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IN VITRO CONTROL OF *Sclerotium rolfsii*, THE CAUSAL AGENT OF COLLAR ROT IN SUNFLOWER USING FUNGICIDES, BOTANICALS AND ORGANIC MATTER

N. A. Sultana[1], M. A. Islam[2], M. S. Islam[2], M. M. Alam[3*], R. W. Bell[4] and M. Mainuddin[5]

[1]Oilseed Research Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur; [2]Department of Plant Pathology, Patuakhali Science and Technology University (PSTU), Patuakhali; [3]Crops Division, Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka; [4]Centre for sustainable farming system, Food Futures Institute, Murdoch University (MU), Murdoch Wa 6050, Australia; [5]Water Security Program, CSIRO, Black Mountain Laboratories, GPO Box 1700, Canberra ACT 2601, Australia.

Abstract

The sunflower (*Helianthus annuus* L.), a vital oilseed crop that significantly reduces production globally, is seriously threatened by collar rot, a disease caused by the soil-borne fungus *Sclerotium rolfsii* reductions. This research sought to assess the effectiveness of various control methods against *S. rolfsii* in laboratory conditions, including nine fungicides, nine plant extracts, and five organic amendments. This study assessed the efficacy of various medicinal plant extracts at different concentrations (5%, 10%, 15%, and 20%) in inhibiting the *in vitro* mycelial growth of *S. rolfsii*. Among the plant extracts tested, garlic demonstrated the most potent antifungal properties, completely suppressing mycelial growth at 10%, 15%, and 20% concentrations. The results indicate that garlic clove extract (98.18-100%) is the most effective at inhibiting *S. rolfsii* growth, with henna (65.92-92.46%) and black cumin extracts (64.80-85.88%) also demonstrating strong efficacy. The fungicide Carboxin and Thiram (Provax 200) were found most effective, achieving total inhibition, followed by Azoxystrobin and Difenoconazole (Amister Top) (91%) and Difenoconazole (Score) (90%). In the case of the extracts of organic amendment material, poultry refuse exhibited the highest inhibition, completely inhibiting the mycelial growth at 20% and 30% concentrations. Among the organic amendments, poultry refuse (94.40-100%), vermicompost (17.55-67.32%), and mustard oilcake (14.88-64.10%), showed strong potential for reducing *S. rolfsii* mycelial growth. The findings of the study suggest that Carboxin and Thiram, poultry refuse, and garlic extract could be effective in managing sunflower collar rot disease.

Keywords: Collar rot, Organic amendment, Plant extract, *Sclerotium rolfsii*.

* Corresponding author: mahfuzbari@gmail.com

Introduction

Sunflower (*Helianthus annuus* L.) is a crucial oilseed crop with considerable potential to decrease the demand for imported vegetable oils. Its oil is considered superior in quality to that of rapeseed and mustard, making it a favored choice among consumers due to its healthy properties. Sunflower ranks fourth globally in vegetable oil production (Pilorgé, 2020), with approximately 30 million hectares harvested and 55 million tons produced (NSA, 2024). This crop is characterized by its short growth cycle (90-110 days), high adaptability, relative drought resistance, and capacity for high yields. Sunflower seeds contain 45-52% edible oil and are rich in protein (23%) (Khan *et al*., 2012). Contributing roughly 13% to global edible oil production (Gabagambi *et al*., 2010), sunflower oil is prized for its nutritional qualities, including its anti-cholesterol properties and high content of essential fatty acids (linoleic acid, 60-73%), making it beneficial for heart health. In Bangladesh, sunflower is a relatively new addition to the oilseed crops, which include mustard, sesame, and groundnut. Although native to the southern United States and Mexico, sunflower cultivation in Bangladesh began on a small scale in 1975 (Habib *et al*., 2017). It has emerged as a promising crop for the dry season in the country's coastal regions. However, its productivity faces threats from both biotic and abiotic factors, with diseases being a significant biotic challenge. One of the major diseases affecting sunflowers is collar rot, caused by the soil-borne fungal pathogen *Sclerotium rolfsii* Sac., which infects over 500 plant species worldwide (Billah *et al*., 2017; Sun *et al*., 2020). This pathogen is particularly harmful to sunflowers, causing rapid wilting and stem lesions that can result in substantial yield losses. Collar rot can cause substantial yield losses in sunflower crops, with the extent of damage influenced by factors such as planting density, soil moisture, and local climate conditions. In severe cases, the disease can decimate entire fields, leading to significant economic losses for farmers. The sunflower plant's susceptibility to collar rot, particularly in its early growth stages, makes early and effective disease management essential. The development of collar rot-resistant sunflower varieties has been slow, partly due to the genetic diversity of *S. rolfsii* and its ability to adapt to environmental conditions.

The characteristic symptoms of collar rot include a brownish lesion at the stem base, which eventually encircles the plant. The infected area becomes covered with white mycelial strands and forms sclerotia, which are small, hard, and resilient structures that allow it to survive in soil for extended periods. The pathogen’s ability to persist in the soil as sclerotia and its wide host range make it challenging to control through traditional crop rotation practices alone. The disease thrives in moist soil conditions, typically appearing within two weeks of planting, and leads to yellowing and eventual plant death. *S. rolfsii* can cause yield losses ranging from 25.60-48.62%, with severe infections resulting in losses of up to 80% (Mehan and McDonald, 1990). The pathogen's persistence in soil and its ability to infect multiple crops make it particularly challenging to control (Sennoi *et al*., 2013).

Managing *S. rolfsii* is complex due to its soil-borne nature, wide host range, ability to produce excessive sclerotia, and the ability to persist in the soil for several years in the form of resistant sclerotia (Billah *et al.*, 2017). Traditional methods for controlling collar rot include crop rotation, field sanitation, and the use of fungicides. Crop rotation, although useful in managing certain pathogens, is less effective against *S. rolfsii* due to its broad host range and ability to persist in the soil. Conventional control methods heavily rely on fungicides, Chemical fungicides, such as Carboxin+thiram, carbendazim, hexaconazole, mancozeb, and thiophanate-methyl, have been widely used to manage collar rot infection in sunflower (Mondal and Khatua, 2013). While effective, the over-reliance on chemical fungicides raises concerns regarding environmental impact and human health risks, and the development of fungicide-resistant strains *of S. rolfsii* has driven research into alternative approaches. Botanicals and organic matter extract present promising alternatives that align with sustainable agriculture practices, offering eco-friendly and potentially more affordable options for collar rot management. Integrating these methods can provide a comprehensive approach to controlling collar rot, improving soil health, and enhancing sunflower plant resilience. Because of the above facts, the present research work was designed to evaluate some fungicides, plant extracts, and extracts of organic amendment materials against collar rot disease of sunflowers.

Materials and Methods

Collection of diseased specimens

Sunflower plants exhibiting collar rot symptoms were collected from agricultural fields across three districts in Bangladesh: Borguna, Potuakhali, and Khulna. The infected samples were brought to the laboratory, cleaned of any soil or debris, and stored in paper bags at 4°C for future analysis. Five samples were collected from each spot to isolate and identify the pathogen responsible for the disease.

Isolation and identification of the pathogen

The pathogen was isolated from the infected sunflower plants using the tissue segment method described by Mian (1995). Infected stem tissues, particularly from the collar area, were thoroughly cleansed, cut into small pieces (4-5 mm), disinfected with 70% ethanol for 5 minutes, washed with sterile distilled water, and dried using sterile absorbent paper. The sanitized tissue fragments were placed on Potato Dextrose Agar (PDA) and kept at 25 ± 1°C for 3- 4 days. Fungal growths that emerged on the PDA plates were transferred to fresh PDA plates and incubated at 25 ± 1°C for 5 days. The extracted pathogen was identified as *Sclerotium rolfsii* based on its morphological characteristics, including white, fluffy mycelia and the formation of brown sclerotia resembling mustard seeds (0.90–1.40 mm in diameter) (Sekhar *et al*., 2017).

Preparation and maintenance of pure culture

Pure culture of *S. rolfsii* was established using the hyphal tip culture method (Alam *et al.*, 2024; Islam *et al*., 2001; Mian, 1995). These cultures were preserved on PDA slants, transferred to fresh media monthly, and stored at 4°C for future use.

Preparation of cold aqueous extracts

Fresh plant components, such as leaves, bulbs, rhizomes, seeds, and cloves, were collected to create botanical extracts. For each extract, 100 g of fresh plant material was blended with 100 mL of sterile distilled water. The mixture was filtered through Whatman No. 1 filter paper, and the resulting liquid was used as the stock solution

(Kamlesh & Gujar, 2002). Extracts from nine plant species viz., ginger, turmeric, onion, garlic, black cumin, henna, neem, tulsi, and eucalyptus were prepared and tested at concentrations of 5%, 10%, 15%, and 20% using PDA as the growth medium (Islam, 2005).

Poisoned food technique

PDA medium was supplemented with botanical extracts at various concentrations (5%, 10%, 15%, and 20%). A mycelial disc of *S. rolfsii* (5 mm diameter) was positioned centrally on each plate and incubated at 25 ± 2°C. Control plates without botanical extracts were also prepared. The fungal colony diameters were recorded daily until complete colonization of control plates occurred, it started one day after inoculation (1 DAI) and continued to four days after inoculation (4 DAI).

In-vitro screening of fungicides against *Sclerotium rolfsii*

The most virulent *Sclerotium rolfsii* isolate (PhaKHaSr1) was tested against nine fungicides encompassing systemic, contact, and combination types, using the poisoned food technique (Islam, 2005) in a grove setting. Fungicide suspensions were prepared following the manufacturer's instructions. For each treatment, 100 ml of PDA was placed in a 250 ml conical flask and autoclaved. The flasks were properly labeled and thoroughly shaken before use. Twenty milliliters of the PDA medium were then poured into 9 cm Petri plates. After solidification, three 5 mm discs were removed from the PDA plate at equal distances from the center using a sterile disc cutter. A measured amount of fungicide (hundred microliters) was placed in each hole, and the plates were refrigerated overnight to allow the fungicide to diffuse into the surrounding medium. The following day, a 5 mm block of a 5-day-old *S. rolfsii* culture grown on PDA was cut with a sterile disc cutter and placed in the center of each plate. The plates were incubated at 25 ± 2°C and monitored daily for fungal growth inhibition. A suitable control was maintained by growing the pathogen on a fungicide-free PDA medium. Four replications were performed for each treatment. Radial mycelial growth of *S. rolfsii* was measured at 24-hour intervals until the colony reached the edge of the Petri dishes in the control plates (Islam *et al.,* 2001; Islam, 2005).

In-vitro screening of the extracts of organic amendment materials

Extracts from five organic amendment materials poultry refuse, vermicompost, mustard oil cake, sawdust, cow dung, and a control were tested against *S. rolfsii* (isolate PhaKHaSr1) at concentrations of 10%, 20%, and 30% using the poisoned food technique (Nene and Thapliyal, 1993). Each amendment was finely ground into powder using a pestle and mortar. For each treatment, 50 grams of powdered material were placed into 250 ml flasks, then 150 ml of sterilized water (w/v) was added and the mixture was left to decompose for 15 days. After 15 days, the mixtures were strained through double-layered muslin cloth, and the filtrates were further filtered using Whatman No. 1 filter paper. The resulting extracts were then autoclaved for 10 minutes to sterilize them, yielding a 100% standard extract solution (Dubey and Patel, 2001). This extract solution was diluted to prepare the required concentrations. PDA plates containing each respective extract concentration were inoculated with 5 mm discs of a 7-day-old *S. rolfsii* culture. Plates without extracts served as controls.

Measurement of radial mycelial growth and growth inhibition

Fungal colony radial growth was measured every 24 hours. The percentage of fungal growth inhibition was determined using the following formula (Hussain *et al*., 2015):

$$\textit{Percent of inhibition (PI)} = \frac{X-Y}{Y} \times 100$$

Where:

Y = Average radial growth (cm) of *S. rolfsii* in control plates.

Z = Average radial growth (cm) of *S. rolfsii* in treated plates.

Experimental design and statistical analysis

The experiments were conducted in a completely randomized design with four replications per treatment. Data were analyzed using R statistical software, and treatment means were compared using the Least Significant Difference (LSD) test at $P \geq 0.05$.

Results and Discussion

Effect of plant extract on in-vitro colony growth of *Sclerotium rolfsii*

The antifungal potential of various plant extracts against *S. rolfsii* was evaluated, and the results demonstrate significant differences in the efficacy of these natural agents (Table 1 and 2, plate. 1). Among all the tested extracts, garlic clove extract showed the highest antifungal activity after four days post inoculation (dpi). At concentrations of 10%, 15%, and 20%, garlic extract completely inhibited the mycelial growth of *S. rolfsii*. Even at 5%, it significantly initiated the growth to 1.63 mm.

Table 1. Efficacy of botanicals on mycelial growth of *S. rolfsii* under in vitro conditions at different concentrations

Botanicals extracts	Radial mycelial growth (mm)			
	5%	10%	15%	20%
Garlic clove extract	1.63 g	0.00 i	0.00 i	0.00 h
Henna leaf extract	30.50 f	15.12 h	7.75 h	6.75 g
Ginger rhizome extract	66.25 e	48.37 f	40.75 f	19.24 e
Turmeric rhizome extract	67.75 e	53.75 e	45.75 b	21.12 e
Onion bulb extract	66.25 e	49.50 f	41.37 f	19.87 e
Black cumin seed extract	31.50 f	20.50 g	14.12 g	12.62 f
Neem leaf extract	74.0 d	64.25 d	53.62 d	25.0 d
Tulshi leaf extract	82.63 c	78.50 c	65.62 c	59.25 c
Eucalyptus leaf extract	85.62 b	84.37 b	78.75 b	75.50 b
Control	89.50 a	89.50 a	89.50 a	89.50 a
SEm ±	1.0124	0.9082	0.8514	0.7071
LSD ($P \geq 0.05$)	2.93	2.63	2.47	2.05
CV (%)	3.39	3.60	3.89	4.30

Values in a column having the same letter(s) did not differ significantly at the 5% level by LSD.

Table 2. Efficacy of medicinal plant extracts on mycelial growth inhibition of *S. rolfsii* under in-vitro

Botanicals extracts	Mycelial growth inhibition (%)			
	5%	10%	15%	20%
Garlic clove extract	98.18	100.0	100.0	100.0
Henna leaf extract	65.92	83.09	91.34	92.46
Ginger rhizome extract	25.97	45.95	54.46	78.49
Turmeric rhizome extract	24.30	39.95	48.87	76.40
Onion bulb extract	25.99	44.70	53.77	77.80
Black cumin seed extract	64.80	77.09	84.21	85.88
Neem leaf extract	17.31	28.21	40.08	72.07
Tulshi leaf extract	7.67	12.27	26.66	33.79
Eucalyptus leaf extract	4.34	5.71	12.02	15.64

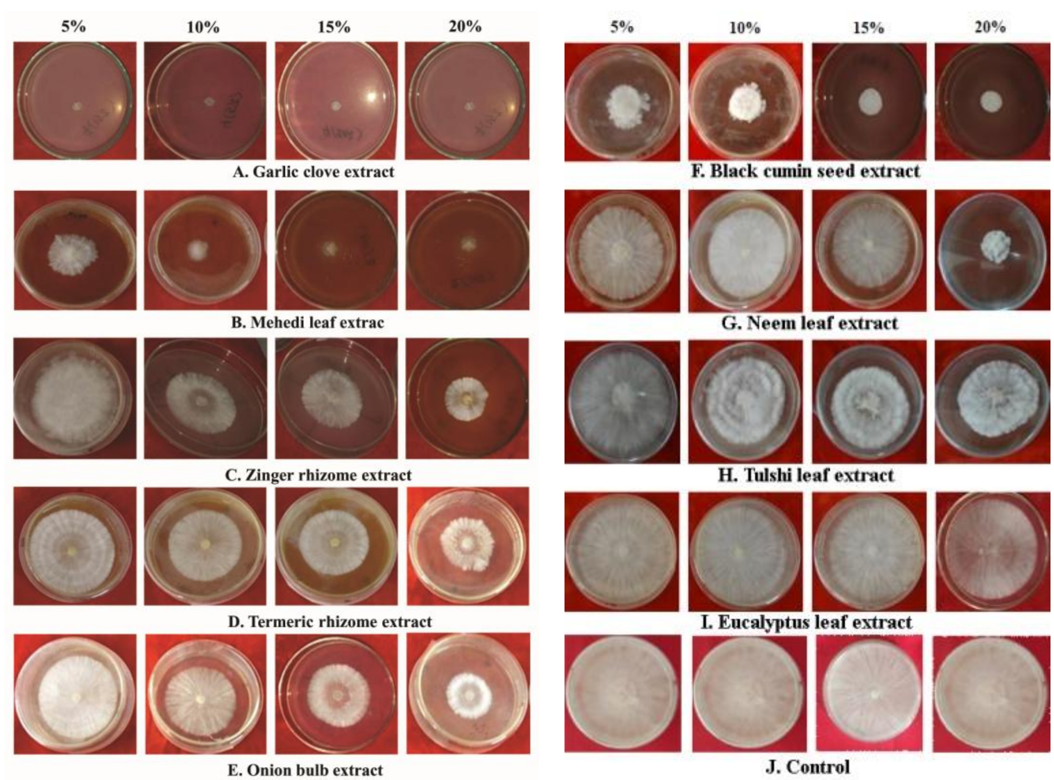

Fig. 1. (a). Pictorial view of radial mycelial growth of *S. rolfsii* in Petri plates containing PDA amended with botanicals (A) Garlic clove extract, (B) Henna leaf extract, (C) Zinger rhizome extract, (D) Turmeric rhizome extract, (E) Onion bulb extract, (F) Black cumin seed extract, (G) Neem leaf extract, (H), Tulshi leaf extract, (I) Eucalyptus leaf extract, and (J) Control at 4 days after inoculation

This profound inhibition is likely due to the presence of sulfur-containing compounds, such as allicin, which has been widely recognized for its antimicrobial properties. Previous studies have shown that garlic has strong inhibitory effects against a wide range of plant pathogens due to its ability to disrupt microbial cell walls and inhibit enzyme activity essential for fungal survival (Benkeblia, 2004). Eucalyptus leaf extract was not as effective as garlic, but still demonstrated significant inhibitory effects. Radial mycelial growth was reduced from 85.62 mm at 5% to 75.50 mm at 20% concentration. Eucalyptus contains bioactive compounds like eucalyptol and flavonoids, which have been documented for their antimicrobial activities. The antifungal mechanism of eucalyptus may involve interfering with fungal cell wall integrity and inhibition of spore germination (Batish *et al*., 2008). Tulsi showed moderate antifungal activity, with mycelial growth decreasing from 82.63 mm at 5% to 59.25 mm at 20% concentration. Tulsi's antifungal properties are attributed to its essential oils, particularly eugenol, which has been found to inhibit fungal growth by disrupting cell membranes and inhibiting enzymatic processes. Previous research supports the moderate antifungal action of Tulsi extracts against a variety of soil-borne pathogens (Prakash and Gupta, 2005). Black cumin seed extract significantly inhibited *S. rolfsii* growth, with a reduction from 31.50 mm at 5% to 12.62 mm at 20% concentration. This suggests that Black cumin has strong potential as a botanical fungicide. Similarly, Henna leaf extract demonstrated substantial inhibition, reducing mycelial growth from 30.50 mm at 5% to 6.75 mm at 20% concentration, suggesting its role as a strong antifungal agent. Other plant extracts such as ginger, onion, neem, and turmeric exhibited moderate inhibition. While garlic, Black cumin, and Henna extracts showed complete inhibition or significant reduction at higher concentrations, these extracts reduced mycelial growth by approximately 85.88-100% at 20% concentration. The antimicrobial properties of these plants are well-documented, with studies attributing their antifungal action to active compounds like curcumin in turmeric, gingerol in ginger, and azadirachtin in neem (Gupta and Sharma, 2017; Tewari *et al*., 2014). In terms of percentage inhibition, garlic extract showed near-complete inhibition (98.18%) at 5%, and complete inhibition at concentrations above 10%. Black cumin and Henna extracts also demonstrated strong antifungal efficacy, achieving 85.88% and 92.46% inhibition, respectively, at 20% concentrations. However, Tulshi and eucalyptus leaf extracts showed comparatively low inhibition rates, indicating limited efficacy against *S. rolfsii*. Tulshi achieved 33.79% inhibition at 20%, and eucalyptus only reached 15.64% at the same concentration. While these plants contain antifungal compounds such as eugenol and eucalyptol, respectively, their concentrations in these extracts may be insufficient for effective suppression of *S. rolfsii*. Their limited efficacy suggests they may not be suitable as primary antifungal agents for managing this pathogen but could be explored in combination with other stronger extracts (Chakrapani *et al*., 2020). These findings align with previous reports indicating variable antifungal efficacy among different plant extracts, depending on their phytochemical composition and mode of action (Pandey *et al*., 2010).

Effects of fungicides on *in-vitro* colony growth of *S. rolfsii*

The efficacy of different fungicidal treatments on the radial mycelial growth of *S, rolfsii* over four days after inoculation was presented in Table 3 and Plate 2. Ipridion

50WP (Rovral) at 0.2% concentration, reduced fungal growth to 70.38 mm by day 4, achieved 21.81% inhibition compared to the control, and demonstrated moderate effectiveness throughout the study period. Metalaxyl and Mancozeb (Newben), applied at 0.25%, showed greater inhibition (36.94%) by day 4, limited growth to 56.75 mm. Similarly, Metalaxyl and Mancozeb were observed as a protective treatment under moderate disease pressure (Ganguly and Banik, 2010). Azostrobin and Difenoconazole (Amistar Top 325 SC), at 0.10% concentration, proved highly effective, restricted the growth to 8.13 mm by day 4 with 90.97% inhibition, ranked it among the most potent treatments in the trial. These fungicides, both of which belong to the triazole class, inhibit the biosynthesis of ergosterol, a vital component of fungal cell membranes, thereby preventing fungal growth (Lamb *et al*., 2000). Carboxin and Thiram (Provax-200 WP) (0.20%) emerged as the most effective, completely restricted fungal growth at all times, and achieved 100% inhibition. These results corroborate earlier studies showing that Carboxin and Thiram are highly effective against soil-borne pathogens like *S. rolfsii* and can be an essential tool in chemical disease management programs (Mahapatra *et al*., 2016). Bavistin, at 0.10%, limited growth up to 65.13 mm by day 4, resulting in 27.64% inhibition, yielded moderately but less effectively than that of Azostrobin and Difenoconazole or Carboxin and Thiram restricted fungal growth to 60.25 mm by day 4, with 33.06% inhibition, yielded moderate results.

Table 3. In vitro efficacy of fungicides on controlling mycelial growth of *Sclerotium rolfsii* in poisoned food technique after 1, 2, 3, and 4 days after incubation (DAI)

Treatments	Dose (%)	Mean radial growth of the fungus (mm) after				% inhibition of mycelial growth over control at 4 DAI
		1 DAI	2 DAI	3 DAI	4 DAI	
Rovral (Iprodion 50% WP)	0.2	20.25b	41.38c	55.88c	70.38bc	21.81
Newben (Metalaxyl 8% + Mancozeb 64%)	0.25	11.62f	29.00e	45.13e	56.75f	36.94
Amistar Top 325 SC (Azostrobin + Difenoconazole)	0.10	2.50g	4.00f	6.63f	8.13g	90.97
Provax-200 WP (Carboxin 37.5% + Thiram 37.5%)	0.20	0.00h	0.00g	0.00g	0.00h	100
Bavistin 50% WP (Carbendazim)	0.10	16.37d	34.63d	49.50d	65.13d	27.64
Cynil 72% WP (Cymoxanil 8% + Mancozeb 64%)	0.25	13.87e	33.00d	45.63e	60.25e	33.06
Oxycob (Copper oxychloride 50% WP)	0.20	20.50b	44.38b	60.00b	72.50b	19.44
Dithen M-45 (Mancozeb 75% WP)	0.25	17.87c	40.13c	53.75c	68.00cd	24.44
Score (Difenoconazole 25% EC)	0.20	2.63g	5.25f	7.50f	9.13g	89.86
Control	-	25.88a	54.25a	75.63a	90.00a	-

Treatments	Dose (%)	Mean radial growth of the fungus (mm) after				% inhibition of mycelial growth over control at 4 DAI
		1 DAI	2 DAI	3 DAI	4 DAI	
SEm ±	-	0.811	0.788	0.981	1.0124	-
LSD (P ≥ 0.05)	-	1.17	2.28	2.84	2.93	-
CV (%)	-	6.19	5.51	4.90	4.04	-

Means followed by the same letter/letters do not significantly differ at the 5% level tested by LSD.

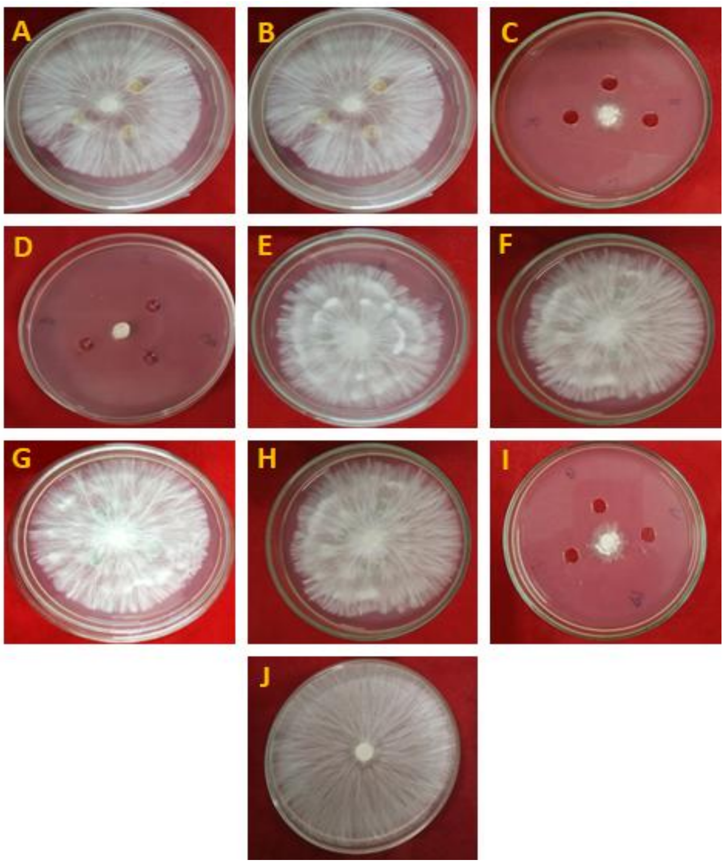

Fig. 2. Radial mycelial growth of *Sclerotium rolfsii* against (A) Rovral 50 WP, (B) Newben (C) Amistar Top, (D) Provax 200 WP, (E) Bavistin, (F) Cynil 72WP, (G) Oxycob, (H) Dithan M-45, (I) Score, and (J) Control at 4 days after inoculation.

