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EVALUATION OF WHEAT GENOTYPES FOR HEAT STRESS AND DISEASE RESISTANCE IN MULTIPLE ENVIRONMENTS OF BANGLADESH

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Abstract

Twenty-seven advanced lines including three exiting varieties (BARI Gom 21, BARI Gom 32 and BARI Gom 33) were evaluated in alpha lattice design with two replications at Bangladesh Wheat and Maize Research Institute, Dinajpur and regional stations at Joydebpur, Jashore and Rajshahi under irrigated timely sown (ITS) and irrigated late sawn (ILS) conditions. The objective of the study was to find out the heat-tolerant wheat lines for future breeding programme to develop heat-tolerant wheat varieties. The genotypes were evaluated for phenological variation such as heading and maturity, yield and yield components, disease reaction, sterility, visual grain quality, etc. The results showed that the highest grain yield (5951 kg ha^{-1}) was recorded with genotype BAW 1415 at Dinajpur under ITS condition and the lowest grain yield (1923 kg ha^{-1}) was observed in BAW 1416 at Rajshahi under ILS condition. Considering the overall performances, genotypes BAW 1402, BAW 1403, BAW 1406, BAW 1407, BAW 1408, BAW 1411, BAW 1422 and BAW 1425 performed better. Considering disease infestation, genotypes BAW 1374, BAW 1393, BAW 1394, BAW 1397, BAW 1399 and BAW 1401 showed a good level of resistance to both leaf rust (LR) and wheat blast (WB). These genotypes could be selected for future breeding programmes to develop heat-tolerant and diseases resistance varieties.

Keywords: Blast, BpLB, Grain yield, Leaf rust, Wheat lines

Introduction

Wheat contributes 20% of the calories and 20% of the protein for daily human consumption (Shiferaw *et al.*, 2013). Among the world's three most important staple food crops, wheat is grown on more than 215 million hectares (ha) worldwide, producing over 735 million tons (t) of grain (Crespo-Herrera *et al*., 2021). Continual heat stress (mean daily temperature of over 17.5^{0}C in the coolest month of the season) affects

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approximately 7 million ha of wheat in developing countries, while terminal heat stress affects 40% of temperate environments, covering 36 million ha (Reynolds *et al.*, 2010). Heat is a non-uniform phenomenon that has a negative impact on plant growth, morphology, physiology, and yield, depending on crop developmental stage, time, and stress severity (Ahmed and Prasad, 2011). Crops at various stages of development require varying temperatures for optimal growth. Under heat stress, plants have limited nutrient uptake capacity and photosynthetic efficiency. Additionally, this stress can shorten the growth period for several developmental phases at the tillering, booting, heading, anthesis, and grain filling stages as well as the size of the organs such as, leaf, tiller, and spikes (Hossain *et al.*, 2013). Plant sensitivity to high temperatures leads to a disrupted metabolic process and lower plant biomass accumulation (Hasanuzzaman *et al.*, 2013).

According to Slafer and Satorre (1999), wheat is extremely susceptible to high temperatures, and trends in rising growing season temperatures have already been noted for the major wheat-producing regions (Gaffen and Ross, 1998; Alexander *et al.*, 2006; Hennessy *et al.,* 2008). Heat stress affects wheat to varying degrees at different phenological stages, but heat stress is more pronounced during the reproductive phase than during the vegetative phase due to the direct effect on grain number and dry weight (Wollenweber *et al.*, 2013).

The optimum time for wheat cultivation in Bangladesh is November. However, due to the late harvesting of the previous crop i.e. Aman rice (monsoon rice), farmers frequently sow the seeds late, even at the end of December. As a result, during the reproductive phase of growth, the wheat plant experiences much higher temperatures than is optimal. High temperatures, during the terminal growth stage, reduce growth, development and finally causes reduce grain yield. However, under high-temperature conditions, there may be varietal differences in growth and yield performance. The purpose of this experiment was to identify heat tolerant wheat genotypes under late sown condition for future wheat breeding programme.

Materials and Methods

Location of the experiment

The experiment was carried out during November to March 2020-21 in four regional stations e,g., Bangladesh Wheat and Maize Research Institute, Nashipur, Dinajpur (25.380 N, 88. 410 E, Elevation 39 M) in AEZ-1(Old Himalayan Piedmont Plain); the Regional Station of BWMRI, Joydebpur (*23.989014 N, 90.418167 E, Elevation 11.54*) in AEZ–28 (Modhupur Tract); the Regional Station of BWMRI, Khaertala-Jessore (23.170664 N *89.212418*, E; *15 m*) in AEZ–11(High Ganges River Floodplain) and the Regional Station of BWMRI, Shampur, Rajshahi (*24.3635886* N, *88.6241351*, Elevation 18 M) in AEZ-11 (High Ganges River Flood plain soil).

Treatments, design and experimental procedures

Twenty-seven promising wheat genotypes including three existing popular wheat varieties namely 'BARI Gom 21', 'BARI Gom 32' and 'BARI Gom 33' were evaluated in this study to identify heat-tolerant genotypes for growing in Bangladesh conditions (Table 1). The experiment was laid out in an alpha lattice design with two replications.

The genotypes were evaluated under irrigated timely sown (ITS) and late sown (ILS) conditions in all locations. In the ITS, all genotypes were sown in lines by hand on November 21-28, whereas in ILS (late sown heat stress) condition, all the genotypes were sown on December 20-25 (Table 2). The seeding rate was 120 kg ha^{-1} for each genotype. Before sowing, seeds of all varieties were treated with a popular fungicide, Provax-200 WP (marketed by Hossain Enterprise Bangladesh Ltd., in association with Chemtura Corp., USA), which contains Carboxin (17.5%) and Thiram (17.5%). For controlling soil-borne insects, Furadan 5G (containing carbofuran, marketed by FMC International S.A. Bangladesh Ltd.) was broadcasted at 15 kg ha^{-1}. Seeds were sown continuously in 5 m long 6 rows plot with a row spacing of 20 cm.

Table 1. Pedigree and selection history of wheat genotypes

Entry	Cross/pedigree
BAW 936	BARI Gom 21 (Shatabdi)
BAW 1202	BARI Gom 32
BAW 1260	BARI Gom 33
BAW-1402	KANCHAN/BAW 1135 BD13DI4S-099DI--050DI-050DI-030DI-4DI
BAW-1403	SHATABDI/BAW 1135 BD13DI13S-099DI-050DI-050DI-030DI-1DI
BAW-1404	BIJOY/BAW 968/SHATABDI BD13DI16S-099DI-050DI-050DI-030DI-19DI
BAW-1405	RODIP/BAW 824 BD13DI22S-099DI-050DI-050DI-030DI-5DI
BAW-1406	BARI Gom 25/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/5. BD13DI34S-099DI-050DI-050DI-030DI-1DI
BAW-1407	BARI Gom 25/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/5. BD13DI34S-099DI-050DI-050DI-030DI-9DI
BAW-1408	HUW234+LR34/PRINIA//KRONSTAD F2004/ SWARNA//BARI Gom 26 BD13DI160T-099DI--050DI-050DI-030DI-11DI
BAW-1409	F1 (CB 7 (Prodip) X CB 42 (BAW 1130 (GOURAB/PAVON 76) Ï CB-90 (BAW1051 (KLAT/SOREN//PSN/3/BOW//4 /VEE #5.10/5 /CNO67 /MFD//MON/3/SERI/6/NL297 NC2142 7B-020B-025B-3B-0B)
BAW-1410	BORL14//BECARD/QUAIU #1 CMSS12Y00070S-099Y-099M-099NJ-099NJ-21Y-0WGY
BAW-1411	MACE/5/TILILA/JUCHI/4/SERI.1B//KAUZ/HEVO/3/AMAD/6/KACHU/B ECARD//WBLL1*2/BRAMBLING CMSS13Y01525T-099TOPM-099Y-099M-0SY-11M-0WGY
BAW-1412	KACHU//KIRITATI/2*TRCH/3/KFA/2*KACHU CMSS13B00118S-099M-0SY-1M-0WGY

Table 1. Contd.

Entry	Cross/pedigree
BAW-1413	WBLL1*2/BRAMBLING*2//BAVIS/3/KACHU #1/KIRITATI//KACHU CMSS13B00377S-099M-0SY-26M-0WGY
BAW-1414	BORL14//BECARD/QUAIU #1 CMSS12Y00070S-099Y-099M-099NJ-099NJ-14Y-0WGY
BAW-1415	SUP152/AKURI//SUP152/3/MUCUY CMSS12Y00300S-099Y-099M-099NJ-099NJ-50Y-0WGY
BAW-1416	MUTUS*2/JUCHI//COPIO CMSS12Y00303S-099Y-099M-099NJ-099NJ-10Y-0WGY
BAW-1417	SUP152/KENYA SUNBIRD//KFA/2*KACHU CMSS13B00156S-099M-0SY-19M-0WGY
BAW-1418	WBLL1*2/BRAMBLING*2//BAVIS/3/KACHU #1/KIRITATI//KACHU CMSS13B00377S-099M-0SY-26M-0WGY
BAW-1419	SOKOLL/WBLL1/4/PASTOR//HXL7573/2*BAU/3/WBLL1 PTSS11Y00144S-0SHB-099SHB-099Y-099B-099Y-19Y-020Y-0B
BAW-1420	BARI GOM-28 / BAW-1051 BD13JA1951S-099JA-50JA-50JA-30JA-06JA
BAW-1421	PRODIP / KINGBIRD #1 BD13JA1972S-099JA-50JA-50JA-30JA-010JA
BAW-1422	Y 3338
BAW-1423	BAW1170/SOURAV BD15JO1800S
BAW-1424	-
BAW-1425	-

Table 2. Sowing dates of wheat genotypes at different locations

Location	Sowing dates	
	Irrigated timely sown (ITS)	Irrigated late sown (ILS)
Dinajpur	21 November 2020	24 December 2020
Joydebpur	28 November 2020	24 December 2020
Jashore	21 November 2020	25 December 2020
Rajshahi	26 November 2020	20 December 2020

Intercultural operations

BWMRI recommended fertilizers such as N, P, K, S and B, respectively were applied at 100, 27, 40, 20, and 1 kg ha^{-1}. During final land preparation, two-thirds of N and the full amount of the other fertilizers were applied as basal. The remaining 1/3 N fertilizer was applied immediately after the first irrigation (16-18 days after sowing, DAS); while second, third and fourth irrigations were applied at 50, 75 and 85 DAS. Mulching was done at 25 DAS and hand weeding at 45 DAS. Phenological data like days to heading and maturity were recorded during the crop growth stage. The crop was harvested at full maturity on 10 April 2021. Grain yield (GY) and yield contributing characters were measured from the middle 4 rows (4 m area) among 6 rows. The harvested samples from each plot were bundled separately, tagged and manually threshed on a threshing floor after drying the bundles thoroughly in bright sunshine. GY and 1000-grain weight (TGW) were measured at 12% moisture in grain (Hellevang, 1995).

Inoculation procedures

At Dinajpur, a mixture of susceptible varieties such as Sonalika, Kanchan, Morocco, Ciano 79 etc was planted around the experimental plots spreader rows. The susceptible mixture acts as a substrate for multiplication and the spread of BpLB and rust inoculum. At the booting stage of the crop in Dinajpur, the spreader rows were inoculated with an aqueous suspension of uredospores of *Puccinina triticina* for disease development of leaf rust. The highly blast susceptible variety 'BARI Gom 26' was sown around the experimental field at Jashore for development of wheat blast diseases. Starting three weeks after sowing and continuing until the primary infection was observed, the spreader rows at Jashore were inoculated with *Magnaporthe oryzae* pathotype Triticum (MoT) spores (20000 spores per mL) for blast symptom development. The inoculum of MoT was multiplied at the plant pathology laboratory of Regional Station, BWMRI, Jashore.

% Diseased Leaf Area (DLA) = $D_1/9 \text{ X } D_2/9 \text{ X } 100$

where, D_1 = First digit, representing relative disease height; D_2 = Second digit, indicating disease severity on the foliage

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+1} + Y_i) \text{ x } 0.5]\ [T_{i+1} - T_i]$$

where, Y_i = Disease severity at the ith observation, T_i = Time (days) of the ith observation and n = Total number of observations (at least 3 observations).

Assessment of Wheat Blast

Wheat blast severity was recorded as per the following equation:

% Disease severity= (%spike incidence/100) x (%diseased area on spike/100)*100

Statistical analysis

Statistical analysis was conducted by the CropStat 7.2 programme with an F-test at 1% and 5% levels.

Results and Discussion

Days to heading

As the young spike expands within the leaf sheaths, it can eventually be felt and seen as a sheath swelling or boot after the flag leaf stage (Acevedo *el at.*, 2002). The length of time required for heading is entirely determined by growth conditions as well as the genetic makeup of specific genotypes (BARI, 2016, Hossain *et al*., 2012; Hossain *et al*., 2013). Days to heading of various genotypes varied significantly depending on genotype, location, and sowing time (Table 3, 4, 5, 6a). All the genotypes headed earlier than check variety Shatabdi except BAW 1410, BAW 1412, BAW 1413, BAW 1414, BAW 1417, BAW 1418 and BAW 1424. All genotypes took a long time to reach heading and maturity in optimum sowing conditions in the case of favorable environmental conditions in Dinajpur, Rajshahi, Jashore, and Joydebpur. All genotypes, however, showed faster heading and maturity at late sowing conditions than at optimum sowing conditions (Table 3). This finding was similar to that of Hossain *et al.*, (2018), who discovered that in some spring wheat genotypes, days to heading were faster in late sowing conditions than in timely sowing conditions. Several studies have confirmed this result (Fischer 1985; Yang *et al.,* 2002; Nahar *et al.,* 2010; Hakim *et al.,* 2012), where they discovered that crops mature significantly more quickly in high temperatures than in normal temperatures. However, genotypes influence the variation of phenological stages (Wahid *et al.,* 2007). Phenological stage is the biological life cycles of wheat such as germination/emergence, tillering, stem elongation, boot, heading/flowering, and grain-fill/ripening etc.

Table 3. Effects of seeding times on yield and other characters of wheat genotypes, 2020-21

Seeding time	Heading (days)	Maturity (days)	Plant height (cm)	Grains spike^{-1}	TGW (g)	Yield (kg ha^{-1})
ITS	65	108	96	52	45	4200
ILS	63	94	92	49	36	3209
CV (%)	1.9	1.2	2.6	9.4	6.8	12.3
LSD (0.05)	1	1	1	1	1	86
F-test	**	**	**	**	**	**

Table 4. Effects of locations on yield and other characters of genotypes

Location	Heading (days)	Maturity (days)	Plant height (cm)	Grains spike^{-1}	TGW (g)	Grain Yield (kg ha^{-1})
Dinajpur	64.2	104.6	98.7	49	44.1	4068
Joydebpur	63.6	98.3	85.8	52.7	36.3	3741
Jashore	64	98.8	96.2	52.4	40.3	3796
Rajshahi	64.2	100.7	94	49.2	40.3	3212
CV (%)	1.9	1.2	2.6	9.4	6.8	12.3
LSD (0.05)	0.3	0.3	0.6	1.3	0.7	122
F-test	**	**	**	**	**	**

Table 5. Performances of genotypes on the yield and other characters of wheat genotypes (Mean results)

Genotype	Heading (days)	Maturity (days)	Plant height (cm)	Grains spike^{-1}	TGW (g)*	Grain Yield (kg ha^{-1})
BARI Gom 21	69	104	100	51	41	3514
BARI Gom 32	58	96	86	45	45	3840
BARI Gom 33	61	100	100	54	44	3723
BAW 1402	57	98	97	50	42	4461
BAW 1403	58	98	89	49	41	4307
BAW 1404	61	99	90	46	38	3442
BAW 1405	58	97	89	50	39	3331
BAW 1406	59	98	91	47	43	3848
BAW 1407	59	98	89	50	42	3833
BAW 1408	63	100	96	54	42	3782
BAW 1409	63	100	94	52	42	3825
BAW 1410	70	103	91	52	40	3835
BAW 1411	66	101	98	53	37	3698
BAW 1412	69	102	90	51	38	3590
BAW 1413	71	103	94	52	36	3455
BAW 1414	70	104	91	52	39	3830
BAW 1415	66	101	97	53	37	3672
BAW 1416	66	102	97	52	37	3031
BAW 1417	71	103	97	55	35	3403
BAW 1418	70	102	88	52	36	3470
BAW 1419	66	101	96	51	34	3343
BAW 1420	61	100	96	42	47	3540
BAW 1421	65	100	97	47	43	3601
BAW 1422	58	99	89	63	40	3998
BAW 1423	62	101	96	44	50	3980
BAW 1424	71	103	99	59	37	3608
BAW 1425	61	101	93	45	41	4056
CV (%)	1.9	1.2	2.6	9.4	6.8	12.3
LSD (0.05)	1	1	2	3	2	317
F-test	**	**	**	**	**	**

*TGW: Thousand Grain Weight

Days to maturity

Days to maturity of wheat, like days to heading, were significantly influenced by sowing times, locations, and genotypes (Tables 3, 4, 5 and 6a). Late sown wheat genotypes completed their life cycle earlier than timely sown wheat genotypes in all four locations, whereas all genotypes took a long time to complete their life cycle under the weather conditions of Dinajpur, followed by Rajshahi, Jashore, and Joydebpur, due to environmental factors, particularly temperature. Among the genotypes, BAW 1402, BAW 1403, BAW 1404, BAW 1405, BAW 1406, BAW 1407 and BAW 1422 took a short time for maturity than the check BARI Gom 21 and BARI Gom 33 might be due to the different genetic makeup. This finding was consistent with the findings of Hossain *et al.*, (2018), who discovered that late-sown wheat completed its life cycle faster than timely-sown wheat. Several studies have found that environmental factors, particularly temperature, influence the days to maturity of wheat genotypes (Spink *et al.,* 1993; Araus *et al.,* 2007 Shahzad *et al.,* 2007).

Plant height (cm)

Plant height is one of the most important parameters of yield contributing characters. In all locations, the highest plant height was in ITS condition (Tables 3 and 6b). In the current study, the favorable environment for plant height was Dinajpur compared to other locations in both ITS and ILS conditions (Table 6b). Considering the genotypes, the lowest plant height was recorded in genotypes BARI Gom 32 and BAW 1418 (Table 5). Plant height data revealed that both sowing dates and varieties had a significant impact on plant height. Anwar *et al.,* (2015) confirmed our findings, observing that plant height was significantly higher under optimal sown conditions.

Grains per spike

The genotypes' performance for trait grains per spike at various sowing times and locations was presented in Tables 3, 4, 5 and 6b. The reproductive stage of wheat is the most temperature sensitive (Hossain *et al*., 2018). In high-temperature stress (above 30 °C) at the flowering stage, nearly all field crops reducing grain set ultimately decreased the grain number per spike due to lower fertilization caused by pollen sterility and/or ovule abortion (Yang *et al.,* 2002; Prasad *et al.,* 2008). In the current study, the highest grain spike was recorded in the ITS condition (Table 3), owing to favorable weather conditions, which ultimately helps to increase grain set. In terms of environmental conditions, Joydebpur's environment was superior to other locations for setting grains per spike (Table 4). BAW1422 and BAW1424 produced more grains per spike than the other genotypes tested in this study. The genotypic difference could be due to genetic variation as well as climatic and edaphic factors as determined by field conditions.

Thousand-grain weight (g)

Heat stress reduces TGW under late sown conditions due to a decrease in individual grain weight, whereas optimum sowing increases TGW (Rahman *et al*., 2018). The highest TGW is obtained as a result of the maximum individual grain weight, which may be due to favorable environmental conditions (Rahman *et al*., 2018). In this study, wheat sown in ITS condition produced the highest TGW, while wheat sown in ILS condition produced the lowest TGW (Tables 3 and 6c). Considering locations, the wheat genotypes produced the highest TGW in Dinapur than other locations (Table 4). The highest TGW was found in BAW 1423 (50 g) followed by BAW 1420 (47g) and BARI Gom 32 (45g) (Table 5). In terms of location, Dinajpur had the highest TGW due to favorable weather conditions during the wheat growth stage (Table 6c).

Table 6a. Interaction effects of location, sowing date and genotype on heading and maturity of wheat genotypes

Genotype	Heading (days)								Maturity (days)							
	Dinajpur		Joydebpur		Jashore		Rajshahi		Dinajpur		Joydebpur		Jashore		Rajshahi	
	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS
BARI Gom 21	75	68	70	66	70	66	75	67	119	101	106	97	110	93	112	99
BARI Gom 32	56	59	58	62	55	60	56	58	108	92	101	94	100	88	100	90
BARI Gom 33	62	62	63	61	61	62	62	61	113	97	103	93	103	91	105	95
BAW 1402	55	57	57	62	56	59	56	58	111	95	102	91	103	89	106	94
BAW 1403	58	59	57	62	57	59	58	59	110	93	103	92	104	87	106	94
BAW 1404	61	61	60	62	60	61	61	62	109	96	104	92	104	90	106	96
BAW 1405	55	58	60	61	58	59	56	58	107	93	103	90	101	88	105	89
BAW 1406	59	58	59	61	58	58	59	58	109	94	103	92	101	89	105	92
BAW 1407	57	58	59	62	60	60	60	59	111	93	103	90	103	89	105	92
BAW 1408	64	64	64	64	64	62	62	62	111	96	106	92	105	91	106	94
BAW 1409	65	63	63	64	64	62	65	62	114	97	103	93	107	91	106	95
BAW 1410	76	67	71	67	76	65	75	65	115	100	106	93	113	93	112	96
BAW 1411	69	63	66	62	70	62	72	62	114	97	104	94	109	91	108	94
BAW 1412	73	67	69	67	75	67	73	66	114	99	105	94	110	94	109	96
BAW 1413	77	68	70	68	78	67	77	67	116	100	105	94	113	93	111	96
BAW 1414	73	66	71	67	76	66	77	66	118	100	106	95	113	94	112	96
BAW 1415	69	65	67	64	70	63	72	63	116	96	102	93	108	93	109	95
BAW 1416	68	64	64	65	71	63	72	62	113	99	105	94	108	93	109	94
BAW 1417	77	68	70	67	78	66	77	67	118	98	105	94	112	94	112	97
BAW 1418	77	67	70	64	76	66	75	66	118	99	105	95	105	93	111	95
BAW 1419	72	63	65	63	69	63	72	63	114	94	103	94	107	90	109	95
BAW 1420	62	57	64	63	63	61	63	61	116	93	104	94	105	90	105	94
BAW 1421	71	63	64	65	68	63	65	60	114	95	105	93	108	93	105	93
BAW 1422	57	59	57	64	57	60	57	58	112	95	102	93	105	90	103	93
BAW 1423	63	63	58	61	61	60	70	61	114	93	103	94	104	102	109	93
BAW 1424	80	68	72	67	77	66	72	67	118	99	105	93	111	93	110	96
BAW 1425	59	64	60	62	61	60	61	60	114	99	104	94	108	90	106	96
CV (%)	1.9								1.2							
LSD (0.05)	2								2							
F-test	**								**							

Table 6b. Interaction effects of location, sowing date and genotype on plant height and grains spike^{-1} of wheat genotypes

Genotype	Plant height (cm)								Grains spike^{-1}							
	Dinajpur		Joydebpur		Jashore		Rajshahi		Dinajpur		Joydebpur		Jashore		Rajshahi	
	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS
BARI Gom 21	108	102	98	86	106	98	101	99	51	43	63	47	61	48	49	49
BARI Gom 32	93	85	81	80	84	90	89	84	44	44	46	44	43	44	44	50
BARI Gom 33	109	105	97	92	100	102	98	100	56	46	61	61	60	52	54	47
BAW 1402	98	99	95	84	99	105	97	100	49	43	54	49	52	54	47	52
BAW 1403	88	91	85	84	91	96	89	90	47	48	52	52	50	50	45	47
BAW 1404	88	91	84	86	95	98	93	89	48	41	52	44	42	45	40	55
BAW 1405	89	88	82	86	93	97	88	90	47	46	56	54	52	47	50	52
BAW 1406	99	86	86	84	90	95	96	91	41	45	55	54	42	45	44	55
BAW 1407	95	85	87	85	90	94	90	89	46	48	55	49	45	51	55	49
BAW 1408	111	96	93	84	96	98	97	95	56	48	59	53	62	58	42	52
BAW 1409	109	94	89	81	96	98	96	94	59	51	58	41	56	56	46	53
BAW 1410	98	95	83	80	98	87	94	92	54	49	59	45	55	47	57	51
BAW 1411	111	99	90	87	106	100	100	95	50	44	65	52	48	58	53	58
BAW 1412	102	90	85	83	92	88	95	91	53	48	61	46	52	56	54	42
BAW 1413	107	91	87	82	102	92	99	92	48	50	71	44	59	56	45	47
BAW 1414	101	95	83	82	101	85	91	90	53	50	55	44	57	48	54	57
BAW 1415	113	100	88	83	104	99	99	96	54	52	60	52	53	60	50	48
BAW 1416	110	102	90	86	103	96	98	94	58	46	59	49	55	48	56	47
BAW 1417	113	98	91	89	102	90	101	95	57	46	62	46	61	56	55	56
BAW 1418	103	89	82	76	91	88	90	89	58	54	60	54	58	51	34	52
BAW 1419	104	99	91	85	100	98	97	97	56	38	60	49	60	53	47	47
BAW 1420	111	92	90	81	97	103	104	93	47	37	47	38	44	44	40	40
BAW 1421	111	98	94	88	99	99	97	91	47	44	50	44	53	48	45	47
BAW 1422	95	93	80	83	90	93	88	89	61	66	71	60	76	64	47	64
BAW 1423	107	100	89	87	98	103	99	91	49	39	47	44	46	41	40	46
BAW 1424	110	106	90	91	107	96	103	93	67	55	62	54	63	57	60	58
BAW 1425	101	97	90	81	95	97	97	92	40	37	49	46	47	49	44	51
CV (%)	2.6								9.4							
LSD (0.05)	5								9							
F-test	**								*							

Table 6c. Interaction effects of location, sowing date and genotypes on TGW and grain yield of wheat genotypes, 2020-21

Genotype	TGW								Grain yield (kg ha^{-1})							
	Dinajpur		Joydebpur		Jashore		Rajshahi		Dinajpur		Joydebpur		Jashore		Rajshahi	
	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS	ITS	ILS
BARI Gom 21	46	41	42	34	45	35	44	41	4447	3244	4137	3469	4001	2721	3528	2564
BARI Gom 32	57	45	47	35	49	34	52	39	5466	3445	4268	3766	4034	3531	3345	2868
BARI Gom 33	46	44	47	38	51	41	48	37	4995	3436	4260	3838	3579	3236	3708	2735
BAW 1402	51	39	42	31	49	40	48	38	5288	3512	4842	4220	5782	4085	4254	3709
BAW 1403	50	42	42	29	47	37	46	37	4789	4162	4833	4342	4532	3605	4328	3868
BAW 1404	28	38	42	33	47	35	47	34	3823	2900	3414	3491	4275	2740	4092	2800
BAW 1405	49	34	44	26	48	39	50	26	3955	2586	3429	3399	4299	3454	3414	2110
BAW 1406	56	39	42	35	49	40	50	30	5068	3354	3916	4252	3929	3418	3520	3330
BAW 1407	56	40	45	29	50	33	49	37	4877	3253	4782	3897	3963	3293	3735	2868
BAW 1408	52	41	43	32	46	39	46	35	4584	2676	4053	4010	4399	3733	3577	3223
BAW 1409	53	42	45	32	49	37	46	38	4970	3751	4117	3573	5104	2909	3499	2679
BAW 1410	45	38	40	32	43	37	43	38	5278	3719	3553	3815	4237	3510	3767	2798
BAW 1411	45	36	38	32	38	35	40	36	4698	2721	4012	3769	4658	3441	3105	3180
BAW 1412	46	40	39	31	41	39	39	32	5219	3195	3701	3207	4058	3080	3138	3120
BAW 1413	48	38	41	26	36	28	39	32	5286	3360	3617	2730	4855	2560	3156	2078
BAW 1414	42	38	39	31	42	40	43	40	5688	3532	3346	3186	4845	2936	3802	3310
BAW 1415	47	35	38	25	42	34	42	36	5951	3438	3492	3225	4033	3082	3435	2719
BAW 1416	49	37	40	23	39	33	41	36	4798	2301	3032	2746	3899	2545	3005	1923
BAW 1417	41	32	36	26	39	35	37	36	4644	1946	4279	3344	4148	2876	3324	2667
BAW 1418	42	40	40	30	39	34	35	32	5660	2797	3932	3238	3133	3164	3204	2633
BAW 1419	40	33	40	21	36	34	35	32	4410	2108	3810	2907	4019	3202	3242	3049
BAW 1420	61	50	46	33	50	41	53	40	5107	2196	3604	3132	4009	3304	4014	2951
BAW 1421	55	44	46	30	47	37	48	39	4811	3679	4128	3337	3728	3060	3659	2407
BAW 1422	52	40	41	28	44	31	44	39	4555	4035	3887	3522	4947	4158	4051	2833
BAW 1423	59	49	51	39	55	48	52	47	5452	3346	4792	4082	4810	3339	3292	2726
BAW 1424	47	40	33	31	38	36	39	37	4264	3705	3534	3219	4201	3788	3289	2863
BAW 1425	44	44	46	35	44	36	43	36	5463	3721	3927	3636	5158	3599	4218	2727
CV (%)	6.8								12.3							
LSD (0.05)	5								896							
F-test	**								NS							

genotypes, BAW 1423 had the highest TGW, followed by check BARI Gom 32, and BAW 1419 had the lowest TGW. All genotypes achieved the highest TGW under ITS conditions, regardless of location. Due to early heading and maturity, high temperatures (soil, air) and a lack of soil moisture (drought) in late sowing reduced individual grain weight (Hossain *et al.,* 2018).

Grain yield

Grain yield was significantly influenced by sowing time, environmental locations and genotypes (Table 3, 4, 5 and 6c). In all locations, the highest yield was obtained in the ITS condition rather than the ILS condition. Wheat that was planted late faced high-temperature stress in the field, followed by drought, which significantly reduced yield. Several reports revealed comparable outcomes (Hossain *et al.,* 2012; Hossain *et al.,* 2013; Hossain *et al.,* 2018). The poor GY of wheat sown in December may be assigned to a decrease in the number of productive tillers/spikes and grains per spike. Considering both seeding time and all the locations, the highest yield was found in genotype BAW 1402 (4461 kg ha^{-1}) followed by BAW 1403 (4307 kg ha^{-1}) and BAW 1425(4056 kg ha^{-1}) and the lowest yield was found in BAW 1416 (3031 kg ha^{-1}). At Dinajpur, the highest grain yield was obtained in BAW 1415 (5951 kg ha^{-1}) in ITS and BAW 1403 (4162 kg ha^{-1}) in ILS condition. The lowest grain yield (1923 kg ha^{-}1) was obtained in BAW 1416 at Rajshahi under ILS conditions. The highest yield loss (37%) due to late seeding was recorded in BAW 1413 while the lowest yield loss (11%) was recorded in BAW 1424 (Table 7).

BpLB, leaf rust and wheat blast

Out of 27 genotypes tested, three genotypes (BAW 1403, BAW 1409 and BAW 1423) were low infection based on area under the disease progress curve (AUDPC) under ITS condition. Singh *et al.,* 2014 also stated that the some inbred recombinant lines of wheat were tolerant to spot blotch in three hotspot regions in India under natural conditions. Under field conditions, one genotype (BAW 1408) was immune to wheat blast, 16 genotypes including three checks were resistant (0.2-10 percent disease index), 4 genotypes were moderately resistant (11-30 percent disease index), 3 genotypes were moderately susceptible (31-50 percent disease index), and 3 genotypes were highly susceptible (76-100 percent disease index). Wheat blast disease was only found in tropical South American regions (Kohli *et al.,* 2011). Wheat blast disease has recently become a major disease in Asia (Islam *et al.,* 2016; Malaker *et al.,* 2016). Among all genotypes, 12 genotypes showed a positive 2NS segment (Alam *et al., 2021*). Under field conditions, the severity of leaf rust varied among advanced genotypes and varieties (Table 7). Varieties/advanced lines demonstrated 0 to 50% severity with various types of disease response, whereas spreader lines demonstrated 80% severity with susceptible reaction. Out of 27 genotypes, 22 genotypes, including three checks, were completely free of leaf rust infection, 3 genotypes displayed moderate resistance (rust severity 11-30%), and 2 genotypes displayed moderate susceptibility (31-50 percent severity). Muhammad *et al.,* (2015) also screened 325 wheat genotypes based on the leaf rust severity scale and discovered that 225 wheat genotypes showed no reaction to leaf rust, 12 genotypes showed a resistant response, 20 moderately resistant, 40 moderately susceptible, 15 moderately resistant to moderately susceptible, and 13 genotypes showed susceptible response.

Table 7. Mean yield, percent yield loss due to late sowing and disease reaction of wheat genotypes

Genotype	ITS	ILS	% Yield loss due to late Seeding	BpLB in Dinajpur (AUDPC)		Wheat Blast index (%) (ILS) at Jashore	2NS	Leaf Rust
				ITS	ILS			
BARI Gom 21	4028	2999	26	47	183	6.65	-	0
BARI Gom 32	4278	3402	20	105	258	5.09	-	0
BARI Gom 33	4135	3311	20	132	217	0.15	2NS	0
BAW 1402	5041	3882	23	121	307	7.45	-	0
BAW 1403	4620	3994	14	91	253	2.13	-	0
BAW 1404	3901	2983	24	200	257	3.87	-	0
BAW 1405	3774	2887	23	224	272	11.62	-	20MSS
BAW 1406	4108	3588	13	177	290	34.25	-	0
BAW 1407	4339	3328	23	229	285	24.18	-	0
BAW 1408	4153	3410	18	103	134	0	-	20MSS
BAW 1409	4422	3228	27	91	157	32.87	-	60s
BAW 1410	4209	3460	18	132	121	0.19	2NS	0
BAW 1411	4118	3278	20	278	210	11.98	2NS	0
BAW 1412	4029	3151	22	372	168	3.54	2NS	0
BAW 1413	4228	2682	37	126	251	3.82	2NS	0
BAW 1414	4420	3241	27	108	168	2.98	2NS	0
BAW 1415	4228	3116	26	200	295	9.51	2NS	0
BAW 1416	3683	2379	35	313	232	25.73	2NS	0
BAW 1417	4099	2708	34	244	226	0.16	2NS	0
BAW 1418	3982	2958	26	198	215	7.44	2NS	0
BAW 1419	3870	2816	27	438	361	32.55	-	0
BAW 1420	4184	2896	31	144	269	100	-	50MSS
BAW 1421	4081	3121	24	264	168	100	-	30MSS
BAW 1422	4360	3637	17	113	173	1.06	-	0
BAW 1423	4586	3373	26	84	182	4.07	-	0
BAW 1424	3822	3394	11	147	301	0.01	2NS	0
BAW 1425	4691	3421	27	102	246	92.3	2NS	0

Conclusion

Based on the overall performance of the experimental results, it can be concluded that irrigated timely sown is better than irrigated late sown conditions for wheat production in Bangladesh. Late planting causes a significant yield loss in every year. Wheat is often late because of delayed harvesting of T. Aman rice, longer time for land preparation, unavailability of labourers, late monsoon and some cases of excess moisture in the soil. As a result, when screening wheat genotypes, late sown conditions are given more weight than optimum sown conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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OVEREXPRESSION OF A DEAD BOX HELICASE *Psp68* GENE AND ITS MUTANT CONFERS SALINITY STRESS TOLERANCE IN BACTERIA

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Abstract

Salinity stress adversely affects crop plant growth and productivity resulting in significant yield losses worldwide. Hence, it is essential to develop stress-tolerant species and as well as to understand the mechanisms behind it. The *Psp68* is a DNA and RNA helicase and it is involved in numerous processes including protein synthesis, maintaining the basic activities of the cell, transcriptional activation/repressors, RNA processing, and abiotic stress response. This study evidence that overexpression of *Psp68* provides salinity (NaCl and LiCl) stress tolerance in bacteria *Escherichia coli* bacterium also. Furthermore, the site-directed mutagenesis technique was applied to create three single mutants namely K168A, Q286G and R461Q, and their expression was also checked in response to the above mentioned stresses. Surprisingly, only the overexpression of single mutant R461Q showed high salinity stress tolerance in *E. coli*. Therefore, this study provides a new tool for developing stress tolerant crop plants and bacteria of agronomic importance in the field of agricultural biotechnology.

Keywords: Cellular stress, *E. coli*, *Psp68*, RNA processing, Salinity stress

Introduction

The helicases are ubiquitous enzymes that catalyze the unwinding of energetically stable duplex DNA or duplex RNA secondary structures (Tuteja, 1997; Tuteja, 2000; Tuteja and Tuteja 2004a and b). These enzymes play an essential role in DNA replication, repair, recombination, transcription, translation, RNA metabolism, and therefore, involved in the basic cellular processes regulating plant growth and development (Kammel *et al.,* 2013; Guan *et al.,* 2013). The helicases play an important role in stabilising growth in plants under stressful environment by regulating stress-induced transcription and translation (Banu *et al.,* 2015; Nidumukkala *et al.* 2019). The p68 is an evolutionarily conserved protein. The human p68 RNA helicase was first identified by immunological cross-reaction with the anti-SV40 large T monoclonal antibody (Crawford *et al.,* 1982). Human p68 cDNA (DDX5) have high homology with eIF4A protein, both have nucleic acid unwinding activity (Scheffner *et al.,* 1989).

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Previous studies with purified p68 protein have shown that it exhibits ATP binding, RNA-dependent ATPase and RNA helicases activities *in vitro* (Iggo and Lane, 1989). The amino acid sequence reveals that p68 protein contains multiple conserved motifs that are well known for all DEAD box RNA helicases (Banu *et al.*, 2015). The overall properties of p68 suggest that it could be an important multifunctional protein involved in protein synthesis, maintaining the basic activities of the cell, transcriptional activation/repressors, RNA processing and abiotic stress response (Banu *et al.*, 2015).

To fight against stresses, plants have develop various unique stress adaptive mechanisms however, the basic cellular responses to stresses are almost similar in prokaryotes, lower eukaryotes and as well as in plants (Kultz, 2003). Therefore, overexpression of the plant stress responsive genes may also provide stress tolerance in the bacteria (Joshi *et al.,* 2009; Joshi *et al.,* 2010; Tajrishi *et al.*, 2011). The objective of this study was to examine whether overexpression of *Psp68* provides salinity stress tolerance in *E. coli* bacterium or not using mutagenesis. Furthermore, the developed single mutations in the conserved motifs of *Psp68* gene were also overexpressed and compared with the growth of the bacteria transformed with mutated and non-mutated *Psp68* genes.

Materials and Methods

Site-directed mutagenesis of *Psp68*

The detail of the *PsP68* gene (Accession number: AF271892.1) was described earlier by Tuteja *et al.*, (2014). The desired point mutations were generated in *PsP68* gene using specific designed forward and reverse primers to develop three mutants viz. K168A, Q286G and R461Q. The Quik Change site-directed mutagenesis kit was used to create the desired point mutations in the *Psp68* gene. The 1.8 kb K168A, Q286G and R461Q mutated *PsP68* DNA fragments were prepared by PCR using normal *Psp68* gene (cloned in pGEM-T easy vector) and appropriate primers (K183A- *Psp68*F, Q286G-*Psp68*F, R461Q- *Psp68*F or *Psp68*R) with desired point mutations designed from *Psp68* gene. Finally, the K183A, Q286G and R461Q, mutated 1.8 kb fragments were cloned in pGEM-T easy vector and the sequence was confirmed by sequencing.

Cloning of mutated and non-mutated *Psp68* in pET28a expression vector

All the mutated (K168A, Q286G and R461Q) and non-mutated *Psp68* genes which were cloned in pGEM-T easy vector were digested with specific restriction enzymes (*Nde*I and *Bam*HI). The digested reaction mixtures were run on the agarose to elute the 1.8 kb fallout from the gel. The eluted fall out band was further ligated into the digested pET28a vector (*Nde*I and *Bam*HI). Ligation reactions were further transformed in DH5α competent cells. The colonies were confirmed by PCR using the gene-specific primers. The recombinant plasmids were again confirmed by sequencing.

Growth of *E. coli* and salt stress responses (NaCl and LiCl) of mutated and non-mutated *Psp68* in transformed cells

The *E. coli* (BL21 cells) were transformed with mutated K168A, Q286G and R461Q and non-mutated *Psp68*-pET28a using the standard technique. The transformed

BL21 cells were grown to log phase OD_{600} = 0.5 and the equal amount of cells were transferred to sterile culture tubes with 10 ml of LB medium containing 50 µg/ml kanamycin, 1 mM IPTG and 200, 400, 800 mM and 1M NaCl or LiCl. The cells were allowed to grow at 37°C and the growth was observed every hour by measuring the OD_{600} using a spectrophotometer.

Results

Preparation of single mutants

The conserved motifs of *Psp68* along with their sequence of motif I (GSGK), motif II (VLDEADRMLDMGFEPQ) and motif VI (YIHRIGRT) are shown in Fig. 1A. On the basis of the characteristics of the conserved motifs, motif I, II and VI were selected for substitution in *Psp68* to create the desired mutants. By using PCR based site-directed mutagenesis, amino acid lysine (K) at position 168 of the motif I, glutamine (Q) at position 286 of motif II and arginine (R) at position 461 of motif VI were substituted to alanine (A), glycine (G) and glutamine (Q) respectively. Finally, the resulted *Psp68* mutants were named as K168A, Q286G, and R461Q.

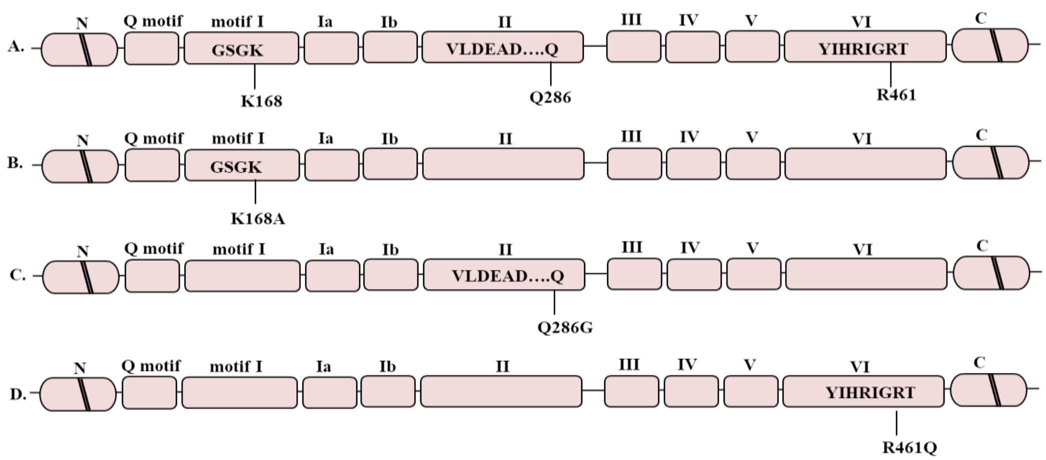

Fig. 1 (A-D). Site-directed mutagenesis in three conserved domains of *Psp68*. A. Schematic diagram showing conserved motifs and the sequence of the three motifs (I, II and VI) on which mutations have been created, B. Schematic diagram of single mutant K168A, C. Schematic diagram of single mutant Q286G, and D. Schematic diagram of single mutant R461Q.

The description of the preparation of single mutants, K168A, Q286G and R461Q were given in the materials and method section. The mutated and non-mutated *Psp68* genes were amplified by using specific primers and first cloned in pGEM-T easy vector. The sequencing results of the cloned single mutants of *Psp68* genes were confirmed the substitution of AAG (code for amino acid K) to GCT (code for amino acid A), CAA (code for amino acid Q) to GGT (code for amino acid G) and AGA (code for amino acid Q) to CAA (code for amino acid Q). Schematic representations of all the single mutants were shown in Fig. 1B-D.

Cloning and restriction analysis of single mutants in pET28a expression vector

All the single mutants of the *Psp68* gene were cloned in *Nde*I and *BamH*I restriction sites of pET28a expression vector under control of T7 promoter and T7 terminator (Fig. 2A). Transformed cells containing recombinant plasmids were identified by colony PCR (Fig. 2B-D). A single colony was re-suspended in 10 µl of water and then boiled for 5 min at 95ºC. After boiling, 2 µl supernatant was used as a template for PCR using gene specific primers. Colony PCR positive clones were further confirmed by restriction analysis (Fig. 2E-G) using *Xho*I restriction enzyme (internal cutter of *Psp68*).

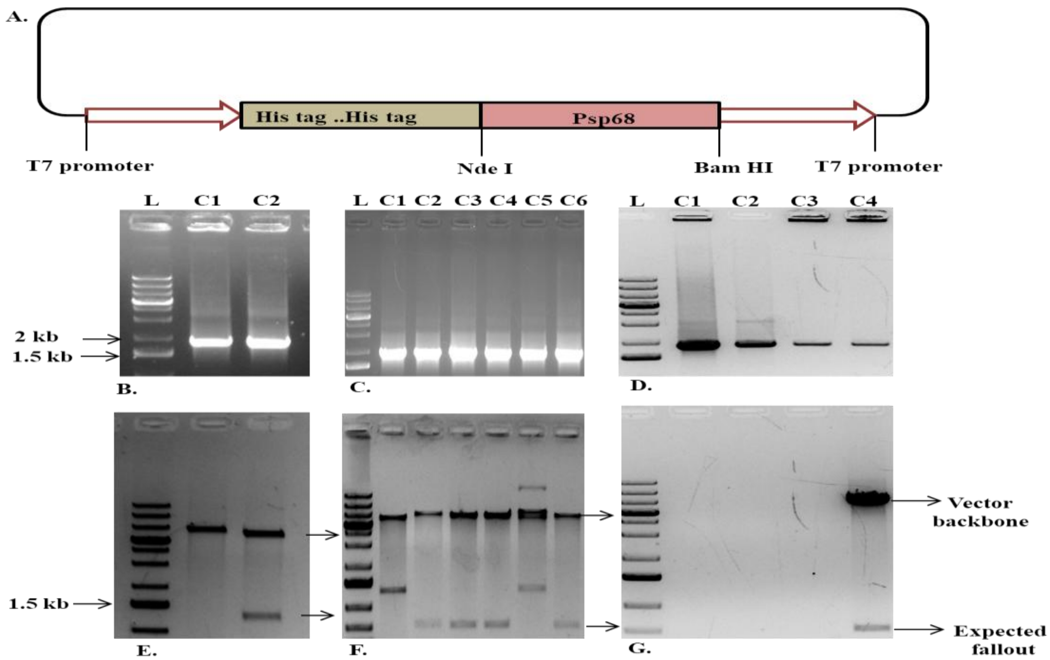

Fig. 2 (A-G). Cloning and restriction analyses of mutants in pET28a. A. Diagrammatic representation of *Psp68*-pET28a construct for expression of *Psp68*. B-D. Confirmation of K168A-*Psp68*-pET28a, Q286G-*Psp68*-pET28a and R461Q-*Psp68*-pET28a respectively by colony PCR. E-G. Restriction analysis of K168A-*Psp68*-pET28a, Q286G-*Psp68*-pET28a and R461Q-*Psp68*-pET28a, respectively by using *Xho*I restriction enzyme**.**

Salinity stress (NaCl and LiCl) responses of mutated and non-mutated *Psp68* in *E. coli*

The *E. coli* (BL21 cells) containing *Psp68*-pET28a and three different single mutated constructs (K168A-pET28a, Q286G-pET28a and R461Q-pET28a) were separately exposed to NaCl salt stress. The cells were grown to log phase OD_{600}= 0.5 and then 1mM IPTG was added to the culture medium to induce gene expression. Along with IPTG, cells were also induced with 200, 400, 800 mM and 1M NaCl concentrations (Fig. 3A). At 200 mM NaCl stress, cells containing *Psp68*-pET28a along with K168A-pET28a and R461Q-pET28a mutants were grown easily while very little growth was observed for Q286G-pET28a mutant (Fig. 3A). No cell growths were detected for K168A-pET28a and Q286G-pET28a mutants at 400, 800 mM and 1M NaCl stress. It is interesting to note that at all the stress treatments (200, 400, 800 mM and 1M NaCl), a high growth was observed in the case of R461Q-pET28a mutant (Fig. 3A). The comparison of the growth curves of the BL21 cells containing wild type *Psp68*-pET28a (non-mutated) along with mutated *Psp68*-pET28a showed that the wild type *Psp68*-pET28a and mutated R461Q-pET28a were able to help *E. coli* tolerate to salinity stress. Whereas, the cells containing K168A-pET28a and Q286G-pET28a mutated proteins were not able to cope up with salinity stress conditions (Fig. 3B).

