in water loss through transpiration was mainly due to the reduction of stomatal conductance to H_2O under salinity and water stress conditions. Genotypes varied in transpiration rates under different stresses. Under only water stress, condition BARI Soybean-6 transpired significantly the highest (11.96 $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) compared to others. Galarsum transpired the lowest (4.01 $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), which was identical with BD 2331 (4.07 $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$). Transpiration rate reduced to lesser extent in the salt stress condition than the combined salt + water stress condition. However, at lower level of salt stress (50 mM NaCl) treatment, significantly the highest

transpiration rate ($7.58 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) was recorded in BD 2331, which was identical ($7.38 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) with the rate of Galarsum. BARI Soybean-6 had the lowest rate ($6.69 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$). At higher level of salt stress (75 mM NaCl), significantly the highest transpiration ($6.66 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) was recorded in BARI Soybean-6 which was identical with the rate of BD 2331 ($6.42 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$). Galarsum showed significantly the least transpiration rate ($6.06 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$). In the combined salt + water stress conditions the transpiration rate was higher in BARI Soybean-6 than other genotypes. At the 50mM NaCl + with water stress, the highest transpiration rate ($2.22 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) was recorded in BARI Soybean-6, which was identical with the rate ($2.73 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) of Galarsum. On the contrary, BD 2331 showed the least rate ($2.55 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$). At higher level of salinity (75mM NaCl) + water stress treatment, BARI Soybean-6 showed the highest transpiration rate ($2.22 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), which was identical to that of BD 2331 ($1.80 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$). At this condition Galarsum showed the lowest transpiration rate ($1.45 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$). The results are in agreement with the findings of Wang *et al.* (2011) in tamarisk (*Tamarix chinensis* Lour.) seedlings. Eisa *et al.* (2012) reported that the transpiration rates were decreased by high salinity in quinoa. Plants subjected to water and salinity stress had lower stomatal conductance resulted in lower rate of transpiration. Water stress and salinity cause stomatal closure which reduced transpiration rate as reported by Halim *et al.* (1990).

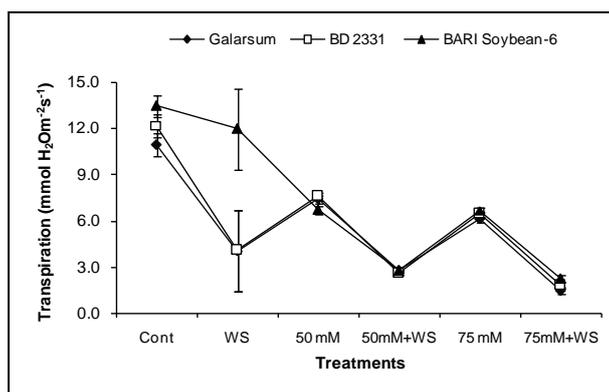


Fig. 4. Leaf transpiration as affected by salinity and water stress treatment. Bar represents mean \pm S.E. of the genotype at the same level of treatment.

Here, Cont = Control, WS = Water stress and mM = NaCl concentration in mM.

Photosynthetic water use efficiency: Water stress, salt stress and, combined salt + water stress led to a strong reduction in soybean plants transpiration rate (E), which reached minimum at higher level of salinity irrespective of water stress and non-water stress conditions (Fig. 5). This led to significantly increase in photosynthetic water use efficiency (PWUE) under different stresses as compared to control. PWUE of soybean genotypes varied under different stresses. Under only water stress condition the highest PWUE (6.35) was

recorded in Galarsum, which was identical with PWUE (5.30) of BD 2331, though the lowest (2.97) was obtained in BARI Soybean-6.

Under salt stress conditions, PWUE was maximum (4.67 and 4.86 in 50 mM and 75mM NaCl, respectively) in Galarsum and minimum (4.44 and 4.10 in 50 mM and 75mM NaCl, respectively) in BARI Soybean-6. At lower level of salinity combined with water stress condition, PWUE was the highest (8.79) in Galarsum, which was identical with the efficiency (8.28) of BD 2331. BARI Soybean-6 showed the lowest efficiency (7.78). At higher level of salinity + water stress conditions, significantly the highest PWUE (14.20) was obtained by Galarsum, which was identical with the efficiency (11.36) of BD 2331 and the lowest (9.19) in BARI Soybean-6. Plants under salt and water deficit conditions usually minimize transpirational water loss and maximize photosynthesis and show higher water-use efficiency (Xu *et al.*, 1994). This is a kind of adaptation mechanism that allows plants to survive water deficit conditions. Salt-induced increase in water use efficiency in *Chenopodium* was also reported by Eisa *et al.* (2012).

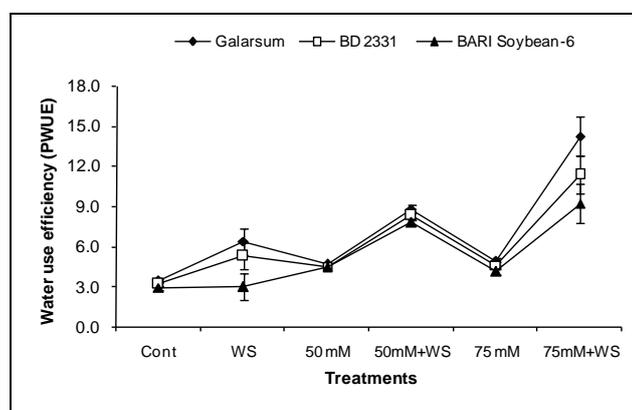


Fig. 5. Photosynthetic water use efficiency (Pn/E) as affected by salinity and water stress treatment. Bar represents mean \pm S.E. of the genotype at the same level of treatment.

Here, Cont = Control, WS = Water stress and mM = NaCl concentration in mM.

Shoot dry weight: Salinity and water stress significantly affected the shoot dry weight of soybean genotypes measured at 63 days after emergence (Table 1). The lowest shoot dry weight (3.38 g) was produced by BARI Soybean-6 at 75 mM NaCl salt + water stress condition which was identical with the dry matter (3.51 g) of BD 2331 and Galarsum (3.54 g) at the same treatment. The shoot dry weights were decreased to 24.58, 23.00 and 21.57% of the control in Galarsum, BD 2331 and BARI Soybean-6, respectively. Shoot dry weight significantly affected in the combined salt + water stress condition as compared to only salt stress in both the salinity levels (50 and 75 mM NaCl). It was drastically reduced with the increasing salinity levels along with water stress. However, all the

genotypes produced identical shoot dry weight at the same level of treatment. At the 75 mM NaCl salt stress condition BD 2331 produced the lowest shoot dry weight (5.68 g) followed by BARI Soybean-6 (6.41 g) and Galarsum (6.94 g). Shoot dry weight of soybean genotypes was significantly affected by water stress as compared to control. The lowest shoot dry weights of 4.63 g, 5.36 g and 5.37 g were recorded in BD 2331, Galarsum and BARI Soybean-6, respectively under water stress conditions. Application of salt water increased the soil salinity and thus decreased the soil water potential though did not decrease water flow to the roots. Root cortical cells can osmotically adjust to some extent allowing water to readily move into the root. Therefore, shoot dry weight of soybean was affected more under salt + water stress conditions than only in salt or water stress conditions. When the soil dries under water stress conditions the soil matric potential is decreased, therefore increased the resistance of water flow to the roots in a non-linear fashion (Homaee *et al.*, 2002). The findings was also an agreement with the findings of Meiri (1984) that the matric potential preferentially affected the shoot growth of bean more than that did the osmotic potential. Wang *et al* (2011) also reported that shoot biomass decreased significantly in tamarisk seedlings due to water severity under

Table 1. Shoot dry weight of soybean genotypes (g/plant) as affected by salinity and water stress at 63 days after emergence

Treatment	Genotypes		
	Galarsum	BD 2331	BARI Soybean-6
Control	14.39 ± 1.54a (100)	15.26 ± 2.00a (100)	15.67 ± 2.22a (100)
Water stress (WS)	5.36 ± 0.98cde (37.25)	4.63 ± 0.65de (30.34)	5.37 ± 0.51cde (34.25)
50 mM NaCl	9.56 ± 0.88b (66.44)	8.73 ± 1.16bc (57.21)	8.36 ± 1.42bc (53.35)
50 mM NaCl + WS	4.82 ± 0.71de (33.47)	4.21 ± 1.04de (27.57)	4.26 ± 0.19de (27.21)
75 mM NaCl	6.94 ± 1.24bcd (48.25)	5.68 ± 0.82cde (37.24)	6.41 ± 0.17bcde (40.88)
75 mM NaCl + WS	3.54 ± 0.43e (24.58)	3.51 ± 0.37e (23.00)	3.38 ± 0.81e (21.57)
HSD (0.05)		3.38	
Std Error		0.90	
CV (%)		15.27	

Different letters indicate a significant difference at $P = 0.05$. Lettering was made for observing the variation in genotype x environmental response.

Data in parenthesis indicate percentage of shoot dry weight to control.

salt and water stress condition. The reduction in shoot dry weight due to salinity was reported by Karim *et al.* (1993) in triticale, Khan *et al.* (1997) in rice, Aziz *et al.* (2005) in mungbean, Chookhampaeng (2011) in pepper plant and Mannan *et al.* (2013a) in soybean.

Sodium accumulation: The accumulation of Na⁺ in the leaves of soybean genotypes was significantly affected by salinity and water stress treatments (Table 2). The highest accumulation (0.171%) was obtained from BARI soybean-6 at 75 mM NaCl salt stress, which was followed by BD 2331 (0.149%). The genotype Galarsum accumulated significantly lower (0.138%) than others at the same treatment. The lowest accumulation (0.061%) was obtained from Galarsum under only water stress treatment, which was identical with BD 2331 (0.066%) in the same treatment and also from Galarsum (0.066%) under control. The results revealed that the accumulation of Na⁺ was higher in the salt stress conditions at both the salinity levels (50 and 75 mM NaCl) than the combined salt + water stress conditions, and the accumulation increased with the rise of salinity level. However, at 75 mM NaCl + water stress treatment, all the three genotypes accumulated identical amount of Na⁺. Among the genotypes, BARI Soybean-6 accumulated maximum (0.116%) followed by BD 2331 (0.113%) and the Galarsum accumulated minimum (0.110%) at the same treatment. The higher accumulation of Na⁺ in leaves under salinity and water stress might be due to higher transpiration rate. Differences in Na⁺ accumulation in soybean genotypes revealed that the genotype Galarsum, which accumulated lower Na⁺ was more tolerant than that of BD 2331 and BARI Soybean-6. The mechanisms of plant responses to salt and water stress have much in common, because salinity leads to many metabolic changes that are identical to those caused by water stress. The results of the study are in agreement with the earlier reports that the tolerant genotypes accumulate less amounts of Na than susceptible ones (Karim *et al.*, 1992; Khan *et al.*, 1997; Ahmadi *et al.*, 2009; Mannan *et al.*, 2013). The excess amount of Na⁺ creates a toxic effect on plant metabolic processes and therefore, the susceptible cultivars having high amounts of Na⁺ suffer more from the effect than the tolerant cultivars (Blum, 1988; Karim *et al.*, 1992). Kao *et al.* (2006) also reported that differences among soybean species in leaf accumulation of Na⁺ might be responsible for the differential sensitivity to NaCl treatments.

Table 2. Sodium (Na) uptake in leaf tissue (%) of soybean genotypes after 6 weeks of imposition of the stress treatments

Treatment	Genotypes		
	Galarsum	BD 2331	BARI Soybean-6
Control	0.066 ± 0.001ij	0.072 ± 0.004hi	0.077 ± 0.005h
Water stress (WS)	0.061 ± 0.003j	0.066 ± 0.001ij	0.072 ± 0.0041hi
50 mM NaCl	0.127 ± 0.002d	0.127 ± 0.002d	0.143 ± 0.003bc
50 mM NaCl + WS	0.094 ± 0.003g	0.099 ± 0.003g	0.101 ± 0.003fg
75 mM NaCl	0.138 ± 0.003c	0.149 ± 0.003b	0.171 ± 0.007a
75 mM NaCl + WS	0.110 ± 0.003ef	0.113 ± 0.004e	0.116 ± 0.004e
HSD (0.05)		0.01	
Std Error		0.003	
CV (%)		3.15	

Different letters indicate a significant difference at P = 0.05. Lettering was made for observing the variation in genotype x environmental response.

Potassium accumulation: The accumulation of K⁺ in the leaves of soybean genotypes was significantly affected by salinity and water stress treatments (Table 3). The highest accumulation (1.25%) was obtained in Galarsum at 50 mM NaCl salt combined with water stress, which was identical with the same genotype at 50 mM NaCl (1.24%), and also BD 2331 (1.22%) and BARI Soybean-6 (1.24%) at only water stress. The accumulation of K⁺ was significantly decreased at 75 mM NaCl salt stress and also combined water stress treatment irrespective of variety. However, the accumulation was to some extent increased in the combined salt + water stress conditions than only the salt stress conditions. The lowest (1.12%) amount of K⁺ accumulation was obtained from BARI Soybean-6 at 75 mM NaCl salt stress, which was identical with the same genotype at 75 mM NaCl salt + water stress (1.13%) and BD 2331 (1.16%) at 75 mM NaCl salt stress. At 75 mM NaCl + water stress treatment, genotype Galarsum accumulated the maximum (1.19%) amount of K⁺, which was identical with the same genotype at 75 mM NaCl salt stress (1.18%) and with BD 2331 both at salt and water stress treatments. Under water and salt stress conditions K⁺ plays an important role in osmoregulation and the tolerant genotype accumulates higher amounts than the susceptible ones (Blum, 1988; Qadar, 1988). Maintenance of high cytoplasmic levels of K⁺ is essential for survival of plants in saline habitats (Chow *et al.*, 1990). Here, the soybean genotype Galarsum accumulated higher amount of K⁺ in leaves than others under salt and water stress conditions. It could be due to high potentiality of the genotype.

Table 3. Potassium (K) uptake in leaf tissue (%) of soybean genotypes after 6 weeks of imposition of the stress treatments

Treatment	Genotypes		
	Galarsum	BD 2331	BARI Soybean-6
Control	1.166 ± 0.006e-g	1.176 ± 0.007d-f	1.155 ± 0.013f-h
Water stress (WS)	1.197 ± 0.009c-e	1.218 ± 0.005a-c	1.239 ± 0.006ab
50 mM NaCl	1.239 ± 0.006ab	1.197 ± 0.009c-e	1.187 ± 0.005c-f
50 mM NaCl + WS	1.250 ± 0.026a	1.208 ± 0.033b-d	1.197 ± 0.009c-e
75 mM NaCl	1.176 ± 0.007d-f	1.155 ± 0.013f-h	1.124 ± 0.006h
75 mM NaCl + WS	1.187 ± 0.005cd-f	1.166 ± 0.007e-g	1.134 ± 0.006gh
HSD (0.05)		0.039	
Std Error		0.01	
CV (%)		1.07	

Different letters indicate a significant difference at P = 0.05. Lettering was made for observing the variation in genotype x environmental response.

Ratio of Potassium and Sodium: The ratio of K⁺ and Na⁺ was decreased under salt and water stress treatments and decreased sharply at the higher salt concentration (Fig. 6). Under only water stress treatment, significantly the highest ratio (17.73) of potassium and sodium was obtained from Galarsum,

which was identical with the ratio (16.45) in BD 2331, while the lowest (15.00) in BARI Soybean-6. Under 75 mM NaCl salt stress treatment, the highest $K^+ : Na^+$ ratio (8.55) was obtained from Galarsum, which was identical with the ratio (7.78) obtained from BD 2331 and the lowest (6.59) from BARI Soybean-6. The ratio was to some extent increased in the combined salt and water stress conditions than only in the salt stress treatments. However, at 75mM NaCl salt + water stress treatment the highest ratio (10.79) was also obtained from Galarsum, and the lowest (9.82) from BARI Soybean-6, which was identical with the ratio (10.31) obtained from BD 2331. The results are in agreement with the findings that tolerant genotypes maintain a higher K^+/Na^+ ratio than susceptible ones (Nair and Khulbe, 1990; Mannan *et al.*, 2013b). A greater degree of salt tolerance in plants was found to be associated with a more efficient system for selective uptake of K^+ over Na^+ (Neill *et al.*, 2002). The selective uptake of K^+ in contrast to Na^+ was considered as one of the important physiological mechanisms contributing to salt tolerance in many plant species (Poustini and Siosemardeh, 2004).

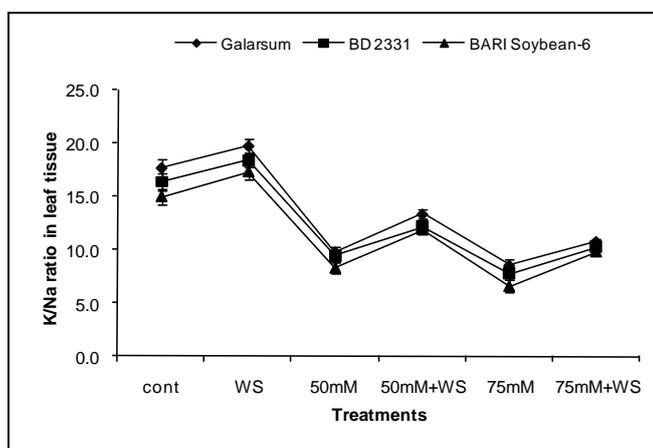


Fig. 6. K/Na ratio in leaf tissue of soybean genotypes after 6 weeks of beginning of the stress treatments. Bar represents mean \pm S.E. of the genotype at the same level of treatment.

Here, Cont = Control, WS = Water stress and mM = NaCl concentration

Conclusion

Salinity and water stress had severe adverse effect on stomatal conductance, photosynthesis and transpiration rate of soybean genotypes. The genotype Galarsum minimized transpirational water loss and maximized photosynthesis as compared to BD 2331 and BARI Soybean-6 under salt and water stress environments. Combined salt + water stress caused greater inhibition of shoot growth than either only salt or water stress in soybean. Galarsum accumulated higher amount of K^+ and lower amount of Na^+ in leaves under salt and water

stress. The results indicated that the genotype Galarsum is more capable in tolerating high levels of salt under water stress condition than BD 2331 and BARI Soybean-6.

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MATING, OVIPOSITION BEHAVIOUR AND BIOLOGY OF HOGPLUM BEETLE

M. M. H. KHAN¹

Abstract

Experiments were conducted to observe mating and oviposition behaviour as well as biology of hog-plum beetle. Results revealed that the average mating frequency, mating duration and mating interval were 11.2, 30.5 hours and 60.3 hours, respectively. The highest number of eggs per clusters was on leaf rachis followed by young stem. The mean number of eggs per cluster was 15.70 while the length and breadth of an egg was 1.95 mm and 0.60 mm, respectively. The mean duration of 1st, 2nd, 3rd and 4th instar larvae were 3.80, 3.40, 3.70 and 3.00 days, respectively. The mean pupal period was 22.74 days. The female beetle lived longer (48.30 days) than the male (45.10 days). The size of female beetle was larger (15.30 mm) than male (11.96 mm). The mean length of antennae was 7.42 mm. The length of fore, mid and hind legs were 9.20, 9.50 and 11.70 mm, respectively. Hind wings were larger (16.10 mm length and 7.85 mm breadth) than fore wings (12.38 mm length and 5.30 mm breadth).

Keywords: Biology, mating, morphometrics, oviposition, *Podontia 14-punctata*.

Introduction

The hog-plum is a deciduous perennial tree with thick succulent leaves. It is very popular fruit in Bangladesh and it grows all over the country. But the quality fruits are produced only in the southern districts especially in Barisal and Patuakhali (Sarder and Mondal, 1983). Its cultivation is seriously hampered by hog-plum beetle or fourteen spotted leaf beetle *Podontia 14-punctata* L. (Chrysomelidae: Coleopteran). The fourteen spotted leaf beetle is distributed throughout South East Asia (Husain and Ahmed, 1977; Howlader, 1993). Mondal (1975) reported that insect appeared in large number during the month from May to June. In Bangladesh, the beetles appear in June, abundant during July to September and disappear in October. The beetles cause serious damage to hog-plum from July to August and the peak defoliation occurred during August to September (Mondal, 1975; Baksha, 1997). Both adults and grubs of this beetle feed on the leaves of the hog-plum and heavy infestation often cause complete defoliation of the tree (Ahmed, 1969; Mondal, 1975; Sarder and Mondal, 1983). Sarder and Mondal (1983) found that the mating of hog-plum beetle took place 2-3 weeks after adult emergence. At the beginning of the mating, the male generally takes initiative and become very aggressive. The males become heated and sometimes moves around the female until they are attracted her for mating.

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The male gets on the back of the female and remains at an angle of 60° , fixing posterior end with the female and start mating. The female also helps in doing so. During mating they cannot fly. Mating generally commenced from the early morning and lasted for 3 to 6 hours. The knowledge of biology of any insect pest helps in developing sound management techniques. Considering the above facts, the research was undertaken to know the mating, oviposition behavior and biology of hog-plum beetle.

Materials and Method

The study was carried out in the laboratory of the Department of Entomology , Patuakhali Science and Technology University (PSTU), Dumki, Patuakhali during April to September, 2012. The experiment was done at normal room temperature, $32 \pm 2^{\circ}\text{C}$ and relative humidity ($85 \pm 5\%$) with a $14 \pm 2:10 \pm 2$ light and dark cycle (L: D).

Observation of Mating and oviposition behavior: Ten pairs of healthy adult beetles were collected from hog-plum trees in couples at mating condition in the morning. After collecting, each couple was released in a separate plastic box ($26.6 \times 14.2 \times 12.2$ cm) provided with a fresh hog-plum leaflet by wrapping the base of leaflet with cotton soaked in 1% sugar solution. The mouth of each box was covered with nylon net (25 mesh) by using rubber band. The fed leaflets were replaced by fresh ones daily and plastic box was cleaned by removing excreta to ensure healthy environment inside the box. Data on the time of beginning and end of mating, mating frequency from 7.00 AM to 10 PM, duration of each mating, interval of mating and oviposition activity were recorded by close observation. The number of eggs per cluster and total number of clusters laid by individual female were counted from different plant parts of hog-plum under natural condition by covering the couple with nylon net.

Observation of developmental stages: Fifty newly hatched larvae were collected from hog-plum trees planted in the hog-plum orchard at Rahamatpur of Barisal district. The newly hatched larvae were yellowish in colour with prominent large black head. Gradually the colour changed to grayish colour following the feeding on hog-plum leaves. After collecting, five newly hatched larvae were placed in a plastic box containing fresh hog-plum leaflets. The larvae were examined daily for larval moults. The second instar larvae came out leaving their exuvae. Before moulting the larvae stopped feeding and remained inactive. Fresh hog-plum leaflets were supplied to replace the used ones regularly till pupation. Developmental period of 1st instar larvae was measured by the time elapsed between egg hatching to 1st moulting. In this way the duration of 2nd, 3rd and 4th (final) instars were recorded. A small quantity of sand mixed with soil was placed at the bottom of the box for pupation. Pupal period was also calculated by the time interval between cessation of feeding and hiding inside the soil for pupation. The longevity of adult male and female beetles was recorded.

Morphometrics of larvae and adult: To find out the length and head width (mm) of different larval instars were measured using a mm scale. Mean values of larval length (mm) and head width (mm) were based on ten observations. Similarly, the length and breadth of adult male and female beetles were measured. For this, ten observations, each for male and female adults were taken. Data on the morphometric measurements of larvae, adult, antennae, legs and wings were made. The weight of full grown larva, adult male and female was measured by using an electric balance. Means and standard errors (SE) were calculated from ten observations.

Results and Discussion

The frequency of mating of 10 couples ranged from 7 to 15 times with mean frequency 11.2 times. Similarly, the duration of mating of 10 couples ranged from 27 to 34 hours with mean duration 30.5 hours. The interval of mating among 10 couples ranged from 57 to 64 hours with mean duration 60.3 hours (Fig. 1). The findings of the present study are in similar trend with findings of Uddin *et al.* (2014) who observed highest frequency of mating 14 times, duration of mating 33 hours and mating interval 63 hours by using 10 couples.

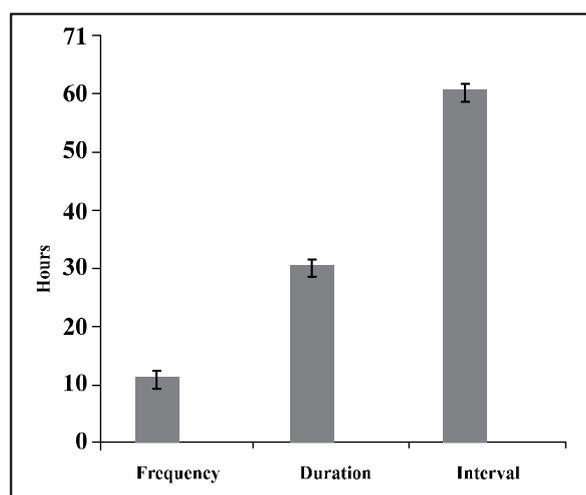


Fig. 1. Mating performances of hog-plum beetle.

In case of leaf, most of the couples laid eggs in clusters on dorsal side (1.57 ± 0.31) and leaf rachis along with young stem (2.1 ± 0.35) of the leaf but only very few clusters were laid on ventral side (1.3 ± 0.00) of the leaf (Fig. 2). From the results it is clear that the maximum egg laying tendency of *P. 14- punctata* was on the leaf rachis along with young stem. Ventral leaf surface was less preferred for laying egg and dorsal side was intermediate preferred. Mondal (1975) observed that female beetles preferably choose stem and bark of the plant near leaves for egg laying. Because heavy bodied adult female probably feel comfortable to lay their eggs on stem and bark. Moreover, females of the second

or latter generations do not get enough leaves because of the complete defoliation. In case of branch, the number of egg clusters per branch ranged 5-9 with mean 6.40 and standard error 0.03 (Fig. 2). The findings are supported by Uddin *et al.* (2014). The observation of Baksha (1997), Singh and Misra (1989) on these behavioural parameters supported the findings of the present study.

Developmental periods of different stages of hog-plum beetle are presented in Figure 3. The mean duration of 1st and 2nd instar larvae was 3.80 ± 0.21 days and 3.40 ± 0.11 days, respectively. The mean duration of 3rd and 4th instar larvae was 3.70 ± 0.21 days and 3.00 ± 0.28 days, respectively. The mean pupal period was 22.74 ± 0.56 days. The mean duration of adult male and adult female was 45.10 ± 3.23 days and 48.30 ± 1.10 days, respectively. The findings are also supported by Uddin *et al.* (2014).