Oxycob at 0.20% concentration allowed growth up to 72.50 mm by day 4, with 19.44% inhibition, it was one of the less effective treatments. Mancozeb (Dithane M-45), at 0.25%, reduced fungal growth up to 68.00 mm by day 4, with 24.44% inhibition, demonstrating a moderate inhibitory effect. Difenoconazole (Score 25EC 0.20%) exhibited high efficacy, inhibited growth up to 9.13 mm by day 4, with 89.86%

inhibition, comparable to Azostrobin and Difenoconazole in effectiveness. Similarly, Suneeta *et al*. (2017) conducted an *in vitro* evaluation of different fungicides against Collar Rot of Gerbera caused by *S. rolfsii*. Difenoconazole 25% EC recorded 100 percent inhibition of pathogen at 500, 1000, and 1500 ppm concentrations. The study concludes that Provax-200 is the most potent fungicide for the growth inhibition of *S. rolfsii*, while Azostrobin Difenoconazole, and Difenoconazole also demonstrate strong efficacy in controlling fungal growth.

In-vitro effects of different extracts of organic amendment materials

An in vitro assessment was conducted to evaluate the effectiveness of organic amendment material extracts on mycelial growth inhibition of *S. rolfsii* and sclerotia production. The study included three concentrations (10%, 20%, and 30%), with results given in Table 4, Table 5, and Fig. 3. Poultry refuse extract demonstrated superior efficacy in inhibiting mycelial growth, reduced it up to 6.75 mm at 10% concentration and completely inhibited growth at 20% and 30%. It was also highly effective against sclerotia production, allowed only 32.25 sclerotia at 10% and entirely suppressed formation of sclerotia at higher concentrations. Vermicompost extract showed moderate effectiveness in controlling mycelial growth, with 73.38 mm, 42.38 mm, and 29.25 mm at 10%, 20%, and 30% concentrations, respectively. The inhibition percentage increased from 17.55% to 67.32% as concentration increased. It also substantially decreased sclerotia production, with counts of 123.50, 88.25, and 73.25 at increasing concentrations, corresponding to inhibition rates between 80.16% and 88.92%.

Table 4. Effect of various organic amendment extracts on the mycelial growth and growth inhibition of *S. rolfsii* under *in-vitro* condition

Treatments	Mycelial growth (mm) of *S. rolfsii* at different concentrations			Percent inhibition at different concentrations		
	10%	20%	30%	10%	20%	30%
Poultry refuse extract	6.75e	0.00e	0.00f	92.40	100.00	100.00
Vermi compost extract	73.38d	42.38d	29.25e	17.55	52.44	67.32
Mustard oilcake extract	75.75cd	44.25c	32.13d	14.88	50.34	64.10
Saw dust extract	77.63c	45.50c	34.88c	12.77	48.94	61.03
Cowdung extract	81.38b	75.75b	72.13b	8.57	15.00	19.40
Control	89.0a	89.13a	89.50a	-	-	-
SEm ±	0.908	0.591	0.5	-	-	-
LSD ($P \geq 0.05$)	2.73	1.78	1.507	-	-	-
CV (%)	2.69	2.39	2.32	-	-	-

Values in a column having the same letter(s) did not differ significantly at the 5% level by LSD.

Table 5. Effect of various organic amendments on Sclerotia production of *S. rolfsii* under *in-vitro* condition

Treatments	No. of mature sclerotia of *S. rolfsii*			Percent inhibition at different concentrations		
	10%	20%	30%	10%	20%	30%
Poultry manure extract	32.25e	0.00e	0.00e	94.82	100.00	100.00
Vermi compost extract	123.50d	88.25d	73.25d	80.16	86.04	88.92
Mustard oilcake extract	130.0d	95.75d	79.75d	79.11	84.86	87.96
Saw dust extract	331.0c	228.50c	183c	46.83	63.87	72.35
Cowdung extract	496.5 b	473.25b	356b	20.24	25.17	46.20
Control	622.50a	632.50a	661.75a	-	-	-
SEm ±	2.958	2.783	3.464	-	-	-
LSD ($P \geq 0.05$)	8.916	8.391	10.441	-	-	-
CV (%)	2.04	2.20	3.07	-	-	-

Values in a column having the same letter(s) did not differ significantly at the 5% level by LSD

Mustard oilcake extract exhibited similar efficacy to vermicompost in restricting mycelial growth, with 75.75 mm, 44.25 mm, and 32.13 mm at 10%, 20%, and 30% concentrations, respectively. Inhibition percentages ranged from 14.88% to 64.10%. Sclerotia production was also significantly reduced, with counts of 130.0, 95.75, and 79.75 at increasing concentrations, resulting in inhibition rates from 79.11% to 87.96%. Sawdust extract had a lesser impact on mycelial growth, with measurements of 77.63 mm, 45.50 mm, and 34.88 mm at 10%, 20%, and 30% concentrations, respectively. Inhibition percentages ranged from 12.77% to 61.03%. Its effect on sclerotia reduction was limited, with counts of 331.0, 228.50, and 183.0 at increasing concentrations, corresponding to inhibition rates between 46.83% and 72.35%. Cowdung extract was found least effective in inhibiting mycelial growth, with measurements of 81.38 mm, 75.75 mm, and 72.13 mm at 10%, 20%, and 30% concentrations, respectively. Inhibition percentages were minimal, ranging from 8.57% to 19.40%. Its impact on sclerotia production was also limited, with counts of 496.5, 473.25, and 356 at increasing concentrations, resulting in inhibition rates between 20.24% and 46.20%.

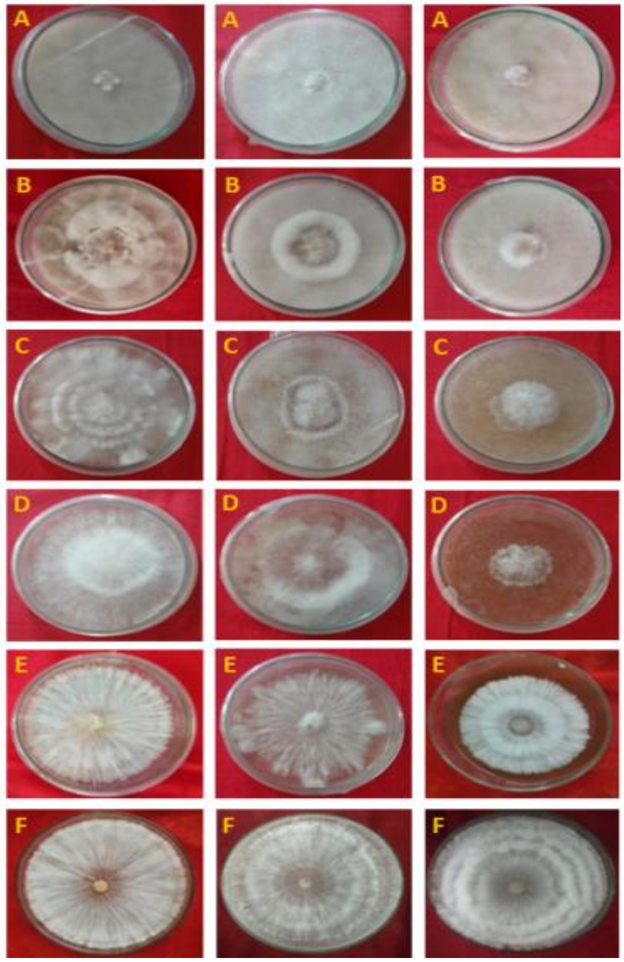

Fig. 3. Radial mycelial growth of *S. rolfsii* in Petri dishes containing PDA amended with an organic amendment at different concentrations, where (A) Poultry manure, (B) Vermicompost, (C), Mustard oil cake (D) Sawdust, (E), Cowdung, and (F) Control

The study concluded that poultry refuse extract demonstrated the highest efficacy in inhibiting both mycelial growth and sclerotia production of *S. rolfsii*, followed by vermicompost and mustard oilcake extract. According to different researchers, organic soil amendments such as Poultry manure, farm yard manure (FYM), Mustard oil cake, compost, at 4 days after inoculation. vermicompost saw dust etc. as a source for inhibition of the fungal growth of *S. rolfsii* (Saha *et al*., 2008). The antifungal activity of the organic amendments may be due to the inclusion of antibiotics and phenolic compounds of unknown nature (Jha *et al*., 2007). Vineela *et al*. (2020) reported that at 10% concentration, FYM and Groundnut cake were found to be more effective in inhibiting the mycelial growth of *S. rolfsii*. Further field studies are recommended to validate the *in vitro* control of *S. rolfsii*, the causal agent of collar rot in sunflowers, using fungicides, botanicals, and organic matter extracts.

Conclusion

Collar rot poses a serious threat to sunflower production, highlighting the need for effective and sustainable management strategies. While, chemical fungicides are effective, they have limitations due to their environmental impact, the risk of resistance development, and associated costs. Botanicals and organic matter extracts offer promising, eco-friendly alternatives that align with sustainable agriculture practices and may also be more affordable for collar rot management. Integrating these methods creates a comprehensive approach to collar rot control, enhancing soil health and boosting sunflower resilience. While challenges exist in standardizing and consistently applying these alternative methods, ongoing research can promote the adoption of integrated disease management strategies. By combining the strengths of fungicides, botanicals, and organic amendments, farmers can more effectively control collar rot, reduce reliance on chemical inputs, and support sustainable sunflower production. Future research should focus on refining these combinations and optimizing their application timing under varying field conditions to achieve consistent, long-term disease management.

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Author’s contribution

The conception, planning, and design of the experiments, as well as the statistical methodology and data analysis, were carried out collaboratively by NAS, NAI, and MSI. All authors equally contributed to the preparation of the manuscript. NAS and MMA conducted the in-vitro culture. All authors actively contributed to the article and have approved the final version for submission.

Conflicts of Interest

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

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IN VITRO SELECTION OF RICE SOMACLONAL VARIANTS FOR SALT TOLERANCE

R. Sharmin[1], M. G. Rasul[1], M. M. Rahman[2], M. A. Hossain[3*] and M. M. Hasan[1]

[1]Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur; [2]Department of Horticulture, BSMRAU, Salna, Gazipur; [3]Biotechnology Division, Bangladesh Rice Research Institute (BRRI), Gazipur. Bangladesh.

Abstract

An experiment was conducted at the Advanced Plant Breeding and Tissue Culture Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University to evaluate the effects of NaCl on rice callus induction and plant regeneration, along with genetic variability assessment through molecular markers. Dehusked seeds of five distinct rice genotypes (Dakshahi, Gondhakasturi, Guamasuri, Duksail, and Khazar) were put on MS medium supplemented with five levels (0, 0.2, 0.4, 0.6 and 0.8%) of NaCl. Developed calli were placed in MS medium for regeneration around 3-6 weeks later. Callus induction and plant regeneration declined with the increase of salinity levels. Dakshahi outperformed the other cultivars in both parameters at different salt concentrations, and it was the only cultivar with callus and regenerated plants at 0.08% NaCl concentrations. To amplify the genomic DNA of parents and somaclones of different varieties, five random amplified polymorphic DNA (RAPD) primers were used. Polymorphic bands were visible in OPD-07 primer. Dakshahi and Khazar were found to have the maximum genetic diversity (0.941). The genetic similarity coefficients between lines demonstrate that the degree of genetic diversity within rice genotypes was quite distinct. The cluster analysis grouped five genotypes into two clusters. The presence of genetic variation in the somaclones of Guamasuri implies the potential of RAPD markers in assessing genetic diversity and validating somaclonal variation. Also, Dakshahi demonstrating the highest tolerance for NaCl stress in both callus induction and plant regeneration, making it a promising candidate for salt-tolerant rice breeding in future.

Keywords: Callus, Cluster, Regeneration, RAPD, Rice, Salt, Somaclone.

Introduction

Rice (*Oryza sativa*) is a staple food consumed by more than half of the world's population. Many people rely on rice as a key source of carbohydrate and protein, particularly in Asia. The land type, ideal atmosphere, and people's preferences for rice as their primary dish create a good market, leading to extensive rice monoculture in many Asian countries including Bangladesh (Shelley *et al*., 2016; Rahman *et al*., 2020). Currently, Bangladesh ranks as the third-largest global rice producer after China and India, covering an area of 11.69 million hectares in 2022, with an average yield of 4.89

* Corresponding author: arafatbsmrau.07@gmail.com

tons/ha (FAOSTAT, 2022). However, in order to fulfill the growing demands due to the rapid population growth, it is estimated that production of rice would need to increase from 6.31 to 7.17 tons/ha by 2030 and 8.06 tons/ha by 2040 (Rabbi *et al.*, 2021). Since Bangladesh is one of the world's most populous countries, there is very little chance that the amount of arable land will be increased, hence high-yielding varieties of rice must be developed (Rabbi *et al.*, 2021).

Rice is grown in almost every part of Bangladesh (Mamun *et al.,* 2021) However, farmers in the southern zone, particularly those in coastal areas, are cautious to grow rice for one key reason is salinity (Shelley *et al*., 2016). Salinity is to blame for the unproductiveness of vast amounts of land in the southern part of Bangladesh (Baten *et al*., 2015; Kabir *et al*., 2021). Salinity tolerance of rice is defined as moderate (Shaheen *et al*., 2005). Rice production in saline soils could not be increased without the development of saline tolerant varieties. It is one of the best options for bringing these lands under cultivation through growing the saline tolerant rice varieties in this area (Kabir *et al*., 2021). So far Bangladesh Rice Research Institute (BRRI) and Bangladesh Institute of Nuclear Agriculture (BINA) have developed six and three saline tolerant rice varieties (BRRI, 2023; BINA 2023). Nevertheless, research to develop salt-tolerant rice varieties must be continued, respectively.

Plant breeders frequently employ local varieties and landraces as parents to generate new varieties since they are prime sources of necessary alleles and traits (Breseghello *et al*., 2013; Hasan *et al*., 2015). Aside from conventional breeding, tissue culture methods are also commonly used in breeding program, particularly in stress tolerance breeding program. Tissue culture is a source of genetic variability that arises from genetic modifications occurred during the *in vitro* culture process, a phenomenon known as somaclonal variation. The technique has been utilized by many researchers to create somaclonal variants and screen salt tolerance (Anwar *et al*., 2010; Pérez-Clemente *et al*., 2012). This screening can be done by selecting variants derived from *in vitro* somaclonal materials such as protoplasts, cell colonies, or callus. Moreover, generating somaclonal variants and combining them with conventional breeding produce fast and effective results (Krishna *et al.,* 2016), making it a powerful strategy for developing salt-tolerant varieties (Pérez-Clemente *et al.,* 2012. Production of new salinity resistant varieties using diverse biotechnological approaches would also require the establishment of regeneration protocols. The goal of this work was to create an effective *in vitro* culture system under various salt concentrations that would serve as a platform for future tissue culture research on improving salt tolerance in rice varieties.

Molecular markers provide genetic information that may be used to determine a species' distinctiveness and rank based on the number of adjacent relatives and phylogenetic relationship (Rahman *et al*., 2007). For determining the level of genetic diversity in rice, different types of molecular markers have been utilized, viz., RFLP (Botstein *et al*., 1980), RAPD (Williams *et al*., 1990), AFLP (Vos *et al*., 1995), SSR (Tautz 1989) and SNP. The RAPD analysis provided a simplistic and rapid way for determining genetic diversity among the genotypes. It nevertheless indicated a significant level of polymorphism even without any prior knowledge of the DNA sequence (Karp *et al*., 1997). Considering the above circumstances, the study was conducted to investigate the consequences of NaCl at various concentrations on callus growth and plant regeneration. Also, the response of five rice genotypes to *in vitro* culture medium, along with their molecular diversity and validate their somaclones by RAPD markers.

Materials and Methods

Experimental location and materials

The experiment was carried out at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur, Bangladesh, at the Advanced Plant Breeding Laboratory of the Department of Genetics and Plant Breeding. In this study, the following rice genotypes were used as experimental materials.

Table 1. List of rice genotypes

SI. No.	Name of genotypes	Place of Collection
1.	Dakshahi	Rice Gene Bank of BRRI
2.	Gondhakasturi	
3.	Gua masuri	
4.	Duksail	
5.	Khazar	

Media preparation and salt concentrations

Healthy, disease-free explants for the study were chosen and then the seeds were dehusked. For callus induction, MS (Murashige and Skoog, 1962) media used supplemented with sucrose (30 g/L), 2, 4-D (2 mg/L), agar (10g/L), and five different salt (NaCl) doses. Callus pieces of suitable size were moved to MS regeneration medium treated with NAA (l mg/L), BAP (1 mg/L), sucrose (30 g/L), agar (l0 g/L), and five different salt concentrations for plant regeneration. The effect of salt was studied using five different amounts of NaCl salt solution (0, 0.2, 0.4, 0.6, and 0.8 %). (0, 34.2mM, 68.4 mM, 102.7 mM and 136.9 mM) or (0. 2000, 4000, 6000 and 8000 ppm)

Callus induction and regeneration

In a laminar air flow cabinet, the dehusked seeds were air dried and sterilized by wiping with 70% ethanol for 10 minutes before being placed on callus induction media. Those seeds were put in the culture to accomplish callus induction while keeping the temperature at 25±2°C in the dark. The seeds of the responsive cultivars began to generate callus after 3-6 weeks of inoculation. The frequency of callus induction was determined using the formula provided. For this calculation, multiple callus generated from a single seed were treated as one. Calli with a minimum diameter of 2 mm were placed in regeneration medium and incubated in a temperature-controlled growing environment to accomplish plant regeneration at 25±2°C under a 16-hour light photoperiod with a light strength of around 2000-3000 lux. Observations were made on a daily basis to track the response. The number of callus-producing plantlets was used to calculate the number of regenerated plants.

Statistical analysis

The data for the callus induction and plant regeneration was statistically examined whenever reasonable. The experiment was carried out with three replications following Factorial Completely Randomized Design (CRD) in a growth room. Duncan's Multiple Range Test (DMRT) was used to compare the means of the Analyses of Variance for dissimilar characteristics.

$$Percent\ callus\ induction = \frac{Number\ of\ seeds\ induced\ calli}{Number\ of\ seeds\ incubated}\ X\ 100$$

$$Percent\ plant\ regeneration = \frac{Number\ of\ calli\ with\ plantlets}{Number\ of\ incubated\ calli}\ X\ 100$$

Molecular characterization

RAPD analysis was done on five parents and three somaclonal genotypes in this study. The modified CTAB (cetyltrimethylammonium bromide) technique of Doyle and Doyle (Doyle and Doyle, 1987) was utilized to extract genomic DNA from leaf samples obtained from two-week-old plants. Five RAPD markers with notable amplifications were used to analyze genetic diversity. Five ten-mer RAPD markers (Chinna Gen, RAPD markers. 2016) were used in the PCR. For the process, a 25 µL mixture containing 25 ng template DNA, 2.5 µL of 2.5 mM dNTPs, 2.5 µL of 10x buffer, 2 µL of 25 mM MgCl2, 2 µL of each of the primers, and 0.25 µL of *Taq* polymerase (add the company of the Taq polemerase) were prepared. The PCR fragment size was estimated using a DNA molecular weight marker (100-bp ladder) (Roche, DNA ladders. 2016). The PCR reaction was carried out at 94°C for 5 minutes, followed by 42 cycles of 94°C for 1 minute, 36°C for 1 minute, and 72°C for 2 minutes, then 72°C for 10 minutes. The final products were electrophoretically analyzed in TAE buffer using a 1% agarose gel dyed with ethidium bromide (5 g/mL). Gels were photographed using a UV transilluminator after being stained with a 0.5 g/mL ethidium bromide solution. The presence or absence of polymorphic bands, as well as the strength of these polymorphic bands, were analyzed in the genomes by Alpha Ease FC 4.0 software. Using NTSYS-pc software an index of genetic variation was determined, and dendrograms were built through the UPGMA method. In the beginning, five decamer random primers (Table 02) were checked for the existence of bands. One primer (OPD 07) was chosen since it showed polymorphic and repeatable banding profiles.

Table 2. List of Primers

SL NO	Primer code	Sequence
01	OPD 02	GGACCCAACC
02	OPD 05	GGACCAACCG
03	OPD 07	TTGGCACGGG
04	OPD 08	GTGTGCCCCA
05	OPD 12	TATTGCCGTT

Results and Discussions

Effects of salt (NaCl) for callus induction and regeneration

The MS medium without salt had the highest frequency of callus induction (90%) and plant regeneration (72%). The lowest result was observed (3 % for callus induction and 1% plant regeneration) in a 0.8% salt concentration. Both callus induction and plant regeneration showed a diminishing trend as salt concentrations increased (Fig. 1.). Similar findings were also stated by Adil *et al.*, (2009); Zinnah *et al.*, 2013; Hasan *et at.*, (2013); Sankepally *et al.*, 2016; Arefin *et al.*, 2018. The effect of NaCl on callus induction and plant regeneration in rice is multifaceted and depends on various factors, including concentration, duration of exposure, and the specific characteristics of the rice cultivar. High concentrations of NaCl can inhibit callus induction in rice. Salinity stress may negatively affect cell division and differentiation, crucial processes in callus formation. The effect of NaCl on plant regeneration is often more complex. While high salt concentrations can hinder regeneration, low to moderate levels may induce stress tolerance mechanisms that lead to improved regeneration efficiency. Moreover, NaCl can induce both osmotic stress and ionic stress. Osmotic stress results from reduced water availability due to increased salt concentration, while ionic stress occurs due to the accumulation of sodium ions. Both types of stress can impact cell physiology and tissue culture responses.

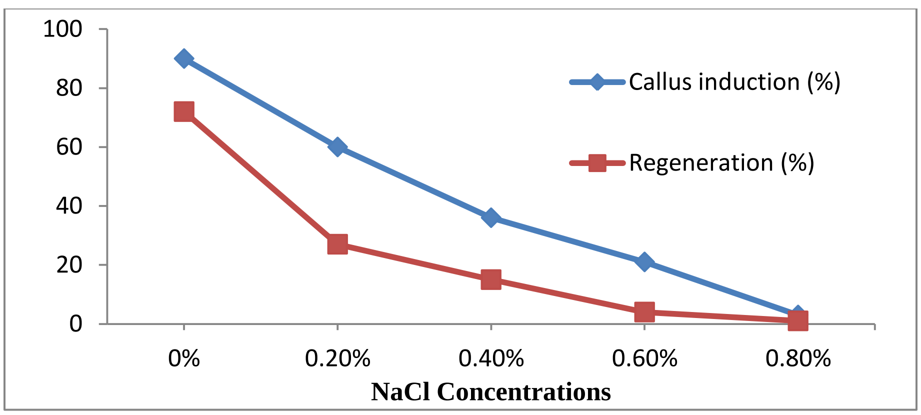


Fig. 1. Mean effects of NaCl salt concentration on callus induction (%) and plant regeneration (%)

Effects of salt on rice cultivars (In vitro)

The selection of an appropriate genotype is a critical factor in the success of in vitro culture techniques in rice. Despite the salt concentrations, Dakshahi had the highest callus induction (53%) and regeneration (31%), while Khazar was found to have the lowest (callus induction 36% and regeneration 17%) (Fig. 2). Guamusuri (42%), showed a moderate result for callus induction, which was statistically similar to Duksail (41%), and Gondhakasturi (38%). Niroula *et al*. (2005), Puhan *et al*. (2013) & Muhammad *et al.* (2013) reported that the callus induction rate varies to ricegenotypes. Similar observations were found in the case of plant regeneration for Guamusuri (26%), Gondhakasturi (24%) and Duksail (21%). Adil *et al.* (2009); Hasan *et al.* (2013); Taratima *et al.* (2022) discovered genotypic alterations and influences in *in vitro* plant regeneration, which matched the present findings.

The response of different genotypes to callus induction and plant regeneration in rice can be varied. Genetic factors play a crucial role in determining the success of tissue culture processes. Some genotypes are more amenable to tissue culture techniques because of their inherent regeneration capacity and natural high propensity for callus induction and plant regeneration, while others may show limited or poor responses. Moreover, some other crucial factors, like response to stress, growth regulator sensitivity, embryonic potential (ability to form somatic embryos), and the molecular and genetic makeup of a cultivar can also influence callus induction and plant regeneration.

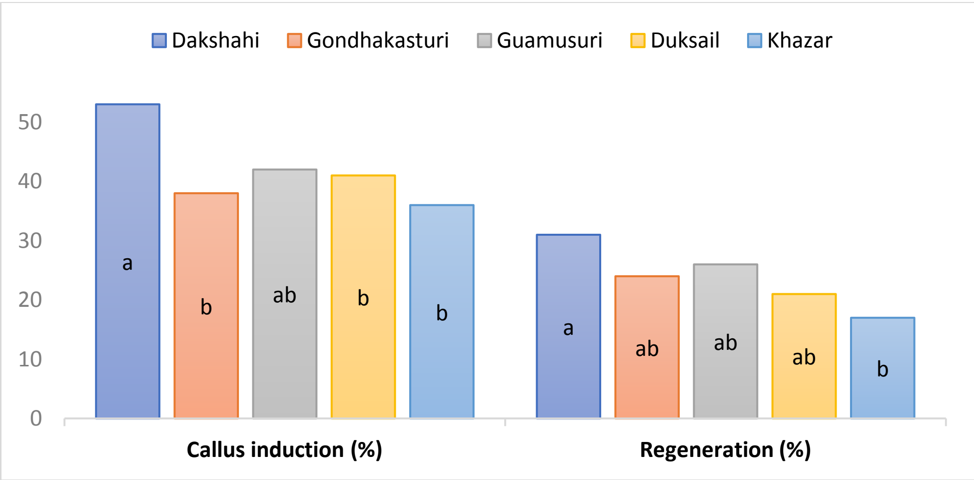


Fig. 2. Mean Effects of different genotypes on callus induction and plant regeneration

Interaction effect of NaCl and genotypes

Guamusuri had the maximum callus induction (100%) in the no salt medium, followed by Dakshahi (95%). The other three cultivars, i.e. Gondhakasturi (85%), Duksail (85%), and Khazar (85%), showed a moderate but statistically similar result in no salt medium. The lowest result was observed in Gondhakasturi (0%), Guamusuri (0%), and Khazar (0%) in 0.8% salt concentrated medium (Table 3). In our study, the callus induction of Dakshahi was better compared to the rest of the cultivars in four salt concentrations, and even in 0.8% NaCL. Duksail performed after Dakshahi and also sowed some results in the top salt concentration.