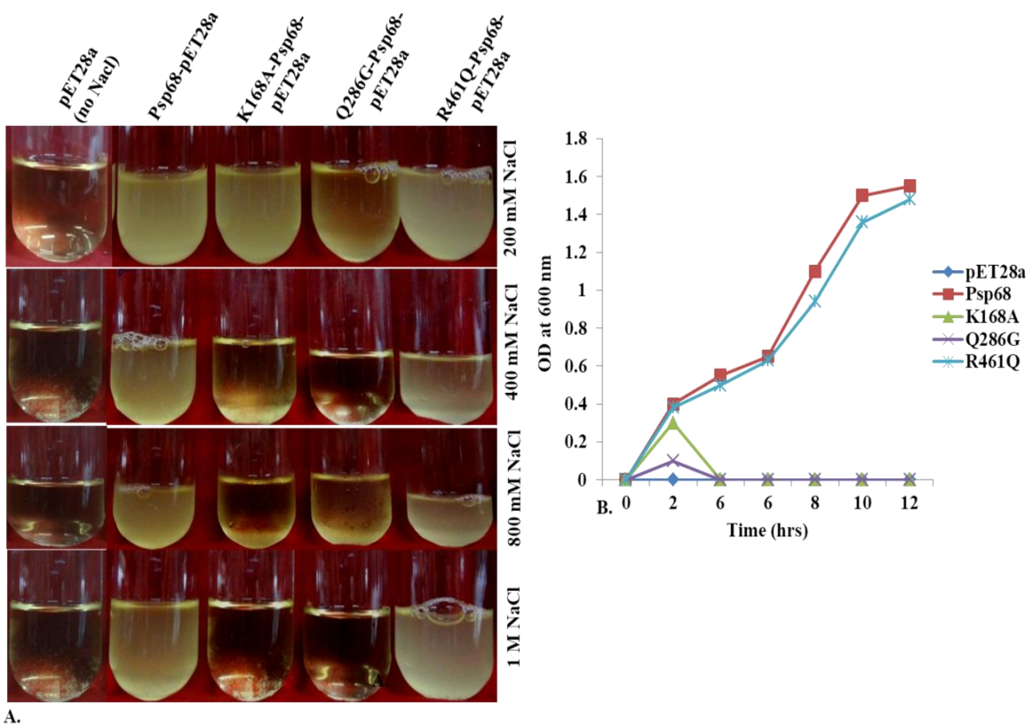

Fig. 3. High salinity (NaCl) stress response of *Psp68* and its mutants in *E. coli*. A. The tubes picture shows the bacterial growth of *Psp68*, single mutants (K168A, Q286G and R461Q), and empty vector after 12 h in the presence of 200 mM, 400 mM, 800 mM and 1M of NaCl. B. Growth curves analysis under salinity stress of *Psp68* and its mutants in *E. coli*.

In another experiment, the *E. coli* (BL21 cells) containing non-mutated (*Psp68*-pET28a) and mutated (K168A-pET28a, Q286G-pET28a and R461Q-pET28a) constructs were separately subjected to grow in the presence LiCl salt stress (200, 400, 800 mM and 1M). The results are shown in Fig. 4, which clearly indicate that the transformed BL21 cells with mutated (R461Q-pET28a) or non-mutated (*Psp68*-pET28a) were equally grow well in presence of all the LiCl stress treatments. The other two mutants (K168A-pET28a and Q286G-pET28a) also showed some growth in presence of 200 and 400 mM LiCl salt stress (Fig. 4).

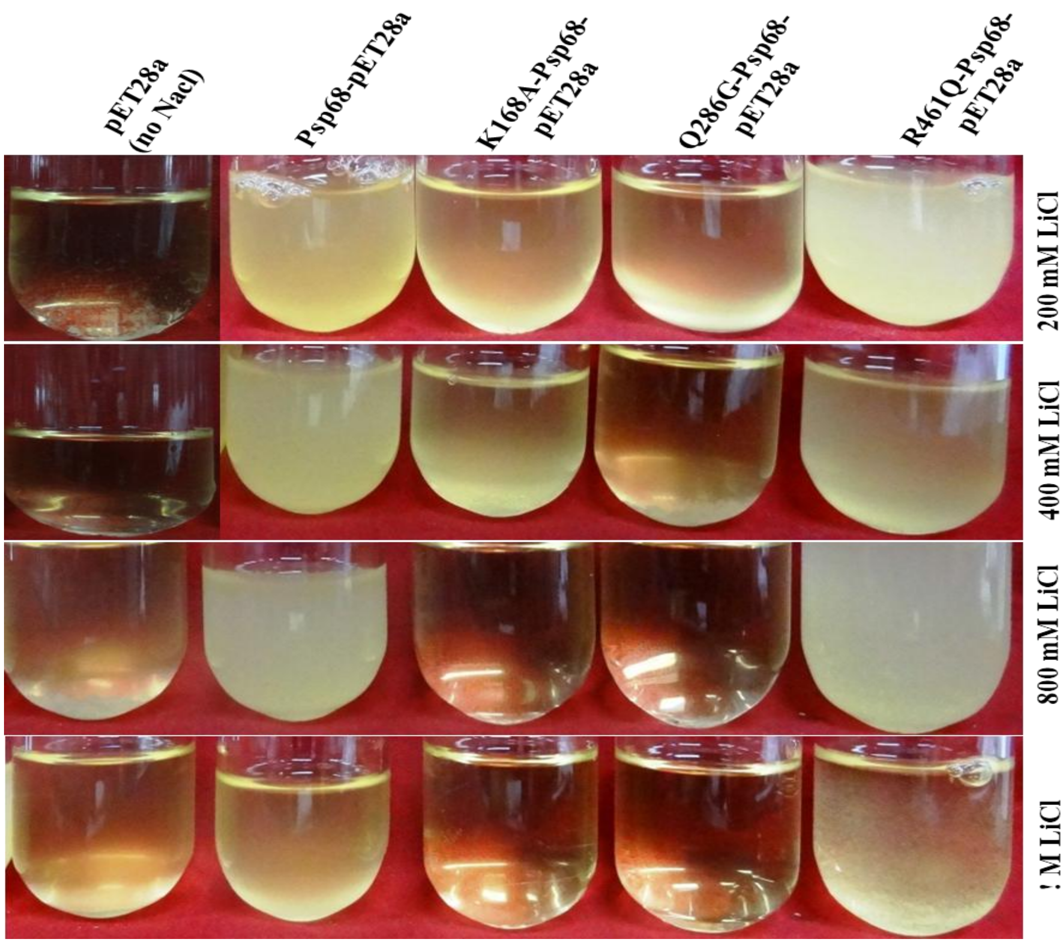

Fig. 4. High salinity (LiCl) stress response of *Psp68* and its mutants in *E. coli*. The tubes picture shows the bacterial growth of *Psp68*, single mutants (K168A, Q286G and R461Q), and empty vector after 12 h in the presence of 200 mM, 400 mM, 800 mM and 1M of LiCl.

Discussion

To adapt to unfavourable environmental conditions, both eukaryotes and prokaryotes have certain stress tolerance mechanism. Cellular stress response in archaea (Macario and Macario 1999), the eubacteria (Hecker and Volker, 2001) and eukaryotes (Pearce and Humphrey, 2001) were always connected with protein and DNA processing and stability (Kültz, 2003). Several sets of homologous/orthologous stress proteins, including chaperones, different cell cycle regulators and DNA repair proteins are induced by environmental stresses in archaea, eubacteria and eukaryotes. Among the various abiotic stresses, the salinity stress adversely affect plant growth and productivity and it is well known that functionally analogous stress resistant genes exist both in unicellular organisms and plants (Serrano *et al.,* 2003). As both the prokaryotes and eukaryotes used the similar basic cellular adaptive mechanisms to tackle the stress, it can be assumed that plant stress tolerance genes can be functionally screened in simple prokaryotic organisms (Joshi *et al.,* 2009; Tajrishi *et al.,* 2011; Xu *et al.,* 2020). This study identified the novel function of *Psp68* in salinity stress tolerance in bacteria with an unidentified mechanism.

The p68 is an evolutionarily conserved member of the DEAD-box protein family of helicases and there are at least five DEAD genes in *E. coli* also. The member of this family has been well characterized by its conserved motifs such as: Q motif, Motif I, Ia and Ib and as GG-doublet- Motif II, III, IV, V and VI (Banu *et al.*, 2015). Each of these conserved motifs has important characteristics. The Q motif is responsible for sensing the nucleotide state of the helicase by forming a stable interaction with Walker A box (P-loop) of other helicase motifs (Strohmeier *et al.,* 2011) while the motif I is crucial for the ATPase and helicase activities by interacting with Mg^{2+} ion (Shi *et al.,* 2004). Mutations in this motif abolish the ATPase activity by reducing the affinity of hydrolysis (Cordin *et al.*, 2004). The motif II is also known as 'Walker motif B' (Venkatesan *et al.*, 1982) and has a role in helicase and ATPase activity (Turner *et al.*, 2007). The DE within this motif is highly conserved and plays a role in DNA and RNA replication (Gorbalenya *et al.,* 1989). The motif VI is important both for ATPase activity and RNA binding (Rogers *et al.,* 2002) and mutation of this motif showed leads to defects in the nucleic acid binding (Pause and Sonenberg, 1992), negative impact on ATP hydrolysis and ligand induced conformational changes in *E. coli* (Tuteja and Tuteja, 2006). In plants *Psp68* has been shown to play an important role in salinity stress tolerance (Banu *et al.,* 2015). The aim of this study was to check whether the same gene can provide the salinity stress tolerance in bacteria or not.

One of the interesting findings of this study is that overexpression of *Psp68* within the *E. coli* bacterium significantly enhanced salinity stress tolerance. More importantly, the mutation of the gene in motif VI provided more salinity stress tolerance than the wild-type *E. coli* suggesting that overexpression of the *Psp68* gene could be utilized for the enhancement of salinity tolerance in organisms including the development of engineered beneficial bacteria with plant growth promoting traits. A considerable genera of plant associated bacteria such as Bacillus, Paraburkholderia Pseudomonas, Delftia etc. have been identified as powerful probiotics that significantly promote growth, yield and quality of crop plants (Khan *et al.,* 2017;Rahman *et al.,* 2018,). Genetic improvement of these natural plant growth promoters for higher salinity tolerance would allow them to use in plant growth promotion under higher level of saline soils.

Salinity stress is one of the most climate change induced abiotic stresses that limit plant growth and productivity. Development of salinity stress tolerant crop plant is an important target to achieve climate-smart agriculture. Our results suggest that overexpression of the *Psp68* gene with mutated motif VI in targeted plant could be an aid for better adaptation of plants to soil salinity (Banu *et al.,* 2015; Nidumukkala *et al.* 2019). Further study is needed to validate this hypothesis by transforming the salinity sensitive crop plants through bioengineering or genome editing.

Conclusion

This study provides a direct evidence that overexpression of *Psp68* and its mutant for motif VI promotes salinity stress tolerance in bacteria. The findings of the current study offer a possibility of the development of salinity tolerant crop plant and also plant growth promoting beneficial bacteria using bioengineering of *Psp68* gene and its motif VI. Further studies are needed to validate this hypothesis that is crucial for the mitigation of soil salinity problem in a large area of cultivated lands.

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Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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EVALUATION OF SHORT DAY LOCAL AND EXOTIC ONION GENOTYPES

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Abstract

Onion is one of the important spices of daily dishes in Bangladesh and are shortage in production of the crop. Due to high photosensitivity in onion, only short-day types are suitable for cultivation in the particular agroclimatic condition of the country. But lack of high yielding potentiality in the existing cultivar along with limited variability within the available germplasm is the major drawback in onion production. To mitigate the problem, the present investigation was done to identify suitable short-day onion genotypes at the Regional Spices Research Centre, BARI, Gazipur during *rabi* 2018-19 and 2019-20. Twenty-nine local and exotic short-day onions were evaluated including two local checks BARI Piaz-1 and BARI Piaz-4. δ^2p and PCV was higher than δ^2g and GCV in almost all the traits studied and higher heritability (h^2b) was observed for total bulb yield. Significant variations were found in morphological and physiological traits for bulb production. Considerably higher bulb length and diameter were found from Ac_G_18_379, Ac_B_18_413, Ac_B_18_420, Ac_B_18_428, BARI Piaz-4, Ac_B_18_419 and Ac_B_18_417. Minimum bulb splitting (%) and bolting (%) were obtained from the genotypes Ac_G_18_379, Ac_G_18_381, Ac_B_18_413 and BARI Piaz-4. Higher dry matter content was noted for the genotypes BARI Piaz-4 (17.9 %), Ac_B_18_425 (21.73 %) and Ac_G_18_384 (21.57 %) along with the TSS ranged from 10.5 to 17.78^{0}Brix. The maximum bulb yield was obtained from the genotype Ac_B_18_413 (20.69 t/ha), followed by Ac_G_18_383 (20.6 t/ha), Ac_B_18_419 (18.48 t/ha) and Ac_B_18_417 (18.2 t/ha). These genotypes could be recommended for commercial cultivation as well as to use in future onion breeding program.

Keywords: Bulb weight, Bulb yield, Short day onion genotypes, TSS

Introduction

Onion (*Allium cepa L.*) is an herbaceous vegetable crop belongs to the family Amaryllidaceous (Alliaceae) which is originated in Iran, western Pakistan and Central Asia (Brewster, 2008) and is widely grown round the globe. Nutritionally it is rich in vitamins, minerals and some soluble sugars (Baliyan, 2014) while also having antioxidant

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and anti-cancerous properties designates as a medicinal crop. Onions are characterized by day length; "long-day" onion varieties will quit forming tops and begin to form bulbs when the day length reaches 14 to 16 hours while "short-day" onions will start making bulbs much earlier in the year when there are only 10 to 12 hours of daylight (Costa *et al.*, 2000). Although it is popular as a vegetable, onion is mostly used as a spice and it is a basic ingredient in Bangladeshi cuisine. Globally onion is grown over 5.4 million hectares with the production over 104.50 million tons annually, where India (26%) and China (23%) account for about half of the world's total onion production (FAO, 2020). Onion ranked first among the spices in Bangladesh, and has been cultivated in 185 thousand hectares of land and the production is 19.54 lakh metric tons (BBS, 2021). Daily per capita onion consumption was 22 grams in 2010, which has increased to 31.04 grams in 2016 (BBS, 2019) indicates an estimated 1.5g increased consumption annually. Every year Bangladesh has to import onion from abroad to fulfill her ever-growing demand. Most of the superior exotic onion genotypes or cultivars are long day (Requiring day length more than 14 hours) which needs longer growing periods for bulb formation and production of larger bulbs. If planted with these high yielding long day varieties in our short-day condition which resembles our winter season, doesn't suit well and generally form only top shoot but bulb formation inhibited which ultimately ends with small sized bulb. Exotic short-day onion genotypes or cultivars (bulb acquiring day length less than 14 hours) can be suited to our climatic condition and form bulbs. The existing available short-day varieties of our country have limited yielding potential much lower than the world average (19.35 t/ha) as well as neighboring countries production, and are not sufficient to fulfill targeted demand. Variability in onion is very scanty in our country. So, introduction of new genetic resources, studying their field level performance, testing the potential to acclimatize in our environmental condition, to select suitable genotype and to recommend for end user level cultivation could be a good strategy to improve the gross onion production. Considering the factors, the present study was undertaken to evaluate some local and exotic short-day onion genotypes along with existing checks for crop improvement and consequently make the suitable potential genotypes available for mass production.

Materials and Methods

A field experiment was carried out at the research station of Regional Spices Research Center, Bangladesh Agricultural Research Institute (BARI), Gazipur, during *rabi* 2018-19 and 2019-20 under irrigated conditions in clay loam soil having soil pH 5.78, organic matter 0.62 %, total N % 0.058 and available P 6.28 µg g^{-1}. A total of twenty-nine short day onion cultivars from different source (local and exotic) were evaluated including two local checks BARI Piaz-1 and BARI Piaz-4 for yield performance (Table 1). The treatments were arranged in randomized complete block design (RCBD) with two replications. Seeds were sown in seedbeds on 1st of November in 2018 and 30th October in 2019 and grown in the seedbeds for 45 days. Day length requirement of the studied genotypes were recorded during growing periods of 2018-19 and 2019-20 and are presented in figure-1. Seedlings were transplanted to the main field at 45 days. The plot size was 3m x 1.2 m and spacing maintained from row to row and plant to plant as 15 cm and 10 cm, respectively. Fertilization was done following recommended dose of cow dung 5t/ha, $N_{115}P_{54}K_{75}S_{20}Zn_3B_2$ Kg/ha (FRG, 2018). The entire quantity of cow dung, P, S, Zn, B and one third of K were applied at the time of final land preparation and the rest K and urea were applied at 25, 50, and 75 days after planting. Irrigation was applied every 15 days interval, and was discontinued 3 weeks before the harvesting. The fungicide (Rovral @ 2.5 g/l) was sprayed at 15 days' interval starting from 45 days after transplanting. The insecticide Admire (Imidacloprid 70 WG) was applied to control thrips. Different onion genotypes were harvested separately on 24 March 2019 and on 28 March 2020 in the respective years. Ten plants from the middle rows were taken for sampling and data recording. Data were recorded on plant height (cm), number of leaves per plants (no.), bulb length (mm), bulb diameter (mm, bulb neck thickness (mm), individual bulb weight (IBW) (g), bulb splitting (%), bolting (%), days to maturity (days), dry matter content of bulbs (%), total soluble solid (TSS) (%) and total bulb yield (t/ha). Whole plot bulb yield was converted into total bulb yield per hectare. Data were analyzed using R platform (R Core Team, 2019).

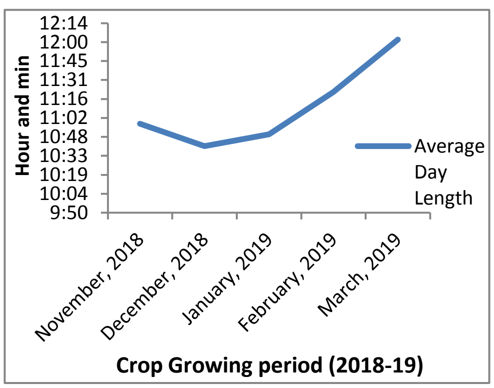
Table 1. Bulb shape and color of 29 short-day onion genotypes collected from different sources

Entry No.	Name of Collection	Source of collection	Bulb shape	Bulb color
1	Ac_B_18_409	IARI, India	Rhomboid	Light Brown
2	Ac_B_18_410	MPUAT, Udaipur, Rajasthan, India	Flat globe	Light Brown
3	Ac_B_18_411	CCS, HAU, Hisar, India	Globe	Light Brown
4	Ac_B_18_412	ICAR-IIHR, Bangaluru, India	Broad elliptic	Red
5	Ac_B_18_413	IARI, India	Globe	Light Brown
6	Ac_B_18_414	ICAR-IARI, India	Rhomboid	Light Red
7	Ac_B_18_415	MPKVP, Rahuri, Maharashtra, India	Rhomboid	Light Brown
8	Ac_B_18_417	IARI, India	Rhomboid	Light Red
9	Ac_B_18_419	May be MP, India or Chennai	Flat globe	Light Brown
10	Ac_B_18_420	PAU, Ludhiana, Panjab, India	Globe	Light Brown
11	Ac_B_18_421	NHRDF, Nashik, India	Flat globe	Light Brown
12	Ac_B_18_422	ICAR-IARI	Flat globe	Light Brown
13	Ac_B_18_424	ICAR-IIHR, Bangaluru, India	Flat globe	Light Brown
14	Ac_B_18_425	NHRDF, Nashik, India	Flat globe	Light Brown
15	Ac_B_18_426	ICAR-IARI, India	Flat globe	Light Brown
16	Ac_B_18_427	PAU, Ludhiana, Panjab, India	Ovate	Light Red
17	Ac_B_18_428	NHRDF, Nashik, India	Flat globe	Light Brown
18	Ac_B_18_429	IIHR, Bangaluru, India	Ovate	Light Brown
19	Ac_B_18_430	ICAR-IARI, New Delhi, India	Flat globe	Light Brown

Table 1. Contd.

Entry No.	Name of Collection	Source of collection	Bulb shape	Bulb color
20	Ac_B_18_431	IIHR, Bangaluru, India	Flat globe	Red
21	Ac_B_18_433	ICAR-IIHR, Bangaluru, India	Globe	Red
22	Ac_G_18_379	Bangladesh	Flat globe	Red
23	Ac_G_18_380	Bangladesh	Ovate	Light Brown
24	Ac_G_18_381	NHRDF, India	Ovate	Light Brown
25	Ac_G_18_382	Bangladesh	Ovate	Light Brown
26	Ac_G_18_383	NHRDF, India	Globe	Light Red
27	Ac_G_18_384	Bangladesh	Flat globe	Light Brown
28	BARI Piaz-1	Regional Spices Research Centre, BARI, Gazipur	Flat globe	Light Brown
29	BARI Piaz-4	Regional Spices Research Centre, BARI, Gazipur	Globe	Red

*Color and shapes of bulbs were recorded following the descriptors for *Allium spp*, IPGRI, 2001



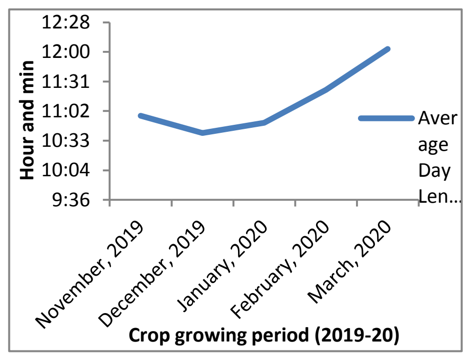


Fig. 1. Average day length of two cropping seasons during *rabi* 2018-19 and 2019-20

Results and Discussion

The analysis of variance for different characters is presented in Table 2 which indicated that there were highly significant differences among the genotypes for almost all of the characters studied except plant height and Total soluble solid (TSS). the variability estimates were presented in Table 3. The genotypic variance revealed that there were significant differences in almost all the characters. Similarly, year × genotype was significant for almost all the characters except for number of leaf and total soluble solute (TSS) indicating greater diversity in the genotypes of the traits but fluctuated over the growing seasons. Multi-environment or multi-year trials are prone to high levels of genotype-environment interaction due to differences in soil types, weather (precipitation, temperature, radiation, evaporation, etc.), and management (fertility levels and levels of protection against pest and diseases) factors (Sangam *et al.,* 2020). In regards to the onion morphological traits, bulb characteristics, and bulb physical and physiological characteristics of the evaluated genotypes showed significant variation which were shown in Table 2.

Genetic variability among genotypes

The variability among the tested genotypes for the target traits allowed for the selection of desirable genotypes for future crop improvement. In the current study, the difference among the genotypes in response to twelve traits over two years were explained, and the results are shown in table 3. It was noted that the highest plant height was obtained in 2019-20 (51.73 cm). No of leaves per plant, days to maturity and bulb length showed almost similar values over two consecutive years. Considering bulb diameter, the maximum value was recorded in 2019-20 (37.83). Considering bulb neck thickness, Bulb Splitting, Bolting, Individual bulb weight, Bulb dry matter content, Total soluble solid and Total bulb yield showed almost similar values over the consecutive two years. The expression of every trait depends on the interaction between genes and environmental factors. Sometimes, more environmental influences hinder the expression of the traits. The variance due to genotype and phenotype indicate the contribution of the heritable part within a trait based phenotypic expression. In the present study the phenotypic variance appeared to be higher than the genotypic variance for all the traits over the years for all the genotypes (Table 3). However, the degree of genetic trait expression depends on the interaction of genotype with environment and farming practices. Previous research results of (Sekara *et al.*, 2017) also agree with the findings of the present study.

In this present investigation, the PCV was comparatively higher than the GCV for all traits, but the closer PCV and GCV for almost all traits over the consecutive two years, indicating the low impact of the environment on the expression of the traits, a symptom of the heritable nature of the traits.

Heritability is a tool that is used to estimate the degree of variation in a group population. The heritability in a group of the population can be classified into three groups (i.e., >80% is high, 40-80% is medium, and low is <40%). In the present investigation over two years' medium to high heritability was observed for almost all the traits.

Plant morphology and bulb characteristics

The mean performance of the genotypes did not vary significantly for plant height (Table 2 & 6). The highest plant height was recorded from Ac_B_18_415 (54.65 cm) which was followed by Ac_B_18_420 (53.63cm), Ac_G_18_382 (53.0 cm), Ac_B_18_419 (52.18 cm) and BARI Piaz-1 (52.50 cm), Whereas the lowest plant height was recorded from Ac_B_18_430 (43.18 cm) which was followed by BARI Piaz-4 (43.75 cm) and Ac_B_18_431 (44.20 cm) (Table 4 & 6). Ibrahim, (2010) as well as Trivedi and Dhumal, (2010) also observed differences in plant height amongst onion genotypes.

Table 2. Full joint combined analysis of variance for bulb yield and desirable traits in onion evaluated at Gazipur during *rabi* 2018-19 and 2019-20

Source of Variation	DF	MSS											
		Plant height (cm)	Number of leaves	Days to maturity	Bulb length (mm)	Bulb diameter (mm)	Bulb neck thickness (mm)	Bulb Spliting	Bolting	Individual bulb weight	Bulb dry matter content	Total soluble solid	Total bulb yield
Year	1	644.38**	3.80**	63.75**	113.33**	1136.32**	9.08**	3.42	5.27	111.05	132.62**	1.53	11.72
Genotype	28	33.96	0.39*	28.57**	39.92**	23.87**	2.63**	304.56**	28.87**	80.2**	21.38**	11.70	58.38**
Year × Genotype	28	37.27*	0.24	27.58**	14.60**	23.91**	2.015**	6.35	32.29**	65.19*	32.54**	10.7	86.48**
Error	56	21.65	0.21	6.68	4.68	7.06	0.76	4.10	12.93	38.42	9.178	7.70	4.06

DF= Degrees of freedom, MSS= Mean sum of square
* and ** indicates significant at 5% and 1% levels.

Table 3. Estimation of genetic parameters in twelve traits of 29 onion genotypes grown in 2018-2019 and 2019-20

Statistics	Plant height (cm)	Number of leaves	Days to maturity	Bulb length (mm)	Bulb diameter (mm)	Bulb neck thickness (mm)	Bulb Spliting (%)	Bolting (%)	Individual bulb weight (g)	Bulb dry matter content (%)	Total soluble solid (%)	Total bulb yield (t/ha)
2018-2019												
x□	47.01	5.96	146.76	29.70	31.57	9.67	9.60	4.66	34.42	19.13	13.40	12.73
h^2b	0.74	0.00	0.97	0.72	0.63	0.42	0.97	0.91	0.93	0.97	0.73	0.92
δ^2g	8.53	0.00	17.04	7.55	6.07	0.22	71.71	17.82	40.42	14.55	3.19	11.60
δ^2p	14.50	0.34	18.02	13.35	13.10	0.83	75.82	21.25	46.26	15.55	5.52	13.72
GCV	6.21	0	2.812	9.25	7.80	4.89	88.18	90.61	18.47	19.94	13.33	26.75
PCV	8.09	9.85	2.89	12.30	11.46	9.45	90.67	98.97	19.76	20.61	17.53	29.09
GA	5.80	0	8.49	5.44	4.72	0.79	17.44	8.66	13.07	7.85	3.55	6.99
Genetic gain	0.12	0	0.06	0.18	0.15	0.08	0	1.86	0.38	0.41	0.26	0.55
2019-2020												
X	51.73	5.59	148.24	31.67	37.83	9.11	9.26	4.23	36.75	16.99	13.63	13.36
h^2b	0.23	0.79	0.41	0.89	0.75	0.75	0.97	0.00	0.707	0.27	0.04	0.86
δ^2g	5.44	0.16	4.35	15.02	10.76	1.34	79.65	0.00	33.678	3.23	0.30	17.85
δ^2p	42.76	0.24	16.74	18.60	17.86	2.25	83.74	22.44	61.46	20.59	13.39	23.86
GCV	4.51	7.08	1.41	12.24	8.67	12.69	96.38	0.00	15.79	10.59	4.03	31.604
PCV	12.64	8.76	2.76	13.62	11.17	16.47	98.83	111.96	21.33	26.713	26.85	36.536
GA	3.04	0.798	3.48	7.94	6.55	2.30	18.38	0.00	11.43	2.58	0.331	8.61
Genetic gain	0.06	0.14	0.02	0.25	0.17	0.25	1.99	0.00	0.31	0.15	0.02	0.644

X: Grand Mean, LSD: Least Significant Difference, CV%: Coefficient of variation, $h^{2:}$ Heritability, δ^2g: Genotypic variance, δ^2p: Phenotypic variance, GCV: genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, GA: Genetic gain

Combined statistics of two years' consecutive study on number of leaves per plant showed significantly different pattern (Table 2 & 6). The number of leaves per plant ranged from 5.28 to 6.58. The results are in agreement with the reports of Boukary, *et al.,* (2012) and Dwivedi, *et al.,* (2012). Ijoyah, *et al*., (2008) observed that the number of leaves per plant in onion is controlled by genetic factors as well as by the environmental factor.

The number of days to maturity is very important as it determines the earliness or lateness of the bulb crop. Marked differences were observed for days to maturity among the genotypes (Table 2). When 80 % of the plant population showed neck fall symptom, we consider that as maturity. Combined analysis (Table 6) Showed that the genotype Ac_G_18_382 (152.25 days) took the maximum days to mature which was followed by Ac_B_18_412 (151.75 days), Ac_B_18_415 (151.75 days), Ac_B_18_420 (151.50 days), Ac_B_18_409 (151.50 days), and Ac_B_18_427 (150.75 days). On the contrary minimum days to maturity were observed in Ac_B_18_425 (142.75 days) which was closely followed by BARI Piaz-4 (143.25 days), Ac_B_18_426 (144.0 days) and BARI Piaz-1 (144.50 days). Days to maturity of onion bulb is influenced by environmental conditions like photoperiod and temperature. Earliness in onions depends on their capacity to initiate bulb formation in a reduced photoperiod and to develop the bulb rapidly after the critical photoperiod is reached. Provided photoperiod and temperature conditions above the critical point, the onion cycle is greatly reduced (Austin 1972).

The bulb length determines the size and shape of onion creating diversity which is very helpful for selecting desirable genotypes. The bulb length varied significantly due to different onion genotypes (Table 3). The highest bulb length was recorded in Ac_B_18_413 (40.0 mm) which was followed by BARI Piaz-4 (39.28 mm), Ac_B_18_419 (34.2 mm) and Ac_G_18_379 (33.18 mm). On the other hand, the genotype Ac_B_18_430 (25.67 mm) produced the lowest bulb length. The findings are agreed with the result obtained by Ishwori *et al.,* (2016), who observed that onion bulbs with high vertical bulb diameter can be stored longer than those with low vertical bulb diameter. The length of the onion bulb is dependent upon the number and size of the green leaves or tops at the time of bulb maturity.

Noticeable variation was observed in bulb diameter in respect of the genotypes (Table 3). Bulb diameter attributes to the size and shape of onion. The genotype Ac_B_18_413 (39.2 mm) produced the highest bulb diameter which was followed by Ac_B_18_420 (38.1 mm), Ac_B_18_417 (37.3 mm) Ac_B_18_433 (37.1 mm), Ac_B_18_428 (37.09 mm) and Ac_B_18_421(37.0 mm). On the contrary, the lowest bulb diameter was recorded in Ac_G_18_380 (27.5 mm). Morozowska, and Holubowicz. (2009) found that diameter of the bulb depends on ring of onion formed by the leaf.

The bulb neck thickness determines the longevity of the stored onion and lesser the thickness is better. The bulb neck thickness ranged from 7.97-11.47 mm (Table 3). The highest neck thickness was recorded in Ac_B_18_420 (11.47 mm) which was followed by Ac_B_18_419 (10.95 mm), Ac_B_18_422 (10.47 mm), Ac_B_18_417 (10.45 mm) and Ac_B_18_424 (10.35 mm). Whereas the lowest neck thickness was recorded in Ac_G_18_380 (7.97 mm). Hirave *et al.,* (2015) reported that, onion bulb with narrow neck thickness stored longer than those with wide collar diameter. The bulb neck thickness is believed to influence the storability of onion.

Table 4. Performances of onion genotypes on plant morphology and bulb characteristics at Gazipur during rabi 2018-19 and 2019-20

Genotype	Plant height (cm)		Number of leaves		Days to maturity		Bulb length (mm)		Bulb diameter (mm)		Bulb neck thickness (mm)	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Ac_G_18_379	49.65	51.20	5.65	5.70	145.00	148.00	37.15	29.21	37.75	35.15	11.36	7.80
Ac_G_18_380	46.00	51.30	6.15	5.40	149.00	142.50	31.00	28.92	24.30	30.75	8.23	7.70
Ac_G_18_381	53.35	49.60	5.80	5.10	149.00	142.50	29.95	34.89	22.90	38.38	7.72	8.70
Ac_G_18_382	51.20	54.80	6.00	6.20	157.00	147.50	32.25	29.10	30.80	34.95	9.76	8.80
Ac_G_18_383	50.85	51.50	6.15	6.00	150.50	146.50	30.70	32.07	28.30	40.60	9.49	7.00
Ac_G_18_384	45.85	51.00	5.50	6.20	142.50	148.50	28.50	24.95	33.40	32.90	9.65	8.10
Ac_B_18_409	45.50	50.80	6.20	5.10	152.50	150.50	28.40	33.08	30.30	41.75	9.06	9.10
Ac_B_18_410	43.15	57.60	5.35	5.20	145.50	149.00	27.70	33.93	31.00	39.80	9.87	7.30
Ac_B_18_411	47.50	48.80	5.70	5.90	148.50	148.00	30.45	28.88	34.40	36.56	9.76	9.50
Ac_B_18_412	48.30	49.20	6.00	5.10	149.50	154.00	28.30	32.12	29.65	43.90	10.02	8.60
Ac_B_18_413	45.85	54.00	6.00	5.30	142.00	148.50	35.45	44.58	35.55	42.89	10.53	7.90
Ac_B_18_414	48.35	47.10	5.85	5.20	149.50	146.00	28.00	29.17	30.60	38.93	9.50	7.90
Ac_B_18_415	48.20	61.10	6.50	5.60	148.50	155.00	26.20	31.70	29.90	35.90	9.11	10.60
Ac_B_18_417	50.85	52.80	6.20	5.50	146.00	145.50	30.65	31.58	34.00	40.68	10.79	10.10
Ac_B_18_419	44.15	60.20	6.35	6.80	149.50	148.00	31.70	36.73	31.25	41.50	10.30	11.60
Ac_B_18_420	48.35	58.90	6.65	5.40	149.50	153.50	29.80	32.85	34.80	41.45	10.45	12.50
Ac_B_18_421	43.50	46.40	6.30	6.20	142.50	150.50	29.20	33.13	34.00	40.00	9.87	8.20
Ac_B_18_422	43.50	50.60	6.35	6.10	141.50	149.00	28.15	28.80	32.55	33.93	10.14	10.80
Ac_B_18_424	44.15	57.60	5.35	5.60	147.50	145.50	27.05	32.25	32.25	35.91	10.00	10.70
Ac_B_18_425	42.00	54.90	6.35	6.30	141.50	144.00	23.80	29.38	29.45	38.71	8.89	9.20
Ac_B_18_426	47.15	49.30	5.50	5.40	142.50	145.50	27.35	30.47	31.15	37.65	9.09	10.20

Table 4. Contd.

Genotype	Plant height (cm)		Number of leaves		Days to maturity		Bulb length (mm)		Bulb diameter (mm)		Bulb neck thickness (mm)	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Ac_B_18_427	45.70	56.60	6.00	5.60	153.50	148.00	28.55	32.77	28.90	35.89	9.17	8.50
Ac_B_18_428	44.65	51.80	5.65	5.60	148.50	147.50	30.15	34.91	30.10	44.08	9.66	9.60
Ac_B_18_429	49.00	48.60	6.00	5.30	146.50	151.50	30.40	31.42	32.30	34.70	9.94	7.90
Ac_B_18_430	46.85	39.50	6.15	5.20	143.50	154.00	28.00	23.35	33.20	29.56	9.43	7.70
Ac_B_18_431	39.20	49.20	5.85	5.40	141.50	150.50	27.60	28.17	33.20	37.10	10.06	9.10
Ac_B_18_433	51.30	52.50	6.00	5.00	149.50	147.50	27.65	33.15	31.70	42.50	9.32	10.40
BARI Piaz-1	53.50	51.50	5.70	5.50	144.00	145.00	27.58	28.04	33.83	35.95	9.31	9.50
BARI Piaz-4	45.80	41.70	5.45	5.30	139.50	147.00	39.55	39.02	33.92	34.94	10.00	9.20
Levlel of significance	**	ns	ns	**	**	ns	**	**	**	**	ns	**
$LSD_{0.05}$	5.01	-	-	0.59	2.03	-	4.93	3.88	5.43	5.46	-	1.96
CV	5.20	11.81	9.85	5.17	0.68	2.37	8.11	5.97	8.40	7.04	8.08	10.49

** 1% level of probability, ns: non-significant LSD: Least Significant Difference, CV%: Coefficient of variation

Table 5. Performances of onion genotypes on physical, physiological and bulb yield at Gazipur during rabi 2018-19 and 2019-20

Genotype	Bulb splitting (%)		Bolting (%)		Individual Bulb Weight (g)		Bulb dry matter content (%)		TSS^0 Brix		Total bulb Yield (T/ha)	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Ac_G_18_379	0.00	0.00	0.00	8.85	29.05	33.22	25.46	12.40	12.78	10.70	16.10	12.67
Ac_G_18_380	4.47	3.90	19.34	0.00	26.55	31.35	16.39	22.16	12.43	15.95	7.61	9.67
Ac_G_18_381	0.00	0.00	0.00	0.00	29.24	32.45	16.01	18.03	11.34	16.70	9.77	17.00
Ac_G_18_382	6.39	2.68	1.19	13.83	37.72	30.58	13.49	23.74	11.35	12.95	10.08	14.00
Ac_G_18_383	2.00	1.32	11.14	6.49	44.10	51.8	15.93	21.46	10.30	12.70	19.45	21.67
Ac_G_18_384	16.47	14.97	0.74	1.08	35.00	27.83	23.76	19.39	15.98	14.30	12.23	9.67
Ac_B_18_409	3.32	4.17	7.50	11.80	37.25	40.86	20.97	15.71	10.63	14.50	9.70	11.77
Ac_B_18_410	4.45	4.50	5.15	6.35	31.50	27.89	15.86	19.08	11.25	15.50	11.22	11.00
Ac_B_18_411	9.16	6.61	12.24	0.87	40.05	30.14	18.71	14.28	13.63	14.70	10.98	12.67
Ac_B_18_412	7.02	3.49	4.20	1.60	40.09	43.23	18.95	12.53	10.90	10.20	13.57	16.85
Ac_B_18_413	0.00	0.00	2.38	2.47	45.50	39.16	27.09	15.83	12.88	9.95	19.39	22.00
Ac_B_18_414	4.45	5.64	3.05	3.52	29.95	27.34	17.96	16.08	15.18	14.95	7.13	10.00
Ac_B_18_415	8.44	5.82	7.35	7.58	38.35	44.88	17.71	14.60	11.33	11.90	15.17	18.00
Ac_B_18_417	12.26	16.33	6.44	3.80	39.35	40.42	12.70	13.77	14.70	15.00	17.08	19.33
Ac_B_18_419	10.16	7.10	3.51	2.44	40.60	38.55	16.32	18.28	12.33	15.45	17.63	19.33
Ac_B_18_420	6.50	1.89	2.44	4.22	41.95	42.35	16.43	18.43	11.38	10.05	9.85	11.67
Ac_B_18_421	19.06	19.03	4.69	6.76	27.50	43.34	25.13	14.80	15.70	10.52	11.74	8.00
Ac_B_18_422	21.02	23.73	0.00	2.55	29.15	29.37	18.86	13.81	16.43	13.90	12.39	7.00
Ac_B_18_424	4.06	4.59	5.94	3.85	33.90	32.89	20.58	16.98	12.78	19.50	13.05	14.33
Ac_B_18_425	20.72	23.51	0.00	2.14	22.85	26.53	19.02	24.45	16.30	19.25	8.54	6.70
Ac_B_18_426	14.12	15.19	1.86	4.78	25.60	27.67	20.13	17.32	14.45	14.80	12.46	9.00
Ac_B_18_427	4.46	1.47	3.30	1.25	36.75	39.87	20.93	14.64	12.85	15.25	11.62	14.33

Table 5. Contd.

Genotype	Bulb splitting (%)		Bolting (%)		Individual Bulb Weight (g)		Bulb dry matter content (%)		TSS⁰ Brix		Total bulb Yield (T/ha)	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Ac_B_18_428	5.92	5.22	6.09	0.75	38.05	33.11	14.99	19.21	11.10	14.70	15.26	17.33
Ac_B_18_429	9.49	13.28	10.40	4.40	28.75	39.71	19.45	12.96	12.65	10.70	6.92	8.00
Ac_B_18_430	31.44	27.17	0.00	2.15	25.15	46.86	20.46	14.91	15.45	10.30	11.70	10.07
Ac_B_18_431	18.48	23.57	3.90	4.20	27.70	40.92	22.76	13.39	15.00	10.30	12.71	7.63
Ac_B_18_433	4.54	5.28	2.96	3.50	41.90	36.9	11.89	13.78	14.15	14.10	13.21	15.33
BARI Piaz-1	30.13	28.11	7.25	6.88	30.55	43.01	23.39	22.66	17.90	12.60	13.17	12.67
BARI Piaz-4	0.00	0.00	2.05	4.60	44.06	43.67	23.35	18.03	15.55	13.90	19.52	20.00
Levlel of significance	**	**	**	ns	**	**	**	ns	**	**	**	**
$LSD_{0.05}$	4.15	4.15	3.80	-	4.95	10.79	2.05	-	3.13	7.41	2.97	5.02
CV	21.10	21.87	19.80	11.96	7.02	14.34	5.22	24.53	11.39	26.54	11.42	18.33

** 1% level of probability LSD: Least Significant Difference, CV%: Coefficient of variation

Table 6. Performances of onion genotypes over the year on plant morphology and bulb characteristics at Gazipur during rabi 2018-19 and 2019-20

Genotype	Plant height (cm)	Number of leaves	Days to maturity	Bulb length (mm)	Bulb diameter (mm)	Bulb neck thickness (mm)
Ac_G_18_379	50.43	5.68	146.50	33.18	36.45	9.58
Ac_G_18_380	48.65	5.78	145.75	29.96	27.52	7.97
Ac_G_18_381	51.48	5.45	145.75	32.42	30.64	8.21
Ac_G_18_382	53.00	6.10	152.25	30.68	32.88	9.28
Ac_G_18_383	51.18	6.08	148.50	31.38	34.45	8.25
Ac_G_18_384	48.43	5.85	145.50	26.73	33.15	8.88
Ac_B_18_411	48.15	5.80	148.25	29.66	35.48	9.63
Ac_B_18_430	43.18	5.68	148.75	25.67	31.38	8.56
Ac_B_18_412	48.75	5.55	151.75	30.21	36.78	9.31
Ac_B_18_420	53.63	6.03	151.50	31.32	38.13	11.47
Ac_B_18_415	54.65	6.05	151.75	28.95	32.90	9.86
Ac_B_18_425	48.45	6.33	142.75	26.59	34.08	9.05
Ac_B_18_424	50.88	5.48	146.50	29.65	34.08	10.35
Ac_B_18_410	50.38	5.28	147.25	30.81	35.40	8.59
Ac_B_18_409	48.15	5.65	151.50	30.74	36.03	9.08
Ac_B_18_428	48.23	5.63	148.00	32.53	37.09	9.63
Ac_B_18_422	47.05	6.23	145.25	28.48	33.24	10.47
Ac_B_18_413	49.93	5.65	145.25	40.01	39.22	9.21
Ac_B_18_431	44.20	5.63	146.00	27.89	35.15	9.58
Ac_B_18_419	52.18	6.58	148.75	34.22	36.38	10.95
Ac_B_18_427	51.15	5.80	150.75	30.66	32.39	8.84
Ac_B_18_421	44.95	6.25	146.50	31.17	37.00	9.04
Ac_B_18_429	48.80	5.65	149.00	30.91	33.50	8.92
Ac_B_18_414	47.73	5.53	147.75	28.59	34.77	8.70
Ac_B_18_417	51.83	5.85	145.75	31.12	37.34	10.45
Ac_B_18_426	48.23	5.45	144.00	28.91	34.40	9.65
Ac_B_18_433	51.90	5.50	148.50	30.40	37.10	9.86
BARI Piaz-1	52.50	5.60	144.50	27.81	34.89	9.41
BARI Piaz-4	43.75	5.38	143.25	39.28	34.43	9.60
$LSD_{0.05}$	6.59	0.66	3.66	3.07	3.76	1.24
CV	9.42	8.01	1.75	7.06	7.66	9.30

LSD= Least significant difference, CV= Co-efficient of variation

Table 7. Performances of onion genotypes over the year on plant physical, physiological and bulb yield at Gazipur during rabi 2018-19 and 2019-20

Genotype	Bulb splitting (%)	Bolting (%)	Individual Bulb Weight (g)	Bulb dry matter content (%)	TSS^0 Brix	Total bulb Yield (t/ha)
Ac_G_18_379	0.00	4.43	31.14	18.93	11.74	14.38
Ac_G_18_380	4.18	9.67	28.95	19.27	14.19	8.64
Ac_G_18_381	0.00	0.00	30.84	17.02	14.02	13.39
Ac_G_18_382	4.53	7.51	34.15	18.61	12.15	12.04
Ac_G_18_383	1.66	8.81	42.95	18.69	11.50	20.56
Ac_G_18_384	15.72	0.91	31.42	21.57	15.14	10.95
Ac_B_18_411	7.88	6.56	35.10	16.49	14.16	11.82
Ac_B_18_430	29.30	1.08	36.01	17.68	12.88	10.89
Ac_B_18_412	5.26	2.90	41.66	15.74	10.55	15.21
Ac_B_18_420	4.20	3.33	42.15	17.43	10.71	10.76
Ac_B_18_415	7.13	7.47	41.62	16.15	11.61	16.59
Ac_B_18_425	22.11	1.07	29.19	21.73	17.78	7.62
Ac_B_18_424	4.32	4.89	33.40	18.78	16.14	13.69
Ac_B_18_410	4.48	5.75	32.20	17.47	13.38	11.11
Ac_B_18_409	3.75	9.65	36.56	18.34	12.56	10.73
Ac_B_18_428	5.57	3.42	35.58	17.10	12.90	16.30
Ac_B_18_422	22.38	1.28	29.26	16.33	15.16	9.69
Ac_B_18_413	0.00	2.43	42.33	21.46	11.41	20.69
Ac_B_18_431	21.03	4.05	34.31	18.07	12.65	10.17
Ac_B_18_419	8.63	2.97	37.08	17.30	13.89	18.48
Ac_B_18_427	2.97	2.28	35.81	17.78	14.05	12.98
Ac_B_18_421	19.04	5.73	35.42	19.96	13.11	9.87
Ac_B_18_429	11.38	7.40	34.23	16.20	11.68	7.46
Ac_B_18_414	5.05	3.29	31.15	17.02	15.06	8.56
Ac_B_18_417	14.29	5.12	37.39	13.23	14.85	18.20
Ac_B_18_426	14.66	3.32	29.14	18.72	14.63	10.73
Ac_B_18_433	4.91	3.23	36.90	12.83	14.13	14.27
BARI Piaz-1	29.12	7.06	36.78	23.02	15.25	12.92
BARI Piaz-2	0.00	3.33	43.87	20.69	14.73	18.76
$LSD_{0.05}$	2.87	5.10	8.78	4.29	3.93	2.85
CV	21.48	28.93	17.51	16.78	20.54	15.44

LSD= Least significant difference, CV= Co-efficient of variation, TSS=Total soluble solid

Physical and physiological characteristics

Splitting of onion bulb greatly reduced the economic value of the crop as well as storability. The genotypes evaluated for bulb splitting (%) showed a great deal of variation (Table 3 & 7). The genotype Ac_B_18_430 (29.3 %) showed highest bulb splitting which was followed by BARI Piaz-1 (29.1%), Ac_B_18_422 (22.38%), Ac_B_18_425 (22.1%) and Ac_B_18_431 (21.0 %). On the other hand, no bulb splitting was recorded from the genotype Ac_G_18_379, Ac_G_18_381, Ac_B_18_413 and BARI Piaz-4. Regarding genotypic effects on bulb splitting the results of the present study were partially in agreement with the report of Jilani and Abdul Ghaffor (2003), where they reported the onion genotypes varied significantly in number of split/double bulbs. The large size sets increased number of doublings, split and early bolting bulbs.

