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PRODUCTIVITY AND RESOURCE USE EFFICIENCY IN LENTIL PRODUCTION IN SELECTED AREAS OF BANGLADESH

M. A. M. MIAH[1], M. A. RASHID[2] AND M. S. RAHMAN[3]

Abstract

Optimum use of resources in crop production is crucial for reducing production costs and getting higher profits. Lentil farmers are traditionally using different inputs without considering their efficient use levels. Hence, the present study estimated the productivities of various lentil varieties and measured the resource use efficiency in lentil production. The study analyzed 360 household data collected from 240 improved variety users and 120 local cultivar users spread in the six lentil growing districts namely Faridpur, Magura, Kushtia, Jhenaidah, Manikgonj, and Sirajganj. Along with descriptive statistics, the study used Cobb-Douglas production and resource use efficiency models for analyzing the data. The average productivity of improved variety (1.63 t/ha) was much higher than local cultivars (1.08 t/ha). The highest productivity was found in medium-intensive growing areas due to the use of better variety (BARI lentil 8) and a higher level of inputs. Human labour, seed, TSP, MoP, other fertilizers, pesticides, irrigation, and variety had a positive and significant effect on lentil production. Farmers were not efficient in using inputs, they used excessive amount of labour but less amounts of seed and fertilizers. Lentil farmers faced the lack of suitable land, biotic and abiotic stresses, and lack of improved seeds. Farmers should be supplied with improved variety and production technology to increase farm profits through the efficient use of resources in lentil production.

Keywords: Lentil, productivity, factors of production, resource use efficiency, Bangladesh.

Introduction

Pulses supply nutrition for human diet (Das et al., 2016.), provide feed for the animal (Miah et al., 2009), increase soil nutrient status by adding nitrogen, carbon and organic matter (Senanayake et al., 1987; Zapata et al., 1987; Sarker and Kumar, 2011), and improves farmers' livelihood through additional income. Because of the high protein content and low cost, pulses are called *poor man's meat* (Sumera and Ali, 2020). So, most of the low-income populations can use this nutritious crop as their staple food. However, the per capita consumption of pulse in our country is 15.7 g/day (HIES, 2016) that is much lower than the desirable intake of 50 g/day (DDP, 2013).

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Lentils (*Lens culinaris*) are protein-rich legumes that provide important micronutrients in a rice-based diet (ISPC, 2018). It is the most consumed pulse in the country and ranks first among the pulses in terms of consumers' preferences (Miah and Rahman, 1991; Afzal et al., 1999). Among the pulse crops in Bangladesh (BBS 2021), lentils placed the 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 metric tonnes with an average yield of 1.26 t/ha. The area and production of lentils were found fluctuating, but the yield registered an increasing trend over the years. 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 2000/01-2019/20 due to the introduction of improved lentil varieties and management technologies (Miah et al. 2021).

The improved varieties of lentil are suitable for the farmers in terms of productivity and profitability (Miah et al., 2021; Sarker et al., 2020; Matin et al., 2018; Tithi and Barmon, 2018; Hossain et al., 2016). The optimum use of resources in crop production is crucial for reducing the cost of production and getting higher profits. Efficient utilization of inputs has also significant impacts on food security (Chiedozie et al., 2010). But lentil farmers are traditionally using different inputs without considering their efficient use levels. Socio-economists always offer the direction of efficient utilization of inputs to the farmers. Resource use efficiency examines the efficiency of each input and indicates the over-utilization or under-utilization of inputs (Ali et al., 2017). In the past, many authors of home and abroad (Khatun et al. 2019; Ali et al., 2017; Chandra et al., 2017; Dhakal et al., 2015; Umar and Kadir, 2015; Akighir and Shabu, 2011; Chiedozie et al. 2010) estimated resource use efficiency in producing various crops (Rice, mustard, cucumber, tomato, strawberry, etc) except lentils.

Therefore, it is crucial to evaluate the efficiency level of input use to maximize profit by minimizing cost. However, the study on resource use efficiency in lentil production is scarce in Bangladesh. Therefore, the present study was designed with the following objectives: 1) to identify the factors influencing the productivity of lentils at the farm level; 2) to measure the resource use efficiency of farmers in lentil production, and 3) to identify the problems of lentil cultivation in the study areas.

Materials and Methods

Sampling technique and sample size

A multi-stage sampling procedure was followed to select study areas and sample households. Based on the crop concentration index (CCI), the study was conducted in purposively selected six lentil growing districts of Bangladesh, taking Faridpur and Magura districts from highly-intensive (**CCI value = 5.54-11.31*), Kushtia

and Jhenaidah districts from medium-intensive (*CCI value = 1.09-4.87*), and Manikgonj and Sirajganj districts from low-intensive growing areas (*CCI value = 0.02-0.83*). Again, in each district two *Upazilas* (administrative unit) and from each *Upazila* one/two Agricultural Blocks (ABs) were purposively selected for collecting data and information from the sample farmers. The *Upazilas* and ABs were chosen in consultation with Agricultural Extension Officer, SAAO, and local BARI scientists. Finally, two lists of lentil growing farmers (adopter and non-adopter) were prepared separately for each AB, and then a total of 30 farmers, taking 20 farmers from adopters and 10 from non-adopters were selected from each *Upazila* for interview. The adopter farmers were those who cultivated improved varieties of lentils and non-adopting farmers cultivated only local cultivars of lentils. Thus, the total numbers of adopting and non-adopting sample farmers were 240 and 120 respectively.

Data collection

Data for the present study were collected by interviewing sample lentil growers with the aid of a pre-designed and pre-tested interview schedule during March to April 2021. Both trained enumerators and researcher collected primary data. Concerning this study, secondary data on lentil area and production were collected and used to supplement the study.

Model specification

The following Cobb-Douglas type production function model was used to estimate the contribution of factors to the productivity of lentils in the study areas. The functional form of the Cobb-Douglas production function model (Gujarati, 2003) is given below (equation 1):

$$Y = \alpha X_1^{b_1} X_2^{b_2} \dots\dots\dots\dots\dots\quad X_n^{b_n} e^{u_i} \quad \text{..... (1)}$$

The production function was converted to logarithmic form (equation 1) so that it could be solved by the least square method, i.e.

$$\ln Y = \ln\alpha + b_1 \ln X_1 + b_2 \ln X_2 + \dots\dots\dots + b_n \ln X_n + Ui \text{..... (2)}$$

The empirical production function model (equation 2) was as follows:

$$\ln Y = \alpha + \beta_1 \ln X_1 + \beta_2 \ln X_2 + \beta_3 \ln X_3 + \beta_4 \ln X_4 + \beta_5 \ln X_5 + \beta_6 \ln X_6 + \beta_7 \ln X_7 + \beta_8 \ln X_8 + \beta_9 \ln X_9$$

$$+ \beta_{10} \ln X_{10} + \beta_{11} \ln X_{11} + \beta_{12} X_{12} + U_i \text{.....} \quad (3)$$

Where,

Y = Yield of lentil (kg/ha)

α = Intercept

β_i = Coefficients of the respective variables to be estimated (i = 1, 2, 3 -----12)

X_1 = Farm size (decimal)

X_2 = Age of the farmer (year)

X_3 = Education (year of schooling)

X_4= Human labour (No./ha)

X_5= Amount of seed (kg/ha)

X_6= Amount of urea (kg/ha)

X_7= Amount of TSP (kg/ha)

X_8= Amount of MoP (kg/ha)

X_9= Amount of other fertilizers (kg/ha)

X_{10}= Cost of pesticides (Tk./ha)

X_{11}= Cost of irrigation (Tk./ha)

X_{12}= Variety dummy (if improved variety=1, Otherwise = 0)

U_i is the error term which is independently distributed with zero mean and constant variance. In addition, one sample t-test was used to find out the significance level of the variation of variables in the regression model.

Resource use efficiency

In order to maximize profit through the efficient allocation of resources, the producer should use more of the variable resource so long as the value of the added production is greater than the cost of the added amount of resource used in the production. The straightforward way of examining such efficiency is to compare the marginal value product (MVP) with marginal factor cost (MFC) of each variable input. The efficiency of inputs used in lentil production was measured by the following equation (4). This approach was used in many past studies (Khatun et al. 2019; Ali et al. 2017; Umar and Kadir, 2015; Dhakal et al. 2015; Abid et al. 2011) for measuring the resource use efficiency.

$$\frac{MVP_x}{MFC_x} = 1 \quad \text{..... (4)}$$

The value of MVP can be estimated using the following equations (5 & 6).

$$MVP_x = MPP_x \times Py \quad \text{..... (5)}$$

$$MPP_x = b_i \times APP_x = b_i \times \frac{Y}{X_i} \quad \text{..... (6)}$$

Where,

MVP_x= Marginal value product of 'X' input

MPPx = Marginal physical product of 'X' input

APPx = Average physical product of 'X' input

MFC_x= PXi = Marginal factor cost of 'X' input (unit price of factor input resource)

Py = Unit price of output

bi = Elasticities or regression coefficients of the various inputs

$\bar{Y}$ = Mean of output

$\bar{X}i$ = Mean of 'X' input factor

The resource is considered to be efficiently used and profit will be maximized when the ratio of MVP to MFC is equal to unity or MVP and MFC for each input are equal. When the ratio is greater than unity, it implies that the resource is underutilized. In that case, there is an ample opportunity to increase total production by increasing the use of specific input in the production process keeping other resources constant. When the ratio is less than unity implying the resource is overused. In that case, it is possible to reduce production cost remains total production unchanged by decreasing the use of specific input.

The relative percentage change in MVP of each resource required to obtain optimal resource allocation, which is MVP = MFC, was estimated using equation 7 below. This formula was also used in different past studies (Khatun et al. 2019; Chandra et al. 2017; Gani and Omonona 2009) in home and abroad.

$$D = [1 - \frac{1}{\frac{MVP}{MFC}}] \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Where, D = Value of percentage change in MVP of each resource. The significance of each explanatory variable was determined using the t-test.

Results and Discussion

Input use pattern

Different types of inputs were used in lentil cultivation. Human labour is one of the crucial inputs that was employed for land preparation, seeding, fertilization, weeding, pesticide spraying, crop harvesting, threshing, drying, and storing. The total number of human labour used for cultivating improved and local lentils was 72 and 63 man-days/ha respectively. The highest number of labour (82 & 74 man-days) was used in the medium-intensive growing areas. They used seeds at the rate of 46 kg and 44 kg per ha for improved and local variety lentils respectively. These rates were a bit higher than the recommended rate (35-40kg/ha). The applications of urea, TSP, and MoP for improved variety lentils in all study areas were a bit higher than the recommended dose. The overall use of inputs was higher for cultivating improved varieties compared to local cultivars and it was true for different growing areas as well (Table 1).

Table 1. Per hectare use of inputs in lentil production in the study areas

Input	High-growing area		Medium-growing area		Low-growing area		All area		Recommended rate
	HYV	Local	HYV	Local	HYV	Local	HYV	Local	
Labour (m-day)	69	61	82	74	66	54	72	63	--
Hired labour	*41*	*38*	*46*	*39*	*42*	*36*	*43*	*38*	--
Family labour	*28*	*23*	*36*	*35*	*24*	*18*	*29*	*25*	--
Seed (kg)	48	47	49	47	42	39	46	44	35-40
Cow dung (kg)	1327	154	4772	4130	718	--	2272	1428	--
Urea (kg)	47	42	48	45	45	41	47	43	40-45
TSP (kg)	92	90	96	91	89	87	92	89	80-90
MoP (kg)	47	40	52	43	46	38	48	40	40-45
Boron (kg)	4.8	2.7	3.9	0.7	1.2	0.2	3.3	1.2	7-10
$ZnSO_4$ (kg)	3.9	2.1	3.8	0.7	0.8	0.6	2.9	1.1	--
DAP (kg)	10.7	8.6	19.0	2.5	7.9	6.8	12.5	6.0	--
Irrigation (Tk.)	2596	2649	1361	681	656	956	1538	1429	*once
Pesticides (Tk.)	1367	1282	1993	1236	770	95	1377	871	--

* Once within 30-40 Days after germination

Productivity of lentils

The average yields of BARI Masur-8, BARI Masur-7, BARI Masur-6, and BARI Masur-4 were 1.86, 1.61, 1.69, and 1.34 tonnes per hectare respectively at the farm level. The average yield gaps of these varieties were found to be 15-31% depending on the variety. However, the average yield of improved varieties (1.625 tonnes/ha) is much higher (33.5%) than that of the local cultivar (Table 2). More-or-less similar yield (1.632 tonnes/ha) was documented in a study conducted in Jashore, Jhenaidah, and Kushtia districts (Hajong et al., 2020). Table 1 further revealed that the yields of both improved and local varieties were higher at the medium-intensive growing areas compared to highly intensive and low-intensive growing areas might be due to the use of higher amounts of inputs and improved varieties.

The productivity of a crop depends on many agro-socio-economic and environmental factors. It varies from variety to variety, location to location, and year to year. Rahman et al. (2012) recorded the average yield of BARI Masur varieties (3, 4, 5, & 6) as 1.733 tonnes/ha in the Jhenaidah and Jashore districts during 2010-11. In the next year (2011-12), Matin et al. (2018) found the average yield of HYV lentils to be 1.479 tonnes/ha in Jashore, Meherpur, and Natore districts. Kazal et al. (2013) recorded lentil yield as 1.160 tonnes/ha in Natore and Bogura districts during 2012.

Table 2. Productivity (kg/ha) of different lentil varieties in the study areas

Lentil variety	High-growing area	Medium-growing area	Low-growing area	All area	**Average potential yield
BARI Masur-8	1832	2017	--	1855 (18)	2250
BARI Masur-7	--	1778	1487	1614 (15)	1900
BARI Masur-6	1676	1793	1445	1692 (21)	2150
BARI Masur-4	--	1456	1326	1339 (31)	1950
All BARI variety	1754	1761	1419	1625 (21)	2063
Local cultivar	1077	1142	1025	1081(33.5*)	--

Note: Figures in the parentheses are percent less yield over potential yield

*Figure in the parenthesis indicate percent lower yield compared to the yield of all improved varieties

** Source: BPH, 2019

Factors influencing the productivity of lentils

The productivity of lentils is likely to be influenced by different factors. The Cobb-Douglas production function model constructed for all areas revealed that the coefficients of human labour, seed, TSP, MoP, other fertilizers, pesticides, and irrigation were positive and significant at 1-10% level, which indicated that 1% increases in those inputs keeping other factors remaining constant would increase the yield of lentil by 0.097%, 0.564%, 0.058%, 0.098%, 0.018%, 0.006%, and 0.005% respectively. It implied that the aforesaid inputs had a positive and significant effect on the yield of lentils. The coefficient of variety dummy was positive and highly significant at the 1% level meaning that 1% increases in the use of improved lentil variety, keeping other factors remaining constant, would increase the yield of lentils by 0.346% (Table 3). The study found some common variables such as seed, TSP, irrigation, and variety dummy in the models constructed for different growing areas which notably influenced the yield of lentils. Only the higher investment in irrigation reduced the yield of lentils in low-intensive growing areas. The results are quite supported by the past studies conducted on lentil production (Rahman et al., 2012; Tithi and Barmon, 2018; Matin et al., 2018). The value of the coefficient of determination (R^2) in model-4 is 0.758 which indicated that around 76% of the variation in output is explained by the independent variables included in the model. The value of F is 90.657 which is significant at 1% level indicates the good fit of the model.

Production function is a functional relationship between outputs and inputs (Jhingan, 2007). There are three stages of production. MPP is negative in stage III and it is not rational to produce with negative MPP (Akighir and Shabu, 2011). However, the returns to scales of lentil production were estimated through the

Table 3. Coefficients of the variables used in the Cobb-Douglas production function

Variables	**Model-1**: High-growing area		**Model-2**: Medium-growing area		**Model-3**:Low-growing area		**Model-4**: All area	
	Coefficient	SE	Coefficient	SE	Coefficient	SE	Coefficient	SE
Constant	***4.030	0.455	***2.766	0.575	***4.401	0.474	***3.642	0.276
LnFsize (dec)	-0.028	0.019	***-0.076	0.024	**0.027	0.012	-0.003	0.011
LnAge (year)	-0.017	0.029	0.172	0.136	-0.069	0.092	0.001	0.030
LnEdu (year)	0.002	0.013	0.011	0.018	-0.013	0.012	-0.005	0.008
LnLabour (No.)	0.017	0.070	0.063	0.067	0.077	0.050	***0.097	0.035
LnSeed (kg)	***0.559	0.093	***0.848	0.126	***0.452	0.089	***0.564	0.061
LnUrea (kg)	0.032	0.064	0.074	0.079	0.007	0.064	0.042	0.043
LnTSP (kg)	*0.093	0.051	0.052	0.065	0.006	0.049	*0.058	0.034
LnMoP (kg)	0.083	0.074	0.054	0.076	***0.151	0.054	**0.098	0.042
LnOthfert (kg)	0.010	0.008	0.011	0.010	*0.016	0.009	***0.018	0.005
LnPesticid (Tk)	0.001	0.004	***0.020	0.006	0.005	0.004	**0.006	0.003
LnIrrigati (Tk)	*0.005	0.003	*0.007	0.004	**-0.006	0.003	***0.005	0.002
Variety dummy (BARI=1, local=0)	***0.488	0.028	***0.353	0.037	***0.185	0.026	***0.346	0.018
Returns to scale	1.246		1.589		0.838		1.227	
F-value	50.668***		38.555***		21.266***		90.657***	
R^2	0.851		0.812		0.705		0.758	
N	120		120		120		360	

Note: Dependent variable = Lentil yield (kg/ha)

***, **, * represent significant at 1%, 5% and 10% level respectively

summation of all the regression coefficients of inputs in the models for determining the stages of production. It is noted that the returns to scales of lentil production were more than unity for all the models except in model-3 implied that the production function exhibited increasing return to scale and lied on the first stage of production. This also implied that when all other variables are held constant, a unit increase in one of them results in higher than proportionate increase in output. However, only the production function of low-growing areas (model-3) showed decreasing returns to scale and reclined in the second stage of production. It indicates that if all the inputs specified in the production function were increased simultaneously by 100%, the yield would increase by 84% (Table 3).

Resource use efficiency

The ratios of MVP and MFC are greater than unity for seed, urea, TSP, MoP, and other fertilizers (Boron, ZnSo4 & DAP) indicating that these inputs were underutilized. The lentil farmers in the study areas used small amounts of these inputs to cultivate lentil meaning that the cost of using these inputs is less than the value of marginal product. The findings suggest that farmers can invest more on these inputs to ensure the use of these inputs efficient. The ratio of MVP and MFC for labour is less than unity implying that such key input was over utilized. This suggests that farmers can reduce the number of labour to make its use efficient. Overall, the study revealed that all the inputs used in lentil production were not optimally utilized (Table 4).

Table 4 further reveals that the adjustment in the MVPs indicated that the level of input use should be increased or decreased for optimal allocation of resources. The level of use of seeds, urea, TSP, MoP and other fertilizers should be increased by 91%, 81%, 57%, 92% and 46% respectively to obtain the optimum profit. On the other hand, human labour was needed to decrease by 160% for getting the highest profit.

Table 4. Estimated resource use efficiency indicators in lentil production

Variable	Coefficient	MPP	Py	MVP	MFC	MVP/ MFC	Adjustment required (%)
Labour (man-day)	0.097	1.944	68.5	133.16	345.60	0.385	-160
Seed (kg)	0.564	16.958	68.5	1161.62	102.50	11.333	91
Urea (kg)	0.042	1.288	68.5	88.23	16.66	5.296	81
TSP (kg)	0.058	0.867	68.5	59.39	25.65	2.315	57
MoP (kg)	0.098	3.014	68.5	206.46	16.11	12.816	92
Other fertilizers (kg)	0.018	1.804	68.5	123.57	67.37	1.834	46

Problems of lentil cultivation

The adopter and non-adopter farmers in the study areas mentioned numerous common issues regarding the problems of lentil production, but the magnitudes of their statements were not the same at all. The majority of the farmers (40-53.3%) opined about the attack of foot rot and stemphylium blight diseases. The leaves of the infected plants become yellow or reddish after 20-25 days of sowing, and the tip of the plant dries slowly due to attack of foot rot disease. This problem was more vital for local cultivars compared to improved varieties. Adverse weather (dense fog, excessive rain, heat, etc.) was another severe problem faced by 15.4-19.2% of respondent farmers. The infestation of lentils by insects (Aphids & cutworm) was reported by 8.3-9.2% of the farmers to be harmful for lentil cultivation. Some respondent farmers were facing the unavailability problem of quality lentil seed in the study areas. The lower yield of local lentils was mentioned as a crucial problem by 15% of non-adopter farmers. The other problems of adopters and non-adopters were lack of cash, the higher price of labour, and the low market price of lentils (Table 5).

Table 5. Problems of lentil cultivation in the study areas

Type of problems	Improved variety user (n=240)		Local cultivar user (n=120)	
	N	%	N	%
1. Infection of foot rot & stemphylium diseases	96	40.0	64	53.3
2. Adverse weather (fog, excessive rain, heat)	37	15.4	23	19.2
3. Lack of irrigation facility	28	11.7	12	10.0
4. Infestation of insects (Aphids, *Katui*)	22	9.2	10	8.3
5. Lack of quality seed	10	4.2	8	6.7
6. Lack of cash	15	6.3	7	5.8
7. Scarcity and higher cost of labour	12	5.0	8	6.7
8. Low yield	7	2.9	18	15.0
9. Low market price	5	2.1	3	2.5
10. Others*	8	3.3	6	5.0

Note:*Higher cost of inputs, low germination of seed, crop dies due to excessive salt, lack of tillage machinery, bad soil quality, etc.

Conclusions

Three variables namely seed, irrigation and variety had a positive and significant effect on lentil production. Farmers used excessive amount of labour but less amounts of seed and fertilizers to produce lentils. However, the level of adjustments for using various resources to earn optimum returns will serve as a bench-mark guideline for the lentil growers, government agencies, and agro-based

companies. However, some lentil farmers in all the study areas were to some extent constrained by suitable land, biotic and abiotic stresses, and quality seeds for the desired level of lentil production.

The study suggests concerned agencies to supply improved and disease registrant lentil varieties and provides sufficient irrigation facilities to increase productivity and farm profits of the farmers. Farmers should be encouraged to use farm machineries for escaping from human labour crisis. Lentil farmers should be given hands-on training for ensuring the efficient use of resources. Thus if proper uses of resources could be ensured, lentil production could be a more viable and attractive commercial enterprise to the farmers.

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PERFORMANCE OF MANDARIN ORANGE GENOTYPES IN NET HOUSE AT JAINTAPUR, SYLHET

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Abstract

A study on performance of seven mandarin genotypes along with BARI Komala-1 as check was carried out at the Citrus Research Station, Bangladesh Agricultural Research Institute (BARI), Jaintapur, Sylhet from January 2016 to December 2018. The average highest number of fruits per plant (37.2), and the maximum fruit yield per plant (6.19 kg) and the maximum fruit yield per hectare (6.87 t) were recorded in the genotype CR Jai-017. The largest fruits (189.67 g) were found in the genotype CR Jai-015 (189.67 g) with the maximum fruit size. Edible portion was found the highest in CR Jai-017 (73.52%) followed by CR Jai-018(72.55%). The genotype CR Jai-016 gave the maximum percent total soluble solids (TSS) (13.58%) and the minimum (9.87) in CR Jai-015, whereas, the titratable acidity (TA) value was the lowest in CR Jai-014 (0.73%) followed by CR Jai-017 (0.75). Maturity index (MI)/sugar acid ratio (17.83) was found in CR Jai-016 followed by CR Jai-017 (15.46). Fruits of CR Jai-017 and CR Jai-018 were dark orange colored. Fruit surface was smooth in BARI Komala-1 and CR Jai-015 whereas other genotypes with pitted skin. The genotype CR Jai-014 has a strong adherence of albedo to pulp, while others were medium to weak. Segment shape of CR Jai-014 and CR Jai-017 were not uniform but the others were uniform. Thickness of segment wall was medium to thick while fruit axis was semi hollow to hollow with light orange to dark orange pulp and pulp firmness was soft to intermediate with medium to high juice content in all the genotypes.

Keywords: Mandarin, Net house, *Citrus reticulata*, titratable acidity, TSS, Sylhet

1. Introduction

Mandarin (*Citrus reticulata* Blanco) locally known as Komala is a popular fruit of Bangladesh. It is a tasty fruit having nutritional and medicinal values, rich in vitamin A, B and C (40 mg $l00g^{-1}$ juice) with recognized immense economic importance (Bhuyan *et al.*, 2016). Mandarin oranges of all kinds are primarily eaten out-of-hand or the sections are utilized in fruit salads, gelatins, puddings or on cakes and very small amounts are canned in syrup (Morton, 1987).

North eastern hilly region of Bangladesh is characterized by small hills and hillocks, wet summer and dry winter, where mandarin is an important crop mostly produced in homesteads along with some commercial fruit crops (Bhuyan

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et al., 2016). However, the productivity is low comparing with other mandarin orange growing countries. One of the main reasons for this low productivity is lack of high yielding varieties. Besides, imbalanced use of nutrients, poor soil management, and lack of irrigation facilities, inadequate management of major pests and diseases as well as low pH of the soil are also responsible to some extent (Nasreen *et al.* 2012).

We conducted the study under net house condition. There are some benefits of net house over the traditional field condition. Previous studies reported that solar radiation and evapotranspiration were 22% and 23.8% lower inside a screen house compared to the open-air, while there was no difference in cumulative rainfall between both production systems (Ferrarezi *et al.*, 2017). On the other hand plants grown inside the enclosed houses had greater canopy area compared to the open-air plots, which is related to higher fruit yield and fruit quality (Schumann *et al.*, 2017). Furthermore, the plants can easily be protected from major pests; therefore, actual potentiality of the plants can be identified without much difficulty. The use of protective netting also prevents yield loss from high temperatures and irradiation, strong wind, and hail (Manja and Aoun, 2019; Mupambi *et al.*, 2018). In addition, protective netting also results in changes in the orchard microclimate that can alter tree physiology and improve tree performance (Manja and Aoun, 2019; Zhou *et al.*, 2018).

Besides, urbanization brings various challenges like increased environmental stressors and massive demand for food.54% of the total world population is urbanized (Pham-Thi *et al.*, 2021). Moreover, many urban residents are facing problems due to lack of space for vegetation. Therefore, rooftop gardening is itself a prodigious idea for pitching a road towards sustainability. The capital city of Bangladesh, the Dhaka is already trying to boost sustainability through urban farming as a possible remedy to these problems (Bhuiyan and Ferdous, 2021; Sultana *et al.*, 2021). Many of the urban farmers are growing different fruits on their rooftops and many of them are trying to produce mandarin orange for getting fresh mandarin oranges on their table. But, still there is a shortage of mandarin variety suitable for rooftop garden, and urban farmers are cheated purchasing mandarin orange seedlings from various nurseries (Islam *et al.*, 2012).

Bangladesh Agricultural Research Institute has already released three varieties of mandarin, which are performing better in different regions of Bangladesh. On the other hand Sylhet and Chottagram region are blessed with a considerable bio-diversity of mandarin (Das *et al.*, 2005). Moreover, in recent years, some exotic germplasm are also collected, which are also good resources for developing more modern varieties of mandarin orange. Therefore, our objectives were to find out suitable mandarin orange genotype(s) with higher yield and quality for in-ground as well as rooftop cultivation. Moreover, we conducted our study under controlled net house condition, which facilitated reduced pests infestation as well

as provided the genotypes showing the maximum potentiality to yield under suitable condition.

2. Materials & Methods

2.1 Experimental location

The experiment was conducted at Citrus Research Station under Bangladesh Agricultural Research Institute (BARI) located at Jaintapur Upazila of Sylhet district (25.13562º N latitude, 92.13217º E longitude, altitude 36 m from mean sea level), in three consecutive years viz. 2016, 2017 and 2018. The experimental location belongs to the northern and eastern piedmont plains (AEZ 22) having sandy loam textured soil with very low (<4.2) pH (Ahmmed *et al.*, 2018).

2.2 Meteorological variables

Subtropical climate is enjoyed by the experimental location, which is characterized by wet summer March to September) and dry winter (November to February). Annual average rainfall ranges from 4500-6000 mm, the average maximum and minimum temperatures are 36ºC and 6ºC in the month of April and January respectively (Bhuyan *et al.* 2016). The weather data for the experimental location is mentioned in Table 1.

Table 1. Weather data for the experimental period 2016-2018

Month	Temperature(°C)						Precipitation (mm)			Relative humidity (%)		
	2016		2017		2018		2016	2017	2018	2016	2017	2018
	Max.	Min.	Max.	Min.	Max.	Min.						
January	27	14	29	14	27	15	4.29	0.50	13.00	61	59	63
February	31	18	31	16	31	17	33.00	32.70	3.80	55	55	54
March	35	21	31	29	35	20	104.51	296.20	53.43	49	62	51
April	34	24	32	23	36	23	498.09	799.30	175.13	74	78	58
May	34	25	35	25	30	24	460.15	308.60	506.80	77	76	77
June	32	26	32	26	34	26	630.39	976.70	726.11	84	86	81
July	32	25	32	25	33	26	464.95	618.20	490.00	87	86	84
August	33	25	33	25	34	26	360.37	555.90	387.40	83	84	83
September	33	25	33	25	33	25	469.61	560.30	346.14	85	85	81
October	32	23	32	23	32	21	318.80	499.08	83.65	83	83	76
November	29	19	31	19	31	18	185.58	25.10	22.90	81	73	72
December	29	17	29	17	27	16	1.70	79.40	21.10	74	75	69

2.3 Experimental condition

The plants were grown using earthen pots (top diameter 18 inches, bottom diameter 12 inches, height 14 inches and approximate volume of 40.5 L).Seven Mandarin genotypes were included in the study along with BARI Komala-1 as check. The saplings were planted in 2013 to initiate the study. At the beginning the potting soil was prepared by mixing sandy loam nursery soil (pH 6.3) and FYM (1:1) on volume basis. Afterwards chemical fertilizers (20 g of triple super phosphate and 12 g of gypsum) were added with 40 liter of potting mix and the pots were filled followed by watering. The pots were left for 15 days; and afterwards the saplings were planted at the middle of each pot. The saplings were previously prepared using pummelo as the rootstock, and 8 months old grafts were selected for planting. The saplings started flowering at the age of two, but we did not allow any fruit on the year 2014 and 2015 in order to facilitate proper growth of the plants. The plants were around 4 years old when we started data collection and evaluation.

2.4 Cultural Management

Nitrogen (30 g urea) and potassium (20 g MoP) were top dressed in every month in the base of each plant to facilitate proper growth of the plants. Moreover, every year, 5cm top soil of each pot was replaced by new growing media (Soil:FYM=1:1). The plants were irrigated 2-3 times in a week for proper flowering and fruit retention in dry season. Pest management, weeding and other intercultural operations were done when necessary. Fruits were harvested when fruits were fully ripened and the skin color of the fruit turned to deep yellow or orange.

2.5 Data Recording

Data were recorded on growth parameters namely, plant height, base girth, canopy spreading (north-south and east-west), fruits per plant, per plant and per hectare yield. Plant height was measured from the base of the plant to the tips of the topmost leaves and expressed in meter, while the base girth was measured 15 centimeter above the soil and expressed in cm. In a similar way the extent of the canopy was measured in both north-south and east-west direction to find out the canopy spreading and expressed in meter. Numbers of fruits per plants were counted and Yield (kg $plant^{-1}$) and yield (t ha^{-1}) was recorded on every commercial harvest.

Every year mature fruits were collected from the plants. Three randomly selected fruits from each plant were harvested for data collection on individual fruit weight, fruit size (length and diameter at equatorial region), segments per fruit, diameter of fruit axis, rind thickness and weight, seeds per fruit, seed weight per fruit, vesicle size, edible portion, total soluble solids (TSS), titratable acidity (TA) and Maturity index.

Total soluble solids content (TSS) was measured with the help of a refractor meter and corrected with temperature factor (Sherwood, 1928). TA was expressed as percent citric acid present in the juice (Hardy and Sanderson, 2010). While, Maturation index (MI) was calculated from the ratio of TSS:TA (Cavalcante *et al.*, 2009).

