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CONTENTS

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POMOLOGICAL TRAITS AND PROFITABILITY OF SELECTED VELVET APPLE GERMPLASM

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Abstract

The research was accomplished during February to August 2021 at Germplasm Center (GC) of Patuakhali Science and Technology University (PSTU) and farmers' homesteads in Patuakhali district to find out the different characteristics, fruit yield and profitability of four velvet apple germplasm. The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. Results manifested that there were significant variations among the germplasm, where local red germplasm exhibited the highest plant height (5.5 m), plant diameter (16.23 cm), number of main branches (12.8), length of the largest branch (3.9 m), plant canopy (8.80 m^2), leaf area (135.2 cm^2) but those traits were minimum in PSTU Bilati gab-2. PSTU Bilati gab-2 emerged floral bud for the longest duration (32.3 days). The measured length of inflorescence before first flowering varied from 5.03 – 6.60 cm where it required 24-28.3 days for development. A range of flowers per inflorescence (7.8 – 11) and duration of flowering (46.5 – 59.3 days) were observed in all the studied germplasm. The percentage of fruit set (18.3 – 30.8) per plant with individual fruit weight 126.70 to 202.43 g. The highest pulp weight (103.9 g) was obtained from PSTU Bilati gab-2 and the lowest (69.25 g) from local yellow germplasm. PSTU Bilati gab-2 was completely seedless. So, it contained the highest edible portion (81.92%). The highest fruit yield (3.5 t/ha) was recorded in PSTU Bilati gab-1 but maximum net profit (251160 Tk/ha) was obtained from PSTU Bilati gab-2 with the highest benefit cost ratio (BCR) of 3.81. Considering percent fruit set, percent edible portion, fruit yield and profitability, PSTU Bilati gab-2 was found to be the best among the four germplasm.

Keywords: Fruit yield, Germplasm, Profitability, PSTU Bilati gab, Velvet apple

Introduction

Velvet apple (*Diospyros discolor* Wild.) is an evergreen tree under the family Ebenaceae (Greuter, 2000), designated as cultivated exotic fruit in Bangladesh (Pasha *et al.*, 2019), which is originated from Philippines (Singh, 1998). Particularly it is native to low and middle altitude of Philippines which later introduced to Java, Malaysia, India and subsequently in all tropical area. It has several English names like 'Mabolo', 'Butter fruit', 'Camogan ebony', 'Velvet persimmon' but in Bangladesh it is termed as 'Gab' (Lim, 2012). It is commonly found in Assam, Bihar and southern part of India. In

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Malaya, it is known as 'buahmantega' or' buahsakhlat' or 'sagalat' and recently it has been reducing in number there. As an ornamental plant, it is seldom planted in India (Haque *et al.*, 2020). Though it has been growing for many decades in Bangladesh but widely grown in the district of Kustia, Jashore, Faridpur, Rajshahi, Barishal, Pirozpur, and Chattogram Hill Tracts region (Ahmed *et al.*, 2011).The plants of velvet apple thrive in a variety of soils and environments, from sea level to 750 meters (Hossain *et al.*, 2015) and they are dioecious, extensively branched, grow to heights of 7–32 m. They have a stout trunk of 50–80 cm diameter, dark brown to black bark with furrows having conical shape crowns. Shape of leaves are categorized as elliptical-oblong to oblong, 20–29 cm length and 6-11 cm width, with obtuse base and acuminate apex, dark green upper surface, glossy, glabrous and lower surface silvery, petiole up to 1.7 cm long and densely pubescent. Leaves at young stage are pinkish to pale green and silky hairy (Haque *et al.*, 2020 and Lim, 2012). The inflorescence of velvet apple is termed as axillary cymes, solitary or cluster where three to seven flowers arises from the leaf axile. Flower are unisexual, incomplete, regular, bracteate, hypogynus, 3-5 sepals, 4-5 petals, 3-5 stigmas with 4 multicarpellary superior ovary. The length of sepal varies between 0.8-1.3 cm and breath 0.8-1.2 cm where the area varies between 0.64 -1.56 cm^2. In case of petal, the length varies between 1.5-2.0 cm, breath 2.0 -2.6 cm and the area varies from 3.01-5.20 cm^2. On the other hand, stigma length varies from 0.3-0.6 cm (Fakir *et al.*, 2018).

The fruit is very tasty and attractive due to its beautiful reddish color (Haque *et al.*, 2020). The surface of the fruit is covered by brown or dull red powdery velvet structure. Although, ripe fruits are delicious, often served as dessert because of its sweet and melting pulp but, immature fruits are astringent. Owing to its sweet taste and aroma, people including children like it too much (Ahmed *et al.*, 2011). Despite being nutritious fruit, it is underutilized as human food because of its nauseous odor comparable to spoiled cheese or cat feces and the rind hair which is irritating to sensitive skin (Pobar, 2013). Fruits are nutritionally rich which contain (per 100 g of edible portion) calories 113 cal, water 69.6 g, carbohydrates 26.6 g, fibre 1.5 g, fat 0.1 g, protein 1.4 g, minerals 0.8 g, calcium 58 mg (Mondal, 2000). The moisture content of different velvet apple germplasm ranges from 77.5-82.5% where range of the approximate dry matter is 17.5-22.5% (Haque *et al.*, 2020). Apart from seed propagation, velvet apple can be propagated by grafting. Seedling plants are normally planted from 25 to 30 feet from each other (Hossain *et al.*, 2015). As a minor tropical fruit, velvet apple is not extensively cultivated, consumed and traded in Bangladesh because of being very limited both geographically and quantitatively, than those of major fruits (Saúco, 2013). Less emphasis on characterization and commercial cultivation as well as lack of proper documentation are the main problems of velvet apple production. Moreover, it is a high yielding plant with minimum investment. Considering the cost of production, the commercial cultivation of velvet apple will be more profitable. In 2015, Patuakhali Science and Technology University (PSTU) registered two varieties of velvet apple, where one having very few seeds namely "PSTU Bilati gab-1" and another was completely seedless variety namely "PSTU Bilati gab-2" but both of these were delicious comparing with local or wild germplasm. As grafted trees both of the germplasm become dwarfed height. Before commercial cultivation, a comparative pomological traits and profitability of the varieties needed to be checked.

Materials and Methods

Experimental materials and procedures

The research was conducted at Germplasm Centre, PSTU and different locations of Patuakhali district (located in between $21^0$48' and $22^0$36' north latitudes and in between $90^0$08' and $90^0$41' east longitudes) of Bangladesh during the year 2021. Four velvet apple germplasm, namely local red, local yellow, PSTU Bilati gab-1 and PSTU Bilati gab-2 were included in this study. The local germplasm were spontaneously grown farmers trees while PSTU varieties were grafted trees. However, the fruiting year-2 was commonly considered for selecting each of the germplasm. The experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. Data were collected following the descriptor (reference) of Ebenaceae family and collected data were analyzed using JMP 14 computer program.

Plant characteristics

The height of plants, bole height, diameter and length of large branches were measured in meter (m), branching pattern and number of main branches were counted from the experimental sites. Considering the width of branches expansion as radius (r), the canopy was measured by this formula of, canopy = πr^2. The leaf area was measured by a leaf area meter (Mod: LAM-B).

Flower and fruit characteristics

Samples of flowers and fruits were collected from each location and carried them to postharvest laboratory of Department of Horticulture, PSTU. Data on flower and fruit characteristics were taken in the laboratory, in addition, data on flowering position, duration of bud emergence, days required for floral development, flowering duration, earliness of flowering, percent fruit set per plant and duration of fruit bearing were collected in the field condition.

Percent fruit set per plant

The numbers of fruit setting were counted from randomly selected 10 inflorescence of every plant and total number of flowers and total fruit sets were recorded. The percent fruit setting was measured according to the formula of Roy (1997), expressed as;

$$Percent\ fruit\ setting\ (\%) = \frac{total\ number\ of\ opened\ flower}{total\ numbrt\ of\ fruit\ beared} \times 100$$

Percent edible portion

It was determined by using this formula of Sharma (2004)-

$$Edible\ portion\ (\%) = \frac{Weight\ of\ the\ edible\ part}{weight\ of\ the\ whole\ fruit} \times 100$$

Determination of dry matter content (%)

Twenty grams (20 g) of fruit flesh was taken in an aluminum foil and kept in an electric oven at 80°C for 72 hours until the weight became constant. Thereafter, the percent moisture content of fruit was calculated using the following formula:

$$Percent\ moisture\ content = \frac{Initial\ weight\ (g) - Final\ weight\ (g)}{Initial\ weight\ (g)} \times 100$$

Percent dry matter content was calculated using the following formula: % dry matter = 100 - % moisture content.

Organoleptic evaluation

It was done at full ripe stage. A panel of 10 members were selected, they were instructed to score the difference of samples by allotting the number from 0 – 5 against the traits. The scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair, 1 for poor and 0 for very poor.

Yield and profitability

The total approximate number of plant per hectare was estimated by the following formula-

$$Desired\ Plant\ Population\ (DPP) = \frac{10000\ m^2}{spacing\ (m^2)}$$

Using this formula, actual number of plants were counted per hectare area. The spacing of velvet apple was considered 6 m × 6 m. In case of PSTU Bilati gab-1 and PSTU Bilati gab-2, 9900 m^2 was allocated for plants population and rest 100 m^2 was allocated for graft production but for other two local varieties total unit of land was allocated. The average fruit yield per plant was calculated for every respective germplasm in kilogram (kg) and then the amounts were converted to per hectare area by the following formula -

$$Per\ hectare\ yield\ of\ velvet\ apple\ fruit\ (t) = \frac{DPP \times yield\ of\ fruit\ per\ plant\ in\ kilogram}{1000}$$

The profitability was measured by analyzing the benefit cost ratio (BCR), where the input cost in taka (Tk) was designated as- i. Labor cost-total number of labor in a year were counted. It was multiplied by the daily wage (600 Tk) per labor, ii. Manure and fertilizer cost, iii. Pesticide cost, iv. Irrigation cost v. Graft making cost and vi. Miscellaneous. As output/ income, the estimation was taken on two products (Fruits and Grafts) - 1. Fruits: In case of local variety, fruit was the only output for commercial cultivation of velvet apple, that's why there was no scope to get other income from it where the considered price of fruit of both local varieties, PSTU Bilati gab-1 and PTU Bilati gab-2 were 40 Tk, 50 Tk and 70 Tk, respectively. 2. Grafts: There is a good demand of PSTU Bilati gab-1 and PSTU Bilati gab-2 to the farmers and entrepreneur. Approximately 250 graft were established in a year which were distributed to the farmers and entrepreneurs @ 100 Tk and 120 Tk, respectively. Income from fruits was recorded by the formula, DPP × Average yield per plant (Kg) × Price of fruit per Kg (Tk)**.** So, Gross income = income from fruits + income from grafts. The net benefit estimated by subtracting the total cost (Tk) from gross income (Tk). So, the benefit cost ratio was obtained by using the following formula:

$$Benefit\ cost\ ratio = \frac{Gross\ income}{Total\ cost\ of\ production}$$

Results and Discussions

Plant characteristics

Plant characteristics varied significantly among the germplasm. The highest plant height (5.54 m), bole hight (1.67 m), diameter of plant (16.23 cm), number of main branches (12.75) (Table 1), length of largest branch (3.89 m), plant canopy (8.41 m^2), leaf area (135.24 cm^2) (Table 2) were observed in local red variety, while the local yellow variety had the similar results. On the other hand, PSTU Bilati gab-2 performed the least in most of the above mentioned traits indicating a highly dwarf plant growth characteristics. These variation among the germplasm might be due to the difference in spontaneously or seeded plant and graft plants. Grafting inhibits apical dominance of scion growth due to the variation in auxin cytokinin ratio (Wang *et al.*, 2017), which in return develop short height plant. Different branching pattern like horizontal, opposite, verticillate and irregular were found accordingly among the germplam. Similar observation was found in the study of Hossain *et al.*, 2015.

Table 1. Plant characteristics of different velvet apple germplasm

Germplasm	Plant height (m)	Bole height (m)	Diameter (cm)	Number of main branches
Local red	5.54 a	1.67 a	16.23 a	12.75 a
Local yellow	5.50a	1.53 a	14.78 ab	11.50 a
PSTU Bilati gab-1	3.33 b	0.58 b	13.10 b	5.25 b
PSTU Bilati gab-2	2.15 c	0.60 b	9.27 c	5.25 b
CV (%)	4.60	11.06	8.31	17.19

In a column, the means bearing similar letter (s) do not differ significantly at 0.05 level by DMRT.

Table 2. Plant characteristics of different velvet apple germplasm

Germplasm	Length of the largest branch (m)	Branching pattern	Plant canopy (m^2)	Leaf area (cm^2)
Local red	3.89 a	Horizontal	8.41 a	135.24 a
Local yellow	2.68 b	Opposite	7.08 b	134.33 a
PSTU Bilati gab-1	2.56 bc	Verticillate	8.80 a	116.49 ab
PSTU Bilati gab-2	2.16 c	Irregular	7.10 b	104.32 b
CV (%)	7.00		9.12	8.71

In a column, the means bearing similar letter (s) do not differ significantly at 0.05 level by DMRT.

Flower and fruit characteristics

The flower traits of velvet apple germplasm were observed meticulously where terminal flowering was observed in both local germplasm and both axile and terminal flowering were found in PSTU Bilati gab-1 and PSTU Bilati gab-2. In addition, local red germplasm produced bud of light green color, local yellow variety of olive green and

both PSTU Bilati gab-1 and PSTU Bilati gab-2 of green flower bud. Wallnöfer (2001) reported that ebenaceae developed bud in the axile of leaves. Maximum duration of floral bud emergence (32.25 days) was observed in PSTU Bilati gab-2 while it was minimum (22 days) in local red germplasm. PSTU Bilati gab-1 produced the largest inflorescence (6.60 cm) but the longest flower (2.70 cm) was produced in local red germplasm where PSTU Bilati gab-2 formed the smallest flower (2.23 cm). The widest diameter (1.11 cm) of flower was recorded in PSTU Bilati gab-1 but the narrowest (0.99 cm) was found in PSTU Bilati gab-2. The longest sepal (1.45 cm) and the longest petal (2.35 cm) were observed in local red germplasm while PSTU Bilati gab-2 gave the smallest value in both cases (1.25 cm and 1.88 cm, respectively) (Table 3).

Table 3. Flowering behavior of different velvet apple germplasm

Germplasm	Duration of bud emergence (Days)	No. of flower per inflorescence	Length of inflorescence (cm)	Days required for development	Length of flower (cm)	Diameter of flower (cm)
Local red	22.00 b	9.75	5.03 c	28.25 a	2.70 a	1.00 b
Local yellow	23.75 b	8.25	6.05 b	26.00 b	2.58 a	1.04 ab
PSTU Bilati gab-1	25.00 b	7.75	6.60 a	29.75 a	2.48 ab	1.11 a
PSTU Bilati gab-2	32.25 a	11.00	5.88 b	24.00 c	2.23 b	0.99 b
CV (%)	16.45		2.55	3.27	8.96	3.89

In a column, the means bearing similar letter (s) do not differ significantly at 0.05 level by DMRT.

Table 4. Flowering behavior of different velvet apple germplasm

Germplasm	Length of sepal (cm)	Length of petal (cm)	Width of sepal (cm)	Width of petal (cm)	Duration of flowering	Earliness of flowering
Local red	1.45 a	2.35 a	1.13	0.64 b	46.50 c	0.00 c
Local yellow	1.25 b	1.89 b	1.13	0.66 b	51.00 b	4.25 b
PSTU Bilati gab-1	1.33 ab	2.15 ab	1.20	0.75 a	57.50 a	5.75 b
PSTU Bilati gab-2	1.25 b	1.88 b	1.33	0.61 b	59.25 a	10.75 a
CV (%)	6.17	6.76		3.72	2.75	20.88

In a column, the means bearing similar letter (s) do not differ significantly at 0.05 level by DMRT

On the other hand, width of petal was statistically identical which ranged from 1.13 – 1.33 cm but PSTU Bilati gab-1 formed the widest petal (0.75 cm) whereas the narrowest (0.61 cm) found in PSTU Bilati gab-2. Fakir *et al*. (2018) mentioned that the length and breadth of sepal were 1.04±0.037 cm and 1.03±0.031 cm, respectively, and the length and breadth of petal were 1.78±0.035 cm and 2.32±0.041 cm, respectively. PSTU Bilati gab-1 required the longest time (29.75 days) for flower development but PSTU Bilati gab-2 needed the least time (24 days). The highest number of flowers

(11.00) were produced in PSTU Bilati gab-2 having statistical no difference with others. Similarly, PSTU Bilati gab-2 had been produced the longest duration of flowering (59.25 days) and performed earliness of flowering (10.75 days) while local red germplasm showed the poorest performance both of those traits (Table 4). These results were supported by the findings of Hossain *et al.* (2015) where they reported 21-26 days required for floral bud development and had been flowered for 50-58 days. The spheroid, ellipsoid, elongated and elliptical shaped fruits were observed in our studied germplasm. The skin color of fruits were differentiated as deep red, yellow, reddish brown and brown. Both the local red germplasm and PSTU Bilati gab-2 consisted of creamy white flesh color where local yellow variety and PSTU Bilati gab-1 exhibited yellowish and white color, respectively (Table 5 and Fig. 1). The research finding had similarity with those traits reported by Haque *et al.*, 2020.

Table 5. Fruit characteristics of different velvet apple germplasm

Germplasm	Shape	Skin color	Flesh color
Local red	Spheroid	Deep red	Creamy white
Local yellow	Ellipsoid	Yellow	Yellowish
PSTU Bilati gab-1	Elongated	Reddish brown	White
PSTU Bilati gab-2	Elliptical	Brown	Creamy white

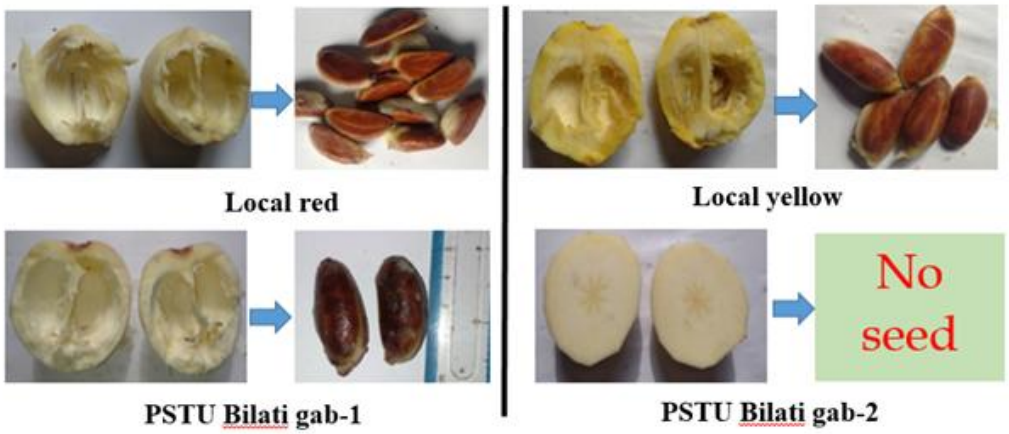


Fig. 1. Pulp and seed of different velvet apple germplasm

The highest fruit set (30.81%), number of fruits per branch (30), the longest fruit (9.78 cm) and the widest fruit (7.04 cm) were found in PSTU Bilati gab-1. Similarly, the highest weight of fruit, the highest weight of peel and the highest weight of pulp were also recorded in PSTU Bilati gab-1 to be 202.43 g, 31.25 g and 145.73 g, respectively but the lowest values were recorded randomly among the germplasm (Table 6).

Table 6. Fruit characteristics of different velvet apple germplasm

Germplasm	Fruit set per plant (%)	No. of fruits per branch	Length of fruit (cm)	Width of fruit (cm)	Weight of fruit (g)	Weight of peel (g)	Weight of pulp (g)
Local red	22.43 b	28.00	8.35 ab	6.26 b	152.58 b	27.83 a	81.04 c
Local yellow	20.63 b	27.50	8.01 b	6.03 b	129.78 b	21.98 b	69.25 c
PSTU Bilati gab-1	27.25 a	30.00	9.78 a	7.04 a	202.43 a	31.25 a	145.73 a
PSTU Bilati gab-2	26.45 a	29.75	5.83 c	6.48 ab	126.70 b	21.83 b	103.85 b
CV (%)	6.47		8.29	4.28	9.01	6.19	8.30

In a column, the means bearing similar letter (s) do not differ significantly at 0.05 level by DMRT

Local red germplasm gave the highest number of seed and maximum weight of seed were 6.49 and 41.68 g, respectively where local yellow germplasm gave statistically similar results of both traits. In addition, the longest (4.95 cm) and the widest (2.20 cm) seeds were obtained from PSTU Bilati gab-1 while local yellow variety gave the smallest (3.89 cm) and the narrowest (1.66 cm) seed (Table 7 and Fig. 1). Hasan *et al.* (2014) mentioned that the length and diameter of velvet apple fruits follow a double sigmoid pattern to attain 9.08 cm and 7.62 cm, respectively whereas flesh weight follows a sigmoid pattern and attained 165 g whose were agreed with our results. As PSTU Bilati gab-2 was seed less, so, the values of all seed traits were designated as null whose indicated it as a superior variety. The superiority of PSTU Bilati gab-2 was also found due to the highest edible portion (81.92%) and dry matter (18.53%).

Table 7. Fruit characteristics of different velvet apple germplasm

Germplasm	No. of seeds	Weight of seed (g)	Length of seed (cm)	width of seed (cm)	Edible portion (%)	Dry matter (%)	Duration of fruit bearing (days)
Local red	6.04 a	41.68 a	4.30 b	1.97 b	53.45 c	16.05 c	139.25 c
Local yellow	5.55 a	36.95 ab	3.89 c	1.66 c	53.21 c	16.52 b	132.25 d
PSTU Bilati gab-1	2.05 b	23.60 b	4.95 a	2.20 a	61.95 b	16.73 b	175.75 a
PSTU Bilati gab-2	0.00 c	0.00 c	0.00 d	0.00 d	81.92 a	18.53 a	158.50 b
CV (%)	16.22	31.25	4.86	6.53	4.88	1.04	11.66

In a column, the means bearing similar letter (s) do not differ significantly at 0.05 level by DMRT

On the other hand, PSTU Bilati gab-1 had been bearing fruits for maximum duration (175.75 days) whereas local yellow variety bears minimum time (132.25 days) (Table 7). Hossain *et al.*, (2015) supported our findings where they reported that one germplasm formed the highest number of seeds (7.14) where another germplasm was seedless. In addition, the highest rind weight (30.27 g), seed weight (86.29 g), non-edible portion (58%) as well as edible portion (87.33%) were found among the germplasm.

Organoleptic evaluation of fruit

PSTU Bilati gab-2 scored the highest result in organoleptic test in respect to all the parameter noted here (i.e., sweetness, aroma, texture, juiciness, fibreness, peeling quality and eye appeal) where other germplasm varied randomly. But the evaluated germplasm were only identical in pulp color (Table 8).

Table 8. Organoleptic evaluation of different velvet apple germplasm

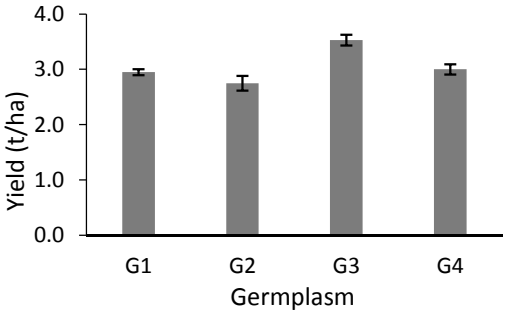
Germplasm	Pulp color	Sweetness	Aroma	Texture	Juiciness	Fibreness	Peeling quality	Eye appeal
Local red	3.55	3.68 b	2.30 ab	2.57 b	2.90 b	3.00 b	2.70 b	3.20 a
Local yellow	3.13	2.90 c	2.07 b	2.60 b	2.90 b	2. 78 c	2.25 c	2.63 b
PSTU Bilati gab-1	3.33	3.17 c	1.95 b	2.87 ab	3.30 a	3.10 b	2.49 bc	3.46 a
PSTU Bilati gab-2	3.15	4.10 a	2.67 a	3.12 a	3.63 a	3.36 a	3.03 a	3.57 a
CV (%)		5.55	8.38	5.97	4.87	2.89	5.30	7.82

In a column, the means bearing similar letter (s) do not differ significantly at 0.05 level by DMRT.

Fruit yield and profitability

The highest fruit yield (3.5 t/ha) was recorded in PSTU Bilati gab-1 followed by PSTU Bilati gab-2 (3.0 t/ha) and local red germplasm (2.95 t/ha) but the least fruit yield (2.8 t/ha) was found in local yellow (Fig. 1). Similar trend of fruit yield was recorded by Ahmed *et al.* (2011) where they reported the fruit yield per plant (at the age of 8 years) to be 7.53 kg which indicated the fruit yield of 2.1 t/ha.

The maximum cost of production (109480 Tk/ha) was estimated from PSTU Bilati gab-1 while the minimum (112320 Tk/ha) from local red germplasm (Fig. 2). The highest gross income (360200 Tk) and net benefit (251160 Tk) achieved from PSTU Bilati gab-2 where those were the lowest in local yellow germplasm (230300 Tk and 124030 Tk, respectively) (Fig. 3 and Fig. 4). PSTU Bilati gab-2 drawn significantly the highest BCR (3.31) whereas it was least (2.17) in local yellow germplasm (Fig. 5). Marri *et al*. (2013) extracted similar profitability in their study on minor fruits in Sindh province of Pakisthan during 2010. They reported that minor fruits cultivation was very profitable but government affiliation would be preferable for promoting and assisting growers and processors of minor fruits.



Legend

G1= Local red

G2= Local yellow

G3= PSTU Bilati gab-1

G4= PSTU Bilati gab-2

Fig. 2. Yield of four velvet apple germplasm in t/ha. Vertical bars represent standard error.

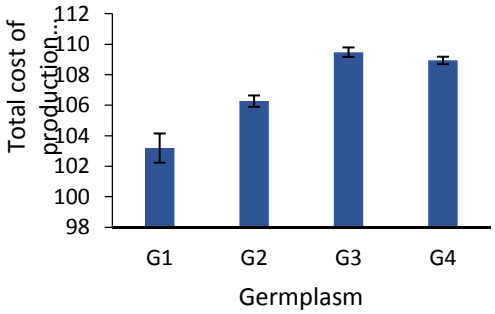


Fig. 3. Total cost of production per hectare area in Taka of four studied germplasm. Vertical bars represent standard error.

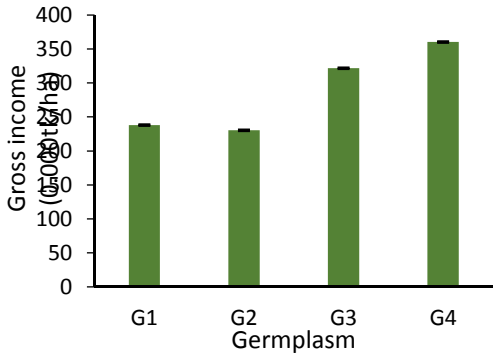


Fig. 4. Gross return in taka per hectare of four studied velvet apple germplasm. Vertical bars represent standard error.

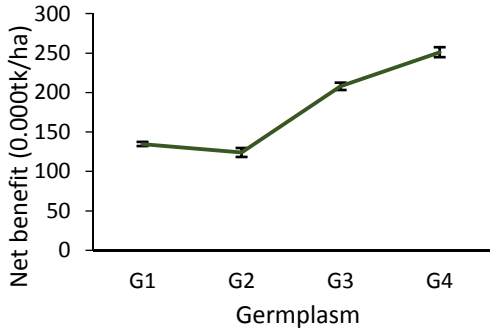


Fig. 5. Net obtained benefit (taka) of four studied germplasm estimated per hectare area. Vertical bars represent standard error.

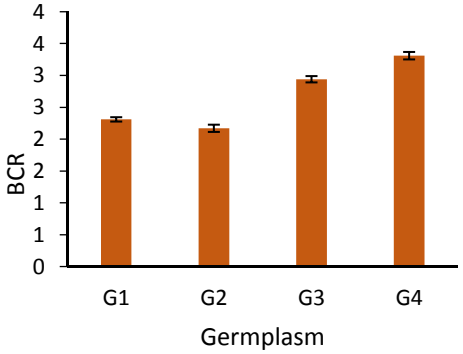


Fig. 6. Benefit cost ratio of four studied germplasm. Vertical bars represent standard error**.**

Conclusion

The findings of this study clearly expressed that the local red germplasm was the best in all parameters of plant characteristics, flower and fruit characteristics as well as yield and profitability. Due to grafting PSTU Bilati gab-2 was induced to be dwarf. During flowering PSTU Bilati gab-2 showed the best performances in forms of duration of flower bud emergence, duration of flowering and earliness of flowering followed by PSTU Bilati gab-1, local yellow germplasm and local red germplasm . PSTU Bilati gab-1 grabbed the superior position in respect to our studied fruit characteristics. Though, PSTU Bilati gab-1 yielded the highest amount of fruits (3.5 t/ha) but ultimate the highest profitability was estimated with PSTU Bilati gab-2. So, overall calculation revealed that PSTU Bilati gab-2 was the best over others. Therefore, PSTU Bilati gab-2 will be recommended for commercial orchard establishment in the southern belt of Bangladesh.

Conflicts of interest

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

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INFLUENCE OF POST-HARVEST APPLICATION OF *Stenotrophomonas rhizophila* ON QUALITY OF MANGO CV. BARI AAM-3

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Abstract

The study was conducted at the Postharvest and Plant Biotechnology Laboratory,of Patuakhali Science and Technology University, Patuakhali, Bangladesh during the period from July to December 2018 to study the biocontrol performances of selected antagonistic bacteria *Stenotrophomonas rhizophila* strain PSTU-Hort-14 on BARI Aam-3**.** All the treatments were arranged in a completely randomized design (CRD) with five replications and repeated twice. The bacterial strain under study was found highly compatible with 20% lemongrass extract and 2% sodium bicarbonate (SBC) or mixture of both which reduced 96.5% of disease over control in naturally infected fruits at the end of 14 days of storage at 12±1ºC and 90±5% RH. The combined treatments of Stenotrophomonas rhizophila-lemongrass extract-SBC showed reduced weight loss by more than 25% compared to the control at 12±1ºC and 90±5% RH. The shelf life was thus extended by 15 days compared to control at 12±1ºC and 90±5% RH. Finally, it was clear that the strain Stenotrophomonas rhizophila strain PSTU-Hort-14 was effective when incorporated with 2% SBC and 20% lemongrass extract to control *C. gloeosporioides* as well as improve the postharvest quality of BARI Aam-3 during cold storage.

Keywords: BARI Aam-3, Mango, Quality, *Stenotrophomonas rhizophila*,, Storability

Introduction

Postharvest pathogens caused significant losses in fruits and they are normally controlled by using synthetic fungicides. Biological control has been developed as an alternative to synthetic fungicide treatment and considerable success has been achieved upon utilizing antagonistic microorganisms to control both pre harvest and postharvest diseases (Janisiewicz and Korsten, 2002). Presently, Edible coatings are traditionally used to improve food appearance and conservation.They act as barriers during processing, handling and storage. They are not solely retard food deterioration and enhancing its quality, but are also safe due to their natural biocide activities or to the incorporation of antimicrobial compounds. Different compounds have been used as

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edible coatings to prevent commodity weight loss, including wax, milk proteins, celluloses, lipids, starch, zein, and alginate (Cha and Chinnan, 2004). Some of the recent findings was the used of chitosan which reduced weight loss and softening with extended shelf life of mango (Ali *et al*., 2011). In addition, edible coatings based on gum arabic alone or in combination with essential oils treatments reduced postharvest anthracnose and delayed ripening of mango and banana (Maqbool *et al.*, 2011).