The mean bolting performance of twenty-nine onion genotypes is presented in table 7. Variability was found among the genotypes and it ranged from 0 to 9.67%. The genotype Ac_G_18_380 (9.67 %) and Ac_B_18_409 (9.65 %) showed the highest bolting which was followed by Ac_G_18_383 (8.8 %), Ac_G_18_382 (7.5 %) and Ac_B_18_415 (7.47 %). Whereas no bolting was recorded in Ac_G_18_381 and Ac_G_18_384. Though all the genotypes received the same fertilizer and cultural management, but varied in their performance to induce bolting. It is more of genotypes feature triggered from the input applied during growing stages. This result is consistent with previous reports indicating that genotype influences onion bolting (Rabinowitch, 1990).

A great deal of genotypic variation was observed in case of individual bulb weight (Table 3 & 7). The mean performance of the onion genotypes showed that the highest Individual bulb weight was produced by the check BARI Piaz-4 (43.87 g) which was followed by Ac_G_18_383 (42.95g), Ac_B_18_413 (42.33 g), Ac_B_18_420 (42.15g), Ac_B_18_412 (41.66 g) and Ac_B_18_415 (41.6 g). The lowest individual bulb weight was recorded from Ac_G_18_380 (28.95 g). Clear sunshine and no foggy weather during vegetative growing period and dry weather and no rain at maturity period favors high photosynthetic rate and higher bulb yield. The maximum individual bulb weight may be due to genotypic character, photosynthetic activity and nutrient availability to the plant, which directly influence on the bulb yield. The variation in individual bulb weight among different genotype might be due to genetic characters of the genotypes. The results were similar to Lakshmipathi (2016) and Suhas (2016), where they found different individual bulb weight from different genotypes.

The genotypes exhibited a wide range of variability in respect of bulb dry matter content (table 3 &7). The maximum dry matter was recorded from the check BARI Piaz-1 (23.02 %) which was followed by Ac_B_18_425 (21.73 %), Ac_G_18_384 (21.57%) and Ac_B_18_413 (21.46 %). On the contrary the lowest dry matter content was recorded from Ac_B_18_433 (12.83 %) and Ac_B_18_417 (13.23%). Preliminary selection of genotypes for good storability based on high bulb dry matter content at harvest could be useful but bulb should be evaluated further after storage. Dry matter content is also believed to influence long storage period of bulb onion in India (Mahanthesh *et al.*, 2008) as well as in Nigeria (Kabura *et al.*, 2008). Genotypes with high dry matter have longer shelf-life and these types of genotypes are recommended for industrial processing.

The genotypes showed wide range of variability in respect of TSS (^{0}Brix) (Table 3 & 7). The maximum TSS (^{0}Brix) was recorded from Ac_B_18_425 (17.78^{0}Brix) which was followed by Ac_B_18_424 (16.14^{0}Brix), BARI Piaz-1 (15.25^{0}Brix), Ac_B_18_422 (15.16^{0}Brix) and Ac_G_18_384 (15.1^{0}Brix). On the other hand, lowest TSS was recorded from Ac_B_18_412 (10.55^{0}Brix) which was followed by Ac_B_18_420 (10.71^{0}Brix). Preliminary selection of genotypes for good storability based on high TSS (^{0}Brix) at harvest could be useful, but bulb should be evaluated further after storage, as TSS level undergoes ups and downs depending upon the storage condition and duration (Sohany *et al*., 2016; Dabhi *et al.*, 2008).

Total bulb yield

The total bulb yield (t/ha) was greatly influenced by different onion genotypes and showed range of variability (Table 3 & 7). The total bulb yield ranged from 7.46-20.69 (t/ha). The maximum bulb yield was recorded from the genotype Ac_B_18_413 (20.69 t/ha) which was followed by the genotype Ac_G_18_383 (20.56 t/ha), BARI Piaz-4 (18.76 t/ha), Ac_B_18_419 (18.48 t/ha) and Ac_B_18_417(18.2 t/ha). However, the minimum total bulb yield was recorded from the genotype Ac_B_18_429 (7.46 t/ha) which was followed by Ac_B_18_425 (7.6 t/ha), and Ac_G_18_380 (8.6 t/ha). The bulb yield is a polygenic character greatly influenced by the genotype and environment interaction. The variation in the total bulb yield per plot could be attributed from weight and size of different onion genotypes which might be contributed towards the production of higher bulb yield per plot. Similar finding was also reported by Lakshmipathi (2016) and Suhas (2016).

Conclusion

The collection of short-day genotypes showed significant variation in terms of morphological and physiological traits. Improvement of onion yield contributing traits was possible using phenotypic selection for Bulb length, Bulb diameter, individual bulb weight, Bulb dry matter content, Total soluble solid and bulb yield, of which showed high value for genotypic and phenotypic coefficient of variation coupled with h^2b (Heritability) and GA (Genetic advance). Compared to the check varieties for above mentioned traits the genotypes Ac_B_18_409, Ac_B_18_413, Ac_B_18_417, Ac_B_18_420, Ac_G_18_383, Ac_B_18_412, Ac_B_18_424, Ac_B_18_415, Ac_G_18_379 and Ac_B_18_419 were promising. It is being suggested that these promising genotypes could be used in the breeding program for crop improvement.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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EFFECTS OF TREE LEAF BIOMASS ON THE YIELD AND ITS YIELD CONTRIBUTING CHARACTERS OF HYBRID RICE

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Abstract

A field experiment was conducted in the Agroforestry Field Laboratory of Bangladesh Agricultural University, Mymensingh during the period from November 2016 to April 2017 to find out the effects of Sadakoroi (*Albizia procera*), Kalokoroi (*Albizia lebbeck*) and Akashmoni (*Acacia auriculiformis*), leaves biomass with different fertilizers dose applications on the yield and yield contributing characters of Chinese Hybrid Rice. There were 10 treatments : T_1 = Sadakoroi (leaf biomass 2 kg /plot) + 15% RFD (Recommended fertilizer dose), T_2= Sadakoroi (leaf biomass 2 kg /plot)+ 30% RFD, T_3 = Sadakoroi (leaf biomass 2 kg /plot)+ 45% RFD, T_4 = Kalokoroi (leaf biomass 2 kg /plot) + 15% RFD, T_5 = Kalokoroi (leaf biomass 2 kg /plot) + 30% RFD, T_6 = Kalokoroi (leaf biomass 2 kg /plot) + 45% RFD, T_7 = Akashmoni (leaf biomass 2 kg /plot) + 15% RFD, T_8 = Akashmoni (leaf biomass 2 kg /plot) + 30% RFD, T_9 = Akashmoni (leaf biomass 2 kg /plot) + 45% RFD and T_{10}= Control (100% RFD). The boro hybrid rice var. SQR6 was used as a test crop. The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications. The result showed that green leaf biomass had a significant effect on the yield contributing characters. The panicle length varied from 21.0 to 25.5 cm. The number of leaves on hill^{-1} varied from 40.2-25.9. The number of effective tillers on hill^{-1} varied from 12.27-10.63. The highest grain yield of 8.87 t ha^{-1}was obtained from treatment T_{10} followed by 8.77 tha^{-1} noted with T_3 where Sadakoroi leaf biomass was applied. Therefore, this study suggests that the green leaf biomass of Sadakoroi and Kalokoroi can be applied to the improvement of yield and yield contributing characters of rice.

Keywords: Agroforestry**,** Akashmoni, Grain yield, Kalokoroi,

Introduction

Bangladesh's geographic and agronomic conditions favor rice cultivation in the world's fourth-largest rice producer. The average yield of rice is low in Bangladesh only 2.74 to 3.74 t ha^{-1} (BRRI, 2020), compared to other rice-yielding countries like South Korea and Japan where the average yield is 3.51 and 7.57 million metric tons respectively (USDA, 2022).

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In Bangladesh, about 60% of arable soils have below 1.5% organic matter whereas productive mineral soil should have at least 2.5% organic matter (Hussain *et al.,* 2013). Repeated tillage continuously reduces soil organic matter content leading to hard soils. Further, soil particles become compact and are not able to retain nutrients due to repeat and overuse of chemical fertilizers. The combined application of organic and inorganic fertilizers was found to increase the grain yield of rice over organic or inorganic fertilizers applied alone. Leaf biomass is a very vital organic source of soil fertility development. The decay of leaf biomass affects the amount of N availability for plant uptake. Rice plant mostly depends on organic N available in the soil and rice crop intakes about (50-80) % of their N from the soil (Hossain and Islam, 2022). The decay of leaf biomass provisions organic carbon, nitrogen, phosphorous, potassium, and other nutrients to the soil that are further measured as an important indicator of soil productivity and ecosystem health. Different leaf biomass such as Akashmoni, Sakoroi, and Kalokoroi leaves biomass are good sources of organic matter (Khan *et al*., 2021) and can play a vital role in soil fertility development as well as providing nutrients, especially N, P, and K. So, the use of Agroforestry tree leaf biomass as a source of organic matter and other nutrients for agriculture, as available in agroforestry, significantly reduces a considerable number of chemical fertilizers. The decomposition of leaf biomass is a basic and significant part of biological nutrient cycling and food webs of floodplain forests. Decomposition refers to both the physical and chemical breakdown of leaf biomass and the mineralization of nutrients (Asigbaase *et al.,* 2021). Climatic factors influencing litter decomposition rates include soil temperature and soil moisture (Petraglia *et al.*, 2019). Agroforestry is a fitting and flexible technology that is environmentally sound and ecologically stable because it allows trees in the crop field to add organic matter to the soil. Farmers have openings to use these leaves as green manure for rice cultivation and it is easy to cultivate rice with green leaf biomass. In Bangladesh, there is scope for using green leaf biomass in rice cultivation. Green leaf biomass recovers the soil fertility status, increases the nutrient availability in soil, reduces the soil acidity, or has a great impact on soil adjustment and increases the yield of rice. The study aimed to the effect of green leaf biomass of different trees on the growth performance, grain yield, and fertility status of the soil of rice.

Materials and Methods

The experiment was conducted at the Agroforestry Farm, Department of Agroforestry, Bangladesh Agricultural University, Mymensingh during the period from November 2016 to April 2017. Tree leaf biomasses of Sadakoroi (*Albizia procera*), Kalokoroi (*Albizia lebbeck*), and Akashmoni (*Acacia auriculiformis*) were used to evaluate the yield and yield contributing characteristics of modern Rice cv. SQR-6 (Jonok Raj) from Chongqing Zhong Yi Seed Co., Ltd. It grows well in the Rabi season. The total growth duration of this variety ranges from 145-150 days with an average grain yield of 10-12 t/ha Islam *et al*., (2013). The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications. The total number of plots was 30 and the unit plot size was 5m x 2 m. The spacing between blocks was 60 cm and the plots were separated from each other by 40 cm. The monthly average temperature, humidity rainfall, and total sunshine hours prevailing at the experimental site during the period of study have been collected from Mymensingh.

Treatments

T1 (T1F1) = Kalokoroi + Leaf biomass 2 kg/plot + 15% RFD (Recommended fertilizer dose), T2= (T1F2) Kalokoroi + Leaf biomass 2 kg/plot + 30% RFD, T3(T1F3) = Kalokoroi + Leaf biomass 2 kg/plot + 45% RFD, T4 (T2F1) = Sadakoroi + Leaf biomass 2 kg/plot + 15% RFD, T5 (T2F2) = Sadakoroi + Leaf biomass 2 kg/plot + 30% RFD, T6 (T1F3) = Sadakoroi + Leaf biomass 2 kg/plot + 45% RFD, T7 (T3F1) =Akashmoni + Leaf biomass 2 kg/plot + 15% RFD, T8 (T3F2) = Akashmoni ++ Leaf biomass 2 kg/plot + 30% RFD, T9 (T3F3) = Akashmoni + Leaf biomass 2 kg/plot + 45% RFD and T10= Control (100% RFD).

Tree leaf biomass collection and maintenance

Tree leaf biomasses like Kalokoroi, Sadakoroi, and Akashmoni leaf biomasses were collected from Bangladesh Agricultural University Campus, Mymensingh and chopped by hand, and mixed uniformly with soil during final land preparation and then left to decompose for ten days.

Tree leaf biomass and fertilizer application

Total tree leaves biomass such as *A.procera*, *A.lebbeck*, and *A.auriculiformis* leaves were incorporated in experimental plots before final land preparation. The recommended doses of fertilizer as urea, TSP, gypsum, MoP, and Zine sulfate at the rate of 120, 90, 60, 80, and 10 kg ha^{-1} respectively were applied as basal. Urea was top dressed in three equal splits i.e.,15, 30, and 55 days after transplanting (DAT).

Transplanting of rice seedlings

Thirty-three (33) day-old seedlings of cv. SQR-6 were transplanted on 05 January 2017 with a hill-to-hill and line-to-line distance of 20 cm x 20 cm. Two or three healthy seedlings per hill were used.

Weeds were controlled by uprooting and removing them from the field. The crop was grown under irrigated conditions. Before each top dressing of urea fertilizers, plots were weeded manually.

The crop was harvested at its full maturity. Harvesting was done on 9 May 2017. The plants of individual treatment as tagged previously were separately harvested and threshed as well as yield contributing components.

Recording data and statistical analysis

The plant height was measured with the help of a meter scale from the ground level of the plant to the tip of the leaf. The number of leaves per hill was considered as the leaves present on the hill. The total number of tillers included effective and non-effective tillers. Panicle length was measured from the neck node to the tip of the panicle. One thousand grains were randomly selected from the harvest of each plot. The weight of grains was recorded by an electrical balance and adjusted to a 14% moisture level and converted to grain yield (kg ha-1). The recorded data were compiled and analyzed and the means for all recorded data were calculated. The mean differences were evaluated by Duncan's New Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Results and Discussion
Plant characteristics of rice
Plant height

Plant height was recorded in three stages such as 30, 60 DAT, and after harvest. At the initial growth stage or 30 DAT, plant height in the treatment was almost similar except for the control (Fig. 1). Heights plant height was observed in the treatment T_{10} (46.26 cm) because recommended fertilizer doses were applied to the soil. The lowest value was found in T_7 (40.52 cm) because Akashmoni leaf biomass with 15% RFD was applied and soil fertility status was also low. The maximum plant height was observed in treatment T_{10} (92.28 cm) where fertilizer supplied sufficient nutrients followed by treatment T_6 (90.15cm) where Akashmoni leaf biomass decomposed and added 45% RFD. The lowest height was observed in treatment T_1 (81.07 cm). AT harvest the maximum plant height of 94.83 cm was observed in the treatment T_{10} because of fertilizer application followed by T_6 (93.37 cm). The lowest height was observed in treatment T_1 (83.63 cm) Akashmoni leaf biomass with 15% RFD was applied. These results are in agreement with that of Win *et al.,* (2019) who reported that the addition of biochar and *Bacillus pumilus* strain TUAT-1 increased plant height significantly.

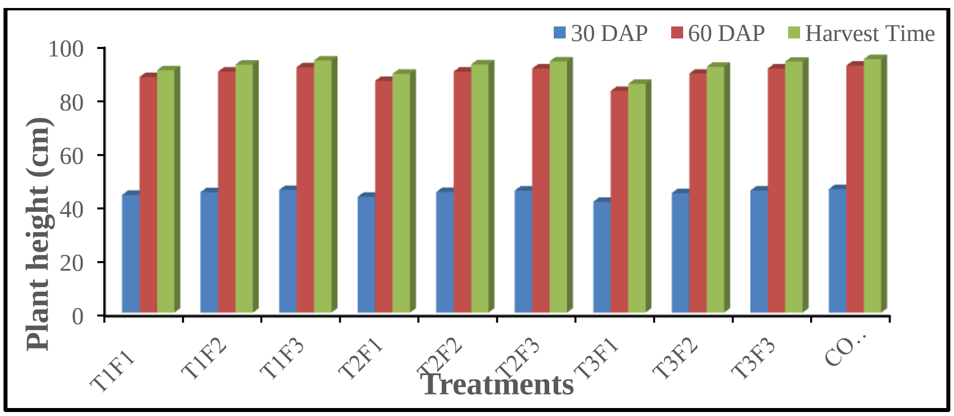

Fig. 1. Plant height of rice due to different treatments

Legend: T1 (T1F1) = Kalokoroi + Leaf biomass 2 kg/plot + 15% RFD (Recommended fertilizer dose), T2= (T1F2) Kalokoroi + Leaf biomass 2 kg/plot + 30% RFD, T3(T1F3) = Kalokoroi + Leaf biomass 2 kg/plot + 45% RFD, T4 (T2F1) = Sadakoroi + Leaf biomass 2 kg/plot + 15% RFD, T5 (T2F2) = Sadakoroi + Leaf biomass 2 kg/plot + 30% RFD, T6 (T1F3) = Sadakoroi + Leaf biomass 2 kg/plot + 45% RFD, T7 (T3F1) =Akashmoni + Leaf biomass 2 kg/plot + 15% RFD, T8 (T3F2) = Akashmoni ++ Leaf biomass 2 kg/plot + 30% RFD, T9 (T3F3) = Akashmoni + Leaf biomass 2 kg/plot + 45% RFD and T10= Control (100% RFD)

Panicle length

The panicle length of rice (cv. SQR6) was significantly influenced by the incorporation of tree leaf biomass. The result showed that the control treatment showed the maximum followed by T_6. The treatment of RFD (T_{10}) produced the highest panicle length of 25.99 cm whereas the lowest panicle length was 20.99 cm from T_7 which was less than all other treatments. These results agree with the report of Wijayanto and Briliawan (2022) that organic manure increased panicle length significantly.

Number of leaves hill^{-1}

The effect of green leaf biomass of different trees on the number of leaves hill^{-1} was non-significant in the treatment T_3 (35.95), and T_9 (36.12) at 30 DAT. At 60 DAT highest value was found in T_{10} (62.00). followed by T_6 (58.33) whereas the lowest was al in treatment T_1 (44.00). Similar results were observed by Islam *et al.*, 2019.

Number of effective tillers hill^{-1}

No. of effective tillers of rice was significantly influenced by the incorporation of tree leaf biomass. The no. of effective tillers varied from 12.27 to 9.72 due to different treatments. The maximum number of tillers was found in RFD (T_{10}) treatment followed by leaf biomass of Sadakoroi+ 45% of RFD. These results were supported by the findings of Bhuiyan *et al.*, (2014).

Table 1. Yield Contributing characters of rice as affected by the incorporation of leaf biomass in the agroforestry system

Treatments	Panicle length (cm)	Leaves/hill^{-1} at 30 DAT	Leaves/hill^{-1} at 60 DAT	Effective tillers hill^{-1}	Non- Effective tillers hill^{-1}
T1:T1F1	22.66e	27.32h	43.67 h	11.01c	1.29e
T2: T1F2	23.01de	32.65e	51.67 e	11.24c	1.59c
T3: T1F3	24.48b	37.37b	58.33 b	11.79b	1.81b
T4: T2F1	22.09f	26.62h	40.00i	10.63d	1.41de
T5: T2F2	22.95de	30.58f	48.33 f	10.99cd	1.77b
T6: T2F3	23.90c	35.49cd	56.33c	11.83b	1.83ab
T7: T3F1	20.99g	25.92hi	39.67i	9.72e	1.35e
T8: T3F2	22.85e	29.35fg	46.67 g	11.01c	1.56cd
T9: T3F3	23.41d	34.49d	54.67d	11.35c	1.79b
T10: Control	25.46a	40.24a	61.00a	12.27a	1.97a
CV (%)	1.18	1.88	1.50	1.94	5.61

Note: Means within the same letter (s) within a column do not differ significantly (P=0.05) according to DMRT.

Legend: T1 (T1F1) = Kalokoroi + Leaf biomass 2 kg/plot + 15% RFD (Recommended fertilizer dose), T2= (T1F2) Kalokoroi + Leaf biomass 2 kg/plot + 30% RFD, T3(T1F3) = Kalokoroi + Leaf biomass 2 kg/plot + 45% RFD, T4 (T2F1) = Sadakoroi + Leaf biomass 2 kg/plot + 15% RFD, T5 (T2F2) = Sadakoroi + Leaf biomass 2 kg/plot + 30% RFD, T6 (T1F3) = Sadakoroi + Leaf biomass 2 kg/plot + 45% RFD, T7 (T3F1) =Akashmoni + Leaf biomass 2 kg/plot + 15% RFD, T8 (T3F2) = Akashmoni ++ Leaf biomass 2 kg/plot + 30% RFD, T9 (T3F3) = Akashmoni + Leaf biomass 2 kg/plot + 45% RFD and T10= Control (100% RFD)

Number of non-effective tillers hill^{-1}

Non-effective tiller was higher in the treatment T_{10} (1.97) whereas lowest in T_1(1.29). The treatments T_3, T_4, T_5, T_9, T_2, and T_8 have no significant differences. Similar results were noted in a study by Barua *et al.*, 2014.

Number tillers hill^{-1}

The number of tillers hill^{-1} of rice (cv. SQR6) was significantly affected by the different treatments. The number of tillers hill^{-1} was initially more or less similar to all treatments. The highest no. of tillers hill^{-1} was found in T_{10} (5) at 30 DAT because of applying REF and the lowest T_7 (3.73) for applying 15% of RFD and leaf biomass of Akashmoni. Treatment $T_{2,}$ $T_{6,}$ T_8, T_3, T_5, and T_7 have no significant difference. At 60 DAP, no. of tillers, hill^{-1}of rice has increased where the maximum number of tillers hill^{-1} was found in T_{10} (14.23) followed by T_6 (13.67) applying 45% of RFD and leaf biomass of Sadakoroi whereas the lowest in T_7 (11) were applying 15% of RFD and leaf biomass of Akashmoni. Treatments T_3, T_6, T_2, $T_{5,}$ and T_9 have no significant difference (Fig. 2). At harvest, the maximum number of tillers hill^{-1} (14) was produced by treatment T_{10} followed by 45% RFD because nutrients were released from Sadakoroi leaf biomass than another leaf biomass. The results are supported by the findings of Bhuiyan *et al.*, (2014).

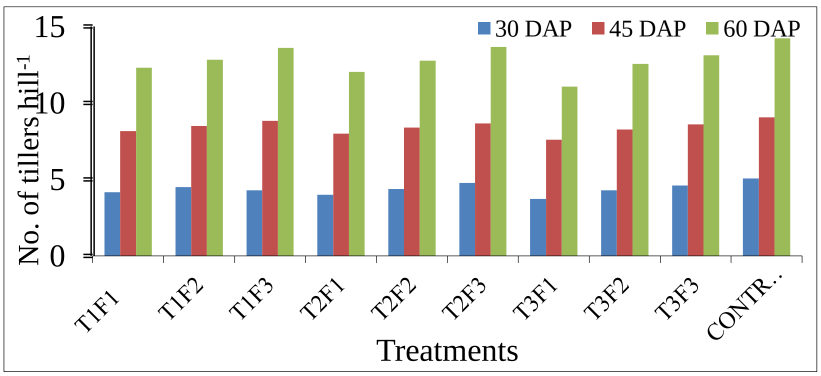

Fig. 2. Number of tillers due to different treatments

Number of spikelet panicle^{-1}

The number of spikelet panicle^{-1} was significantly affected by the different treatments. The number of spikelets panicle^{-1} was divided into three categories such as total, filled, and unfilled. The number of total spikelets in panicle^{-1} varied from 215.13 to 269.07. The highest no. of total spikelet panicle^{-1} (269.07) was found in treatment T_{10} for applying RFD and the lowest one was in treatment T_7 where fertilizer plus 15% leaf biomass of Akashmoni was applied. The treatments were T_2, T_5, T_1, T_4, and T_8 were statistically identical. The highest no. of filled spikelet panicle^{-1} was obtained from the treatment T_{10} (263.07) and the lowest in T_7 (193.07). Unfilled spikelet penile^{-1} was highest in treatment T_7 and lowest in T_{10} (Fig. 3).

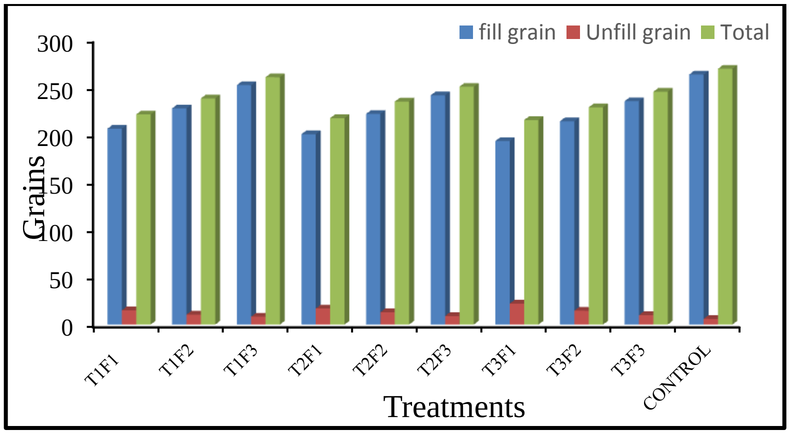

Fig. 3. Grains per panicle rice due to different treatments

1000-grain weight

The maximum fresh weight (34.42 g) of 1000-grain was obtained in treatment T_{10} followed by T_6 (33.48 g). The lowest fresh weight (30.04 g) of 1000 grains was observed in the treatment T_7 plot. Treatments T_3, T_6, and T_9 were statistically significant (Fig. 4). The maximum dry weight (28.40 g) of 1000-grain was obtained in treatment T_{10} followed by treatment of T_3 (28.17 g). The lowest dry weight (23.52 g) of 1000-grain was observed in the treatment T_7 plot. These results are similar to Tian *et al.*, 2017.

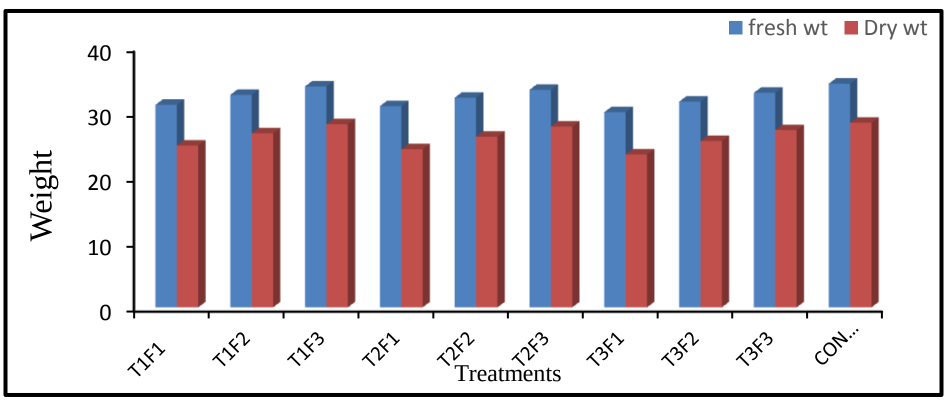

Fig. 4. 1000- grain weight of rice due to different treatments

Grain yield

The highest dry grain yield with the application RFD of rice (cv. SQR6) was found in the treatment T_{10} (8.87 t ha^{-1}) followed by T_7 (8.77 t ha^{-1}) where leaf biomass of Sadakoroi + 45% RFD was applied and the lowest grain yield (7.79 t ha$^{-1)}$ in T_7 (Fig. 5). In Treatment T_{10} and $T_{7,}$ Sadakoroi leaf biomass released nutrients, and 45% RFD applied for that plant got a considerable amount of nutrients which was beneficial for plant growth, development, and physiological process so, grain yield was comparatively better than other Treatments. These results are similar to those of Tian *et al.*, 2017.

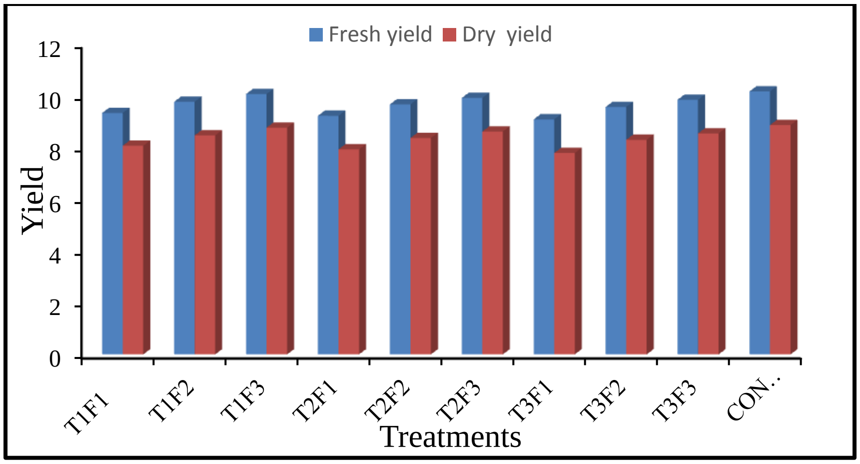

Fig. 5 Grain yield of rice due to different treatments

Legend: T1 (T1F1) = Kalokoroi + Leaf biomass 2 kg/plot + 15% RFD (Recommended fertilizer dose), T2= (T1F2) Kalokoroi + Leaf biomass 2 kg/plot + 30% RFD, T3(T1F3) = Kalokoroi + Leaf biomass 2 kg/plot + 45% RFD, T4 (T2F1) = Sadakoroi + Leaf biomass 2 kg/plot + 15% RFD, T5 (T2F2) = Sadakoroi + Leaf biomass 2 kg/plot + 30% RFD, T6 (T1F3) = Sadakoroi + Leaf biomass 2 kg/plot + 45% RFD, T7 (T3F1) =Akashmoni + Leaf biomass 2 kg/plot + 15% RFD, T8 (T3F2) = Akashmoni ++ Leaf biomass 2 kg/plot + 30% RFD, T9 (T3F3) = Akashmoni + Leaf biomass 2 kg/plot + 45% RFD and T10= Control (100% RFD)

Conclusion

The cultivar SQR6 produced higher grain yield with different doses of RFD, mainly its higher plant height (cm), total number of tillers, number of viable tillers Hill-1, ineffective tillers Hill-1, no. Leaf heel-1, panicle length (cm), number of spikelet panicles-1, full grain panicle-1, incomplete grain panicle-1, and 1000-grain weight (g). It was observed that soil nutrient status improved where green leaf biomass was applied and reduced at low fertilizer levels and maximum grain yield in the control treatment where recommended fertilizer was applied. Rice growth parameters and yield were highest where RFD was applied. Therefore, this study suggests that the green leaf biomass of Sadakoroi and Kalokoroi can be applied to improving soil properties and yielding characteristics of rice.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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GENETIC VARIABILITY STUDIES OF A NOVEL HEALTH PROMOTING LEAFY VEGETABLE RUCOLA *(Eruca sativa)*

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Abstract

Rucola is one of the important leafy vegetables of the 'Brassicaceae' family which is commonly consumed as raw salad. Seven rocula genotypes were evaluated during *Rabi* season 2021-2022 at the experimental farm of Sher-e-Bangla Agricultural University (SAU), Dhaka for genetic variability analysis and were compared with mustard genotype. The results showed that the genotypes were significantly variable for the studied traits. The phenotypic variances and phenotypic coefficient of variations were higher than genotypic variances and genotypic coefficient of variations suggesting the environmental effects on the phenotypic expression of those traits. The high heritability was observed for all the traits except the number of leaves per plant (51.24). Fresh yield per plant showed a significant positive genotypic correlation with number of leaves per plant (0.986) and number of siliquae per plant (0.845) while a significant phenotypic correlation with days to 50% flowering (0.466), plant height (0.473), number of leaves per plant (0.705), leaf length (0.547), days to maturity (0.405) and number of siliquae per plant (0.797). Moreover, seed yield per plant had significant positive correlation with thousand seed weight (0.971) while a non-significant positive correlation with leaf breadth (0.258), number of siliquae per plant (0.030), siliquae length (0.314) and pedicel length (0.168) at both genotypic and phenotypic levels. The results suggest that rucola can be cultivated in the agro-climatic condition of Bangladesh and needs for further genetic improvement of this health promoting crop. This is also the first scientific report of rucola cultivation in Bangladesh.

Keywords: Correlation, Genetic variability, Heritability, Leafy vegetable, Rucola

Introduction

Rucola is a leafy annual vegetable belonging to the mustard family 'Brassicaceae' (Doležalová *et al.*, 2013). The somatic chromosome number of this species is 2n = 22. It is named differently in different countries such as rucola (Italy), arugula (USA), salatrauke (Germany), eruca (Spain) and roquette (France) (Rahim, 2016a). Rucola is native to the Mediterranean region (Goz *et al.*, 2006) and cultivated since Roman times and further introduced and distributed in different countries and continents. It is now commercially grown in Europe (Italy, France and Portugal and

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Czech Republic), Egypt, Turkey, and America (Indiana and Midwest). Rucola is a cool loving plant and grows best in winter. This plant shows rapid leaf growth during winter, but switch to skyward in hot weather and starts to bloom and seed setting. Rucola plants reach about 20-100 cm in height (Rahim, 2016a; Rahim, 2016b). The leaves are succulent, lobed and elongate. All parts of the plant except root are edible (leaves, flowers, siliquae and young and mature seeds). Fresh green rucola leaves are frequently and popularly used in pizza topping and pasta (Doležalová *et al.*, 2013). It is used as a condiment for different delicious meats and fish dishes. Besides, it can be used as salad (raw), cooked and functional plants (Kim *et al.*, 2006) but raw rucola is good for a higher exposure to bioactive phytochemicals like glucosinolates, their hydrolysis products, and also phenolics, flavonoids, and vitamins such as vitamin C (Bennett *et al.*, 2006). It is quite popular as salad with tomatoes, olives and cheese. In addition, the seeds of this species are used to extract edible oil in some part of India (Huang *et al.*, 2014). Now-a-days, the health benefits of vegetables are well-recognized due to the availability of health promoting natural phytochemicals. Rucola possesses a great medicinal value. The capability of this plant to control certain human diseases and disorders like diabetes, cancer, cardiovascular diseases has already been proven from scientific research. According to National Nutrient Database (Standard Reference) of the United States, 100 g of fresh leaves contain only 25 Kcal energy while rich in folic acid (97 µg or 24%), vitamin A (2373 IU), vitamin C (15 mg), vitamin K (108.6 µg) and vitamin B-complexs (Anon., 2016a). Besides, it holds considerable amount of flavonoid compounds specifically flavonol (antioxidant) which act against skin, lung and oral cavity cancers. Rucola leaves also contain ample amounts of minerals like copper and iron and small amounts of calcium, potassium, manganese, and phosphorus. Green rucola leaves are the richest sources of many phytochemicals such as sulfuraphane, glutathione, thiocyanates and isothiocyanates (Barillari *et al.*, 2005; Matthews, 2011). Sulfuraphan can inhibit histone deacetylase, which is known to involve in the development of cancer cells. These phytochemicals are known to fight against prostate, breast, cervical, colon, ovarian cancers. According to the estimation of the Rural Industries Research and Development Corporation (RIRDC) of Australia, green rucola leaves have more anti-cancer potential than other commonly consumed cruciferous vegetable. Since fresh green rucola leaves are usually used in salad, hence its chlorophyll blocks the carcinogenic effects of heterocyclic amines released from fried and grilled meats at higher temperature (Anon., 2016b). It contains alpha-lipoic acid that lowers glucose levels and increases insulin sensitivity (Anon., 2016c). So far, the cultivation of rucola was not noticed anywhere in Bangladesh (Anon., 2016c). However, for the first time, research with this novel health promoting plant has been started by the research group of Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka since 2014. Rucola is suitable for growing around the year in Bangladesh but winter season is the best for leaf growth and production. It starts flowering and seed production at the end of winter and beginning of spring. Rucola produce leaves throughout the year, so a little place in the homestead and 4/5 tubs in balcony or roof top is good enough for year-round leaf production (Anon., 2016c).

A crop improvement program has various activities such as building up a gene pool with variable germplasm, selection of individual from the gene pool and utilization of selected individual to evolve a superior line (Kempthorne, 1957). The genetic variability present in the population, heritability of economically important characters and correlation coefficients of those characters is very important before setting up an effective breeding program. If a plant breeding program is to advance most rapidly and efficiently, knowledge of the phenotypic and particularly of the genotypic interrelationships among and between the yield contributing characters is necessary. The quantification of genotypic correlation for determining the relationships among agronomic characters in diverse population is an effective tool for crop improvement (Bello *et al.*, 2006). Therefore, it is very important to know the genetic variability, heritability, genetic advance and interrelationship among the yield contributing traits of "Rucola" in Bangladesh condition to utilize the potential health benefits of this crop.

Materials and Methods

A total of nine genotypes were used in this experiment (Table 1), of which eight rucola and one mustard. The seeds were collected from Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh. The entire experiment was carried out at the experiment field of Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during *Robi* season 2021-2022. The experiment was laid out at randomized complete bock design (RCBD) with 3 replications and the genotypes were distributed to each plot with each block randomly. The unit plot was 5 m with 4 rows and lines to line and plant to plant distances were 30 cm and 15 cm, respectively. The experimental area was fertilized with chemical fertilizers and cowdung including Urea: TSP: MP @ 55: 160: 160 Kg/ha. Total amount of TSP, MP, along with half of the urea were applied at the time of final land preparation as a basal dose. The second half of the urea was top-dressed at the time of flower initiation. The standard cultural practiced were done during the entire experiment. Plants were harvested at the age 36 days after sowing for fresh vegetative yield which is the economic yield of the plant as well. The siliqua was harvested separately for each genotype when 80% of plants was mature. The data were recorded on ten randomly selected plants for different traits including days to 50% flowering, plant height (cm), number of leaves per plant, leaf length (cm), leaf breadth (cm), fresh yield (g), days to maturity, number of siliquae per plant, siliquae length (cm), pedicel length (cm), number of seeds per siliqua, thousand seed weight (g) and seed yield per plant (g). The recorded data were analyzed using an open source software RStudio. The phenotypic and genotypic variances were determined as per Johnson *et al.,* (1955). Heritability and genetic advance were estimated according to Singh and Chaudhury (1985).

Table 1. Name of the genotypes used in the study

Sl. No.	Advanced populations	Source
1.	Ru/19/GPB-006	GPB[1], SAU[2]
2.	Ru/19/GPB-007	GPB, SAU
3.	Ru/19/GPB-008	GPB, SAU
4.	Ru/19/GPB-009	GPB, SAU
5.	Ru/19/GPB-0010	GPB, SAU
6.	Ru/19/GPB-0011	GPB, SAU
7.	Ru/19/GPB-0012	GPB, SAU
8.	Ru/19/GPB-0015	GPB, SAU
9.	Bra/19/GPB-0089	GPB, SAU

[1]GPB: Department of Genetics and Plant Breeding
[2]SAU: Sher-e-Bangla Agricultural University

Results and Discussion

Morphological characteristics

Rucola is an annual herbaceous plant (Fig. 1). It forms a rosette of basal leaves. They are pinnatifid-oblanceolate with several small lateral lobes and a larger terminal lobe for most of the genotypes except G3 and G5 (Fig. 1). The G3 and G5 showed narrow leaves as compared to rest of the genotypes. Most of the rucola genotypes were late flowering except G3 (42 days) and G5 (42.67 days) compared to mustard genotype G9 (31.33 days) (Table 2). Shinwari *et al.* (2013) reported that a range of 57-99 days for 50% flowering period in rucola genotypes. Similarly, furthermost rucola genotypes were higher in height except G3 (76.45 cm) and G5 (72.37 cm) compared to mustard (G9, 85.07 cm) (Table 2). The maximum plant height 153 cm and minimum 17 cm were reported by Huang *et al.* (2014) in *Eruca sativa*. Shinwari *et al.*, (2013) reported a range 45.0-263.7 cm for plant height in rucola. All the rucola genotypes contained higher number of leaves compared to G9 which had only 12.8 leaves per plant (Table 2). The narrower leaves were recorded in the G3 (1.44 cm) and G5 (1.57 cm) whereas the shorter in leaf length were found in G3 (14.88 cm), G5 (15.59 cm) and G9 (15.59 cm) (Fig. 2 and Table 2). The average leaf length and breadth were 23.83 cm 5.64 cm, respectively (Table 2). The rucola genotype G9 had higher fresh weight of leaves compared to mustard (59.71 g). The maximum fresh weight of leaves was observed in G8 (125.2) harvested at the 35 days after sowing followed by G7 (86.67) and G1 (86.40) (Table 2). Further, the earliest siliqua maturity was recorded in mustard G9 (mustard genotype) which requires only 78.33 days while the rucola genotypes were mostly late maturing except G3 (80.33 days) and G5 (88.33 days) (Table 2). The rucola genotypes had higher number of siliquae per plant than mustard (G9, 98) except G4 (95.60) and G5 (91.67) while the maximum in G8 (173.47) (Table 2). The maximum 474 and minimum only 3 siliquae per plant were reported by Huang *et al.* (2014) in rucola. The highest siliquae length was found mustard (G9, 6.47 cm) followed by G5 (6.41 cm) and G3 (6.35 cm) and lowest in G4 (2.63 cm) followed by G1 (2.72 cm) (Table 2). The maximum pedicel length was found in rucola G5 (2.10 cm) followed by G3 (1.83 cm) and mustard (G9, 1.60 cm) whereas the minimum in G4 (0.44 cm). Among the rucola genotypes, G3 (68.91) and G5 (51.81) showed higher number of seeds per siliqua as compared to mustard G9 (24.64). The remaining rucola genotypes had lower number of seeds per siliqua (Table 2). All the rucola genotypes showed much lower 1000-seed weight as compared to mustard (2.82 g) while the lowest in G3 (0.09 g) and G5(0.09 g) (Table 2). The maximum seed yield per plant was found in mustard G9 (6.69 g) whereas all the rucola genotypes showed much lower seed yield per plant (Table 2). Average 7.9 g seed yield per plant was also reported in rucola by Shinwari *et al.* (2013) which is much more than in Bangladesh. In addition, two rucola genotypes (G3 and G5) exhibited extremely smaller sized seeds (Fig. 3) as compared to other rucola and mustard genotypes analyzed in the study. The higher number of seeds per siliquae in G3 and G5 might be due to their smaller seeds and seed weight. Although they had highest number of seeds per silique but lowest seed yield per plant.

Fig. 1. Rucola genotypes used in the study

Fig. 2. Leaves of rucola genotypes used in the study

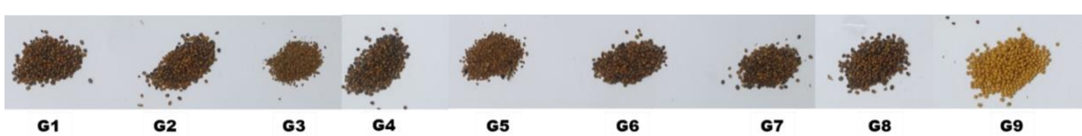

Fig. 3. Seed color of rucola genotypes used in the study

Genetic Variability

The analysis of variance (ANOVA) for 13 quantitative traits, including days to 50% flowering (DFF), plant height (PH), number of leaves per plant (NLP), leaf length (LL), leaf breadth (LB), fresh yield (FY), days to maturity (DM), number of siliquae per plant (NSP), siliqua length (SL), pedicel length (PL), number of seeds per siliqua (NSS), thousand seed weight (TSW), and seed yield per plant (SYP) showed highly significant differences among the tested rucola genotypes (Table 3) which suggests there are inherent genetic variations among tested genotypes. Therefore, the genetic improvement through phenotyping selection for these traits on tested genotypes would be effective. The phenotypic variances (σ^2p) were higher than the genotypic variance (σ^2g) for the evaluated traits suggested the influences of environment on the phenotypic expression of these traits (Table 3). The higher σ^2g were found for DFF, PH, LL, FY, DM, NSP and NSP (Table 3). The results indicating the occurrence of high genetic variability for these traits. The phenotypic coefficient of variation (PCV) was also higher than the genotypic coefficient of variation (GCV) for all the traits, which indicates the variations among genotypes were not only due to the genetic variations but also environmental influences. The PCV ranged from 12.71 to 129.02 whereas the GCV ranged from 12.40 to 128.97 (Table 3). Heritability was classified as low (0-30%), moderate (30- 60%) and high (60% and above) (Robinson *et al.*, 1949). Likewise, the genetic advance as a percentage of the mean (GAPM) was classified as low (0 -10%), moderate (10-20%) and high (20% and more) (Johnson *et al.*, 1955). The high broad sense heritability was detected for most of the evaluated traits except NLP. Nevertheless, the high broad base heritability with high genetic advance in percentage of mean were observed for DFF, PH, LL, LB, FY, DM, SL, PL, NSS, TSW and SYP (Table 3), which indicates that these traits under additive genetic control and selection would be effective (Panse and Sukhatme, 1967; Synrem *et al.*, 2014).

Correlation studies

The correlation among studied characters with yield is the important criteria for phenotypic selection (Mekonnen *et al.*, 2014). Since yield is a polygene character and greatly influenced by the environment. The phenotypic selection based on simply yield is ineffective. Therefore, correlation coefficients among various yield contributing traits aid the selection process in breeding program. The genotypic and phenotypic correlation coefficients among thirteen characters are presented in Table 3 and Table 4. The genotypic correlation coefficients were higher than phenotypic correlation coefficients for the evaluated traits. Fresh yield (FY) of rucola leaves had a highly significant positive correlation with number of leaves per plant (NLP) and number of siliquae per plant (NSP) at the genotypic level, while NLP, NSP and leaf length (LL) at the phenotypic level. It had significant positive correlation with days to 50% flowering (DFF), plant height (PH), days to maturity (DM), whereas significant negative correlation with siliquae length and 1000-seed weight (TSW) at the phenotypic level only. DFF showed significant positive correlation with plant height (PH), NLP, NSP, leaf length (LL), leaf breadth (LB) and DM while significant negative correlation with siliquae length (SL), pedicel length (PL) and number of seeds per siliquae (NSS) at both the genotypic and the phenotypic levels. PH had significant positive correlation with NLP, LL, LB and DM whereas significant negative correlation with SL, PL, and NSS at both the genotypic and the phenotypic levels. Huang *et al.* (2014) found similar results in *Eruca sativa*. Moreover, it had significant positive correlation with FY and NSP at the phenotypic level only.

Table 2. Mean performance of rucola genotypes used in the study

Genotypes	DFF	PH	NLP	LL	LB	FY	DM	NSP	SL	PL	NSS	TSW	SYP
G1	81.33a	144.31a	17.87ab	28.85ab	6.717ab	86.40b	144.33a	117.00c	2.72b	0.49d	15.09d	0.53b	1.63c
G2	81.33a	134.59a	16.67b	27.64bc	6.76ab	70.38c	145.67a	100.72de	2.78b	0.46d	14.59d	0.43d	0.77e
G3	42.00c	76.45b	14.93bc	14.88d	1.44c	79.67b	80.33c	108.00cd	6.35a	1.83b	68.91a	0.09e	0.60e
G4	81.33a	130.85a	17.27ab	25.84c	6.52ab	85.00b	145.67a	95.60de	2.63b	0.44d	15.57d	0.39d	1.38cd
G5	40.67c	72.37b	16.4b	15.59d	1.57c	80.67b	88.33b	91.67e	6.41a	2.10a	51.81b	0.09e	0.58e
G6	80.00ab	139.08a	16.40b	27.41bc	7.12ab	85.00b	141.67a	101.40de	2.76b	0.46d	16.99d	0.47c	1.02de
G7	80.67ab	142.39a	18.07ab	28.35abc	6.87ab	86.67b	142.67a	142.67b	2.81b	0.53d	17.61d	0.50bc	1.72bc
G8	79.00b	144.36a	20.77a	30.37a	7.84a	125.20a	138.67a	173.47a	2.69b	0.55d	15.40d	0.49bc	2.21b
G9	31.33d	85.07b	11.80c	15.59d	5.9b	59.71d	78.33c	98.00de	6.47a	1.60a	24.64c	2.82a	6.69a
Grand mean	66.41	118.83	16.69	23.83	5.64	84.29	122.85	114.28	3.96	0.94	26.74	0.65	1.84

DFF, Days to 50% flowering; PH, Plant height (cm); NLP, Number of leaves per plant; LL, Leaf length (cm); LB, Leaf breadth (cm); FY, Fresh yield (g); DM, Days to maturity; NSP, Number of siliquae per plant; SL, Siliquae length (cm); PL, Pedicel length (cm); NSS, Number of seeds per siliqua; TSW, Thousand seed weight (g) and SYP, Seed yield per plant (g). Letters indicates significant differences among genotypes.