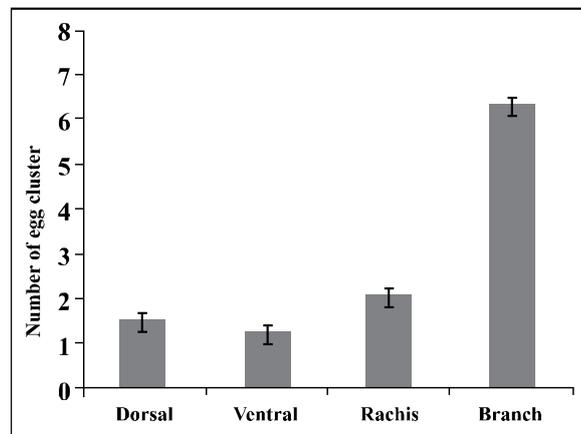


Fig. 2. Oviposition behaviour (number of egg cluster deposited) of hog-plum beetle on leaf surface (dorsal and ventral), leaf rachis and branch of hog-plum tree.

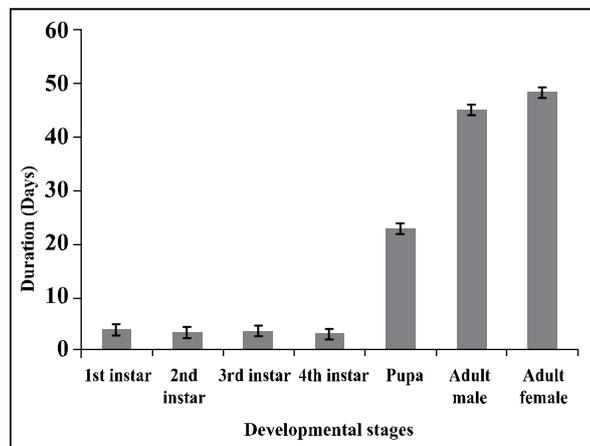


Fig. 3. Duration of different developmental stages of hog-plum beetle under laboratory condition.

The mean length and breadth of egg were 1.95 ± 0.02 mm and 0.60 ± 0.01 mm, respectively.

The mean length and head width of first instar larvae were 2.51 ± 0.18 mm and 0.72 ± 0.00 mm, respectively. The mean length of the 2nd instar larvae was 8.27 ± 0.20 mm and the mean head width was 1.54 ± 0.05 mm. The mean length of the 3rd instar larvae was 16.02 ± 0.23 mm with mean head width was 2.31 ± 0.04 mm. The mean length and head width of 4th instar larvae were 21.80 ± 0.51 mm and 2.81 ± 0.10 mm, respectively. The mean length of male beetle was 11.96 ± 0.15 mm with a mean breadth (across thorax) was 6.1 ± 0.05 mm. The mean length of female beetle was 15.31 ± 0.16 mm with a mean breadth (across thorax) was 8.25 ± 0.21 mm (Table 1).

Similarly, the mean length of the antennae was 7.42 ± 0.20 mm. The mean length of fore, middle and hind legs was 9.20 ± 0.23 mm, 9.50 ± 0.24 mm and 11.70 ± 0.15 mm, respectively. The mean length of fore (elytra) and hind wings were 12.38 ± 0.16 mm and 16.10 ± 0.26 mm, respectively. The hind wings were longer by about 4 mm than the fore wings in length (Table 1). The mean width of fore and hind wings were 5.30 ± 0.14 mm and 7.85 ± 0.41 mm, respectively. Similarly, the hind wings were broader by about 2 mm than the fore wings in breadth (Table 1). According to Mondal (1975) the 1st instar larvae measured 3.53 mm in length and 0.73 mm in head width. The larval length and head width of the 2nd instar was almost similar to those obtained by Mondal (1975). The findings on morphometrics of hog-plum are supported by Uddin *et al.* (2014).

Table 1. Morphometrics of the different life stages and body appendages of hog-plum beetle

Morphometric parameters	Length (mm)		Width (mm)	
	Mean	\pm SE	Mean	\pm SE
Egg	1.95	0.02	0.60	0.01
1 st instar	2.51	0.18	0.72	0.00
2 nd instar	8.27	0.20	1.54	0.05
3 rd instar	16.02	0.23	2.31	0.04
4 th instar	21.80	0.51	2.81	0.10
Adult male (Width across thorax)	11.96	0.15	6.10	0.05
Adult female (Width across thorax)	15.30	0.16	8.26	0.22
Antennae	7.42	0.20	-	-
Fore leg	9.20	0.23	-	-
Middle leg	9.50	0.24	-	-
Hind leg	11.70	0.15	-	-
Fore wing	12.38	0.16	5.30	0.14
Hind wing	16.10	0.26	7.85	0.41

Weight of full grown larva, adult male and female of hog-plum beetle is presented in Figure 4. The mean weight of Full grown larva was 0.366 ± 0.011 g. The mean weight of adult male and female was 0.183 ± 0.004 g and 0.274 ± 0.005 g, respectively. The adult male was lighter in weight than adult female.

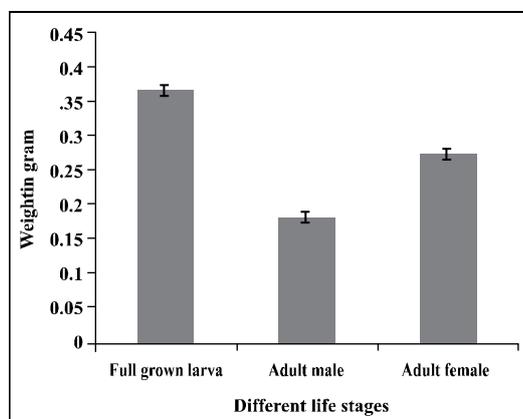


Fig. 4. Weight of Full grown larva, adult male and female of hog-plum beetle

From the findings of the present study it may be concluded that the maximum number of egg clusters was on leaf rachis and young stem compared to leaf. The female beetle lived longer than the male. The size of female beetle was larger than male. The hind pair of leg was longer compared to fore and mid pairs of legs. Hind wings were larger than fore wings.

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SYSTEM PRODUCTIVITY, NUTRIENT UPTAKE AND NUTRIENT BALANCE IN THE WHEAT-MUNGBEAN-T. AMAN RICE CROPPING SYSTEM

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Abstract

System productivity, nutrient uptake and apparent nutrient balance in the wheat-mungbean-T. aman rice cropping system was studied. The experiment comprised four treatments-absolute nutrient control (T₁); farmer's practice (T₂); AEZ basis fertilizer application (T₃) and soil test basis fertilizer application (T₄). The treatments were compared in a RCBD with three replications over two consecutive years, 2008-09 and 2009-10. The experiment was conducted in Chhiata clay loam soil. The average yields of wheat, mungbean and T. aman ranged from 1415 to 3096 kg ha⁻¹, 1020 to 1463 kg ha⁻¹ and 2999 to 4282 kg ha⁻¹, respectively showing T₄ as the best treatment. The same treatment (T₄) demonstrated the highest nutrient uptake by the crops. The apparent balance of N and K (difference between nutrient uptake and nutrient addition) was negative; however it was less negative for T₄ treatment. The P balance was positive for all the treatment except T₁. Positive S balance was observed in T₃ and T₄ but negative in absolute control and farmer's practice. Zinc and B balance was also positive in case of T₃ and T₄, but negative for T₁ and T₂ except B. The study suggests soil test basis fertilizer recommendation for the wheat-mungbean-T. aman rice cropping system.

Keywords: System productivity, nutrient uptake, nutrient balance, wheat-mungbean-T. aman rice.

Introduction

Cropping system in an area depends largely on agro-climatic, technical and institutional factors. Several studies have shown that intensive rice-based cropping system including rice-wheat (RW) causes remarkable depletion of soil nutrients and threat to crop productivity (Timsina and Connor, 2001; Yadvinder-Singh *et al.*, 2005). Besides the farmers are following imbalanced use of fertilizers for crop production which leads to degrade soil fertility (Ali *et al.*, 2010). Farmers generally use fertilizers on single crop basis, not the cropping system. High yielding varieties of crops uptake higher amount of nutrients from soils resulting in depletion of soil organic matter and deterioration of soil fertility, poses a great threat to sustainable crop production. Moreover, continuous

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cropping without adequate replacement of removed nutrients and nutrient loss through erosion, leaching, and gaseous emission have caused depletion soil fertility as well as soil organic matter (Tirol-Padre *et al.*, 2007). The bulk of literature indicates that, apart from residue management, cropping system productivity may become sustainable through integrated use of organic and inorganic sources of nutrients (Singh and Yadav, 1992). Hence, it is important to develop a cropping system based fertilizer dose for specific agro-ecological zone. Quantification of the loss or gain of nutrients under different cropping system has been less attended. Nutrient balance is an important tool for assessing the nutrient reserve in soils. Crop nutrient balance is a differences between nutrients applied to soil in relation to its removal by crops. Negative nutrient balance may limit crop yield and deplete soil fertility and positive nutrient balance shows nutrient accumulation and creates a risk of water and air pollution (Paul Fixen *et al.*, 2014). It is hypothesised that the current fertilizer recommendation could be improved for a definite cropping system. Thus, the aim of this study was to compare system productivity, nutrient uptake and nutrient balance for the wheat-mungbean-T. aman rice cropping system with varying fertilizer management practices.

Materials and Method

Experiment with wheat-mungbean-T. aman rice cropping system was conducted for consecutive two years (2008-09 and 2009-10) at Pulses Research Sub-Station, BARI, Gazipur (24° 0' 13" N latitude and 90° 25' 0" E longitude) which lies at an elevation of 8.4 m above the sea level. The crop field was medium high land with clay loam soil and it belongs to Chhiata series (Soil taxonomy: Udic Rhodustalf) under the agroecological zone Madhupur Tract (AEZ-28).

The experiment consisted of four treatments-absolute nutrient controls (T₁); farmer's practice (T₂); AEZ basis fertilizer application (T₃) and soil test basis fertilizer application (T₄). Descriptions of the different treatments are given in Table 1.

Table 1. Rates of fertilizers (kg ha⁻¹) for wheat, mungbean and T. aman

Treatments	Wheat	Mungbean	T. aman
T ₁	Control	Control	Control
T ₂	N ₈₀ P ₂₀ K ₃₀	N ₆ P ₅ K ₄	N ₆₀ P ₆ K ₂₀
T ₃	N ₉₀ P ₂₀ K ₆₀ S ₁₀ Zn ₀ B ₁	N ₇ P ₇ K ₅	N ₆₅ P ₇ K ₂₈ S ₈ Zn ₁
T ₄	N ₁₁₀ P ₂₅ K ₇₀ S ₂₃ Zn ₂ B ₁	N ₁₅ P ₂₀ K ₁₀ S ₆ Zn ₁ B ₁	N ₇₀ P ₁₂ K ₄₀ S ₁₀ Zn ₁ B ₁

Subscripts represent kg ha⁻¹

The experiment was laid out in a randomized complete block design (RCBD) with three replications. The unit plot size was 4 m × 3 m for all crops having a spacing of 30 cm × 10 cm for wheat, 30 cm × 10 cm for mungbean and 20 cm × 15 cm for T. aman rice. Full amount of fertilizers except urea in wheat and rice was applied to crop during final land preparation. Urea was applied in two equal splits for

wheat and three equal splits for *T. aman* rice. The sources of N, P, K, S, Zn and B were urea, triple superphosphate, muriate of potash, gypsum, zinc sulphate and boric acid, respectively. Wheat (var. BARI Gom-24) seeds were sown on 15 November 2008 and 11 November 2009. Mungbean (BARI Mung-6) seeds were sown on 26 March 2009 and 20 March 2010. *T. aman* rice (var. BRRI dhan33) seedlings (30 days old) were transplanted on 15 July 2009 and 18 July 2010. Intercultural operations were done as and when required. The crops were harvested at maturity. Data on yields (kg ha^{-1}) for all test crops were recorded from whole plot. Analysis of variance (ANOVA) for the yields and different nutrient content was done following the principle of F-statistics and the mean values were separated by DMRT (Gomez and Gomez, 1984) at 5% level of probability using MSTAT-C software.

Soil samples at 0-15 cm were collected before establishing the experiment and after completion of two cycles of the cropping system from each treatment plot. Plant samples (straw and grain) against each treatment plot were oven-dried at 70°C for 48 h and finely ground. The initial and final soil samples were analyzed for soil pH and organic matter by Nelson and Sommers (1982) method; total N by Microkjeldahl method (Bremner and Mulvaney, 1982); exchangeable K by $1\text{N NH}_4\text{OAc}$ method (Jackson, 1973); available P by Olsen and Sommers (1982) method; available S by turbidity method using BaCl_2 (Fox *et al.*, 1964); available Zn by DTPA method (Lindsay and Norvell, 1978); available B by azomethine-H method (Page *et al.*, 1982). Ground plant samples were digested with di-acid mixture ($\text{HNO}_3\text{-HClO}_4$) (5: 1) as described by Piper (1966) for the determination-concentration of N (Micro-Kjeldahl method), P (spectrophotometer method), K (atomic absorption spectrophotometer method), S (turbidity method using BaCl_2 by spectrophotometer), Zn (atomic absorption spectrophotometer method) and B (spectrophotometer following azomethine-H method). Rain and irrigation water samples were analysed for concentration of P, K, S, Zn and B same as plant samples method.

Crop nutrient uptake was calculated from the nutrient (N, P, K, S, Zn and B) concentration and the straw and grain yields (Quayyum *et al.*, 2002). Apparent nutrient balance for the wheat-mungbean-*T. aman* rice cropping system (average of two years) was computed as the difference between nutrient input and output (Paul Fixen *et al.*, 2014). The inputs were supplied from (i) fertilizer (ii) rainfall and (iii) irrigation water and the outputs were estimated from crop uptake in a cycle.

Added cost and added benefit were calculated. Besides, the gross return was calculated on the basis of different treatments which were directly related to the price of product. Cost of cultivation was involved with wage rate (land preparation, weeding, seed sowing and fertilizers application), pesticides, irrigation and fertilizers cost. Land used cost or rental value of land was not considered here. Marginal benefit cost ratio (MBCR) is the ratio of marginal or

added benefit and cost. To compare different treatments combination with one control treatment the following equation was applied (Rahman *et al.*, 2011).

$$\begin{aligned} \text{MBCR (over control)} &= \frac{\text{Gross return}(T_i) - \text{Gross return}(T_o)}{\text{VC}(T_i) - \text{VC}(T_o)} \\ &= \frac{\text{Added benefit(overcontrol)}}{\text{Added cost(overcontrol)}} \end{aligned}$$

Where, $T_i = T_2, \dots, T_4$ treatments; $T_o =$ Control treatment; VC= Variable cost; and Gross return = Yield \times price

Results and Discussion

Crops yield

Nutrient management practices significantly influenced the grain and straw/stover yields of wheat, mungbean and T. aman rice in every year (Table 2). The control (T_1) treatment gave the lowest grain yield of 1415, 1020 and 2999 kg ha⁻¹ (mean of two years) in wheat, mungbean and T. aman rice, respectively. The farmers practice of fertilizer application (T_2) increased grain yield to 2201 kg ha⁻¹ in wheat, 1222 kg ha⁻¹ in mungbean and 3556 kg ha⁻¹ in T. aman rice. Fertilizer dose on AEZ basis (T_3) resulted in further yield increased of 2787 kg ha⁻¹ in wheat, 1346 kg ha⁻¹ in mungbean and 3914 kg ha⁻¹ in T. aman rice. The T_4 treatment (soil test basis fertilizer application) gave the highest crop yields for all the test crops (Table 2). In case of straw/stover yield, the treatments generally statistically differed with one another. The highest value being noted in T_4 treatment. The reasons of yield increase due to fertilizer management might be attributed to deficiency of soil nutrients, particularly N and K. The percent grain yields of wheat, mungbean and T. aman rice increased over control due to different nutrient management practices were 55 to 119%, 20 to 43% and 19-43%, respectively. Islam *et al* (1996) also reported 42% yield increase of rice over control due to balanced fertilization. Most of the yield contributing characters of wheat, mungbean and T. aman rice highly responded to soil test basis fertilization (T_4) followed by AEZ basis fertilization (T_3).

Nutrient uptake

Nutrient management practices had significant effect on the uptake of N, P, K, S, Zn and B by the crops in wheat-mungbean-T.aman rice cropping system in both the years (Table 3). Fertilizer application on soil test basis (T_4) showed significantly higher nutrient uptake by wheat, mungbean and T. aman rice in both the years. The nutrient uptake followed the order: N>K>P>S>Zn>B. The lower nutrient uptake was found in control (T_1) treatment by all test crops. The total uptake of nutrients by crops (wheat+mungbean+T. aman rice) ranged from 160-

260 kg N ha⁻¹, 17.4-32.0 kg P ha⁻¹, 132-194 kg K ha⁻¹, 9.05-17.6 kg S ha⁻¹, 0.47-0.79 kg Zn ha⁻¹ and 0.23-0.40 kg B ha⁻¹ (Table 4).

Table 2. Effects of nutrient management practices on grain and straw/stover yields of crops in the wheat-mungbean-T. aman rice cropping system

Treatment	Grain yield (kg ha ⁻¹)				Straw/stover yield (kg ha ⁻¹)		
	2009	2010	mean	% increase over control	2009	2010	mean
Wheat							
Control (T ₁)	1251d	1580d	1415	-	1984d	2345d	2164
F. practice (T ₂)	2088c	2314c	2201	55	2510c	2800c	2655
AEZ (T ₃)	2565b	3010b	2787	97	3136b	3612b	3374
STB (T ₄)	2898a	3294a	3096	119	3454a	3910a	3682
CV (%)	2.99	2.35	-	-	3.32	2.37	-
Mungbean							
Control (T ₁)	1038d	1003d	1020	-	2156d	2124b	2140
F. practice (T ₂)	1229c	1216c	1222	20	2274c	2235ab	2255
AEZ (T ₃)	1359b	1332b	1346	32	2410b	2389ab	2400
STB (T ₄)	1485a	1440a	1463	43	2531a	2500a	2516
CV (%)	3.65	2.87	-	-	4.23	4.69	-
T. aman							
Control (T ₁)	3022c	2976d	2999	-	3137d	3134c	3135
F. practice (T ₂)	3521b	3591c	3556	19	3736c	3753c	3744
AEZ (T ₃)	3908b	3920b	3914	31	4070b	4094b	4082
STB (T ₄)	4224a	4341a	4282	43	4352a	4443a	4397
CV (%)	3.83	3.94	-	-	3.72	4.74	-

Values within a column with a common letter do not differ significantly ($p=0.05$) by DMRT.

The maximum N uptake was found in STB (260 kg ha⁻¹yr⁻¹) followed by AEZ (T₃). This finding is in agreement with the findings of Timsina *et al.* (2006) where N uptake was consistently and significantly greater under STB than in FP fertilizer management. The minimum N uptake was estimated in control. Shrestha and Ladha (2001) reported that N uptake by sweet pepper-fallow-rice, sweet pepper-indigo-rice, sweet pepper-indigo + mungbean-rice and sweet pepper-corn-rice were 241, 390, 358 and 411 kg N ha⁻¹ yr⁻¹, respectively. The uptake of other nutrients (P, K, S, Zn and B) due to different nutrient management practices followed almost the same trend of N uptake (Saleque *et al.*, 2006; Debnath *et al.*, 2011).

Table 3. Effects of nutrient management practices on nutrient uptake (kg ha⁻¹) by wheat-mungbean-T. aman rice (grain+straw/stover) cropping system

Treatment	N		P		K		S		Zn		B	
	1 st yr	2 nd yr										
Wheat												
Control (T ₁)	33.6d	40.9d	4.75d	5.20d	26.5d	31.2d	2.51c	2.67c	0.15d	0.18d	0.09d	0.08d
F. practice (T ₂)	54.2c	59.5c	8.00c	8.3c	36.1c	39.7c	3.85b	3.76b	0.22c	0.24c	0.11c	0.12c
AEZ (T ₃)	68.0b	78.8b	10.4b	11.2b	45.5b	52.0b	5.33a	5.54a	0.28b	0.33b	0.14b	0.16b
STB (T ₄)	78.5a	88.0a	12.6a	13.3a	51.4a	57.5a	6.59a	6.75a	0.33a	0.37a	0.16a	0.18a
CV (%)	2.14	2.36	8.73	7.65	5.06	4.52	8.16	7.14	5.41	6.11	8.71	7.56
Mungbean												
Control (T ₁)	60.2d	58.0d	5.92c	5.47d	61.7d	59.9d	2.01b	1.65c	0.08	0.07	0.06	0.05
F.practice (T ₂)	68.5c	67.1c	6.90bc	6.40c	67.6c	66.1c	2.47b	2.09b	0.09	0.08	0.06	0.06
AEZ (T ₃)	75.5b	73.6b	7.80b	7.10b	72.9b	71.5b	3.05a	2.39a	0.09	0.09	0.07	0.07
STB (T ₄)	82.7a	80.0a	8.50a	7.90a	78.3a	76.1a	3.51a	2.80a	0.11	0.10	0.08	0.07
CV (%)	2.43	3.11	6.69	4.35	1.23	2.35	9.23	8.54	7.04	6.95	8.19	7.20
T. aman												
Control (T ₁)	63.9d	62.1d	7.04c	6.36c	42.7d	41.3d	5.24c	3.98c	0.23d	0.24d	0.11d	0.10c
F.practice (T ₂)	76.2c	76.5c	9.00b	8.40b	51.7c	51.0c	6.53b	5.88b	0.27c	0.28c	0.12c	0.11b
AEZ (T ₃)	84.8b	84.3b	9.50b	8.70b	57.4b	56.9b	7.20ab	6.02b	0.31b	0.30b	0.15b	0.16a
STB (T ₄)	94.7a	95.0a	11.1a	10.5a	62.3a	62.8a	8.15a	7.47a	0.33a	0.34a	0.16a	0.17a
CV (%)	1.95	1.26	6.27	5.89	1.21	1.09	6.50	5.89	4.04	3.33	3.85	4.97

Values within a column with a common letter do not differ significantly (p=0.05) by DMRT.

Table 4. Effects of fertilizer management practices on total nutrients uptake by crops in the wheat-mungbean-T. aman rice cropping system (mean of two years)

Treatment	N	P	K	S	Zn	B
kg ha⁻¹						
Control(T ₁)	160	17.4	132	9.05	0.47	0.23
F. practice(T ₂)	201	23.5	156	12.3	0.59	0.28
AEZ (T ₃)	233	27.4	178	14.8	0.70	0.37
STB (T ₄)	260	32.0	194	17.6	0.79	0.40

Total input of nutrients

The nutrient input was mainly from fertilizer but in this estimate, the nutrients supply from fertilizer, rainfall and irrigation under wheat-mungbean-T. aman rice cropping system. BNF was not considered. Total input of nitrogen was 146-195 kg N ha⁻¹ of which the major part was added through fertilizer application, except in control treatment. Phosphorus input ranged from 0.48 to 57.5 kg ha⁻¹ yr⁻¹ and K from 9.05 to 129 kg ha⁻¹ yr⁻¹ (Table 5). The S input varied from 5.50 to 44.6 kg ha⁻¹ yr⁻¹. Input of Zn ranged from 0.14 to 4.14 kg ha⁻¹ yr⁻¹. Boron input was estimated 0.36 to 3.36 kg ha⁻¹ yr⁻¹ (Table 5).

Table 5. Total input of N, P, K, S, Zn and B from fertilizer, rainfall and irrigation under wheat-mungbean-T. aman rice cropping system

Treatment	N	P	K	S	Zn	B
	2009-10	2009-10	2009-10	2009-10	2009-10	2009-10
	kg ha ⁻¹					
Control (T ₁)	0.00	0.48	9.05	5.50	0.14	0.36
F. practice (T ₂)	146	31.5	63.1	5.60	0.14	0.36
AEZ (T ₃)	162	34.5	102	23.6	1.14	1.36
STB (T ₄)	195	57.5	129	44.6	4.14	3.36

Apparent nutrients balance

An apparent nutrient balance was calculated considering the amount of added nutrient through fertilizer, rain, irrigation water minus the amount of nutrient removed by crops. However, the nutrient balance did not account for the addition of N from rainfall, irrigation water, or gaseous losses or BNF. Apparent balance of N, P, K, S, Zn and B are shown in Fig. 01 & 02. The balance was mainly affected by different nutrient management practices. The apparent balance of N was negative in all the treatments and the nutrient depletion ranged from -55.0 to -160 kg N ha⁻¹ yr⁻¹. Some researchers supported the results: in rice-maize system in Bangladesh, the apparent nutrient balances have been highly negative for N (-120 to -134 kg ha⁻¹ yr⁻¹) (Timsina *et al.*, 2010). In case of P balance which was negative in control treatment T₁ and the P balance was positive (8.0 to 25.5 kg ha⁻¹) in all the other treatment where P containing fertilizer was utilized. This evident indicated that P depletion was fewer amounts as compared added fertilizer (Ishaque *et al.*, 1998). The balance of K was negative in all the treatments where the K mining ranged from -65.0 to -123 kg K ha⁻¹ yr⁻¹. The results confirmed the declining trends in available soil K in many treatments and they are comparable with many other long-term studies in rice-rice and rice-wheat systems of Asia (Ladha *et al.*, 2003).

The negative S and Zn balance was observed in control (T₁) and farmers practice (I₂) ranged from -3.55 to -6.7 and -0.33 to -0.45 kg ha⁻¹ yr⁻¹, respectively. Remaining treatments showed positive balance ranging from 8.80 to 27.0 and

0.44 to 3.35 kg ha⁻¹ yr⁻¹, respectively. Some workers are in agreement with these findings. Jahan *et al.* (2015a) corroborated that the negative balance was observed in control and farmers practice treatments which was -1 to -8 kg S ha⁻¹ yr⁻¹ and remaining treatments showed positive balance ranged from 1 to 4 kg ha⁻¹ yr⁻¹. Similar result of Zn was found by Jahan *et al.* (2015a) in a monocrop cultivation of T.aman rice where -0.08 to -0.31 kg Zn ha⁻¹ yr⁻¹ was found in control and farmers practice and positive balance (1.12 to 1.61 kg Zn ha⁻¹ yr⁻¹) was found in AEZ and STB treatment. The B balance was found positive (0.08 to 2.96 kg ha⁻¹) in all the treatments. Jahan *et al.* (2015b) observed that positive B balance ranged from 0.86 to 1.17 kg ha⁻¹ and negative B balance was -0.01 to -0.07 kg ha⁻¹ yr⁻¹ in mungbean cultivation.