2.6 Experimental design and Statistical Analysis

The experiment was laid out following completely randomized design (CRD) with three replications. All the recorded data on different parameters were statistically analyzed using Statix10 software and Fisher's LSD Test was performed for mean separations of the studied parameters and interpretation of results (Gomez and Gomez, 1984).

3. Results and Discussion

3.1 Height and tree volume

Variation was observed regarding the plant height and tree volume of mandarin variety/accessions (Table2). In 2016, during the first year of study, the plants of CR Jai-015 grows vigorously and reached up to 193.4 cm in height on an average with 2.71 m^3 of tree volume, which was also emulated in both 2017 and 2018 (212.8 cm height and 3.58 $m^3$of tree volume respectively).But in 2018 during the third year of study more vigorosity (235.0 cm height and 3.36 $m^3$of tree volume) was found from the plants of CR Jai-017. While comparatively weak plants were found from CR Jai-014, Jai-016 and Jai-018. In all three years, CR Jai-011, Jai-013 and BARIKomala-1showed intermediate vigor. This might be due to the genetic make-up of the genotypes under study (Neves *et al.*, 2018).

Table 2. Growth characteristics of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	Plant height (cm)				Tree volume (m^3)			
	2016	2017	2018	Mean	2016	2017	2018	Mean
CR Jai-011	182.6 bc	201.8 b	215.6 bc	200.00 b	2.11 bc	2.29 c	2.63 c	2.34 c
CR Jai-013	188.2 ab	201.6 b	218.8 b	202.87 b	2.15 bc	2.49 c	3.11 b	2.58 bc
CR Jai-014	177.0 c	193.2c	205.6 c	191.93 c	2.20 bc	2.75 b	3.27 ab	2.74 b
CR Jai-015	193.4 a	212.8 a	228.8 a	211.66 a	2.71 a	3.08 a	3.29 a	3.03 a
CR Jai-016	178.0 c	192.8 c	206.6 c	192.45 c	2.22 bc	2.74 b	3.26 ab	2.74 b
CR Jai-017	188.6 ab	211.2 a	235.0 a	211.60 a	2.28 b	2.84 ab	3.36 a	2.83 ab
CR Jai-018	176.2 c	186.4 c	196.4 d	186.33 d	1.89 c	2.22 c	2.27 d	2.13 d
BARI Komala-1	188.4 ab	201.8 b	219.8 b	203.35 b	2.14 bc	2.43 c	3.08 b	2.55 bc
LSD	8.69	8.11	8.63	8.46	0.34	0.25	0.19	0.31
CV%	2.59	02.22	2.18	3.12	8.57	4.55	2.78	8.68

Means were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying LSD test.

3.2 Yield contributing characters and yield

Significant dissimilarities were observed regarding the yield contributing characters and yield of different variety/accessions studied (Table 3). Throughout the experimental period maximum number of fruits plant^{-1} was observed in CR Jai-017 (31.6, 39.0 and 41.0 respectively), while lowest number of fruits plant^{-1} was observed in CR Jai-017 (12.2, 14.2 and 18.4 respectively). On the other hand, CR Jai-015 performed better regarding individual fruit weight in all the experimental years (185.00 g, 192.00 g and 192.00 g in 2016, 2017 and 2018 respectively), where as the smallest fruit was found from CR Jai-016 (125.67 g, 128.00 g and 122.67 g in 2016, 2017 and 2018 respectively). Moreover, CR-016 bears comparatively small fruits (data not presented) in all the studied year. The number of fruits plant^{-1} is very important for mandarin farmer as they sell each fruit by 20-25 BDT of whether it is sweet or not (Gafor and Choudhury, 2011). Similarly, higher individual fruit weight gave a higher biological yield. Therefore, the accessions boar higher number of fruit might be economically advantageous for the farmers. Talukder *et al.* (2015) also postulated similar results when evaluating some genotypes collected from different locations of Bangladesh.

Table 3. Fruits plant^{-1} and Individual fruit weight of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	Fruitsplant^{-1}(No.)				Individual fruit weight (g)			
	2016	2017	2018	Mean	2016	2017	2018	Mean
CR Jai-011	14.5 e	18.3 f	22.7 f	18.4 f	156.33 cd	171.33 cd	174.67 bc	167.44 cd
CR Jai-013	27.0 b	32.0 b	34.7 b	31.2 b	158.67 c	175.33 c	179.00 b	171.00 c
CR Jai-014	12.2 f	14.2 g	18.4 g	15.0 g	172.00 b	184.67 b	192.67 a	183.11 b
CR Jai-015	24.4 c	27.7 c	28.7 cd	26.9 c	185.00 a	192.00 a	192.00 a	189.67 a
CR Jai-016	18.3 d	25.0 d	28.0 d	23.8 d	114.33 f	125.67 g	128.00 f	122.67 g
CR Jai-017	31.6 a	39.0 a	41.0 a	37.2 a	153.67 d	169.33 de	173.00 c	165.33 d
CR Jai-018	23.3 c	26.4 cd	29.7 c	26.4 c	146.33 e	164.67 e	165.00 d	158.67 e
BARI Komala-1	13.7 ef	20.2 e	25.2 e	19.8 e	142.33 e	144.00 f	141.67 e	142.67 f
LSD	1.510	1.371	1.317	1.036	4.92	4.72	4.51	4.12
CV%	4.18	3.09	2.63	2.38	1.83	1.63	1.53	1.45

Means were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying LSD test.

Yield is the most important factor for getting maximum productivity from any genotypes. As the growth parameters boosted up, there was an opportunity for accumulating more food and dry matter that helps in higher production. In the present experiment yield differed significantly in the studied variety/accessions

(Table 4). In the years 2016 and 2018 less productivity was obtained from the check variety BARI Komala-1 (1.94 and 3.59 kg plant^{-1} respectively), whereas, in 2017 lower productivity was found from CR Jai-014 (2.65 kg plant^{-1}). On the other hand, in three consecutive years CR Jai-17 gave maximum yield (1.86 kg, 6.60 kg and 7.09 kg plant^{-1} and 5.40 t, 7.33 t and 6.87 t ha^{-1} respectively). This was due to the genetic potentiality of each genotype. Moreover, this might be due to faster vegetative growth, progress in photosynthesis rate and improvement of the photosynthates translocation (Talukder *et al.*, 2015). Altaf *et al.*, (2008) also obtained 101g to 287 g fruit weight in Kinnow mandarin. But lowest per ha yield was illustrated by CR Jai-014 in three consecutive years (2.94, 3.92 and 3.07 t ha^{-1} respectively). This result is in conformity with other researchers in banana (Chezhigen *et al.*, 1999).

Table 4. Yield plant^{-1} and yield ha^{-1} of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	Yield plant^{-1} (kg)				Yield (t ha^{-1})			
	2016	2017	2018	Mean	2016	2017	2018	Mean
CR Jai-011	2.24 e	3.14 e	3.96 e	3.11 e	2.49 e	3.49 e	4.39 e	3.46 e
CR Jai-013	4.28 c	5.61 b	6.21 b	5.37 b	4.75 c	6.23 b	6.89 b	5.96 b
CR Jai-014	2.12 ef	2.65 g	3.53 f	2.77 g	2.35 ef	2.94 g	3.92 f	3.07 g
CR Jai-015	4.50 b	5.31 c	5.51 c	5.11 c	5.00 b	5.90 c	6.11 c	5.67 c
CR Jai-016	2.10 ef	3.14 e	3.58 f	2.94 f	2.33 ef	3.49 e	3.98 f	3.26 f
CR Jai-017	4.86 a	6.60 a	7.09 a	6.19 a	5.40 a	7.33 a	7.87 a	6.87 a
CR Jai-018	3.41 d	4.34 d	4.89 d	4.21 d	3.79 d	4.81 d	5.43 d	4.68 d
BARI Komala-1	1.94 f	2.93 f	3.59 f	2.82 fg	2.16 f	3.25 f	3.98 f	3.13 fg
LSD	0.183	0.165	0.227	0.135	0.203	0.183	0.251	0.150
CV%	3.29	2.24	2.70	1.89	3.29	2.24	2.70	1.89

Means were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying LSD test.

3.3 Quantitative fruit quality attributes

There were no significant differences among the variety/accessions regarding segments per fruit in the second and third year of study but in the first years significant variations were noticed (Table 5). Maximum number of segments per fruit was counted from CR Jai-011 (12.0 in both 2016 and 2018), whereas both CR Jai-013 and CR Jai-016 showed highest number of segments fruit^{-1} in 2017

(Table 5). The number of segment represents the number of carpels within the ovary. Segment adherence to each other was strong in most of the genotypes of Khasi and Nagpuri mandarins (Talukder *et al.*, 2015). Altaf *et al.* (2008) found 8-12 segments per fruit in Kinnow mandarin. Whereas, Singh and Singh (2004) were noted that 11.5 segments exist per fruit in mandarin. Diameter of the fruit axis is also an important factor for mandarin oranges (Table 4). With the increase of the axis diameter the hollow core inside the fruit increased resulted in lowest edible portion. In our study variety BARI Komala-1 showed the maximum fruit axis diameter in all the study years (1.65cm, 1.66cm and 1.65 cm in 2016, 2017 and 2018 respectively) followed by CR Jai-014 (1.64cm, 1.64cm and 1.64 cm in 2016, 2017 and 2018 respectively); while the lowest fruit axis diameter was found from CR Jai-015 (1.09cm, 1.09cm and 1.09 cm in 2016, 2017 and 2018 respectively). Therefore our study corroborates with the previous study of Talukder *et al.*, (2015), who found solid fruit axis in most of the studied mandarin genotypes and the least with hollow and semi-hollow irrespective of Khasi and/or Nagpuri types.

Table 5. Segments fruit $^{-1}$ and diameter of fruit axis of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	Segments fruit $^{-1}$(No.)				Diameter of fruit axis (cm)			
	2016	2017	2018	Mean	2016	2017	2018	Mean
CR Jai-011	12.0 a	11.3	12.0	11.8 a	1.42 b	1.42 ab	1.42 b	1.42 b
CR Jai-013	10.3 b	10.7	11.3	10.8 c	1.54 ab	1.54 a	1.54 ab	1.54 ab
CR Jai-014	10.7 ab	11.6	12.0	11.4 ab	1.64 a	1.64 a	1.64 a	1.64 a
CR Jai-015	10.6 ab	11.0	11.0	10.9 bc	1.09 c	1.09 cd	1.09 c	1.09 c
CR Jai-016	11.0 ab	11.7	11.7	11.4 ab	0.89 d	0.89 d	0.89 d	0.87 d
CR Jai-017	11.0 ab	11.6	11.7	11.4 ab	1.56 ab	1.56 a	1.57 ab	1.56 a
CR Jai-018	10.6 ab	11.0	11.6	11.1 bc	1.16 c	1.16 bc	1.16 c	1.16 c
BARI Komala-1	11.0 ab	11.0	11.3	11.1 bc	1.65 a	1.66 a	1.65 a	1.66 a
LSD	1.50	1.25	1.05	0.65	0.18	0.27	0.16	0.13
CV%	7.87	6.32	5.16	3.27	7.44	11.16	6.58	5.34

Means were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying LSD test.

The rind is an important quality attribute of mandarin fruit (Table 6). A thick rind is in most of the cases fragile and creates difficulty to peel off the fruit. Thin peels with low average weight also facilitate higher per cent of edible portion. In

all the experimental years, plants of CR Jai-011 showed maximum rind thickness as well as rind weight (3.8 mm, 3.9 mm, 3.9 mm; and 56.39 g, 62.80 g, 64.28 g in 2016, 2017 and 2018 respectively); whereas lowest rind thickness was found from CR Jai-018 (2.3 mm, 2.4 mm and 2.4 mm in 2016, 2017 and 2018 respectively). On the other hand lowest rind weight was found from CR Jai-017 (30.89 g, 32.09 g and 31.50 g in 2016, 2017 and 2018 respectively).Similar result was also found by Altaf *et al.* (2008), who observed 24 to 71 g peel weight in Kinnow mandarin, while rind weight varied from 18.60 to 43.64 g in Khasi type. According to a description given by Mazhar (1959), Kinnow, Feutrell's Early, Coorg and Nagpuri varieties have less percentage of rind.

Table 6. Rind thickness and Rind weight of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	Rind thickness (mm)				Rind weight (g)			
	2016	2017	2018	Mean	2016	2017	2018	Mean
CR Jai-011	3.8 a	3.9 a	3.9 a	3.9 a	56.39 a	62.80 a	64.28 a	61.15 a
CR Jai-013	3.2bc	3.3 cd	3.3 bc	3.2 cd	43.56 c	43.22 c	44.01 c	43.59 c
CR Jai-014	3.4 b	3.5 bc	3.5 ab	3.5 bc	40.27 d	39.110 d	39.11 d	39.50 d
CR Jai-015	3.57 ab	3. 7 ab	3. 7 ab	3.6 ab	46.86 b	50.60 b	51.52 b	49.66 b
CR Jai-016	2.7 d	2.8 e	2.7 de	2.7 ef	34.14 e	34.4 e	35.01 e	34.51 e
CR Jai-017	3.0 cd	3.1 d	3.03 cd	3.0 de	30.89 e	31.52 f	32.09 f	31.50 f
CR Jai-018	2.3 e	2.4 f	2.4 e	2.4 g	41.43 cd	42.88 c	43.66 c	42.66 c
BARI Komala-1	2.6 de	2.6 ef	2.6 e	2.6 fg	27.05 f	29.54 f	29.75 f	28.78 g
LSD	0.37	0.32	0.40	0.36	3.26	2.54	2.55	1.93
CV%	6.86	5.89	7.33	6.60	4.65	3.47	3.43	2.66

Means were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying LSD test.

Seed weight also varied considerably among the treatments exhibiting the genotypic effect of different variety/accessions (Table 7). In three consecutive years–2016, 2017 and 2018 highest average numbers of seeds fruit^{-1} was counted from CR Jai-15 (17.67, 17.67 and 18.00 respectively), followed by CR Jai-011 (17.67, 17.33 and 18.00 respectively), where as lowest number of seeds fruit^{-1} was counted from CR Jai-014 (2.33, 2.67 and 2.33 respectively). Similarly, lowest seed weight fruit^{-1} was found from CR Jai-014 (0.07, 0.07 and 0.11 in 2016, 2017 and 2018 respectively). Contrary highest seed weight fruit^{-1}was measured in CR Jai-016 (0.35, 0.36 and 0.35 in 2016, 2017 and 2018 respectively), followed by CR Jai-017 (0.35, 0.33 and 0.34 in 2016, 2017 and 2018 respectively).

Table 7. Seeds fruit $^{-1}$ and seed weight of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	Seeds fruit $^{-1}$(No.)				Seed weight (g)			
	2016	2017	2018	Mean	2016	2017	2018	Mean
CR Jai-011	17.67 a	17.33 a	18.00 a	17.67 a	0.17 bc	0.18 bc	0.17 bc	0.17 bc
CR Jai-013	7.67 e	7.33 e	7.67 e	7.56 e	0.14 bc	0.15 bc	0.14 b-d	0.14 bc
CR Jai-014	2.33 f	2.67 f	2.33 f	2.44 f	0.07 d	0.07 d	0.11 d	0.08 d
CR Jai-015	17.67 a	17.67 a	18.00 a	17.78 a	0.12 cd	0.12 cd	0.12 cd	0.12 cd
CR Jai-016	11.00 d	11.00 d	11.00 d	11.00 d	0.33 a	0.35 a	0.33 a	0.34 a
CR Jai-017	13.33 c	13.00 c	13.33 c	13.22 c	0.35 a	0.36 a	0.35 a	0.35 a
CR Jai-018	15.00 b	15.00 b	15.00 b	15.00 b	0.14 bc	0.15 bc	0.14 b-d	0.14 bc
BARI Komala-1	11.00 d	11.33 d	11.67 d	11.33 d	0.18 b	0.19 b	0.18 b	0.19 b
LSD	1.53	1.34	1.51	1.40	0.05	0.06	0.06	0.05
CV%	7.33	6.44	7.11	6.68	15.32	16.27	17.69	15.67

Means were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying LSD test.

Total soluble solids (TSS) and titratable acidity (TA) significantly differed among the variety/accession tested (Table 8). Among the variety/accessions CR Jai-016 showed highest TSS in three consecutive years (13.50%, 13.60%, and 13.63% in 2016, 2017 and 2018 respectively), where as lowest TSS was found from CR Jai-015 (9.77%, 9.83%, and 9.87% in 2016, 2017 and 2018 respectively). On the other hand highest percent TA was obtained from CR Jai-018 (0.96%, 0.96%, and 0.97% in 2016, 2017 and 2018 respectively) followed by CR Jai-015 (0.95%, 0.95%, and 0.96% in 2016, 2017 and 2018 respectively), while minimum TA was found from CR Jai-014 (0.73%, 0.73%, and 0.72% in 2016, 2017 and 2018 respectively) followed by CR Jai-017 (0.75%, 0.76%, and 0.75% in 2016, 2017 and 2018 respectively). A total soluble solid along with the TA is important for measuring the fruit quality. The organoleptic sourness of fruit increased with the increasing TA although TSS of that particular fruit is high. Our study unfolded the genotypic effect of different variety/accessions regarding percent TSS, TA and maturity index badly. Babu and Yadav (2002) obtained that 10.48% TSS in Khasi mandarin genotypes and reported that the TSS found from the Khasi mandarin was more than the released variety BARI Komala-1 (8.66%). But in our study the TSS (%) was much higher than the reports of Babu and Yadav (2002). This might be due to the cultural condition of the two studies. As we cultivated the plants under net house condition which facilitated more intensive care and reduced pest infestation, given ultimate

quality of the fruit. Ladaniya (1996) also reported that Nagpur mandarins grown near Nagpur developed minimum 10% TSS after 270-280 days from fruit set, which supported our study.

Table 8. TSS and TA of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	TSS				TA			
	2016	2017	2018	Mean	2016	2017	2018	Mean
CR Jai-011	12.60 ab	12.70 ab	12.71 ab	12.67 ab	0.81 c	0.83 b	0.84 b	0.82 c
CR Jai-013	11.13 b-d	11.24 b-d	11.25 b-d	11.21 b-d	0.94 a	0.94 a	0.96 a	0.95 a
CR Jai-014	11.23 b-d	11.34 b-d	11.35 b-d	11.31 b-d	0.73 d	0.73 c	0.72 d	0.73 d
CR Jai-015	9.77 d	9.83 d	9.87 d	9.82 d	0.95 a	0.95 a	0.96 a	0.95 a
CR Jai-016	13.50 a	13.60 a	13.63 a	13.58 a	0.76 d	0.77 c	0.76 c	0.76 d
CR Jai-017	11.50 bc	11.60 bc	11.60 bc	11.57 bc	0.74 d	0.75 c	0.76 cd	0.75 d
CR Jai-018	10.30 cd	10.37 cd	10.41 cd	10.36 cd	0.96 a	0.96 a	0.97 a	0.96 a
BARI Komala-1	11.43 bc	11.54 bc	11.55 bc	11.51 bc	0.88 b	0.88 b	0.86 b	0.87 b
LSD	1.58	1.59	1.59	1.58	0.044	0.048	0.038	0.041
CV%	7.88	7.88	7.88	7.88	3.00	3.21	2.57	2.74

Means were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying LSD test.

Fruit quality attributes were significantly varied among the variety/accessions studied (Table 4). Edible portion were statistically indifferent among variety/accessions CR Jai-017, CR Jai-018, CR Jai-014 and BARI Komala-1 during 2016, but maximum edible portion was found from CR Jai-017 (73.93%). On the other hand, in the year 2017 and 2018, CR Jai-017, CR Jai-018 and BARI Komala-1 showed statistically similar percentage of edible portion, where CR Jai-017 showed the maximum (72.92% and 73.52% in the year 2017 and 2018 respectively). Regarding maturity index (MI) CR Jai-016 performed better in all the tested year (17.86, 17.76 and 17.83 in 2016, 2017 and 2018 respectively), whereas, the lowest MI were found from CR Jai-015 (10.29, 10.39 and 10.32 in 2016, 2017 and 2018 respectively).This might be due to proper cultural and nutrient management to the genotypes, which gave the genotypes to show their potentiality under controlled condition. Bhuyan *et al.* (2016) found similar result with integrated nutrient management in mandarin orange with higher TSS/TA ratio and better quality. Talukder *et al.* (2015) found more than 70% edible portion in all the studied genotype.

Table 9. Edible portion and MI (TSS/TA) of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	(%)				MI (TSS/TA)			
	2016	2017	2018	Mean	2016	2017	2018	Mean
CR Jai-011	61.52 b	62.80 bc	62.39 c	62.23 e	15.71 ab	15.29 bc	15.20 bc	15.40 b
CR Jai-013	63.94 b	62.59 bc	63.73 bc	63.42 de	11.90 de	11.96 de	11.73 de	11.87 cd
CR Jai-014	72.73 a	63.67 bc	63.12 bc	66.51 c	15.39 bc	15.55 ab	15.69 b	15.54 b
CR Jai-015	64.54 b	64.07 b	64.07 b	64.22 d	10.29 e	10.39 e	10.28 e	10.32 d
CR Jai-016	63.06 b	62.09 c	62.84 bc	62.66 e	17.86 a	17.76 a	17.87 a	17.83 a
CR Jai-017	73.93 a	72.92 a	73.52 a	73.45 a	15.64 ab	15.40 b	15.33 bc	15.46 b
CR Jai-018	72.85 a	72.50 a	72.23 a	72.55 ab	10.77 de	10.84 e	10.74 e	10.78 d
BARI Komala-1	71.04 a	72.03 a	72.61 a	71.89 b	13.06 cd	13.12 cd	13.39cd	13.19 c
LSD	3.29	1.73	1.43	1.29	2.36	2.23	2.00	2.18
CV%	2.76	1.49	1.22	1.10	9.75	9.25	8.31	9.03

Means were calculated from three replications (n = 3) for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying LSD test.

3.4 Qualitative fruit quality attributes

In case of other qualitative fruit characters (Table 5), fruits of CR Jai-017 and CR Jai-018 were found dark orange color in all the experimental period, while greenish yellow color was found in CR Jai-013 and CR Jai-015, but light orange was found in CR Jai-016and CR Jai 011, whereas orange and greenish color fruits were recorded in BARI Komala-1 and the genotype CR Jai-014. Obloid shaped fruits were recorded in all the consecutive years in all genotypes. Fruit surface was found smooth in BARI Komala-1 and CR Jai-015, while other with pitted skin. Strong adherence of albedo to pulp was found in CR Jai-014, while others were medium to weak in every study year. Furthermore, a strong attachment of fruits with stalk was found in CR Jai-013 and CR Jai-016, while others were weak in all the studied year. Rind color of citrus is considered to be one of the most important external factors of fruit quality, as the appearance of fruit greatly influences consumer choice (Olmo *et al.,* 2000). Chahidi *et al.* (2008) also reported various rind colors in Clementine mandarin.

Table 10. Fruit color, fruit shape, fruit surface texture, adherence of albedo to pulp, fruit attachment to stalk of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	Fruit color	Fruit Shape	Fruit surface texture	Adherence of albedo to pulp	Fruit attachment to stalk
CR Jai-011	Light Orange	Obloid	Pitted	Weak	Medium
CR Jai-013	Greenish Yellow	Obloid	Pitted	Medium	Strong
CR Jai-014	Greenish	Obloid	Pitted	Strong	Medium
CR Jai-015	Greenish Yellow	Obloid	Smooth	Weak	Medium
CR Jai-016	Light Orange	Obloid	Pitted	Weak	Strong
CR Jai-017	Dark Orange	Obloid	Pitted	Medium	Medium
CR Jai-018	Dark Orange	Obloid	Pitted	Medium	Medium
BARI Komala-1	Orange	Obloid	Smooth	Medium	Medium

Uniform shape of segments was found in all the genotypes except CR Jai-014 and CR Jai-017 (Table 11). Thickness of segment wall was found medium to thick in all the genotypes except CR Jai 018, which was comparatively thin. Fruit axis was found semi hollow to hollow and pulp color was recorded light orange to dark orange in all the genotypes. Pulp firmness and juice content were recorded soft to intermediate and medium to high respectively in all the genotypes in all the experimental year.

Table 11. Segment shape uniformity, thickness of segment wall, Fruit axis, pulp color, pulp firmness, juice content of mandarin genotypes in net house condition under north eastern hilly region of Bangladesh

Variety/ Accession	Segment shape uniformity	Thickness of segment wall	Fruit axis	Pulp color	Pulp firmness	Juice content
CR Jai-011	Yes	Medium	Semi-hollow	Dark Orange	Soft	High
CR Jai-013	Yes	Thick	Hollow	Light Orange	Intermediate	Medium
CR Jai-014	No	Thick	Semi-hollow	Light Orange	Intermediate	Medium
CR Jai-015	Yes	Medium	Semi-hollow	Light Orange	Intermediate	Medium
CR Jai-016	Yes	Thick	Semi-hollow	Dark Orange	Soft	High
CR Jai-017	No	Medium	Hollow	Dark Orange	Soft	High
CR Jai-018	Yes	Thin	Semi-hollow	Dark Orange	Soft	High
BARI Komala-1	Yes	Medium	Hollow	Dark Orange	Soft	High

Conclusion

From the study, it can be concluded that the genotypes CR Jai-017 and CR Jai-016 were found superior in terms of yield and fruit quality attributes. The genotype CR Jai-016 also may be released as a variety and the both genotypes may be recommended for net house cultivation and roof gardening in controlled condition under north eastern hilly region of Bangladesh.

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HETEROSIS AND COMBINING ABILITY ANALYSIS IN MAIZE USING LINE X TESTER MODEL

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Abstract

Twenty-two lines were crossed with 2 testers in a Line × Tester mating design in 2017-18 and the resulting 44 crosses along with the lines, testers and three checks i.e., BARI Hybrid Maize 9 (BHM 9), 981 and Elite were evaluated in a alpha lattice design with two replications, during rabi, 2018-19. Highly significant differences were found among the genotypes for all the characters studied. Parent and parents vs crosses were significant for all the characters except ASI indicating greater diversity in the parental lines of the traits. Three lines (viz., BMZ 55, BMZ 53, BMZ 4) showed significant negative GCA effect for both days to 50% tasseling and silking, indicating good general combiners for earliness. BMZ 15, BMZ 55, BMZ 53 and BMZ 68 showed significant negative GCA effects for both plant and ear height. BIL 79, Pinacle 17 and BIL 182 exhibited desirable significant positive GCA for grain yield. Considering desirable GCA effects those parents could be used extensively in hybrid breeding program to accumulate those favorable genes. However, two cross combinations BIL 182 x CML 429 and BIL 79 x CML 429 were found promising considering SCA effect, mean performance and could be utilized for enhancing hybrid production. Considering BHM 9 as check, the percent standard heterosis for grain yield varied from -52.6 to 0.6%. None of the crosses showed significant positive heterosis for grain yield except BIL 79 × CML 429.

Keywords: General Combining Ability (GCA), Specific Combining Ability (SCA), Heterosis, maize.

Introduction

Maize (*Zea mays* L.) is a versatile crop with wider genetic variability and able to grow successfully throughout the world covering tropical, subtropical and temperate agro-climatic conditions. Maize acreage and production have an increasing tendency with the introduction of hybrids due to its high yield potential. Efforts are, therefore, required to be made to develop hybrids with high yield potential to increase production of maize. Most efficient use of such materials would be possible only when adequate information on the amount and type of genetic variation and combining ability effects in the materials is available. Heterosis and combining ability is prerequisite for developing a good

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economically viable hybrid maize variety. Combining ability analysis is useful to assess the potential inbred lines and helps in identifying the nature of gene action involved in various quantitative characters. Combining ability is dissected into two parts general combining ability (GCA) and specific combining ability (SCA). Both GCA and SCA variances have been determined and related to the possible types of gene action involved. GCA is a good estimate of additive gene action, whereas SCA is a measure of non-additive gene action (Sharief *et al.*, 2009). This information is helpful to plant breeders for formulating hybrid breeding programmes. A wide array of biometrical tools is available to breeders for characterizing genetic control of economically important traits as a guide to decide upon an appropriate breeding methodology to involve in hybrid breeding. Line × tester mating design developed by Kempthorne (1957), which provides reliable information on the general and specific combining ability effects of parents and their hybrid combinations was used to generate the information. The design has been widely used in maize by several workers like, Joshi et al. (2002) and Sharma *et al.,* (2004) and continues to be applied in quantitative genetic studies. The line×tester analysis provides information on GCA of parents and specific combining ability (SCA) of hybrids which helps to identify good quality inbreds and hybrids, respectively (Silva *et al.,* 2010; Moterle *et al.,* 2011). The present investigation was carried out to determine the nature and magnitude of gene action for yield and other important traits in maize.

Materials and Methods

The experiment was conducted during rabi season, 2018-2019 at the experimental field of Plant breeding division of Bangladesh Agricultural Research Institute (BARI), Gazipur. The institute is located at 23°59' N latitude and 90°25' E longitude. The climate of the area is characterized as tropical with mean monthly maximum and minimum temperature of 28.9°C and 18.8°C, respectively. The soil of the experimental field of BARI, Gazipur is characterized by sandy loam with 62.72% sand, 21.95% silt and 15.33% clay. The soil of the field is slightly acidic to neutral and thus pH varied from 6.1 to 6.9. The organic matter content of the soil is also low which was only1.34% and available phosphorous (P) content is 14.60 ppm. A total of forty-seven (47) entries including 44 test crosses produced by crossing twenty-two elite inbred lines with two testers (CML 429 and CML 425) and three standard checks (BARI Hybrid Maize 9, 981, Elite) were used in this experiment. The lines were obtained from PBD, BARI, but some are originally introduced from CIMMYT breeding program.

The experiment was laid out in alpha lattice design with two replications having plot consisted of two rows of 4-meter lengths with row-to-row distance of 60 cm and plant to plant of 25 cm. Two seeds were planted per hill on 26th of November 2018 and later thinned out to one plant per hill after seedlings were

well established. Fertilizers were applied @ 250, 55, 110, 40, 5 and 1.5 kg/ha of N, P, K, S, Zn and B respectively. Other standard agronomic practices like weeding and pest management have been done manually throughout the entire growing season as required. Data were collected on days to tasseling (DT): number of days from planting to 50% of the plants in a plot shed pollen, days to silking (DS): Number of days from planting to 50% of the plants in a plot produced 2-3 cm long silk, plant height (PH): the average height of five randomly selected plants measured in cm from base of the plant to the first tassel branch, ear height (EH): the average height of five randomly selected plants measured in cm from base of the plant to the node bearing the upper most ear of the same plants used to measure plant height, anthesis silking interval(ASI): number of days interval between days to anthesis or tasseling (DT) and days to silking (DS),grain yield (GY): total grain yield in kg per plot and adjusted to 12.5% moisture level and converted to t/ha.

The data were analyzed for combining ability as per procedure given by Kempthorne (1957). The mean performances of all characters were analyzed using Crop Stat software. Data were analyzed for variance for all the characters studied. Using the mean data of all the single cross hybrid and check variety, the standard heterosis (against the BHM 9; standard check hybrid variety) was estimated and tested. Percent heterosis was calculated by using the following formula:

Standard heterosis (%) = $[(\bar{F}_1 - \bar{C}V)/\bar{C}V] \times 100$

Where, $\bar{F}_1$ and $\bar{C}V$ represent the mean performance of hybrid and standard check variety respectively. The significance test for heterosis was done by using standard error of the value of check variety.

Results and Discussion

The analysis of variance for different characters is presented in Table 1 which indicated that there were highly significant differences among the genotypes for all the characters. ANOVA partitioned the variance into cross/hybrid variance, line variance, tester variance and line × tester variance. All the variance revealed that there were significant differences in all the characters. Similarly, parent and parent's vs crosses were significant for all the characters except ASI indicating greater diversity in the parental lines of the traits. The present observations are in agreement with the earlier report Ali *et.al*. (2012). A comparison of the magnitude of variance components due to GCA and SCA confirms the gene action in controlling the expression of traits. The ratio of GCA and SCA variance for all the traits were less than one, which indicates that all these characters were predominantly governed by non-additive gene effects (Table 1). Similar findings were reported by Kanagarasu *et al.* (2010) and Kumar *et al.* (2014) for grain

yield, cob length, plant height, ear height, 100 grain weight, grain rows per cob, days to 50 per cent tassel and days to 50 per cent silk and Ali *et al.* (2012) for number of grain rows per cob and 100-grain weight in maize in their study.

