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COMPARATIVE STUDY ON THE QUALITY CHARACTERS OF WINTER ONION (*Allium cepa* L.) VARIETIES

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Abstract

The experiment was conducted at Spices Research Sub-Centre (SRSC) of Bangladesh Agricultural Research Institute (BARI), Faridpur, Bangladesh during 2021-22 to compare the quality characters of four existing winter onion varieties such as BARI Piaz-1, BARI Piaz-4, BARI Piaz-6 and Lal Teer King. The field trial was laid out in Randomized Complete Block Design (RCBD) and storage experiment in Completely Randomized Design (RCD). The results of the experiment revealed that the quality parameters studied significant of varied among the varieties except for the days to maturity of bulb.The BARI Piaz-4 given significantly the highest dry yield (3.27t/ha) and the minimum bolting (11.5%) over other three varieties. The BARI Piaz-1 exhibited superior performances in result of dry matter, TSS, bulb firmness, pungency, storage loss and quantity and quality (texture/color) of Beresta (caramelized onions). The BARI Piaz-6 performed in respect of incidence of split bulb and pungency. Lal Teer King showed the highest disease incidence. The BARI Piaz-1, BARI Piaz-6, BARI Piaz-4 and Lal Teer King expressed bronze red, followed by bronze red, pink red and light red skin color, respectively. The flesh color of bulb was reddish in BARI Piaz-4 and Lal Teer King. But BARI Piaz-1 and BARI Piaz-6 white flesh color. Only BARI Piaz-4 had the torpedo shape while other three varieties produced flat shaped bulbs. The BARI Piaz-1, BARI Piaz-6, Lal Teer King gave highly crispy, crispy, light crispy and soft Beresta, respectively. The excellent (richly brown) and good (brown) color of Beresta were observed in BARI Piaz-1 and BARI Piaz-6, respectively. However, the color of Beresta for BARI Piaz-4 and Lal Teer King was very poor (darkish). This present findings suggest that among the varieties, BARI Piaz-1 stands out due to its exceptional quality and beresta characteristics, although it demonstrated the lowest yield. BARI Piaz-4, on the other hand, excels in terms of dry weight and displays minimal bolting tendencies. For those seeking pungency and reduced splitting, BARI Piaz-6 is the preferred choice. Lastly, when considering disease incidence, Lal Teer King demonstrates superior resistance.

Keywords: BARI Piaz, Bulb weight, Dry weight, Storage loss, Winter Onion

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Introduction

In Bangladesh, the Spices Research Centre, BARI, Bogura has developed so far three winter onion varieties viz. BARI Piaz-1, BARI Piaz-4 and BARI Piaz-6. Lal Teer Seed Limited, Bangladesh has also developed a variety namely Lal Teer King which is being grown by the farmers of the country. The quality parameters of a variety are very important aspects for sustaining the variety. Varietal quality depends on different factors such as neck size, individual bulb size, dry matter and total soluble solids (TSS) of bulb, percent of bolting and splitting bulb, bulb firmness, days to maturity, pungency, shape and color of bulb, incidence of insect pest and diseases, bulb dry yield, shelf life of bulbs. So far, no study has been done in the country to compare the quality characteristics of the aforesaid varieties. So, a comparative study on the potential of quality for the aforementioned varieties would be very useful.

The present research work was, therefore, carried out to compare the quality characteristics of four existing varieties such as BARI Piaz-1, BARI Piaz-4, BARI Piaz-6 and Lal Teer King.

Materials and Methods

A field cum storage trial was designed at Spices Research Sub-Centre, BARI, Faridpur, during 2021-22 to compare quality characters for four existing winter onion varieties BARI Piaz-1, BARI Piaz-4, BARI Piaz-6 and Lal Teer King. The field trial was conducted in a Randomized Complete Block Design (RCBD) with four replications. Onion seeds were sown on 14 November 2021. The 42-day-old uniform and healthy seedlings were transplanted on 26 December 2021 in the trial plots with a spacing of 15 cm x 10 cm. The unit plot size was 4 m x 2 m. The experimental field was fertilized with 5000 kg well-decomposed cowdung, 120 kg N, 50 kg P, 85 kg K and 40 kg S per hectare. Nitrogen, phosphate, potash and sulphur were supplied in the form of Urea, TSP, MP and Gypsum, respectively. The entire quantity of cowdung, P, K, S and the one third of N was applied as basal dose during final land preparation. The remaining N was used as top dressed in two equal splits at 20 and 30 days after transplanting. The fungicide mancozeb/iprodione @ 3g/L of water was sprayed at fortnightly interval commencing from one month after transplanting of seedlings. All other recommended management practices were followed for each variety. The data were recorded on neck size (cm), bolting (%), split bulbs (%), individual bulb weight (g), days to maturity (days), bulb dry matter (%), TSS (°brix), incidence of diseases (0-5 scale), dry yield per hectare (t), shape index and shape of bulbs, bulb firmness, skin and flesh color of bulbs, pungency, shelf-life of bulb and percent, texture and color of Beresta (caramelized onion/crispy fried onion). Ten plants were randomly selected from each plot for data recording and averaging. Bulbs were harvested at maturity when the pseudostem became flaccid and unable to support the leaf blades (Brewster, 1990). Bulbs of four varieties were harvested over several days as per maturity symptoms. Days to maturity were recorded considering the days between the transplanting of seedlings and harvesting of bulbs. The leaves of harvested onion were removed five days after curing by cutting 2.0-2.5cm above the bulb. After curing, the total bulb fresh weight was measured for each plot. The number of split bulbs was visually counted in each plot, recorded and expressed in percent in relation to the total number of bulbs per plot. The percent dry matter content of bulbs was calculated on a dry weight basis as per procedure of Walle *et al*. (2018). TSS content of bulbs was recorded by hand refractometer (ATAGO, Master-53M, Japan) with a range of 0-53 °brix. The purple blotch/stemphylium leaf blight severity of onion was scored a 0-5 scale, as described by Sharma (1986). The details of scales are as follows: 0-no disease symptoms, 1-a few spots towards the tip covering 10% of the leaf area, 2-several dark purplish brown patches covering up to 20% leaf area, 3-several patches with paler outer zone covering up to 40% leaf area, 4-leaf streaks covering up to 75% leaf area or breaking of the leaves from centre and 5-complete drying of the leaves or breaking of the leaves from the centre. Observations were made from the first appearance of disease symptoms on leaves; untill the harvest at weekly intervals.

According to Dowker and Fennell (1974), the shape index (SI) of a lightbulb is the ratio of its height (polar diameter) to its equatorial diameter, or polar diameter/ equatorial diameter.Polar diameter is the distance between the onion crown and the point of root attachment to the onion. Equatorial diameter is the maximum width of the onion in a plane perpendicular to the polar diameter. Henceforth shape of the bulb was assessed by using the bulb shape index. Where a shape index smaller than 1 (**<** 1) indicates flat; a shape index equal to 1 indicates globular and a shape index greater than 1 (**>** 1) indicates torpedo (Dowker and Fennell, 1974). Bulb firmness rating was measured in the subjective method by squeezing the bulbs at different points with the hand of testers repeatedly (Larsen and Cramer, 2004). Before measuring bulb firmness, the dry scales of the bulb were removed. Firmness was rated on a scale from 1 to 9, with 1 being the softest or one that gave the the least resistance to squeezing and 9 being the hardest, most firm bulb (Larsen and Cramer, 2004). The Skin and flesh color of bulbs were identified by visual assessment method. The Pungency of onion was evaluated with sensory/flavor perception (organoleptic taste) by a taste panel (Wall and Corgan, 1992). A rating scale for pungency was used, where 1 = extremely mild, 2 = mild, 3 = slightly pungent, 4 = pungent and 5 = extremely pungent. The panel was instructed to taste all of the scales (inner, middle and outer) for each sample and to clear their palates with water and apples between samples. The taste test was conducted over three days with three replications. The Beresta (caramelized onions) was made by slowly cooking the sliced onion in little oil until they were richly browned. The percent of Beresta content was calculated following bulb dry matter estimation procedure. The healthy, sound and uniform bulbs were selected to testing the shelf life of onion bulbs. Twenty-five-kilogram bulbs from each variety were taken for the study. The storage trial was conducted under a completely randomized design with three replications. The onion bulbs were stored in ambient storage for 6 months from 15 April to 15 October- 2022. The total storage loss (%) was calculated from the sum of the sprouting loss (%), physiological loss in weight (%) and rotting loss (%). The recorded data were analyzed statistically as suggested by Gomez and Gomez (1984) and the means were compared by least significant difference (LSD).

Results and Discussion

Quality characters

Neck size, weight, splitting, maturity of bulb, bolting and disease

Quality characteristics such as neck size, individual bulb weight, bolting, splitting and diseases were significantly influenced by varieties except days to maturity (Table 1). The thickest neck diameter (1.31 cm) was observed in BARI Piaz-6 followed by BARI Piaz-4 (1.25 cm) and Lal Teer King (1.23 cm). However, the thinnest (1.18 cm) was attained in BARI Piaz-1. The variation in neck size among the varieties might be attributed to their genetic factor.

Table 1. Quality characters (neck size, bulb weight, bolting, splitting, disease, maturity) as influenced by four varieties of BARI, Faridpur, during 2021-22

Variety	Neck size (cm)	Individual bulb weight (g)	Bolting (%)	Splitting (%)	Disease rating (0-5 scale)	Days to maturity (days)
BARI Piaz-1	1.18	21.68	19.45	15.44	2.89	110.68
BARI Piaz-4	1.25	34.06	11.51	8.42	2.41	114.04
BARI Piaz-6	1.31	26.81	12.21	7.76	2.65	111.55
LalTeer King	1.23	31.16	11.84	8.99	2.12	112.99
CV (%)	9.28	8.61	11.10	22.49	17.31	7.98
LSD (0.05)	0.08	4.34	2.44	1.54	1.03	-
Level of sig.	*	**	**	**	*	NS

** Significant at 1% level of probability, * Significant at 5% level of probability and NS-Non significant

The present finding is in accordance with that of Sirajo and Namo (2019). Neck thickness is believed to influence the storability of onion with better storability from a thinner neck size (Sirajo and Namo, 2019). The heaviest individual bulb (34.1g) was noted from BARI Piaz-4 insignificantly followed by Lal Teer King (31.2g) and significantly followed by BARI Piaz-6 (26.9 g). The lightest bulb (21.7g) was observed in BARI Piaz-1. The difference in bulb weight could be attributed due to the genetic potential of the varieties. The outcome of the current study confirms the previous findings of Sirajo and Namo (2019). The highest bolting (19.5 %) was recorded from BARI Piaz-1 significantly followed by BARI Piaz-6 (12.2%) and Lal Teer King (11.8%). The lowest bolting (2.36%) resulted from BARI Piaz-4. Abu-Rayyan and Abu-Irmaileh (2004) reported that onion required cool weather during inflorescence initiation and seed stalk development. All varieties studied were grown in the same environment. So, this difference in bolting percent could be due to hereditary causes among the varieties. This result corroborates the earlier finding of Lancaster *et al*. (1995). The maximum split bulb (15.4%) was recorded from BARI Piaz-1 significantly followed by BARI Piaz-4 (8.4%). However, the minimum split bulb was counted from BARI Piaz-6 (7.76%) which was statistically similar to that of Lal Teer King (8.99%). The variation in multiplier bulbs was mainly attributed to the genetic variation in varieties. Similar claims were also made by Arya *et al*. (2017). The score of disease incidence ranged from 2.12 to 2.89 with eye estimation. The maximum disease incidence score was observed in BARI Piaz-1 (2.89) consistently followed by BARI Piaz-6 (2.65) and BARI Piaz-4 (2.41). The minimum incidence rating was exhibited in Lal Teer King (2.12). The present evaluation revealed that no variety was recorded in the disease severity categories 0, 1, 4 and 5 under a 0-5 scale (Sharma, 1986). The apparent cause of the variation in disease severity among the varieties might have been due to their genetic potential. The days to maturity ranged from 110.7 to 114.1 days from transplanting to bulb harvest. The variety BARI Piaz-4 took a maximum of days to maturity (114.1 days). The minimum days to mature (110.7 days) was required for BARI Piaz-1. Walle *et al*. (2018) found significant differences in the days to maturity of bulbs among the varieties.

Quality characters

Dry matter, TSS, firmness, pungency, dry yield and storage loss

Considerable variations were observed among the varieties in quality characteristics of dry matter, TSS of bulb, bulb firmness, pungency, dry yield and storage loss of bulb (Table 2). The maximum dry matter (DM) content (19.72%) was obtained from BARI Piaz-1 significantly followed by BARI Piaz-6 (16.70%) and BARI Piaz-4 (16.18%). However, the minimum DM content was noted in Lal Teer King (15.70%). The variation in DM content might be due to characteristic derived genetically from an ancestor. These results agree with those of Arya *et al*. (2017) who reported that the varieties significantly influenced the percent DM of bulbs. The topmost TSS content (19.25 °brix) was obtained in BARI Piaz-1 consistently followed by BARI Piaz-4 (15.08 °brix). The least TSS content (14.58 °brix) was observed from Lal Teer King. The variation in TSS content among the varieties might have been due to their inherent characteristics. Similar variability in TSS content among the varieties was also registered by Arya *et al*. (2017). The variety BARI Piaz-1 exhibited firmer bulbs (9.00) than those of Lal Teer King (8.50) and BARI Piaz-6 (8.63). Moreover, the lowest firmness rating (8.38) was observed in BARI Piaz-4. The firmness score of BARI Piaz-4 was mutually identical to those of BARI Piaz-6 and Lal Teer King. The probable cause of firmness variation might be due to differences in genetic nature among the varieties studied. The Flavor perception score for pungency ranged from 4.60 to 5.00. The strongest pungency (5.00) was recorded in BARI Piaz-1 and BARI Piaz-6 insignificantly followed by Lal Teer King (4.62) and BARI Piaz-4 (4.60). The variation in the pungency of onions among the varieties might be due to their genetic potential. Wall and Corgan (1992) found wide variation in pungency among the varieties. The variety BARI Piaz-4 produced the maximum dry yield of 3.27t/ha which was statistically superior over the remaining three varieties-Lal Teer King (2.84t/ha), BARI Piaz-1 (2.29 t/ha) and BARI

Piaz-6 (2.24 t/ha). The superior performance of BARI Piaz-4 over the remaining varieties was due to its highest fresh yield of onion bulb. In spite of the highest dry matter, BARI Piaz-1 gave the lowest dry yield due to its lowest fresh yield of onion bulb. Walle *et al*. (2018) found variation among the varieties on dry yield. The BARI Piaz-1 had the lowest storage loss (41.81%) insignificantly followed by BARI Piaz-6 (45.12%). The maximum storage loss was observed in the Lal Teer King (48.11%), followed by BARI Piaz-4 (47.05%). The lowest storage loss from BARI Piaz-1 might be due to higher dry matter, TSS, bulb firmness and pungency in BARI Piaz-1. The DM content is also believed to influence the long storage period of onion (Mahanthesh *et al*., 2008). Rabbani *et a1*. (1986) obtained significant variation among the varieties on storage loss.

Table 2. Quality characters (dry matter, TSS of bulb, bulb firmness, pungency, dry yield, storage loss) of four varieties of onion

Variety	Bulb dry matter (%)	TSS of bulb (°brix)	Bulb firmness (1-9 scale)	Pungency (0-5 scale)	Bulb dry yield (t/ha)	Storage loss (%)
BARI Piaz-1	19.7	19.3	9.00	5.00	2.29	41.8
BARI Piaz-4	16.2	15.1	8.38	4.60	3.27	47.1
BARI Piaz-6	16.7	15.0	8.63	5.00	2.24	45.1
LalTeer King	15.7	14.6	8.50	4.62	2.84	48.1
CV (%)	9.2	6.5	3.05	14.23	8.02	4.7
LSD (0.05)	2.5	1.7	0.42	0.28	0.41	4.9
Level of sig.	*	**	*	**	**	*

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

Shape of bulb

A marked variation was recorded in the shape index and shape of bulb among varieties (Table 3). The highest shape index (1.12) was attained in the BARI Piaz-4 significantly followed by BARI Piaz-6 (0.92) and Lal Teer King (0.91). The BARI Piaz-1 showed the lowest shape index (0.81). The shape index might be genetically attributed to the varieties. These results are in harmony with the findings of Sirajo and Namo (2019) and Arya *et al*. (2017) as they recorded a considerable variation in the bulb shape index of onion varieties. The recorded data exhibited that only BARI Piaz-6 had the torpedo shape (Fig.1b). However, BARI Piaz-1 (Fig. 1a), BARI Piaz-6 (Fig. 1c) and Lal Teer King (Fig. 1d) showed the similar shapes like flat. A shape index smaller than 1 ($<$ 1) indicates flat; a shape index equal to 1 indicates globular and a shape index greater than 1 ($>$ 1) indicates torpedo (Dowker and Fennell, 1974).

Table 3. Shape index value and shape of bulb of varieties of onion

Variety	Shape of bulb		
	Index values	Types of index	Shape
BARI Piaz-1	0.81	< 1	Flat shape
BARI Piaz-4	1.12	> 1	Torpedo shape
BARI Piaz-6	0.92	< 1	Flat shape
Lal Teer King	0.91	< 1	Flat shape
CV (%)	12.51	-	-
LSD (0.05)	0.12	-	-
Level of sig.	**	-	-

** Significant at 1% level of probability

Bulb color and beresta

There was a variation among the varieties of color of a bulb and Beresta (caramelized onion) shown in Table 4. The variety BARI Piaz-1, BARI Piaz-4, BARI Piaz-6 and Lal Teer King expressed bronze-red (Fig.1a), pink-red (Fig. 1b), next to bronze-red (Fig. 1c) and light-red skin color (Fig. 1d), respectively. The flesh color of bulb was reddish in BARI Piaz-4 (Fig. 1f) and Lal Teer King (Fig. 1h). But BARI Piaz-1 (Fig. 1e) and BARI Piaz-6 (Fig. 1g) demonstrated white flesh color in the bulb. The recorded variation in color of the bulb among the varieties might be due to their differences in genetic makeup. Similarly, Ratan *et al*. (2017) and Lancaster *et al*. (1995) also published significant variation among the varieties for the color of bulbs.

Table 4. Bulb color and Beresta quality of four varieties of onion

Variety	Color of bulb		Beresta (caramelized onion)		
	Skin	Flesh	Quantity (%)	Texture	Color
BARI Piaz-1	Bronze red	White	38.40	Highly crispy	Excellent
BARI Piaz-4	Pink red	Reddish	29.87	Soft	Darkish
BARI Piaz-6	Next to bronze red	White	32.31	Crispy	Good
Lal Teer King	Light red	Reddish	31.88	Light crispy	Darkish
CV (%)	-	-	3.95	-	-
LSD (0.05)	-	-	2.09	-	-
Level of sig.	-	-	**		-

** Significant at 1% level of probability

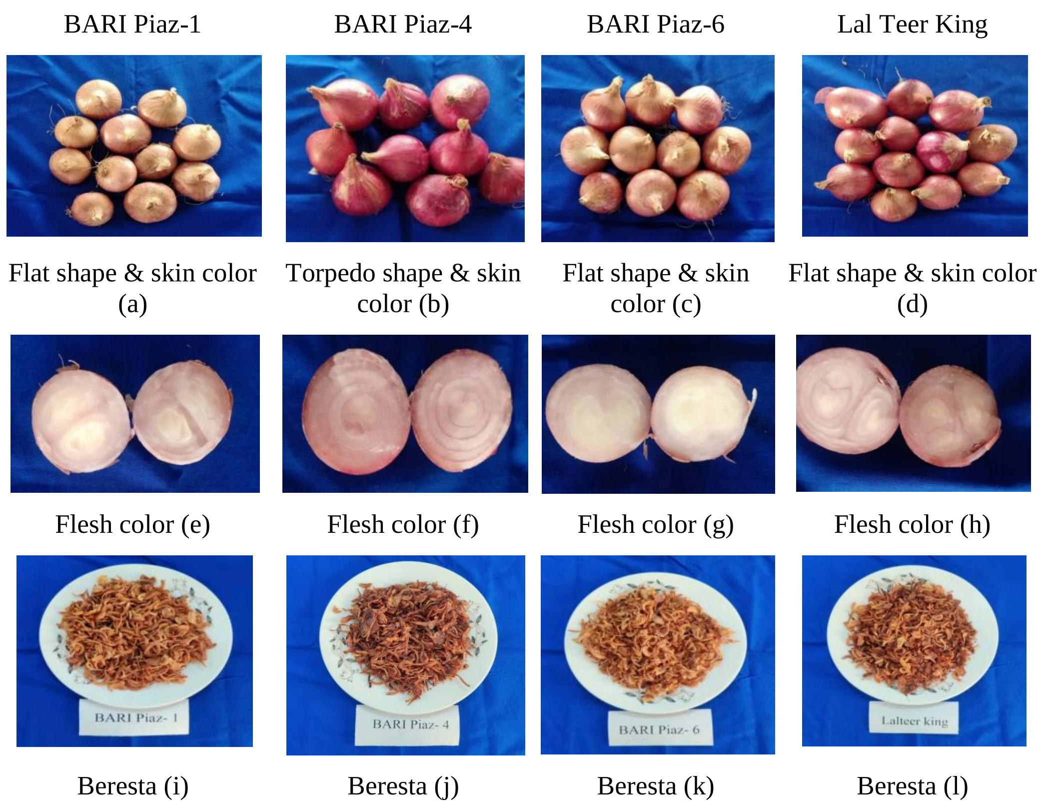

Fig. 1 (a-l)

The highest amount of Beresta (38.4%) was made from BARI Piaz-1 significantly followed by BARI Piaz-6 (32.3%) and Lal Teer King (31.88%). Nonetheless, the lowest percent of Beresta was recorded from BARI Piaz-4 (29.87%). In respect of texture, BARI Piaz-1, BARI Piaz-6, Lal Teer King gave highly crispy, crispy, light crispy and soft beresta, respectively. The excellent (richly brown) and good (brown) colors of Beresta were observed in BARI Piaz-1 (Fig. 1i) and BARI Piaz-6 (Fig.1k), respectively. However, the colors of Beresta for BARI Piaz-4 (Fig.1j) and Lal Teer King (Fig.1l) were very poor (darkish). The darkish color of Beresta in BARI Piaz-4 and Lal Teer King might be due to their reddish flesh color. The variation for the quantity, texture and color of Beresta might be due to varietal characters.

Conclusion

The findings of this study lead to the conclusion that among the varieties examined, BARI Piaz-1 exhibit superior quality and Beresta characteristics, but its yield was low. On the other hand, BARI Piaz-4 demonstrates remarkable dry weight and notable resistance to bolting. In terms of pungency and reduced splitting tendency, BARI Piaz-6 appeared as the top-performing variety. Lastly, when considering disease incidence, Lal Teer King stands out for its exceptional resistance.

Conflicts of Interest

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

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EFFECTS OF SOWING DATE AND CUTTING MANAGEMENT ON THE GRAIN AND FODDER YIELD OF BARLEY

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Abstract

Cereal crops need to be grown for dual purpose to overcome continuous food and feed shortage. Sowing date and cutting management are important to obtain the balanced fodder and grain simultaneously. To address the issue, field experiment was conducted at the agronomy field of Sher-e-Bangla Agricultural University (SAU), Dhaka to find out the effect of optimum sowing date and cutting management on the plant growth, green fodder and grain yield of barley. Five sowing times viz., 30 October, 15 November, 30 November, 15 December and 30 December and four cutting management *viz*., uncut and cutting at Zadoks growth stages, ZGS 19, ZGS29 and ZGS31 were used. Results of the present study revealed that early sowing time, 30 October gave significantly higher plant height, dry matter accumulation, effective tiller, fertile spikelet, spike length, 1000-grain weight, grain yield, straw yield, biological yield and green fodder yield under both uncut (control) and cut conditions. Cutting of barley for green fodder had reduced significantly all growth parameters, yield attributes and yield compared to uncut barley. Among cutting schedules, minimum and maximum reduction in growth parameters, yield attributes and yield were recorded with cutting of fodder at ZGS19 (vegetative growth stage) and ZGS31 (stem elongation stage), respectively compared to uncut. However, cutting of barley for fodder purpose at ZGS29 (tillering stage) was found in a balance between green fodder and grain yield simultaneously. Therefore, the study suggested that early sowing (30 October) can compensate the reduction in barley yield due to cutting for fodder purpose and cutting at maximum tillering stage (ZGS29) can balance having optimum grain and green fodder simultaneously.

Keywords: Barley, Biological yield, Growth parameters, Fodder yield

Introduction

Barley (*Hordeum vulgare* L.) is the world first ranked grain crop in terms of cultivated area, production and productivity. In many countries like USA, New Zealand and Australia, the crop is cultivated for both grain purpose and fodder production. However, in Bangladesh, this crop is cultivated only for grain purpose and the practice for grain and fodder not yet exploited. The production area and productivity of barley in Bangladesh is 0.45 M ha, 1.38 M t and 3.04 t ha^{-1}, respectively (BBS, 2023). Barley is a good source of high-quality forage, containing high amount of nutrients, protein and

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energy when other fodder plant species are low in quantity and quality (Kumar *et al*., 2017; Singh *et al*., 2017).

Time of seed sowing is one of the essential agronomic practices affecting both fodder and grain productivity in barley. Early and optimum seed sowing time has extended growth duration, which subsequently provides an opportunity to accumulate more dry matter as compared to late seed sowing, and henceforward demonstrated in higher grain, biological yield and harvest index. Previous studies suggested that early seed sowing helps increase both grain and fodder yield in dual-purpose system (Alam *et al*., 2005; Sharma, 2007). On the other hand, late seed sowing reduces the time of growth and development phases, which consequently decreases photosynthetic assimilates and source-sink relationship (Alam *et al*., 2007). In addition, late seed sowing may be exposed plants to heat stress during the grain filling stage and hence harmfully affect yield contributing traits, grain yield and biological yield. The adverse impacts of late sowing were significant in dual-purpose system and negatively affected fodder and crude protein Previous research findings suggested that plant growth, biomass, yield traits, and grain yield were higher under early sowing than later sowing (Hameed *et al*., 2003; Singh *et al*., 2019; Mani *et al*., 2009). Deleterious effect of late sowing was reported barley growth, biomass and grain yield and its components (Royo *et al*., 1997; Singh *et al*., 2013; Fayed *et al*., 2015; Farooq *et al*., 2016, Tahir *et al*., 2019). Similarly, fodder and crude protein yields inclined to be progressively declined due to delaying of seed sowing from 25 October to 14 November or 4 December (Moustafa *et al*., 2021). Early seed sowing in late October revealed the highest forage yield and crude protein content related with late sowing date of early December. Singh *et al*. (2013) and Choudhary and Chaplot (2015) reported that seed sowing at the end of October or commencement of November lead to higher nitrogen accumulation and protein content in green fodder, grain and straw compare to late sowing of barley. Moreover, Salama (2019) stated that cutting barley at early growth stages (45 and 55 days after sowing, DAS) increased the fodder yield and crude protein content compared to late cutting at 65 DAS.

On the other hand, cutting height may reduce yield components and grain yield in barley due to the limitation of leaf area and tiller senescence during reproduction stage if barley is not managed appropriately (Waheddullah *et al*., 2018). The regular vegetative growth is obligatory after cutting to produce rational yield, so optimum seed sowing time and management of cutting schedule is essential to appreciate the optimum green fodder and grains yield of barley. Keeping concern about the above points, the present research was conducted to identify the optimum sowing time and cutting schedule of barley for dual purpose cropping system.

Materials and Methods

The study was conducted at the Agronomy field, Sher-e-Bangla Agricultural University, Dhaka during Rabi season (October 2021 to March 2022). The experiment was set as a Factorial Block Design (FBD), with three replications. Factor A and B represented five seed sowing date and four cutting management, respectively.

Barley was sown on five sowing dates *viz.*, 30 October, 15 November, 30 November, 15 December and 30 December. The plant was given four cutting managements *viz.*, no cut for fodder and left for seed only, cut at ZGS19 (maximum seedling growth stage) for fodder and left for seed, cut at ZGS29 (maximum tillering stage) for fodder and left for seed, cut at ZGS31 (stem elongation stage) for fodder and left for seed. The respective cut plots were given cut at 5 cm from a ground level by using sickle. Each of the experimental plots was 4.50 m² (3.00 m × 1.50 m) in size. The experimental plots were prepared by two ploughing and cross-ploughing with rotary plough and finally laddering to ensure a good condition of seed sowing. Seeds of Barley *var*. BARI Barley 9 were sown in row with spacing of 30 cm × 3 cm. The recommended dose of nitrogen (N), phosphorus (P) and potassium (K) 120:50:40 kg ha^{-1} was applied, respectively. Urea was applied in three equal splits i.e. as basal, 25 DAS (crown root initiation) and after 1st cut for fodder. TSP and MoP were applied at the time of final land preparation before seed sowing. All the other agronomic management, e.g. weeding, thinning and pest control were done uniformly to each experimental plot as per requirement. The first cut was done for fodder and the second cut for seed and straw. Data on plant height and dry matter accumulation were recorded of 30 days after cutting (DAC), anthesis and maturity stage of the crop. In case of yield contributing characters and yield, data were recorded on effective tillers per meter row length (mrl^{-1}), length of spike, fertile spikelet $spike^{-1}$, unfertile spikelet $spike^{-1}$, 1000-grain weight (g), green fodder yield (t ha^{-1}) after cut at 80% moisture content, grain yield (t ha^{-1}) at 14% moisture content and straw yield (t ha^{-1}) after sun dry, biological yield (t ha^{-1}) were measured.

Statistical analysis

All data were analyzed by using SPSS 20.0 for windows (SPSS Inc.). The significant differences among the treatment means were compared by Least Significant Difference (LSD) value at 1% level of significance. Different lower case letters in the figures were representing the significant differences among the treatments.

Results and Discussion

Effects of sowing date on plant height

Data presented in Table 1 stated that delay in seed sowing time of barley from 30 October to 30 December had reduced plant height significantly at 30 DAC, anthesis and maturity stage. Plant height ranged from 22.7 cm to 46.3 cm at 30 DAC, 33.5 cm to 80.6 cm at anthesis and 45.6 cm to 92.6 cm at maturity stage in sowing time from 30 October to 30 December (Table 1). Seed sowing at 30 October gave the maximum plant height 46.3 cm, 80.6 cm and 92.6 cm at 30 DAC, anthesis and maturity stage, respectively followed by seed sowing at 15 November where the plant height was 41.5 cm, 75.8 cm, and 88.3 cm at 30 DAC, anthesis and maturity stage, respectively. This may be contributed due to maximum period of time available to early sown crop in contrast to late sown crop for photosynthetic assimilation and translocation for vegetative growth of the barley, resulting in higher plant height. Similar findings was found by Bahadur and Chowdhury (2019) in barley and Waheddullah *et al*. (2018) in wheat, they reported that early seed sowing improved plant height over late seed sowing.

Effects of cutting schedules on plant height

Cutting of barley at different Zadoks growth stage (ZGS) had decreased the plant height meaningfully compared to uncut barley at all the three growth stages of plant (Table 1). Plant height ranged from 20.6 cm to 42.9 cm at 30 DAC, 32.8 cm to 78.3 cm at anthesis period and 45.5 cm to 90.2 cm at maturity stage when the plants were cutting at GS19 (maximum growth stage) to ZGS31 (stem elongation stage). Maximum plant height (67.5 cm, 87.2 cm and 98.3 cm at 30 DAC, anthesis and maturity stage, respectively) was found at uncut plant. The reason might be beheaded the barley plant height causing termination of growth and cutting imposed stress; therefore new shoot growth of plant could not reach the same plant height as that of uncut plant due to shorter growth duration. On the other hand, uncut plant was no disturbance in the vegetative growth and thus brought about the tallest plants. Our study showed significant decreases in plant height by delay cutting compare to uncut, which is in agreement with previous studies on dual purpose wheat and barley (Khalil *et al*., 2011; Iqbal *et al*., 2016; Waheddullah *et al*., 2018).

Effects of sowing date on biomass accumulation

Irrespective of delay in sowing time of barley from 30 October to 30 December had reduced dry matter production significantly at 30 DAC, anthesis and maturity stage (Table 1). Dry matter ranged from 28.5 g mrl^{-1} (mrl, meter row length) to 48.5 g mrl^{-1} at 30 DAC, 204.5 g mrl^{-1} to 275.2 g mrl^{-1} at anthesis and 332.6 g mrl^{-1} to 415.5 g mrl^{-1} at maturity stage in sowing time from 30 October to 30 December. Maximum dry matter was found at 30 October seed sowing at all growth stage of plant. It may be due to the effect of satisfactory environmental conditions at seed sowing time, which resulted in taller and healthier plant. Waheddullah *et al*. (2018) reported that early seed sowing influenced plant biomass that improved the photosynthesis, leaf area, leaf area index and vegetative growth of plants compared to delay seed sowing.

Effects of cutting schedules on biomass accumulation

Cutting of barley at different ZGSs had decreased the dry biomass production significantly compared to uncut barley throughout the growing period (Table 1). Dry matter ranged 36.9 g mrl^{-1} to 47.5 g mrl^{-1} at 30 DAC, 220.3 g mrl^{-1} to 265.8 g mrl^{-1} at anthesis period and 360.6 g mrl^{-1} to 452.3 g mrl^{-1} at maturity stage when the plants were cutting at GS19 (maximum growth stage) to ZGS31 (stem elongation stage). Maximum dry matter accumulation (52.3, 315.2 and 475.5 g mrl^{-1} at 30 DAC, anthesis and maturity stage, respectively) was found at uncut plant. It could be due to the no decapitation stress resulted in higher and vigorous plants which contributed to increase dry matter accumulation in uncut plant compare to cutting at different growth stage for fodder purpose (Waheddullah *et al*., 2018).

Table 1. Effects of sowing date and cutting schedule on growth parameter at different stages of crop

Treatment		Plant height (cm)			Dry matter (g mrl^{-1})		
		30 DAC	Anthesis	Maturity	30 DAC	Anthesis	Maturity
Sowing date	30-Oct	46.3 a	80.6 a	92.6 a	48.5 a	275.2 a	415.5 a
	15-Nov	41.5 b	75.8 b	88.3 b	42.6 b	260.4 b	405.3 b
	30-Nov	35.5 c	63.2 c	76.2 c	36.3 c	245.8 c	385.7 c
	15-Dec	30.2 d	50.7 d	63.7 d	32.8 d	225.7 d	360.3 d
	30-Dec	22.7 e	33.5 e	45.6 e	28.5 e	204.5 e	332.6 e
LSD (0.01)		1.8	1.9	1.4	1.5	3.5	2.8
CV%		5.99	5.03	6.78	8.02	8.11	7.43
Cutting management	Uncut	67.5 a	87.2 a	98.3 a	52.3 a	315.2 a	475.5 a
	Cutting at ZGS19	42.9 b	78.3 b	90.2 b	47.5 b	265.8 b	452.3 b
	Cutting at ZGS29	38.8 c	64.6 c	77.5 c	44.6 c	250.4 c	410.5 c
	Cutting at ZGS31	20.6 d	32.8 d	45.5 d	36.9 d	220.3 d	360.6 d
LSD (0.01)		1.9	1.8	1.4	1.5	2.8	2.6
CV%		7.90	7.14	8.95	7.99	7.35	7.41

Effects of sowing date and cutting managements on yield contributing characteristics

Seed sowing date and cutting managements had significant effect on yield contributing characteristics of barley (Table 2). The effective tillers ranged from 86.75 mrl^{-1} to 145.25 mrl^{-1}, spike length ranged from 5.75 cm to 8.85 cm, fertile spikelet ranged from 20.65 no. spike^{-1} to 38.86 no. spike^{-1}, infertile spikelet 3.05 no. spike^{-1} to 4.75 no. spike^{-1} and 1000-grain weight ranged from 26.75 g to 35.64 g were recorded in different treatments (Table 2). From this study, it was observed that delay in sowing of barley seed from 30 October to 30 December had significantly decreased all the yield attributes. The maximum yield attributes were found in sowing of barley at 30 October. The reason could be the highest period of time of the vegetative growth documented in early seed sown barley which enabled the plant to gain and use maximum resources and harvest the highest number of yield attributes. The maximum number of fertile spikelet (no. spike^{-1}) was obtained in earlier sown crop was possibly due to extended growing season contribute higher photosynthetic assimilation and translocate from source to sink , which caused in higher production of grain along with grain filling. Yield attributes differed significantly in seed sowing times might be clarified that seed sowing at the period of higher temperature, the plant could not get amiable atmosphere for proper vegetative growth and productivity. Previous studies stated that late seed sowing of wheat and barley might be exposed to high temperature after and during flowering stage of crops subsequent in decreased number of fertile spikelet and number grains spike^{-1} and increased number of infertile spikelet and number of unfilled grain spike^{-1} (Fayed *et al*., 2015; Farooq *et al*., 2016).

Cutting of barley for fodder purpose at different Zadoks growth stage had decreased significantly all the yield traits related to uncut barley (Table 2). The effective tillers ranged 105.91 no. mrl^{-1} to 165.6 no. mrl^{-1}, spike length ranged 4.56 cm to 8.78 cm, fertile spikelet ranged 24.4 no. $spike^{-1}$ to 38.5 no. $spike^{-1}$, infertile spikelet 2.86 no. $spike^{-1}$ to d 4.86 no. $spike^{-1}$ and 1000-grain weight ranged from 27.7 g to 36.2 g were recorded from this study. Our results suggested that the highest yield attributes was found in uncut conditions and the lowest was found in cutting at ZGS31 except infertile spikelet. The cause for the lowest number of effective tillers in cut treatments might be due to failure of regeneration of new tillers after cutting the plants and vice-versa. On the other hand, the reason of the lowest spike length and 1000-grain weight in cut treatments might be due to drain on photosynthetic assimilation occurred as a result of regeneration because of cutting thus decreasing translocation of assimilates to spike and grain formation. Furthermore, attaining lower grain weight in cutting treatments may be due to elimination of photosynthetic organs (leaf) by clipping which adversely affected source sink relationship.

Table 2. Effects of seed sowing date and cutting management on yield contributing characters

Treatment		Effective tiller (No. mrl^{-1})	Fertile spikelet (No. $spike^{-1}$)	Spike length (cm)	Infertile spikelet (No. $spike^{-1}$)	1000 grain weight (g)
Sowing date	30-Oct	145.3 a	38.9 a	8.85 a	2.93 e	35.6 a
	15-Nov	135.7 b	36.6 b	8.58 b	3.05 d	34.6 b
	30-Nov	115.3 c	32.6 c	8.25 c	3.25 c	31.6 c
	15-Dec	100.3 d	28.5 d	7.36 d	3.78 b	28.4 d
	30-Dec	86.8 e	20.7 e	5.75 e	4.75 a	26.8 e
LSD (0.01)		2.10	1.40	0.20	0.30	1.2
CV%		7.55	9.03	9.10	8.75	9.01
Cutting management	Uncut	165.6 a	38.5 a	8.8 a	2.7 d	36.2 a
	Cutting at ZGS19	140.8 b	35.6 b	8.6 b	2.7 c	34.4 b
	Cutting at ZGS29	126.5 c	30.8 c	6.8 c	3.8 b	30.6 c
	Cutting at ZGS31	105.9 d	24.4 d	4.6 d	4.9 a	27.7 d
LSD (0.01)		2.20	0.25	0.21	0.20	0.30
CV%		7.90	7.01	7.64	8.60	7.55

Effects of sowing date and cutting management on green fodder and grain yield of barley

Delay in seed sowing from 30 October to 30 December had significantly reduced grain, straw and biological yield (Table 3). The considerably higher grain, straw and biological yield (3.25 t ha^{-1}, 4.5 t ha^{-1} and 7.75 t ha^{-1}, respectively) were recorded in 30 October sown crop, while significantly lower grain, straw and biological yield (1.91 t ha^{1}, 2.5 t ha^{-1} and 4.36 t ha^{-1}, respectively) were recorded in 30 December sown crop. Our study revealed the similar trend with Singh *et al*. (2013) and Choudhary and Chaplot (2015) stated that early seed sowing at the end of October to starting of November lead to in higher yield attributes, grain, straw yield and green fodder yield over late sowing for dual purpose barley.

Among the cutting schedules, the early cutting (ZGS19, maximum growth stage) was recorded with significantly the highest grain (2.75 t ha^{-1}), straw (3.25 t ha^{-1}) and biological yield (6.00 t ha^{-1}), while late cutting (ZGS31, stem elongation stage) resulted with the lowest grain (1.4 t ha^{-1}), straw (1.8 t ha^{-1}) and biological yield (3.2 t ha^{-1}) (Table 3). The causes of significant decrease of yield in cut treatments compared to uncut barley was conceivably due to deduction of photosynthetic organs that lead to in lower vegetative growth rate, net assimilation rate, biomass accumulation, number of effective tillers, 1000-grain weight and the reverse was obtained for uncut treatment.