Presently, there is an increasing interest in the use of lemongrass extract (oil) in the food industry as a source of functional food in drinks, beverages and ice creams. Lemongrass extract, polymers of carbohydrate and inorganic components could also be used as a preservative coating material for fruits. Due to its ability to form a semi-permeable film, lemongrass extract coating might be expected to modify the internal atmosphere of fruit and decrease transpiration losses. In a study, it is reported that lemongrass extract coating had the potential to prolong storage life and control decay of table grape (Castillo *et al*., 2010). Different inorganic salts were used to protect the harvested fruits from fungal pathogens, such as potassium metabisulphite, potassium bicarbonate, sodium bicarbonate, calcium chloride, ammonium molybdate and sodium carbonate (Sharma *et al*., 2009). Among them, sodium bicarbonate (SBC) which is regarded as safe salts with fungistasis property can be used to control postharvest diseases. SBC is able to reduce severity of anthracnose on mango (Hasan *et al*., 2012), apple scab (Ilhan *et al*., 2006) and blue molds of clementine mandarins (Palou *et al*., 2002). It also enhanced the activity of biocontrol agents such as *Trichosporon pullulans* (Yao *et al*., 2004), *Bacillus subtilis* (Obagwu and Korsten, 2003) and *Pseudomonas syringae* (Plaza *et al*., 2001).

The quality of mango fruits is largely dependent on the varieties and various postharvest treatments which are principally applied to increase the storability of fruits. It is essential to understand the physico-chemical changes of mango to improve the postharvest quality of the fruits. A large number of research works on shelf life and quality as influenced by different postharvest treatments has been extensively investigated by a number of scientists in different parts of the world. Although considerable literature dealing with shelf life extension, postharvest loss reduction and physico-chemical changes during storage and ripening of mango is available, but as far we know that there is no work using antagonistic bacterial coating on fruits to extend shelf life have been done in Bangladesh, especially with mango fruits. Thus, this study was conducted to study the biocontrol performance of selected antagonistic bacteria *Stenotrophomonas rhizophila* strain PSTU-Hort-14 with 2% SBC and 20% lemon grass extract on the shelf life and postharvest quality of BARI Aam-3 fruits under refrigerated conditions.

Materials and Methods

Mango fruits of ‘BARI Aam-3’ variety at color stage two (green with tinge of yellow) were used for postharvest treatments. Healthy fruits with uniform size, shape, and maturity were collected from Regional Horticultural Research Station (RHRS), BARI, Lebukhali, Patuakhali. Three treatments were selected as factor A for this study, which were i) combined treatment of SBC, lemongrass extract, bacterial strain PSTU-Hort-14 (cell suspension of bacterial strain PSTU-Hort-14 (10^8 CFU mL^{-1}) in

combination with 20% lemongrass extract and 2% SBC solution); ii) positive control (Dipheniconazol® solution at the rate of 0.50 mlL^{-1}) and iii) negative control (sterilized distilled water). Whereas storage durations act as factor B. Forty four fruits were dipped in each treatment and the total number of fruits were 132. Every week, eight fruits represented five replications (two fruits per replicate) for each treatment were used for determination of physico-chemical characteristics. Data were recorded on 0, 7, 14, 21, and 28 days of storage (12±1ºC and 90±5% RH).

Determination of physical characteristics

Weight loss

To determine the fruits weight loss, fruits weight were measured every alternative day by weighing individual fruit with a top pan electronic balance (Model-668ALED, RFL).

Determination of pulp firmness (N)

Firmness of mango were determined by firmness testing machine (Model: GY 4). This method was mentioned by Hassan (2006).

The pulp to peel ratio was measured with the following formula-

$$Pulp\ to\ peel\ ratio = \frac{Weight\ of\ fruit\ pulp}{Weight\ of\ peel}$$

Determination of glossiness

Glossiness of mango was determined by gloss meter (Model: ETB-0686). Gloss is measured by directing a constant intensity light beam, at a fixed angle, on to the test surface and then monitoring the amount of reflected light from the same angle. This specular reflectance was measured using a gloss meter. A gloss meter provides quantifiable gloss measurements, expressed as gloss units (GU).

Determination of chemical characteristics

Determination of titratable acidity

Titratable acidity (TA) was determined according to the method by Ranganna (1977).

Determination of pH

The remaining of the filtrated juice from TA determination was used to measure the pH of the fruit pulp. The pH was determined by using a glass electrode pH meter (PHS-25 Precision pH/mV meter, LIDA Instrument).

Determination of soluble solids concentration

The soluble solids concentrations of fruits pulp were determined by using a digital refractometer (BOE 32195, BOECO, Germany).

Determination of ascorbic acid

Ascorbic acid was determined according to the dye method by Ranganna (1977).

Determination of total sugar

Sugar content of fruit was estimated by the following procedures described by the Lane and Eynon (1923).

Standardization of fehling's solution

Fifty ml of both fehling's solution A and fehling's solution B was mixed together in a beaker. Ten milliliter of mixed solution was pipette into a 250 ml conical flask and 25 ml distilled water was added to it. Standard sugar solution was taken in a burette. The conical flask containing mixed solution was heated on a hot plate. When the solution began to boil, three drops of methylene blue indicator solution was added to it without removing the flask from the hot plate. Mixed solution was titrated by standard sugar solution. The end point was indicated by decolorization of the indicator. Fehling's factor was calculated by using the following formula:

$$Fehling's\ factor\ (g\ of\ invehrt\ sugar) = \frac{Titre \times 2.5}{1000}$$

Preparation of sample

50 ml fruit juice was mixed with 100 ml of distilled water and 5ml of neutral led acetate solution and then kept for ten minutes and the mixture was homogenized. Then the blended material was transferred to a 250 ml volumetric flask. The volume was made up to the mark with distilled water. The solution was then filtered.

Determination of reducing sugar

Ten ml of mixed fehling's solution was taken in a 250 ml conical flask and made 250 ml with distilled water. Purified juice solution (filtrated) was taken in a burette. Conical flask containing mixed fehling's solution was heated on a hot plate. Three to five drops of methylene blue indicator were added tom the flask when boiling started and titrated with solution taken in burette. The end point was indicated by decolorization of indicator. Percent reducing sugar was calculated according to the following formula:

$$Reducing\ sugar\ (\%) = \frac{F \times D \times 100}{T \times W \times 1000}$$

Where,

F: Fehling's solution

D: Dilution

T: Titre, and

W: Weight of sample

Determination of total invert sugar

Fifty ml of purified solution (filtrated) was taken in a 250 ml conical flask. Five ml citric acid and 50 ml distilled water was added to it. The conical flask containing sugar solution boiled for inversion of sucrose and cooled: Then the solution was transferred to a 250 ml volumetric flask and neutralized by 1N NaOH using phenolphthalein indicator. The volume was made up to the mark with distilled water. Then the mixed fehling's solution was titrated using similar procedure followed as incase of invert sugar (reducing sugar) mentioned earlier. The percentage of total invert sugar was calculated by using the formula used in incase of reducing sugar.

Estimation of non-reducing sugar

Non-reducing sugar was estimated by using the following formula:

Non-reducing sugar (%) = Total invert sugar (%) - Reducing sugar (%)

Sensory evaluation of ripe fruit

Sensory analyses to compare the quality of treated and control mango fruits were carried out by eight trained adults, aged 25-40 years (four male and four female). The panel were trained in a pre-test in which mango fruits with extremely low or high attributes (taste, peel color, pulp color, texture and flavor) were evaluated. Evaluations were based on the Table 3 hedonic scale. After the sensory evaluation, the overall rating of the sensory trail was taken according to Ali *et al*. (2011) with some modifications. The panelists were asked to score the differences between the samples by allotting the numbers from 0 to 5 against Excellent (5), Very good (4), Good (3), Fair (2), Poor (1), and Very poor (0).

Experimental design and statistical analysis

The factorial treatments were laid out in a completely randomized design with five replications. The recorded data on different parameters of the experiment were tabulated and analyzed with appropriate design of experiment (Gomez and Gomez, 1984) adopting a statistical programme MSTAT-C. All the treatment means were calculated and the analyses of variances (ANOVA) of different parameters considered were done by 'F' variance test. The means were separated by Least Significant Difference (LSD) test at 5% level of significance.

Results

Changes in physical characteristics

Weight loss

Mango fruits under all the treatments showed a progressive loss of weight during four weeks of storage at 14 ºC and 95% RH (Fig. 1). However, significantly ($P \leq 0.05$) lower weight loss was recorded with the combination of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* dipped mango compared to negative control and positive control treated fruits. The values ranged between 0.9 to 3% for the combined treatment

after 7 to 28 days of storage. The negative control and positive control treated fruits, on the other hand, exhibited maximum weight loss at each storage interval with the values 5.53% and 3.62%, respectively after the end of storage. 12 ºC and 95% RH for 28 days. Each value is the mean of four replications. Means were separated by Least Significant Difference (LSD) test at 5% level of significance. Vertical bars represent standard error

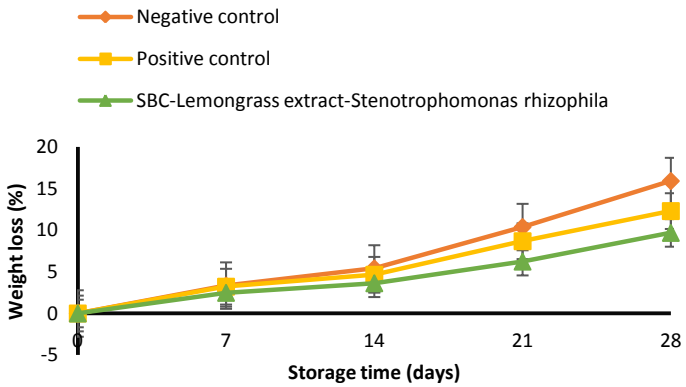

Fig. 1. Effects of different treatments on the weight loss of mango fruits during storage

Flesh firmness

The initial firmness of mango flesh was noted maximum (58.31 N) and the values were noticed similar for control and treated samples. The firmness gradually declined for all fruits with the period of storage advanced; however, the decrease was significantly slower in the combined treatment SBC-Lemongrass extract-*Stenotrophomonas rhizophila* (Fig. 2). The flesh firmness under the combined treatment was consistently higher than those of negative control and positive control treated fruits during entire storage period.

Mango fruits that were subjected to the combination of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* maintained the firmness of 25.70 N after 28 days of storage, which were the readings for the negative control and positive control treated fruits on day 12 or 13 of storage. Hence there was a gain of at least 15 days of extra storage life with the application of this combined treatment.

Glossiness of fruit

Glossiness of mango fruit was measured by a gloss meter during the storage of 28 days at 14 ºC. A gloss meter provides quantifiable gloss measurements, expressed as gloss units (GU). However, the glossiness can be slowed down by the combined treatment of SBC-Lemongrass extract- *Stenotrophomonas rhizophila*.

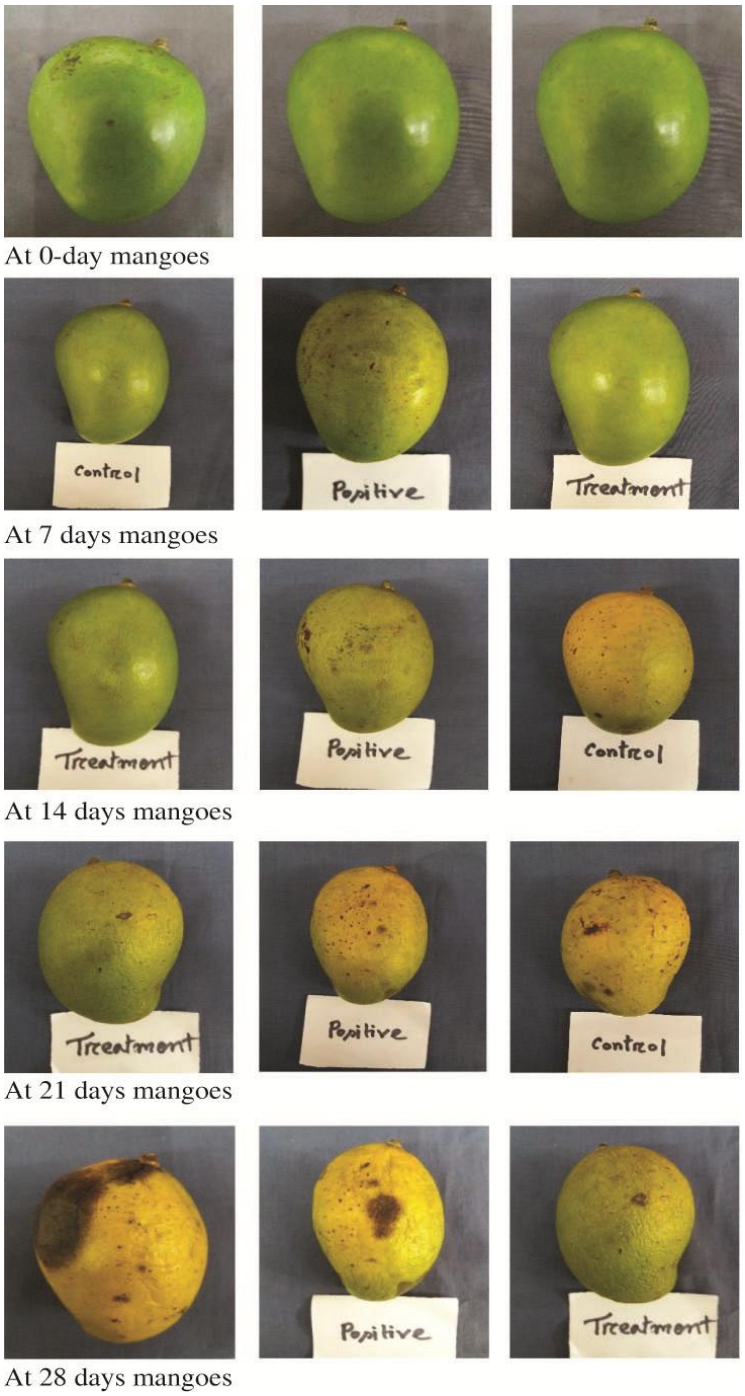

Fig. 2. Effects of combined treatments of SBC-Lemongrass extract-*Stenotrophomonas rhizophila*, positive control (Dipheniconazol®) and negative control (sterilized distilled water) on the physical appearance of mango fruits during storage at 12±1ºC and 90±5% RH for 0, 7, 14, 21 and 28 days

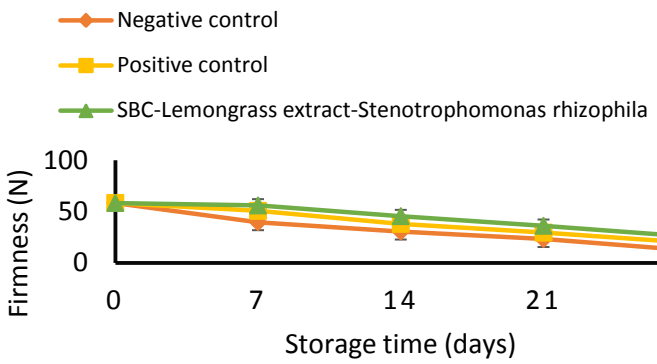

Fig. 3. Effects of different treatments on the flesh firmness of mango fruits during storage at 12ºC and 95% RH for 28 days

Changes in chemical characteristics

Titratable acidity (%)

Titratable acidity of mango fruits gradually decreased with advancement of time during storage (Fig. 4). Significantly the highest TA (1.78%) was recorded in fruits at the beginning of storage (0 days), which declined slowly with storage time and reached at minimum level (0.63%) at the end of storage period. However, fruits that were subjected to the combination of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* exhibited slower decline for the concentration of TA. At the end of storage, the lowest (0.63%) TA was recorded in combined treatment of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* and the highest TA was recorded in negative control (0.94%).

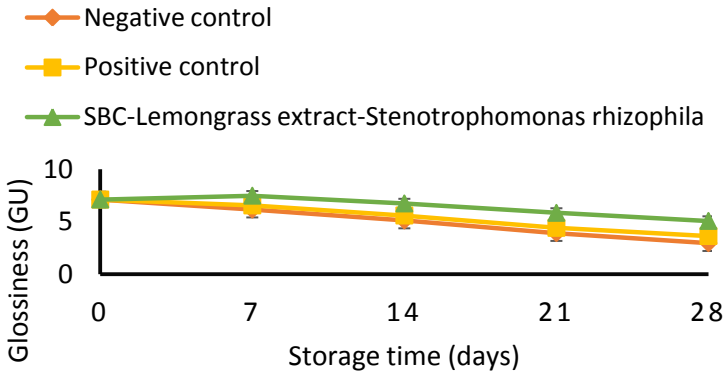

Fig. 4. Effects of different treatments on the glossiness of mango fruits during storage at 12ºC and 95% RH for 28 days

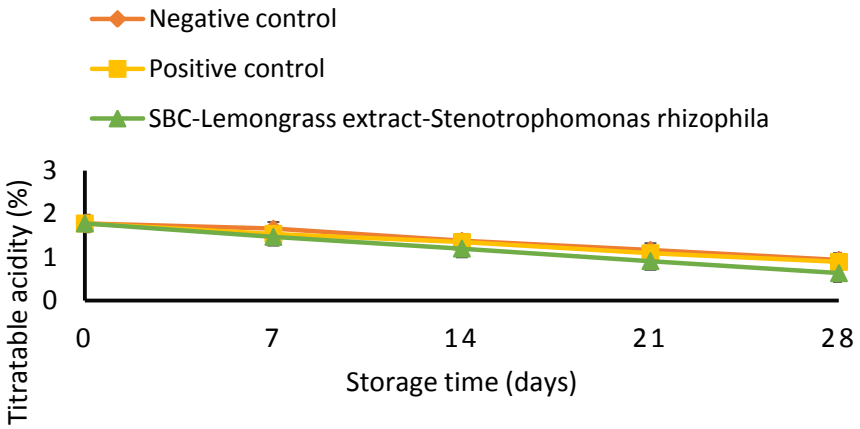

Fig. 5. Effects of different treatments on the titratable acidity of mango fruits during storage at 12ºC and 95% RH for 28 days

pH

The pH of fruit increased gradually as storage progressed with yielding significant differences ($P \leq 0.05$) between the treatments. At the end of storage period of 28 days, pH value was manifested significantly higher (4.86) in fruits subjected to the combination of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* compared to that of water treated control fruits with the value of 4.21.

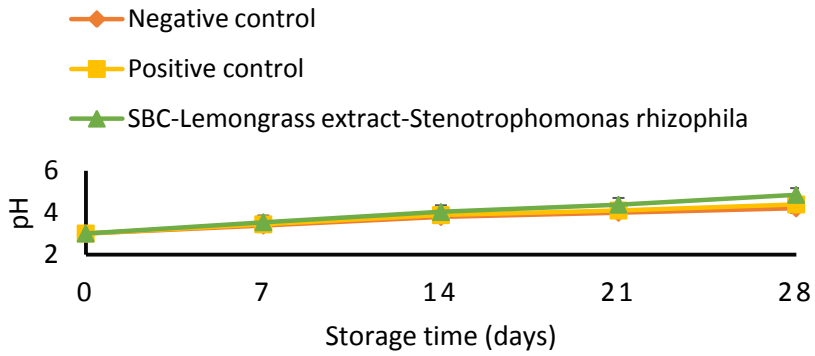

Fig. 6. Effects of different treatments on the pH of mango fruits during storage at 12ºC and 95% RH for 28 days

Soluble solids concentration (SSC)

The SSC of negative control and positive control treated fruits were fairly low initially (1.51) and increased gradually with the advancement in storage period and reached maximum values of 4.06 and 4.22%, respectively after 21 days of storage. After this period, noticeable decrease in SSC was recorded. The sharp decline in SSC values indicated faster metabolic rates of the negative control and positive control treated fruits.

On the other hand, the fruits that were treated with the combination of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* showed gradual changes and reached maximum SSC (4.83%) at the end of 28 days of storage period.

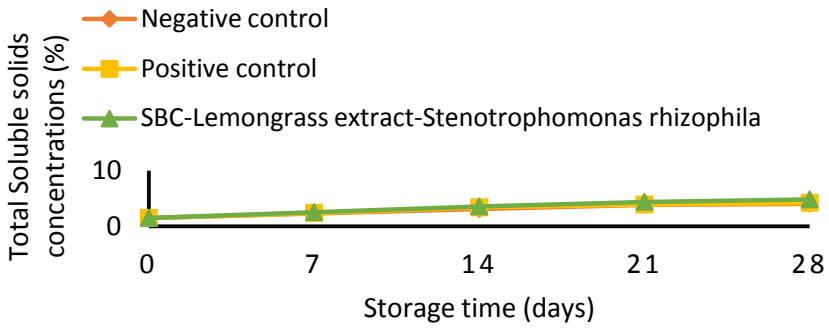

Fig. 7. Effects of different treatments on the TSS of mango fruits during storage at 12ºC and 95% RH for 28 days

Ascorbic acid content

It is evident that the combination of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* induced significant variation in ascorbic acid content of mango fruit during storage. Initially the ascorbic acid content was 10.14 mg 100 g^{-1}. In negative control fruit, the content of ascorbic acid decreased sharply over time and reached minimum value of 6.04 mg 100 g^{-1} after 14 days of storage and thereafter declined until end of 28 days of storage. Almost similar trend was observed for positive control treated fruits. However, fruits that were subjected to the combined treatment SBC-Lemongrass extract-*Stenotrophomonas rhizophila* showed more gradual changes in ascorbic acid content with time and exhibited minimum value of 5.10 mg 100 g^{-1} after 21 days of storage and declined gradually thereafter. After the end of 28 days of storage, there were no statistical

Reducing sugar

In all fruits the reducing sugar values increased with the storage period. However, slower increase was observed with the combined treatment indicating delayed ripening. For all treatments the values of reducing sugar (3.48%) were recorded the same in fruits at the beginning of storage (0 days). At the end of storage, the highest (5.33%) reducing sugar was recorded in the combined treatment of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* and the lowest reducing sugar was recorded in the treatment negative control (4.48%).

Differences regarding ascorbic acid content amongst the treatments.

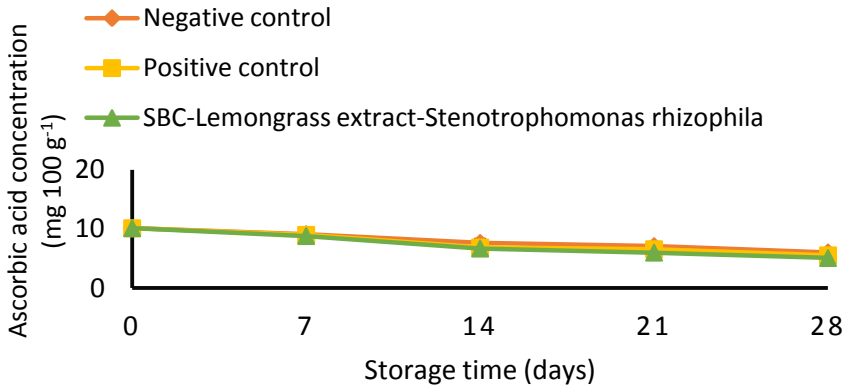

Fig. 8. Effects of different treatments on the ascorbic acid concentration of mango fruits during storage at 12ºC and 95% RH for 28 days

Non reducing sugar

Non reducing sugar values decreased with the storage period. However, slower decrease was observed with the combined treatment indicating delayed ripening. For all the treatments the highest non reducing (3.43%) was recorded in fruits at the beginning of storage (0 days). At the end of storage, the lowest (0.34%) non reducing sugar was recorded in the combined treatment of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* and the highest non reducing sugar was recorded in negative control (0.94%).

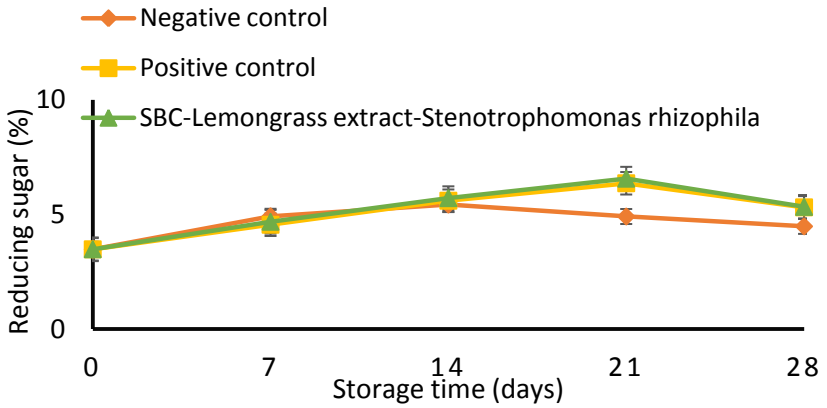

Fig. 9. Effects of different treatments on the reducing sugar of mango fruits during storage at 12ºC and 95% RH for 28 days

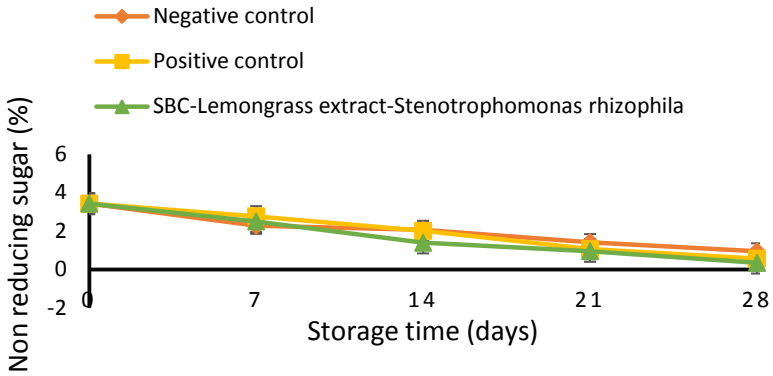

Fig. 10. Effects of different treatments on the reducing sugar of mango fruits during storage at 12ºC and 95% RH for 28 days

Total sugar

The total sugar values decreased with the storage period in all fruits. However, slower decreasing trend was observed with the combined treatment indicating delayed ripening. For all the treatments the total sugar content of mango fruits decreased rapidly during the storage period with significant negative linear relationships. The highest total sugar (6.91%) content was recorded in fruits at the beginning of storage (0 days) for all the treatments. At the end of storage, the highest (5.87%) total sugar was recorded in the combined treatment of SBC-Lemongrass extract-*Stenotrophomonas rhizophila* and the lowest content was recorded in negative control (5.43%).

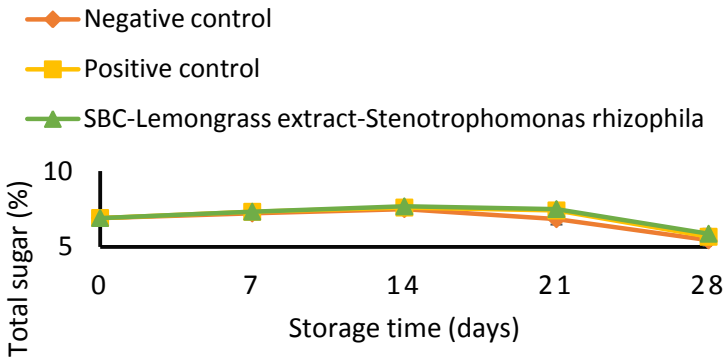

Fig. 11. Effects of different treatments on the total sugar of mango fruits during storage at 12ºC and 95% RH for 28 days

Sensory analysis

Panelists evaluated the visual aspect of the fruits and gave the lowest scores to those of negative control fruits, which ripened after 3 weeks of storage. The fruits began

to decompose after three weeks. The fruits treated with SBC-lemongrass extract-*Stenotrophomonas rhizophila* attained maximum scores by the panelists for all the parameters evaluated. The fruits were glossy and wrinkless, therefore scored 3.85, which was significantly ($P \leq 0.05$) higher than positive control treated fruits. The positive control treated fruits also had a good overall appearance but with some wrinkles. The most attractive pulp with the characteristic of golden yellow color of mango was found in fruits treated with SBC-lemongrass extract- *Stenotrophomonas rhizophila* (3.95), followed by positive control (3.31) treated fruits.

Table 1. Effect of combined treatment SBC-Lemongrass extract-*Stenotrophomonas rhizophila*, positive control (Dipheniconazol®) and negative control (distilled water) on the sensory traits of mango fruits after four weeks of storage at 12±1°C

Treatments	Taste	Peel color	Pulp color	Texture	Flavor
Negative control	0.00 c	0.00 c	0.00 c	0.00 c	0.00 c
SBC- lemongrass extract-*Stenotrophomonas rhizophila*	4.17 a	3.78 a	3.95 a	3.85 a	4.50 a
Positive control	3.50 b	2.90 b	3.31 b	3.65 b	3.90 b

Means in each column followed by the same letter (s) are not significantly different at $P \leq 0.05$ according to Least Significant Difference test

There were significant ($P \leq 0.05$) differences in the texture of the fruits with the different treatments. Fruits with combined treatment were rated the highest (4.17 points), with a firm, crispy pulp and 'melted' in mouth. The flavor was also rated excellent (4.50) because the pulp was not only sweet and pleasant, but also possessed the characteristic aroma.

Discussion

The mechanism for these positive effects is based on the interplay of the biocontrol activity of SBC-*Stenotrophomonas rhizophila* and moisture barrier properties of lemongrass extract and *Stenotrophomonas rhizophila*. In this combination, lemongrass extract acted as a carrier of *Stenotrophomonas rhizophila* and SBC as well as coating, at the same time *Stenotrophomonas rhizophila* produces biofilm which was mentioned by Morikawa (2006) in his review article. The combination of biofilm of *Stenotrophomonas rhizophila* and lemongrass extract coating modified the micro atmospheric environment surrounding the fruits with reduced O_2.

Transpiration is a mass-transfer process in which water vapor moves from the surface of fruits or vegetables to the surrounding air. This process of moisture loss induces wilting, shrinkage, and loss of firmness and crispiness of fruits and vegetables and thus, adversely affects the appearance, texture, flavor and mass of produce (DeEll *et al.*, 2003). Paull and Chen (1989) reported that mango fruits weight loss greater than 8% considerably diminished the postharvest quality. However, weight loss in SBC-lemongrass extract-*Stenotrophomonas rhizophila* treated fruits were significantly reduced

when stored at 12±1ºC for 28 days, which is considered to be acceptable for retailing purposes. This reduction of weight loss coincided with the reduction in respiration rate. As with other edible coatings, lemongrass extract prevented moisture loss and controlled respiratory exchange. In general, this positive effect of edible coatings is based on their hygroscopic properties, which enables formation of a water barrier between the fruit and environment, and thus avoiding its external transference (Morillon *et al.*, 2002). To enhance water barrier efficacy, many formulations of composite coatings are utilized, the most frequently used being polysaccharide-lipid. Thus, increasing lipid content of coating formulations significantly reduced weight loss of mandarins (Pe´rez-Gago *et al.,* 2002). However, for lemongrass extract which the composition is mainly polysaccharides (Ni *et al.,* 2004), was highly effective as a moisture barrier without lipid incorporation. In this study, *Stenotrophomonas rhizophila* and 2% SBC were used with lemongrass extract to make a composite coating with antifungal and moisture barrier properties. Here, *Stenotrophomonas rhizophila* used were for dual purposes. Firstly, to control disease specially postharvest anthracnose disease and secondly, to make a biofilm, which increase the efficiency of lemongrass extract coating. In this formulated coating, SBC used to control anthracnose and increase the concentration of composite coating. Thus, resulting in decreased diffusivity of water vapor through the composite coating and a decreased in hydrophilic tendency of lemongrass extract and increased antifungal properties.