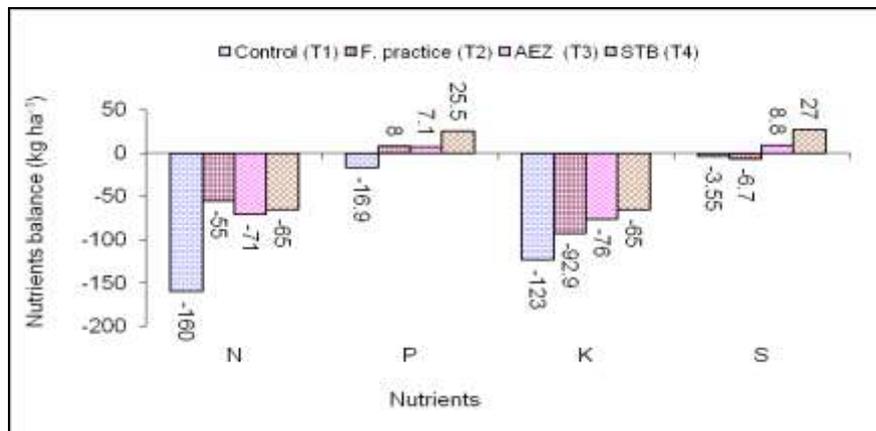


Fig. 01. Apparent nutrient balance of N, P, K and S under wheat-mungbean - T.aman cropping rice pattern.

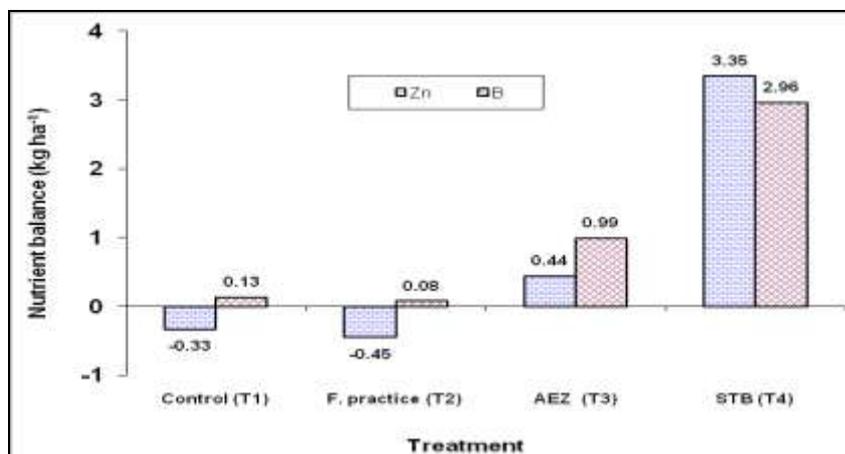


Fig. 02. Apparent nutrient balance of Zn and B under Wheat-Mungbean-T.aman cropping pattern.

Soil Fertility

Initial soil samples were collected from the experimental field and post harvest soil samples were also collected from each treated plot after two cycles of wheat-mungbean-T. *aman* rice cropping system for analyzing different soil properties viz. soil pH, organic matter, total N and available P, K, S, Zn and B. The initial and post harvest soil results are presented in Table 6. Initially the soil pH was 6.1, but after completion of two crop cycles and incorporation of mungbean stover and other crop residues in soil, the pH remained unchanged although minor variation existed. A minor change in soil fertility occurred from initial status due to different fertilizer management practices over two years. Soil test basis fertilizer application (T₄) tended to maintain the initial fertility or increased slightly (Table 6). The treatment T₄ showed an encouraging effect on organic matter, N, P, S, Zn and B only. Such observations are in agreement with the findings of Zaman *et al.* (1994). Potassium (K) slightly decreased in all plots over the initial status. The results are in agreement with the findings of Timsina and Connor (2001). The available Zn and B content of the soil slightly decreased when they were not applied, but remained almost static or increase when applied (Table 6).

Table 6. Initial and post soil fertility status after two cycles of wheat-mungbean-T. *aman* rice cropping system due to different fertilizer management practices

Treatment	pH	OM (%)	Total N (%)	K	P	S	Zn	B
				Meq. 100 g ⁻¹	µg g ⁻¹			
Initial status	6.1	1.28	0.061	0.13	15.0	17.1	1.33	0.16
Control (T ₁)	6.2	1.29	0.060	0.11	15.5	16.5	1.31	0.14
F. practice (T ₂)	6.1	1.31	0.062	0.12	16.1	16.9	1.32	0.14
AEZ (T ₃)	6.1	1.32	0.061	0.11	16.4	17.6	1.35	0.16
STB (T ₄)	6.0	1.34	0.063	0.12	16.8	18.1	1.37	0.17

Economic analysis

The gross margin due to treatment T₄ increased over farmers practice (T₂) for higher crop yield. The highest marginal benefit cost ratio (4.06) was in T₃ followed by T₄ but T₃ was economically viable due to the cost of production of T₃ (Tk. 74248 ha⁻¹ yr⁻¹) was lower than T₄ (Tk. 83967 ha⁻¹ yr⁻¹) (Table 7). Similar observation was made by Malika *et al.* (2015) that the highest marginal benefit-cost ratio of 3.66 was obtained from T₁ (100% RFD) which was followed by T₃ (75% RFD + PM 3 t ha⁻¹). Considering the marginal benefit-cost ratio (MBCR) T₃ ranked the first.

Table 7. Economic analysis of wheat-mungbean-T. aman rice cropping pattern affected by different nutrient managements (after completion of two years cycle)

Treatment	Variable cost	Gross return	Added cost over control	Added benefit over control	Gross margin over control	MBCR
	Tk ha ⁻¹ yr ⁻¹					
Control(T ₁)	59875	151148	-	-	-	
F. practice(T ₂)	69249	185704	9374	34556	25182	3.68
AEZ (T ₃)	74248	209564	14373	58416	44043	4.06
STB (T ₄)	83967	233971	24092	82823	58731	3.44

Note: Input prices: Urea= Tk.12 kg⁻¹, T.S.P= Tk.22 kg⁻¹, MoP= Tk.20 kg⁻¹, Gypsum= Tk.6 kg⁻¹, Zinc sulphate= Tk.120 kg⁻¹, Boric acid= Tk.300 kg⁻¹, Rovral fungicide= Tk.250 100^{-g}, Bavistin fungicide= Tk.200 100^{-g}, Provex fungicide = Tk.3200 kg⁻¹, Ripcord insecticide = Tk.105 100^{-g}, Karate insecticide= Tk.450 500^{-ml}, Plowing= Tk.1400 ha⁻¹(one pass), Labour wage= Tk.125 day⁻¹, Wheat seed= Tk.25 kg⁻¹, Mungbean seed= Tk.60 kg⁻¹, T.aman rice seed= Tk.35 kg⁻¹.

Output prices: Wheat= Tk.18.75 kg⁻¹, Mungbean= Tk.55 kg⁻¹, T.aman rice= Tk.19 kg⁻¹, Wheat straw rate = Tk.1 kg⁻¹, Rice straw= Tk.1.25 kg⁻¹.

Conclusion

Productivity of tested system showed higher through soil test based fertilization. Results clearly indicated that the nutrient uptake by wheat, mungbean and T. aman rice were found to be higher in soil test basis treatment. Nitrogen and K mining occurred remarkably from the soil due to different fertilizer management practices. Results of economic and soil fertility suggest that soil test basis fertilization is good for achieving sustainable crop yield.

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DETERMINATION OF OPTIMUM AND ECONOMIC DOSES OF FERTILIZERS FOR RICE PRODUCTION IN SALINE AND CHARLANDS ECOSYSTEM

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Abstract

On-farm experiment was carried out for four consecutive seasons: *Boro* (dry season) 2011-12, Transplanted Aman (T. Aman, wet season) 2012, *Boro* 2012-13 and T. Aman 2013 at the farmer's field in Londonipara, Sonagazi, Feni to develop fertilizer recommendation for rice-based cropping systems in saline and charland ecosystem in Bangladesh. The experiments were designed with eight treatments and laid out in randomized complete block design (RCBD) with three replications. The treatment combinations were: T₁ = 100% NPKSZn (STB), T₂ = T₁ + 25% N, T₃ = T₁ + 25% NP, T₄ = T₁ + 25% NK, T₅ = T₁ + 25% PK, T₆ = T₁ + 25% NPK, T₇ = 75% of T₁ and T₈ = Absolute control. Results indicated that application of different fertilizers significantly affected the grain yield at all of the seasons. In *Boro* 2011-12, the highest grain yield was found in treatment T₁ (100% STB) while T₃ (T₁ + 25% NP) gave highest grain yield in *Boro* 2012-13. Statistically identical yield was observed in *Boro* 2011-12 with all treatments except control (T₈). Highest grain yield was found with T₆ (T₁ + 25% NPK) treatments in both of T. Aman 2012 and 2013 seasons. Annual straw yield was found more in T₆ (T₁ + 25% NPK) treatment. All the treatment combinations gave significantly higher yield over the control in all seasons. However, on the basis of yield performance, economic analysis and nutrient absorption, the treatment T₆ = T₁ + 25% NPK (N₂₂₅P₃₀K_{17.5}S₁₅Zn₄ for *Boro* and N₁₂₁P₁₅K₉S₁₀Zn₃ for T. Aman) performed the best among the treatments.

Keywords: AEZ-18, crop cycle, cropping system, added return, gross return

Introduction

Applying nutrients to the crop is essential in managing soil fertility so the plants grow and develop normally. A number of crop problems can be related to inefficient management of nutrients and nutrient imbalances in the field. Fertilizer should be applied based on a soil test and the desired yield. Salinity causes unfavorable environment and hydrological situation that restrict normal crop production throughout the year. The freshly deposited alluviums from upstream in the coastal areas of Bangladesh become saline as it comes in contact with the sea water and continues to be inundated during high tides and ingress of sea water through creeks. The factors which contribute significantly to the

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development of saline soils are, tidal flooding during wet season (June-October), direct inundation by saline or brackish water and upward or lateral movement of saline ground water during dry season (November-May). Crop production in this area is dominated by the traditional T. Aman rice with the yield of 2 t ha⁻¹, which is very low due to soil salinity problem, drought in the dry season, lack of sufficient number of saline tolerant rice cultivars as well as lack of appropriate fertilizer management technologies etc. Increased pressure of growing population demand more food. Thus, it has become increasingly important to explore the possibilities of increasing the potential of these (saline) lands for increased production of crops. To meet the food grain requirement for a growing population with limited land resources, an increasing pressure on the land is taking place without adequate compensation of the plant nutrient taken up from the soil. Nutrient mining is one of the major causes for stagnation or decline in yield of major crops of Bangladesh. If this problem of nutrient depletion is not corrected it will cause a serious damage of the soil and to the welfare of mankind. Relatively higher amount of fertilizers need to be used in HYV of different crop cultivation. Fageria *et al.* (1991) stated that supplying of mineral nutrients to crops in adequate amounts is one of the most important factors in achieving higher productivity. Fertilizer has now become a very costly commodity of agriculture in Bangladesh. A huge amount of foreign currency is needed to import different fertilizers in the country. It is, therefore, urgently needed to develop fertilizer management packages in such a way that it suits farmers' resource constraints for ensuring the high use efficiency of fertilizers. Information based on soils, crops and cropping pattern, BARC prepared Fertilizer Recommendation Guide to adopt balanced fertilization for sustaining crop production in the country. This National Fertilizer Recommendation Guide needs to be further updated and verified for different dominant cropping patterns at different AEZs. Therefore, an attempt was made to develop the proper fertilizer management packages for Rice-Fallow-Rice and rice-based cropping systems in saline and charland ecosystem under AEZ 18.

Materials and Method

Experimental sites and seasons: The study was conducted in continuous rice-rice cropping systems for four consecutive seasons, *Boro* (dry season) 2011-12, T. Aman (wet season) 2012, *Boro* 2012-13 and T. Aman 2013 at the farmer's field in Londonipara, Sonagazi, Feni (22.8183°N latitude, 91.38613°E longitude). Two rice crops were grown annually in two seasons known as T. Aman and *Boro*. T. Aman was the wet season (June-July to November-December) in which transplanted rice was grown under partially irrigated conditions in the study area. *Boro* was the dry season (December-January to April-May) in which transplanted rice was grown under fully irrigated conditions. Semi-dwarf, high-yielding rice varieties were grown in the study area; BRRI dhan46 with 150 days duration was grown in T. Aman, and BRRI dhan47 (salt tolerant)/BRRI dhan28 with 152/140 days duration was grown in *Boro* season. The field belongs to agro-

ecological zone (AEZ) number 18 known as Young Meghna Estuarine Floodplain. The clay loam soil having pH 6.79, organic carbon 0.71%, total N 0.07%, available P 9.5 mg kg⁻¹, exchangeable K 0.27 cmol kg⁻¹ and available S 13.1 mg kg⁻¹ was used in the experiment (Table 1).

Experimental design and treatments: The experiment was established in farmer's fields in a randomized complete block design with one complete set of treatments and three replications. Treatments consisted of options for managing fertilizers in rice of saline and char lands ecosystem. The treatments were: T₁ = 100 % N P K S Zn (Soil Test Basis; according to the BARC, 2005); T₂ = T₁ + 25 % N; T₃ = T₁ + 25 % N P; T₄ = T₁ + 25 % N K; T₅ = T₁ + 25 % PK; T₆ = T₁ + 25 % N P K; T₇ = 75 % of T₁ and T₈ = Absolute control (without fertilizer). In Boro season, NPKSZn were applied @ 180-24-14-15-4, 225-24-14-15-4, 225-30-14-15-4, 225-24-17.5-15-4, 180-30-17.5-15-4, 225-30-17.5-15-4, 135-18-10.5-11.25-3 and 0-0-0-0-0 kg ha⁻¹ in T₁, T₂, T₃, T₄, T₅, T₆, T₇ and T₈ treatments, respectively. In T. Aman season, NPKSZn were applied @ 97-12-7-10-3, 121.25-12-7-10-3, 121.25-15-7-10-3, 121.25-12-8.75-10-3, 97-15-8.75-10-3, 121.25-15-8.75-10-3, 72.75-9-5.25-7.5-2.25 and 0-0-0-0-0 kg ha⁻¹ in T₁, T₂, T₃, T₄, T₅, T₆, T₇ and T₈ treatments, respectively. The total amounts of P as triple superphosphate, K as KCl, S as gypsum, and Zn as zinc sulfate were applied as basal-immediately before transplanting of rice. In the recommended practice for rice, N was applied as urea in three equal splits at basal, 25-30 days after transplanting (DAT) and 5-7 days before PI stage and at basal, 20-25 DAT and 5-7 days before PI stage in Boro and T. Aman season, respectively. Unit plot size was 4 × 5 m². All plots were surrounded by permanent bunds to prevent transfer of soil and nutrients between plots. In all cases, rice was transplanted and grown on submerged soil with irrigation. Weeds and insects were controlled to avoid yield losses.

Soil and plant sampling and analysis: Initial soil was collected with a core sampler from the 0 to 15 cm layer of puddled soil before the application of fertilizer. Nine samplings were done from farmer's field. Soil samples were air-dried, ground and passed through a 2 mm sieve and prepared for routine analyses of texture, pH, EC, OC, total N, exchangeable K, available P, S and Zn (Olsen *et al.*, 1954, Page *et al.*, 1982, Islam *et al.*, 2014; Islam *et al.*, 2016 and Saha *et al.*, 2016).

Grain yield was recorded from the central 5 m² harvest area in each plot at maturity and reported on 14% moisture basis. At maturity, 16 hills (four hills from each of the four sides of the grain harvest area) were collected at ground level and fresh straw weight was determined after separating the grains. Grain and straw were dried at 70°C to constant weight and dry weights were recorded. The ratio of fresh and oven-dry weights of straw for 16-hill samples was then used to determine straw yields on an oven-dry basis from fresh straw weights (Islam *et al.*, 2015). Dry grain and straw from the 16-hill samples were ground to pass through a 0.5-mm sieve and analyzed for total N, P and K following standard procedure. The N, P and K contents in grain plus straw were taken as the measure of total N, P and K uptake.

Table 1. Initial Soil Properties of the Experimental Sites

Texture	pH (1:2.5)	Org. C (%)	Total N (%)	Available P (mg kg ⁻¹)	Exchangeable K (cmol kg ⁻¹)	Available S (mg kg ⁻¹)	Available Zn (mg kg ⁻¹)	EC (dS/m)
Clay Loam	6.79	0.71	0.07	9.5	0.27	13.1	Trace	0.65

Table 2. Effect of different fertilizer packages on the yield of rice (t ha⁻¹) in a Boro-Fallow-T. Aman cropping pattern

Treatments	2011-12						2012-13					
	Boro (BRRIdhan47), 2011-12		T. Aman (BRRIdhan46), 2012		Annual yield (t ha ⁻¹ crop-cycle ⁻¹)		Boro (BRRIdhan28), 2012-13		T. Aman (BRRIdhan46), 2013		Annual yield (t ha ⁻¹ crop-cycle ⁻¹)	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T ₁ = NPKS ₁₀ Zn (STB) ¹	5.88	6.51b	3.19	2.60	9.07	9.11	4.67	4.78	4.09	4.35	8.76	9.13
T ₂ = T ₁ + 25% N	5.79	7.27	2.82	2.48	8.61	9.75	5.68	5.24	3.79	4.03	9.47	9.27
T ₃ = T ₁ + 25% NP	5.22	6.21	2.60b	3.07	7.82	9.28	6.62	5.25	3.74	3.91	10.36	9.16
T ₄ = T ₁ + 25% NK	4.99	6.10	2.80	3.13	7.79	9.23	5.53	4.96	4.23	4.70	9.76	9.66
T ₅ = T ₁ + 25% PK	5.82	6.06	2.97	3.00	8.79	9.06	5.36	5.36	4.15	4.37	9.51	9.73
T ₆ = T ₁ + 25% NPK	5.05	7.05	3.82	3.18	8.87	10.23	5.16	4.98	4.67	4.84	9.83	9.82
T ₇ = 75% of T ₁	5.81	6.04	3.01	2.87	8.82	8.91	5.32	5.30	3.45	3.77	8.77	9.07
T ₈ = Control	3.10	4.27	2.05	2.31	5.15	6.58	3.68	4.44	2.58	3.02	6.26	7.46
LSD _{0.05}	0.85	0.60	0.69	0.64	-	-	0.62	0.47	0.45	0.55	-	-
Significant level	**	**	**	*	-	-	**	**	**	**	-	-
CV (%)	9.3	5.6	13.5	12.9	-	-	6.7	5.4	6.6	7.7	-	-

¹Nutrient rates for T₁= N₁₈₀ P₂₄ K₁₄ S₁₅ Zn₄ (Boro) and T₁= N₉₇ P₁₂ K₇ S₁₀ Zn₃ (T. Aman)

** = Significant at 1% level; * = Significant at 5% level.

Total N was determined following Micro Kjeldahl method (Steam distillation method, Bremner 1965). Plant samples were digested using the $\text{HNO}_3\text{-HClO}_4$ (5:2) di-acid mixture. Phosphorous was determined colorimetrically by spectrophotometer (model-V-630, Jasco) and potassium was determined by flame photometer (model-410, Sherwood) according to the procedure described by Yoshida *et al.* (1976).

Data analysis: Analysis of variance (ANOVA) was performed on yield and N, P and K uptake to determine the effects different treatments using the IRRISTAT software version 4.1 (IRRI, 1998). Least significant difference (LSD) at the 0.05 level of probability was used to evaluate the differences among treatment means. Economic analyses were done using standard procedure.

Results and Discussion

Yield: Applied fertilizer significantly influenced the grain and straw yield of Boro and T. Aman rice (Table 2). In Boro season (2011-12), the highest grain yield (5.88 t ha^{-1}) was observed in the treatment T_1 , which was statistically similar to the treatments T_2 , T_3 , T_4 , T_5 , T_6 & T_7 . There were no positive or negative effects on additional fertilizers. The lowest grain yield (3.10 t ha^{-1}) was found in the treatment T_8 (control). The highest straw yield (7.27 t ha^{-1}) was observed in the treatment T_2 , which was statistically similar to the treatments T_6 . The lowest straw yield (4.27 t ha^{-1}) was found in the treatment T_8 (control).

In Boro season (2012-13), the highest grain yield (6.62 t ha^{-1}) was obtained with the treatment T_3 . The lowest grain yield (3.68 t ha^{-1}) was observed in the treatment T_8 (control). Significantly highest straw yield (5.36 t ha^{-1}) was obtained with the treatment T_5 , which was statistically similar to T_2 , T_3 , T_4 , T_6 , and T_7 . The lowest straw yield (4.44 t ha^{-1}) was observed in the treatment T_8 (control), which was statistically similar to T_1 (Table 2).

In T. Aman season (2012), the highest grain yield (3.82 t ha^{-1}) was obtained with the treatment T_6 , which was statistically similar to T_1 . Ali *et al.* (2014) observed the same results on rice yield. The lowest grain yield (2.05 t ha^{-1}) was observed in the treatment T_8 (control), which was statistically similar to T_3 . The highest straw yield (3.18 t ha^{-1}) was obtained with the same treatment T_6 followed by T_4 (3.13 t ha^{-1}), which were statistically similar to T_1 , T_2 , T_3 , T_5 and T_7 . The lowest straw yield (2.31 t ha^{-1}) was observed in the treatment T_8 (control), which was statistically similar to T_1 , T_2 , T_3 , T_5 and T_7 .

In T. Aman season (2013), like first year (T. Aman 2012), the highest grain yield (4.67 t ha^{-1}) was obtained with the same treatment T_6 , which was statistically similar to T_4 . The lowest grain yield (2.58 t ha^{-1}) was observed in the treatment T_8 (control). The highest straw yield (4.84 t ha^{-1}) was obtained with the same treatment T_6 , which was statistically similar to T_1 , T_4 and T_5 . The lowest straw yield (3.02 t ha^{-1}) was observed in the treatment T_8 (control). In first crop-cycle,

the highest annual grain yield (9.07 t ha⁻¹ crop-cycle⁻¹) was obtained with the treatment T₁ while in 2nd crop-cycle, it was obtained with the treatment T₃ (10.36 t ha⁻¹ crop-cycle⁻¹).

Economic analysis: The estimated total variable cost (TVC), gross return, total value of extra production (added return) and marginal benefit cost ratio (MBCR) are presented in Table 3. Economic analysis was done considering the following: fertilizer cost, fertilizer application cost and labor cost for the additional product including by products due to fertilizer application. The application of fertilizer increased the gross and added return in all the treatments (Table 3). The gross return from the control plot per crop-cycle/ha was only about Tk.1,40,643/- and the application of fertilizer increased the gross return, which ranged from Tk 2,07,658/- ha⁻¹ crop cycle⁻¹ in T₇ to Tk.2,23,100/- ha⁻¹ crop cycle⁻¹ in T₆. The highest added-return of Tk. 82,458/- ha⁻¹ crop-cycle⁻¹ was obtained with T₆ followed by T₅ (75,608/- ha⁻¹ crop-cycle⁻¹). The MBCR of all treated plots ranged from 2.43 (T₄) to 3.40 (T₇), which were higher than the permit able limit (2.00).

Table 3. Average yield (t ha⁻¹) and fertilizer use economy as affected by nutrient combinations

Treatment	Average yield (2011-12 & 2012-13)				Gross return** (Tk ha ⁻¹ crop-cycle ⁻¹)	Total Value of Extra production (Tk ha ⁻¹ crop-cycle ⁻¹)	TVC* (Tk ha ⁻¹ crop-cycle ⁻¹)	MBCR
	Boro (BRRI dhan47&28)		T. Aman (BRRI dhan46)					
	Grain	Straw	Grain	Straw				
T ₁ = NPKSZn (STB)	5.28	5.65	3.64	3.48	210528	69885	25249	2.77
T ₂ = T ₁ + 25% N	5.74	6.26	3.31	3.26	214790	74148	28359	2.61
T ₃ = T ₁ + 25% NP	5.92	5.73	3.17	3.49	214265	73623	29395	2.50
T ₄ = T ₁ + 25% NK	5.26	5.53	3.52	3.92	209563	68920	28307	2.43
T ₅ = T ₁ + 25% PK	5.59	5.71	3.56	3.69	216250	75608	26647	2.84
T ₆ = T ₁ + 25% NPK	5.11	6.02	4.25	4.01	223100	82458	29826	2.76
T ₇ = 75% of T ₁	5.57	5.67	3.23	3.32	207658	67015	19715	3.40
T ₈ = Control	3.39	4.36	2.32	2.67	140643	0	0	-

* Total variable cost (TVC) included fertilizer cost (chemical fertilizer), fertilizer application cost and labor cost for additional product.

Price (Taka/kg): Urea=20.00; TSP=22.00; MP=16.00; Gypsum=12.00; ZnSo₄= 180.00.

Labor wage rate = Tk.230/day

**Price (Taka/kg): Paddy=18.50; straw=5.00.

Two additional man-days/ha are required for applying fertilizer and four man-days/ha for per ton additional products including byproducts.