Delay in seed sowing of barley from 30 October to 30 December significantly reduced green fodder yield of dual purpose barley (Table 3). The highest green fodder yield (15.8 t ha^{-1}) was recorded in 30 October sown seed and the lowest (12.4 t ha^{-1}) in 30 December sown seed. The cause is obtainability of optimum growth conditions of temperature, moisture, light and essential plant nutrients to earlier seed sown treatments which lead to in higher fresh fodder production. The delay in cutting of barley for fodder was lead to increasing fodder yield (Table 3). The delay in cutting from ZGS29 to ZGS31 resulted in increasing fodder yield over ZGS19 cutting stage. The highest green fodder yield (10.8 t ha^{-1}) was found in cutting at ZGS31 (stem elongation stage) and the lowest (2.85 t ha^{-1}) in ZGS19 (vegetative growth stage). In case of cutting at ZGS29 (maximum tillering stage), green fodder yield was 6.7 t ha^{-1}. Cutting at maximum tillering stage might be balanced between grain yield and fodder yield compared to cutting at stem elongation stage (Naveed *et al.,* 2015). The reasons are to higher regeneration capacity of plant cutting at tillering stage than stem elongation stage for green fodder. Similar results were found in the study of Salama (2019) and Waheddullah *et al*. (2018) who reported that cutting barley at early growth stages (45 and 55 DAS) caused significant increase in green fodder yield and crude protein content contrast to late cutting at 65 DAS.

Table 3. Effects of sowing date and cutting management on grain yield, straw yield, biological yield and green fodder yield

Treatment		Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Biological yield (t ha^{-1})	Green fodder yield (t ha^{-1})
Sowing date	30-Oct	3.25 a	4.50 a	7.75 a	15.75 a
	15-Nov	2.95 b	4.33 b	7.21 b	15.26 b
	30-Nov	2.80 c	4.20 c	6.80 c	14.80 c
	15-Dec	2.46 d	3.24 d	5.70 d	13.70 d
	30-Dec	1.91 e	2.45 e	4.36 e	12.36 e

Treatment		Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Biological yield (t ha^{-1})	Green fodder yield (t ha^{-1})
LSD (0.01)		0.18	0.27	0.52	0.21
CV%		7.42	8.24	8.50	9.02
Cutting management	Uncut	3.50 a	4.58 a	8.08 a	0
	Cutting at ZGS19	2.75 b	3.25 b	6.00 b	2.85 c
	Cutting at ZGS29	2.50 c	2.65 c	5.15 c	6.65 b
	Cutting at ZGS31	1.40 d	1.76 d	3.16 d	10.75 a
LSD (0.01)		0.21	0.24	0.38	1.05
CV%		6.80	7.45	8.12	7.80

Conclusion

Sowing date and cutting management had significantly influenced the growth factors, yield attributes and yield of barley. This study suggests that early sowing at 30 October and cutting at ZGS29 (maximum tillering stage) is the most suitable management practices for green fodder and grain yield of barley.

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Conflicts of Interest

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

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DEVELOPMENT OF QUASI-AROMATIC HYBRID RICE (*Oryza sativa* L.) THROUGH GENETIC ENHANCEMENT OF LOCAL RICE GENOTYPES IN BANGLADESH

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Abstract

Aromatic rice has high economic and social value, but its commercial production in Bangladesh is limited due to lower yield. In this study, the heterosis of hybrid rice was exploited to improve the yield of aromatic rice. A total of 120 aromatic rice germplasm was tested against five cytoplasmic male sterility (CMS) lines (A) with 40 maintainer (B) and 11 restorer (R) lines and the B lines were converted into CMS lines using backcrossing. The identified 11 restorer lines were being maintained, four local R-lines (Sakkorkhora, Chinigura, Kataribhog and BAU dhan2) were used by assessing pollen and spikelet fertility of the F_1 generation from crosses of 50 selected R-lines against one CMS line (IR58025A). Quasi-hybrid-1 had a yield potential >5.0 t/ha. Growth duration varied between 85-90 days during Aman season, and there was heightened zinc content (>22 mg/kg) in the hybrid grains. Identified restorer and maintainer local genotypes provide broad resources for increasing aromatic rice yield.

Keywords: Aromatic rice, CMS line, Maintainer line, Pollen sterility, Spikelet fertility

Introduction

Rice (*Oryza sativa*) is the third most popular cereal grain in the world (Rahman *et al*., 2021) and is a staple food for over half of the world's population (Kush, 2005). Globally, 503.17 MT of rice is produced, with 29.5% coming from China. The other producers are India (23.8%), Bangladesh (7.0%), Vietnam (5.4%) and Thailand (3.7%) (USDA, 2020). Rice is grown in three distinct seasons namely Aus, Aman and Boro throughout the year. Bangladesh recently ranked the third position globally in rice production, behind China and India, with a production volume of 3.6 crore tonnes (Rahman *et al*., 2021). Among the thousands of local rice cultivars that have been cultivated historically across Bangladesh some 12.16% have been aromatic cultivars cultivated successfully in otherwise ecologically unfavorable growing areas. Besides, it is being socially and economically more appealing, several of these aromatic rice germplasm are well-suited for genetic research (Singh *et al*., 2000). Specifically, more

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than 100 aromatic germplasm have been identified in Bangladesh (Islam *et al*., 2016), with their morphological and genotypic diversity documented. As typically short and bold types with mild to strong aroma (Shahidullah *et al*., 2009), the fragrance of aromatic germplasm results from the presence of a non-functional betaine aldehyde dehydrogenase 2 (BADH2), which is also responsible for low grain yield (Bradbury *et al*., 2005). As such, research into the hybridization of local germplasm becomes necessary not only for the usual goal of enhancing quality traits, but also particularly to increase yields and thus provide strategic breeding information to rice breeders (Travis *et al*., 2015).

Recently, considerable research (in China and other Asian rice-growing countries) to improve coarse rice yields, and to combat chronic food shortages, has employed hybridization using cytoplasmic male sterility (CMS) systems. For production of F_1 seeds using CMS-based hybrid seed technology, a three-line system consisting of a CMS line (A line), a maintainer (B line), and a restorer (R line) is required (Kumar *et al*., 2015). In Bangladesh generally, rice germplasm represents local landraces of Aus, Aman, Boro and HYV types, with more than 100 genotypes that are aromatic and fine. Most of these varieties are also photosensitive and tall statured but, again, have low yield potentials (2-3t/ha). Building on the successes in coarse rice CMS improvements, a similar promise may hold for these fine, aromatic varieties cultivated in smaller areas. However, hybrid rice has>20% yield increases compared to the high yielding inbred varieties from China in the 1970s (Huang *et al*., 2017), few germplasm reveal a strong, efficient restorer capacity for generating three lines of hybrid rice utilizing CMS lines (Islam *et al.*, 2015). The development of such restorers, therefore, represents a key aim for hybrid rice breeding programs and is the purpose of this study.

Materials and Methods

Plant materials

Seed from 113 aromatic rice germplasm were collected from the Bangladesh Rice Research Institute (BRRI) Genebank in Gazipur, Bangladesh; another seven were collected from Bandarban and Sherpur districts during 2013-15. Moreover, a total of five standard non-aromatic CMS lines were collected; four from the International Rice Research Institute (IRRI), Los Baños, Philippines (IR58025A, IR62829A, IR6888A, Gan46A) and one, BRRI1A, from BRRI.

Crossing in source nursery

We grew 120 aromatic rice germplasm (Supplementary information) with the above CMS lines in Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) research field during Aman 2013-15. At flowering stage, crosses of 120 aromatic rice germplasm were conducted with each of the five CMS lines. Fig. 1 depicts the total crossing procedure to develop the new CMS, new purified restorer and quasi-hybrid variety. F_1 seeds were harvested from crosses and stored separately.

Identification of maintainer and restorer lines

F_1 seeds (obtained from the above crosses), along with their respective male parents, were grown in Aman 2014-15 in a test cross nursery. At flowering, pollen sterility/fertility for all F_1s was tested under a compound microscope for identification of genotypes bearing maintainer or restorer genes. F_1s with 100% pollen sterility indicate that the corresponding pollen parents carry maintainer genes (Ali *et al.*, 2014). In contrast, F_1s having 80% or more pollen fertility, as well as spikelet fertility, indicate that the corresponding pollen parent carries the restorer gene (Ali *et al*., 2014; Hossain *et al*., 2018). Four restorer lines (e.g., BUdhan2R, SakkarkhoraR, KataribhogR and ChiniguraR) were identified. These restorer lines were used to develop the quasi-hybrid rice variety, with the male parent as aromatic and the female parent as non-aromatic.

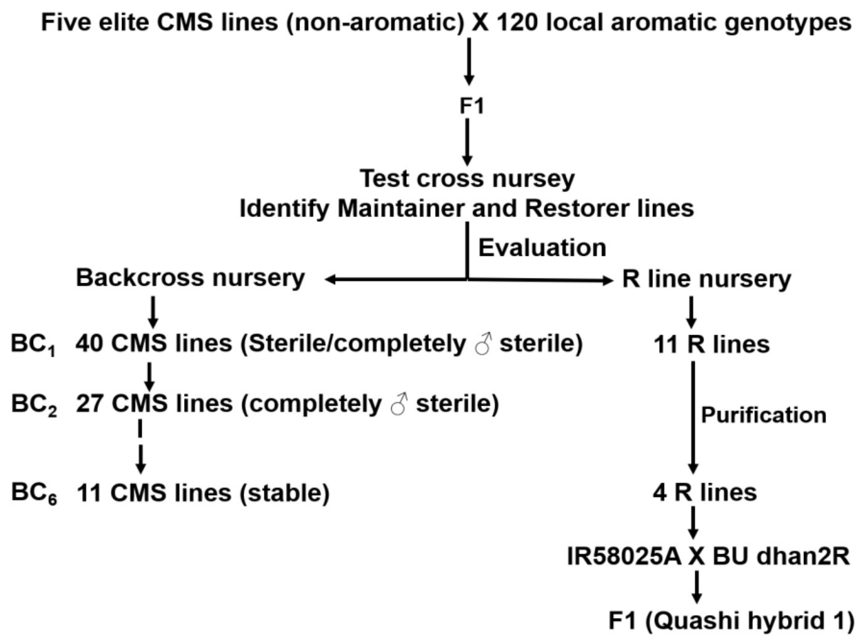


Fig. 1. Schematic diagram of quasi-aromatic hybrid rice development

Synthesis of aromatic CMS lines

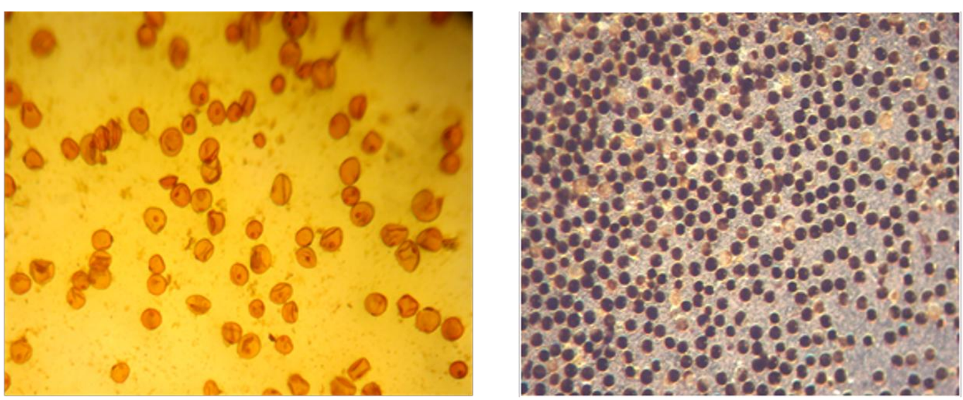
F_1s exhibiting 100% pollen sterility in the test cross nursery were backcrossed with their corresponding maintainer line. Crosses were made between 90 germplasm and IR58025A, 63 germplasm with IR62829A, 62 germplasm with IR6888A, 78 germplasm with Gan46A, and 35 germplasm with BRRI1A in test cross nursery. Maintainer and restorer lines from all these crosses were identified and sorted. F_1s and backcrosses showing 100% pollen sterility (Fig. 2) in the test and backcross nurseries were subsequently backcrossed with their corresponding pollen parents during subsequent rice growing seasons (Table 1 and 2). After 5-6 back crossings, all the maintainer lines were designated as new aromatic CMS lines. Each of the pollen parents will act as a maintainer line for the corresponding aromatic CMS line.

Table 1. Pollen sterility status of 27 BC_2 populations, during Aman season 2013

SL. No.	BC_2 generation	Pollen sterility (%) of F_1
1	IR58052A*2/Elai	100
2	IR58052A*2 / Straw	99.6
3	BRRI 1A*2 / Elai	99.7
4	BRRI 1A*2 / Khazar	100
5	BRRI 1A*2 / Rahduni pagol	99.5
6	BRRI 1A *2 / Noyanmoni	99.6
7	BRRI 1A *2 / Sugandhi dhan2	98
8	Gan 46A *2 / Khazar	99.9
9	Gan 46A*2 / Tilkapur	99.5
10	Gan 46A *2 / Desi katari	98
11	Gan 46A*2 / BR5	97.5
12	Gan 46A*2/ Kaminisoru)	97
13	Gan 46A*2 / Lalsoru	96.5
14	Gan 46A*2 / Gopalbhog	98.5
15	Gan 46A *2 / Baiobhog	96.5
16	Gan46A*2 / Elai	97.7
17	R62829A*2 / Khazar	100
18	IR62829A*2 / Noyanmoni	98.3
19	IR62829A *2 Elai	99.5
20	IR62829A*2 / Dubsail	98.3
21	IR62829A *2 / Dakshahi	99.9
22	IR62829A *2 / Straw	97.8
23	IR6888A *2 / Khazar	100
24	IR6888A*2 / Sugandhi dhan2	97.8
25	IR6888A*2 Dubsail	99.6
26	IR6888A *2 / Elai	100
27	IR6888A*2 Tilkapur	98

Table 2. Fertility restoration ability of test aromatic rice genotypes against 4 alien CMS lines

Pollen parent used	CMS Line Used	Pollen sterility (%) of F_1	Spikelet fertility (%) of F_1	Effective restorers	Status
BU Dhan1	IR58025A	85.8	77.5	BU Dhan1R	PF
BU Dhan2		94.3	81.2	BU Dhan2R	FF
Sakkorkhora		92.5	80.5	SakkorkhoraR	FF
Chinigura		82.5	76.3	ChiniguraR	PF
Kataribhog		86.0	75.2	KataribhogR	PF
Jiradhan	IR 62829A	90.3	80.3	Jiradhan R	FF
BU dhan2		85.3	78.5	BU dhan2R	PF
ChiniguraR		83.5	75.2	ChiniguraR	PF
BaojhakiR		84.3	76.2	BaojhakiR	PF
JirabhogR		88.5	77.3	JirabhogR	PF
Ukknimodhu		85.6	75.5	UkknimodhuR	PF
Jamai aduri		80.3	77.3	Jamai aduriR	PF
Sagordana	Gan 46A	85.3	76.8	Sagordana R	PF
Sagordana	BRRI 1A	81.5	75.3	Sagordana R	PF



Microscopic view of 100% pollen sterility

Microscopic view of >80% pollen fertility

Fig. 2. Microscopic view of pollen fertility restoration pattern in test crosses. Anthers were smeared in solution containing 0.5% iodine in 2% potassium iodide and examined under a light microscope

Identification and development of promising aromatic quasi-hybrids

Four newly identified aromatic restorer lines (e.g., BUdhan2R, SakkarkhoraR, KataribhogR and ChiniguraR) and five non-aromatic CMS lines (e.g., IR58025A, IR62829A, IR6888A, Gan46A, and BRRI1A) were grown in the field at BSMRAU during T. Aman 2014-15. Crosses were made between these restorers and CMS lines. The best crossing material was selected based on yield and other characteristics (grain quality, aroma, plant type, etc.). Materials obtained from two crosses (IR58025A / BUdhan2R and BRRI1A / BUdhan2R) were selected as new quasi-aromatic hybrids. The hybrid developed from a cross between the aromatic CMS line and non-aromatic restorer line is called the Quasi-aromatic hybrid (Zhou, 1995).

Production of F_1 seeds of quasi-aromatic hybrids

Seedlings of two A-lines (IR58025A and BRRI1A) along with the restorer line BUdhan2R were transplanted in a hybrid seed production block at BSMRAU during Boro 2014-15. The ratio of CMS and restorer lines was 12:2. Seedlings of R lines were subsequently transplanted on three different dates to synchronize the flowering time in both the A and R lines. Proper roughing was done both in vegetative and flowering stages, and supplementary pollination methods (like pulling of rope at 10-11 AM during the flowering period) were practiced. F_1 seeds of quasi-aromatic hybrids were harvested from A-lines, and the harvested seed was stored for further field evaluation.

Aroma test

The aroma of the F_1 plant was also identified and confirmed by sniffing and was scored as non-scented, lightly scented and scented using 1.7% KOH-based method (slam *et al.,* 2016).

Evaluation of quasi-aromatic hybrids

Field evaluation was conducted at three locations in Bangladesh, including Gazipur, Bogura and Chattogram. Seeds were grown in the field, along with BRRI dhan34 and BRRI dhan38, as check varieties during Aman 2015, and with BRRI dhan50 during Boro 2014-15, following standard rice cultivation practices (BRRI, 2016). Data on yield, yield-contributing characteristics, and field tolerance to insects and diseases were recorded and analyzed.

Results and Discussion

Identification of maintainer and restorer lines

We determined pollen sterility and spikelet fertility for 328 F_1s obtained from the crosses of five CMS lines and 120 aromatic genotypes during T. Aman 2014. Maintainer and restorer lines from these crosses were identified in successive four years (Fig. 1). In T. Aman 2014, pollen sterility for 40 F_1s ranged from 97.5-100% in the BC_1 population.

These 40 BC_1F_1s populations were further evaluated next season. In Boro 2016-17, pollen sterility ranged from 12.18% to 100%. Considering the results from the two seasons, 11 BC_2F_1s were designated as completely sterile and 16 BC_2F_1s were designated sterile (Table 1). These two categories were taken for further improvement to stabilize the CMS lines. All others including partially sterile, partially fertile, fertile, and fully fertile variants were discarded (Ali *et* al., 2014; Hossain *et al*., 2018). Jayasudha and Sharma (2010) found 10 potential restorers based on pollen and spikelet fertility percent.

Eleven (11) effective restorer lines were identified (Table 2). The highest spikelet fertility (81.15%) was recorded in IR58025A / BU dhan2R. Purification was conducted to identify the most suitable R line (Fig. 1). Crosses of four restorer lines with a common CMS line (IR58025A), the number of plants of each R-line involved in crossing, number of F_1 plants showed 80% and above pollen and spikelet fertility and selection of pure R plants (Table 3). The >80% pollen and spikelet fertility F_1s for 105 plants of SakkarkhoraR, 33 plants of BU dhan2R, 8 plants of ChiniguraR, and 27 plants of KataribhogR were selected as pure R plants. The field performance of the R line during the purification process is shown in Fig. 3. Seeds of individual pure R plants were harvested separately and stored for future use. The progeny of each selected plant of the four R lines will be used as pure R-lines against IR58025A lines in future hybrid programs**.**

Fig. 3. Field performance of R line during purification process at BSMRAU, Gazipur, Boro 2014-15. Upper figure indicates the performance at purification process and lower figure indicates the field performance after purification.

Table 3. Number of plants of four R-lines purified against CMS line IR 58025A

Name of crosses made	The number of plants of R lines crossed	Number of F_1s with >80% pollen and spikelet fertility	Number of plants of R lines selected as pure
IR58025A / SakkarkhoraR	720	105	105
IR58025A / BU dhan2R	108	33	33
IR58025A / ChiniguraR	144	08	08
IR58025A / KataribhogR	120	27	27

Seed multiplication of CMS lines

Seed multiplication of promising CMS lines is a basic requirement for largescale F_1 seed production in any hybrid program. In addition, some sort of seed production through controlled hand-crossing of CMS is necessary for the maintenance of its purity. In light of this, seed multiplication for two promising CMS lines was done during Aman 2014 and Boro 2014-15 (Fig. 4). A total of 18.50 kg and 14.35 kg seeds were harvested from the BRRI1A and IR58025A lines, respectively. A small quantity of seed for each CMS line was also produced by hand-crossing for the maintenance of their purity.

Identification of promising quasi-hybrids

Two promising lines (IR58025A/BU dhan2R and BRRI1A/BU dhan2R) from the crosses were identified and designated as quasi-aromatic hybrids 1 and 2 (QH1 and QH2), respectively. These two quasi-hybrids appear promising for Bangladesh based on overall field performance, which includes plant type, growth duration, and grain quality**.** Fig. 5 depicts the growth performance and shape and size of grain for QH1, for both varieties, growth duration was significantly earlier than standard check varieties, there was >20% heterosis compared to check varieties for grain yield, and both matured approximately 30 days earlier than the local check during Aman, and both contained >21 mg Zn/kg and >9.7 mg Fe/kg in rice grain. QH1 also proved suitable for Boro season. Moreover, we also tested the aroma from the promising lines based on the standard method (Sood and Siddique, 1978). Both the quasi-aromatic hybrid showed scented. The scent was also confirmed by panel test.

Fig. 4. Production F_1 seeds of Quasi-hybrid 1 at BSMRAU, Gazipur, Boro 2014-15.

Multilocation trial of newly developed quasi-hybrid

Table 4 summarizes the performance of quasi-hybrids with respect to growth duration, grain yield, and field tolerance to insect and diseases for Aman and Boro seasons in Gazipur. QH1 yielded 28.86% and 21.89% higher grain over BRRI dhan34 and BRRI dhan38, respectively, while QH2 yielded 42.28% and 32.70% higher grain than the same checks, respectively, during Aman season in Gazipur. In terms of typical heterosis for grain yield, QH2 outperformed QH1, but it also lacked aroma. While both hybrids matured 33 days earlier than the check varieties during Aman season, QH1 performed better than QH2 during Boro; but QH2 was not appropriate for cultivation in the Boro season. Additionally, QH1 showed a 10% standard heterosis in grain yield during the Boro season as compared to BRRI dhan50. With QH1 suitable for two seasons, T. Aman and Boro, both varieties (as noted) are rich in zinc and iron content: 21.80 mg Zn/kg and 9.75 mg Fe/kg (QH1) and 22.85 mg Zn/kg, and 11.15 mg Fe/kg (QH1). However, only QH1 was selected for multilocation field trials due to its aroma and long slender grain. Against standard check varieties at three locations in Bangladesh, QH1 showed a higher yield at all trial locations (Fig. 6). Whereas specific locations showed no significant yield impacts, Bogura showed a slightly higher yield compared to the other two locations.

Subsequently, we sent QH1 to the National Seed Board (NSB) of Bangladesh for release as a commercial variety in Bangladesh. The National Seed Board further recommended and released the variety for cultivation in Bangladesh, so that farmers in the country may benefit from cultivating this healthier, more economically lucrative variety.

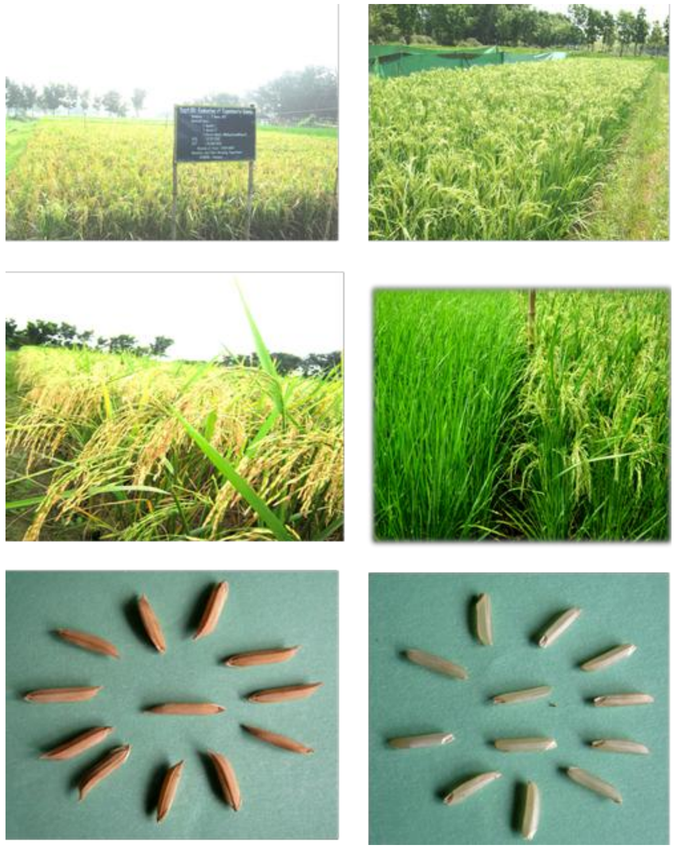


Fig. 5. Field performance and shape and size of grain of Quasi-Hybrid 1 (QH1) at T. Aman 2015

Whereas more than half of the global population depends on rice, with 65% of daily dietary intake coming from rice in developing countries (Sharma *et al*., 2012), the continued, 1.8% population increase in Asia requires another 70% more rice in production in 2025 (compared to 1995). This huge increase in rice demand cannot be produced on limited land, with less water, labor, and chemical resources using inbred rice varieties. Hybrid rice variety can alleviate this problem by increasing yields and land where they can be grown.

Hybrid rice (F1 seeds) offers 15–25% better yields than commercial inbred varieties. However, the development of hybrid rice requires maintainer, restorer, and cytoplasmic male sterility (CMS) lines (Kumar *et al.*, 2015; Rosamma and Vijayakumar, 2006; Sharma *et a*l., 2012). In this study, we identified 27 maintainers (Table 1) and four restorer lines (Table 3). These maintainers and restorers are essential for the commercial exploitation of heterosis breeding programs (Kumar *et al.*, 2015; Rosamma and Vijayakumar, 2006; Sharma *et al.*, 2012); specifically, restorers for various cytosterile sources will increase the cytoplasmic diversification, which in turn can prevent genetic vulnerability due to the use of single CMS source (Kumar *et al.*, 2015).

Table 4. Performance of quasi-aromatic hybrids in field trials during Aman 2016 and Boro 2016-17

Hybrid /Check varieties	Days to flowering		Days to maturity		Grain yield (t/ha)		Insect and disease reaction
	T.Aman	Boro	T.Aman	Boro	T.Aman	Boro	
Quasi-Hybrid 1 (IR58025 / BUdhan2R)	85	123	112	150	4.51	6.25	Field tolerant to pests and diseases
Quasi-Hybrid 2 (BRRI1A / BUdhan2R)	86	115	113	144	4.91	4.41	Field tolerant to pests and diseases
BRRI dhan34	114	-	145	-	3.50		-
BRRI dhan38	116	-	146	-	3.7		-
BRRI dhan50		126		156		6.00	-

These identified maintainers and restorers implicate wild abortive cytoplasmic male sterility among the local and high yielding rice (*Oryza sativa* L.) varieties in Bangladesh. Observing the pollen using a microscope, F_1 plants were obtained rare. This research addresses that gap and provides an asset for future breeding programs in Bangladesh or elsewhere. The availability of such a stable cytoplasmic male sterility and fertility-restoring system is vital for the commercial exploitation of heterosis in rice (Ali *et al*., 2014).

This study found four effective restorers, seven partial restorers, 16 partial maintainers, and 11 complete maintainers. The male parents were identified as effective restorers, partial restorers, weak maintainers, and complete maintainers based on staining (Kumar *et al*., 2015). Pollen fertility was found in the test cross progenies and it had a strong correlation (r = 0.823) with spikelet fertility.

This finding indicates that either pollen or spikelet fertility could be used as a criterion to classify pollen parents as maintainers (Kumar *et al*., 2015). Therefore, the extent of spikelet fertility was used for the classification of pollen parents utilized in test crosses (Joshi *et al*., 2007; Raj and Virmani, 1988). We also found a significant positive correlation between spikelet fertility and stained round fertile pollen, consistent with Kumar *et al.* (2015), Ali *et al.* (2014) and Joshi *et al.* (2007). Surprisingly, spikelet fertility is not correlated with pollen's susceptibility to stain with I-KI solution (Joshi *et al*., 2003). Based on pollen fertility, effective restorer lines were identified when tested by low spikelet fertility restoration in test crosses. High seed yield of hybrid lines relies primarily on high spikelet fertility (Bagheri and Babaeian-Jelodar, 2011), however, because investigation of pollen fertility requires a long time, the classification of pollen parents might be made based on spikelet fertility (Kumar *et al*., 2015).

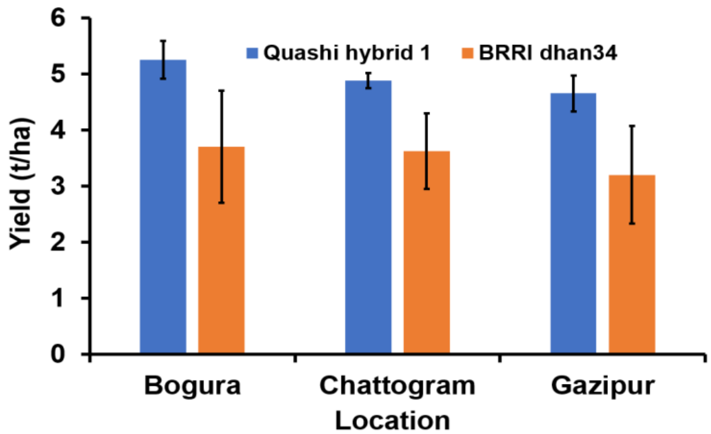


Fig. 6. Yield of Quasi-Hybrid 1 (QH1) at different locations. Error bar indicates standard error

In present study, the pollen parents SakkarkhoraR, BU dhan2R, ChiniguraR, and KataribhogR were identified as effective restorers for the IR58025A CMS line. Pollen parents from Elai, Straw, Khazar, Noyanmoni, Dakshahi and Dubsail yielded completely male sterile progenies in the test crosses (Table 2). All of these pollen parents are local landraces. Surprisingly, these pollen parents could be used as complete sterility maintainers for a WA-type cytosterility system. In our study, one pollen parent, Elai, was crossed with five CMS lines. Elai was identified as a maintainer or partial maintainer. Different outcomes of the same genotype in crosses with several CMS lines have been observed (Hariprasanna *et al*., 2005; Sabar *et al*., 2007; Jayasudha and Sharma, 2010; Umadevi *et al*., 2010; Vanitha *et al*., 2020). Genomic characteristic of female parent influences different expressions of fertility restoration (Hossain and Singh, 2010).

Conclusion

The identified restorers (Chinigura, Kataribhog, Sakkakhora) and maintainers from this study can be locally adopted and utilized to develop short grain hybrids (whereas BU dhan2R could be utilized to develop aromatic long grain hybrids). Notably, local germplasm in this study had a greater frequency as maintainer lines than restorer lines. Identifying a promising maintainer allows backcrossing to develop a CMS line with agronomic superiority and other desirable aromatic traits. We developed Quasi-Hybrid 1 (QH1) through field evaluations at three geographic locations in Bangladesh during two growing seasons (T. Aman and Boro). While QH1 performed better in T. Aman than Boro, significant yield variation was not observed at different locations. Consequently, QH1 was submitted to, and approved by, the National Seed Board of Bangladesh for commercial cultivation.

Acknowledgment

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Conflicts of Interest

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

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PHENOTYPIC VARIATION AND YIELD PERFORMANCE OF PUMPKIN VARIETIES IN COASTAL AREA

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Abstract

The experiment was conducted to evaluate the phenotypic expression and yield attributes of different pumpkin varieties in response to saline stress. The research work was carried out at Satkhira (AEZ-11) in the experimental field of Bangladesh Agricultural Research Institute (BARI), On-Farm Research Division, Khulna from November 2020 to February 2021. The result indicated that the maximum vine length at harvest (330 cm), number of leavesvine^{-1} (40), fresh weight of root (179 g), dry weight of root (14.9 g), fresh weight of shoot (499.8 g), dry weight of shoot (93.7 g), root length (11.8 cm), female flowervine^{-1} (2), number of fruitplant^{-1} (5), individual fruit weight (4230 g), fruit yield (33.9 tha^{-1}), flesh thickness (4.9 cm) were recorded from pumpkin var. BARI Misti Kumra-1 and number of node for 1st male flower (6), number of node for 1st female flower (29), total Soluble Solid (9.9) and β-carotene (110.2 μg/g) were recorded from BARI Misti Kumra-2. The fruit yields of the varieties ranged from 19.4 to 33.9 tha^{-1}. BARI Misti Kumra-1 might have the mechanism that is responsible for salt tolerance as well as will be useful for selection and improvement of Cucurbitaceous species.

Keywords: Coastal saline, Phenotypic variation, Pumpkin,Yield

Introduction

Being a member of Cucurbitaceae family Pumpkin (*Cucurbita moschata*) is a very popular vegetable with great socio-economic importance and is grown extensively in many tropical and sub-tropical countries (Ahamed *et al.*, 2011). Pumpkin (*Cucurbita moschata*: Cucurbitaceae) is a very popular vegetable in many tropical and sub-tropical countries. In Bangladesh it ranks next to brinjal and radish in terms of cultivation area and production. According to the Bangladesh Bureau of Statistics (BBS) the total production of pumpkin was about 140483.9 metric tons (MTs) produced from 29941 acres of land and the average yield of pumpkin 30-35 t ha^{-1} (BBS, 2022).

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Soil salinity is the most brutal environmental factor for crop production worldwide that damages agricultural land. In Bangladesh, the coastal area has been severely impacted by salinity intrusion increasing with 83.3 million hectares to 102 million hectares between1973 to 2010.Over the last 35 years, salinity has increased to 26% within the country (Rabbani et al., 2018). In Bangladesh, 30% of cultivable land is covered by coastal area in addition 1.689 million hectares of coastal land about 1.056 million hectares are affected by various degrees of soil salinity. In coastal region yield is severely decreased i.e. approximately average 20-40% in major crops (cereals, potato, pulses, oil seeds, vegetables, species and fruit crops) (Miah *et al.,* 2020). Most of the literatures indicate that vegetable crops are more sensitive to salt at the vegetative stage than germination stages.

In pumpkins, carotenoid is a natural plant pigment which is responsible to give the orange color. Azizah *et al.,* (2009) reported that pumpkins consist of β-carotene and lycopene.Though soil salinity reduces the productivity of coastal regions but cultivation of pumpkin encouraging but they cannotcultivate extensively due to lack of salt tolerant variety as well as intercultural practices like irrigation, drainage, and mulching area very expensive. Through this research work attempt had been taken to know the considerable level of salinity for better performances of the three varieties.

Materials and Methods

This experiment was carried out in the experimental field of BARI, OFRD, Dawlatpur, Khulna from November 2020 to February 2021. The location of the experimental site was at the High Ganges River Flood Plain (22.8875 N latitude and 89.5167 E longitudes).The soil was clay loam heavy 7.15 P^H and 1.05% organic matter.

In this research work three varieties were used, namely BARI Misti Kumra-1, BARI Misti Kumra-2 and a local one. Local variety was collected from the market of khulna and BARI Misti Kumra-1, BARI Misti Kumra-2 from Bangladesh Agricultural Research Institute, Gazipur. The experiment was laid out in a Randomized Complete Block Design (RCBD) with 4 replications. The field soil was well pulverized and dried in the sun and decomposed cowdung was mixed with the soil. A basal dose of urea, triple super phosphate (TSP), muriate of potash (MP) and molybdenum were used as the source of nitrogen, phosphorus, potassium and molybdenum applied at the rate of @ 1500, 210, 120, 100 and 1kgha^{-1}, respectively. Urea was applied as top dressing in 2 equal splits at 15 and 30 days after transplanting (DAP). The entire amount of cowdung, TSP, MOP and molybdenum were applied at the time of final land preparation. Seeds were treated by provax @ 2 g/kg seed. Treated seeds were sown in the seedbed on 09 October 2020. Healthy and uniform sized 30 days old seedlings were transplanted on 08 November 2020. To prevent the competition of weeds with plants weeding was done 6 times and mulching is also done to keep the soil moist and aerated by breaking the soil crust. Four times irrigation at regular intervals during the growing season was applied. The parameters such as vine length at harvest (cm), number of leavesvine^{-1}, fresh weight of root (g), dry weight of root (g), fresh weight of shoot (g), dry weight of shoot (g), root length (g), number of node for 1st male flower, number of 1st female flower, male flower, female flowervine^{-1}, number of fruitplant^{-1}, individual fruit weight (g), fruit yield (tha^{-1}), flesh thickness (cm), total soluble solid and β-carotene were determined.t. Soil salinity was varied from 2.8 to 9.2 dS m^{-1}. Collected data were statistically analyzed by Software R (version 4.1.2) (R Core Team, 2021) and significance of the difference between pairs of mean by the DMRT test at 5% level of probability.

The soil salinity level during crop growing period is presented in Fig. 1. Soil salinity ranged from 2.8 dS/m to 9.2 dS/m. The lowest salinity 2.8 dS/m showed during month of November and the highest 9.2 dS/m represent at the end of March. It indicates that after the rainy season the salinity level drastically reduced but in the dry season like in march it reaches in its peak marks.

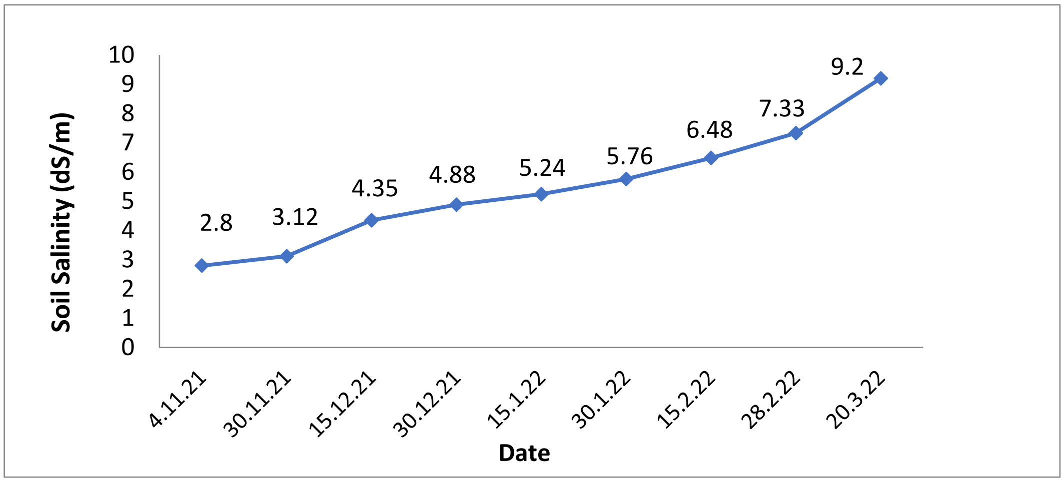

Fig. 1. Soil salinity during crop growing period in the experimental field

Results and Discussion

Pumpkin var. BARI Misti Kumra-1 was high rounded fruit having flesh color deep orange with golden yellow skin color at maturity. While BARI Misti Kumra-2 had the orange patch skin color at maturity, deep orange flesh color, flat rounded shape. Local variety had light yellow colored skin at maturity, slightly rounded in shape having flesh color yellowish. The fruit is typically orange and have many creases running from the stem to the bottom (Table 1).

Table 1. Fruit morphological parameters of different varieties of pumpkin

Variety	Fruit skin color at maturity	Flesh color	Shape
BARI Misti Kumra-1	Golden yellow	Deep orange	High Round
BARI Misti Kumra-2	Orange patch	Deep orange	Flat Round
Local	Light yellow	Yellowish, slightly rounded	High Round

Number of leavesvine^{-1} indicated great phenotypic variability under salinity conditions that has been elucidated in the (Table 2). In this observation, maximum number of leavesvine^{-1} was found in BARI Misti Kumra-1 (40) which was statistically similar with BARI Misti Kumra-2 (34) while in local variety (27). This result is confirmed by a Gabriel Filho*et al.,* 2022; who reported that leaves number reduces due to salinity. In root length of pumpkin, decreased significantly with increasing of salinity levels. The maximum root length (11.8 cm) was obtained from BARI Misti Kumra-1 which was statistically identical to BARI Misti Kumra-2 (10.4 cm) and the lowest value (7.6 cm) from local variety (Table 2). Highest reduction of fresh root weight (g) was found in local variety (135.7 g) which was closely related with BARI Misti Kumra-2 (168.1 g) whereas, lowest growth reduction in BARI Misti Kumra-1 (179.1 g) which was followed by BARI Misti Kumra-2 (168.1 g). The maximum fresh shoot weight (g) was obtained from BARI Misti Kumra-1 (499.8 g) which was parallel with BARI Misti Kumra-2 (492.52g) and minimum in local variety (359.2 g) and it was statistically identical with BARI Misti Kumra-2 (492.5 g). These results were found similar in the previous research findings of Kabir *et. al.,* (2020) in dry weight of root (g), dry weight of shoot (g) the effect of salinity was found to be significant with the increase of salt concentration (Table 2). The maximum fresh weight of root (179.1 g) was observed by BARI Misti kumra-1 as well as the maximum dry weight of root (g) was observed BARI Misti Kumra-1 (14.9 g) which was statistically similar with BARI Misti Kumra-2 (13.2 g) and minimum in local variety (11.2 g). The maximum dry weight of shoot (93.7 g) was observed BARI Misti Kumra-1 which was statistically identical with BARI Misti Kumra-2 (82.9 g) and minimum (48.9 g) was found in local variety. The results are in agreement by Kurum *et al.,* (2013) and Dadashpour (2012).