Table 3. The effect of five genotypes and salt concentrations on callus induction

Genotype	Salt concentrations (%)				
	0	0.2	0.4	0.6	0.8
Dakshahi	95 ab	70 bcd	55 def	35 efg	10 ghi
Gondhakasturi	85 abc	60 cde	30 fgh	15 ghi	0 i
Guamusuri	100a	55 def	35 efg	20 ghi	0 i
Duksail	85 abc	55 def	35 efg	25 ghi	5 hi
Khazar	85 abc	60 def	25 ghi	10 ghi	0 i

In the case of plant regeneration, the highest result was observed in Guamusuri (85%) at 0% salt medium, which was statistically similar to Gondhakasturi (95%) (Table 4). Though Dakshahi (65%) was lower at no salt medium, it was the best performer in all four salt-concentrations. Dukshahi was the only cultivar that regenerated at 0.8% salt concentration. The lowest result was found in Gondhakasturi (0%) and Khazar (0%) in a 0.6% salt concentrated medium. Salinity stress, induced by NaCl, is known to have both positive and negative effects on plant tissue culture processes. Summart *et al.*, (2010) noted that the complex inhibitory action of salt stress on rice is mostly responsible for the decrease in callus induction and plant regeneration. Considering the parameters, it might be said that there is a good chance of having salt stress-responsive genes in Dakshahi, which might trigger cell proliferation in an attempt to survive the stress conditions. This cultivar could also be used in breeding programs to develop salt tolerant rice varieties.

Table. 4. The effect of different genotypes and salt concentrations on plant regeneration

Genotype	NaCl Salt concentrations (%)				
	0	0.2	0.4	0.6	0.8
Dakshahi	65 a-c	45 cd	30 de	10 ef	5 ef
Gondhakasturi	80 ab	25 def	15 ef	0 f	0 f
Guamusuri	85 a	25 def	15 ef	5 ef	0 f
Duksail	70 ab	20 ef	10 ef	5 ef	0 f
Khazar	60 bc	20 ef	5 ef	0 f	0 f

Molecular characterization through RAPD markers

In this study, five decamer operon primers (Table 2) were utilized. One primer (Table 5) generated amplicons and was chosen for genetic diversity analysis after initial screening and taking into account the sharpness, intensity, and repeatability of bands. All the bands produced by the primer were polymorphic. A wide range of polymorphisms in rice genotypes was also found by Zakiyah *et al.* (2019) and Mazumder *et al.* (2020). The somaclone of Guamasuri (0.2%) showed a distinct polymorphic band compared to its parent (Fig. 3). The difference in the callus induction of Guamashuri at 0% and 0.2% level of salt concentrations (Fig. 5), plant regeneration (Fig. 6) and established plant (Fig. 7) reinforce the findings of somaclonal variation. The possible causes of somaclonal variation include chromosome aberrations, DNA amplification, and the occurrence of transposable elements.

Table 5. Selected primer and genetic variations among rice genotypes by RAPD analysis

Primer code	Sequence	Total no. of bands	Polymorphic bands No.	Monomorphic bands No
OPD 07	TTGGCACGGG	3	3	0

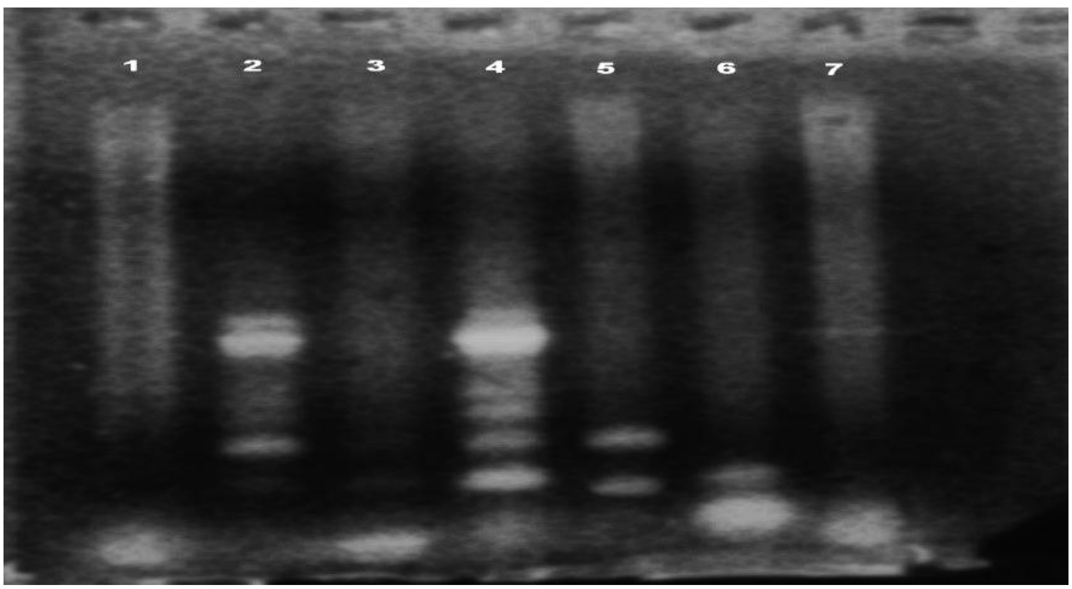


Fig. 3. RAPD profile of five rice varieties and two somaclones generated by OPD 07. Here,1: Guamasuri, 2: Dakshahi, 3: Duksail, 4: Gondhakasturi, 5: Khazar, 6: Somaclone of Guamasuri(0.2%), 7: Somaclone of Guamasuri (0.2%)

Similarity co-efficient analysis

The degree of relatedness between rice genotypes was determined using a similarity matrix based on the fraction of shared RAPD fragments. The similarity estimates for pairs varied from 0 to 0.941 (Table 6). Dakshahi and Khazar were found to have the most genetic diversity (0.941) in RAPD analysis. The genetic similarity coefficients between lines illustrate a lot of genetic variability in rice germplasm. Islam *et al.* (2017) assessed the genetic diversity of ten aromatic rices through RAPD markers and found 80% polymorphism. Again, Mazumder *et al.* (2020) looked over 16 rice genotypes and discovered pair-wise estimations of genetic similarity among the 16 genotypes varied from 0.66 to 0.88. The range of 0.308 to 0.718 was found by Islam *et al.* (2013) during the evaluation of six rice genotypes.

Table 6. Analysis of Similarity matrix via Nei's original procedures of genetic Identity

No.	Variety	Guamasuri	Dakshahi	Duksail	Khazar	Gondhakasturi
01	Guamasuri	1	0.000	0.500	0.200	0.000
02	Dakshahi		1	0.364	0.941	0.500
03	Duksail			1	0.500	0.571
04	Khazar				1	0.462
05	Gondhakasturi					1

Cluster analysis

A cluster diagram was created using genetic similarities acquired from RAPD data. Using UPGMA, a cluster analysis based on Nei's similarity coefficients clustered 5 genotypes into 2 groups (Fig. 4). Cluster I contain only Guamashuri, but Cluster II has two genotypes in each of its two subclusters. Subcluster, I included Gondhakasturi and Duksail, while Dakshahi and Khazar represented Subcluster II (Table 7). Using the UPGMA approach, Islam *et al.* (2013) and Islam *et al.* (2017) analyzed six and ten rice genotypes using RAPD markers and found that the genotypes constituted three and two clusters, respectively. When assessing 16 rice genotypes, Mazumder *et al*. (2020) found four clusters.

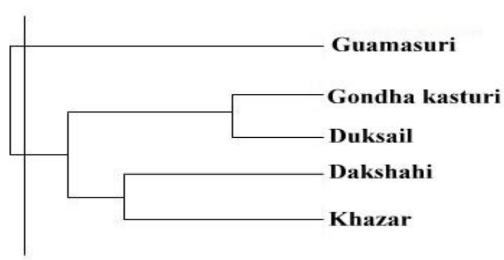


Fig. 4. RAPD analysis dendrogram (UPGMA) pattern in different rice genotypes.

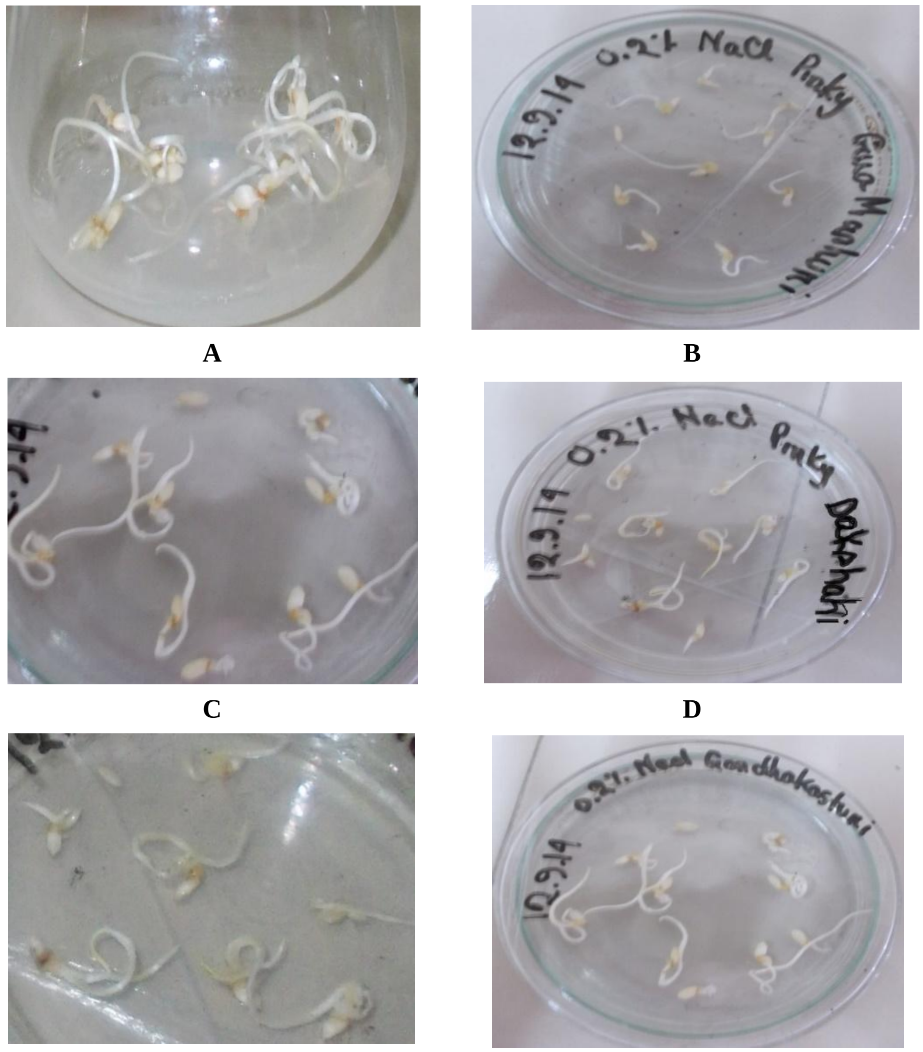
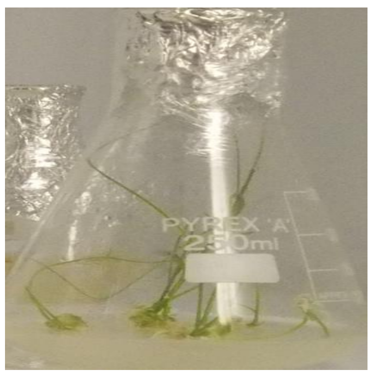


Fig. 5. Callus induction of rice genotypes with and without Salt concentrations

A. Callus of Guamasuri without salt; **B.** Callus of Guamasuri with 0.2 % salt (NaCl) concentration; **C.** Callus of Dakshahi without salt; **D.** Callus of Dakshahi with 0.2% salt (NaCl) concentration; **E.** Callus of Gondha Kasturi without salt; **F.** Callus of Gondha Kasturi with 0.2% salt (NaCl) concentration

A **B**



C **D**

Fig. 6. Plant regenaration of rice genotypes with and without salt concentration

A. Guamasuri without salt; B. Guamasuri with 0.2% salt concentration; C. Dakshahi without salt; D. Dakshahi with 0.2% salt concentration

Fig. 7. Establishment of Guamashuri regenerated plants (0.2% and 0%), concentration of salt

Table 7. Distribution of five aromatic rice genotypes in different clusters

Cluster		Genotypes included in different clusters	No. of genotypes in cluster
I		Guamasuri	1
II	Subcluster I	Gondhakasturi, Duksail	2
	Subcluster II	Dakshahi, Khazar	2

Conclusions

The study underscores the impact of NaCl stress on rice callus induction and plant regeneration, revealing genotypic variability in salt tolerance among different rice genotypes. Dakshahi demonstrated superior performance under salt stress, indicating potential for breeding salt-tolerant rice varieties. Molecular characterization via RAPD markers provided insights into genetic diversity and validated somaclonal variation, with Dakshahi and Khazar showing significant genetic distance. This research highlights the efficacy of integrating tissue culture with molecular tools for developing resilient rice cultivars suitable for saline environments, which can be instrumental in sustaining rice production in salt-affected regions.

Author's contribution

M. M. Hasan and R. Sharmin conceptualized the study and designed the experiments. R. Sharmin and M. M. Hasan collected and analyzed the data and also interpreted the results. R. Sharmin and M. A. Hossain drafted the text. M. M. Hasan, M. G. Rasul, M. M. Rahman, and M. A. Hossain reviewed the results and approved the final version of the manuscript.

Conflicts of Interest

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

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HARNESSING GENETIC DIVERSITY AND VIGOR OF ONION FROM MULTI-GENERATIO STUDY

M. A. Alam[1*], S. Naher[2], M. M. Hasan[3], A. H. F. Fahim[4], M. A. A. Khan[5] M. T. Rahman[6] and S. N. Mozumder[7]

[1]Plant Breeding Division, Spices Research Centre, Bangladesh Agricultural Research Institute (BARI), Bogura; [2]Soil Science Division, Spices Research Centre, BARI, Bogura; [3]Horticulture Division, Spices Research Centre, BARI, Bogura; [4]Agronomy Division, Spices Research Centre, BARI, Bogura; [5]Plant Breeding Division, Regional Spices Research Centre, BARI, Gazipur; [6]Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka; [7]Horticulture Division, Regional Spices Research Centre, BARI, Gazipur. Bangladesh.

Abstract

This study investigates the genetic variation, vigor, and performance of onion generations resulting from crosses. The experiment was conducted at the Spices Research Centre, Shibganj, Bogura, during 2021-22, following diallel mating design with three parental varieties, BARI piaz1, BARI piaz4, and BARI piaz6. The research focuses on evaluating yield and yield-contributing traits, including bulb dimensions, bulbing index, and individual bulb weight. The results demonstrate significant genetic diversity among onion generations, offering opportunities for the breeders to select and develop superior lines. Hybrid vigor and heterosis, particularly evident in F_1 generations, suggest that combining genetically diverse parental lines can lead to progeny with superior performance compared to their parents. However, the choice of parental lines significantly influences the stability and performance of hybrid progeny, with certain varieties demonstrating consistent performance across generations (Parent, F_1, F_2). Trait segregation and variability within F_2 hybrid populations underscore the importance of evaluating large populations to identify individuals with desired trait combinations. Overall, the findings highlight the importance of leveraging genetic diversity and hybrid vigor in onion breeding programs to develop cultivars. By employing breeding strategies the high-performing onion varieties capable of thriving in diverse agro-climatic conditions in onion farming.

Keywords: Bulb dimension, Genetic variation, Heterosis, Parental selection.

Introduction

Onion (*Allium cepa* L.) is one of the most economically important vegetable and spice crops worldwide, valued for its culinary versatility and nutritional benefits (Alam *et al.*, 2023; Alemu et al. 2022; Ochar and Seong 2023; Teshika *et al.*, 2018). It has a wide range of variability regarding bulb shape, size bulb skin color, etc. (Ashagrie *et al.*, 2021; Islam *et al.*, 2019). It is also an important spice crop in Bangladesh and is widely

* Corresponding author: a.alam_83@yahoo.com

cultivated over the country (Alam *et al.,* 2023). It also plays an important role in the country's spices production, contributing significantly to its economy and food security (Alam *et al.,* 2023). However, challenges such as limited genetic diversity, coupled with fluctuating environmental conditions, often hinder efforts to optimize bulb size and yield (Alam *et al.,* 2023; Chowdhury *et al.,* 2019). These challenges could be addressed by developing a diverse source population of onion varieties tailored to the specific agro-climatic conditions of Bangladesh (Biswas 2021; Lyngkhoi *et al.,* 2021; Rivera *et al.,* 2016). In recent years, onion breeding programs have aimed to develop cultivars with improved yield, quality, and resilience to biotic and abiotic stresses to meet the evolving needs of growers and consumers (Alam *et al.,* 2023; Chowdhury *et al.,* 2019). Through the comprehensive selection process, the collection of diverse onion germplasm from various genetic backgrounds and then evaluation for adaptability and performance could facilitate superior parents' identification (Manjunathagowda, 2022). Subsequent breeding efforts focused on hybridization and selection techniques to enhance desirable traits, particularly bulb dimension and yield will provide promising results including sufficient genetic diversity and improved quality (Anand *et al.,* 2023; Goswami 2023; Mwangangi *et al.,* 2019; Salgotra and Bhagirath, 2023).

Through careful investigation and analysis, this research clarifies the implications of genetic diversity and hybrid vigor in onion breeding programs. By evaluating the performance of different generations, from parental lines to F_1 and F_2 hybrids, the study sheds light on the potential for developing superior onion cultivars with enhanced traits for commercial cultivation. Key aspects such as parental selection, trait segregation, and variability in yield performance are examined to provide insights into breeding strategies aimed at improving onion productivity and sustainability. The findings of this study contribute valuable knowledge to the field of onion breeding, offering guidance for breeders in selecting optimal parental lines and designing effective breeding strategies to develop resilient and high-performing onion varieties. Understanding the genetic basis of onion traits and the dynamics of hybridization is crucial for advancing breeding programs and addressing the challenges faced by onion growers in diverse agro-climatic conditions (Khosa *et al.,* 2016; Khosa & Ajmer, 2020; Lyngkhoi *et al.,* 2021; Moran *et al.,* 2021; Singh & Ani, 2020). Ultimately, the goal of the study is to develop onion cultivars that meet the demands of agriculture. So, the present study was undertaken to develop a diverse source population of onions to improve the bulb dimension and yield.

Materials and Methods

Experimental location and plant materials

The study was conducted at the Spices Research Centre in Shibganj, Bogura, during the winter of 2021-22. Three parental varieties, namely BARI piaz1, BARI piaz4, and BARI piaz6, were utilized in the study. These varieties were chosen based on their diverse genetic backgrounds and potential for onion breeding. Major features of the parental genotypes are presented in Table 1.

Table 1. Important characteristics of the parents

Traits	BARI piaz1	BARI piaz4	BARI piaz6
Shape	Round	Oval to spindle	Oval
Skin color	Reddish	Dark reddish	Reddish gray
Single Bulb weight	30-40 g	60-75 g	50-60 g
Bulb Yield	12-16 t/ha	17-22 t/ha	16-20 t/ha
Life cycle	92-105 days	120-135 days	114-125 days

Crossing design and successive steps

A diallel mating design was employed for crossing the parental varieties during the 2018-19 season. The seeds of F_1 hybrids were subsequently grown to produce F_1 bulbs during the 2019-20 season, and superior quality bulbs were selected based on size and skin color. The selected F_1 bulbs were planted during the 2020-21 season to produce F_2 seeds, which were used in the present study.

Seeds sowing and fertilizers application

Seeds of different generations were sown on October 14, 2021, and transplanted on November 24, 2021. Transplantation was carried out using four-week-old seedlings. The unit plot size was 3.0 m × 1.2 m, with a plant spacing of 10 cm × 15 cm. The crop received fertilization with a recommended dose of cow dung (5 t/ha) and $N_{115}P_{54}K_{75}S_{20}Zn_3B_2$ Kg/ha. The application of cow dung, phosphorus (P), sulfur (S), zinc (Zn), and boron (B), along with one-third of nitrogen (N) and potassium (K), was done at the time of final land preparation. The remaining nitrogen (N) potassium (K) and urea were applied at 25, 50, and 75 days after planting. Irrigation was applied at 30, 50, and 70 days after planting, and fungicide was sprayed at seven days intervals to control diseases.

Data collection and analysis

Bulb yield and various morphological traits were recorded for different generations of onion populations. The mean, standard deviation, and range of these traits were calculated separately for parental, F_1, and F_2 generations. Mean, standard deviation, and range were estimated to determine significant differences among the parental and segregating generations for the studied traits.

Results and Discussion

The mean, standard deviation, and range for different yield-attributing traits of parents, F_1's, and F_2's are presented separately in Table 2. On the other hand, performance in regards to fresh bulb yield is presented in Table 3. It was found that the mean performance of F_1'swere superior to the P_1 and P_2. Whereas in F_2, in most of the cases, the mean was lower than the F_1's, while the standard deviation and the range were wider in F_2 compared to F_1's.

Parental generation

The mean of bulb length (BL), bulb diameter (BD), neck diameter (ND), bulbing index (BI), and individual bulb weight (IBW) were 45.06, 51.35, 12.95, 4.02, and 45.6

respectively for parent BARI piaz1 (Table 2). In the case of BARI piaz4 the average BL, BD, ND, BI, and IBW was 64.39, 64.01, 15.09, 4.32, and 96.98, respectively. Whereas for BARI piaz6 the mean of BL, BD, ND, BI, and IBW was 54.45, 56.58, 13.54, 4.26, and 63.98. Variability exists among the parental varieties concerning bulb dimensions, bulbing index (BI), and individual bulb weight (IBW). BARI piaz4 consistently demonstrates larger bulb dimensions and higher IBW compared to the other parental lines, suggesting its potential for potentially more marketable bulbs (Addai 2014). While BARI Piaz-1 and BARI Piaz-6 showed intermediate values for these traits, they still possess respectable sizes, indicating their potential utility in commercial onion production (Addai 2014; Major *et al*. 2023).

Variation in yield attributing traits

Crossing between BARI piaz1 and BARI piaz4 (with reciprocal) through emasculation

In this particular cross combination (BARI piaz1 × BARI piaz4), the performances for different studied traits in F_1 were superior to the parents. Results showed that the average BL, BD, ND, BI, and IBW were 57.7, 55.68, 17.72, 2.26, and 62.77, respectively (Table 2). In F_2, the mean of BL, BD, ND, BI, and IBW was 51.5, 58.98, 14.74, 4.09, and 56.71, respectively (Table 2).

The performance of F_1 of the cross combination (BARI piaz4 × BARI piaz1) was presented in Table 2. Results showed that the average BL, BD, ND, BI, and IBW were 62.77, 50.11, 24.3, 2.09, and 89.09, respectively. In F_2, the mean of BL, BD, ND, BI, and IBW was 61.97, 65.41, 14.11, 4.72, and 79.12, respectively.

Crossing between BARI piaz1 and BARI piaz6 (with reciprocal) through emasculation

The mean performance along with the range of F_1 of the cross combination (BARI piaz 1 × BARI piaz 6) was presented in Table 2. Results showed that the average BL, BD, ND, BI, and IBW were 70.36, 49.6, 23.28, 2.39, and 71.37, respectively. In F_2, the mean of BL, BD, ND, BI, and IBW was 53.02, 58.28, 14.95, 3.99, and 62.26, respectively.

The mean and range of F_1 of the cross combination (BARI piaz6 × BARI piaz1) were presented in Table 2. Results showed that the average BL, BD, ND, BI, and IBW were 62.26, 45.56, 21.26, 2.16, and 55.68, respectively. In F_2, the mean of BL, BD, ND, BI, and IBW were 51.38, 58.06, 14.04, 4.2, and 56.91, respectively.

Crossing between BARI piaz4 and BARI piaz6 (with reciprocal) through emasculation

The mean performance along with the range of F_1 of the cross combination (BARI Piaz-4 × BARI Piaz-6) was presented in Table 2. Results showed that the average BL, BD, ND, BI, and IBW were 52.64, 61.75, 20.75, 2.07, and 100.73, respectively. In F_2, the mean of BL, BD, ND, BI, and IBW was 58.54, 60.95, 14.12, 4.36, and 74.79, respectively.

Table 2. Performance of F_1 and F_2 generations along with parents for different traits

Parents	Item	BL (cm)	BD (cm)	ND (cm)	BI	IBW (g)
Parents						
BARI piaz1	μ ± σ	45.06±4.01	51.35±3.07	12.95±1.03	3.97±0.29	45.6±7.68
	Range	41.02-53.06	47.04-56.97	11.39-14.73	3.58-4.35	34.6-60.56
BARI piaz4	μ ± σ	60.39±3.23	60.01±3.17	15.09±1.65	3.98±0.43	96.98±10.51
	Range	58.5-67.47	58.39-67.28	12.71-16.7	3.8-4.88	82.67-112.98
BARI piaz6	μ ± σ	52.45±5.92	54.58±3.89	13.54±1.76	4.03±0.29	63.98±13.22
	Range	46.63-64.22	52.24-62.64	11.69-15.98	3.93-4.55	45.94-85.71
F_1 generations						
BARI piaz1×BARI piaz4	μ ± σ	57.7±8.1	55.68±12.15	17.72±0.51	2.26±0.26	62.77±24.3
	Range	49.6-65.8	43.53-67.83	17.21-18.22	2-2.53	38.47-87.06
BARI piaz 1×BARI piaz 6	μ ± σ	70.36±4.56	49.6±2.02	23.28±4.05	2.39±0.76	71.37±3.54
	Range	65.8-74.91	47.58-51.63	19.23-27.33	1.63-3.16	67.83-74.91
BARI piaz4×BARI piaz1	μ ± σ	62.77±12.15	50.11±11.64	24.3±1.01	2.09±0.57	89.09±9.11
	Range	50.62-74.91	38.47-61.75	23.28-25.31	1.52-2.65	79.98-98.2
BARI piaz4×BARI piaz6	μ ± σ	52.64±12.15	61.75±2.02	20.75±3.54	2.07±0.18	100.73±5.57
	Range	40.49-64.79	59.73-63.78	17.21-24.3	1.88-2.25	95.16-106.3
BARI piaz6×BARI piaz1	μ ± σ	62.26±7.59	45.56±5.06	21.26±1.01	2.16±0.34	55.68±24.3
	Range	54.67-69.85	40.49-50.62	20.25-22.27	1.82-2.5	31.38-79.98
BARI piaz6×BARI piaz4	μ ± σ	52.64±1.01	60.23±1.52	23.28±1.01	2.26±0.05	88.07±1.01
	Range	51.63-53.65	58.72-61.75	22.27-24.3	2.21-2.32	87.06-89.09
F_2 generations						
BARI piaz1×BARI piaz4	μ ± σ	51.5±3.55	58.98±5.73	14.74±2.03	4.09±0.51	56.71±6.42
	Range	48.07-55.61	50.77-64.1	12.84-17.11	3.61-4.81	48.14-63.7
BARI piaz1×BARI piaz6	μ ± σ	53.02±4	58.28±4.14	14.95±2	3.99±0.81	62.26±5.75
	Range	50.19-55.85	55.36-61.21	13.54-16.36	3.42-4.56	58.2-66.33
BARI piaz4×BARI piaz1	μ ± σ	61.97±3.71	65.41±3.06	14.11±1.51	4.72±0.44	79.12±12.4
	Range	57.75-66.64	63.47-69.93	11.92-15.16	4.35-5.34	71.27-97.33
BARI piaz4×BARI piaz6	μ ± σ	58.54±8.4	60.95±5.76	14.12±1.82	4.36±0.22	74.79±10.68
	Range	44.47-65.52	53.34-65.37	11.84-16.38	3.99-4.54	57.55-85.34
BARI piaz6×BARI piaz1	μ ± σ	51.38±3.48	58.06±2.51	14.04±0.84	4.2±0.39	56.91±9.62
	Range	46.46-56.38	55.04-61.4	12.88-15.26	3.74-4.8	51.03-74.92
BARI piaz6×BARI piaz4	μ ± σ	60.22±3.65	60.41±3.73	15.11±1.26	4.08±0.48	84.78±9.84
	Range	55.74-65.56	54.92-64.2	13.5-16.83	3.34-4.55	67.94-95.68

The mean and range of F_1 of the cross combination (BARI piaz 6 × BARI piaz 4) were onion presented in Table 2. Results showed that the average BL, BD, ND, BI, and IBW were 52.64, 60.23, 23.28, 2.26, and 88.07, respectively. In F_2, the mean of BL, BD, ND, BI, and IBW was 60.22, 60.41, 15.11, 4.08, and 84.78, respectively. The F_1 hybrids (Table 2) generally exhibit intermediate values for bulb dimensions and yield compared to their parental lines, indicating the presence of hybrid vigor or heterosis (Farinati *et al.*, 2023; Wu *et al.*, 2008). Notably, instances of heterosis are particularly notable when

BARI Piaz-4 serves as the female parent, resulting in an elevated yield in the F_1 generation compared to both parental lines. This emphasizes the potential for exploiting hybrid vigor to develop onion cultivars with superior yield potential (Beyene and Mohammed, 2016; Yu *et al.*, 2021). The range of values within each F_1 hybrid combination signifies variability in trait expression, underscoring the necessity of careful selection to identify superior individuals for further breeding (Ahmad *et al.*, 2020; Snowdon *et al.*, 2015). This variability presents an opportunity to select desirable traits and advance promising lines in subsequent generations (Ahmad *et al.*, 2020). Overall, the results suggest the potential for exploiting hybrid vigor in onion breeding programs to develop cultivars with improved bulb characteristics such as size, shape, and weight (Gupta and Singh 2016; Khosa *et al.*, 2016). However, further evaluation of the F_1 hybrids under diverse environmental conditions and across multiple growing seasons would be necessary to assess their stability and suitability for commercial cultivation (Mushtaq *et al.*, 2013; Tignegre *et al.*, 2022).