Table: 4. Effect of different fertilizer packages on the nutrient uptake (kg ha⁻¹) by rice in a Boro – Fallow – T. Aman cropping pattern

Treatments	Boro, 2011-12				T. Aman, 2012				Total nutrient uptake (kg ha ⁻¹ crop-cycle ⁻¹)			
	Nutrient uptake (kg ha ⁻¹)				Nutrient uptake (kg ha ⁻¹)							
	N	P	K		N	P	K		N	P	K	
T ₁ =NPKSZn (STB) ¹	81.17	15.26	144.80		41.88	4.68	61.38		123	20	206	
T ₂ = T ₁ +25% N	92.68	15.20	129.90		42.06	4.15	64.07		135	19	194	
T ₃ = T ₁ +25% NP	74.98	13.82	180.15		36.91	4.31	74.88		112	18	255	
T ₄ = T ₁ +25% NK	77.53	12.72	154.31		39.68	4.45	79.44		117	17	234	
T ₅ = T ₁ +25% PK	82.11	13.71	178.85		42.79	4.75	77.03		125	18	256	
T ₆ = T ₁ +25% NPK	76.05	14.17	180.52		56.26	5.68	84.21		132	20	265	
T ₇ = 75% of T ₁	74.06	13.79	167.20		39.04	4.67	76.31		113	18	244	
T ₈ = Control	50.15	8.64	66.36		25.62	3.42	56.17		76	12	123	
LSD _{0.05}	13.05	1.95	36.31		9.74	0.91	16.96		-	-	-	
Significant level	**	**	**		**	**	*		-	-	-	
CV (%)	9.8	8.3	13.8		13.7	11.5	13.5		-	-	-	

¹Nutrient rates for T₁= N₁₈₀ P₂₄ K₁₄ S₁₅ Zn₄ (Boro) and T₁= N₉₇ P₁₂ K₇ S₁₀ Zn₃ (T. Aman)

** = Significant at 1% level; * = Significant at 5% level

Table 5. Effect of different fertilizer packages on the nutrient uptake (kg ha⁻¹) by rice in a Boro – Fallow – T. Aman cropping pattern

Treatments	Boro, 2012-13		
	Nutrient uptake (kg ha ⁻¹)		
	N	P	K
T ₁ =NPKSZn (STB) ¹	70	15	134
T ₂ = T ₁ +25% N	85	19	149
T ₃ = T ₁ +25% NP	94	19	140
T ₄ = T ₁ +25% NK	73	18	127
T ₅ = T ₁ +25%PK	82	17	152
T ₆ = T ₁ +25% NPK	68	17	145
T ₇ = 75% of T ₁	75	16	145
T ₈ = Control	57	12	125
LSD _{0.05}	8	2	NS
Significant level	**	**	NS
CV (%)	6.1	6.7	8.9

¹Nutrient rates for T₁= N₁₈₀ P₂₄ K₁₄ S₁₅ Zn₄ (Boro) and T₁= N₉₇ P₁₂ K₇ S₁₀ Zn₃ (T. Aman)

** = Significant at 1% level;

Effect on nutrient uptake: Applied fertilizer significantly influenced the nutrient uptake by Boro and T. Aman rice of the cropping pattern (Tables 4 and 5). The highest total N, P and K uptake (kg ha⁻¹ crop-cycle⁻¹) was observed in the treatment T₂, T₁ and T₆, respectively. The lowest total nutrient uptake (kg ha⁻¹ crop-cycle⁻¹) was observed in the treatment T₈ (Table 4).

Conclusion

On the basis of yield performance, economic analysis and nutrient absorption the treatment T₆ = T₁+25% NPK (N₂₂₅ P₃₀ K_{17.5} S₁₅ Zn₄ for Boro season and N₁₂₁ P₁₅ K₉ S₁₀ Zn₃ for T. Aman season) performed the best among the treatments in saline and char lands ecosystem. From the result it is observed that present recommended dose of fertilizer is not sufficient to obtain optimum yield. So, 25% more NPK fertilizer is required to obtain better yield in this ecosystem.

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SEQUENTIAL APPLICATION OF BIO-PESTICIDE, BOTANICAL AND CHEMICAL INSECTICIDE TO MANAGE APHID AND POD BORER ATTACKING YARD LONG BEAN

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Abstract

Present study evaluated the effect of sequential application of a bio-pesticide (*Bacillus thuringiensis* subsp. *Kurstaki*) (*Bt*), a botanical (neem oil) and a chemical insecticide (cypermethrin 25EC) as well as cypermethrin 25EC alone in the management of aphid (*Aphis craccivora*) and pod borer (*Maruca vitrata*) attacking yard long bean (*Vigna unguiculata sesquipedalis*) in the field. In each treatment *Bt*, neem oil and cypermethrin were applied in alternative sequence as well as cypermethrin alone at 07 days intervals. All the treatments reduced aphid population and flower infestation after 24, 48 and 72h of treatment compared to untreated control. Aphid population reduction by the treatments over control was ranged from 34.4 to 71.9%, and the treatment with cypermethrin alone had the lowest abundance of aphid and flower infestation. The *Bt* followed by neem oil and cypermethrin significantly reduced the pod infestation by pod borer and increased yield (38.9–41.4%) over control with BCR: 1.85-2.16. Use of cypermethrin alone was most effective and showed the lowest pod infestation and revealed the increased yield over control (47.7%) with BCR 4.70. Considering the hazardous affect of cypermethrin alone, its application followed by bio-pesticide and botanical sequentially would be promising components for integrated pest management strategies against aphid and pod borer attacking yard long bean.

Keywords: *Bacillus thuringiensis*, cypermethrin, neem oil, yard long bean, sequentially.

Introduction

Yard long bean, *Vigna unguiculata sesquipedalis* is one of the most popular vegetables in Southeast Asia (AVRDC, 2001; Ali *et al.*, 2002). It is a vigorous climbing annual vine crop and known as long poded cowpea or Chinese long bean or snake bean. The tender green pods of yard long bean are a good source of protein, iron, calcium, phosphorus, vitamin A, vitamin C and dietary fiber (Singh *et al.*, 2001). Farmers in Bangladesh grow yard long bean throughout the year to meet high market demand, but the crop in subtropical conditions is highly prone

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to the attack of insect pests. The insects attack at flowering and post-flowering stages is a major factor to limit the production (Jackai *et al.*, 1992). The aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) and pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae) reduce the productivity significantly. The pod borer larvae damage flower buds, flowers, green pods and seeds of this crop thereby reduce 54.4% production (Singh and Jackai, 1988).

The aphid is a polyphagous insect which causes serious damage to bean plants from seedling to the pod bearing stage. They feed on phloem sap with their piercing sucking mouthparts, and their feeding injury causes leaf and fruit growth abnormalities, leaf yellowing and curling, reduction of leaf canopy area, and senescence of premature leaves (Beckendorf *et al.*, 2008). Aphid infestations reduce branching and the infested plants become stunted, suffer from withering or shedding of flowers, and produce lower number of pods (Beckendorf *et al.*, 2008). This insect also plays a role in the transmission of necrotic yellow, mosaic yellow and leaf roll viruses in bean plants (Smith and Boyko, 2007).

To reduce the crop loss, farmers indiscriminately use chemical pesticides. The synthetic chemicals destroy indigenous biodiversity, cause personal and environmental hazards and develop resistance in insects (Hoi, 2010). The lower mammalian toxicity and rapid degradation of botanical pesticides make them a suitable tool in integrated pest management programs (Amin *et al.*, 2000; Roy *et al.*, 2012; Roy *et al.*, 2014). Some essential oils have insecticidal activities on Diptera larvae and adults (Chang *et al.*, 2009). Bio-pesticides are one of the most effective tools available for managing vegetable pests. However, complete reliance on botanical or bio-pesticides alone seems to be less effective in reducing the economic damage caused by insects on vegetables. Hence, this study was conducted to validate an integrated pest management strategy by applying neem oil, *Bacillus thuringiensis* (*Bt*) and cypermethrin at an alternative sequence and cypermethrin alone for the management of aphid and pod borer attacking the yard long beans in the field with special emphasis on the yield and benefit cost ratio.

Materials and Method

Experimental site and duration

The study was conducted in the field laboratory of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during January to May, 2016.

Experimental design and cultivation of yard long bean

Yard long bean seeds were collected from the Horticulture Research Center of Bangladesh Agricultural Research Institute (BARI) and cultivated in a randomized complete block design with a plot size of 4 m × 4 m. Seedlings were grown in a nursery to prevent damage from pests and diseases. Ten day, old

seedlings were transplanted in the plots. The spaces between blocks and between plots were 0.5 m and 1.0 m, respectively. Seedlings were transplanted on 16 January, 2016 in rows. Each plot contained 5 rows and each row had 5 pits separated by 60 cm. At the stage of branching, the plants were supported by bamboo sticks to facilitate creeping. The manures and fertilizers were applied according to the recommended doses of BARI. Weeding, irrigation etc. were done whenever necessary. Pest incidences were monitored from one week after transplanting until last harvest.

Experimental treatments

Five treatments with an alternative sequential application of neem oil, *B. thuringiensis* (*Bt*) and cypermethrin 25EC (one after another) as well as cypermethrin alone were adopted. The *Bt* subsp. *kurstaki* were collected from the Ispahani Agro limited, Gazipur. Neem oil was bought from a pesticide shop in Dhaka. An untreated control was kept for scientific interpretation. Application of the treatments was started at the advent of the infestation of the pest and applied three times at weekly interval. The concentrations of *Bt*, neem oil and cypermethrin were 5 ml l⁻¹ water, 2.5 ml l⁻¹ water and 0.5 ml l⁻¹ water, respectively. The treatments with order of alternate sequence at weekly application are *Bt* → cypermethrin → neem oil; cypermethrin → neem oil → *Bt*; neem oil → *Bt* → cypermethrin; cypermethrin → *Bt* → neem oil; cypermethrin alone (three times), and control.

Data collection

The numbers of aphid per inflorescence were counted at 24, 48, and 72 h after each treatment. The topmost inflorescence from the third plant of the third row was used for counting the aphid population. The total number of bloomed flowers and the number of infested flowers of the topmost inflorescence from the third plant of the third row were counted to calculate the flower infestation level.

Healthy and infested (bore holes with or without frass) pods were plucked and percent pod infestation was calculated at each harvest. The recorded pod infestation at each harvest was pooled for the entire season and the mean pod infestation was derived for each replication. Yield was recorded at every harvest and the yields were categorized as marketable and infested based on the absence or presence of pod borer infestation. The pod yield recorded during each observation was pooled for the entire season and the total pod yield was derived for each replication, which was then used for estimating the total yield in tons per hectare for each treatment.

Data analysis: Data were analyzed by general linear model (GLM) followed by analysis of variance (ANOVA) and then mean comparisons were done by Duncan's Multiple Range Test (DMRT) through SPSS (IBM SPSS statistics 21).

Results and Discussion

Result

The effects of bio-pesticides and chemical insecticide on the population abundance of aphid attacking yard long bean inflorescence (number of aphid inflorescence⁻¹) are presented in Table 1. The treatments had significant effect ($F_{5, 144} = 33.4$, $p < 0.001$) on the abundance but the effects of hours after treatment ($F_{2, 144} = 2.8$, $p = 0.06$) and interaction between treatments and hours after treatment ($F_{10, 144} = 0.28$, $p = 0.99$) were insignificant. Aphid population abundance at 24, 48 and 72 hours after treatment varied from 2.1 ± 0.6 to 6.4 ± 1.4 , 1.8 ± 0.7 to 6.4 ± 1.6 and 1.6 ± 0.5 to 6.4 ± 1.4 inflorescence⁻¹, respectively. The mean aphid population among the treatments varied from 1.8 ± 0.6 to 6.4 ± 1.5 inflorescence⁻¹ and the results differed significantly ($F_{5, 156} = 34.2$, $p < 0.001$). The lowest and the highest populations were shown by cypermethrin → cypermethrin and control, respectively. Aphid population reduction over control varied from 34.4 to 71.9% and the treatment with cypermethrin alone showed the highest reduction.

Table 1. Effect of *Bacillus thuringiensis* (Bt), neem oil and cypermethrin applied sequentially at 7 days interval (3 times) on the population abundance of aphid attacking yard long bean inflorescence in the field

Treatments with order of alternative application sequence	Number of aphid inflorescence ⁻¹			Mean population	% Population reduction
	24 HAT	48HAT	72HAT		
Bt → cypermethrin → neem oil	3.9±1.7bA	3.7±1.2bcA	2.8±1.5bcA	3.4±1.5c	46.9
Cypermethrin → neem oil → Bt	3.7±1.2bA	3.0±1.0cA	3.0±1.5bcA	3.2±1.3c	50.0
Neem oil → Bt → cypermethrin	4.4±1.7bA	4.4±1.3bA	3.7±1.6bA	4.2±1.5b	34.4
Cypermethrin → Bt → neem oil	3.7±1.4bA	3.2±1.4cA	3.0±1.9bcA	3.3±1.6c	48.4
Cypermethrin alone (three times)	2.1±0.6cA	1.8±0.7dA	1.6±0.5cA	1.8±0.6d	71.9
Control	6.4±1.4aA	6.4±1.6aA	6.4±1.4aA	6.4±1.5a	

Data expressed as mean ± SD. Means followed by the same lowercase letter(s) in the same column or by the same uppercase letter(s) in the same row are not significantly different, as assed by DMRT ($p \leq 0.05$).

Table 2 shows the yard long bean flower infestation levels caused by aphid in different treatments. The infestation levels ranged from 20.3 ± 1.7 to $52.6 \pm 10.3\%$ and the results differed significantly ($F_{5, 48} = 4.2$, $p < 0.01$). All the treatments revealed significantly lower percentage of infestation compared to the untreated control. The treatment with cypermethrin alone applied three times resulted

significantly lower level of infestation than its sequential application with *Bt* and neem oil.

There was significant difference ($F_{5,48} = 8.2, p < 0.001$) in pod infestation levels of yard long beans caused by pod borer (Table 2). Pod infestation levels ranged from 14.8 ± 1.3 to $40.3 \pm 4.9\%$, and the treatments had significantly lower percentage of infestation compared to control. The lowest infestation level was occurred when cypermethrin alone was applied three times.

Table 2. Effect of *Bacillus thuringiensis* (*Bt*), neem oil and cypermethrin applied sequentially at 7 days interval (3 times) on the flower and pod infestation of yard long bean by aphid and pod borer, respectively

Treatments with order of alternative application sequence	% Infestation	
	Flower infestation by aphid	Pod infestation by pod borer
<i>Bt</i> → cypermethrin → neem oil	35.1±2.7 bc	22.2±2.3 bc
Cypermethrin → neem oil → <i>Bt</i>	25.9±3.9 bc	23.5±2.3 bc
Neem oil → <i>Bt</i> → cypermethrin	38.5±6.0 ab	25.0±2.6 b
Cypermethrin → <i>Bt</i> → neem oil	30.7±4.6 bc	22.1±3.0 bc
Cypermethrin alone (three times)	20.3±1.7 c	14.8±1.3 c
Control	52.6±10.3 a	40.3±4.9 a

Data expressed as mean ± SD. Means followed by the same lowercase letter(s) in the same column or by the same uppercase letter(s) in the same row are not significantly different, as assed by DMRT ($p \leq 0.05$).

Table 3. Yield of yard long bean obtained by the management of aphid and pod borer using the sequential application of *Bacillus thuringiensis* (*Bt*), neem oil and cypermethrin at 7 days interval (3 times)

Treatments with order of alternative application sequence	Yield (t/ha)			% Marketable yield over control
	Marketable	Infested	Gross	
<i>Bt</i> → cypermethrin → neem oil	9.9±0.7ab	2.5±0.2b	12.4±0.8a	41.4
Cypermethrin → neem oil → <i>Bt</i>	9.7±0.4ab	2.5±0.3b	12.3±0.5a	40.2
Neem oil → <i>Bt</i> → cypermethrin	9.8±1.4ab	2.6±0.3ab	12.5±1.3a	40.8
Cypermethrin → <i>Bt</i> → neem oil	9.5±0.8b	2.6±0.2ab	12.1±0.9a	38.9
Cypermethrin alone (three times)	11.1±0.4a	1.9±0.3c	13.0±0.5a	47.7
Control	5.8±0.9c	3.0±0.2a	8.8±0.8b	-

Data expressed as mean ± SD. Means within a column followed by the same letter(s) are not significantly different as assessed by DMRT ($p \leq 0.05$).

There are significant differences in the marketable yield ($F_{5, 12} = 14.4, p < 0.001$), infested yield ($F_{5, 12} = 6.7, p < 0.01$) and gross yield ($F_{5, 12} = 9.5, p < 0.01$) of the yard long bean pods (Table 3). The marketable yield, infested yield and gross yield of the pods ranged from 9.5 ± 0.8 to 11.1 ± 0.4 , 1.9 ± 0.3 to 3.0 ± 0.2 and 8.8 ± 0.8 to 13.0 ± 0.5 ton ha⁻¹, respectively. All the treatments revealed significantly higher

marketable yield and gross yield than untreated control. The infested yield was the highest ($3.0 \pm 0.2 \text{ t ha}^{-1}$) in the control treatment. The marketable yield and gross yield were the highest in the treatment with cypermethrin alone applied three times. Marketable yield over control varied from 38.9 to 47.7% and the treatment with cypermethrin alone showed the highest level.

The costs of the different treatments for aphid and pod borer management and the return obtained from the yard long bean yield are presented in Table 4. The cost was the lowest (TK. 18580/=) for the treatment with cypermethrin alone applied three times accordingly its gross return (TK. 222000/=), net return (TK. 203420/=), adjusted net return (TK. 87420/=) and BCR (4.70) were the highest.

Table 4. Benefit-cost ratio analysis of *Bacillus thuringiensis* (*Bt*), neem oil and cypermethrin applied sequentially at 7 days interval (3 times) for management of aphid and pod borer in yard long bean field

Treatments with order of alternative application sequence	Management Cost (Taka)	Gross return (Taka)	Net return (Taka)	Adjusted net return (Taka)	BCR
<i>Bt</i> → cypermethrin → neem oil	25930/=	198000/=	172070/=	56070/=	2.16
Cypermethrin → neem oil → <i>Bt</i>	25930/=	194000/=	168070/=	52070/=	2.0
Neem oil → <i>Bt</i> → cypermethrin	25930/=	196000/=	170070/=	54070/=	2.08
Cypermethrin → <i>Bt</i> → neem oil	25930/=	190000/=	164070/=	48070/=	1.85
Cypermethrin alone (three times)	18580/=	222000/=	203420/=	87420/=	4.70
Control	---	116000/=	116000/=	-	

1 Kg price TK. 20 only.

Discussion

This study clearly indicated that the abundance of aphid on bean inflorescence varied among the treatments. The application of cypermethrin alone and sequential application of cypermethrin, *Bt* and neem oil significantly reduced aphid population as well as flower infestation, but cypermethrin applied alone was found to be the most effective in reducing aphid population and flower infestation. The present findings corroborate with Weaver *et al.* (1997) who reported that the bio-pesticides and oils were subjected to simple chemical fractionation and had great potential for insect control.

Yule and Srinivasan (2014) reported that *B. thuringiensis* followed by cypermethrin reduced 23-85% yard long bean pod damage by *M. vitrata*. In the present study, sequential application of cypermethrin, *Bt* and neem oil showed significantly lower level of pod damage compared to untreated control. However, treatment with sequential use of cypermethrin followed by *Bt* and neem oil was not found to be superior compared to three times application of cypermethrin alone.

Chemical pesticides or *Bt* or neem oil could stress the exposing insect pests and subsequent application of *Bt* and neem oil on stressed pests could lead to high

mortality rates (Khalique and Ahmed, 2001). But in this study, *Bt*, neem oil and chemical were used, because using the same pesticide or *Bt* or neem oil repeatedly might lead to the development of resistance in insects (Ekesi, 1999). Many studies confirmed moderate to high effectiveness of *B. thuringiensis* against *M. vitrata* in field conditions (Sunitha *et al.*, 2008; Sreekanth and Seshamahalakshmi, 2012). However, an earlier study by Yule and Srinivasan (2013) revealed that *B. thuringiensis* alone could not reduce yield losses, and neem is effective only at higher concentrations for lepidopteran pests.

In this study, highest yield was obtained from the plants that were treated with only cypermethrin followed by the treatments with *Bt* and neem oil, and cypermethrin applied sequentially one after another at 7 days interval for three times. Yule and Srinivasan (2014) reported that *B. thuringiensis* in combination with cypermethrin reduced pod damage by *M. vitrata* in yard long bean and substantially increased yield 22-190%. The present study showed that *Bt*, neem oil and cypermethrin applied sequentially reduced aphid populations from 34.4 to 50.0% over control. The treatment combination reduced flower infestation by aphid, and pod infestation by borer, and resulted higher yield (38.9 to 41.4% over control). Annan *et al.* (1995) observed reduced plant growth and yield in the cowpea, *Vigna unguiculata* due to the infestation of *A. craccivora*. The gross return, net return, adjusted net return, and benefit-cost ratio of the treatments varied with their management expenditure and yield.

This study revealed that an integrated strategy based on *Bt*, neem oil and cypermethrin applied sequentially could effectively curtail yield losses caused by aphid and pod borer in yard long bean. The sequential application of chemical insecticide, *Bt* and neem oil reduces the amount of chemical pesticides significantly in the environment, which might avoid the development of resistance and loss in biodiversity. Because of the global focus on improving the safety of pesticides for human health and the environment, it is necessary to replace chemical insecticides by bio-pesticides.

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**MANAGEMENT OF BRINJAL SHOOT AND FRUIT BORER
LEUCINODES ORBONALIS GUENEE USING
DIFFERENT APPROACHES**

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Abstract

The present study was conducted during October 2014 to June 2015 in the farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur to find out the most effective management option (s) against brinjal shoot and fruit borer (BSFB) with 6 treatments namely, *Beauveria bassiana*, 1×10⁹ CFU @ g l⁻¹, Bioneem 0.3 EC (Azadirachtin) @ 1.5 ml l⁻¹, Tracer 45 SC (Spinosad) @ 0.4 ml l⁻¹, Vertimec 1.8 EC (Abamectin) @ 1.2 ml l⁻¹ of water, Mechanical control (hand picking) with clean cultivation and untreated control. Results revealed that shoot infestation at pre-fruiting stage was the lowest in Bioneem 0.3 EC (4.34%) and at fruiting stage in Tracer 45 SC treated plot (7.75%) and the highest was in untreated control plot (17.94% at pre-fruiting stage and 39.46 at fruiting stage). The lowest fruit infestation by BSFB was obtained with Tracer 45 SC (8.16% n/n and 10.0% w/w) followed by *B. bassiana*, 1×10⁹ CFU (23.23% n/n and 18.27% w/w) and the highest infestation was observed under untreated control plot (48.59% n/n and 32.09% w/w). Percent reduction of infested fruit by number (83.21%) and weight (68.84%) over untreated control was higher in Tracer 45 SC treated plot resulting significantly higher marketable yield. Therefore, the significant highest marketable yield (34.39 t ha⁻¹) was harvested in the plot treated with Tracer 45 SC followed by 22.78 t ha⁻¹ with *B. bassiana*, 1×10⁹ CFU and 19.26 t ha⁻¹ from Vertimec 1.8 EC treated plot. The highest benefit cost ratio of 3.05 was obtained from Tracer 45 SC followed by 2.93 in *B. bassiana*, 1×10⁹ CFU and 2.89 with Vertimec 1.8 EC sprayed plot.

Keywords: *Leucinodes orbonalis*, microbial pesticide, biopesticide, mechanical control.

Introduction

Brinjal (*Solanum melongena* L.) is one of the popular vegetables favored by the people of many countries viz., Central, South and South East Asia, some parts of Africa and Central America (Harish *et al.*, 2011). It is native to India and is grown all over Bangladesh (Pareet, 2006). Eggplant is extensively damaged by the brinjal fruit and shoot borer (BSFB) (*Leucinodes orbonalis*) which caused 31-86% fruit damage in Bangladesh (Alam *et al.*, 2003). It is the most noxious and

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destructive pest of brinjal. It is reported that BSFB attacks either sporadically or in outbreak every year throughout the Indian subcontinent (Chakraborti and Sarkar, 2011).

To overcome this loss, different types of insecticides are used by the farmers of Bangladesh and they apply indiscriminately even at fruiting stage (Zafar *et al.*, 2012). Different combinations of management packages were used by our Bangladeshi farmer to manage BSFB. The combination of Chlorpyrifos 50% EC + Cypermethrin 5% EC, being the most effective and economically viable insecticide to manage *L. orbonalis* in eggplant crop (Sharma *et al.*, 2012). A survey of pesticide use in Bangladesh indicated that farmers sprayed chemical insecticides up to 180 times during a cropping season to protect their eggplant crop infested by the eggplant fruit and shoot borer, *L. orbonalis* (SUSVEG-Asia, 2007). Spraying frequency was reported 140 times or more in the 6-7 months cropping season and contributed to 32% of total cost of production (Alam *et al.*, 2003).

However, indiscriminate and non-judicious use of insecticides may result in a series problem related to both loss of their effectiveness and in the long run, it develops insect resistance (Alam *et al.*, 2003), pollution and health hazards (FAO, 2003). Again the question of residual toxicity of pesticides in brinjal is another big threat to our vegetable exports in the foreign markets (Islam *et al.*, 1999). Therefore, environment friendly management approach is a must to reduce environment and health hazards and to manage this pest eco-friendly. Various non-chemical approaches like biopesticides, botanicals, clean cultivation, mechanical control like hand picking and destroying of infested plant parts particularly shoots and fruits are common practices used for suppressing the insect pests (Hassan, 1994).

Due to lack of knowledge and unavailability of non-chemical pest management approaches, growers of Bangladesh mostly depend on insecticides to keep the crop production steady. Appropriate knowledge and availability of botanical pest management approaches and their integration with selective chemicals may give better results against brinjal shoot and fruit borer. Considering the circumstances, the present study was conducted to evaluate the effectiveness of some eco-friendly management approaches against *L. orbonalis* in brinjal field.

Materials and Method

The experiment was conducted in the experimental farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during October 2014 to June 2015 following 6 treatment and three replications with Randomized Complete Block Design (RCBD). The treatments were as follows:

T₁= *Beauveria bassiana*, 1×10⁹ CFU @ g l⁻¹, T₂= Bioneem 0.3 EC (Azadirachtin) @ 1.5 ml l⁻¹, T₃= Tracer 45 SC (Spinosad) @ 0.4 ml l⁻¹, T₄=

Vertimec 1.8 EC (Abamectin) @ 1.2 ml l⁻¹ of water, T₅= Mechanical control (hand picking) with clean cultivation, T₆= Untreated control.

BARI Brinjal-2 (Tarapuri) were used in this study. Manures and fertilizers were applied according to Rashid (1999) and intercultural operations such as irrigation, weeding, mulching, thinning and other operations were done throughout the cropping season for proper growth and development of the plants.

When the pest caused approximately 2% shoot infestation, the application of treatments was started. All the treatments were applied at 10 days interval and repeated 6 times up to last harvest. Data on shoot and fruit infestation, yield by number and weight were recorded. Total number of shoots and the number of infested shoots were recorded from 5 plants randomly selected in each plot at before and after flowering to fruit initiation and at fruiting stages at 7 days interval. The number of healthy and infested fruits and their weight per plot were recorded at each harvest. The infestation of the pest was expressed in percentage based on total number of shoot and fruit (n/n) and weight (w/w) of fruit.

The cumulative yield in Kg per plot of healthy as well as infested fruits was computed. For economic analysis, Marginal Benefit Cost Ratio (MBCR) was calculated on the basis of total expenditure of the respective spray schedule along with the total return from that particular spray schedule. The data were analyzed by using MSTAT-C software for analysis of variance (ANOVA) and the means were separated by using Duncan's Multiple Range Test (DMRT) at 5% level.