Table 2. Effects of varieties on morphological characters of pumpkin as affected by salinity during growth phases

Variety	Number of leavesvine^{-1}	Vine length at harvest (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)	Root length (cm)
BARI Misti Kumra-1	40 a	330.1 a	499.8 a	93.7 a	179.1 a	14.9 a	11.8 a
BARI Misti Kumra-2	34.2 ab	272.5 b	492.5 a	82.9 a	168.1 ab	13.2 ab	10.4 ab
Local	27.1 b	177.7 c	359.2 b	48.9 b	135.7 b	11.2 b	7.6 b
CV (%)	14.7	12.5	8.6	16.7	14.7	9.0	16.7

The number of node for 1st male flower was found significant (Table 3) where maximum number of node for 1st male flower was 6 that from BARI Misti Kumra-1 but identical to BARI Misti Kumra-2 (5). Local variety had lowest number of node for 1st male flower (4) and decreased in number of node for 1st female flower was found in local variety (17). The maximum number of node for 1st female flower was found in BARI Misti Kumra-1(22), which was statistically identical to BARI Misti Kumra-2 (20); Similar result was recorded by Kumanan and Devi (2021). Flesh thickness ranged from 2.9 cm to 4.9 cm. The thickest flesh was recorded from BARI Misti Kumra-1 (4.9 cm), which was statistically identical to BARI Misti Kumra-2 (4.5 cm). On the contrary, thinnest flesh was recorded in local variety (2.9 cm). An increase of the total soluble solid content was observed the highest in BARI Misti Kumra-1 (9.9) while salinity caused a highest reduction in local variety (7.3). These findings are supported by Mahmud *et al.*, (2016) and Del Amor *et al.*, (1999) indicating the phenotypic variability, heritability. Vine length ranged from 177.67 cm to 330.1 cm. The longest vine length at harvest (cm) was found in BARI Misti Kumra-1 (330.1cm) while the lowest from local variety (177.7cm). Number of fruit/plant was observed maximum in BARI Misti Kumra-1 (5), which was statistically identical to BARI Misti Kumra-2 (5) whereas local variety had minimum value (3) in number of fruitplant^{-1}. Tamilselvi and Jansirani (2017) corroborates with the present findings. In male flowervine^{-1}, the maximum increase was observed in BARI Misti Kumra-1 (29) while minimum in BARI Misti Kumra-2 (19).

Considering the female flowervine^{-1}, no significant difference was observed among the varieties. Sultana et al. (2015) also examined high variability in number of female flowers plant^{-1}, number of male flowers plant^{-1}.Significant differences were observed in TSS (%) which ranged from 7.3 to 9.9 %. The maximum TSS was found in BARI-Mistikumra-2(9.90%) which was statistically similar to BARI Misti kumra-1 (8.89%) while he lowest was found in local variety (7.3 %). Ahmed *et al.*, 2017 reported that TSS ranged from 7.38 to 10.75 % in 19 pumpkin genotypes; Rouf *et al.*, (2011) also found TSS in pumpkin varied from 6.10 to 9.10% which supports the present findings. The highest content of β-carotenoids was found in the BARI Mistikumra-2 (110.2 µg/g) and the lowest content was in local cultivar (43.8 µg/g). (Kulczynski and Gramza-Michałowska, 2019) also reported the variation in β-carotene from eleven cultivars ranged from 38.7 to 115.3 µg/gwhere the highest β-carotene was found from Melonowa Zolta (115.29 ± 0.95) and lowest was Porcelain Doll (38.67 ±1.7).

Table 3. Effects of varieties on yield and yield contributing characters of pumpkin as affected by salinity during their growth phase.

Variety	Number of node for 1st male flower	Number of node for1st female flower	Male flower vine^{-1}	Female flower vine^{-1}	Number of fruit plant^{-1}	Flesh thickness (cm)	Totale Soluble Soid	β-carotene (µgg^{-1})
BARI Misti Kumra-1	6 a	22 a	24 b	2.3	5 a	4.9 a	8.9 b	87.0 b
BARI Misti Kumra-2	5 a	20 a	29 a	2.3	5 a	4.5 a	9.9 a	110.2 a
Local	4 b	17 b	19 c	1.5	3 b	2.9 b	7.3 c	43.8 c
CV (%)	7.47	6.85	9.61	26.33	14.70	16.4	6.51	7.57

Some variations appeared in individual fruit weight (Fig. 2 (a)). The maximum weight of individual fruit was 4230 g which was recorded from BARI Misti Kumra-1 while minimum in local variety (1535 g). Variations were observed for fruit yield among the varieties under salinity conditions (Fig. 2(b)). Fruit yield (tha^{-1}) ranged from 19.4 to 33.87 tha^{-1}. The figure presented maximum fruit yield was obtained from BARI Misti Kumra-1 (33.8 tha^{-1}) and minimum was from local variety (19.4 tha^{-1}).

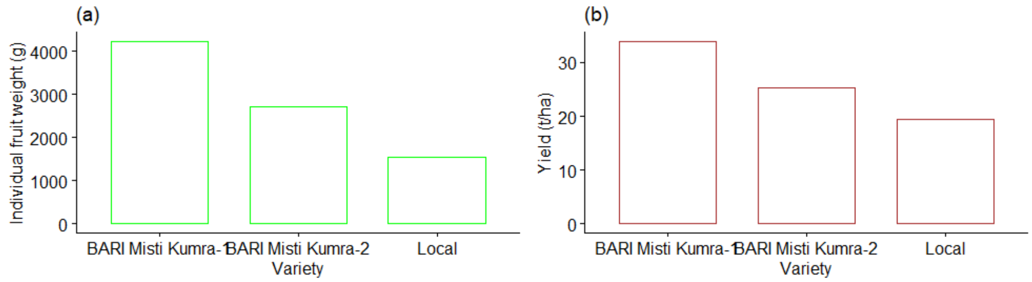

Fig. 2. (a) Individual fruit weight (b) Total Yield of different varieties affected by salinity in their growth phase

Pearson correlation analysis showed that maximum parameters have the strong positive correlation among them though they are non-significant in nature (Fig. 3). However, the highest positive correlation was found between the correlation of individual fruit weight and yield.

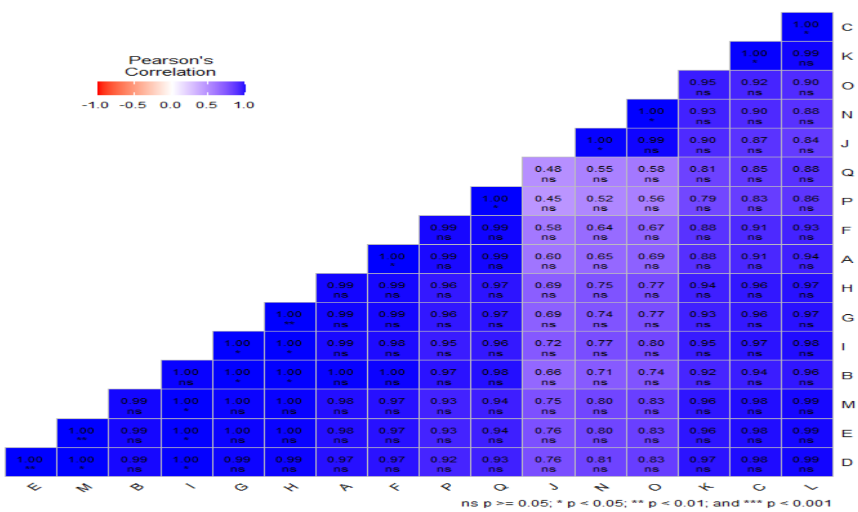

Fig. 3. Pearson correlation co-efficient of recorded parameters of pumpkin varieties.

A=Number of leaves vine^{-1}, B=Vine length at harvest (cm), C=Fresh weight of shoot (g), D=Dry weight of shoot (g), E=Fresh weight of root (g), F=Dry weight of root (g), G=Root length (cm), H=Number of node for 1st male flower, I=Number of node for1st female flower, J=Male flower vine^{-1}, K=Female flower vine^{-1}, L=Number of fruitplant^{-1}, M=Flesh thickness(cm), N=Total Soluble Soid, O=β-carotene (μg g^{-1}), P=Yield (t ha^{-1}), Q= Individual fruit weight (g)

Conclusion

This study examined the effects of salt concentrations on three pumpkin varieties (BARI Misti Kumra-1, BARI Misti Kumra-2 and local) in coastal saline area of Bangladesh. Though pumpkin is moderately salt tolerant but productivity decreases with increasing salinity. From the findings of this investigations, it may be concluded that in saline conditions, among three varieties, BARI Misti Kumra-1 renders the highest phenotypic attributes such as vine length,number of fruitplant^{-1},individual fruit weight, fruit yield, flesh thickness, TSS and β-carotene performance. On the other hand, the most affected pumpkin variety was local variety.

Conflicts of Interest

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

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DEVELOPMENT OF BLAST-RESISTANT RICE LINES THROUGH MARKER-ASSISTED SELECTION

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Abstract

Rice blast (*Pyricularia oryzae)* is a major constraint for rice productivity in Bangladesh and many other countries. An experiment was conducted from November 2022 to April 2023 in the Biotechnology Division of the Bangladesh Institute of Nuclear Agriculture (BINA) aimed at developing blast-resistant rice varieties using marker-assisted selection. Three rice varieties were used: BRRI dhan48, BRRI dhan58, and IRBL9-W. SSR markers were employed for F_1 confirmation, selecting 14 plants for backcrossing. PCR analysis and gel electrophoresis confirmed F_1 plants through polymorphic markers. A total of 97 BC_1F_1 seeds were produced. The study contributes to developing blast-resistant rice lines, crucial for mitigating yield loss caused by *Pyricularia oryzae*, a significant threat to rice production in Bangladesh.

Keywords: Backcrossing, Marker-assisted selection, *Pyricularia oryzae*, SSR Marker

Introduction

In Bangladesh, rice production is significantly harmed by blast disease. Blast is one of the most devastating diseases in rice-growing regions worldwide, responsible for 11-15% yield loss annually (Jabeen *et al.*, 2012). Rice is cultivated in both paddy and upland conditions. The relationship between lower water conditions and increased incidence of rice diseases, such as those caused by *Pyricularia oryzae*, has been extensively documented (Khan *et al.*, 2021). Environmental factors, including climatic changes leading to water scarcity, have been found to create conditions favorable for the proliferation of rice pathogens (Ramona *et al.*, 2021). Specifically, reduced water availability can stress rice plants, compromising their natural defenses and making them more susceptible to diseases (Zampieri *et al.*, 2023). Therefore, investigating production technologies aimed at cultivating rice under lower water conditions is crucial for understanding and mitigating the increased incidence of rice diseases in such environments. The pathogen might infect at any of the growth stages of the rice plant, from seedling to the pre-maturity stage of the crop (Yaduraju, 2013). Rice blast (*Pyricularia oryzae*) poses a vicious threat to the country's economy (Ganesh *et al.*, 2012).

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It is estimated that each year enough rice is destroyed by rice blast alone to feed 60 million people over three years. Out of the total yield loss due to diseases in rice, 35% is attributed to blast, 25% to sheath blight, 20% to BLB, 10% to tungro, and the remaining 10% to other diseases (Prasanna *et al.*, 2013). The disease causes yield losses ranging from 1-100% in Japan, 70% in China, 21-37% in Bali Indonesia, and 30-50% in South America and Southeast Asia (Kato, 2001). In 2017, Bangladesh experienced significant yield losses attributed to an epiphytotic outbreak of blast diseases caused by *Pyricularia oryzae*, affecting various regions including Dinajpur, Rangpur, Thakurgaon, Panchghar, Kushtia, Jashore, Pabna, Barishal, Mymensingh, Munshigonj, Chuadanga, among others. This outbreak led to substantial reductions in the production of Boro rice and transplanted Aman, with recorded disease severities of 21.19% and 11.98%, respectively. The most affected Boro rice varieties were BRRI dhan28 (29.6% disease severity), followed by BRRI dhan29 (25.9% disease severity) and BRRI dhan61 (21.9% disease severity), while the most affected T. Aman rice was BRRI dhan34 (22.9% disease severity) (Hossain and Ali, 2017). The major symptoms of this disease are found on leaves, with brownish spots having a grey center, a sunken lesion on the node, and a brown or black lesion found on the neck of the panicle. Among them, panicle blast is the most devastating and sometimes causes 80-100% yield loss (Pagliaccia *et al.*, 2018). The occurrence of this disease increased the market value of rice, posing a threat to national food security and the economy.

To identify blast-resistant F_1 genotypes, the marker-assisted selection technique was performed in the laboratory. Simple Sequence Repeat (SSR) markers are widely used as they are highly polymorphic in nature. They are powerful genetic markers due to their abundance, genetic co-dominance (identification of loci in either homozygous or heterozygous condition), dispersal throughout the genome, multi-allelic variation, and high reproducibility (Gao *et al*., 2005; Zhang *et al*., 2007; Thomson *et al*., 2009). In the present study, SSR markers were used to identify blast-resistant gene-containing varieties using Marker-Assisted Backcrossing (MABC).

Materials and Methods

Plant materials and crossing scheme

This experiment was conducted from November 2022 to April 2023 at the research field and laboratory of the Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. Three different rice varieties were obtained from the Bangladesh Rice Research Institute (BRRI) and the Bangladesh Institute of Nuclear Agriculture (BINA). Among them, the blast-resistant and high-yielding breeding line IRBL9-W was used as the donor parent. On the other hand, two popular high-yielding and blast-susceptible varieties, BRRI dhan48 and BRRI dhan58, were used as recipient parents (Nihad *et al*., 2022). Seeds of all these parental lines were sown in the experimental field at BINA. Subsequently, a primer survey was conducted among the parental lines to identify polymorphic primers, and conventional breeding was carried out to produce F_1 seeds. Two months later, F_1 seeds of two cross varieties, BRRI dhan48×IRBL9-W and BRRI dhan58×IRBL9-W, were soaked in petri plates for 48 hours to break dormancy. Seeds of each variety were placed in distinct, tagged petri plates. The bottom of each petri dish was filled with sterilized tissue paper, and a certain amount of water was added to each plate. Once the seeds started sprouting, they were removed from the water and air-dried for germination. After germination, the seeds were dispersed in the main seedbed. At 30 days of age, seedlings were transplanted into the research field at BINA, and the plants were labeled with stick labels.

PCR analysis for SSR markers

Vigorous 21-day-old leaves were carefully cut with sterilized scissors and washed in 70% ethanol and distilled water. The collected leaf samples were then placed in polythene bags with proper labeling and stored in an icebox to prevent damage to the leaf tissues during transportation to the laboratory. Subsequently, the samples were stored in a -80°C freezer. DNA was extracted from the leaves of each genotype using the DNA extraction miniprep method. The simplified mini-scale methodology of DNA extraction for PCR analysis recommended by IRRI was followed. The quality of the isolated DNA was confirmed to be satisfactory for PCR analysis (Zhang *et al*., 2009). For quantification of DNA concentration, a NanoDrop 2000c Spectrophotometer was used (Desjardins and Conklin, 2010). The total volume of PCR master mix for the study was 10 µl per sample, containing 1 µl of DNA. The components of the PCR cocktail preparation included Go Taq Green master mix (5.0µl), ddH_2O (3.0 µl), Primer Forward (0.5µl), Primer Reverse (0.5 µl), and DNA (100ng/ µl) (1.0 µl). The thermal profile of PCR cycles consisted of an initial denaturation at 94°C for 5 minutes, followed by denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, repeated for 35 cycles. The final extension was performed at 72°C for 5 minutes. After PCR, the products were mixed with 3 µl of 2X gel loading dye. The reagents used for Polyacrylamide Gel included 40% Acrylamide, 10% APS, 10X TBE Buffer, and TEMED (N,N,N',N'- tetramethylethane-1,2-diamine). For the preparation of an 8% Polyacrylamide Gel, premix (40% Acrylamide, 10X TBE Buffer, dH2O), APS (10%), and TEMED (N,N,N',N'- tetramethylethane-1,2-diamine) were mixed in the appropriate proportions. Approximately 2.5 µl of each PCR product was loaded into each well, and a 25bp DNA ladder was used for size determination. Electrophoresis was run for approximately 1.5 hours at 70 volts for each loaded gel. Subsequently, the gel was soaked in ethidium bromide (10mg/mL) solution for 20-30 minutes and placed on a high-performance ultraviolet light box (UV trans-illuminator) of a gel doc for analysis of the DNA bands. The DNA bands were observed using Alpha Easefc 4.0 software and the records were saved (Nadim *et al.*, 2022).

Confirmation strategy of F1 plants through MAS

Markers were evaluated based on the intensity of bands, consistency within the individual, presence of smearing, and potential for population discrimination. In this experiment, 96 random SSR markers were screened for two rice genotypes to evaluate their suitability for amplifying DNA sequences that could be accurately scored. The list of markers is provided in Table 1.

Table 1. List of SSR Markers

Sl. No.	Primer Name	Sl. No.	Primer Name	Sl. No.	Primer Name
1	RM271	37	RM34	73	RM103
2	RM515	38	RM101	74	RM86
3	RM316	39	RM112	75	RM47
4	RM408	40	RM39	76	RM3330
5	RM205	41	RM40	77	RM7023
6	RM17	42	RM41	78	RM115
7	RM171	43	RM132	79	RM7102
8	RM5493	44	RM109	80	RM1359
9	RM544	45	RM55	81	RM48
10	RM407	46	RM136	82	RM519
11	RM258	47	RM100	83	RM206
12	RM292	48	RM52	84	RM158
13	RM11	49	RM5501	85	RM1178
14	RM123	50	RM83	86	RM23818
15	RM18877	51	RM84	87	RM26063
16	RM23679	52	RM116	88	RM300
17	RM283	53	RM70	89	RM528
18	RM5953	54	RM3843	90	RM26416
19	RM166	55	RM107	91	RM445
20	RM131	56	RM80	92	RM27694
21	RM7	57	RM1377	93	RM7643
22	RM4	58	RM106	94	RM5404
23	RM49	59	RM6836	95	RM556
24	RM8	60	RM1361	96	RM7175
25	RM9	61	RM88		
26	RM50	62	RM53		
27	RM13	63	RM54		
28	RM14	64	RM87		
29	RM16	65	RM108		
30	RM19	66	RM5473		
31	RM126	67	RM8225		
32	RM22	68	RM117		
33	RM23	69	RM5961		
34	RM27	70	RM113		
35	RM29	71	RM82		
36	RM2	72	RM114		

DNA samples from 54 F_1 plants (39 plants of BRRI dhan48 × IRBL9-W and 15 plants of BRRI dhan58 × IRBL9-W) were collected for extraction. Using representative polymorphic markers as shown in Table 1, Polymerase Chain Reaction (PCR) was performed. Each F_1 genomic DNA was compared with its respective parental DNA. F_1 plants containing sharp parental DNA bands (both donor and recipient) were confirmed as true F_1 plants and subjected to subsequent background selection. The selected 28 F_1 plants were transferred to plastic pots with proper labeling and kept under shed for emasculation.

Backcrossing process for BC1F1 seeds production

For producing BC_1F_1 seeds, F_1 plants were kept in labeled pots. Two sets of BRRI dhan48 and BRRI dhan58 were seeded to synchronize flowering with F_1 plants for producing BC_1F_1 seeds. F_1 plants were emasculated, and labeled pots were kept in a glasshouse. The next morning, pollination was performed by dusting male flowers from the donor line onto the female stigma of F_1 plants and bagging them with glassine plastic bags with proper tagging. After a few days, when the seeds became mature, they were carefully collected and stored with proper tagging.

Statistical analysis

While traditional statistical tests were not the focus of this study, molecular marker analysis and selection processes played a critical role in achieving the study's goal of developing blast-resistant rice varieties through marker-assisted breeding. These genetic analyses are fundamental in plant breeding programs to select and propagate desirable traits efficiently. However, only the bar graph demonstrating comparison among the number of survived F_1 plants, selected F_1 plants for backcross breeding and BC_1F_1 seeds was constructed through Microsoft Excel 2019 software.

Results and Discussion

The primary aim of this study was to develop blast-resistant characteristics in a blast-susceptible rice variety through marker-assisted breeding. The selected plants were subsequently backcrossed to retain all desirable features. Two crossed rice genotypes were utilized for this experiment, namely F_1 genotypes of BR48× IRBL9-W and BR58× IRBL9-W, for the improvement of advanced blast-resistant lines. Initially, 96 primers were employed for the primer survey. Out of these, a total of 30 and 34 primers exhibited polymorphism in the two crosses, respectively. Ultimately, 5 primers showing highly polymorphic bands were selected and utilized for F_1 confirmation (Table 2). One of the primary challenges in rice production in Bangladesh is the prevalence of various diseases. In recent years, rice blast caused by *Pyricularia oryzae* has emerged in epidemic proportions, resulting in significant yield losses and posing a serious threat to our nation's food security and economy (Jabeen *et al*., 2012). To ensure food security, it is imperative to take necessary measures to manage this disease. Consequently, enhancing rice blast resistance has become one of the most crucial breeding objectives. This study aimed to develop blast-resistant rice varieties with the goal of producing blast-resistant advanced rice lines in the BC_1F_1 generation.

Primer survey

In this study, a total of 96 SSR primers were surveyed on both parental DNA to identify polymorphic markers. Primers containing heterozygous alleles (in comparison to one donor and one recipient parent) in the banding pattern of sample DNA were utilized for the selection process. Each number in the gel represented two parental DNA bands for one SSR primer. Both corners (right and left) of the gel contained various DNA ladders (20 bp and 100 bp) as indicators. DNA samples were isolated from both donor and recipient parental plants, and PCR was conducted using 11 polymorphic SSR markers. PCR bands from all parental plants were scored for heterozygous alleles for the donor and recipient parents.

Survey analysis

The survey was conducted with one recipient and one donor parent each, such as BRRI dhan48 and IRBL9-W, BRRI dhan58 and IRBL9-W, for each marker. A total of 48 primers were used for survey 1, and initially, 16 primers were selected, which exhibited a "Circular marking" in the gel. The primarily selected polymorphic primers from survey 1 were RM271, RM408, RM205, RM5493, RM258, RM11, RM123, RM23679, RM7, RM4, RM9, RM13, RM16, RM19, RM23, and RM55. Similarly, 14 primers were initially chosen from survey 2, with selected polymorphic primers including RM1377, RM1361, RM5473, RM8225, RM5961, RM3330, RM7102, RM519, RM206, RM26063, RM300, RM528, RM26416, and RM7175. Additionally, 20 primers were initially selected in the gel, with chosen primers from survey 3 being RM271, RM408, RM205, RM5493, RM407, RM258, RM11, RM23679, RM166, RM7, RM4, RM9, RM13, RM16, RM19, RM22, RM23, RM29, RM101, and RM55. Out of the 48 primers, 15 were primarily chosen, with primers from survey 4 being RM5501, RM107, RM1361, RM87, RM8225, RM5961, RM82, RM7102, RM519, RM206, RM1178, RM26063, RM528, RM27694, and RM7175.

Marker-Assisted Selection (MAS) is a molecular breeding process whereby a molecular marker, based on DNA variation, is used for the indirect selection of an agronomic trait of interest. MAS is not influenced by environments and can be conducted at any plant growth stage. It is particularly useful for the selection of recessive genes and biochemical traits in heterozygous plants. Undesirable genotypes can be quickly eliminated by MAS. This feature is particularly important and useful for some breeding schemes such as backcrossing and recurrent selection, which require crossing with or between selected individuals (Luo *et al*., 2014).

Highly polymorphic primer selection

Following primary selection, highly heterozygous band-showing primers were selected to identify true F_1 plants. Among these primers, five highly polymorphic markers were selected based on their sharp banding pattern and heterozygosity (Table 2).

Table 2. Highly polymorphic SSR markers with their representative parental genotypes

Parental genotypes	Highly polymorphic loci
BRRI dhan48 and IRBL9-W	RM23679, RM26063
BRRI dhan58 and IRBL9-W	RM13, RM9, RM101

A similar study was conducted by Xiao *et al*. (2019), in which they developed seven improved lines, comprising three monogenic lines, three two-gene pyramids, and one three-gene pyramid, by introgressing R gene(s) into a common genetic background using marker-assisted backcross breeding (MABB). They screened 302 SSR markers. All the improved lines conferred a wider resistance spectrum compared with their current parents. However, the three monogenic lines did not perform well under the field conditions of the two nurseries. Given their similar performances on the main agronomic traits as the recurrent parent, the two-gene pyramids achieved the breeding goals of a broad resistance spectrum and effective panicle blast resistance.

Another similar study was conducted by Singh *et al*. (2015). In that study, molecular screening and genetic diversities of major rice blast resistance genes were determined in 192 rice germplasm accessions using simple sequence repeat (SSR) markers. Two parents were screened for parental polymorphism using 96 SSR markers, of which 5 markers exhibited high polymorphism (Table 2). These markers were: RM23679= Chr. 9; RM26063= Chr. 11; RM101= Chr. 12; RM9= Chr. 1; RM13= Chr. 5. These loci were selected based on the polymorphic markers found in this study, which were used to confirm the F_1 plants.

F_1 plants generation

F_1 seeds of two crossed rice genotypes were sown in the experimental field of BINA with proper spacing and line maintenance. The number of F_1 seedlings that survived is presented in Fig. 3.

After the primer survey, five highly polymorphic primers were selected and tested for those F_1 genotypes (BRRI dhan48 and IRBL9-W= RM23679, RM26063; BRRI dhan58 and IRBL9-W= RM13, RM9, RM101). F_1 confirmation was conducted by analyzing DNA bands. F_1 plants carrying both parental DNA were confirmed as true F_1. Besides the selected F_1 plants, residual F_1 plants were removed. Out of 54 plants, only 14 F_1 plants were confirmed (BRRI dhan48 and IRBL9-W= 11 plants, BRRI dhan58 and IRBL9-W= 3 plants) (Fig. 3). Hasan *et al*. (2015) conducted a similar study. Leaf blast resistance line D521, neck blast resistance line D524, and BB resistance were developed through the introgression of leaf resistance gene *Pi1*, neck blast resistance gene *Pi2* derived from donor BL122, and bacterial resistance gene *Xa23* derived from donor CBB23 into an elite, early maturing maintainer line of hybrid rice susceptible to both blast and blight, Ronfeng B hybrid rice through marker-assisted backcross breeding programs. By using three SSR markers (MRG4766, AP22, and RM206), *Pi1*, *Pi2*, and *Xa23* were identified and used to test the recovery of the genetic background for the improved lines by using 131 polymorphic markers. Primer survey plays an important role, which is necessary for successful foreground, recombinant, and background selection. Polymorphic markers are essential for a marker-assisted selection process. They are required for confirming F_1 genotypes and improving advanced rice lines.

Confirmation of F_1 plants through polymorphic markers

DNA samples were collected from 54 F_1 plants. PCR was carried out using 5 polymorphic SSR markers. The following figures show the gel picture of F_1 confirmation. A particular SSR marker was used to compare alleles of each F_1 plant DNA with their parental DNA. Among all the F_1 plants, only a few were confirmed as true F_1. For F_1 confirmation of BRRI dhan48 × IRBL9-W cross generation using the RM23679 marker: P_1 and P_2 stand for BRRI dhan48 and IRBL9-W, respectively, and both corners of the gel contain 1kb DNA ladder (L) (Fig. 1). A total of 39 F_1 plants were compared with their parental DNA using the RM23679 marker, and out of these, 19 F_1 plants (plant no. 1, 8, 9, 10, 11, 12, 14, 15, 16, 19, 24, 26, 27, 28, 29, 31, 33, 35, and 39) were selected (Fig.1).

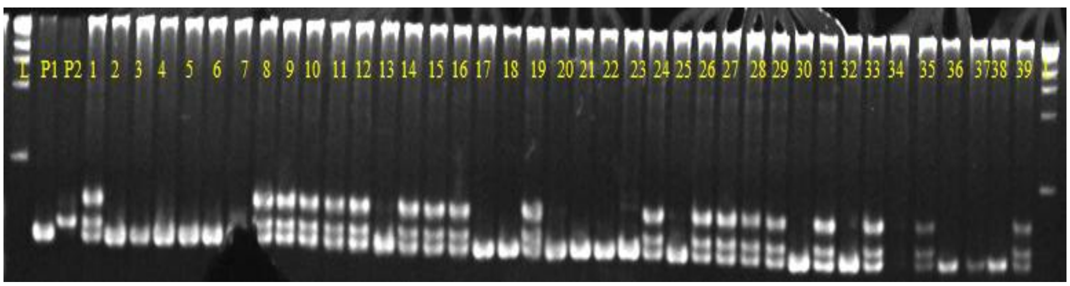

Fig. 1. F_1 confirmation using RM23679 marker for BRRI dhan48 × IRBL9-W cross generations

F_1 confirmation for the BRRI dhan48 × IRBL9-W cross generation using the RM26063 marker: BRRI dhan48 × IRBL9-W is marked as P_1 and P_2, respectively, and both corners of the gel contain a 1kb DNA ladder (L). The RM26063 marker was used to compare among 39 F_1 plants with their parental DNA, and out of these, 11 F_1 plants (plant no. 1, 9, 10, 11, 14, 15, 16, 19, 26, 28, and 31) were selected (Fig. 2).

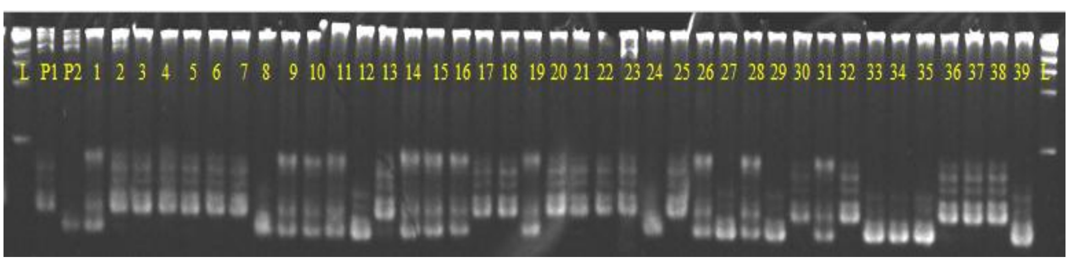

Fig. 2. F_1 confirmation using RM26063 marker for BRRI dhan48×IRBL9-W cross-generation

After analyzing the data from two gel docs (Fig. 1 and 2), 11 F1 plants were confirmed for the BRRI dhan48 × IRBL9-W genotype. True F_1 plants were confirmed based on the presence of double bands, each corresponding to the parental bands shown by the polymorphic primers. The confirmed F_1 plants for BRRI dhan48 × IRBL9-W were 1, 9, 10, 11, 14, 15, 16, 19, 26, 28, and 31.

For F_1 confirmation of the BRRI dhan58 × IRBL9-W cross generation using the RM13 marker: P_1 and P_2 represent BRRI dhan58 and IRBL9-W, respectively, and both corners of the gel contain a 1kb DNA ladder (L) (Fig. 2). A total of 15 F_1 plants were compared with their parental DNA using the RM13 marker, and out of these, 5 F_1 plants were selected.

Similarly, for F_1 confirmation of the BRRI dhan58 × IRBL9-W cross generation using the RM9 marker: A total of 15 F_1 plants were compared with their parental DNA using the RM9 marker, and out of these, 4 F_1 plants were selected.

For F_1 confirmation of the BRRI dhan58 × IRBL9-W cross generation using the RM101 marker: A total of 15 F_1 plants were compared with their parental DNA using the RM101 marker, and out of these, 3 F_1 plants were selected. After analyzing the data from three gel docs, 3 F_1 plants were confirmed for the BRRI dhan58 × IRBL9-W genotype. True F_1 plants were confirmed based on the presence of double bands, each corresponding to the parental bands shown by the polymorphic primers. The confirmed F_1 plants for BRRI dhan58 × IRBL9-W were 5, 6, and 10.

BC_1 F_1 seeds production

To produce BC_1F_1 seeds, F_1 plants were kept in labeled pots. Two sets of IRBL9-W were seeded for synchronization of flowering with F_1 plants to produce BC_1F_1 seeds. Subsequently, F_1 plants were emasculated, and then pots were placed in the greenhouse. The next morning, pollination was performed by dusting male flowers onto the female stigma and then bagged with a glassine plastic bag with proper tagging. After a few days, unpolished seeds matured. A total of 97 BC_1F_1 seeds were produced from 14 F_1 plants (Fig. 3).

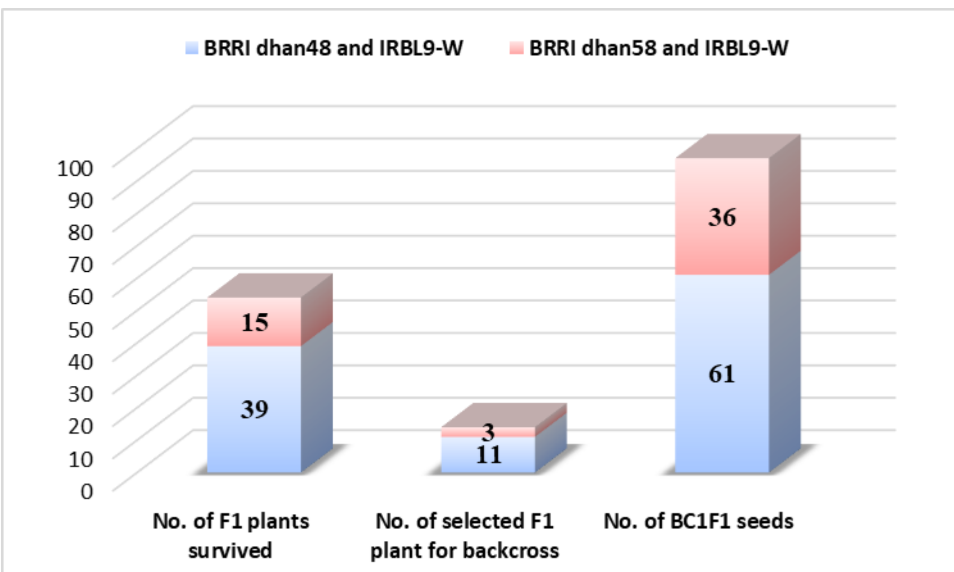

Fig. 3. Number of survived F_1 plants, selected F_1 plants for backcross breeding and BC_1F_1 seeds produced from BRRI dhan48 × IRBL9-W & BRRI dhan58 × IRBL9-W cross

The confirmed 14 F_1 plants were individually planted in plastic pots with appropriate labeling. Artificial emasculation was performed on the selected F_1 plants, which were then backcrossed with the recurrent parents (BRRI dhan48 and BRRI dhan58). Upon reaching maturity, BC_1F_1 hybrid seeds were collected, dried, and stored in paper bags with proper labeling. A total of 97 BC_1F_1 seeds were obtained from the selected cross combinations during the reporting period. The outcomes of the experiment mirror those of previously conducted studies aimed at developing blast-resistant varieties through marker-assisted selection processes. This study may offer unique opportunities for researchers to enhance blast-resistant advanced rice lines using various approaches.

Conclusion

The study involved primer surveys, PCR analysis for SSR markers, confirmation of F_1 plants, and backcrossing for BC_1F_1 seed production. Through meticulous selection processes and molecular marker analysis, 14 true F_1 plants were confirmed, leading to the production of 97 BC_1F_1 seeds. This approach holds promise for improving blast-resistant rice lines, crucial for ensuring food security amid disease threats.

Conflicts of Interest

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

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DETERMINATION OF THE POTENTIALITY OF DIFFERENT SESAME (*Sesamum indicum* L.) GENOTYPES

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Abstract

A field experiment was carried out in Randomized Complete Block Design (RCBD) with three replications at the experimental field of Sher-e-Bangla Agricultural University (SAU), Dhaka during the *Kharif* season of 2022 to examine the genetic variability, correlation, and path analysis based on 12 characters of 43 sesame genotypes. Analysis of variance exhibited significant differences among the genotypes for most of the characters except the number of capsules per plant. The phenotypic variance and phenotype coefficient of variation were relatively higher than the respective genotypic variance and genotypic coefficient of variation for all the characters. High broad sense heritability together with high genetic advance in percent of mean was observed for number of secondary branches per plant (90.1%), 1000-seed weight (99.3%), and seed yield per plant (97.9%) while moderate heritability for days to 50% flowering (59.8%). The correlation coefficient analysis revealed that seed yield per plant had a significant positive correlation with plant height, days to 50% flowering, days to 80% maturity, number of primary branches per plant, number of capsules per plant, number of seeds per capsule, height of the first capsule, and 1000-seed weight. It appears from path co-efficient analysis that plant height (0.856), days to 80% maturity (0.227), number of primary branches per plant (0.467), number of secondary branches per plant (0.441), capsule length (0.258), and number of seeds per capsule (0.213) had a positive direct effect on the yield per plant whereas, internode length (-0.799) followed by number of capsules per plant (-0.370), and days to 50% flowering (-0.198) had a negative direct effect. Based on mean performance, heritability, and interrelationship, the genotypes G6, G12, and G36 for seed yield per plant, and the genotypes G26, G27, and G37 for early maturity could be selected for further varietal improvement of sesame.

Keywords: Correlation, Genetic variability, Heritability, Sesame genotype

Introduction

Sesame (*Sesamum indicum* L., 2n = 26) is an annual flowering plant of the Pedaliaceae family which is an ancient oilseed crops (Abdipour *et al.*, 2018). It is also known as “Till’ in Bangladesh. Several wild relatives occur in Africa and a smaller number in India (Nayar and Mehra, 1970). It exhibits drought tolerance, grows well in

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most of the well-drained soils and various agro climatic regions, and is well adapted to different crop rotations (Tripathi *et al.*, 2013). The largest area of production is currently believed to be in India, but the crop is also grown in Myanmar, Sudan, Uganda, Ethiopia, Nigeria, Tanzania, Pakistan, Korea, Russia, Turkey, Mexico and South America (Kumar *et al.*, 2012). Sesame ranks sixth in the world in respect of edible oil seeds production 6172.32 thousand metric tons from 13965.844 thousand ha of land of which 70% was produced in Asia and 25% in Africa (www.fao.org/faostat/). The average sesame yield in Bangladesh is about 944 kg per ha. Sesame can be cultivated in both *Kharif* and *Robi* seasons in Bangladesh; nonetheless, more is produced (about two-third) in *Kharif* season (Chowdhury and Hassan, 2013). It loves high land with sandy loam soil. Sesame seeds containing 50% oil and 25% protein are used in baking, candy making, and some other food industries (Manikantan *et al.*, 2015). High quality edible and medicinal oil can be extracted from sesame seed, which can be stored for a long time. Sesame oilcake (byproduct) is used as feed for poultry, fish, cattle, goat and sheep (Khan *et al.*, 2009). Moreover, sesame oil contains about 47% oleic and 39% linoleic acid (Sultana *et al.*, 2019). It is used in cooking, salad and margarine as well as ingredients for pharmaceutical and cosmetic industries, and synergist for insecticides (Salunkhe and Desai, 1986). It also contains lignans and sesamol which are known to have anticancer activities (Kapadia *et al.*, 2002). Bangladesh has been suffering from serious deficit of edible oil since past several decades. To cope with this shortage, every year, huge amount of foreign currency is being spent for importing edible oil from abroad. The sesame production is still lower than other oilseed crops mainly due to the lack of high yielding varieties (HYV), biotic and abiotic stresses, and shattering problem. Sesame is treated as less input intensive crop, hence, breeding improved varieties could be a promising approach (Ashri, 1988). A very little research has been done on improvement of the sesame in Bangladesh. Few sesame varieties have been released by this time from different research institutes but this is not enough to face the future challenges. Therefore, development of high yielding sesame variety having resistance/tolerance to biotic and biotic stresses is very much essential.

Up to now majority of the released sesame varieties in different countries are the product of selection and pedigree breeding. This is due to the lack of sufficient genetic variation within the existing germplasm collections, especially for traits such as resistance to various diseases and seed retention capacity etc. (Zanten, 2001). However, selection for high yield is made difficult due to its complex nature in sesame. The polygenic inheritance of yield components makes accurate selection more difficult. Moreover, these complex traits are highly influenced by environment, which reduces the progress to be achieved through direct selection. In such cases, there is another option to hasten the genetic improvement which is known as indirect selection for yield. So, the phenotypic and genotypic variability studies play an important role to select the better sesame crop. Besides, knowledge of the naturally occurring diversity in a population helps to identify diverse groups of genotypes that can be useful for the breeding program. Therefore, the present research considered on genetic variability, heritability and genetic advance among different sesame germplasm to select potential genotypes for utilization in further varietal improvement of sesame.