In general, surface coatings have been applied to many fruits and vegetables to maintain or enhance gloss and improve storage quality. Dang *et al.*, (2008) also reported that the external colour of mango fruit is generally retained when coated with lemongrass extract. Similarly, in this study, the extent of skin color development of mango fruit was significantly slower when treated with the combination of SBC-lemongrass extract-*Stenotrophomonas rhizophila* compared to negative control or positive control treated fruits. This combined treatment showed the best control of skin color change throughout the storage, suggesting a delay in the ripening of the fruits. The delay in skin color development of mango exposed to the combination of SBC- lemongrass extract - *Stenotrophomonas rhizophila* in this investigation could also be related to its effect on in modification of internal atmosphere of the fruits. These conditions delayed ripening and senescence process, thus resulting in retention of greenish yellow color and firmness of fruits.

The combination of SBC- lemongrass extract -*Stenotrophomonas rhizophila* slowed down the increase in concentration of total soluble solids and reduced the decrease in content of titratable acidity of mango during storage. These might be due to the semi-permeable coating formed by the combination of lemongrass extract - *Stenotrophomonas rhizophila* around the fruit surfaces, which was very effective in reducing disease incidence and severity, delayed the ripening process and consequently all other activities associated with ripening were also suppressed. Lower respiration rate and ethylene production of the mango fruits treated with the treatment were observed during storage at 12°C for 28 days. Thus, the slower increase in SSC and higher level of titratable acidity in the pulp of mango fruits that were subjected to the treatment could probably due to reduction of oxygen supply on the fruit surface, which inhibited

respiration rate and the growth of spoilage organisms (Yonemoto *et al.*, 2002). The incorporation of 2% SBC in the combined treatment in this work presumably reduced the rate of ripening and eventually the gradual changes in soluble solids concentration and sugars seem to be under the direct control of interaction with the disease of mango (Hasan *et al*., 2012). Results of this study are in agreement with the findings of previous works on various fruits coated with lemongrass extract-based coatings, such as Sweet cherry mango (Dang *et al.,* 2008). Decrease in acidity during storage and ripening most probably due to utilization of organic acids in the respiration resulting increase in pH of the fruits. In another study, Ghanta *et al.* (1994) also reported that the titratable acidity in mango fruit decreases gradually throughout the fruit development until it reaches the full ripe stage. In this study, the increased in pH and decrease in titratable acidity of mango followed a linear trend with increased in storage period for both coated and uncoated fruits. However, the combined treatment in the current work slowed the changes of pH and titratable acidity during storage which effectively delayed fruit senescence. Slowing down the respiration rate in fruits by means of this treatment could explain the delay in the use of organic acids in the enzymatic reactions of respiration, thus retarding ripening (Hernandez-Munoz *et al.,* 2006).

The trend of development of ascorbic acid in mango fruit increased during ripening but, declined by the end of storage. However, postharvest application of SBC-lemongrass extract *Stenotrophomonas rhizophila* exhibited a best effect on the content of ascorbic acid by slowing down its increasing rate and no any decreasing trend like negative and positive controls treated fruits with storage time, which can be attributed to low respiration rate (Jiang *et al.,* 2001). The content of ascorbic acid in fruits that were subjected to the treatment increased steadily until 21 days of storage and then decreased slightly till the end of storage. This slower increase in ascorbic acid content might be due to reduced internal oxygen that resulted in retarding the oxidation of ascorbic acid.

Moreover, the lemongrass extract coating imparted an attractive natural-looking sheen to the mango fruit, almost liked a freshly harvested fruit with lower changes in both skin color and dehydration. The visual aspect of the fruit is usually correlated to the overall quality, where firmness, crunchiness, juiciness and sweetness of fruit were significantly higher in SBC- lemongrass extract -*Stenotrophomonas rhizophila* treated fruit compared with the negative control or positive control treated fruits. It is interesting to point out that none of the panelists could discern any bad odor or "off-flavor" attributed to the SBC- lemongrass extract -*Stenotrophomonas rhizophila* treatment.

Conclusion

Utilization of biocontrol agents as the postharvest treatment in managing disease of tropical fruits is more acceptable for marketing in the developed countries where their phytosanitary regulations is stringent because they are moving toward reducing use of synthetic fungicides. It was found that lemongrass extract and SBC not only enhanced the biocontrol efficacy of *stenotrophomonas rhizophila* strain PSTU-Hort-14, but also manage to improve the storability of mango by reducing moisture loss without compromising the fruit quality. Fruits treated with the combination of SBC- lemongrass extract -*Stenotrophomonas rhizophila* reduced transpiration rate stored at 12±1^{o}C and

90±5% RH. This suggest that the treatment significantly retained fruit firmness, decreased weight loss and delayed the changes in external color, soluble solids concentration and ascorbic acid contents of the fruit during storage. It also suggests that this composite coating is promising as a natural, harmless and eco-friendly coating to be used in commercial postharvest applications for prolonging the shelf life of mango.

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Conflicts of interest

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

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GROWTH AND INSTABILITY ANALYSIS OF BLACK GRAM (*Vigna mungo* L.) IN BANGLADESH

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Abstract

Growth and instability of crops are two crucial aspects that significantly impact the contributions to enhance agricultural resilience, food security, economic development, risk management, income volatility, and sustainable practices to meet increasing demands. Black gram is one of the high-value pulse crops in Bangladesh, and is essential for human and animal nutrition, and also contributes to soil health improvement through adding nitrogen, carbon and organic matter. This study presents the growth and instability in area, production, and productivity of black gram along with the contributory factors affecting the growth and instability production based on secondary data for the last 40 years (1981-2020). The entire period was divided into four sub-periods: 1981-1990, 1991-2000, 2001-2010 and 2011-2020 for analysis through different statistical tools. Growth rates were calculated by fitting an exponential growth function, and instability was analyzed by generating the Cuddy-Della Valle index. The analysis presented highlights with a significant decrease in the area of black gram cultivation and production, even though there has been a notable increase in productivity. However, this increase in yield is not sufficient to meet the overall demand for black gram in the country. The study also points out that the growth rate of yield is low compared to the increase in demand. Throughout the study period, the contribution of the area was -3.29%, while the yield contribution was 142.25% in the average growth of black gram production at the national level. The analysis underscores the lack of stability in black gram cultivation, production, and yield during this period. Notably, there were instabilities of 4.37%, 4.89%, and -25.7% in area, production, and productivity of black gram at the national level, respectively. Hence, researchers, policymakers, and farmers must prioritize advancing improved technologies to boost black gram production in the country. This enhancement holds the promise of improving food and nutritional security in Bangladesh. Encouragingly, there are signs that the Bangladesh Agriculture Research Institute (BARI) has strengthened its varietal development research, and the government has recently implemented incentives to support the cultivation of pulse crops including black gram.

Keywords: Black gram, Cuddy-Della Valle index, Growth decomposition, Instability

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Introduction

Pulses are important food crops in Bangladesh. They are an important source of essential nutrients in the human diet (Das 2016.), provide feed for animals, and generate good profits for farmers (Miah *et al.*, 2009). Pulses contribute to agricultural and environmental sustainability through adding nitrogen, carbon, and organic matter in soil (Senanayake *et al.*, 1987; Zapata *et al.*, 1987; Sarker and Kumar, 2011). A favorable climatic condition exists in Bangladesh for growing different types of pulses all over the country. Because of the high protein content and low-price, pulses are called *poor man's meat*. So, most of the low-income people use these nutritious crops in daily diet. However, per capita consumption of pulses in our country is only 17.1 g/day (HIES, 2022) which is much lower than the desirable intake of 50 g/day (DDP, 2013). The local production of pulses almost remained static in the last couple of years, causing a rise in imports to meet the increasing demand (Miah *et al.*, 2022). Bangladesh had to import a huge quantity of pulses to meet the demand. Bangladesh imported 1363.39 thousand tons of pulses valuing USD 707.172 million in 2021 (FAOSTAT, 2021). According to Bangladesh Bank's statistics, Bangladesh spent Tk. 6,185 crores to import pulses in the last fiscal year of 2021-22, an increase of 11% year-on-year.

Black gram (locally called *Mashhkalai* is a leguminous plant under the *Vigna mungo* species. It is a drought-tolerant crop predominantly grown during Kharif-II season. Its seed is generally eaten as whole seed, flour for various snacks or after splitting cooked as *Dal*. Protein, fiber, vitamins, and minerals like calcium and iron are abundant in whole black grams (Reddy *et al.*, 1982 and Salunkhe *et al.*, 1985). The composition of black gram residue is approximately as follows: cellulose (26.8% ± 2.3%), hemicellulose (32.48% ± 3.0%), lignin (23.14% ± 2.1%), crude protein (16% ± 0.8%), and ash content (5.1% ± 1.2%) (Ilyas *et al.*, 2012). Black gram is also used as a green manure and cover crop or fodder crop and as a short-lived forage. In 2021-22, it was cultivated in about 41.3 thousand hectares of land producing 39.00 thousand MT with an average yield of 0.94 t/ha (BBS, 2022), the demand for black gram was 1.3 lakh MT in 2021 (Bokhtiar *et al.*, 2022). Therefore, decent growth in black gram is also necessary to contribute to the overall GDP of Bangladesh. However, instability in agricultural production is on the rise due to several factors, such as erratic rainfall patterns, low irrigation coverage, and an increase in the frequency and severity of natural disasters, among others. Instability also exists in the area regarding the production and yield of black gram. This instability has also had adverse effects on food management and macroeconomic stability in the country. There has been some economic and agronomic study in Bangladesh focusing on aspects other than the growth and instability of black gram, and therefore, we have focused on these areas. In this context, this paper attempts to analyze these two aspects, as balancing these is essential for building resilient and productive agricultural systems. The specific objectives of this study are to determine the growth rates of area, production, and yield of black gram in Bangladesh; and to measure the change and instability in area, production, and yield of black gram.

Materials and Methods

Data and its sources

The study was based on secondary data collected from various published sources. Times series data on the area, production, and yield of black gram for the last 40 years from 1981-82 to 2019-20 were collected from different issues of the Yearbook of Agricultural Statistics of Bangladesh.

Analytical procedures

Various statistical tools were used to analyze the data to examine the nature of change, instability, and degree of relationship in area, production, and yield of black gram in Bangladesh.

Trend analysis

Trend analysis aims to find out the extent and causes of instability of area and production of black gram over time. This information may lead researchers as well as policymakers to prepare appropriate policy documents and priority research areas for the improvement of black gram in the country. A simple line graph and bar diagram were used to show the trends in area, production, and yield of black gram in Bangladesh.

Index number

The relative changes in area, production, and yield of black gram that occur within a specified timeframe can be quantified using an index number. At first, the entire study period is divided into four sub-periods such as 1981-1990, 1991-2000, 2001-2010, and 2011-2020. The reason for the division was to know the changes that occurred in the area, production, and yield of black gram in every decade. The average value of area, production, and yield of the first 10 years sub-period (e.g. 1981-1990) provided the base information.

Annual growth rates

Growth rates are the percent change of a variable over time. It is important because it can help researchers and policymakers to predict future production growth. For simplicity and widely used even in the recent past (Rahman *et al.*, 2011; Das and Mishra 2020; Chaudhary *et al., 2016*) the compound growth rates of area, production, yield, and price of black gram were worked out by fitting an exponential function of the following type:

$$Y = ae^{bt} \text{ or } LnY = Lna + bt \text{----------(1)}$$

Where Y is the area/production/yield of black gram, 't' is the time in a year, and 'a' is the constant, e^b -1 is the compound growth rate which is expressed in percentage. The component analysis model has been used to measure the relative contributions of area and yield toward the overall output change with regard to individual crops. The growth performance of the crops has been studied using this model by numerous researchers (Gupta and Saraswat, 1997; Singh and Ranjan, 1998; Siju and Kombairaju, 2001; Kakali and Basu, 2006).

$\Delta P = A.\Delta Y + Y.\Delta A + \Delta A.\Delta Y$---------- (2)

Change in production = Yield effect + Area effect + Interaction effect

Thus, the total change in production is attributed to area and yield that can be decomposed into three effects viz; yield, area and interaction effects.

Instability index

Instability means the quality or state of being unstable or lack of stability. Agricultural instability can be measured by different methods, such as the coefficient of variation (CV), dispersion, Cuddy Della Valle Index (CDI), Coppock Instability index, etc. The present study applied the Cuddy and Valle (1978) Index to examine the nature and degree of instability in the area, production, and yield of black grams in Bangladesh. The use of CV as a measure to show the instability in any time series data has some limitations. It does not explain properly the trend component inherent in the time series data. If the time series data exhibit any trend, the variation measured by CV can be overestimated, i.e. the region which has growing production at a constant rate will score high in instability of production if the CV is applied for measuring instability. As against that, CDI first attempts to de-trend the CV by using the coefficient of determination (R^2). Thus, it is a better measure to capture instability in agricultural production. A low value of this index indicates low instability in production and vice-versa. The estimable form of the equation is as follows:

$CV_t = (CV) \times \sqrt{1 - R^2}$ ---------- *(3)*

Where, CV_t is the coefficient of variation around the trend; CV is the coefficient of variation around the mean in percent; and R^2 is the coefficient of determination from time trend regression adjusted by the number of degrees of freedom.

$$CV = \frac{Standard\ deviation}{Mean} \times 100$$

$$R^2 = 1 - \frac{Unexplained\ variation}{Total\ variation}$$

Results and Discussion

Trends of area, production and yield of black gram in Bangladesh

The trends in the area, production and yield of black gram in Bangladesh have shown fluctuations over the decades. The cultivated area and production of black gram in the first decade initially showed a declining trend until 1985-86, after which there was an increasing trend from 1986-87 to 1989-90. This increase in area during the second decade, up to 1998, was driven by the black gram's resilience to waterlogging, lesser susceptibility to diseases and pests, consistent yields, and minimal production costs (Rahman, 1989). This led to a period of higher area and production between 1987-88 and 1997-98. The second decade, starting from 1998-99 to 1999-2000, once again witnessed a decline. In the third decade, spanning up to 2004-05, the trend of decreased area and production persisted. This decline was attributed to cultivation of local cultivars with traditional farming methods towards low yields, shortage of essential inputs, and (Rahman and Baten, 2016). However, starting in 2005-06 of the third decade witnessed a

resurgence in black gram cultivation, and this upward trend continued into the fourth decade. While the yield remained relatively stable during the first and second decades, it showed an increasing trend during the third and fourth decades, albeit with some minor fluctuations.

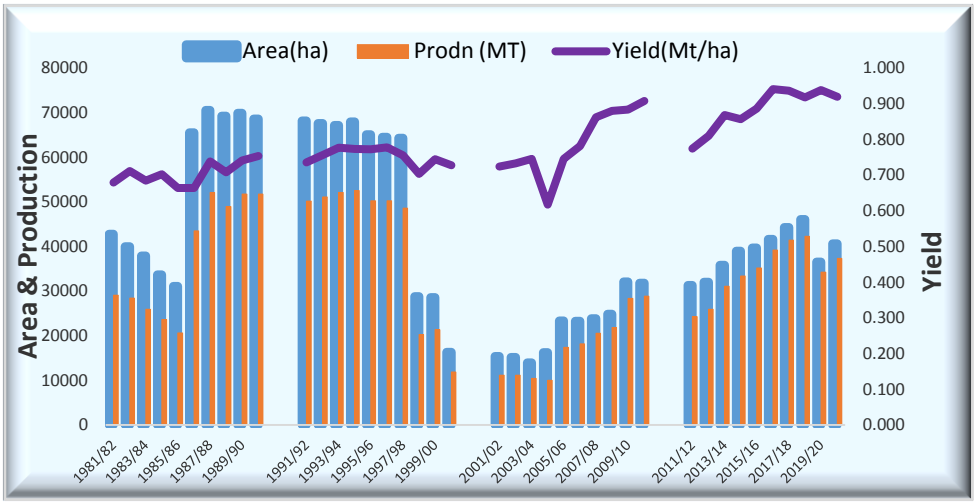

Fig. 1. Trends of area, production and yield of black gram during 1981-82 to 2019-20

Source: Using data from various issues of BBS in different years.

The overall indices showed that the area and production of black gram increased to some extent from its base period of 1981-1990 during 1991-2000. But the overall indices of area and production indicated a decreasing trend over the period from 2001-2010 to 2011-2020. On the other hand, the productivity indices revealed an increasing trend during the period from 1981-90 to 2011-20 (Table 1.). Despite the decrease in area, the yield of black gram has gone up in those periods which was mainly due to the cultivation of improved varieties (BARI Mash-1, -2, -3, -4 & -5) along with adoption of appropriate management technologies.

Table 1. Index of area, production and yield of black gram in Bangladesh

Period	Area (%)	Production (%)	Yield (%)
1981-1990	100 (52914)	100 (37565)	100 (0.704)
1991-2000	101.8	108.7	106.8
2001-2010	41.6	47.3	111.8
2011-2020	73.1	91.6	125.6

Note: Figures within parentheses indicate the 10-year average value in the base year of the indices.

Source: Various issues of BBS

Annual growth of black gram production

The overall annual growth rates scenario reveals that the area and production of black gram registered a negative growth rate during 40 years' period (1981-2020)

although the production growth rate was not significant at all (Table 2). On the other hand, black gram yield had a significant positive growth rate during the 40-year period. The growth rates of different periods show that some growth rates registered both in area and production were found positive and significant from 1981-1990, 2001-2010, and 2011-2020. Both area and production growth rates were found negative during 1991-2000 due to the limited adoption of HYV black gram technology (BARI released 3 black gram varieties during 1990-1996). However, the growth rates of yield were positive and highly significant for all periods except the period 1991-2000. The highly significant growth rates of productivity were mainly due to the adoption of improved black gram varieties and matching production technologies. This indicates that adoption of newly developed high yielding to be realized in farmers' fields.

Table 2. Annual growth rates of area, production and yield of black gram (1981-82 to 2019-20)

Period	Area (ha)	Production (mt)	Yield (t/ha)
1981-1990	8.57***	9.50***	0.93*
1991-2000	-14.13***	-14.60	-0.47
2001-2010	9.60***	12.77***	3.17***
2011-2020	3.01**	4.92***	1.92***
1981-2020	-1.43**	-0.68	0.76***

Note: '***' '**' & '*' represent 1%, 5% and 10% level of significant

Sources of growth of black gram production

Change in the mean area appeared to be the largest source of change in the mean production of black gram in all the periods. At the national level, changes in the mean yield were the main source of changes in black gram production in Bangladesh. The change in yield contributed 142.25% of the changes in the mean production of black gram at the national level. The overall scenario indicates that the positive change in production has been attributed to the positive change in the area (Table 3).

Table 3. Growth decomposition in the production of black gram (1981-82 to 2019-20)

Period	Effect (%)				
	Area (A)	Yield (Y)	Interaction	Residual	Total
	$\Delta A*Y$	$A*\Delta Y$	$\Delta A*\Delta Y$	$\Delta COV(A,Y)$	ΔQ
1981-1990	75.26	26.62	1.89	-3.77	100
1991-2000	93.71	1.14	-5.15	10.30	100
2001-2010	72.99	31.04	4.03	-8.06	100
2011-2020	89.73	11.20	0.93	-1.86	100
1981-2020	-3.29	142.25	38.97	-77.93	100

Source: Author's calculation using BBS data of different years

Instability of black gram cultivation

The estimates of instability in area, production, and yield of black gram are presented in Table 4. The instabilities of the black gram area (4.37%) and production (4.89%) at the national level were not so high, but the instability of production was little bit higher than the area instability. On the other side, the instability related to productivity was about -25.7% during 1981-82 to 2019-20 meaning that black gram productivity was almost stable and increasing over the stipulated period. The reason for the stability is due to release of three high yielding varieties (1990-1996)990-96 and their adoption at farm level between 2001-2020, resulted in increase in production.

Table 4. Instability indices for area, production and yield of black gram (1981-82 to 2019-20)

Period	Instability (%)		
	Area (ha)	Production (t)	Yield (t/ha)
1981-1990	2.03	2.20	-10.21
1991-2000	2.72	3.01	-10.52
2001-2010	1.03	1.45	-29.96
2011-2020	0.86	1.08	-26.21
1981-2020	4.37	4.89	-25.70

Source: Author's calculation using BBS data of different years

Conclusion

Over the study period from 1981-82 to 2019-20, an analysis of black gram cultivation revealed significant fluctuations in cultivation area, production levels, and yield. However, a closer examination of the last ten years presents a distinct perspective, showing a consistent upward trend in the cultivation area, production, and yield of black gram. This recent pattern indicates a positive shift in how black gram is cultivated and managed. The increase in yield can be attributed to the widespread adoption of improved varieties and advanced management technologies. These advancements have likely played a pivotal role in enhancing overall black gram productivity, contributing to the upward trajectory observed in both cultivated area and production. The success of sustainable agricultural practices relies on achieving a balance between high growth rates and low production instability, a critical consideration with significant implications for researchers and policymakers. In the context of this study, a comprehensive investigation into black gram production in Bangladesh has yielded insightful findings. The analysis of growth in black gram production underscores a positive and substantial increase in yield. However, it's important to note that this growth is modest, suggesting a need of genetic innovation and agronomic research in future. In contrast, a different trend emerges concerning black gram cultivation area and production, both showing negative growth rates. This decline raises concerns about the potential shrinking of cultivable land or declining interest among farmers in cultivating black gram. The vulnerability of agricultural production to natural disasters remains an enduring reality in Bangladesh,

introducing an element of instability. A national-level perspective reveals relatively lower degrees of instability when analyzing the extent of instability in black gram cultivation. This highlights the resilience of black gram cultivation in the face of climatic fluctuations, providing a sense of stability within the broader agricultural landscape.

Recommendations

Based on the conclusions drawn from this study, several recommendations can be put forth to foster sustainable growth in black gram production in Bangladesh. To begin with, agricultural researchers should release farmer-preferred black gram varieties that are both more climate-resilient and high-yielding, aligning with the domestic demand. Secondly, the improved black gram varieties and technologies already developed by BARI should be introduced to farmers through massive deployment initiatives. Thirdly, research and policy support are needed to increase the acreage and yield of black gram which will help increase the per capita availability of black gram, reduce import dependence, and to some extent stabilize prices. Ultimately, fostering collaboration between research institutions, quality seed production agencies, non-governmental organizations, agricultural marketing and private sector participants should be prioritized, as it holds the key to augmenting the growth and stability of black gram production in Bangladesh.

Conflicts of Interest

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

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CORRELATION AND PATH COEFFICIENT ANALYSIS OF DIFFERENT GROWTH AND YIELD COMPONENTS OF KIDNEY BEAN (*Phaseolus vulgaris* L.)

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Abstract

An investigation was undertaken to determine the major yield contributing traits based on their direct and indirect effects on yield through the correlation and path coefficient analysis. The research of kidney bean germplasm was based on the evaluation of germplasm collected from Sylhet, Bandarban hilly regions and Bangladesh Agricultural Research Institute, considering 17 yield and its contributing characters. The study unraveled that yield per plant had highly significant positive correlation with number of leaves (0.83, 0.78), number of pods per plant (0.78, 0.73), pod length (0.86, 0.76), dry weight of pod (0.97, 0.90), number of seeds per pod (0.79, 0.72) at both genotypic and phenotypic correlation levels. It showed significant and negative association with pod diameter (-0.67) at the genotypic level. In path coefficient analysis high positive direct effect was found in seed yield towards yield per hectare. While, it was observed negligible positive indirect effect towards yield per hectare via, days to first flowering, days to maturity, plant height, number of pods per plant, petiole length, pod diameter, and number of seeds per pod. The overall results suggest that the yield contributing traits such as number of leaves, number of pods per plant, pod length, dry weight of pod, and number of seeds per pod should be considered as selection index for yield improvement of kidney bean.

Keywords: Correlation, Germplasm, Kidney bean, Path coefficient

Introduction

Kidney bean *(Phaseolus vulgaris* L.) is cultivated widely due to its high nutritional composition, especially good source of protein in dry seed, and higher content of fiber in fresh pod. The bean is usually consumed either as fresh pod or dry bean (pulse). This pulse has different names such as Bush bean, French bean, Haricot bean, Snap bean, Navy bean, Pinto bean, Rajma, Green bean, while in Bangladesh it is known as '*Forshisheem* or *Jarsheem*'. The crop is very popular among the ethnic people, especially in the hilly areas of Sylhet, Bandarban, Rangamati and Khagrachari districts, where the bean is consumed as the main sources of protein (Fatema *et al.,* 2019). Besides

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the hilly areas, the crop is being also cultivated in Jashore, Rangpur, Cumilla, Cox's Bazar, etc., districts. Being a short duration crop kidney bean fits well in three and four crop-based cropping patterns both in hills and plains areas of Bangladesh. Recently, BRAC and Hortex Foundation are trying to extend the cultivation area of the kidney bean because the crop is an exportable vegetable now and has a huge demand in the foreign countries (FAOSTAT, 2021). Thus, the popularity of kidney bean cultivation is increasing in Bangladesh due to its high demand as an export product. Yield is an important quantitative character that is greatly depended on several attributing characters. Some characters affect directly, while some affect indirectly to the yield. For the improvement of yield and other important characters, a knowledge of the extents of variation in the available germplasm, the correlation of associated characters with yield, degrees of environmental influences on these traits and the heritability of the characters is necessary (Saifullah and Rabbani, 2009). Generally, correlation coefficient shows association among yield related characters (Toker and Cagirgan, 2004). However, correlation studies provide the relative effects to the yield on the other hand, path analysis is used as a supplementary component of correlation coefficients. Thus, path analysis allows partitioning of components as their direct and indirect effects and it uses a standard partial regression co-efficient (Shabana *et al.,* 1990). The path analysis method gives the clear idea about the effects of every distinct characteristic on yield (Onder and Babaoglu, 2001). Path analysis has been used by the breeders to recognize the traits that are useful selection criteria to improve crop yield (Shompa *et al*., 2020).

Kidney bean is getting popularity in Bangladesh due to its high nutritional values and export values, however, till date only three varieties of kidney bean have been so far released in Bangladesh (Rahman *et al*., 2022), hence the scope of genetic improvement of the bean is very ample. The literature survey indicates that scanty researches have been carried out on kidney bean improvement in Bangladesh. Therefore, the present investigation was undertaken to determine the major of yield contributing traits of kidney bean. and their direct and indirect effects on yield to select the highly correlated. Thus, the breeders can use those characters as a selection index to improve the yield.

Materials and Methods

Location of experimental site and soil

The present investigation was carried out at the experimental field of Sher-e-Bangla Agricultural University, Dhaka. The area situated at 23°46'16" N latitude and 90°22'46" E longitude at an altitude of 4 meter above the sea level with sub-tropical climate. Soil of the experimental site belongs to the general soil type, Shallow red brown terrace soils under Tejgaon Series. Topsoil is clay loam in texture, the pH ranged from 6.0- 6.6 and had organic matter 0.84%. The experimental area was flat which facilitated irrigation and drainage system easily. Soil samples from 0-15 cm depths were collected from experimental field. The soil analyses were done by Soil Resource and Development Institute (SRDI), Dhaka. The experimental field belongs to Agrological Zone Madhupur Tract (AEZ 28) having soil properties as 6.62 pH; 0.84 organic matter; 0.046 % total N; 21 ppm available P and 0.41 cmolc kg^{-1} exchangeable K (Roy *et al.,* 2019).

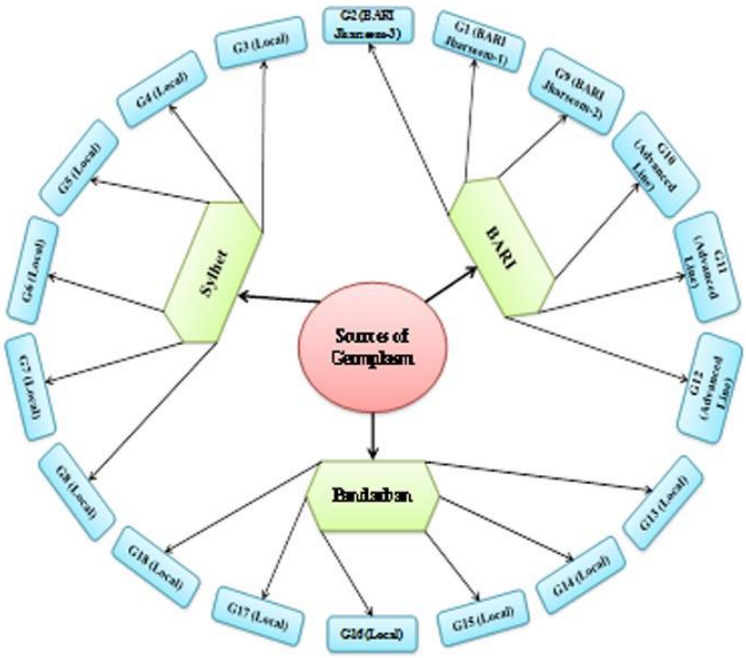

Fig. 1. Different sources of 18 germplasms of kidney bean

Plant materials

Total 18 genotypes of kidney beans were collected from hilly areas of Sylhet and Bandarban districts. Three advanced lines and three released varieties were collected from Bangladesh Agricultural Research Institute (BARI). The sources of the collected Kidney bean germplasm are shown in Fig. 1.

Land preparation, seed sowing and crop raising

The land was prepared through ploughing and it was leveled to avoid water logging condition. The field experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The experimental field was irrigated to have optimum level of moisture condition. Recommended doses of fertilizers were applied (Azad *et al.*, 2020). The seeds were treated with Vitavax 200 and water priming for 12 hours to get good field emergence before sowing. The seed was sowing on 16 November 2016. Total land area was 210 m^2 where each genotype occupied three rows. Plant to plant and row to row distance followed were 25 cm and 50 cm, respectively. Staking was done using bamboo sticks to keep the plants erect. The experimental plot was irrigated during the cropping period with light irrigation.

Recording of growth and yield contributing parameters

Five individual plants of each treatment from each replication were selected randomly at the time of recording the data on various characters. Mean data of five plants for each replication was used for statistical analysis. The observations were

recorded on Days to 5-leaves stages, Days to 1st flowering, Days to 50% flowering, Days to pod maturity, Days to 1st pod setting, Plant height (cm), Number of leaves plant^{-1}, Number of pods plant^{-1}, Leaf area (cm^2), Petiole length (cm), Pod length (cm), (Fig. 2), Pod diameter (cm), Dry weight of pod (g), Number of seeds pod^{-1}, 1000 seed weight (g), Seed weight (g/plant) and Seed yield (t/ha). For each parameter data recording methods were described in Table 1.