Table 1. Mean squares and estimates of variance for grain yield and yield components in maize evaluated at Gazipur during *rabi* 2018-19

Sources	df	DT (days)	DS (days)	PH (cm)	EH (cm)	ASI (days)	Y (t/ha)
Genotypes	67	116.51**	112.54**	2752.89**	787.93**	2.88**	17.58**
Parents	23	37.08**	38.04**	1379.12**	488.52**	1.03	0.60
P vs C	1	5897.97**	5749.76**	133049.84**	31238.30**	0.94	941.32**
Crosses	43	24.55**	21.29**	457.54**	239.94**	3.91**	5.18**
Lines	21	36.75**	34.85**	791.29**	395.55**	4.68**	7.34**
Testers	1	145.10**	39.56**	125.28	166.38**	33.14**	31.66**
Lines x Testers	21	6.60	6.87	139.62	87.83	1.76	1.76*
Error	67	7.85	9.17	95.61	74.21	1.22	0.90
Estimate of component of variance							
σ^2 g (line)		7.54	7.00	162.92	76.93	0.73	1.39
σ^2 g (tester)		3.15	0.74	-0.32	1.79	0.71	0.68
σ^2gca		0.27	0.22	4.85	2.32	0.03	0.05
σ^2sca		-0.62	-1.15	22.00	6.81	0.27	0.43
σ^2gca/ σ^2sca		-0.43	-0.19	0.22	0.34	0.11	0.11

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

DT=days to 50% tasseling, DS=days to 50% silking, PH=plant height, EH=ear height, ASI= anthesis silk interval, Y= yield.

The proportional contributions of lines (female), testers (male) and their interactions (crosses) to total variance for different traits revealed that female lines (maternal) contributed much higher compared to male lines (paternal) in all studied traits (Table 2). Results showed that maternal parents play the most important role for those traits. Similar conclusion was reported by Amiruzzaman (2010) who observed the greater effect of female lines for grain yield and other traits.

Table 2. Proportional contribution of lines, testers and their interactions to total variance in maize

Sources	DT (days)	DS (days)	PH (cm)	EH (cm)	ASI (days)	Y (t/ha)
Line (L)	73.12	79.93	84.46	80.51	58.40	69.18
Tester (T)	13.75	4.32	0.64	1.61	19.69	14.22
Line x Tester	13.14	15.75	14.90	17.88	21.91	16.60

DT=Days to Tassel, DS=Days to Silk, PH=Plant Height, EH= Ear Height, ASI= anthesis silk interval

General combining ability (GCA) effects

The general combining ability (GCA) effects of lines (females) and testers (males) are presented in Table 3. Among the parents, three lines (viz., BMZ 55, BMZ 53, BMZ 4) showed significant and negative GCA effect for both days to 50% tasseling and silking, indicating good general combiners for earliness. Bhavana *et al*. (2011) and Jawaharlal *et al*. (2012) also reported the additive gene action for days to 50 per cent tassel and silk. Lines BMZ 15, BMZ 55, BMZ 53 and BMZ 68 showed significant and negative GCA effects for both plant and ear height. The lines (BMZ 55, BMZ 53) also recorded negative GCA effect for days to tasseling, and silking indicated that these parents were suitable for earliness and/or short stature breeding. Similar observations in maize were reported by Motamedi *et al.* (2014) and Premlatha and Kalamani (2010). Three parental lines (BIL 79, Pinacle 17 and BIL 182) exhibited desirable significant positive GCA for grain yield. These lines could be desirable parents for hybrids as well as for inclusion in breeding program, since they may contribute favourable alleles in the synthesis of new varieties. The parents exhibited significant and positive GCA for yield, were good general combiner and those could be used for exploiting more positive alleles for yield (Table 3). Significant GCA effect for yield in maize was also reported by Amin *et al.* (2014), Kumar *et al.* (2014), Ivy and Hawlader (2000) and Amiruzzaman (2010). As GCA is generally associated with additive gene action in inheritance of characters, the lines with high GCA may be utilized in hybridization program to improve a particular trait through transgressive segregation.

Specific combining ability (SCA) effects

The Specific combining ability effects are presented in the Table 4. In respect of days to tassel and days to silk, no cross combination recorded significant and negative SCA effects. In case of maize, significant and negative value is expected for plant and ear height to develop short stature plant. The lowest days for 50% tasseling and silking was found in the cross BMZ 53 × CML 425. Lowest plant height and ear height was observed in cross BMZ15 × CML 425. Positive SCA effect is expected for yield and yield components. In case of grain yield, only one cross (CML 451 × CML 429) exhibited significant positive SCA effects. Among the cross combination highest yield (13.3 t/ha) was produced by BIL 79 × CML 429 followed by BIL 182 × CML 429 (12.6 t/ha).

Table 3. General combining ability (GCA) effects and mean of parents for grain yield and yield components and other characters in maize

Parents	DT (days)		DS (days)		PH (cm)		EH (cm)		ASI (days)		Y (t/ha)	
	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean
Testers												
1.CML 429	1.28**	94	0.67	99	1.19	83	-1.38	42	-0.61**	5	0.60**	4.0
2.CML 425	-1.28**	83	-0.67	87	-1.19	99	1.38	55	0.61**	4	-0.60**	4.6
SE (gi)	0.42		0.46		1.47		1.30		0.17		0.14	
SE (gi-gj)	0.60		0.65		2.08		1.84		0.24		0.20	
Lines												
1. Pinacle 20	-1.011	98	-1.85	104	8.22	106	0.81	44	-0.84	6	0.19	4.6
2. BMZ 15	-5.261**	98	4.60**	103	-22.28**	79	-14.19**	27	0.66	5	-1.93**	3.5
3. BIL 79	3.239*	99	1.90	104	17.72**	124	9.06*	66	-1.34*	6	2.92**	4.8
4. Pinacle 17	1.989	101	3.15*	104	-6.78	89	-6.19	40	1.16*	4	2.59**	3.6
5. BMZ 55	-4.761**	95	-3.35*	100	-21.28**	57	-15.19**	28	1.41*	5	-1.11*	3.6
6. Pinacle 10	4.239**	101	3.40*	105	-1.53	81	1.06	36	-0.84	4	0.85	3.8
7. Pinacle 12	-0.761	100	0.15	104	16.97**	95	9.06*	45	0.91	4	0.46	4.0
8. BMZ 68	-3.761**	98	-2.85	101	-17.78**	80	-9.69*	33	0.91	4	-0.01	3.4
9. CML 481	4.239**	100	5.40**	104	6.97	69	11.81**	29	1.16*	4	-1.07*	3.5
10. CML 451	1.989	97	3.90*	101	-1.03	122	-4.94	73	1.91**	4	-0.08	4.7

Table 3. Continued

Parents	DT (days)		DS (days)		PH (cm)		EH (cm)		ASI (days)		Y (t/ha)	
	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean
11. BMZ 25	1.739	100	1.15	105	-9.28	48	0.56	19	-0.59	5	-0.07	3.7
12 BMZ 56	-1.011	97	0.15	103	-2.78	88	-3.44	35	1.16*	6	-1.96**	3.5
13. BMZ 53	-4.261**	96	-4.35**	99	-16.03**	58	-16.94**	22	-0.09	4	-0.63	3.6
14. BMZ 4	-3.761**	94	-4.35**	99	-14.28**	48	-7.69	22	-0.59	5	-1.22**	3.6
15. 900M 1	-0.011	103	-0.85	109	-7.03	120	-0.69	52	-0.84	6	-0.40	4.9
16. 900M 4	3.489*	103	3.15*	107	24.72**	92	15.81**	30	-0.34	4	0.67	4.0
17. CML 496	3.989**	105	2.65	109	22.72**	94	21.06**	33	-1.34*	5	0.64	4.0
18. BIL 182	-1.011	99	-2.10	102	-4.03	76	-1.44	37	-1.09	4	2.38**	3.7
19. Pinacle 3	-1.511	101	-2.35	105	8.47	94	-4.19	32	-0.84	4	-0.80	4.1
20. CML 487	-0.511	97	-0.10	102	-5.03	83	0.81	26	0.41	5	-1.13*	3.8
21. 900M 10	0.739	101	1.65	106	10.72*	99	6.06	45	0.91	5	-1.10*	4.2
22. E 34	1.989	100	0.15	104	12.72*	165	8.56	77	-1.84**	4	0.78	5.5
SE (gi)	1.40		1.51		4.89		4.31		0.55		0.47	
SE (gi-gj)	1.98		2.14		6.91		6.09		0.78		0.67	

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

DT=Days to Tassel, DS=Days to Silk, PH=Plant Height, EH= Ear Height, ASI= Anthesis silking interval, Y=Yield.

Table 4. Specific combining ability (SCA) effects and mean of the crosses for grain yield and yield contributing characters in maize

Crosses	DT (days)		DS (days)		PH (cm)		EH (cm)		ASI (days)		Y (t/ha)	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
1.Pinacle 20 × CML 429	-1.53	83	-1.17	87	-8.69	156	-2.875	68	0.36	4	-1.04	9.3
2.Pinacle 20 × CML 425	1.53	84	1.17	88	8.69	171	2.875	76	-0.36	4	1.04	10.1
3.BMZ 15 × CML 429	0.22	81	0.58	86	4.81	139	3.125	59	0.36	5	-0.78	7.4
4.BMZ 15 × CML 425	-0.22	78	-0.58	83	-4.81	127	-3.125	55	-0.36	6	0.78	7.8
5.BIL 79 × CML 429	-0.28	89	0.08	92	0.31	174	-1.625	77	0.36	3	0.24	13.3
6.BIL 79 × CML 425	0.28	87	-0.08	90	-0.31	171	1.625	83	-0.36	4	-0.24	11.6
7.Pinacle 17 × CML 429	0.47	88	1.33	94	-3.19	146	2.125	66	0.86	6	-0.82	11.9
8.Pinacle 17 × CML 425	-0.47	85	-1.33	90	3.19	150	-2.125	64	-0.86	6	0.82	12.3
9.BMZ 55 × CML 429	-0.28	81	-0.17	86	7.81	143	6.625	61	0.11	6	-0.25	8.7
10.BMZ 55 × CML 425	0.28	79	0.17	85	-7.81	125	-6.625	51	-0.11	7	0.25	8.0
11.Pinacle 10 × CML 429	1.72	92	1.58	95	-10.44	144	-10.125	61	-0.14	3	-1.12	9.8
12.Pinacle 10 × CML 425	-1.72	86	-1.58	90	10.44	163	10.125	84	0.14	5	1.12	10.9
13.Pinacle 12 × CML 429	1.22	86	1.83	92	-4.44	169	-5.625	73	0.61	6	-0.93	9.6
14.Pinacle 12 × CML 425	-1.22	81	-1.83	87	4.44	175	5.625	87	-0.61	6	0.93	10.3
15.BMZ 68 × CML 429	2.72	85	1.83	89	-3.69	135	-6.375	54	-0.89	4	-0.42	9.7
16.BMZ 68 × CML 425	-2.72	77	-1.83	84	3.69	140	6.375	69	0.89	7	0.42	9.3
17.CML 481 × CML 429	0.72	91	-0.92	94	-6.44	157	3.625	85	-1.64*	4	0.34	9.4
18.CML 481 × CML 425	-0.72	87	0.92	95	6.44	167	-3.625	81	1.64*	8	-0.34	7.5
19.CML 451 × CML 429	-0.53	87	-0.42	93	6.06	161	5.875	71	0.11	6	1.29*	11.3
20.CML 451 × CML 425	0.53	86	0.42	93	-6.06	147	-5.875	62	-0.11	7	-1.29*	7.5
21.BMZ 25 × CML 429	-1.28	86	-1.17	90	3.81	151	-6.125	64	0.11	4	-0.09	10.0
22.BMZ 25 × CML 425	1.28	86	1.17	91	-3.81	141	6.125	79	-0.11	5	0.09	8.9

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

DT=Days to Tassel, DS=Days to Silk, PH=Plant Height, EH= Ear Height, ASI= Anthesis silking interval, Y=Yield.

Table 4. Continued

Crosses	DT (days)		DS (days)		PH (cm)		EH (cm)		ASI (days)		Y (t/ha)	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
23. BMZ 56 x CML 429	-2.03	83	-3.67	86	14.31*	168	6.375	73	-1.64*	4	0.67	8.8
24. BMZ 56 x CML 425	2.03	84	3.67	92	-14.31*	137	-6.375	63	1.64*	8	-0.67	6.3
25. BMZ 53 x CML 429	1.72	83	1.33	87	2.06	142	2.875	56	-0.39	4	0.25	9.7
26. BMZ 53 x CML 425	-1.72	77	-1.33	83	-2.06	136	-2.875	53	0.39	6	-0.25	8.0
27. BMZ 4 x CML 429	-0.28	82	-0.17	85	2.81	145	1.625	64	0.11	4	0.79	9.7
28. BMZ 4 x CML 425	0.28	80	0.17	84	-2.81	137	-1.625	63	-0.11	5	-0.79	6.9
29. 900M 1 x CML 429	-1.53	84	-1.17	88	-4.44	145	-3.875	65	0.36	4	0.31	10.0
30. 900M 1 x CML 425	1.53	85	1.17	89	4.44	151	3.875	76	-0.36	4	-0.31	8.2
31. 900M 4 x CML 429	0.47	90	0.83	94	-3.69	177	-3.875	82	0.36	4	1.08	11.9
32. 900M 4 x CML 425	-0.47	86	-0.83	91	3.69	182	3.875	92	-0.36	5	-1.08	8.5
33. CML 496 x CML 429	-0.53	89	-0.17	92	-0.19	179	-3.125	88	0.36	3	0.17	10.9
34. CML 496 x CML 425	0.53	88	0.17	91	0.19	177	3.125	97	-0.36	4	-0.17	9.4
35. BIL 182 x CML 429	0.47	85	0.58	88	5.56	158	2.375	71	0.11	3	0.06	12.6
36. BIL 182 x CML 425	-0.47	82	-0.58	86	-5.56	144	-2.375	69	-0.11	4	-0.06	11.2
37. Pinacle 3 x CML 429	-1.03	83	-0.67	87	-5.44	159	1.625	67	0.36	4	0.45	9.8

Table 4. Continued

Crosses	DT (days)		DS (days)		PH (cm)		EH (cm)		ASI (days)		Y (t/ha)	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
38. Pinacle 3 x CML 425	1.03	83	0.67	87	5.44	168	-1.625	67	-0.36	4	-0.45	7.7
39. CML 487 x CML 429	-2.03	83	-1.42	88	3.56	155	0.125	71	0.61	5	-0.19	8.8
40. CML 487 x CML 425	2.03	85	1.42	90	-3.56	145	-0.125	73	-0.61	5	0.19	8.0
41. 900M 10 x CML 429	0.22	87	0.33	92	1.31	168	5.375	81	0.11	5	-0.10	8.9
42. 900M 10 x CML 425	-0.22	84	-0.33	90	-1.31	163	-5.375	73	-0.11	6	0.10	7.9
43. E 34 x CML 429	1.47	89	0.83	91	-1.69	167	1.875	80	-0.64	2	0.11	11.0
44.E 34 x CML 425	-1.47	84	-0.83	88	1.69	168	-1.875	79	0.64	4	-0.11	9.6
45.BHM 9(check 1)		85		91		195		90		6		14.19
46.981(check 2)		87		95		197		92		8		12.76
47.Elite (check 3)		86		93		184		80		8		14.05
$SE_{(Sij)}$	1.98		2.14		6.91		6.09		0.78		0.61	
$SE_{(Sij-Skl)}$	2.80		3.03		9.78		8.61		1.11		0.95	

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

DT=Days to Tassel, DS=Days to Silk, PH=Plant Height, EH= Ear Height, ASI= Anthesis silking interval, Y=Yield.

Heterosis

The percent standard heterosis expressed by F_1 hybrids over the commercial hybrid check variety BHM9 for yield and different yield contributing characters are presented in Table 5. The degree of heterosis in F_1 hybrids varied from character to character or from cross to cross.

Table 5. Percent heterosis over the best check hybrid (BHM 9) for different characters of maize

Crosses	DT	DS	PH	EH	ASI	Y(t/ha)
1.Pinacle 20 × CML 429	-2.4	-4.9	-20.3**	-25.0*	-41.7*	-29.8**
2.Pinacle 20 × CML 425	-1.8	-3.8	-12.6*	-15.6	-33.3	-23.2**
3.BMZ 15 × CML 429	-5.3	-6.0	-29.0**	-35.0**	-16.7	-43.9**
4.BMZ 15 × CML 425	-8.8*	-8.8**	-35.1**	-38.9**	-8.3	-41.2**
5.BIL 79 × CML 429	4.1	0.5	-10.8*	-14.4	-50.0**	0.6
6.BIL 79 × CML 425	1.8	-1.1	-12.3*	-7.8	-41.7*	-12.1
7.Pinacle 17 × CML 429	3.5	3.3	-25.1**	-27.2**	0.0	-9.9
8.Pinacle 17 × CML 425	-0.6	-1.1	-23.1**	-28.9**	-8.3	-6.6
9.BMZ 55 × CML 429	-5.3	-5.5	-26.9**	-32.2**	-8.3	-33.7**
10.BMZ 55 × CML 425	-7.6*	-6.6	-36.2**	-43.9**	8.3	-39.0**
11.Pinacle 10 × CML 429	7.6*	3.8	-26.2**	-32.8**	-50.0**	-25.4**
12.Pinacle 10 × CML 425	0.6	-1.1	-16.7**	-7.2	-25.0	-17.6*
13.Pinacle 12 × CML 429	1.2	0.5	-13.6**	-18.9	-8.3	-27.0**
14.Pinacle 12 × CML 425	-4.7	-4.9	-10.3*	-3.3	-8.3	-21.9**
15.BMZ 68 × CML 429	-0.6	-2.7	-31.0**	-40.6**	-33.3	-26.6**
16.BMZ 68 × CML 425	-10.0**	-8.2*	-28.5**	-23.3*	16.7	-29.4**
17.CML 481 × CML 429	6.5	3.3	-19.7**	-5.6	-41.7*	-29.0**
18.CML 481 × CML 425	1.8	3.8	-14.4**	-10.6	33.3	-43.1**
19.CML 451 × CML 429	2.4	2.2	-17.4**	-21.7*	0.0	-14.3
20.CML 451 × CML 425	0.6	1.6	-24.9**	-31.78**	16.7	-42.9**
21.BMZ 25 × CML 429	1.2	-1.6	-22.8**	-28.9**	-41.7*	-24.6**
22.BMZ 25 × CML 425	1.2	-0.5	-27.9**	-12.2	-25.0	-32.3**
23. BMZ 56 x CML 429	-2.9	-5.5	-14.1**	-19.4*	-41.7*	-33.1**
24. BMZ 56 x CML 425	-1.2	1.1	-30.0**	-30.6**	33.3	-52.4**
25. BMZ 53 x CML 429	-2.4	-4.9	-27.2**	-38.3**	-41.7*	-26.2**

Crosses	DT	DS	PH	EH	ASI	Y(t/ha)
26. BMZ 53 x CML 425	-9.4**	-9.3**	-30.5**	-41.7**	-8.3	-39.2**
27. BMZ 4 x CML 429	-4.1	-6.6	-25.9**	-29.4**	-41.7*	-26.6**
28. BMZ 4 x CML 425	-6.5	-7.7*	-30.0**	-30.0**	-25.0	-47.7**
29. 900M 1 x CML 429	-1.2	-3.8	-25.9**	-27.8**	-41.7*	-24.1**
30. 900M 1 x CML 425	-0.6	-2.7	-22.6**	-16.1	-33.3	-37.9**
31. 900M 4 x CML 429	5.3	2.7	-9.2	-9.4	-33.3	-10.1
32. 900M 4 x CML 425	1.2	-0.5	-6.7	2.2	-25.0	-35.6**
33. CML 496 x CML 429	4.7	1.1	-8.5	-2.8	-50.0**	-17.2*
34. CML 496 x CML 425	2.9	0.0	-9.5	7.2	-41.7*	-28.9**
35. BIL 182 x CML 429	0.0	-3.3	-19.2**	-21.7*	-50.0**	-4.9
36. BIL 182 x CML 425	-4.1	-6.0	-26.2**	-23.9*	-33.3	-14.9*
37. Pinacle 3 x CML 429	-2.4	-4.9	-18.5**	-25.6**	-41.7*	-26.1**
38. Pinacle 3 x CML 425	-2.9	-4.9	-14.1**	-26.1**	-33.3	-42.0**
39. CML 487 x CML 429	-2.4	-3.3	-20.8**	-21.7*	-16.7	-33.4**
40. CML 487 x CML 425	-0.6	-1.6	-25.6**	-18.9	-16.7	-39.5**
41. 900M 10 x CML 429	1.8	0.5	-13.8**	-10.0	-16.7	-32.5**
42. 900M 10 x CML 425	-1.8	-1.6	-16.4**	-18.9	0.0	-40.0**
43. E 34 x CML 429	4.7	-0.5	-14.4**	-11.1	-75.0**	-16.6
44.E 34 x CML 425	-1.8	-3.8	-13.8**	-12.2	-33.3	-27.4**

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

DT=Days to Tassel, DS=Days to Silk, PH=Plant Height, EH= Ear Height, ASI= Anthesis silking interval, Y=Yield

Days to pollen shedding and silking determine the maturity of the hybrid. For heterosis Days to tasseling and silking ranged from -10.0 to 7.6 % and -9.3 to 3.8% respectively. Negative heterosis is desirable for these two characters. Considering commercial hybrid BHM9 as a check four crosses BMZ 15 × CML 425, BMZ 55 × CML 425, BMZ 68 × CML 425, BMZ 53 x CML 425 showed significantl and negative heterosis for days to pollen shedding. Maximum negative heterosis was observed in the cross BMZ 68 × CML 425 for this trait. For days to silking three crosses BMZ 15 × CML 425, BMZ 68 × CML 425, BMZ 53 x CML 425 exhibited significantly and negative heterosis and highest negative heterosis was observed in the cross of BMZ 53 x CML 425.

Negative heterosis is desirable for plant height and ear height which helps for developing short statured plant leading tolerant to lodging. Heterosis for different

crosses ranged from -36.2 to -6.7 % and -43.9 to 2.2%, respectively, for plant and ear height. Significant and negative heterosis for both these traits were reported by Uddin et al. (2006), Alam et al. (2008) and Amiruzzaman (2010).

In case of grain yield, the percent of standard heterosis varied from -52.6% to 0.6%. Most of the crosses showed significant and negative heterosis except BIL 79 × CML 429 which showed positive value.

Conclusion

Good general combining ability effects for yield and important yield contributing characters were noticed in the lines *viz.* BMZ 55, BMZ 53, BMZ 4 (earliness), BMZ 68, BMZ 15, BMZ 53, BMZ 55 (dwarf character) and, BIL 79, BIL 182 and Pinacle 17 (higher yield). These parents could result in the production of superior single crosses. Four crosses BMZ 15 × CML 425, BMZ 55 × CML 425, BMZ 68 × CML 425, BMZ 53 x CML 425 showed significant and negative heterosis for days to pollen shedding. For days to silking three crosses BMZ 15 × CML 425, BMZ 68 × CML 425, BMZ 53 x CML 425 exhibited significant and negative heterosis. In case of grain yield most of the crosses showed significant and negative heterosis except BIL 79 × CML 429 which showed positive value (0.6%), almost similar to the check variety. Hybrid BIL 182 x CML 429 and BIL 79 x CML 429 could be advanced for commercial hybrid development after verifying the performances over locations.

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SIX GENERA OF PLANT-PARASITIC NEMATODES FROM BANGLADESH

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Abstract

The presence of plant-parasitic nematodes causing crop loss every year had been overlooked in the existing fruits, vegetables and flowers cultivation system in Bangladesh. Therefore, the study was conducted to isolate and identify plant-parasitic nematodes from soil and infected plant-parts of fruits (banana, orange), vegetables (pointed gourd, tomato and brinjal) and flower (gerbera) from the districts of Jashore, Jhenaidah, Gazipur and Sylhet during 2017-18 and 2018-19 cropping seasons. A total of 68 samples of which 20 fruit samples, 45 vegetables samples and 3 flower samples were collected and observed for nematode infestation. Baermann funnel method was used to extract the active nematodes from plant parts and Cobb's method was used for the isolation of nematodes from soil samples. Different morphological structures of the nematodes viz. esophagus, median bulb, basal bulb, intestine, reproductive structures (vulba, bursa), etc. were used to identify the nematodes. To identify *Meloidogyne* spp. from root knot of tomato, brinjal and gerbera, juvenile larvae J_2 and perineal pattern of female nematodes were used. A total of six genera of nematodes were identified and all of them were under the order Tylenchida. The nematode genera were *Tylenchorhynchus* sp., *Tylenchus* sp., *Hoplolaimus* sp., *Helicotylenchus* sp., *Pratylenchus* sp. and *Meloidogyne* spp. The root-knot nematode, *Meloidogyne incognita* was recorded for the first time in flower crop gerbera in Bangladesh.

Keywords: Plant-parasitic nematode, infestation, isolation, identification, Baermann funnel, Cobb's method, morphology.

Introduction

Plant parasitic nematodes are most neglected problem of agricultural productivity in Bangladesh. They infect flowers, fruits, vegetables and forest trees. It has been reported that the economic loss for important crops (vegetables, fruits and edible field crops) are 14% for a total of over $80 billion annually (Agrios, 2004). About fifteen thousand species of nematodes have been reported around the world. Among them 2200 species are identified as plant parasitic (Goodey *et al*., 1965). Globally plant-parasitic nematodes considered as one of the major pests of vegetable crops. Damage caused by nematodes could reach up to 30% of total production of tomato, eggplant and melon (Janati *et al*., 2018).

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In Bangladesh, several studies were conducted on the presence of plant parasitic nematodes in potato, banana, fruits and vegetables (Timm and Ameen, 1960; Mian, 1986 and Mian, 1987). In those studies, several plant parasitic nematodes were recorded and they were *Aphelenchoides, Aphelenchus, Belonolaimus, Criconema, Criconemoides, Ditylenchus, Helicotylenchus, Hemicyclophora, Hirschmanniella, Hoplolaimus, Longidorus, Meloidogyne, Paratylenchus, Pratylenchus, Radopholus, Rotylenchulus, Rotylenchus, Scutellonema, Trichodorus, Tylenchorhynchus, Tylenchulus, Tylenchus* and *Xiphinema*. Among them *Aphelenchoides* sp., *Ditylenchus destructor*, *Helicotylenchus* sp., *Meloidogyne* sp., *M. incognita*, *M. javanica*, *Neotylenchus* sp., *Pratylenchus coffea*, *P. penetrans*, *Rotylenchulus* sp., *Rotylenchus* sp., *Tylenchorhynchus claytoni* and *Xiphinema* sp. were found with rhizosphere soils of potato. Other four types of nematodes such as burrowing (*Radopholus*), spiral (*Helicotylenchus* sp.), root knot (*Meloidogyne* spp.) and lesion (*Pratylenchus*) were found in the fields of banana. In another study, some important genera of nematode were identified in banana cultivation at Joydebpur (Choudhury *et al*., 1981). They recorded *Helicotylenchus* sp., *Hoplolaimus*, *Hirschmanniella*, *Tylenchorhynchus claytoni* and *Meloidogyne* spp. associated with the root damage of banana plantations. However, no nematode infestations have been recorded yet in flower cultivation system in Bangladesh.

Considering the above facts, this study was conducted from 2017-2019 for collection and identification of plant-parasitic nematodes from commercially grown fruits (banana, orange), vegetables (pointed gourd, tomato and brinjal) and flower (gerbera) samples collected from Jashore, Jhenaidah, Gazipur and Sylhet districts of Bangladesh.

Materials and Methods

I) Samples collection

A total of 68 samples (symptomatic root and soil from rhizosphere) were collected in 2017-2019 to identify the associated plant-parasitic nematodes. Among 68 samples, 20 from fruits, 45 from vegetables and 3 from gerbera were investigated. All the fruits and vegetables samples were collected from Jashore, Jhenaidah, Gazipur and Sylhet districts (Table 1 and figure 1). Samples of gerbera were collected from floriculture section of Horticultural Research Centre (HRC), BARI, Gazipur. For each type of crop, the rhizosphere soil was taken from the suspected root infection to make a composite sample following zig-zag pattern. Two kg soil was collected from each composite sample and kept in polythene bag for identification of nematodes.

Table 1. Particulars of soil and plant samples collected for identification of plant-parasitic nematodes in different crops grown in four districts of Bangladesh

Host plant/ soil sample	Production system	Location	Geographic location
Banana rhizospehere soil	Orchard	Gazipur, Sylhet	23° 59' 59.7876" N and 90° 25' 12.9828" E
Orange rhizospehere soil	Orchard	Sylhet	24° 53' 11.1696" N and 91° 52' 50.5992" E
Pointed gourd infected roots	Field	Jashore	23° 10' 14.3904" N and 89° 12' 44.7048" E
Tomato root gall and rhizosphere soil	Field	Jashore	23° 10' 14.3904" N and 89° 12' 44.7048" E
Brinjal root gall and rhizosphere soil	Field	Jhenaidah	23° 38' 1.8276" N and 89° 4' 0.6816" E
Gerbera root gall	Experimental field	Horticultural Research Centre, Gazipur	23° 59' 20.4504" N and 90° 25' 5.4012" E

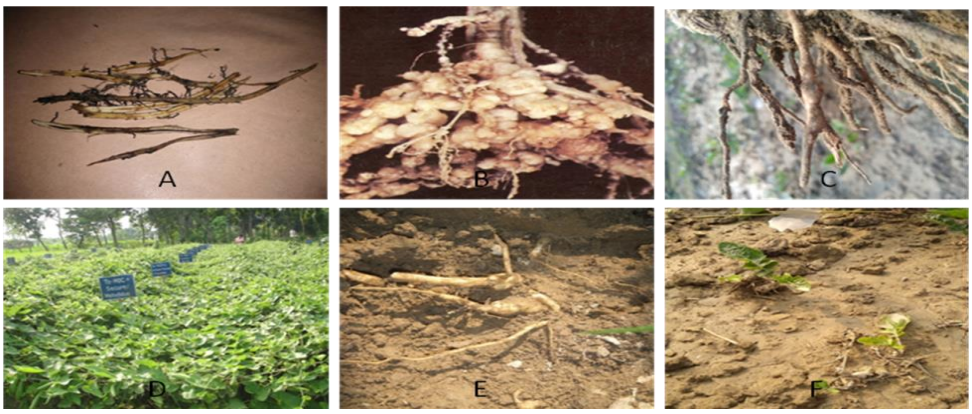

Fig. 1. Plant-parasitic nematodes infected plant samples. A) Nematode infected banana sample B) Root-knot nematode infected tomato sample C) Root-knot nematode infected gerbera sample D) Pointed gourd field E) Root-knot infected pointed gourd sample F) Root-knot nematode infected gerbera field.

II) Nematode extraction

a) Infected roots: The Baermann funnel method was used for the extraction of active nematodes from infected plant samples. The collected plant samples were cut into pieces of about 1 cm size. Root sub-samples (10 g) were wrapped with cheesecloth and formed a ball shaped structure. A clean funnel was placed in a

stand filling with water until it reaches up to 1 cm below the rim. Bubble formation inside the tubes was avoided and the clips were closed well. Samples were submerged in water of funnel for 48 hours. Active nematodes were moved to the end of the rubber tube leaving the debris behind. Syracuse dish was used to collect the nematodes from the rubber tube for identification under stereo and compound microscope.

b) Rhizosphere soil: Cobb's decanting and sieving method was used for the isolation of nematodes from soil samples (Van, 2006). The sample (maximum 100 g) was stirred in a water-filled beaker. After heavy particles had settled down, the nematode suspension was poured and sieved. Sieving was carried out with a series of sieves of decreasing mesh size i.e 500 µm, 350 µm 100 µm and 45 µm so that nematodes of different sizes could be collected separately.