Results and discussion

Rate of shoot infestation

Efficacy of the environment friendly management approaches to reduce the infestation varied appreciably at both pre-fruiting and fruiting stages. All the environment friendly management approaches significantly reduced percent shoot infestation in both stages compared to untreated control plot (Table 1). Shoot infestation ranged 5.08-17.94% at pre-fruiting and 7.75-39.46% at fruiting stages under different treatments. At pre-fruiting stage, the highest shoot infestation was recorded in untreated control plot (17.94%) and the lowest infestation (5.08%) was obtained with Bioneem 0.3 EC which was statistically similar to Tracer 45 SC (5.19%), and *B. bassiana*, 1×10⁹ CFU (5.51%) treated plots followed by in Vertimec 1.8 EC (8.57%) and mechanical control (9.43%) plots. The highest shoot infestation reduction over untreated control was 71.68% under Bioneem 0.3 EC followed by Tracer 45 SC (71.05%) and *B. bassiana*, 1×10⁹ CFU (69.29%) treated plots. The least shoot infestation reduction over untreated control was found in mechanical control plot (47.40%) followed by Vertimec 1.8 EC (52.23%) treated plot (Table 1).

At fruiting stage, the highest shoot infestation was observed in untreated control plot (39.46%) and the lowest infestation (7.75%) was obtained with Tracer 45 SC

which was statistically significant from other approaches followed by *B. bassiana*, 1×10⁹ CFU (16.20%), Vertimec 1.8 EC (20.69%) and Bioneem 0.3 EC (22.08%) treated plots followed by in mechanical control (30.33%) plots. The highest shoot infestation reduction over untreated control was 80.36% under Tracer 45 SC followed by *B. bassiana*, 1×10⁹ CFU (58.95%), Vertimec 1.8 EC (47.57%) and Bioneem 0.3 EC (44.04%) treated plots. The least shoot infestation reduction over untreated control was found in mechanical control plot (23.14%) (Table 1).

Chatterjee *et al.* (2009) revealed that the lowest mean shoot as well as fruit infestation (7.47 and 9.88%) was recorded in the plots treated with Spinosad 2.5 SC (50 g a.i. ha⁻¹). Singh *et al.* (2009) observed that Profenofos @ 0.1% and Spinosad @ 0.01% were most effective in reducing shoot infestation by *L. orbonalis* and resulted higher brinjal fruit yield.

Islam *et al.* (1999) observed the minimum acceptable level of shoot infestation reduction over untreated control was 80.00% by applying different insecticides. Devi *et al.* (2014) conducted their experiment on comparative efficacy of some biorational insecticides for management of shoot and fruit borer (*Earias Vittella* Fab.) attacking okra. Pooled data of two years of experiment (2012 and 2013) expressed 8.30% shoot and fruit infestation and 59.88 (q ha⁻¹) yield was obtained due to application of *B. bassiana* @ 300 (g a.i. ha⁻¹).

Table 1. Effect of different treatments on brinjal shoot infestation by BSFB at pre-fruiting and fruiting stages during October 2014 to June 2015

Treatments	Pre-fruiting stage		Fruiting stage	
	% shoot infestation	% shoot infestation reduction over untreated control	% shoot infestation	% shoot infestation reduction over untreated control
<i>Beauveria bassiana</i> , 1×10 ⁹ CFU	5.51cd	69.29	16.20c	58.95
Bioneem 0.3 EC	5.08d	71.68	22.08c	44.04
Tracer 45 SC	5.19d	71.05	7.75d	80.36
Vertimec 1.8 EC	8.57bc	52.23	20.69c	47.57
Mechanical control	9.43b	47.40	30.33b	23.14
Untreated control	17.94a	-	39.46a	-
CV (%)	9.65	-	8.42	-

Means within a column with a common letter(s) do not differ significantly (P=0.05) according to DMRT at 5% level.

Rate of fruit infestation (n/n and w/w)

In entire cropping season, significantly the lowest rate of fruit infestation (n/n) was found in Tracer 45 SC (8.16%) treated plot. The highest fruit infestation was

obtained from untreated control plot (48.59%) followed by mechanical control plot (41.35%) with no significant difference between them. Percent infested fruit was recorded from *B. bassiana*, 1×10^9 CFU, Vertimec 1.8 EC and Bioneem 0.3 EC were 23.23%, 26.40% and 27.62%, respectively with no statistical difference among them. The percent reduction of fruit infestation (n/n) over control was 52.19%, 43.16%, 83.21%, 45.67% and 14.90% utilizing *B. bassiana*, 1×10^9 CFU, Bioneem 0.3 EC, Tracer (Spinosad) 45 SC, Vertimec 1.8 EC and mechanical control, respectively (Table 2).

In entire cropping season, the lowest rate of fruit infestation (w/w) was found in Tracer 45 SC (10.00%) treated plot. The highest fruit infestation was found in untreated control plot (32.09%) which was followed by mechanical control plot (26.47%), Bioneem 0.3 EC (24.08%), Vertimec 1.8 EC (23.57%) and *B. bassiana*, 1×10^9 CFU (18.27%) treated plot, respectively. No statistical difference was noted in fruit infestation (w/w) by the treatments of mechanical control, Vertimec 1.8 EC and Bioneem 0.3 EC. The percent reduction of fruit infestation (w/w) over control was 43.07%, 24.96%, 68.84%, 26.55% and 17.51% obtained in plots treated with *B. bassiana*, 1×10^9 CFU, Bioneem 0.3 EC, Tracer 45 SC, Vertimec 1.8 EC and mechanical control, respectively (Table 2).

The results of the present study can be compared to those obtained by the finding by Aparna and Dethé (2006) who studied the bioefficacy of Spinosad during Kharif 2005 and 2006. The lowest fruit infestation percentage was found of 13.34%, 13.69%, 7.89% and 8.21% on number and weight basis. The results of the mean fruit damage (%) by *L. orbonalis* in first (2005) and second (2006) trials indicated that Spinosad was found to be effective in suppressing the fruit damage among the treatments tested. The higher percent reduction of fruit damage over control was observed in Spinosad treated plants, which were 90.71% and 86.81% in 2005 and 2006, respectively.

Mamun *et al.* (2014) reported that the lowest fruit infestation was observed from the plot with Spinosad + pheromone trap. The combined management practices of setting pheromone trap with Spinosad spraying reduced the brinjal fruit infestation (5.95%) significantly than other treatments (14.09% for Spinosad and 19.18% for pheromone trap). Sparks *et al.* (1995) reported that Spinosad has relatively broad spectrum activity and has been effectively used for the control of many species of insect pests of Lepidoptera attacking various crops.

The present findings are comparable to the findings by Mittal and Ujagir (2005) who evaluated the newer molecule Spinosad (Tracer) 45% SC along with other insecticides. The results showed that among different treatments, the lower pod damage and lower number of *Helicoverpa armigera*, *Maruca vitrata* (Geyer) and *Melanagromyza obtusa* (Malloch) larvae were recorded in Spinosad 90 g ha^{-1} and Spinosad 73 g ha^{-1} treated plot.

Table 2. Effect of different treatments on the rate of brinjal fruit infestation by BSFB during October 2014 to June 2015

Treatments	Rate of fruit infestation (n/n)		Rate of fruit infestation (w/w)	
	% fruit infestation	% fruit infestation reduction over untreated control	% fruit infestation	% fruit infestation reduction over untreated control
<i>Beauveria bassiana</i> , 1×10 ⁹ CFU	23.23 c	52.19	18.27 c	43.07
Bioneem 0.3 EC	27.62 c	43.16	24.08 b	24.96
Tracer 45 SC	8.16 d	83.21	10.00 d	68.84
Vertimec 1.8 EC	26.40 c	45.67	23.57 b	26.55
Mechanical control	41.35 b	14.90	26.47 b	17.51
Untreated control	48.59 a	-	32.09 a	-
CV (%)	7.83	-	5.28	-

Means within a column with a common letter(s) do not differ significantly (P=0.05) according to DMRT at 5% level.

Yield of brinjal (t ha⁻¹)

All the treatments produced significant quantity of marketable yield and decreased the quantity of infested yield compared to untreated control plot. Significantly the highest marketable yield (34.39 t ha⁻¹) was recorded from Tracer 45 SC plot which was higher than that of other treatments. The second highest yield of healthy fruits was found in *B. bassiana*, 1×10⁹ CFU sprayed plot (22.78 t ha⁻¹) which was statistically similar to Bioneem 0.3 EC (19.06 t ha⁻¹) and Vertimec 1.8 EC (19.26 t ha⁻¹) treated plots. The lowest yield of marketable fruits (15.94 t ha⁻¹) was recorded from untreated control which was statistically similar to that of mechanical control (17.28 t ha⁻¹), Vertimec 1.8 EC and Bioneem 0.3 EC plots (Table 03).

Significantly the lowest weight of infested yield was recorded from Tracer 45 SC treated plot (3.82 t ha⁻¹) which was statistically similar to that of *B. bassiana*, 1×10⁹ CFU sprayed plot (5.09 t ha⁻¹). Weight of infested yield was found in Vertimec 1.8 EC, Bioneem 0.3 EC and mechanical control with clean cultivated plot were 5.94 t ha⁻¹, 6.05 t ha⁻¹ and 6.22 t ha⁻¹, respectively. The highest weight of infested fruits was harvested from the untreated control plot (7.53 t ha⁻¹) which was statistically different from all other treatments (Table 3).

Significantly the highest total yield was recorded from Tracer 45 SC treated plot (38.21 t ha⁻¹) followed by *B. bassiana*, 1×10⁹ CFU sprayed plot (27.87 t ha⁻¹) which was statistically similar to that of Bioneem 0.3 EC (25.11 t ha⁻¹) and Vertimec 1.8 EC (25.20 t ha⁻¹) treated plots. The lowest weight of healthy fruits yield was recorded from untreated control plot (23.47 t ha⁻¹) which was

statistically similar to that of mechanical control (23.51 t ha⁻¹), Vertimec 1.8 EC and Bioneem 0.3 EC treated plots (Table 3).

Adiroubane and Raghuraman (2008) reported that Spinosad (45 SC @ 225 g a.i. ha⁻¹) was effective along with Oxymatrine (1.2 EC @ 0.2%) applied against *L. orbonalis*. Spinosad was superior in producing marketable yield as it reduced the fruit damage and was at par with oxymatrine. Similarly, Awal (2012) reported that the highest healthy fruit yield in brinjal 20.70 t ha⁻¹ was obtained in the plots treated with IPM package comprising Tracer 45 SC 0.4 ml l⁻¹ + Pheromone trap + Mechanical control and field sanitation followed by IPM packages consisting Tracer 45 SC 0.4 ml l⁻¹ + Pheromone trap (18.56 t ha⁻¹), and in sole use of Tracer 45 SC @ 0.4 ml l⁻¹ (16.78 t ha⁻¹). Similarly, Chatterjee *et al.* (2009) also reported that Flubendiamide, Spinosad and Chlorfenapyr were highly effective in reducing the damage caused by *L. orbonalis* on brinjal and led to increases in yield.

Effectiveness of Spinosad (Tracer) 45 EC along with other standard insecticides was tested against pigeon pea pod borer by Vishal and Ram (2005). Lower pod damage was observed in Spinosad 90g, Spinosad 73g, Spinosad 56g and Spinosad 45g treated plots compared to untreated control plot over two years. Accordingly, greater grain yields were also obtained in Spinosad 90g (1741 kg ha⁻¹), Spinosad 73g (1463 kg ha⁻¹), Spinosad 45g (1218 kg ha⁻¹) and Spinosad 56g (1213 kg ha⁻¹) treated plots as compared to untreated control (768 kg ha⁻¹) plot.

Table 3. Effect of different treatments against BSFB on fruit yield (t ha⁻¹) during October 2014 to June 2015

Treatment	Yield (t ha ⁻¹)		
	Marketable	Infested	% marketable yield increase over untreated control
<i>Beauveria bassiana</i> , 1×10 ⁹ CFU	22.78b	5.09c	27.87b
Bioneem 0.3 EC	19.06bc	6.05b	25.11bc
Tracer 45 SC	34.39a	3.82c	38.21a
Vertimec 1.8 EC	19.26bc	5.94b	25.20bc
Mechanical control	17.28c	6.22b	23.51c
Untreated control	15.94c	7.53a	23.47c
CV (%)	9.67	6.78	-

Means within a column with a common letter(s) do not differ significantly (P=0.05) according to DMRT at 5% level.

Economic analysis

The management cost of different treatments used against brinjal shoot and fruit borer was calculated and presented in Table 4. The untreated control plot did not require any pest management cost. Although the marketable yield differed in

various control measures but the calculated benefit cost ratio (BCR) provided separate scenarios for variation in pest management cost.

The highest gross return of Tk. 756637.00 ha⁻¹ was found in Tracer 45 SC treated plot followed by Tk. 462799.00 in *B. bassiana*, 1×10⁹ CFU applied plot and Tk. 423704.00 in Vertimec 1.8 EC sprayed plot. On the other hand, the lowest gross return Tk. 350696.00 was calculated in untreated control plot followed by Tk. 380247.00 in the mechanical control plot and Tk. 419412.00 in Bioneem 0.3 EC treated plot (Table 4).

The highest net return of Tk. 656443.00 ha⁻¹ was found in Tracer 45 SC treated plot followed by Tk. 501111.00 in *B. bassiana*, 1×10⁹ CFU applied plot and Tk. 404940.00 in Vertimec 1.8 EC sprayed plot. On the other hand, the lowest net return of Tk. 350696.00 was calculated in untreated control followed by Tk. 401188.00 in Bioneem 0.3 EC and Tk. 367927.00 in the mechanical control plot (Table 4).

The highest adjusted net return of Tk. 305747.00 ha⁻¹ was found in Tracer 45 SC treated plot followed by Tk. 112103.00 in *B. bassiana*, 1×10⁹ CFU applied plot and Tk. 54243.00 in Vertimec 1.8 EC sprayed plot. On the other hand, the lowest adjusted net return of Tk. 17231.00 was calculated in the mechanical control plot followed by Tk. 50492.00 in Bioneem 0.3 EC treated plot (Table 4).

The highest benefit cost ratio of 3.05 was obtained from the treatment with Tracer 45 SC and the second highest benefit cost ratio of 2.93 was recorded from the plot treated with *B. bassiana*, 1×10⁹ CFU followed by Vertimec 1.8 EC sprayed plot (2.89). The lowest benefit cost ratio of 2.44 was found with the mechanical control and clean cultivated plot. The benefit cost ratio in the Bioneem 0.3 EC applied plot was 2.77 (Table 4).

Table 4. Benefit cost ratio analysis of selected treatments against BSFB during October 2014 to June 2015

Treatments	Management cost (Tk)	Gross return (Tk)	Net return (Tk)	Adjusted net return (Tk)	BCR
<i>Beauveria bassiana</i> , 1×10 ⁹ CFU	38312.00	501111.00	462799.00	112103.00	2.93
Bioneem 0.3 EC	18224.00	419412.00	401188.00	50492.00	2.77
Tracer 45 SC	100194.00	756637.00	656443.00	305747.00	3.05
Vertimec 1.8 EC	18764.00	423704.00	404940.00	54243.00	2.89
Mechanical control	12320.00	380247.00	367927.00	17231.00	1.40
Untreated control	0.00	350696.00	350696.00	-	-

Market value of brinjal = 25Tk./kg, cost of *Beauveria bassiana*, 1×10⁹ CFU per spay = 5628 Tk ha⁻¹, Bioneem 0.3 EC per spay = 2577 Tk ha⁻¹, Tracer 45 SC per spay = 16357 Tk ha⁻¹, Vertimec 1.8 EC per spay = 2667 Tk ha⁻¹, mechanical control = 2050 Tk ha⁻¹, Labor cost = 220 Tk. day⁻¹, sprayer rent = 20-25 Tk. day⁻¹.

The findings of the present study indicated that the Tracer 45 SC was most effective to manage infestation of BSFB in brinjal. The results of this study revealed that Tracer 45 SC, *B. bassiana*, 1×10^9 CFU and Vertimec 1.8 EC were also satisfactorily effective in suppressing BSFB infestation and found as cost effective. Benefit cost ratio studied by other researchers are also in agreement with this findings. Awal (2012) reported that in suppressing of BSFB, the BCR was the highest (7.15) in an IPM package consisting of Tracer 45 SC 0.4 ml l^{-1} + Pheromone trap + Mechanical control and field sanitation followed by BCR 6.72 and 5.47 in the IPM packages consisting of Tracer 45 SC 0.4 ml/liter + Pheromone trap and in sole use of Tracer 45 SC 0.4 ml l^{-1} , respectively which is comparable to the present findings.

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ECONOMICS OF MAIZE GRAIN STORAGE AT HOUSEHOLD LEVEL IN CHUADANGA DISTRICT OF BANGLADESH

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Abstract

There is a common perception among farmers and concerned professionals that maize grain cannot be stored like paddy or other cereals in conventional rural storage devices. As such, farmers generally sell their produce at harvest for any price offered to avoid quality deterioration and losing total return after harvest, which leads to lower profitability. To counter this situation, in the recent years, some progressive farmers at Chuadanga district (one of the concentrated maize growing area of Bangladesh) have been storing their maize grain within their household by using traditional bamboo granaries (*Golas* in the Bengali language) with the aim to capture higher future prices, while these *Golas* are traditionally used for storing paddy. Through the Cereal Systems Initiative for South Asia in Bangladesh (CSISA-BD) project, the International Maize and Wheat Improvement Center (CIMMYT) also helps further more to disseminate this technologies in the Chuadanga area. Given the uptake of this profit-increasing strategy by farmers, it is essential to document and evaluate the knowledge learned in order to inform scale-out efforts designed to reach farmers in other areas. A survey was conducted to assess maize grain storage systems in use at the household level in Chuadanga District farmers, and to estimate the overall profitability of storing maize grain. The survey results showed that approximately 80% of the total maize grain produced in 2014 (5.93 t/ha, on average) by sampled farmers was stored; most households employed *Gola* (62%) and/or jute sacks (55%), among other options. *Golas* were their first preference, followed by storage systems that pair polythene bags with (respectively) jute sacks and plastic bags, and then several other options. On average, farmers stored their maize grain 25.6 weeks (2014), 21 weeks (2013), and 20.9 weeks (2012) while realizing a price benefit of BDT 2.16/kg, BDT 4.72/kg, and BDT 5.35/kg in the respective years. Despite the fact that average storage costs and average quantities stored both varied by less than 7% between 2012 and 2014, the profit per unit (100 kg) and profit per household was significantly less in 2014 (BDT 10,161) than in 2013 (BDT 24,445) and 2012 (BDT 30,255), due to the much lower price differential obtained from maize grain storage in 2014. Examination of the total unit cost of storage reveals that the *Golas* were the most profitable storage system employed by the farmers and its profits helps farmers to improve their livelihood. Proper dissemination of

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these practices (storing and *Gola*) could be helpful for the farmers at the intensive maize growing area. Limitation of drying space and higher labour cost were the two major problems faced by the farmers, introducing quick-drying devices (flatbed dryers) can solve both the problems. It can be established either by local service providers, or farmers organizing themselves for collective ownership of such equipment.

Keywords: Bangladesh, cost analysis, grain storage, *Gola*, granary, maize grain, profitability.

Introduction

Maize (*Zea mays*) is a cereal crop that can be grown in a fairly broad range of agro-climatic conditions. It is relatively a new crop within the rice-based cropping patterns of Bangladesh; until 2001-02, there were only trivial areas under cultivation at the national level (BBS, 2014). Due to high demand resulting largely from the poultry industry (Karim *et al.*, 2010, Miah *et al.*, 2014), which has burgeoned to meet the change in Bangladeshi diets towards greater protein content, maize is now an important cash crop for the farmers who grow it. During 2013-14, area under maize was 307287 hectares and production was 2.124 million mt (BBS, 2016), which was ranked third after rice and wheat in area and was second only to rice in terms of production. This cereal crop is a direct food substitute for both rice and wheat and, farm households in Bangladesh begin to consume more of it, maize would benefit the undernourished and malnourished people of rural Bangladesh due to a higher nutritional content (Karim *et al.* 2010)^a. With a very high yield potential, maize must be considered an important crop for ensuring food security in a not-too-distant future that will hold 200+ million people.

Maize is acceptable to its growers mainly due to a lower cost of production and higher yields in comparison to that of rice (Rahman, 2011, Karim *et al.*, 2010, Moniruzzaman *et al.*, 2009, Uddin, 2008, Mohiuddin *et al.*, 2007). Nevertheless, the challenge of increasing the rate of adoption and sustainability of maize depends on social acceptance and economic profitability (Miah *et al.*, 2013). In addition, there is a common perception among farmers and concerned professionals that maize grain cannot be stored like paddy or other cereals in conventional rural storage devices (maize grain has a large embryo and more fat content, inviting insects more quickly). Since maize is generally not consumed by Bangladeshi farmers', they never made any serious effort to store it. Thus, farmers generally sell their produce at harvest for any price offered to avoid quality deterioration and losing total return after harvest, which leads to lower

^a Current human consumption of maize is very limited outside of the Chittagong Hill Tracts area, but there are places where maize is consumed in the form of flour added to wheat flour, as popcorn (especially in Dhaka), as roasted kernels (sold as a crunchy snack by nut vendors), and boiled or roasted on the cob.

profitability. In turn, they then lose out when the off-season price is much higher later in the year. To counter this situation, some progressive farmers have been storing their maize grain within their household by using traditional bamboo granaries (*Golas* in the Bengali language) with the aim to capture anticipated higher future prices (Momin *et al.*, 2014).

Under the Cereal Systems Initiative for South Asia in Bangladesh (CSISA-BD) project, the International Maize and Wheat Improvement Center (CIMMYT), as part of its agricultural development interventions, conducted a maize storage trial in Chuadanga District during the period July–October 2012. The objective was to compare three maize grain storing systems against locally-made bamboo granaries in order to evaluate and select a suitable method of storing maize grain by Bangladeshi farmers that could be promoted by the project. The *Golas* featured in the trial included project-promoted improvements in the form of tin sheet floors (and other upgrades) in order to deter rodents.



Figure 1. Locally-made bamboo *Gola* (left); inside view of a *Gola* with a tin floor improvement (right).

Overall the *Golas* performed well in terms of grain quality, color, and rate of pest/disease infestation when compared to other devices like poly bags (Momin *et al.*, 2014). Subsequently, the results were shared widely with all farmers/groups working with CSISA-CIMMYT in Jessore (especially Chuadanga district) during different project events such as trainings, farmers' field days, cross visits, etc. Given the uptake of this profit-increasing strategy by farmers in the Chuadanga area, it is essential to document and evaluate the knowledge learned in order to inform scale-out efforts designed to reach farmers in other areas. As such, this study has been taken with the following specific objectives.

- a. to identify and catalog the common maize storage systems in use at the household level,
- b. to performing a cost analysis and estimating the profitability of maize grain stored for each type of storage device and utilization of increased income from maize grain storage for house hold welfare,
- c. to document the problems that farmers still encounter with respect to maize grain storage at the household level, and

- d. to suggest some recommendation to overcome the problems and scale out the locally made maize grain storage device for the welfare of maize growers in other maize growing areas.

Materials and Method

Sampling procedure and sample size

Table 1. Survey details by storage system

Storage system	Targeted	Sampled	% of targeted
<i>Gola</i> (granary)	88	63	72
Storage in jute sacks	51	36	71
Storage in poly & plastic bags	6	4	67
Storage in a pucca room	8	6	75
Total	153	109	71

Data for the present study were gathered from both primary and secondary sources. A sample frame was developed that consisted of 153 maize grain-storing households from 13 villages within 7 unions (a political administrative unit under upazila) under the Chuadaga Sadar and Alamdanga upazilas of Chuadanga District. Due to the limited numbers of farmers engaging in this activity, these villages were purposively selected considering the spatial concentration of households storing maize grain and/or their affiliation with project activities. As presented in Table 1, farmers were categorised into different groups by storage device; a total of 109 households were randomly selected from these groups. Focus Group Discussions (FGD) was also conducted to verify the collected quantitative data.

Method of data collection and period of study

Primary data were collected from selected households in Chuadanga District of western Bangladesh (approximately 160 km west of Dhaka) during September 2014; a revisit was done in January 2015 to collect missing data, especially price data from farmers that had not yet sold any grain. Data collection was conducted by the principal investigator (lead author) and a collaborator from the Bangladesh Agricultural Research Institute (BARI). The household-level survey was based on a 3-page questionnaire comprising 40 questions; each interview lasted an average of 70-80 minutes. Two FGDs were conducted using a list of 20 structured questions; on average, each FGD lasted approximately 90 minutes and included 8-9 participants. Secondary data were also collected from different published sources, such as the Bangladesh Bureau of Statistics (BBS), research reports, and project reports in order to compare against the primary data and to enable a more comprehensive understanding of the overall situation.

Analytical techniques

The collected quantitative data were compiled; corrected, analyzed and descriptive statistical data are presented in tabular form and via graphs. A simple statistical regression model was also employed to analyse the data, as well. Finally, the cost analysis with regard to maize grain storage considering cost of the storage device, cost of grain drying and winnowing, the cost of pest protection measures used, etc. These costs were calculated for each individual household and then averaged on a categorical (device) basis. The returns from storing maize were calculated by considering the price benefit (differences in price between beginning time and grain sales time) and quantity stored. The profitability of maize grain storage was also estimated by calculating the gross margin (i.e. returns over variable costs).

Results and Discussion

Storage Data and System Preferences

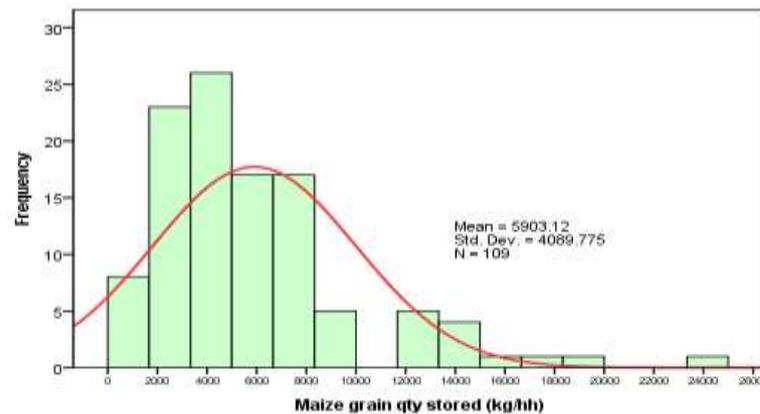
The data on maize cultivation in 2013-14 by the sampled farmers (by farm size^b) were collected and presented in Table 2. The mean cultivated area under maize was 0.958 hectare (ha) per household, with a mean yield of 10.1 tons per hectare (t/ha). This yield is considerably higher than the national average of 6.61 t/ha for 2012-13 (BBS, 2014), which is unsurprising because Chuadanga farmers consistently outperform the rest of the country in terms of maize yield. Across the farm size categories of the study, however, there is an overall difference of only slightly less than 1 t/ha. Excluding the *Large* category, the difference narrows to only 0.18 t/ha, suggesting that observed differences in production are mainly due to difference in area cultivated. Note that the area under maize cultivation is greater for *Marginal & Landless* farmers (0.69 ha) as compared to *Small* farmers (0.56 ha); this leads to a higher mean production for the *Marginal & Landless* farmers even though the *Small* farmers have a slightly higher mean yield. Overall mean production was 7.42 tons per household, which is heavily influenced by the *Large* farmers in terms of area cultivated and (to a lesser degree) higher yield.