The F_2 generations demonstrate significant variability in bulb morphology and yield, typical of segregating populations like F_2 generations (Table 2). While generally exhibiting a wider range of values compared to F_1, instances of strong hybrid vigor are observed, particularly in combinations involving BARI Piaz-4. This highlights the potential for identifying and selecting superior lines with enhanced traits for further cultivation and breeding efforts (Kopecký *et al.*, 2022; Gautham and Thangappan, 2018. The variability within each F_2 population combination underscores the importance of evaluating large populations to identify superior lines with desirable characteristics for commercial production (Kopecký *et al.*, 2022; (Kopecký *et al.*, 2022; Gautham and Thangappan, 2018). Careful selection and evaluation are essential to advance promising lines and develop improved onion cultivars (Gupta *et al.*, 2021). Therefore, the F_2 generations represent a diverse population with the potential for the development of improved onion cultivars through selective breeding and further evaluation (Khosa *et al.*, 2016; Mitrova *et al.*, 2015).

Bulb yield in different generations

The bulb yield for different crosses in different generations is presented in Table 3. Variation was observed in the yielding behavior for different cross combinations. The higher mean bulb yield was recorded in F_1 than in the parental genotypes as expected. While most of the cases in F_2 , a reduction in mean bulb yield was observed compared to F_1's. At the same time, wider ranges are also observed in F_2's for the yielding performances. The cross combination (BARI piaz1 × BARI piaz4) produced the maximum yield (21.39 t/ha) in F_1 whereas the combination (BARI piaz6 × BARI piaz1) produced the minimum bulb yield (14.39 t/ha). Overall, crossing between BARI piaz1 and BARI piaz6 had the least production ability compared to other combinations. Comparison of different generation's yielding abilities were depicted in figures 1, 2, and 3 for different cross combinations.

The range of fresh yield values within each cell indicates the variability observed in yield performance within that specific cross combination and generation. A wider range suggests greater variability in yield performance within the population (Amir *et al.*, 2023). Comparing the mean fresh yield values across different generations within the same cross combination allows for the assessment of changes in yield performance over generations (Abdalla *et al.*, 2022; Alemu *et al.*, 2022). For instance, comparing the mean fresh yield of the F_1 and F_2 generations resulting from the same cross combination revealed potential differences in yield stability or improvement. Additionally, comparing mean fresh yield values across different cross combinations provides perceptions of the influence of parental genetics on yield performance (Khaki *et al.*, 2020; Patil and Subramaniam, 2020). Though the yielding ability was a little superior in F_1 it was not reduced much in F_2 as of BARI piaz1, so if the trend continues in further subsequent generations as F_3, $F_{4,}$ etc. then mass selection will be advantageous from those cross combinations involving BARI piaz4 as the female parent (Pike 1986; Shokrgozar *et al.*, 2021; Singh 1997).

Table 3. Performance of F_1 and F_2 generations along with parents for fresh yield (t/ha) of onion

Cross combinations	Mean (μ) ± Standard Deviation (σ)				Range			
	P_1	P_2	F_1	F_2	P_1	P_2	F_1	F_2
BARI piaz1xBARI piaz4	11.07±3.63	18.2±4.77	21.39±1.94	13.04±2.9	10.11-16.05	13.17-25.94	19.44-23.33	9.98-16.2
BARI piaz1xBARI piaz1	18.2±4.77	11.07±3.63	19.64±0.19	18.82±3.04	13.17-25.94	10.11-16.05	19.21-19.83	15.6-22.84
BARI piaz1xBARI piaz6	11.07±3.63	15.47±4.4	15.56±0.38	13.4±0.91	10.11-16.05	13.61-19.52	14.8-16.17	12.89-15.17
BARI piaz6xBARI piaz1	15.47±4.4	11.07±3.63	14.39±2.33	11.36±1.95	13.61-19.52	10.11-16.05	12.06-16.72	8.95-13.74
BARI piaz4xBARI piaz6	18.2±4.77	15.47±4.4	17.98±2.33	17.89±5.95	13.17-25.94	13.61-19.52	15.56-20.22	12.71-25.64
BARI piaz6xBARI piaz4	15.47±4.4	18.2±4.77	17.11±0.78	16.44±7.64	13.61-19.52	13.17-25.94	16.33-17.89	6.2-26.13

μ=Mean value; σ=Standard deviation; P_1=Parent one; P_2=Parent two; F_1=First filial generation; F_2=Second filial generation/segregating generation

Impact of parental selection

The choice of mother had a significant effect on the performance of progeny and segregating generation (Fig. 1, 2, 3). For instance, if BARI piaz1 is used as a mother the fresh bulb yield will be superior in F_1 in comparison to using as pollen donor (father) of the same parents' combination (i.e., BP1 x BP4 is superior to BP4 x BP1 in F_1). But in F_2, the performance became reversed, i.e., the fresh yield drastically reduced in F_2 when BARI piaz1 was used as a mother. In contrast, when BARI piaz4 was used as the mother plant then the F_1 performance was slightly superior to parents but not as high as BARI piaz1. Though the superior yield ability in $F_{1,}$ it was not reduced much in F_2 as of BARI piaz1, so if the trend continues in further subsequent generations as F_3, $F_{4,}$ etc., then mass selection will be advantageous from those cross combinations involving BARI piaz4 as female parent. When it comes to BARI piaz6, it showed better heterosis with BARI piaz4 rather than with BARI piaz1. Even in the case of the cross-combination BARI piaz6 × BARI piaz4, it showed an elevated yielding ability in F_2 which is superior to F_1. The choice of the mother plant significantly influences the yield performance of the progeny (Baskin and Carol, 2019; Mazer & David, 1996). For example, when BARI Piaz1 was used as the mother, the F_1 generation generally showed superior fresh bulb yield compared to using it as the donor in the same cross combination. Interestingly, while BARI piaz4 may not yield as high as BARI piaz1 in the F_1 generation, its performance does not decrease drastically in the subsequent F_2 generations. This suggests that BARI piaz4 may offer more stable and consistent yield performance over generations, making it a favorable choice for breeding programs (Pike 1986; Shokrgozar *et al.*, 2021; Singh 1997).

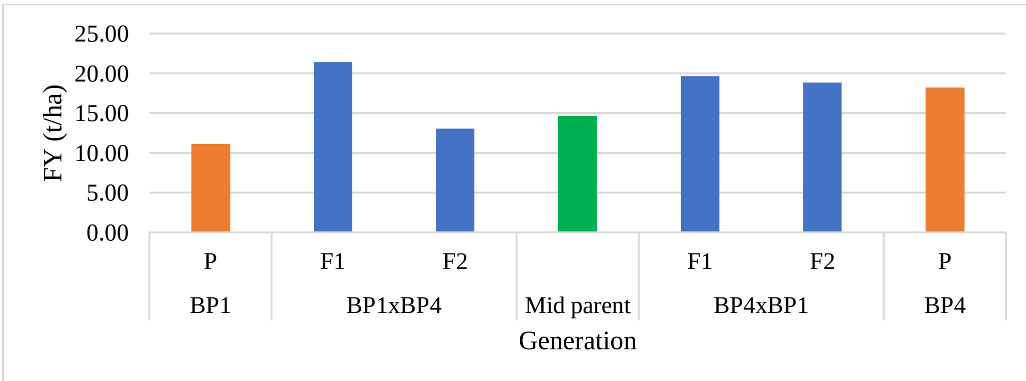

Fig. 1. Comparison of different generation's fresh yielding ability of the crosses between BARI piaz1 and BARI piaz4

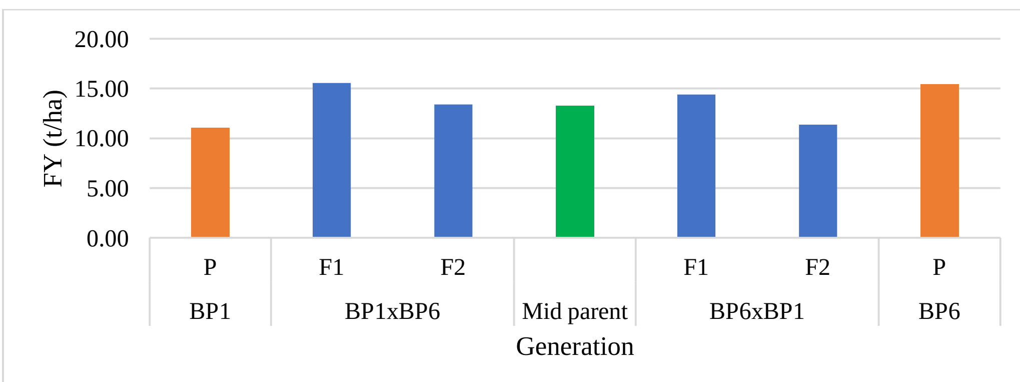

Fig. 2. Comparison of different generation's fresh yielding ability of the crosses between BARI piaz1 and BARI piaz6

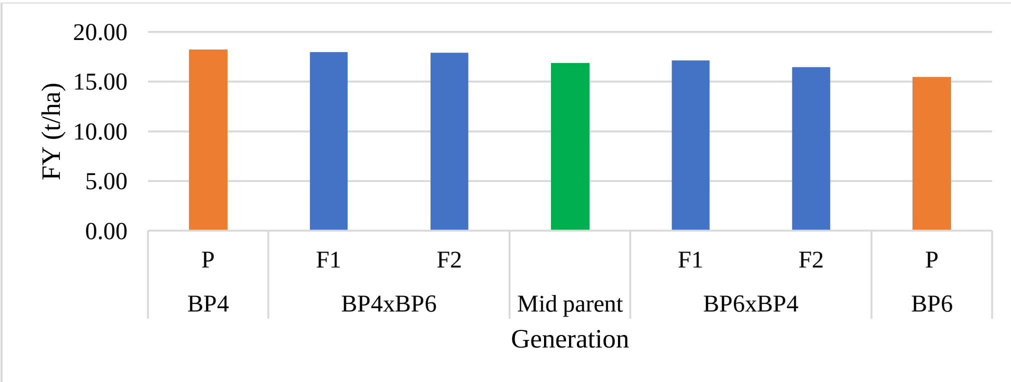

Fig. 3. Comparison of different generation's fresh yielding ability of the crosses between BARI piaz4 and BARI piaz6

Heterosis and inbreeding depression

Table 4 presents data on heterosis and inbreeding depression in F_1 and F_2 generations of onion hybrids involving BARI piaz1, BARI piaz4, and BARI piaz6. In terms of heterosis over mid-parent, F_1 hybrids frequently show positive heterosis for neck diameter (ND) and individual bulb weight (IBW), meaning these traits exceed the mid-parent average. For instance, the BARI piaz1 x BARI piaz6 hybrid exhibits high heterosis in ND (75.76%) and IBW (30.26%). Conversely, many hybrids show negative heterosis in the bulbing index (BI), indicating a lower BI than the mid-parent, which could result in more elongated bulb shapes. Regarding heterosis over a better parent, some F_1 hybrids, such as BARI piaz1 x BARI piaz6, outperform the superior parent in ND (71.93%), indicating an improved neck diameter compared to both parents. For individual bulb weight (IBW), certain hybrids (e.g., BARI piaz6 x BARI piaz1) do not exceed the better parent, showing any additional benefit in bulb weight.

In contrast, inbreeding depression generally leads to decreased performance in F_2 generation, with notable negative values in traits like field yield (FY). This reduction suggests that yield and bulb quality are adversely affected by inbreeding. In summary, some F_1 hybrids exhibit strong heterosis for neck diameter and bulb weight, which are valuable traits for commercial onion production. However, inbreeding depression negatively impacts yield and bulb quality in F_2 generations. These findings can guide parent selection in breeding programs aimed at improving yield, bulb shape, and quality.

Table 4. Heterosis and inbreeding depression analysis in F_1 and F_2 generations of onion

Cross combinations	BL	BD	ND	BI	IBW	FY
Heterosis over mid-parent						
BARI piaz1 x BARI piaz4	9.44	0.00	26.39	-43.09	-11.95	46.16
BARI piaz 1 x BARI piaz6	44.31	-6.35	75.76	-40.22	30.26	17.26
BARI piaz4 x BARI piaz1	19.05	-10.00	73.32	-47.37	24.97	34.20
BARI piaz4 x BARI piaz6	-6.70	7.78	44.95	-48.30	25.16	6.80
BARI piaz6 x BARI piaz1	27.70	-13.98	60.51	-45.97	1.62	8.44
BARI piaz6 x BARI piaz4	-6.70	5.12	62.63	-43.56	9.43	1.63
Heterosis over better parent						
BARI piaz1 x BARI piaz4	-4.45	-7.22	17.43	-43.17	-35.28	17.53
BARI piaz 1 x BARI piaz6	34.15	-9.12	71.94	-40.71	11.55	0.58
BARI piaz4 x BARI piaz1	3.94	-16.50	61.03	-47.45	-8.14	7.91
BARI piaz4 x BARI piaz6	-12.83	2.90	37.51	-48.65	3.87	-1.21
BARI piaz6 x BARI piaz1	18.70	-16.53	57.02	-46.42	-12.97	-6.98
BARI piaz6 x BARI piaz4	-12.83	0.37	54.27	-43.93	-9.19	-5.99
Inbreeding depression						
BARI piaz1 x BARI piaz4	-10.75	5.93	-16.82	80.97	-9.65	-39.04
BARI piaz 1 x BARI piaz6	-24.64	17.50	-35.78	66.95	-12.76	-13.88
BARI piaz4 x BARI piaz1	-1.27	30.53	-41.93	125.84	-11.19	-4.18
BARI piaz4 x BARI piaz6	11.21	-1.30	-31.95	110.63	-25.75	-0.50
BARI piaz6 x BARI piaz1	-17.48	27.44	-33.96	94.44	2.21	-21.06
BARI piaz6 x BARI piaz4	14.40	0.30	-35.09	80.53	-3.74	-3.92

BL=Bulb length; BD=Bulb Diameter; ND=Neck Diameter; BI=Bulbing Index; IBW=Individual Bulb Weight; FY=Fresh Yield

The text also highlights instances of heterosis, particularly with BARI piaz4 as the female parent. The cross-combination involving BARI piaz6 and BARI piaz4 showed elevated yield ability in the F_2 generation compared to the F_1 generation, which is a different result from earlier studies (Engelen *et al.*, 2004; Gogas and Metaxia, 2014). Such observations suggest the potential for early generation selection of superior segregating lines with desirable yield characteristics for further cultivation and breeding efforts (Gogas & Metaxia, 2014).

Overall, the research underscores the significant genetic variability within onion populations resulting from diverse parental crosses, providing breeders with ample opportunities to develop superior cultivars tailored to specific needs (Bertan and Oliveira, 2007). Understanding the dynamics of parental selection and the cross combination is crucial for onion breeders to develop cultivars with improved yield and other desirable traits (Ochar and Seong, 2023; Swarup *et al.*, 2021). The presence of hybrid vigor and heterosis in F_1 generations indicates the potential for combining diverse parental lines to produce offspring with enhanced traits, including yield, quality, and resilience. The observed variability in trait expression within F_2 hybrid populations emphasizes the importance of evaluating large populations to identify individuals with desired trait combinations, enabling breeders to advance breeding objectives effectively. Continued evaluation and selection within populations are necessary to identify and advance superior lines for commercial cultivation and breeding programs (Santantonio *et al.*, 2020). Careful selection of parental lines, such as prioritizing varieties like BARI Piaz4 with consistent performance, is crucial for ensuring stability and success in hybrid progeny across generations.

Conclusion

This study, based on crosses between three onion varieties viz., BARI piaz1, BARI piaz4, and BARI piaz6 offers an understanding of effective onion breeding strategies. The significant genetic diversity observed within these populations provides ample opportunities for breeders to select and develop superior lines with enhanced traits. The F_1 generations consistently outperformed their parental lines, demonstrating the benefits of hybrid vigor and heterosis in boosting yield, quality, and resilience. Parental selection played a key role, with varieties like BARI piaz4 showing stable performance across multiple generations, making it ideal for breeding programs focused on high-yielding, stable cultivars. Furthermore, the trait segregation seen in F_2 populations highlights the importance of evaluating large populations to identify individuals with desirable combinations of traits. This study emphasizes the value of harnessing genetic diversity, hybrid vigor, and careful parental selection in developing resilient, high-performing onion varieties that thrive in diverse environments, thereby contributing to sustainable agricultural productivity.

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Author’s contribution

M.A.A: Conceptualization, data curation, formal analysis, investigation, methodology, resources, software, validation, visualization, writing – original draft. M.S.N: Investigation, methodology, validation, writing – original draft. M.M.H: Methodology, resources, validation, supervision. reviewed, revised and edited the content. AHFF: Investigation, methodology, validation. M.A.A.K: Methodology, validation. snm: funding acquisition, project administration, resources, supervision, writing – review & editing. M.T.R.:Reviewed, revised and edited the content.

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EVALUATION OF SWEET POTATO VARIETIES BASED ON GROWTH AND YIELD

S. Nasrin[1*], M. A. Mannan[2], S. I. Shorna[2], K. Islam[1] and M. Rahman[3]

[1]Breeding Division, Bangladesh Jute Research Institute (BJRI), Manik Mia Avenue, Dhaka; [2]Agrotechnology Discipline, Khulna University, Khulna; [3]Bangladesh Agricultural Research Institute (BARI), Gazipur. Bangladesh.

Abstract

The research was conducted at Germplasm Centre of Agrotechnology Discipline, Khulna University, Khulna during the period from November 2021 to April 2022, to evaluate the growth and yield performance of selected six sweet potato varieties**.** The experiment consisted of six sweet potato varieties: BARI misti alu10, BARI misti alu11, BARI misti alu12, BARI misti alu14, BARI misti alu15 and local variety (check). The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The parameters measured included number of sprouts, plant height, number of branches, branch length, number of leaves, length of leaves, breadth of leaves, number of tubers, weight of tubers and yield (t ha^{-1}). The results of the study revealed that, BARI misti alu10 showed maximum yield (54.33 t ha^{-1}) followed by BARI misti alu14 (44.66 t ha^{-1}). Based on the findings of the experiment, BARI misti alu10 and BARI misti alu14 was found better in respect yield and yield contributing characters. The outcome of this study could be helpful in germplasm management, hybridization programs and crop improvement program.

Keywords: Evaluation, Growth, Sweet potato, Yield.

Introduction

Sweet Potato (*Ipomoea batatas* L.) belongs to family Convolvulaceae is one of the most important root crop and major food, feed, and vegetable crop in many tropical developing nations. In Bangladesh, it is known as " misti alu " (Mahmud *et al.*, 2021). The children in char and rural areas of Bangladesh are affected mainly by vitamin A deficiency which causes 88 children to become blind each day (Banglapedia, 2019) can be alleviated by sweet potato consumption. It also provides vitamin B complex, C, and E along with K, Mn, Cu, Fe, crude protein, β carotene, phenolic compounds, and beneficial fibers (Szalay, 2017; Sun *et al.*, 2019). Bangladesh's climate is favorable for the growth of sweet potatoes. In Bangladesh, sweet potato is grown in the riverbank areas. Currently, about 23,571 tons of sweet potato are being produced from an area of 23,014 hectares of land with an average yield of 10.25 t ha^{-1} (Alam *et al.*, 2023). Between 2000 to 2013, total sweet potato production of Bangladesh increased from 92,479 to 104,000 MT (FAOSTAT 2014). Despite the nutritional advantages and additional benefits of

[*] Corresponding author: shamimakbd.seema@gmail.com

producing sweet potatoes, biotic and abiotic stress factors have a significant negative impact on their productivity (Guo *et al.*, 2006). In Bangladesh, the coastline region occupies 30% of the total area and about 0.83 million hectares of cultivable land of Bangladesh (Haque, 2006). Different levels of soil salinity have an impact on the cultivable regions in coastal districts. In the coastal region of Bangladesh, research experiment with sweet potato varieties is meager due to soil and climatic limitations so ensure better yield is a demand of time. So, the present study was carried out to evaluate the growth and yield of the selected potato varieties.

Materials and Methods

The field experiment was conducted at Germplasm Centre of Agro-technology Discipline, Khulna University, Khulna during the period from November 2021 to April 2022 to study the growth and yield performance of selected sweet potato varieties. The climate of the experimental area is sub-tropical in nature. It was carried out on a medium high land, fairly leveled, well drained silty loam soil. The treatments included six varieties (V_1 = BARI misti alu10, V_2 = BARI misti alu11, V_3 = BARI misti alu12, V_4 = BARI misti alu14, V_5 = BARI misti alu15 and V_6 = Local Variety). Sweet potato cuttings were used as planting material these were collected from BARI sub-station, Satkhira. The experiment was laid out in the Randomized Complete Block Design (RCBD) with three replications. The unit plot was 4.8 m × 2.4 m in size and each plot contained 32 hills. Ridges and furrows were formed at 60 cm distance. The manures and fertilizers were used according the doses BARI, 2022. Intercultural operations and plant protections activities were done when it's necessary. Five plants were selected randomly from each unit plot. The number of sprouts hill^{-1} from the selected plants were counted and their average was recorded at 15 DAP. Plant height**,** number of leaves hill^{-1}, No of branches hill^{-1}, number of leaves, length of leaves (cm), and breadth of leaves (cm) was recorded at 15, 30, 45 and 60 DAP. Number of tuber hill^{-1}, number of tuber plot^{-1} weight of tuber hill^{-1}, weight of tuber plot^{-1} were taken using electric balance and their average was recorded in kg. The tuber yield plot^{-1} of sweet potato was converted into tha^{-1} by using the following formula:

$$\text{Yield (t/ha)} = \frac{\text{Yield/plot (kg)} \times 10000\ \text{m}^2}{\text{Area of plot (m}^2\text{)} \times 1000\ \text{kg}}$$

The collected data were statistically analysed for analysis of variance using computer software "Statistix-10". The means were compared by Duncan's Multiple Range Test (DMRT) at 5% and 1% levels of probability.

Results and Discussions

Number of sprouts

Statistically non-significant variation observed the number of sprouts hill^{-1} at 15 DAP. However, numerically the maximum number of sprouts hill^{-1} were observed in BARI misti alu14 (6.67) followed by BARI misti alu12 (6.33) and the local variety (6.33) whereas lowest (4.67) in BARI misti alu10 (Table 1).

Plant height

At 15 and 45 DAP, the varieties were not differed significantly for plant height. At 45 DAP, there were significant variation among the varieties in respect of plant height. The maximum plant height (61.33 cm) was observed in local variety followed by BARI misti alu15 (55.33 cm) and BARI misti alu12 (48.33 cm) while the shortest (47.67 cm) in BARI misti alu11 (Table 1). At 60 DAP, the plant height varied significantly among the varieties. The maximum height (77.33cm) was observed in the local variety followed by BARI misti alu12 (70.17cm) and BARI misti alu10 (60.5 cm) and that was the lowest (50 cm) in BARI misti alu11. There was tired to increase plant height with the advancement of date of planting (Table 1).

Table 1. Number of sprouts hill^{-1} and plant height of sweet potato varieties at different days after planting (DAP)

Variety	No. of sprouts/hill	Plant height (cm)			
		15 DAP	30 DAP	45 DAP	60 DAP
BARI misti alu10	4.67	19.67	26.67	44.33c	60.50b
BARI misti alu11	8.00	21.00	35.17	42.67c	50.00c
BARI misti alu12	6.33	20.00	33.50	48.33bc	70.17a
BARI misti alu14	6.67	21.50	34.50	48.00bc	57.00bc
BARI misti alu15	4.74	31.50	51.33	55.33ab	59.83b
Local (check)	6.33	17.33	37.50	61.33a	77.33a
LSD $_{(0.05)}$	NS	NS	NS	**	**

Means with common letters do not have any significant difference at 5% level of significance.
**Significant at 1% level of probability, LS: Level of significance, NS: Not significant

Number of branches

At 15, 30 and 45 DAP, there were no significant variation among the varieties in respect of number of branches. At 60 DAP, the number of branches varied significantly among the varieties. The maximum number of branches (7.00) were observed in BARI misti alu14 followed by BARI misti alu-10 (5.67) and the lowest number (4.33) were found in BARI misti alu11 followed by BARI misti alu12. (Table 2).