Materials and Methods

The experiment was conducted at the experimental field of SAU, Dhaka during *Kharif* season 2022 with forty-three sesame genotypes with three replications following the randomized complete block design (RCBD). The sesame genotypes were from Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute (BARI) and different sesame growing regions of Bangladesh (Table 1). All the genotypes were randomly distributed to each plot with each block as per experimental design. The recommended dose of fertilizers and cow dung were applied and standard agronomic management was practiced. The total amount of TSP, MP, along with 50% of the urea were applied during final land preparation while rest amount of the urea was top-dressed at flower bud initiation stage. The data were collected on days 50% to flowering, days 80% to maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, capsule length (cm), number of seeds per capsule, height of first capsule (cm), internode length (cm), thousand seed weight (g) and seed yield per plant (g). Analysis of variance (ANOVA), correlation coefficients were determined by R Studio package (https://www.rstudio.com). Phenotypic and genotypic variance was determined as per Johnson *et al.* (1955). Heritability and genetic advance were assessed following Singh and Chaudhury (1985) and Allard (1960). Genotypic and phenotypic co-efficient of variation were estimated by the formula of Burton (1952).

Table 1. Different genotypes of sesame with their source of collection

Sl. No.	Genotype	Accession Number	Source of collection	Sl. No.	Genotype	Accession Number	Source of collection
1	G1	BD-10643	PGRC[1]	23	G23	BD-11637	PGRC
2	G2	BD-10645	PGRC	24	G24	BD-11638	PGRC
3	G3	BD-10648	PGRC	25	G25	BD-11639	PGRC
4	G4	BD-10652	PGRC	26	G26	BD-11640	PGRC
5	G5	BD-10654	PGRC	27	G27	BD-11641	PGRC
6	G6	BD-10661	PGRC	28	G28	BD-11642	PGRC
7	G7	BD-11621	PGRC	29	G29	BD-11643	PGRC
8	G8	BD-11622	PGRC	30	G30	BD-11644	PGRC
9	G9	BD-11623	PGRC	31	G31	BINA Til-2	BADC[2]
10	G10	BD-11624	PGRC	32	G32	BINA Til-4	BADC
11	G11	BD-11625	PGRC	33	G33	BARI Til-3	BADC
12	G12	BD-11626	PGRC	34	G34	BARI Til-4	PGRC
13	G13	BD-11627	PGRC	35	G35	Si/GPB/22/00041	Rajbari
14	G14	BD-11628	PGRC	36	G36	Si/GPB/22/00042	Pabna
15	G15	BD-11629	PGRC	37	G37	Si/GPB/22/00043	Chuadanga
16	G16	BD-11630	PGRC	38	G38	Si/GPB/22/00044	Chuadanga

Sl. No.	Genotype	Accession Number	Source of collection	Sl. No.	Genotype	Accession Number	Source of collection
17	G17	BD-11631	PGRC	39	G39	Si/GPB/22/00045	Kurigram
18	G18	BD-11632	PGRC	40	G40	Si/GPB/22/00046	Rangpur
19	G19	BD-11633	PGRC	41	G41	Si/GPB/22/00047	Moulovibazar
20	G20	BD-11634	PGRC	42	G42	Si/GPB/22/00048	Bagura
21	G21	BD-11635	PGRC	43	G43	Si/GPB/22/00049	Gazipur
22	G22	BD-11636	PGRC				

[1]PGRC: Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur; [2]BADC: Bangladesh Agricultural Development Corporation

Results and Discussion

Genetic variability

The analysis of variance (ANOVA) showed highly significant variation among the sesame genotypes for all the traits (Table 3) suggesting a wide scope of selection for these characters which provides a good opportunity for improving traits of interest through breeding programs. The result showed that the genotypes G26 and G27 were early flowering that required only 50 days for 50% flowering, while the genotypes G40 (56.7 days) and G43 (56.7 days) were late in flowering (Table 2). Phenotypic and genotypic variance for 50 days to 50% flowering (DFF) was 2.99 and 1.79, respectively (Table 3). The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for DFF were 2.46 and 3.18, respectively. The PCV was higher than GCV indicated environmental influence on the phenotypic expression of this trait. A moderate heritability (59.8%) with low genetic advance (2.13) and low genetic advance in percent of mean (3.91) was observed for this trait. Robinson *et al.* (1949) categorized the heritability as low (0-30%), moderate (30-60%) and high (60% and above) while the genetic advance as a percentage of the mean (GAPM) was classified by Johnson *et al.* (1955) as low (0-10%), moderate (10-20%) and high (20% and more). However, a high heritability with high genetic advance in percent of mean was reported by Pavani *et al.* (2020) for DFF which is dissimilar with the present results. The genotypic and phenotypic variances for days to 80% maturity (DEM) were 1.5 and 2.3, respectively (Table 3). The GCV (1.2) was lower than the PCV (1.5). The high heritability (65.95%) with low genetic advance (2.05) and genetic advance in percent of mean (2.07) were found for DEM indicating that the phenotypic selection would not be effective. Nonetheless, Thouseem *et al.* (2022) also reported low heritability for days to maturity in sesame. The maximum and minimum plant height (PH) were recorded in G12 (154.47 cm) and G33 (115.5 cm), respectively with the mean value of 132.9 cm (Table 2). The phenotypic variance (191.5) was higher than genotypic variance (40.5) (Table 3) suggested that there was influence of environment on the phenotypic expression of the genes controlling plant height. The PCV and GCV were 10.41and 4.79, respectively. Low heritability (21.16%) with low genetic advance (6.03) and low genetic advance in percent of mean (4.54) (Table 3) suggesting that PH was governed by non-additive gene. On the contrary, Durge *et al.* (2022) reported a high heritability coupled with moderate to high genetic advance for plant height. The lowest number of primary branches per plant (NPBP) was found in G19 (3.7) followed by G32 (4.1) whereas the highest was observed in G22 (7.5) with the mean value of 5.2 (Table 2 and Table 3). The phenotypic variance (1.4) was higher than genotypic variance (0.37) (Table 3) suggested that there was influence of environment on the phenotypic expression of this character. The PCV and GCV were 22.6 and 11.6, respectively. Low heritability (26.4%) with low genetic advance (0.65) and moderate genetic advance in percent of mean (12.3) (Table 3) indicated this character was governed by non-additive gene. But, high heritability for NPBP was reported by Kumar *et al.* (2022) in sesame. The lowest number of secondary branches per plant (NSBP) was observed in G38 (2.13) whereas the highest was observed in G20 (7.7) with the mean value of 4.2 (Table 2 and Table 3). The phenotypic variance (2.5) was slightly higher than genotypic variance (2.2) suggested that there was few influence of environment on the expression of the genes controlling this trait. The PCV and GCV were 32.1 and 30.5, respectively. High heritability estimated (90.06%) was recorded for NSBP with high genetic advance in percent of mean (59.6) (Table 3) which revealed that this character was governed by non-additive gene but high genetic advance in percentage of mean which indicated that possibility of predominance of additive gene, so much scope to improve this trait through phenotypic selection. Similar to our result Roy *et al.* (2022) also reported high heritability coupled with high genetic advance for number of branches per plant. The highest number of capsules per plant (NCP) found in G22 (219.40) while the lowest was observed in G31 (105.20) (Table 2). The NCP showed the highest phenotypic variance (1703.13) and highest genotypic variance (183.78) which indicates large environmental influence over genotypes. The PCV (29.05) was higher than the GCV (9.5) suggesting that the presence of adequate variation between the genotypes (Table 3). The heritability estimates for this trait was low (10.79%) with low genetic advance (9.17) and low genetic advance in percent of mean (6.46) suggesting non-additive gene effects for this trait. However, Paramasivam and Prasad (1981) reported opposite to our result as high heritability estimates for NCP. The capsule length (CL) ranged from 1.31 cm (G22) to 4.08 cm (G28) (Table 2). The phenotypic and genotypic variances for this trait were 0.27 and 0.11, respectively. The PCV and GCV were 23.43 and 14.76, respectively for CL indicated that moderate variation exists among sesame genotypes for this trait (Table 3). The moderate heritability (39.69%) was observed for CL with moderate genetic advance in percent of mean is (19.16) suggesting that the additive genes controlling this trait. Therefore, phenotypic selection based for CL would be effective. Kumar *et al.* (2022) also reported a similar result as high heritability for capsule length. The maximum number of seeds per capsule (NSC) was recorded in G5 (69.4) while the minimum in G16 (44.3) (Table 2).

The phenotypic variance (43.6) was much higher than the genotypic variance (24.2) indicated a great influence of environment on the phenotypic expression of this trait. The PCV (11.5) was also higher than the GCV (8.6) for NSC suggesting a moderate variation among the sesame genotypes (Table 4). This character also showed a moderate heritability (55.4%) with moderate genetic advance in percent of mean (13.1). The result revealed that the additive genetic effect plays an important role for the phenotypic expression. Thus, phenotypic selection for this character would be so rewarding. Srikanth and Ghodke (2022) also reported high heritability coupled with high genetic advance as percentage of mean for number of seeds per capsule in sesame. The maximum values for height of first capsule (HFC) was found in G38 (73.9 cm) whereas the minimum was in G26 (31.2 cm) with the mean vale of 53.2 cm (Table 2). The phenotypic and genotypic variances for HFC were 68.96 and 78.46, respectively (Table 3). The PCV and GCV were 22.8 and 15.61, respectively (Table 4). The moderate heritability (46.78%) was found for HFC with moderate genetic advance (11.70) and moderate genetic advance in percent of mean (22.00). Pavani *et al.* (2020) found high heritability and high genetic advance for HFC in sesame. The highest internode length (IL) was recorded in G9 (13.46 cm) whereas the lowest was in G32 (5.4 cm) with the mean vale of 8.6 cm (Table 2). The phenotypic and genotypic variances were 3.83 and 1.47, respectively (Table 3). The PCV and GCV were 22.8 and 14.11, respectively. The moderate heritability (38.45%) was found for IL with moderate genetic advance in percent of mean (18.02). Nevertheless, high genetic advance in per cent of mean was observed by Kumar *et al.* (2022) in sesame. The highest thousand seed weight (TSW) was found in G14 (4.3) and G23 (4.3) whereas the lowest in G29 (0.72) with the 2.7 g mean value (Table 2). There was a few difference between genotypic (0.66) and phenotypic (0.69) variances suggested that environmental factors have less effect on the phenotypic expression of TSW.

The PCV and GCV were 30.92 and 30.81, respectively (Table 4). There was also a very few difference between PCV and GCV, indicating less environmental influence on this trait. A very high heritability (99.31%) with high genetic advance in percent of mean (63.25). It is suggested that the phenotypic selection for TSW would be rewarded. A similar result was also reported by Srikanth and Ghodke (2022) and Kumar *et al.* (2022) for TSW in sesame. The highest seed yield per plant (SYP) was found in G6 (18.07 g) while the minimum in G3 (2.74) with the 13.60 g mean value (Table 2). The phenotypic variances and genotypic variances for this trait were 12.95 and 12.67, respectively. The PCV and GCV were 32.01 and 31.7, respectively. The less differences between genotypic and phenotypic variances as well as PCV and GCV indicated that there is less influence of environmental factors on this trait. The SYP showed a very high heritability (97.89%) with high genetic advance in percent of mean (64.54) (Table 3). Thus suggesting that the major role of additive genetic effects and less environmental influence for the phenotypic expression of this trait and the phenotype selection would be rewarded for SYP. Roy *et al.* (2022), and Srikanth and Ghodke (2022) also reported high heritability coupled with high genetic advance for seed yield per plant in sesame.

Table 2. Mean performances of 43 sesame genotypes in respect of 12 traits

Genotypes	DFF	DEM	PH	NPBP	NSBP	NCP	CL	NSC	HFC	IL	TSW	SYP
G1	54.67c-f	99.00b-g	134.44b-f	6.33a-d	7.47ab	150.93b-h	2.52b	54.40i-m	59.20b-g	8.94b-j	2.09no	14.89cd
G2	55.33a-d	102.33a	140.37a-d	5.33c-j	5.73d-g	181.47a-c	2.50b	58.60c-k	46.40g-o	9.17b-j	1.81qr	9.17lm
G3	55.33a-d	100.67a-d	147.57a-d	5.67c-i	6.47cd	124.13c-h	2.18b-e	54.87h-m	69.53a-c	10.03b-f	2.48j	2.74s
G4	53.00f-h	97.67f-i	126.33f-h	4.27h-j	7.33ab	131.67c-h	2.23b-e	65.20abc	42.07k-p	7.40g-l	2.57ij	10.24ijk
G5	55.67a-d	99.33b-f	140.78a-d	4.87d-j	4.53j-n	139.33c-h	2.23b-e	69.40a	47.27g-o	7.86f-l	2.20mn	10.99i
G6	55.00a-e	100.67a-d	130.84b-h	5.40c-i	7.53ab	208.33ab	2.10b-e	65.80ab	44.07i-p	8.14e-k	4.12b	18.07a
G7	54.00d-f	101.33ab	140.18a-f	4.40g-j	2.47st	130.53c-h	2.18b-e	57.53e-l	49.67f-n	8.74b-j	2.60i	10.77i
G8	55.00a-e	99.33b-f	117.23gh	4.87d-j	5.67d-g	126.40c-h	1.87b-f	62.67a-g	55.13d-l	7.46g-l	3.47e	7.42pq
G9	55.67a-d	98.33d-i	134.97a-h	4.80d-j	6.87bc	137.40c-h	2.17b-e	64.07a-e	57.47b-i	13.46a	1.30t	12.17gh
G10	54.67b-f	100.33a-e	128.78c-h	4.87d-j	7.53ab	141.47c-h	2.15b-e	57.07e-l	50.60e-n	8.24c-k	2.31kl	12.05h
G11	54.00d-f	100.00a-f	131.02b-h	5.00c-j	5.33f-j	172.53a-f	2.16b-e	54.53i-m	47.13g-o	6.90j-l	4.20b	14.12de
G12	56.00a-c	99.67b-f	154.47a	6.27a-e	5.53f-h	186.53a-c	2.29bc	64.80a-d	63.93a-f	10.60b-e	4.20b	17.39ab
G13	54.00d-f	98.67c-h	140.38a-f	4.87d-j	4.67i-n	138.27c-h	2.08b-e	61.07b-j	46.00g-o	8.19d-k	2.36k	9.81kl
G14	54.33cd-f	100.00a-f	136.67a-g	5.27c-j	3.27q-s	117.60d-h	2.39bc	62.47a-g	57.87b-i	10.12b-f	4.32a	12.51gh
G15	54.00d-f	98.67c-h	123.03e-h	4.60f-j	5.00g-l	146.13b-h	2.22b-e	54.73h-m	40.67m-p	7.05i-l	1.54s	7.69op
G16	53.33e-g	99.00b-g	117.57gh	4.73d-j	5.13f-k	101.20h	2.27b-d	44.27o	46.47g-o	8.43b-j	1.98op	8.09op
G17	54.67b-f	98.33d-i	133.65b-h	5.40c-i	7.47ab	174.47a-e	2.30bc	61.20b-i	51.80e-n	8.18d-k	3.02f	15.34c
G18	54.33c-f	98.67c-h	142.32a-e	6.07a-f	5.93d-f	149.33b-h	2.31bc	52.67k-n	63.53a-f	9.42b-i	1.98op	10.70ij
G19	54.67b-f	99.00b-g	123.51e-h	3.73j	3.53o-r	107.60gh	2.26b-e	51.73k-n	51.67e-n	8.32c-k	2.53ij	8.28no
G20	54.00d-f	99.33b-f	132.87b-h	5.53c-i	7.73a	159.33a-h	2.19b-e	46.60no	44.73h-p	8.78b-j	1.54s	8.31no
G21	54.67b-f	100.00a-f	150.38ab	5.20c-j	3.27q-s	137.80c-h	2.13b-e	57.07e-l	64.93a-e	8.53b-j	2.31klm	16.65b
G22	55.00a-e	100.00a-f	126.57d-h	7.53a	6.40c-e	219.40a	1.31f	57.00e-l	56.73c-j	6.81jkl	3.96c	13.82e
G23	55.00a-e	99.33b-f	124.09e-h	4.47f-j	3.53o-r	112.40e-h	1.36f	58.40c-k	55.13d-l	7.20h-l	4.32a	9.91j-l
G24	54.33c-f	100.00a-f	122.85e-h	5.73b-i	3.27q-s	127.40c-h	1.61d-f	61.87b-h	50.93e-n	7.12i-l	2.55ij	12.95fg
G25	54.67b-f	101.00a-c	142.67a-e	5.00c-j	4.40k-n	128.67c-h	2.47b	62.87a-f	58.67b-h	9.75b-g	1.75r	14.13de
G26	50.00i	96.33hi	117.12gh	4.67e-j	4.33k-o	129.13c-h	1.61ef	58.00d-k	31.20p	5.87kl	1.90pq	7.42pq
G27	50.00i	96.67g-i	121.44f-h	4.73d-j	5.47f-i	149.13b-h	1.80c-f	62.47a-g	34.13op	6.95i-l	1.87q	5.10r
G28	54.00d-f	98.00e-i	141.12a-f	4.47f-j	3.53o-r	169.47a-g	4.08a	54.47i-m	41.87l-p	8.31c-k	3.12f	10.23ijk
G29	52.00gh	96.00i	127.33d-h	4.40g-j	4.20l-p	143.73c-h	2.43bc	52.53k-n	39.87n-p	8.38b-j	1.72r	6.77q
G30	54.67b-f	99.67b-f	141.25a-f	5.73b-i	5.93d-f	137.87c-h	2.36bc	54.73h-m	58.80b-h	9.63b-h	3.75d	13.74ef
G31	55.67a-d	100.00a-f	133.59b-h	5.07c-j	3.47p-r	105.20h	2.38bc	53.67k-n	64.87a-e	9.30b-j	2.35k	12.67gh
G32	54.33c-f	99.67b-f	126.74d-h	4.13ij	2.80r-t	143.93c-h	2.47b	54.00j-m	42.60j-p	5.43l	2.26k-m	9.10lmn
G33	54.33c-f	98.67c-h	115.47h	6.60a-c	3.27q-s	124.20c-h	2.37bc	58.60c-k	59.87a-g	7.24h-l	2.81gh	8.10op
G34	56.00a-c	99.33b-f	135a-h	5.87b-h	4.20lm-p	108.60gh	2.43bc	55.53g-m	67.60a-d	8.34b-k	3.08f	14.03e
G35	56.00a-c	101.00a-c	132.74b-h	5.73b-i	3.53o-r	138.60c-h	1.92b-f	66.47ab	56.53c-j	7.15h-l	3.91c	14.27de
G36	56.33ab	101.00a-c	124.87d-h	7.33ab	4.58j-n	179.27a-d	2.39bc	62.87a-f	55.53c-l	10.65b-d	2.21lm	17.24ab
G37	51.33hi	96.00i	128.87c-h	4.67e-j	4.73h-m	109.53f-h	2.32bc	52.73k-n	54.73d-m	10.82b	2.59ij	5.90r
G38	55.67a-d	100.00a-f	144.58a-d	4.60f-j	2.13t	125.07c-h	2.25bcde	60.87b-j	73.93a	10.73bc	2.89g	7.67op
G39	56.00a-c	100.00a-f	131.76b-h	6.00a-g	5.60e-g	150.73b-h	2.20b-e	48.53m-o	59.27b-g	8.75b-j	2.51ij	12.60gh
G40	56.67a	100.00a-f	137.53a-f	4.80d-j	3.87n-q	141.60c-h	2.34bc	52.80k-n	49.60f-n	9.23b-j	2.86gh	12.54gh
G41	54.67b-f	99.33b-f	123.81e-h	4.53f-j	3.93m-q	146.20b-h	2.34bc	53.60k-n	46.93g-o	8.52b-j	2.53ij	8.46m-o
G42	54.00d-f	100.00a-f	144.35a-d	4.27h-j	4.00m-q	129.47c-h	2.45bc	50.47l-o	56.40c-k	9.75b-g	2.75h	13.90e
G43	56.67a	100.00a-f	142.65a-e	6.53a-c	5.13f-k	125.40c-h	2.43bc	56.53f-l	71.33ab	10.19b-f	2.35k	15.49c

PH = Plant height (cm), DFF = Days to 50% flowering, DEM = Day of 80% maturity, NPBP = Number of primary branches per plant, NSBP = Number of secondary branches per plant, NCP = Number of capsules per plant, CL = Capsule length (cm), NSC = Number of seeds per capsule, HFC = Height of first capsule (cm), IL = Internode length (cm); TSW = Thousand seed weight (g), SYP = Seed yield per plant (g).

Table 3. Estimation of genetic variability for yield contributing characters related to yield of sesame genotypes

Parameters	Range		MS	Mean	CV (%)	σ^2p	σ^2g	σ^2e	PCV	GCV	h^2_b	GA	GA (% mean)
	Max	Min											
DFF	56.67	50.00	6.58**	54.50	2.01	2.99	1.79	1.20	3.18	2.46	59.81	2.13	3.91
DEM	102.33	96.00	5.29**	99.20	1.58	2.28	1.50	0.89	1.52	1.24	65.95	2.05	2.07
PH	154.47	115.47	272.52*	132.88	9.25	191.49	40.51	150.98	10.41	4.79	21.16	6.03	4.54
NPBP	7.53	3.73	2.12**	5.22	19.37	1.39	0.37	1.02	22.58	11.60	26.40	0.65	12.27
NSBP	7.73	2.13	9.45**	4.24	10.14	2.48	2.23	0.25	32.14	30.50	90.06	2.92	59.63
NCP	219.40	101.20	2070.7**	142.03	27.44	1703.13	183.78	1519.35	29.05	9.54	10.79	9.17	6.46
CL	4.08	1.31	0.49**	2.23	18.20	0.27	0.11	0.16	23.43	14.76	39.69	0.43	19.16
NSC	69.40	44.27	91.98**	57.43	7.68	43.64	24.17	19.48	11.50	8.56	55.37	7.53	13.12
HFC	73.93	31.20	285.34**	53.18	16.66	147.41	68.96	78.46	22.83	15.61	46.78	11.70	22.00
IL	13.46	5.43	6.79**	8.61	17.86	3.83	1.47	2.36	22.76	14.11	38.45	1.55	18.02
TSW	4.32	1.30	2.04**	2.67	2.56	0.69	0.66	0.03	30.92	30.81	99.31	1.69	63.25
SYP	18.07	2.74	38.30**	13.60	4.65	12.95	12.67	0.27	32.01	31.66	97.89	7.26	64.54

*, ** indicate significant at 5% and 1% level of probability, respectively.

PH = Plant height (cm), DFF = Days to 50% flowering, DEM = Day of 80% maturity, NPBP = Number of primary branches per plant, NSBP = Number of secondary branches per plant, NCP = Number of capsules per plant, CL = capsule length (cm), NSC = Number of seeds per capsule, HFC = Height of first capsule (cm), IL = Internode length (cm), TSW = Thousand seed weight (g) and SYP = Seed yield per plant (g); σ^2p = Phenotypic variance, $\sigma^2 g$ = Genotypic variance, $\sigma^2 e$ = Environmental variance, PCV = Phenotypic co-efficient of variation, GCV = Genotypic co-efficient of variation, h^2_b = Broad sense heritability, GA = Genetic advance, GA (% mean) = Genetic advance in percent of mean.

Correlation studies

The genotypic and phenotypic correlation coefficients among thirteen characters were presented in Table 4. Days to 50% flowering (DFF) showed significant positive correlation with DEM (rg=0.999; rp =0.458), NPBP (rg-0.595; rp=0.276), HFC (rg=0.847; rp=0.440), IL (rg=0.521; rp=0.215) and SYP (rg=0.587; rp=0.448) at both the genotypic and phenotypic level (Table 4). It had also significant positive correlation with TSW (rp=0.226) at phenotypic level but non-significant correlation (rg=0.291) at genotypic level. Besides, DFF showed non-significant positive correlation with number of capsules per plant (NCP) (rg=0.265; rp=0.085), CL (rg=0.186; rp=0.070), NSC (rg=0.175; rp=0.105) both the genotypic and phenotypic level. Moreover, it had a non-significant negative correlation with NSBP (rg=-0.026; rp=-0.010) at both the genotypic and phenotypic level. Aye and Htwe (2019) also reported that DFF had a significant positive correlation with SYP. Days to 80% maturity (DEM) exhibited a significant and positive correlation with HFC (rg=0.707; rp=0.211), TSW (rg=0.336; rp=0.180*) and SYP (rg=0.615; rp=0.321) at both the genotypic and phenotypic level (Table 5). It had significant positive correlation with NPBP (rg=0.647), NCP (rg=0.403) and IL (rg=0.337) at genotypic level while non-significant positive correlation at phenotypic level. Sumathi and Muralidharan (2011), and Lalpantluangi, and Shah (2018) were also found a significantly positive correlation of DEM with YPP in sesame. Plant height (PH) had a significant positive correlation with days to 50% flowering (DFF) (rg=0.754; rp=0.208), days to 80% maturity (DEM) (rg=0.797; rp=0.176) capsule length (CL) (rg=0.567; rp=0.245), height of first capsule (rg=0.491; rp=0.558), internode length (IL) (rg=0.846; rp=0.384) and yield per plant (YPP) (rg=0. 546; rp=0.246) at both the genotypic and phenotypic level. It had also a non-significant positive correlation with number of seeds per capsule (NSC) (rg=0.198; rp=0.045) and thousand seed weight (TSW) (rg=0.140; rp=0.060) at both the genotypic and phenotypic level. Moreover, it had highly significant positive correlation with number of primary branches per plant (NPBP) (rg=0.500) at the genotypic level while non-significant negative correlation with (rp=-0.074) at phenotypic level. In addition, PH showed a non-significant negative correlation with number of secondary branches per plant (NSBP) (rg=-0.004; rp = -0.024) at both the genotypic and phenotypic level. The results suggested that the increase of PH will also increase the SYP, and the phenotypic selection for PH would meaningful for sesame improvement. Goudappagoudra *et al.* (2011), Sumathi and Muralidharan (2011), Sultana *et al.* (2019) were also reported a significantly positive correlation of PH with YPP in sesame. Similar result was also reported by Sumathi and Muralidharan (2010). Number of primary branches per plant (NPBP) had significant positive correlation with NSBP (rg = 0.389; rp = 0.220), NCP (rg=0.675; rp = 0.368), HFC (rg=0.769; rp = 0.261) and SYP (rg=0.650; rp = 0.344) at both the genotypic and phenotypic level. This character also showed a non-significant negative correlation with CL (rg = -0.216; rp = -0.097) at both the genotypic and phenotypic level. Besides, NPBP had a significant posative correlation with NSBP (rg=0.389; rp = 0.220) at both the genotypic and phenotypic level. Our results are in agreement with the findings of Meenakumari and Ganesamurthy (2015) for NPBP in sesame. Number secondary branches per plant (NSBP) had a highly significant positive correlation only with NCP (rg = 0.748; rp=0.371) at both the genotypic and phenotypic level. NSBP also showed a non-significant positive correlation with NSC (rg = 0.196; rp=0.145), IL (rg = 0.167; rp = 0.060) and SYP (rg = 0.135; rp=0.123) at both the genotypic and phenotypic level. Moreover, NSBP had a non-significant negative correlation with CL (rg = -0.141; rp = -0.070) and TSW (rg = -0.102; rp = -0.020) at both the genotypic and phenotypic level. Sumathi and Muralidharan (2010) observed that SYP had significantly positive correlation with number of branches per plant. Number of capsules per plant (NCP) showed significant positive correlation with NSC (rg= 0.307; rp= 0.195) and SYP (rg= 0.775; rp= 0.275) at both the genotypic and phenotypic level (Table 5). NCP also showed a significant positive correlation with TSW (rg= 0.433) whereas highly positive with HFC (rg= 0.433) at the genotypic level. Moreover, NCP had a non-significant negative correlation with IL (rg=-0.107; rp = -0.089) at both the genotypic and phenotypic level. The result suggested that the increase of NCP will also increase the SYP and phenotypic selection for this trait would be effective. Meenakumari and Ganesamurthy (2015), Aye and Htwe (2019), Sultana *et al.* (2019) also reported the significant positive correlation of NCP with SYP in sesame. Capsule length (CL) had a highly significant positive correlation only with IL (rg = 0.480) at genotypic level. It showed non-significant positive correlation with SYP at both the genotypic (rg = 0.068) and phenotypic (rp = 0.033) level. It had negative correlation with NSC (rg = -0.287; rp = -0.160) and TSW (rg = -0.190; rp = -0.112). Number of seeds per capsule (NSC) showed significant positive correlation with SYP (rg = 0.320; rp = 0.240) at both the genotypic and phenotypic level. It had significant positive correlation with TSW (rp = 0.214) at phenotypic level only. The result suggested that SYP increase with the increase of NSC. Goudappagoudra *et al.* (2011), Meenakumari and Ganesamurthy (2015), Sultana *et al.* (2019) also reported the significant positive correlation of NSC with SYP in sesame. Height of first capsule (HFC) showed highly significant positive correlation with IL at both genotypic (rg = 0.707) and phenotypic (rp = 0.434) level. It also showed significant positive correlation with SYP at genotypic (rg = 0.376) and phenotypic (rp = 0.254) level. Moreover, it had also a significant positive correlation with TSW (rp = 0.182) at phenotypic level. Internode length (IL) had non-significant positive correlation with SYP (rg = 0.228; rp = 0.141) at both genotypic and phenotypic. It also had non-significant negative correlation with TSW (rg = -0.178; rp = -0.108) at both the genotypic and phenotypic level. Thousand seed weight (TSW) had significant positive correlation with SYP (rg = 0.377; rp = 0.373) at both genotypic and phenotypic level. Sumathi and Muralidharan (2011) and Kehie *et al.* (2020) also reported similar result for TSW and SYP in sesame.

Path co-efficient analysis

Path co-efficient analysis partitioned the correlation coefficient as direct and indirect effect of yield of different yield contributing traits. The direct and indirect effects of path co-efficient analysis for sesame are presented in Table 5. Days to 50% flowering (DFF) had the direct negative direct effect (-0.198) on SYP. DFF had positive indirect effect on SYP via PH (0.645), DEM (0.246), NPBP (0.278), CL (0.048), NSC (0.037), HFC (0.009) and TSW (.045). Moreover, it had indirect negative effect on SYP via NSBP (-0.011), NCP (-0.097) and IL (-0.416). Ultimately, it made positive significant correlation with SYP (0.587**). It revealed that relationship between these traits and selection for this trait will be rewarding for yield improvement. Days to 80 % maturity had direct positive effect (0.227) on SYP. It had indirect positive effect on SYP through PH (0.682), NPBP (0.302), NSC (0.037), HFC (0.007) and TSW (0.052). It had indirect negative effect on SYP via DFF (-0.214), NSBP (-0.053), NCP (-0.149), CL (-0.008) and IL (-0.269). Eventually, it made positive significant correlation with SYP (0.615**). Plant height (PH) had maximum direct positive effect (0.856) followed by number of primary branches per plant on seed yield per plant. It had indirect positive effect on SYP via days to 80 % maturity (0.181), NPBP (0.234), capsule length (CL) (0.147), number of seed per capsule (NSC) (0.042), height of first capsule (HFC) (0.005), and thousand seed weight (TSW) (0.021). Furthermore, it had indirect negative effect on SYP via days to 50% flowering (DFF) (-0.150), NSB (-0.001), NCP (-0.114) and IL (-0. 675). Finally, it made positive significant effect with SYP (0.546**). The result indicated that if PH increases then SYP also increases through the positive indirect effect of PH with other traits. The phenotypic selection based on PH would be effective. Number of primary branches per plant had second highest direct positive effect (0.467) on SYP. This trait also showed indirect positive effect on yield via PH 0.428), DFF (0.118), DEM (0.147), NSBP (0.171), NSC (0.020), HFC (0.008) and TSW (0.042). NPBP had indirect negative effect on SYP via NCP (-0.249), CL (-0.055) and IL (-0.213). Finally, it made positive significant effect with seed YPP (0.650**). Thus indicated that if NPBP increases then SYP also increases through the positive indirect effect of other traits. Number of secondary branches per plant (NSBP) had positive direct effect (0.441) on SYP. It had indirect positive effect on yield via DFF (0.005), NPBP (0.182), NSC (0.001) and HFC (0.001). NSBP had indirect negative effect on SYP via PH (-0.003), DEM (-0.027), NCP (-0.276), CL (-0.036), IL (-0.133) and TSW (-0.016). Lastly, it made NON-significant but positive effect with seed YPP (0.135). Number of capsules per plant (NCP) had the direct negative effect (-0.370) on SYP followed by indirect positive effect via PH (0.264), DEM (0.091), NPBP (0.315), NSBP (0.330), NSC (0.065), HFC (0.004), IL (0.070) and TSW (0.067) (Table 6).

NCP had indirect negative effect on yield via DFF (-0.052) and CL (-0.003). Finally, it had highly significant positive genotypic correlation with SYP (0.775**). The results indicated that correlation was mainly due to the direct effect of the trait and it was realized via indirect effects. Similar to our result for NCP was reported by Sumathi and Muralidharan (2010). Capsule length (CL) had direct positive effect (0258) on SYP. It also exhibited indirect negative effect via all the traits except via PH (0.485) and NCP (0.005). CL finally exhibited a non-significant positive correlation SYP (0.068). Number of seeds per capsule (NSC) had direct positive effect (0.213) on SYP. It had also exhibited indirect positive effect via all the traits except via DFF (-0.034) and NCP (-0.113). This trait finally exhibited significant positive correlation with seed yield per plant (0.320). Height of first capsule (HFC) had direct positive effect (0.011) on SYP. It had indirect positive effect via PH (0.419), DEM (0.160), NPBP (0.359), NCP (0.157), NSC (0.009) and TSW (0.042) whereas indirect negative effect via DFF (-0.167), NSBP (-0.047), CL (-0.004) and IL (-0.565). This trait also highly correlated with SYP (0.376**). Internode length (IL) had direct negative effect (-0.799) on SYP. It also showed indirect positive effect via PH (0.723), DEM (0.076), NPBP (0.125), NSBP (0.073), NCP (0.032), CL (0.124) and HFC (0.007) while indirect negative effect via DFF (-0.103), NSC (-0.006) and TSW (-0.027). IL had non-significant but positive genotypic correlation with SYP (0.228). Thousand seed weight (TSW) had a direct positive effect (0.156) on SYP. It also showed indirect positive effect via PH (0.119), DEM (0.076), NPBP (0.128), CL (0.049), NSC (0.063), HFC (0.003) and IL (0.142) whereas indirect negative effect via DFF (-0.057), NSBP (-0.045) and NCP (-0.160). Finally, TSW had highly significant positive genotypic correlation with SYP (0.377). The lower residual effect (R) of 0.147 indicated that the contribution of component characters was 85.3 percent. The rest 14.70 percent was the contribution came from other factors.

Table 4. Genotypic and phenotypic correlation coefficients among various yield and its contributing characters of sesame genotype

Characters		PH	DFF	DEM	NPBP	NSBP	NCP	CL	NSC	HFC	IL	TSW
PH	r_g											
	r_p											
DFF	r_g	0.754**										
	r_p	0.208*										
DEM	r_g	0.797**	0.999**									
	r_p	0.176*	0.458**									
NPBP	r_g	0.500**	0.595**	0.647**								
	r_p	-0.074	0.276**	0.120								
NSBP	r_g	-0.004	-0.026	-0.122	0.389**							
	r_p	-0.024	-0.010	-0.044	0.220*							
NCP	r_g	0.309*	0.265	0.403**	0.675**	0.748**						
	r_p	0.081	0.085	0.138	0.368**	0.371**						
CL	r_g	0.567**	0.186	-0.034	-0.216	-0.141	-0.015					
	r_p	0.245**	0.070	-0.012	-0.097	-0.070	0.035					
NSC	r_g	0.198	0.175	0.177	0.196	0.047	0.307*	-0.287				
	r_p	0.045	0.105	0.091	0.145	0.005	0.195*	-0.160				
HFC	r_g	0.491**	0.847**	0.707**	0.769**	-0.107	-0.426**	-0.018	0.042			
	r_p	0.558**	0.440**	0.211*	0.261**	0.113	-0.150	0.073	-0.027			
IL	r_g	0.846**	0.521**	0.337*	0.267	0.167	-0.107	0.480**	0.049	0.707**		
	r_p	0.384**	0.215*	0.030	0.076	0.060	-0. 089	0.165	-0.031	0.434**		
TSW	r_g	0.140	0.291	0.336*	0.274	-0.102	0.433**	-0.190	0.298	0.274	-0.178	
	r_p	0.060	0.226*	0.180*	0.130	-0.020	0.134	-0.112	0.214 *	0.182*	-0.108	
SYP	r_g	0.546**	0.587**	0.615**	0.650**	0.135	0.775**	0.068	0.320 *	0.376*	0.228	0.377*
	r_p	0.246**	0.448**	0.321**	0.344**	0.123	0.275**	0.033	0.240 **	0.254**	0.141	0.373**

* ,** = Significant at 5% and 1% level of significance, respectively. Here, PH = Plant height (cm), DFF = Days to 50% flowering, DEM = Days of 80% maturity, NPBP = Number of primary branches per plant, NSBP = Number of secondary branches per plant, NCP = Number of capsules per plant, CL = Capsule length (cm), NSC = Number of seeds per capsule, HFC = Height of first capsule (cm), IL = Internode length (cm), TSW = Thousand seed weight (g) and SYP = Seed yield per plant (g).

Selection of sesame genotypes through cluster analysis

Cluster analysis was carried out using 43 sesame genotypes for yield and its contributing traits by an online clustering tool (http://www2.heatmapper.ca). The results revealed that 43 sesame genotypes were grouped into four clusters (Figure 1 and Table 6). The maximum genotypes were included in cluster III (14) followed by cluster IV (12) and cluster II (9) while minimum genotypes in cluster I (8) (Table 6). The sesame genotype G6, G12 and G36 which were grouped in cluster IV exhibited better performance for yield per plant (Figure 1, Table 2 and Table 6). Moreover, the genotype of cluster II, G26 and G27 showed minimum mean values for days 50% flowering and days to 80% maturity suggesting early maturing genotypes belongs to this cluster. Sultana *et al.* (2019) reported similar results in sesame.

Table 5. Partitioning of genotypic correlations into direct (bold) and indirect effects of 12 important traits by path analysis of sesame genotypes

Characters	PH	DFF	DEM	NPBP	NSBP	NCP	CL	NSC	HFC	IL	TSW	SYP (r_g)
PH	0.856	-0.150	0.181	0.234	-0.001	-0.114	0.147	0.042	0.005	-0.675	0.021	0.546**
DFF	0.645	-0.198	0.246	0.278	-0.011	-0.097	0.048	0.037	0.009	-0.416	0.045	0.587**
DEM	0.682	-0.214	0.227	0.302	-0.053	-0.149	-0.008	0.037	0.007	-0.269	0.052	0.615 **
NPBP	0.428	0.118	0.147	0.467	0.171	-0.249	-0.055	0.020	0.008	-0.213	0.042	0.650**
NSBP	-0.003	0.005	-0.027	0.182	0.441	-0.276	-0.036	0.001	0.001	-0.133	-0.016	0.135
NCP	0.264	-0.052	0.091	0.315	0.330	-0.370	-0.003	0.065	0.004	0.070	0.067	0.775**
CL	0.485	-0.036	-0.007	-0.101	-0.062	0.005	0.258	-0.061	-0.001	-0.383	-0.029	0.068
NSC	0.169	-0.034	0.040	0.045	0.002	-0.113	0.074	0.213	0.001	0.024	0.046	0.320*
HFC	0.419	-0.167	0.160	0.359	-0.047	0.157	-0.004	0.009	0.011	-0.565	0.042	0.376*
IL	0.723	-0.103	0.076	0.125	0.073	0.032	0.124	-0.006	0.007	-0.799	-0.027	0.228
TSW	0.119	-0.057	0.076	0.128	-0.045	-0.160	0.049	0.063	0.003	0.142	0.156	0.377*

Residual effect (R) = 0.147

* ,** = Significant at 5% and 1% level of significance, respectively

PH = Plant height (cm), DFF = Days to 50% flowering, DEM = Days of 80% maturity, NPBP = Number of primary branches per plant, NSBP = Number of secondary branches per plant, NCP = Number of capsules per plant, CL = Capsule length (cm), NSC = Number of seeds per capsule, HFC = Height of first capsule (cm), IL = Internode length (cm), TSW = Thousand seed weight (g) and SYP = Seed yield per plant (g)

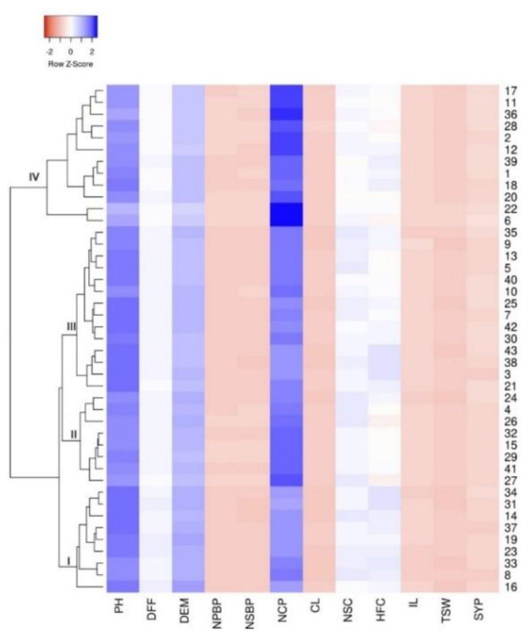

Fig. 1. Heatmap representation of 43 sesame genotypes into four clusters in respect of 12 traits.