Table 1. Name of parameters and data recording methods

Sl No	Traits/Parameters	Method of data recording
1.	Days to 5-leaves stages	Whole plot basis
2.	Days to 1st flowering	Whole plot basis
3.	Days to 50% flowering	Whole plot basis
4.	Days to pod maturity	Whole plot basis
5.	Days to 1st pod setting	Whole plot basis
6.	Plant height(cm)	5-plant basis
7.	Number of leaves plant^{-1}	5-plant basis
8.	Number of pods plant^{-1}	5-plant basis
9.	Leaf area (cm^2)	5-plant basis by using leaf area meter
10.	Petiole length (cm)	5-plant basis
11.	Pod length (cm)	5-pod basis
12.	Pod diameter(cm)	5-pod basis
13.	Dry weight of pod (g),	5-pod basis
14.	Number of seeds pod^{-1}	5-pod basis
15.	1000-seed weight(g)	Total of 1000 dry seed
16.	Seed weight plant^{-1} (g/plant)	5-pod from each genotype
17.	Seed yield hectare^{-1} (t/ha)	Seed yield plot-1 is converted to seed yield hectare^{-1} (t/ha)

Statistical analysis

The mean values of each trait from all the genotypes were subjected to statistical analysis. The correlation coefficients were estimated to determine the degree of association of characters with yield and also among the yield components; Both genotypic and phenotypic coefficients of correlation between two characters were determined by using the variance and covariance components as suggested by Al-Jibouri *et al.,* (1958). Path coefficient analysis was carried out using phenotypic correlation values of yield components on yield as suggested by Wright (1921) and Dewey and Lu (1959). Standard path coefficients which are the standardized partial regression coefficients were obtained using statistical software packages OPSTAT (Pal *et al*., 2017)

Results and Discussion

Phenotypic correlation analysis

The phenotypic correlation between yield and its contributing traits of available kidney germplasm found in Bangladesh are presented in the Table 2. Seed yield per plant was highly significant and positively correlated with number of leaves (0.781), leaf area (0.440), petiole length (0.474), number of pod per plant (0.776), pod length (0.772), pod dry weight (0.909), number of seed per pod (0.719), 1000 seed weight (0.677) (Table 3). While the negative phenotypic correlations were found with plant height (-0.128), days to 5 leaves stages (-0.502), days to 1st flowering (-0.179), days to maturity (-0.401), days to 50% flowering (-0.049), days to 1st pod setting (-0.106), pod diameter (-0.404) (Table. 2). The correlation of days to first pod setting with days to 1st flowering (0.902), days to 50% flowering (0.940), days to maturity (0.728), plant height (0.498) and pod length (0.611) was positive and highly significant at the phenotypic levels (Table 2).

Number of pods per plant displayed high significant and positive correlation with number of leaves (0.821), pod length (0.732), dry weight of pod (0.769), number of seeds per pod (0.845), seed yield (0.734) and yield (0.735) at phenotypic levels (Table 3). Pod length is one of the main yield components in Kidney bean. Pod length showed similar association at phenotypic levels. It was recorded that pod length had positive and high significant correlation with number of leaves (0.827), dry weight of pod (0.759), number of pods per plant (0.732), number of seeds per plant (0.682), seed yield (0.767) and yield (0.76) (Table 2). Number of seeds per pod was found positive association with number of leaves (0.84), Number of pods per plant (0.845), pod length (0.682), dry weight of pods (0.804), seed yield (0.720) and yield (0.720). This also had significant and positive correlation with leaf area (0.520). All these associations were observed at phenotypic levels (Table 2).

Genotypic correlation analysis

The seed yield per plant was positively correlated number of leaves (0.837), number of pod per plant (0.793), leaf area (0.391), petiole length (0.371), pod length (0.861), pod dry weight (0.976), number of seed per pod (0.799),1000 grains weight (0.463). While the seed yield was negatively correlated with days to 5 leaves stages (-0.559), days to 1st flowering (-0.217), days to 50% flowering (-0.142), days to maturity (-0.462), days to 1st pod setting (-0.176), plant height (-0.186), pod diameter (-0.677) (Table 3). The correlation of days to first pod setting with days to 1st flowering (0.952), days to 50% flowering (0.990), days to maturity (0.791), plant height (0.557) and pod length (0.635) was positive and highly significant at the genotypic levels (Table 3). Number of pods per plant displayed high significant and positive correlation with number of leaves (0.855), pod length (0.795), dry weight of pod (0.802), and number of seeds per pod (0.903), seed yield (0.793) and yield (0.785) at genotypic levels (Table 3). But it showed negatively significant association with pod diameter (-0.676) at genotypic level. Pod length is one of the main yield components in kidney bean. Pod length showed similar association in case of genotypic levels. Results also showed that pod length had

Fig. 2. Green pod length variation of different genotypes of kidney bean

positive and high significant correlation with number of leaves (0.909), dry weight of pod (0.816), number of pods per plant (0.795), number of seeds per plant (0.742), seed yield (0.861) and yield (0.861) (Table 3). The number of seeds per pod was found in significant and positive association with number of leaves (0.885), Number of pods per plant (0.903), pod length (0.742), dry weight of pods (0.816), seed yield (0.799) and yield (0.798). This also had a significant and positive correlation with leaf area (0.537). All these associations were observed at genotypic level (Table 3).

Several correlation studies were conducted in kidney bean and found similar trends of results. A group of scientists found that number of pods per plant and number of seeds per pod were positively associated with seed yield in kidney bean (Atuahene-Amankwa and Michaels, 1997; Chand, 1999; Coimbra *et al.,* 1998; Coyne, 1968; Duarte and Adams, 1972; Nienhuis and Singh, 1986; Samal *et al*., 1995; Immaculee, 2011; Murry *et al*., 2022. Again, in case of first pod setting, similar result also observed by Aggarwal *et al*., 1973; Chand (1999), Coimbra *et al*., 1998. The positive and significant association of pod per plant with seeds per pod has also been reported by Mishra *et al*., (1996), Singh (2000) and Prasad (1995). Singh (2000) reported pod length showed significant and positive association with plant height, number of pods per plant. Apart from that, Alemu *et al.* (2017) reported that there was negative and significant association between green pod width and green pod length. However, pod length was negatively associated with seed yield observed by Immaculee (2011) in kidney bean, Venkatkrishnakishore *et al.* (2002) and Mittal and Singh (2005) in Mungbean. Pod length had non-significant correlation with days to maturity in the investigation confirmed by Narsinghani and Saxena (1991). Seeds per pod were recorded significant and positive association with pod length in accordance with Sharma *et al.*(1998) and Basavaraja *et al.* (2021). Similarly, the significant association of seeds per plant with pod length was reported by Singh *et al*., 1994; Kumara *et al*., 1997; in Rice bean and Chauhan *et al*., 2003; in cowpea.

Path coefficient analysis

Correlation coefficients show relationships among independent variables and the linear relationship between these variables. However, it is not sufficient to describe these relationships when the causal relationship among variables is needed. Yield components have either a direct or an indirect effect on seed yield, or both. Therefore, we calculated the direct and indirect effects of yield components on seed yield through the other components in Kidney bean germplasm. A high positive direct effect was found in seed yield (1.00) towards yield per hectare. It was recorded as the highest positive effect on yield per hectare. However, Immaculee (2011) found pods per plant having the highest direct effect on yield per hectare. Further, it was recorded negligible positive indirect effect towards yield per hectare via days to first flowering (0.01), days to maturity (0.01), plant height (0.015), number or pods per plant (0.012), petiole length (0.005), pod diameter (0.017), number of seeds per pod (0.026). It also reported negligible negative indirect effect towards yield per hectare via days to 5- leaves stage (-0.009), days to 50% flowering (-0.013), days to 1st pod setting (-0.017), number of leaves (-0.019), leaf area (-0.016), pod length (-0.002), dry weight pod (-0.019), 1000 seed weight (-0.012) (Table 5). The genotypic correlation (1) with yield was positive and significant. Karasu and Oz

Table 2. Phenotypic correlation coefficient for 17 characters of kidney bean germplasm

	FF	50%F	M	FPS	PH	NLP	NPP	LA	PL	PdL	PdD	DWP	NSP	1000SW	SY	Y
LS	0.410	0.267	0.165	0.379	0.059	-0.495*	-0.330	-0.018	-0.119	-0.494*	0.378	-0.407	-0.423	0.050	-0.504*	-0.504*
FF		0.956**	0.628**	0.902**	0.531*	-0.244	-0.095	0.267	0.481*	-0.177	0.273	-0.109	-0.164	-0.077	-0.196	-0.196
50%F			0.674**	0.940**	0.584*	-0.052	0.057	0.399	0.614**	-0.064	0.203	0.027	0.031	-0.130	-0.096	-0.096
M				0.728**	0.571*	-0.287	-0.279	0.205	0.419	-0.486*	0.273	-0.200	-0.228	-0.383	-0.376	-0.376
FPS					0.498*	-0.105	-0.071	0.367	0.611**	-0.201	0.232	-0.015	-0.070	-0.041	-0.139	-0.139
PH						0.072	0.144	0.572*	0.676**	0.102	0.107	0.082	-0.049	-0.407	-0.156	-0.155
NLP							0.821**	0.451	0.468	0.827**	-0.266	0.825**	0.844**	0.195	0.781**	0.781**
NPP								0.462	0.419	0.732**	-0.361	0.769**	0.845**	-0.065	0.734**	0.735**
LA									0.810**	0.499*	-0.008	0.604**	0.520*	0.024	0.366	0.367
PL										0.423	0.024	0.551*	0.429	0.081	0.349	0.349
PdL											-0.236	0.759**	0.682**	0.300	0.767**	0.767**
PdD												-0.322	-0.344	0.133	-0.411	-0.411
DWP													0.804**	0.288	0.902**	0.902**
NSP														0.074	0.720**	0.720**
1000SW															0.348	0.348
SY																1.000**

** = Significant at 1% ; * = Significant at 5% ; LS= Days to 5 leaves stage ; FF=Days to 1st Flowering 50% ; F=Days to 50% flowering; M=Days to Maturity; FPS=Days to 1st Pod Setting; PH=Plant Height (cm); NLP=No of Leaves/plant; NPP=No of Pod/Plant; LA=Leaf A;rea (cm^2); PL=Petiole Length (cm); PdL=Pod Length (cm); PdD=Pod Diameter (cm); DWP=Dry Weight of Pod; NSP=No of Seeds/Pod; 1000SW=1000 Seed Weight; SY=Seed Yield (g/Plant); Y=Seed Yield (g/Plant)

Table 3. Genotypic correlation coefficient for seventeen characters of kidney bean germplasm

	FF	50%F	M	FPS	PH	NLP	NPP	LA	PL	PdL	PdD	DWP	NSP	1000SW	SY	Y
LS	0.423	0.275	0.176	0.385	0.066	-0.515*	-0.337	-0.017	-0.126	-0.532*	0.678**	-0.417	-0.456	0.087	-0.559*	-0.560*
FF		0.988**	0.699**	0.952**	0.556*	-0.257	-0.112	0.273	0.495*	-0.193	0.435	-0.105	-0.163	-0.139	-0.217	-0.217
50%F			0.773**	0.990**	0.607**	-0.068	0.048	0.417	0.640**	-0.072	0.309	0.023	0.038	-0.202	-0.142	-0.142
M				0.791**	0.703**	-0.288	-0.324	0.227	0.460	-0.559*	0.370	-0.204	-0.247	-0.580*	-0.462	-0.462
FPS					0.557*	-0.127	-0.085	0.386	0.635**	-0.213	0.331	-0.011	-0.067	-0.076	-0.176	-0.172
PH						0.077	0.148	0.601**	0.721**	0.112	0.283	0.090	-0.028	-0.506*	-0.186	-0.185
NLP							0.855**	0.469*	0.483*	0.909**	-0.523*	0.848**	0.885**	0.253	0.837**	0.831**
NPP								0.471*	0.428	0.795**	-0.676**	0.802**	0.903**	-0.125	0.793**	0.785**
LA									0.821**	0.536*	0.029	0.616**	0.537*	0.033	0.391	0.391
PL										0.447	0.044	0.559*	0.441	0.093	0.371	0.372
PdL											-0.408	0.816**	0.742**	0.359	0.861**	0.861**
PdD												-0.554*	-0.631**	-0.092	-0.677**	-0.677**
DWP													0.816**	0.352	0.976**	0.976**
NSP														0.083	0.799**	0.798**
1000SW															0.463	0.462
SY																1.000**

** = Significant at 1% ; * = Significant at 5%; LS =Days to 5 leaves stage ; FF = Days to 1st Flowering 50% ; F=Days to 50% flowering; M=Days to Maturity; FPS=Days to 1st Pod Setting; PH =Plant Height (cm); NLP=No of Leaves/plant ; NPP=No of Pod/Plant; LA=Leaf Area (cm^2); PL=Petiole Length (cm); PdL=Pod Length (cm); PdD =Pod Diameter (cm);DWP=Dry Weight of Pod; NSP=No of Seeds/Pod; 1000SW=1000 Seed Weight; SY=Seed Yield (g/Plant); Y=Seed Yield (t/ha)

(2010) found high positive direct effect of seed yield per plant with yield per hectare. He also found positive significant correlation between the traits. Acharya (2013) reported seed weight had negligible positive direct effect on toward the yield, and he also reported high positive indirect effects towards pod yield per hectare via pod weight. Seeds per plant (0.442) exhibited the highest positive direct effect on seed yield found by Mehra *et al.,* (2016) and kalauni (2020). Previous studies indicated that seed yield per hectare in the bean was positively correlated with number of pods per plant, number of seeds per pod and seed yield per plant (Duarte and Adams, 1972; Westerman and Crothers, 1977; Prakash and Ram, 1981. From the investigation, it can be concluded that seed yield per plant and number of seeds per pod can be considered the vital traits in selection of desirable genotypes of kidney bean for their genetic improvement.

The days to 1st pod setting recorded negligible positive direct effect (0.004) towards yield per hectare. Further, it was recorded negligible positive indirect effect towards yield per hectare via days to first flowering (0.020), days to maturity (0.013), plant height (0.011), number of pod per plant (0.011), pod diameter (0.008) and number of seeds per plant (0.008) (Table 5). However, it was recorded negligible negative indirect effect towards yield per hectare via days to 5 leaves stage (-0.026), days to 50% flowering (-0.037), number of leaves (-0.019), leaf area (-0.020), petiole length (-0.001), pod length (-0.026), dry weight of pod (-0.020), 1000 seed weight (-0.019), seed yield per plant (-0.080). These results are in agreement with results of Fisher (1918), Pande *et al.,* (1975), Shinde and Dumbre (2001), and Tamilselvan *et al.,* 1994. The number of pods per plant recorded negligible positive direct effect (0.020) towards yield per hectare. On the other hand, Kumar *et al.,* 2014; found number of pods per plant showed high positive indirect effect through number of seeds per pod. Mishra *et al*., 1996; reported high positive direct effect of pods per plant on seed yield which was an indication of improvement of high pod yield through selection of these characters.

Further, it was recorded negligible positive indirect effect towards yield per hectare via. days to 1st flowering (0.022), days to maturity (0.023), plant height (0.027), petiole length (0.016), pod length (0.008), pod diameter (0.028), number of seeds per pod (0.041) and significant, high, positive indirect effect toward seed yield per plant (0.641) (Table 4). Number of pods per plant did not have any significant effect on yield per hectare through days to 5- leaves stage. However, it was found negligible negative indirect effect towards yield per hectare via, days to 50% flowering (-0.003), days to 1st pod setting (-0.010), number of leaves (-0.010), leaf area (-0.006), dry weight pod (-0.009) and 1000 seed weight (-0.004). The correlation of number of leaves was highly significant (0.785) with yield per hectare. Highly positive indirect effect of pod per plant was recorded through seed per pod whereas indirect effects of seeds per pod through other character were negligible (Immaculee, 2011). Pod length observed negligible positive direct effect (0.031) towards yield per hectare. However, Ulukan *et al.,* (2003) and Sharifa (2014) found that pod length had high positive direct effect on seed yield per plot in broad bean. It was also recorded negligible negative indirect effects to yield per hectare via days to days to 1st pod setting (-0.006), number of leaves (-0.004), leaf area (-0.001), dry weight pod (-0.004) (Table 4). On the other hand, it was found negligible positive indirect effect toward yield per hectare via days to 5- leaves stage (0.006), days

Table 4. Path coefficient analysis showing direct and indirect effects of different characters on the yield of kidney bean

Characters	Direct effect	Indirect effect															Genotypic correlation with yield
		LS	FF	50%F	M	FPS	PH	NLP	NPP	LA	PL	PdL	PdD	DWP	NSP	1000SW	SY	
LS	-0.036		0.015	-0.029	0.009	-0.011	0.007	-0.019	0.010	-0.022	0.011	-0.032	0.006	-0.020	0.001	-0.022	-0.429	-0.560*
FF	0.031	-0.020		-0.032	0.019	0.004	0.019	-0.012	0.018	-0.014	0.010	-0.020	0.015	-0.013	0.013	-0.013	-0.222	-0.217
50%F	-0.040	-0.022	0.025		0.015	0.001	0.018	-0.017	0.013	-0.018	0.003	-0.021	0.012	-0.017	0.013	-0.018	-0.087	-0.142
M	0.016	-0.023	0.015	-0.030		-0.009	0.010	-0.019	0.011	-0.021	0.006	-0.031	0.008	-0.019	0.005	-0.023	-0.358	-0.462
FPS	0.004	-0.026	0.020	-0.037	0.013		0.011	-0.019	0.011	-0.020	-0.001	-0.026	0.008	-0.020	0.008	-0.019	-0.080	-0.172
PH	0.026	-0.028	0.003	-0.033	0.002	-0.027		-0.029	0.001	-0.029	-0.009	-0.024	0.000	-0.029	0.000	-0.030	0.021	-0.185
NLP	-0.010	0.003	0.023	-0.003	0.025	-0.006	0.030		0.023	-0.004	0.013	0.011	0.028	-0.007	0.043	-0.002	0.663**	0.831**
NPP	0.020	0.000	0.022	-0.003	0.023	-0.010	0.027	-0.010		-0.006	0.016	0.008	0.028	-0.009	0.041	-0.004	0.641**	0.785**
LA	-0.016	-0.013	0.016	-0.018	0.015	-0.011	0.025	-0.017	0.013		-0.003	-0.005	0.017	-0.018	0.027	-0.013	0.393	0.391
PL	-0.012	-0.014	0.017	-0.024	0.015	-0.006	0.024	-0.020	0.012	-0.018		-0.008	0.014	-0.019	0.024	-0.014	0.401	0.372
PdL	0.021	0.006	0.026	0.003	0.027	-0.006	0.035	-0.004	0.027	-0.001	0.019		0.031	-0.004	0.041	0.002	0.636**	0.861**
PdD	-0.037	-0.052	-0.019	-0.051	-0.020	-0.047	-0.018	-0.050	-0.020	-0.050	-0.020	-0.052		-0.050	-0.023	-0.047	-0.120	-0.677**
DWP	-0.005	0.006	0.028	0.001	0.029	-0.001	0.032	-0.005	0.027	-0.001	0.015	0.014	0.033		0.046	0.003	0.756**	0.976**
NSP	0.053	0.007	0.030	0.001	0.031	0.000	0.030	-0.003	0.028	0.001	0.020	0.013	0.035	-0.002		0.003	0.549*	0.798**
1000SW	0.003	-0.006	0.024	-0.005	0.022	-0.004	0.018	-0.008	0.023	-0.007	0.019	-0.002	0.018	-0.008	0.024		0.351	0.462
SY	1.000	-0.009	0.011	-0.013	0.013	-0.017	0.015	-0.019	0.012	-0.016	0.005	-0.002	0.017	-0.019	0.026	-0.012		1.000**

Residual Effect= 0.11597014; ** = Significant at 1% * = Significant at 5% ; LS=Days to 5 leaves stage; FF=Days to 1st Flowering; 50%F=Days to 50% flowering; M=Days to Maturity; FPS=Days to 1st Pod Setting; PH=Plant Height (cm); NLP=No of Leaves/plant; NPP =No of Pod/Plant; LA =Leaf Area (cm²); PL=Petiole Length (cm); PdL=Pod Length (cm); PdD =Pod Diameter (cm); DWP = Dry Weight of Pod; NSP =No of Seeds/Pod; 1000SW =1000 Seed Weight; SY = Seed Yield (g/Plant); Y=Seed Yield (g/Plant)

to 1st flowering (0.026), days to 50% flowering (0.003), days to maturity (0.027), plant height (0.035), number of pods per plant (0.027), petiole length (0.019), pod diameter (0.021), number of seeds per pod (0.041) 1000 seed weight (0.002) and high positive indirect effect of seed yield per plant (0.636). The genotypic correlation of leaf area (0.861) with yield per hectare was positive and significant. Pod length recorded low positive indirect effects towards pod yield per hectare via pod weight (Acharya, 2013).

Number of seeds per pod showed negligible positive direct effect (0.053) towards yield per hectare. In previous studies, it was reported that seed number per pod had highest and positive direct effect on seed yield (Amini *et al*., 2002; Dursun, 2007). Shinde and Dumbre (2001) also found it on consonance. Further, it was recorded negligible positive indirect effect towards yield per hectare via, days to 5- leaves stage (0.007), days to first flowering (0.030), days to 50% flowering (0.001), days to maturity (0.031), plant height (0.030), number or pods per plant (0.028), leaf area (0.001), petiole length (0.020), pod length (0.013), pod diameter (0.035), 1000 seed weight (0.003) and high positive indirect effect of seed yield per plant (0.549) (Table 4). It also found negligible negative indirect effect towards yield per hectare via, number of leaves (-0.003) and dry weight pod (-0.002). It had significant and positive genotypic correlation (0.79) with yield per hectare. Dursun (2007) found that number of seeds per pod showed the highest indirect effect on yield via wet pod weight. Number of seed per pod exhibited high positive indirect effects towards pod yield per hectare via pod weight and moderate negative indirect effect number of pods per plant observed by Acharya (2013).

Conclusion

The genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients for all the characters under study. The association of the characters showed that seed yield per hectare was highly significant and positive with number of leaves, number of pods per plant, pod length, dry weight of pod, number of seeds per pod and seed yield per plant at both genotypic and phenotypic levels. The path co-efficient analysis for seed yield per hectare revealed that seed yield per plant exerted direct effect on seed yield, number of seeds per pods. Thus the results suggest that number of leaves, number of pods per plant, pod length, dry weight of pod, number of seeds per pod and seed yield per plant can be used as selection index to maximize the yield of kidney bean in Bangladesh.

Conflicts of Interest

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

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INFLUENCE OF CHEMICALS AND CRUDE PLANT MATERIALS AS PRE-STORAGE TREATMENT ON SEED QUALITY OF ONION

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Abstract

Fresh onion seeds dried to 7.0% seed moisture content were stored with crude plant materials (red chili powder, neem leaf powder or lemon leaf powder each @ 20g/kg seed), and chemicals (common bleaching powder and mancozeb each @ 2g/kg of seed). The germination potential of onion seeds was found satisfactory in every treatment. Water uptake during imbibition was maximum in lemon leaf treated seed which indicates better germination as the imbibition of water is an essential part of germination. There was high correlation between EC measurements and germination indicated that conductivity readings have the potential to provide a rapid assessment of standarding laboratory germination. In terms of seed-associated pathogens during storage, chemicals have shown better results in suppressing pathogens.

Keywords: Crude plant materials, Neem leaf, lemon leaf, Quality, Seed germination

Introduction

Seed vigor consists of those properties, which conclude the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions. The main factors affecting seed storage capability are the content of high temperature, ambient relative humidity, and seed moisture (Abdul-Baki, 1980). The loss of viability in onion seed is very fast, normally within a year (Singh and Bhonde, 2003).The onion (*Allium cepa* L.) is a crop of major economic and dietary importance in all parts of the world. Compared with many other crops, the onion has a fairly complex life cycle involving several distinct development phases. It is generally known that onion seed is one of the shortest-lived seeds of the common vegetable crops (Angel *et al*., 2014) rapidly losing viability after harvest unless special precautions are taken in its storage. It is therefore generally recommended that only fresh onion seed should be used for crop production (Riekels *et al*., 1976) and only seeds of high germination percentage should be sold. The percentage and rate of germination of onion seeds also vary considerably among seed lots (Bedford & MacKay, 1973) and this leads to difficulties in establishing optimum plant populations in the field. It has long been known that the factors, which

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have the greatest influence on the longevity of seeds in storage are moisture, temperature, and oxygen partial pressure. It is usually agreed that the moisture content of the atmosphere is the most critical factor, with a rise in air moisture being more damaging than rising temperatures. However, it has been recommended that dry and very dry seeds should be humidified before germination to raise their moisture contents slowly in the initial stages (Powell & Matthews, 1979; Ellis *et al*., 1985 a & b). Several other workers have also attempted to store the onion seeds under different storage conditions for different periods (Caneppele *et al*., 1995; Pandey, 1996; Stumof *et al*., 1997; Yanping *et al.*, 2000). Therefore, it becomes necessary to evaluate the physiological quality of commercially available onion (*Allium cepa* L.) seed stored for different periods for proper crop stand and performance in the field. In the present study, the experiment was conducted to find out possible way to improve the storability of onion seeds by using crude plant materials and chemicals as a pre-storage seed treatment.

Materials and Methods

Seed source and storage

Seeds of onion (*Allium cepa* L.) variety BARI Peaj-1 was obtained in April 2020 from the research field of Seed Technology Division, Bangladesh Agricultural Research Institute, Gazipur. After collection, seeds were cleaned and dried in the sunlight for 5-6 days to a moisture content of 7.0 % for safe storage. Seeds were then stored in the 500ml capacity rubber stopper glass bottles under ambient conditions in the laboratory till seed invigoration treatment. After cleaning and drying seeds were divided into six lots for Pre-storage seed invigoration treatments. After treatments seeds were stored in a 100 ml. capacity test tube, each containing 50 g seeds. The test tubes were then air-tied primarily with aluminum foil paper and covered again with polythene to control moisture absorption of the treated seed. Onion seeds were dry-dressed with three crude plant material, viz. neem leaf powder (*Azadirachta indica* L.) at 20g/kg of seed, red chili powder (*Capsicum frutescens* L) at 20g/kg of seed, lemon leaf powder (*Citrus limon* L.) at 20g/kg of seed, and two chemicals viz., mancozeb at 2 g/kg and bleaching powder at 2g/kg of seed in an airtight test tube at room temperature. After treatment, test tubes were shaken twice a day for up to 7 days for thorough mixing of crude plant materials and chemicals with the seeds, and the test tubes were kept in the laboratory under ambient conditions (25-30^0 C).

Determination of seed moisture content

Three replicate samples, each of 1000 seeds were taken and weighed (M2), evenly spaced in 90 mm glass Petri dishes and placed in an oven at 103±2°C for 17 ± 1 h, cooled in a silica gel container for 15–30 min, after which they were reweighed (M3). The moisture content was expressed as a percentage of their wet weight in accordance with the International Rules for Seed Testing (ISTA, 1985) and calculated as:

$$\textit{Percentage moisture content (\% MC.)} = \frac{M2-M3}{M2-M1} \times 100$$

Where, M1 is the weight of the dish, M2 is the weight of the dish and its contents before drying and M3 is the weight of the dish and its contents after drying.

Water uptake during the imbibition period

Seed samples were counted, weighed (W_2), and then imbibed in Petri dishes on double sheets of filter paper moistened with 10 ml of distilled water in ambient conditions at 25±2°C. Dishes were removed from the series at intervals, and the seeds were drained and surface water removed by blotting between sheets of paper towel. The seeds were then immediately weighed (W_1), and the percentages of water taken up were recorded and calculated as:

$$\textit{Percentage water uptake} = \frac{W1-W2}{W2} \times 100$$

Where,

W1 is the weight of the seeds after imbibition and

W2 is the weight of seeds before imbibition.

Germination test

The germination potential of the onion seeds was estimated (ISTA, 1985). Germination percentages, using four replicates of 100 seeds, were determined by placing the seed samples in 120 mm Petri dishes on filter papers (Whatman No. 1) moistened with 3 ml of distilled water. Seeds were distributed evenly within each dish. Petri dishes were covered with their lids and then placed in a germination room, maintained at 20±2°C temperature. Each Petri dish was watered daily with an amount of distilled water according to its requirement. The first counting of germination was recorded on the 6th day and the final counting was recorded on the 12th day. The germination percentage was recorded by the following formula

$$\textit{Germination percentage} = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ placed\ for\ germination} \times 100$$

The following recognizable abnormalities of onion seedlings were recorded as abnormal seedling, as listed in Section 5.8.2 (ISTA, 1966).

Ia - no primary root

Ib - primary root short, stunted and weak or spindly

IVc - poorly developed leaf-like cotyledon without a definite bend or "knee"

Va - decayed cotyledon

Ve - decayed primary root

Vg - completely decayed seedling (an additional category to those in Section 5.8.1)

VIa - short and weak, or spindly, or watery seedling

Electrolyte leakage

Solute leakage of treated seeds was estimated by placing triplicates of 2 g seeds each in 20 ml of distilled water for 12 h at 25^{0}C. Electrical conductivity of the medium was measured with a conductivity meter (Model EC-407L, NeoMet., USA).

Tetrazolium viability test

1g of 2,3, 5-triphenyl tetrazolium chloride (TTC) was dissolved in 100 ml of distilled water to make a 1% solution of the tetrazolium salt. The test was conducted with

two replicates of 100 seeds soaked in distilled water for 18 - 20 h. Each seed was cut longitudinally without completely separating the two halves. The seeds were submerged in 1% TTC solution for at least 8 h at 25°C in darkness, after which the staining patterns were recorded.

Seed health

A total of six different treatments (viz. T_1= lemon leaf powder, T_2= neem leaf powder, T_3= red chili powder, T_4= mixture of T_1, T_2 & T_3, T_5= bleaching powder, T_6= Mancozeb) along with control were applied to assess the quality of onion seeds in storage. The prevalence of seed-associated pathogens was identified in two different ways; a) growing treated seeds in PDA plates, b) growing untreated seeds in PDA plates prepared with treatment elements. Pathogens were observed after 72 hours of incubation at 25ºC. The prevalence of pathogens was documented from different treatments.

Results and Discussion

Moisture content of seeds after storage

The initial moisture of the seed was 7.0%. Seeds were stored after harvesting for up to the next growing season for 8 months.

Replicate samples of the onion seeds of different treatments were withdrawn from the test tube in which they had been stored. These were weighed, and their water contents were determined on a dry-weight basis (ISTA, 1985). The lowest moisture content (7.24%) was recorded when the seed was treated with lemon leaf powder which was probably due to the high absorption ability of lemon leaf powder from the air and the highest moisture content (9.17%) was recorded when the seed treated with Mancozeb which was statistically identical to the moisture content of seeds treated with red chili powder (9.13%). The moisture content of the other seed treatments varied from 7.43-7.97% (Table 1).

Moisture content and water uptake during imbibition

Replicate samples of onion seeds were tested for their moisture contents and the amounts of water required for full imbibition were determined. The results presented in Table 1, showed that the seed moisture content after imbibition differs among the seed lots tested. However, the percentages of water taken up did not differ depending upon the seed lot (different seed treatments). Water uptake during imbibition was maximum (50.89%) in lemon leaf treated seed, which contained the lowest moisture content before imbibition. The minimum water uptake (44.08%) was recorded in the untreated seed. Treatments and initial moisture content (after storage) of seeds may cause differences in the process of imbibition, and the amount of water required for the initiation of metabolism and germination. The imbibition of water into the dry seed is an essential part of germination; which has also been documented as a potentially hazardous period (Woodstock, 1988). The imbibition of water converts the seed from a quiescent body with a very low or non-detectable respiratory rate into a metabolizing organism, active in respiration and in biosynthesis, and capable of growth (Mayer & Poljakoff-Mayber, 1974). The amounts of water taken up by the seeds during imbibition prior to germination

in the present study appeared within the expected range depending on their initial moisture contents (Table 1).

1000 seed weight before and after imbibition

1000 seed weight before and after imbibition were not varied significantly among the treatments. The lowest (2.996 g) 1000 seed weight before imbibition was recorded in mixture of leaves and the highest (3.047 g) was recorded in bleaching powder treatment. The lowest (4.328g) 1000 seed weight after imbibition were recorded in untreated seed and the highest (4.568g) was recorded in neem leaf powder treatment (Table 1).