Following the harvest for the 2013-14 growing season, on average 80% of the total maize grain produced was stored by the sampled farmers –this shows that these farmers are actively pursuing higher prices through storage. Interestingly, in terms of percentage stored, the *Marginal & Landless* farmers stored the most grain (89%), followed by the *Large* (87%), and *Medium* farmers (80%). On average, 5.9 tons per household (t/hh) were stored. As shown in Figure 2, the majority (85 households) stored between 2-8 t/hh of grain, with only 8 households storing less than 2 tons, and 4 households storing more than 16 tons.

^b Farm size is determined from farm holdings (i.e. total land owned + area leased/shared in – area leased/shared out to others). The categories specified (e.g. Small = 0.21-1.0 ha) are based upon those used by the Bangladesh Bureau of Statistics (BBS, 2013).

Table 2. Area, production, and storage statistics per household, by farm size category (2014)

Particulars	Marginal (< 0.21 ha)	Small (0.21-1.0 ha)	Medium (1.01-3.0 ha)	Large (> 3.0 ha)	Average
Respondent (number)	17	49	39	4	--
Maize area cultivated (ha)	0.686	0.556	0.920	1.255	0.958
Yield (t/ha)	9.91	10.10	9.92	10.88	10.07
Production (t/hh)	6.80	5.61	9.13	13.57	7.42
Storage (t/hh)	6.06	4.23	7.27	11.82	5.93
Stored (% of total production)	89.2	75.3	79.6	86.5	80.0

**Fig. 2. Frequency distribution of maize grain quantity stored.**

The study identified eight different systems used by sampled farmers to store maize grain.^c Table 3 displays the name of each storage system, as well as results of a simple preference ranking across the different options. The scale ranges from 1 to 7 (1 = least preferred; 7 = most preferred); respondents were asked to take into consideration the availability, value, duration, cost, etc. of each system. On average, it was found that *Golas* were the most preferred storage option, followed by the poly bag/jute sack system, the poly bag/plastic bags system, and jute sacks. Although metal/plastic drums are air-tight and a very good option for storing grain, they were ranked last due to their high cost. Note that, while each of the surveyed farmers used at most two devices at a time, they all stated that they had knowledge about each of the storage options under consideration.

^c A storage system may include an individual device (e.g. a *Gola*) or a combination of devices (e.g. plastic bag inside a jute sack).

Table 3. Average choice score and preference ranking of storage systems known/used

Ranking score	Storage system						
	Jute sack	Plastic sack	Poly bag only	Poly+ Plastic bag	Poly+ Jute sack	<i>Gola</i> (granary)	Metal/ Plastic drum
Avg. choice score ¹	4.01	2.58	3.10	4.62	4.86	6.68	2.12
Preference ranking	4	6	5	3	2	1	7

¹The preference score ranges from 1 (least preferred) to 7 (most preferred).

Table 4. Quantity stored, duration, and grain prices by principal storage systems utilized

Year	System	HHs (%)	Qty. stored		Storage duration (weeks)	Maize grain price (BDT/100kg)		
			t / hh	% stored		At storage time	After storage	Price diff.
2014	<i>Gola</i>	62	4.81	47	25.6	1,517	1,733	216.1
	Jute sack	55	7.34	41	26.3	1,511	1,735	224.3
	Plastic & poly bags	7	6.07	4	25.5	1,500	1,694	193.8
	Pucca room	6	9.10	8	22.3	1,513	1,688	175.0
	Average		5.93		25.6	1,514	1,730	215.7
2013	<i>Gola</i>	62	5.26	55	21.4	1,571	2,045	474.2
	Jute sack	45	6.63	33	21.2	1,586	2,063	477.5
	Plastic & poly bags	9	5.75	5	19.2	1,575	2,030	455.0
	Pucca room	6	6.83	7	19.2	1,585	2,013	427.1
	Average		5.77		21.0	1,576	2,048	471.5
2012	<i>Gola</i>	67	5.54	59	21.4	1,414	1,958	544.1
	Jute sack	50	7.33	34	20.5	1,403	1,923	519.8
	Plastic & poly bags	6	7.07	4	20.0	1,400	1,917	516.7
	Pucca room	2	9.20	4	16.0	1,400	1,913	512.5
	Average		6.19		20.9	1,410	1,947	535.4

As shown in Table 4, among the principal devices used by the sampled farmers to store their maize grain, 62% of farmers used a *Gola* to store 47% of the total stored grain in 2014. In 2013, *Golas* contained approximately 55% of total stored grain, and 59% in 2012. Jute sacks were the second most popular device, used by 55% farmers in 2014, 45% in 2013, and 50% in 2012.

Price Differentials and Maize Storage Costs

The duration of maize grain storage depends, in part, on price trends that vary from year to year. Table 4 shows that the holding period was greater in 2014 (25.6 weeks, ranging from 17 to 43 weeks) than in 2012 (20.9 weeks, ranging from 6 to 34 weeks). Nevertheless, the average storage price differentials (grain price at the time of storage less the sale price after storage) for each device were highest in 2012 and lowest in 2014. This indicates that year-to-year price variability—both at harvest time and subsequent sale prices—is more influential in determining the price spread than either the length of the storage period, or the type of storage device employed. For example, the length of storage time in 2014 was (on average) a month longer than in the two previous years, yet the average price differential was 54% and 60% less than in 2013 and 2012, respectively. Figure 3 also illustrates this with regressions contrasting 2014 with 2012. While the quadratic regression curve of 2014 data clearly shows that the positive rate of return of storage is increasing with time, the group of farmers who stored the longest (around 40 weeks) still received less of a spread than what the 2012 farmers obtained at 21 weeks.

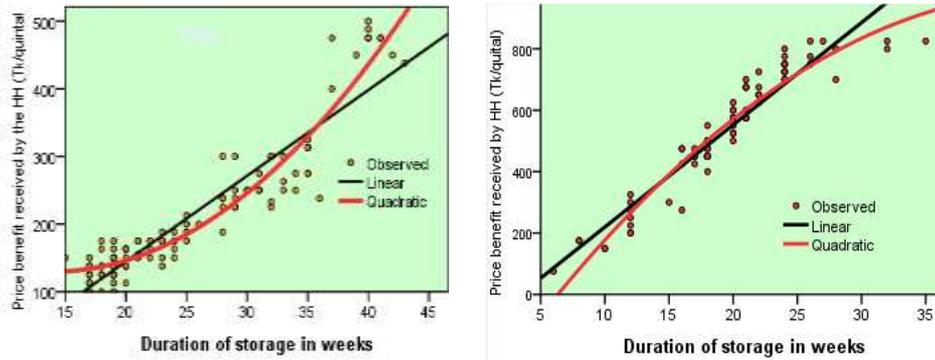


Figure 3. Regression of storage period against price benefit in 2014 (left) & in 2012 (right).

The cost of the storage device was estimated considering the capacity of the container/device, unit price, longevity, cost of repairs, and the salvage value (if any). Note that only the rental cost of the room was considered for farmers storing maize in a pucca room. The average capacity was highest for pucca rooms (10.4 tons), followed by *Golas* (4.63 tons); the average device cost was greatest for jute sacks, at BDT 16.7 per 100 kg, and least for *Golas* at BDT 12.9/100 kg (Table 5).

In addition to device cost, other costs involved with storage include the cost of drying and winnowing, the cost of pest protection, and grain loss costs. Before storing the grain, however, first it must be dried. Households reported a scarcity of open space where they can easily dry their maize grain. Maize grain was dried to approximately 12% moisture content after 3-4 days of exposure to sun on the

roof of a building, on a pucca chatal/road, or on the ground either directly, or on top of a Trepal/cloth or a synthetic net. The majority of the farmers used the roof of their house (35%) for maize grain drying, followed by a pucca road/chatal (31%), and a Trepal/cloth (19%) on the ground. Approximately 12% of farmers dried maize directly on the ground, while 3% used synthetic nets. Labour is the main component of the cost of drying and winnowing, most of which is shared by family members—especially women. Drying directly on the ground (BDT 29.38/100 kg) and using a net (BDT 30.29/100 kg) were the two most expensive options, because they required more time to dry the grain and therefore involved more labour, which ultimately increased the cost. The least expensive options were drying on the roof of a building (BDT 24.14/100 kg), followed by drying on a pucca road/chatal (BDT 24.33/100kg).

Table 5. Component costs and other data used to estimate total unit cost of storage (2014)

Storage system	Capacity (kg/unit)	Unit price of device (BDT)	Device longevity (years)	Repairing cost, if any (BDT/yr)	Salvage value (BDT)	Total unit cost (BDT/100 kg)
<i>Gola</i>	4,629	10,587	76.1	429.4	1,051	12.93
Jute sack	60	20.50	2.06	0	0	16.70
Plastic & poly bags	58	18.75	2.25	0	0	14.88
Pucca room	10,400	n/a	n/a	1,267*	n/a	13.68

* The cost was for room rent

Table 6. Cost of storing maize grain at the household level (2014), in BDT / 100 kg

Storage system	Device cost	Drying and winnowing cost	Cost for protection measures	Cost of grain loss	Total storage cost of maize grain
<i>Gola</i>	12.93	25.63	4.95	0	43.51
Jute sack	16.70	24.60	4.88	1.23	47.41
Plastic & poly bags	14.88	25.00	4.33	0	44.21
Pucca room	13.68	26.79	3.13	0	43.60
Average	14.28	25.35	4.80	0.02	44.45

Approximately 83% of the sampled farmers took preventative action against pests, of which 72% used Phostoxin (a fumigant that produces hydrogen phosphide gas), while 6% sprayed an insecticide, and 5% used both Phostoxine and an insecticide sprayed outside the granary and sacks (data not shown). Eighty-six percent of farmers using jute sacks applied one of the protection measures described above, followed by 83% of farmers using *Golas*, and 80% of

farmers using plastic/poly bags. Farmers, those storing their maize in a pucca room, two-thirds of them took preventative action against pests. Across all protection methods, an average of BDT 4.80/100 kg was required for grain protection against pests. Overall, an extremely low amount of maize grain was damaged by insects/pests, affecting only the farmers using jute sacks or pucca rooms. In 2014, storage losses were only 0.10%, which is equivalent to 5.9 kg per household. Storage losses were 0.48% in both 2013 and in 2012, which equals 27.7 kg and 29.7 kg grain lost per household, respectively. In monetary terms, grain losses per household were BDT 90, BDT 439 and BDT 417 in 2014, 2013 and 2012, respectively. Table 6 summarizes the various costs involved in storing maize grain during 2014 for each storage device. The jute sack system was the costliest (BDT 47.41/100 kg), while *Golas* (BDT 43.51/100 kg) were the most economical system, although storage in a pucca room was nearly identical in cost (BDT 43.60/100 kg).

Profitability of Maize Storage

The amount of profit earned through is presented in Table 7 for each system. Profits were estimated by deducting the total costs of storage from the price differential obtained (from Table 4) through household storage. The average profit obtained, across all storage types, was BDT 171.3/100 kg. The jute sack system was the most profitable system, and was 35% higher than the pucca room system (the least profitable). Storage in *Golas* ranked second in terms of profitability, only slightly behind (2% less) the jute sacks.

Table 7. Profit earned from storage of maize grain by system, in BDT/100 kg (2014)

Storage system	Price benefit	Total cost for storing maize grain	Profit earned
<i>Gola</i>	216.1	43.51	172.6
Jute sack	224.3	47.41	176.9
Plastic & poly bags	193.8	44.21	149.5
Pucca room	175.0	43.60	131.4
Average	215.7	44.45	171.3

Table 8 shows the profit earned by household for each of the three years examined. The highest average profit per household was BDT 30,255 in 2012, followed by BDT 24,445 in 2013, and BDT 10,161 in 2014. As mentioned previously, year-to-year price fluctuations largely determine the magnitude of the price differential obtained from storage in any given year; as such, this will of course have the greatest influence on profitability. This is clearly illustrated in the table: average storage costs did not vary much by year (less than 7% between 2013 and 2014), nor did the average quantities stored at the household level (less than 7% between 2012 and 2013), yet the profit per unit (100 kg) and profit per

household is significantly less in 2014 than the two previous years because of the much lower price differential.

Table 8. Household-level profit earned from storing maize grain (2012-2014)

Storage year	Price benefit (BDT/100 kg)	Storage cost (BDT/100 kg)	Storage profit (BDT/100 kg)	Qty. stored (kg/hh)	Net profit (BDT/hh)
2014	215.7	44.45*	171.3	5,933	10,161
2013	471.5	47.69	423.8	5,768	24,445
2012	535.4	46.95	488.5	6,194	30,255

* Including storage losses.

Profits earned by storing maize grain were used to purchase/mortgage in additional land, invest in business and/or deposit savings for future needs purchased essential household needs such as food, clothes, health and educational costs, and it also enables the purchase of agricultural inputs and equipment, as well as items such as motorcycles, electronic equipment/appliances (e.g. radios, televisions, fans) etc. Clearly, profits from maize grain storage have improved the welfare of these farmers and their families.

Constraints in storing maize grain

Although the respondent farmers were very happy to generate income from storing maize grain, and they will continue this activity in the future, they still faced some problems—mainly in terms of space limitations for grain drying and winnowing. There is a scarcity of open space where they can easily conduct this work. Whereas larger farmers use the concrete roof of their house as a drying area, many farmers are constrained in terms of efficient drying because they do not have such type of house. Labour and time required for drying and winnowing was the second-most indicated problem with storage, followed by insect infestation, and then liquidity constraints. The latter problem manifests as an urgent need for cash at the time of harvest (or sometime thereafter) that precludes farmers from storing maize grain (or reduces the storage time). Finally, about 10% of the cost of storing maize grain is due to pest prevention measures, farmers reported insect infestation as the third ranked problem they faced.

Conclusions and Recommendations

a. Conclusions: Considering the total cost of storage, *Golas* are the most profitable storage system, followed closely by pucca rooms. Plastic/poly bags remain in the third-ranked position, although they are quite competitive with *Golas* and pucca rooms. The advantage of *Golas* resides in the fact that many farmers already own these traditional devices within which they store rice—thus it is simply a matter of awareness (perhaps a few small modifications, as well) that they can also be used to successfully stored maize. Storing maize grain in HH level shows very good income generating potentials; farmers can earn a good

amount of profit without investing much capital. They felt wealthy with their income flow and improving their livelihood by using the profit.

The scarcity of open space for efficient drying of maize grain, more labour and time required for drying and winnowing were the main problems followed by insect infestation. Liquidity constraints were also problems to smaller farmers to continue storing longer period.

b. Recommendations: The systems of storing was found best in *Gola* coupled with its better return having no cost of plastic and poly-bags and being of very long life period. So, proper dissemination of these practices (storing and *Gola*) could be helpful for the farmers at the intensive maize growing area. An action plan to demonstrate and disseminate these low cost technologies by taking much motivational and orientation program is very essential; Government (through BARI, DAE) can take this responsibility by involving location specific NGOs also.

By introducing quick-drying devices can solve both two major problems of drying space and labour cost. Establishment of drying facilities (e.g. low-tech flatbed dryers, or perhaps more advanced and expensive blow dryers) can either by local service providers, or farmers organizing themselves for collective ownership of such equipment. Involvement of lower levels of financing (i.e. micro-credit with reasonable terms/conditions) will help farmers address the liquidity constraint they reported, which would allow them to store grain in pursuit of higher prices instead of forcing them to sell at the time of harvest (or sell early if they manage to store).

More research is required to define the specific insect-pest problems and the best alternatives for addressing them, while also taking into consideration the various chemicals used as treatment agents. Ideally, any such study examining storage pests and chemical responses would simultaneously examine the issue of aflatoxin, with respect to its prevalence in stored maize grain, as this poisonous mold is known to propagate in improperly stored staple commodities and has been detected at high levels in food items and poultry feed Bangladesh (Roy *et al.*, 2013).

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**EFFECT OF SOME NEW GENERATION INSECTICIDES ON THE
POPULATION ABUNDANCE OF APHID AND DIFFERENT
BENEFICIAL INSECTS IN MUSTARD CROP**

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Abstract

The present study evaluated the efficacy of some new generation insecticides namely Clothianidin 48 SC, Fenpyroximate 5 SC, Pyridaben 20 WP and Methoxyphenozide 24 SC along with a synthetic pyrethroid insecticide, Fenpropathrin 20 EC against mustard aphid and their effects on beneficial insects i.e. syrphid flies and foraging honeybees during 2015 - 2016 in Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. Tested six treatments were: Clothianidin 48 SC 1ml l⁻¹, Fenpyroximate 5 SC 0.25 ml l⁻¹, Pyridaben 20 WP 1g l⁻¹, Methoxyphenozide 24 SC 1ml l⁻¹, Fenpropathrin 20 EC 1ml l⁻¹ and an untreated control, replicated three times in RCBD. It was found that, Methoxyphenozide 24 SC was found to be the most effective against aphid offering lower aphid population (1.42/ top 10 cm central twig) at 7 days after spraying (DAS) which was statistically identical to Clothianidin 48 SC (1.50/top 10 cm central twig). Among the insecticides, Methoxyphenozide 24 SC was also found to be safer to the populations of syrphid flies revealing flight activity of 6.85 adults (plot/5 min) and honeybees of 8.82 workers (plot/5 min), respectively at 7 DAS. Whereas, Clothianidin 48 SC and Pyridaben 25 WP treated plots were found to be highly toxic to these beneficial insects. Consequently, the highest yield was obtained from Methoxyphenozide 24 SC (1.55 t/ha) followed by Fenpyroximate 5 SC (1.42 t/ha) treated plots.

Keywords: New generation insecticides, aphid, beneficial insects, mustard crop.

Introduction

In Bangladesh, Rapeseed-mustard (*Brassica rapa*) is an important oil yielding crop. But its production is seriously hampered due to attack of various insect-pests. Among them mustard aphid, *Lipaphis erysimi* (Kalt.) is the most destructive pest in all the mustard growing regions of the country. Aphid population and rate of infestation are very much dependent on sowing time (Islam *et al.*, 1991). Aphid sucks the cell sap from the stems, twigs, buds, flowers and developing siliqua causing a significant loss in yield. On the other hand, aphid produces honey dew which facilitates the growth of the fungus that makes the plant parts black (Awasthi, 2002). *Lipaphis erysimi* may cause upto 96 %

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yield loss (Bakhetia and Sekhon 1989, Bakhetia and Arora 1986) and 15 per cent oil loss ((Verma and Singh1987) of mustard.

Currently, growers rely heavily on conventional and broad spectrum insecticide use to suppress attack of mustard aphid. The pesticidal sprays have become a threat to mustard ecosystem causing resurgence of pest and threat to natural enemy fauna. Indiscriminate use of insecticides has many side effects, such as development of insecticide resistance in insect pests, adverse effects on friendly organisms, environmental pollution and accumulation of toxic elements in food and ultimately pesticide residue-induced diseases in human beings (Ambethger, 2009). Against this backdrop, the use of new generation insecticides could be considered as possible alternative for controlling aphid.

Syrphid fly (*Allograpta obliqua*) larva are generally important predators of aphid and adults are considered important agents in the cross pollination of mustard. Honeybees are primary pollinators of mustard crop and hence they are important to increase productivity of mustard crop (Hayter and Cresswell, 2006). Coccinellid beetles (Lady bird beetle), *Coccinella septempunctata* are important entomophagous predators upon many species of aphids and observed as an efficient and mightiest predator of *L. erysimi* in field conditions (Singh and Singh 2013). However, there are scanty information about the adverse effects of the new generation insecticides on these predatory arthropods and pollinating agents.

Keeping the above points under consideration, the present studies were undertaken to evaluate the effect of certain new generation insecticides on the population abundance of aphid and the predator syrphid fly and the pollinator honeybee.

Materials and Method

Experimental design and treatments

The experiment was carried out in the research field of Bangladesh Agricultural Research Institute (BARI) during 2015-16. The experiment was laid out in a randomized complete block design (RCBD) with 3 replications having plot size of 4 × 3m and spacing between row to row and plant to plant as 30 cm and 10 cm, respectively. The mustard cultivar BARI- Sarisha 14 was sown on November 16, 2015. There were six treatments including control. Four new generation insecticides, Clothianidin 48 SC @ 1 ml/ litre water, Fenpyroximate 5 SC @ 0.25 ml/ litre water, Pyridaben 20 WP @ 1 g/ litre water and Methoxyphenozone 24SC @ 1 ml/ litre water along with a synthetic pyrethroid insecticide, Fenprothrin 20EC @ 1 ml/ litre water were applied to evaluate their effectiveness against mustard aphid and relative toxicity to predator syrphid fly and foraging honeybee.

In the present investigation coccinellid beetle population was very low throughout the cropping season. Hence, toxicity of the tested insecticides to coccinellid beetle could not be studied.

The test insecticides were sprayed twice with the help of an air compression high volume sprayer at spray volume rate of 500 litres/ha. The first spray of insecticides was given when the aphid population reached ETL (50 aphids / plant) and second after an interval of 10 days. During application care was taken to maintain the distance around 25 cm between the nozzle and plant parts. The whole plant was thoroughly covered by spray fluid. Adequate safety measures were taken during spraying. Necessary agronomic practices were done to raise a good crop.

Recording of data

The data on different parameters were recorded following Dutta *et al.* 2016. The aphid population was recorded on 10 randomly selected plants per plot. On each plant, 10 cm top central twigs were observed to record aphid. The data on surviving aphid population was recorded. Population of foraging honeybee and syrphid fly were counted separately from whole plot during peak foraging time for 5 minutes and was recorded as mean population per plot.

Pre-count observation of aphid, syrphid fly and honeybee were recorded one day before spraying and subsequent post count data at 3 and 7 days after spraying (DAS) were also recorded. Three species (*Apis mellifera*, *A. dorsata* and *A. cerena indica*) of honey bee were noticed but data were taken together as honeybees. The seed yield of mustard was taken

from the each treated and untreated control plot.

Statistical analysis

Data were analyzed by using MSTAT-C software for analysis of variance and treatment means were separated by applying Duncan's Multiple Range Test (DMRT) at 1 % level of significance.

Results and Discussion

Result

Population reduction of aphids as influenced by different insecticidal treatments: It is evident from Table 1 that all the insecticidal treatments were significantly superior to untreated control in reducing aphid population. At 3 days after spraying (DAS), methoxiphenozide showed lowest population with 3.20 aphids/ top 10 cm in central twig as against 25.68 aphids/ top 10 cm in central twig of untreated control plot. But Efficacy of methoxiphenozide was statistically similar to clothianidin. Similarly, percent reduction of aphid population over pretreated at 7 DAS was recorded highest from methoxiphenozide (94.83%) followed by clothianidin (94.48%), while this was lowest in untreated control (3.18%) were only water was sprayed.

Table 1. Effect of different on the population reduction of aphid in mustard crop.

Treatments	Mean Aphid population/ top 10 cm central twig of plant			Per cent reduction of aphid population over pretreated at 7 DAS
	1 DBS	3DAS	7 DAS	
Clothianidin 48SC	27.15	3.86d	1.50d	94.48
Fenpyroximate 145SC	27.42	6.50c	3.62c	86.79
Pyridaben25WP	27.22	6.56c	3.98c	85.37
Methoxyphenozide 24SC	27.45	3.20d	1.42d	94.83
Fenpropathrin 20EC	28.21	10.25b	6.21b	77.98
Untreated control (water spray only)	28.25	25.68a	27.35a	3.18
CV%	8.95	11.02	12.65	-

Means having same letter(s) in a column are not significantly different at $P > 0.01$ followed by DMRT. DBS= Day before spray; DAS= Day after Spray

Population of adult syrphid fly as influenced by different insecticidal treatments:

The results presented in Table 2 indicated that there were significant variations in syrphid fly population in different treatments. At 3DAS, among the insecticides, significantly the highest population of syrphid fly was observed in plots treated with Methoxyphenozide (7.35 flies/ plot / 5 min) which was followed by Fenpyroximate (4.95 flies/ plot / 5 min). The similar result was found at 7 DAS. Among the insecticides, the syrphid fly population reduction over pretreated at 7 DAS was found highest (83.31%) in clothianidin while this was the lowest in methoxyphenozide (44.08%) treated plots.

Population of foraging honey bee as influenced by different insecticidal treatments:

The results presented in Table 3 indicated that population of foraging bee was significantly different among the treatments after insecticidal applications at both 3 DAS and 7 DAS. At 3 DAS, among the tested insecticides Methoxyphenozide recorded the maximum bee population (8.32 bees/plot/5 min) which was followed by Fenpyroximate (6.43 bees/plot/5 min). The lowest bee population (3.43 bees/plot/5 min) was obtained from Clothianidin treated plots. A similar trend was also observed at 7 DAS. Consequently, Clothianidin recorded highest decrease of honey bee population over pretreatment at 7 DAS (86.44%) indicating its higher toxicity to bee pollinators followed by Pyridaben (83.54%). However, in control plots bee population remained almost same showing a little increase (2.43%) at 7 days after spray.

Table 2. Effect of different insecticides on the population of adult syrphid fly in mustard crop.

Treatments	Mean syrphid fly population/plot/5 min			Per cent decrease(-) / increase (+) of syrphid fly population over pre treatment at 7 DAS
	1 DBS	3DAS	7 DAS	
Clothianidin 48SC	12.05	2.25d	2.01d	(-)83.31
Fenpyroximate 145SC	12.84	4.95c	4.87c	(-)62.07
Pyridaben 25WP	11.82	2.01d	2.42d	(-)79.53
Methoxyphenozide 24SC	12.25	7.35b	6.85b	(-)44.08
Fenpropathrin 20EC	11.65	4.62c	4.04c	(-)65.32
Untreated control (water spray only)	12.12	12.25a	12.45a	(+)2.72
CV(%)	8.55	10.15	11.65	-

Means having same letter(s) in a column are not significantly different at $P > 0.01$ followed by DMRT. DBS= Day before spray; DAS= Day after Spray

Table 3. Effect of different insecticides on the population of foraging honeybee in mustard crop.