NLP had significant positive correlation with LL, FY, DM and NSP while significant negative correlation with SL, PL, 1000-seed weight (TSW) at both the genotypic and the phenotypic levels. LL had significant positive correlation with LB and DM whereas significant negative correlation with SL, PL and NSS at both the genotypic and the phenotypic levels. It had also significant positive correlation with FY and NSP at the phenotypic level only. LB had significant positive correlation with DM and NSP while significant negative correlation with SL, PL and NSS at both the genotypic and the phenotypic levels. DM had significant negative correlation with SL, PL and NSS at both the genotypic and the phenotypic levels. SL had significant positive correlation with PL and NSS at both the genotypic and the phenotypic levels. PL had significant positive correlation only with NSS at both the genotypic and the phenotypic levels. The rucola seed yield per plant (SYP) had a highly significant positive correlation with TSW at both the genotypic and the phenotypic level. It had a non-significant positive correlation with LB, NSP, Sl and PL at both the genotypic and the phenotypic levels. A similar result was reported by Banglian *et al.* (2014) and Shinwari *et al.* (2013) reported a non-significant positive correlation in *Eruca sativa*. The results disagreed with the finding of Gnanasekaran *et al.*, (2008) and Yol *et al.*, (2010) who observed negative and significance correlation of these traits with seed yield per plant in sesame. Moreover, SYP had significant negative correlation with DFF and NLP at the phenotypic level only. Plant breeders looks for genetic variation among plant characters to select desirable ones as the traits are correlated one with another and directly associated with yield, hence the correlation among these traits with yield would be helpful for selection suitable rucola genotypes in Bangladesh condition.

Table 3. Estimation of genetic parameters for different traits of rucola genotypes

Traits	GMS	CV (%)	$σ^2_g$	$σ^2_P$	$σ^2_e$	GCV	PCV	h^2_b	GA	GA (%)
DFF	1388.81**	1.74	462.49	463.83	1.34	32.38	32.43	99.71	44.24	66.62
PH	2907.09**	7.05	945.66	1015.78	70.12	25.88	26.82	93.10	61.12	51.44
NLP	17.77**	12.40	4.50	8.78	4.28	12.40	12.71	51.24	3.12	18.74
LL	125.92**	6.15	41.26	43.41	2.15	26.95	27.64	95.05	12. 90	54.12
LB	17.24**	18.48	5.39	6.47	1.08	41.17	45.13	83.24	4.36	77.38
FY	943.94**	6.19	305.55	332.82	27.26	20.74	21.64	91.81	34.50	40.93
DM	2805.18**	3.71	928.12	948.94	20.83	24.80	25.07	97.81	62.07	50.52
NSP	2186.60**	6.70	709.35	767.89	58.54	23.31	24.25	92.38	52.73	46.14
SL	10.15**	4.22	3.37	3.40	0.03	46.40	46.59	99.18	3.77	95.18
PL	1.42**	8.68	0.47	0.48	0.01	73.13	73.64	98.61	1.41	149.58
NSS	1172.25**	10.22	388.26	395.73	7.47	73.70	74.41	98.11	40.21	150.38
TSW	2.08**	3.85	0.693	0.694	0.001	128.97	129.02	99.91	1.72	265.56
SYP	10.82**	17.75	3.57	3.67	0.11	102.57	104.09	97.10	3.84	208.20

DFF, Days to 50% flowering; PH, Plant height (cm); NLP, Number of leaves per plant; LL, Leaf length (cm); LB, Leaf breadth (cm); FY, Fresh yield (g); DM, Days to maturity; NSP, Number of siliquae per plant; SL, Siliquae length (cm); PL, Pedicel length (cm); NSS, Number of seeds per siliqua; TSW, Thousand seed weight (g) and SYP, Seed yield per plant (g).

Table 4. Genotypic correlation among different traits of rucola genotypes

	DFF	PH	NLP	LL	LB	FY	DM	NSP	SL	PL	NSS	TSW
DFF												
PH	0.973**											
NLP	0.858**	0.776*										
LL	0.974**	0.984**	0.876 **									
LB	0.774*	0.941**	0.510	0.894**								
FY	0.485	0.495	0.986**	0.565	0.338							
DM	0.997**	0.982**	0.832**	0.983**	0.811**	0.444						
NSP	0.403	0.516	0.830*	0.560	0.454	0.845 **	0.362					
SL	-0.994**	-0.995**	-0.831**	-0.987**	-0.860**	-0.484	-0.999 **	-0.428				
PL	-0.959**	-0.991**	-0.684*	-0.966**	-0.930**	-0.372	-0.972**	-0.379	0.983**			
NSS	-0.746*	-0.869**	-0.442	-0.836**	-0.999**	-0.219	-0.807**	-0.298	0.820**	0.875**		
TSW	-0.444	-0.217	-0.753*	-0.295	0.244	-0.457	-0.374	-0.132	0.334	0.173	-0.231	
SYP	-0.434	-0.192	-0.617	-0.263	0.251	-0.308	-0.372	0.018	0.321	0.173	-0.234	0.985**

DFF, Days to 50% flowering; PH, Plant height (cm); NLP, Number of leaves per plant; LL, Leaf length (cm); LB, Leaf breadth (cm); FY, Fresh yield (g); DM, Days to maturity; NSP, Number of

Table 5. Phenotypic correlation among different traits of rucola genotypes

	DFF	PH	NLP	LL	LB	FY	DM	NSP	SL	PL	NSS	TSW
DFF												
PH	0.934**											
NLP	0.622**	0.596**										
LL	0.949**	0.966**	0.645**									
LB	0.707**	0.789**	0.292	0.773**								
FY	0.466*	0.472*	0.705 **	0.547**	0.291							
DM	0.986**	0.929**	0.575**	0.938**	0.752**	0.405*						
NSP	0.384*	0.494**	0.566**	0.534**	0.435*	0.797**	0.346					
SL	-0.988**	-0.956 **	-0.585**	-0.967**	-0.767**	-0.461*	-0.982**	-0.401*				
PL	-0.948**	-0.956**	-0.477*	-0.936**	-0.826**	-0.367	-0.949**	-0.369	0.9767**			
NSS	-0.738**	-0.826**	-0.308	-0.802**	-0.907**	-0.195	-0.799**	-0.289	0.805**	0.855 **		
TSW	-0.4423*	-0.210	-0.530**	-0.287	0.226	-0.439*	-0.370	-0.127	0.332	0.171	-0.228	
SYP	-0.429*	-0.189	-0.454*	-0.253	0.258	-0.281	-0.360	0.030	0.314	0.168	-0.219	0.971**

DFF, Days to 50% flowering; PH, Plant height (cm); NLP, Number of leaves per plant; LL, Leaf length (cm); LB, Leaf breadth (cm); FY, Fresh yield (g); DM, Days to maturity; NSP, Number of siliquae per plant; SL, Siliquae length (cm); PL, Pedicel length (cm); NSS, Number of seeds per siliqua; TSW, Thousand seed weight (g) and SYP, Seed yield per plant (g).

Conclusion

In the present study, genetic variability, heritability, genetic advance and correlation among yield and yield contributing trait of rucola genotypes were assessed which was newly introduced in Bangladesh by the research group of the Department of Genetics and Plant Breeding, SAU. The results suggest that the rucola genotypes is suitable for cultivation in Bangladesh. The genetic variability analysis showed that there was a significant variation among the studied genotypes for each character. The higher genotypic variance was found in days to 50% flowering, plant height, leaf length, fresh yield, days to 80% maturity, number of siliquae per plant and number of seeds per siliqua. The high broad sense heritability was detected for most of the evaluated traits except number of leaves per plant. Nevertheless, the high broad sense heritability with high genetic advance in percentage of mean were observed for days to 50% flowering, plant height, leaf length, leaf breadth, fresh yield, days to 80% maturity, siliquae length, pedicel length, number of seeds per siliqua, 1000-seed weight and seed yield per plant. Fresh yield of rucola leaves had a highly significant positive correlation with number of leaves per plant and number of siliquae per plant at the genotypic level, while number of leaves per plant, number of siliquae per plant and leaf length at the phenotypic level. Therefore, phenotypic selection would be effective for these traits. Further research can be carried out based on present findings for the genetic improvement of the novel health promoting crops in Bangladesh.

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Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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IMPACT OF CLIMATE CHANGE ON LEMON (*Citrus limon* L.) PRODUCTION IN EASTERN BANGLADESH

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Abstract

The study was conducted to assess the impact of climate change on lemon production at Sreemangal of eastern Bangladesh as the area is moderately vulnerable to climate change. The study sample consisted of 80 randomly selected lemon growers who were interviewed and the data were collected to identify their perceptions, social characteristics, and the impact of climate change on lemon production. The results of the survey indicated that the grower's perception of climate change was impactful as a majority of growers claimed increased annual precipitation (48.8%), increased summer temperature (48.8%), and reduced winter temperature (46.3%). In the case of extreme events, 56.3% of growers mentioned that the intensity of storms has increased in the last 5 years (2015-2019) and 58.8% of lemon growers said that the intensity of rainfall had increased substantially. However, in terms of environmental hazards, the findings indicate that excess rainfall, pest infestation, cloudy skies, hail storms, and drought are the major problems in lemon production. On the surface, overcast skies cause most of the damage to lemon production. Apart from this, soil fertility, pests, diseases, excess temperature, crop sowing time, maturity period, and drought had a significant impact on lemon production in the study area. From the correlation coefficient table, it appears that many socioeconomic characteristics were also influenced by the impact of climate change on lemon production. In the study area, there is a positive significant relationship between changes in lemon production and changes in environment and risks. The lemon crop requires moderate temperatures and average rainfall for improved production.

Keywords: Citrus fruit, Ecological suitability, Rainfall, Temperature

Introduction

Citrus is mainly grown in tropical and subtropical areas of the world. Lemon production depends on suitable climatic conditions. Climatic factors include temperature, rainfall, and wind. Climate change is one of the foremost serious threats to sustainable development (Khan *et al*., 2021). Certain human activities have also been identified as significant causes of recent climate change, often referred to as "global warming". The climate of Bangladesh can be characterized by high temperatures, heavy rainfall, high humidity, and fairly marked three seasonal variations hot summer, shrinking winter, and

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medium to heavy rains during the rainy season Khan (2021). In general, maximum summer temperatures range between 38-41°C. April and January is the hottest and coolest month in most parts of the country, and the average coolest temperature is about 16–20°C and around 10°C at night. According to IPCC (2007), climate change affecting the sea level in the coastal region of Bangladesh has been predicted to rise to 80 cm by 2100. Climate change in Bangladesh has become a threat to rural farmers and agricultural workers.

Among the horticultural crops like pineapple, banana, mango, and other fruits, lemon required less cultural practices and hence reduces the labor cost. The area now under the lemon productivity was remaining fallow earlier in lemon cultivation. The environment of Bangladesh is favorable for lemon cultivation. With the globalization of world trade and the establishment of the WTO, the export opportunities for citrus fruits increased significantly. Hence, citrus growers faced increased competition at the world market level. The demand for lemons is increasing day by day, for which it is necessary to know the effect of climate change on the productivity of lemons as well as the current methods of production. In Bangladesh, productivity per unit and overall productivity need to be increased substantially to compete in the international markets. Climate alarm, in the form of unreliable weather patterns, weather excesses, and usual climate variability can potentially affect people's livelihoods adversely, which in turn can induce additional stress and result in vulnerability (Asaduzzman *et al.,* 2005). Bangladesh is a disaster-prone country, despite the fertile land it is subjected to food shortages because of the heavy dependence on agricultural productivity and the vagaries of weather and natural disaster (Paramanik, 1991). Environmental change presents nowadays danger for most agrarian areas and sustenance security among all other influenced divisions. Crop yields are predicted to fall up to 30%, creating a very high risk of hunger and only sustainable climate-resilient agriculture is the key to enabling farmers to adapt and increase food security (World Bank, 2001).

Despite promising climatic conditions for year-round citrus production in the country, its production continues to face many difficulties such as insect infestation (Haque *et al*., 2019), postharvest losses, the glut in peak season, and information gap in domestic and export markets (HORTEX Foundation, 2010). The study area is mainly affected by different types of climatic hazards. So, the environmental impact is effective in the agricultural sectors, especially for lemon cultivation. So, in this situation, it is necessary to know the extent of climate change perception and the impact of climate change on lemon productivity. The study aimed to assess the perceived impact of climate change, analyze the selected characteristics, explore the relationship between selected traits and the impact perceived by growers on lemon productivity due to climate change, and ascertain the environmental hazards faced by lemon farmers.

Materials and Methods

The experiment was conducted in an area of 450.74 square kilometers at Sreemangal in Moulvibazar, eastern Bangladesh. This study used both quantitative and qualitative research methods to get a comprehensive view of the perceived impact of climate change on the lemon productivity of Bangladesh. A qualitative method of key informant interviews was used while a quantitative survey method was used. The key informants were the Agriculture Extension Officers of the unions under model farmers. Keeping the objectives of the study in mind an interview schedule was prepared to collect information. The questions and statements in the schedule were simple and easily understood by the respondents. Both English and Bengali language versions were used with the respondents or the lemon growers.

For this study, researchers prepared an up-to-date list of leguminous growers in the study area with the help of local leaders and concerned SAAOs. The total lemon growers interviewed were 320, among which 72, 64, 60, 48, and 40 were from Sreemangal, Rajghat, Asidron, Sindurkhan, kali-ghat, and Mirzapur respectively. The sample size was determined as 80.

Measurement of the dependent variable

Impact of climate change perceived

The extent of overall lemon productivity in response to climate change is the dependent variable of this study. For this study, we followed the climate change impact index (CCII) developed by Rahman (2005) and the formula was below:

$CCII = I_{no} \times 0 + I_{low} \times 1 + I_{mid} \times 2 + I_{high} \times 3$

Note: II= Impact Index; I_{no}= Frequency of respondents that have no impact; I_{low}= Frequency of respondents that have a low impact; I_{mid}= Frequency of respondents that have a medium impact; I_{high}= Frequency of respondents that have high impact

Measurement of independent variables

The farmers were classified into three categories according to the National Youth Policy, young (18-35 yr), middle (36-50 yr), and old age (above 50 yr).

The education of respondents was classified under Primary education (Grade l-5), Secondary education (Grade 6-10), and higher secondary (Grade 10-12) and graduated as 13 or above.

The occupation was classified (1, 2, 3) as agro-farmer, entrepreneur, and businessman, respectively. The family size was classified as small family (1-7 persons), medium family (7-11 persons), and large family (above 11 persons).

The farm size was classified into four categories marginal (land ownership up to 0.20 ha), small (land ownership 0.201-1.00 ha), medium (land ownership of 1.013.00 ha), and large (land ownership above 3.00 ha). The farm size was measured using the formula below:

Farm Size = A + B + (C + D) + E

Note: A= Own land under own cultivation; B= Land taken from others as a lease; C= Own land given to others as a lease; D= Mortgages; E= Taking mortgages

The method of ascertaining income involved two phases. The actual amount of annual family income of the respondent is calculated by using the formula below:

Total annual income = Agricultural income + Income from the non-agricultural source.

Respondents were classified into three categories as Low income (less than Bangladeshi Taka 1100000), Medium income (Taka 1100000-1700000), and High income (above Taka 1700000). Respondents were classified into three categories of communication levels with the extension personnel or agencies and they are low (score up to 14), Medium (14-21), and high (score above 21).

Environmental hazards faced by the farmers

The **r**espondents were classified into three categories of exposure to environmental hazards such as low hazardous (score up to 6), medium hazardous (6-9), and high hazardous (score above 9). For this study, Rahman (2005) categorization of environmental hazards was followed. An environmental hazard index (EHI) was developed to fulfill this objective using the following formula:

$$EHI = EHI_{no} \times 0 + EHI_{low} \times 1 + EHI_{mid} \times 2 + EHI_{high} \times 3$$

Note: EHI= Environmental Hazards Index; EHI_{no}= Frequency of respondents that have experienced no hazards; EHI_{low} = Frequency of respondents that have experienced low hazards; EHI_{mid} = Frequency of respondents that have experienced medium hazards; EHI_{high} = Frequency of respondents that have experienced high hazards; The EHI for each of the environmental hazards ranged from 0 to 240.

Statistical analysis

The data collected were analyzed, coded, transferred from the interview schedule to a master sheet, summarized, categorized, and entered into a database using Microsoft Excel 2019. The data were analyzed using SPSS (Version 16.0) which was used to perform all statistical analyses.

Results and Discussion

Personal and socio-economic profile of lemon growers

The observed age of the growers ranged from 29 to 59 Years (Table 1). Among the respondents, the highest proportion was 48.8%, the median 32.4% young, and 18.8% old. Most middle-aged growers are involved with lemon productivity. Mahmood (2011) reported that age is an important factor regarding knowledge because age had a significant negative correlation with horticultural adaptation.

The educational background of the cultivators ranged from 5 to 13 (Table 1). Similar results were also observed by Sarker *et al.*, 2017 and the findings where the levels of education were (23.81, 28.57, 19.05, 23.81, and 4.76) % of the respondents were illiterate, don't complete primary education, completed primary education, primary level to SSC, and more than SSC.

The occupation of cultivators were agro-farmer, entrepreneurs, and businessmen (Table 1). The highest 41.2% of people were businessmen, 37.4% were an entrepreneur and 21.2% were agro-farmers. A recent research appraisal of the number of environmental and sustainability degree conceding programs has more than doubled over the last two decades from around 500 in 1990 to over 1200 today, and further, that jobs in these fields between 2008–2018 are projected to increase at a rate of around 28%, which is faster than the average for all occupations (Vincent, 2010).

The family size of the respondents varied from 5 to 13 (Table 1). Medium family size was 55%, followed by small family and large family (32.5 and 12.5) %. Rashid (2014) found similar results and results were marginal, small, and large farm holders 37.5, 30, and 13.7%; respectively in Bagerhat.

The farm size of the growers ranged from 0.31 to 6.25 ha (Table 1). The medium farm holder 78.8% constituted the highest proportion 78.8% and the lowest 7.4% in small farm holders and 13.8% had a large farm. The highest proportion was 78.8% for medium farms, followed by 13.8% for large farms, and the lowest farm size was 7.4% for small farms. Rashid (2014) observed similar results and the farm size ranged from 0.203-4.182 ha in Bagerhat.

Table 1. Distribution of the growers according to their personal profile

Age				
Categories (year)	Number	Percent	Mean	Standard deviation
Young age (18-35)	26	32.4		
Middle age (36-50)	39	48.8	41.36	8.180
Old age (above 50)	15	18.8		
Total	80	100		
Education				
Categories (year)	Number	Percent	Mean	Standard deviation
Primary (1-5)	1	1.2		
Secondary (6-10)	46	57.5		
Higher Secondary (11-12)	23	28.8	9.88	2.213
Graduate (13 above)	10	12.5		
Total	80	100		
Occupation				
Categories (Score)	Number	Percent	Mean	Standard deviation
Agro-Farmer	17	21.2		
Entrepreneur	30	37.4	2.20	0.770
Businessmen	33	41.2		
Total	80	100		
Family size				
Categories (year)	Number	Percent	Mean	Standard deviation
Small (1-7)	26	32.5		
Medium (7-11)	44	55	8.80	2.016
Large (11 or above)	10	12.5		
Total	80	100		

Table 1. Contd.

Farm size				
Categories (ha)	Number	Percent	Mean	Standard deviation
Small (>1)	6	7.4		
Medium (1-3)	63	78.8	2.2519	0.65156
Large (3<)	11	13.8		
Total	80	100		
Annual family income				
Categories (ha)	Number	Percent	Mean	Standard deviation
Low income (>1100000)	11	13.8		
Medium income (1100000-1700000)	34	42.5	1702378	612385.705
High income (1700000<)	35	43.7		
Total	80	100		
Communication media exposure				
Categories (Score)	Number	Percent	Mean	Standard deviation
Low contact (>14)	19	23.8		
Medium contact (14-21)	47	58.7	17.54	3.680
High contact (<21)	14	17.5		
Total	80	100		

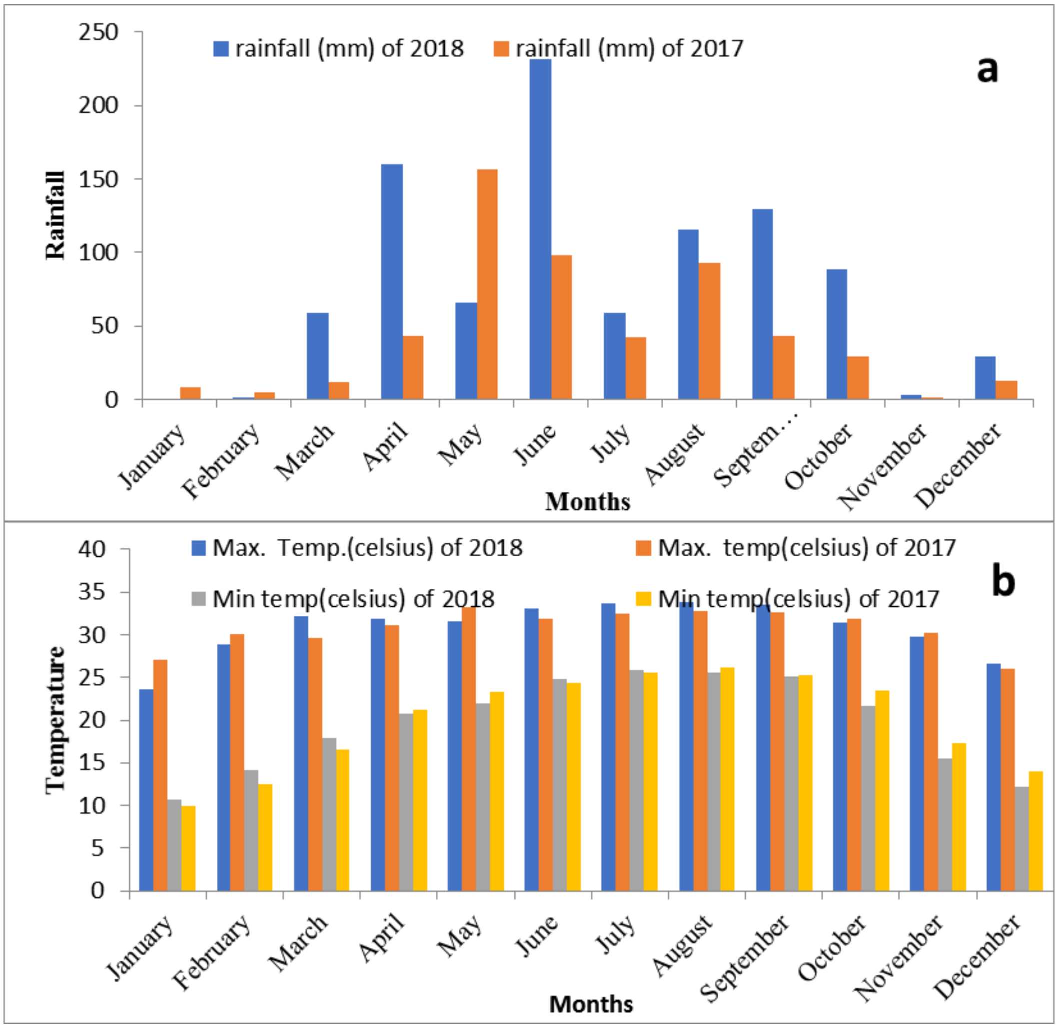
The annual family income of the growers ranged from Taka 456000-3205113 (Table 1). The majority (43.7) % of the respondents was a high annual family income, followed by 42.5% medium annual family and the lowest annual family income was 13.8%. Sarker *et al*., (2017) observed similar results and results were that 56% have their cultivated land where they produce lemon and 44% of farmers have no own land, and earn their livelihood by working on another's land.

The agricultural extension contact score of the growers ranged from 10 to 23 against the possible range from 0 to 33 (Table 1). The highest proportion of respondents was medium media exposure (58.7%), followed by 23.8% for low media exposure and the lowest was 17.5% for high media exposure.

Meteorological data (rainfall and temperature)

Total rainfall at Sreemangal was 2420 mm in 2018-19, as per the records of the meteorological department. Approximately 86% of the annual average rainfall occurs between April and September (Fig. 1). The maximum and minimum rainfall were 231.2 mm and 0 mm in April and January in 2018 and 156.25 and 1 mm in May and November in 2017. The maximum and minimum temperature was 33.8 and 10.70^{0}c respectively in August and January of 2018 and the maximum and minimum temperature was 33.1 and 9.90 ^{0}c respectively in May and January of 2017. Sarker *et al*., (2017) found that 2030-

2290 mm annual rainfall humidity was between 60 and 86%, the duration of sunshine from 5-9 hours, and this study also found annual temperature from 10-37°C where the minimum and maximum temperature prevail in January and May in Muktagacha.



Source: Bangladesh Meteorological Department (year 2017-2018)

Fig. 1. a). Annual rainfall, b) Annual temperature of 2017-2018

Grower's perception of climate change

Farmers' perception of climate change has been studied (Table 2). The data showed that farmers revealed information on average annual rainfall patterns, from 2015-2019. However, the lemon growers revealed the monsoon length as shown in Table 2 for the period 2015-2019. Most of the farmers (52.5%) said that the annual mean temperature has not changed since the previous 5 years and only 13.75% of farmers said that the annual average temperature has decreased since the previous 5 years.

Farmers (46.25%) said that the annual average winter temperature has decreased, and only 11.25% of farmers mentioned that the winter temperature increased 5 years ago. Farmers (48.75%) said summer temperature increased 5 years ago and only farmers (16.25%) said summer temperature decreased. Most of the farmers (56.25%) said that storm intensity has increased and farmers (10%) said that storm intensity has decreased over the previous 5 years. Martínez *et al*., (2020) noted that climate change was influencing the citrus management practices of the producers.

Table 2. Distribution of the respondents based on their perception of climate change

Sl. No.	Name of the statement		The extent of perception (No=Number)							
			Increased		Decreased		No changed		Don't know	
			No	%	No	%	No	%	No	%
1.	Precipitation	Annual	39.00	48.75	6.00	7.50	25.00	31.25	10.00	12.50
		In rainy season	16.00	20.00	18.00	22.50	34.00	42.50	12.00	15.00
		In dry season	32.00	40.00	10.00	12.50	24.00	30.00	14.00	17.50
		Rainy season length	21.00	26.25	13.00	16.25	36.00	45.00	10.00	12.50
		Summer season length	11.00	13.75	38.00	47.50	24.00	30.00	7.00	8.75
2.	Temperature	Annual	15.00	18.75	11.00	13.75	42.00	52.50	12.00	15.00
		Winter season	9.00	11.25	37.00	46.25	21.00	26.25	13.00	16.25
		Summer season	39.00	48.75	13.00	16.25	18.00	22.50	10.00	12.50
3.	Extreme events	Intensity of storms	45.00	56.25	8.00	10.00	16.00	20.00	11.00	13.75
		Intensity of hotness	13.00	16.25	36.00	45.00	25.00	31.25	6.00	7.50
		Intensity of rainfall	47.00	58.75	9.00	11.25	13.00	16.25	11.00	13.75

Environmental hazards faced by lemon growers

Environmental hazard scores faced by the growers ranged from 5 to 12 against the possible range from 0 to 15. The distribution of Environmental hazards faced by growers was depicted according to their experience (Table 3). The majority (43.8%) of the growers in Sreemangal had medium environment hazard scores while 37.5% had low environment hazard scores and 18.8% had high environmental hazards. Ahmed *et al*., (2020) observed similar results and the farmers perceived Nor'wester (77.9 %) as the most frequent hazard causing crop damage followed by heavy rainfall, hailstorm, heavy wind, flashflood, heavy fog, and floods.

Impact of climatic change as perceived by lemon growers

The data in Table 3 indicated the impact of climatic change on the growers in Sreemangal with an average of 30.25. The majority (53.7%) of the growers in Sreemangal observed high impact towards climate change while about 32.5% observed low impact and 13.8% observed medium impact was found in the study area. During 2003-2013, the total damage and losses to the crop subsector amounted to about 13$ billion. Almost 60% of this damage and losses were caused by floods, followed by storms with 23% (FAO, 2015).

Table 3. Distribution of environmental hazards and impacts of climate change

Environmental hazards faced				
Categories	Number	Percent	Mean	Standard deviation
Low hazardous (>6)	30	37.5		
Medium hazardous (6-9)	35	43.7	7.50	1.909
High hazardous (9<)	15	18.8		
Total	80	100		
Impacts of climate change				
Categories (Days)	Number	Percent	Mean	Standard deviation
Low impact (>28)	26	32.5		
Medium impact (28-30)	11	13.8	30.25	2.698
High impact (30<)	43	53.7		
Total	80	100		

The overall rank order of hazardous events experienced by growers

The percentage of distribution of the growers in Sreemangal according to environmental hazards is provided in Table 4. Along with the EHI and rank order of each environmental hazard, the environmental hazards index of the respondents of the 5 items ranged from 0 to 240. The problems identified by the growers in Sreemangal were listed according to their importance. Most of the growers of the study area experienced excess precipitation to a considerable extent rather than other environmental hazards. The highest hazard index (180) was found in the case of precipitation. The next index was found in the case of the cloudy sky (168). During the period 2003-2013, 3.4% of all humanitarian aid went to agriculture, while this sector absorbed about 22% of total damage and losses caused by natural hazards (FAO, 2015).

Table 4. Overall rank order of hazardous events experienced by growers

Problems	Growers (N=80)				EHI	Rank order
	High	Medium	Low	Not at all		
Drought	0	7	31	42	45	4th
Spread of pest	25	42	10	3	169	2nd
Hail storm	2	5	18	55	18	5th
Cloudy sky	28	38	8	6	168	3rd
Excess precipitation	33	34	13	0	180	1st

The rank order of climate change on lemon productivity

The impact of climate change on lemon productivity score ranged from 85 to 176 against the possible score of 0 to 240 (Table 5). The growers marked extra cloudy sky mostly affected lemon productivity and scored highest to 176, while the lowest ranked drought on citrus growth and productivity scored 85. The study of OĞUZ *et al*., (2017) supported this study.

Table 5. The rank order of climate change of lemon productivity

Impacts	Growers (N=80)				CCII	Rank Order
	High	Medium	Low	Not at all		
Due to excess temperature, the amount of lemon productivity increased before	25	28	16	11	147	4th
The fertility of the soil has decreased compared to before	35	28	10	7	171	2nd
The sowing time of lemon has changed	26	22	18	14	140	5th
Crop productivity takes longer than before	20	30	15	15	135	6th
Extra cloudy sky cause damage to lemon productivity	38	25	12	5	176	1st
Drought on citrus growth and productivity	10	15	25	30	85	7th
New pests and diseases are seen in lemon fields	30	23	13	14	149	3rd

Relationship between selected socio-economic characteristics and their impact of climate change on lemon production

From Table 6 it was found that education and communication media exposure of the lemon growers had a negatively significant relationship with the impact of climate change. Similarly, age, occupation, family size, farm size, and annual family income had a positively significant relationship with the impact of climate change on lemon productivity in the study area. In the country bean, the insect of aphid and pod borer showed a significant positive relationship with temperature and pod numbers in Sylhet (Khan *et al*., 2020).

The calculated value of (r=0.516) was found greater than both the tabulated value of 0.05 and 0.01 levels (Table 6). It was concluded that there was a positive significant relationship between the age of the lemon growers and the impact of climate change on lemon productivity. Age had a positive significant relationship with agricultural adaptation to climate change (Santa, 2013).

The calculated value of (r=-0.627) was found smaller than both the tabulated value of 0.05 and 0.01 levels (Table 6). It was concluded that a negative significant relationship between education level and the impact of climate change on lemon productivity. Education had a positive significant relationship with "Perception of the positive effect of climate change" as well as higher education may be ensured a higher perception of a positive effect of climate change (Santa, 2013).

Table 6. Correlation Co-efficient between Selected Characteristics of Respondents and their Impact of Climate Change on Lemon Production

Dependent variable	Independent variable	Correlation coefficient (r) values with 98 d.f	Tabulated value of 'r'	
			0.05 level	0.01 level
Impact of Climate Change on Lemon Productivity	Age	0.516**	0.197	0.257
	Level of education	-0.627**		
	Occupation	0.408**		
	Family size	0.570**		
	Annual Family Income	0.567**		
	Farm size	0.440**		
	Communication Media Exposure	-0.450**		

Note: ** = Correlation is significant at 0.01 level of probability
* = Correlation is significant at 0.05 level of probability

The calculated value of (r=0.408) was found greater than both the tabulated value of 0.05 and 0.01 levels (Table 6). It was concluded that there was a positive significant relationship between the occupation of the lemon growers and the impact of climate change on lemon productivity. Occupation of the household head, gender of household age, and family type had a negative relation to an adaptation of climate change strategies (Dahal *et al*., 2019).

The computed Correlation coefficient value of (r=0.570) was found greater than both the tabulated value of 0.05 and 0.01 levels and was statistically positively significant (Table 6). It was concluded that family size had a positive significant relationship between family size and the impact of climate change on lemon productivity. When the family size was large, most of the family members did not find the minimum facilities to lead a life. Most of the members had less education and communication facilities. The study of Shafqat *et al*., (2021) supported this study for the farm size and lemon production.

The calculated value of (r = 0.440) was found greater than both the tabulated value of 0.05 and 0.01 levels (Table 6). Growers having large farm sizes are expected to have higher productivity of lemon. It was concluded that there was a positive significant relationship between the farm size of the lemon growers and the impact of climate change on lemon productivity. The farm size had a positive significant relationship with the agricultural adaptation to climate change (Santa, 2013).

Indicate that the computed Correlation coefficient value of (r = 0.567) was found greater than both the tabulated value 0.05 and 0.01 level was statistically positively significant (Table 6). It was concluded that annual family income could vary positively with the variation of climate change impact. The annual family income had a positive significant relationship with the agricultural adaptation to climate change Santa (2013).

The calculated value of (r = -0.450) was found smaller than both the tabulated value of 0.05 and 0.01 levels (Table 6). It was concluded that a negative significant relationship between communication media exposure and the impact of climate change on lemon productivity. A study was conducted on the participation of rural women in income-generating activities on the agricultural farm and found that communication media exposure had no significant relationship with the extent of participation in activities (Haque, 2008).

Conclusion

Lemon (*Citrus limon*) needs average temperature and adequate rainfall for maximum productivity. However, excess precipitation and cloudy sky were the main climatic factors affecting the lemon quality and spreading pests respectively. It is found from the study that the climatic parameters have changed within the study period. The temperature is found to increase in the summer season, whereas decreasing in the winter season. On the other hand, the trend of annual rainfall and the number of storm occurrences has increased in the last 5 years. Growers of the study area are more or less concerned about the impact of climate change on lemon productivity in Sreemangal Upazila. For the last few years, lemon has been growing vastly as a consequence of adopting strategies to cope up with climate change. However, most of the growers rely mostly on applying chemical fertilizers for high productivity and ignore environmental sustainability. Cooperation and coordination of DAE and NGOs are required for the success of good agricultural practice (GAP) in lemon production. A balance is to be maintained among the quality and supply of planting materials, maintaining the sustainability of the environment and natural resources to cope up with climate change.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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IDENTIFICATION OF *Lasiodiplodia theobromae* [(Pat.) Griff. & Maubl] AS A CAUSAL PATHOGEN OF RAIN TREE GUMMOSIS [*Samanea saman* (Jacq.) Merr.] AND ITS CONTROL MANAGEMENT

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Abstract

Gummosis in a Rain tree [*Samanea saman* (Jacq.) Merr.] is a new disease in Bangladesh. The prevalence of this disease is increasing over time. An exploratory survey was made to find out the extent of this disease in different areas of Bangladesh during 2017 and 2018. Infested samples of wood were collected to find out associated pathogens. After the isolation of the fungi in the Forest Pathology Laboratory of the Bangladesh Forest Research Institute, the optimal conditions for the growth of the pathogen was determined. Subsequently, the suitable control method was developed. It was found that the roadside plantation of Mongla Sadar Thana, Bagerhat district had the highest disease incidence and severity (42.93 and 54.38 %), and the lowest (12.63 and 18.34 %) was recorded at Satkhira Sadar Thana, Satkhira district, respectively. *Lasiodiplodia theobromae* [(Pat.) Griff. & Maubl)] was found associated with gummosis in the affected trees. The result of the pathogenicity test revealed that there was a similarity in symptoms that arise between artificial inoculation and natural symptoms in the field. The optimal condition of conidial germination, mycelial growth, and sporulation of *L. theobromae* was observed at pH 6-8, 90-95 RH, and 25-30°C temperature. The concentration of 2.5 % glucose and sucrose was the best for conidial germination, mycelial growth, and sporulation, and sucrose was better than glucose. PDA medium had the maximum mycelial growth (72.18 mm) and excellent sporulation, while the YEA medium had the lowest mycelial growth (59.19 mm) and poor sporulation. The fungicides Knowin (Carbendazim), ARBA (Carbendazim), and Autostin (Carbendazim) were found to completely inhibit pathogen mycelial growth and sporulation (100%) at 50, 100, and 150 mg/L concentrations. Furthermore, spraying these fungicides and the Bordeaux mixture at a rate of 2 % in the field resulted in the development of the smallest gummosis lesions. *L. theobromae* was a pathogen causing gummosis disease of rain tree, proven by morphology and Koch's postulates. Different environmental and nutritional factors affect the growth and sporulation of the pathogen and the application of Knowin, ARBA, Autostin, and Bordeaux mixture at 2 % can help control the disease at the field level.

Keywords: Bordeaux mixture, Disease incidence, *Lasiodiplodia theobromae*, Pathogenicity test

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Introduction

Rain tree [*Samanea saman* (Jacq.) Merr.] is a fast-growing multipurpose exotic tree species in Bangladesh that have been widely planted in village woodlots, dams, roadside, community forests, private forests, and homesteads in many districts of the country (Zabala, 1991). The tree is used for various purposes including shade, ornamental, furniture, animal feed, and medicine (Ferdous *et al*., 2010). This tree species has a hugely beneficial contribution to the socio-economic development of the country. Bangladesh is a densely populated country having more than 1000 people per square kilometer with only 12.8% forest cover is not enough to meet the demand for forest products (GoB, 2020). Government and private organizations have taken the initiative in the last 10 to 15 years to plant a variety of tree species on the roadside, fallow lands, and marginal lands to expand the number of forested areas in the country. Due to this initiative of the government, a large number of rain trees are being planted in almost all parts of the country. This is one of the most planted tree species in the Barisal and Khulna districts, contributing approximately 19 % of the total area (Mondal, 2016).

Rain tree is susceptible to various pests and diseases in both nursery and plantation stages. Dieback and gummosis disease are very significant in the Indian sub-continent among plantation diseases (Mondal, 2016). In recent years, rain tree plantations in different areas of Bangladesh suffered from a declining disease showing symptoms of drying branches from the tip accompanied by a heavy exudation of yellowish-brown gum from the stem and its branches and browning of vascular tissues. Infected wood and the defoliation that may occur weaken the trees but if the disease infects the trunk, the tree may die. Recently, 25-30 % rain tree mortality has been recorded due to gummosis in major rain tree plantation areas of Bangladesh (Papia, 2018).

L. theobromae [(Pat.) Griff. & Maubl] has been known as a fungus with a wide host range, estimated at more than 280 plant species, and with varied pathological effects on its hosts (Domsch *et al*., 2007; Khanzada *et al.*, 2004). In tropical areas, *L. theobromae* is known to cause major losses to mango, cocoa, banana, and sweet potato farmers (Rieger, 2006; Amusa *et al*., 2003). It is the causal agent of gummosis of branches and trunks of citrus, mango, cashew, and neem (Hasan *et al*., 2020; Khanzada *et al*., 2018; Twumasi, 2014; Cardoso *et al*., 2006; Khalil, 2012). In Bangladesh, there has been no information about this fungus as the disease agent in rain trees and whether *L. theobromae* from various other hosts can infect rain trees. Thus, the main objective of this study was to find out the causal organism associated with the gummosis disease of rain trees and to develop a suitable control method for rain tree gummosis.

Materials and Methods

Survey for the incidence of gummosis disease of rain trees in different parts of Bangladesh

In this present study, a survey was carried out in three districts of Bangladesh namely; Chattogram, Satkhira, and Bagerhat with the view to documenting quantitatively the incidence and severity of the gummosis disease of rain trees from July 2017 to December 2018. From each district, samples from 50 rain trees were collected and a gummosis incidence was assessed. The age of the trees was obtained from the local forest office and validated by the people living around the spots. The disease incidence and severity were assessed in different planting type's viz. nursery plantation, roadside plantation, orchard plantation, and individual tree planting. Gummosis incidence was determined as the proportion of plants showing gummosis symptoms and expressed as a percentage of the total number of plants assessed (Jagtap *et al.*, 2012). A tree was defined and recorded as having gummosis when it had any of the following symptoms: discoloration of the bark surface, discoloration of the underlying tissues, the dried whole part of the plant, and the exudation of the gum from infected tissues.

Calculating the disease incidence (Jagtap *et al.*, 2012)

$$\text{Percent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plant } examine} \times 100$$

Disease severity index was calculated as:

$$\text{(DSI)} = \frac{Sum\ of\ all\ disease\ rating}{Total\ number\ of\ assed\ plants \times maximum\ rating\ value} \times 100$$

Disease scale description of disease status

0 = Tree with no symptom associated with gummosis; 1 = Decline symptom associated with gummosis up to 25 % of the branch affected; 2 = widespread decline of the branch associated with gummosis up to 25-50 % of the branch affected; 3 = Decline and death of the branch associated with gummosis up to 50-75 % of the branch affected; 4 = Decline of 75-100 % tree, including the dead tree.

Collection of samples

Stem and branches of rain trees that were showing gummosis were collected from the survey areas and used for pathogen isolation. Both the aerial as well as underground portions of the trees were carefully studied for any pathogenic infection. Disease symptoms were found at the junction of dead and healthy portions of the stems and branches. Diseased specimens were collected in polythene bags and brought to the forest pathology laboratory of Bangladesh Forest Research Institute (BFRI).

Isolation and identification of the pathogen responsible for the disease

In the laboratory, infected tissues were excised with a sterilized scalpel at the point of disease symptom progression, then surface sterilized for 2 minutes with a 70 % ethanol solution. The tissues were then washed three times with sterilized water and then dried on sterile paper towels followed by incubation on PDA (Hi-Media, India) medium at 35°C in the darkroom for 3 days. Lactic acid (1 mL) and streptomycin sulphate (0.5 g/L, Sigma-Aldrich, USA) was incorporated into the medium as supplements. Using a single spore technique, the mycelium from the diseased sample was re-isolated and transferred to a petridish containing fresh PDA media. The Petri dishes were kept in a dark room at 25ºC for 7 days. For pure culture, the experiments were carried out at different times. To grow, the isolated fungus was sub cultured on PDA media. For further experiments, the sub-cultured plates were kept in a refrigerator at 4ºC. Identification of fungi was based on morphological and microscopic characteristics.

Pathogenicity test of the causal organism

The pathogenicity test was conducted at Forest Pathology Laboratory and Nursery at BFRI Campus, Chattogram. One-year-old seedlings of the rain trees were selected as a host for conducting pathogenicity tests. Using a sterile knife, a 1 x 2 cm inoculum block was made into the stem of the rain tree seedlings. A 5 mm inoculum disc from 5- day-old culture of a test fungus on PDA was placed in the gap and the inoculated portion and wrapped with Parafilm. In the control plants, a 5 mm PDA block without fungus was placed. Seedlings were irrigated after inoculation and the wrapping material was removed from the stems after 2 weeks of inoculation. Seedlings were monitored for the development of disease symptoms and isolations were made from the stem of the test plants to confirm the pathogenicity. The experiments were carried out in a randomized complete block design with three replications. Ten plants were used in each replication.

Influence of different nutrition, physical and environmental parameters on the growth and development of pathogen

Environmental and nutritional factors

Conidial germination (CG)

Relative humidity (70, 75, 80, 85, 90, 95 and 100), pH (4, 5, 6, 7, 8, 9 and 10), temperatures (5, 10, 15, 20, 25, 30 and 35ºC), Glucose and Sucrose solutions (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 % concentrations) were used to get suitable condition for CG. Conidia were collected from a 10-day-old PDA plate culture, and suspension (10^3/ml) was prepared using sterilized distilled water (for RH, pH, and temperature)/different concentrations of glucose and sucrose solution separately. A drop of the conidial suspension was placed on a separate groove slide and stored at 25ºC in a moisture chamber for 24 hours after being taken in a sterilized watch glass. After the incubation period, a drop of lactophenol cotton blue was used to cover the conidial suspension on the slide, and the percentage of CG was calculated using (x 40) power microscopes. Three replications were used for each particular treatment.

Mycelial growth and sporulation

Relative humidity, pH, temperature, glucose, sucrose (same range as CG), and seven solid media (Yeast extract Agar, Potato Dextrose Agar, Malt extract agar, Oatmeal, Richards, Sabourauds, and Czapeks) were evaluated to get favourable MG and sporulation of the pathogen. Effect of RH, pH, temperature, glucose, and, sucrose on MG of the pathogen was done using a PDA medium. Different media were autoclaved at 121°C/15lbs/inch^2 pressure; these were poured into sterilized Petri dishes. Agar discs (5 mm) were taken from 10-d10-day-oldture of pathogen and placed separately with respect to each treatment in the centre of each petridish and incubated in the respective conditions. After seven days of incubation, radial mycelium growth was measured by following Brown (1923) methods. Three replications were used for each particular treatment. After seven days, two discs (5 mm in size) were cut selectively and shaken vigorously in a test tube containing 5 ml of distilled sterile water to investigate the sporulation of the pathogen. To facilitate conidia counting easier, a forty- µl cotton blue solution was poured into it. The ten-µl conidial suspension was put on slides from each treatment with the help of a micropipette. The conidia were (conidia/microscopic field under 40 X) counted for each treatment under a compound microscope.

In-vitro evaluation of fungicides against mycelial growth, sporulation, and conidial germination inhibition of pathogen

Mycelial growth inhibition

Fourteen commercial fungicides were tested *in vitro* for their effects on conidial germination and mycelial growth of the pathogen followed by the poisoned food technique. The radial growth of the colony was recorded on the 7th day when maximum growth was observed, and percent inhibition was calculated using the formula given by Vincent (1927).

$I = \frac{C-T}{C} \times 100$; Where I = Percent Inhibition; C = Radial growth of fungus in control; T= Radial growth of fungus in treatment. The details of the fungicides used against the pathogen are given in Table 1.

Table 1. The details of the fungicides used against the pathogen

Sl. No.	Trade name	Chemical name
1.	Indofil	Mencozeb
2.	Knowin	Carbendazim
3.	Ridomil	Manocozeb
4.	Oxyvit	Copper oxychloride
5.	Cupravit	Copper oxychloride
6.	Aimcozim	Carbandazim
7.	Champion	Copper hydroxide
8.	Sunvit	Copper oxychloride
9.	Diathane M 45	Mancozeb
10.	Thiovit	Sulpher
11.	Autostin	Carbendazim
12.	Amivit	Copper oxychloride
13.	Rovral	Eprodion
14.	ABRA	Carbendazim

Conidial germination inhibition

Conidia of *L. theobromae* cultured on PDA plates were taken and suspensions (10^5/ml) were made separately with different concentrations of different fungicides. These suspensions (1.25 ml) were taken in small sterilized Petridishes (65 mm) and were kept at 28±2°C for 5-30 minutes. A drop of treated conidial suspension (from different concentrations of fungicide) was taken on separate slides to continue for 5 min. interval and was kept at 28±2°C in a humidity chamber for 24 hrs of incubation. Then a drop of lactophenol cotton blue was placed on the conidial suspension on the slides. The slides were examined under the high-power microscope (×40) for recording the percentage of conidial germination. Three replications were used for each particular treatment. Percentage inhibition of conidial germination (PICG) using the formula by Skidmore and Dickinson (1976). Where PICG = C_1-C_2/C_1 × 100.

C_1 = Total number of conidia in the control treatment.

C_2 = Germination of conidia in fungicidal treatment.

Inhibition of the sporulation

Once the control had reached maximum growth, circular portions of 1 cm in diameter were taken from the active growth site of each treatment and the corresponding repetitions and placed on Petri dishes containing 5 mL sterile distilled water; the mycelium was gently taken with the help of a sterilized glass handle, and the conidia were counted with a hemocytometer by-product, treatment, and repetition. The control proceeded through such a process. Three replications were used for each particular treatment.

Efficacy of chemical fungicides to control rain tree gummosis under field conditions

The experiments were carried out in the Forest Protection Division Nursery (FPD) at BFRI. Six-month-old rain tree seedlings were used in this study. Seedlings were previously inoculated with agar culture discs containing the mycelium of *L. theobromae* at the stem as described before (Pathogenicity test). After 30 days of inoculation when the disease is in progress, plants were then either sprayed with the fungicide (2 %; treatment) or with sterilized distilled water (control). The application of fungicide on the stem started in May to August 2018. Fourteen commercial fungicides (Indofil, Sunvit, Diathene M45, Oxyvit, Rovral, Aimcozim, Thiovit, Ridomil, Amivit, Cupravit, Champion, Knowing, Arba, and Autostin) and Bordeaux mixture (2 %) were sprayed for control of gummosis disease of rain tree under field condition. A total of three replications were at individual treatment and lesion size was recorded after 30 days interval. Ten plants were used in each replication.