III) Sample preparation for microscopic observation and identification

The following simple method was used for permanent slide preparation to identify the nematodes (Ryss, 2003).

a) Killing: A drop of 10 µl distilled water was placed at the bottom of one 0.5 ml tube. Alive nematodes were transferred in the tube with the needles. Another Eppendorf tube containing 0.5 ml 4% hot formalin was pour down in the water containing tube. The tube was closed and was shaken to prove the nematodes were not attached to the wall.

b) Fixation: After killing the nematodes, the tube was placed in water bath at 80°C for 30 min. After fixation, the nematodes were placed at room temperature**.**

c) Processing in glycerin: After reaching the tube in room temperature, the content was shaken and transferred to a glass container. The nematodes were picked out and transferred to a glass slides containing a drop of glycerin and distilled water in a proportion of 1:20. Then the slides were placed in a hotplate at 70°C for 15 min.

d) Slide preparation: The nematodes were transferred from hotplate to a drop of pure glycerin on a glass slide. A cover slip was placed over the glass slide. The border of glass slide was sealed with paraffin for preserving the slide to observe in future.

IV) Perineal pattern sample preparation for compound microscopic study

Galled roots of brinjal, tomato and gerbera flower were gently washed with tap water and were placed in 0.9% NaCl solution. Egg masses were collected using needle and placed in Eppendorf tubes under a stereomicroscope. Nematodes were dissected under compound microscope. The female nematodes were transferred

to a small drop of 45% lactic acid in plastic petri-dishes. The female body was pushed out in the solution, so that it could be held in the surface tension. A dissecting needle was used to remove the posterior part of female nematode and the cuticle was trimmed in a square pattern in the center. The body was transferred in a glycerol solution. This process was done for up to ten patterns for the same *Meloidogyne* species. Finally, the slide was covered with cover slip and sealed with nail polish (Sasser *et al*., 1983).

V) Nematode identification

After collecting the nematodes in both Baermann funnel and Cobb's extraction methods, they were observed under both stereo and compound microscopes. Most of the measurements were recorded by ocular micro meter at X40 objective. This morphological identification was done based on the book 'pictorial key to the genera of plant-parasitic nematodes' (Mai and Lyon, 1975). Identification of plant-parasitic nematodes was based on morphological characters of nematodes such as second stage juveniles J2, body length, perineal pattern, head and tail, excretory pore, dorsal esophageal gland, spicule, median bulb, basal bulb, intestine, reproductive structures (vulba, bursa).

Results and Discussion

Six species/genera of plant parasitic nematodes were identified from 68 samples collected from the infected roots and soil rhizosphere of banana, orange, pointed gourd, tomato, brinjal and gerbera samples from Gazipur, Sylhet, Jashore and Jhenaidah districts (Table 2). They were *Helicotylenchus* sp. (spiral nematode), *Hoplolaimus* sp. (lance nematode), *Pratylenchus* sp. (lesion nematode), *Tylenchus* sp., *Tylenchorhynchus* sp. (stunt nematode) and *Meloidogyne* spp. (root-knot nematode). *Pratylenchus* sp. was the key nematode in orange and banana orchard whereas for brinjal and tomato it was *Meloidogyne javanica* and *Hoplolaimus* sp. as supported by the previous findings of Bahadur (2021). *Helicotylenchus* sp., and *Tylenchorhynchus* sp. were present only on the rhizosphere soil of banana orchard. Presence of these two nematodes species on banana orchard confirms the findings of earlier researchers (Choudhury *et al*., 1981). The *Tylenchus* sp. was only present in pointed gourd field. In addition, *Meloidogyne incognita* was isolated only from the gerbera root samples. There was no previous record of plant parasitic nematode *M. incognita* in gerbera. The finding of the present study was supported by the research report of India (Meena *et al*., 2015). They reported the presence of *Meloidogyne incognita* in gerbera. This nematode caused prominent root galls in the infection site and reduced both quality and quantity of gerbera in the field.

Table 2. Particulars of plant-parasitic nematode genera identified from different crops of four districts of Bangladesh

District/ Location	Host plant	Production system	Identified genera from soil sample	Identified genera from plant root sample
Gazipur	Banana	Orchard	*Tylenchorhynchus* sp., *Pratylenchus* sp., *Helicotylenchus* sp.	-
Sylhet	Banana	Orchard	*Tylenchorhynchus* sp., *Pratylenchus* sp., *Helicotylenchus* sp.	-
Sylhet	Orange	Orchard	*Pratylenchus* sp.	-
Jashore	Pointed gourd	Crop field	-	*Tylenchus* sp.
Jashore	Tomato	Crop field	*Meloidogyne javanica, Hoplolaimus* sp.	*Meloidogyne javanica, Hoplolaimus* sp.
Jhenaidah	Brinjal	Crop field	*Meloidogyne javanica, Hoplolaimus* sp.	*Meloidogyne javanica, Hoplolaimus* sp.
Horticultural Research Centre, Gazipur	Gerbera	Research field	-	*Meloidogyne incognita*

Morphological features of nematodes:

The morphological characteristics of different nematodes isolated from different crop plants and rhizosphere soils were noted and used to identify them up to genera. The following six different nematodes genera were identified on the basis of their respective morphological characters observed under microscope.

1. *Helicotylenchus* sp.: Heat-killed nematode was C-shaped, stylet was well developed, 21-24 µm long, with 5-6 µm basal knob. Tail was slightly tapered, with anus was marked by a slight depression; terminus annulated and hemispherical in shape**.** Mail tail was similar to females, except for genital characters the spicules was present (Fig. 2).

2. *Hoplolaimus* sp. (Lance nematode): Spear knob was anchor shaped with distinct anterior projection. Tail was hemispherical and shorter than anal body diameter. The female had a short, and rounded tail (Fig. 3).

3. *Pratylenchus* sp. (Lesion nematode): Low and flattened head region was noticed with distinct head skeleton. The stylet was around 20 µm, and

moderately developed with distinct basal knobs. The esophagus had a well-developed median bulb. The female had posterior vulva and tail was tall, cylindrical to conoid. The male tail was conical with a distinct bursa that reached the tail tip (Fig. 4).

4. *Tylenchus* sp.: Female body was slender with hooked or curled tip of tail. Body was open or close C-shaped after fixation. Labial region was slightly narrower than the rest of body. Stylet was delicate, with small and rounded knobs. Tail was slightly acute to strongly curved ventrally with finely rounded to acute terminus (Fig. 5).

5. *Tylenchorhynchus* sp.: This nematode had a medium sized body. Stylet was around 20µm thin to slender, had strong knobs and cone with a long shaft. Esophageal glands were bound by a membrane into a large basal bulb, tail was round. Body was medium in size and the distance from anterior end of esophagus to median bulb less than distance from median bulb to intestine (Fig. 6).

6. Root-knot nematodes:

(a) Meloidogyne incognita-*Meloidogyne incognita* nematode was isolated from root samples of gerbera flower collected from HRC, Gazipur. The perineal region generally had an angularly oval structure with a high dorsal arch. Inverted-V shape was formed by striae in the dorsal to the tail. Striae were in distinct waves which bent towards the lateral lines. Striae were straighter with an oval appearance in ventral region (Jepson, 1987). Striae were straighter with an oval appearance in ventral region (Fig. 7. E).

From the collected soil and root samples, *Meloidogyne incognita* J2 juvenile were isolated and identified based on tail length and stylet length. The stylet of *M. incognita* was robust and distinct. The stylet length was 13.3 µm and tail length 57.6 µm.

(b) Meloidogyne javanica- *Meloidogyne javanica* root knot nematode was isolated from tomato and brinjal root samples. Distinct lateral fields formed by double incisures are typically in the perineal patterns of *Meloidogyne javanica* (Fig. 7. F). *M. javanica* had a general oval or oval to pyriform with a medium height and occasionally compressed dorsal arch in perineal regions. The tail length of second stage juvenile J2 was 55.2 µm and stylet length was 14.0 µm (Whitehead, 1968).

Fig. 2. A) C-shaped *Helicotylenchus* sp. B) Anetrior portion with stylet and basal knob. C) Spicule of male tail.

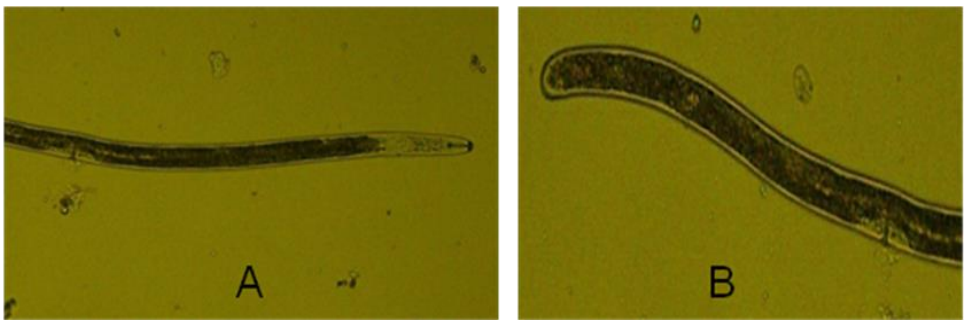

Fig. 3. A) Anterior portion of *Hoplolaimus* sp. B) Short rounded tail of female*Hoplolaimus* sp. nematode.

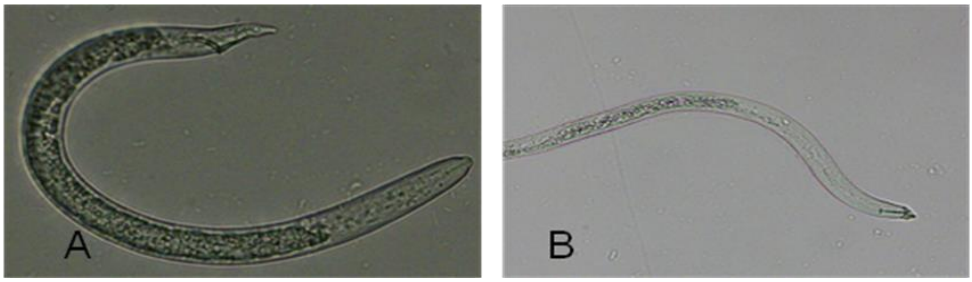

Fig. 4. A) Male *Pratylenchus* sp. with distinct bursa B) Anterior portion of female *Pratylenchus* sp.

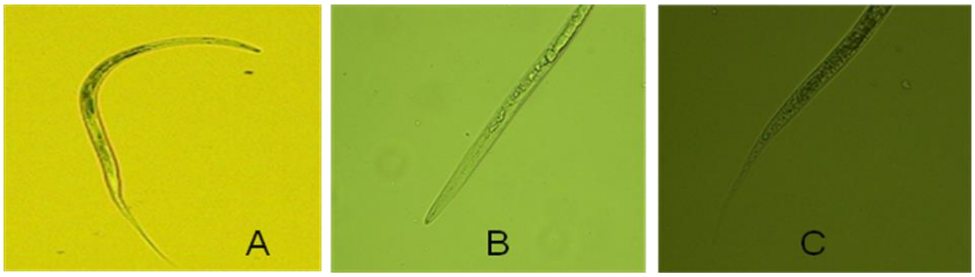

Fig. 5. A) Female *Tylenchus* sp. B) Slender female body with anterior portion C) Posterior portion.

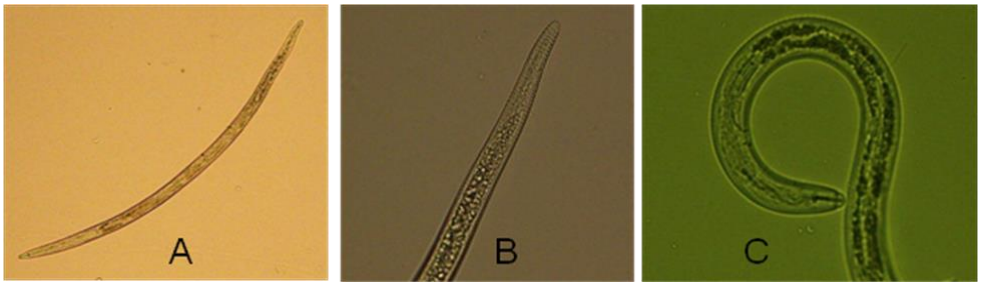

Fig. 6. A) Female *Tylenchorhynchus* sp. B) Round tail C) Anterior portion with strong basal knob.

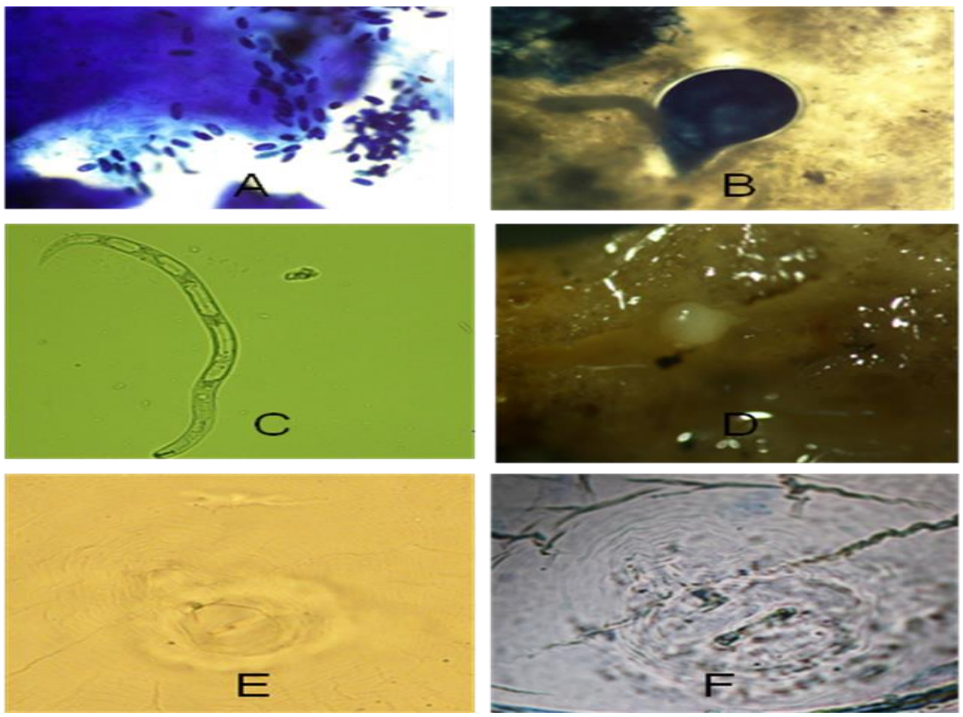

Fig. 7. A) Stained egg mass of root knot nematodes B) Immature female observed with lactophenol cotton blue C) II stage free juvenile (J2) D) Female mature *Meloidogyne javanica* nematode inside of a tomato root E) Perineal pattern of female *Meloidogyne incognita* F) Perineal pattern of female *Meloidogyne javanica.*

Conclusion

In conclusion, *Tylenchorhynchus* sp. and *Helicotylenchus* sp. of plant-parasitic nematodes were found only in banana plantations whereas, *Pratylenchus* sp. were found in both banana plantations and orange orchards. In contrast, *Tylenchus* sp. nematodes were recorded only in pointed gourd infceted root samples. *Hoplolaimus* sp. and *Meloidogyne javanica* were commonly found nematodes both in root galls and rhizosphere soil of tomato and brinjal. There were spherical galls in the roots of gerbera flower. *Meloidogyne incognita* was recorded for the first time in gerbera roots and this nematode might lower the production of gerbera flower that commericaly grown in some parts of Jashore, Bogra, Rangpur, Kushtia, Chuadanga and Jhenaidah districts of Bangladesh.

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MATING AND OVIPOSITION BEHAVIOUR OF BRINJAL SHOOT AND FRUIT BORER, *LEUCINODES ORBONALIS* GUENEE

M. A. MANNAN[1], K. S. ISLAM[2] AND M. JAHAN[3]

Abstract

Mating and oviposition behaviour of brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* Guenee (Lepidoptera:Pyralidae) were carried out in the laboratory on brinjal plants maintaining the temperature (20-25^{0}C) and relative humidity (80-85%). The moths were found active at night for mating and oviposition. Mating usually occurred at late night but a few moths were found to continue mating up to 8.0 hour of the day. Maximum moths completed mating between 4.00 to 6.00 hour of the day. Mating period ranged from 28-50 minutes. Mating occurred in first and second night of the adult emergence. Majority of the moths (90.80%) were found to mate in the first night and the rest went for mating in the following night. Oviposition always occurred at night. Females were always found to oviposit at night with an average 86.62% oviposition during the first half of the night. Again 60% egg deposition of first half of the night occurred during 20.00 to 22.00 hours. A female laid 241.50 eggs in an average of 2.70 days of oviposition. The egg laying pattern indicates that the egg laying continued for a maximum of 4 days showing a decreasing pattern of deposition with the increase of age. About 50% of the total eggs were deposited on the first day. The BSFB females preferred brinjal leaves for oviposition. Distribution of the eggs on the upper and lower surfaces of the leaves was found 1:2.74. Females showed higher preference for upper canopy of the brinjal plant as oviposition site.

Keywords: Brinjal, *Leucinodes orbonalis*, mating, fecundity.

Introduction

Brinjal (*Solanum melongena* L) is an economically important vegetable crop grown throughout Bangladesh. It is the leading vegetable in the country and ranks first among summer and winter vegetables in terms of total acreage. Brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* Guenee is the most destructive pest of brinjal and has become a serious production constraint in all brinjal growing countries (Alam *et al*. 2003). The BSFB is very active during the rainy and summer seasons and often causes more than 86% damage (Prodhan *et al*., 2018). The yield loss has been estimated to be about 86% (Ali et al. 1996) and more than 85% (Rashid *et al*. 2003) in Bangladesh. BSFB starts the damage from seedling stage but severe attack occurs soon after reaching the maximum vegetative stage and continues till the last harvest of the fruits and is very

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difficult to control since it feeds inside the shoots and fruits (Ghosh and Senapati 2009).

Farmers are applying toxic chemical pesticides indiscriminately to control the pest. Frequent use of systemic insecticides makes the vegetables poisonous, ecologically unsafe and economically unviable. It is easy to replace the poisonous chemicals to control the pest with the knowledge through understanding the nature and behaviour of insect. The behavioral activities of insect such as adult emergence, oviposition site, mating frequency & time and duration of neonate larvae development can help to apply IPM approach. Many researchers had few knowledge on mating, oviposition, preference of egg laying site, distribution of eggs at different plant canopies, fecundity and viability of eggs of BSFB. Appropriate knowledge on these parameters can contribute to develop an IPM strategy for the effective management of BSFB. It is also important to know the life cycle of an insect so that control mechanisms may be undertaken during the most susceptible stage of the life cycle. Therefore, a study was undertaken on the mating and oviposition behavior of BSFB.

Materials and Methods

Mating behaviour

To study the mating behaviour, twenty field collected pupae of BSFB were kept in Petri dish (14.0 cm x 1.5cm) and were under close observation for adult emergence. From the observation of its biology in the laboratory, Department of Entomology, Bangladesh Agricultural University, Mymensingh, it was found that BSFB adult started to emerge from the pupae within 10-12 days at 20-25^{0}C temperature with relative humidity of 78-86%. About 75-85% adults were emerged from pupae. Usually it occurred just after sunset. Therefore, the freshly emerged adults were observed for mating into two groups and it started from 8.0 PM. Grouping was made according to the time of adult emergence. The population which were emerged during the first half of the night were kept in one group and another group was made with the population emerged during the second half of the night. One group is kept under observation for the period of 8.0 PM to 8.0AM and another group is from 8.0 AM to 8.0 PM. To record the number of mating of the same group it was left for up to two days. Starting time and duration of mating of the above two groups were recorded through video camera. The mating frequency at different time was calculated.

Oviposition behaviour

Adult behavior and oviposition were recorded in the cylindrical jars (40 cm x 10cm). BSFB adult usually lays eggs on the brinjal leaves and twigs both of which are rough surfaced. In the laboratory, the fine meshed net was used to cover both open sides of the cylinder to encourage the females to lay eggs on it.

BSFB female adult was transferred into a both side open cylindrical glass jar containing a few pieces of green papers inside. The inner surface of the glass jar was wrapped with green papers to make the environment of the jar somewhat green like brinjal leaves. Sugar solution of 5% soaked in cotton was placed inside the cylinder to provide nourishment for the adults during the period of oviposition. Immediately after transferring the adult both the open ends were covered carefully with small pored net so that the moths were not injured during the process. The moth was transferred to another similar jar (40 cm x 10cm) after 24 hours of the placement of the adult following the same procedures. This was continued till the moths survived. Twenty mated females from the Petri dish (14.0 cm x 1.5cm) were used separately as 20 replications for recording the egg laying trend (Plate 1). After removal of adults the green papers and the fine meshed net were checked thoroughly for the presence of BSFB eggs.

Plate 1. Oviposition chamber wrapped with green paper inside the glass cylinder.

Plate 2. Eggs laid by BSFB female on the nylon net.

As expected the female laid all the eggs on the net (Plate 2). There was no trace of eggs on the green papers. The eggs on the net were very tiny and were clearly seen with the help of magnifying glass. Then those were counted and it was continued until the death of the female. This counting was done at midnight and early in the morning as it was observed to lay eggs after sunset. From the keen observation it was found that no eggs were laid in day time. After counting of eggs, the net with eggs was placed in a plastic film container (Plate 3) and allowed to hatch. For all the batches of eggs, the same procedure was maintained. All the batches of eggs of an individual were added to get the fecundity of the female. Twenty mated females were observed for laying their eggs and mating was observed through Camera. The age specific egg laying pattern of the female was determined. The eggs laid by a female per day from the first oviposition day

to the last oviposition day were counted daily. The longevity of the adults was recorded also.

Net with eggs were cut into several pieces carefully with minimum damage and kept in plastic film container and allowed to hatch. The eggs were observed for hatching everyday. The time of egg hatching was also recorded. Fecundity was recorded from 6:00 to 14:00 hour of the day. Once the eggs hatched into neonate larvae the duration of the incubation period was recorded. The mean incubation period and percentages of hatching were calculated.

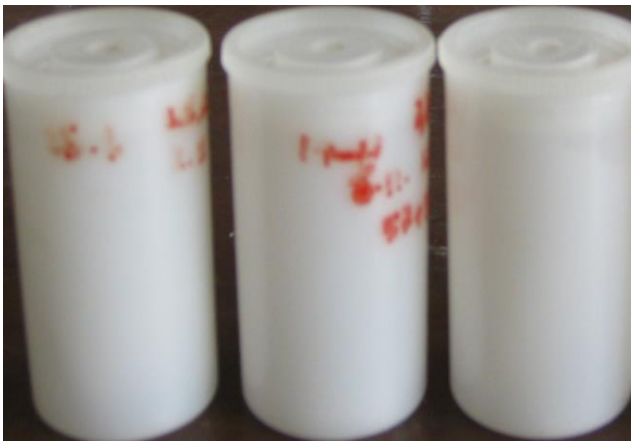

Plate 3. Eggs of BSFB on piece of net kept in film container for hatching.

Preference of egg laying site

To study the preference of egg laying on various brinjal plant locations, a tub grown brinjal plant was kept in a large bowl and covered with polythene in such way so that mated females were not able to go outside. Then one mated female was released in one brinjal plant with fruits. The eggs deposited on the different parts of the brinjal plant were recorded. To determine the egg laying preference of BSFB at three plant canopies the plant was divided vertically in three equal portion named upper, middle and lower canopy. The number of eggs deposited on three canopies were recorded. The percentage of egg laying at different locations and plant canopy was calculated. There were 10 replications in each experiment of complete randomized design. The data were analyzed statistically using ANOVA in MSTAT-C and compared using LSD values.

Results and Discussions

Mating behavior

BSFB moths were found active at night when the mating usually took place. The percentage of adult emergence was 88.90±1.07 at first half of the night (Table 1). Mating frequency was found to vary with the adult emergence time. The

percentage of mating was 90.80±0.59 in the night of adult emergence and it was 9.20±0.59 in the next night (Table 2). It was also revealed from the study that the mating of BSFB moths generally occurred at late night usually between 4.0 to 6.0 AM. (Fig. 1). In some occasions mating was noticed in the early morning up to 8.0 AM. Maximum mating frequency (53.90%) was observed during the period of 5.0-6.0 AM and minimum (4.40%) was in the 7.0-8.0 AM. The moths went for mating only once either on the same day of emergence or a day after emergence. The average mating period was 43.27±1.07 minutes (Table 3). Singh and Singh (2001a) reported mating more than once in the life span of female which occurred at night or very early hours in the morning. Yasuda and Kawashaki (1994) observed the copulation of male and female at 4.40 AM which lasted for 43 minutes. Das and Islam (1982) showed that the virgin 1-day old females began calling from 18:15 to 23.45 hours and duration was 33 minutes. Kavitha *et al.* (2008) showed that mating took place on the same or next day after emergence. Prabhat and Johnsen (2000) reported that the feeding and mating activities occurred during night and mating lasted for about 16 minutes. The findings of the above authors supported the present investigation on mating behavior of adult moths of *L. orbonalis.*

Table 1. Adult emergence and oviposition behaviour of brinjal shoot and fruit borer

Parameters	Mean percentage± SE	
	First half of the night	Second half of the night
Adult emergence	88.90±1.07	11.10± 1.07
Oviposition	86.62±2.53	13.38±2.53

*SE Values were determined from the mean of 20 individuals

Table 2. Mating and oviposition behaviour of brinjal shoot and fruit borer

Parameters	Mean percentage± SE			
	First night	Second night	3rd night	4th night
Mating	90.80±0.59	9.20±0.59	-	-
Oviposition	47.05±2.88	23.11±2.88	23.06±2.88	6.78±2.88

*SE Values were determined from the mean of 20 individuals

Table 3. Some biological parameters of brinjal shoot and fruit borer

Biological parameters observed	Mean±SE (range)
Copulation period in minutes	43.27±1.07 (28-50)
Pre-oviposition period in days	1.10±0.52 (1.08-1.21)
Oviposition period in days	2.70±0.04 (1-4)
Fecundity	241.50±2.32 (149-334)
Percentage of egg hatching	77.43±0.30 (54-88)
Egg hatching period	3.52±0.53 (3-5)

*SE Values were determined from the mean of 20 individuals.

The present results and the findings of different authors confirm that BSFB moths undergo mating at night preferably during the later part of the night. In most studies it revealed that the duration of mating is more than half an hour except the finding of Prabhat and Johnsen (2000). Duration of mating in the present study is same as reported by Singh and Singh (2001a) but it varies from the findings of Das and Islam (1982) and Prabhat and Johnsen (2000). The probable reason of this variation might be due to variation of environmental condition and the ability of mating of the studied insect(s).

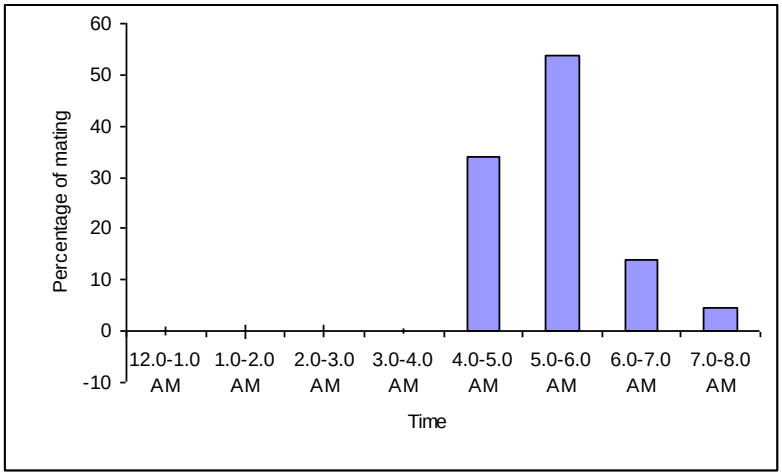

Fig. 1. Mating frequency of brinjal shoot and fruit borer at different hours from midnight to morning.

Oviposition behaviour

BSFB usually lays eggs on the brinjal leaves and twigs both of which are rough surfaced. In the laboratory, it was evident that BSFB female prefers to deposit its eggs in a place of rough surface. The fine meshed net was found to be a preferred surface for egg deposition of BSFB. Generally oviposition started during the next night after emergence and mating. On an average 47.05±2.88% female were found to start egg laying in the first night and 23.11±2.88% were in the second night (Table 2). Egg laying always occurred during the night and from the observation it was found to start egg laying just after sunset. Maximum percentage of (86.62±2.53) egg laying occurred at first half of the night and rest of them were in the second half of the night (Table 1). The egg laying frequency of BSFB at different hours during the first half of the night was shown in Figure 2. Maximum percentage (60.00%) of eggs were laid from 20.00 to 22.00 hours followed by 24.34% and 15.66% at 18.00 to 20.00 and 22.00 to 24.00 hours, respectively. The oviposition period was 2.70±0.04 days while the preoviposition period was 1.10±0.52 days (Table 3). The egg laying continued for 4 days. The egg laying pattern of female BSFB was shown in Figure 3. The number of eggs

laid in different days varied significantly ($p \geq 0.01$). The number of eggs laid daily decreased with the day followed by. The highest number of eggs was laid on the first day (139.14). A certain decrease of egg laying was found in the second day. Then it showed a similar trend with the third day. Thereafter a sharp decrease of egg laying occurred at 4th day reaching a few (21.50) number of eggs. On the second and third day, the female laid on an average of 68.50 and 67.67 eggs, respectively which were statistically similar. Only a few (21.50) number of eggs were laid on the 4th day. Almost 50% of the total fecundity was found on the first day. Although females were found to survive about a week but effective egg laying period was first three days. On an average 275.00 eggs were laid by a female. The eggs were distributed in mass or singly in a scattered form. A range of 40-60 eggs were found in a mass.

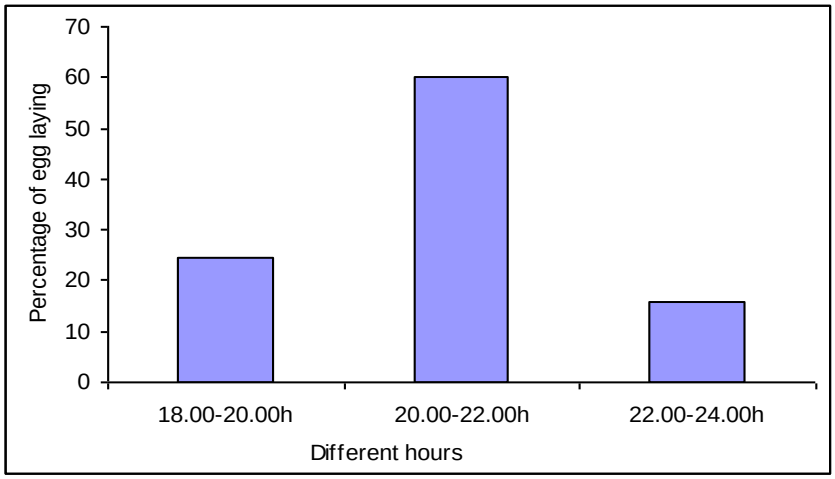

Fig. 2. Egg laying frequency of brinjal shoot and fruit borer at different hours in the first half of the night.

Singh and Singh (2001b) reported that the female laid eggs within a day or second day after the mating. Singh and Singh (2001a) reported that the laying of eggs started on the same day of mating and continued till fourth day with an average preoviposition and ovipositional period 1.35 and 2.09 days, respectively. The number of eggs gradually decreased by each day. The egg laying activities of the female was reported by Prabhat and Johnsen (2000), Alam *et al*. (2003) and Rahman (2005). Rahman (2005) reported that eggs were laid during the later part of the night to the early hours of the morning. Gupta and Kauntey (2007) reported that the average oviposition periods of BSFB was 2.46 days. Harit and Shukla (2003) expressed the similar opinion indicating that the female BSFB moth starts laying eggs on the same day or a day after mating and has an average oviposition period of 2.1±0.171 days.