Table 1. Initial moisture content and extra water are taken up during imbibition of onion seeds of different treatments stored at ambient conditions

Pre-storage seed invigoration treatment	% moisture before imbibition	% moisture after imbibition	Water uptake (%)	1000 seed wt. (g) before imbibition	1000 seed wt. (g) after imbibition
Bleaching Powder (T1)	7.97	38.01	48.526	3.0477	4.525
Mancozeb (T2)	9.17	39.613	49.511	3.021	4.515
Red Chilli Powder (T3)	9.13	39.99	46.785	3.046	4.4713
Neem Leaf Powder (T4)	7.57	39.707	49.205	3.062	4.5687
Lemon Leaf Powder (T5)	7.24	42.74	50.892	3.0207	4.558
Mixture of leaves (T6)	7.43	38.233	47.843	2.9967	4.4303
Control (Untreated) (T7)	7.92	37.703	44.087	3.005	4.328
LSD 5%	* 0.57	* 1.96	NS	NS	NS
CV (%)	4.05	2.85	11.61	1.41	3.42

* Significant at 5% level; NS= Not significant. *Values are the means of four replicates, each of 1000 seeds (Initial moisture of seed during storage was 7.0%)

Seed viability

Samples of the seeds from the different treatments available were subjected to the tetrazolium test. The seed samples tested showed good viability. The highest viability was recorded when the seeds were treated chemically (97% and 96% viability in bleaching powder and Mancozeb respectively) (Table 2) The tetrazolium test is a biochemical test, which differentiates the living and dead tissues of seed by the presence or absence of a red stain known as formazan. A germination test can give an apparently erroneous result because some seeds may be in a state of dormancy, but the tetrazolium test includes all the seeds, which are alive either active or dormant (Porter *et al*., 1947).

Fully stained seed Partially stained seed Dead seed/ Non-viable

Fig. 1. Different stained condition of onion seed after viability test

Germination test (Normal seedling, abnormal seedling, and dormancy)

Laboratory germination tests are performed in optimum conditions, but field conditions are seldom optimal. Some indications of the probable performance of a seed lot in the field may be obtained by subjecting the seeds to some degree of stress before or during the laboratory germination period. A comparison of germination percentages in Tables II shows that seed from all treatments had slightly changed their relative positions (initial germination percentage before storage was 99%) with the best performance in the germination test (86%) shown in the lemon leaf powder treated seed. Seeds treated with neem leaf powder, a mixture of leaves, and bleaching powder were in the middle, and seeds stored with mancozeb and without any treatment gave the poorest performance. Seedling abnormalities vary slightly with the treatments. The highest abnormalities were recorded in the seedlings resulting from treatment T6 (mixture of leaves) and the lowest in the T4 treatment (Mancozeb). Abnormalities were similar to those from all other treatments. Considering the level of dormancy chemically treated seeds showed the highest dormant condition (17% and 16% in bleaching powder and Mancozeb, respectively). This might be due to some chemical interactions between the treatments along with the seed. Seed treated with lemon leaf powder and neem leaf powder resulted in the lowest (5% and 6%, respectively) dormant condition of the seed (Table 2).

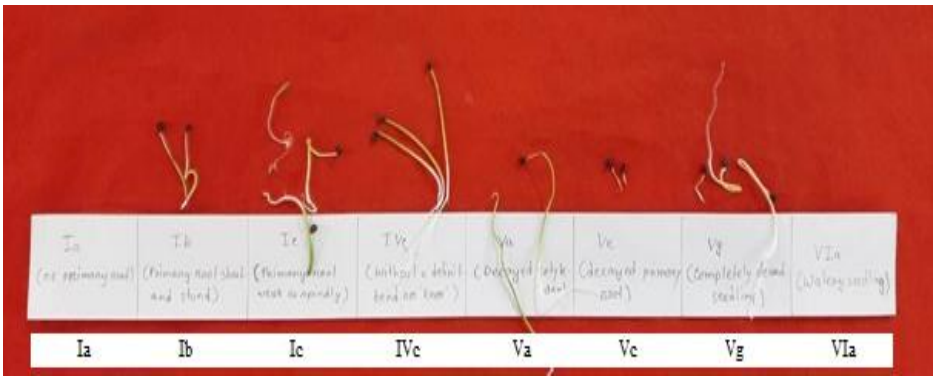

Fig. 2. Different types of abnormal seedlings of onion that were found at the period of last counting days of germination test

Table 2. Germination potential after twelve days of germination

Pre-storage seed invigoration treatment	Viability (tetrazolium test)	Number of Normal Seedling	Number of abnormal Seedling	Dormancy
Bleaching Powder	97	74	6	17
Mancozeb	96	77	3	16
Red Chilli Powder	90	75	5	10
Neem Leaf Powder	92	80	6	6
Lemon Leaf Powder	94	83	6	5
Mixture of leaves	90	75	8	7
Control (Untreated)	90	72	5	13
LSD 5%	6	5	3	2
CV (%)	3.66	3.41	27.57	9.86

*Fig. are the means of four replicates of 100 seeds for germination test, and two replicates of 50 seeds each for tetrazolium test.

The close relationship between the EC of different treatment's germination percentages was illustrated in Fig. 3. The highest (1087µs/cm) solute leakage was recorded in the untreated seed which indicates the lowest germination percentage which was statistically identical with the chemically treated seeds. And the lowest (914µs/cm) solute leakage was recorded in Lemon leaf powder treatment (T5) which was statistically identical with all the treated seeds of crude plant materials. (Fig. 3). The relation between solute leakage and normal germination percentages was highly negative in all treatments. This shows that conductivity detects that the germination differences among the treatments.

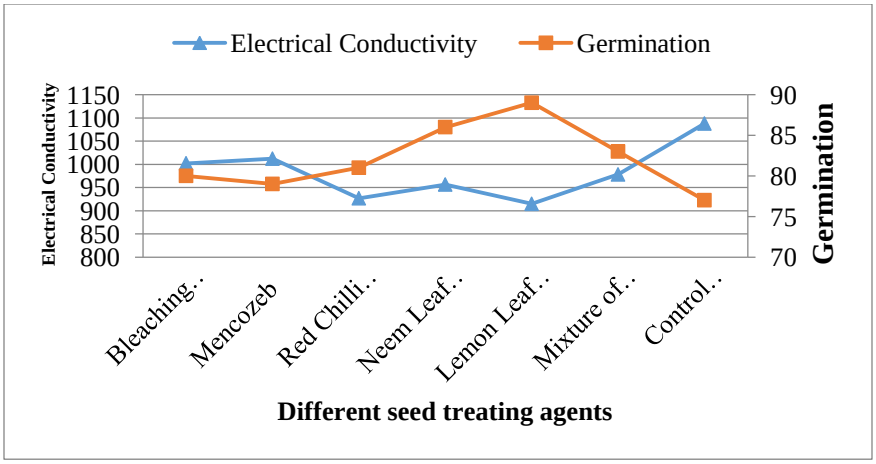

Fig. 3. Relationship between electrical conductivity and germination percentage

We measured the leakage of all treated seed samples measuring the overall mean of all seed leakage. Obviously, higher solute leakage would occur in samples with a high proportion of dead seeds (low germination percentages). Demir *et al*. (2008) and Mavi *et al. (*2016) were also reported that the average conductivity reading per gram seed weight in the bulk conductivity method is successful. A high correlation between EC measurements, germination, and dead seeds indicates that conductivity readings have the potential to provide a rapid assessment of standard laboratory germination. Information about the standard germination percentages of any lot can be obtained within 24 hours, compared to the 12-day germination test in this work for cress. It is easy to correlate directly germination with solute leakage and germination with the tetrazolium test. But it is highly difficult to correlate these three parameters. In this experiment, a high EC value was found in chemically treated seeds which indicates a low germination percentage, more precisely which indicates more dead seeds. But in the tetrazolium salt test, these two treated seeds showed high value, which indicates having minimum dead seeds. So it can be concluded like that, EC test will provide an assessment of germination but cannot provide the assessment of dead seeds.

Conclusion

Considering the experimental results, it could be concluded that pre-storage seed treatment with lemon leaf powder is an appropriate corrective measure to be chalked out to maintain the germination potential of onion seeds during storage.

Conflicts of Interest

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

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COASTAL FARMERS' KNOWLEDGE ON CLIMATE SMART AGRICULTURE IN BANGLADESH

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Abstract

Climate smart agriculture (CSA) is highly knowledge-intensive and innovative by nature. This study aimed to assess the extent of knowledge of coastal farmers about CSA and explore the contributions of the selected characteristics of the coastal farmers to their knowledge. Data were collected by using an interview schedule from 354 coastal farmers from 3 districts, namely Satkhira, Khulna, and Bagerhat, through the multistage random sampling method from December 2021 to March 2022. Descriptive and inferential statistics were used. To explore the contribution of the predictor variables to the outcome variables, full model regression analysis was employed. Results indicate that about 14.1% of the farmers had poor knowledge, 75.1% had medium-level knowledge, and the rest, 10.7%, had high-level knowledge of CSA. Farmers' education, annual agricultural income, extension contact, decision-making ability, and benefit obtained from CSA had significant positive contributions to their CSA knowledge. The findings indicate that the government should invest in improving farmers' decision-making ability and education, including agricultural extension and advisory services, which are the cornerstones of knowledge improvement regarding CSA and like other new approaches.

Keywords: Coastal farmers, CSA, Knowledge, Intensive, Innovative

Introduction

The coastal zone contributes approximately 16 percent of the total rice production of the country, covering about 70 percent of the total paddy-cropped area (Huq *et al*., 2005). The entire coastal regions of Bangladesh are increasingly susceptible to flooding tropical cyclones and associated saltwater intrusion (Roy *et al.*, 2019; Ramírez-Villegas and Thornton, 2015). The impacts of coastal hazards have been diminishing the potentials of these regions and thus drawing national and international concerns for protecting coastal agriculture through implementing numerous initiatives such as formulating the Master Plan for the Southern Agricultural Development (MoA

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and FAO, 2013). Addressing climatic challenges will require radical changes in agricultural systems. These systems have to become more efficient and resilient at every scale from the farm level to the global level. They have to become more efficient in resource use (use less land, water, and inputs to produce more food sustainably) and become more resilient to changes and shocks. In this situation, FAO has introduced the concept of climate smart agriculture (CSA) as a way forward for food security in a changing climate. CSA aims to improve food security, help communities adapt to climate change and contribute to climate change mitigation by adopting appropriate practices, developing enabling policies and institutions and mobilizing needed finances (Mahashin and Roy, 2018). CSA is an approach for transforming and reorienting agricultural development under the new realities of climate change (Lipper *et al.*, 2014). FAO (2013) defined CSA as "agriculture that sustainably increases productivity, enhances resilience (adaptation), reduces and removes Greenhouse gases (mitigation) where possible, and enhances achievement of national food security and development goals". In these definitions, the principal goal of CSA is identified as food security and development (Lipper *et al.*, 2014; FAO, 2013); while productivity, adaptation, and mitigation are identified as the three interlinked pillars necessary for achieving this goal.

Knowledge is a key factor in adoption of climate-smart technologies. Knowledge refers to what the respondents know about it (IDAF, 1994). It is the result of some activity such as generation, storage, dissemination and utilization of something that entails either information or data. It is usually based on learning, thinking, and a proper understanding of the problem area (Azad, 2013). If a farmer does not have proper knowledge on CSA, he or she will not be interested in practicing CSA. Any farmer practicing CSA without proper knowledge on it, he or she will face a lot of problem and his farming will be difficult and consequently he will lose hope and discontinue practicing CSA and finally cannot cope with changing climate, his production will be reduced. Therefore, assessing knowledge on CSA will make us understood its level in the farmers and thus help us to take necessary intervention for them. This study carried out the extent of assessing the farmers' knowledge on CSA, describing, and exploring the contributions of the selected characteristics of the coastal farmers to their knowledge on CSA.

Materials and Methods

Study area

The study area of this research was three coastal upazilas namely Tala, Dacope and Morrelgonj of the districts of Satkhira, Khulna and Bagerhat respectively. Some basic facts of the study area like agroecological zone (AEZ), area, population, literacy rate, major crops, etc. are presented in Table 1 as stated in BBS (2021).

Population and sample of the study

Out of 19 coastal districts of Bangladesh 3 districts, viz. Satkhira, Khulna and Bagerhat were purposively selected as study area. Three upazilas were randomly selected from these districts taking one upazila from each district. Nine villages from these three upazilas were again selected randomly taking 3 villages from each upazila. A total of 4489 farm families were found from selected nine villages and this number was considered as the population of the study. As the number of farmers in each of the villages was not the same, from each of the locations a 'proportionate random sampling' technique was used and the sample size was found 354. To make a respective sample from the population the formula was used as developed by Kothari (2004).

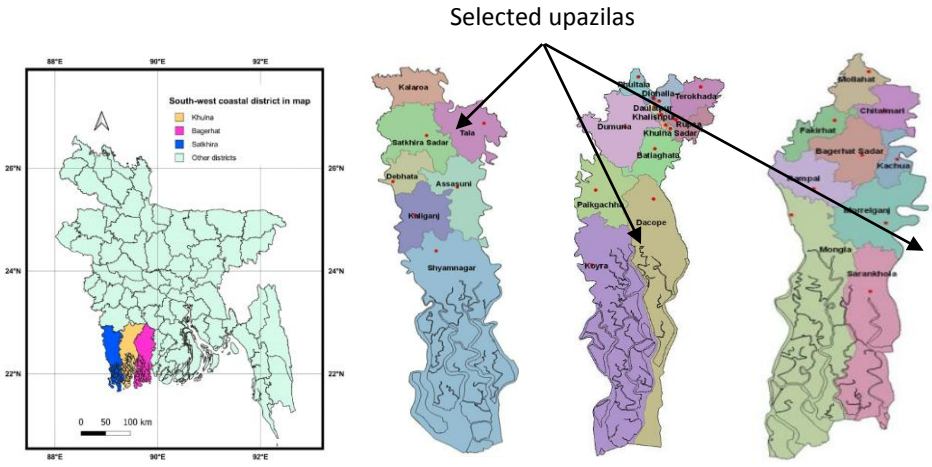

Fig. 1. A map of Bangladesh (left side) with its administrative districts. Right side: maps of three districts (Satkhira, Khulna and Bagerhat) with three upazilas (Tala, Dacope and Morrelgonj) from where data were collected

Table 1. Basic facts of the study area

Study area	AEZ	Area (km^2)	Population (000)	Literacy	Major crops	Existing major CSA practices	Cropping intensity
Morrelgonj, Bagerhat	13	460.90	295	60.7%	Paddy, Potato, sugarcane	Plastic irrigation pipe, Salinity resistant variety, Mulching, etc.	132
Dacope, Khulna	13	991.58	152	56%	Paddy, Watermelon,Potato, pumpkin	Plastic irrigation pipe, Rain water harvesting, Watermelon cultivation, etc.	114
Tala, Satkhira	11	344.15	300	50.9%	Paddy, Jute, Brinjal, Sugarcane	Ridge planting, raised bed planting, mulching, etc.	198

$n = [Z^2 P QN] / [(N-1) e^2 + Z^2 P Q]$

Where, n = Sample size

Z = Table value at 1 d.f. (1.96)

P = Probability (assume 0.5)

Q = Remaining from probability (1-P) = 0.5

N = Total population = 4489

e = The level of precision (5%)

By putting the values in the above formula, the sample size was determined as follows-

$$n = \frac{Z^2 PQN}{(N-1)e^2 + Z^2 PQ}$$

$$n = \frac{(1.96)^2 \times 0.5 \times 0.5 \times 4489}{(4489-1) \times (0.05)^2 + (1.96)^2 \times 0.5 \times 0.5}$$

$$n = 353.95 \approx 354$$

Variables and instruments for data collection

Data were collected by the household' survey, using an interview schedule from 354 coastal farmers during December 2021 to March 2022. Coastal farmers' knowledge on CSA was the main focus of this study and it was considered as the dependent variable. Education, farm size, annual agricultural income, farming experience, extension media contact, training exposure, innovativeness, credit availability, access to market, access to ICTs, decision making ability and benefit obtained from CSA were considered as the predictor/independent variables of this study.

Measurement of the variables

Measurement of knowledge on CSA

The content of the knowledge test is composed of questions called items. Items for the test were collected from different sources, such as literature, agronomists, horticulturists, soil scientists, agricultural economists, entomologists, plant pathologists, agri-environmentalists, and agricultural extension personnel, NGO professional and progressive farmers. The questions were designed to test the climate-smart agricultural knowledge of the coastal farmers. The items were collected and prepared in relation to climate change and its impact on agriculture, productivity, adaptation and mitigation strategies for food production. According to Anderson and Krathwohl (2001), a set of 20 questions taking 4 from remembering, 4 from understanding, 4 from applying, 4 from analyzing, 2 from evaluating and 2 from creating related to CSA were asked to the respondents and a score of 2, 1 or 0 were assigned for each of the correct, partially correct and wrong answers, respectively. Then the possible knowledge score of a farmer could range from 0 to 40 where 0 indicated very poor knowledge and 40 indicated very high knowledge on CSA. Based on the previous studies, for example, Roy *et al.*, 2021; the measurement procedure of independent variables is given in Table 2 below.

Data entry and analysis

Data from all the interview schedules were coded, tabulated and analyzed in accordance with the objectives of the study. Data checking tools like outliers checking and removing multi-collinearity were employed. Pearson product-moment correlation test was initially done and found no strong correlation ($r > 0.8$) between two or more predictors in the regression model. The analysis was performed using SPSS software version 21. Descriptive analysis such as range, numbers and percentage distribution, mean and standard deviation (SD) were used. Full model regression analysis was used to find out the contribution of the predictor variables to the outcome variable.

Table 2. Measurement of independent variables

Variables	Measurement
Education	Number of years of schooling
Farm size	Total quantity of farming land in ha, including gardening and fishery
Annual agricultural income	Total yearly earning from farming
Farming experience	Number of years a farmer was involved in farming
Extension media contact	Total scores of a respondent on his nature and frequency of 14 selected extension media
Training experience	Total number of days that a respondent had undertaken different types of training related to agriculture/climate smart agriculture
Innovativeness	Scores assigned for respondent farmer as 5, 4, 3, 2, and 1 for innovators, early adopters, early majority, late majority and laggards respectively
Credit availability	Percentage of loan received against his/her sought amount
Access to the market	Score by using a 3-point rating scale of buying inputs and selling goods for his farming activities
Access to ICTs	Score using 4-point rating scale of selected five technologies
Decision making ability	Score obtained by using a 3-point rating scale of the six selected items
Benefit obtained from CSA	Score obtained using 4-point rating scale of 20 selected benefits

Result and Discussion

Extent of knowledge on climate smart agriculture

Coastal farmers' knowledge scores could range from 0 to 40. However, their observed knowledge scores ranged from 17 to 32, the mean was 25.45 and standard deviation was 3.86. The distribution of the farmers according to their knowledge level is shown in Table 3. The Table reveals that the majority (75.14%) of the farmers had medium-level knowledge followed by 14.13% had poor knowledge and 10.73% had high level knowledge on CSA. Farmers having poor to medium-level of knowledge constitute 89.27% of the total farmers. The adverse climatic conditions compelled majority of the farmers to practice several CSA technologies available to them and by practicing CSA they acquired some knowledge on it.

Table 3. Distribution of the coastal farmers according to their knowledge on CSA

Categories	Number	Percent	Mean	SD
Poor knowledge (<50% marks obtained)	50	14.13	25.45	3.86
Medium-level knowledge (50-75% marks obtained)	266	75.14		
High level knowledge (>75% marks obtained)	38	10.73		
Total	354	100		

Source: Authors

The education of the farmers might influence their knowledge as 12.72% (Table 3) of the farmers were illiterate this portion might have poor knowledge on CSA. Furthermore, extension contact might influence the majority of the farmers (89.27%) acquiring poor to medium-level knowledge on CSA as majority (86.15%) of the farmer had low to medium extension contact (Table 4) while extension contact had a significant positive contribution to their knowledge on CSA (Table 5). Israel (2019) and Ochieng (2015) found almost similar results regarding knowledge on climate change that the majority (72% and 81% respectively) of the respondents had poor to medium-level knowledge on climate change. Rahman (2015) and Hassan (2004) also found almost similar results regarding the knowledge of farmers that the majority (75% and 70.4% respectively) of them had medium-level knowledge on rice cultivation and partnership extension approach in their respective studies.

Selected characteristics of the coastal farmers

Table 4 indicates that only 12.71% of the farmers were illiterate and the rest 87.29% were literate which was composed of secondary education (59.32%), primary education (20.06%), higher secondary education (5.37%) and tertiary education (2.54%). Over time, through the government's initiatives, different NGOs' education programmes (e.g., BRAC school) and social involvement and the need of the farmers, they somehow obtained literacy for which literacy is little greater than the national average. About half of the respondents (50.56%) had low annual agricultural income; their annual agricultural income was up to Tk.150000. The next group were medium-income farmers (40.68%) while the lowest proportion belonged to high-income group (8.76%). Mittra and Akanda (2019) found similar results in their study that the majority (62.2%) of the coastal farmers had low annual income. The majority (68.64%) of the farmers had medium extension contact followed by low media contact (17.51%) and high media contact (13.85%). Rahman (2018) found similar results that the majority (65.1%) of the farmers had medium extension contact. The majority (69.49%) of the respondents had medium decision-making ability, while 19.49% and 11.02% had high and low decision-making ability, respectively. Hossain (2017) found almost similar results that the majority (62.9%) of the respondents had medium decision-making ability. The highest proportion

(75.42%) of the farmers belonged to the category of medium-benefits obtained from CSA, while 9.32% and 15.26% belonged to low-benefit obtained and high-benefits obtained from CSA categories, respectively.

Contribution of selected characteristics of the farmers to their knowledge on CSA

Results presented in Table 5 show the summarized results of full model multiple regression analysis with 12 independent variables on the farmers' knowledge on CSA. The value of R2 is 0.494, which means that all of the 12 variables accounted for 49.4% of the variation in knowledge on CSA of the coastal farmers. The regression equation obtained is presented below-

Table 4. Salient features of the selected characteristics of the farmers (n=354)

Characteristics	Measuring unit	Range		Categories	Number	Percent	Mean	SD
		Possib	Obser ved					
Education	Year of schooling	Unknown	0-15	Illiterate (0-0.5)	45	12.71	7.53	3.51
				Primary education (1-5)	71	20.06		
				Secondary education (6-10)	210	59.32		
				Higher secondary education (11-12)	19	5.37		
				Tertiary education (>12)	9	2.54		
Farm size	Score	1-5	2-5	Marginal farmer (0.021-0.2)	36	10.20	3.26	0.73
				Small farmer (0.21-1.0)	214	60.5		
				Medium farmer (1.01-3.0)	80	22.6		
				Large farmer (> 3.0)	24	6.8		
Annual agricultural income	Score	1-10	1-10	Low-income farmer (<150)	179	50.56	3.94	1.85
				Medium income farmer (151-300)	144	40.68		
				High income farmer (>300)	31	8.76		
Farming experience	Year	Unknown	10-50	Low experienced farmer (<15)	65	18.36	24.60	9.9
				Medium experienced farmer (15-35)	247	69.77		
				High experienced farmer (>35)	42	11.87		
Extension media contact	Score	0-42	15-31	Low contact farmer (< 18)	62	17.51	23.13	4.66
				Medium contact farmer (18-28)	243	68.64		
				High contact farmer (>28)	49	13.85		
	No	Un	0	No trained farmer (0)	260	73.45		

Characteristics	Measuring unit	Range		Categories	Number	Percent	Mean	SD
		Possib	Obser ved					
Training exposure				Low trained farmer (1-2)	71	20.06	0.61	1.26
				Medium trained farmer (3-4)	14	3.95		
				High trained farmer (>4)	9	2.54		
Innovativeness	Score	1-5	1-5	Innovator (5)	39	11.03	3.39	0.92
				Early adopter (4)	122	34.46		
				Early majority (3)	140	39.54		
				Late majority (2)	45	12.71		
				Laggard (1)	8	2.26		
Credit availability	Score	0-100	0-83	No credit farmer (0)	288	81.36	9.84	21.16
				Low credit farmer (<50)	18	5.08		
				Medium credit farmer (50-70)	43	12.15		
				High credit farmer (>70)	5	1.41		
Access to market	Score	0-20	10-17	Low access (<11)	29	8.19	13.47	1.79
				Medium access (11-15)	266	75.14		
				High access (>15)	59	16.67		
Access to ICT	Score	0-15	3-10	Low access (<5)	47	13.28	6.27	1.55
				Medium access (5-8)	274	77.4		
				High access (>8)	33	9.32		
Decision making ability	Score	6-18	11-17	Low decision making (<12)	39	11.02	13.76	1.77
				Medium decision making (12-15)	246	69.49		
				High decision making (>15)	69	19.49		
Benefit obtained from CSA	Score	0-60	34-55	Low benefit (< 40)	33	9.32	45.91	5.09
				Medium benefit (40-51)	267	75.42		
				High benefit (> 51)	54	15.26		

Source: Author

$$Y = b_{0+} b_1X_1 + b_3X_3 + b_5X_5 + b_{11}X_{11} + b_{12}X_{12} + E$$

$$Or,\ Y = 0.01 + 0.177X_1 + 0.244X_3 + 0.112X_5 + 0.817X_{11} + 0.278X_{12}$$

i.e., Knowledge = 0.01+ 0.177 (education) + 0.244 (annual agricultural income) + 0.112 (extension contact) + 0.817 (decision making ability) + 0.278 (benefit obtained from CSA)

For every 1 year of passing in schooling, an extra 0.177 knowledge score was obtained. Rahman (2015) and Mondal (2014) found that the education of the farmers had a significant positive relationship with their knowledge. It might be due to that education makes awareness in a person and leads him to acquire knowledge on a matter that he is involved. For increasing the annual income of every 1 score (Tk.50000), an extra 0.244 knowledge score was obtained. Mandal (2016), Dhali (2013) and Sharif (2011) also found in their respective studies that the annual income of the farmers had a positive significant relationship with their knowledge. This might be due to that the increased income of a farmer can inspire him to spend money to acquire knowledge on a certain subject. For increasing every 1 score of extension media contact, an extra 0.112 knowledge score was obtained. The more the number of extension media and frequency of contact is used by the respondents, the more they will obtain knowledge. Mondal (2014) found that extension contact had 1.3% of the total variation in knowledge of strawberry cultivation. This might be due to that through extension contact knowledge was disseminated and farmers were motivated to acquire knowledge on farming issues.

For increasing every 1 score of decision-making ability, an extra 0.817 knowledge score was obtained. That means that, farmers having high decision-making ability tends to have high knowledge on CSA. This might be due to that a person with higher decision-making ability can confidently involve himself in any knowledge-acquiring activity without any hesitation. For increasing every 1 score of benefit obtained from CSA, an extra 0.278 knowledge score was obtained. The reason might be that whenever a farmer gets any benefit from CSA practice, he tends to be inspired to know about it.

Table 5. Regression analysis showing the contribution of selected characteristics of the farmers to their knowledge on CSA

Variable entered	'b' Value	Value of 't' (with probability level)
Education (X_1)	0.177**	3.240 (0.001)
Farm size (X_2)	0.091	0.294 (0.769)
Annual agricultural income (X_3)	0.244*	1.977 (0.049)
Farming Experience (X_4)	-0.024	-0.840 (0.401)
Extension contact (X_5)	0.112**	2.685 (0.008)
Training exposure (x_6)	0.123	1.126 (0.261)
Innovativeness (x_7)	-0.160	-0.891 (0.374)
Credit availability (x_8)	-0.001	-0.073 (0.942)
Access to market (x_9)	0.120	1.279 (0.202)
Access to ICTs (X_{10})	0.092	0.814 (0.416)
Decision making ability(X_{11})	0.817**	7.063 (0.000)
Benefit obtained from CSA (X_{12})	0.278**	7.325 (0.000)

Multiple R = 0.703, R-square = 0.494, Adjusted R-square = 0.473, F-ratio = 23.663 at 0.000 level of significance, Standard error of estimate = 2.803, Constant = 0.010

*Significant at 0.05 Level, **Significant at 0.001 Level

Conclusion and recommendations

Three-quarters of farmers had medium-level knowledge on CSA while one-tenth had high-level knowledge. Farmers' education, annual agricultural income, extension contact, decision-making ability and benefit obtained from CSA had positive significant contributions to their knowledge on CSA. Based on the findings of the study, the following recommendations can be made-

Farmers' knowledge about CSA can be increased by investing in non-formal education. Key approaches and techniques of non-formal education include demand-driven training, commodity interest group (CIG) farmer training, exposure visits, etc. Farmers' agricultural income needed to be increased by providing subsidies or other financial support or by ensuring reasonable prices of agricultural products and services. Knowledge level on CSA can be increased through extension contact like, motivational campaigns and result and method demonstrations. A number of extension media and frequency of extension communication are to be increased for those who has less contact or who are beyond extension contact. Agricultural extension and advisory services should play a major role in improving farmers' decision-making abilities. To enhance these abilities, extension service providers should facilitate farmers' access to climate-smart productive technologies; provide knowledge on management of these technologies, and share tips and tricks for combining the available resources in an optimal way.

Conflicts of interest

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

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IN VITRO REGENERATION OF BLACK PEPPER (*Piper nigrum* L.)

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Abstract

This investigation was done to determine how various plant growth regulators such as benzyl adenine (BA) and indole-3-butyric acid (IBA) affected the *in vitro* plant regeneration of the black pepper plant. The largest number of shoots was produced when the shoot tips and nodal segments of black pepper were utilized as explants and inoculated in Murashige Skoog (MS) media supplemented with 1.5 mg/L BA. The treatment with 2.0 mg/L BA at 8 weeks after induction (WAI) produced the most leaves (3.2). In the 2.0 mg/L treatment of IBA, the greatest number of roots was regenerated. The treatment 2.0 mg/L BA + 2.0 mg/L IBA produced the highest results for shoot induction, shoot length, and overall leaf production when the effects of both hormones were combined. The same treatment also produced the highest proportion of root induction and longest roots. Regenerated plantlets survive at a 46.7% rate in a shaded building and at a 57.1% rate in the open air under bright sunlight. In order to produce black pepper on a big scale, an effective approach for *in vitro* regeneration of black pepper has been established.