Treatments

There were the following 14 treatments. T_0 = Control (Without fungicide), T_1 = Indofil, T_2 = Sunvit, T_3 = Diathene M45, T_4 = Oxyvit, T_5 = Rovral, T_6 = Aimcozim, T_7 = Thiovit, T_8 = Ridomil, T_9 = Amivit, T_{10} = Cupravit, T_{11} = Champion, T_{12} = Knowing, T_{13} = Arba, T_{14} = Autostin, T_{15} = Bordeaux mixture.

Statistical analysis

All data were analyzed by DMRT using the help of the computer package program SPSS (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Survey of disease incidence in different rain tree growing areas of Bangladesh

The incidence and severity percentages of rain tree gummosis in different planting types were shown in Table 2. The highest incidence and severity percentage assessed in roadside plantation were at Mongla Sadar Thana, Bagerhat district (42.93 and 54.38 %), and the lowest incidence and severity percentages (12.63 and 18.34 %) were recorded at Satkhira Sadar thana, Satkhira district, respectively.

Table 2. Incidence and severity of rain tree gummosis in a different part of Bangladesh.

Districts	Thana/union	Planting type	Age (Year/Month)	Incidence (%)	Disease severity (%)
Chattogram	Ramgarh, Fatikchari	Orchard plantation	15-38 years	27.75 c	29.38 d
	Udalia, Katirhat, Fatikchari	Orchard plantation	25-45 years	24.85 de	26.52 f
	Dantmara, Bhojpur, Fatikchari	Orchard plantation	25-40 years	28.85 bc	32.65 g
	Chattogram sadar, BFRI, campus	Nursery	6-12 month	14.79 h	19.18 h
Satkhira	Shyamnagar	Road-side plantation	10-45 years	25.72 d	28.47 e
	Kalaroa	Road-side plantation	15-50 years	23.28 ef	26.79 ef
	Satkhira Sadar	Road-side plantation	15-45 years	12.63 i	18.34 i
Bagerhat	Chila, Mongla	Single plantation	15-50 years	18.32 g	22.71 g
	Chandpai, Mongla	Single plantation	15-60 years	22.46 h	29.18 d
	Sundarban 89	Road-side plantation	7-45 years	29.61 b	35.89 b
	Mongla sadar	Road-side plantation	15-65 years	42.93 a	54.38 a

In a column, the same letters are not significantly different by DMRT at the 5 % level.

Symptoms of gummosis disease on rain tree

The affected trees initially had sunken lesions on the trunks, twigs, and branches. These recessed lesions develop darker in color over time, and exudation of yellowish, white, or transparent gum through them becomes more noticeable, it spreads throughout the body within three to six months and then the tree dies. Diseased trees suffer from defoliation, but if the disease spreads to the trunk, the tree may die. Infected trees frequently had additional symptoms, such as vascular discoloration beneath the gummosis. Canker develops on the stem and branches of plants as they grow older. Infected trees show signs of different levels of dieback (Fig. 1 A-D).

Isolation and identification of the pathogen

On PDA, *L. theobromae* (Pat.) Griff. & Maubl, synonym *Botryodiplodia theobromae* colonies had white aerial mycelia that eventually turned dark olivaceous mycelium (Fig.1E). Conidia ranging in color from dark brown to black were produced by mycelium (Fig. 1G).

Fig. 1. Gummosis disease symptoms and causal organism of rain tree gummosis. A: An affected mature rain tree showing dead branch at a road site plantation in Shyamnagar, Satkhira; B: Gum exudation in the main trunk; C: bark cracking symptoms in a stem in a younger rain tree; D: Xylem necrosis of the gum-secreting incision; E: *L. theobromae* cultured on PDA medium after 7 days; F & G: Mycelium and conidia of *L. theobromae.*

Mycelial growth and production of immature and mature conidia have also been observed. Conidia were sub-ovoid or ellipsoid, thick-walled, hyaline, and one-celled when immature, but matured to dark brown, two-celled, and with irregular longitudinal striations. The size of mature conidia averaged 24.6 ± 0.24 µm long and 13.9 ± 0.16 µm wide (Fig.1G). Pycnidia contained septate paraphyses. Based on the morphological and microscopic characters observed and by comparing with those previously reported (Auger *et al*., 2004; Larignon *et al.*, 2001; Phillips, 2002; Punitthalingam and Waller, 1976; Taylor *et al.*, 2005; Úrbez-Torres *et al.*, 2006; Pavlic *et al.*, 2007), the fungus was confirmed as *L. theobromae*.

Pathogenicity test of the causal organism

L. theobromae was found associated with the gummosis disease of rain tree. After 15 days of inoculation in rain tree plants, *L. theobromae* showed typical symptoms of the disease. No vascular browning was observed in control plants. Likewise, dyeing of tips, internal browning, death of leaves at the branch apices, and gum exudation were observed where plants were inoculated with pathogen after 15 days (Fig. 2 A-I and Table 3). Previous studies have noted that *L. theobromae* is a wound parasite of plants (Punithalingam, 1976), although it is a rare source of human infection (VSlez and Diaz, 1985). This fungus attacks more than 500 species of plants in different parts of the world (Punithalingam, 1980). It is a common tropical and subtropical plant pathogen with a wide host range associated with different decline syndromes, including *Acasia confusa*, *Albizia falcataria (Paraserianthus falcataria)*, *Eucalyptus* sp., *Mangifera sylvatica*, *M. indica*, *Mangnolia candolii, Paulownia fortune*, *Vitis vinifera*, *Prunus domestica*, *Citrus lemon*, and *Vitis vinifera*, among others (Alves *et al*., 2008; Abdollahzadeh *et al*., 2010; Trakunyingcharoen *et al*., 2018; Pipattanapuckdee *et al*., 2019; Pillay *et al*., 2013; Slippers and Wingfield, 2007; Shahbaz *et al*., 2009; de Silva *et al*., 2019). This pathogen has become important because it causes numerous diseases, including seed rot (Gure *et al*., 2005), and stem canker, dieback, root rot, fruit rot, leaf spot, and witches' broom (Punithalingam, 1980).

Pathogen re-isolation and confirmation by microscopic observation

At the end of the experiment of pathogenicity, re-isolation of the pathogen was carried out for identification. The mycelia and conidia of the re-isolated pathogen were observed under a compound microscope. Based on Koch's postulate examination, it was possible to reproduce the disease by artificially inoculating the rain tree plant with pathogens to cause the recurrence of the disease (Khalil, 2012). It indicated that the gummosis disease is caused by *L. theobromae* and it plays a significant role in disease development. Re-isolation from the dead and green branches of *L. theobromae* inoculated plants showed up to 95 % recovery of the fungus (Data not shown).

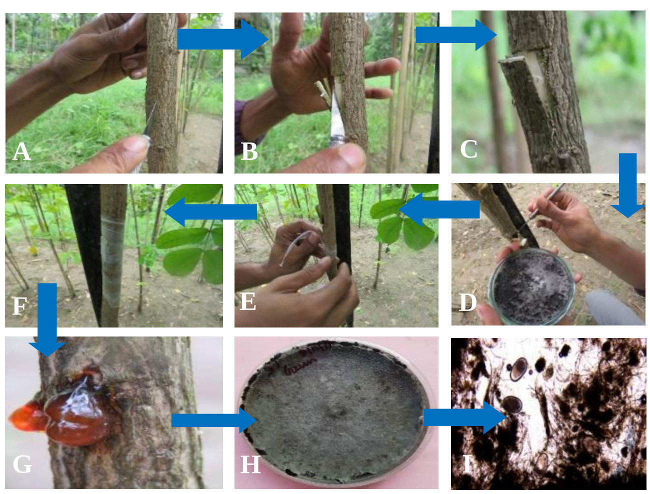

Fig. 2. Pathogenicity tests of *L. theobromae*, the causative organism rain tree gummosis at different stages. A: block was made using a sterilized knife (1 x 2 cm size); (B & C) Remove the bark from the cut portion; (D) Inoculation of fungus disc in the cut portion; (E & F) Wrapped by parafilm; (G) Gum oozing from inoculated portion after 15 days of inoculation; (H) Re-isolated of *L. theobromae* from infected portion; I: Microscopic view of *L. theobromae.*

Influence of different nutrition, physical and environmental parameters on conidial germination, mycelial growth, and sporulation of *L. theobromae*

According to the results, pH has a significant impact on mycelial growth, conidia germination, and sporulation of *L. theobroma*. The highest CG, MG, and sporulation of *L. theobromae* were observed at 6-8 pH. Relatively less growth was obtained at the 4 and 10 pH levels (Table 4 & 5). Filamentous fungi are known to be acid-tolerant, with most of them preferring a pH of 5.0 to 6.0 for cellular development and various metabolic functions (Rosfarizan *et al*., 2000). This studies have similarities with the records of Baloch *et al*., (2018) who recorded the maximum mycelial growth of *L. theobromae* on media in which pH levels were adjusted at 7 and 8. The results of *L. theobromae* conidia formation at various pH levels were similar to those reported by Zhao *et al*., (2010).

Table 3. The severity of symptoms on rain tree plants inoculated with *L. theobromae*

Treatments	Symptoms produced on rain tree plant			
	Dyeing of tips	Gum exudation	Internal browning	Death of leaves at the branch apices
L. theobromae	1	3	2	2
Control	0	0	0	0

0 = No symptoms, 1 = Very light, 2 = Moderate, 3 = Severe symptoms.

Relative humidity is one of the major limiting factors determining pathogens' growth, conidial germination, and disease development. In the present study, *L. theobromae* showed variation in its mycelial growth, conidial germination, and sporulation at different relative humidity levels. Among the seven relative humidity levels tested, the maximum mycelial growth, conidial germination, and excellent sporulation were recorded at 95 % relative humidity, followed by a 90 % relative humidity level (Table 4 & 5). Similarly, the earlier reports of Udhayakumar (2018) observed the highest percentage of conidial germination, and mycelial growth of *Colletotrichum falcatum* at 90 to 100 % relative humidity. In another study, Gadgile *et al*., (2009) stated that the development of *B. theobromae* rot is dependent on relative humidity.

On the PDA medium, the colony growth of *L. theobromae* varied in response to temperature changes. The temperature ranges of 25°C and 30°C were shown to be optimal for the fungus's fastest mycelial growth, conidial germination, and sporulation. The influence of other temperature ranges was moderate (Tables 4 and 5). These findings are in full agreement with Rehman *et al*., (2011) who observed the highest growth of *L. theobromae* when it was incubated at 30ºC and 25ºC, and the minimum growth was obtained when *L. theobromae* was incubated at 15ºC. Fernández *et al*., (2014) found that temperature highly affected the mycelial growth of *B. cinerea* isolates and discriminate isolates based on their temperature optima.

Different media had a significant impact on the mycelial radial growth rate and sporulation of *L. theobromae*. The highest mycelial colony growth and sporulation of the test fungus were observed on the PDA medium, whereas the minimum mycelial colony growth and poor sporulation were seen on the YEA medium (Table 5). The presented results were consistent with those of Alam *et al.,* (2001), who found that *L. theobromae* mycelium growth was highest on Potato Dextrose Agar and Czapek Dox agar media. Likewise, on Potato Dextrose Agar, Baloch *et al.*, (2018) observed the quickest mycelial development of this fungus. Several other workers also stated that PDA was the best media for the mycelial growth of *L. theobromae* (Maheshwari *et al.*, 1999).

Different concentrations of glucose and sucrose significantly inhibited MG, CG, and sporulation in *L. theobromae*. The highest MG, CG, and excellent sporulation, were in 2.5 of glucose and sucrose solution. Sucrose has shown better results than glucose (Tables 4 and 5). Jash *et al*., (2003) observed that sucrose is the best carbon source for the growth of *Alternaria zinniae* followed by starch and maltose. In another study, Ray (2004) showed that lactose and glucose had a similar effect on the growth of *L. theobromae*.

In-vitro* evaluation of fungicides against mycelial growth, conidial germination and inhibition of sporulation of *L. theobromae

The percent inhibition of mycelial growth, conidial germination, and sporulation of *L. theobromae* by different fungicides (Indofil, Sunvit, Diathene M45, Oxyvit, Rovral, Aimcozim, Thiovit, Ridomil, Amivit, Cupravit, Champion, Knowin, Arba, and Autostin) varied significantly ($p \leq 0.05$) affected at different concentrations *in vitro*. The highest percent inhibition of mycelial growth, conidial germination, and sporulation (100 %) were observed with Knowin, ARBA, and Autostin at 50, 100, and 150 mg/L concentrations (Fig. 3, 4 and 5). These results agree with those of Pitt *et al.*, (2010) who also reported that mycelial growth of *Diplodia seriata*, *Neofusicoccum parvum*, *Lasiodiplodia theobromae,* and *Botryosphaeria dothidea* was significantly inhibited by carbendazim, tebuconazole, procymidone, iprodione, and fluconazole. Khanzada *et al.*, (2004) also found that Carbendazim and Thiophanate-methyl were highly effective in inhibiting the growth of the *Lasiodiplodia theobromae*. Saeed *et al*., (2017) found that the systemic chemical fungicides, Score, Cidely Top, and Penthiopyrad, significantly inhibited the mycelial growth of *L. theobromae* in *in vitro*.

Efficacy of chemical fungicides for control of rain tree gummosis under field condition

Results presented in Table 6 indicates that fungicide treatment significantly reduced the percent development of the lesion of gummosis with the untreated control. The lowest percent development of the lesion was observed when 2 % Knowin, Arba, Autostin, and Bordeaux mixture were sprayed (Fig. 6). The efficacy of the benzimidazole fungicides against a broad group of wood pathogens was demonstrated by Luque *et al.*, (2008), who reported that carbendazim and thiophanate methyl were the most effective in reducing mycelial growth of *Diplodia corticola* isolated from oak trees. In their subsequent field experiments, they also observed that carbendazim was the most effective fungicide and that thiophanate methyl was the next most effective at reducing numbers of surface lesions caused by *D. corticola* on oak trees in Spain. Similarly, Carbendazim, Sodium orthophenylphenate, Potassium metabisulfite, Mancozeb, Carboxin, Dodine, Iprodione, and Thiabendazole were evaluated for control of *B. theobromae*on mango cv.

Dashehari by Sharma *et al.*, (1994). They found that 0.1 percent Carbendazim (dip treatment) was the most effective fungicide for control of *B. theobromae.* Assuah, (1997) worked on the etiology and control of citrus gummosis disease at the University Agriculture Station, Kade, Ghana, and observed that Bordeaux mixture (1:4) and Bavistin (50% carbendazim) at 2 gm /L were effective against the disease.

Table 4. Effect of different environmental and nutritional factors on conidial germination of *L. theobromae*

pH		RH (%)		Temperature (°C)		Glucose/Sucrose (%)		
Rate	CG (%)*	Rate	CG (%)*	Rate	CG (%)*	Rate	CGG (%)*	CGS (%)*
4	45.12 f	70	20.12 g	05	45.32 f	0.5	20.26 f	48.14 f
5	52.38 d	75	45.72 f	10	53.86 e	1.0	25.42 e	52.28 e
6	74.95 b	80	69.53 d	15	69.28 d	1.5	47.78 c	57.31 c
7	85.15 a	85	73.28 c	20	73.92 c	2.0	52.31 b	61.28 b
8	69.53 c	90	75.42 b	25	85.29 a	2.5	56.16 a	63.14 a
9	48.85 e	95	85.29 a	30	82.14 b	3.0	51.38 b	55.23 d
10	25.34 g	100	58.24 e	35	45.12 f	3.5	45.92 d	51.18

*Mean of three replications

RH: Relative Humidity, **CG:** Conidial Germination, **CGG**: Conidial Growth in Glucose, **CGS:** Conidial Growth in Sucrose. All treatments were observed (except temperature effect) at 25°C. In a column, the same letters are not significantly different by DMRT at the 5 % level.

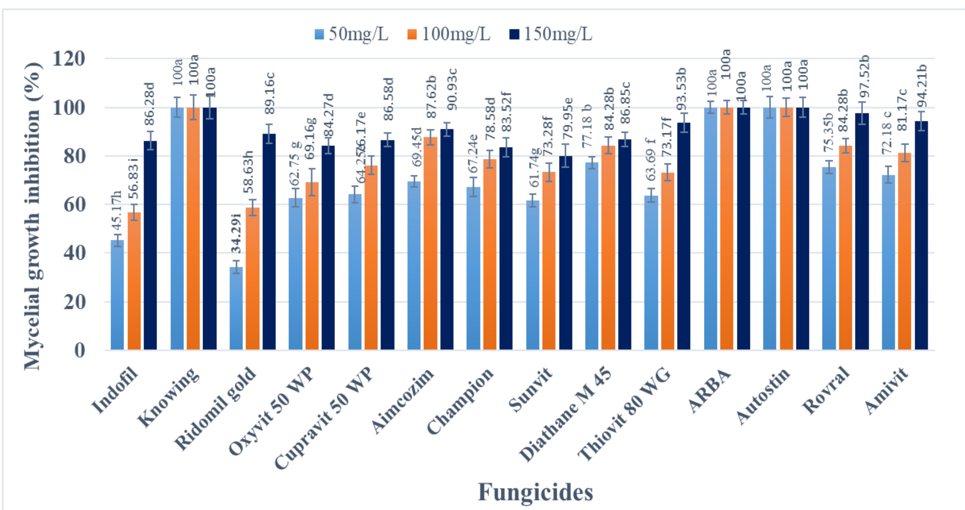

Fig. 3. Effect of different concentrations of the fungicides on the mycelial growth inhibition of L. theobromae. Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.

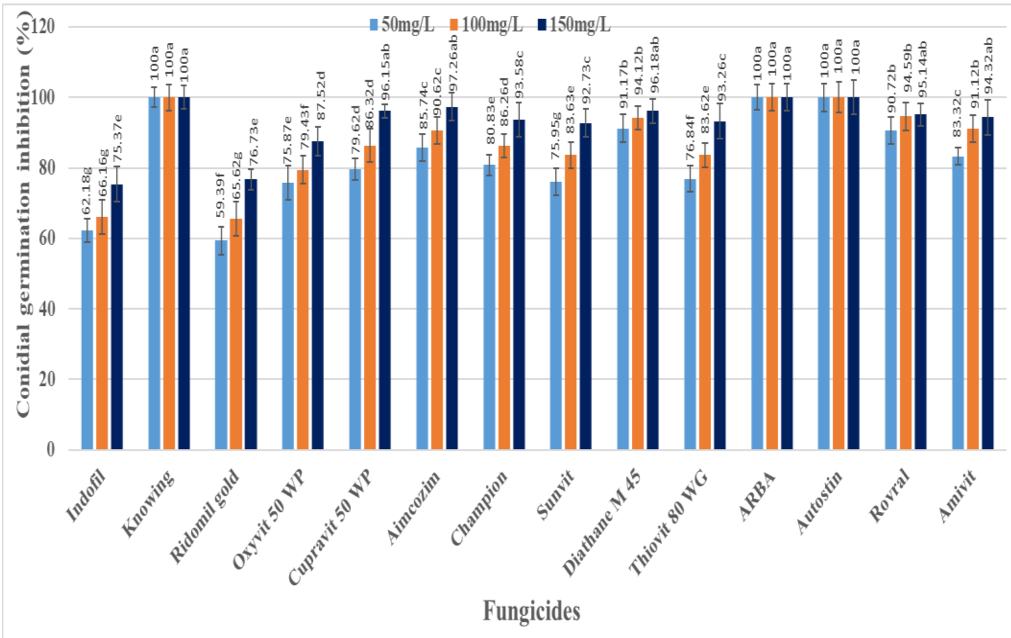

Fig. 4. Effect of different concentrations of the fungicides on the conidial germination inhibition of *L. theobromae.* Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.

Table 5. Effect of different environmental and nutritional factors on mycelial growth (mm) and sporulation of *L. theobromae* after 7 days

Mycelial growth (mm) and sporulation of *L. theobromae*																	
pH			RH (%)			Temperature (°C)			Media			Glucose (%)			Sucrose (%)		
Rate	MG* (mm)	Spor	Rate	MG* (mm)	Spor	Rate	MG* (mm)	Spor	Media name	MG* (mm)	Spor	Rate	MGG* (mm)	Spor	Rate	MGS* (mm)	Spor
4	68.21 e	-	70	49.72 f	-	05	00 g	-	Richards	65.17 c	+++	0.5	58.16 f	+	0.5	62.75 g	+
5	72.31 d	++	75	52.41 g	+	10	25.13 f	+	MEA	69.38 b	+++	1.0	65.27 e	+	1.0	69.42 f	+
6	85.18 b	++++	80	58.16 e	++	15	35.14 e	++	Oatmeal	63.27 d	++	1.5	78.82 d	+	1.5	74.38 e	++
7	**89.82 a**	++++	85	69.18 c	+++	20	69.37 d	+++	PDA	**72.18 a**	++++	2.0	85.98 c	++++	2.0	92.29 c	++++
8	78.12 c	++++	90	72.27 b	++++	25	**82.16 a**	++++	Sabourauds	62.26 d	++	2.5	**92.38 a**	++++	2.5	**96.27 a**	++++
9	65.91 f	+	95	**79.38 a**	++++	30	79.18 b	++++	Czapeks	58.17 e	++	3.0	88.19 b	++++	3.0	94.17 b	++++
10	58.41 g	-	100	63.14 d	+++	35	72.12 c	+++	YEA	59.19 f	++	3.5	83.28 c	+++	3.5	89.28 d	+++

*Mean of three replications
MG = Mycelial growth, MGG = Mycelial growth in glucose, MGS = Mycelial growth in sucrose
Spor = Sporulation, - = Nil, + = Poor, ++ = Fair, +++ = Good, ++++ = excellent
In a column, the same letters are not significantly different by DMRT at the 5 % level.

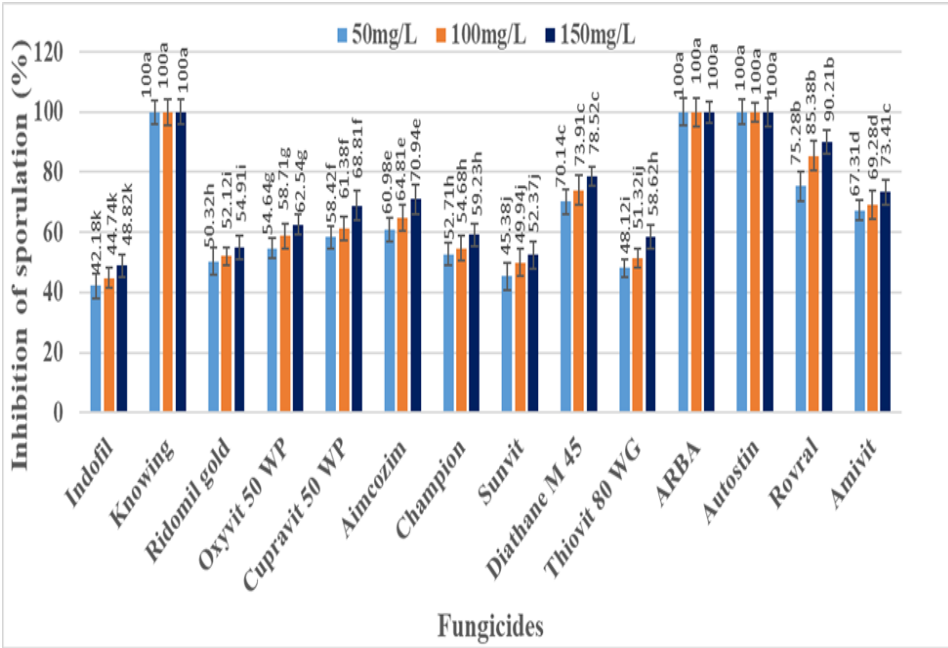

Fig. 5. Effect of different concentrations of the fungicides on the sporulation inhibition of *L. theobromae*. Bars marked by the same letters are not significantly different ($p < 0.05$) by DMRT analysis.

Table 6. Influence of the fungicidal treatment on the development of the lesion size on the stem of *S. saman* at 2 % concentration

Treatments	Lesion size before spray (cm) (April)*	Development and size of the lesion (cm)				Percent development of the lesion*
		May*	June*	July*	August*	
T_0	5.4	5.8	6.2	6.8	7.3	35.18 a
T_1	7.8	7.9	8.3	8.9	9.12	16.92 f
T_2	7.4	7.8	8.2	8.4	8.6	16.21 f
T_3	6.8	7.1	7.5	8.3	7.9	16.18 f
T_4	6.3	6.6	6.9	7.4	7.6	20.63 e
T_5	5.3	5.6	5.8	6.3	6.6	24.53 d
T_6	5.8	6.2	6.4	6.9	7.4	27.59 c
T_7	6.9	7.3	7.4	7.8	8.1	17.39 f
T_8	6.4	7.3	7.6	7.9	8.4	31.25 b
T_9	5.9	6.2	6.5	6.9	7.2	22.04 e
T_{10}	7.9	8.1	8.4	8.6	8.9	12.65 g
T_{11}	7.4	7.6	7.9	8.2	8.7	17.57 f

Table 6. Contd.

Treatments	Lesion size before spray (cm) (April)*	Development and size of the lesion (cm)				Percent development of the lesion*
		May*	June*	July*	August*	
T_{12}	8.4	8.2	7.9	8.1	8.2	2.44 j
T_{13}	8.2	7.8	7.6	7.7	7.8	4.87 i
T_{14}	7.9	7.6	7.4	7.1	7.2	8.86 h
T_{15}	7.2	6.8	6.4	6.6	6.8	5.56 i
SD	0.998	0.828	0.81	0.78	0.75	9.35
CV (%)	14.39	11.64	11.13	10.26	9.61	53.51

*Mean of three replications

In a column, the same letters are not significantly different by DMRT at 5% level

T_0 = Control (Without fungicide), T_1 = Indofil, T_2 = Sunvit, T_3 = Diathene M45, T_4 = Oxyvit, T_5 = Rovral, T_6 = Aimcozim, T_7 = Thiovit, T_8 = Ridomil, T_9 = Amivit, T_{10} = Cupravit, T_{11} = Champion, T_{12} = Knowing, T_{13} = Arba, T_{14} = Autostin, T_{15} = Bordeaux mixture

Fig. 6. Effectiveness of chemical fungicides spraying in 2 % on lesion development of rain tree gummosis under field condition (before and after spray). (A_1& A_2) Control; (B_1& B_2) Indofil; (C_1& C_2) Sunvit; (D_1& D_2) Diathene M45; (E_1 & E_2) Oxyvit; F_1& F_2: Rovral; (G_1& G_2) Aimcozim; (H_1& H_2) Thiovit; (I_1&I_2) Ridomil; (J_1& J_2) Amivit; (K_1& K_2) Cupravit; (L_1& L_2) Champion; (M_1& M_2) Arba; (N_1& N_2) Autostin; (O_1& O_2) Bordeaux mixture; (P_1& P_2) Knowing.

Conclusion

The morphological observation indicated that the pathogen causing gummosis disease on rain trees was *L. theobromae*. The result of the pathogenicity test showed that there was a similarity in symptoms that arise between artificial inoculation and natural symptoms in the field. This test indicated that *L. theobromae* was the causal agent of rain tree gummosis disease. This fungus grows well at a pH of 6-8, relative humidity of 90-95 %, a temperature of 25-30°C, and a glucose and sucrose concentration of 2.5 %. The PDA medium is suitable for the growth and sporulation of this fungus. The fungicides Knowing, ARBA, Autostine, and the Bordeaux mixture have shown good results in controlling gummosis disease. In the future, this preliminary study would help in the development of long-term management strategies to control gummosis disease in rain trees in Bangladesh.

Acknowledgment

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Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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EFFECTS OF ROW SPACING ON DIFFERENT LENTIL VARIETIES UNDER STRIP TILLAGE SEEDING SYSTEM

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Abstract

Lentil is an important legume crop grown by three or four time tillage operations with post sowing irrigation after monsoon rice harvest. Strip tillage is a climate smart tillage technology where residual soil moisture can be utilized for plant growth and development. This study was conducted at Regional Agricultural Research Station, Ishurdi, Bangladesh in two consecutive years of 2020 and 2021 to find out the optimum row spacing for specific lentil variety under strip tillage seeding system. Four row spacing (S_1= 20 cm, S_2= 25 cm, S_3= 30 cm, S_4= 40 cm) and three varieties of lentil (V_1= BARI Masur-8, V_2= BARI Masur-7, V_3= BARI Masur-6) were assigned in a factorial Randomize Complete Block Design with three replications. The trend of lentil seed yield was recorded as BARI Masur-8 > BARI Masur-7 > BARI Masur-6 and seed yield decreased with increasing row spacing for all varieties. The results showed that 25 cm row spacing for BARI Masur-8, 20 cm row spacing for BARI Masur-6 and BARI Masur-7 were found suitable row spacing at strip tillage seeding systems. Among the lentil varieties, BARI Masur-8 showed better performance than others due to higher yield attributes and seed yield.

Keyword: BARI Masur, Seed yield, Strip tillage system, Soil moisture

Introduction

Recently, conventional as well as deep tillage system is being replaced by strip tillage technology in worldwide, including Bangladesh. Strip tillage is a climate-smart tillage technology that increases crop productivity while decreasing planting costs. Residual soil moisture can also be harvested using the strip tillage technique by planting rabi crops after the monsoon rice harvest.

Strip tillage technologies are more viable in drought stress areas where seeding operations and initial plant establishment can be carried out with residual soil moisture available immediately after monsoon rice harvest (Bell and Johansen, 2009). Many research reports suggest that strip tillage lentil cultivation is possible (Zaman *et al.*, 2019 and 2020). Lentils are placed in first position according to area coverage (40% of total pulse area) and production (45% of total pulse production). It is cultivated across the country, covering an area of 1.41 lakh hectares with a production of 1.77 lakh

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metric tons with an average yield of 1.26 t/ha (BBS 2021). However, the annual growth rates of the area decreased by 0.152%, while the growth rates of production (2.62%) and yield (2.77%) significantly increased during 2000-01 to 2019-20 due to the introduction of improved lentil varieties and management technologies (Miah *et al.*, 2021). Due to lack of desired plant population, its average yield in the country1130 kg ha^{-1} is quite low as compared to its yield potential 1800-2000 kg ha^{-1}. Optimum plant population density of lentil is an important factor to realizing the potential yields as it directly affects plant growth. According to Parveen and Bhuiya (2010), seed rate is one of the most important factors influencing lentil growth, yield, and quality. The choice of sowing row spacing is an important agronomic practice influencing plant density and crop establishment. Yield of lentil can be increased by using proper row spacing. Though Pulse Research Center, Bangladesh Agricultural Research Institute (BARI) suggested row spacing of lentil for deep tillage or conventional tillage where 3 to 4 times tillage operation was done. But in strip tillage seeding system, there was no finding of row to row spacing for lentil. Since information on these aspects is lacking in Bangladesh, therefore the present investigation was carried out to find out the optimum row spacing of specific variety under conservation agriculture system specially strip tillage.

Materials and Methods

Experimental site

The experiment was conducted in two consecutive years of 2019-20 and 2020-21 at the agro ecological zone of High Ganges River Floodplain (AEZ # 11), Regional Agricultural Research Station, Ishurdi, Pabna (24.03° N; 89.05° E; 16 AMSL) in Bangladesh.

Soil characteristics

The textural class of soil is sandy clay loam. The soil pH was 7.2 that represented neutral soil according to their pH value, organic matter 0.98% that indicated poor organic matter content soil, field capacity of soil 28.5%, permanent wilting point of 13% and bulk density of 1.49 g cm-3 were observed at the experimental plot. Available reports indicate that most soils of Bangladesh have low organic matter content. About 70% of the net cultivable areas in high and medium-high lands have a soil organic matter content of less than 2% (Banglapedia 2021).

Climatic parameter

Climatic parameter like monthly decade wise average maximum-minimum temperature and average total rainfall of the study area are shown in figure 1. Temperature data are collected from the experimental plot and rainfall data are collected from weather station of Agricultural Research Station, BARI, Ishurdi which is located around 300 meter distance from the experimental plot. Precipitation is very low and unevenly distributed. In this area of Bangladesh, winter crops are fully dependent on irrigation except pulses crop. Post-sowing irrigations are required for pulse crops when seeds are sown using both conventional and deep tillage methods. However, strip tillage systems do not require post sowing irrigation for soil moisture utilization.

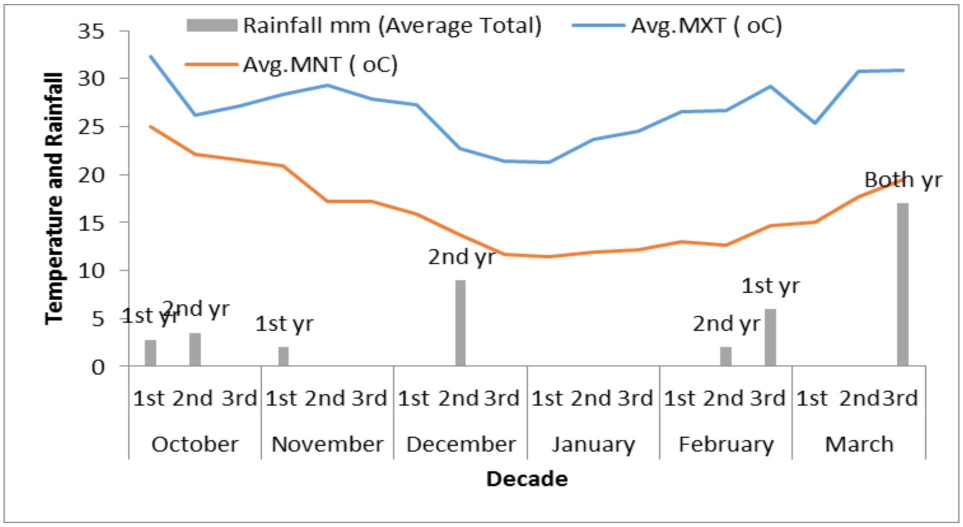

Fig. 1. Climatic parameter of growing period

Soil moisture determination

Soil samples were taken from the effective root zone of the lentil plant, which is 0 - 45 cm. The root zone was divided into three sections, *viz.* 0 - 15, 15 - 30 and 30 - 45 cm. Soil samples were collected from these three sections with the help of an auger. Collected sample was mixed each other's and the fresh weight of the soil sampled was recorded immediately with the help of a portable weighing balance. After being weighed, the samples were stored in soil sampling cores, which were then placed in an electric oven for 24 h at 100°C. The dry weight of the samples was recorded after oven drying. Soil moisture contents were then calculated as under:

$$Soil\ moisture\ content(\%) = \frac{Fresh\ weight\ of\ soil\ sample}{Dry\ weight\ of\ soil\ sample} \times 100$$

Water table depth was also measured about 9.62 m in dry season (Mid-January to April) and day by day declining.

Treatments and experimental design

Four row spacing (S_1= 20cm, S_2= 25cm, S_3= 30cm, S_4= 40cm) and three varieties (V_1= BARI Masur-8, V_2= BARI Masur-7, V_3= BARI Masur-6) were assigned in a factorial Randomize Complete Block Design with three replications. In this study skip the harmony of selection row spacing because of handling BARI strip tillage machine. Use row spacing 40 cm instead of 35 cm cause the limitation of furrow opener arrangement and maintain 35 cm row spacing. The furrow opener arrangement for different row spacing are shown in figure 2. The number of line for different row spacing like 20 cm, 25 cm, 30 cm, and 40 cm is 6, 5, 4 and 3 lines respectively. Seeding operation can be done by using inclined plate type seeding mechanism. Lentil are sown in continuous seeding system by this machine.

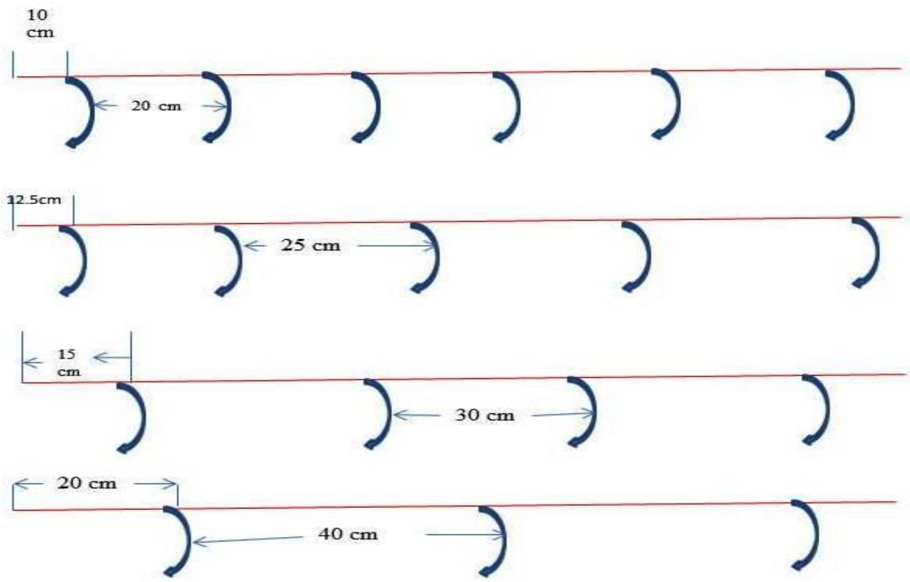

Fig. 2. Furrow opener arrangement for different row spacing

Fertilizer management

The crop was fertilized with 40-40-40-55-10 kg ha-1 as form of urea, murieate of potash, gypsum, boron, respectively. Only DAP (Di ammonium phosphate) was applied with machine and other fertilizer was broadcasted in land surface before seeding operation. Glyphosate @ 6ml per litter of water was sprayed before one day of seeding operation.

Cultural practices

Seed of different selected varieties of lentil were sown in the unit plot of size of 10 × 6 m by BARI developed strip tillage seeding machine. In strip tillage system maintained 20 cm, 25 cm, 30 cm and 40 cm distance between two rows. In strip tillage system, rotating blades were reduced where 4 blades in a face to face configuration remain in the gang at front position of seed furrow opener for tilling in strip 4 cm to 6 cm and creating tilt soil just in front of furrow openers. Between the two furrow openers the soil remained untilled. Strip tillage seeding system that utilizes residual soil moisture by tilling the soil just in front of furrow opener and place seed, and fertilizer in line at the appropriate depth in a single operation just after monsoon rice harvest. Monsoon rice residue was also used and maintain height 20−25 cm for lentil. At the sowing condition soil moisture was recorded 27.5% in first year and 29% in 2nd year. Lentil seeds were sown on 6th November and harvested on 4th March in both years. All the agronomic practices were carried out uniformly. The seed yield was recorded after harvest from unit plot size 5 × 6 m. Data was analyzed by using R software.

Results and Discussions

Machine performance on lentil

Total time requirement and fuel consumption of BARI strip tillage machine for lentil seed sowing are given in figure 3. These result was found 66% lower time with 68% of lower fuel than that of conventional tillage for lentil where using two wheeler. The conventional tillage performance data are collected from secondary sources of different research paper. In conventional tillage system, lentil cultivation was required time about 18.5 hr/ha and about 17.5 li/ha of fuel (Zaman et al., 2019). In strip tillage seeding, 57% lower fuel consumption were recorded than conventional tillage (Hossain et all,.2014).

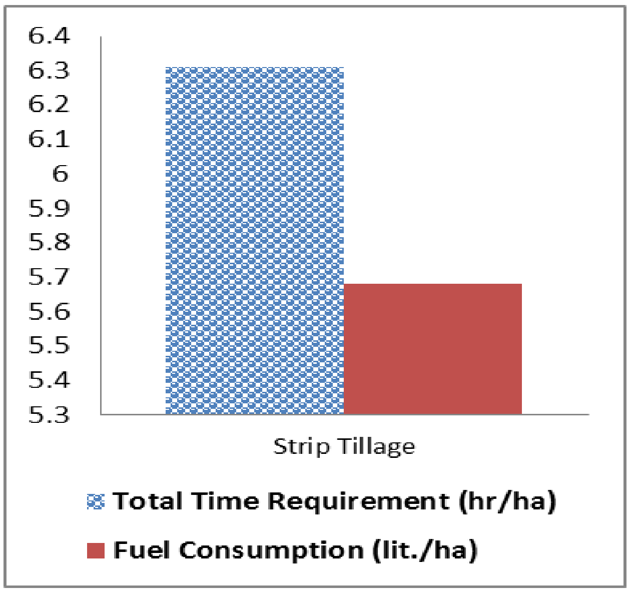

Fig. 3. Machine Performance on lentil

Performances of lentil varieties under strip tillage system

Effect of seed yield and plant population on different varieties were shown in figure 4. From the results, it was found that plant population was statically identical among the varieties but yield varied significantly. The highest seed yield was recorded from BARI Masur-8 which was significantly different from other variety and lowest from BARIMasur-6.

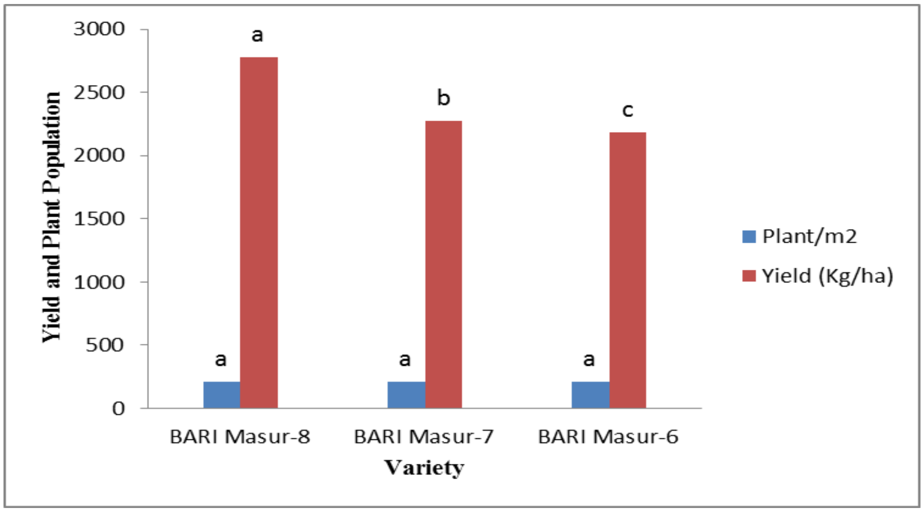

Fig. 4. Performance of different varieties on strip tillage

Relation between row spacing and seed yield

Row spacing and seed yield relations are shown in figure 5. The result was observed that plant population was decreased with increasing the row spacing for all variety. The plant population was observed about 220, 211, 200 and 194 at 20 cm, 25 cm, 30cm and 40cm respectively. Similar trend was observed in case of seed yield. Among the spacing 20 cm row spacing gave highest seed yield for all variety due to higher plant population.

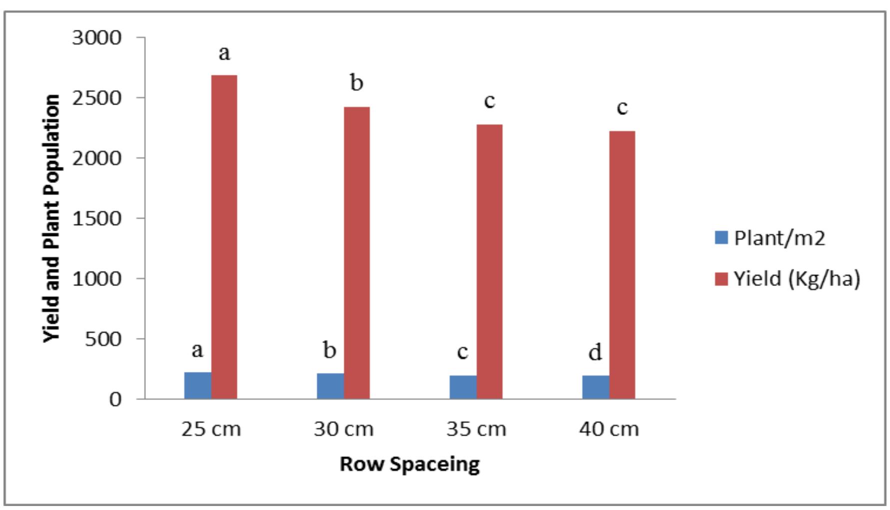

Fig. 5. Relation between row spacing and seed yield

Combined effect of spacing and varieties on strip tillage lentil

Combined effect of spacing and varieties are described in Table 1.

Plant population

The variation of plant per square meter was obtained S1 > S2 > S3 > S4. There was a trend to decrease plant population with the increase of row spacing in all the variety. Population did not influence any of the variety. Optimum row spacing have optimally utilized the growth resources, particularly solar radiation as compared to narrow row spacing where plants might have suffered due to mutual shading in case of adjoining rows and more plants within case of wider spacing. Optimum plant population density in lentil is an important factor for realize the potential yields as it directly affects plant growth and development (Turk *et al.,* 2003).

Pod per plant

Different row spacing significantly influenced number of pods /plant and seed yield of lentil. From this study, highest pod per plant was observed in highest row spacing treatment. Highest pod per plant was observed at BARI Masur-8 for all row spacing. The variety BARI Masur-6 and 7 showed similar trends in pods/plant but much lowers than former variety. Higher spacing can be related to intensify completion of plants and the decrease in over ground space for light interface.

Seed weight

Thousand seed weight of different varieties on different row spacing are given in Table 1. There were no significant variation was found. Seed weight was identically among the treatment.

Seed yield per hectare

Seed yield variation was obtained among the selected lentil varieties and different row spacing. The trend of seed yield was recorded as follows: BARI Masur-8 > BARI Masur-7 > BARI Masur-6. From the results, seed yield was decreased with increasing row spacing for all varieties. This could be clarified by the dominant effect of terminal bud lessens at lower densities and plants produce more auxiliary branches. So, they have better conditions for utilizing environmental conditions and produce more flowers. Consequently, increases pod number per plant. The increasing of row spacing is directly decreased plant population that leads to yield reduction.

From above results, it could be concluded that, BARI Masur-8 is found suitable than others lentil varieties due to steam blight tolerance and vigorous growth characters. There was no significant yield different between 20 cm and 25 cm row spacing for BARI Masur-8 except others combination. The result supports that 25 cm row spacing for BARI Masur-8; 20 cm for BARI Masur-6 and BARI Masur-7 are more suitable row to spacing at strip tillage seeding system. This results are consistent with the results of the of Idri's (2008) on faba been and Seyyed et al. (2014) on lentil that indicated increasing plant spacing increased number of pods per plant and consequently gave the highest seed yield.

Table 1. Combined effect of spacing and varieties on strip tillage lentil

(Pooled average of 2019-2020 and 2020-2021)Treatment	Plant per m^2	Pods/plant	1000 seed weight (gm)	Seed yield (kg/ha)
V1 XS1	221 a	115 abc	19.79	2930 a
V1 X S2	212 abc	134 ab	19.94	2943 a
V1 XS3	201 ef	135 a	20.38	2670 b
V1 X S4	193 f	136 a	20.11	2563 bc
V2X S1	221 a	110 c	20.89	2443 cd
V2X S2	212 bc	112 c	20.78	2286 de
V2XS3	199 ef	112 c	20.95	2190 ef
V2XS4	195 ef	112 bc	21.40	2187 ef
V3X S1	220 ab	109 c	19.79	2670 b
V3XS2	211 cd	111 c	20.89	2037 fg
V3XS3	202 de	110 c	21.12	1973 g
V3XS4	197 ef	112 c	21.50	1913 g
LSD (0.05)	8.83	22.09	2.05	160.41
CV%	4.88	11.12	NS	3.95

Spacing (S_1= 25cm, S_2= 30cm, S_3= 35cm, S_4= 40cm) and three varieties (V_1= BARI Masur-8, V_2= BARI Masur-7, V_3= BARI Masur-6)

Soil moisture profile

The Soil moisture profile of different experimental plot is shown in figure 6 (a-d). From the figure, it was found that there was no significant variation was found among the varieties as well as row spacing plot. Soil moisture was gradually decreased from sowing to harvest period for all variety at entire row spacing. Lentil faced permanent wilting point after 75 DAS.