Table 6. Distribution of 43 sesame genotypes into four clusters

Cluster	No. of Genotypes	Genotypes
I	8	G8, G18, G19, G23, G31, G33, G34 and G37
II	9	G4, G15, G24, G26, G27, G28, G29, G32 and G41
III	14	G3, G5, G7, G9, G10, G13, G21, G25, G30, G35, G38, G40, G42 and G43
IV	12	G1, G2, G6, G11, G12, G17, G18, G20, G22, G28, G36 and G39

Conclusion

The genetic variability, correlation and path analysis of yield and yield contributing traits of 43 sesame genotypes were evaluated. The result revealed significant differences for most of the characters except number of capsules per plan. High broad sense heritability together with high genetic advance in percent of mean was observed for number of secondary branches per plat, thousand seed weight and seed yield per plant while the lowest heritability was found for number of capsule per plant. The significant positive correlation with seed yield per plant was found for plant height, days to 50% flowering, days to 80% maturity, number of primary branches, number of capsules per plant, number of seeds per capsule, height of first capsule and 1000-seed weight. Plant height, days to 80% maturity, number of primary branches per plant, number of secondary branches per plant, capsule length and number of seeds per capsule had the positive direct effect on yield per plant whereas, internode length followed by number of capsules per plant and days to 50% flowering had the negative direct effect. Based on mean performance, heritability and interrelationship, genotype G6, G12 and G36 for seed yield per plant, and G26, G27 and G37 for early maturity could be selected for utilization in future varietal improvement of sesame.

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Conflicts of Interest

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

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ASSESSING SEED HEALTH AND RESISTANCE TRAITS IN WATERMELON AGAINST GUMMY STEM BLIGHT

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Abstract

Water melon is a popular summer fruit, and gummy stem blight (GSB) disease, caused by the fungal pathogen *Didymella bryoniae,* is this fruit.. Using resistant sources is the most effective disease management strategy. The seed health test revealed the presence of *D. bryoniae* and *F. oxysporum f.sp. niveum* fungi associated with water melon seeds imported from different counties. BARI line 03-144x21 and BARI line 02-07x08 showed the lowest lesion symptomof 25.67 x 25.17mm and 24.17 x 23.67mm in the laboratory detached leaf test in the artificially inoculated with *D. bryoniae* isolates S002 and S005, respectively. In pot evaluation test, all BARI lines showed lower gummy stem blight disease incidence and severity which indicated moderate resistant (MR) against the disease and all the imported hybrid varieties showed moderate susceptible to susceptible the disease. In the field experiments, four BARI inbreed lines and two commercial varieties (Black Diamond (Metal Seed) and Black Diamond (Alamgir Seed) showed resistance (MR) against the disease. Thus, it can be concluded that BARI inbred lines, viz., BARI line 01-08X07, BARI line 02-07X08, BARI line 03-144X21BARI line 04-21X144, are moderate resistant against stem blight disease in water melon with the commercial varieties and the advanced line is susceptible to stem blight disease in water melon.

Keywords: BARI inbred line, Gummy stem blight (GSB) and Resistant variety

Introduction

Watermelon holds the distinction of being the most widely consumed cucurbit worldwide, with cucumber and melon following closely behind (FAO, 2005). In Bangladesh, watermelon cultivation covers an extensive area of 12,246.59 hectares, resulting in a substantial production of 254,814.25 metric tons (BBS, 2020). This fruit crop carries significant economic importance and is valued for its abundance of lycopene, citrulline, and essential minerals and vitamins. The presence of pathogens in infected seeds can significantly diminish germination rates, weaken plant vigor, and ultimately reduce potential yields by transferring the pathogen from seed to plants. The most severe consequence of seed-borne pathogens is their ability to contaminate disease-free areas,

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serving as the primary source of inoculation for disease outbreaks. Seed infection typically occurs during three distinct phases: seed production, development, and maturation. Pathogens can play a role in each of these growth stages and can be transmitted from one crop to the next, establishing systemic infections that can colonize the seeds (McGee, 1995).

Gummy stem blight, caused by *Didymella bryoniae* (anamorph *Phoma cucurbitacearum* (Fr. Fr) Sacc.), is a widely prevalent disease affecting cucurbits globally (Sitterly and Keinath, 1996). Cucurbita spp. are particularly vulnerable to black rot, which directly diminishes both pre and post-harvest yields (Keinath *et al*., 1995; Zitter and Kyle, 1992). The pathogen resides both on and within the seed coat, transmitting from seed to seedling (Lee *et al*., 1984). Cotyledons and young leaves of watermelon and are equally susceptible to gummy stem blight. Although the leaves of young squash and cucumber plants initially display resistance, they become susceptible with age, particularly under high temperature and humid conditions (Prasad and Norton, 1967). Vansteekelenburg (1983) investigated the epidemiology of *D. bryoniae* and the occurrence of ascospores in glasshouses, outdoor environments, and controlled conditions. In a study conducted by Vansteekelenburg (1985), the impact of humidity on the occurrence of *D. bryoniae* on cucumber leaves and growing tips was examined under controlled settings. It was reported that a 10-fold increase in conidial concentrations was required to achieve the same level of infection as with leaf wetting. The disease is known to spread in greenhouses during the growing season through airborne ascospores and conidia transported by water on plant surfaces, as well as through contact between plants or between plants and humans or tools.

Effectively managing gummy stem blight (GSB) through fungicide applications and suitable cultural practices is challenging, especially during periods of rainfall when high humidity persists for prolonged durations. There's also growing concern among pathologists and breeders regarding the potential development of resistance by *D. bryoniae* to fungicides. This issue has been a focus of attention since the 1970s, as there's interest in exploring resistance to GSB as an alternative to chemical control. Variations in gummy stem blight (GSB) resistance have been noted among different commercial watermelon cultivars, with 'Congo' displaying the least susceptibility, 'Fairfax' showing intermediate susceptibility, and 'Charleston Gray' being the most susceptible, as reported by Schenck in 1968. Despite these efforts, no watermelon cultivars with high levels of resistance to natural GSB epidemics have been released. The rising challenges posed by GSB outbreaks in the southeastern United States prompted further studies on genetic resistance. Gusmini *et al.* in 2005 exploring new genetic sources of resistance to GSB. The watermelon breeding program at North Carolina State University developed efficient screening methods for testing watermelon germplasm, including systems for mass production of inoculum and disease assessment scales.

To address the challenges posed by GSB in watermelon cultivation and enhance production while boosting the income of watermelon growers, various strategies from previous literature were considered. These included implementing pathogen-free seeds and selecting resistant varieties. Given the importance of managing GSB, it is essential to observe the seed health status of the watermelon commercial varieties and to evaluate them against wilt and stem blight disease.

Materials and Methods

Evaluation seed health status of commercial varieties of watermelon against stem blight disease

Watermelon seeds of imported commercial hybrid variety (Table 1) were collected from the seed market, while BARI inbred lines were acquired from HRC, BARI, and kept at 4°C. The seeds underwent sterilization using a 2% NaOCl solution for 5 minutes, followed by rinsing with sterilized distilled water three to four times. After sterilization, the seeds were air-dried and subjected to the standard blotter method to assess mycoflora incidence and germination rates.

Table 1. Commercial hybrid varieties/inbreed lines including type and colour of watermelon seeds used in the study

Varieties / line	Type	Colour
Black supper	Hybrid	Black
Black diamond	Hybrid	Black
Black Diamond	Hybrid	Black
Sweet dragon	Hybrid	Green with stripe
Tropical dragon	Hybrid	Green with stripe
Big family	Hybrid	Green with stripe
World queen	Hybrid	Green with stripe
Jumbo jaguar	Hybrid	Black
Black giant	Hybrid	Black
Thailand 2	Hybrid	Black
Black bull	Hybrid	Black
Dragon beauty	Hybrid	Green with stripe
Sweet green	Hybrid	Green with stripe
Sugar emperor	Hybrid	Green with stripe
BARI line 01-08x07	Inbreed	Green with stripe
BARI line 02-07x08	Inbreed	Green with stripe
BARI line 03-144x21	Inbreed	Green with stripe
BARI line 04-21x144	Inbreed	Green with stripe

Screening of watermelon commercial varieties for evaluating against D. bryoniae by detached leaves method

The study was carried out in the Plant Pathology Division laboratory at BARI following the procedure of Alam *et al*. 2014, 2015 and 2020. Two isolates *D. bryoniae*, namely S002 and S005 were employed in this investigation. Healthy, fully grown water melon leaves were used and subjected to surface sterilization using a 2% NaOCl solution for 90 seconds, followed by rinsing in sterilized water three times. After air drying on paper towels, the leaves were laid out, and wounds were created with a sterile needle before inoculation with a 4 mm mycelial plug from 7-day-old *Didymella bryoniae* (S002 and S005) cultures grown on PDA plates. The inoculated leaves were then placed on wet blotting paper in a 30 cm sterilized petri dish within a humid chamber. This chamber, measuring 170x240x80 mm, contained 5 ml of distilled water and was sealed with a tight lid. The setup was incubated at 25±2 °C with a 16-hour photoperiod. Disease symptoms were evaluated three days post-inoculation by measuring the length and width of lesions extending beyond the mycelial plugs. Control leaves were inoculated with sterilized water after wounding. To prevent cross-contamination, different isolates of *D. bryoniae* were incubated separately in their own chambers, with three replicate chambers per isolate, each containing three leaves.

Evaluation of commercial watermelon varieties against gummy stem blight under artificially inoculated condition (Pot culture experiment)

This experiment was conducted in the pot house of Plant Pathology Division, BARI, Gazipur, Bangladesh. In this experiment, S002 of *D. bryoniae* was used for evaluation of commercial verities. S002 were grown in water-soaked mixture of ground corn, wheat bran and grass pea seed coat (1:10: 5, w/w) for 10 days. Thirty-five mycelial blocks (5 mm in diameter) of PDA cultures (7-day-old) were used to inoculate 1 kg of mixture and incubated for 10 days at room temperature with a 12-h photoperiod. To confirm their pathogenicity, 15-day-old seedlings (10 seedlings/isolate, repeated three times) of watermelon were transplanted into 20-cm-diameter pots filled with soil (mixed with 10 g of inoculum/kg of soil).

On appearance of symptoms, the disease incidence was recorded by using following formula. Disease rating was made on weekly basis to find out the percentage of disease incidences among these cultivars by following the scale of Thompson and Jenkins (1985) with slight modification (Table 2).

Evaluation of commercial varieties of watermelon against stem blight diseases under natural field condition

A disease screening nursery was established to identify source of resistance against stem blight diseases of water melon in the Plant Pathology Division, BARI, Gazipur and RHRS (Regional Horticultural Research Station), BARI, Patuakhali. Collected eighteen watermelon varieties/lines were evaluated against wilt and stem blight disease under natural condition in field (Table 1). All the recommended horticultural practices were followed to maintain the crop in good condition.

Table 2. Disease rating scale (Thompson and Jenkins, 1985)

Scale	Disease incidence (%)	Grading
0	0 %	Immune
1	up to 10 %	Resistant/Moderately resistant
2	11 – 25 %	Moderately Resistant
3	26 – 45 %	Moderately Susceptible
4	46 – 70 %	Susceptible
5	71 –100%	Highly Susceptible

Disease incidence was recorded using the following formula:

$$\text{\% Disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total of observed plants}} \times 100$$

Disease severity for each location was calculated following the equation:

$$\text{\% Disease severity} = \frac{\Sigma \text{ infection frequencies} \times \text{ number of leaves of each class}}{\text{Total of observed leaves} \times \text{ highest value of the evaluation scale}} \times 100$$

Both the categorized data and calculated disease severity % were presented.

Results and Discussion

Seed health of commercial varieties and BARI advanced lines of watermelon against *Didymella bryoniae*

To know the incidence of mycoflora, germination and seedling vigor, respectively seed samples of popular variety of watermelon listed in Table 1 was collected from seed market and Horticultural Research Center (HRC), BARI. Fungi such as *Didymella bryoniae*, *Fusarium oxysporum* f.sp. *nevium* (Fon) and other seed borne fungi (*F. oxysporum* f.sp. *nevium* (Fon) and *Penicillium* sp.) was isolated from the incubated seeds of watermelon and their cultures were maintained on Potato Dextrose Agar plates for the purpose of identification. The seed health status of fourteen commercial varieties and four BARI inbreed lines was assessed, as detailed in Fig. 1. The primary seed-borne fungal pathogens identified were *D. bryoniae*, *F. oxysporum* f.sp. *nevium* (Fon) and *Penicillium* sp. Among the evaluated varieties/genotypes, Sweet Dragon watermelon varieties exhibited the highest germination rate of 97.28%, while Dragon Beauty displayed the lowest germination rate. Regarding seed infection, the World Queen variety had the highest incidence of 47.82% (all seed borne fungi), whereas Sweet Dragon had the lowest (18.3%) (all seed borne fungi). Sweet Green had the highest *Didymella* infection, 14.3%, while Sweet Dragon had the lowest (3.2%). *Penicillium* infection was most pronounced in Black Giant (9.2%) and least in Black Diamond (2.3%).

The current investigation clearly demonstrates that *D. bryoniae* acts as seed-borne pathogen, capable of transferring inoculum from the seed to the plant. The study revealed that *D. bryoniae* predominantly resides in the seed coat, a finding supported by similar observations in cucumber and pumpkin by Lee *et al.* (1984). Additionally, research conducted elsewhere indicates that in watermelon *D. bryoniae* can infiltrate the epidermis, cotyledons, and embryos (Rankin, 1954). Agarwal and Sinclair (1997) noted that seed-borne pathogens can be transmitted through infection of the embryo, endosperm, or contamination of the seed coat. During seed development and maturation, there is a significant potential for *D. bryoniae* spores to disperse, germinate, and systematically invade the seeds (Rankin, 1954).

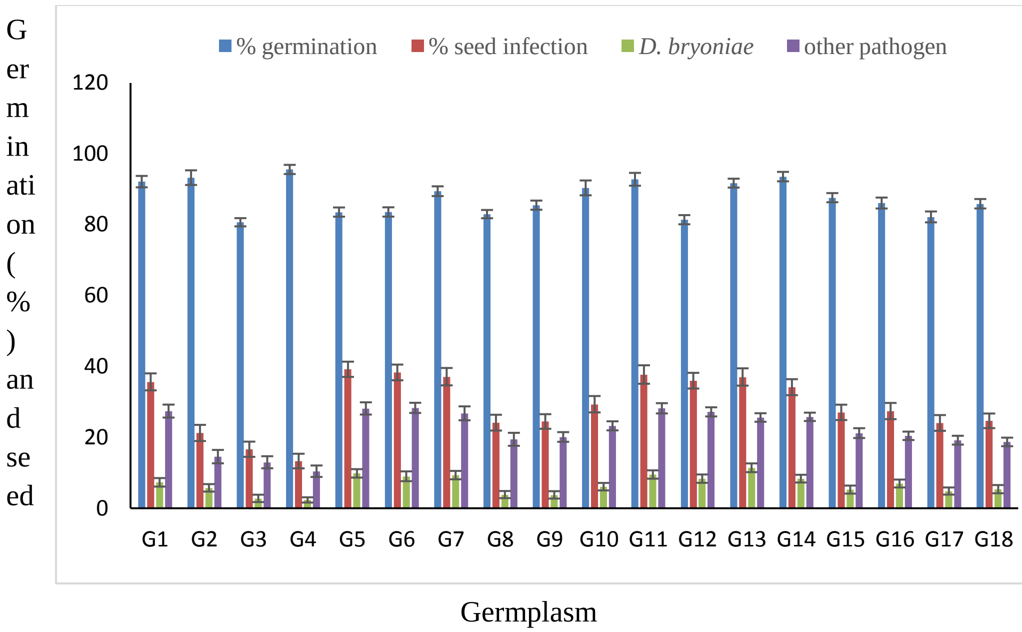

Fig. 1. Seed born pathogens of commercial varieties and BARI advanced lines of watermelon showing %germination, seed infection due to *D. bryonae* and other pathogen. G1=Black Super, G2=Black Diamond (Metal seed), G3=Black Diamond (Alamgir Seed),G4=Sweet Dragon, G5=Tropical Dragon, G6=Big Family, G7=World Queen,G8=Jumbo Jaguar, G9=Black Giant, G10=Thailand 2, G11=Black Bull, G12=Dragon Beauty, G13=Sweet Green, G14=Sugar Emperor, G15=BARI line 01-08x07, G16=BARI line 02-07x08, G17=BARI line 03-144x21, G18=BARI line 04-21x144

Evaluation of watermelon commercial varieties against D. bryoniae by detached leaves method in laboratory

By using isolates S002 and S005 of *D. bryoniae* all varieties of watermelon developed lesion in the inoculated leaf. Susceptibility to *D. bryoniae* isolates was varied among the varieties (Fig. 2, 3 and 4). In case of S002, Black bull verity developed the highest lesion (length 36.33mm and width 33.83mm) and inbreed line BARI line 03-144x21 showed the lowest lesion development (length 25.67mm and width 25.17mm). In case of S005, world queen developed the highest (length 36.83mm and width 35.50mm) black lesion and BARI line 02-07x 08 developed the lowest lesion (length

24.17 mm and width 23.67mm (Fig. 2 and 3). Detached-leaf tests offer several benefits compared to alternative methods like multi-race or multi-pathogen testing. These advantages include avoiding issues related to systemic acquired resistance, the capacity to assess and preserve susceptible plants crucial in genetic studies, and the opportunity for enhanced replication by utilizing more leaves when evaluating individual plants of distinct genotypes. Amand and Wehner (1995) briefly noted the susceptibility of cucumber detached leaves to *D. bryoniae*. Detached-leaf tests, while convenient and exhibiting lower coefficients of variation compared to greenhouse tests, did not yield results correlated with field outcomes. Unlike greenhouse screening where supplemental nutrients were provided, detached-leaf tests didn't include them to avoid encouraging secondary organism growth. The practice of rinsing detached leaves before inoculation aimed to decrease surface organisms but might have inadvertently removed guttation exudates, potentially contributing to the disparity between field and detached-leaf infection (Amand and Wehner, 1995).

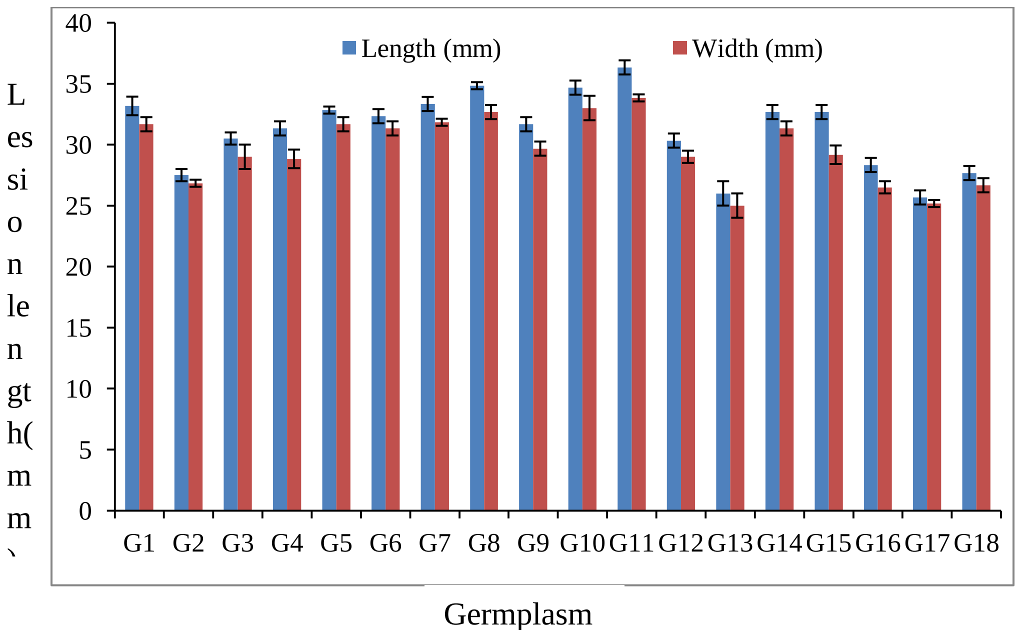

Fig. 2. Leaf lesion developed 72 hours after inoculation of *D. bryoniae* S002. G1=Black Super, G2=Black Diamond (Metal seed), G3=Black Diamond (Alamgir Seed),G4=Sweet Dragon, G5=Tropical Dragon, G6=Big Family, G7=World Queen,G8=Jumbo Jaguar, G9=Black Giant, G10=Thailand 2, G11=Black Bull, G12=Dragon Beauty, G13=Sweet Green, G14=Sugar Emperor, G15=BARI line 01-08x07, G16=BARI line 02-07x08, G17=BARI line 03-144x21, G18=BARI line 04-21x144

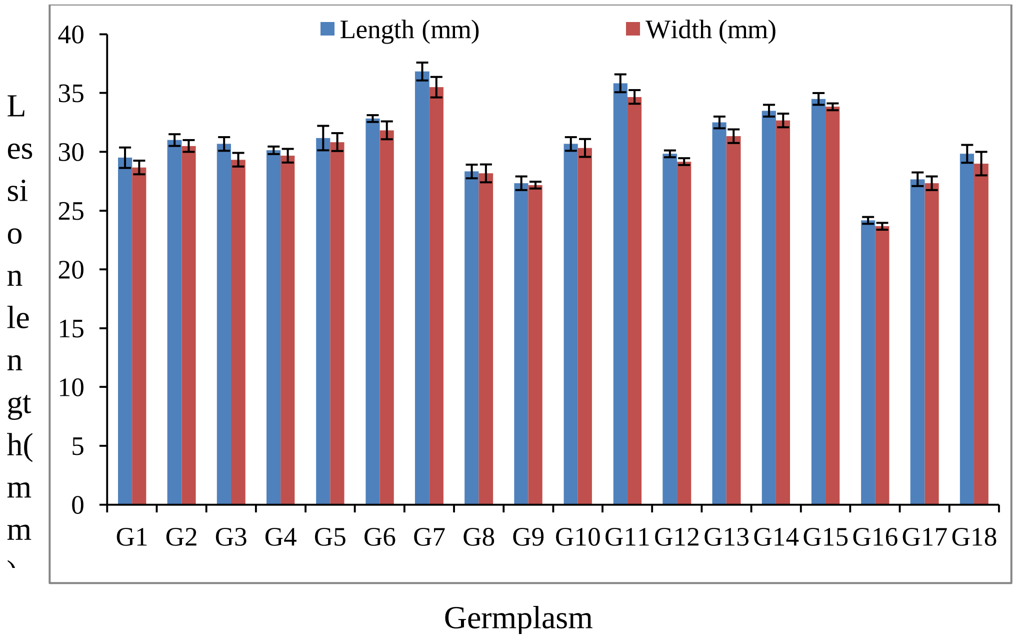

Fig. 3. Leaf lesion developed 72 hours after inoculation of *D. bryoniae* S002. Showing the length and width of lesion. G1=Black Super, G2=Black Diamond (Metal seed), G3=Black Diamond (Alamgir Seed), G4=Sweet Dragon, G5=Tropical Dragon, G6=Big Family, G7=World Queen,G8=Jumbo Jaguar, G9=Black Giant, G10=Thailand 2, G11=Black Bull, G12=Dragon Beauty, G13=Sweet Green, G14=Sugar Emperor, G15=BARI line 01-08x07, G16=BARI line 02-07x08, G17=BARI line 03-144x21, G18=BARI line 04-21x144

Evaluation of imported varieties and BARI inbred lines against gummy stem blight under artificially inoculation condition in pot

Under artificial inoculation condition, the incidence of stem blight in watermelon varied from 20.64% to 56.05% (Table 3). The highest incidence, 56.05%, was observed in the Black Giant (G_9) variety, which statistically similar to Jambo Jaguar (G_8), Dragon Beauty(G_{12}), Big Family (G_6), Tropical Dragon (G_5) Black Bull (G_{11}), and World Queen (G_7) varieties, all those varieties were categorized as susceptible (S). Conversely, the lowest incidence, 20.64%, was recorded in BARI line 04-21X144 (G18), which was statistically similar to BARI line 03-144X21 (24.04%), BARI line 01-08X07 (24.71%) and Black Diamond (Metal Seed) (G_2) and classified as moderately resistant (MR). The severity of stem blight followed a similar trend, with BARI line 04-21X144 (G18) exhibiting the lowest severity of 10.68%, statistically close to BARI line 03-144X21 (12.38%) and BARI line 01-08X07 (14.49%) all the imported varieties showed moderately susceptible to susceptible against stem blight disease (Table 3). Plants grown in the greenhouse and inoculated at dawn showed severe infection, whereas those inoculated in the field exhibited fewer visible symptoms of GSB. The timing of inoculation had a significant impact, with plants inoculated in the greenhouse being more susceptible compared to those inoculated in the field.

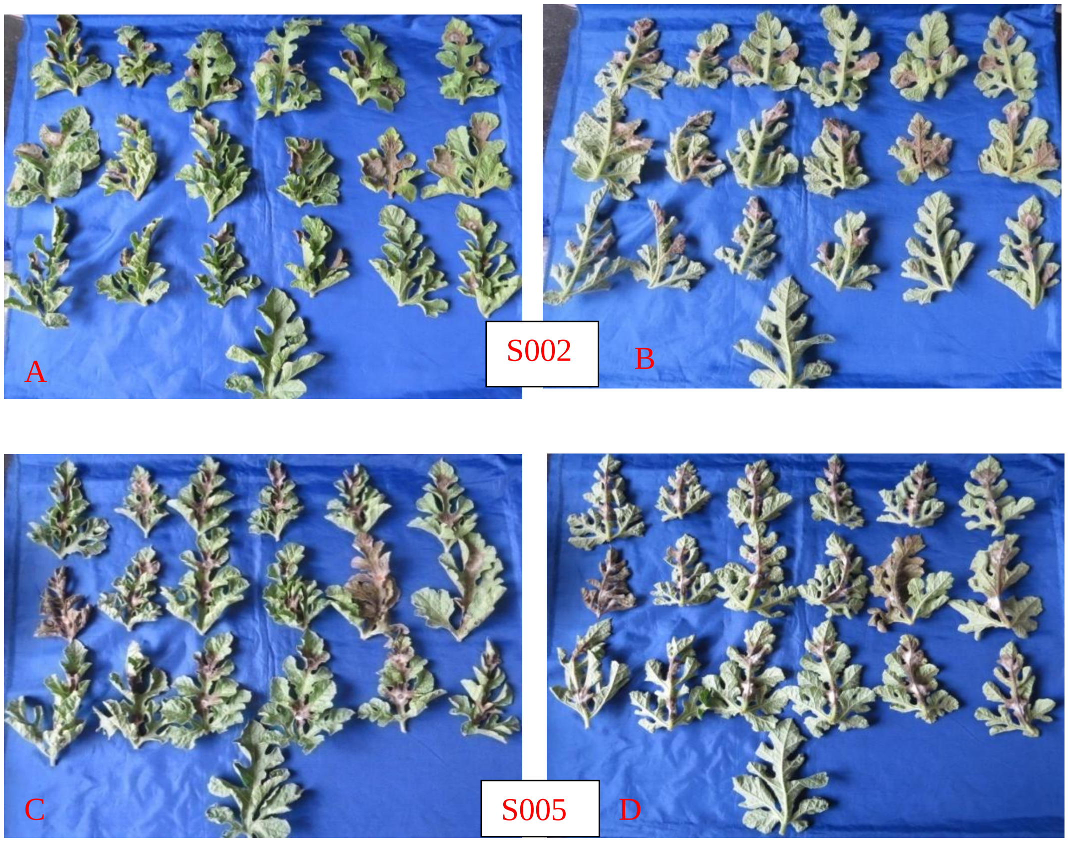

Fig. 4. Symptom developed on upper and lower surface of different commercial varieties against isolate S002 and S005 of *D. Bryoniae* in detached leaves assay. A, upper surface of S002 inoculated leaves of G_1-G_{18} and control; B, lower surface of S002 inoculated leaves of G_1-G_{18} and control; C, upper surface of S005 inoculated leaves of G_1-G_{18} and control; D, lower surface of S005 inoculated leaves of G_1-G_{18} and control.

Table 3. Incidence, severity and disease reduction of stem blight disease of watermelon in 18 varieties/lines at Pot house, PPD, BARI Gazipur.

Variety/Germplasm	% Stem blight incidence	% Stem blight severity	Disease reaction
G1=Black Super	37.57bc	25.66ab	MS
G2=Black Diamond (Metal Seed)	24.89de	18.30de	MS
G3=Black Diamond (Alamgir Seed)	27.20d	16.71de	MS
G4=Sweet Dragon	42.79b	22.78bc	MS
G5=Tropical Dragon	53.03a	25.81ab	S

Variety/Germplasm	% Stem blight incidence	% Stem blight severity	Disease reaction
G6=Big Family	55.01a	27.76a	S
G7=World Queen	52.23a	26.18ab	S
G8=Jumbo Jaguar	55.12a	27.38a	S
G9=Black Giant	**56.05a**	29.45a	**S**
G10=Thailand 2	46.92b	27.79a	S
G11=Black Bull	54.37a	27.85a	S
G12=Dragon Beauty	55.08a	27.15a	S
G13=Sweet Green	39.18bc	20.08cd	MS
G14=Sugar Emperor	32.40c	17.67de	MS
G15= BARI line 01-08x07	24.71de	14.49ef	MR
G16= BARI line 02-07x08	26.16d	17.10de	MR
G17= BARI line 03-144x21	24.04de	12.38f	MR
G18= BARI line 04-21x144	20.64e	10.68f	MR
CV (%)	9.96	11.82	

Evaluation commercial varieties of watermelon against stem blight disease under natural field condition

A field experiment was carried out at two locations: RHRS in Patuakhali and the Plant Pathology research field of BARI in Gazipur, to assess various commercial watermelon varieties and BARI lines against stem blight disease under natural field condition. The findings from this experiment were documented and presented through Tables 4 to 6.

First male flower opening days and numbers

In the field at RHRS in Patuakhali, it was observed that the earliest male flower opening occurred at 40 days after seed sowing in genotypes BARI line 03-144x21, Sugar Emperor, and World Queen, while the Big Family genotype exhibited male flower opening by 49 days later. The range of male flower opening among the 18 genotypes varied from 40 to 49 days (Table 4). Similarly, at BARI in Gazipur, the earliest male flower opening was recorded at 39 days in BARI line 03-144x21 followed by the varieties Black Diamond and Black Giant where male flower opened at 40 days and the Big Family variety displaying later male flower opening at 48 days (Table 6). The range of male flower opening among the 18 genotypes ranged from 40 to 48 days.

At RHRS in Patuakhali, on the first flowering, the highest number 9.67 of male flowers was recorded in the Big Family genotype while the lowest number 5.00 was observed in the genotypes Jumbo Jaguar, Black Ball, BARI line 01-08x07, and BARI line 02-07x08 (Table 6). Similarly, at BARI in Gazipur, the highest number of male flowers on the first day of observation was noted in the Big Family genotype at 8.67, while the lowest number of 4.33 was observed in the genotypes Black bull, BARI line 01-08x07 and BARI line 02-07x08 (Table 6).

First female flower opening days and numbers

At RHRS in Patuakhali, the World Queen genotype showed the earliest female flower opening on the 42nd day of seed sowing, while the Big Family genotype exhibited later openings on the day 49. On the first day of female flower emergence, Black Diamond (Alamgir Seed), Tropical Dragon, and BARI line 03-144x21 displayed higher number of flowers at 17.33 whereas Black Diamond, Sweet Dragon, Sweet Green, and BARI line 02-07x08 had a lower count of 13.33 (Table 4). At BARI in Gazipur, the World Queen genotype exhibited the earliest opening of female flowers on the 41st day, while the Big Family genotype showed later openings on day 48. On the initial day of female flower emergence, Tropical Dragon displayed a higher count of flowers of 17.00, while Black Diamond, Sweet Dragon, and Sweet Green varieties had a lower count of 12.00 (Table 6).

Number of fruit per plant (NFPP)

In the field at RHRS in Patuakhali, the highest number of fruits per plant was observed in BARI line 01-08x07 at 3.95 followed by Black Bull, Black Super, World Queen, Sugar Emperor and BARI line 04-21x144 where the fruit number per plant was 3.10, 3.06, 3.05, 3.03 and 2.97, respectively. and the lowest number was recorded in the Big Family variety of 1.89 (Table 4).

Individual fruit weight (IFW)

The Thailand-2 genotype had the highest individual fruit weight (6.63 kg), while the lowest weight of 3.03 kg was observed in the BARI line 01-08x07 (Table 4).

Fruit length (cm) and fruit breadth (cm)

Thailand 2, Sweet Green and exhibited higher fruit length at 31.20 and 30.74 cm, respectively followed by Black Giant with fruit length 29.65 cm. while BARI line 04-21x144 displayed the shortest length at 17.15 cm (Table 4). Thailand 2 and Super Emperor showed the widest fruit breadth of 25.22 and 25.58 cm, respectively, whereas BARI line 01-08x07, BARI line 02-08x07, BARI line 03-144x21 and BARI line 04-21x144 had the narrowest breadth (Table 4).

Fruit yield (t/ha)

At RHRS in Patuakhali, Fruit yield ranged from 21.16 to 65.13 t/ha. The variety Sugar Emperor exhibited the highest fruit yield 65.13 t/ha followed by Black Bull, Dragon Beauty and Sweet Green where the yield was 53.45, 50.82 and 50.60 t/ha, respectively. The lowest yield was recorded from Big Family variety of 21.16 t/ha (Table 4).

Field Evaluation of different cultivar/ varieties/lines against the stem blight disease of watermelon

Two field experiments were conducted at RHRS, Patuakhali and Plant Pathology Division, BARI, Gazipur. At RHRS, Patuakhali, results revealed that, various varieties, or lines exhibited significant differences in both the incidence and severity of stem blight diseases (Table 5). Stem blight disease incidence ranged from 13.5% to 47.5%. Black Giant (G9) showed the highest disease incidence at 47.5%, which was statistically comparable to Jambo Jaguar (46.4%), Big family (46.2%), Black Bull (44.9%), and Tropical Dragon (44.2%). Conversely, the lowest disease incidence was noted in BARI line 04-21X144 at 13.5%, which was statistically similar to BARI line 03-144X21 (15.0%), Black Diamond (16%), and BARI line 01-08X07 (16.3%). Stem blight severity in field observations ranged from 10.3% to 26.6%. The highest disease severity was observed in Jambo Jaguar (G8), statistically a kin to Black Giant (G9), Big family (G6), Dragon Beauty (G16), Tropical Dragon (G5) watermelon varieties (Table 5).

At Plant Pathology Division, BARI, Gazipur, stem blight incidence ranged from 10.4% to 45.9% ((Table 6). Black Giant (G9) variety exhibited the highest disease incidence of 45.9%, which was statistically similar to Jambo Jaguar (45.2%), Big Family (45.3%), Black Bull (43.7%), and Tropical Dragon (43.2%). The lowest incidence was observed in BARI line 04-21X144 at 10.4%, followed by BARI line 03-144X21 (12.9%) and Black Diamond (14.9%). At Plant Pathology Division, BARI, stem blight disease severity ranged from 9.2% to 25.6%. Jambo Jaguar (T8) had the highest disease severity followed by Black Giant (G9), Big Family (G6), Dragon Beauty (G12), Tropical Dragon (G5), among other watermelon varieties. The lowest disease severity was recorded in BARI line 04-21X144, similar to BARI line 03-144X21 (9.9%) and BARI line 01-08X07 (11.69%) (Table 6). Based on these findings, it was concluded that BARI lines demonstrated moderate resistance against stem blight disease under both artificial conditions and inoculum pressure. However, hybrid varieties from abroad displayed susceptibility to the disease.

The assessment of GSB resistance shows variability ranging from 11-70% (2 to 4 rating units) among different plants and replications (Wehner and Amand, 1993). They observed variability in GSB outbreaks may stem due to genetic or environmental factors (Wehner and Amand, 1993), which can influence pathogen aggressiveness, leading to variations across years and between field and greenhouse tests (Gusmini and Wehner, 2002; Stewart *et al*., 2015). Recent findings indicate that genetically distinct fungal species serve as causal agents of GSB, suggesting that resistance variability across different years and environments, such as greenhouses and fields, may arise from interactions between the environment and fungal species (Stewart *et al*., 2015). The inconsistency in observed ratios may be due to interactions among multiple genetic loci, as well as interactions with environmental factors (Kumar, 2009). In the controlled environment of the greenhouse, conditions were standardized for both plant and pathogen development. However, in the field, where a large number of cultivars were evaluated, the need for extensive space led to increased environmental variability across different sites and years.

Table 4. Performance of water melon 18 varieties/lines at RHRC research field, Patuakhali.

Variety/Germplasm	1stMFO	NO1stMF	1stFFO	NO1stFF	NFPP	IFW(kg)	FL(CM)	FB (CM)	YTH
G1=Black Super	44	7.67^{bc}	46	15.3^{bc}	3.06^{b}	4.35^{ef}	27.4^{bc}	19.17^{cd}	38.11
G2=Black Diamond(Metal Seed)	41	5.67^{d-e}	44	13.0^{d}	2.45^{ef}	4.08^{f}	28.3^{bc}	21.95^{de}	31.06
G3=Black Diamond (Alamgir Seed)	42	6.00^{c-e}	44	17.3^{a}	2.74^{b-d}	4.94^{b-e}	26.8^{cd}	21.91^{de}	43.80
G4=Sweet Dragon	43	8.00^{ab}	46	13.0^{d}	2.93^{bc}	4.74^{c-e}	19.2^{d}	20.95^{cd}	50.49
G5=Tropical Dragon	47	7.67^{bc}	48	$17.3^{\mathbf{a}}$	2.79^{b-d}	4.64^{d-f}	27.8^{bc}	22.36^{cd}	40.21
G6=Big Family	49	9.67^{a}	49	14.0^{bc}	2.13^{ef}	4.74^{c-e}	26.1^{d}	20.75^{de}	21.16
G7=World Queen	40	7.00^{b-d}	42	16.3^{a}	3.05^{b}	4.86^{b-e}	28.3^{bc}	20.55^{de}	40.91
G8=Jumbo Jaguar	44	5.00^{e}	46	13.3^{d}	2.60^{b-e}	4.91^{b-e}	30.4^{a}	20.68^{de}	49.28
G9=Black Giant	41	6.67^{b-e}	44	16.3^{a}	2.37^{df}	4.59^{d-f}	29.7^{ab}	21.96^{de}	32.69
G10=Thailand 2	45	6.00^{c-e}	47	16.3^{a}	2.10^{ef}	6.63^{a}	31.2^{a}	25.22^{a}	43.70
G11=Black Bull	44	5.00^{e}	45	14.0^{bc}	3.10^{b}	4.99^{b-e}	27.3^{cd}	22.55^{bc}	53.45
G12=Dragon Beauty	40	6.00^{c-e}	43	17.0^{a}	2.76^{b-d}	5.07^{b-d}	28.0^{bc}	22.25^{cd}	50.82
G13=Sweet Green	45	6.67^{b-e}	46	13.0^{d}	2.80^{b}	5.31^{b-d}	30.7^{a}	23.69^{bc}	50.60

Variety/Germplasm	1stMFO	NO1stMF	1stFFO	NO1stFF	NFPP	IFW(kg)	FL(CM)	FB (CM)	YTH
G14=Sugar Emperor	40	5.67^{de}	46	17.0^{a}	3.03^{b}	5.49^{b}	28.2^{bc}	25.58^{a}	65.13
G15=BARI line 01-08x07	42	5.00^{e}	45	14.0^{bc}	3.95^{a}	3.03^{g}	19.4^{e}	16.19^{e}	37.39
G16=BARI line 02-07x08	44	5.00^{e}	45	13.0^{a}	2.07^{f}	3.20^{g}	18.9^{e}	18.35^{e}	22.40
G17=BARI line 03-144x21	40	6.67^{b-e}	43	17.3^{a}	2.83^{b-d}	4.48^{g}	19.1^{e}	17.91^{e}	38.12
G18=BARI line 04-21x144	45	7.00^{b-d}	46	15.33^{ab}	2.97^{b}	4.02^{d-f}	17.2^{f}	16.81^{e}	34.54
CV (%)	-	5.32	-	6.26	3.37	3.46	7.54	8.37	7.77

1stMFO= First male flower opening, NO1stMF= No of First Male Flower, 1stMFO= First female flower opening, NO1stFF= No of First Female Flower, IFW= Individual Fruit Weight, NFPP= No of fruit per plant, FL(cm.) = Fruit length (cm.), FB(cm.) = Fruit breath (cm.) and YTH= Yield (ton/ha)

Table 5. Incidence and severity of stem blight disease of water melon in 18 varieties/lines at RHRC research field, Patuakhali.