Keywords: Benzyl adenine, Indole-3-butyric acid, *In vitro* regeneration

Introduction

A flowering plant in the Piperaceae family known as black pepper (*Piper nigrum* L.) is grown for its fruit, sometimes known as a peppercorn. It is mostly used as a spice and seasoning after it is dried. The fruit has a solitary seed and measures approximately 5 mm (0.20 inch) in diameter when it is fresh and fully grown. Black pepper (cooked and dried unripe fruit), green pepper (dried unripe fruit), and white pepper (ripe fruit seeds) are the more accurate names for peppercorns and the ground pepper that is made from them (Harrison and Paul, 2016). Old English "pipor," Latin "piper," and Sanskrit "pippali," which means "long pepper," are the origins of the word pepper. People started referring to the unrelated new world chili pepper (genus *Capsicum*) when they used the word pepper in the 16th century. It is primarily referred to as "Golmorich" in Bangladesh. It is indigenous to the West Indies, South America, Indonesia, Malaysia, and India. However, it is also frequently grown in tropical areas. The 'King of Spices' is the name given to it (Srinivasan, 2007; Mathew *et al.*, 2001). Since ancient times, peppercorns have been ground, dried, and fried for flavor and as a traditional remedy. One of the most

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popular spices used in cuisines worldwide and one of the most traded spices in the world is black pepper. It is spicy because of the chemical molecule piperine, which differs from the capsaicin found in chili peppers in terms of heat. The scavenging of superoxide anion, hydrogen peroxide, and nitric oxide are just a few of the reactive oxygen and nitrogen species that it has been shown in studies to have antioxidant effects against. It has also been found that antioxidant enzymes improve *in vivo*. Through several pathways such as cytotoxicity, apoptosis, autophagy, and interference, *Piper nigrum* also demonstrated anticancer effects against numerous cell lines from the breast, colon, cervical, and prostate. It is the excellent source of manganese, iron, vitamin K, and good source of dietary fiber. In various areas of Bangladesh, particularly in hilly terrain, black pepper is grown. However, there are no statistics accessible in our nation on the area and black pepper production. Black pepper is used extensively in our nation each year for both culinary and medical purposes. The majority of them are imports. Cuttings, layering, and grafting are all methods for multiplying black pepper. The creation of recombinants during seed propagation frequently leads to genetic variety, but other techniques of propagating black pepper are slow and time-consuming. Therefore, it is necessary to develop effective techniques for the quick spread of black pepper. The fastest and most dependable approach for producing disease-free, genetically stable, and identical children under this situation is plant tissue culture (Hussain *et al.,* 2011). The enhancement of black pepper plants, the preservation of germplasm, and clonal propagation have all benefited greatly from tissue culture techniques (Sajc et al., 2000). If a method could be developed, it would enable quick clonal replication of planting materials in Bangladesh in a relatively short amount of time. Plants create signal molecules called phytohormones, or plant hormones, which are found in incredibly tiny amounts. All aspects of plant growth and development, including embryogenesis (Méndez Hernández *et al.,* 2019), the control of organ size, pathogen defence (Shigenaga *et al.,* 2016; Burger *et al.,* 2019), stress tolerance (Ku *et al.,* 2018; Ullah *et al.,* 2018), and reproductive development (Pierre-Jerome *et al.,* 2018) are governed by plant hormones. Depending on their chemical compositions, different hormones can be grouped into various classes. Each class of hormones can have a variety of chemical configurations, but they all share a common set of physiological functions. Abscisic acid, auxins, cytokinins, ethylene, and gibberellins were the first five major families of plant hormones to be identified through research (Thomas et al., 1979). Compounds called auxins have a favorable impact on cell growth, bud development, and root initiation. To promote root growth, auxins, particularly indole-3-acetic acid (IAA), 1-naphthaleneacetic acid (NAA), and indole-3-butyric acid (IBA), are frequently used. A class of substances known as cytokinins affect how cells divide and how shoots form. Taking into account the aforementioned facts, the current inquiry has been carried out to determine the effectiveness of various hormones with the ideal concentration of BA and IBA for *in vitro* testing.

Materials and Methods

Piper nigrum (black pepper) planting supplies were gathered from a variety of nurseries in Agargaon, Sher-e-Bangla Nagar, Dhaka 1207. In the current study, experimental materials included nodal segments and shoot tips. Black pepper plants were harvested for their shoots with young leaves. In order to use the shoot as an explant, the

superfluous leaves were taken off and the shoot was pruned to a size of 1-2 cm. The nodal segments and shoot tips of the explants were thoroughly cleaned under running water. Trix is soaked in with the explants for ten minutes. then repeatedly rinsed with diluted water. Explants were then submerged for 20 minutes in a 100 mg/L ascorbic acid solution. In the Laminar Air Flow Cabinet, the remaining tasks were completed. The explants were taken out of that solution after 20 minutes and repeatedly rinsed with distilled water then sterilized for 1 minute with 70% ethanol, followed by 1 minute of distilled water washing. Once more, the explants were sterilized for 5 minutes with a 0.2–0.5% $HgCl_2$ and Tween 20 solution. The explants were then cleaned at least three times with distilled water. The explants' final sizes ranged from 0.5 to 1 cm. The explants were finally prepared for careful insertion into the culture vessel.

Surface sterilized nodal explants (2 to 3 cm) were individually inoculated on liquid MS media (Fig. a) supplemented with various doses of BA (BA (0.5, 1.0, 1.5, and 2.0 mg/L) and control (0.0 mg/L)). For direct shoot induction, four levels of IBA (1.0, 1.5, 2.0, and 2.5 mg/L) were practiced with each level of BA (1.0, 1.5, 2.0, and 2.5 mg/L). Extracted induced axillary shoots were grown for root induction on four concentrations of IBA (0.5, 1.0, 1.5, and 2.0 mg/L) and control (0.0 mg/L) in an aseptic environment. The culture vials with fully formed plantlets were moved to normal room temperature after 2.5 months. The rooted plantlets were taken out of the culture vials the following two to three days, and the medium that was still attached to the roots was carefully rinsed away with tap water. Individual plantlets were transplanted into plastic containers filled with a 1:1:1 mixture of soil, sand, and cow dung. For seven days following transplantation, a moist, clear poly bag was placed over the plants and pot to avoid desiccation. The plantlets were housed in a shade house for 12 days to lessen unexpected shock. Plantlets were then moved to the field after 12 days.

Results and Discussions

Significant variations were observed among different concentrations of BA on days to shoot induction. Minimum 9.4 days were required in the treatment 1.5 mg/L BA (Fig. b). Legesse *et al.,* 2017; found the lowest response of number of shoots in BA 2.0 mg/L while the highest response was observed in medium supplemented with BA 5.0 mg/L in black pepper. The highest number of shoot was obtained (1.8, 2.6 and 3) at 3 WAI, 5 WAI and 8 WAI respectively at 1.5 mg/L (Table 1) Whereas shoot regeneration was not observed in control treatment at 3 WAI, 5 WAI but very incipient shoot found at 8 WAI. Soniya and Das (2002) reported that maximum number of shoot was recorded on MS medium supplemented in 2mg/L BA for *Piper nigrum*. It partially contradicts with the result. Legesse *et al.,* 2017; reported that 4mg/L BA was best for shoot proliferation of *Piper nigrum*. Shoot regeneration response is less in Black pepper. It might be the genetical ability of the spices crop. The treatment BA 2.0 mg/L (Fig. c) gave the maximum number of leaves (1.8, 2.0 and 3.2) at 3 WAI, 5 WAI and 8 WAI, respectively. No shoot was produced in controlled treatment at 3 WAI and 5 WAI. Lowest number of leaves (1.2) was found at control treatment at 8 WAI (Table 2).

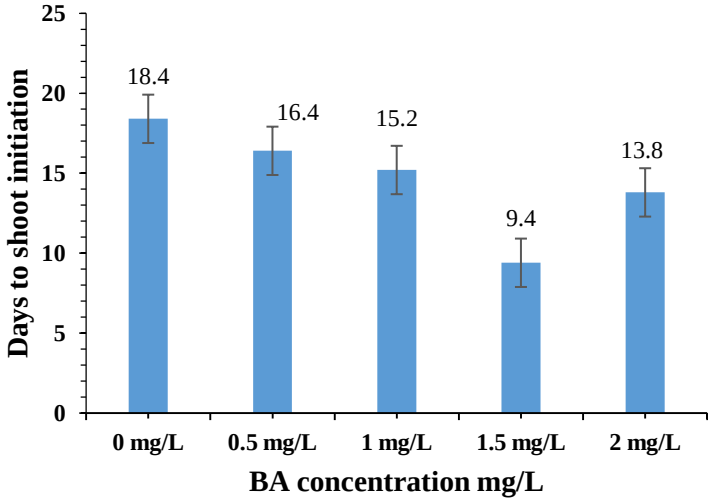


Fig. 1. Effects of BA on days to shoot induction in Black pepper

Table 1. Effects of different concentration of BA on number of shoot at different weeks after induction

(WAI)

BA mg/L	Number of shoot per plant		
	3 WAI	5 WAI	8 WAI
0	0 c	0 d	1 c
0.5	1b	1.2 cd	2 b
1.0	1b	1.6 bc	2 b
1.5	1.8 a	2.6 a	3 a
2.0	1.2 b	2 b	2 b
CV percent	28.28	27.03	15.81
LSD (0.05)	0.4	0.5	1

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

Table 2. Effects of different concentration of BA on number of leaf at weeks after induction (WAI)

BAmg/L	Number of leaf		
	3 WAI	5 WAI	8WAI
0	0 b	0b	1.2 c
0.5	1.0b	1.2 b	1.8b
1.0	1.0b	1.2b	2.2b
1.5	1.2 b	1.2 b	2.2b
2.0	1.8 a	2.0 a	3.2a
CV%	28.28	30.93	21.09
LSD(0.05)	0.4	0.5	0.6

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

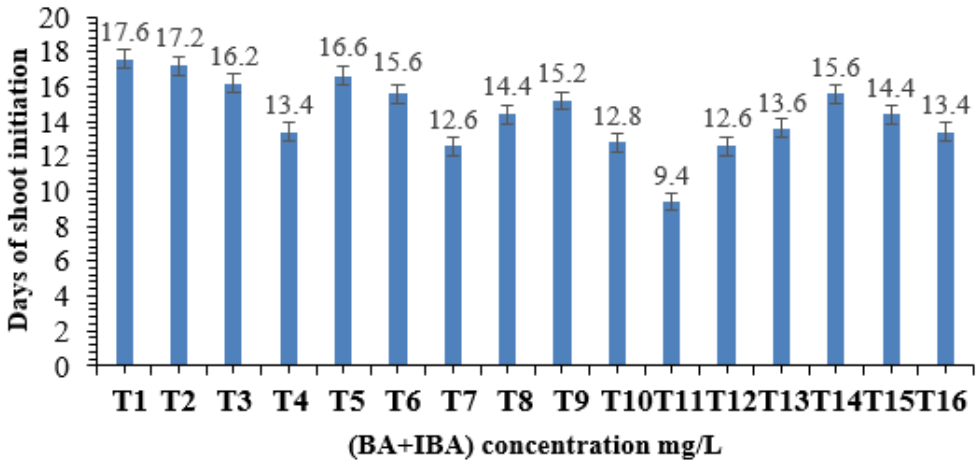
Legesse *et al.* (2017) reported the maximum number of leaves (3.16) on BA concentration on 3mg/L of BA, but the minimum number of leaves (1.62) was recorded on controlled treatment in Black pepper. Increasing the amount of cytokinins like BA up to 5 mg/L gave as the maximum number of leaves per shoot in *Piper nigrum* as reported by Soniya and Das (2002) which is partially similar with the result. There was significant influence of different concentrations of IBA on the number of roots per shoot. The treatment 2.0 mg/L (Fig. d) gave the highest number of root (2.0, 3.0 and 4.0) at 3 WAI, 5 WAI and 8 WAI (Table 3). Furthermore, indicated that IBA interacted significantly with the culture medium and the materials, having a strong influence for plantlet rooting. Significant variations were observed among the combined effect of different concentrations of BA and IBA on days to shoot induction. The minimum duration 9.4 days was obtained in BA 2.0 mg/L+ IBA 2.0 mg/L than rest of the treatments.

Table 3. Effect of different concentration of IBA on number of root at different weeks after induction

(WAI)

IBAmg/L	Number of roots per shoot		
	3WAI	5WAI	8WAI
0.5	1.2bc	1.8bc	2.2c
1.0	1.0 c	2.0bc	2.0 c
1.5	1.6 ab	2.2 b	3.0 b
2.0	2.0 a	3.0 a	4.0 a
CV%	24.38	21.08	7.99
LSD(0.05)	0.5	0.6	0.3

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.



*I= Standard error bar

Fig. 2. Effect of (BA+IBA) on days to shoot initiation in black pepper

Khan *et al.*, 2016; reported that in BAP 1.0 mg/L and IAA 1 mg/L supplemented medium 10 - 20 days were required for shoot initiation in black pepper. The highest number of shoot (1.8, 2.4 and 3.2) was noticed from the BA 2.0 mg/L + IBA 2.0 mg/L (Fig. e). (Monney *et al.*, 2016; reported that highest number of shoot 1.28 was found in BA 3.0 mg/L+ IBA 0.1 mg/L in *Cryptolepis sanguinolenta*. Which is contradicts with the result (Table 4). The highest length of shoot (1.91 cm, 3.23 cm and 5.10 cm) at 3 WAI, 5 WAI and 8 WAI, respectively was noticed from the BA 2.0 mg/L + IBA 2.0 mg/L (Table 5).

Table 4. Effects of BA and IBA on the number of shoot at different weeks after induction (WAI)

BA+IBA (mg/l)	Number of shoot		
	3 WAI	5 WAI	8 WAI
1+1	1.2 b	1.4 cd	1.6 ef
1+1.5	1 b	1.2 d	1.4 f
1+2	1.2 b	1.4 cd	2 cde
1+2.5	1.4 ab	1.4 cd	1.6 ef
1.5+1	1.4 ab	1.8 bc	2 cde
1.5+1.5	1.2 b	1.8 bc	1.8 def
1.5+2	1.4 ab	2 ab	2 cde
1.5+2.5	1.2 b	2 ab	2 cde
2+1	1.4 ab	2 ab	2.4 bc
2+1.5	1.4 ab	1.6 bcd	2 cde

BA+IBA (mg/l)	Number of shoot		
	3 WAI	5 WAI	8 WAI
2+2	1.8 a	2.4 a	3.2 a
2+2.5	1.2 b	1.6 bcd	2 cde
2.5+1	1.2 b	1.6 bcd	2.2 bcd
2.5+1.5	1.2 b	1.4 cd	2.2 bcd
2.5+2	1.2 b	1.6 bcd	2.2 bcd
2.5+2.5	1.4 b	1.8 bc	2.6 b
CV percent	36.49	27.72	19.05
LSD (0.05)	0.6	0.6	0.5

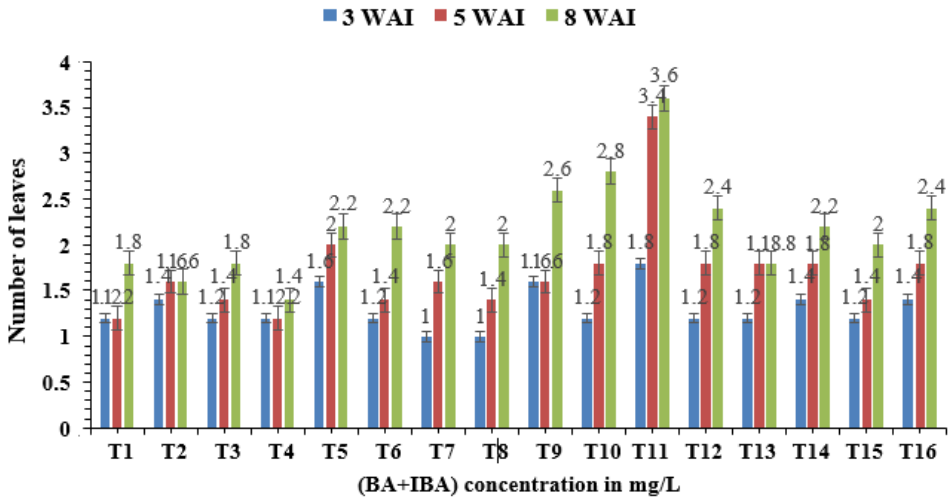
Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

Table 5. Effect of different concentration on BA and IBA on the length of shoot

BA+IBA (mg/L)	Length of shoot		
	3 WAI	5 WAI	8 WAI
1+1	1.36 jk	2.16 d	2.40 g
1+1.5	1.34 l	2.32 c	2.73 de
1+2	1.34	2.33 c	2.75 cde
1+2.5	1.37 j	2.34 c	2.72 e
1.5+1	1.71 e	2.53 b	2.83 bcd
1.5+1.5	1.74 d	2.52 b	2.83 bcd
1.5+2	1.75 c	2.54 b	2.83 bcd
1.5+2.5	1.75 cd	2.52 b	2.82 bcde
2+1	1.41 I	2.33 c	2.85 b
2+1.5	1.41 i	2.34 c	2.85 bc
2+2	1.90 a	2.71 a	3.23 a
2+2.5	1.85 b	2.32 c	2.86 b
2.5+1	1.50 g	2.14 de	2.50 fg
2.5+1.5	1.60f	2.16 d	2.53 f
2.5+2	1.43 h	2.15 de	2.53 f
2.5+2.5	1.35 kl	2.12 e	2.54 f
CV percent	0.66	1.35	3.02
LSD (0.05)	0.0129	0.0400	0.1045

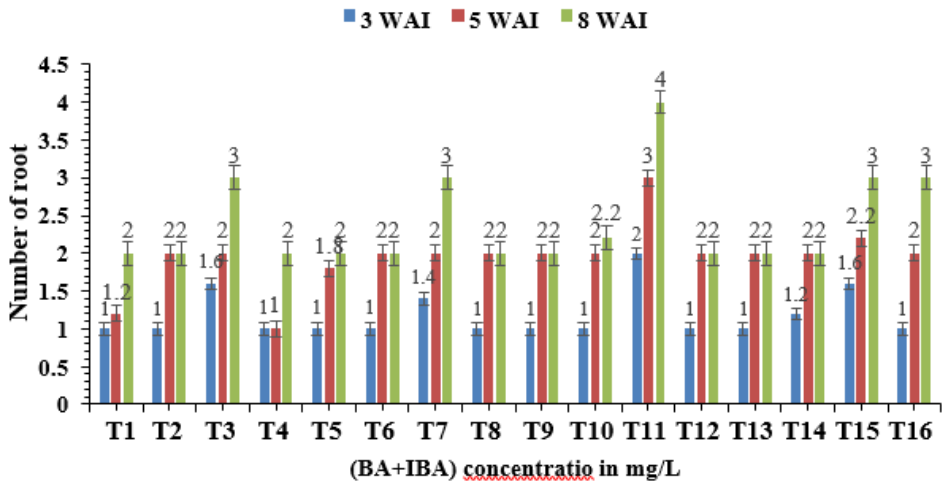
Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

The treatment BA 2.0 mg/L+ IBA 2.0 mg/L (Fig. f) gave the highest number of leaves (1.8, 3.4 and 3.6) at 3 WAI, 5 WAI and 8 WAI respectively. The treatment 2.0 mg/L BA + 2.0 mg/L IBA (Fig. g) gave the highest number of root (2.0,3.0 and 4.0).



*I=Standard error bar

Fig. 3. Combined effects of BA and IBA on number of leaves in black pepper



*I=Standard error bar

Fig. 4. Combined effects of BA and IBA on number of root in black pepper

After considerable number of shoots and roots were developed at 8 weeks of culture. The plantlets were removed from vial carefully without any root damage. In the growth cabinet and in the shade house, plants (Fig. h) were acclimatized and hardened before being transferred to the field conditions. At first 15 plants were transplanted and 7 were survived in shade condition (46.67%). Finally, in normal atmospheric condition 7 plants (Fig. i) were transplanted among them 4 survived and survival rate was 57.14%. Anand *and Rao,* 2000; reported that in natural condition 75% plantlets survived. observed 90% plantlets survival in soil in shade house.

Fig. a. Inoculation of explants in culture media

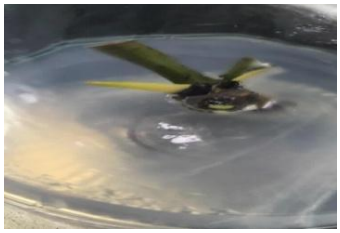


Fig. b. Number of shoot in MS medium supplemented with BA1.5mg/L

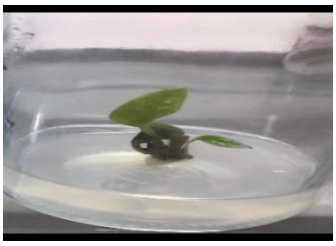


Fig. c. Number of leaves at 8 WAI in the treatment of 2.0mg/L BA

Fig. d. Root development in the treatment of 2.0mg/LIBA

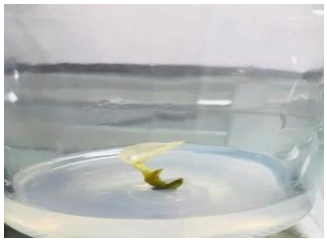


Fig. e. Number of shoot at 8 WAI in the treatment of BA2.0mg/L+IBA 2.0mg/L

Fig. f. Number of leaves at 8 WAI in the treatment of 2.0mg/LBA+ 2.0mg/LIBA

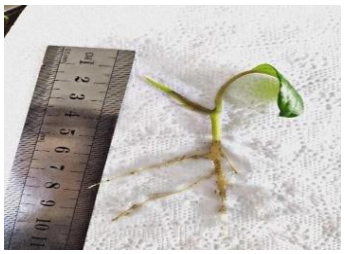


Fig. g. Number of root at 5 WAI in the treatment of 2.0mg/LBA+2.0mg/LIBA

Fig. h. Acclimatization of plantlets in the shade condition

Fig. i. Establishment of plantlet in natural condition

Conclusion

In the current study, a successful regeneration and multiplication procedure for *Piper nigrum in vitro* clonal propagation using nodal segments is described. For the growth of black pepper, other explants such meristems and root tips can be tested. Further study can be done with different concentrations and combinations of auxin and cytokinine group of hormones for the micropropagation of black pepper.

Conflicts of Interest

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

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EXPLORING GENOTYPIC VARIATION IN GROWTH AND YIELD TRAITS OF BETEL VINE (*Piper betle* L.) GENOTYPE

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Abstract

The present research spans two consecutive growing seasons (2021-22 and 2022-23) at the Spices Research Centre, Shibganj, Bogura to evaluate the growth and yield attributes of the betel vine genotypes. The inaugural season (2021-22) focused solely on line BL0027, while the following season (2022-23) included multiple betel vine lines, BL0025, BL0027, BL0028, BL0030, and BL0040, with BARI Pan-3 as check variety. Experimental plots were arranged in a Randomized Complete Block Design (RCBD) with three replications. Data were collected three times throughout the leaf harvesting period, encompassed fifteen morphological traits. The results highlight significant genotypic variations in vine growth, morphology, and yield characteristics. During season 1 (2021-22), genotypes exhibited varying vine lengths, daily growth rates, internode dimensions, and leaf traits, underscoring genetic diversity. BL0027 consistently displayed superior growth and yield attributes, and closely followed by BARI Pan-3 in the season 2 broad assessments. However, genotypic variations persisted, emphasizing the influence of genetics on betel vine attributes. These findings are crucial for betel vine breeding programs and agricultural practices, offering insights into genetic diversity and potential for tailored cultivation. Future research should explore the genetic and environmental factors underlying these traits to optimize betel vine cultivation and management.

Keywords: BARI Pan-3, Betel vine, Genetic diversity, Leaf production,Variability

Introduction

The betel vine, scientifically known as *Piper betle* L. belongs to the Piperaceae family, has held a unique cultural and economic significance across Asia for centuries (Bar *et al.*, 2020). The origin of betel vine is thought to be Central or Eastern Malayasia (Chattapadhyay and Maity, 1967). Bangladesh, a land deeply rooted in tradition and cultural heritage, has long embraced the betel vine as an integral part of its social tapestry. The vibrant green leaves of this plant, which are chewed for their stimulating and aromatic qualities, have been cherished across generations, gracing ceremonial

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occasions and everyday life alike. It is primarily consumed in South Asia and worldwidely known as betel quid or paan, in combination with areca nut or fermented tobacco leaf (Saraswat *et al.,* 2020; Shah *et al.,* 2021). The leaves exhibit antioxidant, anticarcinogenic, anti-inflammatory, antibacterial, antifungal and nematicidal properties and its essential oils known for biological activities (Kumar *et al.,* 2010; Rai *et al.,* 2011). About 30% of adults chew betel quid in Bangladesh and the world context it is approximately 10-20% (Gupta and Warnakulasuriya, 2002; Flora *et al.,* 2012). Additionally, its numerous health benefits have been documented in traditional medicine systems, further enhancing its importance. It has the property of antacid, carminative, and tranquilizer which helps in digestion, removes the bad smell of the mouth, improves taste and appetite, and strengthens the teeth (Islam *et al.,* 2015). In recent years, the global demand for betel leaves and their associated products has surged, resulting in an increased need for the cultivation of this versatile plant. This heightened demand has prompted a growing interest in the genetic improvement of betel vine genotypes to meet both quantity and quality requirements. The total betel leaves production in Bangladesh in 2021-22 was estimated at 206993.70 (tons) and total cultivated area was about 21838.19 ha (BBS, 2022).

The meticulous evaluation of betel vine genotypes should focus within this unique geographical context, emphasizing the importance of morphological traits as key indicators of the plant's adaptability and productivity. Bangladesh's varied climatic zones, ranging from the lush plains to the hilly terrain, provide a dynamic backdrop for the cultivation of betel vine, giving rise to a rich tapestry of genotypes with distinct morphological characteristics. Morphological traits, including leaf shape, size, color, vine structure, and flowering patterns, serve as crucial markers for discerning and classifying betel vine genotypes. These traits not only reflect the genetic diversity within the betel vine but also hold the key to optimizing cultivation practices for better yields, quality, and resilience to local environmental conditions. In a nation where betel vines play a vital role in cultural rituals, traditional medicine, and culinary traditions, the study of these morphological traits not only has economic implications but also preserves and enhances the cultural heritage of Bangladesh. As Bangladesh stands on the cusp of harnessing the full potential of betel vine cultivation, the evaluation of genotypes based on morphological traits becomes a vital tool for ensuring productivity, quality, and the conservation of traditional practices. This comprehensive exploration within the Bangladeshi context seeks to empower stakeholders with the knowledge needed to make informed decisions and forge a path towards a thriving betel vine industry that not only supports livelihoods but also celebrates the country's rich cultural heritage. Therefore, the present study has been undertaken, for understanding and evaluating the diverse betel vine genotypes through the lens of morphological traits takes on paramount significance in the Bangladeshi context.

Materials and Methods

Site and genotypes

The experiment was conducted at the Spices Research Centre, Shibganj, Bogura, spanning two consecutive growing seasons: 2021-22 and 2022-23. In the inaugural

season (2021-22), the experiment was conducted an assessment solely on line BL0027 with check. Subsequently, during the ensuing season 2 (2022-23), a broader evaluation was undertaken, encompassing multiple betel vine lines, namely BL0025, BL0027, BL0028, BL0030, and BL0040. The genotypes were collected from betel vine growing region of Rajshahi, Bangladesh. Throughout both seasons, we utilized BARI Pan-3 was utilized as the reference or check variety to facilitate comparative analysis.

Experimental plan

The experimental plots were established with a standardized unit plot size of 3 square meters, each accommodating four betel vine plants per hill, while maintaining a consistent plant spacing of 10 cm by 10 cm. To optimize the experimental design for efficient management and data collection, nine hills were arranged within a single bed, with a one-meter gap separating adjacent beds. The study adopted a Randomized Complete Block Design (RCBD) with three replications. To ensure optimal growth and development, irrigation and various intercultural operations were executed as required during the experimental period.

Observations recorded and statistical analysis

Data were meticulously recorded three times annually, specifically during the leaf harvest periods. The parameters under evaluation encompassed a range of morphological traits, including vine length (VL), internode length (IL), internode diameter (ID), peduncle length (PL), peduncle diameter (PD), leaf length (LL), leaf width (LW), number of leaves (NL), and weight of leaves (WL) of the betel vine plants. In addition to these primary observations, calculated vine growth (VG) (difference between the base height and vine length after four months of growth), vine growth per day (VGD), weight of single leaf (WSL), number of leaves per meter vine (NLM), number of leaves per hectare per year (NLHY), and weight of leaves per hectare per year (WLHY) were based on the recorded data. NLHY and WLHY represent cumulative observations for leaves harvested three times in a single year, converted to leaves per hectare. All other observations were averaged from the three data collection points within a year. These recorded data form the basis subsequent analysis and findings. To analyze variance based on the extensive dataset obtained from these observations, employed the R platform for statistical analysis (R Core Team, 2022).

Results and Discussion

In this study, the result of an extensive study aimed at characterizing the growth and yield attributes of vine genotypes during the season 1 (2021-22) and season 2 (2022-2023), respectively. Summary of the individual genotype's performances for the growing season 1 and 2 were presented in Table 1 and 2, respectively. From the table 1 it was indicated that the studied genotypes were significantly varied except for ID, LL, LW, NL and NLM. The coefficient of variation was ranged from 3.21 to 13.32 for different studied traits. On the other hand, during season 2, all the genotypes were significantly varied for the studied traits. The range of coefficient of variation for different studied traits was 0.86 to 13.4 (Table 2).

Vine growth

In season 1, the average vine length for BARI Pan-3 was 169.34 cm, in which between the leaf harvests a total vine growth was 104.06 cm at four-month tenure. The daily growth rate for this genotype was approximately 1.11 cm per day. On the contrary, the average vine length for BL0027 was 174.57 cm. The daily growth rate for this genotype was approximately 1.16 cm per day which in turn extended on an average to a total vine growth of 109.16 cm during four months periods.The average internode length measured for BARI Pan-3 was 7.54 cm while average diameter of internodes in this genotype was 0.71 cm. In case of BL0027, average internode length and diameter were 8.31 and 0.70 cm, respectively. In betel vines, having longer vines with shorter gaps between the nodes is considered desirable because it leads to a higher leaf count, thanks to more nodes (Rahman *et al.,* 2020). On the other hand, during season 2, BARI Pan-3 exhibited the highest vine length at 188.72 cm, while BL0040 had the shortest vine length at 169.62 cm. These variations highlight genotypic differences in vine elongation potential. Similar to the vine length BARI Pan-3 displayed the highest mean vine growth, reaching 123.72 cm, while BL0040 had the lowest growth at 103.59 cm. This variation in vine growth underscores the importance of genetic diversity in determining vine development. Quite the similar, BARI Pan-3 exhibited a mean daily growth rate of 1.03 cm, as did BL0025 and BL0027, while BL0028, BL0030, and BL0040 had slightly lower daily growth rates. These findings indicated that certain genotypes exhibit faster daily growth rates than others. Variations in vine length likely stemmed from a combination of factors, including seasonal fluctuations in temperature and atmospheric humidity, as well as inherent genetic diversity among the different cultivars (Pariari and Imam, 2012). BL0030 had the longest average internode length at 9.09 cm, while BARI Pan-3 had the shortest at 7.02 cm. Differences in internode length can influence vine structure and overall growth. BL0025 displayed the smallest internode diameter (0.38 cm), whereas BL0030 had the largest (0.44 cm). This parameter reflects genotypic variations in stem thickness.

Leaf size

In season 1, peduncles of BARI Pan-3 had an average length of 7.56 cm and diameter was approximately 0.38 cm. The leaf length and width were 15.21 and 11.23 cm, respectively. Alternatively, peduncles of BL0027 had an average length of 10.24 cm and the diameter was approximately 0.52 cm. Leaves of the genotypes had an average length of 16.01 cm whereas average width was 11.91 cm. Rahaman *et al.,*1997 was found that variation in leaf length between 6.2 cm to 15.3 cm among 27 genotypes of betel vine. For the season 2, BL0027 exhibited the longest peduncles with an average length of 9.88 cm, while BL0040 had the shortest peduncles at 8.50 cm. On the other hand, BL0025 had the smallest peduncle diameter (0.34 cm), while BL0030 had the largest (0.77 cm). Peduncle length and diameter are crucial for nutrient transport and leaf development and varies among these genotypes. BL0027 exhibited the longest leaves (14.88 cm) and widest leaves (11.18 cm) on average. Leaf dimensions contribute to the photosynthetic capacity and overall productivity of the vines. Pariari and Imam (2012) reported the result which indicated that leaf width ranged from 8.65 - 10.45 cm which was supported to present study.