Length of branches

At 15 DAP, the varieties were differed significantly in respect of branch length. The maximum branch (20.67 cm) was found in BARI misti alu15 followed by BARI misti alu14 (17.16 cm) and BARI misti alu11 (16.67 cm) and that was the shortest (9.83 cm) in the local variety (Table 2). At 30 and 45 DAP, the length of branches was not varied significantly among the varieties. At 60 DAP, the branch length varied significantly among the varieties. The maximum length of branches was observed in the local variety (52.67 cm) followed by BARI misti alu10 (50.33 cm) and BARI misti alu14 (43.67 cm) and that was the lowest (31.5 cm) in BARI misti alu11 (Table 2).

Table 2. Number of branches and length of branches of sweet potato varieties at different days after planting (DAP)

Variety	Number of Branches				Branch length (cm)			
	15 DAP	30 DAP	45 DAP	60 DAP	15 DAP	30 DAP	45 DAP	60 DAP
BARI misti alu10	5.00	3.33	4.67	5.67ab	11.67ab	21.33	34.00	50.33ab
BARI misti alu11	4.00	3.67	4.00	4.33b	16.67ab	18.83	26.33	31.50d
BARI misti alu12	3.00	2.67	3.33	4.33b	14.50ab	27.67	30.17	36.33cd
BARI misti alu14	4.00	2.33	4.67	7.00a	17.16ab	25.33	36.50	43.67abc
BARI misti alu15	3.67	3.00	4.00	4.67b	20.67a	28.67	33.33	38.33bcd
Local Variety	2.67	2.67	3.67	4.67b	9.83b	15.33	37.33	52.67a
LSD $_{(0.05)}$	NS	NS	NS	*	*	NS	NS	*

Means with common letters do not have any significant difference at 5% level of significance by DMRT. *Significant at 5% level of probability and NS: Not significant

Number of leaves

At 15, 30, 45 and 60 DAP, the number of leaves were found statistically non-significant among the varieties. However, numerically the maximum number of leaves were observed in BARI misti alu10 and that was the minimum in BARI misti alu12 in all date of planting (Fig. 1).

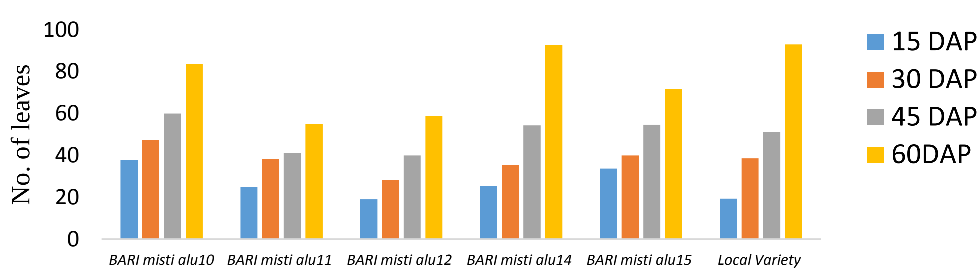

Fig. 1. Number of leaves of sweet potato varieties at different days after planting (DAP)

Length of leaves

At 15, 30 and 45 DAP, there were no significant difference among the varieties in respect of leaf length. However, numerically the maximum length of leaves were produced in BARI misti alu-15 and that was the minimum in the local variety in all date of planting (Fig. 2). At 60 DAP, there was significant variation among the varieties in respect of leaf length. The maximum length of leaves (11.2 cm) was observed in BARI misti alu15 followed by BARI misti alu12 (9.67cm) and BARI misti alu14 (9.50 cm) and that was the shortest (8.50 cm) in BARI misti alu10 (Fig. 2).

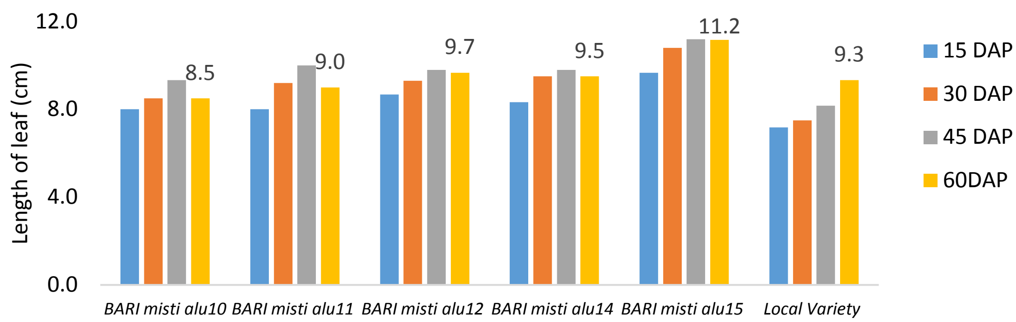

Fig. 2. Length of leaves of sweet potato varieties at different days after planting (DAP)

Breadth of leaves

At 15, 30 and 45 DAP, the varieties were not differed significantly for leaf breadth. However, numerically the broadest leaves were found in BARI misti alu15 and that was the narrowest in BARI misti alu11 in all date of planting (Fig. 3). Similar trend was recorded at 60 DAP, the varieties were not differed significantly for leaf breadth. However, numerically the broadest leaves were produced in BARI misti alu10 and that was the smallest in BARI misti alu12 (Fig. 3)

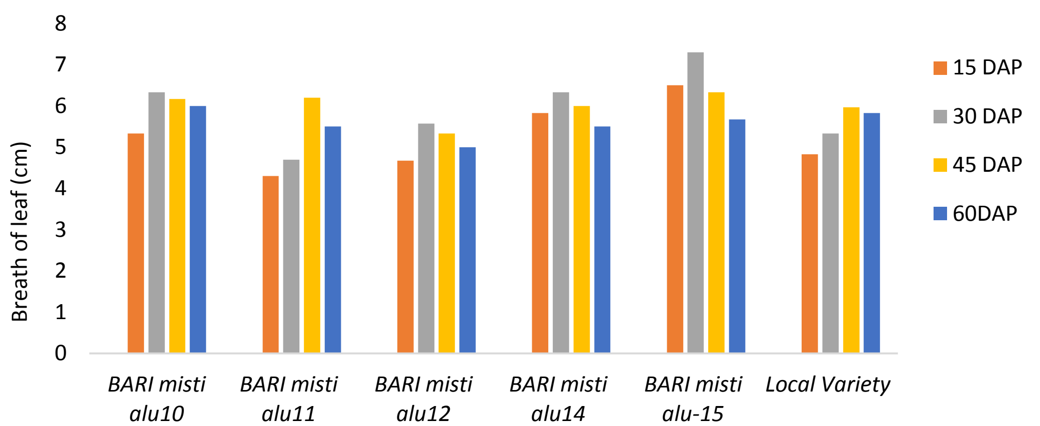

Fig. 3. Breadth of leaves of sweet potato varieties at different days after planting (DAP)

Number of tubers plot-1

The number of tubers of sweet potato varieties was found statistically significant. The maximum number of tubers per plot were obtained from BARI misti alu10 (405.33) followed by BARI misti alu14 (309.33) and BARI misti alu11 (202.67) and lowest number of tuber per plot (138.67) was found in the local variety (Table 3).

Weight of tuber plot^{-1}

Statistically significant variation found from weight of tubers of sweet potato varieties. The maximum weight of tubers per plot (28.52 kg) was obtained from BARI misti alu10 followed by BARI misti alu14 (23.45 kg) and local variety (23.24 kg) and the lowest (15.12 kg) in BARI misti alu12 (Table 3).

Tuber yield

The tuber yield of sweet potato was converted into per hectare and has been expressed in ton. Varietal difference in relation to yield was highly significant. The most productive variety was BARI misti alu10 (54.3 t ha^{-1}) followed by BARI misti alu14 (44.6 t ha^{-1}) and that was the lowest (28.8 t ha^{-1}) in BARI misti alu12 preceded by BARI misti alu15 (29.5 t ha^{-1}) (Table 3).

Table 3. Number of tubers, weight of tuber and tuber yield of sweet potato varieties

Variety	No. of tubers/plot	Wt. of tuber/plot (kg)	Tuber yield (t/ha)
BARI misti alu10	405.33a	28.520a	54.33a
BARI misti alu11	202.67c	17.301bcd	32.96bcd
BARI misti alu12	164.00c	15.139d	28.84d
BARI misti alu14	309.33b	23.445ab	44.66ab
BARI misti alu15	196.00c	15.496cd	29.51cd
Local Variety	138.67c	23.236abc	44.26abc
LSD $_{(0.05)}$	**	*	*

Means with common letters do not have any significant difference at 5% level of significance by DMRT. *Significant at 5% level of probability ** = Significant at 1% level, LS= level of significance.

Correlation

All the yield contributing characters of sweet potato varieties were positively correlated with yield. Among them, plant height, number of branches, number of leaves, length of branches, breadth of leaves and number of tuber hill^{-1} were correlated significantly with yield among the varieties. But the weight of tuber hill^{-1} was not significantly correlated with yield (Table 4.).

Table 4. Correlation coefficient of plant height, number of branches, length of branches, leaf number, breadth of leaf, no. of tuber and weight of tuber with yield

Yield contributing character	Tuber yield	Level of significance
Plant height	0.07	**
Number of branches	0.17	**
Length of branches	0.59	*

Yield contributing character	Tuber yield	Level of significance
Number of leaves	0.43	**
Breadth of leaves	0.16	**
Number of tuber/hill	0.56	*
Weight of tuber/hill	0.64	NS

*Significant at 5% level of probability, **Significant at 1% level of probability, NS-Not significant

Positive correlations exist between stem length and aboveground biomass yield, leaf number and biomass yield followed by tuber number and tuber yield (George *et al.*, 2002). BARI misti alu10 was the maximum branch producing variety at 15 DAP and 45 DAP and it was the highest yielder variety also. On the other hand, BARI misti alu14 was the second most branch producer variety and second higher yielder variety at the same time.

There are also positive and significant correlations between stem length and number of tubers, number of leaves and number of tubers, stem length and tuber yield, number of leaves, and tuber yield (Zelalem *et al*., 2009). This research study also agrees with those positive correlations. Here, the sweet potato varieties having longer stem, more branches, more and broader leaves gave more tuber and higher yield. There are direct relationships between the leaves and tubers of the plant. These relationships are indicated by strong positive correlations between the number of tubers and the number of leaves (Lahlou & Ledent, 2005). Correlation analysis of the present study also revealed the same relationship.

Conclusion

The experiment data revealed that, BARI misti alu14 was superior in number of sprouts. The longest plant and longest branch were observed in the local variety at 45 and 60 DAP. The longest leaf was observed in BARI misti alu15 in at all the DAP. BARI misti alu10 was the maximum leaf producer at 45 DAP, while it was the local variety at 60 DAP. In case of sweet potato tuber, the highest number of tubers, maximum weight of tubers and also the maximum yield BARI misti alu10 followed by BARI misti alu14. BARI misti alu10 and BARI misti alu14 found better in respect of growth, yield contributing characters and yield.

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Author’s contribution

The authors confirm contribution to the paper as follows: study conception and design: M. A. Mannan, S. Nasrin; data collection, analysis and interpretation of results: S. I. Shorna, M. Rahman; draft manuscript preparation: S. Nasrin, K. Islam. All authors reviewed the results and approved the final version of the manuscript.

Conflicts of Interest

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

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INFLUENCE OF MULCHING AND FERTILIZERS ON GROWTH, YIELD AND QUALITY OF SUGAR BEET

N. Jahan[1*], A. H. M. Solaiman[1], N. Islam[1], F. Hossain[2], and S. Choudhury[1*]

[1]Department of Horticulture, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka; [2]Department of Agroforestry and Environmental Science, SAU, Dhaka. Bangladesh.

Abstract

An experiment was carried out during the period from October 2022 to April 2023 to investigate the effect of different mulching materials and fertilizers on sugar beet growth, yield, and quality. The experiment consisted of mulch materials: no mulch, black polythene mulch, and rice straw mulch; and four fertilizer treatment: control (no fertilizer), 100% RCF (recommended chemical fertilizer), 100% vermicompost @ 10 t/ha and 50% vermicompost @ 5 t/ha + 50% RCF. Treatment with 50% vermicompost + 50% inorganic fertilizer (RCF) produced significant results than other treatments. Among the mulch materials, black polythene performed better than the other mulch materials. Accordingly, 50% vermicompost + 50% inorganic fertilizer and black polythene mulch may be recommended to obtain better sugar beet yield and quality.

Keywords: Black polythene, Rice straw, Sugar beet, Vermicompost.

Introduction

Tropical sugar beet, scientifically known as *Beta vulgaris* (L.), is a biennial, herbaceous tuber crop grown in temperate climates that yields sugar. It is one of the most widely grown crop in the Chenopodiaceae family (Chawla *et al.*, 2016). The current production of sugar in Bangladesh meets about 5% of total demand and 20% of total requirement covers with jaggery mainly from sugarcane and the rest 75% sugar demand is fulfilled by import (Rahman *et al.*, 2016). The area under cane cultivation is drastically reduced due to pressure of cereals and other short-duration crops, which cause lower amount of sugarcane production. Sugar beet has got many benefits compared to sugarcane due to its short duration with high sucrose contents (Paul *et al.*, 2018).

Soil fertility is being depleted due to increased cultivation in the country driven by population growth, while the use of mineral fertilizers to restore nutrients has drawbacks such as high costs and harmful effects on soil health and the environment (Abd-Elrahman, 2017). Fertilizers, both organic and inorganic, may influence the growth and development of crops. Imbalanced nutrition is one of the important constraints towards higher productivity and other quality parameters of crops. According to Islam *et al.* (2016), an integrated strategy to fertilizer management might significantly reduce leaching losses of nitrogen (N), phosphorous (P), potassium (K), and sulfur (S). Manures

[*] Corresponding author: nusrat2923@gmail.com; shormin2000@gmail.com

provide all the necessary nutrients, enhance the physical, chemical, and biological characteristics of soils, and may even aid increase agricultural yield while preserving the environment. According to Kamal *et al*. (2012), deteriorated soil can be productively restored with organic fertilizer. Integrated nutrients management have a major impact on tomato (*Lycopersicon esculentum* L.) agronomical growth and crop yield (Chopra *et al.*, 2017). Mulching, particularly polyethylene mulch, which raises soil temperature and modifies the microclimate (Malik *et al*., 2018), can improve the available soil moisture condition by capturing micro efficient or ineffective precipitation, decreasing soil evaporation, and limiting runoff (Chen *et al*., 2019). Mulching is essential for improving crop productivity by raising soil temperature, conserving moisture, and reducing weed infestation, particularly in winter, leading to lower costs (Chakraborty *et al*., 2008). The use of black polyethylene film mulch was also found to be superior to that of straw mulch (Moursy *et al*., 2021).

Crop yield potential can be achieved by the effective and balanced use of both organic and inorganic fertilizer sources as well as the application of appropriate agronomic package practices (Meena *et al*., 2016). A limited amount of information has been published on sugar beet production in terms of the best mulch material and suitable nutrient management. Therefore, this experiment has understood to determine the effect of mulching and nutrient management on growth and yield of sugar beet and to identify the suitable treatment combination for quality sugar beet production.

Materials and Methods

The study was carried out at the central farm of Sher-e-Bangla Agricultural University Dhaka during November 2022 to April 2023. The farm is situated at an elevation of 8.2 meters above sea level, at latitude 23°47′ N and longitude 90°35′ E. The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications. This study was set up using a two-factorial design with Factor A: M_0 (No mulch), M_1 (Black polythene mulch) and M_2 (Rice straw mulch); and F_0=Control (no fertilizer), F_1=100% RCF (recommended chemical dose), F_2= 100% Vermicompost @ 10 t/ha, and F_3= 50% Vermicompost @ 5 t/ha + 50% RCF. The chemical composition of vermicompost was analyzed with the help of SRDI (Table 1). The initial soil nutrient status was also explored in the laboratory of SRDI (Table 2). In case of mulching, 15μM of black high-density polyethylene (HDPE) film was used and the thickness of rice straw mulch was maintained 4-5 cm (3t/ha) as a layer, which helped to control soil temperature and moisture. Well-decomposed vermicompost was applied 15 days before seeding. Nitrogen, phosphorus, potassium, zinc, sulfur and boron were applied in the forms of urea, TSP, MP, Zinc sulphate, gypsum and boric acid @ 260, 100, 225, 10, 100 and 20 kg ha^{-1}, respectively. Full dose of phosphorus was applied as basal dressing at the time of sowing. Nitrogen and potash were split into three applications: one at the base and two side dressings at 30 and 60 days after sowing (FRG, 2018). The tropical sugar beet genotype SV889 was used. Seeds treated with Vitavax-200 fungicide, were sown on November 10, 2022, in bed method, at a spacing of 50 cm × 20 cm. Sugar beets were harvested by hand when 70–80% of the leaves were dried. Irrigation was stopped 15 days before harvesting to allow the land to dry.

Table 1. Nutrient composition of vermicompost

Sample	Chemical composition		
	N (%)	P (%)	K (%)
Vermicompost	1.32	1.16	1.27

Table 2. Initial soil fertility status of the experimental plot

Soil characters	Value
pH	6.7
Organic matter	1.35
Nitrogen	0.11%
Phosphorus	20 ppm
Potassium	0.12 meq/100 g soil
Sulphur	44 ppm
Boron	0.19 ppm
Zink	8.52 ppm

Fig. a. Research field with treatment combination

Fig. b. Sugar beet data collection

Measurement of growth parameters

Five plants in each treatment and replication were used to measure different growth parameters. The height of the plant, length of leaf petiole, length of leaf blade, width of leaf blade, root length was measured with the help of a measuring tape and root diameter measured by a slide caliper. Plant height and no. of leaves data were taken on the dates of harvest as well as at 30, 60, 90 and 120 DAS. Number of leaves were counted individually. Root length and root diameter were measured on the dates of 60 DAS to harvest.

SPAD value

The initial fully inflated leaf content was measured using a Minolta, Tokyo, Japan, SPAD-502 chlorophyll meter. All treated and control plants had measurements made from the middle of the leaf lamina.

Measurement of yield and yield attributes

Sugar beet yield

Five plants were randomly counted from each plot, and the mean weight of shoot and root was recorded (g) using a digital electric balance. The shoot and root were dried for 48h at 70ºC in a convection oven, then transferred into desiccator and allowed to cool down at room temperature, final weight was recorded (g) as shoot and root dry weight.

Sugar beet yield was calculated using the formula:

$$Sugar\ beet\ yield\ (t/ha) = \frac{Beet\ yield\ (kg/plot)}{1000\ x\ Net\ plot\ size}\ x\ 10000$$

Sugar yield

Sugar yield was calculated using the formula:

$$Sugar\ yield\ (t/ha) = \frac{Sucrose\ \%}{100}\ x\ beet\ yield\ (t/ha)$$

Measurements of quality attribute

TSS

By using a hand refractometer (Hanna Instruments, HI96801, Romania) at room temperature, the percentage of brix was measured after harvest.

Sucrose %

Pol or percent sucrose is the only sucrose content in the juice measured by polarimeter. Pol percent juice was measured by using automatic polarimeter (Model AP-300, Atago Co., Ltd., Japan).

Purity%

Apparent purity percentage was determined as a ratio of sucrose % divided by TSS% of roots as the method outlined by Carruthers and Old Field (1960). The purity percentage was calculated from the data of brix and sugar percentage by using the following formula:

$$Purity\ \% = \frac{Sucrose\ \%}{TSS\ (Brix\ reading)}\ X\ 100$$

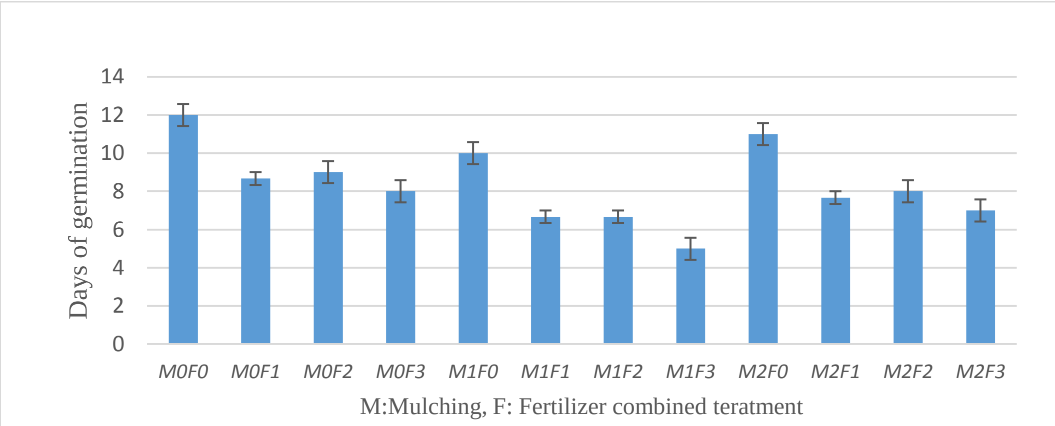
Statistical analysis

The collected data were analyzed statistically by using the “Statistix 10” computer package. Least Significant Difference (LSD) technique at 0.05% level of significance was used to compare the mean differences among the treatments (Gomez and Gomez, 1984).

Results and Discussion

Seed germination

The use of mulching improved nutrient availability, soil structure, reduce nitrate leaching, improved the physical characteristics of the soil, increased biological activity, supply organic matter, regulate temperature and water retention, and lessen erosion (El-Beltagi *et al*., 2022). The combined use of mulching and fertilizers significantly ($P \leq 0.05$) increased the days of seed germination (Figure 1). The shortest beet root germination time (5 days) was achieved with black polythene mulch and a mixture of 50% vermicompost and 50% RCF. Treatments M_1F_1 and M_1F_2 both resulted in germination within 6.67 days, while the slowest germination (12 days) occurred with no treatment. Ferdousi *et al*. (2010) reported similar results in potato seed germination (maximum time 17.00 days), where organic and inorganic fertilizers combined with black polythene mulch sped up the process.

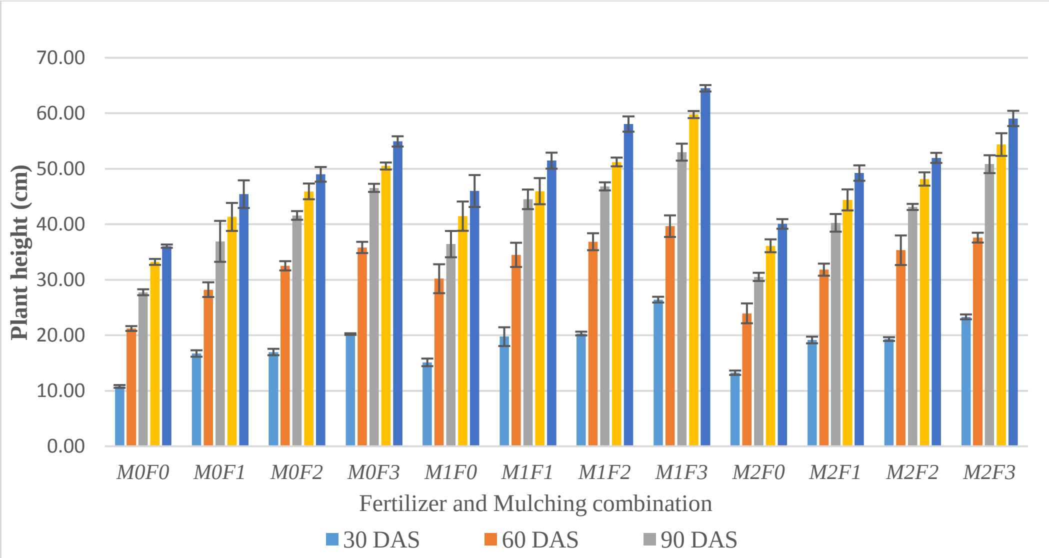


Here, M_0=No mulch, M_1=Black polythene mulch, M_2=Rice straw mulch, F_0=Control (No fertilizer), F_1=100% RCF (Recommended chemical fertilizer), F_2=100%Vermicompost @ 10 t/ha, and F_3=50%Vermicompost @ 5 t/ha + 50% RCF.

Fig. 1. Effect of mulching and fertilizer management on days to seed germination of sugar beet

Plant height (cm)

Sugar beet plant height was significantly influenced by mulching and nutrient management. The impact of different mulching and fertilizer treatments on plant height at various growth stages (30, 60, 90, 120, and 155 DAS) is shown in Figure 2. During 30 to 155 DAS, plant height ranged from 26.43 to 64.50 cm in the M_1F_3 treatment and 23.33 to 59.07 cm in the M_2F_3 treatment. In comparison, plant height in the M_0F_3 treatment ranged from 20.23 to 54.93 cm, while the control (M_0F_0) ranged from 10.80 to 36.07 cm. The M_1F_3 treatment produced the tallest plants, with a maximum height of 64.50 cm at 155 DAS, followed by M_2F_3 at 59.07 cm and M_1F_2 at 58.07 cm. The shortest plants (36.07 cm) were observed in the control treatment at 155 DAS. The use of black polythene mulch with 50% RCF and 50% vermicompost @ 5 t/ha resulted in the maximum plant height of sugar beet.



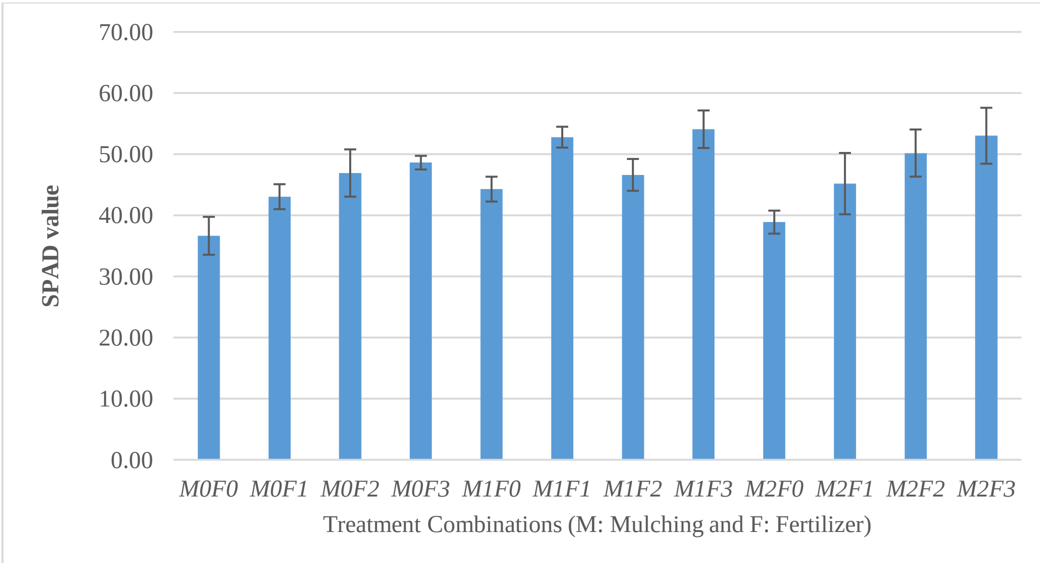
Here, M_0=No mulch, M_1=Black polythene mulch, M_2=Rice straw mulch, F_0=Control (No fertilizer), F_1=100% RCF (Recommended chemical fertilizer), F_2=100%Vermicompost @ 10 t/ha, and F_3=50%Vermicompost @ 5 t/ha + 50% RCF.