Variety/Germplasm	Stem blight incidence (%)	Stem blight severity (%)	Disease reaction
G1=Black Super	27.8e	20.39cd	MS
G2=Black Diamond(Metal Seed)	16fg	16.38fg	MR
G3=Black Diamond (Alamgir Seed)	18.2f	14.32fg	MR
G4=Sweet Dragon	34.0d	19.95de	MS
G5=Tropical Dragon	44.2ab	24.07ab	MS
G6=Big Family	46.2ab	24.93ab	S
G7=World Queen	43.3bc	22.70bc	MS
G8=Jumbo Jaguar	46.4ab	26.57a	S
G9=Black Giant	47.5a	26.41a	S
G10=Thailand 2	40.4c	21.24cd	MS
G11=Black Bull	44.9ab	23.04bc	MS
G12=Dragon Beauty	46.4ab	24.41ab	S
G13=Sweet Green	34.3d	17.34ef	MS
G14=Sugar Emperor	27.0e	16.55fg	MS

Variety/Germplasm	Stem blight incidence (%)	Stem blight severity (%)	Disease reaction
G15= BARI line 01-08x07	16.3fg	11.80hi	MR
G16= BARI line 02-07x08	18.3f	14.74fg	MR
G17= BARI line 03-144x21	15.0fg	10.91i	MR
G18= BARI line 04-21x144	13.5g	10.27i	MR
CV (%)	6.47	8.42	

MR= moderately resistant, S= Susceptible, MS= moderately susceptible

Table 6. Incidence and severity of stem blight disease of water melon in 18 varieties/lines at BARI research field Gazipur.

Variety/Germplasm	1stMFO	NO1stMF	1stFFO	NO1stFF	% Stem blight incidence	% Stem blight severity	Disease reaction
G1=Black Super	43	7.00bc	45	13.7cd	27.1f	20.5c-e	MS
G2=Black Diamond(Metal Seed)	40	5.00d-e	43	12.0e	15.0hi	14.8gh	MR
G3=Black Diamond (Alamgir Seed)	41	5.33d-e	43	16.3ab	17.2h	13.3hi	MR
G4=Sweet Dragon	42	7.33bc	45	12.0e	33.1e	19.0ef	MS
G5=Tropical Dragon	46	7.00bc	47	17.0a	43.2a-c	23.2a-c	MS
G6=Big Family	48	8.67a	48	13.0de	45.3ab	23.5ab	MS
G7=World Queen	41	6.33b-e	41	15.7b	42.3c	21.8b-d	MS
G8=Jumbo Jaguar	45	5.00de	45	13.3de	45.2ab	25.6a	S
G9=Black Giant	40	6.00b-e	43	16.0ab	45.9a	24.8a	S
G10=Thailand 2	44	5.33de	46	15.7b	37.2d	20.0de	MS
G11=Black Bull	45	4.33e	44	13.0de	43.7a-c	23.8ab	MS
G12=Dragon Beauty	42	5.33de	42	16.0ab	45.3ab	24.9a	S
G13=Sweet Green	44	6.00b-e	45	12.0e	32.4e	16.9fg	MS
G14=Sugar Emperor	41	5.00de	45	16.0ab	22.8g	15.4gh	MR
G15=BARI line 01-08x07	41	4.33e	44	13.7cd	14.7hi	11.7ij	MR
G16=BARI line 02-07x08	43	4.33e	44	12.7de	16.6h	14.9gh	MR
G17=BARI line 03-144x21	39	6.00b-e	42	16.3ab	10.0j	MR	
G18=BARI line 04-21x144	44	6.33b-e	45	14.3c	10.4j	9.1j	MR
CV (%)	-	6.67	-	5.43	4.97	8.79	

1stMFO= First male flower opening, NO1stMF= No of First Male Flower, 1stMFO= First female flower opening, NO1stFF= No of First Female Flower

Relationship between individual fruit weight (kg) and yield (tha-1) of watermelon

Fig. 4 indicates a linear correlation between individual watermelon fruit weight (kg) and their yield per hectare (measured in t). This suggests a positive relationship between fruits weight and their yield. A regression line was devised to represent this relationship as y =7.801x + 4.8849, where 'y' stands for yield (tons/ha) and 'x' represents individual fruit weight (kg). The coefficient of determination, R^2 = 0.3252, implies that 32.52% of the variation in watermelon yield per hectare can be explained by differences in individual fruit weight.

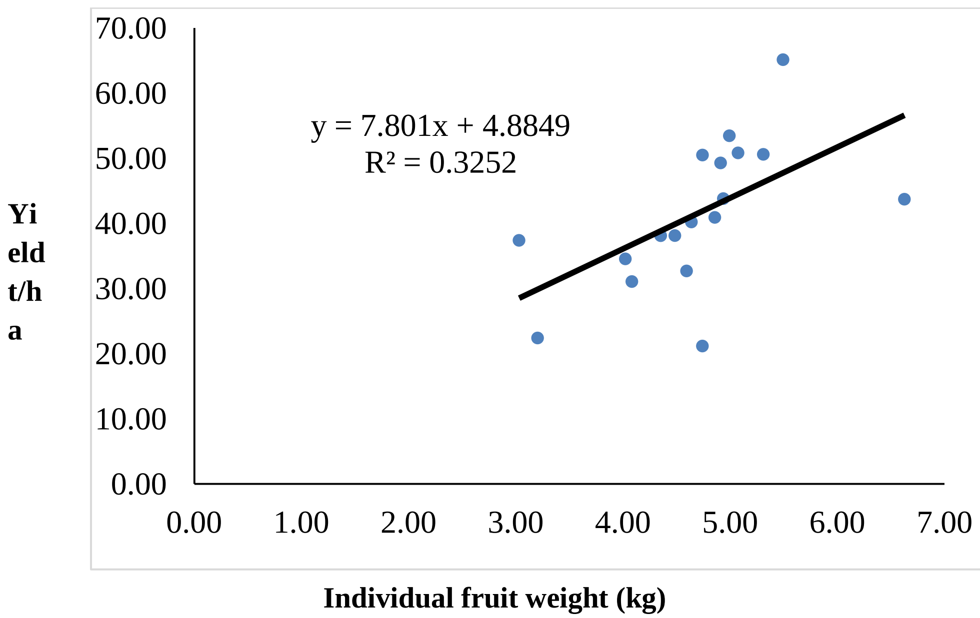

Fig. 4. Relationship between individual fruit weight (kg) and yield (t/ha) of watermelon.

Water melon seeds may be contaminated with seed borne fungi like *Didymella bryoniae, Fusarium oxysporum* f.sp. *niveum* and other seed borne fungi. BARI lines 03-144x21, and 02-07x08 showed the lowest gummy stem blight disease symptom development in the laboratory detached leaf test and the lowest disease incidence and disease severity under an artificially inoculated condition in a pot house. All the commercial varieties and the advanced line have susceptibility to the gummy stem blight disease of water melon. From these studies, it can be concluded that the BARI lines, viz., BARI line 01-08X07, BARI line 02-07X08, BARI line 03-144X2 and BARI line 04-21X144, showed moderately resistance against stem blight disease of water melon and all the commercial varieties and advanced lines showed susceptibility of GBD of water melon.

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Conflicts of Interest

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

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EDIBLE WILD FRUITS USED BY THE TRIBAL COMMUNITIES OF ROWANGCHHARI UPAZILA IN BANDARBAN

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Abstract

Wild edible fruits play a vital role in the daily lives of the ethnic people living in Chittagong Hill Tracts. It has great nutritional and medicinal values. The present study deals with the identification, documentation, and exploration of wild edible fruits consumed by tribal communities in the Rowangchhari upazila of Bandarban Hill District, Bangladesh. Information on wild edible fruit tree species was collected through structured and semi-structured interviews. A total of 35 wild edible fruits belonging to 23 families were recorded with their scientific name, family, local name, time of availability, and mode of consumption. These fruits are generally eaten fresh and raw. *Dillenia indica*, *Diospyros malabarica*, *Ficus racemosa*, *Flacourtia jangomas*, *Haematocarpus validus*, *and Syzygium fruticosum* are some fruits that are commonly used by the local inhabitants, and some of these fruits are also used to treat different diseases traditionally.

Keywords: Bandarban, Hills, Medicinal plants, Tribal people, Wild fruit

Introduction

Wild edible fruits have played a very vital role in supplementing the diet of the ethnic people in the hilly areas of Bangladesh. Some of them are sold in local markets or preserved for use during prolonged droughts. Wild edible fruits are excellent sources of fiber, vitamins, minerals, and polyphenols, all of which have health advantages. The risk of many illnesses, including diabetes, cancer, and coronary heart disease, is decreased by eating wild fruits (Brahma *et al*., 2013). It has been used as a source of food, medicine and nutritional supplements by many people in rural areas, tribal and indigenous societies from ancient times and continue to be widely consumed today (Nahar *et al*., 1990). Wild edible fruits have a high nutritional and therapeutic value and are a good source of vitamins and minerals (micronutrients) like copper, zinc, iron, manganese, magnesium and some hormones, as well as protein and energy, all of which are needed for the human body. Many of these wild fruits have higher nutritional and mineral content than commercial fruits in some contexts (Nahar *et al*., 1990, Seal *et al*., 2014).

Wild fruits are a valuable source of carbohydrates, proteins, lipids, vitamins, minerals, fibers and other nutrients (Deshmukh and Waghmode, 2011). Wild edible fruit

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plants are essential for improving rural livelihoods and providing food, nutrition and sustenance to poor populations around the world (Mishra *et al*., 2008; Tiwari *et al*., 2010; Badhani *et al*., 2011). According to Johns and Eyzaguirre, (2007) indigenous populations in East Africa use dietary supplements made from wild plant material that contains antioxidants to break down cholesterol from traditional foods including meat, milk, and blood. However, due to overexploitation and different anthropogenic activities such as forest and jungle cutting, these wild fruits producing plants are currently diminishing and becoming increasingly threatened, with some even becoming extinct. During seasonal food shortages or calamities such as droughts and floods, wild edible fruit trees provide crucial protection against starvation or famine. The tribal people of Rowangchhari upazila of Bandarban Hill District collect a diverse range of wild edible fruit plants from their natural state. It is a part of their culture to eat wild edible fruit plants on a daily basis. It is necessary to explore present status and future availability of wild fruits under growing population. Apart from this, no significant work has been done on the availability of wild edible fruits in Rowanchhari. Considering this fact, the present research work has been undertaken on the documentation of wild edible fruits grown in the Rowangchhari upazila of Bandarban Hill District.

Materials and Methods

The present investigation was conducted for about two years, from July 2019 to June 2021 in Rowangchhari upazila in the Bandarban Hills District of Bangladesh. Rowangchhari Upazila has a total area of 442.89 sq km and located in between 22°03' and 22°20' north latitudes and in between 92°14' and 92°30' east longitudes. The upazila consists of 4 Unions/Wards, 15 Mauzas/Mahallas. The total population of Roangchhari upazila is 27,264. Of these, 14,243 are males and 13,021 are females. The upazila is inhabited by Marma, Chakma, Tripura, Thanchangya, Murang, Bawm, Kheyang, Khumi and other ethnic groups. The Marmas are the largest tribe inhabiting the forested hilly region in the Rowangchhari upazila of Bandarban Hill District. Four different paras namely Dalujhiri para, Mandui para, Rowangchhari bazar para and Bijoy para were selected to execute the present investigation. These areas have been selected because they have representatives of four communities namely Chakma, Marma, Tripura and Tanchangya. The field works were conducted in three prominent seasons (winter, summer and monsoon) in a year for better information about wild edible fruits. Twenty elderly ethnic people who depend on gathering wild fruits from the forest for their subsistence have been identified. Furthermore, five such ethnic individuals who gather wild fruits from the forest and resell them in the neighborhood market have been chosen. Most of them have primary level education. In order to ensure proper data collection in the field about wild edible fruit, data collectors were initially provided with a number of concepts. The information was gathered by local tribal people through scheduled interviews, questionnaires, informal meetings, and local market visits. During survey, live specimens along with photographs were taken and interacted with local wild edible fruit vendors and villagers for local identification and to assess the traditional knowledge on wild edible fruits. Questionnaire was prepared for the collection of data such as local name, habit of plants, plants type, and time of availability and mode of consumption as food. The information was cross-checked after discussions with several tribal people, the village head, elder women, and other local informants. The common plant samples were identified in the field by the authors and the unidentified species were preserved in the Bangladesh Forest Research Institute and finally identified with the help of plant taxonomists of Forest Botany Division of Bangladesh Forest Research Institute, Chattogram and Bangladesh National Herbarium, Dhaka. The tribal name in this paper is abbreviated and placed in parenthesis (M stands for Marma, Ch for Chakma, Tr for Tripura, and Ta for Tanchangya).

Results and Discussion

In the present study, a total of 35 wild edible fruit plants have been collected belonging to 23 families and 28 genera. Moraceae, Euphorbiaceae and Flacourtiaceae were found to be the largest families, containing 3 species each, followed by Anacardiaceae, Boraginaceae, Dilleniaceae, Fabaceae, Myrtaceae, Rubiaceae and Sterculiaceae (2 species each), and the rest of the families were found in single species (Table 1). The collected plants are arranged in alphabetical order with their common name, tribal name, family, time of availability and mode of consumption (Table 1).

Table 1. List of wild edible fruits in Rowangchhari upazila of Bandarban Hill District

Sl. no.	Scientific name	Family	Common name	Tribal name	Time of availability	Mode of consumption
1.	*Alangium salvifolium* (L.f.)	Alangiaceae	Ankar kata	Ankura (Ch).	June-July	Ripen fruit pulp is eaten
2.	*Antidesma ghae-sembilla* Gaertn.	Euphorbiaceae	Elena	Parajam (Ch), Baro vongor (Ta).	July-August	Ripen fruit is eaten fresh, roasted or jams and jellies
3.	*Artocarpus chama* Buch.-Ham. *ex* Wall	Moraceae	Chapalish	Bon kanthal (Ma), Bathagola (Ch).	June-August	Young fruit is eaten by cooking
4.	*Artocarpus lacucha* Buch.	Moraceae	Dewa	Bhorta gula (Ma), Momichi (Ch).	June-July	Ripen fruit is eaten
5.	*Bouea oppositifolia* (Roxb.) Meissner	Anacardiaceae	Uriam	Uriaam (Ch), Jaraboo aam (Ma), Moyaam (Tr).	May-June	Ripen fruit is eaten raw, and young fruit is eaten as a vegetable.
6.	*Buchanania lanzan* Speng.	Anacardiaceae	Nala amsi	Pival (Ma).	April-May	Seed kernel is eaten raw
7.	*Calamus tenuis* L.	Arecaceae	Jali bet	Jai bet (Ch).	February-April	Ripen fruit is eaten raw

Sl. no.	Scientific name	Family	Common name	Tribal name	Time of availability	Mode of consumption
8.	*Cardiospermum halicacabum* L.	Sapindaceae	Noaphutki	Hedaboksa (Ch), Nala maiachi (Ma), Kerapoksak (Ta).	May-November	Ripen fruit is eaten raw
9.	*Citrus medica* L.	Rutaceae	Adha jamir	Haidda lebu (Ch).	September-November	Green fruit is eaten raw
10.	*Cordia dichotoma* G. Frost.	Boraginaceae	Kalauza	Chaine (Ma), Bohalli (Ch).	April-May	Ripen fruit is eaten raw and young fruit is eaten as a vegetable
11.	*Dillenia indica* L.	Dilleniaceae	Chalta	Ulugach (Ch), Kraaming (Ma), Thaiplaw (Tr).	July-October	Mature fruit is used in curries to add flavor and sourness
12.	*Dillenia pentagyna* Roxb.	Dilleniaceae	Hargeza	Ulu (Ch), Jange bring (Ma), Thaiplaw (Tr).	May-June	Ripen fruit is eaten raw and young fruit is used to make pickles or cooked
13.	*Diospyros malabarica* (Desr.)	Ebenaceae	Deshi gaab	Keth gula (Ch), kock (Tr), Gaab gaith (Ta).	June-July	Ripen fruit is eaten raw
14.	*Ehretia acuminata* R.Br.	Boraginaceae	Kala-huza	Kala-ujja (Ch).	May-July	Ripen fruit is eaten raw and young fruit is used to make pickles
15.	*Ficus racemosa* L.	Moraceae	Jagadumu r	Noroputitida (Ch), Sanak (Ma).	May-August	Ripen fruit is eaten raw and young fruit is eaten in curries
16.	*Flacourtia indica* (Burm. f.) Merr.	Flacourtiaceae	Baichi	Benchi (Ch), Binja (Ma), Katai (Tr).	June-August	Ripen fruit is eaten raw
17.	*Flacourtia inermis* Burm.f.	Flacourtiaceae	Loai	Tomytomy (Tr).	May-December	Ripen fruit is eaten raw and young fruit is also cooked
18.	*Flacourtia jangomas* Lour.	Flacourtiaceae	Paniala	Painnya gula (Ch), Tamagry	July-August	Ripen fruit is eaten raw

Sl. no.	Scientific name	Family	Common name	Tribal name	Time of availability	Mode of consumption
				(Ma), Painna mola (Tr).		
19.	*Garcinia cowa* Roxb.	Clusiaceae	Kau	Kaogula (Ch), Tahgala (Ma).	June-August	Ripen fruit is eaten raw
20.	*Gardenia coronaria* Buch-Hum.	Rubiaceae	Kannyari	Rekphul gach (Ch), Rangkhu (Ma).	June-September	Ripen fruit is eaten raw
21.	*Haematocarpus validus* Bakh.f.*ex*	Menispermaceae	Lalgula	Roseco (Ch), Ranguichi (Ma).	July-August	Ripen fruit is eaten raw
22.	*Musa ornata* Roxb.	Musaceae	Bonkala	Bizi kola (Ch), Ramanigi bela (Ma), Li phang (Tr).	Through the year	Ripen fruit is eaten raw and young fruit is also cooked
23.	*Phyllanthus acidus* (L.) Skeel.	Euphorbiaceae	Arbori	Fungleosasi (Ch), Dendalum (Ma).	March-May	Fruit is used in curries to add a sour flavor
24.	*Phyllanthus emblica* L.	Euphorbiaceae	Amloki	Hadamola (Ch), Soi sha (Ma), Omloki (Tr), Kalamabagula (Ta).	September-November	Fruit is eaten raw or dried and is also taken as pickles
25.	*Pithecellobium dulce* Roxb.	Fabaceae	Khoiababla	Quamochil (Tr), Jilapi gach (Ch).	April-July	Fruit is eaten raw
26.	*Protium serratum* Wall. *ex* Colebr.	Burseraceae	Gutguttya	Gutguittiya (Ch), Shu dui shi (Ma), Thai cherem (Tr).	July-August	Fruit is eaten raw
27.	*Randia spinosa* Poir.	Rubiaceae	Monkata	Mainphal (Ch).	December-February	Mature seed is eaten after roasting
28.	*Solanum torvum* Sw.	Solanaceae	Tit begun	Bigal biji (Ch), Kajo ba (Ma), Titar berul (Ta).	Through the year	Fruit is eaten fried as a vegetable

Sl. no.	Scientific name	Family	Common name	Tribal name	Time of availability	Mode of consumption
29.	*Sterculia foetida* L.	Sterculiaceae	Jangla badam	Yaa-hea (Ch), Letpan-show (Ma).	July-August	Mature seed is eaten after roasting
30.	*Sterculia villosa* Roxb. *ex*. smith	Sterculiaceae	Udal	Udal pata (Ch), Chambai (Ma), Naichini udal (Tr).	March-May	Ripen fruit is eaten raw
31.	*Syzygium clavifolium* Roxb.	Myrtaceae	Pania jam	Lamba jam (Ch).	May-June	Well ripe fruit is eaten raw
32.	*Syzygium frutocosum* Roxb.	Myrtaceae	Khidu jam	Potti jam (Ch), Ta sabbi (Ma).	May-June	Fruit is eaten raw
33.	*Tamarindus indica* L.	Fabaceae	Tentul	Tedoy (Ch), Gayosi si (Ma).	February-March	Both unripe and ripe fruit is eaten raw or as pickles
34.	*Terminalia catappa* L.	Combretaceae	Kat badam	Badam gach (Ch).	May-August	Seed kernel is eaten raw
35.	*Ziziphus oenoplia* (L.) Mill.	Rhamnaceae	Bon boroi	Si mo thaui (Ma), Mon boroi (Tr).	November-December	Ripen fruit is eaten raw

Based on habit, there are trees (70%) being the most commonly used plants, followed by shrubs (18%), herbs (7%) and climbers (5%) (Fig. 1).

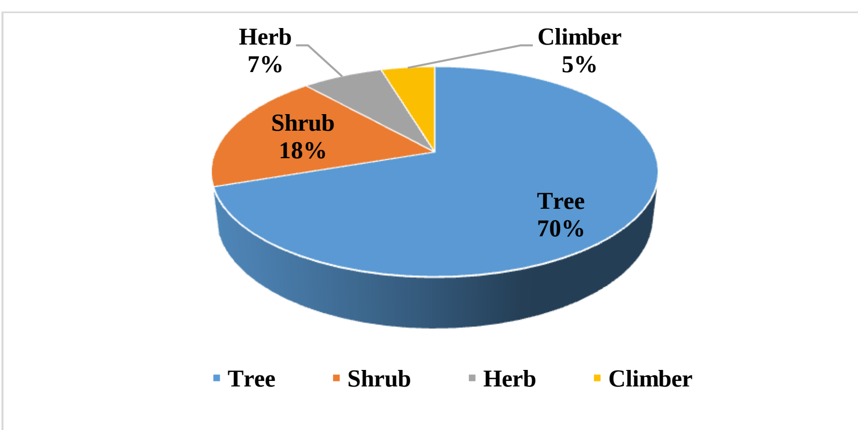

Fig. 1. Classification of wild edible fruits by its habit form

The abundance of wild edible fruits in Rowangchhari upazila of Bandarban Hill District varies with the seasons. During the study 19 fruit plant species were collected during the summer season (April-June); 09 fruit species in monsoon (July-August); 04 fruit species in winter (November-March) and 03 fruit species were collected round the year. Most of the fruits are only available in the summer due to their seasonality in fruiting. In Bangladesh, the main fruit season is summer and in winter, there are very few fruits available in Bangladesh (Pasha *et al*., 2015). The findings indicate that while people eat a sufficient amount of fruits during the summer and monsoon, there is a severe lack of local fruit during other seasons.

Das, (1987) reported that there are 60 wild edible fruit species in the forests of Bangladesh. Khatun, (2016) reported that the tribal people in Khagrachari District used 100 different species of wild fruit. Chowdhury, (2015) listed 400 fruits and medicinal plants, including herbs, climbers, shrubs, and trees from Bangladesh's southwest coast. Alam and Mohiuddin, (2021) documented 90 tree species, 84 herb and shrub species from Rowangcharri upazila of Bandarban district although there is no separate list for wild edible fruit. According to Paul *et al*., (2020) there are 49 species of wild edible fruits belonging to 25 families, most of which are consumed by tribal people in Bangladesh's Central and Highland Triangle. In the Shikkim Himalayas, Bhutia *et al*., (2015) recorded 26 wild edible fruits, 14 of which were identified as the most desirable. According to Singh *et al*., (2014), the Meitei population in Manipur's Imphal valley uses 39 types of small edible fruits from 29 genera and 23 families as medicine.

Since the world's population is expanding quickly at the moment, current agricultural production cannot meet everyone's food needs. Moreover, less land is being used for agriculture due to a variety of factors, including building and urbanization. The world will then be facing a nutrition and health crisis. Wild edible fruits species provide a superior source to meet the demand for food in these conditions. Wild edible fruits are a good source of vitamins and minerals and require little or no maintenance. Wild edibles were the only source of food during famines and before to the regularization of traditional agriculture. To ensure food security and sustainability in the near future, it will be crucial to document unconventional wild edible resources. It will provide for the nutritional needs of future generations. But regrettably, only the elderly remember this untamed treasure, and its existence is under jeopardy. The current study on edible wild fruit will contribute to the documentation of this unusual information and be useful in the development of domesticating these species for agricultural purposes, which will create jobs for those living in hilly areas.

Conclusion

Wild edible fruit plants are affordable, readily available, and extremely valuable to society because of their nutritional and therapeutic qualities. With the change in the socioeconomic situations of people during recent years, some of the information has been lost, and the population of some of the wild plant species is also shrinking due to habitat loss. Poor people rely on gathering these wild edible fruits for their livelihood since they sell them in the surrounding marketplaces. The population of wild edible fruit trees in Rowangchhari upazila of Bandarban Hill District is deteriorating primarily due to human and livestock population pressure resulting from their overexploitation, severe forest degradation, and related agricultural expansion. Mass attention is needed to protect and popularize the wild edible fruit among the local tribal people. Therefore, it is necessary to address the conservation of these plant species and the popularization of their use.

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Conflicts of Interest

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

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EXPLORING GENETIC VARIABILITY OF CHILLI GENOTYPES IN RELATION TO YIELD AND ASSOCIATED TRAITS

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Abstract

Chilli peppers, integral to Capsicum spp., are globally vital crops valued for culinary, economic, and nutritional contributions. Assessing genotypes is essential for improving varieties with traits like higher yield, disease resilience, and enhanced nutritional value. This study was conducted at the Spices Research Centre in Shibganj, Bogura, during the period of 2019-20 to evaluate 20 genotypes of chilli. Employing an alpha lattice design with two replications, the evaluation considered genetic diversity, variation, heritability, clustering, and trait associations as key components. All the traits exhibited remarkable significance ($P< 0.01$) for the studied genotypes, underscoring the genetic variability inherent to the traits in focus. Heritability varied from 79% to 99% for the traits investigated. Foremost in yield was found in the AVPP 1111 genotype (21.62 t/ha), trailed by the Indch 39 (21.19 t/ha), and A1511050 (20.57 t/ha). The cluster analysis dendrogram visually demonstrated the proximity among different genotypes concerning their similarities, ultimately forming six distinct clusters. While the studied traits displayed higher genotypic co-efficient of variation (GCV), and phenotypic co-efficient of variation (PCV) values, however, GCV values were closely aligned with PCV values across the traits. Notably, a robust positive correlation existed between yield and both single fruit weight and the weight of fruit per plant. The outcomes of this study, i.e., the promising genotypes AVPP 1111, Indch 39, A1511050 etc.; grouping; and significant positive correlations of different traits with grain yield, hold valuable insights for future chili improvement initiatives.

Keywords: Chilli, GCV, Genetic diversity, Heritability, PCV, Variability

Introduction

Chilli (*Capsicum annuum* L.) is an important valuable commercial spice-cum-vegetable crop belonging to the family Solanaceae, and originated in Latin American regions of New Mexico, Guatemala and Bulgaria (Prajapati *et al.,* 2020). Chilli is diversely used as a spice, condiment, culinary supplement, medicine, vegetable and

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ornamental plant. It is also one of the most widely used spice crop in Bangladesh. It is widely cultivated throughout the year. It is a self-pollinated crop but chances of cross-pollination are also high. It has wide variability especially on shape, size, skin color, hotness etc. Germplasm collection followed by evaluation is a continuous process in crop breeding program and is also important for the maintenance of biological diversity and food security. Improvement of any crop depends on the extent of genetic variation present, and the degree of improvement depends on magnitude of the available beneficial genetic variability. Therefore, it is necessary to explore the mutual relationship between yield and yield components for efficient utilization of the genetic stock in crop improvement program of chilli.

Heritability is used to denote the relative degree to which a character is transmitted from parent to offspring. The magnitude of such estimates suggests the extent to which improvement is possible through selection (Nechifor *et al.*, 2011). It also indicates how much of the genetic variability has a genetic origin and gives necessary information for the genetic selection process (Falconer, 1981). The correlation between the yield and its component characters are not often real because of inter-relationship existing between the component characters themselves. Therefore, analysis of inter component correlation is very essential to expose the direct and indirect contributions of each component (Wright, 1921; Srinivas *et al*., 2020). In Bangladesh, the cultivated area of chilli is 1.03 lakh hectare, and the total production is 1.41 lakh metric tons (dry chilli) with an average yield of 1.37 t/ha (BBS, 2018). Though there are quite a few popular chilli varieties introduced by public research institutes and private seed companies, has higher yield potential, but still we are well short in production than the expected demand. So, we are trying to develop new varieties with higher yields and better quality that will compete with the existing popular chilli varieties. By keeping the view in consideration, the present experiment was conducted to evaluate chilli genotypes collected from different agro-ecological zones and to identify the potential genotype(s) in terms of yield and its attributing traits suitable for mass production while conserve the land races for future research purposes.

Materials and Methods

Germplasm

A total of 20 chilli lines collected from all over the Bangladesh were included in the study. Commercially available chilli variety developed by public research institute were used as standard check. Details of the germplasm were given in supplementary table S1.

Location

The chilli lines were evaluated at Spices Research Center, BARI, Bogura during winter (Rabi) season of 2019-20. The weather details prevailed during cropping seasons of different years at the location were given in supplementary table S2.

Experimental plan

The field trial was laid out in Alpha lattice design accommodating the genotypes under study with 2 replications. The seeds of the different genotypes were sown in seed bed on 26 September, 2019 and four weeks old seedlings were transplanted on well-prepared raised bed in the field on 31 October, 2019. The unit plot size was 3 m x 1 m keeping 0.5 m space between beds. A 50 cm x 50 cm spacing was maintained for row to row and hill to hill during planting. The crop was fertilized with recommended dose of cow dung 5t/ha, $N_{100}P_{52}K_{100}S_{22}Zn_3B_2$ kg/ha. Other intercultural practices were done as and when required in which timely irrigation was provided to ensure moisture availability and plant protection measures were taken to repel pest infestation.

Observations recorded

Various morpho-physiological traits observations were recorded using standard protocol for chilli phenotyping (IPGRI, AVRDC and CATIE. 1995). Plant height, number of fruits per plant, single fruit weight, weight of fruits per plant, was recorded on randomly taken five plants, and then averaged. Fresh yield (Green chilli) was recorded from field weight on whole plot basis at harvestand converted to tons per hectares.

Statistical analysis

The analysis of variance for individual traits was carried out using R software (R Core Team 2021). The theoretical formula for calculation of ANOVA implemented in R was in accordance as for Alpha lattice design (Patterson and Williams 1976). Analysis was required to test whether the genotypes differed significantly among themselves or not. Clustering of genotypes were done using 'Dendextend' package (Tal Galili, 2015) in R software (R Core Team 2021).

Linear model of observations in alpha lattice design as follows

$$Yijk=\mu + ti + rj + bjk + eijk \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(1)$$

Where,

Yijk- observed trait for i-th treatment received in the k-th block within j-th replicate

ti - fixed effect of the i-th treatment

rj- effect of the j-th replicate

bjk- effect of k-th incomplete block within the j-th replicate

eijk- experimental error

Phenotypic and genotypic variance were calculated according to the formula given by (Lush , 1949). Heritability in broad sense for all the characters was computed as suggested by (Lush, 1949). Heritability was classified in to low (0-30 %), moderate (30-60 %) and high (>60 %) as suggested by (Robinson et al, 1949). Correlation analysis was performed in 'R' software (R Core Team 2021) using 'Agricolae' package (de Mendiburu, 2015).

Results and Discussion

Variability estimates

Twenty chilli genotypes were studied for estimating genetic diversity and variability based on some morphological traits. All the morphological characters studied in this study showed highly significant variability (GV) ($P < 0.01$) (Table 1). These significant differences indicate that the genotypes were genetically variable for the studied traits. Variations in growth, and yield components have been reported in many studies (Sharma *et al.*, 2010; Thul *et al.*, 2009; Alam et al., 2022; Alam et al., 2023; Khan et al., 2022).

In the present study, it was found that the GCV and PCV value was higher for all the traits (Table 1); however, GCV values were near to PCV values for all the studied traits. Higher values of PCV and GCV indicated that there was high variability exiting among the genotypes. High genotypic coefficients of variation (GCV) were observed for the traits single fruit weight (54.6%), weight of fruit per plant (35.5%), fresh yield (26.57%), and number of fruits per plant (22%). On the contrary, the lowest genotypic coefficient of variation was exploited by the trait plant height (11.3%). High phenotypic coefficients of variation (PCV) were observed for traits single fruit weight (55.3%), weight of fruit per plant (35.6%), fresh yield (27.7%) and number of fruits per plant (23.9%). On the contrary, the lowest phenotypic coefficient of variation was exploited by the trait plant height (14.0%).

The results from all the genotypes depicted that, phenotypic variances (PV), and phenotypic coefficient of variation (PCV) were higher than genetic variances (GV) and genotypic coefficient of variation (GCV) for all the studied characters suggesting some environmental influence on those characters. Similar result was found by Yanti (2016) with sixteen genotypes of chilli indicating high contribution of genotypic effect for phenotypic expression of such characters. Kannan *et al*., (2016) conducted a study on evaluating eight diverse genotypes of chill, and they found that high genotypic and phenotypic coefficient of variation, heritability and genetic advance. Similarly, high heritability found in the studied genotypes for fruits per plant, fruit weight, flowers per branch, fruits per branch and clusters per plant, revealed these traits are under the control of additive gene action. This indicated high response to selection for genetic improvement of chilli genotypes.

Table 1. Co-efficient of variance and heritability of the different traits in chilli

Trait	h^2_b	GV	PV	GCV	PCV	GA	GG
PH	0.79	140.82^{**}	215.78	11.34	14.04	19.75	18.87
NF	0.92	779.75^{**}	920.03	21.97	23.87	52.96	41.67
SFW	0.99	5.28^{**}	5.41	54.61	55.29	4.68	111.11
WFP	0.99	29834.38^{**}	30037.85	35.49	35.61	354.61	72.86
FY	0.96	16.52^{**}	17.96	26.57	27.70	8.03	52.51

Note: PH=Plant height; NF=Number of fruits per plant; SFW=Single fruit weight; WFP=Weight of fruit per plant; FY=Fresh yield; h^2b=Heritability; GV=Genetic variance; PV=Phenotypic variance; GCV= genotypic coefficients of variation; PCV= phenotypic coefficients of variation; GA=Genetic advance; GG=Genetic gain.

Heritability and genetic advance

Heritability estimates often as a measure of precision of trials (Schmidt et al., 2019), is of tremendous significance to the breeder, as its magnitude indicates the accuracy with which a genotype can be recognized by its phenotypic expression. Heritability of the studied traits in the present study was ranged from 79-99%. The higher values of the estimates indicated that majority of the variation in a trait is due to variation in genetic factors (Wray and Visscher 2008; Visscher et al., 2008), which means traits are less influenced by the surrounding environment, ultimately reflects the precision/ accuracy of the trial (Schmidt et al., 2019). Most of the traits such as single fruit weight (h^2b=99%), weight of fruit per plant (h^2b= 99%), fresh yield (h^2b= 96%), number of fruits per plant (h^2b= 92%) and plant height (h^2b= 79%) exhibited high heritability (>60%) accompanied with high to moderate genotypic and phenotypic coefficient of variance (Table 1). High heritability of those traits indicated that influence of environment on these characters was negligible or low. Therefore, selection can be effective on the basis of phenotypic expression of those traits in the individual plant by implementing simple selection methods. High heritability does not always indicate a high genetic gain; heritability should be used together with genetic advance in predicting the ultimate effect for selecting superior varieties. (Muchie and Fentie, 2016).

The estimates of high heritability (>60%) coupled with high genetic advance (>20%) were recorded in weight of fruit per plant (h^2b=99 %, GA=354.61%), in Table 1 which exhibited good scope for improving these traits through phenotypic selection due to additive gene action. High genetic advance associated with high heritability of fruit weight, fruit weight per plant, single fruit weight, fresh yield, number of fruits per plant and plant height suggested appreciable level of improvement could be possible for these characters subjected to selection. High estimates of heritability for these characters suggested that the selection based on phenotypic performance would be effective as propounded by Johnson *et al*., (1955). High heritability coupled with high genetic advance has been reported for yield and fruit weight per plant in chilli (Munshi and Behra, 2000; Sreelathakumary and Rajamony, 2004; Singh and Yadav, 2008).

Means and range

Plant height ranged from 82.86 cm to 127.12 cm, with a mean of 104.65 cm, (Table 2). The tallest genotype was Indch 36 (127.12 cm) followed by TOZP 11 (117.08 cm) and the dwarf one was BARI Morich-3 (82.86 cm). Average number of fruits per plant varied from 80.08 to 187.86 with a mean of 127.09. Most profuse bearing genotype was found in EW 1009 while least one was found in Indch 41. Single fruit weight ranged from 1.51 g to 10.45 g with a mean of 4.21 g. Most light weight fruit was found from genotype VTNMCH 2, whereas healthy one was from genotype Indch 41. Average weight of total fruits per plant varied from 211.31 g to 781.37 g with a mean of 486.72 g. The maximum fruit weight per plant was observed in genotype Indch 41 and the minimum was found in genotype VTNMCH 2. Fresh yield ranged from 9.12 t/ha to 21.62 t/ha with a mean of 15.30 t/ha. The highest yielding genotype was AVPP 1111 (21.62 t/ha) followed by genotypes Indch 39 (21.19 t/ha), and A1511050 (20.57 t/ha). In contrast, the genotype AVPP 1236 was recorded for the lowest yield (9.12 t/ha) (Fig. 1).

Table 2. Performances of twenty chilli genotypes evaluated during 2019-2020

Genotype	PH	NF	SFW	WFP	FY
AVPP 1111	90.4	103.6	7.6	777.4	21.6
Indch 39	100.9	99.3	6.3	602.1	21.2
A1511050	103.7	148.5	4.2	625.7	20.6
EW 2050	109.7	111.7	6.5	720.3	19.2
LTMCH	114.3	120.9	3.8	465.1	19.2
Indch 23	94.2	172.21	3.2	563.0	18.6
TOZP 11	117.1	141.3	3.3	468.8	17.6
Indch 41	98.5	80.1	10.5	781.4	17.8
Indch 33	104.6	109.2	6.4	698.0	16.6
F1HC	102.9	130.4	3.2	410.6	14.5
LTMHCH	112.0	141.7	3.3	464.9	14.4
Indch 36	127.1	122.7	4.4	523.6	13.9
KSMRCH 1	115.0	132.9	2.9	387.6	12.8
Mohona 2	91.2	83.9	5.7	450.8	12.7
AVPP 1245	95.9	136.6	2.2	296.6	12.2
BARI Morich-3	82.7	122.1	2.6	315.9	11.5
VTNMCH 1	110.1	144.4	1.9	264.7	11.5
VTNMCH 2	104.8	139.7	1.5	211.3	10.6
EW 1009	109.5	187.9	2.2	422.8	10.2
AVPP 1236	108.0	113.17	2.6	283.8	9.1
Grand Mean	104.7	127.1	4.21	486.7	15.3
Min.	82.9	80.1	1.51	211.3	9.1
Max.	127.1	187.9	10.45	781.4	21.6
LSD (1%)	11.87	18.82	0.55	27.02	1.9
CV%	8.27	9.32	8.66	2.93	7.83

Note: PH=Plant height; NF=Number of fruits per plant; SFW=Single fruit weight; WFP=Weight of fruit per plant; FY=Fresh yield. LSD=Least significant difference; CV%=Coefficient of variation

Clustering pattern

Cluster dendrogram from cluster analysis showing the closeness of different genotypes in terms of their similarity (Fig. 1). The phenotypic relatedness was found in cluster analysis, with two major clusters. Furthermore, the grouping of evaluated genotypes, clusterted into six sub-groups. This may probably have genotypes with less diversity, so the base gene pool was narrow (Madu and Uguru, 2006). The top yielding genotypes AVPP 1111, Indch 39 and A1511050 were in a same cluster, and entirely different from other genotypes. The check entry BARI Morich-3 were in a cluster with four other genotypes. The dendrogram provides insights into the genetic diversity of the chilli genotypes and can be useful in selecting suitable parents for breeding programs to improve the yield and quality of chilli crops. The phenotypic relatedness also evidenced by the narrow range o similarity coefficient (0.637-0.866) (Votava *et al*., 2005).

Correlation analysis

The interrelationship among traits is utmost importance for effective selection in cultivar development. Correlation coefficients give reliable and useful information on the relationship between the traits in terms of the nature, extent and direction of selection (Zeeshan *et al*., 2013). The type of genotypic (r_g) and phenotypic (r_p) correlations in plant breeding can have important implications for the selection of breeding lines and the development of new cultivars. Table 3 showed the correlation coefficients (r_g and r_p) between different traits. For number of fruits per plant, both r_g and r_p were positive for plant height and yield indicating a moderate correlation with the other traits. For example, there was a positive correlation between number of fruits per plant and yield, both in terms of r_g and r_p (r_g=0.26, r_p=0.33). This suggests that the plants with a higher number of fruits per plant tend to have a higher yield.