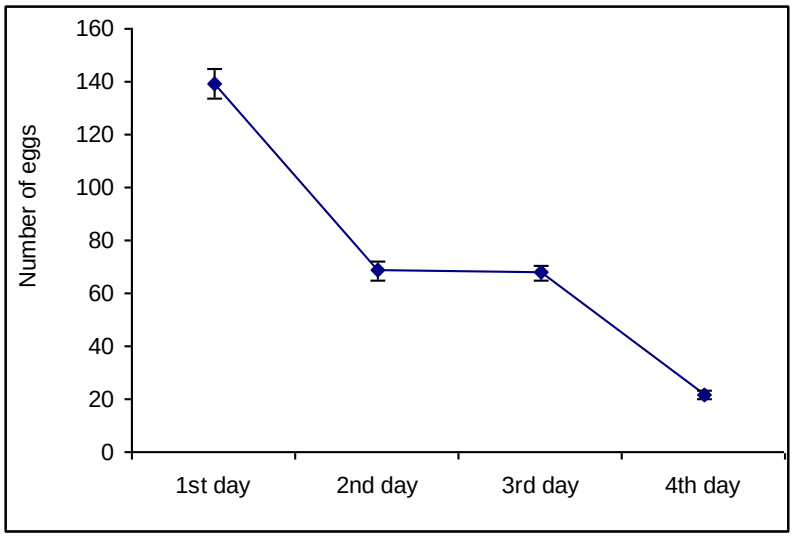

Fig. 3. Egg laying pattern of BSFB female at different days after emergence. Vertical bars represent number±SE.

The findings of the above authors are similar with the results of the present study. However, the findings of Rahman (2005) is different. The present findings on the oviposition time is not in full agreement with the findings of Rahman (2005). No females laid eggs in the late hours of night or in the early morning while Rahman (2005) indicated that egg laying occurred during the period mentioned. On an average the mean oviposition period was observed in this study was 2.70 days. But Gupta and Kauntey (2007) reported it as 2.46 days. Harit and Shukla (2003) reported the oviposition period of BSFB as 2.1 days. A little difference in the oviposition period found in the present study and the two authors might be due to variation of environmental condition. However, all the findings showed that the oviposition period of BSFB is very short. This indicates that the female emerged with full compliment of eggs which are deposited in short time starting as early as possible.

Preference of egg laying site

BSFB was found to deposit its eggs on different parts of brinjal plant such as leaf, petiole, calyx and stem. The females preferred to lay eggs on the leaves (Fig. 4). It is noted that the moth showed higher preference for tender leaves as oviposition site. Maximum eggs (90.51%) were recorded from the leaf of brinjal plant. Only a few eggs were found in stem, calyx and petiole. There was no significant difference among the number of eggs laid on stem, calyx and petiole. A distinct preference was also observed for deposition of eggs on two surfaces of the leaves. The ratio of the eggs laid on upper and lower surface of the leaf was 1:2.74.

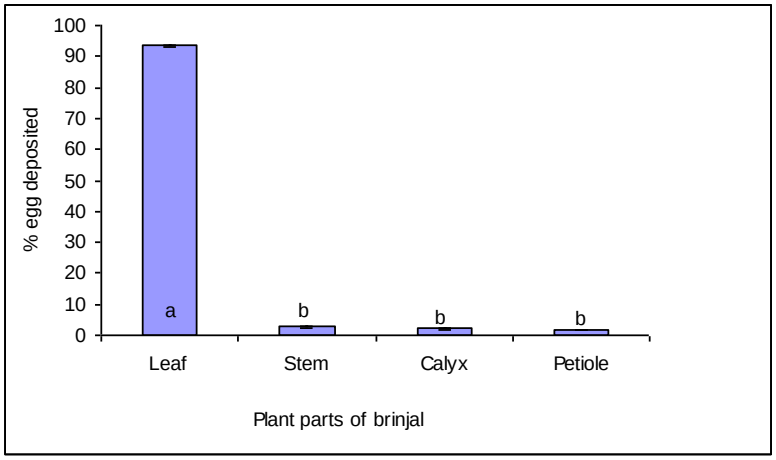

Fig. 4. %Egg deposited of BSFB moth at different plant parts of brinjal. Vertical bars represent number± SE. Same letter(s) within the bars do not differ significantly.

Ardez *et al*. (2008) showed that the eggs were mostly found on the lower leaf surface of top most open and middle leaves although few were also deposited on the upper leaf surface and petiole. The preference of egg laying in the present study is similar to the findings of Ardez *et al*. (2008). Alam *et al*. (2003) reported that eggs were laid singly on the lower surface of the young leaves, green stems, flower buds or calyces of the fruits. Rahman (2005) reported that eggs were laid singly on shoots, flower buds, near the peduncle of fruits and on the lower surface of the leaves. The present findings are in agreement with the report of above authors.

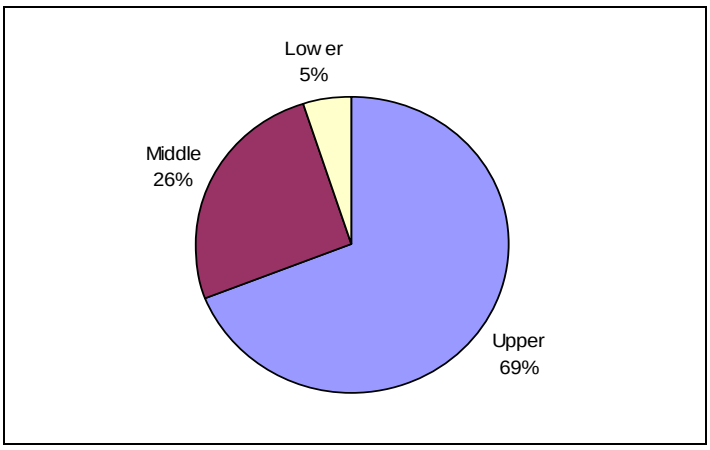

Fig. 5. Distribution of eggs of BSFB at three different plant canopies. A plant was divided into three vertical sections. Each section was considered as a level of canopy.

Distribution of BSFB eggs at different plant canopies

Percentage of egg deposition by BSFB at three different plant canopies varied significantly ($p \geq 0.01$) and was shown in Figure 5. The highest percentage (69%) of eggs was deposited in the upper plant canopy and lowest (5%) was in the lower canopy. Twenty six percent egg deposited was in the middle part of the plant. Upper canopy had a higher number of young leaves, shoots, flower buds, calyces of the fruits than the middle and lower canopy which explained the reasons for deposition of higher number of eggs in upper canopy.

Fecundity and viability

After mating the female moth of *L. orbonalis* laid on an average 241.50±2.32 eggs (Table 3). The overall mean percentage of egg hatching was 77.43±0.30 (54-88). The egg hatching period was 3.52±0.53 days (3-5). It is important to note that eggs were hatched early in the morning. Usually in the morning 87.41% eggs were hatched at 7.0-9.0 AM. Rest of the eggs were hatched at 9.0 AM-2.0 PM. Egg laying was started just after sunset and no egg was laid during day time.

Singh and Singh (2001a) reported that on an average 174.95 eggs were laid by a female and the viability of eggs was 82.61%. Alam *et al*. (2003) reported that the number of eggs laid by a female varies from 80 to 253. An adult female laid as few as 8 to as many as 295 eggs during its lifetime with an average of 118 eggs (PhilRice, 2007). Kavitha *et al.* (2008) reported that the average number of eggs laid by an individual female was 170.

The number of eggs laid per female and %egg hatching reported by the above authors were more or less same found in the present study. The similarity and difference found in the present study and reports of the above authors could be for the variation of environmental conditions. Environmental conditions influence the biology of many insects including the fecundity. It is clearly reflected from the fecundity data of BSFB in the present study. A large variation (8 to 295 eggs) in the egg laying of BSFB reported by PhillRice (2007) might be related to the environmental variations.

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IMPACT OF FORAGING INSECT POLLINATORS ON CHILI PRODUCTION

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Abstract

The abundance, foraging behavior, and diurnal and seasonal dynamics of the pollinator insects in chili ecosystem, and the impact of the pollinator insects on chili production was studied at the Bangabandhu Sheikh Mujibur Rahman Agricultural University, (BSMRAU) Gazipur, Bangladesh using the chili variety BARI Morich 2. The crop was cultivated in natural, supplemented insect and self-pollination conditions. The lime butterfly, honey bee, carpenter bee and sweat bee were found in the chili ecosystem. Honey bee depicted significantly the highest abundance and visitation frequency and carpenter bee was found as the most rapid forager. The diurnal and seasonal abundance of the pollinator insects was fluctuated and the peak abundance was found at 11.00 hour of the day. Abundance of lime butterfly, honey bee and carpenter bee revealed negative relationship with maximum and minimum temperatures, and positive relationship with relative humidity and rainfall. Abundance of sweat bee was positively correlated with maximum and minimum temperatures and rainfall, and negatively correlated with relative humidity. The chili plants exerted vulnerable response to insect pollination producing significantly the higher number of fruits per plant, fruit length, diameter and weight, number of seed per fruit, seed weight and yield.

Keywords: Abundance, behavior, *Capsicum frutescens*, insect pollinators, yield.

Introduction

Chili (*Capsicum* spp.*)* belongs to the family Solanaceae is nutritionally rich in vitamin A, B and C. Chili fruits are pungent because of the constituent of capsaicin, however many varieties are non-pungent which are used as vegetables (Kim *et al*., 2002). Chili was originated from South and Central America and it was first cultivated in Peru where it harbors the greatest diversity of cultivated chili in the world (Meckelmann *et al.*, 2013). Five species of chili especially *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* have been domesticated and cultivated all over the world.

Chili flowers are self-pollinating and chasmogamous those carry out pollination at the time of the opening of petals. Various pollinating insect species forage in the chili ecosystem and there is substantial possibility of cross pollination by insects of the chasmogamous flowers of chili. Honey bees and other bees visit chili flowers on warm bright days or during dry periods (Vishwakarma, 2018). Insect

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pollinators on chili flowers can improve the quality of fruits, which ultimately affect the yield of chili (Aminatun *et al*., 2019).

Pollinator insects increase fertilization rate in flowering plants by transferring pollen from anther to stigma within a flower or between flowers of the same plant or different plants of the same species. Aminatun *et al.* (2019) reported that nine species of pollinator insects visited the flowers of chili plants with a view to feeding, pollen collection, and warmth.

Insect pollination increased viability of seed, formation of more nutritious fruits, increased seed yield and fruit set (Pudasaini *et al*., 2014; Nancy *et al*., 2019). Foraging behavior, frequency of visitation and pollination efficiency vary among the insect species as they respond to the varying flowering plants, time of the day and season of the year (Amin *et al*., 2015; Amin *et al*., 2019a).

Climate change and intensification of agriculture created alarming circumstances against insect pollinators and their pollination services (Kremen *et al*., 2002; Klein *et al*., 2007). Crop pollination and global food production is at the risk due to declining of many pollinator species (Gallai *et al*., 2009). Now-a-days, different pollinator insects specially honeybees and bumblebees are being commercially reared for pollination of vegetable and fruit crops, and investigations are done to find out the pollination efficiency and impact of the native pollinator insects on various crop plants. Considering the above mentioned points, the study was undertaken to find out the insect pollinator species and their abundance and foraging behavior in chili ecosystem, and their impact on yield and seed quality of chili.

Materials and Methods

The study was conducted from January to June 2019 in the field and laboratory of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, (BSMRAU) Gazipur (25°25’ N and 89°5’ E), Bangladesh. Climate of the location is subtropical with mean annual rainfall of 2376 mm, relative humidity of 65.8%, and temperature of 24.4°C (Amin *et al*., 2018). Out of two experiments, one experiment was conducted in 25 sq.m. large plots with three replications to find out the insect pollinator species, their abundance and foraging behavior in chili ecosystem. The other experiment was conducted with three treatments in randomized complete block design and five replications to estimate impact of insects on yield and seed quality of chili. The seeds of chili (*Capsicum frutescens)* variety BARI Morich 2 were collected from the Spices Research Center of Bangladesh Agricultural Research Institute, Bogura. The seedlings were raised in polythene bags and forty days old seedlings were transplanted in the experimental plots on February 25, 2019. The chili plants were cultivated in 5m × 5m plots with three replications to observe the abundance and foraging behavior of the pollinator insects. In this plot, seedlings were transplanted

in 10 rows and each row contained 10 plants apart from 50 cm. To investigate the impact of pollinators on fruit set, chili plants were also cultivated in another 15 plots. The size of these plots was 1.5m × 2.5m and the seedlings were transplanted in rows apart from 50 cm. Each of these plots contained 3 rows and each row had five plants. Manures (cow dung 5 $t.ha^{-1}$ and poultry manure 2 $t.ha^{-1}$) and fertilizers (NPKS @ 32, 16, 25 and 3 $kg.ha^{1}$) were applied according to the Fertilizer Recommendation Guide (FRG, 2018). Irrigation and weeding were done as and when needed.

The experimental 15 plots (1.5m × 2.5m) were arranged into three treatments following randomized complete block design with five replications. The treatments were (i) open plot (natural pollination), (ii) enclosed plot (covered with white mosquito net) having ten supplemented insect pollinators i.e. lime butter fly (2), honey bee (3), carpenter bee (3), and sweat bee (2) per week, and (iii) self-pollination (covered with white mosquito net).

Abundance and foraging behavior of the pollinators

The abundance and foraging behavior of the pollinator insects was observed throughout the flowering period. Free-living insects were collected from each of the 5m × 5m experimental plot using a 30 cm diameter sweep net having 1.5 mm mesh, and attached with a 1.5 m long handle. Every week sweeping was done at 07.00, 09.00, 11.00, 01.00 and 03.00 h of the day, and each sample consisted of 30 sweeps. The collected insects were brought to the Entomology Laboratory of BSMRAU and the pollinator insects were separated. The abundance of the pollinator insects, their diurnal dynamics, seasonal incidence and visitation frequency were calculated. *Frequency of the pollinator insects was calculated using the following formula- F* $(\%) = \Sigma \frac{nt}{Nt} \times 100$, where, F = Percentage of frequency, nt = Number of individuals belong to a visiting insect species, Nt = Total number of insect species included in the census.

Landing duration of the pollinator insects on chili flowers was measured manually using a stop watch. Arrival and departure times of the visiting-insects on the flowers were recorded and landing duration was calculated. Observations on landing were done in between 10:00 to 11:30 h of the day and data were recorded 50 times for each species.

Effect of pollinators on chili fruit and yield

The impact of insect pollinators on fruit set and number of fruits per plant under the three pollination conditions was evaluated. For this purpose, 2 plants from every plot of each category of pollination condition were randomly selected at the first harvest of fruit (45 days after transplanting) and number of fruits per plant was counted. Five ripe fruits from each of the plot of each pollination condition were randomly selected and their individual lengths and widths were measured using slide-calipers, and the

weights were taken using a digital balance (AG204, Mettler Toledo, Switzerland). After that the fruits were sliced with a knife and the number of seeds of each fruit was counted. At every harvest, the plucked fruits of each pollination condition were kept separately and their weights were measured. The fruit yield of each plot was converted into kg/ha. Thousand seed weight of all plots of each pollination condition was taken from sun dried fruits using a digital balance.

Economic value of pollination and crop vulnerability ratio

The economic value of the pollination service was estimated by the contribution of pollinators to the market value of chili production intended for human consumption, and the value was calculated using the formula developed by Gallai *et al.* (2009). The formula for Insect Pollination Economic Value (IPEV) was IPEV $=\sum(P \times Q \times D)$, where, IPEV = Insect Pollination Economic Value, P = Price per unit, Q = Quantity produced due to insect pollination, D = Pollination dependency ratio. The dependency ratio enables the calculation of the production loss in case of a complete disappearance of pollinators. The dependency ratio for chili is of 0.05 (Klein *et al*., 2007; Gallai *et al*., 2009).

Crop vulnerability ratio provides a measure of the potential relative production loss attributable solely to the lack of insect pollination. Crop vulnerability ratio was computed according to the formula of Gallai *et al.* (2009). The formula for Crop Vulnerability Ratio (CVR) was $CVR = \Sigma \frac{IPEV}{EPV}$, where, CVR= Crop Vulnerability Ratio, IPEV = Insect Pollination Economic Value, EPV = Economic Production Value.

Weather information and data analysis

The meteorological data were obtained from the weather station of BSMRAU located 250 m away from the experimental field. One-way analysis of variance (ANOVA) followed by Tukey honestly significant difference test was used for analyzing the abundance and foraging behavior of the pollinator insects, and their impact on chili production. Correlation coefficient (r) values were calculated for pollinator population with meteorological parameters. All the analyses were performed using IBM SPSS 20.0.

Results and Discussion

Four species of insects namely lime butterfly (*Papilio demoleus),* honey bee (*Apis florea),* carpenter bee (*Xylocopa violacea)* and sweat bee *(Halictus* sp.) were found in the chili ecosystem (Table 1). Abundance, landing duration and visitation frequency rate of the pollinator insects varied from 2.5±0.2 to 3.5±0.3/30 sweeps, 17.7±1.3 to 25.1±1.5 second, and 21.5±1.9 to 29.8±1.8%, respectively, and the results differed significantly among different species of pollinators. Honey bee showed the highest abundance and visitation frequency rate and the lime butterfly revealed the longest landing duration. Sweat bee and carpenter bee depicted the

lowest abundance and landing duration, respectively. Lime butterfly and sweat bee exerted statistically similar and the lowest visitation frequency rate.

Tables 1. Abundance and foraging activity of recorded pollinator insect species in chili ecosystem

Observed pollinator insects	Abundance/ 30 sweeps	Landing duration (Seconds)	Visitation frequency rate (%)
Lime butterfly (*Papilio demoleus)*	2.7±0.1 ab	25.1±1.5a	23.2±0.9b
Honey bee (*Apis florea)*	3.5±0.3a	21.2±1.6ab	29.8±1.8a
Carpenter bees (*Xylocopa violacea)*	2.9±0.2ab	17.7±1.3b	25.5±1.1ab
Sweat bee *(Halictus* sp.*)*	2.5±0.2b	23.1±2.1ab	21.5±1.9b

Abundance, visitation frequency and landing duration of the pollinator insects vary with their behavioral characteristics, growth, survival and reproduction strategies, nesting sites, flowering plant species and geographic locations (Mandal *et al*., 2018).

Diurnal dynamics of the pollinator insects in chili ecosystem showed that the abundances of the insects were increasing from 07.00 hour of the day and reached to the peak at 11.00 hour and then declined (Figure 1). Abundance of honey bee was maximum all the daylong followed by carpenter bee, lime butterfly and sweat bee. In the peak foraging hour, the abundances of honey bee and sweat bee were 6.2 and 5.0 individuals/30 sweeps, respectively. This finding shows agreement with the report of Amin *et al*. (2015) who observed peak foraging activity of the pollinators at 11.0 h of the day. Ahmad and Aslam (2002) reported the peak foraging of pollinator insects on carrot at 8.0 - 9.0 h of the day whereas Kumar and Jaiswal (2012) found the increasing trend of pollinators on coriander from 09.00 to 13.00 h and thereafter declined.

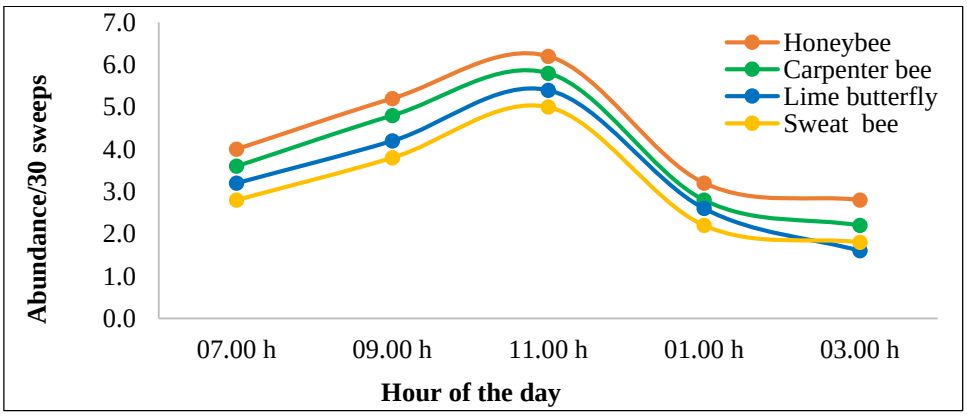

Fig. 1. Diurnal dynamics (sedentary nature) of the insect pollinators in chili ecosystem.

The pollinator insects showed fluctuation in their abundances in the chili ecosystem and they foraged from 1st week of April to the 1st week of May, which coincided with blooming of flower (Figure 2). Honey bee exhibited higher abundance from beginning to end of the blooming season. The peak abundance of carpenter bee (3.7 individuals/30 sweeps) was found in the second week of April when the maximum and minimum temperatures, relative humidity and rainfall were 26°C, 19°C, 91% and 48.7 mm, respectively (Table 2). Lime butterfly and honey bee showed the highest abundance in the third week of April when the maximum and minimum temperatures, relative humidity and rainfall were 34°C, 23°C, 85% and 1.62 mm, respectively.

Table 2. Weather information at Gazipur in Bangladesh from April to May 2019

Observation day	Weather parameters			
	Max. Temperature (˚C)	Min. Temperature (˚C)	Relative humidity (%)	Rainfall (mm)
03 April	31.0	19.0	91	20.13
10 April	26.0	19.0	91	48.7
17 April	34.0	23.0	85	1.62
24 April	35.0	23.5	85	0.0
01 May	36.0	28.0	78	0.0

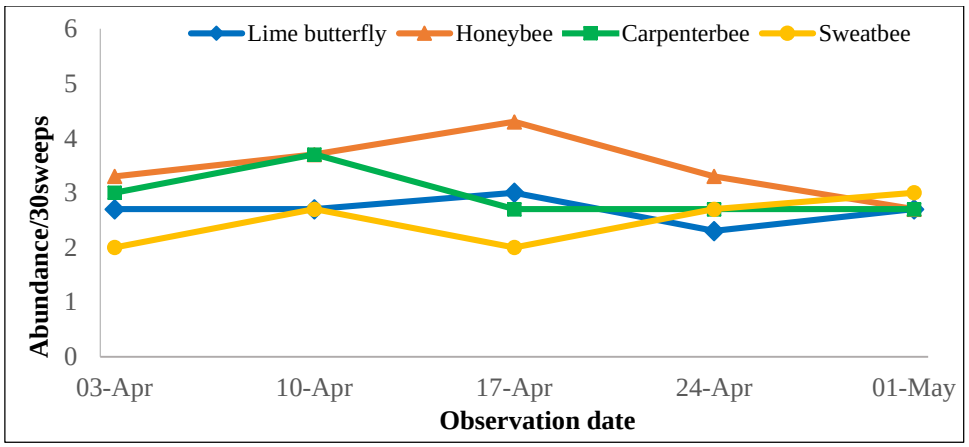

Fig. 2. Seasonal (weekly) dynamics of the pollinator insects in chili ecosystem

Variable correlation coefficient values were existed between pollinator population on chili and weather parameters of the experimental site (Table 3). Abundance of lime butterfly, honey bee and carpenter bee showed negative relationship with maximum and minimum temperatures, and positive relationship with relative humidity and rainfall. But significant correlation was found between the abundance of carpenter bee and maximum temperature. Sweat bee population

showed positive but insignificant correlation with maximum and minimum temperatures, and insignificant negative correlation with relative humidity.

Table 3. Correlation coefficient (r) values between the abundance of the pollinator insects in chili ecosystem and weather parameters

Parameters	Maximum Temperature(°C)	Minimum Temperature(°C)	Relative humidity(%)	Rainfall (mm)
Lime butterfly	-0.139^{NS}	-0.067^{NS}	0.019^{NS}	0.090^{NS}
Honey bee	-0.327^{NS}	-0.476^{NS}	0.456^{NS}	0.197^{NS}
Carpenter bees	-0.974**	-0.701^{NS}	0.696^{NS}	0.994**
Sweat bee	0.128^{NS}	0.551^{NS}	-0.531^{NS}	0.007^{NS}
Max. Temperature (°C)		0.836^{NS}	-0.828^{NS}	-0.988**
Min. Temperature (°C)			-.999**	-0.759^{NS}
Relative humidity (%)				0.752^{NS}

NS Non-significant, * Significant, ** Highly significant.

Table 4. Impact of insect pollination on chili production

Parameters	Pollination conditions		
	Enclosed	Insect supplemented	Natural
Number of fruits per plant	165.2±3.1b	177.2±2.4a	168.8±2.2b
Fruit length (cm)	6.7±0.1c	7.4±0.1a	7.1±0.1b
Fruit diameter (mm)	7.2±0.1b	7.8±0.1a	7. 7±0.1a
Fruit weight (g)	2.22±0.05b	2.43±0.01a	2.33±0.01ab
Number of seeds per fruit	63.9±1.4b	72.1±0.5a	68.5±1.1a
Weight of 1000 seeds (g)	3.3±0.1b	4.5±0.1a	4.4±0.1a
Yield (t/ha)	14.7±0.1c	16.4±0.2a	15.6±0.1b
Yield increases due to insect pollination (t/ha)	-	1.65	1.13
Insect pollination exclusion reduced yield (%)	10.09	-	5.38
Insect pollination economic value (US$)	-	82.5	56.5
Crop vulnerability ratio	-	0.005	0.004

Price of green chili = 1 US$/kg

The effect of insect pollination on fruit and seed set, fruit and seed characteristics, yield, economic value and crop vulnerability ratio varied significantly (Table 4). Number of fruits per plant, fruit length (cm), fruit diameter (mm) and fruit weight (g) among the three pollination conditions varied from 165.2±3.1 to 177.2±2.4, 6.7±0.1 to 7.4±0.1, 7.2±0.1 to 7.8±0.1, 2.22±0.05 to 2.43±0.01, respectively and the results differed significantly among the three conditions. The number of seeds

per fruit, weight of 1000 seeds (g) and yield (t/ha) of chili of the pollination conditions varied from 63.9±1.4 to 72.1±0.5, 0.32±0.01 to 0.46±0.01 and 14.7±0.1 to 16.4±0.2, respectively and the results differed significantly. Insect supplemented condition resulted significantly the highest number of fruits per plant, fruit length and weight, and yield. Fruit diameter, number of seed per fruit and weight of 1000 seeds were statistically similar in insect supplemented and natural pollination conditions. Increased yields due to insect pollination were 1.65 and 1.13 tons/ha in supplemented and natural conditions, respectively and insect pollination exclusion reduced yields in enclosed and natural conditions were 10.09% and 5.38%, respectively. Insect pollination economic value in insect supplemented condition was of 82.5 US$ and that was of 56.5 US$ in natural pollination condition. Chili plants revealed vulnerability ratio of 0.005 and 0.004 in insect supplemented and natural pollination condition, respectively.

Divergent pollinator insects and their varied level of abundance and visitation frequency carry out inconsistent pollination efficiency that consequently enumerates disparate quantity and quality of yields in various crops (Amin *et al*., 2019b). They also reported significant effect of supplemented insect pollinators on the yield and seed quality of eggplant. Naturally occurring pollinator insects contributed on the siliqua production and yield of mustard (Mandal *et al.*, 2018). Pudasaini *et al.* (2014) found 30%- 40% increased yield and higher weight of seeds in *A. cerana* and *A. mellifera* pollinated plants of rapeseed. Vishwakarma (2018) observed that number of seeds/fruit, fruit weight and 213.90% yield increased in chili due to honey bee pollination.

With supplemented insect pollinators, flowers get chance for pollination in the phase of fully functional generative organs thus produce increasing quantity and quality of fruits (Bieniasz, 2007; Bozek, 2012). Kwon and Saeed (2003) found the increase in fruit weight of 27.2% and number of seeds of 47.8% in capsicum due to pollinating by bees. Jacquemin (2017) reported insect pollination vulnerability scale from 0.0% to 41.1% and on average 11.1% for the crops that are used for human food.

The findings of this study showed that four species of pollinator insects visited the chili field and they exhibited distinctive foraging behavior. The pollinators were most abundant at 11.0 and 15.0 h of the day, and the daily maximum and minimum temperatures, relative humidity and rainfall affected their abundance. The pollinator insects revealed significant effect on quality and quantity of chili.

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GGE BIPLOT ANALYSIS FOR YIELD STABILITY OF LENTIL GENOTYPES

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Abstract

The experiment was conducted at three different locations of Bangladesh Agricultural Research Institute viz., at PRC, Ishurdi, Pabna, at PRSS, Joydebpur, Gazipur and RARS, Jashore, during the period November to March in 2014-15 and 2015-16. The experiment was laid out in Randomized Complete Block Design with three replications. Variance analysis showed significant interaction between genotype and environment on yield and its related traits. The highest yield was recorded in genotypes BARI Masur-7 followed by BARI Masur-6, BARI Masur-5, BARI Masur-4 and the lowest was in BLX-66004-12. The PCA (Principal component analysis) scores of a genotype in the GGE analysis indication of the stability or adaptation over environments. GGE biplot analysis related that the PC1 and PC2 for different traits, i.e. 80.8% and 11.7% for days to flowering, 72.4% and 18.7% for days to maturity, 66.3% and 15.4% for plant height, 73.8% and 17.3% for pods/plant, 70.1% and 22.8% for 100 seed weight and 38.37% and 31.04% for yield of the total variation, respectively. Considering regression co-efficient values and also biplot analysis most stable variety was BARI Masur-4 followed by BARI Masur-5 and BARI Masur-6 and lowest stable variety was BLX-05002-6. Among the six environments, Ishurdi 2014-15 and Gazipur 2014-15 were most discriminating (informative) and Jashore 2014-15 and Jashore 2015-16 were less discriminating. Among two years (2014-15 and 2015-16) at different location 2015-16 was found favorable for lentil production.

Keywords: PCA (Principal component analysis), GGE biplot, genotype × environment interaction, lentil.

Introduction

Lentil (*Lens culinaris* M.) is a diploid (2n=14), self-pollinating, winter grain legume with a slender semi erect winter leguminous crop . It is one of the oldest grain legumes having remains dated to 11,000 BC from Greece's Franchthi cave, is originated from Near East and Central Asia (Sandhu and Singh, 2007). Lentil is a vital elemental source of energy, protein, carbohydrates, fiber, minerals, vitamins and antioxidant compounds (Urbano *et al.*, 2007). It is having low level of fat and sodium, high in protein and is an excellent source of both soluble and insoluble fiber, complex carbohydrates, vitamins and minerals, especially B

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vitamins, potassium, phosphorus and cholesterol-lowering fiber (Yadav *et al.*, 2007). In 2011-2012 Bangladesh produce 1.76 lakh mt of lentil from 1.58 lakh ha of land with an average yield 1.11 t/ha (Anonymous, 2013). The most important goal of lentils improvement programs not only high yield, biotic and abiotic stresses tolerant cultivars, but also wide adaptability and stability (Hamdi *et al.*, 2002; Dehghani *et al.*, 2008). Genotype which can adjust its phenotypic state in response to environmental fluctuation in such a way that is gives maximum stable economic return, can be termed as well "buffered" or stable (Allard and Bradshaw, 1964). It is necessary to identify the stable genotypes suitable for wide range of environments. Stability analysis helps in the identification of location specific and widely adaptable genotype. The additive main effects and multiplicative interaction (AMMI) model has been suggested as efficient means in determining stable and high yielding genotypes (Zobel *et al*., 1988). Yan *et al*. (2000) adopted GGE biplot is a graphical tool which displays, interprets and explores two important sources of variation, namely genotype main effect and GE interaction of multi-environmental trails (MET) data.