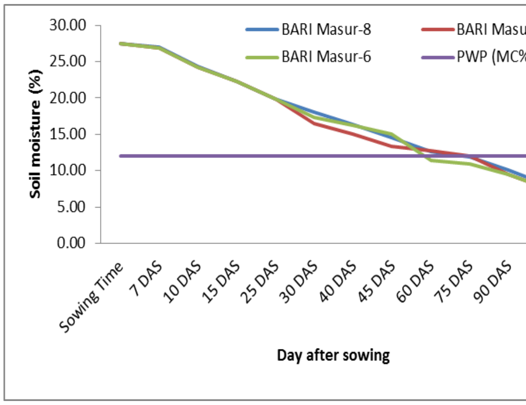

Fig. 6a. 20 cm row spacing plot

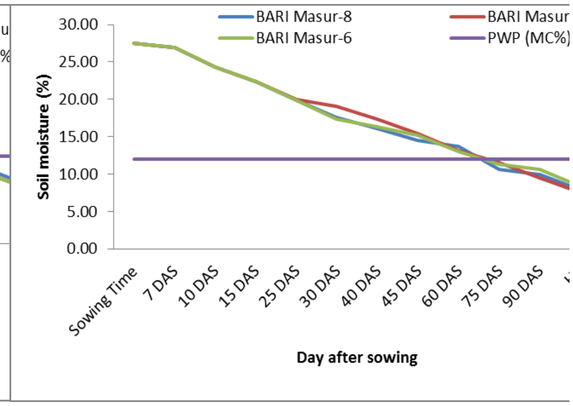

Fig. 6b. 25 cm row spacing plot

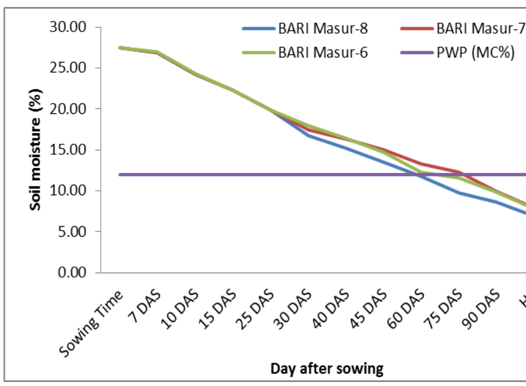

Fig. 6c. 30 cm row spacing plot

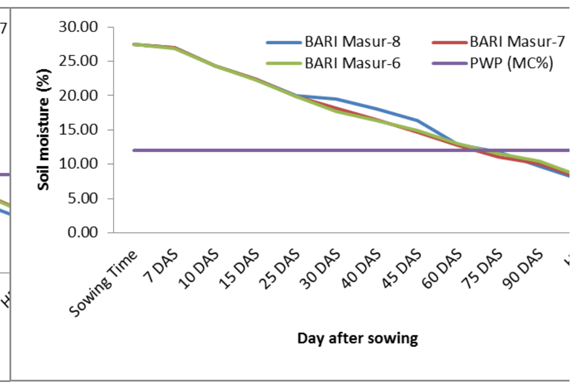

Fig. 6d. 40 cm row spacing plot

Fig. 6. Soil moisture profile of different experimental plot

Conclusion

From the study, the seed yield of lentil could be improved by seeding at optimum density. So, 25 cm row spacing for BARI Masur-8; 20 cm for BARI Masur-6 and BARI Masur-7 for lentil could be suitable row spacing at strip tillage seeding system. But lentil var. BARI Masur-8 could be cultivated under strip tillage seeding system for higher productivity in Ishurdi region.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper**.**

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INTERCROPPING PULSES WITH MULBERRY ON SERICULTURE PRODUCTIVITY AND PROFITABILITY

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Abstract

This study was conducted during 2020-2021 in three locations viz: research field of Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, five farmer's fields of Bholahat, Chapainawabganj and Paba, Rajshahi to evaluate the effect of growing pulses intercrops with mulberry productivity, silkworm rearing, soil properties and economy. The experiments were laid out in RCBD methods with three replications and six treatments.The growth and yield parameters of mulberry like average branch number/plant, total leaf number/plant, total branches length/plant, nodes/ meter/plant, length of longest shoot, leaf present/branch, 10 leaves area, total leaf weight/plant, total shoots weight/plant and total leaf yield were higher in T_4. Leaf quality viz: moisture content, total chlorophyll, crude protein, total sugar, reducing sugar and mineral in percentage (%) were significantly greater in T_4. The cocoon attributes like weight of 15 larvae, single cocoon weight, shell weight, cocoon shell ratio, highest filament length, renditta and cocoon productivity/100 dfls were better also in T_4 (54.68, 33.75, 0.27, 21.98, 990.41, 10.29 and 72.67) as compared to control (51.23, 31.21,0.19, 19.05, 962.96, 12.44, 69.07) respectively. Chickpea as an intercrop was given higher benefit: cost (1.30) due to increased soil fertility, higher leaf yield (except control), leaf quality, cocoon yield and additional income as compared with other intercrops (1.14, 1.07, 1.01, 0.93 and 0.86 for pea, grasspea, mugbean, sole mulberry and lentil respectively).

Keywords: Chickpea, Cocoon yield, Grasspea, Mugbean, Pruning, Renditta

Introduction

Sericulture is an agro-based industrial plantation crop with deep rooted culture and ritual of Bangladesh society artistic with decent climate. It is an art of systematic cultivation of mulberry and rearing of silkworms for the production of silk. Mulberry (*Morus* spp.) is a sole food plant for silkworm, *Bombyx mori* L. for commercial production of raw silk in sericulture industry. It is a deciduous or moist deciduous tree species originated from slopes of Himalayas that can be endured and grown-up to an elevation of 9000 mean sea level (msl). Sericulture is also an incredible for its low investment, swift and high returns as well as generating self-employment opportunity.

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Furthermore, this industry is also gorgeous mostly to small and marginal farmers, mainly steady sources of income.

Sericulture is facing harsh competition due to restricted land resources and competition with other agricultural crops. Therefore, it is a crucial requisite to advance joint harmony between sericulture and agriculture for sustainable co-existence. Generally, most of sericulture farmers have limited land holdings and depends mainly upon family labor and simple tools, they neither have the capability to take risk, nor have adequate land to expand its cultivation. Thus, by growing of other short duration crops, farmers can gets extra profits from intercrops (Ahsan *et al.*, 1989). The eternally swelling need for food, clothing and shelter from the inadequate land on account of rising population, has obligatory man to progress increasing the financial profits from unit area of land. In this regard, multi-cropping and intercropping are cautiously feasible options that mainly emphasize on crops diversification and amplification of land use. Mulberry cultivation is a main component for financially viable and success of sericulture as well as it would be more remunerative, if intercropped with short term crops, than as a mono crop (Ramamurthy *et al.*, 2006).

Intercropping with mulberry is increased productivity per unit area of land and time as well as also helps in impartial and judicious application of land and farming inputs including labour through cultivation of short duration crops between the rows of mulberry without affecting the quantity and quality of mulberry leaf (Vishaka *et al.*, 2017). Lots of study has already been conducted for mixing of Sericulture with agriculture and horticulture (Gargi *et al.*, 1997). In Kashmir saffron intercropping with mulberry yielded a good quality of leaf from the same field where saffron was cultivated alone to generate work as well as good deal of returns to farmers during lean period when there is no operations related to saffron cultivation (Kaur *et al.*, 2002). Several recent studies also suggest that mulberry can be successfully intercropped with medicinal plants (Madhusudan *et al.*, 2015).

The information especially on leguminous crops intercropping with mulberry was unavailable. Therefore, prospect of leguminous crops intercropping with mulberry was a scorching researchable issue in Bangladesh. This study was conducted to estimate the impact of legumes intercropping with mulberry production, silk cocoon productivity, and soil fertility status as well as sericulture economy. It was hypothesized that legumes intercropping with mulberry will be more profitable for sericulture farmers.

Materials and Methods

Location

The Experiment was carried out at the research field of Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi in the Agro-Ecological Zones (AEZ-10 and AEZ-11), Farmers' field of Bholahat, Chapainawabganj (AEZ-11 and AEZ-26) and Paba, Rajshahi (AEZ-26) during 2020-2021.

Plantation system and variety

Mulberry variety BM-11 and paired row high bush mulberry plantation system maintaining spacing between plant to plant (61cm × 61cm), line to line (92 cm × 92 cm) and row to row (183 cm × 183 cm) were used for this study. Intercrops were sowed in lines between the rows and maintaining standard spacing for respective pulses.

Garden management

All cultural practices were done as per requirements. Each experimental treatment was applied individually in definite farmer's field according to the farmer's perception at pruned garden. Intercrops seeds were sown in prepared bed between the rows and maintaining standard spacing for respective pulses after 2-3 days of mulberry garden pruning through broadcasting method. Only the BSRTI recommended basal fertilizer dose ($N_{300}P_{150}K_{100}$ kg/ha/year) were used for mulberry cultivation. The mulberry leaves were harvest 75-80 days after pruning and respective intercrop was harvested depend on maturity.

Experimental design and treatments details

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications and six treatments. The treatments were 1) T_0 = sole mulberry (control), 2) T_1 = mulberry + pea, 3) T_2 = mulberry + grasspea, 4) T_3 = mulberry + lentil, 5) $T_{4\,=}$ mulberry + chickpea and 6) $T_{5\,=}$ mulberry + mugbean.

Measurement of soil properties

The soil pH was determined by using the glass electrode method (Haber *et al.*, 1909). Soil organic C was determined by chromic acid digestion and spectrophotometric analysis (Heanes, 1984). Soil organic matter content was determined by multiplying the percent value of organic carbon with the conventional Van-Bemmelen's factor of 1.724 (Piper, 1950). The nitrogen content of the soil sample was determined by distilling soil with alkaline potassium per manganate solution (Subhaiah and Asija, 1956). The distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N sulphuric acid (H_2So_4) (Podder *et al.*, 2012). The soil available K was extracted with 1N NH_4OAC and determined by an atomic absorption spectrometer (Biswas *et al.*, 2012). The available P of the soil was determined by spectrophotometer at a wavelength of 890 nm. The soil sample was extracted by Olsen method with 0.5 M $NaHCo_3$ as outlined by Huq and Alam (2005). Zn in the soil sample was measured by an atomic absorption spectrophotometer (AAS) after extracting with DTPA Soltanpour and Workman (1979). Initial soil properties of the experimental soil are presented in Table-1.

Recorded growth and yield parameters

The recorded growth and yield parameters were total leaf number/branch, leaf present/branch, total branch height/plant (cm), length of longest shoot (cm), total shoot weight/plant (g), node/meter, 10 leaves area/plant (cm^2), total leaf weight/plant (g) and leaf yield (t)/hectare/crop followed by the respective procedure after 90 days of pruning.

Table 1. Average of initial physical and chemical properties of the experimental soil

pH	OM (%)	N (%)	P (kgha^{-1})	K (me/ 100 g soil)	Ca (me/ 100 g soil)	Mg (me/ 100 g soil)	Zn (ppm)	Fe (ppm)	Cu (ppm)
8.35	1.01	0.06	11.3	0.33	28.036	2.86	0.94	2.65	0.48

Analysis of leaf quality

The mulberry leaf samples at different heights of the plant (top, middle and bottom) were collected in poly bags at 75 days after pruning (DAP) and composite leaf samples were made. The moisture (%) was determined by followed the Vijayan *et al*., (1996), total Chlorophyll content Hiscox and Israelstam (1979) using the spectrophotometer and were computed using the standard formulae (Arnon, 1949), total mineral (%) AOAC (1980), protein (%) Kjeldahl's method (Wong 1923), total sugar and reducing sugar (%) followed by the Miller (1972) and Loomis *et al.*, (1937) procedure and methods.

Recorded silkworm rearing attributes

The leaves from mulberry were fed to silkworms and yield contributes viz. weight of 10 matured larvae (g), single cocoon weight (g), single shell weight, cocoon shell ratio, highest filament length (m), renditta and yield of cocoon/100 disease free laying eggs (dfls) and economics of mulberry leaf production with intercrops were also recorded during the study period.

Economics

The prices of inputs were used at the time of their use and selling prices of seeds based on prevailing market rates at the time of harvest of the produce will be taken into account.

Net returns

The net profit/ha was calculated by deducting cost of cultivation/ha from gross returns/ha.

Benefit- Cost Ratio (BCR)

BCR= Net returns (Tk /ha/crop) / Cost of cultivation (Tk/ha/crop)

Statistical analysis

The collected data were statistically analysed and mean values were evaluated by Duncan's Multiple Range Test (DMRT) through using the Statistic10 software. In the case of soil, the mean values of post-harvest soil properties were recorded for this study through using the Genstat 12.1thed^{n} for Windows (Lawes Agricultural Trust, UK) software.

Results and Discussion

Effect of pulses intercropping on growth performances of mulberry plant

Number of branches/plant

The number of branches/plant of mulberry was statistically significant for the treatment of T_0 which was statistically similar with the treatment of T_4 (Table 2). However, the maximum branch number/plant was 12.30 for sole mulberry (T_0) cultivation followed by T_4, T_1, T_5, T_2 and T_3 respectively.

Table 2. Growth performances of different pulses intercropped with mulberry garden

Treatments	Branch number/ Plant	Total leaf number/ Plant	Total branch height/ plant (cm)	Node/ meter/ Plant	Length of longest shoot (cm)	Leaf present/ branch	Leaf area (cm^2)	Total leaf weight/ plant (g)	Total shoot weight/ plant (g)	Total leaf yield/ hectare/year (t)
T_0	12.30 a	1354.3 a	961.60a	24.77 a	132.59 a	25.37 a	61.32a	923.21 a	435.69 a	11.08a
T_1	11.55 bc	1296.9 b	882.66b	23.33 c	124.58 b	21.81 b	56.59b	896.70b	413.03 b	10.76b
T_2	11.10 c	1258.0 c	799.95c	23.23 c	122.79 b	21.47 c	54.34c	872.84 c	407.46 b	10.4 c
T_3	9.89 d	1152.0 d	720.94d	22.20d	113.26 c	18.83 d	52.38d	820.79d	347.13 c	9.85d
T_4	12.08 ab	1349.2 a	885.90b	23.64b	126.55ab	21.87 b	56.74b	899.03b	413.78 b	10.81b
T_5	11.49 bc	1296.0 b	885.35b	23.28 c	123.44 b	21.75 b	56.45b	894.65b	412.82 b	10.75b

Here, T_0 = sole mulberry (control) T_1 = mulberry + pea, T_2 = mulberry + grasspea, T_3 = mulberry + lentil, T_4 = mulberry + chickpea and T_5 = mulberry + mugbean

Total leaf number per plant

The significant trend was observed for total leaf number/plant of mulberry through pulses intercropped with mulberry (Table 2). Statistically significant total leaves were recorded for the T_0 treatment that was similar with the treatment of T_4 (Table 2). However, the maximum leaves number/plant was 1354. 28 for sole mulberry (T_0) plant followed by the other treatments.

Total branches height per plant

Total branches height/plant of mulberry was significantly greater for the T_0 treatment (Table 2). The recorded maximum branch height was 961.6 cm for sole mulberry (T_0) cultivation.

Nodes per meter per plant

The intercropping treatments had a significant effect on nodes/meter of mulberry (Table 2). However, the maximum nodes / meter was 24.77 for sole mulberry (T_0) which was statistically significant followed by the T_4, T_1, T_5, T_2, respectively while least was found in T_3 (22.20).

Length of longest shoot

The length of longest shoot of mulberry was significantly greater for the T_0 treatment which was statistically similar with the treatment of T_4 (Table 2). However, the recorded maximum length of longest shoot was 132.59 cm in sole mulberry (T_0) plant followed by T4 (126.55), T_1 (124.58), T_5 (123.44), T_2 (122.79), respectively while least was found in T_3 (113.26).

Leaf per branch

The presence of mulberry leaves/branch markedly varied for intercropping treatments. The recorded maximum leaves present/branch was 25.37 for sole mulberry (T_0) cultivation that was statistically highest among all treatments (Table 2). However, in case of pulses intercropping with mulberry where leaves /branch was obtained in T_4 (21.87), T_1 (21.81), T_5 (21.75), T_2 (21.47) and T_3 (18.83), respectively.

Leaf area

The significantly greater 10 leaves area of mulberry was recorded for the treatment of T_0 (Table 2). However, the maximum leaf area was 61.32 cm^2 for sole mulberry (T_0) cultivation followed by the treatments of T_4 (56.74), T_1 (56.59), T_5 (56.45), T_2 (54.34), respectively while least in T_3 (52.38).

Total leaf weight per plant

The intercropping treatments had a highly significant trend on total leaf weight of mulberry plant. However, the maximum total leaf weight/plant was found 923.21g for sole mulberry (T_0) plant that was statistically highest followed by the treatment of T_4 (899.03g), T_1 (896.70g), T_5 (894.65g), T_2 (872.84g) and T_3 (820.79g), respectively (Table 2).

Total shoots weight per plant

The total shoot weight/plant of mulberry was highly significant for the treatment of T_0 (Table 2). However, the maximum total shoot weight/plant was found 435.69g for cultivation of sole mulberry (T_0) plant followed by T_4 (413.78g), T_1 (413.03g), T_5 (412.82g), T_2 (407.46g) and T_3 (347.13g) treatments respectively.

Effect of pulses intercropping on productivity

The mulberry leaf productivity was statistically differed by the intercropping of pulses with mulberry plant. Among the treatments average leaf yield was greater in sole mulberry (11.08 t ha^{-1}) compared to rest of intercropping treatments was mainly due to increasing all yield attributes characters (Table 3). Vishaka *et al.*, (2017) reported that in sole mulberry at 60 days after pruning compared to other intercropping treatments the growth parameters were significantly greater due to no competition from the intercrops for various inputs in sole mulberry. However, the mulberry leaf yield was T_4 (10.81 t ha^{-1}), T_1 (10.76 t ha^{-1}), T_5 (10.75 t ha^{-1}), T_2 (10.47 t ha^{-1}) and T_3 (9.85 t ha^{-1},) respectively with reasonable yield due to better growth and yield contributing characters (Table 3). Rajegowda *et al.*, (2020) also found that intercropping of mulberry with legumes performed better growth attributes and produced higher leaf yield due to enhancement of soil fertility.

In case of pod production for various pulses the maximum pod production was 1 ton/hectare/crop for the treatment of T_4 (chickpea) which was statistically highest than the other treatments. The pod production for grasspea and mugbean was statistically similar but the lowest pod production was found for lentil. However, the recorded pod production for mugbean were (0.80 t), grasspea (0.75 t), pea (0.60 t) and lentil (0.50 t) per hectare per crop respectively (Table 3).

Table 3. Growth performances of different pulses intercropped with mulberry garden

Treatments	Mulberry leaf yield (t/ha/crop)	Yield of intercrops (t/ha/crop)
T_0	11.08a	-
T_1	10.76b	0.60c
T_2	10.47c	0.75b
T_3	9.85d	0.50 d
T_4	10.81b	1.00a
T_5	10.75b	0.80b

Here, T_0 = sole mulberry (control), T_1 = mulberry + pea, T_2 = mulberry + grasspea, T_3 = mulberry + lentil, T_4 = mulberry + chickpea and T_5 = mulberry + mugbean

Performance of intercropping on mulberry leaf quality

The leaf quality of mulberry, moisture, total chlorophyll, crude protein, total sugar, reducing sugar and mineral contain in mulberry leaf were significantly improved by pulses intercropped with mulberry. Among treatments the recorded maximum moisture, total chlorophyll, crude protein, total sugar, reducing sugar and mineral were (76.78%), (39.74), (20.86%), (6.41%), (4.17%) and (12.77%), respectively for T_4 treatment followed by the T_1, T_2, T_5 and T_0 respectively (Table 5). Intercropping of pulses with mulberry had a possessive impact on improvement of mulberry leaf quality viz. moisture, total chlorophyll, protein, total sugar, reducing sugar and mineral. But better performance was found for the mulberry + chickpea intercropped followed by the mulberry + pea, mulberry + mugbean and mulberry + grasspea, respectively. However, the moisture and total chlorophyll contain in T_4 treatment were statistically differed compared to all treatments (Table 4).

Intercropping effect on silkworm rearing attributes

The silkworm rearing performance was statistically differed to feed on different types of pulses intercropped mulberry leaf. However, intercropping treatments, the silkworm rearing attributes viz., weight of single larvae (3.65 g), single cocoon weight (33.75g), shell weight (0.27g), cocoon shell ratio (21.98), highest filament length (990.41m), renditta (10.29) and cocoon productivity/100 dfls (72.67 kg), respectively were better in treatment T_4 (mulberry + chickpea) followed by the T_1 (mulberry + pea), T_2 (mulberry + grasspea), T_5 (mulberry + mugbean), T_0 (sole mulberry) and T_3 (mulberry + lentil) treatments, respectively (Table 5).

Table 4. Average leaf quality performances of different pulses intercropped with mulberry garden

Treatments	Moisture (%)	Total chlorophyll (SPAD value)	Protein (%)	Total sugar (%)	Reducing sugar (%)	Mineral (%)
T_0	75.57 d	38.51 d	20.13 d	6.03 c	3.63 ab	11.77 d
T_1	76.69 b	39.66 b	20.77 ab	6.38 a	4.05 a	12.74 ab
T_2	76.53 c	39.59 b	20.75 bc	6.34 ab	4.03 a	12.71 b
T_3	74.83 e	37.98 e	19.72 e	5.52 d	3.44 b	11.11 e
T_4	76.78 a	39.74 a	20.86 a	6.41 a	4.17 a	12.77 a
T_5	75.62 d	38.76 c	20.66 c	6.28 b	3.84 ab	12.60 c

T_0 = Sole mulberry (Control) T_1 = mulberry + pea, T_2 = mulberry + grasspea, T_3 = mulberry + lentil, T_4 = mulberry + chickpea and T_5 = mulberry + mugbean

Table 5. Average silkworm rearing performances of different pulses intercropped with mulberry garden

Treatments	Weight of single larvae (g)	Single cocoon weight (g)	Shell weight (g)	Cocoon shell ratio	Highest filament length (m)	Renditta	Cocoon productivity /100 dfls (kg)
T_0	3.56 c	32.40 d	0.20 bc	20.13 bc	978.14 b	11.13 b	70.55 c
T_1	3.64a	33.65 a	0.26 a	21.36 ab	986.30 ab	10.44 d	72.62 a
T_2	3.62 b	33.36 b	0.25 a	21.05 ab	985.09 ab	10.80 c	71.36 b
T_3	3.42d	31.21 e	0.19 c	19.05 c	962.96 c	12.44 a	69.07 d
T_4	3.65a	33.75 a	0.27 a	21.98 a	990.41 a	10.29 e	72.67 a
T_5	3.57c	32.62 c	0.22 b	20.36 b	979.70 b	11.13 b	71.10 b

Here, T_0 = sole mulberry (control) T_1 = mulberry + pea, T_2 = mulberry + grasspea, T_3 = mulberry + lentil, T_4 = mulberry + chickpea and T_5 = mulberry + mugbean

Intercropping effect on soil properties

The results of the study indicated that post-harvest soil properties were not affected due to intercropping of pulses with mulberry plant except nitrogen and potassium (Table 5). The initial soil analysis showed average soil pH of 8.77, organic matter contents 1.01%, nitrogen N 0.06%, P 11.3 kg/ha, K 0.33 me/100g soil, Ca 28.036 me/100 g soil, Mg 1.96 me/100 g soil, Zn 0.94 ppm, Fe 2.63 ppm and Cu 0.48 ppm (Table 1). After harvest of intercrops, the post-harvest soil properties showed average soil pH 7.82, organic matter contents of 1.38%, nitrogen N 0.13%, P 14.89 kg/ha, K 0.19 me/100g soil, Ca 28.92 meq/100 g soil, Mg 3.11 meq/100 g soil, Zn 3.45 ppm, Fe 3.59 ppm and Cu 0.55 ppm. (Table 6).The maximum organic matter (1.58%), N (0.22%), P

(17.50 kg/ha), K (0.20 meq/100g soil), Ca (30.55 meq/100 g soil), Mg (3.44 meq/100 g soil), Zn (5.17 ppm), Fe (4.12 ppm) and Cu (0.64 ppm) respectively was obtained from T_4 (mulberry + chickpea) followed by other treatments as well as maximum soil pH was 8.03 for T_0. Similarly, the recorded average maximum nitrogen (0.22%) and potassium (0.20 meq/100 g soil) contain was also in mulberry + chickpea where recorded minimum in soil of T_3 (mulberry + lentil) treatment as well as soil pH 7.57 for T_4 treatment (Table 6).

Table 6. Average post-harvest soil properties of different pulses intercropped with mulberry garden

Treatments	pH	OM (%)	N (%)	P (kg/ha)	K (meq/ 100 g soil)	Ca (meq/ 100 g soil)	Mg (meq/ 100 g soil)	Zn (ppm)	Fe (ppm)	Cu (ppm)
T_0	8.03 a	1.31 cd	0.08a	14.00 c	0.18 a	28.10 d	2.87 c	2.69 d	3.13 c	0.51 cd
T_1	7.77 bc	1.45 b	0.15 a	17.33 a	0.20 a	29.71 b	3.42 a	3.56 b	4.08 a	0.57 b
T_2	7.77 bc	1.34 c	0.13 a	14.60 b	0.20 a	28.66 c	3.06 b	3.31 c	4.01 b	0.56 bc
T_3	8.00 ab	1.30 d	0.07 a	11.40 d	0.17 a	28.08 d	2.84 c	2.67 d	3.06 d	0.50 d
T_4	7.57 c	1.58 a	0.22 a	17.50 a	0.20 a	30.55 a	3.44 a	5.17 a	4.12 a	0.64 a
T_5	7.77 bc	1.32 cd	0.12a	14.53 c	0.19 a	28.43 c	3.04 b	3.30 c	3.15 c	0.51 cd
Average	7.82	1.38	0.13	14.89	0.19	28.92	3.11	3.45	3.59	0.55

Here, T_0 = Sole mulberry (control), T_1 = mulberry + pea, T_2 = mulberry + grass pea, T_3 = mulberry + lentil, T_4 = mulberry + chickpea, T_5 = mulberry + mug bean, OM= Organic matter, N = Nitrogen, P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium, Zn = Zinc, Fe = Iron and Cu = Copper

The average nitrogen contains in intercropped soil T_1 (0.15), T_2 (0.13), T_4 (0.22) and T_5 (0.12%) respectively greater than the control (0.08%) which may be due to the atmospheric N_2 fixation through nodule formation of legumes intercrops. The other soil nutrients viz: phosphorus (P), Potassium (K), calcium (Ca), magnesium, zinc (Zn), iron (Fe) and cupper (Cu) were grater in intercropped soil compared to sole mulberry growing soil. However, the obtained maximum P (17.50 kg/ha), K (0.20 me/100g soil), Ca (30.55 me/100 g soil), Mg (3.44 me/100 g soil), Zn (5.17 ppm), Fe (4.12 ppm) and Cu (0.64 ppm) in T_4 (mulberry + chickpea) treatment followed by other treatments. These could be greater biomass production in intercropped soil least of sole mulberry growing soil during the co-growing stage, resulting attributed soil nutrients improvement as corroborates with findings of Zheng *et al.*, (2011).

Cost benefit analysis as intercropping of mulberry with pulses

Total cost of cultivation was more in mulberry + mug bean intercropping (Tk. 133500 ha/crop) followed by mulberry + lentil (Tk. 111500 /ha/crop), mulberry + chickpea (Tk. 103300 /ha/crop), mulberry + pea (Tk. 102500 /ha/crop) and mulberry + grasspea (Tk. 101700 /ha/crop), respectively, while least in sole mulberry cultivation TK. (95000 /ha/crop). The gross return was ranged from Tk. 88,750 /ha/crop to Tk. 1, 34,450 /ha/crop. The maximum gross return of Tk. 1, 34,450 /ha/crop was obtained from intercropping of mulberry + chickpea followed by mulberry + mug bean (Tk. 1, 34,250/ ha), mulberry + pea (Tk. 1, 17,250/ha), mulberry + grasspea (Tk. 1,09,050/ha) and mulberry + lentil (Tk. 96,250/ha), respectively The lowest gross returns of Tk. 88,750 /ha/crop was found in sole mulberry cultivation (Table 7). The recorded higher BCR (1.30) was found in mulberry intercropped with chickpea which was at par with mulberry + pea (1.14), mulberry + grasspea (1.07), but mulberry + mugbean (1.01), sole mulberry (0.93) and mulberry + lentil (0.86), respectively (Table 7). In this study it was found that better cocoon yield, market price as well as additional income for chickpea and pea. These findings are in conformity with previous findings of Ahsan *et al.*, (1989), Kabir *et al.*, (1991), Gargi *et al.*, (1997) an Shankar *et al.*, (2000) found higher net returns and B: C ratio in mulberry and legume intercropping system compared to sole mulberry cultivation. Similarly, Ramamurthy *et al.*, (2006) also reported that intercropping of mugbean and chickpea with mulberry, the net returns was Tk. 35,552/ha/year and income equivalent ratio 1.9 whereas in case of sole mulberry crop net returns was Tk. 18,712 /ha/year.

Table 7. Economics of mulberry leaf production with pulses intercrops

Treatments	Gross return (Tk. /ha)			Cost of cultivation (Tk./ha)				Gross margin (Tk./ha)	BCR
	Cocoon	Intercrop	Total	Mulberry	Silkworm rearing	Intercrop	Total		
T_0	183750	-	183750	45000	50000	-	95000	88750	0.93
T_1	183750	36000	219750	45000	50000	7500	102500	117220	1.14
T_2	183750	27000	210750	45000	50000	6700	101700	109050	1.07
T_3	183750	24000	207750	45000	50000	16500	111500	96250	0.86
T_4	183750	54000	237750	45000	50000	8300	103300	134450	1.30
T_5	183750	84000	267750	45000	50000	38500	133500	134250	1.01

Here, T_0 = Sole mulberry (Control) T_1 = Mulberry + Pea, T_2 = Mulberry + Grasspea, T_3 = Mulberry + Lentil, $T_{4\,=}$ Mulberry + Chickpea, $T_{5\,=}$ Mulberry + Mugbean

Conclusion

The results of the present study exposed that intercropping in mulberry provides in utilization of space between mulberry plants. Intercropping of pulses could increase the income of sericulture farmers along with sericulture activities. In the long run, farmer's income as well as productivity could be increased by growing chickpea as intercrop in mulberry and enhanced soil fertility.

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Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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EFFECT OF DIETARY PHOSPHORUS AND RESTRICTED FEEDING ON PERFORMANCE, EGG QUALITY AND SERUM BIOCHEMICAL TRAITS OF LAYING HENS AT THE SECOND PRODUCTION PHASE

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Abstract

The effect of available phosphorus and restriction of feeding on the performance, egg quality, serum biochemical and yolk fatty acid profiles in laying hens at 40 to 60 weeks of age was studied. A total 540 laying hens (Lohmann Brown) aged at 40 weeks were randomly placed in a 2×3 factorial arrangement of dietary treatments including two levels of available phosphorus (AP, 0.32 and 0.45%) and three restricted feeding levels (RFL, 90, 95 and 100%) having 5 replicates in each treatment, and the experiment were conducted for twenty weeks. The results showed that dietary interaction between AP and RFL had significant effects on egg production percentage ($P<0.01$), egg weight ($P<0.05$), daily egg mass ($P<0.01$) and feed conversion ratio ($P<0.01$). Production performance was significantly ($P<0.01$) affected by RFL; and both 95% and 100% RFL had showed similar mean value in egg production and egg weight. Egg quality traits: eggshell color and eggshell breaking strength were differed ($P<0.05$) among the treatments. Moreover, albumen height, Haugh unit and eggshell breaking strength significantly ($P<0.05$) increased in diet contained 0.45% AP. Dietary limitation of feed did not alter the egg quality except eggshell color. Serum albumin was influenced among the treatments and the mean value of albumin and total protein were same in sole effect of 95% and 100% RFL. Higher serum glucose was obtained in response to the primary effect of 0.32% AP. The total concentration of yolk fatty acid profiles was not assorted in the impacts of AP, RFL and in their interaction. Therefore, the results suggest that 95% feeding was sufficient to hens during second production period, for optimum production performance and egg quality as well as diet containing 0.45% inorganic phosphorus could have influenced positively on egg quality.

Keywords: Albumen, Egg mass, Egg quality, Feed restriction, Yolk fatty acid

Introduction

For raising commercial poultry industry, cost of production is considered to be a crucial factor since total expenses enormously influenced by feed price that around 65 to 75% of total cost of poultry production (Abdurofi *et al*., 2017). Rising costs of feed, labor

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and miscellaneous items without a corresponding increase in the price of eggs have made feed restriction one of the ways of reducing production costs in commercial layer farms. Productivity and profitability widely depend to a larger extent on the genetic make-up or constitution of an animal being reared for either meat or egg production. In addition, other important factors influencing productivity include prevailing environmental conditions, management practices such as feed and feeding management and the technical knowledge of the farmers (Bell and Weaver, 2002). Dietary feed restriction has, therefore, been carried out by limiting the amount of feed given to birds each day (Scott *et al*., 1999) or fixed time restricted for access to feed (Sandoval and Gernat, 1996). Reducing feed supply whether qualitative or quantitative is usually practiced to limit feed intake of birds in order to improve efficiency of feed utilization as well as reduce feed and production costs (Zhan *et al*., 2007), thereby, increased economic benefit (Olawumi, 2014). Moreover, control feeding program has been associated to keeping up body weight during laying period and also assured that birds do not make abundance fat in abdominal cavity (Kostal *et al*., 1992). Agreeing to Batonon *et al*., (2014) also reported that marginal reduction of feed when offered to hens during production period that was able to perform at an equivalent level of full fed control diet without any unfavorable effects with their performance.

Phosphorus is an essential mineral after that calcium in laying hen diets. It plays a key role in numerous body functions including bone development, cellular digestion system, cellular regulatory mechanism and formation of egg (Sohail and Roland, 2002). Laying hens fulfill their requirements from the formulated corn and soybean-based diet. Various concentration of plant based feed ingredients are used for ration formulation therein present approximately two thirds of total phosphorus as insoluble complex form (phytic acid), which cannot utilize by poultry due to the lack of insufficient of phytase enzyme in the digestive tract (Singh, 2008). Although, phosphorus requirement of laying hens varies depending on the composition of diet (Skrivan *et al*., 2010; Kim *et al*., 2017) and calcium level (Pelicia *et al*., 2009), vitamin D3 (Keshavarz, 1996) and phytase enzyme availability (Kim *et al*., 2017). The level of phosphorus either over or low in diet can negatively be influenced on the egg production and egg quality. As a result, required dietary phosphorus according to hen ages must be maintained in diets for ideal production and good management. Swiatkiewicz *et al*., (2010) reported that eggshell thickness and breaking strength were decreased by reducing the dietary levels of calcium and phosphorus. On the other hand, Li *et al*., (2007) shown that production performance of layers fed diet containing available phosphorus of 2.9 and 4.0 g/kg diet from 23 to 47 weeks of age were similar. However, the establishment of available phosphorus requirement for commercial layers is a persistent challenge that might be due to the fundamental fact of instability of variable amount of phytate phosphorus in the diet and moreover, research information is very scanty on relation between available phosphorus and egg quality of hens at older ages. Therefore, the main focus of this study was therefore to know the egg quality, serum and yolk fatty acid composition of laying hens at second phase production period with different dietary available phosphorus and restricted feeding levels.

Materials and Methods

Birds and experimental design

A total of 540 commercial laying hens (Lohmann Brown) 40 weeks of age were arbitrarily assigned to six treatments with five replicates and 18 hens in each replicate, the experimental period was 20 weeks from 40 to 60 weeks of age. Before starting the experiment, hens were selected based upon their similar body weight and egg production. And, hens were subjected to seven days adaptation period, provided mash feed (115g/hen/day) according to the breeders' manual. Treatments were assigned in a completely randomized deign 2 × 3 factorial arrangement, consisting of two AP (0.32 and 0.45) and three RFL were set at 90, 95 and 100%. Hens were housed in 3-tier A-shaped metal cages stand totaling 6 lines with two hens per cage. Mortality was recorded properly and all dead birds were replaced from spare hens and kept up identical treatments.

Table 1. Ingredients and nutrient composition of diet

Ingredients	Percent (%)
Corn	67.23
Soybean meal (44%)	17.38
Corn gluten meal	4.24
Limestone	9.42
Calcium-phosphate	0.94
Salt	0.38
L-Lysine	0.05
DL-Methionine	0.03
Vitamin premix	0.18
Mineral premix	0.15
Calculated nutritional levels	
Metabolizable energy (ME, kcal/kg)	2800.00
Crude protein (CP, %)	16.00
Lysine (%)	0.74
Methionine (%)	0.32
Calcium (%)	3.8
Available phosphorus (%)	0.32

[1]Contains per kg: vit. A 5500 IU; vit. D3 1100 ICU; vit. E 11 mg; vit. B12 0.0066 mg; vit. K3 1.1 mg; riboflavin 4.4 mg; pathothenic acid 11 mg (calciumpantothenate:1.96 mg); choline 190.96 mg; folic acid 0.55 mg; pyridoxine 2.2 mg; biotin 0.11 mg; thiamine 2.2mg; ethoxyquin 125 mg. [2]Contains per kg: Cu 10 mg; Fe 60 mg; I 0.46 mg; Mn 120 mg; Zn 100 mg.

Experimental diet

The basal diet content 2800 Kcal/kg Metabolizable energy (ME), Crude protein (CP) 16%, Calcium (Ca) 3.8%, AP 0.32% and other dietary nutrient contents in diet showed in Table 1. According to the treatment, experimental diets were prepared with level of Ca 4.0%, AP 0.32 and 0.45%. Fine limestone as Ca source that contained 38.5% Ca and monodicalcium phosphate (MDCP) that comprised 18% Ca and 21% phosphorus (P) were added in diet for the adjustment of Ca and AP levels. In the entire experimental period, house temperature was maintained at 22±3^{o}C with photoperiod was fixed at 16 h light and 8 h dark.

Production performance and egg quality of laying hens

Throughout the experimental period, the number of eggs laid, cracked and broken eggs per replica in each group were registered daily evening at 5.00 pm and egg production was expressed as percentage of hen day egg production. The egg weight was taken every two days interval excluding cracked and abnormal eggs. Feed conversion ratio (FCR) was expressed as the ratio of gram of feed consumed and divided by daily egg mass. It was calculated by multiplying the average hen day production percentage by the average weight of eggs. Cracked and broken eggs were recorded daily in proper spreadsheets, and were evaluated as the total number of cracked or broken eggs divided by the number of produced eggs, and the result was multiplied by 100. Thirty eggs from each group were collected at the termination of every four weeks to determine exterior and interior egg quality. Eggshell strength was measured using egg multi tester instrument (QC-SPA, Technical Services and Supplies, TSS, UK). The shell thickness was measured at different three points using micrometer (Digimatic micrometer, series 293-330, Mitutoyo, Japan) after removing the inner shell membrane. An average of three different thickness measurements of an egg was described as eggshell thickness. Albumen height, Haugh unit, eggshell and yolk color were measured using semi-automated egg multi tester equipment (QCM+, TSS, UK).

Serum biochemical analysis

At the termination of the experimental period, blood samples were collected from the wing vein of two hens per replication and transferred into non-heparinized tubes. The collected blood samples were centrifugation at 3000 rpm for 15 minutes at 4^{o}C, serum was transferred into eppendorf tube and kept at -20^{o}C until the laboratory analysis. Serum albumin, triglycerides, cholesterol, high density lipoprotein (HDL), total protein, glucose, AST and ALT were measured by Konelab 20 analyzer (Thermo Fisher Scientific, Vantaa, Finland) following the manufacturer's instructions.

Yolk fatty acids profile

Towards the end of the experiment, eight eggs from each group were picked randomly in order to determine their fatty acids profile. Yolks from collected eggs were separated and 0.5g of fresh yolk was weighed into test tube. Analysis of the fatty acid composition of the total lipid was carried out by 7683B Series Injector and using 1µl 6890N Network GC system (Agilent Technologies, Santa Clara, USA) gas chromatography equipped.

Statistical analysis

The data were analyzed by two-way analysis of variance (ANOVA) using General Linear Models (GLM) Procedure of SAS (Statistical Analysis System, version 9.1, 2002) included the main effects of AP, RFL and the interaction of these effects. Duncan's multiple-range test was used to detect significant probability value (P<0.05).

Table 2. Effect of available phosphorus and restricted feeding levels on production performance of laying hens

AP (%)	RFL (%)	Egg Production (%)	Egg weight (g)	Daily egg mass (g)	FCR	Broken egg (%)	Cracked egg (%)
40-60 weeks							
0.32	90	89.21^{c}	60.86^{c}	54.29^{b}	1.91^{c}	0.01	1.09
	95	89.97^{b}	61.48^{abc}	55.31^{ab}	1.98^{b}	0.18	1.47
	100	90.33^{a}	62.16^{ab}	56.15^{a}	2.05^{a}	0.18	1.18
0.45	90	88.92^{c}	61.19^{bc}	54.41^{b}	1.90^{c}	0.16	1.16
	95	89.85^{b}	61.89^{abc}	55.50^{a}	1.97^{b}	0.16	1.07
	100	89.83^{b}	62.66^{a}	56.29^{a}	2.04^{a}	0.12	0.81
Main effects							
AP (%)	0.32	89.83	61.50	55.25	1.98	0.13	1.25
	0.45	89.53	61.91	55.40	1.97	0.15	1.02
RFL (%)	90	89.06^{b}	61.02^{b}	54.35^{c}	1.91^{c}	0.09	1.13
	95	89.91^{a}	61.68^{ab}	55.40^{b}	1.98^{b}	0.17	1.27
	100	90.08^{a}	62.41^{a}	56.22^{a}	2.05^{a}	0.15	0.99
SEM		0.11	0.19	0.20	0.01	0.02	0.10
P value							
AP x RFL		<.0001	0.04	0.002	<.0001	0.30	0.68
AP		0.16	0.28	0.73	0.81	0.68	0.27
RFL		<.0001	0.005	<.0001	<.0001	0.32	0.59

AP, available phosphorus; RFL, restricted feeding levels; FCR, feed conversion ratio; SEM, standard error of the means; [a,b,c] means in the same column with different supercripts differ (P<0.05).

Results

Egg production, egg weight, feed conversion and broken eggs

The production performance of layers fed different levels of AP and access of feeding was presented in Table 2. Significant (P<0.05) difference shown in egg production, egg weight, daily egg mass and FCR in response to the main effects of RFL and the dietary interaction between AP and RFL treatment groups. The egg production of laying hens tended higher between the interaction of 0.32% AP and full-fed control (100%) group than that of other dietary treatments. As for main effect of AP, production performances of layer were not significantly affected but marginally decline egg production percentage and increase egg weight, daily egg mass were observed in high AP level. Comparable mean value was found in egg production percentage between 95 and 100% access of feeding group as well as egg weight in 90% and 95%; 95 and 100% feeding group. Egg production had significantly (P<0.01) inferior in 90% feeding group. Increasing level of restricted feed had linear effect on daily average egg mass and feed conversion ratio. Percentage of broken and cracked eggs in the present study was not influenced by the effect of AP or RFL and in their interaction.

Albumen height, haugh unit, yolk color and eggshell breaking strength

No significant (P>0.05) interaction was observed in egg quality traits between the restricted feeding and the AP levels without exception of eggshell color and eggshell breaking strength (Table 3). Hens fed diet containing 0.45% AP significantly (P<0.5) increased albumen height, haugh unit and eggshell breaking strength (P<0.01) than hens fed of 0.32% AP. Besides, egg yolk color and eggshell thickness was tended to be increased numerically in the main effect of high AP in diet. Restricted feeding level did not influence the egg quality traits except egg shell color (P<0.05).

Table 3. Effect of available phosphorus and restricted feeding levels on egg quality of laying hens at 60 weeks

AP (%)	RFL (%)	Eggshell color	Albumen height (mm)	Haugh Unit	Yolk Color	Eggshell breaking Strength (kg/cm^2)	Eggshell thickness (mm)
	90	26.73^a	8.18	89.48	4.03	2.74^b	0.369
0.32	95	25.37^{ab}	7.92	88.12	4.17	2.87^b	0.366
	100	25.73^{ab}	8.20	89.54	4.10	3.03^{ab}	0.3726
	90	27.07^a	8.64	91.90	4.27	3.39^a	0.372
0.45	95	26.27^{ab}	8.27	90.34	3.93	3.16^{ab}	0.370
	100	24.57^b	8.50	90.92	4.27	3.47^a	0.377
Main effects							
AP (%)	0.32	25.94	8.10^b	89.05^b	4.10	2.88^b	0.369
	0.45	25.97	8.47^a	91.05^a	4.16	3.34^a	0.373
	90	26.90^a	8.41	90.69	4.15	3.07	0.371
RFL (%)	95	25.82^{ab}	8.09	89.23	4.05	3.02	0.368
	100	25.15^b	8.35	90.23	4.18	3.25	0.375
S EM		0.25	0.09	0.50	0.05	0.06	0.002
P value							
AP x RFL		0.04	0.24	0.33	0.21	0.004	0.719
AP		0.96	0.04	0.04	0.54	0.0003	0.359
RFL		0.02	0.30	0.48	0.46	0.30	0.365

AP, available phosphorus; RFL, restricted feeding levels; SEM, standard error of the means; ^{a,b} means in the same column with different supercripts differ (P<0.05).

Serum biochemical indices

The effects of dietary available phosphorus and restricted feeding on serum biochemical parameters of laying hens had been shown in Table 4. None of the serum biochemical parameters were unaffected except albumin, glucose and total protein in the study. As the effect of dietary AP, higher glucose concentration was found in 0.32% AP content in diet. Serum albumin and total protein were differed significantly (P<0.05) by the sole effect of access of feeding levels. However, statistically similar difference was noticed in between 95 and 100% feeding group. In addition, serum AST and ALT level were not altered by the dietary AP, RFL and their interaction.

Table 4. Effect of available phosphorus and restricted feeding levels on serum characteristics of laying hens aged at 60 weeks

AP (%)	RFL (%)	Alb (g/dl)	Chol (mg/dl)	Glu (mg/dl)	AST (IU/l)	ALT (IU/l)	HDL (mg/dl)	TP (mg/dl)	TG (mg/dl)
0.32	90	1.95[b]	134.11	276.59	167.99	0.46	6.54	5.94	1338.71
	95	2.07[ab]	157.33	273.81	181.65	0.90	10.15	6.25	1925.22
	100	2.15[a]	173.94	263.48	166.08	1.14	11.72	6.62	2094.23
0.45	90	1.97[b]	152.02	250.83	160.65	0.49	9.75	6.20	1804.16
	95	2.08[ab]	152.97	264.14	167.64	0.75	10.63	6.50	1785.39
	100	2.12[a]	139.50	263.40	165.38	0.59	7.71	6.67	1620.75
Main effects									
AP (%)	0.32	2.06	155.13	271.29[a]	171.91	0.83	9.47	6.27	1786.05
	0.45	2.06	148.16	259.46[b]	164.56	0.61	9.36	6.46	1736.76
RFL (%)	90	1.96[b]	143.07	263.71	164.32	0.47	8.15	6.07[b]	1571.43
	95	2.08[a]	155.15	268.98	174.64	0.82	10.39	6.38[ab]	1855.30
	100	2.13[a]	156.72	263.44	165.73	0.86	9.72	6.65[a]	1857.49
SEM		0.02	6.12	2.79	3.28	0.08	0.71	0.10	121.16
P value									
AP x RFL		0.02	0.52	0.10	0.59	0.12	0.29	0.20	0.60
AP		0.98	0.58	0.03	0.27	0.18	0.94	0.33	0.84
RFL		0.02	0.62	0.67	0.39	0.11	0.42	0.05	0.55

AP, available phosphorus; RFL, restricted feeding levels; SEM, standard error of the means; [a,b] means in the same column with different supercripts differ (P<0.05).