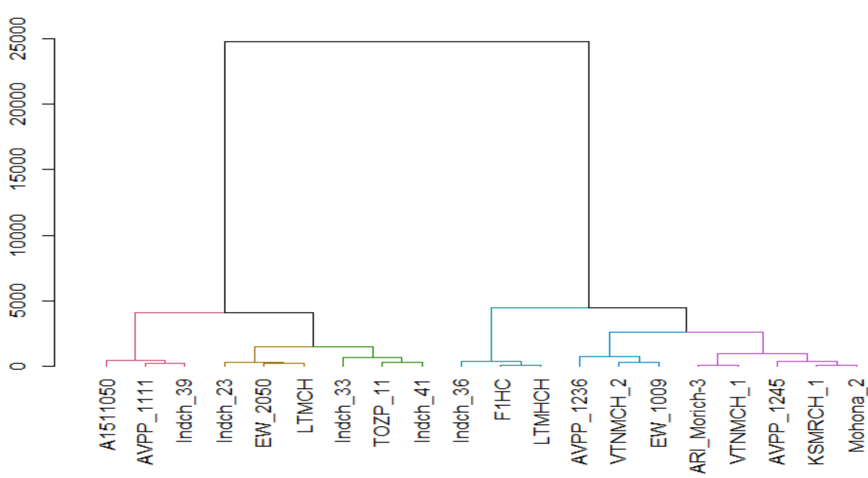

Fig. 1. Dendrogram showing the cluster pattern of genotypes of chilli

Table 3. Correlation among different yield and yield contributing traits of chilli genotypes

Traits	Type	PH	NF	SFW	WFP
NF	r_g	0.31			
	r_p	0.25			
SFW	r_g	-0.23	-0.73**		
	r_p	-0.21	-0.73**		
WFP	r_g	-0.09	-0.42	0.89**	
	r_p	-0.09	-0.44	0.90**	
FY	r_g	0.15	0.26	0.64**	0.84**
	r_p	0.08	0.33	0.67**	0.81**

Note: PH=Plant height; NF=Number of fruits per plant; SFW=Single fruit weight; WFP=Weight of fruit per plant; FY=Fresh yield; $r_{g=}$Genotypic correlation; $r_{p=}$Phenotypic correlation

From the study, it was also found that the weight of fruit per plant and single fruit weight were strongly positively associated with yield, while weight of fruit per plant and single fruit weight are negatively associated with plant height and number of fruits per plant. Similar results have been reported in chili by Hosamani (2008) where they observed significant correlation of various yield attributing traits with fruit yield. In case of weight of fruit per plant, both r_g and r_p were negative, indicating a weak negative correlation with the other traits. For example, there was a negative correlation between weight of fruit per plant and number of fruits per plant (r_g=-0.42, r_p=-0.44) and weight of fruit per plant and plant height (r_g=-0.09, r_p=-0.09). But there was a strong positive correlation between single fruit weight and yield (rg=0.84, r_p=0.81). For single fruit weight, both r_g and r_p were negative with number of fruits per plant, plant height and positive with yield, indicating a moderate to strong correlation with the other traits. For example, there was a strong positive correlation between single fruit weight and yield (r_g=0.64, r_p=0.67). There was a negative correlation between single fruit weight and plant height, meaning that as the plant height increases, the single fruit weight tends to decrease. There was also a negative correlation between single fruit weight and number of fruits per plant, suggesting that as the number of fruits per plant increases, the single fruit weight tends to decrease. In case of yield, both r_g and r_p were positive with all the other traits, indicating moderate to strong correlations. For example, there was a strong positive correlation between yield and weight of fruit per plant (r_g=0.84, r_p=0.81).

Overall, the correlation coefficients suggest that weight of fruit per plant and single fruit weight were strongly positively associated with yield, while weight of fruit per plant and single fruit weight were negatively associated with plant height and number of fruits per plant. These relationships can be useful in plant breeding programs for selecting traits that are likely to result in higher yields.

Conclusion

Evaluation of 20 chili genotypes has unveiled significant genetic diversity and variability in morphological traits, which will enhance chili breeding programs. Heritability estimates ranging from 79% to 99% suggest a predominant genetic influence on the studied traits, with minimal environmental impact. Traits such as fresh fruit yield, weight per plant, number of fruits per plant, and single fruit weight exhibit high genetic and phenotypic variability, coupled with moderate to high heritability. These insights provide valuable guidance for breeders aiming to optimize quantitative characteristics in C. annuum crosses. Hierarchical clustering techniques identified six clusters among the accessions, offering a roadmap for selecting ideal parents in breeding programs to enhance productivity and quality. Notably, genotypes such as AVPP 1111, Indch 39, and A1511050, clustered closely with lower genetic distances, displayed the highest yields, emphasizing the significance of genetic relatedness in yield performance. Overall, this study's comprehensive findings hold substantial value for both researchers and producers, contributing to the advancement of chilli cultivation and ultimately enhancing livelihoods of farmers.

Conflicts of Interest

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

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PROXIMATE COMPOSITION AND NUTRIENT CONTENT OF VARIOUS SELECTED VARIETIES OF SOYBEAN (*Glycine max* L.)

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Abstract

The physico-chemical characteristics of five soybean cultivars were evaluated in this study. Seed weight, moisture percentage, ash percentage, oil percentage, protein percentage, chemical constants (saponification, acid, and iodine value), fatty acid composition, and mineral composition of the seeds of five soybean genotypes and their cakes were estimated. BINA Soybean-3 had the highest seed weight (159 g) among the five varieties. The moisture level of the beans ranged from 9.06% (BARI Soybean-6) to 9.84% (BINA Soybean-3). The variety, Shohag, had the highest concentration of ash (5.20%). The oil content ranged from 14.88% in BINA Soybean-3 to 16.87% in BU Soybean-2. The maximum amount of oil cake was found in BU Soybean-2 (83.23%). The percentage of protein ranged from 38.01% (BINA Soybean-5) to 52.20% (BU Soybean-2). BINA Soybean-3 has the highest iodine number (79.65) , while BU Soybean-2 has the highest saponification value (189.9), and BINA Soybean-3 has the lowest acid value (0.90). The largest amounts of linoleic acid (54.39%), linolenic acid (9.56%), and palmitoleic acid (0.18%) were found in BU Soybean-2. The variety Shohag had the highest levels of phosphorus (0.84%), copper (29.24 ppm), calcium (0.23%), iron (86.75 ppm), and sulfur (0.09%) in terms of mineral composition. This study found that Shohag had remarkable mineral compositions, while BU Soybean-2 outperformed the other soybean varieties in terms of quality.

Keywords: Fatty acids, Mineral composition, Protein percentage, Soybean

Introduction

Soybean (*Glycine max* L.), also known as the golden miracle bean, belongs to the Leguminosae family (sub-family Papilionoidea). The crop is widely cultivated around the world for both human consumption and animal feed. Soybean is the world's most important oilseed crop and soybean seeds have an oil content of 42-45 percent and an edible oil content of 22 percent (Yaklich *et al.,* 2002). Soybeans are a healthy source of protein for diabetics because they are low in starch. Soy sauce is a common component in Asian cuisine and is a salty, dark liquid made from crushed soybeans and wheat that has been fermented with yeast in salt water for six months to a year or more. Miso, tempeh, and fermented bean paste are other fermented soy products. (Muzaiyanah *et al.,* 2020; Khan *et al.,* 2012; Hosseini *et al.,* 2002).

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With a population of over 170 million, Bangladesh consumes about 3.0 million tons of edible and non-edible fats every year. The annual needs for oils and fats are met through the importion of 90 to 92% of requirements. According to 2019 statistics, palm oil, soybean oil, and two types of rapeseed oil are currently the three main edible oils consumed in the country, with import shares of each being at a ratio of 58:37:5. Palm and soybean oils are two of the three types of oils that are imported in crude form and processed locally before being sold in refined form (Alam, 2020). Soybeans are exceptionally nutrient-dense foods. It contains about 20% oil and 40% high-quality protein, while rice, wheat, maize, and pulses contain only 7.0%, 12%, 10%, and 20-25 % respectively. Most cereals are low in essential amino acids e.g. lysine, while soybean contain abundant essential amino acids . Additionally, the soybean seed's chemical qualities can substantially impact the soymilk's flavor characteristics (Terhaag, *et al.*, 2013).

In addition to these, soybeans contain isoflavone which affects cell physiology, proliferation, growth, and maturity and also functions as a key regulators to preserve health. Soybean oil is primarily composed of polyunsaturated fatty acids (PUFAs), particularly linoleic acid (C18:2), an omega-6 (6) fatty acid that accounts for 55% of soybean oil. Soybean oil also contains omega-3 fats, which are heart-healthy fats found in salmon and sardines but the fat is less common in plant-based foods (Deol *et al.*, 2017).

Six high-yielding soybean varieties were developed by the BARI ORC in 1981, and two of those types were made available for farm-level cultivation. Since 2011, BINA has also released six soybean cultivars as Brag, Devis, Shohag, etc. BARI developed the Soybean-4, BARI Soybean-5, and BARI Soybean-6 types, and BINA developed the BINA Soybean-1, BINA Soybean-2, BINA Soybean-3, BINA Soybean-4, BINA Soybean-5, and BINA Soybean-6 varieties. In 2020, BSMRAU released the BU Soybean-2 cultivar, which has a high protein content. Hence, the general objective of this study was to assess the physical and chemical characteristics, mineral content, oil percentage, and fatty acid profiles of the collected varieties of soybean and compare the physico-chemical parameters and nutritional quality of the collected six varieties of soybean.

Materials and Methods

Five varieties of Soybean namely BINA Soybean-3, BU Soybean-2, Shohag, BINA Soybean-5, and BARI Soybean-6 were collected for the study. The seeds were from BARI, Joydebpur, Gazipur, and BINA, Mymensingh. Seeds were cleaned sun-dried and stored in a plastic container in a cool place until used for the chemical analysis.

The experiment was carried out at Central Laboratory, BINA, BAU campus, Mymensingh, Bangladesh from April 2020 to October 2020. The experiment was conducted in a Completely Randomized Design (CRD) with three replications.

Parameter of physical analysis

Determination of 1000-grain weight

The mass was determined by randomly selecting 1000 seed samples and weighing in an electronic balance of 0.00 g sensitivity.

Percent moisture content

Empty aluminum moisture dish was weighted (W 1) and a 2 g sample was taken in a moisture dish and weighted (W 2). The sample was spread evenly and placed without a lid in the oven and dried samples overnight at 100° C. The Aluminum dish was weighed after cooling (W 3). The moisture was determined from the following formula

Moisture = W 2−W 1/ W 2−W 3× 100

Determination of ash

The temperature of the muffle furnace was fixed to 600 ^{0}C and the crucible was heated for 1 h and transferred into a desiccator; cooled to room temperature and weighted (W1). About 2 g sample was put into the crucible weighted (W2). The sample was burned in a muffle furnace at 600oC for about 2 hrs. The crucibles were transferred into the desiccator and cooled to room temperature and weighed (W3) (Ranganna *et al.*, 1986).

% Ash = wt. of ash/wt of sample taken x100

Weight of the sample taken = W2–W1 Weight of the ash obtained = W3–W1

Chemical analysis

Estimation of oils/fats

Soybean oil was extracted by using the Soxhlet method described by Aziz *et al.*, (2018). Dried and ground soybean samples were weighed out into an extraction thimble. The weight of the thimble and sample were recorded in the laboratory workbook. The thimble was placed into the Soxhlet. 250 ml petroleum ether was added to the Soxhlet flask, then it was connected to the holder and condenser. Soxhlet flask was placed on the hot plate and distilled at low temperature (40-60oC) for 3-4 hours for each sample. After extraction, it was turned off and allowed to cool. When distillation was ceased, the extraction thimble was removed and allowed to air dry for 30-40 minutes the thimble was weighed out. The loss of weight was crude fat.

Estimation of protein

% N = (14.007) x (normality of the acid 0.02) / wt. of the sample taken (mg) x 100

Where 14.007 is the equivalent weight of nitrogen. Nitrogen percent is converted into protein by multiplying with a factor of 6.25 for cereals and pulses (AOAC, 2010).

Chemical constant

Saponification value (SPV)

Saponification value = $(B-T)\times 0.5\times 56.1\ /wt.of\ o$ Here, B = ml of HCl required for each blank

T = ml of HCl required for each oil sample

Iodine value

The percent weight of I (iodine) absorbed by the oil was calculated by the following formula: Iodine number = $(B-S)\times N\times 0.127\ /W \times 100$

Where, B = ml 0.1 N Na2S2O3 required by blank

S = ml 0.1 N $Na_2S_2O_3$ required by oil sample N = Normality of $Na_2 S_2O_3$

W = Weight of sample in grams.

Acid value

Acid value = $56.1 \times N \times V$ WHere, N = Normality of KOH

W = wt. of sample in grams.

V = Variation (Final reading – initial reading)

Estimation of fatty acid composition

The gas-liquid chromatographic method was used to determine the fatty acid content (Uppstrom and Aa, 1978).

Estimation of minerals

By using a combination diluter-dispenser, 1 ml aliquot was taken from the filtrate and 19 ml water (dilution 1) was added. The other dilutions were made in the following order. For P and K determination, 1 mL aliquot from dilution 1, 9 mL of water, and 10 mL of color reagent were mixed. It was allowed to stand for about 20 min and a reading was taken by a spectrophotometer at 680 nm for P and by using a flame photometer (Model AnA-135, OSK, Japan) at 766.5 nm for K. For S determination, 7 ml of aliquot from dilution 1, 9 ml of acid seed solution and 4 ml of turbid metric solution were mixed thoroughly. It was allowed to stand for 20 minutes and not longer than one hour. The reading was taken in a turbid meter or a colorimeter at 535 nm using a cuvette with a 2 cm light path.

For Ca and Mg determination, 1 ml aliquot from dilution 1, 9 ml of water, and 10 ml of 1 percent lanthanum solution were mixed. It was analyzed by AA procedure 700 (ANALYTIK JENA).

For Fe, Mn, and Zn determination, the original filtrate was used to analyze these elements by AA (Atomic Absorption spectrophotometer) procedure. (Sharif *et al.*, 2017).

Statistical analysis

Statistics 10 was used for the analysis of the experiment-recorded data for each character. One-way ANOVA was followed to calculate the variations of experimental treatments. The mean values for each treatment were computed, and the F variance test was used to analyze the variance of the study's features.

Results and Discussion

Physical characteristics of different varieties of soybean

The weight of 1,000 grains, the moisture content, and the ash percentage of five different soybean varieties (Table 1) were collected. It was found that seeds' weight varies based on their size and shape. The highest thousand-grain weight (159.1) belongs to BINA Soybean-3, which is followed by BU Soybean-2 (151.7). Weight-wise, BARI Soybean-6 was the lightest (115.6).

In moisture content the varieties from 9.06 to 9.84%. The two varieties of soybeans with the highest moisture content were BINA Soybean-3 (9.84%) and Shohag (9.46 %). BARI

Soybean- 6 (9.06%) and BINA Soybean-5 (9.32%) had the lowest moisture content. The current findings are supported by (Rani *et al.,* 2008; Anwar *et al.,* 2016; Kulkarni *et al.(*2019).

Considering % of ash, the variety Shohag (5.20%) got the highest value, followed by BINA Soybean-5 (4.68%) and BU Soybean-2 (4.32%). The current findings are supported by Anwar *et al.(*2016); Rani *et al.(*2008); Gupta *et al.(*2013), and Kuzniar *et al.(*2013) among others.

Table 1. Weight of 1000 seeds, moisture percentage, and Dry matter percentage of different varieties of soybean

Name of the released cultivars (Treatments)	Wt. of 1000 seeds (gm)	Moisture %	Ash %
		mean	
BINA Soybean-3	159.1a	9.84a	4.8c
BU Soybean-2	151.7b	9.71a	4.32e
Shohag	122.4c	9.46b	5.20a
BINA Soybean-5	121.7c	9.32b	4.68d
BARI Soybean-6	115.6d	9.06c	5.06b
% CV	4.25	4.26	3.42
LSD (0.05)	0.4897	0.1873	0.5031

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at a 5 % level of significance.

Chemical characteristics of different varieties of soybean

Oil content

The soybean varieties with the highest oil content were BU Soybean-2 (16.87%) followed by BINA Soybean-5 (15.85%), and Shohag (15.83 %). BINA Soybean-3 has the least amount of oil % in it (14.88 %). According to Anwar *et al.* (2016) found a higher content of oil than the content of oil we found..

Oil cake

The highest oil cake was obtained in BINA Soybean-3 (85.12 %). The content of oil cake for BU Soybean-2 (83.13%), BINA Soybean-5 was the lowest (84.15 %) while Shohag had the lowest (84.15%) oil cake. Similar results were reported by Sharma *et al. (2014)* and Anwar *et al.(2016).*

Protein

The protein content of the soybean cultivars lines is presented in (Table 2). The maximum amount of protein was found in BU Soybean-2 (52.20%), followed by Shohag (40.10 %). The lowest protein level was found in BINA Soybean-5 (38.01%), which was statistically equivalent to BINA Soybean-3(38 %).

Table 2. Proximate analysis of oil content, oil cake , and protein of different varieties of soybean

Name of the released cultivars (Treatments)	Oil %	Oil cake %	Protein %
		mean	
BINA Soybean-3	14.88c	85.12a	38.29c
BU Soybean-2	16.87a	83.13c	52.20a
Shohag	15.83b	84.17b	40.10b
BINA Soybean-5	15.85b	84.15b	38.01c
BARI Soybean-6	15.42bc	84.58b	39.80b
%CV	1.85	0.91	0.68
LSD (0.05)	0.615	1.41	0.49

Mean values in columns marked with the same letter(s) do not differ significantly by LSD at a 5 % level of significance.

Chemical constant

Saponification value

The value of saponification ranged from 187.11 to 189.85 was found in different soybean varieties. The highest saponification values were found in BU Soybean-2 (189.85), which was followed by BARI Soybean-6 (188.61) and BINA Soybean-5 (189.17). The lowest amount of saponification value was found in Shohag (187.17). The values that were observed were comparable to findings reported by Anwar *et al.(* 2016)

Iodine value

The highest level of iodine value was found in BU Soybean-2 (79.65), which is statistically similar to BINA Soybean-3 (79.49) and Shohag (78.58). The lowest iodine value was found in BINA Soybean-5 (73.56). The published values of 73.02 g/100 g oil by Amos-Tautua *et al.*, (2015) support the current figures, however, the values are also lower than the values discovered by Prodhan *et al.*, (2015), Anwar et al. (2016), and (Abitogun *et al.*, 2008).

Acid value

Several varieties of soybean seeds have an acidity that ranges from 0.90 to 0.99. The BINA Soybean-5 contained the most acidic levels (0.99). The lowest value was discovered in BU Soybean-2 (0.90). The current value is supported by Belsare *et al.* (2017) who found that the acid value varied from 0.56 to 1.12 mg KOH/g. The data, however, is higher than the values published by Prodhan *et al.* (2015) and lower than those reported by Abitogun *et al.*(2008).

Table 3. Proximate analysis of Saponification value, Iodine value, and Acid value of different varieties of soybean

Name of the released cultivars (Treatments)	Saponification value (mg of KOH/gm)	Iodine value (gm of I/100gm)	Acid value (mg of KOH/gm)
		mean	
BINA Soybean-3	188.34b	79.49a	0.93bc
BU Soybean-2	189.85a	79.65a	0.90c
Shohag	187.11c	78.58b	0.94bc
BINA Soybean-5	189.17ab	73.76d	0.99a
BARI Soybean-6	188.61b	77.72c	0.97ab
% CV	0.85	0.45	3.45
LSD (0.05)	1.033	0.604	0.05

Mean values in columns marked with the same letter do not differ significantly by LSD 5% level of significance.

Saturated fatty acid composition

Palmitic acid (C16:0) was found in the highest concentrations in BINA Soybean-3 (10.82%), BU Soybean-2 (10.63%), and BARI Soybean-6 (10.62%). (10.49 %). Shohag had the lowest levels of palmitic acid (C16:0) (9.33 %). Shohag had a stearic acid (C18:0) level between 3.64 and 4.81 percent and BINA Soybean-3 (0.13 %) and BINA Soybean-5 (0.23 %) had an arachidic acid (C20:0) level between 0.13% and 0.23 %. The content of behenic acid (C22:0) in BINA Soybean- 5 was the greatest (0.87 %), followed by BARI Soybean- 6 (0.77 %), and BINA Soybean-3 had the lowest concentration (0.55 %). These results are similar to those from Martin *et al.(*2008). Reduced value was presented by Aboitogun *et al.* (2008).

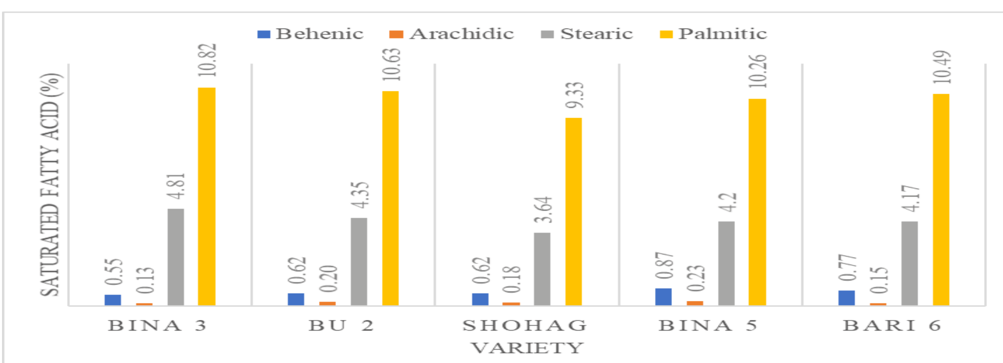

Fig. 1. Saturated fatty acid comparison of five varietiesof soybean

Unsaturated fatty acid composition

The highest concentration of palmitoleic acid (C16:1) was found in BU Soybean-2 (0.18%), followed by BINA Soybean-3 (0.13%), which is statistically equivalent to BARI Soybean-6 (0.17%). The least amount of palmitoleic acid (C16:1) was present in Shohag (0.09 %). Oleic acid (C18:1) was present in the highest concentrations in BINA Soybean-5 (30.71%) and the lowest concentrations in Shohag (16.99 %). Linoleic acid (C18:2) content varied from 44.63% to 54.39 %. The amount of linoleic acid (C18:2) in BINA Soybean-5 was the lowest and the highest in BU Soybean-2 (54.39 percent). The amount of linolenic acid (C18:3) in BARI Soybean-6 and BU Soybean-2 was significantly different (9.56%). The data described here is identical to that presented by Aboitogun *et.al.(*2008).

Mineral composition phosphorus (P)

The most phosphorus (P) concentration was found in Shohag (0.84 %), followed by BU Soybean-2 (0.81 %), and BARI Soybean-6 (0.60 %) (0.82 %). The lowest phosphorus content was found in BINA Soybean-3 (0.69 %), followed by BINA Soybean-5 (0.73 %). Rani *et al. (*2008) have reported values that are comparable to the current findings.

Potassium (K)

The soybean variety with the highest K concentration was found in BU Soybean-2 (0.77%), which was statistically equivalent to BINA Soybean-5 (0.76%) and Shohag (0.76 %). The lowest percentage was found in BINA Soybean-3 (0.69 %), followed by BINA Soybean-5 (0.76 %).

According to Ozcan *et al.* (2014), the potassium content of soybean seeds ranged from 16,375 mg/kg (raw Adasoy) to 20,357 mg/kg (sprouted A3127), which was higher than the results of the present study.

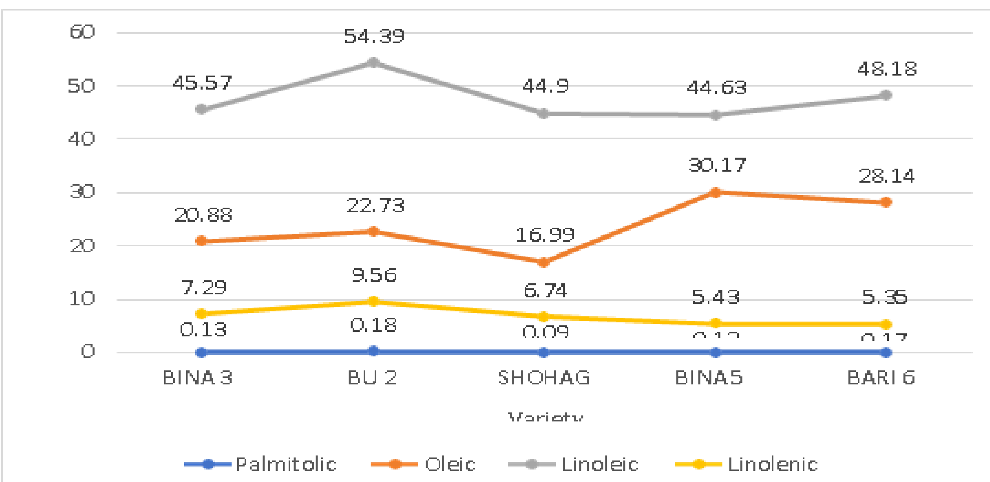

Fig. 2. Unsaturated fatty acid comparison of five soybean varieties

Magnesium (Mg)

Shohag (0.465 %), which is statistically equivalent to BINA Soybean-3 (0.43 %), had the highest content of magnesium, whereas BU Soybean-2 had the lowest concentration (0.32 %). The current value exceeds what (Etiosa *et al.,* 2018) and (Uwem *et al.*, 2017).

Calcium (Ca)

The highest calcium content was found in Shohag (0.23 %), while BINA Soybean-3 came in second (0.21 %). The lowest value was discovered in BINA Soybean-5 (0.14 %). The findings are similar to those of Rani *et al.* (2008).

Iron (Fe)

Shohag (86.75 ppm) has the greatest level of Fe, followed by BU Soybean-2 (72.42 ppm). The lowest Fe value was found in BINA Soybean-5 (56.05 ppm), which was followed by BARI Soybean-6 (68.55 ppm). Iron concentration was determined to be similar by Rani *et al.(*2008). reported higher iron concentrations than the data currently available.

Manganese (Mn)

Shohag had the second-highest level of Mn (32.05 ppm), followed by BU Soybean-2 (31.71 ppm). The lowest level was found in BINA Soybean-5 (24.03 ppm), followed by BARI Soybean-6 (29.13 ppm). The Mn concentration in soybean cultivars was approximately 0.651 (mg/100g), according to (Uwem *et al*., 2017).

Zinc (Zn)

BARI Soybean-6 (54.85 ppm), followed by BINA Soybean-3, had the highest amount of zinc found (36.12 ppm). The least amount was recorded by Shohag (23.92), followed by BU Soybean- 2 (24.52 ppm). Zinc levels range from 7.16-7.89 mg/100 g and 2.7 mg/100 g, respectively, according to (Rani *et al.,* 2008) and (Etiosa *et al.,* 2018).

Conclusion

Shohag exhibited the lowest concentration in saponification value and the highest concentration of ash, phosphorus, potassium, sulfur, magnesium, and calcium among these five soybean types. In addition, BU Soybean-2 had the lowest concentration of oil cake but the highest concentration of protein, saponification value, linoleic acid, linolenic acid, and palmitolic acid as well as mineral content such as iron, manganese, copper, and zinc. According to this study, Shohag has a unique mineral constitution. However, in terms of characteristics, BU Soybean-2 did better than the other types.

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Conflicts of Interest

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

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EXPLICATING THE SALINITY TOLERANCE OF COWPEA (*Vigna unguiculata* L. Walp.) GENOTYPES AT SEED GERMINATING STAGE

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Abstract

Cowpea is an important food and fodder legume in the arid and semi-arid tropics of the world. Soil salinity adversely affects seed germination, which ultimately reduces crop yield. The present study was carried out to screen the cowpea germplasm and identify the salinity tolerant genotypes at the germination stage. Initially, an experiment with five salinity levels (0, 50, 100, 200, and 250 mM of NaCl) was conducted, and the results showed that 200 mM of NaCl concentration was found to be a sensible salinity stress to assess the salinity tolerance of cowpea genotypes. A total of 29 cowpea genotypes were evaluated under a 200 mM NaCl concentration. Four salinity tolerance indices such as absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) were calculated from germination percentage without stress (GC) and germination with 200 mM NaCl (GS). All the indices showed significant variation in responses to salinity stress among the cowpea genotypes. The results revealed that salinity stress significantly reduced the germinating percentage in cowpea. Total seven cowpea genotypes (G4, G9, G12, G15, G26, G27, and G32) were found to be salinity tolerant lines at the germination stage. The seven genotypes were clustered in the same group. which had the higher RST (G4: 0.58, G9: 0.80, G12: 0.50, G15: 0.60, G26: 0.50, G27: 0.60, and G32: 0.80) and STI (G4: 0.58, G9: 0.80, G12: 0.50, G15: 0.60, G26: 0.50, G27: 0.60, and G32: 0.79) under salinity stress. However, this is a preliminary screening against salinity at the germination stage, and further research is required at the seedling vegetative and reproductive stages for validation.

Keywords: Cowpea, Salinity tolerance index, Seed germination, *Vigna unguiculata*

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is an important food and fodder legumes in the arid and semi-arid tropics of the world. The diploid chromosome number of cowpea is 2n = 22 and belongs to the family Fabaceae (Paudel *et al.*, 2021). This crop grows well in temperature of about 28ºC (Craufurd *et al.*, 1997). Cowpea provides food for millions of people, mostly in developing countries, with an annual worldwide production is about 4.5 million metric tons (Animasaun *et al.*, 2015). The young leaves,

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immature pods, and peas are used as vegetables, whereas some appetizers and main dishes are prepared from the grain (Singh and Tarawali, 1997). It is not only grown for leafy vegetable and grain legumes for human consumption but also for livestock feed (Gerrano *et al.*, 2015). It is a good source of protein, vitamins, and minerals. In dry basis, cowpea grain contains about 23-32% protein, 50–60% carbohydrate and about 1% fat (Jayathilake, *et al.*, 2018). The consumption of cowpea exerts protective effects against several chronic diseases, including gastrointestinal disorders, cardiovascular diseases, hypercholesterolemia, obesity, diabetes and several types of cancer (Forta *et al.*, 2008, Rotimi *et al.*, 2013, Trehan *et al.*, 2015). Therefore, this crop is important for human nutrition. Cowpea requires less input to cultivate and able to fix atmospheric nitrogen to soil. It can be adapted to adverse environmental conditions. Although cowpea is nutritious and favorable for growing in the agro-climate of Bangladesh but production is still lower compared to other countries.

Salinity is one of the most serious limiting factors for cowpea production in the arid and semi-arid tropics of the world. In Bangladesh, coastal area constitutes 20% to 30% of the agricultural land (https://www.thedailystar.net/news-detail-145077). Salinity alters the morphology, physiology, and metabolism of plants and severely affects growth and yield of crop plants. Grain yield is frequently used in crops such as cowpea as the main criteria for salt tolerance. In the view of some researchers only physiological markers such as content of Na, K, the ratio of the potassium to sodium, and the proline accumulation pattern are less feasible and are not promising (Shannon, 1984). The physiological tolerance along with agronomic traits has been shown to be applicable and their relationship with salinity stress tolerance indices are considered strong enough to be exploited as a selection tool in the breeding of salinity tolerant cultivars. Therefore, the relationship of physiological markers with grain yield in saline conditions is very important.

The salinity-affected soils are predominant in the southern coastal areas of the country, including Khulna, Satkhira, Bagerhat, Pirozpur, Jhalakathi, Patuakhali, Chittagong, Cox's Bazar, Noakhali, Barguna and Bhola. The farming communities of these areas need to introduce salinity tolerant crop cultivars. Exploiting genetic variability of available cowpea germplasm which have the salinity tolerance mechanism is vital for crop yield. Moreover, very little research has been carried out on improvement of the cowpea in Bangladesh. Considering the above problem and prospects, the proposed research is planned to evaluate the potential for salinity tolerance of cowpea genotypes at seed germinating stage.

Materials and Methods

Plant materials and experimental design

Twenty-nine cowpea genotypes were used in this study (Table 1) which were collected from Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University (SAU) and Plant Genetic Resource Centre (PGRC), Bangladesh Agricultural Research Institute (BARI). The experiment was conducted in a factorial experiment with two factors (genotype × salinity stress) following completely randomized design (CRD) with three replications in the laboratory of Department of Genetics and Plant Breeding, SAU, Dhaka, Bangladesh during Rabi season December 2022 to February7 2023.

Salinity treatment and screening of tolerant genotypes

Initially, five genotypes comprised G1, G3, G5, G6 and G31 and the five salinity levels were 0, 50, 100, 200, and 250 mM were considered to find out the optimum seed germination under different salinity levels. Three replications for both the genotypes and salinity levels were maintained. Different salinity levels of 50, 100, 200 and 250 mM were prepared by dissolving 2.92, 5.84, 11.68 and 14.612 g of pure NaCl in one liter distilled water, respectively while distilled water (0 mM) was used as control for the in vitro experiment (Asfaw, 2011). The germination experiment was conducted in the laboratory at room temperature as previously described Mamo *et al.* (1996). Petri dishes with a diameter of 10 cm were lined with Whatman No. 3 filter paper supplied with 8 ml of each treatment and control solutions. The seeds were surface sterilized 60 s with 2% bleach followed by 70% ethanol and rinsed with distilled water (Ravelombola *et al.*, 2017). The optimum NaCl concentration for assessing salinity tolerance at the germination stage was considered where cowpea seed germination percentage between genotypes were significantly different. Afterward, ten uniform seeds of each cowpea genotypes were placed on each petri dish consisted 8 ml of 100 mM. Moreover, the petri dishes were covered with a polyethylene sheet to avoid the loss of moisture through evaporation. The seed was considered as germinated when both the plumule and radicle had emerged 2 mm long. The germination percentage (GP) was determined using the following formula at day eight after sowing (Islam *et al.*, 2019).

Table 1. List of cowpea genotypes used in the study

Sl. No.	Genotypes	Accession	Sl. No.	Genotypes	Accession
1	G1	Vu/19/GPB-0001	16	G16	Vu/19/GPB-0017
2	G2	Vu/19/GPB-0002	17	G17	Vu/19/GPB-0020
3	G3	Vu/19/GPB-0003	18	G18	Vu/19/GPB-0021
4	G4	Vu/19/GPB-0004	19	G19	Vu/19/GPB-0022
5	G5	Vu/19/GPB-0005	20	G20	Vu/19/GPB-0023
6	G6	Vu/19/GPB-0006	21	G21	Vu/19/GPB-0024
7	G7	Vu/19/GPB-0007	22	G22	Vu/19/GPB-0026
8	G8	Vu/19/GPB-0008	23	G25	Vu/19/GPB-0029
9	G9	Vu/19/GPB-0009	24	G26	Vu/19/GPB-0030
10	G10	Vu/19/GPB-0010	25	G27	Vu/19/GPB-0032
11	G11	Vu/19/GPB-0011	26	G28	Vu/19/GPB-0033
12	G12	Vu/19/GPB-0012	27	G30	Vu/19/GPB-0035
13	G13	Vu/19/GPB-0013	28	G31	Vu/19/GPB-0036
14	G14	Vu/19/GPB-0015	29	G32	Vu/19/GPB-0037
15	G15	Vu/19/GPB-0016			

$\%\ G = (\text{No. of seeds germinated/No. of total of seeds for germination}) \times 100$

The salinity tolerance of the cowpea genotypes under NaCl stress was determined by calculating absolute decrease (AD), inhibition index (II), relative salt tolerance index (RST), salinity tolerance index (STI) following the previously described formulas (Saad *et al.*, 2014; Ravelombola *et al.*, 2017).

$$AD = GC - GS$$

$$II = 100 \times (GC - GS)/GC$$

$$RST = GS/GC$$

$$STI = (GS \times GC)/(GC_{av})^2$$

Where, GC = Germination percentage without NaCl stress, GS = Germination percentage under NaCl stress, and GC_{av} = Average germination percentage of a cowpea genotype without NaCl stress.

Data analysis

Mean data for each variable were worked out by dividing the total corresponding number of observations. Differences between cowpea genotypes for different variables and treatments were tested for significance by using analysis of variance and mean separations were carried out by using Statistix 10.0 program.

Results

Optimum NaCl concentration at seed germination stage

At first, an experiment with five salinity levels consisted of 0, 50, 100, 200, and 250 mM of NaCl was conducted to determine the optimum NaCl concentration for assessing salinity tolerance of cowpea genotypes (Fig. 1). The germination percentage (GP) revealed a significant variation among cowpea genotypes as well as salinity levels. The results suggest that the seed germination (GP) was decreased with the increase of salinity levels (Figs. 2-3). The highest seed germination (over 80 %) was observed at 0 mM (control without NaCl stress) while lowest at 250 mM of NaCl. The seed germination of cowpea genotypes varied significantly at 200 mM of NaCl.

Germination percentage (GP) of cowpea genotypes

All cowpea genotypes differ significantly for GP at both non-stressed ($F = 1.79$ and $P = 0.0321$) and NaCl stressed ($F = 1.79$ and $P = 0.000$) condition (Table 4). The GP of cowpea genotypes ranged from 61.11% to 94.44% at non-stress condition (0 mM NaCl) while 8.33% to 75.00% at 200 mM of NaCl (Table 2).

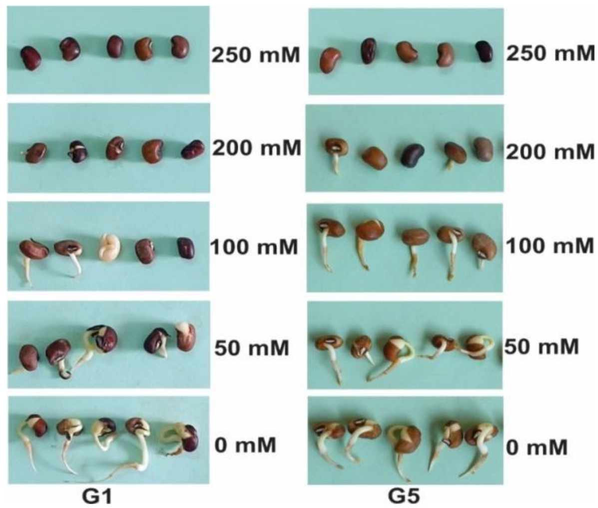

Fig. 1. Germinated cowpea seeds at different salinity levels (0 mM, 50 mM, 100 mM, 200 mM and 250 mM of NaCl) at 48 h after sowing

The result showed that salinity stress significantly decreases the GP in cowpea genotypes. The cowpea genotypes G3 (86.11%), G5 (88.89%), G6 (88.89%) and G32 (94.44%) had the maximum GP, while G13 (61.11%) and G19 (66.67%) had the minimum GP at non-stressed condition (Table 2). On the other hand, the highest GP was recoded in G32 (75%) followed by G9 (66.67%) at salinity stress (200 mM NaCl) (Table 2). On the contrary, the lowest GP was observed in G1 (8.33%), G2 (8.33%), G3 (8.33%), G6 (8.33%), G11 (8.33%), G13 (8.33%), G19 (8.33%) and G20 (8.33%) at salinity stress (200mM NaCl) (Table 2).

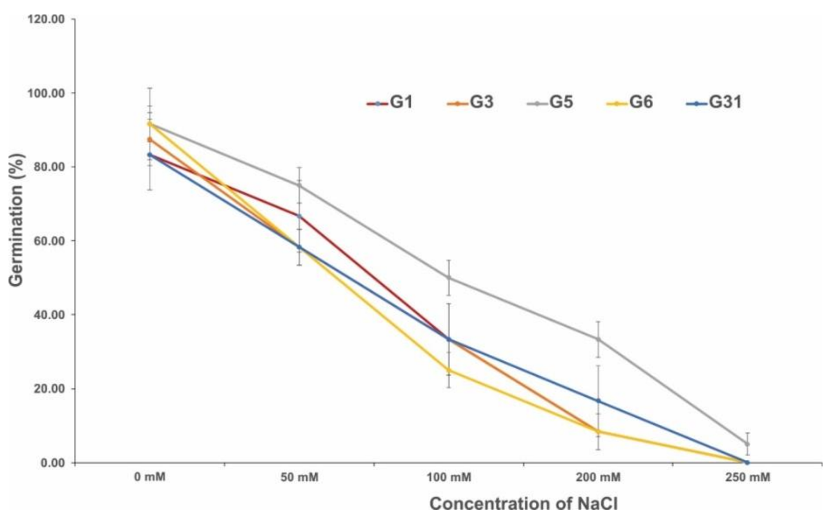

Fig. 2. Germination percentage of five cowpea genotypes under different salinity levels (0 mM, 50 mM, 100 mM, 200 mM and 250 mM of NaCl)

Absolute decrease (AD) and inhibition index (II) of cowpea genotypes

The absolute decrease (AD) and inhibition index (II) of germination between the non-stressed and salinity stressed condition of cowpea genotypes were determined (Table 2). The result showed that salinity stress caused by 200 mM NaCl significantly decreased the AD (F = 4.38 and P = 0.0000) which ranged from 16.67% to 80.56%.