Fig. 2. Effect of mulching and fertilizer management on sugar beet plant height at different days after sowing

Similar findings were reported by Jagadeesh *et al*. (2018), Maloisane *et al*. (2022) and Sarker *et al*. (2023) on sugar beet. Plant height was 10.90 to 12.33% higher with different treatments compared to the control. The combination of vermicompost, inorganic fertilizer and mulching enhanced plant growth by providing both immediate and sustained nutrient release.

SPAD value

The revealed data showed statistically significant ($P \leq 0.05$) variations on SPAD value of sugar beet under interaction effects of different mulching and fertilizer management treatment. The combined treatments, M_1F_3 (BPM + 50% RCF and 50% vermicompost @5t/ha) and M_2F_3 (RSM + 50% RCF and 50% vermicompost @5t/ha) produced the maximum SPAD values (54.7 and 53, respectively). Meanwhile, the control treatment (No mulch + no fertilizer) gave the lowest, 36.63 SPAD value (Figure 3). SPAD value increased in fully vegetative stage that means from 60 DAS to 120 DAS.

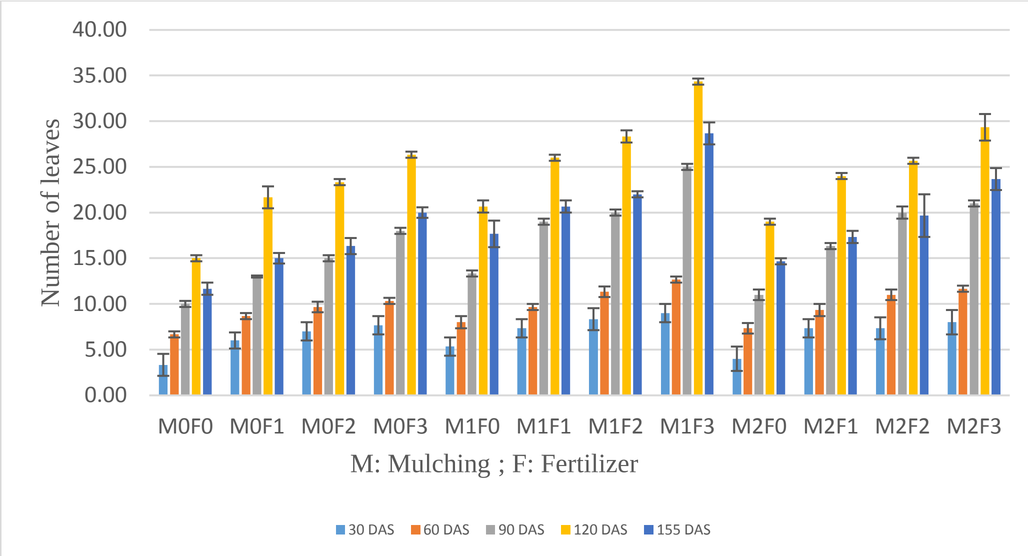


Here, M_0=No mulch, M_1=Black polythene mulch, M_2=Rice straw mulch, F_0=Control (No fertilizer), F_1=100% RCF (Recommended chemical fertilizer), F_2=100%Vermicompost @ 10 t/ha, and F_3=50%Vermicompost @ 5 t/ha + 50% (RCF).

Fig. 3. Effect of Mulching and Fertilizer management on SPAD value of sugar beet

Number of leaves/plants

The number of leaves is one of the major growth-attributing parameters. The interaction of different mulching types and organic and inorganic fertilizer management significantly ($P \leq 0.05$) influenced the number of sugar beet leaves (Figure 4) and data were recorded from 30 DAS to 150 DAS**.** The M_1F_3 treatment resulted in the highest leaf count of 28.67 leaves per plant, while the control (M_0F_0) had the lowest at 11.67 leaves per plant. The combined treatments M_0F_3, M_1F_1, M_1F_2, and M_2F_3 also showed superior leaf numbers (20.00, 20.67, 22.00, 23.67 leaves per plant, respectively) with no significant differences among them. The result obtained shows that the number of leaves decreased at harvest due to drying of older leaves. The combined application of vermicompost and chemical fertilizer produced more leaves per plant than vermicompost alone (Zaman *et al*., 2015; Lasmini S A *et al*., 2019).

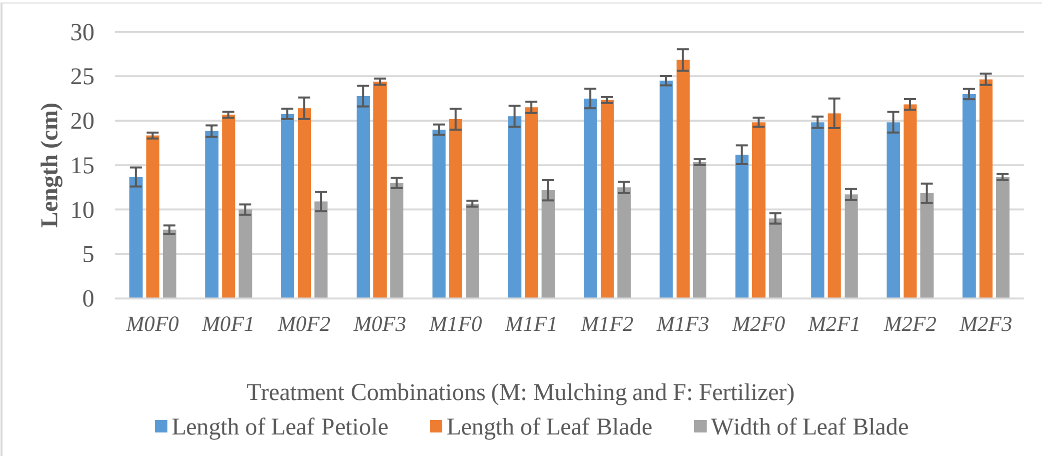


Here, M_0=No mulch, M_1=Black polythene mulch, M_2=Rice straw mulch, F_0=Control (No fertilizer), F_1=100% RCF (Recommended chemical fertilizer), F_2=100%Vermicompost @ 10 t/ha, and F_3=50%Vermicompost @ 5 t/ha + 50% RCF.

Fig. 4. Effect of mulching and fertilizer management on no. of leaves of sugar beet at different days after sowing

Leaf characters

The combined application of mulching and fertilizer have significant ($P \leq 0.05$) effect on the leaf characters (length of leaf petiole, length of leaf blade and width of leaf blade) of sugar beet and the results are showed in the Figure 5. The longest leaf twig length and leaf blade length were 24.5 cm and 26.83 cm, recorded from M_1F_3 treatment. The lowest length of leaf petiole 13.67 cm and length of leaf blade was 18.33 cm, recorded from M_0F_0 (control) treatment. The results are in agreement with the finding of Ruksun *et al.* (2022) in spinach. Similarly, the largest leaf blade width of 15.33 cm was recorded in the M_1F_3 treatment, while the second largest, 13.67 cm was observed in the M_2F_3 treatment. The smallest width, 7.73 cm, was recorded in the control (M_0F_0) treatment. Similar results were found by Dulal *et al*. (2021) in radish.

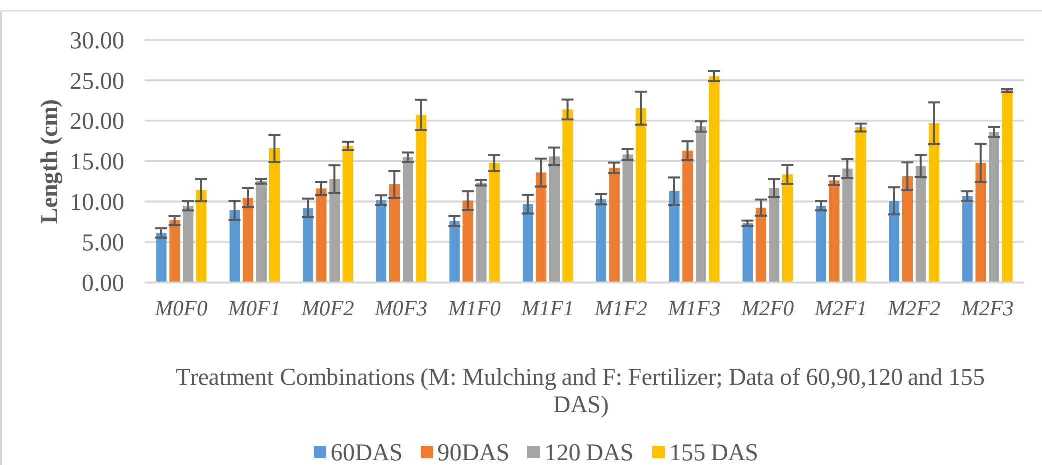


Here, M_0=No mulch, M_1=Black polythene mulch, M_2=Rice straw mulch, F_0=Control (No fertilizer), F_1=100% RCF (Recommended chemical fertilizer), F_2=100%Vermicompost @ 10 t/ha, and F_3=50%Vermicompost @ 5 t/ha + 50% RCF

Fig. 5. Effect of mulching and fertilizer management on leaf characters of sugar beet

Root length (cm)

The interaction of mulching and nutrient management significantly ($p<0.05$) influenced root length (Figure 6). The longest root (25.53 cm) was obtained with black polythene mulch and M_1F_3. Treatments M_1F_1 and M_1F_2 had similar lengths (21.40 cm and 21.57 cm, respectively). The shortest beet root length (11.43 cm) was observed in the M_0F_0 treatment (no mulch, no fertilizer). Similar results were reported by Maloisane *et al*. (2022) in sugar beet.

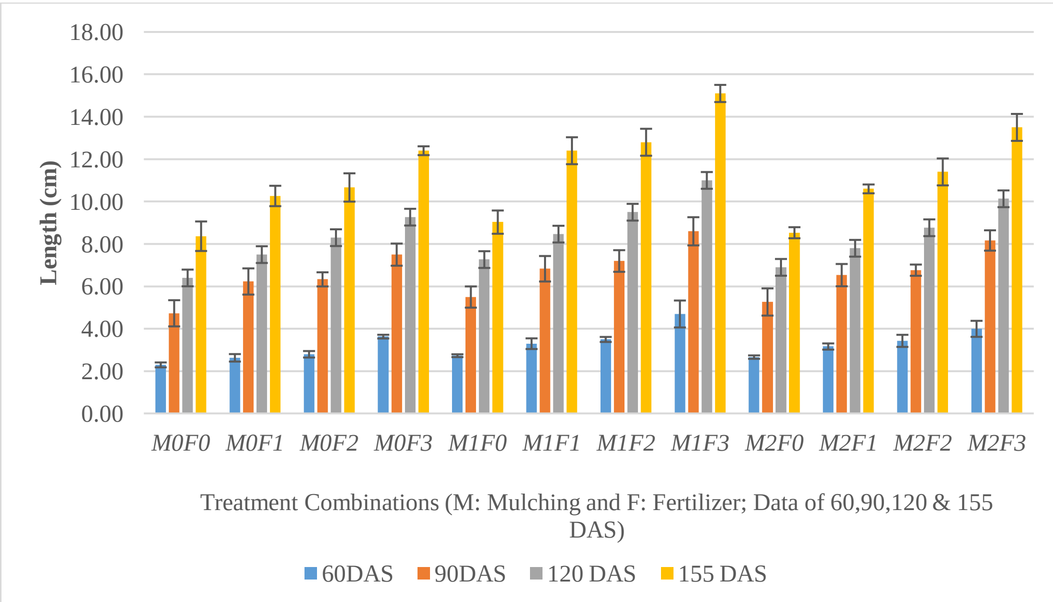


Here, M_0=No mulch, M_1=BPM, M_2=RSM, F_0=Control (No fertilizer), F_1=100% RCF (Recommended chemical fertilizer), F_2=100%Vermicompost @ 10 t/ha, and F_3=50% Vermicompost @ 5 t/ha + 50% RCF.

Fig. 6. Effect of mulching and fertilizer management on root length of sugar beet at different days after sowing

Root diameter (cm)

Sugar beet root diameter increased gradually from 60 DAS to 155 DAS with both individual mulching and fertilizer treatments (Fig. 7). The largest root diameter (15.1 cm) was recorded in the M_1F_3 treatment, followed by 13.5 cm in M_2F_3. The smallest diameter (8.37 cm) was observed in M_0F_0 (no mulch, no fertilizer) at harvest. Similar results were reported by Dulal *et al*. (2021) in radish and Maloisane *et al*. (2022) in sugar beet.



Here, M_0=No mulch, M_1=BPM, M_2=RSM, F_0=Control (No fertilizer), F_1=100% RCF (Recommended chemical fertilizer), F_2=100%Vermicompost @ 10 t/ha, and F_3=50%Vermicompost @ 5 t/ha + 50% RCF.

Fig. 7. Effect of mulching and fertilizer management on root diameter of sugar beet at different days after sowing

Shoot fresh and dry weight (g)

Data on Table 3 represent that, at harvest, the M_1F_3 treatment produced the maximum fresh shoot weight of 450 g, while the M_0F_0 treatment had the lowest (115.33 g). The combined effects of mulching and fertilizer management significantly influenced sugar beet shoot dry weight. Similarly, the M_1F_3 treatment recorded the highest shoot dry weight at 78.6 g, while the control had the lowest at 16.26 g (Table 2). These results align with Alam *et al*. (2007), who found that the best shoot dry matter was obtained with the combined application of vermicompost and chemical fertilizer.

Table 3. Effect of mulching and integrated fertilizer management on plant yield components of sugar beet

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Beet root fresh weight (g)	Root dry weight (g)
M_0F_0	115.33	16.26	390.70	40.31
M_0F_1	169.00	28.09	588.00	101.61
M_0F_2	230.00	32.65	673.70	119.67
M_0F_3	306.00	45.66	803.70	173.95
M_1F_0	179.33	28.77	749.00	109.97
M_1F_1	265.67	37.50	781.00	168.11
M_1F_2	280.67	40.28	966.70b	159.18
M_1F_3	450.00	78.60	1520.70	434.39
M_2F_0	166.00	23.97	568.30	76.58
M_2F_1	253.33	33.56	754.70	130.16
M_2F_2	238.67	40.28	735.70	159.18
M_2F_3	384.00	60.92	1176.70	286.95
CV%	14.29	9.06	13.01	10.69
LSD (0.05)	61.25	6.02	177.47	30.39

Here, M_0=No mulch, M_1: Black polythene mulch, M_2: Rice straw mulch. F_0: Control (No fertilizer), F_1: 100% vermicompost @ 10t/ha, F_2: 100% Recommended chemical dose, and F_3: 50% Vermicompost @ 5t/ha and 50% RCF.

Root fresh and dry weight (g)

At harvest, the M_1F_3 treatment produced the highest root fresh weight of 1520.70 g, while the M_0F_0 treatment had the lowest at 390.7 g (Table 3). Enhancing root quality and output is mostly dependent on diet. The root dry weight (Table 2) of sugar beet was affected by the combined application of vermicompost and RCF during the cropping season. At harvest, the M_1F_3 treatment had the highest root dry weight at 434.39 g, while M_0F_0 had the lowest at 40.31 g. According to Ferdoushi *et al*. (2010), black polythene mulch and a combination of organic and chemical fertilizers produced the highest potato yields.

Sugar beet yield (t/ha)

The combined application of mulching and fertilizer significantly ($P \leq 0.05$) increased sugar beet yield (Table 4). It is evident from the data that the highest sugar beet yield, 121.52 t/ha, was recorded in the M_1F_3 treatment, while the lowest, 31.25 t/ha, was in the M_0F_0 treatment. Gross yield was highest with M_1F_3, 288.86% greater than the control (M_0F_0). Nutrition plays an important role in improving productivity and quality of beet root (Hussain & Kerketta, 2023). The treatment 50% vermicompost and 50% NPK supplies higher macro and micronutrients to the soil and plants get it in the available from which results in better growth, yield and quality of crops (Manivannan *et al.* 2009). Moursy AMM *et al*. (2021) reported that, root yield of sugar beet increased by 16.8 and

51.6%, as well for rice straw mulch and BPFM (black polythene film mulch) treatment compared to no mulch (NM). Black polythene mulch with mineral fertilizer and vermicompost showed the best performance for growth and yield of carrot by Biswas *et al*. (2019).

Sugar yield (t/ha)

The combined effect of mulching and fertilizer management significantly (P≤0.05) influenced sugar yield. Table 4 shows that the highest yield, 18.30 t/ha, was recorded from the M_1F_3 treatment (BPM + 50% VC @ 3 t/ha and 50% RCF), followed by M_2F_3 (13.36 t/ha). Other yields were M_1F_2 (10.69 t/ha), M_1F_1 (9.13 t/ha), and M_0F_3 (8.74 t/ha). The lowest (3.76 t/ha) sugar yield was recorded from control treatment. The best treatment combination (M_1F_3) gave 386.7% increased sugar yield compared to control. Sugar yield increased by 25.8 and 101.3% as well for RSM and BPFM treatment compared to no mulch (Moursy M A *et al*., 2021).

Table 4. The combined effect of mulching and integrated fertilizer management on yield and quality attributes of sugar beet

Treatment combinations	Beet yield (t/ha)	Sugar yield (t/ha)	TSS (°Brix)	Sucrose (%)	Purity (%)
M_0F_0	31.25	3.76	15.00	10.02	66.80
M_0F_1	49.71	6.11	18.60	12.31	66.78
M_0F_2	53.89	6.83	19.00	12.68	66.73
M_0F_3	64.3	8.74	19.60	13.60	69.38
M_1F_0	54.88	7.04	18.00	12.84	71.33
M_1F_1	67.92	9.13	19.50	13.45	68.97
M_1F_2	77.33	10.69	20.07	13.89	69.24
M_1F_3	121.52	18.30	20.60	15.06	73.37
M_2F_0	44.13	5.49	18.00	12.45	69.16
M_2F_1	55.31	6.89	18.80	12.47	66.32
M_2F_2	58.85	8.17	19.30	13.86	71.81
M_2F_3	94.13	13.36	20.17	14.20	70.64
CV%	13.01	13.05	2.95	0.14	0.19
LSD (0.05)	14.19	1.92	0.94	0.03	0.22

Here, M_0=No mulch, M_1: Black polythene mulch, M_2: Rice straw mulch. F_0: Control (No fertilizer), F_1: 100% vermicompost @ 10 t/ha, F_2: 100% Recommended chemical dose, and F_3: 50% Vermicompost @ 5 t/ha and 50% RCF.

Total soluble solid

Total soluble solid (TSS) was significantly (P≤0.05) affected by the combined application of mulching and fertilizer (Table 4). The highest TSS values were 20.60, 20.07 and 20.04°Brix, recorded in the M_1F_3, M_1F_2, and M_2F_3 treatments, respectively.

The lowest TSS (15°Brix) was observed in the M_0F_0 treatment (control). These results highlight that black polythene mulch with 50% vermicompost and 50% RCF produced the highest TSS in sugar beet. The similar result was reported by Kondal *et al*. (2024) on sugar beet.

Sucrose (%)

The combined effects of mulching and fertilizer management significantly ($P \leq 0.05$) impacted sucrose % (Table 4). The highest sucrose level, 15.06%, was found in the M_1F_3 treatment, followed by 14.2% in M_2F_3. Other sucrose levels were M_1F_2 (13.89%), M_2F_2 (13.86%), and M_0F_3 (13.6%). The lowest sucrose level, 10.02%, was in the M_0F_0 treatment (control). Black polythene mulch with 50% vermicompost and 50% RCF yielded the highest sucrose content. These findings were in harmony with those of Moursy *et al*. (2021).

Purity (%)

The purity (%) of sugar beet was influenced by the combined application of mulching and fertilizer, as shown in Table 4. Data show that the highest purity of 73.37% was recorded in the M_1F_3 treatment, followed by 71.81% in M_2F_2. Other treatments had purities of 71.33% (M_1F_0) and 70.64% (M_2F_3). The lowest purity, 66.32%, was found in M_2F_1. Black polythene mulch with 50% vermicompost and 50% RCF produced the highest juice purity. Positive effect of mulching and integrated fertilizer apply on juice quality might be due to the promotional effect metabolic process and translocation of carbohydrates from tops to roots (Helaly *et al.*, 2017).

Correlation matrix

In current study, a positive linear relationship between the growth and yield parameters was observed, the correlation matrix among different plant growth, yield and quality parameters have been presented in Table 5. The correlation matrix showed that plant height of sugar beet had significantly strong and positive correlation with shoot length of plant (r = 0.984***), root length (r = 0.976***), root diameter (r = 0.965***), plant weight (r = 0.941***), shoot fresh weight (r = 0.948***), root fresh weight (r = 0.920***), TSS (^{O}Brix) (r = 0.911***), sucrose% (r = 0.924***), root dry weight (r = 0.881**), shoot dry weight (r = 0.924***), root yield (r = 0.928***), shoot yield of sugar beet (r = 0.949***), and sugar yield (r = 0.918***). These results indicated that root yield of sugar beet depends on plant height, shoot length, root length, root diameter, plant weight of sugar beet, shoot and root fresh weight, shoot and root dry weight of beet. Root fresh weight has a strong correlation with shoot fresh weight (0.991***). The sugar beet quality parameters, such as, brix%, pol% and purity% also correlated with root yield of sugar beet. Some pairs show moderate correlations (e.g., values between 0.5 and 0.7), indicating a weaker but still positive relationship. TSS (^{o}Brix) has moderate correlations with purity% (0.597**) and high correlations with pol% (0.952***). Correlation studies provides a measure of association between the characters and reveals that character that might be useful as an index for selection.

Table 5. Correlation matrix among different parameters of sugar beet as influenced by treatments

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Plant H	1																		
Shoot L	0.984	1																	
Root L	0.976	0.968	1																
Root D	0.965	0.941	0.970	1															
Plant W	0.941	0.965	0.943	0.926	1														
Sh F W	0.948	0.968	0.947	0.932	0.975	1													
R F W	0.920	0.955	0.918	0.895	0.991	0.954	1												
Brix	0.911	0.908	0.873	0.869	0.812	0.830	0.781	1											
Sucrose	0.924	0.945	0.899	0.892	0.875	0.863	0.858	0.952	1										
R DW	0.881	0.914	0.898	0.895	0.980	0.954	0.971	0.733	0.820	1									
Sh D W	0.924	0.947	0.926	0.929	0.983	0.978	0.969	0.789	0.862	0.989	1								
R & S	0.943	0.955	0.943	0.908	0.943	0.894	0.933	0.889	0.923	0.883	0.887	1							
RDM%	0.922	0.943	0.933	0.926	0.994	0.963	0.987	0.771	0.850	0.989	0.986	0.922	1						
Beet Y	0.928	0.954	0.931	0.914	0.998	0.959	0.993	0.796	0.866	0.977	0.974	0.948	0.994	1					
Shoot Y	0.949	0.968	0.947	0.932	0.974	1.000	0.953	0.831	0.863	0.953	0.978	0.893	0.962	0.958	1				
Sugar Y	0.918	0.944	0.926	0.912	0.996	0.954	0.990	0.773	0.856	0.983	0.977	0.936	0.997	0.998	0.953	1			
SPAD V	0.891	0.891	0.902	0.858	0.839	0.863	0.790	0.862	0.880	0.809	0.841	0.874	0.811	0.820	0.864	0.809	1		
Purity	0.597	0.663	0.606	0.599	0.679	0.595	0.700	0.496	0.736	0.699	0.693	0.653	0.694	0.688	0.594	0.712	0.582	1	
nL	0.960	0.980	0.964	0.942	0.979	0.953	0.969	0.849	0.927	0.944	0.956	0.968	0.969	0.976	0.953	0.974	0.881	0.743	1

*** Significant at 0.05% level of probability

Conclusion

The study demonstrates that the combined application of mulching and integrated fertilizer management significantly enhances the growth, yield, and quality of sugar beet. The M_1F_3 treatment (black polythene mulch with 50% vermicompost and 50% RCF) produced the highest values across all growth parameters, including seed germination, plant height, number of leaves, leaf dimensions, root diameter and length, and total dry matter over the treatment combination M_2F_3. This treatment also yielded the highest beet production, sugar yield, TSS, purity, and sucrose percentage. Conversely, the control treatment showed the lowest values in these parameters. These findings highlighted the effectiveness of combined application of M_1F_3 in optimizing sugar beet production and quality.

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Author’s contribution

A. H. M. Solaiman and N. Islam were responsible for the study's conception and design; A. H. M. Solaiman verified the analytical methods; A. H. M. Solaiman, N. Islam, and S. Choudhury encouraged, investigated, and supervised the work's findings; N. Jahan collected, analyzed, and interpreted the data; and F. Hossain, S. Choudhury, and N. Jahan prepared the draft manuscript. Each author evaluated the findings, offered insightful criticism, influenced the direction of the study, and gave their approval to the manuscript's final draft.

Conflict of Interest

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

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EFFECTS OF VERMICOMPOST AND RICE HUSK ASH ON THE YIELD OF SWEET GOURD

M. M. Khanum[1*], M. A. A. A. Muzahid[2], M. Nuruzzaman[1], M. Akter[3] and M. S. Huda[1]

[1]Agronomy Division, Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Rajbari, Dinajpur; [2]Department of Agricultural Extension (DAE), Khamarbari, Dhaka; [3]Soil Science Division, BARI, Gazipur. Bangladesh.

Abstract

A field experiment was carried out at the research field of Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Rajbari, Dinajpur (Latitude: $25^0$388.9″ N, Longitude: $88^0$395.79″ E) during rabi season of 2020-21 and 2021-22 to see the response of vermicompost and rice husk ash on the yield of sweet gourd. Five treatments were included in the experiment as T_1=100% RCF (Recommended Chemical Fertilizer) + Vermicompost 3 t/ha, T_2=100% RCF+ Rice husk ash 2 t/ha, T_3= 100% RCF+ Vermicompost 1.5 t/ha + Rice husk ash 1.5 t/ha, T_4=100% RCF +Vermicompost 3 t/ha +Rice husk 1 t/ha, T_5=100% RCF + 50% extra potassium. The results revealed that the highest fruit yield was recorded in (T_4) 100% RCF +Vermicompost 3 t/ha +Rice husk 1 t/ha followed by that in (T_1) 100% RCF+ Vermicompost 3 tha^{-1} and (T_3) 100% RCF + Vermicompost 1.5 t/ha + Rice husk ash 1. t/ha and the lowest in (T_5) 100% RCF+50% extra potassium. The highest gross return (Tk. 328500 ha^{-1}), as well as gross margin (Tk. 214600 ha^{-1}), was obtained in (T_4) 100% RCF +Vermicompost 3 t/ha +Rice husk 1 t/ha and the lowest gross return (Tk. 151200 ha^{-1}) and gross margin (Tk. 78675 ha^{-1}) were obtained from the treatment (T_5) 100% RCF + 50% extra potassium. The highest BCR (2.88) was also obtained from the treatment (T_4) 100% RCF + Vermicompost 3 t/ha + Rice husk 1 t/ha combination could be effective for overall productivity and monetary return.