Fatty acid composition of egg yolk

The effect of AP and RFL on fatty acid composition of egg yolk had been summarized in Table 5. The egg yolk lipid profile was not significantly (P>0.05) differed in the interaction of AP and feed restriction groups. The significantly (P<0.05) more

Table 5. Effect of available phosphorus and restricted feeding levels on yolk fatty acid composition of laying hens at 60 weeks

AP (%)	RFL (%)	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:1	C20:4	C22:6	MUFA	PUFA	UFA	SFA	UFA/SFA
0.32	90	0.49	27.30	3.50	9.78	45.18	13.05	0.18	0.29	0.17	0.07	48.97	13.46	62.43	37.57	1.66
	95	0.46	27.54	3.58	9.55	47.14	11.03	0.16	0.30	0.17	0.08	51.02	11.44	62.45	37.55	1.68
	100	0.44	27.15	3.54	9.61	45.33	13.26	0.15	0.27	0.18	0.07	49.14	13.65	62.80	37.20	1.70
0.45	90	0.48	27.18	3.55	9.58	45.20	13.34	0.16	0.30	0.15	0.07	49.05	13.72	62.77	37.24	1.69
	95	0.44	27.06	3.50	9.46	46.57	12.28	0.14	0.33	0.16	0.06	50.39	12.64	63.04	36.96	1.71
	100	0.47	26.97	3.36	9.55	45.05	13.87	0.14	0.34	0.19	0.07	48.75	14.27	63.02	36.99	1.71
Main effects																
AP (%)	0.32	0.46	27.33	3.54	9.65	45.88	12.44	0.16	0.29[b]	0.17	0.07	49.71	12.85	62.56	37.44	1.68
	0.45	0.46	27.07	3.47	9.53	45.61	13.17	0.15	0.32[a]	0.17	0.07	49.39	13.54	62.94	37.06	1.70
RFL (%)	90	0.48	27.24	3.52	9.68	45.19	13.20	0.17	0.30	0.16[b]	0.07	49.01	13.59	62.60	37.40	1.68
	95	0.45	27.30	3.54	9.50	46.86	11.66	0.15	0.31	0.16[b]	0.07	50.71	12.04	62.75	37.26	1.69
	100	0.45	27.06	3.45	9.58	45.19	13.56	0.15	0.30	0.18[a]	0.07	48.94	13.96	62.91	37.09	1.70
SEM		0.01	0.16	0.08	0.14	0.34	0.36	0.004	0.008	0.004	0.002	0.356	0 .36	0.258	0.26	0.02
P value																
AP × RFL		0.27	0.95	0.99	0.99	0.35	0.25	0.09	0.15	0.12	0.59	0.36	0.26	0.98	0.98	0.97
AP		0.98	0.44	0.67	0.67	0.69	0.32	0.06	0.02	0.67	0.18	0.66	0.34	0.47	0.47	0.51
RFL		0.15	0.83	0.91	0.87	0.06	0.07	0.10	0.63	0.04	0.95	0.07	0.07	0.89	0.89	0.83

AP, available phosphorus; RFL, restricted feeding level; SEM, standard error of the means; [a,b] means in the same column with different supercripts differ ($P<0.05$).

eicosenoic acid (20:1) was found in the sole level of 0.45% AP content diet and arachidonic acid (20:4) in 100% full-fed control group, and comparable mean value was obtained in 90 and 95% restricted feeding group. Linoleic acid (C18:2) increased 5.68% when hens given 0.45% AP content diet than 0.32% AP that level was 12.22%. Furthermore, the total proportion of polyunsaturated fatty acids (PUFA) 5.37% and unsaturated fatty acids (UFA) 0.61% were increased in 0.45% AP containing diet as well as decreased 1.01% saturated fatty acids (SFA) as compared that of diet containing 0.32% AP.

Discussion

The present results showed that there is no significant difference on performance of laying hens as the effect of AP, which conforms to the result obtained by Boling *et al*. (2000) who reported that hens fed diet containing AP levels from 0.15 to 0.45% had no altered production performance during the entire experimental period (20 to 70 weeks). The results were also consistent with previous findings of (Ceylan *et al*., 2003). Another study, it has been found that production performance was not significantly high when laying hens fed a diet containing either levels of 2.5g/kg or 3.5g/kg AP at 21 to 61 weeks (Hughes *et al*., 2008). Thus, several reports indicate that productivity of laying hens had not changed when non-phytate phosphorus was higher than 2.0g/kg in corn soybean based diet even neither phosphorus deficiency symptoms (Mansoori and Modirsanei, 2015; Kozlowski and Jeroch, 2011). Francesch *et al*., (2005) found that egg weight was not affected by AP levels but reducing AP level resulted in a decrease egg weight which was partly in accordance with our results. Production performances of layer in the present study had greatly reduced with increasing level of feed restriction, which is in agreement to the previous study (Scott *et al*., 1999; Olawumi, 2014) who noted that levels of feed restriction either short-term or long term has significant effect on productive performance parameters during laying period. They also proposed that moderate quantitative feed restriction could not have negative effect on egg production performance, egg weight and it can be used for reducing feed and production costs and also get profit maximization. However, during the laying stage, the energy is the most important factor to ensure optimal production rates (Costa *et al*., 2009).Therefore, the reduction of production performance in laying hens in this study may be attributed due to the daily imposition of restricted feeding, which might be related to the limited daily energy, calcium, phosphorus and other nutrient intake of laying hens.

The results of egg quality traits are in agreement with the findings of Englmaierova *et al*., (2012) who reported that high level of non-phytate phosphorus (4g/kg) in diet significantly increased albumen height, haugh unit and eggshell thickness as well as other quality traits (yolk color, eggshell breaking strength and shell thickness) were positively influenced. Moustafa *et al*., (2015) also reported that high dietary AP level numerically increased eggshell thickness as compared to low. It was also shown that high AP in diet decreased haugh unit, which was opposed with our present result. In another study of Park *et al*., (2009) found that egg quality parameters were tended to be increased in aged laying hen when dietary levels of non-phytate phosphorus were 0.30 to 0.40%. However, the results obtained in the present study indicate that high dietary AP

(0.45%) improved egg quality, it possibly due to the fact that phosphorus may have influenced metabolic process and blood phosphorus concentration in aged hens. As to the effects of RFL, there was no effect of restriction level in egg quality traits that findings were similar with Osman *et al.*, (2010) who observed that feed restriction during laying period had no impact on egg quality. Nevertheless, significant effect was obtained in egg shell color, albumen height and eggshell breaking strength in dietary interaction between AP and the RFL treatment groups; it may be associated due to insufficient nutrient elements for restriction of feeding.

Kaya *et al.*, (2014) reported that laying hens fed diet containing 0.37% AP supplemented with organic acid mixture had significantly increased serum glucose concentration than that of control. It could be attributed to the dietary acidification that might stimulate glycogenesis pathway through the conversion of glucose 6-phosphate (G-6-P) to glycogen when the glucose in blood was high (Fushimi *et al*., 2001). Furthermore, it can be associated with stress on account of dietary restricted feeding may be elevated secretion of plasma corticosterone hormone. Previous study revealed that effect of energy restriction in poultry reduced serum total cholesterol and high-density lipoproteins (Chen *et al*., 2012). Rajman *et al*., (2006) stated that quantitative feed limitation in poultry had an effect on plasma biochemistry and also appeared that protein, albumin and triglyceride concentration were decreased in blood. On the other hands, Mellouk *et al*., (2018) observed that total cholesterol and triglyceride concentration was decreased when the quantitative restriction feed given to birds. According to Ei-Far (2014) conducted a study to investigate the effects of feed restriction on ducks and found that serum triglyceride and total cholesterol was significantly lower in restricted feeding groups that was partly same trend to the current study, although our results did not show significant effect. However, it revealed that restriction of diet could be modified the plasma lipids concentration and reduced the basal metabolism (Weyenberg *et al*., 2008). The turnover of adipose tissue triglyceride is responsive to change in macronutrient intake in which fasting increases lipolysis (Varady *et al*., 2007).

Generally, yolk fatty acid profiles vary depending on number of factors including improvement of nutrients in diet and management strategies and/or dietary manipulation which have been carried out different dietary oil sources supplementation in layer diets such as flaxseed, garlic, black cumin, fish and vegetable oil that those have given some promising effects. Several studies have previously shown that lipid composition of eggs yolk is fairly easy to change with dietary additives (Sing and Sachan, 2010), farming conditions (Rizzi *et al*., 2007) and housing system (Hidalgo *et al*., 2008), and also influenced by the laying hen breed (Kiani and Gharooni, 2016). The results of the present study cannot be comparable with the finding of previous literature since there is no distinct illustrative related evidence of the effects of AP on yolk fatty acids in laying hens. Linoleic acid was higher ($P<0.05$) in the egg yolk of hens fed 0.45% AP in our current study. According to the findings of Fendri *et al*., (2012) the linoleic acid of egg yolk increased by the supplementation of natural mineral (zeolite) 1 or 2% in layer diets from that of control. It was shown that selenium supplementation from 0.15 to 0.30 mg/kg in diet of laying hens significantly increased percentage of total unsaturated fatty acids, in this way increasing or decreasing the concentration of n-3 or n-6 in the total fatty acids pool (Zdunczyk *et al*., 2013). A significant increase ($P<0.05$) of arachidonic acid (20:4) was found 12.5% higher in full-fed control (100%) group. It could be due to the activity of delta-6 desaturase enzyme that promotes the desaturation of linoleic acid into arachidonic acid (Vidal *et al*., 2013). Although, the present results were not comparable with Li *et al*., (2016) who reported that higher phosphorus level in diet was significantly reduced fatty acid content of broilers meat than deficient and normal phosphorus diet. Therefore, the present investigation revealed that results indicating that higher level of dietary phosphorus influenced the long chain fatty acids profile of egg yolk.

Conclusion

The current study demonstrated that 95% feeding could be followed in hens during second production period without any adverse effects statistical change on the performance and egg quality. Dietary inorganic phosphorus at the level of 0.32% was sufficient to maintain the production performance, whereas 0.45% for egg quality.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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EEECT OF TREE LEAF BIOMASS ON SQR6 HYBRID RICE IN AGROFORESTRY SYSTEM

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Abstract

A field experiment was conducted in the Agroforestry Field Laboratory of Bangladesh Agricultural University (BAU), Mymensingh, during the period from November 2016 to April 2017 to find out the response of Rain tree (*Samanea saman*), Ipil-Ipil (*Leucaena leucephala*), and Minjiri (*Senna siamea*), leaves biomass with different fertilizers dose applications to yield and yield contributing character of Chinese Hybrid Rice cv. SQR6. Ten treatments were used for the Randomized Complete Block Design (RCBD) with three replications. The results showed that green LB had a significant effect on crop characters viz., plant height, panicle length, no. of tillers hill^{-1}, no. of leaves hill^{-1}, no. of panicles hill^{-1}, no. of effective tillers hill^{-1}, no. of non-effective tillers hill^{-1}, no. of spikelets panicle^{-1} and 1000-grain weight. The panicle length varied from 22.30 to 25.11 cm. The number of leaves on hill^{-1} varied from 62.00-26.00. The number of effective tillers on hill^{-1} varied from 12.23-10.24 whereas the number of non-effective tillers on hill^{-1} varied from 3.10-1.29. The maximum grain yield of 8.96 t ha^{-1} was obtained from Treatment T_{10} followed by T_6 where Ipil-Ipil of LB with 45% RFD was applied with a grain yield of 8.63 t ha^{-1}. Therefore, this study suggests that the green LB of Ipil-Ipil and Minjiri could be used the improvement of yield contributing characteristics of rice.

Keywords: Agro-forestry, Ipil-Ipil, Minjiri, Rice Yield, Yield attributes

Introduction

Bangladesh is a small country with a large population. The current population of Bangladesh is 165.15 million and the growth rate is 1.22% (BBS, 2022). Most of the people of our country depend on agriculture. Most farmers cannot always afford the high input cost of cultivation or modern high-yielding varieties. Sustainable crop production gets more attention in Bangladesh through the introduction of an agroforestry system, whereby tree litter is used as a supplement and to enhance crop production.

Exhaustive soil farming has worldwide resulted in the degradation of agricultural soils, with decreases in soil organic matter and loss of soil structure, adversely affecting

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soil functioning and causing a long-term threat to future yields (Pagliai *et al.,* 2004). Moreover, intensive tillage operations over a long period cause a detrimental effect on surface soil as well as hastening the decomposition of soil organic matter (Slentel *et al*., 2007).

Organic matter is called the life of the soil and plays an important role in sustainable soil fertility and crop productivity. The organic matter of Bangladesh soils is decreasing day by day. Different tree leaf biomasses such as the Rain tree, Ipil-Ipil, and Minjiri, etc. are good sources of organic matter and can play a vital role in soil fertility improvement as well as supplying nutrients, especially N, P, K, and S (Khan *et al*., 2020). The leaf litter supplies the carbon, nitrogen, phosphorus, potassium, and other nutrients in the soil that are further considered important indicators of soil productivity and ecosystem health. Through decomposition, the nutrients within leaf litter are converted into an available form for uptake by vegetation and thereby exercising a critical control on vegetation productivity (Chatzistathis, and Therios, 2013). In an agroforestry system, nutrients may be released from leaf litter by leaching or mineralization.

Now farmers are more attentive to practicing agroforestry systems in homestead areas, fallow land, etc. In the homestead, there are many trees such as kalo koroi, mahogoni, neem, sada koroi, raintree, krishnochura, and Ipil-Ipil, etc. for various purposes. Farmers have opportunities to use these leaves as green manure for rice cultivation and it is easy to cultivate rice with green leaf biomass. In Bangladesh, there is scope for using green leaf biomass in rice cultivation. It is so much proven that decomposed leaves have a good impact on the yield of rice. Besides the farmers can avoid the cost of fertilizer, organic matter, pesticides, etc. The study aimed to the effect of green leaf biomass of different trees on the growth performance, yield, and physical and chemical properties of Chinese hybrid rice.

Materials and Methods

Experimental site and design

The experiment was conducted at the Agroforestry Farm, Department of Agroforestry, Bangladesh Agricultural University (BAU), Mymensingh from November 2016 to May 2017 of AEZ-09 (Old Brahmaputra Floodplain). The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications.

Test variety

Rice cv. SQR6 (Jonok Raj) a modern variety of rice, was used as the test crop in this experiment. The variety was released from Chongqing Zhong Yi Seed Co., Ltd.

Treatments and the tree LB collection

The treatments as the tree LB like Rain tree (*S. saman*), Ipil-Ipil (*L. leucephala*), and Minjiri (*S. siamea*) LB with 15, 30, and 45% RFD and the LB were collected from the trees of BAU, Mymensingh (Table 1). These LB were chopped by hand and mixed uniformly with soil during final land preparation and then left to decompose for ten days.

Table 1. Treatments of the study

Treatments	Dose
T1 = Raintree	LB 2 kg /plot + 15% RFD
T2 = Raintree	LB 2 kg/plot + 30% RFD
T3 = Raintree	LB 2 kg/plot + 45% RFD
T4 = Ipil-Ipil	LB 2 kg/plot + 15% RFD
T5 = Ipil-Ipil	LB 2 kg/plot + 30% RFD
T6 = Ipil-Ipil	LB 2 kg/plot + 45% RFD
T7 = Minjiri	LB 2 kg/plot + 15% RFD
T8 = Minjiri	LB 2 kg/plot + 30% RFD
T9 = Minjiri	LB 2 kg/plot + 45% RFD
T10= Control (100% RFD)	-

Note: RFD=Recommended fertilizer dose, LB= Leaf biomass

Tree leaf biomass and fertilizer application

The whole amount of various tree LB such Ipil-Ipil, Minjiri, and Rain tree leaves were incorporated in experimental plots before final land preparation. The recommended doses of all fertilizers were applied in control plots during final land preparation. Urea was top dressed in three equal splits i.e.,15, 30, and 55 days after transplanting (DAT).

Chinese transplanting and method of seedlings

Thirty-three (33) day-old seedlings of cv. SQR-6 was collected from the Agronomy Field of BAU. Seedlings were uprooted with care from the slightly irrigated seedbed and transplanted on 05 January 2017 with a hill-to-hill and line-to-line distance of 20cm x 20cm and 33cm distance after two rows.

Harvesting and yield data collection

Harvesting was done on 9 May 2017 at its full maturity. The plants of individual treatment as tagged previously were separately harvested and threshed as well as yield contributing components as per requirement for each treatment. The ten hills were randomly selected from each plot at maturity to record the yield and yield contributing characters.

Recording data

The plant height was measured with the help of a meter scale from the ground level of the plant to the tip of the leaf. The number of leaves per hill was considered as the leaves present on the hill. The total number of tillers included effective and non-effective tillers. Panicle length was measured from the neck node to the tip of the panicle. One thousand grains were randomly selected from the harvest of each plot. The weight of grains was recorded by an electrical balance and adjusted to a 14% moisture level and converted to grain yield (kg ha^{-1})

The recorded data were compiled and analyzed and the means for all recorded data were calculated. The mean differences were evaluated by Duncan’s New Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Results and Discussion
Characteristics of rice
Plant height

Plant height was recorded in three stages such as 30, 60 DAT, and after harvest (Fig. 1). At the initial growth stage or 30 DAT, the height plant was noted in treatment T_{10} (46.26) cm because recommended fertilizer doses were applied to the soil. The lowest value was found in T_7 (40.52) cm because Raintree LB with 15% RFD was applied where soil fertility status was also low. At 60 DAP, the height plant was observed in treatment T_{10} (92.28) cm and followed by treatment T_6 (90.15) cm where Raintree, LB decomposed and added 45% RFD. The lowest height was observed in treatment T_1 (81.07) cm. At the final growth stage or harvest stage, the highest plant height of 94.83cm was observed in the treatment T_{10} because of RFD. The second highest plant height in T_6 (93.37) cm where Raintree, LB decomposed and added 45% RFD. The lowest height was observed in treatment T_1 (83.63) cm Raintree LB with 15% RFD applied. These results are in agreement with that of Win *et al*., (2019) who reported that the addition of biochar and *Bacillus pumilus* strain TUAT-1 increased plant height significantly.

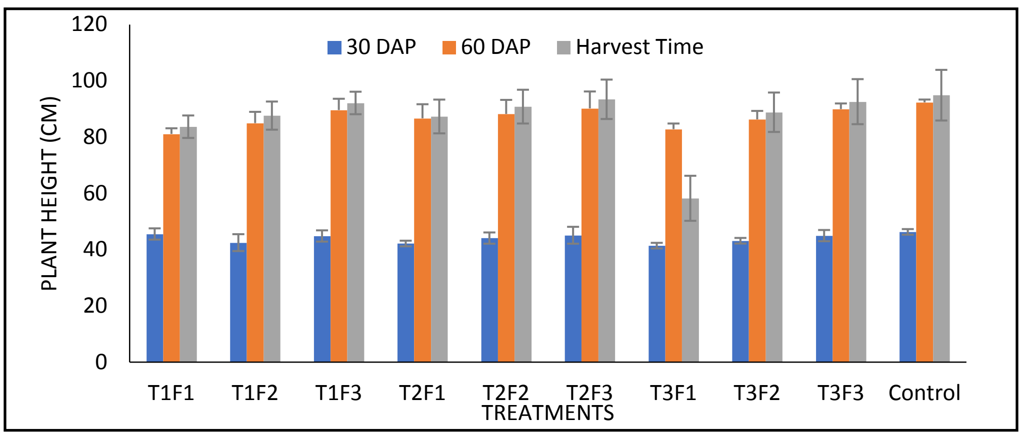

Fig. 1. Plant height of rice in different treatments

Note: T1F1=Raintree (LB 2 kg/plot) + 15% RFD, T1F2= Raintree (LB 2 kg/plot) + 30% RFD, T1F3= Raintree (LB 2 kg/plot) +45% RFD, T2F1=Ipil-Ipil (LB 2 kg/plot) + 15% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) + 30% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) +45 % RFD, T3F1=Minjiri (LB 2 kg/plot) + 15% RFD, T3F2=Minjiri (LB 2 kg/plot) + 30% RFD and T3F3=Minjiri (LB 2 kg/plot) +45 % RFD

The number of leaves per hill

The effect of green LB of different trees on the number of leaves on hill^{-1} varied from (41.56-26.00, and 62.00-44.00) in the treatment of T_{10} and T_1 at 45 and 60 DAT, respectively (Table 2). The highest number of leaves hill^{-1} was observed in T_6 (38.43) because Ipil-Ipil LB with 45% RFD and the lowest number of leaves hill^{-1} was observed in T_1 (26.00). The highest number of leaves hill^{-1} was observed in T_6 (58.33) because of Ipil-Ipil LB with 45% RFD and the lowest in the treatment T_1 (44.00). Similar results were observed by Islam *et al*., 2019 and the number of tillers on hill^{-1} varied from 11.67- 21.00.

Panicle length

The panicle length of rice (cv. SQR6) was significantly influenced by the incorporation of tree LB. The control treatment produced the maximum length of panicle followed by (T_6). The panicle length varied from 22.30 to 25.11 cm (Table 2). The treatment of RFD (T_{10}) produced a panicle length of 25.11 cm followed by 24.59, 24.41, 24.21, 24.08, 23.87, 23.77, 23.46, and 22.92 in the treatments of ($T_{6,}$ T_9, T_3, T_5, T_8, T_2, T_4, T_7). The lowest panicle length was 22.30 cm in the treatment of T_1.

The number of effective tillers per hill

The number of effective tillers of rice was significantly influenced by the incorporation of tree LB which varied from 12.27 to 10.24 in T_{10} and T_1 due to different treatments (Table 2). The highest number of tillers was found at 11.82 in the treatment of T_9 because of the use of Minjiri LB with 45% RFD and the lowest number of tillers was found at 10.24 in T_1. These results were supported by the findings of Bhuiyan *et al*., 2014.

The number of non-effective tillers per hill

The non-effective tiller hill^{-1} varied from 2.10 to 1.29 (Table 2). The non-effective tiller was higher in the treatment T_9 (1.88) and the lowest number of non-effective tillers was T_1 (1.29) in the LB. The treatments T_3, $T_{4,}$ T_5, $T_{9,}$ T_2, and T_8 have no significant differences.

Table 2. The effect of leaf biomass on vegetative characteristics of rice production provenance and spacing in agroforestry systems

Treatments	No. of leaves hill^{-1} 45 DAT	No. of leaves hill^{-1} 60 DAT	Panicle length (cm)	Effective tillers hill^{-1}	Non-effective tillers hill^{-1}
T1F1	26.00h	44.00h	22.30i	10.24f	1.29f
T1F2	30.50f	50.33ef	23.77f	11.38bcd	1.59d
T1F3	35.95d	55.67c	24.21cd	11.92ab	1.84c
T2F1	27.92g	48.00fg	23.46g	11.45bcd	1.45e
T2F2	32.60e	54.00cd	24.08de	11.32cde	1.81c
T2F3	38.43b	58.33b	24.59b	12.13a	1.97b
T3F1	27.10g	46.67g	22.92h	10.79ef	1.41e
T3F2	30.90f	52.33de	23.87ef	10.95de	1.68d
T3F3	36.12cd	56.00bc	24.41bc	11.82abc	1.88bc
Control	41.56a	62.00a	25.11a	12.23a	2.10a
CV%	2.76	2.92	0.52	2.86	3.44
Significance Level	**	**	**	**	**

Note: Means within the same letter (s) within a column do not differ significantly (P=0.05) according to DMRT.

T1F1=Raintree (LB 2 kg/plot) + 15% RFD, T1F2= Raintree (LB 2 kg/plot) + 30% RFD, T1F3= Raintree (LB 2 kg/plot) +45% RFD, T2F1=Ipil-Ipil (LB 2 kg/plot) + 15% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) + 30% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) +45 % RFD, T3F1=Minjiri (LB 2 kg/plot) + 15% RFD, T3F2=Minjiri (LB 2 kg/plot) + 30% RFD and T3F3=Minjiri (LB 2 kg/plot) +45 % RFD

The number of tillers per hill

The number of tillers hill^{-1} of rice (cv. SQR6) was significantly affected by the different treatments (Fig. 2). The maximum number of tillers hill^{-1} was found in T_{10} (7.07) at 30 DAT because of applying RFD followed by 6, 5.63, 5.33, 5.13, 4.73, 4.70, 4.57, and 4.17 in the treatment of T_6, T_9, T_3, T_5, T_2, T_8, T_4, and T_7. The lowest T_1 (3.83) for applying Raintree LB with 15% of RFD at 30 DAT. The maximum number of tillers hill^{-1} was found in T_{10} (13.33) at 45 DAT because of applying RFD followed by 13.00, 12.77, 12.53, 12.07, 11.90, 11.77, 11.50, and 11.13 in the treatment of T_6, T_9, T_3, T_5, T_2, T_8, T_4, and T_7. The lowest T_1 (9.8) for applying Raintree LB with 15% of RFD at 45 DAT. The maximum number of tillers hill^{-1} was found in T_{10} (14.33) at 60 DAT because of applying RED followed by 14.10, 13.77, 13.70, 13.13, 12.97, 12.90, 13.63, and 12.20 in the treatment of T_6, T_3, T_9, T_5, T_2, T_4, T_8, and T_7. The lowest T_1 (11.53) for applying Raintree LB with 15% of RFD at 60 DAT. The results are supported by the findings of Bhuiyan *et al*., 2014.

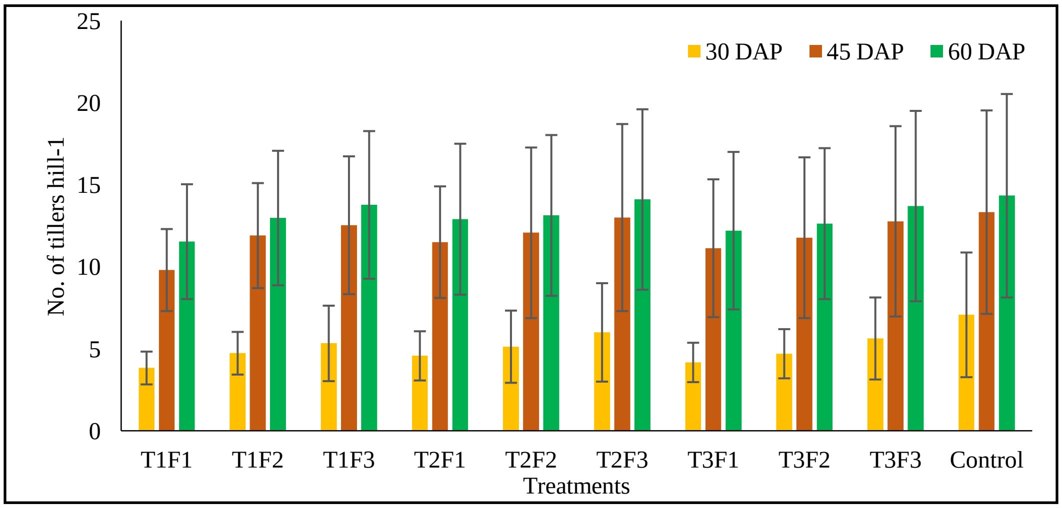

Fig. 2. Number of tillers in different treatments

Note: T1F1=Raintree (LB 2 kg/plot) + 15% RFD, T1F2= Raintree (LB 2 kg/plot) + 30% RFD, T1F3= Raintree (LB 2 kg/plot) +45% RFD, T2F1=Ipil-Ipil (LB 2 kg/plot) + 15% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) + 30% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) +45 % RFD, T3F1=Minjiri (LB 2 kg/plot) + 15% RFD, T3F2=Minjiri (LB 2 kg/plot) + 30% RFD and T3F3=Minjiri (LB 2 kg/plot) +45 % RFD

The Number spikelet per panicle

The number of spikelet panicle^{-1} was significantly affected by the different treatments. The number of spikelet panicle^{-1} was divided into three categories such as total, filled, and unfilled. The number of total spikelets in panicle^{-1} varied from 265.27 to 291.87. The highest number of total spikelet panicle^{-1} was found in the treatment T_{10} (291.87) for applying RFD and followed by 288, 281.27, 280.07, 279.67, 267.73, and 267.70 in the treatments of T_6, T_9, T_3, T_5, T_2, and T_4, and T_7, respectively. The lowest one in the treatment T_1 (265.27) where fertilizer plus 15% LB of Raintree was applied. The highest number of filled spikelet panicle^{-1} ^{was} obtained from the treatment T_{10} (277.67) and the lowest in T_1 (220.70). The unfilled spikelet panicle^{-1} was highest in treatment T_7 (44.57) and the lowest in T_{10} (14.20) (Fig. 3).

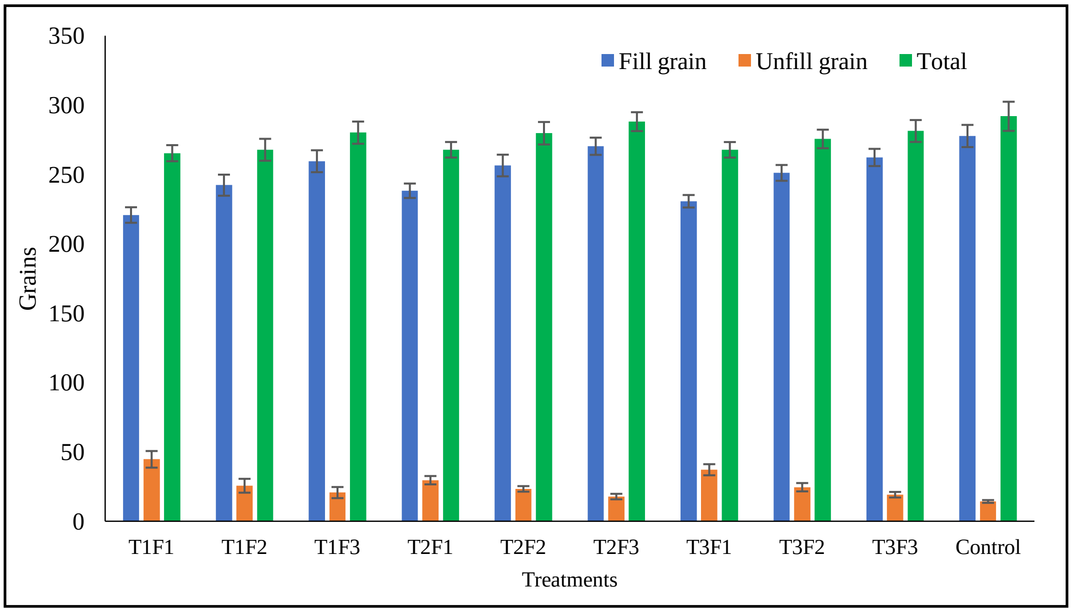

Fig. 3. Grain performance of spikelet rice in different treatments

Note: T1F1=Raintree (LB 2 kg/plot) + 15% RFD, T1F2= Raintree (LB 2 kg/plot) + 30% RFD, T1F3= Raintree (LB 2 kg/plot) +45% RFD, T2F1=Ipil-Ipil (LB 2 kg/plot) + 15% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) + 30% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) +45 % RFD, T3F1=Minjiri (LB 2 kg/plot) + 15% RFD, T3F2=Minjiri (LB 2 kg/plot) + 30% RFD and T3F3=Minjiri (LB 2 kg/plot) +45 % RFD

1000-grain weight

The highest fresh weight varied from 35.00 to 29.00 g of 1000-grains (Fig. 4). The maximum fresh weight was 35.00 in T_{10} and followed by 33.83, 33.13, 32.53, 32.17, 31.80, 31.77, 30.97, and 30.10 in the treatment of T_6, T_9, T_3, T_2, T_5, T_8, T_7, and T_4, respectively. The lowest fresh weight was 35.00 in T_1. The higher dry weight varied from 26.97 to 23.23 g of 1000-grains. The maximum fresh weight was 26.97 g in T_{10} and followed by 26.17, 25.93, 25.60, 25.30, 25.13, 24.90, 24.53, and 23.83 in the treatment of T_6, T_9, T_3, T_2, T_5, T_8, T_7, and T_4, respectively. The lowest fresh weight was 23.23 in T_1. These results are in agreement with that of Tian *et al*., 2017.

Grain yield

The maximum dry grain yield with the application RFD of rice was found in the treatment T_{10} (8.96 t ha^{-1}) followed by 8.63, 8.46, 8.15, 7.59, 7.11, 6.60, 6.47, and 6.16, in the treatment of T_6, T_9, T_3, T_5, T_8, T_2, T_4, and T_7. The lowest yield (5.87) t ha^{-1} was found in T_1 (Fig. 5). The maximum fresh grain yield with the application RFD of rice.

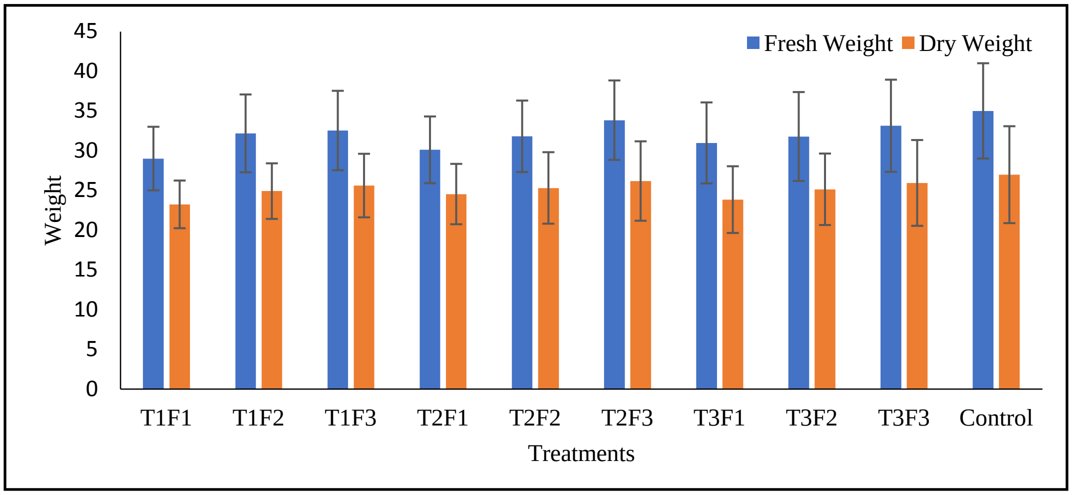

Fig. 4. 1000- grains weight of rice in different treatments

Note: T1F1=Raintree (LB 2 kg/plot) + 15% RFD, T1F2= Raintree (LB 2 kg/plot) + 30% RFD, T1F3= Raintree (LB 2 kg/plot) +45% RFD, T2F1=Ipil-Ipil (LB 2 kg/plot) + 15% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) + 30% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) +45 % RFD, T3F1=Minjiri (LB 2 kg/plot) + 15% RFD, T3F2=Minjiri (LB 2 kg/plot) + 30% RFD and T3F3=Minjiri (LB 2 kg/plot) +45 % RFD

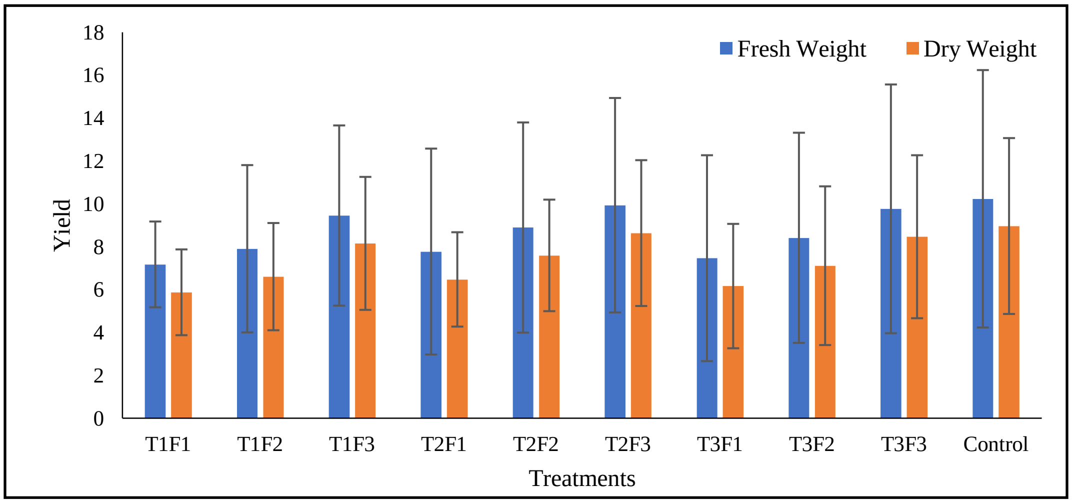

Fig. 5. Grain yield of rice in different treatments

Note: T1F1=Raintree (LB 2 kg/plot) + 15% RFD, T1F2= Raintree (LB 2 kg/plot) + 30% RFD, T1F3= Raintree (LB 2 kg/plot) +45% RFD, T2F1=Ipil-Ipil (LB 2 kg/plot) + 15% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) + 30% RFD, T2F2=Ipil-Ipil (LB 2 kg/plot) +45 % RFD, T3F1=Minjiri (LB 2 kg/plot) + 15% RFD, T3F2=Minjiri (LB 2 kg/plot) + 30% RFD and T3F3=Minjiri (LB 2 kg/plot) +45 % RFD SQR6) was found in the treatment T_{10} (10.23 t ha^{-1}) followed by 9.93, 9.76, 9.45, 8.89, 8.41, 7.90, 7.77, and 7.46 in the treatment of T_6, T_9, T_3, T_5, T_8, T_2, T_4, and T_7. The lowest yield (7.17) t ha^{-1} was found in T_1. These results are similar to Tian *et al*., 2017.

Conclusion

The Chinese cultivar SQR6 produced a much higher grain yield than inbred rice cultivars at similar leaf biomass with dissimilar doses of RFD rate, due mainly to improve rice yielding characteristics per unit area caused by more rice yield per panicle in leaf biomass. The outcome of the experiment exposes that the growth parameter and yield of rice gave the highest value in the Ipil-Ipil leaf biomass. It is experiential that soil nutrient status was amended where green leaf biomass was applied and reduced fewer fertilizer doses were applied and the highest yield in the control treatment where endorsed fertilizer doses were practical. The growth parameter and yield of rice were highest in the treatment where RFD was applied. Therefore, this study proposes that the green leaf biomass of Ipil-Ipil and Minjiri can smear to the development of soil properties and yield contributing characteristics of rice.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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EFFECTS OF SALINITY ON SOIL PROPERTIES OF COASTAL AREAS OF BAGERHAT AND PIROJPUR DISTRICTS

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Abstract

A study was conducted to observe the soil properties under naturally occurring saline soil conditions in Bagerhat and Pirojpur. Soil samples at 0-20 cm depth were collected from Bagerhat sadar and Khachua upazila under Bagerhat district and Projpur sadar and Nazirpur upazila under Projpur district. Observations were made on soil pH, organic matter, cation exchange capacity (CEC), electrical conductivity (EC), exchangeable sodium percentage (ESP), total N, available P, S and exchangeable K, Ca, Na and Mg contents. Result indicates that pH value ranged from 6.70 to 7.40 and 6.60 to 7.79, organic matter 1.75 to 3.61% and 1.57 to 2.90 %, total N 0.11 to 0.18% and 0.08 to 0.97 %, available P 7.40 to 39.18 mg/kg and 2.92 to 23.40 mg/kg and 0.20 to 1.04%, available S 15.53 to 66.82 mg/kg and 3.50 to 35.53 mg/kg in Bagerhat and Pirojpur districts, respectively. In Bagerhat and Pirojpur districts, EC varied from 2.84 to 7.10 ds/m and 1.16 to 4.90 ds/m, CEC 9.77 to 36.35 and 10.34 to 48.70 $meq100g^{-1}$ soils, exchangeable Na 0.62 to 2.80 $meq100g^{-1}$ soil and 0.75 to 2.11 $meq100g^{-1}$ soil, exchangeable K 4.20 to 22.68 $meq100g^{-1}$soil and 3.64 to 41.04 $meq100g^{-1}$ soil, exchangeable Ca 0.84 to 8.37 $meq100g^{-1}$ and 0.80 to 17.82 $meq100g^{-1}$, exchangeable Mg 0.13 to 0.60 $meq100g^{-1}$ soil 0.17 to 0.38 $meq100g^{-1}$ soil and ESP 4.36 to 11.72 and 3.12 to 7.34, respectively. In Bagerhat, a positive significant correlation of CEC was found with total N, K, EC, Na, K, Ca and Mg contents. In Pirojpur, a negative significant correlation of CEC was found with OM, S and Na contents. The paired shows that S, EC, CEC, K, Ca and ESP will significant between the two locations. These results would be useful for predicting crop production and varietal response to soil nutrient conditions and developing fertilizer management in the coastal areas of Bangladesh.

Keywords: CEC, EC, OM, Salinity, Soil nutrients

Introduction

A number of environmental issues and problems are hindering the development of coastal livelihood of Bangladesh. Salinity is one of them, which is expected to be aggravated by climate change and sea level rise and eventually which affects crop production. Bangladesh has 147,570 km^2 land area that includes 710 km coastal line

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along the Bay of Bengal equivalent to 47,201 km^2 areas (Alam *et al*., 2017). The cultivable land covers 59% in which 16% area is under rice cultivation (Ahmed, 2011). Salinity has serious negative impacts on agriculture (Hossain, 2009) which otherwise may enhance crop production and national economy. In Bangladesh about 0.883 million hectares of the arable lands, which constitutes about 52.8 percent of the net cultivable area in 64 Upazilas of 13 districts, are affected by varying degrees of soil salinity (Alam *et al*., 2017). A recent study indicates that the salinity affected area has increased from 8,330 km^2 in 1973 to 10,560 km^2 in 2009 (Soil Resource Development Institute (Mahmuduzzaman *et al*., 2014). In shrimp cultivation area soil salinity gradually increased since 1990. This salinization accelerates which may be due to the effect of saline water flooding for long period, slow permeability, presence of highly saline ground water at shallower depth (SRDI, 2018). Tidal flooding occurs during wet season (June-October), direct inundation by saline water and upward on lateral movement of saline ground water during the dry season (November-May) (Alam *et al*., 2017). In addition, cyclone and tidal surge is accelerating this problem (Abedin, 2010). Most of the river water remains saline throughout the year and is not suitable for irrigation (SRDI, 2020). The saline water as irrigation in the coastal areas reduces the growth of most agricultural crops (Murtaza *et al.*, 2006). Liang *et al.*, (2005) also stated soil salinization is one of the most serious types of land degradation as well as and a major obstacle to the optimal utilization of land resources.

Salt affected area are estimated approximately 952 million ha and this area is increasing year after year all over the world including Bangladesh (Wang *et al.,* 2012). Degraded in respect to salinization is around 1.02 million ha which amounts to 6.9% of the geographical area of the country (SRDI, 2020). Soil with an electrical conductivity of saturation extracts above 4 dS m^{-1} is called saline soil (Flowers and Yeo, 1995). Soil salinity (electrical conductivity: $EC > 4$ dS m^{-1}) is a major abiotic stress which limits plant growth and development, causing yield loss in crop species (Corwin and Yemoto, 2017). Salt-affected soils are identified by excessive levels of water-soluble salts, especially sodium chloride (NaCl) (Tanji, 2002). Salinity causes decline in the crop productivity and yield of the crop which results in severe degradation of bio-environment and ecology (Hoque *et al.*, 2013) which is responsible for low cropping intensity in coastal area (SRDI, 2020).

In an indirect way, soil salinization can abruptly affect plant growth, due to destruction of the soil configuration and its consequent compacting. This occurs due to a dispersion of the clay particles caused by substitution of the calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions present in the complex by sodium (Na^{+}), resulting in an increase in soil solidity, which is, in the percentage of exchangeable sodium (PES), that, in the last instance, is the main factor responsible for the deterioration of the physical properties of salt-affected soils. The excessive amounts of salts provided by irrigation waters can have adverse effects on the chemical and physical properties of the soils and on their biological processes (SRDI, 2020). These effects include mineralization of the carbon (C) and nitrogen (N) and the enzymatic activity, which is crucial for the decomposition of organic matter and release of the nutrients necessary for sustainability of the production (Wong *et al.*, 2008). In addition, the agricultural practices can increase or reduce the microbial population, thus altering the activity, source and persistence of the enzymes in the soil. (Gupta *et al*., 2022).

Research has been carried out on naturally occurring saline soils, and the detrimental influence of salinity on the microbial communities of soil and their activities reported in the majority of studies (Sardinha *et al*., 2003). Increase in salinity intrusion and increase in soil salinity will have critically bad impacts on agriculture. The aim of the present study was therefore, to study the soil properties in the south west coastal soils of Bangladesh.

Materials and Methods

Study area

The south western coastal zone is covered by the Sundarban's mangrove forest, covering greater Khulna. Greater Khulna district consists of 9 (nine) Upazilas. Out of them two upazillas namely Bagerhat sadar and Kachua were selected as study area. In Pirojpur district 2 (two) upazillas were selected, namely, Pirojpur sadar and Nazirpur as study areas. From each upazilla (3) three unions were identified for the study. 8 (eight) land sides agriculture field and 4 (four) river sides agriculture field were selected as study sites

Bagerhat District (Khulna division) comprises an area of about 3959.11 square kilometer (sq. km), locates in between 21°49' and 22°59' North latitudes and in between 89°32' and 89°98' East longitudes. It is bounded by Gopalganj and Narail districts on the North, The Bay of Bengal on the South, Gopalganj, Pirojpur and Barguna districts on the East, Khulna district on the West. Kachua is located at 22°39′00″N 89°53′00″E/ 22.6500°N 89.8833°E/22.6500; 89.8833. It has a total area of about 131.62 sq. km.

Pirojpur District comprises with an area of about 1307.6 sq. km, is a district in south-western Bangladesh. It is bounded by Gopalganj and Barisal districts on the north, Barguna district on the south, Jhalokati district on the east, Bagerhat district on the west. Geographically the study area falls in between 22.576475 N 89.9896735 E. Pirojpur (Town) stands on the bank of the Damodor river. Nazirpur Upazila is located in between 22°40' and 22°52' north latitudes and in between 89°52' and 90°03' east longitudes. It has a total area of about 233.63 sq. km.

The study sites are highlighted in Figure 1and 2. The areas lied at 0.9 to 2.1 m above mean sea level. Soil characteristics of the western coastal zone were silty loams or alluvium.

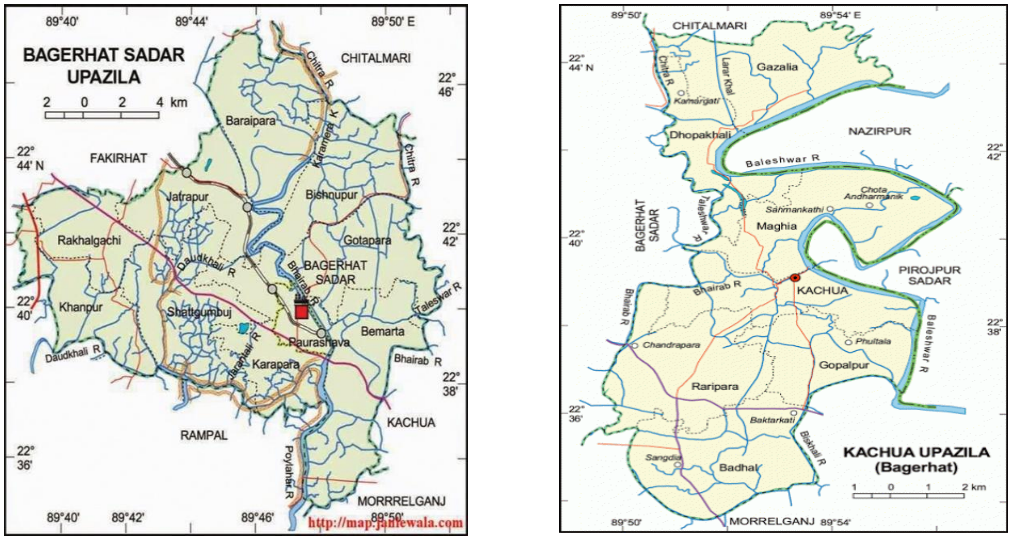

Fig. 1. Study area at Karapara,Gotapara and Bagerhat Sadar in Bagerhat sadar Upazilla and Badhal, Kachua Sadar and Raripara in Kachua Upazilla.

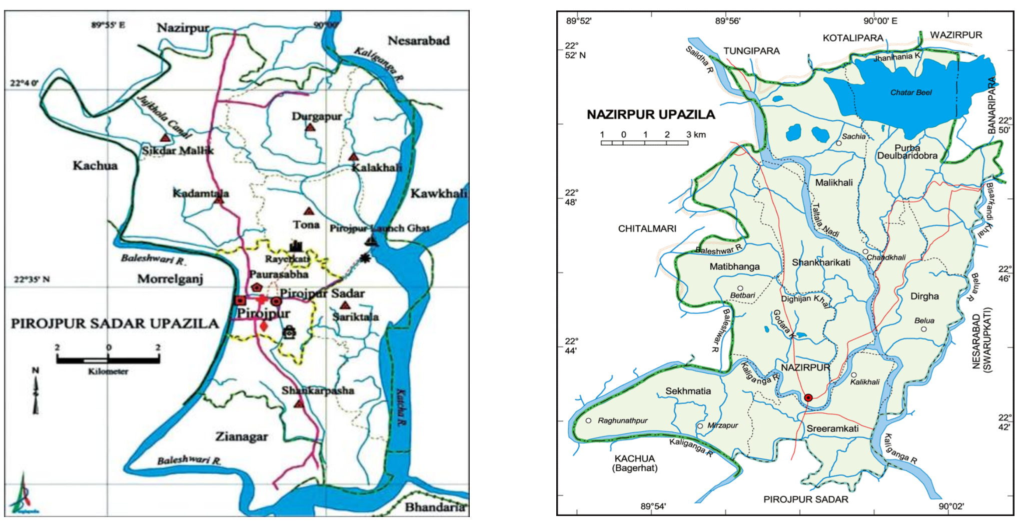

Fig. 2. Study area at Shankorpasa, Pourosova and Sariktola dumuritola in Pirojpur sadar upazilla and Sheikhmatia, Siramkathi and Mativanga in Nazirpur upazilla.