Treatments	Mean honey bee population/plot/5 min			Per cent decrease(-) / increase (+) of honey bee population over pre treatment at 7 DAS
	1 DBS	3DAS	7 DAS	
Clothianidin 48SC	16.60	3.43d	2.25d	(-)86.44
Fenpyroximate 145SC	15.32	6.43b	6.07bc	(-)60.74
Pyridaben25WP	16.10	3.13d	2.65d	(-)83.54
Methoxyphenozide 24SC	15.02	8.32c	8.82b	(-)41.28
Fenpropathrin 20EC (water spray only)	16.23	5.62bc	5.64b	(-)65.25
Untreated control	15.64	15.95a	16.02a	(+)2.43
CV(%)	6.85	11.24	10.26	-

Means having same letter(s) in a column are not significantly different at $P > 0.01$ followed by DMRT. DBS= Day before spray; DAS= Day after Spray

Seed yield of mustard as influenced by different insecticidal treatments:
Table 4 indicated that there were significant variations of seed yield of mustard

due to spraying of different insecticides. Significantly the highest yield was obtained from Methoxyphenozone (1.55 t/ha) and this was followed by Fenpyroximate (1.42 t/ha) treated plots. However, the lowest yield was obtained from untreated control plots (1.02 t/ha) followed by clothianidin treated plots (1.25 t/ha). Similarly, Methoxyphenozone treated plots offered the highest (51.56%) yield increase over control followed by Fenpyroximate (38.86%) treated plots. On the contrary, Clothianidin 48SC provided significantly the lowest (22.36%) yield increase over control followed by Pyridaben 25WP (24.51%).

Table 4. Effect of different insecticides on seed yield of mustard crop

Treatments	Seed yield (t/ha)	% Yield increase over control
Clothianidin 48SC	1.25b	22.36
Fenpyroximate 145SC	1.42ab	38.86
Pyridaben25WP	1.28b	24.51
Methoxyphenozone 24SC	1.55a	51.56
Fenprothrin 20EC	1.41ab	37.30
Untreated control (water spray only)	1.02c	-
CV (%)	7.52	-

Means having same letter(s) in a column are not significantly different at $P > 0.01$ followed by DMRT.

Discussion

Over the years many insecticide groups having diverse mode of action has been studied to control mustard aphid. Chandra *et al.* (2014) evaluated different insecticides for the management of aphid and found imidacloprid 17.8 SL to be most effective followed by acetamiprid 20 SP. However, Rajesh *et al.* (2013) obtained maximum protection of mustard crop and highest yield by applying thiamethoxam 25% WDG @100 g/ha. Youn *et al.* (2003) also observed that Thiamethoxam was most effective in controlling *L. erysimi* population.

Maula *et al.* (2010) found that Oxydemeton methyl 25EC was most effective among the three insecticides causing the highest mortality of mustard aphid followed by Dimethoate 40EC. But they observed lowest mortality of *Coccinella septempunctata* in Dimethoate 40EC treated plots. Singh and Lal (2011) also found Oxydemeton methyl 25 EC @ 0.05% as the most effective against mustard aphid resulting in significantly higher yield as compared to other tested insecticides. Rajagopal and Kareen (1984), and Tripathi *et al.* (1988) observed that Dimethoate was relatively safe to the predator.

Kumar and Kumar (2016) studied the efficacy of several biopesticides and chemical insecticides against mustard during Rabi season 2012-2015 in Allahabad, India and obtained significantly higher seed yield and net return with spraying of dimethoate 30 EC followed by malathion 50 EC and neem oil (0.5%), respectively.

Dutta *et.al.* (2016) evaluated some new generation insecticides and a botanical against mustard aphid and assessed the effect of insecticides on the abundance of coccinellid predators and foraging honeybees. They observed Buprofezin 40 SC and Diafenthiuron 500SC were most effective against aphids while Azadiractin 1EC appeared to be safest to coccinellid beetles and foraging honeybees. They also reported that the Indoxacarb 145SC was most toxic against honeybees.

The findings of the present study reveal that, Methoxyphenozide insecticide showed higher effectiveness in controlling mustard aphid and provided higher seed yield as compared to other tested products. In view of safety to syrphid fly and honeybees Methoxyphenozide proved to be relatively safest insecticide. On the other hand, clothianidin and pyridaben was highly toxic to both syrphid fly and foraging honeybees. The highest yield was also obtained from Methoxyphenozide treated plots. More effective and safer insecticides must be introduced for aphid management in mustard to provide alternatives to conventional insecticides. So, from the present study, it could be concluded that, Methoxyphenozide might be a viable component in mustard aphid IPM program. Widespread adoption of Methoxyphenozide among mustard IPM programs across the country will benefit producers by reducing total insecticide applications and subsequent costs for aphid control, as well as limiting further resistance development in pest populations. However, the efficacy of Methoxyphenozide across different locations with varied ecology in Bangladesh should be evaluated.

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CHARACTER ASSOCIATION AND PATH CO-EFFICIENT ANALYSIS IN WHEAT (*Triticum aestivum* L.)

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Abstract

The experiment was carried out with 50 wheat lines to study their mean, range, cv (%), correlation co-efficient, and path co-efficient considering 14 different morphological characters at the experimental field of Regional Wheat Research Centre (RWRC), Bangladesh Agricultural Research Institute (BARI), Gazipur during December 2010 to April 2011. Significant variation was observed among the genotypes for all characters studied. In general, genotypic correlations were higher than the phenotypic correlations. It indicates that there was an inherent association among them which was adversely influenced by the environment. The correlation coefficients showed that, seed yield was negatively and significantly correlated with days to heading (DTH), plant height (PHT), days to anthesis (DTA), days to physiological maturity (DPM), and canopy temperature at anthesis stage (CT_{anth.}) but only negatively correlated with canopy temperature at vegetative stage (CT_{veg.}), canopy temperature at grain filling stage (CT_{gf.}), spikelets per spike both genotypically and phenotypically and grain per spike showed genotypically negative correlation. Path analysis showed that plant height (PHT), days to physiological maturity (DPM), canopy temperature at grain filling stage (CT_{gf.}), and thousand grain weight (TGW) influenced seed yield directly in positive direction but days to heading (DTH), days to anthesis (DTA), grain filling duration (GFD), grain filling rate (GFR), Chlorophyll content at anthesis stage (CHL_A), canopy temperature at vegetative stage (CT_{veg.}), canopy temperature at anthesis stage (CT_{anth.}), spikelets per spike, and grains per spike had negative direct effect on seed yield. Considering analytical findings of correlation co-efficient, path co-efficient analysis and field performance, the genotypes G 3, G 10, G 11, G 12, G13, G 21, G 29, G 35, G 38, G 40, G 46 and G 48 were found suitable for future breeding programme.

Keywords: Character association, Path co-efficient analysis, Wheat, genotypes.

Introduction

Wheat in Bangladesh has been established as second most important cereal crop next to rice. To meet the increasing demand of wheat in Bangladesh and to decrease import, local production needs to be increased faster. Wheat Research

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Centre (WRC) of Bangladesh Agricultural Research Institute (BARI) now has a wide range of spring wheat germplasm collection from different sources. Most of these have been collected from International Maize and Wheat Improvement Centre (CIMMYT), Mexico and few from Nepal, India, Pakistan, Australia etc. Determination of correlation co-efficient between the characters has a considerable importance in selecting breeding materials. The path co-efficient analysis gives more specific information on the direct and indirect influence of each of the component characters upon seed yield (Behl *et al.*, 1992). Selection of grain yield becomes difficult unless the association among the yield contributing characters are known. So estimation of correlation helps to identify the relative contribution of component characters towards yield (Panse, 1957). As yield is the main objective to a breeder, it is important to know the relationship among various characters that have direct and indirect effect on yield. Yield components influence the ultimate yield of a crop both directly and indirectly (Turkey, 1954). Path analysis specifies the causes and effect relationship and measures the relative importance of each variable (Wright, 1959). Therefore, correlation in combination with path co-efficient analysis will be an important tool to find out the association between direct and indirect effects and quantify the direct and indirect influence of one character upon another (Dewey and Lu, 1959). The correlation co-efficient between yield and yield contributing characters usually show a complex chain of interacting relationship. Path co-efficient estimates the direct and indirect effects of different yield contributing characters towards the yield through correlation co-efficient analysis and visualize the relationship in more meaningful way. Therefore, the present study was undertaken to find out and establish suitable selection criteria for higher yield through study of relationship between yield and yield components in spring wheat.

Materials and Method

Fifty wheat genotypes were grown in a randomized complete block design with three replications at the experimental field of Bangladesh Agricultural Research Institute, Gazipur during first week of December 2010 to first week of April 2011. The experimental site was at 23.46° N latitude and 90.23° E longitude with an elevation of 8 meter from sea level. The experimental field was prepared thoroughly by ploughing with tractor followed by harrowing and removing the stubbles. The crop was fertilized with NPKS and B @ 100, 28, 40, 20 and 2.5 kg ha⁻¹ respectively to ensure proper growth and development. The elements N, P, K, S and B were applied in the form of Urea, Triple Super Phosphate,

Muriat of Potash, Gypsum and Boric acid respectively. Two-third of urea and the entire quantity of other fertilizers were applied at final land preparation along with Furadon 3G @ 8 kg ha⁻¹ (Anon. 2011). The rest one-third urea was top-dressed at crown root initiation stage (17-21 days after sowing) following first irrigation. Data were collected on days to heading (DTH), days to anthesis (DTA), days to maturity (DTM), grain filling duration (GFD)[(days], grain filling

rate (GFR) [$\text{g m}^{-2} \text{d}^{-1}$], plant height (PHT) in cm, chlorophyll content at anthesis (CHL_A) in SPAD unit, canopy temperature ($^{\circ}\text{C}$) at vegetative ($\text{CT}_{\text{veg.}}$), anthesis ($\text{CT}_{\text{anth.}}$) and grain filling ($\text{CT}_{\text{gf.}}$) stage, spikelets spike^{-1} (no.), grains spike^{-1} (no.), thousand grain weight (TGW) [g], and grain yield m^{-2} (g). Grain yield m^{-2} of each genotype was converted into grain yield per hectare (Kg/ha). The data were analyzed for different components. Phenotypic and genotypic variances were estimated by the formula used by Johnson et al. (1955). Phenotypic and genotypic co-efficient of variation were calculated by the formula of Burton (1952). Simple correlation co-efficient was obtained using the formula suggested by Clarke (1973); Singh and Chaudhary (1985). Genotypic and phenotypic correlation co-efficient were calculated following Miller et al. (1958). Path co-efficient analysis was done following the method outlined by Dewey and Lu (1959).

Results and discussion

The mean, range, and CV of seed yield and yield contributing characters of 50 genotypes of spring wheat are presented in Table 1. Variations were observed among the lines for all the characters studied. The average days to heading (DTH) across genotypes was 72.77 days and ranged from 61 to 79 days (Table 2). The minimum DTH was observed in genotype G 21 (61.67 days) and the maximum (79 days) in genotype G 27 (Table 2). Days to anthesis (DTA) ranged from 65 to 84 (Table 1). Shorter vegetative period was observed in the genotype G 21 (65.33) and the longer (84.33) in the genotype G 27 (Table 2). Days to physiological maturity (DPM) ranged from 98 to 111 (Table 1). The lowest days to physiological maturity was observed in the genotype G 21 (98.33) and the highest (111.33) in the genotype G 27 (Table 2). Grain filling duration (GFD) ranged from 23 to 34 days (Table 1). Shorter GFD was recorded in genotype G 29 (23.33 days) and the longer one (34.00 days) in genotype G 44 (Table 1). The average Grain filling rate (GFR) across genotypes was $14.43 \text{ g}^{-2}\text{d}^{-1} \text{ m}^{-2}$ and ranged from 10.07 to 18.98 (Table 1). Minimum GFR was observed in genotype G 3 ($10.07 \text{ g}^{-2}\text{d}^{-1} \text{ m}^{-2}$) and the maximum ($18.98 \text{ g}^{-2}\text{d}^{-1} \text{ m}^{-2}$) in genotype G 40 (Table 2). Plant height (PHT) ranged from 80 to 98 cm (Table 1). The minimum plant height was observed in the genotype G 3 (80.33cm) and the maximum (97.67cm) in the genotype G 4 and G16 (Table 2). The average Chlorophyll content at anthesis stage (CHL_A) across genotypes was 49.51 SPAD unit for all genotypes and ranged from 43.50 to 54.53 (Table 1). The highest chlorophyll content (54.50 SPAD unit) was recorded in genotype G 45 while the lowest chlorophyll content (43.50 SPAD unit) was recorded in G 2 (Table 2). The canopy temperature measured by infrared thermometer has been used to evaluate genotypes for their ability to keep their canopy cool with less impaired assimilation processes as suggested by Reynolds *et al.*, (1994). Canopy temperature at vegetative stage ($\text{CT}_{\text{veg.}}$) ranged from 19.90 to 22.50 $^{\circ}\text{C}$ (Table 1). Minimum average canopy temperature at vegetative stage was recorded in the genotype G 46 (19.90 $^{\circ}\text{C}$) and the maximum (22.50 $^{\circ}\text{C}$) was in the genotype G 7

(Table 1). Canopy temperature at anthesis stage ($CT_{anth.}$) ranged from 21.10 to 23.50 °C (Table 1). Minimum average canopy temperature was recorded in the genotype G 39 (21.10 °C) and the maximum (23.50 °C) was in the genotype G 15 (Table 2). Canopy temperature at grain filling stage ($CT_{gf.}$) ranged from 21.50 to 24.90 °C (Table 1). The minimum average canopy temperature was recorded in the genotype G 44 (21.50 °C) and the maximum (24.90 °C) was in the genotype G 15 (Table 2). Spikelets spike⁻¹ ranged from 13.90 to 18.40 (Table 1). The minimum spikelets spike⁻¹ produced by the genotype G 3 (13.90) and the maximum (18.40) by genotype G 38 (Table 2). The average Grain spike⁻¹ across genotypes was 50.74 and ranged from 40.70 to 57.90 (Table 1). Genotype G 49 produced minimum grains spike⁻¹ (40.70) while G 35 produced the maximum (57.90) grains (Table 2). Thousand grain weight (TGW) ranged from 30.50 to 45.90 g (Table 1). The lowest 1000-grain weight was recorded in the genotype G 4 (30.50g) and the highest (45.90g) was in genotype G 13 (Table 2). The average Grain yield was 3810.14 kg ha⁻¹ for all genotypes and ranged from 2552 to 4998 kg ha⁻¹ (Table 1). G 41 was the lowest yielder (2552.00 kg ha⁻¹) while G 40 was the highest yielder (4998.00 kg ha⁻¹) among the genotypes studied (Table 2). The highest co-efficient of variation (CV%) was recorded in the character grain filling rate (13.94) followed by grain yield (13.68), grain filling duration (9.32), and TGW (8.86) (Table 1). Considering the plant height, days to heading, days to maturity, grain filling duration, grains per spike, spikelets per spike, thousand grain weight, seed yield and other yield contributing characters, the genotypes G 3, G 13, G 21, G 29, G 35, G 38, G40, and G 46 were selected for future breeding programme.

Character association

Yield is a complex character and related with several yield contributing attributes. Selection for yield would not be effective unless other yield components influence it directly or indirectly. If selection pressure is given for improvement of any character highly associated with yield it will affect other correlated traits. Thus, interrelationship among different characters with yield will help breeders to improve the desired traits through selection.

Correlation co- efficient

Genotypic (r_g) and phenotypic (r_p) correlation coefficients between pairs of fourteen quantitative traits are presented in Table 3. In general, the genotypic correlation coefficient values were higher than the phenotypic values. This indicated that strong intrinsic associations were somewhat masked at phenotypic level due to environmental effects. Many investigators (Barma *et al.*, 1990; Barma *et al.*, 2002 and Rahman, 2009) had reported high estimates of genotypic correlation than phenotypic correlation. It revealed that inherent relationships between two traits were suppressed by the environmental influence at phenotypic level. The results are discussed character wise as follows:

Table 1. Mean, Range, and CV (%) of seed yield and yield contributing characters of fifty spring wheat genotypes

Component	M _{Sp}	M _G	M _E	Mean	Range	CV%	F-value
DTH	41.693	41.088	0.605	72.77	61-79	5.12	**
DTA	50.398	49.804	0.594	76.83	65-84	5.34	**
DPM	22.278	21.684	1.372	103.35	98-111	2.64	**
GFD	18.348	17.754	1.739	26.55	23-34	9.32	**
GFR	12.091	10.52	15.76	14.3	10.07-18.98	13.94	**
PHT	41.854	40.115	4.765	91.83	80-98	4.07	**
CHL _A	19.984	18.75	1.234	49.51	43.5-54.5	5.22	**
CT _{vg} (°C)	0.585	0.312	0.273	21.03	19.9-22.5	2.14	**
CT _{anth} (°C)	0.514	0.236	0.278	22.08	21.1-23.5	1.89	**
CT _{gf} (°C)	0.786	0.463	0.323	23.75	21.5-24.9	2.15	**
Splet/spk.	3.946	3.458	0.488	16.02	13.9-18.4	7.17	**
Gr./spk.	46.76	31.88	14.872	50.74	40.7-57.9	7.78	**
TGW	32.652	25.727	6.925	37.21	30.5-45.9	8.86	**
Grain yield (kg/ha)	815481	709547	105934	3810.14	2552-4998	13.68	**

** Significant at 1% level of probability

DTH= Days to heading, DTA= Days to anthesis, DPM=Days to physiological maturity (days); GFD= Grain filling duration, GFR= Grain filling rate, PHT= Plant height (cm), CHL_A=Chlorophyll content at anthesisstage (SPAD unit), CT_{vg}=Canopy temperature at vegetative stage (°C); CT_{anth}= Canopy temperature at anthesis stage (°C); CT_{gf}=Canopy temperature at grain filling stage (°C); Splet/spk.= Spikelets per spike, Gr./spk.= Grains per spike, TGW= Thousand grain weight, M_{Sp}= Mean sum of squares due to phenotype, M_G= Mean sum of squares due to genotype; M_E= Mean sum of squares due to error.

Days to heading showed significant negative correlation with grain yield kg ha⁻¹ at genotypic and phenotypic level ($r_g = -0.402^{**}$ and $r_p = -0.381^{**}$). This trait had significant negative correlation with grain filling duration ($r_g = -0.823^{**}$ and $r_p = -0.747^{**}$), and canopy temperature at vegetative stage ($r_g = -0.691$ and $r_p = -0.318$) (Table 3). Barma *et al.* (1990) mentioned that the longer the vegetative period the shorter the grain-filling period. This trait had strong positive association with days to anthesis ($r_g = 0.996^{**}$ and $r_p = 0.991^{**}$) and days to maturity ($r_g = 0.808^{**}$ and $r_p = 0.725^{**}$) at genotypic and phenotypic level. Burio *et al.* (2004) found negative correlation with days to heading and grain yield.

Plant height showed significant positive association with grain filling rate ($r_g = 0.393^*$), TGW ($r_g = 0.285^*$), and grain yield ($r_g = 0.355^*$) at genotypic level (Table 3). Several investigators found plant height to be correlated significantly and positively with grain yield (Hassan *et al.* 1996; Khan and Bejwa, 1999). But Tila *et al.*, (2005) reported that plant height was negatively correlated with harvest index and grain yield both at genotypic and phenotypic levels.

Table 2. Mean performance of the 50 genotypes for phenological, physiological, and yield contributing traits.

Gen.	DTH	DTA	DPM	GFD	GFR	PHT	CHLA	CT _{vg} (°C)	CT _{anth} (°C)	CT _{g f} (°C)	Splet/spk.	Gr./spk.	TGW	Yield kg/ha
1	66.33	69.30	100.67	31.3	13.90	90.00	45.90	21.70	22.20	23.60	16.30	46.80	40.40	4365
2	75.00	79.70	106.00	26.3	14.90	89.00	43.50	21.60	22.10	24.10	16.50	51.30	32.30	3911
3	71.00	75.70	104.67	29.0	10.10	80.33	44.50	21.40	22.20	23.90	13.90	45.00	31.90	2918
4	73.00	77.30	104.00	26.7	15.80	97.67	47.00	20.80	21.90	23.70	14.20	53.60	30.50	4229
5	74.00	78.70	106.67	28.0	10.90	96.00	45.30	20.80	21.90	23.80	17.60	53.80	34.70	3049
6	74.67	79.70	105.67	26.0	14.30	93.67	51.20	22.00	22.80	24.40	15.00	41.20	41.20	3723
7	67.67	71.00	101.00	30.7	11.20	94.00	49.80	22.50	22.80	24.30	15.20	52.10	33.90	3418
8	62.33	66.00	99.67	33.7	11.20	92.33	46.40	21.90	22.90	24.40	17.00	51.30	38.40	3766
9	70.33	73.70	100.67	27.0	16.20	96.00	53.60	21.40	22.00	23.70	15.80	51.90	34.90	4367
10	69.67	73.00	100.67	27.7	16.00	94.67	51.60	21.50	21.90	23.90	14.90	47.00	32.80	4424
11	69.67	73.30	101.33	28.0	17.60	91.33	52.50	21.30	22.00	23.80	16.60	54.30	35.70	4905
12	70.00	73.70	102.67	29.0	16.90	95.67	53.70	21.30	22.00	23.70	14.50	50.30	40.50	4899
13	72.33	76.00	100.67	24.7	14.90	91.33	50.70	21.10	22.10	23.70	16.30	50.70	45.90	3678
14	74.33	78.30	105.67	27.3	13.80	92.33	50.40	21.00	21.90	23.90	17.30	52.70	41.70	3762
15	74.00	77.70	102.33	24.7	14.30	92.00	50.50	21.00	23.50	24.90	15.70	47.90	35.40	3516
16	72.00	76.00	101.33	25.3	13.70	97.67	52.40	21.10	22.20	23.70	16.50	51.40	36.90	3458
17	71.00	74.70	100.00	25.3	15.80	94.33	51.30	20.80	21.80	23.80	17.30	50.90	42.20	4000
18	73.33	77.00	102.00	25.0	11.90	95.00	48.60	20.90	21.70	23.80	16.10	45.30	38.00	2983
19	72.33	76.00	102.33	26.3	15.20	89.67	48.00	21.20	21.80	23.90	15.00	49.00	35.60	3987
20	72.33	76.30	101.33	25.0	14.40	92.00	47.20	21.40	21.90	23.10	16.80	50.70	38.00	3600
21	61.67	65.30	98.33	33.0	12.70	83.00	48.50	21.50	22.30	23.80	16.40	54.70	32.80	4199
22	74.33	78.00	103.00	25.0	15.10	93.67	51.10	20.70	21.80	23.70	16.20	51.20	38.30	3781
23	73.00	77.00	104.33	27.3	14.70	90.67	49.80	20.50	21.90	23.80	14.30	52.10	36.80	4012
24	75.33	79.30	105.00	25.7	12.80	91.00	46.40	21.10	22.00	24.20	14.00	46.70	35.00	3282
25	76.67	82.70	108.00	25.3	11.70	88.00	49.80	21.00	22.00	22.50	15.70	56.90	39.90	2966
26	76.00	80.70	105.00	24.3	13.80	88.00	50.70	20.80	22.10	24.30	16.40	43.10	32.30	3355
27	79.00	84.30	111.33	27.0	13.00	89.33	46.50	20.70	22.20	23.90	16.30	52.20	37.10	3502

Table 2. Cont'd.

Gen.	DTH	DTA	DPM	GFD	GFR	PHT	CHL _A	CT _{vg} (°C)	CT _{anh} (°C)	CT _{gf} (°C)	Splet/spk.	Gr./spk.	TGW	Yield kg/ha
28	72.00	75.70	101.33	25.7	14.20	92.33	50.20	20.80	22.40	24.20	14.40	51.00	38.40	3636
29	77.00	81.70	105.00	23.3	14.20	94.33	48.20	21.30	22.00	24.10	17.30	52.30	36.60	3333
30	75.00	79.70	107.33	27.7	13.20	96.67	51.40	20.80	21.70	23.60	17.40	56.80	37.90	3654
31	75.33	79.70	107.00	27.3	13.70	95.67	53.30	20.80	22.10	23.70	16.20	52.60	39.10	3746
32	77.33	81.70	107.00	25.3	16.30	91.00	47.40	20.90	21.80	23.70	17.20	48.80	33.10	4132
33	76.33	80.70	105.00	24.3	15.80	91.67	46.90	21.00	21.70	23.50	16.80	51.00	38.10	3799
34	71.67	75.30	100.00	24.7	16.20	92.33	52.00	20.80	21.60	23.50	14.90	56.00	36.60	3994
35	71.00	74.70	100.67	26.0	17.30	87.67	52.30	20.80	22.00	23.70	14.70	57.90	36.40	4495
36	73.00	76.70	102.00	25.3	15.60	88.67	52.60	20.80	22.00	23.90	15.10	55.60	30.90	3953
37	74.33	78.70	106.00	27.3	12.80	94.00	48.60	20.90	21.80	23.50	16.50	55.10	36.40	3504
38	75.33	79.30	105.67	27.0	15.60	93.33	47.70	20.40	21.30	23.10	18.40	46.30	41.80	4212
39	74.67	79.00	106.33	27.3	14.70	93.67	48.90	20.40	21.10	23.30	17.40	56.40	39.60	4048
40	69.00	73.30	99.67	26.3	19.00	96.33	46.50	20.80	21.90	23.80	14.90	50.00	42.40	4998
41	76.00	80.70	104.33	23.7	10.80	81.33	50.30	21.00	22.60	24.20	17.10	55.50	37.00	2552
42	74.33	78.30	103.33	25.0	16.70	96.33	45.90	20.80	22.60	24.10	18.10	47.90	38.10	4148
43	75.00	78.70	103.67	25.0	14.70	90.67	48.20	20.60	22.70	24.40	15.90	49.40	38.40	3671
44	62.67	66.00	100.00	34.0	11.70	91.33	51.20	21.10	21.80	21.50	16.60	48.40	37.10	3991
45	73.33	77.00	101.33	24.3	12.80	91.33	54.50	21.20	22.70	23.90	16.70	55.00	34.60	3107
46	74.33	78.30	105.00	26.7	14.60	88.67	51.50	19.90	22.10	23.30	17.40	51.60	42.00	3890
47	76.33	80.30	104.67	24.3	14.70	91.67	48.80	20.50	22.10	23.40	14.30	48.60	36.50	3577
48	71.33	75.00	100.00	25.0	18.20	92.00	50.30	20.80	22.50	23.70	14.60	48.10	36.10	4546
49	76.00	80.00	103.67	23.7	17.20	94.00	50.30	20.40	21.90	23.50	15.60	40.70	40.40	4053
50	76.00	80.70	104.33	23.7	14.40	88.00	51.50	21.00	21.80	23.50	15.70	47.90	39.30	3410

DTH= Days to heading, DTA= Days to anthesis, DPM=Days to physiological maturity (days); GFD= Grain filling duration, GFR= Grain filling rate, PHT= Plant height (cm), CHL_A=Chlorophyll content at anthesisstage (SPAD unit), CT_{vg}=Canopy temperature at vegetative stage (°C); CT_{anh}= Canopy temperature at anthesis stage (°C); CT_{gf}=Canopy temperature at grain filling stage (°C), Splet/spk.= Spikelet per spike, Gr./spk.= Grain per spike, TGW= Thousand grain weight.