The highest AD was recorded in G6 (80.56%) while the lowest in G9 (16.67%) (Table 2). The II of germination was varied significantly among the cowpea studied genotypes (F = 5.74, P = 0.0000) (Table 4). The II varied from 20% to 91.11% (Table 2) demonstrating a wide range of variation among the cowpea studied genotypes against salinity stress. The higher II was exhibited in cowpea genotypes, including G1 (91.11%), G3 (91.11%), G2 (90%), G6 (90%) and G20 (90%) while lower II was recorded in G32 (20%), G9 (20%), G27 (40%), G15 (40%), G4 (41%), G26 (50%) and G12 (50%) under salinity stress (Table 2).

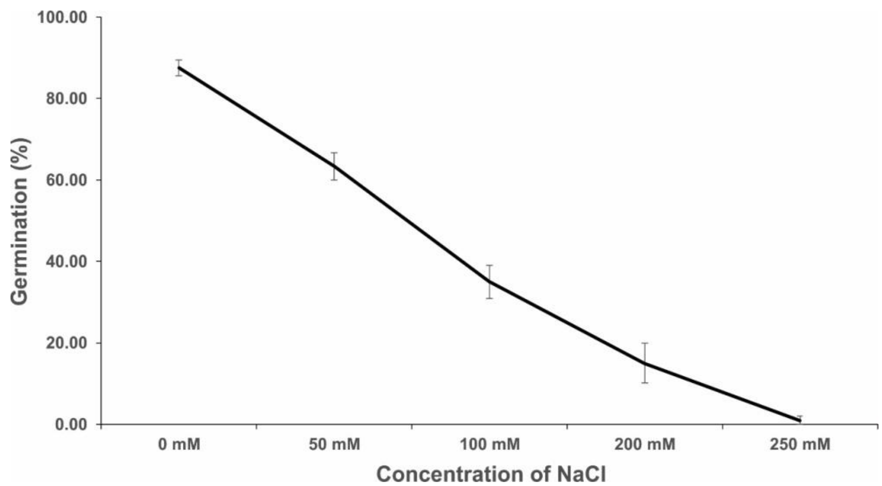

Fig. 3. Pooled germination percentage of five cowpea genotypes of different salinity levels (0 mM, 50 mM, 100 mM, 200 mM and 250 mM of NaCl)

Salinity stress tolerance of cowpea genotypes

The relative salinity tolerance (RST) and salinity tolerance index (STI) of cowpea genotypes which are commonly to measure salinity tolerance in plant were determined. The RST differed significantly for the studied genotypes (F = 5.71 and P = 0.0000) (Table 4). The higher RST was detected in G32 (0.80), G9 (0.80), G27 (0.60), G15 (0.60), G4 (0.58), G26 (0.5) and G12 (0.5) whereas the lower RST was found in G1 (0.09), G2 (0.10), G3 (0.09), G6 (0.10) and G20 (0.10) under salinity stress (200 mM NaCl) (Table 2). The cowpea genotypes showed a significant variation for STI (F = 6.46, P = 0.0000). The maximum STI was recorded in G9 (0.80), G32 (0.79), G27 (0.60), G15 (0.60), G4 (0.58), G26 (0.5) and G12 (0.5) while minimum in G6 (0.09) followed by G1 (0.11), G2 (0.11), G3 (0.11) and G20 (0.11) salinity stress (200 mM NaCl) (Table 2).

Clustering of different cowpea genotypes

The cowpea genotypes were further clustered using 'ClustVis: a web tool for visualizing clustering of multivariate data' available at https://biit.cs.ut.ee/clustvis/. The result showed that there were two cluster and cluster I exhibited a total of seven genotypes, including G27, G15, G4, G26, G12, G32 and G9 which were shown to be salinity tolerant at 200 mM NaCl (Fig. 4).

Discussion

In the first study, five different salinity levels were used to find out optimal salinity stress level in cowpea (Figs. 1-2). Gogile *et al.* (2013) also used four different concentrations of NaCl (0, 50, 100 and 200 mM) to screen salinity tolerant cowpea genotypes. The highest seed germination percentage (GP) was recorded in control (0 mM NaCl) and the lowest at 250 mM of NaCl as anticipated (Figs. 2-3). Therefore, no salinity tolerance can be assessed at those salinity levels. Besides, the GP was decreased with the increase of salinity levels in cowpea (Figs. 2-3). Thiam *et al.* (2013) and Ravelombola *et al.* (2017) also previously reported a similar finding in cowpea. Ravelombola *et al.* (2017) showed that 150 mM Concentration of NaCl to analyze the performance of cowpea genotypes against salinity stress at seed germination stage**.** However, the present study revealed that 200 mM of NaCl concentration could be a sensible salinity stress to assess the salinity tolerance of cowpea genotypes. Thus, 200 mM NaCl concentration was used for evaluation salinity tolerance of 29 cowpea genotypes in screening study.

Table 2. Germination percentage (without stress, GC; with 100 mM of NaCl, GS, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST) and salt tolerance index (STI) in cowpea genotypes

Genotypes	Germination without stress (GC)	Germination with 200 mM of NaCl (GS)	AD (%)	II (%)	RST	STI
G1	83.33	8.33	75.00	91.11	0.09	0.11
G2	77.78	8.33	69.44	90.00	0.10	0.11
G3	86.11	8.33	77.78	91.11	0.09	0.11
G4	72.22	41.67	30.56	41.67	0.58	0.58
G5	88.89	33.33	55.56	62.22	0.38	0.38
G6	88.89	8.33	80.56	90.00	0.10	0.09
G7	72.22	16.67	55.56	76.67	0.23	0.23
G8	83.33	16.67	66.67	80.00	0.20	0.20
G9	83.33	66.67	16.67	20.00	0.80	0.80
G10	72.22	16.67	55.56	76.67	0.23	0.23
G11	72.22	8.33	63.89	88.33	0.12	0.12

Genotypes	Germination without stress (GC)	Germination with 200 mM of NaCl (GS)	AD (%)	II (%)	RST	STI
G12	83.33	41.67	41.67	50.00	0.50	0.50
G13	61.11	8.33	52.78	85.56	0.14	0.14
G14	83.33	16.67	66.67	80.00	0.20	0.20
G15	83.33	50.00	33.33	40.00	0.60	0.60
G16	72.22	25.00	47.22	65.56	0.34	0.36
G17	77.78	25.00	52.78	68.33	0.32	0.33
G18	72.22	16.67	55.56	75.56	0.24	0.23
G19	66.67	8.33	58.33	88.33	0.12	0.14
G20	77.78	8.33	69.44	90.00	0.10	0.11
G21	77.78	16.67	61.11	80.00	0.20	0.23
G24	77.78	16.67	61.11	78.33	0.22	0.21
G25	83.33	16.67	66.67	80.00	0.20	0.20
G26	83.33	41.67	41.67	50.00	0.50	0.50
G27	83.33	50.00	33.33	40.00	0.60	0.60
G28	83.33	16.67	66.67	80.00	0.20	0.20
G30	66.67	16.67	50.00	71.11	0.29	0.23
G31	83.33	16.67	66.67	80.00	0.20	0.20
G32	94.44	75.00	19.44	20.00	0.80	0.79

The result indicated that genotype G32 and G9 exhibited higher GP 75% and 66.67%, respectively (Table 2). The highest GP of these genotypes suggesting that they were tolerant to salinity stress (200 mM NaCl) at seed germination stage (Table 2). Furthermore, the lowest GP (8.33%) was detected in eight cowpea genotypes, including G1, G2, G3, G6, G11, G13, G19 and G20 at salinity stress (Table 2). The result demonstrating that these genotypes were very susceptible to 200 mM NaCl salinity stress () at seed germinating stage. Genotypes with higher AD value indicated higher susceptibility to salinity stress while with the lower II value indicated higher level of salinity tolerance (Ravelombola *et al.*, 2017). Five cowpea genotypes including G1, G2, G3, G6, and G20 showed over 90% II values (Table 2) indicated that these genotypes were highly susceptible to salinity stress at seed germination stage. On the other hand, seven cowpea genotypes such as G32, G9, G27, G15, G4, G26 and G12 exhibite lower II under salinity stress (200 mM NaCl). This results suggested that they could be utilized as salinity tolerant cowpea genotypes at germination stage for further genetic improvement program for cultivation in the salinity prone areas. The present results of AD and II were in agreement with the previous findings reported by Ravelombola *et al.* (2017) for cowpea genotypes under salinity stress.

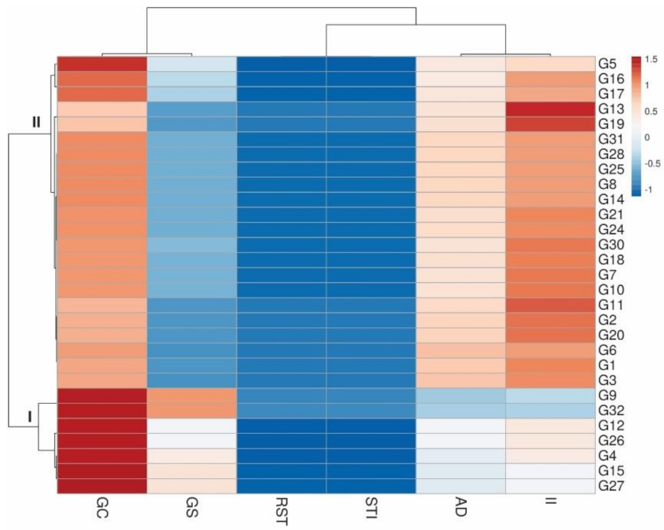

Fig. 4. Heatmap represents clustering cowpea genotypes in respect of six parameters. GC: Germination without stress (No stress), GS: Germination with 100 mM of NaCl (stress), AD: absolute decrease, II: inhibition index, RST: relative salt tolerance and (STI) salt tolerance index. This heatmap was generated using online tool freely available at https://biit.cs.ut.ee/clustvis/

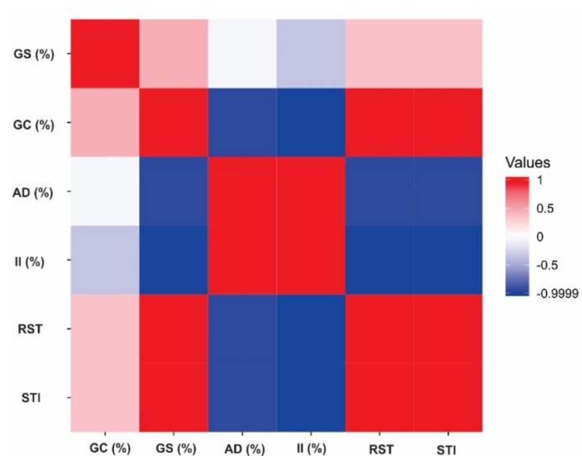

Fig. 5. Correlation matrix showing correlation between different parameters of 29 cowpea genotypes. The blue and red colors in the legend indicates highest and lowest values, respectively. GC: Germination without stress (No stress), GS: Germination with 100 mM of NaCl (stress), AD: absolute decrease, II: inhibition index, RST: relative salt tolerance and (STI) salt tolerance index. This heatmap was generated using online tool freely available at http://www.heatmapper.ca/

The genotypes with higher the RST and STI values are considered as salinity tolerant the genotypes (Saad *et al.*, 2014). The higher RST (0.5 or over) of G32, G9, G27, G15, G4, G26 and G12 (Table 2) under salinity stress (0 Mm NaCl) indicating that they were salinity tolerant genotypes at seed germination stage. On the other hand, the lower RST of G1, G2, G3, G6 and G20 (Table 2) indicating that they were susceptible to salinity stress at seed germination stage. A similar result for RST and STI was reported in cowpea by Ravelombola *et al.* (2017) under salinity stress.

Furthermore, the correlation studies of six parameters were performed using online correlation matrix visualization tool available at http://www.heatmapper.ca/. The result indicated that there was a lower relationship between the germination percentages (GC) under non-stressed condition (Fig. 5). Thus revealed that salinity tolerance at seed germination stage had a poor relationship with the GP non-stressed conditions. Conversely, the germination percentage under salinity stress (GS) had a strong negative correlation with AD and II. Nonetheless, GS had a very strong positive correlation RST and the STI indicating that salinity tolerance at seed germination stage is greatly associated with GS (Fig. 5). Seven cowpea genotypes such as G27, G15, G4, G26, G12, G32 and G9 clustered in the same cluster (I) which had the highest RST and STI which shown to be salinity tolerant at 200 mM NaCl (Table 2 and Fig. 4). The results revealed that the stress tolerance parameters like RST and STI could be very important parameters for evaluation of salinity tolerance at seed germination stage in cowpea.

Conclusion

Significant variation in responses to salinity stress was observed for all variables among the cowpea genotypes. Salinity stress significantly affected the germination percentage of the cowpea genotypes. Considering all six variables including germination without stress (GC), germination with 200 mM of NaCl (GS), absolute decrease (AD), inhibition index (II), relative salt tolerance (RST) and salt tolerance index (STI), total seven cowpea genotypes, including G4, G9, G12, G15, G26, G27 and G32 were found to be salinity tolerant under salinity stress (200 mM NaCl) at seed germination stage. These genotypes although showed tolerance to salinity at germination stage needs further screening against salinity stress at seedling, vegetative and reproductive stages to confirm the salinity tolerance. However, this screening technique may be used for preliminary screening for salinity tolerance.

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Conflicts of Interest

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

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OPTIMIZING CABBAGE YIELD THROUGH INTEGRATED BIOCHAR AND COW URINE APPLICATIONS

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Abstract

This investigation was carried out at the Germplasm Center of Patuakhali Science and Technology University (PSTU) from mid November 2019 to early February 2020 with an obejective is to enhance cabbage (cv. F1 Atlas 70) yield by combined use of fertilizers, biochar and cow urine. The treatments consisted of T_1 = Control, T_2 = Biochar + Cow urine, T_3 = Recommended manures, T_4 = Recommended fertilizers, T_5 = Recommended fertilizers + biochar + cow urine, and T_6 = Recommended manures and fertilizers. The result showed a substantial impact of combined use of biochar, cow urine and recommended dose of fertilizers (T_5) on the growth and yield of cabbage. Significant improvements were noted for plant height (33.6 cm), leaf no. (22.8), plant spread (64.52 cm^2), leaf weight (0.25 kg $plant^{-1}$), and length of the stem (4.77 cm) as well as cabbage head characteristics such as head length (20.0 cm), diameter (24.6 cm), weight (2.17 kg), and overall head yield (77.9 t/ha). Next to T_5, T_6 i.e., combined use of recommended manure and fertilizers showed identical performances. The findings underscore the potential of integrating fertilizers, biochar and cow urine to enhance cabbage growth and yield..

Keywords: Biochar, Cabbage yield, Cow dung, Cow urine, Fertilizers

Introduction

Cabbage (*Brassica oleracea* var. capitata) is one of the most economically and nutritionally important vegetables globally (Weerakkody *et al.*, 2020). However, as the world population surpasses 9.7 billion by 2050 (FAO, 2017), the demand for sustainable agricultural practices intensifies, compelling agricultural researchers and practitioners to explore innovative approaches to optimize crop yield while mitigating environmental degradation (Lindblom *et al.*, 2017). In this context, the integrated application of biochar and cow urine along with chemical fertlizers emerges as a promising strategy to enhance cabbage cultivation practices and address the challenges posed by conventional agricultural methods (Mithu *et al.*, 2022).

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Biochar, a carbon-rich substance produced through the pyrolysis of organic materials, has gained recognition for its multifaceted benefits in soil improvement (Khan *et al.*, 2024). Studies have demonstrated that biochar application enhances soil structure, promotes water retention and improves nutrient availability (Lehmann *et al.,* 2011; Jeffery *et al.*, 2017). Cow urine, a natural byproduct of bovine metabolism, has long been utilized in traditional agriculture for its rich nutrient content and plant growth-promoting properties (Singh *et al.,* 2023). Cow urine contains a variety of essential nutrients, including nitrogen, phosphorus, potassium, and micronutrients, which serve as vital elements for plant growth and development (Pandey *et al.*, 2017; Singh ., 2020). Moreover, cow urine exhibits bio-stimulant properties that enhance nutrient uptake, stimulate root growth, and improve plant vigor, thereby reducing the need for synthetic fertilizers and minimizing environmental pollution (Chaudhari *et al.*, 2023).

Despite the individual merits of biochar and cow urine in agriculture, limited research has explored their effects on cabbage cultivation. Understanding the synergistic interactions between biochar and cow urine and their impact on cabbage growth and yield deserves attention for developing sustainable land management (SLM) practices that optimize resource utilization and environmental stewardship. Hence, the present study was undertaken to examine the effect of biochar and cow urine together with chemical fertilizers on the growth parameters and yield of cabbage.

Materials and Methods

The experiment was carried out at the Germplasm Center within the premises of the Department of Horticulture at Patuakhali Science and Technology University (PSTU) Campus, situated at coordinates 22°27'53.9"N and 90°23'06.8"E. The experiment was aid out in a randomized complete block design (RCBD) with six treatments and seven replications. The treatments consisted of control (T_1), biochar + cow urine (T_2) where biochar rate was 1 ton per hectare and cow urine application 1000 liters per hectare, 20 ton/ha cow dung for manure application (T_3), recommended fertilizers per hectare - 300 kg Urea (N), 300 kg TSP (P), and 250 kg MoP (K) (T_4). recommended fertilizers along with biochar and cow urine (T_5), and recommended manure and fertilizers (T_6). Biochar used in this experiment was produced through a process of pyrolysis. Biomass feedstock, sourced locally, underwent thermal decomposition in the absence of oxygen at temperatures ranging from 300 to 700 degrees Celsius.

The resulting biochar was finely ground and applied to the soil. Fertilizers were applied in the root zone during land preparation. The recommended full quantity of phosphorus and potassium, as well as half of the nitrogen dose were evenly distributed in the root zone. The remaining half of the nitrogen was applied in two equal splits at 20 and 35 days after transplanting (DAT) as a local application. Each experimental unit consisted of 10 cabbage plants. Transplantation of 25-day old cabbage seedlings was done in mid November 2019 and harvesting was in early February 2020. The cabbage variety used F1 Atlas 70. Each field plot measured 2.8 m², with plant spacing set at 30.48 cm x 30.48 cm. All necessary intercultural operations and plant protection measures were done. The data were subjected to analysis of variance (ANOVA) at 5% level of significance. Means were separated using Tukey's Honestly Significant

Difference (HSD) test at a 5% level of significance. This data analysis was performed using an automated software, 'JMP 8.'

Results

Impact of recommended fertilizers, biochar, and cow urine treatment on cabbage growth

Plant height exhibited significant differences across the treatments (Table 1). At 30 DAT, the recommended fertilizers + biochar + cow urine treatment (T_5) showed the highest height (23.8 cm), with the increase of approximately 94.5% over the control (T_1). This trend persisted at 45 DAT, with T_1 measuring 19.2 cm and T_5 reaching 27.8 cm, indicating a significant 44.0 % increase. At 60 DAT, T_1 maintained a height of 22.7 cm, and T_5 exhibited the highest height of 30.4 cm, showcasing a 34.1% increase. At, 75 DAT, T_1 recorded a height of 26.1 cm, while T_5 showed the maximum height (33.6 cm), reflecting 28.4% increase.

Similarly, the number of leaves per plant showed significant variations among the treatments (Table 2). At 30 DAT, treatment (T_1) exhibited an average of 10.5 leaves per plant, while T_5, showed the highest leaf count of 12.9, indicating a 22.1% increaseover control. This trend continued at 45 DAT, where T_1 had 13.5 leaves per plant, and T_5 had 16.6,with an increase of 23.2% . At 60 DAT, T_1 maintained 16.2 leaves per plant, and T_5 exhibited the highest (18.5), indicating 14.1% increase. The pattern persisted at 75 DAT, with T_1 having 18.9 leaves per plant where T_5 maximum count at 22.8, showed 20.2% increase over control.

Table 1. Plant height (cm) of cabbage at different days after transplantation (DAT) for various treatments (Mean±SE)

Treatment	Days after transplanting			
	30	45	60	75
T_1	12.27±0.38c	19.18±0.42b	22.67±0.73b	26.13±0.63c
T_2	13.17±0.98c	19.34±1.15b	25.46±1.57ab	29.52±1.44c
T_3	19.42±0.25b	22.86±1.25ab	25.95±2.23ab	29.66±1.89ab
T_4	19.57±0.70b	22.81±0.93ab	27.62±0.89ab	30.66±0.91ab
T_5	23.81±1.14a	27.78±2.25a	30.40±2.17ab	33.55±1.85ab
T_6	22.62±0.69ab	26.70±0.64a	28.40±0.36a	32.20±0.79b
CV (%)	15.45	18.14	14.73	11.98
Level of significance	**	**	**	**

T_1= Control, T_2= Biochar + Cow urine, T_3= Recommended manures, T_4= Recommended fertilizers, T_5= Recommended fertilizers + biochar + cow urine, T_6=Recommended manures and fertilizers, SD= Standard deviation, CV= Coefficient variance, *= 5% Level of significance and **= 1% Level of significance, NS= Non-significance

Table 2. Number of leaves per cabbage at different days after transplantation (DAT) for various treatments (Mean±SE)

Treatment	Days after transplanting			
	30	45	60	75
T_1	10.50±0.49	13.48±0.24b	16.24±0.85ab	18.92±0.05c
T_2	11.04±0.70	13.84±0.39b	15.72±0.28b	19.24±0.32bc
T_3	12.32±0.10	15.20±0.21ab	17.00±0.62ab	20.40±0.06bc
T_4	11.96±0.62	15.16±0.35ab	15.92±0.48b	20.56±0.23b
T_5	12.88±1.02	16.60±0.63a	18.52±0.64a	22.84±0.77a
T_6	12.38±0.95	16.36±0.47a	17.56±0.35ab	22.68±0.24a
CV (%)	14.16	9.55	9.12	8.27
Level of significance	NS	**	**	**

T_1= Control, T_2= Biochar + Cow urine, T_3= Recommended manures, T_4= Recommended fertilizers, T_5= Recommended fertilizers + biochar + cow urine, T_6=Recommended manures and fertilizers, SD= Standard deviation, CV= Coefficient variance, *= 5% Level of significance and **= 1% Level of significance, NS= Non-significance

Leaf breadth, measured in centimeters (cm), exhibited significant variations across treatments (Table 3).There was trend of increasing leaf breadth with the advancement of days after plaanting from 30 to 75 DAT. The maximum breadth increased at 30 DAT (66.4%) while minumum at 75 DAT (24.3%) over control. These results showed the positive effect of the recommended fertilizers + biochar + cow urine treatment on promoting broader leaf breadth throughout the cabbage cultivation period.

Impact of recommended fertilizers, biochar, and cow urine on cabbage diameter, length, weight, dry weight and head yield

The characteristics of cabbage heads, including diameter, head length, head weight, and dry weight, exhibited significant variations across the different treatments (Table 4). At the final harvest, T_5 showed the largest cabbage heads with a diameter of 24.6 cm with increase of 27.6% over the control (T_1) . Similarly, T_5 showed the longest head length (20.0 cm), significant improvement of about 20.8% compared to T_1. In terms of head weight, T_5 recorded the highest weight (2.17 kg), indicating a remarkable increase of approximately 24% over T_1. Additionally, T_5 exhibited the highest dry weight of the head (fresh 20g) at 5.53, showed an increase of 22.4% over T_1..

Table 3. Leaf breadth (cm) of cabbage at different days after transplantation (DAT) for various treatments (Mean±SE)

Treatment	Days after transplanting			
	30	45	60	75
T_1	8.60 ±0.26d	13.76±0.46d	19.24±0.33c	26.68±0.39c
T_2	9.16±0.70d	13.88±1.13d	19.68±0.86c	26.64±0.76c
T_3	11.76±0.18c	17.60±0.36c	23.16±0.07b	30.92±0.24b
T_4	11.88±0.42bc	18.48±0.85bc	23.20±0.62b	30.96±0.64b
T_5	14.28±0.52a	21.52±0.48ab	27.52±0.89a	33.08±0.39ab
T_6	13.76±0.25ab	21.16±0.13a	27.16±0.25a	32.56±0.16b
CV (%)	10.09	12.25	14.96	9.3
Level of significance	**	*	*	*

T_1= Control, T_2= Biochar + Cow urine, T_3= Recommended manures, T_4= Recommended fertilizers, T_5= Recommended fertilizers + biochar + cow urine, T_6=Recommended manures and fertilizers, SD= Standard deviation, CV= Coefficient variance, *= 5% Level of significance and **= 1% Level of significance

Table 4. Effect of recommended fertilizers, biochar, and cow urine treatment on diameter, length, weight, and dry Weight of cabbage (Mean±SE)

Treatment	Diameter of cabbage (cm)	Head Length (cm)	Head Wt. (kg)	Dry Wt. of the head (Fresh 20 gm)
T_1	19.28±0.34c	16.56±19d	1.75±0.02c	4.53±0.02c
T_2	19.08±0.89c	16.55±53d	1.78±0.07c	4.52±0.07c
T_3	22.04±0.29b	18.11±04c	1.98±0.01b	5.00±0.06b
T_4	22.56±0.64ab	18.31±0.27bc	1.99±0.03b	5.07±0.11b
T_5	24.63±0.35a	20.00±0.37a	2.17±0.04a	5.53±0.09a
T_6	24.32±0.19a	19.62±0.06ab	2.13±0.01ab	5.49±0.03a
CV(%)	11.15	8.20	9.06	8.70
Level of significance	*	*	**	*

T_1= Control, T_2= Biochar + Cow urine, T_3= Recommended manures, T_4= Recommended fertilizers, T_5= Recommended fertilizers + biochar + cow urine, T_6=Recommended manures and fertilizers, SD= Standard deviation, CV= Coefficient variance, *= 5% Level of significance and **= 1% Level of significance

Table 5. Impact of recommended fertilizers, biochar, and cow urine treatment on head weight per plot and hectare (Mean±SE)

Treatment	Head weight per plot (kg)	Head yield (ton /ha)
T_1	17.48±0.22c	62.72±0.77c
T_2	17.76±0.67c	63.72±2.41c
T_3	19.84±0.07b	71.19±0.27b
T_4	19.92±0.27b	71.47±0.95b
T_5	21.72±0.38a	77.93±1.37a
T_6	21.32±0.14ab	76.50±0.49ab
CV(%)	9.06	9.06
Level of significance	*	*

T_1= Control, T_2= Biochar + Cow urine, T_3= Recommended manures, T_4= Recommended fertilizers, T_5= Recommended fertilizers + biochar + cow urine, T_6=Recommended manures and fertilizers, SD= Standard deviation, CV= Coefficient variance, *= 5% Level of significance and **= 1% Level of significance

Treatment T_5 exhibited the highest head weight per hectare(77.9 ton) with the increase of 24.4% compared to the control (62.7 ton) (Table 5). These results highlight the efficacy of the recommended fertilizers + biochar + cow urine treatment in enhancing cabbage yield, emphasizing its potential for optimizing production..

Discussion

The findings of this study reveal a significant and consistent enhancement in various growth parameters of cabbage plants when subjected to the recommended fertilizers, biochar, and cow urine treatment. The substantial increase in plant height observed in the recommended fertilizers + biochar + cow urine treatment with highlighting the benefits of biochar in improving soil structure and nutrient retention, consequently promoting plant growth (Lehmann *et al.,* 2011). Cow urine, known for its rich nutrient content, can contribute essential elements for plant development (Pandey *et al.,* 2017). The synergistic effects of these components might have played a crucial role in the observed superior plant height throughout the cultivation period. Cow urine, with its nitrogen-rich composition, could have contributed to the increased leaf count, as nitrogen is a key element in leaf formation (Kumar *et al.*, 2015). Cow urine, acting as a natural growth promoter, could have also played a role in stimulating lateral growth (Bakshi, 2017). Leaf breadth, an essential indicator of plant health, demonstrated consistent improvement with the recommended fertilizers + biochar + cow urine treatment. The larger cabbage heads observed in the recommended fertilizers + biochar + cow urine treatment reflect the combined benefits of enhanced nutrient availability and improved soil structure. Regarding stem and root development, the treatment's positive effects of biochar in promoting root growth and nutrient In terms of cabbage yield,emphasizing the positive impact of organic and bio-based treatments on overall crop productivity (Elad *et al.,* 2010). The synergistic effects of biochar and organic inputs, particularly cow urine, demonstrate the potential of integrated and sustainable approaches for optimizing crop productivity.

Conclusion

This study showed a high productivity of integrating biochar and cow urine with fertilizers in the cabbage cultivation practices. For optimal cabbage growth parameters, including plant height, leaf development, and head characteristics, the combined use of fertilizers, biochar and cow urine (T_5) was proven to be very effective and thus it is recommended for adoption.

Conflicts of interest

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

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Short Communication

MOLECULAR IDENTIFICATION OF ROOT-KNOT NEMATODES (*Meloidogyne* spp.) OF TOMATO AT GAZIPUR DISTRICT IN BANGLADESH

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Abstract

Four root-knot nematode infected tomato root samples were collected from different locations of Gazipur, Bangladesh. Two methods of PCR were used to detect *Meloidogyne* spp. in tomato roots. One is molecular identification by DNA sequencing of the ribosomal DNA (rDNA) 28SD2/D3 gene, and another is PCR using species-specific SCAR primers. In the phylogenetic tree, the obtained sequences; OR351387, OR351388, OR351389, and OR351390 clustered with specific *Meloidogyne incognita* clade. Species-specific primers produced a fragment of 399 bp for *Meloidogyne incognita.* Thus, utilizing species-specific markers and analyzing the results of a phylogenetic tree made from the amplified 28S D2/D3 gene region revealed the association of *Meloidogyne incognita* on tomato root-knot disease in Bangladesh.

Keywords: *Meloidogyne incognita*, Root-knot disease, 28SD2/D3 gene, SCAR primers

Introduction

Meloidogyne spp. are highly damaging and yield reducing plant-parasitic nematode of tomato (*Solanum lycopersicum* L.) production. The average loss of crop due to Root-knot nematode (RKN) infestation could be reach up to 15% (Timm and Ameen, 1960). The species which is responsible for the gall formation on majority of crop roots are *M. incognita, M. arenarea, M. hapla* and *M. javanica* (Moens *et al.,* 2009). However, the species of *Meloidogyne* that causes root-knot of tomato has not been properly investigated in Bangladesh. It is assumed that *M. incognita* causes root-knot disease of tomato. For the proper identification of RKN species of tomato, molecular tools have been progressively developed in past 20 years. PCR and DNA sequencing methods are very fast, sensitive, and applicable for any stage of nematodes population (Ye *et al.*, 2019). So, the current study was proposed to identify the causal agent of root-knot of tomato utilizing PCR and DNA sequencing.

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Materials and Methods

In October-December 2022, four RKN infected tomato root samples were collected from Kapashia, Dhirasrom and BARI campus of Gazipur. The samples showed typical symptoms; stunted plants, pale yellow leaves and big galls on the roots (Yigezu Wendimu, 2021) (Fig.1.A-B). For molecular identification, SCAR primer pair; Inc-K14-F/Inc-K14-R (CCCGCTACACCCTCAACTTC/GGGATGTGTAAATGCTCCTG) and a universal primer pair; RK28SF/R (CGGATAGAGTCGGCGTATC/GATGGTTC GATTAGTCTTTCGCC) were used to target gene 28S D2/D3. DNA was isolated from approx. 150 second stage juveniles (J2) (Figure 1.C) and was extracted following Wizard genomic DNA purification kit (Promega Corporation, Madison, WI, USA). DNA quality was measured using spectrophotometer at A260/280 (1.91). PCR were performed in a thermal cycler and per PCR reaction containing 12.5 µL GoTaq Green master mix, 9.5 µL nuclease-free water, 1 µL of each primer (10 µM), and 1µL template DNA. The PCR condition for universal primer RK28SF/R was: one cycle of denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 53 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 8 min (Manojkumar and Somashekharappa, 2022). Moreover, the PCR condition for *M. incognita* specific primers (SCAR) was: one cycle of denaturation at 95 °C for 5 min, followed by 40 cycles at 94°C for 30 s, 55 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min (Randig *et al.*, 2022).

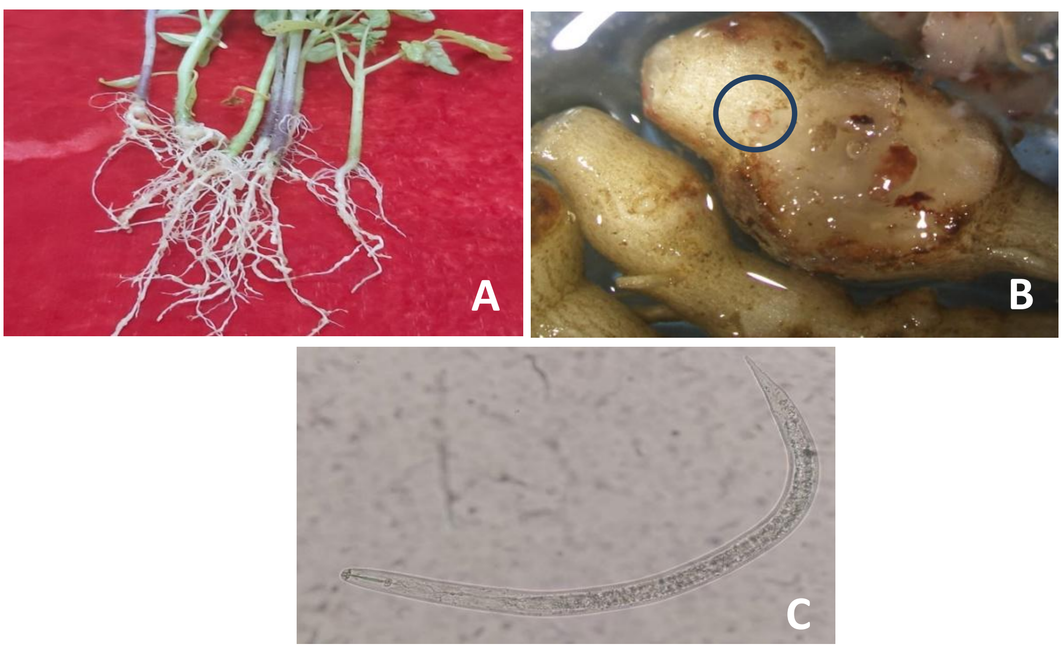

Fig. 1. (A). Root-knot nematode infected tomato roots (B). Female eggs inside the roots (C). Second satge juvenile of *Meloidogyne* spp.

Results and Discussion

SCAR primer pair produced a specific fragment of 399 bp for *M. incognita* (Fig. 2.). RK28SF/R primer pair amplified PCR 612 bp products for the isolates and the purified DNA was successfully sequenced (National Institute of Biotechnology, Bangladesh) and deposited to GenBank. The possible identities of the isolates were established by comparing sequences with those in Gen Bank database (National Center for Biotechnology Information [NCBI]) under US National Institute of Health, Bethesda, MD, USA. Blast analysis of the obtained sequences of the isolates were found 97-99% nucleotide homology with different *M. incognita* isolates that were previously submitted in GenBank database (NCBI), MD, USA (Table 1). Phylogenetic analysis of the 28S gene sequence data was done by means of Maximum Composite Likelihood method using MEGA 10.0 software. The sequence distance was calculated by Tamura and Nei, 1993; parameter model (Tamura and Nei, 1993). Bootstrap values were obtained 500 replicates to determine the support from each group. In the Phylogenetic tree, the isolates of Bangladesh; OR351387, OR351388, OR351389 and OR351390 were placed in distinct *M. incognita* group (Fig. 3.). Traditionally, morphology-based identification such as body length, morphology of sexual organs, mouth and tail parts provide inadequate information. Previously in Bangladesh, root-knot nematodes species have been identified from different vegetables and fruit crops based on only morphological characters (Elahi *et al.*, 2021).

Table 1. Blast results of isolates after sequencing and their highest homology with gene bank strains

Isolates	BLAST result	BLAST homology	Query cover	Type strains
OR351387	*Meloidogyne incognita*	99.13%	100%	OM522593
OR351388	*Meloidogyne incognita*	99.13%	100%	MF177881
OR351389	*Meloidogyne incognita*	97.92%	100%	MF177882
OR351390	*Meloidogyne incognita*	98.72%	100%	MF177880

However, this method cannot provide clear variation among closely related taxa and also for this work, required highly skilled taxonomist (Oliveira *et al.*, 2011). On the other hand, there are several advantages of PCR method in molecular diagnosis of *Meloidogyne* spp. Utilizing DNA sequencing and species-specific PCR for the detection of nematodes are the better options compared to conserved ITS regions or morphological identification (Tesarova *et al.*, 2003). Moreover, for the identification of tropical root-knot nematode, species-specific PCR is recommended. Because of the genes of tropical root-knot nematodes are too conserved to identify only with DNA sequencing method and BLAST search (Danso *et al.*, 2023).

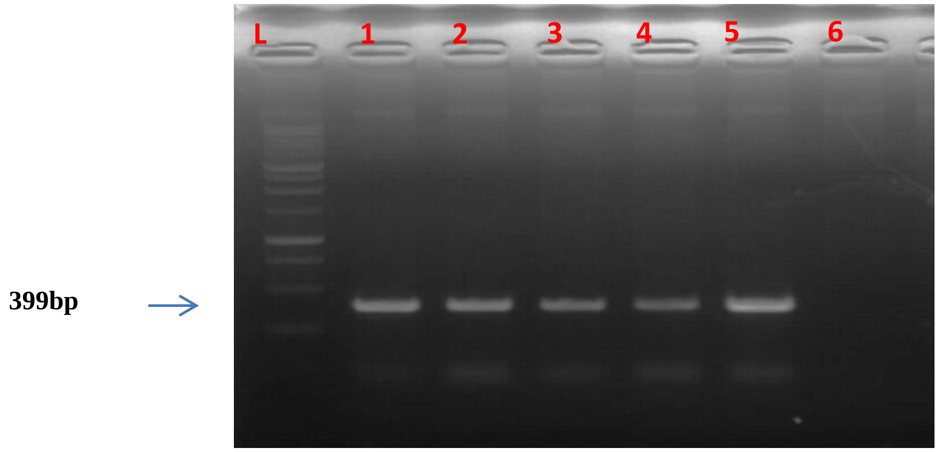

Fig. 2. Specificity of Inc-K14-F/Inc-K14-R-based PCR assay for the detection of *Meloidogyne incognita* in symptomatic tomato plants. L denotes 1 kb ladder, 1= positive control, 3-5= samples, 6= negative control.

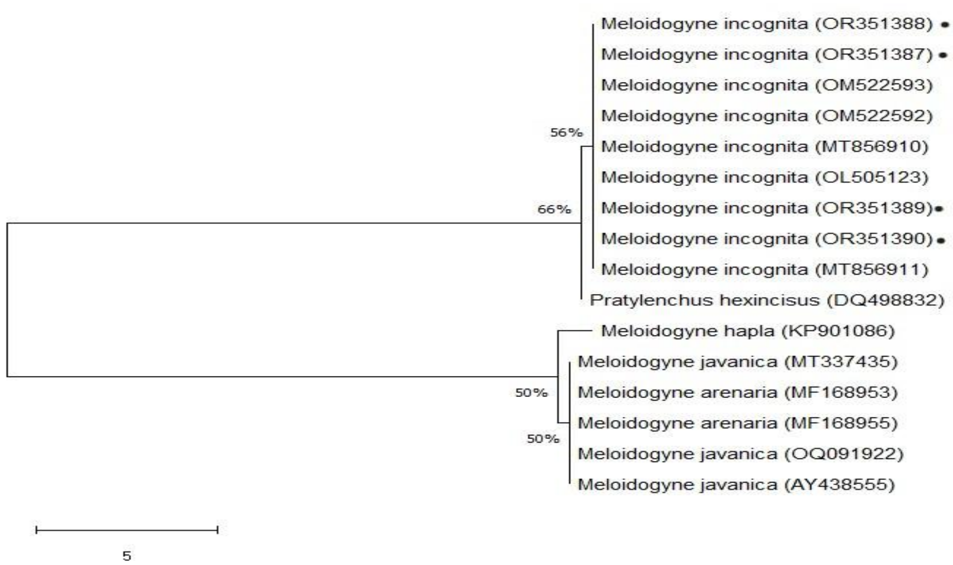

Fig. 3. Phylogenetic relationship of root-knot nematodes infecting tomato concluded by Tamura-Nei model of the 28S gene sequences. *Pratylenchus hexincisus* (DQ498832) was used as the out-group. Bootstrap support values for maximum likelihood (ML) greater than 50% are given at the nodes. Isolates obtained in this study are indicated with black dot.

Conclusion

It is the first report of molecular characterization for root-knot nematodes of tomato in Bangladesh. The results showed that *M. incognita* was present in several tomato-growing fields in the Gazipur district of Bangladesh.

Conflicts of Interest

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

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