Genotype-environment (G×E) interaction is essential particularly for the selection of location specific genotypes, genotype-environment interaction is of major consideration to the breeders (Eberhart and Russell, 1966). GGE biplot methodology allows visual examination of GE interaction pattern of multi-environmental data based on two concepts. First, yield is measured as the combined effect of G, E and GE. Only G and GE are relevant to and considered in genotype evaluation. The yield of each cultivar in a tested environment is a result of genotypic main effect (G), environmental main effect (E) and genotype × environment (GE) interaction (Yan and Kang, 2003). Second, GGE biplot technique separates two principal components, PC1 and PC2, which are also referred to as primary and secondary effects, respectively. The principal components are derived from subjecting environment-centered yield data (the yield variation due to GGE) to singular value decomposition. Then the pattern of genotypic response across environments can be graphically determined in a GGE biplot (Yan and Tinker, 2006). For cultivar evaluation, G and GE are important components for explaining a meaningful relationship between genotypes and environments from the GGE biplot. GGE biplot is exploited for graphical display of G×E pattern of yield trial data with several advantages. Selecting genotypes with high yield and yield stability in a wide range of environments become important as reliable production in quantity (Gauch *et al*., 2008). Understanding genotype by environment (GE) interactions is necessary to accurately determine stability in lentil genotypes and help breeding programs by increasing efficiency of selection (Sabaghnia *et al.*, 2008). So the present study is undertaken with the objectives of (a) To analysis yield stability and adaptability of newly lentil genotypes and (b) To evaluate discrimination and representativeness of test locations.

Materials and Methods

Planting materials: Twelve lentil genotypes BARI Masur-1, BARI Masur-2, BARI Masur-3, BARI Masur-4, BARI Masur-5, BARI Masur-6, BARI Masur-7, BLX-05008-05, BLX-05002-6, BLX-05008-21, BLX-66004-12 and ILL-5134 genotypes of lentil were evaluated in this study. Among them seven released variety and other four advanced lines of lentil were collected from Pulse Research Centre (PRC), Ishurdi, Pabna, whereas another advanced line ILL-5134 was collected from ICARDA, Aleppo, Syria.

Field Trails: The study was conducted at three diverse experimental sites of Bangladesh Agricultural Research Institute viz., Pulses Research Centre (PRC), Ishurdi, Pabna; Pulse Research Sub-station (PRSS), Joydebpur, Gazipur; and Regional Agricultural Research Station (RARS), Jashore. Trials were carried out in two consecutive season viz., during the period Rabi (November to March) 2014-15 and 2015-16. Three sites and two years combinations were considered as 6 environments- Environment-1: Gazipur' 14-15, Environment-2: Gazipur' 15-16, Environment-3: Ishurdi' 14-15, Environment-4: Ishurdi' 15-16, Environment-5: Jashore 14-15 and Environment-6: Jashore 15-16. The experiments were laid out in a Randomized Complete Block Design (RCBD) with 3 (three) replications. In each replication, a lentil genotypes was sown in a size of 4 m long with 2 rows. Row to row distance was 40 cm with 80 cm spacing between the adjacent plots. In each row, spacing between the adjacent plants was 6-8 cm. The entire quantity of N, P, K, S, Zn and B @ 20-20-20-10-2-1 kg/ha were applied during final land preparation. Intercultural operations were done as necessary during the growing period for proper growth and development of the plants.

Table 1. Characteristics of the three tested locations

Site	Soil type	AEZ	Soil pH
PRC, Ishurdi	Silty loam	High Ganges River Floodplain of Agro-ecological Zone (AEZ-11)(Anonymous, 2004)	6.91
PRSS, Gazipur	Clay loam	Madhupur tract of Agro-ecological Zone (AEZ- 28) (Brammer, 1971)	5.7
RARS, Jashore	Sandy loam	High Ganges River Floodplain of Agro-ecological Zone (AEZ- 11)	8.2

Trait measurement: Plant height, days to flowering, days to maturity, pod per plant, seeds per pod, weight of 100 seed and yield per plant were recorded. Variance and GGE biplot analysis were done accordingly. All graphic summaries were done using the 'GGE biplot' package in 'R' program.

Results and discussion

Yield and other traits was highly influenced and this might be due to the effects of environments. There was significant variation also found for days

to flowering, days to maturity, plant height, pods/plant, 100 seed weight and yield (kg/ha) (Table 3). For days to flowering BLX-66004-12 and BLX-05002-6 was found earlier at Gazipur and Ishurdi during 2015-16 compare to other genotypes and years (Table 4). Considering year for all locations, in 2015-16 all the genotypes was flowered earlier, this might be due to environmental effect on genotypes. In case of days to maturity BLX-05008-05 was found earlier at Jashore (72 days) for both years. But at Ishurdi and Gazipur BLX-05008-05 was also found earlier in 2015-16 compare to 2014-15. Among the 12 genotypes BARI released variety was found late compare to advance line for days to maturity. Plant height also showed significant difference at three locations for both the years at Ishurdi 2014-15 and Gazipur 2014-15, all the genotypes have longest plant compare to 2015-16. Apart from this pods/plant and 100 seed weight varied significantly both the years at three- locations.

Table 3. Combined Analysis of Variance (ANOVA) of lentil genotypes for yield related traits at location during 2014-15 & 2015-16

Source of variance	DF	DM	PHT	PP	100 Swt. (g)	Yield (kg/ha)
Environment	318.24***	695.64***	1329.81***	17147.9***	6.32***	407987.5*
Rep. (Env.)	3.49	7.51	14.06	218.5	0.23*	71454.1***
Genotype	236.72***	462.23***	53.60***	1536.7***	1.12***	69183.3***
Env.× Genotype	72.13***	111.29***	31.39***	499.9***	0.51***	39916.6*
Residuals	18.24	18.36	8.11	152.2	0.10	200041.00

NB:* Significant at $P \leq 0.05$, ****** Significant at $P \leq 0.01$ and ******* Significant at $P \leq 0.001$

DF= Days to flowering, DM= Days to maturity, PHT= Plant height, PP=Pods per plants, Swt.= Seed weight

Highest number of pods/plant was found in BARI Masur-6 which was followed by BARI Masur-3 at Jashore in 2015-16. Similar trend was also observed at Ishurdi for genotypes BARI Masur-6 and BARIMasur-7. For 100 seed weight, highest seed weight was in BLX-05008-05 over three locations followed by BLX-05002-6, BARI Masur-7, ILL-5134 and BARI Masur-3. The highest mean yield (1390 kg/ha) was found in BARI Masur-7 followed by BARI Masur-6 (1390 kg/ha), BARI Masur-5 (1380 kg/ha) and BARI Masur-4 (1320 kg/ha). The genotypes BARI Masur-3 (1290 kg/ha), ILL-5134 (1250 kg/ha) and BCX-05008-05 (1250 kg/ha) are performed comparatively well. Each genotype was defined in respect of stability by three values 1) Mean yield across environment 2) The linear regression (b values) of genotype mean yield in each environment and 3) The mean square deviation from the regression of each genotype.

Table 4. Mean performance of 12 Lentil genotypes for yield contributing traits at three locations during 2014-15& 2015-16, Conducted at Gazipur, Ishurdi, and Jashore in Bangladesh

Sl. No	Genotypes	Days to flower						Mean & Rank	Days to maturity						Mean & Rank
		Gazipur		Ishurdi		Jashore			Gazipur		Ishurdi		Jashore		
		2014-15	2015-16	2014-15	2015-16	2014-15	2015-16		2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	
1	BLX-05008-21	62	43	46	41	44	42	51 d	95	91	94	91	89	78	98 f
2	ILL-5134	64	48	50	43	45	51	45 g	101	94	96	88	89	101	95 i
3	BLX-05002-6	50	40	46	42	43	51	46 f	104	94	97	92	89	99	97 g
4	BLX-66004-12	55	39	46	41	50	52	47 e	115	101	93	95	93	102	100 e
5	BLX-05008-05	58	43	45	46	35	41	45 g	93	91	93	93	83	72	96 h
6	BARIMasur-1	55	46	62	55	50	51	53 b	104	95	109	93	102	101	101 d
7	BARIMasur-2	56	43	61	54	51	52	53 b	105	93	112	93	101	100	101 d
8	BARIMasur-3	51	44	62	56	52	51	53 b	103	94	112	93	101	101	101 d
9	BARIMasur-4	51	42	62	54	52	52	52 c	105	93	112	95	102	102	102 c
10	BARIMasur-5	53	47	63	54	52	51	53 b	103	99	113	97	103	102	103 b
11	BARIMasur-6	52	49	62	54	52	52	54 a	104	100	111	95	103	101	102 c
12	BARIMasur-7	52	49	62	53	53	52	54 a	106	100	113	96	104	101	104 a
Average		54.91	44.41	55.58	49.41	48.25	59.83		103.16	95.41	95.16	85.66	88.08	91.5	
CV (%)		1.2	1.4	1.9	1.6	1.7	0.67	-	0.72	0.92	0.65	0.55	-	-	-
LSD (0.05)		1.88	1.34	1.41	1.22	1.36	1.08	-	1.99	1.67	1.96	1.41	NS	NS	-

Table 4. Cont'd

Sl. No	Genotypes	Plant height						Mean & Rank	Pods/Plant						Mean & Rank
		Gazipur		Ishurdi		Jessore			Gazipur		Ishurdi		Jessore		
		2014-15	2015-16	2014-15	2015-16	2014-15	2015-16		2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	
1	BLX-05008-21	27	23	32	37	34	33	32 d	14	14	11	26	38	43	24 k
2	ILL-5134	25	22	36	31	32	42	31 e	23	16	27	31	34	78	35 h
3	BLX-05002-6	31	29	34	30	36	45	34 b	12	14	18	37	59	75	36 g
4	BLX-66004-12	27	24	31	31	35	44	32 d	11	16	23	31	47	72	33 i
5	BLX-05008-05	24	23	30	32	25	30	27 f	26	13	15	27	31	36	25 j
6	BARIMasur-1	33	24	35	30	37	42	34 b	35	14	43	35	60	78	38 f
7	BARIMasur-2	27	25	36	31	38	40	33 c	22	12	47	24	53	68	45 c
8	BARIMasur-3	31	26	41	33	39	46	36 a	37	14	44	36	61	79	43 e
9	BARIMasur-4	28	24	38	31	37	40	33 c	31	16	38	34	53	84	47 b
10	BARIMasur-5	28	24	41	28	34	40	33 c	32	19	53	32	75	71	48 a
11	BARIMasur-6	28	25	36	33	37	39	33 c	30	20	59	37	60	83	43 e
12	BARIMasur-7	30	26	41	31	37	41	34 b	38	19	23	39	61	75	44 d
Average		28.25	24.58	35.91	31.5	35.08	40.16		25.91	15.58	33.41	32.41	52.66	70.16	
CV (%)		3.5	3.6	2.2	2.8	5.6	6.7	-	16.2	15.4	14.5	16.5	12.1	14.2	-
LSD (0.05)		7.82	8.33	6.6	4.2	ns	ns	-	18.3	20.05	31.2	33.6	3.06	2.05	-

Table 4. Cont'd

Sl. No	Genotypes	100 seed weight						Mean & Rank	Grain yield (Kg ha^{-1})						Mean & Rank
		Gazipur		Ishurdi		Jashore			Gazipur		Ishurdi		Jashore		
		2014-15	2015-16	2014-15	2015-16	2014-15	2015-16		2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	
1	BLX-05008-21	1.6	1.7	2.2	2.3	2.2	2.4	2.0 e	880	1020	930	770	1020	1730	1060 h
2	ILL-5134	2.1	2.0	1.8	2.8	1.9	1.8	2.1d	980	1800	1320	1170	1280	920	1250 e
3	BLX-05002-6	2.1	1.6	2.0	3.9	1.8	2.1	2.3 b	560	500	1620	1040	880	2170	1130 g
4	BLX-66004-12	2.2	2.2	1.6	2.2	1.7	1.9	1.9 f	400	520	1470	900	810	1170	880 i
5	BLX--05008-05	2.3	1.9	3.0	2.2	3.1	2.6	2.5 a	1080	1570	1020	1300	750	1800	1250 e
6	BARIMasur-1	1.5	1.5	1.8	2.3	1.4	1.5	1.7 h	850	1430	1250	1090	1360	1320	1220 f
7	BARIMasur-2	1.7	1.6	1.6	2.6	1.5	1.5	1.8 g	840	1100	1300	1030	1230	1300	1130 g
8	BARIMasur-3	2.0	1.9	2.1	2.9	1.9	2.0	2.1 d	1190	1300	1300	1210	1290	1450	1290 d
9	BARIMasur-4	1.6	1.7	2.0	3.2	1.7	1.9	2.0 e	880	1190	1380	1220	1470	1770	1320 c
10	BARIMasur-5	1.8	1.7	2.3	2.8	1.9	1.6	2.0 e	850	1230	1470	1350	1530	1830	1380 b
11	BARIMasur-6	2.0	1.6	1.8	2.7	1.9	1.9	1.9 f	730	1640	1530	1110	1300	2050	1390 a
12	BARIMasur-7	1.9	1.7	2.0	3.2	1.8	2.8	2.2 c	560	1510	1600	1410	1300	1980	1390 a
Average		1.9	1.75	3.59	2.75	3.1	2.0		817	1234	1240	1055	1092	1524	
CV (%)		2.44	4.21	3.22	3.56	7.33	8.91	-	18.52	15.32	9.56	7.43	12.44	14.65	-
LSD (0.05)		0.19	0.17	0.26	0.29	ns	ns	-	7.33	8.33	14.23	12.11	4.42	5.23	-

Regression co-efficient ranged from 0.8562 (for genotype BLX-05008-6) to 1.3833 (for genotype BARI Masur-2). Among the 12 genotypes, seven genotypes had regression co-efficient greater than 1.0 indicate sensitive to environment changes in respect of yield but five genotypes (BARI Masur-4, BARI Masur-6, BARI Masur-7, BLX-05008-05 and BLX-05002-6) had regression co-efficient less than 1.0 (Table 5). These genotypes were relatively better adapted to poor environment and were insensitive to environment changes in respect of yield. Such genotypes could be recommended only for cultivation for unfavorable conditions. However on of the genotypes BARI Masur-4 having regression co-efficient closer to unity (0.9862) with highest yield than the overall genotypes mean suggest that it could be recommended for cultivation under any type of environments for higher yield. Therefore, this genotype can be selected as stable over the environments. Regarding Mean square Deviation the genotypes BARI Masur-4, BARI Masur-6, BARI Masur-7 and BLX-05008-05 possess minimum values therefore, these four genotypes are more stable across the environments. If we consider year, most of the genotypes showed better yield in Ishurdi 2015-16 compare to 2014-15 and similar results was also obtained by Jashore. Apart from these Ishurdi locations showed better yield in 2015-16 compare to 2014-15. This may be due to favorable or in favorable environmental effects on genotypes.

Table 5. Regression Co-efficient and Mean Square Deviation of 12 lentil genotypes

Sl. No	Genotypes	G×E Mean Yield	Regression Coefficient	Mean Square Deviation
1.	BLX-05008-21	1060	1.2033	1873
2.	ILL-5134	1250	1.0343	2890
3.	BLX-05002-6	1130	0.8562	967
4.	BLX-66004-12	880	1.1520	8932
5.	BLX--05008-05	1250	0.9632	825
6.	BARIMasur-1	1220	1.1855	6114
7.	BARIMasur-2	1130	1.3833	67893
8.	BARIMasur-3	1290	1.3452	68363
9.	BARIMasur-4	1320	0.9862	234
10.	BARIMasur-5	1380	1.0028	56734
11.	BARIMasur-6	1390	0.8812	113
12.	BARIMasur-7	1390	0.9784	214

GGE Biplot analysis

GGE biplot analysis was done to find out stable genotypes among locations. GGE biplot is exploited for graphical display of G×E pattern of yield trial data with several advantages. The yield of each genotype in a tested environment is a result of genotypic main effect (G), environmental main effect (E) and genotype

× environment (GE) interaction (Yan and Kang, 2003). The stability performance of the lentil genotypes is presented in the biplot based on grain yield and other yield contributing traits data (Figure 1-5). The horizontal axis (PC1) indicates the main effect of genotype while the vertical axis (PC2) shows the interaction of genotype and environment which is the basic criterion for judging genotypic stability. The lines passing from the origin (0.0) of the coordinate of a location and genotype are referred to as environmental vector and genotype vector, respectively. The average environmental axis (AEA) is the line that passes through the coordinates of all the locations and the biplot origin. The length of the environmental vector from the origin to its coordinate is used to measure the discriminating ability of the location. In this experiment, the principal component 1 (PC1) and PC2 obtained from all the six observed characters accounted for 80.8% and 11.7% for days to flowering, 72.4% and 18.7% for days to maturity, 66.3% and 15.4% for plant height, 73.8% and 17.3% for pods/plant, 70.1% and 22.8% for 100 seed weight and 38.37% and 31.04% for yield of the total variation, respectively. Seed yield showed as high as 38.37% variation that can be explained by PC1.

Days to flowering: According to Figure 1 genotypic main effect explain 80.8% of total variation and G × E interaction explain 11.7% of total variation for days to flowering. Six locations fall into two sector of polygon. Among twelve genotypes, five genotypes are corner of the polygone to days to flowering indicates that these are vertex genotypes. The polygon also showed that Ishurdi location for both the years was found earlier compare to best for other locations.

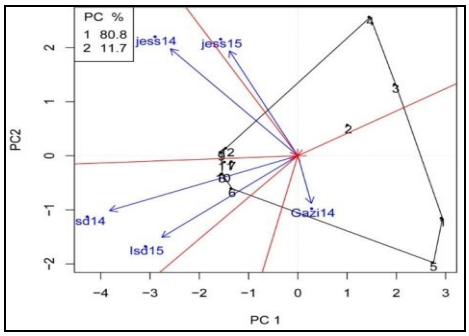

Fig. 1. GGE Biplot for Days to flowering showing the interaction of PC2 against PC1 scores12 lentil genotypes in three locations (six environments) at 2014-15 and 2015-16

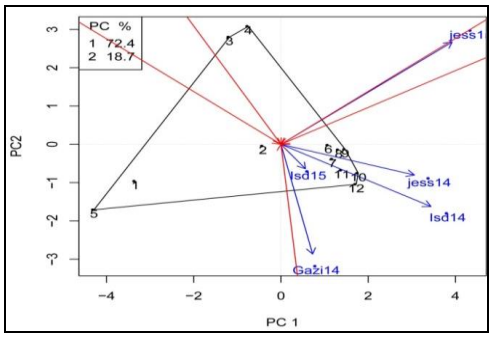

Fig. 2. GGE Biplot for Days to maturity showing the interaction of PC2 against PC1 scores12 lentil genotypes in three locations (six environments) at 2014-15and and 2015-16

Days to maturity: Figure 2 showed that the genotypes BARI Masur-1(101 days), BARI Masur-2(101 days), BARI Masur-3(101 days), BARI Masur-4 (102

days), BARI Masur-5 (103 days), BARI Masur-6 (102 days) and BARI Masur-7 (104 days) are the better performance for days to maturity in Ishurdi and Jashore location comparatively other genotypes. Genotypes ILL-5134 (95 days) are the better performance for days to maturity as it is closer to the origin. So the genotype can be selected for the earlier genotype.

Pods per plant: Figure 3 explained the genotype and environment interaction. According to fig 4.3 showed that the genotypes BARI Masur1, BARI Masur-3, BARI Masur4, BARI Masur5, BARI Masur-6 and BARI Masur-7 are the better performance for pods per Plant in Ishurdi 2014-15,Ishurdi 2015-16, Gazipur 2014-15 and Jashore 2014-15 locations comparatively other genotypes. On the other hands the genotypes BARI Masur-4 and BARI Masur-7 was found as stable for pods per plant as it is closer to the origin. Genotypes BARI Masur-4 and BARI Masur-7 close to the origin of axes had wider adaptation i.e, most stable genotypes.

Seed weight: Figure 4 explained the genotype and environment interaction. According to fig 4 we showed that the genotypes BARI Masur-4 and BARI Masur-7 are the better performance for seed weight in Gazipur location comparatively other genotypes. On the other hands BARI Masur-3, BARI Masur-5 and BARI Masur-6 were found as stable for SWT as those genotypes positioned near to origin.

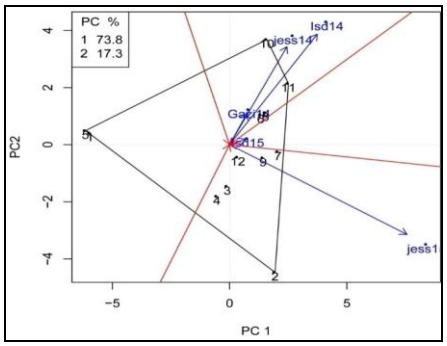

Fig. 3. GGE Biplot for pods per plants showing the interaction of PC2 against PC1 scores 12 lentil genotypes in three locations (six environments) at 2014-15 and 2015-16

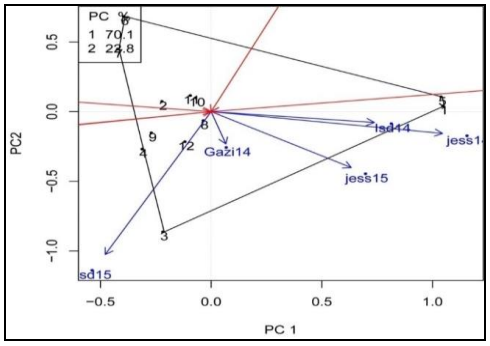

Fig. 4. GGE Biplot for seed weight showing the interaction of PC2 against PC1 scores 12 lentil genotypes in three locations (six environments) at 2014-15 and 2015-16

Yield (kg/ha): Figure 5 explained the genotype and environment interaction. The first two PCs explained 69.41% (PC1 = 38.37% andPC2 = 31.04%) of total variation for lentil multi-environmental trials. Twelve genotypes represent a polygon and the genotypes corner of the polygon most responsive genotypes. Most of the genotypes situated in mega environment which show the Ishurdi and Jashore locations. The genotypes BARI Masur-4, BARI Masur-5, BARI Masur-6

and BARI Masur-7 showed most interaction with environment followed by others. The genotypes BARI Masur-4, BARI Masur-5, BARI Masur-6 and BARI Masur-7 were the better performance show for yield in Ishurdi and jashore comparatively other genotypes. All entries distributed in to four sectors. Among the tested locations, Ishurdi and Jashore had larger environmental vectors indicating high discriminating ability.

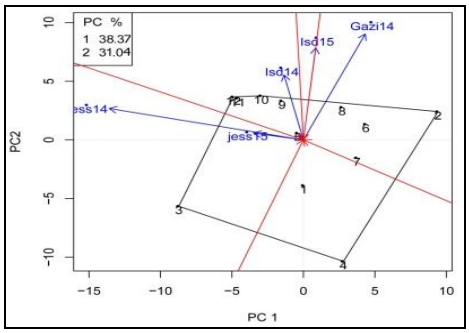

Fig. 5. GGE Biplot for Yield (kg/ha) showing the interaction of PC2 against PC1 scores 12 lentil genotypes in three locations (six environments) at 2014-15 and 2015-2016

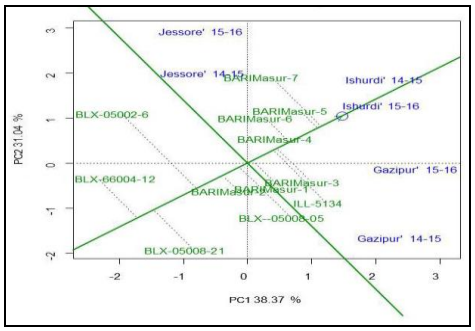

Fig. 6. GGE Biplot for yield mean and Stability performance

Biplot for yield mean and Stability performance

In Figure 6, a vector is drawn from the biplot origin to each marker of the stability statistics to facilitate visualization of the relationship among different stability statistics. The correlation coefficient between any two stability statistics is approximated by the cosine of the angle between the vectors. Therefore, the most stable variety was BARIMasur-4 and second one BARIMasur-5 and third one BARIMasur-6 and lowest stable variety was BLX-05002-6. Although, multi-environment trials are used for genotype evaluation, they can also be used in stability statistics evaluations. Ideal stability statistics should be highly differentiating of the genotypes and at the same time identifying high yielding genotypes. In Figure 6, the stability statistics are ranked based on both discriminating ability and representativeness. The center of the concentric circles is where an ideal stability statistics should be; its projection on the average tester coordinate PC1 was designed to be equal to the longest vector of all stability statistics; therefore, it is the most discriminating; its projection on the average tester coordinate PC2 was obviously zero, meaning that it is absolutely representative of the average stability statistics. Therefore, the closer stability statistics are to this mean yield, the better it is as stability statistics.

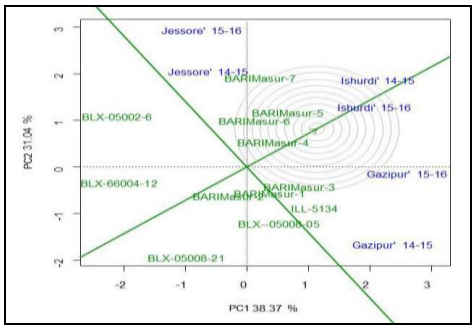

Fig. 7. Ranking of Genotypes based on Yield and Stability performance

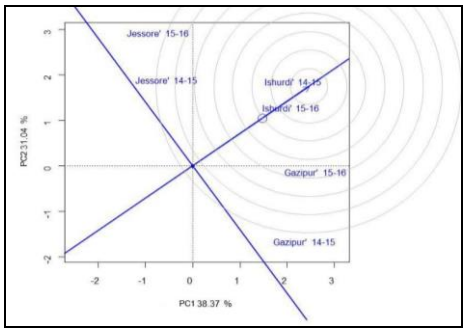

Fig. 8. Ranking of environment based on yield and stability performance

Ranking of Genotypes based on Yield and Stability performance

The estimation of yield and stability of genotypes (Figure 7) were done by using the average co-ordinates of the environment (AEC) methods (Yan, 2001; Yan and Hunt, 2002). The average environment is defined by the average values of PC1 and PC2 for the all environments and it is presented with a circle. The average ordinate environment (AOE) was defined by the line which is perpendicular to the average environment axis (AEA) line and pass through the origin. This line divided the genotypes in to those with higher yield than average and in to those with lower yield than average. By projecting the genotypes on AEA axis, the genotypes are ranked by yield, where the yield increases in the direction of arrow. In this study the highest yield had genotypes BARI Masur-5, BARI Masur-6, BARI Masur-4, BARI Masur-7 and the lowers was in BLX-66004-12. In this study, the greatest stability in the high yielding group had genotypes BARI Masur-4, BARI Masur-5 and BARI Masur-6, while the most stable of all was BARIMasur-4. These results are in agreements with those obtained by Naheif (2013) in wheat.

Ranking Environment

Stability performance of genotypes is an important consideration in breeding programs (Kang and Pham 1991, Kang 2002). According to Yan (2002), discriminating ability and representativeness are the important properties of a test location, an ideal location should be highly differentiating of the tested genotypes and at the same time representative of the target locations. According to Figure 8, location Ishurdi 2014-15 is more desirable test environment than the other test locations. Thus, genotype evaluation in Ishurdi 2014-15 maximizes the observed genotypic variation among genotypes for grain yield of lentil. The discriminating ability of a location can show the comparison of genotypes, but the presence of GE interaction complicates the identification of genotypes in the ideal test

location (Yan *et al.* 2000). Usually non-additive or crossover GE interaction was observed in the most MET and it is essential to reveal the nature of GE interaction. GGE methodology is suitable tool to analyze these kinds of interactions and partitioning them into their PCs. The test location should has large PC1 scores in order to discriminate genotypes in terms of the genotypic main effect and absolute small PC2 scores in order to be more representative of the overall locations (Yan and Rajcan, 2002).

Conclusion

It was revealed that the most stable varieties were BARI Masur-4, BARI Masur-5 and BARI Masur-6 across the six environments. The varieties BARIMasur-3, BARIMasur-4, BARIMasur-5, BARIMasur-6 and BARIMasur-7 exhibited comparatively higher mean yield (>1.30 t/ha) and stable performance across the environments and the location Ishurdi 2014-15 was stable for most of the genotypes of lentil.

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EVALUATION OF GLADIOLUS GENOTYPES AT AKBARPUR, MOULVIBAZAR

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Abstract

Twelve gladiolus genotypes were collected and evaluated in the Research Field of Regional Agricultural Research Station, Akbarpur, Moulvibazar, during November 2018 to May 2020 for identifying suitable line(s) for commercial cultivation in north eastern region of Bangladesh. The genotype that produced flower stalk within minimum days of planting was GL-Akb-012 (66.16 days.The longest rachis length was recorded in GL-Akb-003 (80.12 cm) which was statistically significant from other eleven genotypes. Plant height varied considerably with the highest plant height in GL-Akb-012 (145.67 cm) and the lowest plant height was in GL-Akb-011 (89.33 cm). All of the genotypes in general, produced more than 12 florets per spike, however GL-Akb-001 produced the highest number of florets per spike (17.33). Vase life of the genotypes showed variation, GL-Akb-012 had the highest vase life of 9 days. The highest weight of corm found in GL-Akb-004 (320.00 g). In respect of variation in plants, for instance, flower quality, corm and cormel production characters, the gladiolus genotype GL-Akb-012 was most promising for commercial cultivation.

Keywords: Gladiolus, flower, corm, cormel, genotype, evaluation.

Introduction

Gladiolus (*Gladiolus grandiflorus L.*), popularly known as sword Lily, is an ornamental bulbous plant native of South Africa (Sharma and Sharma, 1984). It belongs to the monocot family Iridaceae. It is a very popular flowering plant in international cut flower trade grown throughout the world in a wide range of climatic conditions. In Bangladesh, the agro ecological conditions are very conducive for the survival and culture of gladiolus. Regarding area and production of gladiolus flowers, no authentic reports are available in the country. It was estimated that, currently about 10,000 ha of land was under this flower cultivation (Rakibuzzaman *et al*., 2018). In Bangladesh, commercial floriculture is expanding very rapidly. Today, floriculture has emerged as a lucrative profession in Bangladesh with a much higher potential for returns than most other fields and horticultural crops (Sultana, 2003).

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The major production belts of this flower are found in Jessore Sadar, Sharsha, Chowgacha, Kushtia, Chuadanga, Chattogram, Mymensingh, Dhaka, Savar and Gazipur regions. Recently, cultivation of this crop in other parts of the country has been started in a small scale. It has great economic value as a cut flower and its cultivation is relatively easy. Studies have established by Momin (2006) that income from gladiolus flower production is six times more than the returns from rice. Its elegant spikes, varieties of colour with long vase life are the reason for its ever-increasing demand. Apart from ornamental value, gladiolus have extensively utilized in medicines for headache, lumbago, diarrhoea, rheumatism and allied pains (Bose *et al*., 2003). Flower and corm of some gladiolus are used as food in many countries (Guillarmod, 1977; De Meyer, 1982). The average cropping intensity in Sylhet region is low (167%) compared to the national average (190%). A survey conducted by DAE showed nearly 1.64 lakh hectares of land in Sylhet division remains fallow during winter (rabi season), (BARI, NATP phase 2, 2018). To utilize this vast fallow land a new crop with considerable commercial acceptance can be introduced in this region and gladiolus flower could be that crop. Considering the ever-growing popularity of gladiolus flower, it deserves evaluation in this area to know the possibility of adaptation and to undertake necessary future improvement for both quantitative and qualitative characters to increase the cropping intensity as well as utilization of large fallow lands.

Materials and Methods

The experiment was conducted at the research field of Regional Agricultural Research Station, Akbarpur, Moulvibazar, Sylhet, during the Rabi season of 2018-2020. Twelve genotypes of gladiolus viz. GL-Akb-001, GL-Akb-002, GL-Akb-003, GL-Akb-004, GL-Akb-005, GL-Akb-006, GL-Akb-007, GL-Akb-008, GL-Akb-009, GL-Akb-010, GL-Akb-11 and GL-Akb-012 were collected from Floriculture division of Bangladesh Agricultural Research Institute, Jhikargacha upazila of Jessore district, and Jhenaidah district and were included in the experiment (Table 1). Before planting, land was prepared properly and manured with cowdung (10 t/ha), TSP and MoP were also applied @ 225 and 125 kg/ha at the time of final land preparation. Urea 200 kg/ha was applied in two equal installments of 25 and 45 days after emergence (Azad, 2017). Other intercultural operations such as watering, weeding, earthing up, stacking, plant protection measures etc. were applied as and when necessary. The unit plot size was 1.2 m*1.0 m. On October of the years 2018 and 2019, medium sized (3.5-4.0 cm) corms of different gladiolus germplasm were planted at about 6-9 cm depth in the plot. Distances between row to row and plant to plant were 20 cm and 20 cm, respectively. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Data were recorded on different parameters like plant height, no. of leaves/plant, days to spike initiation, days to flowering, spike length, rachis length, number of floret/rachis, weight of single stick, number of

spike/hill, vase life, number of corms, number of cormel/hill and weight of corm. The spikes were cut when lower one or two florets showed colour but still in tight bud stage. The cut spikes were wrapped initially with newspaper in the field and then kept into water to study the vase life. Corms and cormels were harvested only when the leaves turned into brown color (Mukhopadhyay, 1995). The collected data were statistically analyzed using statistics 10 software.