Keywords: Benefit-cost**,** Rice husk ash, Sweet gourd, Vermicompost.

Introduction

Bangladesh ranked 3rd in global vegetable production, alongside China and India. Farmers are making a big profit from the cultivation of vegetables, which is changing their lives. Vegetables are gradually acknowledged as crucial for food and nutrition security (Schreinemachers *et al.*, 2018). Today, the cheapest source of vitamins and minerals which are required for good health are vegetables. Sweet gourd is an important vegetable crop grown extensively throughout the tropical and subtropical countries. Due to its high nutritional content and lucrative market price, sweet gourd may be considered as a high-value crop. In our country, both immature and mature fruits are used as vital

* Corresponding author: mahbuba.bari27@gmail.com

ingredient for several culinary preparations. The sweet gourd is rich in carbohydrates and minerals and a cheaper source of vitamins, especially carotenoid pigments, which have a major role in nutrition in the form of pro-vitamin-A, antioxidants, when used at the ripening stage (Dutta *et al.,* 2006). Thus, this vegetable can contribute to improving nutritional status of the people of Bangladesh, particularly the vulnerable group in respect of vitamin-A requirement. It is easy to cultivate and requires limited resources and time, which makes cultivating sweet gourd very profitable. Most vegetables require fertilizer (organic and inorganic) for growth and optimum yield. The nutrient requirement of sweet gourd is generally high due to lots of biomass being produced by the plant (Oloyede *et al.,* 2013). Organic fertilizers release nutrients over time thereby creating a healthy growing environment, while a fast release of nutrients is provided by inorganic fertilizers. Manures are a good source of fertilizer as they are natural products used to provide nutrients to plants by farmers. Inorganic fertilizers application tends to release fast nutrients to sustain soil fertility and crop production and it has led to reduced crop yield, soil acidity, and nutrient imbalance (Agbede *et al.,* 2008; Uyovbisere *et al.,* 2000). Fertility and nutrient status of the soil a major factor for improving crop yield and quality (Kolodziej, 2006). The amount of fertilizer used as a soil amendment, including organic and inorganic fertilizer have a positive impact on the availability of nutrients for the crop and nutrient status of the soil (Bijlsma *et al.,* 2000). Thus, the use of different organic fertilizers solely or in combination with inorganic fertilizers enhanced the yield and quality of crops (Zhang and Fang, 2007). Soil amelioration and improvement via integrated soil fertility management strategy including organic and inorganic fertilizer is a major intervention component that has improved crop production worldwide (Chand *et al.,* 2006; Urmi *et al.,* 2022). Vermicompost enhances the effect of soil microbial activity, increases oxygen supply, retains normal soil temperature, increases soil porosity and water penetration, improves nutrient content, and increases plant growth, yield, and quality (Arora *et al.,* 2011; Rehman *et al.,* 2023; Oyege *et al.,* 2023). In Sri Lanka, rice husk ash is highly available amendment in large quantities. It has reasonable quantities of cations Ca, Mg, K, Na, and other essential elements including P and very little N. Rice husk ash from various locations contains 0.72–3.84% K_2O and 0.23-1.59 MgO . Bronzeoak Ltd (2003) reported that potassium and phosphorous contents of rice husk ash were 0.01-2.69% P_2O_5 and 0.1-2.54% K_2O respectively and the pH was 08.1-11.0. The ash increases the soil pH, thereby increasing available phosphorous, it improves the aeration in the crop root zone, and also increases the water holding capacity and level of exchangeable potassium and magnesium (AICOAF, 2001;Yuan and Xu, 2011; Singh *et al.*, 2018 ; Oladele *et al.,* 2019; Yin *et al.*, 2022). The amount of potassium varies with the temperature and time at which the husk burns, therefore it can be used as a potassium source for crop production. Potassium is one of sixteen essential nutrients required for plant growth and reproduction and it is classified as a macronutrient, as are nitrogen and phosphorus (Marschner, 1995; Rawat *et al.,* 2016; Torabian *et al.,* 2021). Potassium is supplied by inorganic fertilizers such as muriate of potash sulphate of potash or complex fertilizers, or by some organic sources. Potassium is one of the key limiting nutrients for plant growth and development. Due to intensive cropping, mining of potassium from soil reserves is now a great concern to researchers. Rice husk ash is now available in rural areas and is considered a good source of potassium and silica. Sweet gourd is an important and nutritious vegetable in our country. Therefore it needs to evaluate the effect of ash as a source of potassium and silicon on this vegetable. Hence the experiment needs to be conducted to ascertain the effect of potassium and silica on yield components and yield of sweet gourd.

Materials and Methods

Experimental site description

The experiment was conducted at the research field of Agricultural Research Station, BARI, Rajbari, Dinajpur, Bangladesh during winter (rabi*)* season of 2020-21 and 2021-22. The experimental site was located at Latitude: 25.63671^0 N and Longitude: 88.65269^0 E at an elevation of 38 m above mean sea level and it belongs to the Agro-ecological Zone-1 (Old Himalayan Piedmont Plain) in Bangladesh (FRG, 2018). The initial soil sample (0-15 cm) was tested at the Soil Resources Development Institute (SRDI), Dinajpur, Bangladesh. The soil in the experimental area was medium-high and clay loam texture having 2.16% organic matter, pH 6.07, 0.10% total nitrogen (N), 0.14 meq 100 g^{-1} soil potassium (K), 48.16 μg/g phosphorus (P), 8.15 μg/g sulfur (S), 0.89 μg/g zinc (Zn) and 0.35 μg/g boron (B). During crop growth period, Monthly weather data on temperature (maximum and minimum) and total rainfall (mm) were recorded in the both years (Fig.1). The average maximum and minimum temperature in the crop season (November to April) were ranged 22.69°C-33.99°C and 11.15°C-21.76°C during in 2020-21 and 22.25°C-33.43°C and 11.4°C-23.28°C in 2021-22 respectively. The weather of the experimental site is hot sub-humid with total rainfall of 25 mm in 2020-21 and 106 mm in 2021-22 during crop season.

Experimental treatments details

The experiment was laid out in a randomized completely block design (RCBD) with three replications. The unit plot size was 4 m×4 m. Five treatments viz., T_1=100% RCF (Recommended Chemical Fertilizer) +Vermicompost 3 tha^{-1}, T_2=100% RCF+ Rice husk ash 2 t/ha, T_3=100% RCF +Vermicompost 1.5 t/ha + Rice husk ash 1.5 t/ha, T_4=100% RCF +Vermicompost 3 t/ha +Rice husk 1 t/ha, T_5=100% RCF+50% extra potassium was tested.

Crop husbandry

The land of the experimental plot was prepared with a power tiller by ploughing and cross ploughing followed by laddering and the soil was brought into a good tilth. Sweet gourd var. BARI Mistikumra-2 was used in the experiment. Fifteen days old seedlings of sweet gourd were transplanted on 07 November 2020 and 10 November 2021 according to treatments. The land was fertilized with @ $N_{80}P_{36}K_{100}S_{24}Zn_4B_2$ kg/ha respectively (FRG, 2018). The source of N, P, K, S, Zn, and B were urea, triple super phosphate (TSP), Muriate of potash (MoP), gypsum, zinc sulphate, and boric acid, respectively. All organic manure rice husk ash and all PKSZnB were applied in the pit 5-7 days before planting and mixed thoroughly with the soil. N was applied around the plant as a side dressing at 15, 30, 50, and 70 DAT under moist soil conditions and mixed thoroughly with the soil as soon as possible. Following urea application, four irrigations were given. To control powdery mildew on sweet gourd, fungicide Indofil M @ 0.2% was sprayed at every 15 days interval. Pheromone traps (Cue lure) @ 100 traps per hectare were used to control cucurbit fruit fly in the sweet gourd field from 30 days after planting till the sweet gourd harvest. (Cork *et al.*, 2003). Sweet gourd harvesting started at 120 DAT and was carried out four times. Yield components of sweet gourd were taken from randomly selected 4 plants from each plot. Fruit yields were taken from the whole plot. Collected data were analyzed statistically by using R software packages and mean differences for each character were compared by the Least Significant Difference (LSD).

Table 1. Nutrient status of vermicompost and rice husk ash used in experiment field

Name of the manure	PH	OM	K	Total N	P	S	B	Zn
					%			
Vermicompost	5.3	14.2	0.30	1.71	0.304	0.698	0.008	0.014
Rice husk ash	5.5	12.3	0.70	0.52	0.518	0.586	0.005	0.012

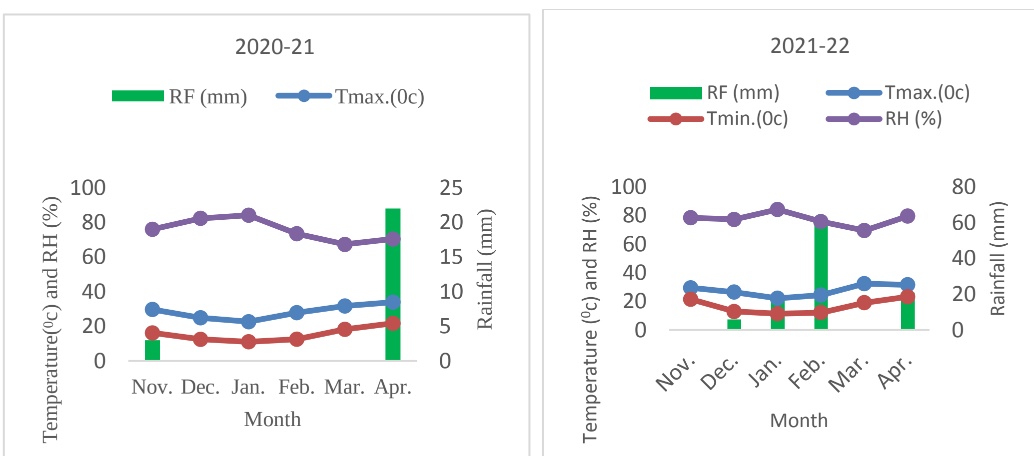


Fig. 1. Monthly average maximum temperature, minimum temperature, Relative Humidity and rainfall during the growing period (2020-21 and 2021-22)

Results and discussion

Yield and yield contributing characters of sweet gourd

The vine length at 1st fruiting, fruits plant^{-1}, fruit length and diameter, flesh thickness, single fruit weight, and fruit yield of sweet gourd had significant differences among the fertilizer doses except for days to 1st male and female flowering (Table 1). The plant required 51.00-56.50 days for 1st male flowering and 67.66-72.66 days for 1st female flowering. The longest length of the vine was recorded from T_4 (283.67 cm) followed by T_1 (250.31 cm) and the shortest vine was recorded from T_5 (210.22 cm) at 1st fruiting. This is in agreement with the findings of Bello *et al.* (1995). The highest number of fruits plant^{-1} (5.08) was obtained from T_4 followed by T_1 (4.70), while the lowest number of fruits plant^{-1} (3.43) was found in the treatment T_5. The treatment T_4 gave the highest fruit length (28.66 cm) and diameter (21.06 cm) which was followed by the T_1 treatment (27.20 cm length and 20.54 cm diameter) and the lowest fruit length (24.33 cm) and diameter (17.93 cm) were measured in T_5. The highest single fruit weight (2714.03 g) was observed from T_4 followed by T_1 (2106.54 g). Nahar (2016) stated that the fruit length and diameter were positively and significantly correlated with individual fruit weight. Fruit yield was recorded as the highest in T_4 (32.85 tha^{-1}) followed by T_1 (25.34 tha^{-1}) and T_3 (21.18 t/ha) and producing the lowest in T_5 (15.12 t/ha). The results expressed that a higher fertilizer dose increased the fruit yield of the sweet gourd as compared to the recommended dose of fertilizers. The results are in agreement with the findings of other researches (Anonymous, 2017 and Rathod, 2018).

Table 2. Yield and yield contributing character of sweet gourd at different treatment combinations (pooled data of 2 years)

Treatments	1st male flower (days)	1st female flower (days)	Vine length at 1st fruiting (cm)	Fruits/ Plant (no.)	Fruit length (cm)	Fruit diameter (cm)	Flesh thickness (cm)	Single fruit wt. (g)	Yield (t/ha)
T_1	51.33	68.66	250.31	4.70	27.20	20.54	4.36	2106.54	25.34
T_2	52.33	71.00	230.86	3.80	25.86	18.56	3.87	1841.60	16.58
T_3	51.33	69.33	246.33	4.33	26.40	20.17	4.12	1960.26	21.18
T_4	51.00	67.66	283.67	5.08	28.66	21.06	4.50	2714.03	32.85
T_5	56.50	72.66	210.22	3.43	24.33	17.93	3.65	1760.90	15.12
LSD (0.05)	2.54	6.75	17.09	0.68	2.94	1.20	0.33	275.92	2.63
CV (%)	2.57	5.13	3.72	8.37	1.48	3.21	4.31	6.98	6.29

T_1=100% RCF+ Vermicompost 3 tha^{-1}, T_2=100% RCF+ Rice husk ash 2 tha^{-1}, T_3=100% RCF + Vermicompost 1.5 tha^{-1}+ Rice husk ash 1.5 tha^{-1}, T_4=100% RCF +Vermicompost 3 tha^{-1}+Rice husk 1 tha^{-1}, T_5=100% RCF + 50% extra potassium

Correlation study

The correlation coefficient among different characters has been presented in Table 3. Fruit yield obtained a positive response in all the characters, whereas a significant positive correlation was showed with vine length at 1st fruiting (0.83***), fruit/plant (0.89***), fruit diameter (0.80***), flesh thickness (0.87***) and Single fruit weight (0.93***). Single fruit weight and flesh thickness showed a significant positive correlation in almost all the characters except fruit length (0.36). Fruit diameter showed a strong positive and significant relationship in all the traits, except fruit length (0.24). Fruit length showed a positive and insignificant relationship in all the traits. Fruit per plant showed a positive and significant relationship with almost entire the study traits, except fruit length (0.19).

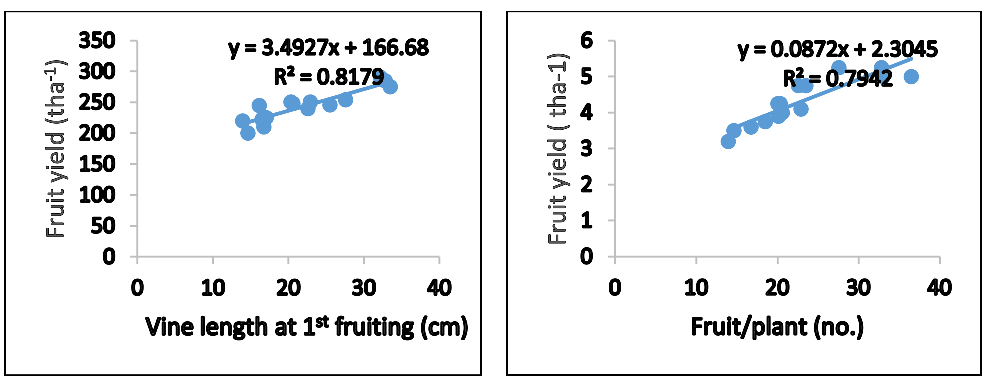
Table 3. Correlation among the study characters

	VL	FPP	FL	FD	FT	SFW	FY
VL	1						
FPP	0.82***	1					
FL	0.19	0.24	1				
FD	0.79***	0.86***	0.24	1			
FT	0.83***	0.86***	0.26	0.80***	1		
SFW	0.86***	0.68**	0.36	0.65**	0.65**	1	
FY	0.90***	0.89***	0.35	0.80***	0.87***	0.93***	1

*significant at = 0.05; **significant at = 0.01; ***significant at = 0.001; VL= Vine length; FPP= Fruit per plant; FL= fruit length; FD=Fruit diameter; FT=Flesh thickness; SFW= Single fruit weight; FY= Fruit yield

The functional relationship of different yield contributing traits on bulb yield of sweet gourd

The functional linear analysis was performed using the data of yield contributing characters along with the fruit yield. From the relationship, it was displayed that the studied characters had a positive contribution to the fruit yield, which indicated that the fruit yield was dependent on those characters. To evaluate the role of those characters, linear regressions were done. The results exposed that yield contributing characters like vine length at 1st fruiting, number of fruits per plant, fruit length, fruit diameter, flesh thickness, and single fruit weight accounted for 82, 79, 12, 76, 65 and 88% of the total fruit yield variation, respectively (Fig. 2).



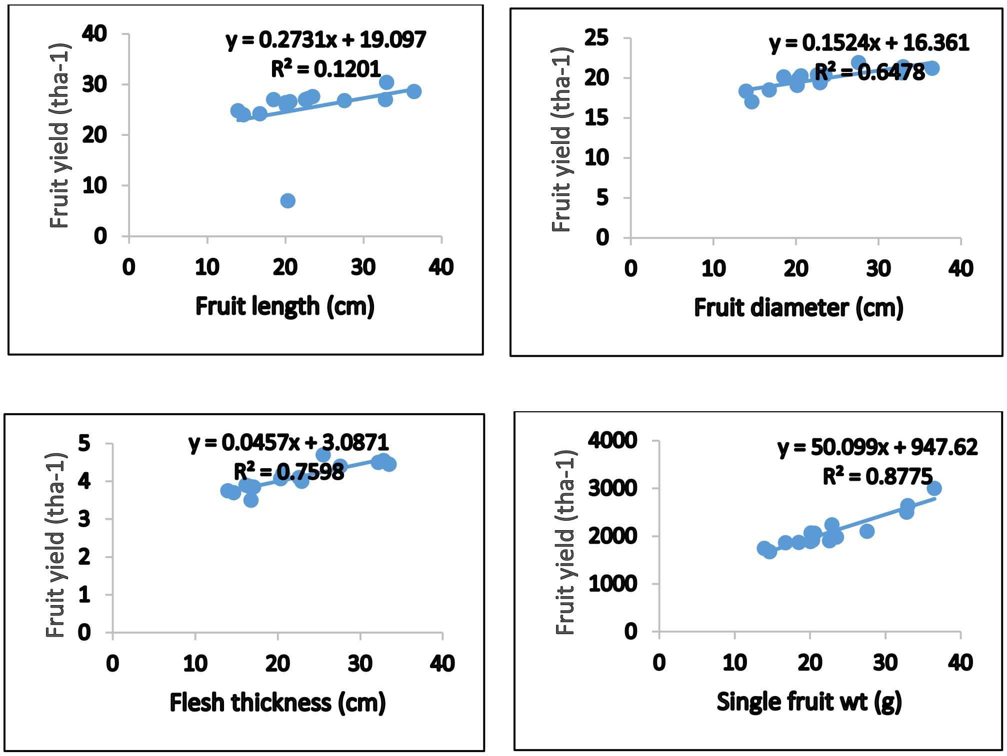


Fig. 2. Relationship of different yield contributing characters on fruit yield of sweet gourd

Cost and return

The maximum gross return was obtained from T_4 treatment (Tk. 328500 ha^{-1}) followed by T_1 treatment (Tk. 253400 ha^{-1}) and T_3 treatment (Tk. 211800 ha^{-1}), while the minimum (Tk. 151200 ha^{-1}) from T_5 treatment (Table 4). The highest gross margin was also recorded from the T_4 treatment (Tk. 214600 ha^{-1}) followed T_1 treatment (Tk. 141000 ha^{-1}) and T_3 treatment (Tk. 114900 ha^{-1}), while the lowest (Tk. 78675 ha^{-1}) was from T_5 treatment. The benefit-cost ratio (BCR) was also the highest in the T_4 treatment (2.88) followed by T_1 (2.25) and T_3 (2.19) treatments, while the lowest in local T_5 (2.08) treatment.

Table 4. Cost and return for sweet gourd cultivation at ARS, Rajbari, Dinajpur

Treatments	Gross return (Tk ha^{-1})	TVC (Tk ha^{-1})	Gross margin (Tk ha^{-1})	BCR
T_1	253400	112400	141000	2.25
T_2	165800	77400	88400	2.14
T_3	211800	96900	114900	2.19
T_4	328500	113900	214600	2.88
T_5	151200	72525	78675	2.08

Sweet gourd @ Tk. 10 kg^{-1}

Conclusion

The results revealed that the highest fruit yield was recorded in (T_4) 100 % RCF +Vermicompost 3 t/ha + Rice husk 1 t/ha followed by that in (T_1) 100 % RCF + Vermicompost 3 tha^{-1} and (T_3) 100 % RCF + Vermicompost 1.5 t/ha + Rice husk ash 1.5 t/ha and the lowest in (T_5)100 % RCF + 50% extra potassium. The highest gross return (Tk. 328500 ha^{-1}) as well as gross margin (Tk. 214600 ha^{-1}) was obtained in (T_4) 100 % RCF + Vermicompost 3 t/ha + Rice husk 1 tha^{-1} and the lowest gross return (Tk. 151200 ha^{-1}) and gross margin (Tk. 78675 ha^{-1}) were obtained from the treatment (T_5) 100 % RCF + 50% extra potassium. The highest BCR (2.88) was also obtained from the treatment (T_4) 100 % RCF +Vermicompost 3 t/ha + Rice husk 1 t/ha. The overall results indicated that among the treatments (T_4) 100 % RCF + Vermicompost 3 t/ha + Rice husk 1 t/ha and (T_1) 100 % RCF + Vermicompost 3 t/ha were found suitable for total productivity and economic return of the system.

Author's contribution

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Conflicts of Interest

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

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EFFECT OF SULPHUR AND BORON ON THE GROWTH AND SEED YIELD OF FENUGREEK (*Trigonella corniculata* L.)

M. Khatun[1], K. Khatun[1], T. Mostarin[1], M. J. Hasan[2], M. K. A. Nadim[3], S. M. A. Chowdhury[4] and S. E. Akter[5*]

[1]Department of Horticulture, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka; [2]Entomology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh; [3]Biotechnology Division, BINA, Mymensingh; [4]Agronomy Division, BINA, Mymensingh; [5]Crop Physiology Division, BINA, Mymensingh. Bangladesh.

Abstract

A field experiment was conducted at Horticulture farm of Sher-e-Bangla Agricultural University, Dhaka, during the Rabi season of November, 2021 to April, 2022 to study the effects of different level of sulphur and boron on the yield and yield components of fenugreek (*Trigonella corniculata*) seed. The experiment consisted of two factors: Factor A: 4 Levels of sulphur (S); $T_0 = S_0$ kg ha^{-1}, $T_1 = S_{10}$kg ha^{-1}, $T_2 = S_{15}$kg ha^{-1}, $T_3 = S_{20}$kg ha^{-1} and Factor B: 4 levels of boron (F); $F_0 = B_0$kg ha^{-1}, $F_1 = B_{1.0}$kg ha^{-1}, F2 = $B_{1.5}$kg ha^{-1}, $F_3 = B_{2.0}$kg ha^{-1}. The experiment was laid out in randomized complete block design (RCBD) with three (3) replications. Data on different growth, yield contributing and yield parameter of fenugreek were recorded and significant variation was observed from different treatments. T_3F_3 treatment showed better performance over other treatment combination. Maximum plant height (50.08 cm), number of primary branches per plant (10.41), secondary branches per plant (5.09), number of seeds per pod (8.91), number of pod per plant (1192.30), weight of seeds per plant (2.50 g), weight of seed per plot (75.00 g), maximum seed yield per hectare (625.00 kg) was obtained from T_3F_3 treatment combination.

Keywords: Chlorophyll, Combination, Fenugreek, Flowering, Micronutrient.

Introduction

Fenugreek (*Trigonella corniculata* L.) is a semi-arid crop belonging to the family Fabaceae. It is commonly known as ‘Champamethi’ and ‘Marwari methi’, is a diffused sub erect and strongly scented annual herb. In Bangladesh, it is also known as Firingi. The green leaves contain several alkaloids like trigonelline, choline, gentianine and carpain. The leaves and seeds of fenugreek are used as spice and condiments and as flavoring agents due to their characteristic pleasant odor. Improper nutrient management is one of the major reason which causes lower yield and poor quality seed in fenugreek. So, the integrated nutrient management approach could be a rational way to increase herbage yield and seed quality. Sulphur is a plant nutrient with a crop requirement similar

[*] Corresponding author: sayedeshtiak@bina.gov.bd

to that of phosphorus. Sulphur is essential for production of protein, fats and oils, promotes enzyme activity and helps in chlorophyll formation, improves root growth and grain filling resulting in vigorous plant growth. It has been observed when Sulphur is present in critical amount of soil (less than 10 ppm), the plant growth, quality and total production of crop is adversely affected (Jones *et al*., 1972). Sulphur also helps in improving the nutrient content and uptake of nutrients in legume crops (Singh and Singh, 1992). Protein, tryptophan, lysine, methionine, globulin, and egg white content all fundamentally increased with rising sulfur levels up to 30 kg ha^{-1}. Boron (B) is the only non-metal among the plant essential micro-nutrients, quite rare and occurs chiefly as borates of calcium and sodium. Boron deficiency has been proved to be of the major constraints for crop production. Boron is involved directly and indirectly in the cell growth of new shoots and root as it is also highly important for boll formation, flowering, pollination, and seed development (Dordas *et al*., 2007). It also increases the utilization of macro-nutrients by plants and promotes the translocation of photosynthetic products from the source towards the sink during the crop life cycle (Ali *et al*., 2009). Studies revealed that deficiency of boron cause prominent reduction of growth, nodulation, yield percentage, vigour and viability in legume and cereal crops (Ahmad *et al.*, 2012). Boron supply increases the uptake and reutilization of N, P, K, Na, Ca and other (Yaseen *et al*., 2004). Under the above mention context and situation, the present experiment was conducted to find out the optimum level of sulphur and boron for maximum growth and seed yield of fenugreek.

Materials and Methods

The experiment was conducted at the Horticultural Farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from November, 2021 – April, 2022 to find out the optimum level of sulphur and boron for maximum growth and seed yield of fenugreek. The seeds of Fenugreek (var. BARI firingi-1) were collected from Horticultural Division of Bangladesh Agricultural Research Institute (BARI), Gazipur. The experiment consists of two factors i.e., Factor A: 4 Levels of Sulphur; $T_0 = S_0$kg ha^{-1}(Control), $T_1 = S_{10}$kg ha^{-1}, $T_2 = S_{15}$kg ha^{-1} and $T_3 = S_{20}$kg ha^{-1}; Factor B: 4 levels of Boron; $F_0 = B_0$kg ha^{-1}(Control), $F_1 = B_{1.0}$kg ha^{-1}, $F_2 = B_{1.5}$kg ha^{-1} and $F_3 = B_{2.0}$kg ha^{-1}.