Leaves number and weight

In case of season 1, on average, there were 12.56 leaves per plant while total weight of leaves of BARI Pan-3 was 39.67 g during a single harvest. An individual leaf from this genotype had an average weight of 3.16 g. Considering average potentials, BARI Pan-3 exhibited an average of 12.11 leaves per meter of vine. The total number of leaves produced by BL0027 was 12.94 leaves per plant which was equivalent to 43.01 g. The individual leaf weight was 3.32 g. This genotype exhibited the potential of producing an average of 11.87 leaves per meter of vine. Rahman *et al.*(2020) reported that Gayasur pan produced significantly highest number of leaves (16.35 no.) per meter vine. In season 2, BL0027 had the highest mean number of leaves (17.50), while BL0028 had the lowest (11.83) during a single harvest. It also exhibited the highest average number of leaves per meter of vine (14.21), while BL0040 had the lowest (10.46). Contrary to this, BARI Pan-3 produced the heaviest leaves (49.90 g on average), whereas BL0040 had the lightest leaves (23.26 g). Similarly, BARI Pan-3 had the highest mean weight per single leaf (2.89 g), while BL0030 had the lowest (2.14 g). Leaf number plays a vital role in canopy development and light interception, while, leaf weight directly influences the biomass and potential yield of the vines. This parameter provides insights into vine canopy density and nutrient allocation. Das *et al.*(1995) recorded that maximum fresh weight (380.75 g) and dry weight (44.60 g) in Ghanagette cultivar and produced highest number of leaves (88) per vine.

Fresh yield

Results of season 1 revealed that, the estimated leaf yield for BARI Pan-3 was 43.59 lakhs leaves/ha in terms of number which was approximately 13.77 t/ha in a year. The estimated number of leaves for BL0027 was 46.60 lakhs/ha in a year which was approximately 15.45 t/ha. Season 2 result exhibited that, BL0027 had the highest estimated number of leaves per hectare per year (51.16 lakhs) closely followed by BARI Pan-3 (50.19 lakhs), while BL0040 had the lowest (37.65 lakhs). Quite the similar BL0027 produced the highest estimated weight of leaves per hectare per year (15.67 t/ha) followed by BARI Pan-3 (14.52 t/ha), while BL0040 had the lowest (8.08 t/ha). Guha (2006) reported that annual yield of a good crop of betel vine was 60-70 leaves/ plant and 6 -7 millions/ha. The number and weight of leaves per hectare is a critical factor in determining overall productivity. This parameter directly impacts the potential yield and economic value of the betel vine. Sheet (2002) reported that the highest number of leaves (62.66 lakh ha^{-1}) in cv. Chandrakona. Rahman *et.al.* (2020) reported that the cultivar PB 006 (Misti pan) and PB 009 (BARI Pan-1) produced significantly higher yield as 23.77 t/ha and 23.82 t/ha, respectively.

These comprehensive results highlighted significant genotypic variations in vine growth, morphology, and yield characteristics among the studied betel vine genotypes, offering a comprehensive understanding of its variability and potential for agricultural applications. This information is valuable for betel vine breeding programs, management practices, and the selection of suitable genotypes for specific agricultural contexts. Further research into the genetic and environmental factors influencing these traits is recommended for a more in-depth understanding of vine variability and potential improvements in viticulture.

Table 1. Performances of betel vine genotypes evaluated during 2021-22

Genotype	VL	VG	VGD	IL	ID	PL	PD	LL	LW	NL	WL	WSL	NLM	NLHY	WLHY
BARI Pan-3	169.34	104.06	1.11	7.54	0.71	7.56	0.38	15.21	11.23	12.56	39.67	3.16	12.11	43.59	13.77
BL0027	174.57	109.16	1.16	8.31	0.70	10.24	0.52	16.01	11.91	12.94	43.01	3.32	11.87	46.60	15.45
SE	5.51	5.57	0.06	0.58	0.04	0.51	0.05	1.71	1.54	1.06	4.57	0.11	1.18	4.25	1.68
T-test	*	*	*	**	NS	**	**	NS	NS	NS	*	**	NS	**	**

VL=Vine length; VG=Vine growth; VGD= Vine growth per day; IL=Internode length; ID=Internode diameter; PL=Peduncle length; PD=Peduncle diameter; LL=Leaf length; LW=Leaf width; NL=Number of leaves; WL=Weight of leaves; WSL=Weight of single leaf; NLM=Number of leaves per meter vine; NLHY=Number of leaves per hectare per year; WLHY= Weight of leaves per hectare per year; SE=Standard error;

Table 2. Performances of betel vine genotypes evaluated during 2022-23

Genotype	VL	VG	VGD	IL	ID	PL	PD	LL	LW	NL	WL	WSL	NLM	NLHY	WLHY
BARI Pan-3	188.72	123.72	1.03	7.02	0.61	8.28	0.39	10.72	8.90	17.25	49.90	2.89	13.94	50.19	14.52
BL0025	187.03	121.03	1.01	7.87	0.38	7.88	0.34	9.79	7.89	15.08	45.25	3.00	12.46	44.86	13.46
BL0027	189.13	123.13	1.03	7.00	0.54	9.88	0.41	14.88	11.18	17.50	53.62	3.06	14.21	51.16	15.67
BL0028	166.81	100.81	0.84	8.21	0.37	7.04	0.31	9.54	6.18	11.83	25.37	2.14	11.74	42.25	9.06
BL0030	166.58	100.58	0.84	9.09	0.44	8.78	0.77	10.50	6.74	10.75	25.80	2.40	10.68	38.45	9.23
BL0040	169.62	103.59	0.86	9.05	0.42	8.50	0.38	9.47	7.77	10.83	23.26	2.15	10.46	37.65	8.08
Mean	177.98	112.14	0.93	8.04	0.46	8.39	0.43	10.82	8.11	13.88	37.20	2.61	12.25	44.09	11.67
SE	2.53	2.53	0.02	0.37	0.05	0.82	0.04	0.94	1.06	0.71	1.83	0.02	0.57	2.04	0.50
CV	1.42	2.26	2.14	4.57	10.44	9.71	8.33	8.71	13.04	5.12	4.92	0.86	4.64	4.64	4.30
LSD	2.06	2.06	0.02	0.30	0.04	0.67	0.03	0.77	0.86	0.58	1.50	0.02	0.46	1.66	0.41
F-TEST	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**

VL=Vine length; VG=Vine growth; VGD= Vine growth per day; IL=Internode length; ID=Internode diameter; PL=Peduncle length; PD=Peduncle diameter; LL=Leaf length; LW=Leaf width; NL=Number of leaves; WL=Weight of leaves; WSL=Weight of single leaf; NLM=Number of leaves per meter vine; NLHY=Number of leaves per hectare per year; WPHY= Weight of leaves per hectare per year; SE=Standard error; CV=Coefficient of variation; LSD=Least significant difference;

Conclusion

In this research, was conducted an extensive study to assess the growth and yield attributes of various betel vine genotypes. The results of this study revealed significant genotypic variations in vine growth, morphology, and yield characteristics among the studied betel vine genotypes. Notably, different genotypes exhibited varying levels of performance across these traits, highlighting the importance of genetic diversity in determining betel vine development and productivity. In season 1 (2021-22), BARI Pan-3 and BL0027 exhibited distinct growth patterns, with varying vine and leaf characteristics. During season 2 (2022-23), the expanded assessment involving additional genotypes further emphasized the influence of genetics on betel vine attributes. The genotype BL0027 consistently demonstrated superior vine growth, leaf characteristics, and yield potential when compared to the other genotypes. But genotypic differences continued,

with BARI Pan-3, in particular, showing unique characteristics including yield and daily growth rates and vine length. These differences underscored the genetic variability within the betel vine species. This research contributes valuable insights into the growth and yield attributes of betel vine genotypes, shedding light on their genetic diversity and potential for agricultural applications. The findings of this study have significant implications for betel vine breeding programs and agricultural practices. Future studies should delve deeper into the genetic and environmental determinants of these traits to further refine betel vine cultivation and management practices.

Conflicts of Interest

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

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ENHANCING AGRICULTURAL PRODUCTIVITY THROUGH A SEMI-AUTONOMOUS IOT ROBOT IN SMART FARMING SYSTEMS

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Abstract

In addressing the challenge of enhancing agricultural productivity in developing countries, this research introduces a semi-autonomous IoT robot designed to modernize traditional farming practices in regions like Bangladesh. The study explores whether such a robot can effectively integrate with existing farming practices and assesses its impact on agricultural productivity, resource optimization, and most importantly cost-efficiency. The literature reveals a push towards smart farming technologies, but their adoption in less affluent regions is hindered by cost and resource constraints. Employing a mixed-methods case study approach, the research developed and tested a robot equipped with an NPK sensor for detecting levels of nitrogen, phosphorus, and potassium in the soil, a water level indicator to measure flood water levels in millimeters, and a soil moisture sensor. These data were transmitted to the user's phone over the internet, allowing for remote monitoring of fertilizers and water levels. Additionally, the system included a remote-controllable water dispenser for irrigation and a fruit-picking mechanism for harvesting. The results indicated that all intended data collection was executed accurately, enabling users to remotely monitor soil conditions and effectively control the robot's actions. However, the initial cost of the robot may be slightly expensive for individual farmers, though mass production is anticipated to reduce the price to a level that is reasonably affordable for widespread adoption. However, limitations in sensor calibration for different soil types are acknowledged. Future research suggest exploring sensor calibration precision, extending system capabilities, and integrating predictive AI for a comprehensive agricultural solution.

Keywords: Agriculture, IoT, Sensor, Semi-autonomous, Smart farming, Web server

Introduction

Agriculture plays a pivotal role in shaping the economy and ensuring sustenance in many developing countries, including Bangladesh. Traditional agricultural practices in these regions often result in inefficiencies and suboptimal yields, highlighting the need for modernization. The emergence of smart farming, driven by technological

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advancements, promises to enhance agricultural productivity. However, these state-of-the-art systems, while promising, often remain inaccessible to less affluent regions due to their high costs and resource requirements. The existing literature highlights various technological initiatives aimed at improving agricultural productivity. Studies such as those by (Oliveria *et al.,* 2020), and (Malavazi *et al.,* 2018), have introduced monitoring systems and agrovot robots, respectively, with a focus on weed management and autonomous operation in crops. While these solutions are innovative, they are often constrained by crop specificity and high costs. (Birrell *et al.,* 2020) also designed a robotic lettuce harvesting system, emphasizing precision in handling delicate vegetables. The importance of smart agriculture, employing technologies like AI, IoT, and robotics, is underscored by works like (Subeesh and Mehta, 2021), (Said Mohamed *et al.,* 2021), and (Ummadi *et al.,* 2022). These studies explore the automation and digitization of agriculture, emphasizing the potential of IoT and AI in developing smart farm machinery and real-time crop health monitoring. Furthermore, (Dhanaraju *et al.,* 2022), and (Krishnan *et al.,* 2020), delve into the convergence of robots, AI, IoT, and cloud computing in agriculture, underscoring the advancements in smart farming and precision agriculture. Recognizing this gap, our study aims to design, develop, and test a cost-effective, semi-autonomous IoT robot that seamlessly integrates with existing farming practices in developing regions like Bangladesh. This research is designed to perform tasks ranging from monitoring soil moisture levels to precise pesticide dispensing, ensuring real-time data transmission for immediate action.

Materials and Methods

Our proposed system hinges on the integration of key components, central to which is the Arduino Pro Mini, orchestrating various functionalities. Accompanying units include the Esp8266 and ESP32cam, essential for communication and imaging tasks, respectively. The system's acumen in environmental adaptation is enhanced by sensors such as an infrared sensor for navigational aid, NPK sensor for soil fertility, Soil moisture sensor, and Water level detector. A Robotic arm enables task-specific manipulations, while an LCD display offers immediate system feedback.

Arduino pro mini

This is a microcontroller board based on the ATmega328P. From the 14-digital input/output pins of the Arduino Pro Mini, 6 PWM pins can be used as outputs. It also has 6 analogue inputs, an onboard resonator, a reset button, and holes for mounting pin headers. In the prototype robotic vehicle, most and components devices were controlled by the Arduino Pro Mini (www.etechnophiles.com),

Node MCU Esp8266 and ESP32cam

It was used as a Wi-Fi module and microcontroller in this prototype. It was used to collect data using digital and analogue pins from various sensors (Joshi *et al.,* 2015). After calibrating those data points, the collected data was sent to the Arduino Pro Mini.It was used to stream data from the field with the help of external devices such as mobile phones or computers (Mehendale, 2022).

Sensors utilized in the system

NPK Sensor measures the levels of nitrogen, phosphorus, and potassium in the soil, providing values in milligrams per kilogram (mg/kg). Soil Moisture Detector (YL69) measures soil moisture, with readings ranging from 0 to 1023. Lower readings signify higher soil moisture content. Although the ideal metric is percentage, the sensor doesn't provide this directly. The readings vary with different soil types, necessitating initial manual calibration for each new soil context. The water level Indicator tool gauges flood water levels above the ground. Readings below 100 suggest minimal water presence. A reading around 300 indicates the water has reached half the sensor's height, set at 40mm. Values exceeding 500 denote water levels surpassing 40mm. These sensors collectively inform the system's responses, ensuring it reacts appropriately to various environmental conditions.

Block diagram

The whole working process of (Fig.1) IoT-based autonomous integrated smart farming system for agricultural farms system is mainly an IoT-based robotic vehicle..

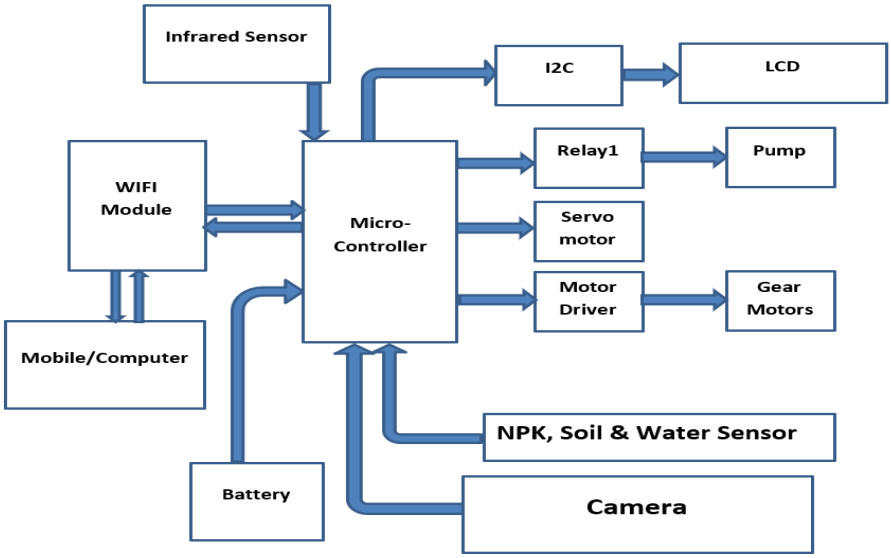

Fig. 1. System block diagram

In the schematic representation, several components are linked to a central microcontroller, encompassing elements like the I2C, LCD display, and relay module—the latter essential for regulating the pump's operations. The system incorporates two kinds of servo motors for diverse functionalities, particularly in maneuvering mechanical limbs, through a motor driver and a gear motor. The vehicle's motion is propelled by these motors, steered by inputs from various sensors monitoring soil nutrients, moisture, and water levels. Additionally, a camera is interfaced with the Arduino Pro Mini, while data communication is facilitated through a Wi-Fi module, enabling real-time data transmission to a central server and subsequent access via digital devices. The setup also includes infrared sensors to monitor road conditions and vehicle movement, providing a

holistic overview of the system's operation as depicted in the comprehensive block diagram.

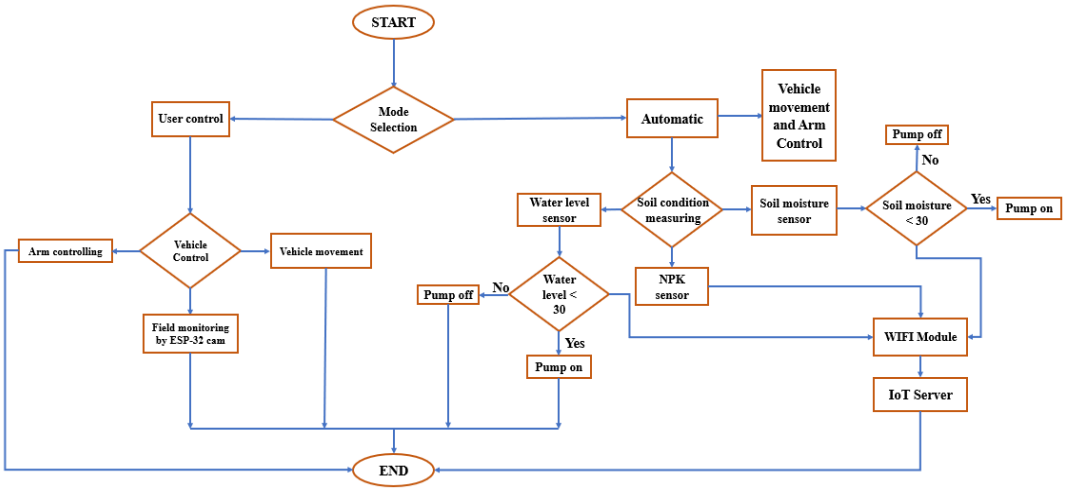

Fig. 2. Overall system flowchart

The flowchart presented illustrates the architectural blueprint of the proposed system, simplifying the detection framework to enhance comprehension of the project's operational mechanics. It details the process initiated by input data derived from the potentiometer readings, followed by the application of specific algorithms to manage water and fertilizer distribution. The system's versatility is evident, with the robotic vehicle's control shifting between manual and automatic. Specifically, a potentiometer reading of zero implies manual control via a local server, allowing user intervention. Conversely, a reading exceeding three hundred triggers the vehicle's autonomous function. This mode involves data transmission to the server for analysis, with particular attention to soil moisture levels. Should these levels fall below 30, the system autonomously activates the pump, ensuring adequate water supply without necessitating manual oversight. The foundation of the server underscores its role in overseeing and maintaining agricultural data storage. At the heart of this system is a Wi-Fi module, functioning as the primary microcontroller that bridges the sensor data with the server. Data storage is facilitated through the Blynk software, a specialized Internet of Things (IoT) platform that streamlines the creation of associated mobile and web applications (Serikul *et al.*, 2018). This innovative platform swiftly integrates IoT mobile applications for devices like iOS and Android with various hardware, including Arduino, ESP8266, and ESP32. Essential parameters such as NPK levels, soil moisture, and water levels are interfaced with the Wi-Fi module, enabling real-time monitoring. Users can conveniently access this crucial data via the Blynk server on personal devices like smartphones or computers, provided there is an internet connection between the user's device and the Wi-Fi module. Users can see the data from their mobile device or computer when the device and the automated vehicle WiFi module are connected to the internet. Without the internet, this procedure will not run.

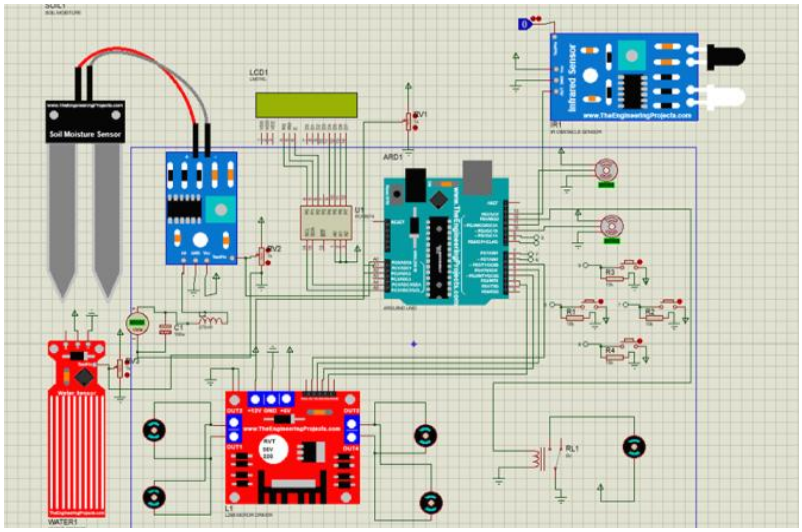

Fig. 3. Simulated diagram in Proteus

Software implementation

A simulation model was shown which the complete simulation model of this project is. Various sensors are used here, like soil, water, NPK, etc. No library is available for the NPK sensor Proteus. So, its work is shown by using a potentiometer as an alternative. The camera module is used in hardware. But since there is no camera in Proteus, only other activities are shown. Here, the display is connected through the display driver. And there are four buttons through which the car will be driven. Four motors are connected to the motor driver, and the vehicle will be controlled by pressing the buttons through the mobile server. As there is no Wi-Fi connection, the opposite button is shown as physically attached. Thus, the simulation model is created using Arduino Pro Mini by connecting each sensor. After designing the whole circuit in Proteus, the simulation was run to check whether it worked properly or not. In the simulation, soil and water levels were set to 29%. When the soil and water level value is less than 30%, the pump will be on, which means it will start pumping. These conditions were set in code on the microprocessor. So, the user can see that when soil and water values are 29%, the relay is on, which is pointed with an arrow, and the pump starts. LCD display shows that when the soil and water sensor value is 31%, the relay is OFF, and the pump stops. As there is no NPK sensor in the Proteus library, a potentiometer was used here instead of an NPK sensor, which represents the NPK sensor with the potentiometer. By changing the potentiometer value, the user can change the NPK sensor value.

In this project, four wheels were used to move a robotic vehicle. In the simulation, the four remote switches were used to control the wheels virtually. This remote switch is not connected to the circuit. These are connected virtually. Arduino cannot take 12V directly; that's why a motor driver was connected to it for controlling the wheels. Also, two voltmeters were connected to the wheels. When any of the remote switches are pressed, Arduino sends a signal to the motor driver, which regulates voltage, and the motor rotates. In the figure, the regulation voltage is shown as 3.75V on the

voltmeter. In the simulation circuit, a switch was used to control the robotic arm and clamping. Two servo motors were used to control the robotic arm and clamp. The upper servo motor controls the robotic arm, and the lower servo motor controls the clamping. When the switch is pressed, the servo motors rotate and control the robotic arm and clamping. The 3D design of the prototype model for this project was designed with the help of Solid Work software. Solid works is a computer-aided design and engineering software for solid modeling.

Results and Discussion

This is the final implementation of the prototype robotic vehicle. Here, this prototype was designed the same as the 3D design, which was designed on Solid Work. It was implemented with four wheels, an Arduino Pro Mini, three sensors, a water pump, a robotic arm, and an ESP32cam.

Hardware results

In this research, the amount of fertilizer in the raw soil was measured using an NPK sensor, the moisture content of the soil was determined using a soil sensor, and the water level of the soil; particularly in the paddy field, which is constantly in need of water, was determined using a water level sensor. Three different fertilizers (nitrogen, potassium, and phosphorus) that are commonly used in the agricultural sector were used here. Urea is the most concentrated solid nitrogen fertilizer, a white, crystalline solid with a nitrogen content of 46%. The focus here will be on its use as a nitrogen fertilizer. Triple Super Phosphate, also known as TSP, is a widely used Phosphorus (P) source for several benefits. Triple Super Phosphate (TSP) has a phosphate content of 20%. So, TSP fertilizer was used for Phosphorus measuring purposes, and Potassium was used. For the measurement of the appropriate value of the NPK sensor, a mixture of soil was created with urea, TSP, and Potassium fertilizer. Since Urea contains nitrogen, TSP contains phosphorus, and Potassium fertilizer (Potash) contains potassium only, they are mixed together and tested. As these fertilizers do not have other elements that would obstruct the reading theoretically, the mixture is ideal for testing the NPK sensor's ability to sense all three components. After mixing, the sensor detected the amount of fertilizer in the soil, and then the user received or saw the result value on the LCD display and mobile device via the BLYNK server. This research was divided into the following eight cases to be tested.

Case 1 and Case 2 (raw- soil, soil with water)

In Case 1, a sample of soil from an area where no crops had been grown was tested. This soil had minimal moisture content, leading to low readings from both the water level sensor and the soil moisture sensor. As the soil was naturally low in Nitrogen (N), Phosphorus (P), and Potassium (K), the sensors reflected this with negligible values for these nutrients. In Case 2, the same soil sample was used, but with a significant change: water was added to it. This addition of water was crucial for allowing the NPK sensor to effectively measure the nutrient values in the soil. After adding water, the NPK sensor displayed values of 173 mg/kg for nitrogen (N), 51 mg/kg for phosphorus (P), and 70 mg/kg for potassium (K), as indicated on the LCD display and the BLYNK server.

The addition of water was essential in facilitating the sensor's ability to accurately assess the nutrient content of the soil.

Case 3 (fertilizer and water mixture with soil)

Here, fertilizer and water were mixed with the soil and measured with all three sensors. This time, the data on fertilizer and water amounts was taken only from the BLYNK server; that shows the mobile display. Since there was an issue, the LCD display has less capacity for counting and displaying digits. In this case, the value for Nitrogen, Phosphorus, and Potassium are 229 mg/kg, 143 mg/kg, and 196 mg/kg, as shown in the figure below. The water and soil sensor values were 53 and 24 percent, respectively.

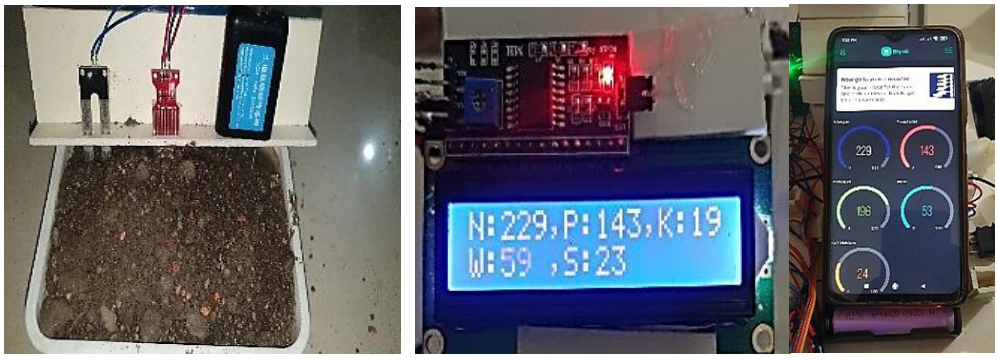

Fig. 4. Soil mixture with Fertilizer and Water (Real Data) a) Sensors connected to sample soil, b) Output values from sensors, c) Values displayed in mobile phone over internet

Case 4 (Live streaming with ESP 32 Cam)

In this scenario, a command was initiated to navigate the robotic vehicle toward a specified target, identified here as a fruit-bearing plant. Upon reaching the intended location, the integrated ESP-32 camera enabled live streaming of the plant's surface directly to a local server. However, accessing this live feed required adherence to certain prerequisites, most notably, the viewing device had to be connected to the same Wi-Fi network as the server. The vehicle was equipped with a specialized arm mechanism, designed for the precise task of grasping and relocating objects, demonstrating its utility in agricultural settings like this.

Case 5 (Picking fruit or vegetables)

In this specific case, sample fruit plants were used as a part of the robotic arm to pick up sample fruit. The following figure demonstrates how the robotic arm is harvesting sample fruits from the plant and placing them, with the assistance of clamps, in the box where they will be stored. Additionally, the entire harvesting process is manually controlled from a mobile device, allowing precise and targeted picking of fruits.

Case 6 (Vehicle moving)

This prototype vehicle is an automatic vehicle. It can move automatically in the field. An IR sensor was used for automatic movement. When the IR sensor detects an obstacle, it changes direction automatically. The figure below represents the vehicle movement. At first, the vehicle goes forward, but when the IR sensor senses the obstacle, the vehicle changes its direction automatically and turns right.

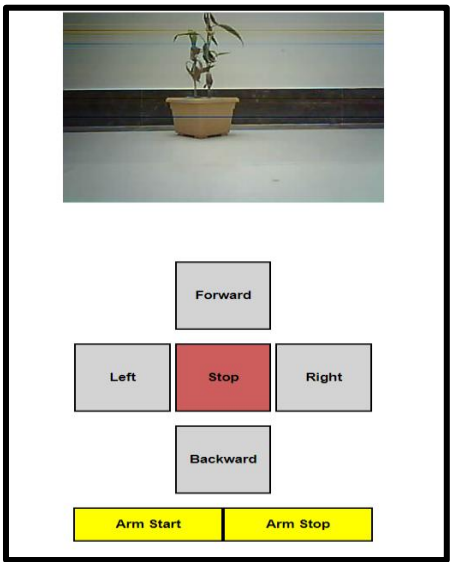

Fig. 5. View of Live Streaming with ESP 32 Cam

Case 8 (Irrigation system)

The proposed design model is engineered to autonomously irrigate the field, utilizing data derived from the soil via moisture and water level sensors. As the vehicle traverses the field, it employs these sensors to assess soil conditions. Specifically, if soil moisture falls below the 30 percent threshold, the system activates the water pump, triggering immediate irrigation. Conversely, if moisture levels exceed this preset limit, the system refrains from engaging the pump, thereby preventing unnecessary water usage. This mechanism ensures an efficient response to real-time soil moisture data, optimizing water resource management. When the soil moisture sensor detects a moisture level of 1 % as shown in the display, the water pump automatically runs and instantly provides water. But the pump will stop providing water when the moisture increases by up to 30 percent.

Results of comparison

In the first scenario, when the raw soil was measured, the values for nitrogen (N), phosphorous (P), and potassium (K) all came out to be zero. This is shown in above figures which also illustrates how the NPK sensor value rises when water is added to soil and the two are mixed together. It is possible to draw the conclusion that the NPK sensor

cannot detect elements of fertilizer until water is mixed in with the soil first. When compared to Cases 1 and 2, Case 3's NPK, soil, and water level detector sensor values were higher after the addition of TSP, urea, and potassium fertilizer. The sensor data from the experiment in different cases is shown in the table below.

Table 1. Sensor data

Sensor name		Case 1	Case 2	Case 3
NPK	Nitrogen (N) (mg/kg)	0	173	229
	Phosphorus (P) (mg/kg)	0	51	143
	Potassium (K) (mg/kg)	0	70	196
Soil moisture (%)		1	1	53
Water Level (%)		12	0	24

The vertical axis in this bellow graph shows the quantity of each of the five soil components (water level, nitrogen, phosphorus, potassium, and soil moisture level), while the horizontal axis shows time. The data from the soil moisture sensor, water level detector, and NPK were stored on the BLYNK server. Through the BLYNK server, users can view this recorded data as a graph or chart. From there, users track how much the sensor value has increased or decreased and examine the sensor's value in this graph.