Soil sample collection

Systematic random sampling techniques were used for sample collection from different paddy fields. Soil samples were collected from 0-20 cm depth. A total number of 12 samples (with 3 replications for each sample) were collected to determine soil salinity and fertility status. Samples were collected from 15-16 March, 2020. Global

Positioning System (GPS) was used to record the absolute positions of collected samples (Table 1). For soil samples, a transparent polythene bags were used to preserve the samples and each bag was labeled. About 1kg of soil was collected from each place to prepare a representative sample. Samples were placed in sealed polythene bags that were labeled to avoid any damage. The level contained the name of the places, date of collection and code number of soil sample. Samples were dried in laboratory at room temperature (25 ºC) for 20 days and then ground. The ground samples were then sieved through a 20-mesh sieve (< 2 mm diameter) to make the samples suitable for chemical analyses (Petersen, 2002). The labeled samples were analyzed in the central lab of the Soil Resource Development Institute, Dhaka, Bangladesh.

Table 1. Sampling locations in the study area

Site No.	Sample no.	Sampling Station	Latitude	Longitude
Site 1	S_1	Karapara, Bagerhat Sadar, (Agriculture field)	22º39´49˝N	89º46´16˝E
Site 2	S_2	Gotapara, Bagerhat (Agriculture field)	22º39´59.748˝N	89º50´47.682˝E
Site 3	S_3	Bemorta, Bagerhat Sadar (River bank agriculture field)	22º63´16˝N	89º82´24˝E
Site 4	S_4	Badhal, Kachua (Agriculture field)	22º38´33.37962˝N	89º51´30.02828˝E
Site 5	S_5	Sadar, Kachua (Agriculture field)	22º38´10.96476˝N	89º51´37.08216˝E
Site 6	S_6	Raripara, Kachua (River bank agriculture field)	22º36´0.57107˝N	89º50´6.97538˝E
Site 7	S_7	Shankorpasa, Pirojpur (Agriculture field)	22º32´3.42144˝N	89º57´20.34396˝E
Site 8	S_8	Pourosova , Pirojpur Sadar (Agriculture field)	22º35´2.77814˝N	90º1´31.74766˝E
Site 9	S_9	Sariktola dumuritola, Pirojpur (River bank agriculture field)	22º34´50.73542˝N	90º1´32.00696˝E
Site 10	S_{10}	Siramkathi, Nazirpur (Agriculture field)	22º42´13.824˝ N	89º57´56.124˝ E
Site 11	S_{11}	Sheikhmatia, Nazirpur (Agriculture field)	22º43´29.028˝ N	89º 54´42.66˝ E
Site 12	S_{12}	Mativanga, Nazirpur (River bank agriculture field)	22º42´13.86˝ N	89º 57´56.16˝ E

Soil analysis

Soil pH was determined by glass electrode pH meter as described by Jackson, (1962) with soil water ratios of 1:2.5. Soil electrical conductivity (EC) was measured with the EM-38 instrument, a conductivity meter (Rhoades, 1982) and with four-electrode techniques in field plots having salinity adjusted to different levels but uniform with depth and organic carbon was determined by wet-oxidation method Walkley and Black, (1934) as modified by Allison, (1965). The organic matter was obtained by multiplying the content of organic carbon by Van Bemmelen, factor of 1.73 Page *et al.* (1982). Total N was determined by micro-Kjeldahl digestion by using $CuSO_4$-$NaSO_4$ catalyst mixture was used to determine total N. The ammonia (NH_3) from the digestion was distilled with 40% NaOH into 5% Boric acid and determined by titrating with 0.01 N H_2SO_4 (Jackson, 1973). Available P in the soil sample was measured colorimetrically by the phospho-vanadomolybdate method (Hanson, 1950). Consentration of exchangeable K, Ca and Mg of the soil samples were determined after the soil by mixing 10 milliliters of 1 normal, pH-7, ammonium acetate with a 1 gram scoop of air-dried soil sample and shaking for 5 minutes, the filtered extract is analyzed with an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Chintala *et al*., 2014). For both soil sample; Na was determined by flame emission spectrophotometer and S was determined by turbid metric method with the help of a spectrophotometer. CEC was reported as milliequivalents per 100 grams of soil (meq/100g) Reganold and Harsh, (1985). The ESP is an already familiar ion-exchange parameter: the exchangeable sodium equivalent fraction multiplied by 100. A common alternative parameter is the exchangeable sodium ratio (ESR) also found. (Bleam, 2017). Data were analyzed with SPSS 26.0 and correlation among soil properties were performed with Microsoft Office Excel spreadsheet.

Results and Discussion

Determination of chemical properties of soil

The pH, OM and the concentration of total N, available P and S in soil different soil samples of Bagerhat district varied significantly (Table 2). In Bagerhat, the highest pH value was (7.40) found in S_6 sample and the lowest (6.70) was found S_1 sample which is statistically similar with S_2, S_3, S_4 and S_5 samples. In Pirojpur, the pH value ranged from 6.60 to 7.79 with a mean of 7.16. The highest pH value (7.97) found in S_{10} sample and the lowest (6.70) was found in soil sample S_7. The concentrations of soil nutrients (e.g., organic C, N, P, and K) are good indicators of soil quality and productivity because of their favorable effects on the physical, chemical, and biological properties of soil (Cao *et al*., 2011). Saline soils vary widely in their physical and chemical properties as well as hydrology (Ikehashi and Ponnamperuma, 1978). The pH of the present study corroborates with some other studies (Uddin *et al*., 2014).

In Bagerhat, the OM concentration of soil samples ranged from 1.75 to 3.61% with a mean of 2.47%. The highest OM was found in S_{11} and lowest was recorded in S_7 soil sample. In Pirojpur, the OM range of soil sample was from 1.57 to 2.90 % with a mean of 2.12%. The highest OM found in S_7 sample and the lowest was recorded in S_{11} soil sample.

Although the results represent OM content at considerable status in some of the study areas but it was not the case for every sample location. Hossain (2001) reported that the low OM content of soils in Bangladesh is one of the most serious threats to the sustainability of agriculture and application of OM improves crop growth and yield. The low OM content in Bangladesh soils may be due to the rapid decomposition of OM because of tropical monsoon climate, rapid removal of mineralized products through leaching and crop removal, high cropping intensity and low return of crop residues to the soil (Karim and Iqbal, 2001).

The total N in the studied soils ranged from 0.11 to 0.18% in Bagerhat district and the maximum content of total N was found in S_3, S_4, S_5 and S_6 whereas the minimum was found in S_1 which is statistically similar to S_2. The highest total N (0.97 %) was found in S_8 whereas the lowest (0.08) was found in S_7 in Pirojpur. Soil N content was low in the study area (M = 0.08, Table 2) (Chowdhury *et al*., 2011). Nitrogen status in the studied soils was less fertile and farmers need to use different organic and inorganic fertilizers in paddy fields. The result of the present study showed similarities with several researches, as of Islam *et al*., (2014) and Maliwal, and Somani, (2010) found most soils had very low amounts of total N. Like other tropical and subtropical soils, Bangladesh soils have long been categorized as poor in soil N fertility because of low N supplying capacity (Islam, 1983). Patcharapreecha *et al.,* (1989) reported that total N contents in saline soils (0.005-0.043%) were very low in all the soils they studied. Several studies have shown that salinity reduces N uptake (Al-Rawahy *et.al.*, 1992) by crops and do not support plant growth due to a higher osmotic pressure in the plant soil system (Bhumbla, 1977) despite adequate nutrient levels being available in the soil. Nitrogen availability in wet soils prevailing in the saline areas is sensitive to various environmental factors, including air temperature, water tables, flooding periods and soil properties (Ehrenfeld and Yu, 2012).

Available P content of soils varied from 39.18 mg/kg (S_1 sample) to 7.40 mg/kg (S_4 sample). In Bagerhat, the concentration of available P was highest in S_1 (39.18 mg/kg) and that of the lowest in S_4 (7.40 mg/kg). However, the mean of total concentration of P of the samples was 19.01 mg/kg. In case of Pirojpur, the concentration of available P was highest in S_8 sample (23.4 mg/kg) and that of the lowest in S_9 sample (2.92 mg/kg) whereas, the mean of total concentration of P was 13.85 mg/kg. available P in soils are classified in four groups such as low (< 12), medium (12.1-24.00), high (24.0-30.00) and very high (> 30.0) (Chowdhury *et al.,* 2011). The critical limit for P in Bangladesh soils is considered to be 10.0 mg/kg for neutral and calcareous soils and 7.0 mg /kg for acid soils (FRG, 2018). Thus, the concentration of % of total P were very low (Tables 3 and 6) in study area according to BARC (2018) and Chowdhury *et al*., (2011) who reported that 41% of the soils of Bangladesh contained P below the critical level and 35% of the soils contained P in between the critical level the optimum level. The P content in soils depends largely on the application of fertilizers for agricultural practices and it present in soil as solid phase with varying degree of solubility. When water soluble P is added to the soil, it is converted very quickly to insoluble solid phase by reacting with soil constituents. These may include calcium (Olsen, 1953), Fe and Al oxides (Dean and Rubin, 1947) and partly OM. The added P is more likely to be absorbed on hydrated Fe and Al oxides or on the edge of the clay minerals in neutral to acidic range of soils (Russell, 1988). These reactions affect the availability of P and as a result of these reactions, a very small amount of total P is present in soil solution at any time reflected by soil testing. However, a low to medium range of available P in soils of the under-study area may be mostly affected by previous fertilization, pH, OM content, texture, various soil management and agronomic practices (Verma *et. al*., 2005).

In Bagerhat, S content ranged from 15.5 to 66.82 mg/kg with an average content of 38.71 mg/kg, whereas the highest S (66.82 mg/kg) was recorded in S_2 sample (Table-2) and the lowest (15.53 mg/kg) in S_1 sample. In Pirojpur, the total S content ranged from 3.5 to 35.5 % with an average content of 16.83 mg/kg, whereas the highest amount (35.5 %) was found in S_{10} sample and lowest amount (3.50 mg/kg) in S_9 sample. Except coastal saline areas, most soils in Bangladesh react to K and S. The critical limits of S are 0.12 meq/100 g soil and 10 mg/kg soil. Considering these critical limits, coastal soils usually have higher concentrations of S than its corresponding critical limits (Huq and Shoaib, 2013).

Table 2. Soil nutrient content (%) in different soil samples from Bagerhat district

Sample no.	pH	Organic matter (%)	Total N (%)	Available P	S
				(mg/kg)	
S_1	6.70b	1.75f	0.11b	39.18a	15.53f
S_2	6.90b	1.95e	0.13b	11.60e	66.82a
S_3	6.80b	2.15d	0.18a	22.68b	45.92c
S_4	6.93b	2.89b	0.18a	7.40f	37.23d
S_5	6.87b	3.61a	0.18a	12.47d	16.83e
S_6	7.40a	2.52c	0.18a	20.73c	49.92b
Mean	6.94	2.47	0.16	19.01	38.71
CV (%)	2.01	3.05	10.59	0.14	0.95

Note: S_1 =Karapara, Bagerhat Sador (agriculture field) S_2=Gotapara, Bagerhat (Agriculture field), S_3= Bemorta, Bagerhat Sadar (River bank Agriculture field). S_4= Badhal, Kachua (Agriculture field), S_5= Sador, Kachua (Agriculture field) and S_6= Raripara, Kachua (River bank Agriculture field)

Table 3. Soil nutrient content (%) in different soils samples from Pirojpur district

Sample no.	pH	Organic Matter (%)	Total N (%)	Available P	S
				(mg/kg)	
S_7	6.60e	2.90a	0.19b	8.60e	25.15b
S_8	6.80d	1.96d	0.97a	23.40a	19.60c
S_9	6.70de	2.37b	0.12c	2.92f	3.50f
S_{10}	7.97a	2.05c	0.12c	20.02b	35.53a
S_{11}	7.80b	1.57f	0.08d	11.37d	6.57e
S_{12}	7.12c	1.84e	0.09cd	16.80c	10.50d
Mean	7.16	2.12	0.21	13.85	16.81
CV (%)	1.19	0.45	0.033	0.75	1.92

Note: S_7= Shankorpasa, Pirojpur (Agriculture field), S_8= Pourosova, Pirojpur Sadar (Agriculture field), S_9= Sariktoladumuritola, Pirojpur (River bank agriculture field), S_{10}=Siramkathi, Nazirpur (Agriculture field), S_{11}= Sheikhmatia, Nazirpur& S_{12}= Mativanga, Nazirpur (River bank agriculture field)

The EC, CEC, exchangeable Na, K, Ca, Mg and ESP value of different soil sample varied significantly (Table 4). Results of the present study Result showed that in Bagerhat, the highest EC value recorded in S_3 and S_5 samples (7.10 ds/m) and the lowest was recorded S_4 samples (2.84 ds/m) which is statistically similar with S_1 samples with a mean EC value of 4.68 ds/m. In Pirojpur, the highest EC value was recorded in S_{12} sample (4.90 ds/m) and the lowest was recorded in S_8 sample (1.61 ds/m) with a mean value of EC 3.19 ds/m. The higher EC values means transpiration of salts in this area with negligible surface runoff. Instead evaporation of surface and groundwater at shallow depth leave behind the salts which appear as encrustation on soils. On the other hand, lower values of EC were recorded for upstream and topographically higher areas can be attributed to the rolling topography, relatively higher gradient, seasonal irrigation and alternating cropping patterns. About 33% of studied soil samples were saline according to the acceptable range as indicated by Allotey *et.al.*, (2009) and Indonesian Agency for Agricultural Research and Development, Indonesia and NSW Department of Primary Industries, Australia (2008). Almost similar findings were reported by Uddin and Islam (1998) in different coastal agricultural saline soils. Soil EC is a measure of the amount of salts in soil (salinity of soil). It is an important indicator of soil health and it affects crop yields, crop suitability, plant nutrient availability, and activity of soil microorganisms which influence key soil processes including the emission of greenhouse gases such as N oxides, methane, and carbon dioxide (Smith and Doran, 1996).

The mean value of CEC was 20.7 meq100g^{-1}and the highest CEC was recorded in S_3 sample (36.4 meq100g^{-1}) and the lowest was recorded in S_2 sample (9.77 meq100g^{-1}) in Bagerhat. In case of Pirojpur, the mean value of CEC was 37.99 meq100g^{-1} and the highest was recorded in S_{11} sample (48.7 meq100g^{-1}) and the lowest was recorded in S_7 sample (10.34 meq100g^{-1}). CEC is a fundamental soil property used to predict plant nutrient availability and retention in the soil. It is the potential of available nutrient supply, not a direct measurement of available nutrients (Barker, *et al.*, 2017).

In Bagerhat, the total amount of exchangeable Na was found highest in S_{11} sample (2.8 meq100g^{-1}) and that of the lowest in S_7 sample (0.62 meq100g^{-1}) with an average of 1.42 meq100g^{-1}. The total amount of exchangeable sodium was found highest in S_{10} sample (2.11 meq100g^{-1}) and that of the lowest was recorded in S_7 sample (0.75 meq100g^{-1}) in Pirojpur. Incase of Bagerhat, the highest amount of exchangeable K was observed in S_3 sample (22.68 meq100g^{-1}) and the lowest in S_1 sample (4.20 meq100g^{-1}) with an average of 12.7 meq100g^{-1}. In Pirojpur, the highest amount of exchangeable K was observed in S_{10} sample (41.04 meq100g^{-1}) and the lowest was recorded in S_7 sample (3.64 meq100g^{-1}) with an average of 12.7 meq100g^{-1}. Adequate level of exchangeable K in the study area may be attributed to the prevalence of K-rich clay minerals like Illite and Kaolinite. Besides farmers use of different types of organic and inorganic fertilizers that include K containing fertilizers (BARC, 2018; Islam *et.al*.,1985), the decomposition of the minerals containing K are mentionable worthy reasons of increasing K in saline soils (Sharpley,1989). However, K can be found rich in saline soils (Maliwal and Somani, 2010).

Calcium (Ca) is the predominant positively charged ion (Ca^{++}) held on soil clay and OM particles. Soils normally have large amounts of exchangeable Ca (300-5000 ppm). Exchangeable Ca of soils collected from Bagerhat area ranged from 0.84 to 8.37 meq100g^{-1} soil with an average content of 4.45 meq100g^{-1} soil. In Pirojpur, exchangeable Ca ranged from 0.80 to 17.82 meq100g^{-1} soil with an average content of 8.33 meq100g^{-1} soil. Other reports also show low soil Ca content in the different soil samples in the saline areas (BARC 2018; Chowdhury *et al*., 2011). This lower Ca content may be attributed to changes in osmotic and ion-specific effects that can produce imbalances in plant nutrients, including deficiencies of several nutrients or excessive levels of Na^+ (Kaya *et.al*., 2001).

Table 4. Chemical properties of different soil samples from Bagerhat district

Sample no.			Exchangeable cations (meq^{-1}100 g soil)				
	EC	CEC	Na	K	Ca	Mg	ESP
S_1	2.96e	9.77c	0.62e	4.20f	1.11d	0.24c	6.41d
S_2	3.20c	11.47c	1.33c	7.38d	0.93e	0.23c	11.72a
S_3	7.10a	36.35a	2.80a	22.68a	7.97b	0.50b	7.71c
S_4	2.84e	9.80c	0.80de	6.83e	0.84f	0.13c	8.22b
S_5	7.10a	27.58b	1.20cd	15.81c	8.37a	0.60a	4.36e
S_6	4.90b	29.14b	1.80b	19.39b	7.48c	0.47b	6.18d
Mean	4.68	20.68	1.42	12.71	4.45	0.36	7.43
CV (%)	0.82	6.73	6.63	0.55	1.49	7.23	7.16

Note: S_1 =Karapara, Bagerhat Sador (agriculture field) S_2=Gotapara, Bagerhat (Agriculture field), S_3= Bemorta, Bagerhat Sadar (River bank agriculture field). S_4= Badhal, Kachua (Agriculture field), S_5= Sador, Kachua (Agriculture field) and S_6= Raripara, Kachua (River bank agriculture field). EC=Electrical conductivity. CEC=Cation Exchange Capacity, ESP= Exchangeable sodium percentage.

In case of Bagerhat, the highest amount of exchangeable Mg was found in S_5 sample (0.60 meq100g^{-1} soil) and the lowest in S_4 sample (0.13 meq100g^{-1} soil) (Table 4).

In case of Pirojpur the highest amount of exchangeable Mg was found in S_8 sample (0.38 meq^{-1}100 g soil) and the lowest in S_{11} sample (0.17 meq^{-1}100 g soil). Mg content was very low in soil samples of Pirojpur district (Table 5). Similarly Mg content was low in soil sample of Bagerhat district (Table 5). Similar results were also recorded in the study of Chowdhury *et. al.,* (2011). Differences in osmotic and ion-specific effects as found in saline soils resulted in the imbalances in plant nutrients that caused nutrient deficiencies in soil (Kaya *et al.,* 2001). Mg is located both in clay minerals and associated with cation exchange sites on clay surfaces. The primary and secondary minerals are important sources of Mg for plant nutrition, especially in unfertilized soil. But plant-available Mg concentrations cannot be accurately predicted based only on the parent material composition due to differences in mineral weathering rates and leaching (Chowdhury *et. al*., 2011).

The highest ESP value was observed in S_2 sample (11.72) and the lowest in S_5 sample (4.36) in Bagerhat. The highest ESP value was observed in S_1 sample (7.34) which was statistically similar with S_4 sample and the lowest in S_3 sample (3.12) in Pirojpur. When the values of ESP in soils are greater than 15, the soils are said sodic soils and considered as problem soils (Osman, 2013). All collected soil samples were in the category of non-sodic as ESP was found to be less than the critical sodicity values. Highly significant positive relationship of CEC with clay content and CEC has already been observed by several authors (Wang *et al.*, 2005). The positive relationship between clay content and exchangeable Ca and Mg may be the resultant effects of negatively charged sites of clays which adsorb positively charged ions (Mckenzie *et. al.*, 2004).

Table 5. Chemical properties of different soil samples from Pirojpur district

Sample no.			Exchangeable cations (meq^{-1}100 g soil)				
	EC	CEC	Na	K	Ca	Mg	ESP
S_7	2.18d	10.34e	0.75e	3.64e	0.80f	0.35b	7.34a
S_8	1.61f	47.77ab	1.62c	33.03d	10.34c	0.38a	3.39bc
S_9	2.12e	46.40bc	1.45d	23.18d	17.82a	0.35b	3.12c
S_{10}	4.29b	29.47d	2.11a	23.98d	3.06e	0.32c	7.16a
S_{11}	4.06c	48.70a	1.83b	41.04a	5.66d	0.17e	3.77bc
S_{12}	4.90a	45.29c	1.92b	30.84c	12.31b	0.22d	4.25b
Mean	3.19	37.99	1.61	25.95	8.33	0.29	4.83
CV (%)	0.66	2.58	3.017	2.72	2.82	4.99	10.33

Note: S_7= Shankorpasa, Pirojpur (Agriculture field), S_8= Pourosova, PirojpurSadar (Agriculture field), S_9= Sariktoladumuritola, Pirojpur (River bank agricure field), S_{10}=Siramkathi, Nagirpur (Agriculture field), S_{11}= Sheikhmatia, Nazirpur& S_{12}= Mativanga, Nazirpur (River bank agriculture field. EC=Electrical conductivity. CEC=Cation Exchange Capacity, ESP= Exchangeable sodium percentage.

Correlation

The correlations among the studied parameters were done to observe the relationship. In Bagerhat, in case of EC the positive significant relation was found with total N, total K, CEC, exchangeable Na, exchangeable K, exchangeable Ca and exchangeable Mg whereas the negative non-significant relation was found with total P and ESP (Tabel-6). In CEC the positive significant relation was found with total N, total K and EC exchangeable Na, exchangeable K, exchangeable Ca and exchangeable Mg whereas the negative non-significant relation was found with total P and ESP (Tabel-6).

In Pirojpur, in case of EC, the positive significant relation was found with total pH and CEC whereas the significant negative relation was found with OM, N, K, S and Ca. In CEC, the negative significant relation was found with total OM, total S and exchangeable Na whereas the significant positive relation was found with exchangeable Ca (Tabel-8). These results were in conformity with the results reported by several researchers (Pan *et.al.*,2013; Eltaib, 2003).

Table 6. Correlation in different field and riverbank field soil of Bagerhat district

	pH	OM (%)	N	P	K	S	EC	CEC	Na	K	Ca	Mg	ESP
pH	1												
OM (%)	.206	1											
N	.293	.613**	1										
P	-.237	-.608**	-.510*	1									
K	.174	.449	.459	.032	1								
S	.375	-.367	.072	-.412	-.189	1							
EC	.030	.464	.543*	-.078	.933**	-.159	1						
CEC	.219	.274	.625**	.002	.890**	.057	.916**	1					
Na	.174	.449	.459	.032	1.000**	-.189	.933**	.890**	1				
K	.360	.269	.657**	-.083	.832**	.178	.867**	.982**	.832**	1			
Ca	.301	.468	.616**	-.031	.960**	-.112	.939**	.956**	.960**	.932**	1		
Mg	.170	-.112	.436	-.024	.563*	.442	.671**	.824**	.563*	.852**	.660**	1	
ESP	.007	-.442	-.336	-.242	-.503*	.646**	-.419	-.369	-.503*	-.290	-.497*	.181	1

Note: OM=Organic Matter, EC=Electrical conductivity. CEC=Cation Exchange Capacity, ESP= Exchangeable sodium percentage.

Table 7. Correlation of different soils sample of Pirojpur District, Bangladesh

	pH	OM (%)	N	P	K	S	EC	CEC	Na	K	Ca	Mg	ESP
pH	1												
OM (%)	-.649**	1											
N	-.359	-.059	1										
P	.345	-.466	.582*	1									
K	-.573*	.669**	.541*	.055	1								
S	.255	.308	.166	.537*	.426	1							
EC	.733**	-.572*	-.622**	.220	-.773**	.027	1						
CEC	.167	-.793**	.214	.152	-.363	-.671**	.151	1					
Na	-.567*	.667**	.539*	.040	.997**	.411	-.783**	-.355	1				
K	.507*	-.969**	.167	.369	-.576*	-.451	.382	.898**	-.570*	1			
Ca	-.373	-.207	.105	-.244	.074	-.719**	-.160	.726**	.081	.360	1		
Mg	.760**	-.850**	-.086	.533*	-.451	.004	.689**	.616**	-.454	.764**	.192	1	
ESP	.208	.512*	-.286	.094	.214	.815**	.161	-.896**	.203	-.682**	-.801**	-.231	1

Note: OM=Organic Matter, EC=Electrical conductivity. CEC=Cation Exchange Capacity, ESP= Exchangeable sodium percentage.

Paired t-test

In pair sample t test of studied parameters of both Bagerhat and Pirojpur soils, S, EC, CEC, exchangeable K, exchangeable Ca and ESP were found significant while other parameters did not vary significantly (Table-8).

Table 8. Soil nutrient content (%) in different field and riverbank field soils from Bagerhat and Pirojpur districts

		Mean	T value	Significance
pH	Bagerhat	6.93	-1.735	NS
	Pirojpur	7.16		
OM (%)	Bagerhat	2.47	1.503	NS
	Pirojpur	2.11		
N (%)	Bagerhat	.16	-1.242	NS
	Pirojpur	.26		
P	Bagerhat	19.00	1.354	NS
	Pirojpur	13.85		
K	Bagerhat	.36	1.143	NS
	Pirojpur	.29		
S	Bagerhat	38.70	4.103	**
	Pirojpur	16.80		
EC	Bagerhat	4.68	2.955	**
	Pirojpur	3.19		
CEC	Bagerhat	20.68	-6.501	**
	Pirojpur	37.99		
Na	Bagerhat	.36	1.121	NS
	Pirojpur	.29		
K	Bagerhat	12.71	-5.161	**
	Pirojpur	25.95		
Ca	Bagerhat	4.45	-3.423	. **
	Pirojpur	8.33		
Mg	Bagerhat	1.42	-.939	NS
	Pirojpur	1.61		
ESP	Bagerhat	7.43	3.082	**
	Pirojpur	4.83		

Conclusion

Coastal field soils and Riverbank agriculture field soils of Bagerhat district in Bangladesh can be characterized as nearly neutral to basic in soil reaction (pH ranged 6.70 to 7.40%) and soil salinity fell in low to medium (EC ranged 2.84 from 7.10). Soils of Shankorpasa, Pirojpur (Agriculture field), Pourosova, Pirojpur Sadar (Agriculture field) and Sariktoladumuritola, Pirojpur (River bank agriculture field), can be characterized as slightly acidic to basic (pH ranged 6.60 to 7.8), EC low to normal range (EC ranged from 1.16 to 4.90) in soil reaction. The OM level of both districts exhibited lower to medium than good agricultural soil (OM ranged 1.75 to 3.61% and 1.57 to 2.90 %).The soils of Bagerhat district showed low concentrations of total N, available P, exchangeable Ca, low exchangeable Mg and high level of exchangeable K. In Piropur district total N, K, S concentrations were low and exchangeable K and Ca was medium to high. In Bagerhat, CEC had positive significant relation with total N, total K, EC, exchangeable Na, K, Ca and Mg. In Pirojpur, CEC had the significant positive relation with pH, OM, total N, K, exchangeable Na and exchangeable Ca. Further investigation can be performed to justify these significant correlations between chemical characteristics of soil.

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Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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EVALUATION OF *Brassica rapa* GENOTYPES SUITABLE FOR RICE BASED CROPPING PATTERN IN BANGLADESH

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Abstract

The field experiment was conducted with 15 *Brassica rapa* genotypes to estimate the genetic variability and correlation of yield contributing traits. The results indicated that the phenotypic variance for all the characters was considerably higher than the genotypic variance denoting little influence of environmental factors. Low genotypic and phenotypic coefficient of variation showed in plant height (6.36, 8.20) and thousand seed weight (4.58, 11.63). While moderate genotypic and phenotypic coefficient of variation was observed in seed yield (12.68, 18.09), number of branches per plant (13.71, 25.18), number of seeds per siliqua (20.20, 28.86). High genotypic (40.65) and phenotypic coefficient of variation (52.85) was observed for number of siliquae per plant. Low heritability with high genetic advance showed in plant height (0.60%, 8.85), number of branches per plant (0.29%, 0.54) and number of seeds per siliqua (0.48%, 6.75) indicating the possibility of non-additive gene action. High heritability with high genetic advance and high genetic advance in percentage of mean were observed in plant height (0.60%, 8.85, 10.16), number of siliquae per plant (0.59%, 31.93, 64.42), number of seeds per siliqua (0.48%, 6.75, 29.12) and seed yield (0.49%, 260.64, 18.32) which revealed the possibility of predominance of additive gene effects. Number of branches per plant had showed significant positive association with number of siliquae per plant (rg= 0.850**, rp= 0.795**) and number of seeds per siliqua (rg= 0.821**). On the other hand, it had significant negative association with thousand seed weight (rg= -0.912**) and non-significant positive and negative association showed with others characters. The results of the path analysis revealed that plant height (0.818) had the maximum direct effect and maximum negative direct effect was observed for number of seeds per siliqua (-2.558). However, the results suggested that some yield related traits such as plant height and thousand seed weight could be used in breeding program for the development of high yielding short duration *B. rapa* variety development in Bangladesh.

Keywords: Correlation coefficient, Genetic variability, Germplasm, Path coefficient

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Introduction

Oilseed Brassicas have been ranked after soybean and palm oil in edible oil production. Brassica species have played an important role in agriculture and contributed to the economy and health in the world. The family Brassicaceae, containing about 350 genera and 3500 species, is one of the ten most economically important plant families with a wide range of agronomic traits. The Brassica genus generally has been categorized into three categories *viz;* Mustard, Rapeseed, and Cole. The Brassicaceae is distinguished on the basis of the presence of conduplicate cotyledons (i.e., the cotyledons are longitudinally folded around the radical) and two segmented fruits (siliquae), which contain seeds in one or both segments with simple hairs if present. These characteristics separate the mustard family from all other plant families. *B. rapa*. is an important widely cultivated crop comprised of a genome n=10 with various forms or morphotypes such as leafy vegetables, turnips, and oilseed rape. At a present large number of commercial varieties are available, and their characterization, differentiation and plant varieties were made by a set of descriptors/ characters.

In Bangladesh rapeseed and mustard is the first leading oil crops in respect of productivity land coverage of oilseed (BBS, 2021). Rapeseed is the most important sources of edible oil in Bangladesh. *B .rapa*, *B. napus* and *B. juncea* are three major cultivated species of Bangladesh. Rapeseed is the most important sources of edible oil in Bangladesh. Rapeseed oil used for both culinary and industrial purpose. The mustard cake contains high protein (37%) rich feed which is highly palatable to livestock (Anil Kumar *et al.*, 2002). Now Bangladesh is facing shortage of edible oil. At present, production of oilseed is about 0.99 million tons (BBS, 2021), which covers only 10% of the domestic need. About 90% of requirement of oil has been imported every year by spending huge amount of foreign currency (BBS, 2021). In Bangladesh, the national grain yield of mustard/rapeseed is about 950 kg/ha which is very low in comparison to other developed countries (2400 kg/ha) (Food and Agriculture Organization of the United Nations, 2020). On the other hand, the area of cultivation of mustard in Bangladesh is lower due to rice based cropping system which is very challenging for increasing overall production of mustard. Henceforth, for a sustainable food security the thrust is to develop short duration high yielding rapeseed variety in between the Transplanted Aman and Boro Rice, for a sustainable food security.

Seed yield in rape is a complex and highly variable character and is being associated with a number of component characters (Varshney *et al.*, 1986). Yield improvement is one of the major goals in rapeseed breeding. Information related to genetic variability and character association is a prerequisite for initiating a successful breeding program aiming to develop high yielding and short duration varieties. Correlation and path coefficient are used to assess the relative contribution of different components on yield (Sachan and Sharma, 1971 and Jatasra and Paroda, 1978). The path coefficient analysis has been found to give more specific information on the direct and indirect influence of each of the component characters upon seed yield (Behl *et al.*, 1992). Thus, the present study was undertaken to find out suitable genotypes for higher seed yield through study of genetic variability, heritability, genetic advance, correlation among different characters and the direct and indirect effect of these characters towards seed yield with short duration lines for further use in variety development research.

Materials and Methods

The experiments were conducted at the research fields of Oilseed Research Centre of Bangladesh Agricultural Research Institute, Gazipur (latitude: 23°99′N, longitude: 90°41′E), during Rabi season 2020 (winter season in Bangladesh). The area is characterized by subtropical monsoon climate, with average annual rainfall of about 1,898 and 1,895 mm, respectively. The soil characteristics of the experimental field is sandy clay loam and silty clay loam in texture, respectively. The field capacity, permanent wilting point, and bulk density were 0.295 cm^3 cm^{-3}, 0.141 cm^3 cm^{-3}, and 1.50 g cm^{-3}, respectively. Fifteen *B. rapa* genotypes were used in this experiment. The experiment was set up in a RCBD with three replications, following 30 cm × 10 cm spacing. The unit plot size was 5 m × 1.5 m and block to block distance was 1.5 m. The plot was fertilized with 250, 170, 85, 150, 5 Kg/ha Urea, TSP, MOP, Gypsum and Borax respectively. Standard agronomic practices were carried out to raise healthy crop. Harvesting was done when 80% of the plants showed symptoms of maturity i.e. straw colour of siliquae, leaves, stem and desirable seed colour in the matured siliquae. Ten plants were selected at random from all genotypes in each plot and data were recorded on Plant height (cm), Number of branches per plant (no.), Number of siliqua per plant (no.), Number of seeds per siliqua (no.), Thousand seed weight (g), Seed yield (kg/ha). The data were analyzed for different genetic components. Phenotypic and genotypic variance was estimated by the formula used by (Johnson et al., 1955). Heritability and genetic advance were measured using the formula given by (Singh and Chaudhary, 1985) and (Allard, 1960). Genotypic and phenotypic coefficient of variation was calculated by the formula of (Burton, 1952). Simple correlation 17 coefficient was obtained using the formula suggested by (Singh and Chaudhary, 1985) and path co-efficient analysis was done following the method outlined by (Dewey and Lu, 1959).

Results and Discussion

The results of analysis of variance (ANOVA) for all the traits under study are presented in Table 1. According to the table there was a significant difference among the genotypes and replications for the traits suggesting there were significant differences among the genotypes and replications for the characters. It was found that the tallest plant of 97.26 cm was observed in G11 while the shortest plant of 77.30 cm was in G4 (Table 2).

The maximum number of primary branches per plant (4.83) and number of siliqua per plant (92.60) were recorded in G11 and the lowest number of primary branches per plant (2.23), and number of siliqua per plant（26.20）was in G13. On the other hand, number of seed per siliqua was observed the highest in G2 (34.96) and the lowest in G8 (15.20). Maximum thousand seed weight (4.00 g) was recorded in G4, and the minimum 3.00g was in G6 and G10. The highest seed yield (1761.85 kg/ha) was recorded in G5 and the lowest (1137.85 kg/ha) was recorded in G2 in Table 2.

Table 1. Analysis of variances of seven important characters in respect of *Brassica rapa*

Source	df	DM	PH	NBPP	NSPP	NSPS	TSW	SY
Replication	2	16.98	17.64	0.58	259.25	129.87**	2.12***	91720
Genotypes	14	114.2**	112.40***	1.28*	1498.69***	88.70**	0.13	131380**
Error	28	7.88	8.79	0.25	26.48	10.34	1.15	19.29
CV%		25.87	27.84	20.75	33.40	20.42	10.06	13.79

DM, Days to maturity (day); PH, Plant height (cm); NBPP, Number of branches per plant (no.); NSPP, Number of siliqua per plant (no.); NSPS, Number of seed per siliqua (no.); TSW, Thousand seed weight (g): SY, Seed yield (kg/ha)

The phenotypic variance was considerably higher than the genotypic variance for all the characters studied indicating less environmental influence of these characters (Table 3). Deshmukh *et al.,* (1986) also reported that phenotypic coefficient of variation was higher than the genotypic coefficient of variation. Least difference between phenotypic variance and genotypic variance were observed in number of branches per plant, number of siliqua per plant, number of seed per siliquae and thousand seed weight which indicated low environmental influence on this character which might be due to their genetic control. Relatively high phenotypic variation was observed in plant height (51.02), number of siliquae per plant (686.41), number of seed per silique (44.81) and Seed yield (66246.8) which indicated large environmental influence on these characters. Characters like plant height (6.36, 8.20) and thousand seed weight (4.58, 11.63) showed low genotypic and phenotypic coefficient of variation indicated that the genotype has considerable variation for these traits. Moderate genotypic and phenotypic coefficient of variation was observed in number of seed per siliqua (20.20, 28.86), number of branches per plant (20.22, 22.16) and seed yield (12.68, 18.09) which indicated moderate variability were present among the genotype for these characters. Number of siliquae per plant showed high genotypic and phenotypic coefficient of variation (40.65) and (52.85) respectively indicated that the genotype were highly variable for this trait (Table 3). Plant height (0.60%, 8.85), number of branches per plant (0.29%, 0.54) and number of seed per siliqua (0.48%, 6.75) showed low heritability with high genetic advance which indicated the possibility of non-additive gene action. The high heritability was due to favorable influence of environment rather than genotype and selection for such traits might not be rewarding. Plant height (0.60%, 8.85, 10.16), number of siliqua per plant (0.59%, 31.93, 64.42), number of seed per siliqua (0.48%, 6.75, 29.12) and seed yield (0.49%, 260.64, 18.32) showed high heritability with high genetic advance and high genetic advance in percentage of mean revealed the possibility of predominance of additive gene effects and selection should lead to a fast genetic improvement of the material.

Table 2. Mean performances of seven important traits in *Brassica rapa*

Codes	Genotype	DM	PH	NBPP	NSPP	NSPS	TSW	SY
G1	BC-2014-Y01	78.00bc	81.10fg	3.76bc	47.80bc	23.96d	3.33b	1267.86bc
G2	BC-2014-Y02	77.00c	86.26cd	3.50cde	41.63cd	34.96a	3.66ab	1137.85cde
G3	BC-214-Y-6	77.33c	91.86bc	4.63ab	108.43a	18.50ef	3.33b	1169.63cd
G4	2014-Y11	77.00c	77.30h	3.73bc	38.76ef	19.16e	4.00a	1496.08b
G5	BC-2014-B08	76.00cd	84.50cde	3.56bcd	38.50ef	21.36e	3.66ab	1761.85a
G6	BC-2014-B10	75.33cd	93.00ab	3.10cde	41.50cd	24.03cd	3.00bc	1282.97bc
G7	BC-2014-B14	73.00d	86.83cd	3.10cde	40.06cde	25.30c	3.33b	1299.00fg
G8	BS-14X BS 15-1	75.33cd	79.83def	3.96b	64.66b	15.20fgh	3.66ab	1269.18bc
G9	BS-14X BS 15-3	80.00bc	82.63de	2.86cdef	46.00bc	17.50fg	3.66ab	1694.45a
G10	BS-14X BS 15-4	80.33b	87.60c	3.86bc	41.80cd	21.96e	3.00bc	1218.89bcd
G11	BS-14X BS 15-1 (NET)	73.00d	97.26a	4.83a	92.60a	18.36g	3.33b	1697.80a
G12	BS-14X BS 15-3 (NET)	75.33cd	96.96a	3.83bcd	41.96c	30.30ab	3.33b	1238.52bcd
G13	BC-2014-Y02 (NET)	83.6a	92.16b	2.23 cdef	26.90efgh	30.43ab	3.30bc	1336.67bc
G14	BC-2014-Y03 (NET)	81.4ab	80.70gh	3.20def	36.00efg	22.00e	3.66ab	1697.78a
G15	BS-14- (CH)	78.66bc	81.66gh	3.36de	36.93ef	24.80def	3.33b	1561.48ab
	F-Test	**	**	**	**	**	**	**
	LSD $_{(0.05)}$	17.77	18.11	0.55	12.36	3.53	0.25	146.54
	Sx/sd	7.10	7.03	0.89	25.82	6.97	0.49	259.62

In a column means having similar letter(s) or without letter is identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability; CV%, Percentage of co-efficient of variation; LSD, Least significant difference; Sx/sd, Standard deviation; DM, Days to maturity (day); PH, Plant height (cm); NBPP, Number of branches per plant (no.); NSPP, Number of siliqua per plant (no.); NSPS, Number of seed per siliqua (no.); TSW, Thousand seed weight (g): SY, Seed yield (kg/ha)

Table 3. Estimation of some genetic parameters in *Brassica rapa*

Parameters	DM	PH	NBPP	NSPP	NSPS	TSW	SY
σ^2g	29.80	30.68	0.23	406.13	21.94	0.02	32566.6
σ^2p	49.70	51.02	0.80	686.41	44.81	0.15	66246.8
GCV	6.03	6.36	13.71	40.65	20.20	4.58	12.68
PCV	7.90	8.20	25.18	52.85	28.86	11.63	18.09
h^2b (%)	0.55	0.60	0.29	0.59	0.48	0.15	0.49
GA	8.12	8.85	0.54	31.93	6.75	0.12	260.64
GA (%)	9.87	10.16	15.38	64.42	29.12	3.72	18.32

σ^2g, Genotypic variance; σ^2p, Phenotypic variance; GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation; h^2b, Broad sense heritability; GA, Genetic advance; GA (%), Genetic advance in percent of mean; DM, Days to maturity (day); PH, Plant height (cm); NBPP, Number of branches per plant (no.); NSPP, Number of siliqua per plant (no.); NSPS, Number of seed per siliqua (no.); TSW, Thousand seed weight (g): SY, Seed yield (kg/ha)

Table 4. Correlation co-efficient among different characters of the *Brassica rapa*

Characters	Correlation	PH	NBPP	NSPP	NSPS	TSW	SY
DM	r_g	0.039	0.287	0.298	0.26	0.12	0.15
	r_p	0.399	0.422*	0.325	0.34	0.23	0.21
PH	r_g		0.037	0.291	0.310	-1.284**	-0.418
	r_p		0.421**	0.435**	0.352*	-0.308*	-0.085
NBPP	r_g			0.850**	0.821**	-0.912**	-0.131
	r_p			0.795**	-0.122	0.001	0.146
NSPP	r_g				-0.696**	-0.583*	0.037
	r_p				-0.295*	-0.037	0.142
NSPS	r_g					-0199	-0.809
	r_p					-0.213	-0.200
TSW	r_g						0.076
	r_p						0.182

**, Significant at the 0.01 level of probability; *, Significant at the 0.05 level of probability; DM, Days to maturity (day); PH, Plant height (cm); NBPP, Number of branches per plant (no.); NSPP, Number of siliqua per plant (no.); NSPS, Number of seed per siliqua (no.); TSW, Thousand seed weight (g): SY, Seed yield (kg/ha)

Plant height showed phenotypic level highly significant positive association with number of branches per plant, number of siliquae per plant and number of seeds per siliqua found significantly positive. On the other hand, plant height showed highly significant negative association with thousand seed weight and seed yield found non-significant negative correlation with genotypic and phenotypic level. Singh *et al.,* (1987) also found a similar result. The result revealed that the tallest plant initiated with an increase of number of primary branches per plant. Number of branches per plant had showed significant positive association with number of siliquae per plant (r_g= 0.850**, r_p= 0.795**) and number of seeds per siliqua (r_g= 0.821**). On the other hand, it had significant negative association with thousand seed weight (r_g= -0.912**) and non-significant positive and negative association found with others characters. This result is disagreement with Singh *et al.*, (1969) who got negative association between numbers of branches per plant. Number of siliquae per plant had significant negative association both genotypic and phenotypic level with number of seeds per siliqua (r_g= -0.696**; r_p= -0.295*) and thousand seed weight (r_g= -0.583*). Number of seeds per siliqua showed non-significant negative association with thousand seed weight (r_g= -0199; r_p= -0.213) and seed yield (r_g= -0.809; r_p= -0.200) indicated that as the 1000 seed weight and seed yield would decrease. Thousand seed weight had non-significant positive association with seed yield (kg/ha) both genotypic and phenotypic level (0.076; 0.182) indicated that as the thousand seed weight increases, the seed yield (kg/ha) would increase.

Table 5. Partitioning of genotypic correlation with seed yield (kg/ha) into direct (bold) and indirect components of *Brassica rapa*

Character	DM	PH	NBPP	NSPP	NSPS	TSW	SY
DM	**0.831**						
PH	0.054	**0.818**	-0.007	-0.484	-0.794	-0.949	-0.418
NBPP	0.498	0.067	**-0.212**	-1.413	2.101	-0.674	-0.131
NSPP	0.52	0.530	-0.181	**-1.661**	1.780	-0.431	0.037
NSPS	0.531	0.564	0.174	1.156	**-2.558**	-0.147	-0.809
TSW	0.12	-2.336	0.194	0.968	0.510	**0.739**	0.076
SY							-0.332

DM, Days to maturity (day); PH, Plant height (cm); NBPP, Number of branches per plant (no.); NSPP, Number of siliquae per plant (no.); NSPS, Number of seeds per siliqua (no.); TSW, Thousand seed weight (g): SY, Seed yield (kg/ha)

The results of the path analysis revealed that plant height (0.818) had the maximum direct effect followed by thousand seed weight (0.739). Maximum negative direct effect was observed for number of seeds per siliqua (-2.558) followed by number of branches per plant (-0.212) and number of siliquae per plant (-1.661). Number of seed per siliqua had negative direct effect as well as negative and non-significant genotypic correlation with yield (-0.809). The contributions of yield components like plant height and thousand seed weight were higher in the present study. Singh (1985) observed high positive direct effect on 50% flowering, plant height, numbers of branching, number of siliquae per plant, number of seeds per siliquae on yield. Varshney (1986) working with several strains of *B. rapa* found the negative direct effect of plant height, siliquae per plant, seeds per siliqua and 1000 seed weight on yield. The residual effect was (-0.332) indicated that about 72% of the variability was contributed by six quantitative characters studied in path analysis. This low residual effect might be due to characters not studied, environmental factors, sampling error etc. The results revealed that the germplasm possesses expected variations in the examined traits. Variation among the genotypes are desirable for breeding works toward developing target oriented varieties. Present investigation depicts that a wide variation existed among the *B. rapa* genotypes which are potential resources to develop short duration (all the studied genotypes are short durated) and high yielding varieties from further experimentations. In addition, there was correlation of different yield components with the yield of *B. rapa*.

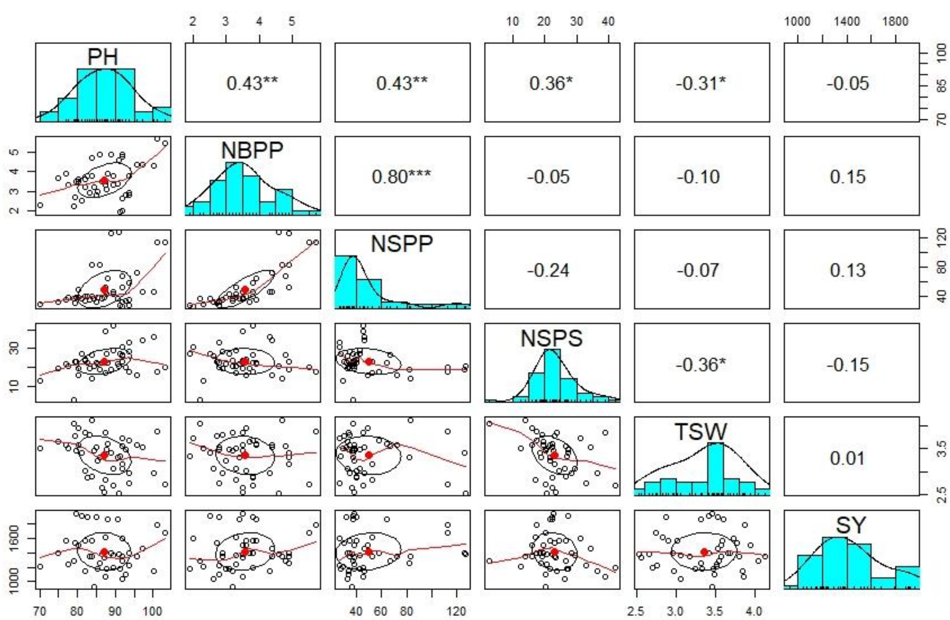

Fig. 1. Pearson correlation with p values among six characters of the *B. rapa*

Conclusion

This present study revealed that the agronomic and yield contributing traits has potential for developing short and high yielding varieties. Generally *B. rapa* varieties cultivated throughout the country in T. aman rice-Fallow-Boro rice based cropping patterns. From the combined analysis we found significant variations among the traits in the germplasm. From the mean performance of yield and other related contributing characters, genetic parameters, G5. G14, G11 and G9 found promising genotypes and these could be brought to the breeding programs in future. They will be able to release new varieties after further experimentation, evaluation and adaptive experiments.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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