Table 3. Genotypic (r_g) and phenotypic (r_p) correlation coefficient among yield and yield related heat tolerance traits in spring wheat

Traits	PHT	DTA	DPM	GFD	GFR	CHL _A	CT _{veg.}	CT _{Anth.}	CT _{gf}	Splet. spk. ⁻¹	Gr.spk. ⁻¹	TGW	GY
DTH	r_g 0.055	0.996**	0.808**	-0.823**	0.097	-0.062	-0.691**	0.241	0.154	0.131	-0.072	0.103	-0.402**
	r_p 0.036	0.991**	0.725**	-0.747**	0.052	-0.045	-0.318*	0.137	0.127	0.110	-0.040	0.025	-0.381**
PHT	r_g 0.026	-0.031	-0.073	-0.073	0.393*	0.234	0.031	-0.133	0.038	0.162	0.036	0.285*	0.355*
	r_p 0.005	-0.047	-0.056	-0.056	0.278	0.030	-0.141	-0.203	-0.086	0.075	-0.123	0.154	0.253
DTA	r_g 0.844**	-0.791**	0.048	-0.097	-0.659**	-0.097	-0.659**	-0.275	0.127	0.133	-0.057	0.094	-0.432**
	r_p 0.766**	-0.719**	0.017	-0.076	-0.301*	-0.076	-0.301*	-0.107	0.112	0.122	-0.020	0.028	-0.401**
DPM	r_g 0.339*	-0.339*	-0.239	0.281*	-0.450*	-0.281*	-0.450*	-0.363**	-0.061	0.238	0.051	0.040	-0.439**
	r_p -0.108	-0.108	-0.272	-0.244	-0.203	-0.244	-0.203	-0.116	0.056	0.205	0.004	-0.023	-0.325*
GFD	r_g -0.356*	-0.154	0.649**	0.062	-0.289*	0.062	0.649**	0.062	-0.289*	0.044	0.143	-0.115	0.257
	r_p -0.326*	-0.143	0.255	0.044	-0.179	0.044	0.255	0.044	-0.179	0.040	0.038	-0.067	0.265
GFR	r_g 0.229	-0.446**	-0.311*	-0.002	-0.210	-0.311*	-0.002	-0.311*	-0.002	-0.210	-0.144	0.154	0.682**
	r_p 0.188	0.188	-0.213	0.017	-0.079	0.188	-0.213	-0.148	0.017	-0.079	-0.029	0.187	0.995**
CHL _A	r_g -0.179	-0.179	-0.179	-0.020	-0.144	-0.179	-0.179	-0.020	-0.200	-0.144	0.234	0.153	0.159
	r_p -0.055	-0.055	-0.055	0.083	-0.022	-0.022	-0.022	0.083	-0.022	-0.026	0.195	0.124	0.123
CT _{veg.}	r_g 0.425**	0.357*	-0.255	0.425**	0.357*	-0.255	0.425**	0.425**	0.357*	-0.255	-0.229	-0.175	-0.048
	r_p 0.428**	0.237	-0.054	0.428**	0.237	-0.054	0.428**	0.428**	0.237	-0.054	-0.032	-0.288*	-0.076
CT _{anth.}	r_g 0.622**	-0.252	-0.252	0.622**	-0.252	-0.252	0.622**	0.622**	-0.252	-0.252	-0.284*	-0.183	-0.298*
	r_p 0.575**	-0.080	-0.080	0.575**	-0.080	-0.080	0.575**	0.575**	-0.080	-0.080	-0.092	-0.107	-0.147
CT _{gf}	r_g -0.283*	-0.055	-0.055	-0.283*	-0.055	-0.055	-0.283*	-0.283*	-0.055	-0.055	-0.285*	-0.239	-0.176
	r_p -0.059	-0.059	-0.059	-0.059	-0.059	-0.059	-0.059	-0.059	-0.059	-0.059	-0.059	-0.205	-0.089
Splet/spk.	r_g 0.124	0.331*	0.124	0.124	0.331*	0.124	0.331*	0.124	0.331*	0.124	0.124	0.331*	-0.187
	r_p 0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.223	-0.058
Gr./spk.	r_g -0.204	-0.204	-0.204	-0.204	-0.204	-0.204	-0.204	-0.204	-0.204	-0.204	-0.204	-0.204	-0.029
	r_p -0.034	-0.034	-0.034	-0.034	-0.034	-0.034	-0.034	-0.034	-0.034	-0.034	-0.034	-0.034	0.013
TGW	r_g 0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091
	r_p 0.155	0.155	0.155	0.155	0.155	0.155	0.155	0.155	0.155	0.155	0.155	0.155	0.155

DTH= Days to heading, DTA= Days to anthesis, DPM=Days to physiological maturity (days); GFD= Grain filling duration, GFR= Grain filling rate, PHT= Plant height (cm), CHL_A=Chlorophyll content at anthesisstage (SPAD unit), CT_{veg}=Canopy temperature at vegetative stage (°C); CT_{anth}= Canopy temperature at anthesis stage (°C); CT_{gf}=Canopy temperature at grain filling stage (°C),TGW= Thousand grain weight r_g = Genotypic correlation, r_p = Phenotypic correlation.

Days to anthesis showed significant negative correlation with GFD ($r_g = -0.791^{**}$ and $r_p = -0.719^{**}$) and grain yield kg ha^{-1} ($r_g = -0.432^{**}$ and $r_p = -0.401^{**}$) at genotypic and phenotypic level. This trait had significant negative correlation with canopy temperature at vegetative stage ($r_g = -0.659^{**}$ and $r_p = -0.301^*$) (Table 3). Barma *et al.* (1990) mentioned that the longer the vegetative period the shorter the grain-filling period. Days to anthesis had strong positive association with days to maturity ($r_g = 0.844^{**}$ and $r_p = 0.766^{**}$) at genotypic and phenotypic level. All these associations indicate that long vegetative growth coupled with delayed anthesis was detrimental trait in late sown condition. Longer vegetative growth period only could increase seed setting to some extent but the ultimate result was negative. Delayed anthesis exposes the crop to sharply rising high temperature which cut the grain filling period causing inadequate grain filling. Burio *et al.*, (2004) observed negative correlation between days to anthesis and grain yield. Amin *et al.*, (1992) reported significant negative correlation between days to anthesis and grain filling period.

Days to physiological maturity showed significant negative correlation with grain yield kg ha^{-1} at genotypic and phenotypic level ($r_g = -0.439^{**}$ and $r_p = -0.325^{**}$). It showed negative correlation with all the characters except spikelet per spike ($r_g = -0.238$ and $r_p = -0.205$) and grain per spike ($r_g = -0.051$ and $r_p = -0.004$) both at genotypic and phenotypic levels (Table 3). This association indicates that late maturing genotypes had longer duration of grain filling. Rahman (2009) reported significant positive correlation of grain filling period with maturity period. Sangam (1994) reported that this trait exhibited negative association with grain yield but Jadhav (1994) reported positive and significant association.

Grain filling duration (GFD) showed significant positive correlation at genotypic level and insignificant positive correlation at phenotypic level ($r_g = 0.649^{**}$ and $r_p = 0.255$) with Canopy temperature at vegetative stage (Table 3). But canopy temperature at grain filling stage ($r_g = -0.289^*$ and $r_p = -0.179$) showed significant negative correlation at genotypic level and insignificant negative correlation at phenotypic level and GFR ($r_g = -0.356^*$ and $r_p = 0.326^*$) showed significant negative correlation at genotypic and phenotypic level (Table 3). Rahman (2009) observed significant positive correlation for GFD with grain yield and insignificant positive correlation with biomass. He also reported GFD was negatively correlated with grain filling rate.

Grain filling rate (GFR) ($\text{g d}^{-1} \text{m}^{-2}$) showed very strong positive correlation with grain yield ($r_g = 0.682^{**}$ and $r_p = 0.995^{**}$). The result revealed that faster grain filling rate increased the grain yield significantly (Table 3). There was a significant negative association of this trait with canopy temperature at vegetative stage ($r_g = -0.446^{**}$ and $r_p = -0.213$) and canopy temperature at anthesis stage ($r_g = -0.311^*$ and $r_p = -0.148$) at genotypic level and insignificant negative association at phenotypic level. Barma (2005) and Rahman (2009) reported strong and positive correlation of grain filling rate with grain yield.

Chlorophyll content of flag leaf at anthesis stage (CHL_A) showed insignificant positive correlation with grain yield ($r_g = 0.159$ and $r_p = 0.123$), TGW ($r_g = 0.153$ and $r_p = 0.124$), and grain per spike ($r_g = 0.234$ and $r_p = 0.195$) both at genotypic and phenotypic level (Table 3). Hede *et al.*, (1999) found highly significant correlations between leaf chlorophyll content and 1000-grain weight. Rahman (1996) reported that flag leaf chlorophyll content measured at 2nd and 3rd week after anthesis showed significant and positive correlation with grain yield.

Canopy temperature at vegetative stage (CT_{vg}) had significant negative correlation with TGW ($r_g = -0.175$ and $r_p = -0.288^*$) at phenotypic level and insignificant at genotypic level (Table 3). CT_{vg} showed significant positive correlation with CT_{anth} ($r_g = 0.425^{**}$ and $r_p = 0.428^{**}$) at genotypic and phenotypic level but CT_{gf} ($r_g = 0.357^*$ and $r_p = 0.237$) showed significant positive correlation with this trait at genotypic and insignificant positive correlation at phenotypic level. Rees *et al.*, (1993) found canopy temperature depression (CTD) and photosynthetic activity to be positively correlated with grain yield. Rahman *et al.*, (1997) observed that high yielding genotypes possess significantly low canopy temperature and medium chlorophyll content. Balota *et al.* (2007) also observed significant correlation coefficients of CTD at three developmental stages i.e. pre-heading, heading and post-anthesis stages with grain yield. CIMMYT began CTD measurements on different irrigated experiments in Northwest Mexico and it was found that phenotypic correlations of CTD with grain yield were occasionally positive (Reynolds *et al.*, 1994; Fischer *et al.*, 1998). They also reported that CTD has been used as a selection criterion for tolerance to drought and high temperature stress in wheat breeding and the used breeding method generally comes by mass selection in early generations like F_3 .

Canopy temperature at anthesis stage ($CT_{anth.}$) had significantly negative correlation with grain yield ($r_g = -0.298^*$ and $r_p = -0.147$) and grain per spike ($r_g = -0.284^*$ and $r_p = -0.092$) at genotypic level but insignificant at phenotypic level (Table 3).

Canopy temperature at grain filling stage (CT_{gf}) had significant negative correlation with spikelet per spike ($r_g = -0.283^*$ and $r_p = -0.055$) and grain per spike ($r_g = -0.285^*$ and $r_p = -0.059$) at genotypic and insignificant at phenotypic level (Table 3). Rahman (2009) reported strong and negative correlation of CT_{gf} with grain yield.

Spikelets per spike had significant positive correlation with TGW ($r_g = 0.331^*$ and $r_p = 0.223$) at genotypic level and insignificant positive correlation at phenotypic level (Table 3). The insignificant negative correlation was noticed between grains spike^{-1} and grain yield ($r_g = -0.029$ and $r_p = 0.013$) at genotypic level while it was positive and insignificant at phenotypic level. Singh & Sharma (1999) observed negative correlation of grains per spike and grain yield but several authors (Gautam *et al.*, 2002; Bergalie *et al.*, 2001; Shukla *et al.*, 2005)

had reported that significant and positive correlation of grains per spike and grain yield. However, negative insignificant correlation between grains spike⁻¹ and TGW ($r_g = -0.204$ and $r_p = -0.034$) was obtained in the tested experiment. The negative correlation between grains spike⁻¹ and TGW indicates a competitive demand of both sinks (grain number and size) for photosynthates from a common source, which expressed through a compensating balance between two traits under stress condition.

Thousand grains weight (TGW) (g) showed insignificant positive correlation with grain yield ($r_g = 0.091$ and $r_p = 0.155$) both at genotypic and phenotypic level (Table 3). Guttieri *et al.*, (2001) observed that decrease in grain weight of each spike due to drought during grain filling period. Wardlaw *et al.*, (1989) suggested individual grain weight as to be the most sensitive yield component to high temperature. Several authors (Singh *et al.*, 1997; Narwal *et al.*, 1999; Nayeem *et al.*, 2003; Jat *et al.*, 2003; Zecevic *et al.*, 2004) had reported significant and positive correlation of 1000-grain weight with grain yield.

Path coefficient analysis

Association of characters determined by correlation co-efficient may not provide an exact picture of the relative importance of direct and indirect influence of each of yield components on seed yield. In order to find out a clear picture of the inter-relationship between seed yield and other yield attributes, direct and indirect effects were worked out using path analysis at phenotypic level which also measured the relative importance of each component. Seed yield was considered as a resultant (dependent) variable and others were causal (independent) variables. Estimates of direct and indirect effects of path coefficient analysis are presented in Table 4. Residual effects which have influenced yield to a small extent have been denoted as “R” The results are discussed character wise as follows:

Days to heading (DTH) had high negative direct effect on grain yield ha⁻¹ (-0.9562). It contributed high negative indirect effect on yield via days to anthesis (-10.6496), spikelets spike⁻¹ (-0.0692), grain filling rate (-0.0332), and canopy temperature at anthesis stage (0.0145). Days to heading however showed positive indirect effect on grain yield through days to maturity (5.4769), grain filling period (4.2476), canopy temperature at vegetative stage (1.4034), canopy temperature at grain filling stage (0.0973), plant height (0.0451), grain per spike (0.0433), chlorophyll content at anthesis stage (0.0068), and thousand grain weight (0.0003). Which ultimately resulted the total significant negative correlation with grain yield (-0.4020**) (Table 4).

Plant height (cm) showed positive direct effect on yield (0.8232). It also showed positive indirect effect via grain filling period (0.3745), canopy temperature at grain filling stage (0.0237), canopy temperature at anthesis stage (0.0080), and

thousand grain weight (0.0009). The highest indirect negative effect on yield through days to anthesis (-0.2809), days to maturity (-0.2117), grain filling rate (-0.1341), spikelets per spike (-0.0858) canopy temperature at vegetative stage (-0.0629), days to heading (-0.0524), chlorophyll content at anthesis stage (-0.0259), and grain per spike (-0.0219). Which led to significant positive correlation with grain yield at genotypic level (0.3548*) (Table 4). Chaturvedi *et al.* (1995) and Halloli (1997) reported positive direct effect on grain yield. But Ehdai and Waines (1989) found plant height to be negatively correlated with grain yield at genotypic level.

Days to anthesis (DTA) had very high negative direct effect on grain yield (-10.694). It contributed high negative indirect effect on yield via days to heading (-0.952), spikelets per spike (-0.070), and grain filling rate (-0.016). Days to anthesis however showed positive indirect effect on grain yield through days to maturity (5.7182), grain filling period (4.0811), canopy temperature at vegetative stage (1.3389), canopy temperature at grain filling stage (0.0799), grain per spike (0.0341), plant height (0.0216), canopy temperature at anthesis stage (0.0166), chlorophyll content at anthesis stage (0.0107), and thousand grain weight (0.0003). Which ultimately resulted the total significant negative correlation with grain yield (-0.4319**) (Table 4). Burio *et al.*, (2004) found significant negative correlation between days to anthesis and grain yield.

Days to physiological maturity (DPM) showed high positive direct effect on grain yield (6.7791). The indirect effect via grain filling period (1.747), grain filling rate (0.0817), chlorophyll content at anthesis stage (0.0311), canopy temperature at vegetative stage (0.9136), canopy temperature at anthesis stage (0.0218), and thousand grain weight (0.0001) was positive. There was a strong negative indirect effect through days to anthesis (-9.0206) on yield followed by days to heading (-0.7725). The indirect effects of other traits on yield were also negative. It resulted ultimately the total genotypic correlation significantly negative with yield (-0.4393**) (Table 4).

Grain filling duration (GFD) showed high negative (-5.1588) direct effect on yield. The indirect effect via plant height (-0.0598), days to physiological maturity (-2.2957), canopy temperature at vegetative stage-1.3179, canopy temperature at anthesis stage (-0.0038), canopy temperature at grain filling stage (-0.1828), spikelets per spike (-0.0232), grain per spike (-0.0859), and thousand grain weight (-0.0004) was negative. There was a strong positive indirect effect through days to anthesis (8.46) on yield followed by days to heading (0.7873). The indirect effects of other traits on yield were also positive. It resulted ultimately the total genotypic correlation positive with yield (0.2574) (Table 4). Gashaw *et al.*, (2007) reported that grain filling period had negative direct effect (-0.52) on yield.

Table 4. Direct and indirect effects of thirteen traits on grain yield

Traits	DTH	PHT	DTA	DPM	GFD	GFR	CHL _A	CT _{veg.}	CT _{Anth}	CT _{gf}	Splet/ spk.	Gr./spk.	TGW	Genotypic correlation with yield
DTH	-0.9562	0.0451	-10.6496	5.4769	4.2476	-0.0332	0.0068	1.4034	-0.0145	0.0973	-0.0692	0.0433	0.0003	-0.4020**
PHT	-0.0524	0.8232	-0.2809	-0.2117	0.3745	-0.1341	-0.0259	-0.0629	0.0080	0.0237	-0.0858	-0.0219	0.0009	0.3548*
DTA	-0.9522	0.0216	-10.6942	5.7182	4.0811	-0.0164	0.0107	1.3389	0.0166	0.0799	-0.0704	0.0341	0.0003	-0.4319**
DPM	-0.7725	-0.0257	-9.0206	6.7791	1.7470	0.0817	0.0311	0.9136	0.0218	-0.0382	-0.1260	-0.0305	0.0001	-0.4393**
GFD	0.7873	-0.0598	8.4600	-2.2957	-5.1588	0.1214	0.0170	-1.3179	-0.0038	-0.1828	-0.0232	-0.0859	-0.0004	0.2574
GFR	-0.0929	0.3235	-0.5148	-1.6233	1.8352	-0.3411	-0.0254	0.9062	0.0187	-0.0015	0.1111	0.0861	0.0005	0.6823**
CHL _A	0.0590	0.1926	1.0345	-1.9051	0.7924	-0.0783	-0.1105	0.3630	0.0012	-0.1263	0.0763	-0.1405	0.0005	0.1587
CT _{veg.}	0.6609	0.0255	7.0515	-3.0499	-3.3483	0.1523	0.0198	-2.0306	-0.0256	0.2256	0.1346	0.1371	-0.0006	-0.0479
CT _{Ant}	-0.2307	-0.1091	2.9434	-2.4588	-0.3224	0.1060	0.0023	-0.8640	-0.0602	0.3925	0.1332	0.1701	-0.0006	-0.2982*
CT _{gf}	-0.1474	0.0309	-1.3541	-0.4101	1.4935	0.0008	0.0221	-0.7255	-0.0374	0.6313	0.1496	0.1711	-0.0008	-0.1759
Splet/ spk.	-0.1253	0.1336	-1.4243	1.6164	-0.2266	0.0717	0.0160	0.5171	0.0152	-0.1787	-0.5285	-0.0745	0.0011	-0.1869
Gr./spk	0.0690	0.0300	0.6078	0.3449	-0.7390	0.0490	-0.0259	0.4641	0.0171	-0.1801	-0.0657	-0.5998	-0.0007	-0.0293
TGW	-0.0986	0.2344	-1.0029	0.2708	0.5919	-0.0527	-0.0169	0.3553	0.0110	-0.1511	-0.1752	0.1221	0.0033	0.0914

Residual effect R = 0.3744

DTH= Days to heading, DTA= Days to anthesis, DPM=Days to physiological maturity (days); GFD= Grain filling duration, GFR= Grain filling rate, PHT= Plant height (cm), CHL_A=Chlorophyll content at anthesisstage (SPAD unit), CT_{veg}=Canopy temperature at vegetative stage (°C); CT_{anth}= Canopy temperature at anthesis stage (°C); CT_{gf}=Canopy temperature at grain filling stage (°C),TGW= Thousand grain weight r_g= Genotypic correlation, rp= Phenotypic correlation.

The direct effect of grain filling rate (GFR) [$\text{g d}^{-1}\text{m}^{-2}$] was negative (-0.3411) on grain yield although it showed highly significant positive correlation with yield (0.6823) (Table 4). The indirect effect through days to maturity (-1.6233) and days to anthesis (-0.5148) on yield was very high and negative. But indirect effect through grain filling rate and canopy temperature at vegetative stage on yield (0.9062) was positive and high. The indirect effect of grain filling rate via all other characters on yield was also positive. Thus, negative direct effect was counter balanced by high positive indirect effects and total correlation coefficient was significantly positive (0.6823) between grain filling rate and grain yield. So, indirect causal factors should be considered simultaneously for selection. Barma (2006) reported significant positive correlation of grain filling rate with grain yield.

Chlorophyll (CHL) content of flag leaf at anthesis stage (CHL_A) had negative direct effect (-0.1105) on yield. But indirect effect on yield via days to maturity (-1.9051) was high followed by grains per spike (-0.1405), and canopy temperature at grain filling stage (-0.1263). The indirect effect via grain filling rate (-0.0783) was negative but low in magnitude. But indirect effect through days to anthesis (1.0345), grain filling duration (0.7924) and plant height (0.1926) on yield was positive and high. The indirect effect of chlorophyll (CHL) content of flag leaf at anthesis stage (CHL_A) via all other characters on yield was also positive (Table 4). This resulted positive genotypic correlation of chlorophyll content (0.1587) with yield. Barma (2006) found significant genotypic correlation of chlorophyll content with grain yield.

Canopy temperature at vegetative stage (CT_{veg}) had highly negative direct effect (-2.0306) on yield. But indirect effect on yield via grain filling period (-3.3483) was high followed by days to maturity (-3.0499). The indirect effect via canopy temperature at anthesis stage (-0.0256) and thousand grain weight (-0.0006) was negative but low in magnitude. But indirect effect through days to anthesis (7.0515) and days to heading (0.6609) on yield was positive and high. The indirect effect of canopy temperature at vegetative stage via all other characters on yield was also positive. This resulted negative genotypic correlation of canopy temperature at vegetative stage (-0.0479) with yield (Table 4).

Canopy temperature at anthesis stage ($\text{CT}_{\text{anth.}}$) had negative direct effect (-0.0602) on yield. But indirect effect on yield via days to maturity (-2.4588) was high followed by canopy temperature at vegetative stage (-0.8640), grain filling period (-0.3224), and days to heading (-0.2307). The indirect effect via plant height (-0.1091) and thousand grain weight (-0.0006) was negative but low in magnitude. But indirect effect through days to anthesis (2.9434) on yield was positive and high. The indirect effect of canopy temperature at anthesis stage via all other characters on yield was also positive. This resulted significant negative genotypic correlation of canopy temperature at anthesis stage (-0.2982) with yield (Table 4).

Canopy temperature at grain filling stage ($^{\circ}\text{C}$) had positive direct effect (0.6313) on yield. But indirect effect on yield via grain filling period (1.4935) was high followed by grains per spike (0.1711) and spikelets per spike (0.1496). The indirect effect via plant height (0.0309) and grain filling rate (0.0008) was positive but low in magnitude. But indirect effect through days to anthesis (-1.3541) on yield was negative and high. The indirect effect of canopy temperature at grain filling stage via all other characters on yield was also negative. This resulted negative genotypic correlation of canopy temperature at grain filling stage (-0.1759) with yield (Table 4).

The direct effect of spikelets per spike on yield was negative (-0.5285) but moderate in magnitude (Table 4). The indirect effect via days to anthesis (-1.4243) was high followed by grain filling duration (-0.2266), canopy temperature at grain filling stage (-0.1787), and days to heading (-0.1253). The indirect effect via grains per spike (-0.0745) was negative but low in magnitude. There was a strong positive and indirect effect through days to maturity (1.6164) on yield followed by canopy temperature at vegetative stage (0.5171) and plant height (0.1336). The indirect effects of other traits on yield were also positive. This resulted negative genotypic correlation of spikelets per spike (-0.1869) with yield

Grains spike⁻¹ had direct negative effect on yield (-0.5998). This trait had maximum indirect negative effect on yield via grain filling period (-0.7390) followed by canopy temperature at grain filling stage (-0.1801), spikelets per spike (-0.0657), chlorophyll content of flag leaf at anthesis stage (-0.0259), and TGW (-0.0007). A number of positive indirect effects on yield were observed for this trait via days to anthesis (0.6078), canopy temperature at vegetative stage (0.4641), days to maturity (0.3449), days to heading (0.0690), grain filling rate (0.0490), plant height (0.0300), and canopy temperature at anthesis stage (0.0171). All these ultimately led to negative correlation with yield (-0.0293) at genotypic level (Table 4). But Subhani *et al.*, (2000) observed highly positive association between grains spike⁻¹ and grain yield in bread wheat.

Thousand grains weight (TGW) [g] showed direct positive effect on grain yield (0.0033) but low. This trait had maximum indirect positive effect on yield via grain filling period (0.5919) followed by canopy temperature at vegetative stage (0.3553), plant height (0.2344), days to maturity (0.2708), grains per spike (0.1221), and canopy temperature at anthesis stage (0.0110). There was a strong negative and indirect effect through days to anthesis (-1.0029) on yield followed by spikelets per spike (-0.1752) and canopy temperature at grain filling stage (-0.1511). The indirect effects of other traits on yield were also negative. This resulted positive genotypic correlation of thousand grains weight (g) with yield (0.0914) (Table 4). Ibrahim (1994) and Deshmukh *et al.* (1990) observed in direct positive effect of thousand grains weight (g) on grain yield in bread wheat and durum wheat respectively. The residual effect (0.3744) indicated that the character under study contributed 62.56% of the yield. It is

suggested that there are some other characters those contributed 37.44% to the grain yield m^{-2} (Table 4). Considering analytical findings of correlation coefficient, path co-efficient analysis and field performance, the genotypes G 3, G 10, G 11, G 12, G13, G 21, G 29, G 35, G 38, G 40, G 46 and G 48 were found suitable for future breeding programme.

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