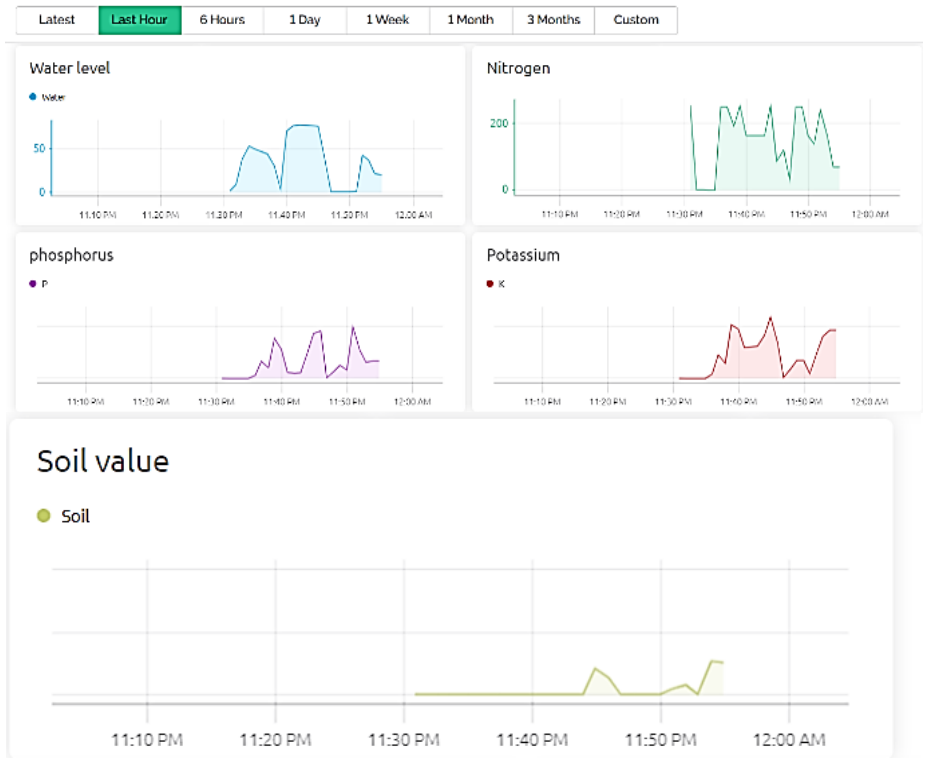

Fig. 6. Graph from BLYNK server

In-depth analysis of results

Our analysis began with an evaluation of soil's natural state (Case 1) and proceeded through various scenarios, each adding a new variable (water, fertilizers) and monitoring changes through the sensors. The most significant leap in nutrient values was observed in Case 3, where the integration of fertilizers and water dramatically altered the readings, underscoring the sensors' sensitivity and the soil's responsive nature.

Sensor efficiency and accuracy

The consistency observed in sensor readings across different cases establishes their reliability. However, the disparity between natural soil nutrient levels and those recorded post-fertilization indicates not just the impact of agricultural supplements but also the precision required in calibrating these sensors, particularly for varying soil types and conditions.

Real-time monitoring and responsiveness

The system's ability to transmit data for real-time analysis (as seen in the live streaming case) is crucial for immediate decision-making. The rapid response of the irrigation system and the robotic arm's successful field operations demonstrate the system's potential for timely interventions, directly influencing crop health and yield.

Automated navigation and task execution

The vehicle's navigation and obstacle detection, essential for its autonomous functions, were tested rigorously. Its successful maneuvering and task execution, even in non-uniform terrains, highlight its adaptability and functionality in real-world scenarios.

Sensor Accuracy (NPK Sensor Specifications)

- Measure Range: 0-1999mg/kg
- Accuracy: ±2% Full scale (F.s)
- Resolution: 1mg/kg (mg/l)

The NPK sensor's specifications indicate a high level of precision in nutrient measurement within the soil, essential for accurate agricultural adjustments. The consistency observed in sensor readings across different cases establishes their reliability in the field. However, the disparity between natural soil nutrient levels and those recorded post-fertilization highlights the impact of agricultural supplements and the precision required in sensor calibration, particularly for varying soil types and environmental conditions. The integration of our semi-autonomous IoT robot into farming practices marks a transformative approach to agriculture, with substantial implications for efficiency, productivity, and economic viability. By employing real-time data, the system optimizes resource use, potentially reducing water and fertilizer usage by a significant margin, thereby minimizing environmental impact and expenditure. This precision in resource distribution not only curtails waste but also fosters ideal crop-growing conditions, directly contributing to an expected rise in yields. Furthermore, the

automation of mundane tasks alleviates labor requirements, decreasing human error and operational costs, which translates into enhanced overall productivity. Currently valued at Tk. 28,300, the initial cost of implementing this system might appear prohibitive for individual farmers. However, the project's true economic appeal lies in its scalability. As the technology is adapted for mass production, there's a realistic projection that costs could plummet substantially, rendering this innovative solution more accessible and financially feasible for widespread adoption, even within economically constrained regions. This transition not only democratizes advanced farming practices but also fortifies the agricultural sector against future challenges.

Conclusion

The findings of this study clearly expressed that the robot effectively monitors and controls farming activities in real-time, showcasing reliability and precision in collecting soil parameters. Notably, the system demonstrated its capability to accurately detect variations in nutrient levels and soil moisture, thereby optimizing resource use and aligning with sustainable practices. Comparing with previous studies, the robot's successful navigation and task execution in various field conditions confirm its adaptability and potential for broader application. However, the study acknowledges certain limitations, such as the need for precision in sensor calibration across different soil types. Therefore, a promising step towards revolutionizing smart farming in developing regions, providing a cost-effective and technologically advanced solution and significant contribution to future agricultural innovations.

Conflicts of Interest

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

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VARIABILITY AND HERITABILITY STUDY IN SOME SELECTED GENOTYPE OF RAPESEED (*Brassica rapa* L.)

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Abstract

A field experiment was conducted in the research field of Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh to study variability and heritability in *Brassica rapa* L. for developing short durable, high yielding varieties. The experiment had laid out in a randomized complete block design with three replications and consisted of seven genotypes viz., G_1 (BARI Sarisha-14), G_2 (Brown Special), G_3 (Yellow Special), G_4 (Tori-7), G_5 (BARI Sarisha-17), G_6 (BARI Sarisha-15) and G_7 (BARI Sarisha-6). The result revealed that G_7 had the highest yield plant^{-1} (6.91 g) and longest duration (108 days) while G_4 had the lowest yield plant^{-1} (4.12 g) and G_2 had the shortest duration (79 days). High genotypic and phenotypic coefficient of variation for number of secondary branches plant^{-1} (95.4 % and 99.4 %), number of siliqua plant^{-1} (29.6 % and 30.3%), plant height (25.2 % and 25.4 %) and number of seeds siliqua^{-1} (26.76% and 27.2%) had estimated. High heritability with high genetic advance and gain had observed for days to 80% maturity (99.9%, 21% and 23.4 %), plant height (98.55%, 53.99% and 51.6 %) and number of siliqua plant^{-1} (95.4 %, 101.3 % and 59.4 %). While high heritability with low genetic advance and high genetic gain were observed for number of primary branches plant^{-1} (92.1%, 0.70% and 188.4%), number of secondary branches plant^{-1} (92.02%, 7.47 % and 188.43 %), length of siliqua (98.3%, 1.104% and 29.96), 1000 seed weight (84.6 %, 1.23% and 32.01%) and yield plant^{-1} (97.4 %, 2.01% and 38.47%). Days to 80% maturity, plant height and number of siliqua plant^{-1} showed high heritability with high genetic advance and genetic gain indicated additive gene action and selection could be useful for these traits. While high heritability with low genetic advance had observed for number of primary and secondary branches plant^{-1}, length of siliqua, 1000 seed weight and yield plant^{-1} indicated non additive gene action and selection might be ineffective for these traits.

Keywords: Additive gene, Genetic advance, Genetic gain, Heritability, Variance

Introduction

Mustard and rapeseed have become the major oilseed crops occupying the third position among the oilseed crops in the world and the world area harvested under mustard

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and rapeseed is 38,509,853 MT, and production is 75,711,806 MT (FAOSTAT, 2020). The climate and soil conditions of Bangladesh is also preferable for mustard and rapeseed production (Chowdhury *et al.*, 2014). In Bangladesh total cultivated area under rapeseed and mustard cultivation is 0.589 million hectares which produces 1.34 ton ha^{-1} in 2020-21 and *B. rapa* occupies the 1st position in respect of area and production (AIS, 2022). Although short durable, low yielding and pest susceptible variety (Tori-7) of *B. rapa* is popular in Bangladesh but there is still lack of improved short durable and high yielding varieties. As a result, Bangladesh is suffering from an acute shortage of edible oil and therefore, it is very important to develop new varieties with such desirable traits. Varietal improvement requires a laborious and long term breeding activities, which depends on the extent and nature of genetic variability, heritability, and genetic advance present in the genotypes (Islam *et al.*, 2020 and Snehi *et al.*, 2020). The more the variability in the breeding population the more effective selection will be achieved (Rauf and Rahim, 2018). Genetic variability and heritability had estimated by different morphological parameters that involve polygenic inheritance of gene action (Ali *et al*., 2013, Azam *et al.*, 2013 and Iqbal *et al*., 2014). Polygenic inheritance of gene action has higher environmental influences so heritability estimation is very much important to ensure an effective selection process as it shows the influence of genes and environment on different morphological traits (Sultana *et al.*, 2021). Heritability and phenotypic variance along with the selection intensity can give the most accurate estimate of genetic advance for effective selection (Parvin *et al*., 2020). High heritability along with high genetic advance for a particular trait indicated most effective selection condition (Bibi *et al*., 2016; Rauf and Rahim, 2018 and Mazurkievicz *et al*., 2019). For developing a high yielding variety, it is very important to estimate the individual role of different morphological traits on higher yield (Ejaz-Ul-Hasan *et al.*, 2014). Therefore, the present study had conducted to estimate the variability, heritability and genetic advance for yield and yield related attributes in *B. rapa* (main oil yielding species in Bangladesh) for developing short durable and high yielding genotypes. This would provide a guide for effective selection in the breeding population in formulating the most appropriate breeding technique for improvement of various traits.

Materials and Methods

Study area

The experiment had conducted in the research field of SAU, Dhaka, Bangladesh from October/20 to March/21.

Plant materials

The plant materials of the present study consisted of seven genotypes of *B. rapa* collected from Bangladesh Agricultural Research Institute (BARI) and Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, which included G_1 (BARI Sarisha-14), G_2 (Brown Special), G_3 (Yellow Special), G_4 (Tori-7), G_5(BARI Sarisha-17), G_6 (BARI Sarisha-15) and G_7 (BARI Sarisha-6).

Materials and Methods

The experiment had laid out in a randomized complete block design (RCBD) with three replications. The genotypes had randomly distributed to each replication having row spacing of 30 cm and plant distance of 10 cm. Three rows of each accession had sown in each replication. Ten plants had selected at random from each replication and data on ten quantitative parameters namely; days to 50% flowering, days to 80% maturity, plant height, number of primary branches plant^{-1}, number of secondary branches plant^{-1}, number of siliqua plant^{-1}, siliqua length, number of seeds siliqua^{-1}, 1000 seed weight and seed yield plant^{-1} had noted.

Statistical analysis

Means separated using Least Significant Difference test. The analysis of variance for different characters had carried out using mean data in order to assess the genetic variability among populations as given by Cochran and Cox (1957). The broad sense heritability (h^2_{bs}) was estimated for all characters as the ratio of genotypic variance to the total of phenotypic variance as suggested by Lush (1949) and Hanson *et al*. (1956) and had categorized according to Robinson *et al*. (1966). Genetic advance had measured and categorized using the formula given by Johnson *et al.* (1955). Genotypic and phenotypic co-efficient of variation had been calculated by the formula of Burton (1952) and had categorized as suggested by Sivasubramanian and Madhamenon (1973).

Results and Discussion

Analysis of variance

Analysis of variance was performed for ten quantitative characters including yield and yield-attributing traits for selected *B. rapa* genotypes (Table 1) and observed that mean sum of squares due to genotypes were significant for all the studied traits at 1% level of significance, thus exhibiting the presence of considerable genetic variability except primary branches plant^{-1}. Singh *et al.,* (2013), Tripathi *et al.,* (2013) and Shekhawat *et al*., (2014) also reported considerable genetic variability for all these traits in their experiments, as mean sum of squares due to genotypes were significant. Abideen *et al.,* (2013) also studied non-significant differences in primary branches plant^{-1} among the genotypes. This might be due to environmental effects.

Mean performance

Significant variation in days to 50% flowering and 80% maturity had observed which ranged from 34.33 to 58.86 days and 79.33 to 108.00 days respectively (Table 2). Days to 50% flowering was maximum in G_7 (58.86 days) and the minimum in G_4 (34.33 days) while days to 80% maturity was maximum in G_7 (108.00 days) and the minimum in G_2 (79.33 days). The result matched with the findings of Karmokar (2018) and Ullah

(2018) who reported days to 50% flowering for different lines and varieties of *B. rapa* ranged from 33.00 to 57.33 days and 27.33 to 55.66 days respectively while days to 80% maturity ranged from 78.00 to 89.67 and 78.33 to 87.33 days respectively. Minimum days of 50% flowering indicated short durable population. Plant height ranged from 67.86 to 149.23 cm. The highest plant height was recorded in G_7 (149.23 cm) and lowest in G_4 (67.86 cm) (Table 2). The result differed from the findings of Karmokar (2018) and Ullah (2018) who reported that, the plant height for different lines and varieties of *B. rapa* ranged from 80.77 to 111.47 cm and 94.56 to 107.73 cm respectively. This might be due to environmental effects. The number of primary and secondary branches plant^{-1} ranged from 6.53 to 9.13 and 0.13 to 9.80 respectively (Table 2). The maximum primary branches plant^{-1} had recorded in G_6 (9.13) and the minimum was in G_5 (6.53) while the secondary branches plant^{-1} had found to be the maximum in G_4 (9.80) and the minimum was in G_6 (0.13). The more branches plant^{-1} indicating more siliqua that ultimately increased yield plant^{-1}.The result had supported by Karmokar (2018) who reported that number of primary and ranged from 59.48 to 124.29, 96.54 to 124.44 and 78.00 to 180.33 respectively. Number of seeds siliqua^{-1} ranged from 11.85 to 30.97 (Table 2). It was maximum in G_5 (30.97) followed by G_6 (24.11) which was statistically similar with G_1 (23.99) while the minimum number was in G_4 (11.85). Ali *et al.,* (2002) observed that the hybrid of *B. rapa* produced an excellent number of seeds siliqua^{-1} (25.06) while Karmokar (2018) and Ullah (2018) found that the seeds siliqua^{-1} for different lines and varieties of *B. rapa* ranged from 11.98 to 16.22 and 12.83 to 20.87 respectively. The result of the present study exceeded the range of this finding. Which might be due to the differences in size and shape of siliqua in different genotypes. The siliqua length ranged from 3.04 to 4.57 cm. The highest siliqua length had measured in G_2 (4.57 cm) followed by G_7 (4.14 cm) while the lowest in G_4 (3.04 cm) preceded by G_1 (3.18 cm) and G_5 (3.36 cm) (Table 2). secondary branches plant^{-1} for different lines and varieties of *B. rapa* ranged from 5.13 to 10.33 and 0.50 to 10.93 respectively but higher than the findings of Ullah (2018) who estimated that the range was between 5.67 to 4.12 and 1.45 to 2.27 respectively. This might be due to environmental effects. Number of siliqua plant^{-1} ranged from 90.64 to 246.53 (Table 2). It was maximum in G_4 (246.53) followed by G_2 (219.66) while the minimum number was in G_1 (90.64). The findings exceeded the range reported by Naznin *et al.,* (2015), Karmokar (2018) and Ullah (2018) who reported that the number of siliqua plant^{-1} for different lines and varieties of *B. rapa*.

Table 1. Analysis of variance for seed yield and seed yield attributing traits in *Brassica rapa* genotype

Source of variation	Df	Days to 50% flowering	Days to 80% maturity	Plant height (cm)	No. of primary branches plant^{-1}	No. of secondary branches plant^{-1}	No. of siliqua plant^{-1}	Siliqua length (cm)	No. of seed siliqua^{-1}	1000 Seed Weight (g)	Seed yield plant^{-1} (g)
Genotypes	6	235.11**	311.89**	2101.65**	2.24	44.13**	7726.9**	0.88**	101.46**	1.35**	2.96**
Replications	2	26.09	20.67	1.15	0.66	0.69	404.7	0.02	1.18	0.02	0.58
Error	12	0.09	0.08	10.24	1.02	1.24	124.2	0.01	0.91	0.08	0.03
CV (%)		0.66	0.32	3.05	13.69	28.06	6.54	1.97	4.41	7.20	3.09
LSD		0.52	0.51	5.70	1.80	1.98	19.83	0.13	1.70	0.50	0.29

Df = Degree of freedom, CV = Co-efficient of variation, LSD = Least Significant Difference, ** = Significant at 1%

Table 2. Mean performance for yield and yield contributing traits for seven genotypes of *Brassica rapa* L.

Sl. No.	Genotypes	Days to 50% flowering	Days to 80% maturity	Plant height (cm)	Number of primary branches plant^{-1}	Number of secondary branches plant^{-1}
1.	G_1	39.11 e	84.33 e	85.36 d	7.06 b	3.13 c
2.	G_2	34.33 g	79.33 g	109.73 b	7.26 b	7.66 b
3.	G_3	42.00 d	87.00 d	114.10 b	7.40 ab	0.46 d
4.	G_4	36.00 f	81.33 f	67.86 e	6.66 b	9.80 a
5.	G_5	53.33 b	98.33 b	97.33 c	6.53 b	3.73 c
6.	G_6	45.33 c	90.66 c	114.86 b	9.13 a	0.13 d
7.	G_7	58.86 a	108.00 a	149.23 a	7.66 ab	0.83 d
	Min.	34.33	79.33	67.86	6.53	0.13
	Max.	58.86	108.00	149.23	9.13	9.80
	Mean	44.13	89.85	104.78	7.39	3.96
	CV%	0.66	0.32	3.05	13.68	28.06
	LSD	0.51	0.50	5.69	1.79	1.97

G_1 (BARI Sarisha-14), G_2 (Brown Special), G_3 (Yellow Special), G_4 (Tori-7), G_5 (BARI Sarisha-17), G_6 (BARI Sarisha-15) and G_7 (BARI Sarisha-6). (Note: BARI: Bangladesh Agriculture Research Institute)

Sl. No.	Genotypes	Number of siliqua plant^{-1}	Siliqua length (cm)	Number of seeds siliqua^{-1}	1000 seed weight (g)	Seed yield plant^{-1} (g)
1.	G_1	90.64 e	3.18 e	23.99 b	3.33 ef	4.93 c
2.	G_2	219.66 b	4.57 a	19.06 d	3.52 de	5.22 c
3.	G_3	145.99 d	3.74 c	20.61 cd	4.55 ab	5.11 c
4.	G_4	246.53 a	3.04 f	11.85 e	2.86 f	4.12 d
5.	G_5	106.56 e	3.36 d	30.97 a	3.90 cd	6.09 b
6.	G_6	161.63 cd	3.73 c	24.11 b	4.10 bc	5.20 c
7.	G_7	172.73 c	4.14 b	20.80 c	4.75 a	6.91 a
	Min.	90.64	3.04	11.85	2.86	4.12
	Max.	246.53	4.57	30.97	4.75	6.91
	Mean	170.54	3.68	21.63	3.86	5.22
	CV%	6.53	1.96	4.40	7.20	3.08
	LSD	19.82	0.13	1.69	0.49	0.28

G_1 (BARI Sarisha-14), G_2 (Brown Special), G_3 (Yellow Special), G_4 (Tori-7), G_5 (BARI Sarisha-17), G_6 (BARI Sarisha-15) and G_7 (BARI Sarisha-6). (Note: BARI: Bangladesh Agriculture Research Institute)

Similar result had been observed by Karmokar (2018) and Ullah (2018) who reported that siliqua length for different lines and varieties of *B. rapa* ranged from 4.67 to

5.96 cm and 5.07 to 6.38 cm respectively. 1000 seed weight ranged from 2.86 to 4.75. The highest 1000 seed weight had recorded in G_7 (4.75 g) and the lowest in G_4 (2.86 g) while significant variation for seed yield plant^{-1} was observed among the genotypes and it ranged from 4.12 to 6.91 g (Table 2). The highest yield was recorded in G_7 (6.91 g) followed by G_5 (6.09 g) and the lowest yield was recorded in G_4 (4.12 g) preceded by G_1 (4.93 g). The higher 1000 seed weight indicated that the seeds are bigger and contained higher amount of oil. These result matched with the findings of Karmokar (2018) and Ullah (2018) who reported that 1000 seed weight for different lines and varieties of *B. rapa* ranged from 3.33 to 4.53 g and 2.50 to 3.63 g respectively and for seed yield plant^{-1} it ranged from 3.53 to 7.31 g and 5.65 to 7.48 g respectively.

Variability

For days to 50% flowering and days to 80% maturity phenotypic variance (78.42 and 104.02 respectively) were higher than the genotypic variance (78.34 and 103.94 respectively). High values of phenotypic coefficient of variation (PCV) (20.12 %) and genotypic coefficient of variation (GCV) (20.11 %) had observed for days to 50 % flowering while 80 % maturity exhibited moderate GCV and PCV of 11.34 and 11.35 % respectively (Table 3). The difference between genotypic and phenotypic variances and PVC and GVC were relatively low for these traits. Hussain *et al.,* (2014) and Rout *et al.,*(2019) also found similar result that indicated the less influence of environment on the expression of the characters. The high value for PVC and GVC had estimated here but Sikarwar *et al.,* 2017; found low PCV and GCV that may be due to environmental factors. For plant height, Genotypic and phenotypic variance were 697.14 and 707.37 respectively with large environmental influence (10.23). The PCV and GCV also had the higher values, 25.38 and 25.19 % respectively (Table 3). Iqbal *et al.,* (2015), Naznin *et al.,* (2015) and Aktar *et al*., (2019) also found the similar results. High PCV and GCV values for this traits had supported by Gupta *et al.,* (2019), indicated the existence of inherent variability among the studied genotypes for this trait. The genotypic and phenotypic variance were recorded as 0.40 and 1.43 respectively along with low GCV (8.64 %) and moderate PCV (16.18 %) for of primary branches plant^{-1} while for secondary branches plant^{-1}, the genotypic and phenotypic variance were recorded as 14.30 and 15.53 respectively along with very high GCV (95.35 %) and PCV (99.39 %) (Table 3). Relatively low differences between genotypic and phenotypic variance for number of primary branches plant^{-1} indicated less environmental influences, this result matched with Hussain *et al.,* 2014; and Naznin *et al.,* 2015; Iqbal *et al.,* 2015; and Rout *et al.,* 2019; also estimated low GCV and moderate PCV but Sikarwar *et al.,* 2017; and Gupta *et al.,* 2019; reported high GCV and PCV for this trait. For number of siliqua plant^{-1}, phenotypic variance (2658.44) was higher than genotypic variance (2534.23) (Table 3).

Table 3. Genetic variance estimation for ten yield and yield contributing traits of *Brassica rapa L. genotype*

Traits	Phenotypic Variance (σ^2p)	Genotypic Variance (σ^2g)	Environ-mental Variance (σ^2e)	Phenotypic coefficient of variation (PCV) (%)	Genotypic coefficient of variation (GCV) (%)
Days to 50% flowering	78.42	78.34	0.08	20.12	20.11
Days to 80% maturity	104.02	103.94	0.08	11.35	11.34
Plant height (cm)	707.37	697.14	10.23	25.38	25.19
Number of primary branches plant^{-1}	1.43	0.40	1.03	16.18	8.64
Number of secondary branches plant^{-1}	15.53	14.30	1.23	99.39	95.35
Number of Siliqua plant^{-1}	2658.44	2534.23	124.21	30.23	29.52
Length of siliqua (cm)	0.297	0.292	0.005	14.80	14.67
Number of seeds siliqua^{-1}	34.42	33.52	0.9	27.12	26.76
1000 seeds weight (g)	0.50	0.42	0.08	18.36	16.89
Seed yield plant^{-1} (g)	1.004	0.978	0.026	19.17	18.92

High genotypic variance indicates the better transmissibility of the character from parent to their offspring. Higher value of PCV (30.23%) and GCV (29.52%) had also estimated for this trait indicated the existence of inherent variability among the studied genotypes but differences between phenotypic and genotypic variance was relatively higher (124.21) indicated influence of environment (Table 3). The result matched with Aktar *et al*., (2019), Gupta *et al.,* (2019) and Rout *et al.,* (2019). The genotypic variance (34.42), phenotypic variance (33.52), environmental variance (0.90), high GCV (26.76) and high PCV (27.12) had estimated for number of seeds siliqua^{-1} (Table 3). Very low environmental influences and high GCV, PCV indicated presence of additive gene effects and hence the selection might be effective for this trait. Sikarwar *et al.,* (2017), Aktar *et al*., (2019) and Rout *et al.,* (2019) also estimated similar result for this trait. The genotypic and phenotypic variance for siliqua length was 0.297 and 0.292 respectively with environmental variance 0.005 (Table 3). Very low difference between genotypic and phenotypic variance indicated very low environmental influences and the preponderance of additive gene effects, hence, the selection, based on these traits might be effective. Naznin *et al.,* (2015) and Rout *et al.,* (2019) also found least difference between phenotypic and genotypic variances for this trait. Moderate GCV (14.67%) and PCV (14.80 %) values were estimated for this trait (Table 3). Salam *et al.,* (2017) also reported moderate GCV and PCV values for this trait. Very low genotypic, phenotypic and environmental variance (0.42, 0.50 and 0.08 respectively), moderate GCV and PVC (16.89 % and 18.36 % respectively) had observed for 1000 seed weight (Table 3). Yield

plant^{-1} also exhibited low genotypic, phenotypic and environmental variance (1.00, 0.98 and 0.02 respectively) with moderate estimates of PCV (19.17%), low GCV (18.92%) (Table 3). Low environmental influences focused on additive gene effects. Aktar *et al*. (2019) and Rout *et al.*, 2019; also reported less environmental influences for these traits.

Heritability and genetic advance

Days to 50% flowering and 80% maturity showed high heritability (99.89 % and 99.92 % respectively) with high genetic advance (20.22 % and 20.99 % respectively) and high genetic gain (41.40 % and 23.36 % respectively) (Fig. 1). High heritability and high genetic advance and gain indicating that this trait was under additive gene control and selection for genetic improvement for these traits would be effective. Sikarwar *et al.*, (2017), Salam *et al.*, (2017), Singh *et al.*, (2018), Aktar *et al.*, (2019) and Gupta *et al.*, (2019) also observed similar result. High heritability (98.55%) coupled with high genetic advance (53.99 %) and genetic gain (51.53 %) had estimated for this trait (Fig. 1). High heritability coupled with high genetic advance and high genetic gain implied that this trait had governed by additive gene action and selection might be effective for further genetic improvement of this trait. Similar result had observed by Bibi *et al.*, (2016), Salam *et al.*, (2017), Singh *et al.*, (2018) and Gupta *et al.*, (2019) for plant height. Low heritability 28.49 % with low genetic advance (0.70%) and low genetic gain 9.50% had estimated for number of primary branches plant^{-1} that indicated non-additive gene effects and selection might be ineffective for this trait. The result agreed with Mekonnen *et al*., (2014) but did not matched with Naznin *et al.*, (2015), Sikarwar *et al.*, (2017) and Rout *et al.*, (2019) who studied high heritability with high genetic advance for number of primary branches plant^{-1}. While for number of secondary branches plant^{-1}, high heritability 92.02 % with low genetic advance (7.47%) and very high genetic gain 188.43% were estimated (Fig.1).

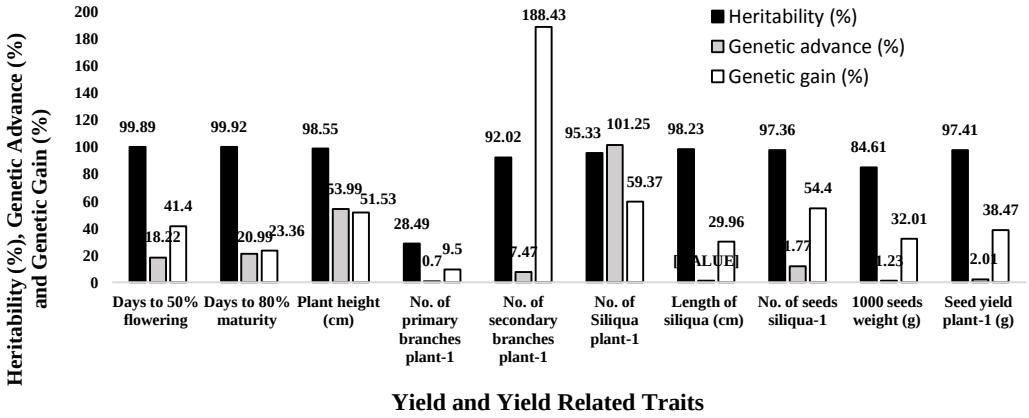

Fig. 1. Heritability, genetic advance and genetic gain for yield and yield related traits of *B. rapa* genotype

Which reflected non-additive gene action, as high estimates of heritability and low genetic advance had found. The result agreed with Khan *et al*.(2013) but did not matched with Sikarwar *et al.* (2017), Singh *et al.* (2018), Gupta *et al*.(2019) and Rout *et al*.(2019) who studied high heritability with high genetic advance for number of secondary branches plant^{-1}.Therefore, selection might be ineffective for this trait. High heritability (95.33%) with very high genetic advance (101.25%) and high genetic gain (59.37%) had estimated for number of siliqua plant^{-1} (Fig. 1). Which indicated selection for this trait could be useful for future breeding program due to prevalence of additive gene action. The result of the present study supported by Sikarwar *et al.*, 2017; Rauf and Rahim (2018), Aktar *et al*., 2019; Gupta *et al.*, 2019; and Rout *et al.*, 2019). High heritability (97.36%), moderate genetic advance (11.77%) and high genetic gain (54.40%) had recorded for number of seeds siliqua^{-1} indicated presence of additive gene effects and hence the selection might be effective for seeds siliqua^{-1}. Sikarwar *et al.*, 2017; Aktar *et al*., 2019; Gupta *et al.*, 2019; and Rout *et al.*,2019; also estimated similar result for this trait. While high heritability (98.23%) with low genetic advance (1.10%) and high genetic gain (29.96%) had observed for siliqua length (Fig. 1).High heritability estimates with high genetic gain speculate the presence of additive gene effects but very low genetic advance with high heritability indicated that high heritability occurs due to environmental effects, so, selection for genetic improvement of this trait would be ineffective. Khan *et al.*, 2013; also found similar results for siliqua length but Bibi *et al.*, (2016), Salam *et al.*, 2017; Sikarwar *et al.*, 2017; and Singh *et al.*, 2017; reported high heritability estimates with high genetic gain for this trait. High heritability (84.61%), low genetic advance (1.23%) and high genetic gain (32.01%) had observed for 1000 seed weight while seed yield plant^{-1} also exhibited high heritability (97.40%), low genetic advance (2.01) and high genetic gain (38.47%) (Fig.1). High heritability with high genetic gain focused on additive gene effects but very low genetic advance with high heritability indicated that high heritability occurs due to environmental effects, so, selection for genetic improvement of these traits would be ineffective for future breeding program. The result of the present study matched with Khan *et al.*, 2013; while Yared and Misteru (2016), Salam *et al.*, 2017; Singh *et al.*, 2018; Aktar *et al*., 2019; and Rout *et al.*, 2019; reported high heritability with high genetic advance for these traits.

Conclusion

Significant variations were observed among the genotypes for all the studied traits. All the characters except plant height, number of siliqua plant^{-1} showed least difference between genotypic and phenotypic variances, which indicated low environmental influence on those characters. Hence, selection will be beneficial for those traits. The high genotypic and phenotypic coefficient of variation were observed for number of secondary branches plant^{-1}, number of siliqua plant^{-1}, plant height and number of seeds siliqua^{-1} indicated these characters could be improved by phenotypic selection. High heritability coupled with high genetic advance and genetic gain had observed for days to 80% maturity, plant height and number of siliqua plant^{-1} indicated selection for these traits could be useful for future breeding program due to prevalence of additive gene action. While high heritability coupled with low genetic advance and high genetic gain were observed for number of primary branches plant^{-1}, number of secondary

branches plant^{-1}, length of siliqua, 1000 seed weight and seed yield plant^{-1} indicated that, high heritability occurs due to environmental effects, so, selection for genetic improvement of these traits would be ineffective. However, high heritability coupled with moderate genetic advance and high genetic gain were estimated for days to 50% flowering and number of seeds siliqua^{-1} indicated medium possibility of selection.

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Conflicts of Interest

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

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