Table 1. Source of collection and colour of gladiolus genotype

Genotype	Source of collection (Location)	Colour
GL-Akb-001	Floriculture division, BARI	Magenta
GL-Akb-002	Jhikargacha, Jessore	Purple
GL-Akb-003	Jhenaidah	White
GL-Akb-004	Floriculture division, BARI	Pink in periphery with red at center
GL-Akb-005	Jhenaidah	Red
GL-Akb-006	Jhikargacha, Jessore	Orange
GL-Akb-007	Jhikargacha, Jessore	Yellow-orange
GL-Akb-008	Floriculture division, BARI	Vermilion
GL-Akb-009	Floriculture division, BARI	Light-pink
GL-Akb-010	Jhikargacha, Jessore	Pink-red
GL-Akb-011	Jhikargacha, Jessore	Cream
GL-Akb-012	Floriculture division, BARI	Yellow

Results and Discussion

Vegetative and floral characteristics of Gladiolus genotype

In two years consecutive study the statistical comparative data showed almost similar pattern of result for all parameters taken. As regards to the color of flower, the genotypes showed remarkable variation which are shown in Table 1.

The plant height among the different genotypes varied significantly. The highest plant height was produced by the genotype GL-Akb-012 (145.44cm) which was followed by GL-Akb-003(144.15cm) and GL-Akb-005 (138.67cm). On the other hand, the lowest plant height was obtained by the genotype GL-Akb-011 (89.22cm). Variations in vegetative characters may be due to their genetic make-up as well as varietal differences. Similar, results were observed by Pragya *et al*. (2010) and Neha *et al*. (2012) in gladiolus.

Days to spike initiation is very important as it determines the earliness or lateness of the flower crop. Marked differences were observed for days to spike initiation among the genotypes (Table 2). The genotype GL-Akb-011 (67.17 days) took maximum days to spike initiation. On the other hand, the genotype GL-Akb-009 initiated spike within minimum days (50.17 days) followed by GL-Akb-007 and

GL-Akb-008 (53.17 days). Tirkey *et al.* (2018) reported to have spikes of gladiolus after 53.4-67.0 days of planting. Variation in days to spike initiation seem to be genetically controlled as reported by Pragya *et al*. (2010) in gladiolus.

Noticeable variations were observed in spectrum of days required for floral bud initiation in respect of germplasms (Table 2). The lowest days for flowering was observed in case of GL-Akb-009 (63.49 days) followed by GL-Akb-007 (64.16 days). In contrast, germplasm GL-Akb-011 (73.66 days) which was followed by GL-Akb-005 (73.16 days). This variation can be implied for larger duration of flower supply in the market.

Significant variation in respect of spike length was found among the genotypes (Table 2). The longest spike (106.15 cm) was produced by genotype GL-Akb-003 while the shortest spike (60.33 cm) was produced by GL-Akb-011. Whereas, Bhagur (1989) recorded spike length ranged from 50 to 120 cm in varietal evaluation of gladiolus.

The length of rachis varied widely from 45.66 cm to 80.00 cm (Table 2). The highest length was in GL-Akb-003 (80.12 cm). The lowest length (45.33 cm) was in genotype GL-Akb-008. Anuradha and Gowda (1994) observed the highest rachis length in gladiolus of about 50 cm. This variation in spike length and rachis length might be attributed to the inherent genetic characters associated with the genotypes. Similar, observations were reported earlier by Kishan *et al*., (2005); Manjunath and Jankiram (2006) and Neha *et al*. (2012).

Table 2. Vegetative and flowering parameters of Gladiolus genotype

Genotype	Plant height (cm)			Days to spike initiation			Days to flowering		
	Year 1	Year 2	Mean	Year 1	Year 2	Mean	Year 1	Year 2	Mean
GL-Akb-001	118.0	118.21	118.10	58.0	58.21	58.11	69.33	68.00	68.66
GL-Akb-002	110.33	110.12	110.23	59.66	59.00	59.33	70.0	70.66	70.33
GL-Akb-003	144.0	144.30	144.15	59.0	59.33	59.17	70.66	71.00	70.83
GL-Akb-004	114.0	114.55	114.28	54.0	54.66	54.33	66.33	66.99	66.66
GL-Akb-005	138.67	138.31	138.49	62.66	62.33	62.50	73.0	73.33	73.16
GL-Akb-006	110.33	110.42	110.38	59.33	59.00	59.17	69.0	70.00	69.5
GL-Akb-007	110.0	110.30	110.15	53.33	53.00	53.17	64.0	64.33	64.16
GL-Akb-008	112.67	112.13	112.40	54.0	54.33	54.17	66.33	66.00	66.16
GL-Akb-009	107.0	107.34	107.17	50.33	50.00	50.17	63.33	63.66	63.49
GL-Akb-010	105.33	105.12	105.23	63.0	63.66	63.33	72.66	72.00	72.33
GL-Akb-011	89.33	89.10	89.22	67.33	67.00	67.17	73.66	73.66	73.66
GL-Akb-012	145.67	145.21	145.44	54.0	54.66	54.33	66.33	66.00	66.16
CV (%)	3.83	3.81	3.82	4.01	4.03	4.02	2.96	2.98	2.97
LSD (0.05)	7.59	7.41	7.50	3.92	3.94	3.93	3.44	3.46	3.45

Appreciable variation regarding the number of florets per spike among the genotypes was observed and varied from 11.83 to 17.16. The highest number of

florets per spike was produced by GL-Akb-001 (17.16), which was closely followed by GL-Akb-003 (16.49). The genotype GL-Akb-012 produced the lowest number of floret (12.33) per spike. The number of florets per spike varied from 7-17 as reported by Negi *et al.,* (1982) and Lal and Plant (1989).

Table 2. Vegetative and flowering parameters of Gladiolus genotype (cont.)

Genotype	Length of spike (cm)			Length of rachis (cm)			No. of floret/stick		
	Year 1	Year 2	Mean	Year 1	Year 2	Mean	Year 1	Year 2	Mean
GL-Akb-001	100.67	100.0	100.33	67.00	67.33	67.16	17.33	17.00	17.16
GL-Akb-002	89.33	89.20	89.26	56.33	56.00	56.16	13.00	13.00	13.00
GL-Akb-003	106.0	106.30	106.15	80.00	80.25	80.12	16.33	16.66	16.49
GL-Akb-004	84.33	84.00	84.16	50.33	50.00	50.16	13.66	13.33	13.49
GL-Akb-005	74.0	74.63	74.315	56.66	56.96	56.81	14.33	15.00	14.66
GL-Akb-006	69.67	69.21	69.44	53.00	53.86	53.43	15.00	14.66	14.83
GL-Akb-007	67.33	67.00	67.16	49.00	49.69	49.34	14.00	14.33	14.16
GL-Akb-008	71.33	71.52	71.42	45.66	45.00	45.33	11.66	12.00	11.83
GL-Akb-009	69.0	69.21	69.10	51.66	51.00	51.33	13.00	13.00	13.00
GL-Akb-010	71.0	71.20	71.1	47.66	47.23	47.44	12.33	12.66	12.49
GL-Akb-011	60.33	60.23	60.28	51.00	52.14	51.57	13.66	13.33	13.49
GL-Akb-012	93.0	93.10	106.15	58.33	59.00	58.66	12.00	12.66	12.33
CV (%)	3.9	3.92	3.91	3.59	3.62	3.60	5.32	5.41	5.365
LSD (0.05)	5.25	5.23	5.24	3.37	3.39	3.38	1.24	1.25	1.245

Table 2. Vegetative and flowering parameters of Gladiolus genotype (cont.)

Genotype	Weight of single stick (gm)			Vase life (days)		
	Year 1	Year 2	Mean	Year 1	Year 2	Mean
GL-Akb-001	84.67	85.00	84.83	8.33	9.00	8.66
GL-Akb-002	80.33	80.00	80.16	7.33	7.66	7.49
GL-Akb-003	97.67	98.21	97.94	8.0	8.33	8.16
GL-Akb-004	106.67	106.00	106.33	8.33	8.00	8.16
GL-Akb-005	91.00	91.21	91.10	9.0	9.00	9.00
GL-Akb-006	73.33	74.00	73.66	8.0	8.33	8.16
GL-Akb-007	64.00	63.50	63.75	8.66	8.33	8.49
GL-Akb-008	63.33	63.00	63.16	8.66	8.66	8.66
GL-Akb-009	49.33	50.12	49.72	8.0	8.33	8.16
GL-Akb-010	62.67	62.85	62.76	8.0	8.00	8.66
GL-Akb-011	72.33	72.00	72.16	7.66	7.66	7.49
GL-Akb-012	96.33	95.82	96.07	9.0	8.66	8.16
CV (%)	3.64	3.60	3.62	NS	NS	NS
LSD (0.05)	4.83	4.81	4.82	NS	NS	NS

Genotypes showed a wide range of variation among them in respect of spike weight. It ranged from 49.72g to 106.33g (Table 2). Maximum spike weight (106.33 g) was recorded in the genotype GL-Akb-004 and the minimum (49.72 g) was in GL-Akb-009.

A great deal of genotypic variation was observed in case of vase life. Among genotypes, vase life varied from 7.33 to 9 days. The maximum vase life (9 days) was found in the genotype GL-Akb-005 and closely followed by GL-Akb-008 (8.66 days) while the minimum (7.49 days) in genotype GL-Akb-002 and GL-Akb-011. In a varietal performance trial, Lal and Plant (1989) reported that the vase life of gladiolus ranged from 8 to 15 days. Negi et al., (1982) indicated that vase life was an essential character for selection of gladiolus varieties. The difference in vase life might be due to different genetic configuration of the genotypes.

Table 3. Corm and cormel production influenced by gladiolus genotypes

Genotype	No. of corm/plant			No. of cormel/plant		Mean
	Year 1	Year 2	Mean	Year 1	Year 2	
GL-Akb-001	1	1	1	63.66	64.0	63.83
GL-Akb-002	1.33	1.66	1.49	38.33	39.0	38.66
GLAKB 003	1.33	1.33	1.33	75.00	74.15	74.57
GL-Akb-004	1.33	1.0	1.16	54.66	53.36	54.01
GL-Akb-005	1.33	1.33	1.33	79.66	79.25	79.45
GL-Akb-006	2.00	2.00	2	69.33	68.75	69.04
GL-Akb-007	1.33	1.0	1.16	65.33	65.0	65.16
GL-Akb-008	1.33	1.66	1.49	60.66	60.0	60.33
GL-Akb-009	1.33	1.0	1.16	73.33	73.85	73.59
GL-Akb-010	1.66	1.33	1.49	70.33	70.21	70.27
GL-Akb-011	2	2	1	73.33	72.96	73.14
GL-Akb-012	1.66	1.66	1.66	71.00	71.25	71.12
CV (%)	NS	NS	NS	15.39	15.41	15.4
LSD (0.05)	NS	NS	NS	17.25	17.12	17.18

Table 3. Corm and cormel production influenced by gladiolus genotypes (Cont.)

Genotype	Wt. of single corm (g)			Wt. of cormel (g)		
	Year 1	Year 2	Mean	Year 1	Year 2	Mean
GL-Akb-001	128.33	128.00	128.16	2.70	7.75	5.22
GL-Akb-002	145.00	145.25	145.12	1.83	1.80	1.81
GLAKB 003	151.67	151.12	151.39	2.90	2.95	2.92
GL-Akb-004	320.00	319.00	319.50	1.96	1.92	1.94
GL-Akb-005	175.00	175.21	175.10	4.16	4.14	4.15
GL-Akb-006	71.67	70.75	71.21	0.73	.76	0.74
GL-Akb-007	81.67	81.00	81.33	1.50	1.52	1.51
GL-Akb-008	130.00	129.25	129.62	1.06	1.05	1.05
GL-Akb-009	145.00	145.20	145.10	0.73	.75	0.74
GL-Akb-010	96.67	96.45	96.56	1.03	1.00	1.015
GL-Akb-011	207.00	206.78	206.89	0.76	.80	0.78
GL-Akb-012	286.67	285.96	286.31	2.23	2.25	2.24
CV (%)	29.11	30.06	29.58	28.23	28.26	28.24
LSD (0.05)	79.64	79.46	79.55	0.86	.87	0.86

Corm and cormel characteristics in gladiolus genotype

Data on corm production of twelve lines of gladiolus genotypes are presented in Table 3. The number of corm per plant was the highest in GL-Akb-006 and GL-Akb-011 (2.00). The genotype GL-Akb-001produced 1 corm per plant, and GL-Akb-12 produced 1.66 corm per plant. The variation in corm production among the genotypes might be due to difference in genetic constituents as well as environmental effects. Variation (1.0 to 4.0) in corm production among some genotypes of gladiolus was observed at Bangalore in India by Anuradha and Gowda (1994).

Number of cormel per plant was significantly affected by the genotypes (Table 3). The highest number of cormels per plant was obtained from the genotypes GL-Akb-005 (79.45), which was closely followed by GL-Akb-003 (74.57) the lowest was in GL-Akb-002 (38.66). Misra and Saini (1988) recorded 5 to 20 cormels per plant in gladiolus genotypes in a trial.

Genotypes showed a wide range of variability in respect of corm weight. It ranged from 71.21 g to 319.50g. The maximum corm weight was recorded from the genotype GL-Akb-004 (319.50 g) closely followed by GL-Akb-012(286.31 g) and the minimum (71.21 g) in genotype GL-Akb-006. Sharma and Sharma (1984) reported that corm weight varied from 15 g to 60 g in varietal trial of gladiolus conducted in India which was more or less in consonance with the present investigation

Genotypes exhibited a wide range of variability in respect of cormel weight that ranged from 0.74 g to 4.17 g. The maximum weight was found in GL-Akb-005 (4.15 g). The minimum weight 0.74 g was in the genotype GL-Akb-006 and GL-Akb-009 which was closely followed by GL-Akb-011 (0.78 g). Negi *et al.*, (1982) reported that cormel weight in gladiolus ranged from 0.5 g to 1.7 g which is more or less similar with the findings of the present investigation.

Conclusion

The collected gladiolus genotypes showed substantial variation in terms of different characteristics. From two years study, it may be concluded that the gladiolus genotypes such as GL-Akb-001, GL-Akb-003, GL-Akb-005, GL-Akb-008 and GL-Akb-012 were promising in respect of vibrant colours, floret number, rachis length, spike length, vase life and cormel production and can be suggested for cultivation in north eastern climatic condition of Bangladesh.

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DETECTION OF MYCOVIRUS IN *Sclerotium rolfsii*, A PHYTOPATHOGENIC FUNGUS IN BANGLADESH

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Abstract

A research project was conducted to detect presence of mycovirus, a probable virocontrol agent in *Sclerotium rolfsii*, a destructive soil-borne plant pathogenic fungus. A total of 500 isolates of the pathogen was isolated from infected wheat, bush bean, tomato, eggplant, lentil, chickpea, eggplant, chilli and okra grown Bangladesh following tissue planting methods and brought to the Virology laboratory of Okayama University, Japan. After brought to Japan, a total of 472 isolates were re-isolated and the fungal isolates were cultured on potato dextrose agar for extraction of DNA and dsRNA. The extracted nucleic acids were tested for the presence of viruses using rolling circle amplification (RCA) (for DNA viruses) and cellulose column chromatography (for RNA viruses). The detected *S. rolfsii* viruses were further characterized molecularly. For detection of DNA virus, a total of 120 isolates were tested, none were found positive for DNA viruses. For detection of dsRNA virus, a total of 472 isolates were tested, 7 isolates showed possible dsRNA positive band. The partial cDNA sequences of the dsRNA segments isolated from the strain SR336 were obtained. BlastX database search with the partial sequence from SR336 isolates showed similarities with randorna like virus. To screen the presence of randorna like virus in *S. rolfsii* isolates, 64 isolates were selected from 472 isolates of *S. rolfsii*. The isolates of *S. rolfsii* were tested by RT-PCR using randorna virus specific primers**.** Out of 64 isolates 13, (20.31%) isolates were randorna virus positive strain and rest of the isolates were absent of randorna virus.

Introduction

Mycoviruses are viruses which infect fungi. The majority of mycoviruses have double stranded RNA genomes and isomeric particles, but approximately 30% have positive sense, single-stranded RNA genomes. True mycoviruses have an ability to infect healthy plant pathogenic fungi causing mypovirulence and death. Studies in fungal and viral interaction can lead to the development of novel biological control strategies (Cho *et al*., 2013; Longkumer *et al*., 2020). The search of mycoviruses having potential virocontrol properties is focused mainly on identifying and characterizing those viral species in naturally infected fungal pathogens to be controlled (Garcia-Pdrajas *et al*., 2019). Fungal pathogens such as species of *Sclerotium rolfsii, Sclerotinia, Rhizoctonia* and *Fusarium* are ubiquitous and have broad host range enabling them to cause a severe infection resulting in huge yield losses of important crops. Various control tactics like cultural, mechanical are used to control those pathogens. However, effectiveness

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of those techniques may not be satisfactory. From eco-friendly view point, biological control can play vital role in infecting the virulent fungal pathogen by reducing their virulence giving to a phenomenon known as hypovirulence (Longkumer *et al*., 2020). *S. rolfsii* is a ubiquitous, widely distributed, major soil-borne fungal pathogen attacking more than 500 crop species under about 100 families including cereals, vegetables, pulses and oil seeds in the world. The fungus produces resistance spores called sclerotia, which help to survive for long time under adverse condition. It is very difficult to eradicate the fungus from infected fields (Aycock, 1966). In Bangladesh, the destructive pathogen occurs throughout the country and attacks most of the important crops causing considerable yield losses (Mondal *et al*., 1996). To control the diseases mainly cultural and fungicidal management are recommended. However, fungicides sprays often exhibit an associated risk of developing fungicide-resistant fungal isolates (Ma *et al*., 2009; Kuang *et al*., 2011) and may pose potential negative impacts on both environment and food safety (Punja *et al.*, 1982). *Trichoderma* species have long been known for their capacity to reduce plant diseases caused by soil-borne fungi (Whipps and Lumsden, 2001) and some commercial formulations of these bio-control agents are available in many countries of the World. "Virocontrol" is defined as a form of biocontrol of fungal plant pathogens using mycoviruses that infect and weaken target pathogenic fungi. It is environmentally friendly and durable. Many researchers have been inspired by the success of virocontrol of the chestnut blight disease using fungal viruses in Europe. The hypovirulence-associated mycoviruses have potential for exploitation as alternative biological control agents. Mycoviruses are viruses that selectively infect fungi and are ubiquitous in all major groups of filamentous fungi. The presence of some mycoviruses have been associated with particular phenotypic traits, including killer toxin production in *Saccharomyces cerevisiae, Ustilago maydis* and *Zygosaccharomyces bailii* (Schmitt and Neuhausen, 1994), debilitated diseases in *Helminthosporium victoriae* (Nuss and Koltin, 1990) and virulence reduction (hypovirulence) in *Sclerotinia sclerotiorum* (Boland, 1992), *Fusarium graminearum* (Chu *et al*., 2002), *Cryphonectria parasitica* (Anagnostakis 1982) and so on. Identifications of new mycoviruses from *S. rolfsii* will offer new insights into implementation of virocontrol of *S. rolfsii*. The presence piece of research was under taken to detect mycovirus, a probable virocontrol agent in *S. rolfsii* a destructive soil-borne phytopathogenic fungus.

Materials and Methods

Isolation of *S. rolfsii* isolates, their culture and preservation

Virus infected or virus-free *S. rolfsii* isolates were isolated from different infected host plants such as tomato, eggplant, wheat, cucurbits, lentil and chickpea collected from various locations of Bangladesh (Table 1) and cultured on potato dextrose agar (PDA) medium at 25°C±2°C. Growing mycelium tips were sub-

cultured for purification of isolates. The colonies were developed within 3-4 days and sclerotial formation started within a week. A total of 500 isolates of *S. rolfsii* were collected from Bangladesh and brought to the Virology laboratory of Okayama University, Japan. Out of five hundred isolates 472 isolates were re-isolated, preserved and used for further study.

Table 1. Particulars of Sclerotium *rolfsii* isolates

Name of dstrict and upazilla	Name of crops	Total number of isolates	Remarks
Gazipur, Sadar	Wheat	76	The disease infected plant samples were collected during seedling stage of the crops except bush bean, brinjal and chilli where the disease infected plant samples were collected both seedling and maturity stage of the crops
	Barley	58	
	Bush bean	18	
	Lentil	51	
	Chickpea	24	
	Chilli	16	
Jamalpur, Sadar	Brinjal	12	
	Lentil	43	
	Chickpea	06	
Jashore, Sadar	Wheat	45	
	Barley	18	
	Lentil	29	
	Chickpea	19	
Pabna, Ishurdi	Wheat	07	
	Lentil	14	
	Chickpea	11	
Madaripur, Sadar	Lentil	21	
	Chickpea	11	
Barisal, Rahmatpur	Bush bean	04	
	Lentil	12	
	Chickpea	05	
Total		500	

Morphological and molecular characterization of isolates

Initially the *S. rolfsii* isolates were identified based on the morphological characterization. Finally molecular studies were performed by PCR based partially amplification of ITS region and sequencing for identification of *S. rolfsii* isolates.

DNA isolation

Total nucleic acids were prepared from fungal mycelia as described by Suzuki *et al*. (2003). Seven days old fungal mycelia cultured in 20 ml potato dextrose broth (PDB) were harvested onto two layers sterilized Miracloth (Calbiochem). The harvested mycelia were homogenized using a mortar and pestle in the presence of liquid nitrogen and suspended in 4 ml of extraction buffer composed of 100 mM Tris-HCl (pH 8.0), 200 mM NaCl, 4 mM EDTA and 2% sodium dodecyl sulfate (SDS). The suspended solution was transferred to 15 ml Corex tubes containing 4 ml of water saturated phenol/chloroform followed by phenol/chloroform and of chloroform-isoamyl alcohol extraction. The nucleic acid solution was transferred to clean tubes containing 0.1 volumes of 3M NaOAc and 2 volumes of cold ethanol and incubated at 4 ^{0}C for 1 hr. Total nucleic acids were collected by centrifugation at 10000 rpm for 10 min, washed with 70% ethanol, dried, and re-suspended in water.

PCR amplification of ITS region

Polymerase Chain Reaction (PCR) amplification of Internal Transcribed Spacers (ITS) region of rDNA was performed using universal primers ITS-1 (5' - TCC GTAGGT GGA CCT GCG G - 3') as forward primer and ITS-4 (5' -TCC TCC GCT TAT TGA TAT GC - 3') as reverse primer (White *et al*., 1990) in eppendorf PCR master cycle. Amplification was carried out in 0.2 ml eppendorf tubes with 25 µl reaction mixture containing 2.5 µl of 10x Taq buffer, 2.5 µl of 25 mM MgCl2, 2.0 µL of ITS1 primer, 2.0 µl of ITS-4 primer, 0.5 µl of 100 mM dNTP mix, 0.125 µl of Taq polymerase and 14.37 µl of sterile PCR water and 3 µl of DNA sample. The PCR amplification was carried out by 35 cycles, of which denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1.5 min with initial denaturation at 94°C for 4 min before cycling and final extension at 72°C for 6 min after cycling. Amplified PCR products were observed in 1.0 per cent agarose gel in 0.5% TAE buffer and visualized under UV transilluminator with ethidium bromide staining. The size of the PCR product was estimated by comparison with known DNA marker of 1 kb DNA ladder. The banding profiles of ITS-PCR products were documented in gel documentation system.

dsRNA isolation and cDNA library construction

Total nucleic acid was extracted by the method as described by Sun and Suzuki (2008). For dsRNA extraction, all the fungal strains of *S. rolfsii* were cultured on cellophane membranes on potato dextrose agar (PDA) plates for 4 to 5 days at room temperature. The mycelia were collected and ground to a fine powder in liquid nitrogen. Nucleic acid fractions were obtained by treatments with phenol, phenol/chloroform and chloroform. Double-stranded RNA was further purified

by binding with CC41 cellulose. Total nucleic acids were incubated with CC41 cellulose in STE buffer containing 15% (v/v) ethanol for 1 hour. After washing the cellulose with the STE-15% ethanol for 3 times, dsRNA was eluted by STE buffer and precipitated by the addition of 2 volumes of ethanol followed by digestion with S1 nuclease and subsequently with DNase I, as described by Suzuki *et al*. (2003).

A cDNA library of total dsRNA was constructed using a classic non-PCR based method to minimize misincorporations during cDNA synthesis (Hillman *et al*., 2004). After denaturation at 65ºC in 90% dimethyl sulfoxide (DMSO), dsRNA was used as a template for cDNA synthesis with random hexamers using a Time Saver TM cDNA synthesis kit. The resulting cDNA was ligated into the cloning site of pGEM-T Easy vector in Rapid Ligation Buffer with T4 DNA ligase. The resulting ligates were transformed into competent *E. coli* strain DHα5 and incubated in a series of conditions such as on ice for 30 min followed by at 42 ^{0}C for 60 sec and then 3 min on ice. The recombinant was grown in 80 µl SOC medium at 37 ^{0}C for 60 min and spread on LB plate. The plates were incubated overnight for growing the recombinant plasmid. Selected individual plasmid clone was cultured in 3 ml LB containing 50 µg/ml ampicillin for overnight. The plasmids were then purified from *E. coli* using QIAprep Spin Miniprep Kit (QIAGEN) according to the protocol provided by the manufacturer.

Rolling circle amplification (RCA) for detection of ssDNA viruses

Amplification of small circular DNA was performed by rolling circle amplification (RCA) using a TempliPhi™ Kit following the manufacturer's protocol. Master Mix 1 was prepared by mixing 5 µl of TempliPhi Reaction Buffer and 0.2 µl of Tem-pliPhi Enzyme Mix. Five microliters of this TempliPhi premix was mixed with 1 ul of DNA template each fungal isolates then incubated at 30°C for 18 hours. After this incubation period, the enzyme was heat-inactivated at 65°C for 10 minutes. The samples were cooled and stored at 4°C. The RCA products were digested with different restriction enzyme in 10 µl final volumes for 2 h at 37°C, following the manufacturer's protocol, separated in 1 % agarose gels in 0.5 % TAE buffer and stained with 0.5 µg/ml ethidium bromides. The unit-length linear genomes purified from the agarose gels by a Wizard SV gel and PCR clean-up system kit (Promega) that was cloned in specific vector and were transformed into competent *E. coli* strain DHα5. Selected individual plasmid clone was cultured in 3 ml LB containing 50 µg/ml ampicillin for overnight. The plasmids were then purified from *E. coli* using QIAprep Spin Miniprep Kit (QIAGEN) according to the protocol provided by the manufacturer and were sequenced.

Sequencing and sequence analysis

Plasmid DNA was prepared by spin columns and sent to Institute of Plant Science and Resources (IPSR), Japan for sequencing. Sequences were analyzed using the Genetyx DNA-processing software (SDC, Tokyo). Sequence fragments were assembled with AutoAssembler™ 2.0 ABI Prism and analyzed using Genetyx Mac 10.0. Homology search was performed with the BLAST suite of programs from National Center for Biotechnology Information (NCBI) and sequence was aligned following the program CLUSTALW (Thompson *et al*., 1997).

Results and Discussion

Morphological characterization of *S. rolfsii* isolates

A total 500 isolates of *S. rolfsii* were isolated from infected plant parts grown in Bangladesh and brought to the Virology laboratory of Okayama University, Japan. After brought to the Japan, a total of 472 isolates were re-isolated and identified on the basis of morphological characterization.

Molecular characterization of *S. rolfsii* isolates by ITS-PCR

The structure of rDNA cluster and the expected amplified products with ITS-1 and ITS-4 primers are shown in Fig. 1. The primers ITS-1 and ITS-4 were used for PCR amplification of ITS region of rDNA cluster which included ITS-1 and ITS-2 regions of all 472 isolates. Both the primers produced amplified product size of 650-700 bp in all the 472 isolates. These results confirmed that all the isolates belonged to genus *Sclerotium*. Harlton *et al*. (1995) screened a worldwide collection of *S. rolfsii* which revealed variation in ITS regions of 12 sub-groups of *S. rolfsii*. Almeida *et al*. (2001) studied variability among 30 isolates of *S. rolfsii* by RAPD and were differentiated into distinct groups by ITS-PCR.

Detection of double-stranded RNA fragments/mycovirus in *S. rolfsii* isolates

All 472 *S. rolfsii* isolates were tested for the presence of putative dsRNA viruses. Extracts of dsRNA from mycelia of all 472 *S. rolfsii* isolates were treated with DNase I and S1 nuclease, and then subjected to agarose gel electrophoresis. One or two distinct dsRNA segments/band was observed in several strain of *S. rolfsii* (Fig. 2). DsRNA of selected 7 fungal strain possible virus origin of those fungal strains were reverse-transcribed and used for library construction for sequence analysis. The partial cDNA sequences of the dsRNA segments isolated from the strain SR336 were obtained (Fig. 3). BlastX database search with the partial sequence from SR336 isolates revealed that the isolated dsRNA was the partial sequence showed similarities with randorna like virus. Two pairs of primer from the partial sequence of SR336 isolates were desinged. To search the presence of

randorna like virus in *S. rolfsii* isolates, randomly 64 isolates were selected from 472 isolates of *S. rolfsii*. The selected isolates of *S. rolfsii* were tested by RT-PCR using randorna virus specific primers**.** Results showed that out of 64 isolates *S. rolfsii*, 13 (20.31%) showed randorna virus positive strain (Fig. 4). Subsequent necessary analyses were carried out by the host laboratory.

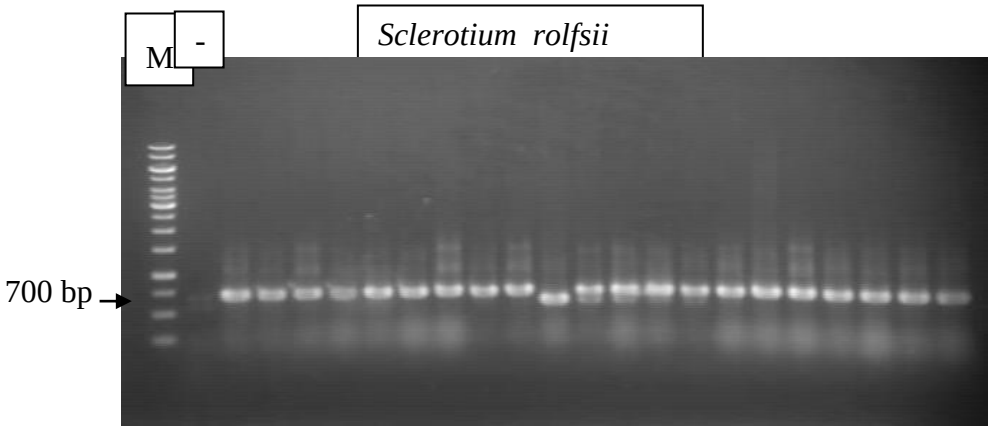

Fig 1. Amplification product of Internal Transcribed Spacer (ITS) with ITS-1 and ITS-4 ribosomal DNA primers; M=1 kb DNA ladder. All Lanes represented *S. rolfsii* isolates.

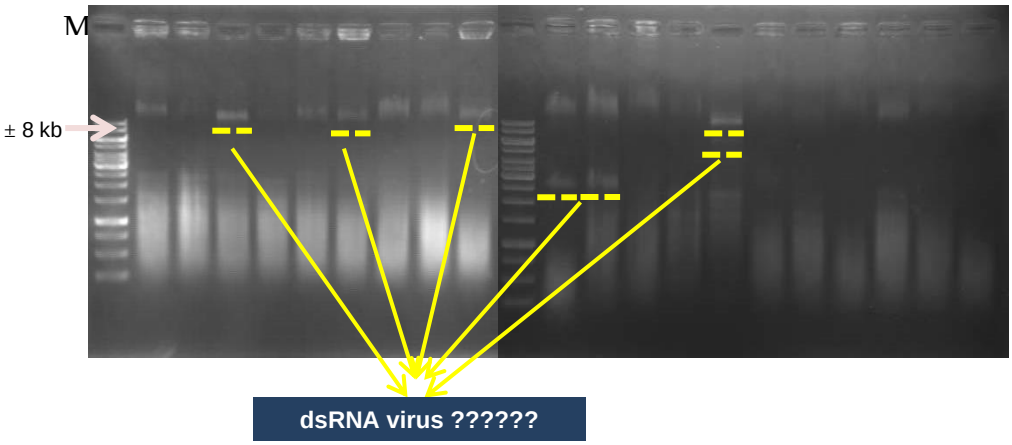

Fig. 2 Gel electrophoretic profiles of dsRNAs isolated from *S. rolfsii* isolates. DsRNAs purified from different strains of *S. rolfsii* and were analyzed in 1% agarose gel electrophoresis. A GeneRuler 1-kb DNA ladder (Thermo Scientific) was also used as a marker.

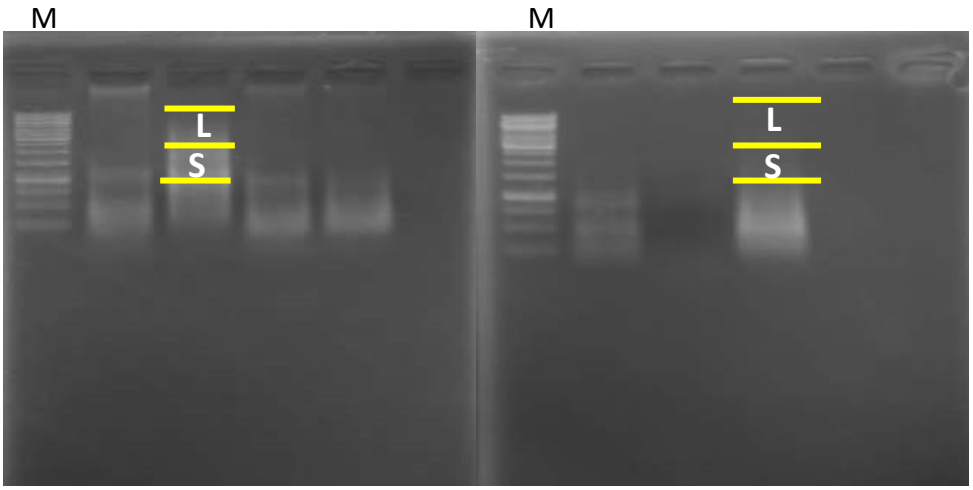

Fig. 3 Gel electrophoretic profiles of constructed cDNA of *S. rolfsii* isolates. For construction of cDNA purified dsRNA from different strains of *S. rolfsii* were used and analyzed in 1.5% agarose gel electrophoresis. A GeneRuler 1-kb DNA ladder (Thermo Scientific) was also used as a marker.

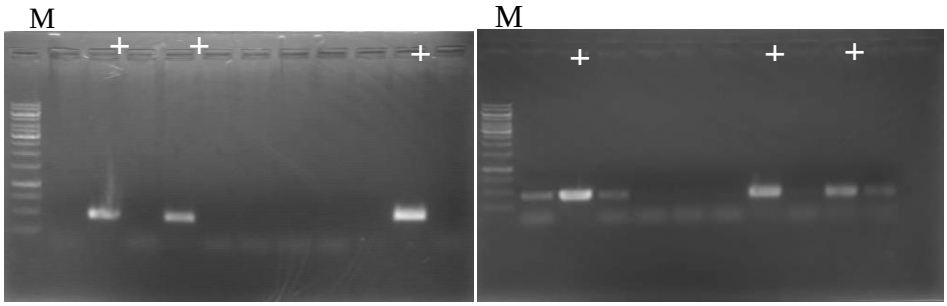

Fig. 4 Gel electrophoretic profiles of constructed cDNA of *S. rolfsii* isolates. For RT-PCR purified dsRNA from selected different strains of *S. rolfsii* were used with rendorna virus specific primers and analyzed in 1.5% agarose gel electrophoresis. A GeneRuler 1-kb DNA ladder (Thermo Scientific) was also used as a marker.

Detection of ssDNA viruses by Rolling Circle Amplification in *S. rolfsii* isolates

To detect the presence of in different strains of *S. rolfsii* isolates, rolling circle amplification (RCA) by PCR with restriction enzyme (RE) digestion was employed. A total of 35 isolates of *S. rolfsii* were randomly selected from 472 isolates of *S. rolfsii* for detection of ssDNA viruses associated with *S. rolfsii* strains. The RCA products were directly Hind III digested, and restriction products were separated by gel electrophoresis (Fig. 5). The resulted fragments were purified by a Wizard SV gel and PCR clean-up system kit (Promega). The

purified fragments were ligated into the cloning site of pGEM-T Easy vector (Promega) with T4 DNA ligase (Promega). The resulted ligates were transformed into competent *E. coli* strain DHα5 and the selected transformants were then used for colony PCR using M13F and M13R primers set to identify the positive transformants. Results showed that none of the transformants were found positive transformants which indicated that selected RCA fragments were not ssDNA virus (Fig 6). The results of the present study indicated that ssDNA virus was not associated with the tested 35 isolates of *S. rolfsii*. Therefore, more fungal strains should be tested the host laboratory for this purpose.

Conclusion

The present finding is the first evidence of mycovirus in a plant pathogenic fungus *S. rolfsii* from Bangladesh. This report might be helpful for more study of mycovirus in other plant pathogenic fungi occur in Bangladesh.

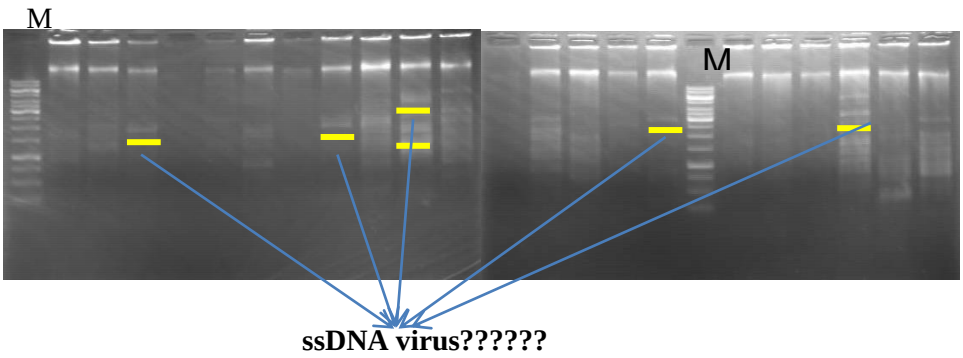

Fig. 5 Gel electrophoretic profiles of RCA products digested with Hind III. DNA purified from different strains of *S. rolfsii* used as a template for RCA reaction by TempliPhi kits and were analyzed in 1% agarose gel electrophoresis. A Gene Ruler 1-kb DNA ladder (Thermo Scientific) was also used as a marker.

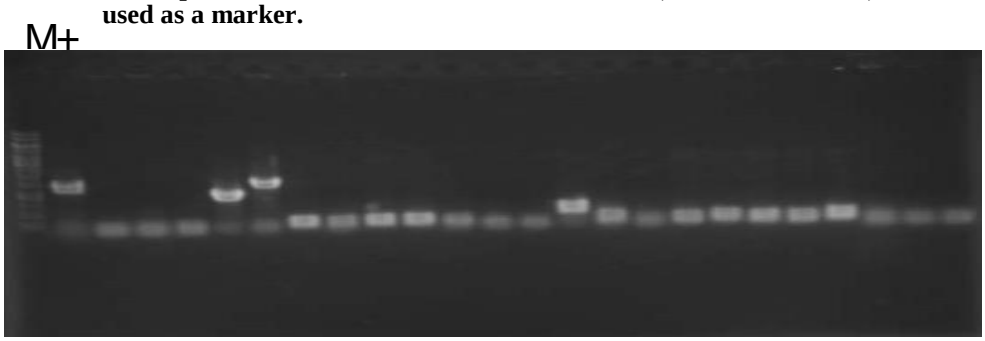

Fig. 6. Gel electrophoretic profiles of Colony PCR of *E. coli* transformants. The purified ssDNA fragment of RCA reaction were ligated into the cloning site of pGEM-T and transformed into competent *E. coli* strain DHα5. The selected colony of *E. coli* transformants were used for PCR with M13F and M13R primers and were analyzed in 1% agarose gel electrophoresis. A Gene Ruler 1-kb DNA ladder (Thermo Scientific) was also used as a marker.

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QUALITY ASSESSMENT OF THE FARMER'S PRODUCED SEED POTATOES

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A.T.M. S. ISLAM[4] AND A. HANNAN[5]

Abstract

The experiment was conducted at the research field and post graduate laboratory of Plant Pathology Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur, during 2015 - 2016 to find out the performance of different source's seed potatoes and select suitable methods for improvement of farm saved seed potatoes. In the experiment, farm saved seed was compared with the certified seed, positive selection's seed, seed plot technique's (STP) seed Potato and Truthfully level seed potato as of BARI Alu-8(Cardinal). The maximum yield (27.72 t ha^{-1}) was recorded from positive selection's seed, which was similar to Certified and SPT seeds. The maximum plants of farmer's seed potatoes (≥ 8 %, 7.33 % and 4.17 %) were infected by PVY, PLRV and PMV, respectively. The seed potatoes of positive selection and seed plot technique were the best alternatives to supplement the certified seed.

Keywords: Positive selection, certified seeds, seed plot technique, seed potatoes.

Introduction

Potato (*Solanum tuberosum* L.) belonging to the family Solanaceae is an economically important crop of the world. It is also very popular in Bangladesh because of its high profit return. It gives remarkably high yield and produces more palatable energy per unit area and time than many other crops. The adaptation of potato cultivation in developing countries is increasing as food crop (Naik and Karihaloo, 2007). Potato is the 2nd most important food crop of Bangladesh next to rice; it is mainly used as a vegetable crop, auxiliary to the main food. It is also world leading crop that furnishes appreciable amount of vitamin-B and Vitamin-C as well as some minerals (Thomson and Kelly, 1997). In Bangladesh, total land area under potato crop in 2019-20 was 4,61,538 hectares compared to 4,23,887 hectares in 2009-10, which is 8.88% higher. Average yield rate of potato has been estimated 20.81 metric tons per hectare and total potato production has been estimated 9606 thousand metric tons at 2019-20 compared to 7930 thousand metric tons of the year 2009-10 which was 21.13 % higher (BBS, 2020). The seed rate of potato is very high which increases the total production cost, and it is counted as a major inhibitor to use the quality seeds by the growers. The major constraints in potato production have been identified as the unavailability of quality and healthy

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seed potatoes, difficulties in the production and distribution of disease free seeds, wide range of pest and diseases, inadequacy of cold storage facilities, etc. resulting in rotting and sprouting, and violent price fluctuation (Hoque and Sultana, 2012). So, it is essential to ensure quality seed potato within farmer's ability. Use of low quality seed is one of the main reasons for low yield of potato. Only 10% quality seed potato of the total requirement is being supplied by different government and other private organizations. The remaining 90% seed requirement is being mitigated by the farmers' retained seed which is usually of poor quality (Kabir and Haque 2012). On the other hand, improving the quality of seed potato is considered as a pathway to improve farmers potato yields and income (Getachew and Mela, 2000; Tindimubona *et al.,* 2000; Eshetu *et al.,* 2005; Hirpa *et al.,* 2010). For these reason, it is essential to increase the quality of farmer's own seed potato, which can directly decrease the yield gap between the farmers and potential yield. A large group of potato growers use their farm saved low quality seed tubers. Considering these the experiment was undertaken to find out the performance of different source's seed potatoes and select suitable methods for improving farm saved seed potatoes in Bangladesh.

Materials and Methods

The experiment was conducted at the research field and post graduate laboratory of Plant Pathology Division, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, during November 2015 to March 2016. Maximum temperature, minimum temperature, humidity (%) and total rainfall during the cropping period are presented in Fig. 1. Soil texture of the experiment field was clay loam to sandy loam having P^H of 6.25. The experiment was laid out in a Randomized Complete Block Design (RCBD) with 03 (three) replications. The unit plot size was 2.4 m × 4.0 m with 60 cm × 25 cm spacing. Treatments were as (i) Farmer's seed, (ii) Seeds of Positive selection, (iii) Certified seed, (iv) Seeds of seed plot technique and (v) Truthfully level seed of the most popular variety BARI Alu-8 (Cardinal). The farm saved seed potatoes was collected from farmer's stock who used certified seed. The seed potatoes of positive selection was collected directly from the farmer's (who used certified seed) fields by selecting healthy and vigorous vegetative mother plants at 65 days after planting. The seed potatoes of seed plot technique (SPT) was collected from a farmer's (who used certified seed) separately cultivated plot, situated at multi-locational trial (MLT) site of Ranigonj, On-farm Research Division (OFRD), Bangladesh Agricultural Research Institute (BARI), Dinajpur. The truthful level seed potato was collected from a local seed company (M N Agro & Traders, Thakorgong). The certified seed potatoes were collected from Bangladesh Agricultural Development Corporation (BADC), Thakorgong. The tillage operations were done for 4-5 times during 25 Oct to 03 November 2015. Fertilizers were applied @ N-P-K-S-Mg-Zn-B as of 90-20-90-10-5-2-0.5 $kgha^{-1}$ and compost of cowdung @ 5 t ha^{-1}. The rest half of N and half of K and full dose of other fertilizers were applied as basal dose before final land

preparation. The rest half of N and K was side-dressed at 35 DAP (FRG 2012). The well sprouted whole tubers were planted as per treatments on 4 November 2015. Planting was done at the depth of 5-7 cm at 25 cm × 60 cm spacing. After planting the tubers was covered with soil. Earthing up and weeding were done two times during growing period. The first earthing up was done after planting and the second earthing up was done at 35 DAP (Days after planting). Irrigation was provided thrice throughout the growing period at 10, 35 and 50 DAP. Dursban @ 5 ml per litre and Metasystox @ 1ml per litre was applied respectively to control cut worm and aphid. The fungicide Mencozeb @ 2.0 gm per liter were sprayed from 100% plant emergence and at 10 days interval as routine spray for seed potato production to keep the potato plants free from late blight infection. The crop was haulm pulled at 75 DAP. After 10 days the crop was harvested. The ten plants were harvested at the first time from each plot and then the rest of the plants were harvested with help of a country plough and spade. Care was taken to avoid injury in potatoes during harvesting. Emergence percentage was recorded by eye observation at 20 DAP and 30 DAP. The percent disease incidence of different diseases was recorded by observing the symptoms of diseases like Potato leaf roll virus (PLRV), Potato mosaic virus (PMV), Potato virus Y (PVY), Late blight (LB) of potato and Bacterial wilt, etc. For confirmation of viral diseases ELISA (enzyme-linked immunosorbent assay) test were done at Tuber Crop Research Centre, Bangladesh Agricultural Research Institute, Gazipur. The incidence of PLRV, PVY and PMV and bacterial wilt was assessed with the following formula-

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

The yield was recorded by calculation of weight of tubers per plot after harvest. Tubers were graded into four categories namely over size (> 55 mm), "B" grade (40-55 mm), "A" grade (28-40 mm) and small size (< 28 mm) tuber (Sarker *et al.*, 2018). Tuber number and weight per plant, seed tuber number and weight per plant were determined. The number of infected tubers by Common scab, *Rhizoctonia* canker and deformed tuber were counted. The disease incidence (%) was calculated according to the equations given below.

$$\text{Disease incidence (\%)} = \frac{\text{Total no. of infected tubers}}{\text{Total number of tubers}} \times 100$$

Percentage of deformed tuber were calculated using the following formula:

$$\text{Deformed tuber (\%)} = \frac{\text{Number of deformed tuber}}{\text{Number of total tuber}} \times 100$$

Data were subjected to statistical analysis to find out the levels of significance of the experimental results. The mean of all the treatments were calculated and analysis of variance was performed and the mean differences were adjudged by

Duncan's Multiple Range Test (DMRT) at 5% level of probability using MSTATC, the statistical computer package program (Russell, 1986).

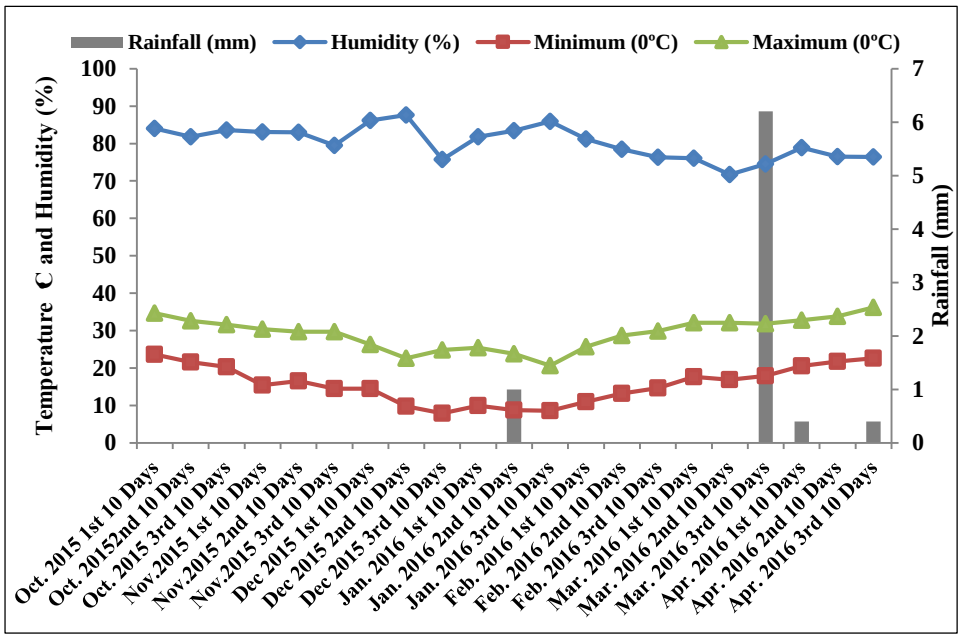

Fig. 1. Ten days average maximum temperature, minimum temperature, humidity and rainfall during the cropping period of 15-16 at HSTU, Dinajpur.

Results and Discussions

The quality of farmer's seed potato is most remarkable and noticeable subjected to low cost quality seed potato production.

Emergence of seed potato

The maximum emergence percentage of potato was remarkably influenced by the quality of seed which is directly related with the source of seeds. The maximum emergence percentage (98.96) at 20 days after planting (DAP) was recorded from positive selection's seeds which was statistically similar to the certified seed and seeds of seed plot techniques (SPT) and the minimum emergence percentage (89.38) was recorded from farmer's seed (Table 1). The emergences of positive selection's seed potatoes were faster than others. The result was supported by the findings of Okeyo *et al.,* (2018), where they stated that positive selection was a good management strategy to manage the seed borne viruses of potato.

Plant population at haulm pulling time

The healthy potato plants can survive more time in the field than the diseased ones. The highest plant (98.96%) at haulm pulling time was recorded from positive

selection's seeds. The lowest plant (85.82%) recorded from Farmer's seed (Table 1). Good quality seed tuber has the potentiality to good field performance. The result was corroborated with the findings of Okeyo *et al.,* (2018) and Gildemacher *et al.,* (2007**),** where they stated that positive selection seed potatoes were better than normal seed tubers.

Tuber number per plant

The tuber number per plant is very important for yield and quality of seed potato. The significant variation was also observed in tuber number per plant among the different sources of seed potatoes. The maximum tuber number (8.24) per plant was recorded from positive selection's seed potatoes, which was statistically similar with the certified seed and seed plot technique's seed potato. The minimum tuber number (5.96) per plant was counted from the truthfully labelled seed tuber which was statistically similar with farmer's own seed potato (Table 1). The seed potatoes of positive selection produced maximum tuber number per plant might be due to good vegetative growth and low disease susceptibility then other source's seed potato. The result was supported by the findings of Okeyo *et al.,* (2018). Where they stated that the field of positive selected seed potato had low visual virus incidences, higher number of tubers per hill and yield (t ha^{-1}) and low virus incidences of PVS (47%), PVY (0.0%), PLRV (0.0%) and PVM (0.0%), tested by ELISA from the plots of positive selection's seed potato.

Table 1. Status of different sources seed potatoes on plant population and tubers per plant

Treatments	Emergence 20 DAP (%)	Emergence 30 DAP(%)	Plants at Haulm pulling (%)	Number of tubers /plant
Farmer's seed	89.38	97	85.42	6.18
Positive selection's seeds	98.96	100	98.96	8.24
Certified seed	95.83	100	98.96	8.22
Seeds of SPT	96.67	100	97.92	7.46
TLS	90.62	99	95.83	5.96
$LSD_{0.05}$	4.69	NS	3.17	1.14
CV (%)	2.64	1.57	1.76	8.39

Performances of different sources of seed potatoes on grading of tubers and yield

Significant differences were observed in different sources of seed potatoes regarding weight of different sized tuber per plant and yield (Table-2). The maximum weight of "A" grade seed tubers were observed from the seeds of SPT which was similar to certified and positive selection's seeds. But the maximum

weight of "B" grade (257.26 g) tubers were observed from the positive selection's seed potato and the minimum weight (198.54 g) from farmer's seed. The maximum weight (70.31 g) of over sized tubers were measured from farmer's seed, which was similar to positive selection's seeds. The maximum weight of tuber per plant was measured in positive selection's seeds which were similar to certified and seeds of SPT and the minimum weight (329.48 g) was recorded in farmer's seed. The maximum yield (27.72 t ha^{-1}) was recorded from positive selection's seeds which was similar to certified and seeds of SPT and the minimum weight (21.97 t ha^{-1}) was observed in farmer's seed (Table 2). The positive selection's might maximize tuber weight per plant and increase yield. The result were supported by the findings of Okeyo *et al.,* (2018), Gildemacher *et al.,* (2011) and Anonymous 2012. Where it was stated that positive selection plots had low visual virus incidences, high number of tubers per hill and increase average yield.

Table 2. Status of different sources of seed potatoes regarding grades of tubers and yield

Treatment	Weight of tuber per plant (g)					Yield (t ha^{-1})
	<28 mm	28-40 mm	40-55 mm	>55 mm	total	
Farmer's Seed	13.13	47.50	198.54	70.31	329.48	21.97
Positive Selection's Seed	17.60	76.04	257.26	64.86	415.77	27.72
Certified Seed	16.25	77.34	250.49	38.59	382.68	25.51
Seeds of SPT	17.42	91.77	229.58	45.21	383.98	25.60
TLS	9.90	54.27	207.29	62.92	334.37	22.29
$LSD_{0.05}$	NS	16.34	22.05	18.31	34.41	2.29
CV (%)	22.44	12.51	5.12	17.24	4.95	4.95

Status of different source's seed potatoes on viral diseases

The field performance of different seed sources on viral diseases like potato virus Y (PVY) potato leaf role virus (PLRV) and potato mosaic virus (PMV) are shown in Fig. 2. The maximum plants (more than 9%) were infected by PVY in Farmer's seed. The minimum plants (2.1%) were infected by PVY in positive selection seed or certified seed (2.1%). Considering PLRV infected plant, severely infection was observed in TLS (8.3 %) and farmer's seeds (7.3%). the minimum number of PLRV infected plants were counted from positive selection seed or certified seed or TLS seeds. The similar trend was also recorded in PMV infected plant, where the maximum (5.2 %) infection was observed in farmer's seeds. The minimum (1.0%) PMV infected plants were recorded from certified seed. The healthy seed potato collecting from seed plot technique or positive selection was comparatively disease free and alternate option for different virus management. These results were supported by Anonymous 2012, Okeyo *et. al*., (2018), Thomas-Sharma *et al.,* (2016), and Schulte-Geldermann *et al.,* (2012), where they stated that different

viral diseases of potato were managed through use of positive selection seed, or SPT seed or certified seed.

Status of different source's seed potato of bacterial wilt and black scarf

The maximum plants were infected by wilt at 60 DAP in farmer's seed (3.13%), the minimum plants are infected in the field of certified (0.00%) and seeds of SPT (0.00%). On the other hand, the maximum plants were infected by Black scarf **(***Rhizoctonia* canker) in the plot of farmer's seed (3.13%), the minimum plants infected by Black scarf **(***Rhizoctonia* canker) both in the field of positive selection seed (0.00%) (Fig 3). Positive selection decrease seed borne diseases of potato. These result were supported by Gildemacher *et al.*, (2011), Anonymous 2012 and Hossain *et al.*, (2010) they found that incidence of virus or bacteria in potato field were very low in the field of healthy and clean mother seed potato and positive selection can reduce bacterial wilt.

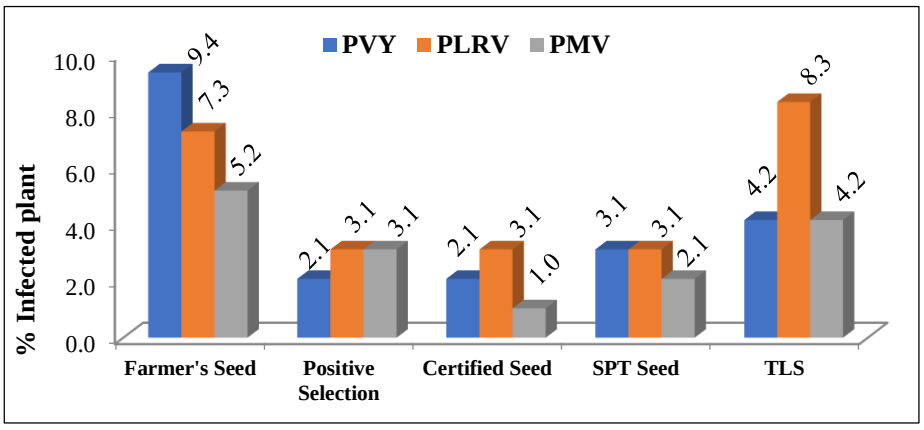

Fig. 2. Status of different sources of seed potatoes regarding viral diseases.

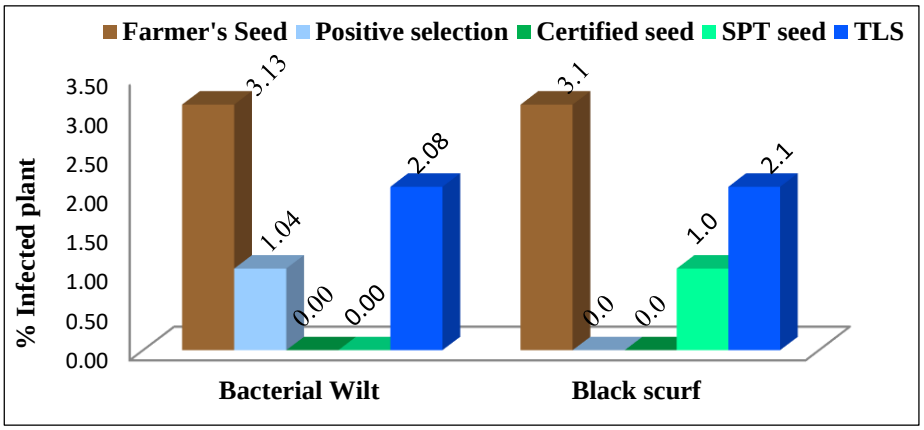

Fig. 3. Status of different sources of seed potatoes regarding bacterial wilt and black scurf.

Status of different sources of seed potatoes on tuber diseases and marketable potato

Potato tubers, from the seeds of different sources were infected significantly by *Rhizoctonia* canker and common scab. The highest infected tuber (1.00%) of *Rhizoctonia* canker were found in the field of TLS which was similar with farmer's seed. The lowest infected tuber (0.00%) were found in the field of certified seeds. Considering the common scab, the maximum (2.01%) infected tuber recorded from farmer's seed, which was similar with TLS (1.56%). In case of deformed or physiological disorder tuber, largest amount (7.36%) was counted in farmer's seeds. The lowest amount (1.55%) of deformed tubers was found in positive selection seed, which was similar with the tubers of certified or SPT seeds (Table 3). The quality of mother seed potato is related to produce marketable potato. The results was also supported by Anonymous (2012), where it was reported that incidence bacterial diseases and common scab were very low in seed plot techniques compared to farmer's practice.

Table 3. Status of different sources seed potatoes on tuber diseases and marketable Potato

Treatments	Infected tuber (%) (wt. basis)		Deformed tuber (%) (wt. basis)	Non-marketable Tuber (%) (wt. basis)	Marketable Potato (%) (wt. basis)
	Rhizoctonia Canker	Common Scab			
Farmer's Seed	1.00	2.01	7.36	14.22	85.78
Positive Selection's Seeds	0.39	0.91	1.55	4.02	95.98
Certified Seed	0.00	0.77	1.68	3.36	96.64
Seeds of SPT	0.42	0.42	1.81	3.62	96.38
TLS	1.39	1.56	6.65	12.54	87.46
$LSD_{0.05}$	0.56	1.37	2.70	5.97	5.97
CV (%)	46.73	53.59	37.65	42.01	3.43

Farmer's seeds were infected with different diseases. So, the maximum (14.22%) tubers were nonmarketable and minimum (85.78%) tubers were marketable. On the other hand, the minimum non marketable (3.36%) tubers and maximum marketable (96.64%) tubers produced by certified seeds which was similar to positive selection or SPT seed potatoes.

Economic analysis

Benefit-cost analysis of potato production from different sources seed potatoes are presented in Table 4. The highest gross return (266056.6 $Tkha^{-1}$), net return (144056.6 $Tkha^{-1}$) and benefit cost ratio (2.2) was recorded from positive selection's seeds. But, closest benefit cost ratio (2.1) was recorded in seeds of seed plot technique. The lowest gross return (73958.66 $Tkha^{-1}$) was calculated in farmer's seed potatoes.

Table 4. Partial benefit cost ratio of potato production from different sources of seed potatoes

Treatments	Yield (t ha^{-1})	Marketable yield (t ha^{-1})	Gross return (Tk ha^{-1})	Cost (Tk ha^{-1})	Net return (Tk ha^{-1})	BCR
Farmer's seed	21.97	18.85	188458.7	114500	73958.6	1.6
Positive selection's seeds	27.72	26.61	266056.6	122000	144056.6	2.2
Certified seed	25.51	24.65	246528.6	137000	109528.6	1.8
Seeds of SPT	25.60	24.67	246732.8	117500	129232.8	2.1
TLS	22.29	19.49	194948.3	129500	65448.34	1.5

Here, certified seed potato = 35.00 Tk kg^{-1}, TLS= 30.00 Tk kg^{-1}, Positive Selection's seed potato= 25.00 Tk kg^{-1}, Farmer's Seed potato = 20.00 Tk kg^{-1}, Seed potatoes of SPT= 22.00 Tk $kg^{-1}$and Ware Potato = 10.00 Tk kg^{-1}, Urea 16 Tk kg^{-1}, TSP 22 Tk kg^{-1}, MoP 15 Tk kg^{-1}, Gypsum 25 Tk kg^{-1}, $ZnSO_4$ 220 Tk kg^{-1}, Boric Acid 420 Tk kg^{-1}, Labour 400 Tk Day^{-1} $Person^{-1}$ and Land rent 15,000 Tk $season^{-1}$ ha^{-1}.

Conclusion

From the aforementioned results and discussions, it can be concluded that the tuber yield was higher from the positive selection's or seed plot technique's or certified seed potato. The disease development was lower in positive selection's or seed plot technique's or certified seed potatoes. Moreover, considering the gross return and benefit-cost ratio, the seed potato of positive selection or seed plot technique are the best alternatives to supplement the certified seed. Potato farmers can now choose to either buy commercial seed potatoes or practice positive selection or seed plot technique (SPT).

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