Table 1. Chemical characteristics of the initial soil of the experimental field

pH	6.48
Organic Matter (%)	0.86
Total N (%)	0.079
Total P (ppm)	15
Exchangeable K (meq/100 g dry soil)	0.12
Available S (meq/100 g dry soil)	0.119

The experiment was laid out in Randomized Complete Block Design (RCBD) with 3 replications. The size of unit plot was 1.2 m × 1 m. Manures and fertilizers were applied such as Cowdung, @ 2 t ha^{-1}, Urea 125, TSP 100, MoP 100 kg ha^{-1}. The desired population density was maintained by thinning plants. Irrigation, mulching, weeding and plant protection measures etc., were performed for proper plant growth. Data were recorded on plant characters, yield and yield attributes. Land preparation, fertilizer, irrigation and labor costs for all treatments, from seeding to harvesting, were recorded per experimental plot and converted to cost per hectare The cost was calculated using market rates. Data were analyzed using analysis of variance (ANOVA) technique with the help of R software package (version: 4.2.2) and the mean differences were adjudged by least significant difference test (LSD) at 5% level of probability Gomez and Gomez (1984).

Results and Discussion

Combined effect of sulfur and boron showed statistically significant variation on plant height (Table2). The longest plant height (50.08 cm) was recorded at T_3F_3 (S_{20} kg ha^{-1} + $B_{2.0}$kg ha^{-1}) while the lowest (43.00 cm) at T_0F_0 treatment (Table 2). These results obtained are closely similar by Mehta *et al.* (2013). Number of maximum primary branches $plant^{-1}$ was observed from T_3F_3 (10.41) while T_0F_0 (control) showed minimum number of primary branches $plant^{-1}$. Number of maximum secondary branches $plant^{-1}$ (5.09) was observed from T_3F_3 followed by T_3F_2 treatment but T_0F_0 (control) treatment combination showed minimum number of secondary branches $plant^{-1}$.The T_3F_3 treatment took minimum days to flower initiation (43 days) which was almost similar to T_3F_2 treatment (44 days) while the control treatment took maximum days (53 (Table 2).This might be due to fact that adequate supply of sulphur and boron can promote the growth and development of reproductive organs and improved metabolic activities resulting in earlier flowering. Lal (2015) stated earlier that increased level of sulphur and boron influence in early flowering of legume plants. Both the control application dose combination of sulphur with boron took maximum days to 50% flowering where control treatment took maximum (69 days) to 50% flower initiation. The highest level of sulphur with boron application dose took minimum days for 50% flowering but T_3F_3 treatment took minimum (61 days) to 50% flowering (Table 2). The t study was observed that increased level of Sulphur and boron reduced the time to 50% flowering .Similar results was recorded by Kalaiyarasan *et al.*(2020) that effect between sulphur with boron was significant on growth attributes at all stages of crop growth.

Table 2. Effects of sulphur and boron on plant characters, days to first and 50% flowering of fenugreek

Treatment	Plant height (cm)	Primary branches $plant^{-1}$ (no.)	Secondary branches $plant^{-1}$ (no.)	Days to first flowering	Days to 50% flowering
T_0F_0	43.00 o	7.14 l	2.94 bc	53.17 a	68.58 a
T_0F_1	43.48 no	7.49 kl	3.02 bc	50.2 de	66.18 de
T_0F_2	44.88 kl	8.07 ij	3.22a-c	47.27 gh	64.30 hi
T_0F_3	45.83 ij	8.27 h-j	3.58a-c	45.43 jk	62.89 kl
T_1F_0	43.94mn	7.75 jk	2.88 c	52.27 b	67.59 b
T_1F_1	46.32 hi	8.63 f-h	3.76a-c	49.67 ef	65.71 ef

Treatment	Plant height (cm)	Primary branches $plant^{-1}$ (no.)	Secondary branches $plant^{-1}$ (no.)	Days to first flowering	Days to 50% flowering
T_1F_2	47.26 fg	9.05 d-f	4.12a-c	46.63 hi	63.83 ij
T_1F_3	48.18 de	9.38 c-e	4.49a-c	44.80kl	62.42 lm
T_2F_0	44.32 lm	7.87 jk	3.04 bc	51.43 bc	67.12 bc
T_2F_1	46.76 gh	8.84 e-g	3.94a-c	49.07 f	65.24 fg
T_2F_2	48.63 cd	9.58 b-d	4.66a-c	46.00ij	63.36 jk
T_2F_3	49.12 bc	9.79 bc	4.85 ab	44.27 lm	61.95 mn
T_3F_0	45.32 jk	8.45g-i	3.40a-c	50.83 cd	66.65 cd
T_3F_1	47.71 ef	9.21 de	4.31a-c	47.83 g	64.77 gh
T_3F_2	49.57 ab	9.97 ab	5.05 a	43.63 mn	61.47 no
T_3F_3	50.08 a	10.41 a	5.09 a	43.00 n	61.07 o
LSD (0.05)	0.61	0.28	0.98	0.43	0.37
CV%	4.02	5.36	4.56	8.25	7.25

Here, T_0=control (no sulphur), $T_1=S_{10}$kg ha^{-1}, $T_2=S_{15}$kg ha^{-1}, $T_3=S_{20}$kg $ha^{-1}$and F_0 = control (no boron), F_1= $B_{1.0}$kg ha^{-1}, F_2= $B_{1.5}$kg ha^{-1}, F_3 = $B_{2.0}$kg ha^{-1}

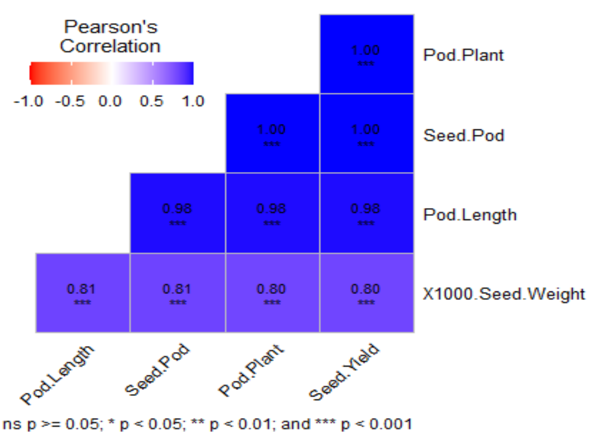
The difference in pod number per plant was significantly influence by application of sulphur and boron t (Table 3). The maximum pod number $plant^{-1}$ (1192.30) was recorded from T_3F_3 which was statistically similar to T_3F_2 (1160.30). On the other hand, the lowest pod number $plant^{-1}$ (722.0) was found from T_0F_0 (control) (Table 3). Maximum length of the pod was noticed with T_3F_3 (2.51 cm), while the minimum length of pod was 1.71 from treatment T_0F_0.The number of seeds per pod ranged from 5.10 to 8.91. The maximum number of seeds per pod (8.91) was obtained from the T_3F_3which was statistically similar (8.66) to T_3F_2.The lowest number of seeds per pod (5.10) was obtained from the T_0F_0 treatment. This might be due to optimum sulphur and boron attributed to lesser flower drop and enhanced pollen germination and pollen tube growth probably restricted fertilization. Treatment T_3F_3 recorded higher 1000- green seeds weight (1.76 g) and lowest weight in T_0F_0 (control) treatment (1.45 g) which was statistically similar to T_0F_1 (1.47).Wide variation was found with the application of different levels of sulphur and boron in seed yield per hectare (Table 3). It ranged from 463.70 to 625.00 kg per hectare. The highest average seed yield of 625.00 kg ha^{-1} was obtained in the T_3F_3while the lowest yield of 463.70 kg ha^{-1} from the T_0F_0 treatment. Shivran (2000) reported that combined application of 20 kg sulphur and 2 kg boron ha^{-1} significantly increased plant height, number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of seeds/pod, 1000-seed weight and seed yield of Fenugreek.

Table 3. Effect of sulphur and boron on yield and yield attributes of Fenugreek

Treatment	Pods plant^{-1} (no.)	Pod length(cm)	Seed pod^{-1} (no.)	1000- Seed weight (g)	Seed yield (kg ha^{-1})
T_0F_0	722.00 o	1.71	5.10 o	1.45	463.70 o
T_0F_1	753.40 no	1.76	5.27 no	1.47	473.53 no
T_0F_2	847.40 kl	1.90	6.05 kl	1.53	506.23 kl
T_0F_3	910.00 ij	2.07	6.57 ij	1.58	527.93 ij
T_1F_0	783.20 mn	1.81	5.60mn	1.50	485.20 mn
T_1F_1	941.80 hi	2.06	6.82 hi	1.60	538.60 hi
T_1F_2	1003.80 fg	2.16	7.35 fg	1.63	560.33 fg
T_1F_3	1066.90 de	2.26	7.87 de	1.68	581.73 de
T_2F_0	815.70 lm	1.86	5.79 lm	1.51	494.50 lm
T_2F_1	973.20 gh	2.12	7.08 gh	1.62	549.40 gh
T_2F_2	1098.00 cd	2.33	8.13 cd	1.70	592.97 cd
T_2F_3	1129.80 bc	2.39	8.39 bc	1.72	603.80 bc
T_3F_0	878.90 jk	1.96	6.30 jk	1.56	517.00 jk
T_3F_1	1035.40 ef	2.21	7.61 ef	1.66	571.17 ef
T_3F_2	1160.30 ab	2.45	8.66 ab	1.74	614.97 ab
T_3F_3	1192.30 a	2.51	8.91 a	1.76	625.00 a
LSD (0.05)	6.27	1.02 ^{NS}	2.64	0.36 ^{NS}	9.78
CV%	4.95	5.36	6.45	7.25	8.75

Here, T_0=control (no sulphur), $T_1=S_{10}$kg ha^{-1}, $T_2=S_{15}$kg ha^{-1}, $T_3=S_{20}$kg ha^{-1}and F_0 = control (no boron), $F_1= B_{1.0}$kg ha^{-1}, $F_2= B_{1.5}$kg ha^{-1}, $F_3 = B_{2.0}$kg ha^{-1}

From the correlation study, it was observed that yield showed significant and positive correlation with podsplant^{-1} (no.), Seedspod^{-1} (no.), pod length (cm) and seed yield (kg ha^{-1}) (Fig. 1). Latye *et. al*. (2 016) was also found the similar results.



Here, Pod.Plant- Pod plant^{-1} (no.), Seed.Pod- Seed pod^{-1} (no.), Pod.Length- Pod length (cm), X1000.Seed.Weight- Seed yield (kg ha^{-1})

Fig. 1. Correlation among the yield contributing attributes of fenugreek.

In the combination of different levels of sulphur and boron, maximum gross return (Tk. 61803) was obtained from the T_3F_3 treatment while lowest gross return (Tk. 36819) was obtained in T_0F_0 treatment**.** The highest gross margin (Tk 109375) was obtained from the T_3F_3 treatment and second highest gross margin (Tk. 107620) was obtained in T_3F_2. The lowest gross margin (Tk. 81148) was obtained from the treatment combination of T_0F_0. Variation was observed in BCR of different level of nutrient combination. The highest benefit cost ratio (2.30) was attained from the T_3F_3 treatment and the lowest benefit cost ratio (1.83) was obtained from of T_0F_0 (Table 4).

Table 4. Cost and return analysis of different levels of sulphur and boron application of Fenugreek

Treatment	Gross return (Tk.)	Cost of production(Tk)	Gross margin (Tk.)	BCR
T_0F_0	36819	44329	81148	1.83
T_0F_1	38369	44499	82868	1.86
T_0F_2	43922	44668	88590	1.98
T_0F_3	47550	44838	92388	2.06
T_1F_0	39214	45696	84910	1.86
T_1F_1	48389	45866	94255	2.06
T_1F_2	52023	46035	98058	2.13
T_1F_3	55598	46205	101803	2.20
T_2F_0	40158	46380	86538	1.87
T_2F_1	49596	46549	96145	2.07
T_2F_2	57051	46719	103770	2.22
T_2F_3	58777	46888	105665	2.25
T_3F_0	43411	47064	90475	1.92
T_3F_1	52722	47233	99955	2.12
T_3F_2	60217	47403	107620	2.27
T_3F_3	61803	47572	109375	2.30

Here, T_0=control (no sulphur), $T_1=S_{10}$kg ha^{-1}, $T_2=S_{15}$kg ha^{-1}, $T_3=S_{20}$kg $ha^{-1}$and F_0 = control (no boron), $F_1= B_{1.0}$kg ha^{-1}, $F_2= B_{1.5}$kg ha^{-1}, $F_3 = B_{2.0}$kg ha^{-1}. Total cost of production was done in details according to the procedure of Alam *et al.* (1989).

Where, Sale of marketable yield at175 Tk/kg. Gross income = Marketable yield × Tk/kg, Gross margin = Gross income **-**Total cost of production. Benefit Cost Ratio (BCR) = Gross return ÷ Cost of production

Conclusion

The highest growth, yield and yield contributing parameter was recorded from S_{20} with $B_{2.0}$ kg ha^{-1}along with Cowdung,@ 2 t ha^{-1}, Urea 125,TSP100, MoP100kg ha^{-1}.

Further study may be needed at different level of sulphur and boron combination in relation to growth and seed yield and quality performance of fenugreek in different agro-ecological zones (AEZ) of Bangladesh.

Author's contribution

M. Khatun, K. Khatun, and T. Mostarin designed and developed the study; M. Khatun collected the data; M. Khatun, K. Khatun, T. Mostarin, and S. E. Akter analyzed and interpreted the results; and M. J. Hasan, M. K. A. Nadim, S. M. A. Chowdhury, and S. E. Akter prepared the draft paper. The results were evaluated by all authors, who then approved the final version of the paper.

Conflicts of Interest

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

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EFFECTS OF BORON AND CALCIUM ON SEED YIELD AND QUALITY OF ONION

M. K. Islam[1*], M. G. Morshed[2], J. Uddin[2], S. Nasrin[1], A. Hasan[1] and M. A. S. Jiku[3]

[1]Breeding Division, Bangladesh Jute Research Institute (BJRI), Manik Mia Avenue, Dhaka; [2]Department of Horticulture, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka; [3]Agronomy Division, BJRI, Manik Mia Avenue, Dhaka. Bangladesh.

Abstract

A field experiment with onion var. BARI piaz1 was conducted in the research farm of Sher-e-Bangla Agricultural University, Dhaka, during the winter season (October to February) to find out the response of boron and calcium on seed yield and quality of onion. The experiment was carried out with four levels of boron viz., B_0 (0 ppm), B_1 (500 ppm), B_2 (1000 ppm) and B_3 (2000 ppm); and three level of calcium viz., Ca_0 (0 ppm), Ca_1 (2500 ppm) and Ca_2 (5000 ppm). This experiment was laid out in RCBD with three replications. The results showed that the treatments significantly influenced the plant height and number of leaves at all growth stages except harvesting time. At harvest, boron and calcium significantly increased the umbel per plot, flowers per umbel, fruits per umbel, fruit set (%), yield per plant, 1000-seed weight, seed yield per ha, germination and normal seedling percentage; and reduced the abnormal seedling percentage. Combined application of boron and calcium also significantly increased the all above parameters mentioned except abnormal seedling percentage of onion. The highest result of all parameters including yield showed from B_3Ca_2 but this treatment reduced production of abnormal seedling. Besides, combined of boron and calcium treatment B_3Ca_2 gave the highest plant height, leaf number and chlorophyll content. The application of 2000 ppm of boron together with 5000 ppm of calcium (B_3Ca_2) treatment gave the best performance showing the yield and quality of onion seed.

Keywords: Boron, Calcium, Onion seed, Quality, Yield.

Introduction

In Bangladesh, onion stands out as the foremost spice crop in terms cultivation area of 86,429 hectares and total onion production is 5,89,410 m tons. BBS (2017) reported that the average yield of onion in Bangladesh was 10.27 tons per hectare being produced from1.85 lakh hectares of land in 2016-17 with production 19 lakh tons of onion but imported 15 lakh tons of onion in the same year and there was a shortage of 8.05 lakh tons in that particular year. Seeds are produced by limited number of farmers in particular areas such as Faridpur, Natore and Rajshahi districts of Bangladesh (BBS, 2016). Bangladesh requires some 1,300 tons of onion seed per year, of them, about 300

* Corresponding author: kamrulmk717@gmail.com

tons are managed through farmer-to-farmer exchange while seed firms can supply only 100 tons and the remaining seed from import (BBS, 2017). There are still opportunities to increase the yield of onion seed by modifying the cultivation practices including fertilization in particular with boron and calcium and also by adopting cultural management practices.

Boron is a vital micronutrient for increasing the onion seed production. Many researchers have the opinion that some secondary and trace elements like boron (B) and manganese (Mn) can play vital role in escalating the yield of onion seed (Rao and Deshpande, 1971). Micronutrients play a vital role in the plant metabolic process from cell wall development to respiration, photosynthesis, chlorophyll formation, enzymes activity, nitrogen fixation etc., they play an essential role in improving yield and quality (Alam *et al*., 2010). Manna and Maity (2016) reported that foliar applications of micronutrient such as boron was effective in improving growth, yield and quality of onion. Boron at different doses had significant effects on the production of leaf, plant height, root numbers, seed yield and 1000- seeds weight (Bhonde *et al.,* 1999). Macronutrient like Calcium, plays a vital role in crop growth and development. It maintains cell wall structure, stabilizes membranes, enhances pollen germination, regulates enzymes, and promotes overall plant health and vigor. Additionally, calcium boosts root growth, early crop development, and disease resistance. Sullivan *et al.* (1974) showed that calcium deficiency has also been shown to decrease seed quality by inhibiting plumule development.

Onion var. BARI piaz1 is a popular onion variety extensively cultivated in Bangladesh. Its seeds are primarily produced using the bulb to seed method in specific regions. However, the impact of plant nutrients, especially boron (B) and calcium (Ca), on the seed production of BARI piaz1 in Bangladesh on seed production in Bangladesh is currently lacking. This study aims to investigate the impact of varying levels of B and Ca on the growth, yield, and quality of onion seed production.

Materials and Methods

The experiment was conducted at the Experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, from October 2018 to February 2019. The soil, classified as non-calcareous dark grey soil, belonged to the Madhupur tract (AEZ No. 28), with a pH value of 5.7. The experimental area experienced a sub-tropical climate characterized by high temperature, humidity, heavy precipitation, and occasional winds. Onion var. BARI piaz1 is a Rabi season crop with a life cycle of 130-140 days, reaching a height of 50-55 cm. Its yield ranges from 12-16 tons of bulbs per hectare and 800-1000 kg of seeds per hectare. The experiment was followed a Randomized Completely Block Design (RCBD) with three replications. The trial was conducted with four levels of boron. 1.0 ppm (B_0), 2. 500 ppm (B_1), 3.1000 ppm (B_2) 4. 2000 ppm (B_3). The source of boron was borax ($Na_2 B_4O_7.10 H_2O$) and three levels of calcium. 1. 0 ppm (Ca_0), 2. 2500 ppm (Ca_1) 3. 5000 ppm (Ca_2). The source of calcium was gypsum. Medium-sized bulbs, approximately 3.5 cm in diameter, were planted in 6 rows with 7 bulbs each. A total of 42 bulbs were planted per 1.5m^2 plot on October 16, 2018, maintaining row and bulb distances of 25 cm and 20 cm, respectively. Ca or damaged bulbs were replaced with healthy plants from the border as needed. Weeding and mulching were performed to maintain soil moisture and aeration. The crop was irrigated eight times by using a water can. Control measures were applied against cutworms and field crickets. Germination tests for harvested seeds were conducted in the laboratory using petri dishes and filter paper soaked with water. Data collection occurred 10 days after germination, with seedlings classified as normal or abnormal following ISTA guidelines (1999).

Plant height and no. of leaves was measured from 10 sample plants at 30, 45, 60, 75, and 90 days after planting. Additionally, five leaves per pot were randomly selected, and their top, middle, and base lengths were measured using a Leaf device. The average length was then used to calculate total chlorophyll content in SPAD units. The number of emerged umbels per plot was counted before flowering, while the total number of flowers per umbel was determined at flowering. Seeds from 15 randomly selected sample plants were weighed individually to determine the average seed weight per plant. Additionally, for each treatment, the average weight of 1,000-seed was counted. Seed yield per hectare was calculated by converting the seed yield per plot value. Statistical analysis was conducted using computer-based software statistix 10.0 and mean separation was performed by using LSD at 5% level of significance.

Results and Discussions

Crop Morphological parameters

Plant height (cm)

A significant variation was found in plant height from combined application of boron and calcium (Table 1.) whereas plant heights at 30 DAP ranged from 8.03 to 13.10cm. The maximum plant height (13.10cm) was recorded from B_3Ca_2 (2000ppm boron and 5000ppm calcium) treatment, whereas the minimum plant height (8.03cm) in B_0Ca_0 (0ppm Boron and Calcium) treatment i.e., plant height increased 63.14% over (control treatment. At 45DAP, plant height ranged from 12.43 to 16.23 cm. The maximum plant height (16.23 cm) was recorded from B_3C_2 treatment whereas the minimum plant height (12.43cm) in B_0Ca_0 treatment where plant height increased 30.57% over B_0Ca_0 treatment. At 60DAP, plant height ranged from 20.47 to 25.03 cm. The highest plant height (25.03cm) was recorded from B_3Ca_2) treatment whereas the lowest plant height (20.47cm) in B_0Ca_0 i.e., plant height increased 22.28% over B_0Ca_0 treatment. At 75 DAP, plant height ranged from 29.57 to 38.43cm. The highest plant height (38.43cm) was recorded from B_3Ca_2 whereas the lowest plant height (29.57cm) in B_0Ca_0 treatment. Plant height increased 29.96% over control treatment. At 90 DAP, plant height ranged from 42.07 to 53.67 cm. The maximum plant height (53.67cm) was recorded from B_3Ca_2 whereas the minimum plant height (42.07cm) in B_0Ca_0 treatment.

After application of B_3Ca_2 treatment, Plant height increased 27.57% over B_0Ca_0 treatment. At harvest, plant height ranged from 41.97 to 53.97cm. The maximum plant height (53.97cm) was recorded from B_3Ca_2 whereas the minimum plant height (41.97cm) in B_0Ca_0 treatment. After application of B_3Ca_2 treatment, plant height increased 29.27% over B_0Ca_0 (control) treatment.

Table 1. Effects of boron and calcium on plant height of onion at different days after planting

Treatment	Plant height (cm)					
	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	At Harvest
B_0Ca_0	8.03g	12.43h	20.47h	29.57i	42.07g	41.97h
B_0Ca_1	8.70f	12.83gh	20.80gh	29.97hi	43.33fg	43.13gh
B_0Ca_2	9.03f	13.17fg	21.63fg	30.57ghi	43.67efg	43.60fg
B_1Ca_0	9.43e	13.50ef	21.80f	31.03fgh	44.27def	44.43efg
B_1Ca_1	10.47d	14.53d	22.83de	32.03ef	44.93def	45.17def
B_1Ca_2	11.27c	15.27bc	23.13cd	33.13de	45.43d	46.40cd
B_2Ca_0	9.80e	13.77e	22.10ef	31.33fg	44.80def	45.13def
B_2Ca_1	11.37c	15.70ab	23.93bc	33.67cd	45.90cd	46.17cd
B_2Ca_2	11.97b	15.90a	24.17ab	34.87bc	47.30bc	47.37bc
B_3Ca_0	10.77d	14.87cd	22.83de	32.57de	45.17de	45.43de
B_3Ca_1	11.93b	15.80ab	24.15ab	35.70b	48.10b	48.47b
B_3Ca_2	13.10a	16.23a	25.03a	38.43a	53.67a	53.97a
$LSD_{(0.05)}$	0.3762	0.5789	0.8952	1.2188	1.6955	1.5748
CV (%)	2.12	2.36	2.32	2.20	2.19	2.02

Number of leaves

A significant variation in the total number of leaves per plant was found among the treatments at all growth stages (Table 2.) 30 DAP, leaf numbers ranged from 2.37 to 2.95. The maximum leaves (2.95) were recorded from B_3Ca_2 whereas the minimum leaves (2.37) in B_0Ca_0 treatment. After application of B_3Ca_2 treatment leaf numbers increased 24.47% over B_0Ca_0 (control) treatment. At 45 DAP, leaf numbers ranged from 4.20 to 6.73. The maximum leaves (6.73) were recorded from B_3Ca_2 whereas the minimum leaves (4.20) in B_0Ca_0 treatment. The B_3Ca_2 treatment leaf numbers increased 60.23% over B_0Ca_0 treatment. At 60 DAP, leaf numbers ranged from 5.23 to 7.47. The highest leaves (7.47) were recorded from B_3Ca_2 whereas the lowest leaves (5.23) in B_0Ca_0 treatment. After application of B_3Ca_2 treatment leaf numbers increased 42.82% over B_0Ca_0 (control) treatment. At 75 DAP, leaf numbers ranged from 6.33 to 8.63. The highest leaves (8.63) were recorded from B_3Ca_2 whereas the lowest leaves (6.33) in B_0Ca_0 (0ppm Boron and Calcium) treatment. After application of B_3Ca_2 treatment leaf numbers increased 36.33% over B_0Ca_0 treatment. At 90 DAP, leaf numbers ranged from 7.20 to 9.03. The maximum leaves (9.03) were recorded from B_3Ca_2 whereas the minimum leaves (7.20) in B_0Ca_0 treatment. After application of B_3Ca_2 treatment leaf numbers increased 25.42% over B_0Ca_0 treatment. At harvest, leaf numbers ranged from 5.20 to 8.17. The maximum leaves (8.17) were recorded from B_3Ca_2 whereas the minimum leaves (5.20) in B_0Ca_0 treatment. After application of B_3Ca_2 treatment leaf numbers increased 57.11% over B_0Ca_0 treatment.

Table 2. Effects of boron and calcium on leaf number of onion at different days after planting

Treatment	Leaf number at					
	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	At Harvest
B_0Ca_0	2.37h	4.20j	5.23h	6.33i	7.20g	5.20i
B_0Ca_1	2.44g	4.60i	5.53g	6.66gh	7.53efg	6.30fg
B_0Ca_2	2.47g	4.87h	5.67g	6.76gh	7.90cd	6.60de
B_1Ca_0	2.58f	5.00gh	5.81fg	6.49hi	7.37fg	5.90h
B_1Ca_1	2.64e	5.27ef	6.00 ef	7.13de	7.73cde	6.80d
B_1Ca_2	2.72d	5.73d	6.33d	7.43d	7.97cd	7.23c
B_2Ca_0	2.58f	5.15fg	6.10de	6.80fg	7.50efg	6.13gh
B_2Ca_1	2.72d	5.90cd	6.73c	7.77c	8.03c	7.47bc
B_2Ca_2	2.79c	6.00c	6.97bc	8.00bc	8.43b	7.63b
B_3Ca_0	2.69de	5.43e	6.33d	7.10ef	7.67def	6.47ef
B_3Ca_1	2.85b	6.27b	7.03b	8.10b	8.70ab	7.73b
B_3Ca_2	2.95a	6.73a	7.47a	8.63a	9.03a	8.17a
$LSD_{(0.05)}$	0.0579	0.2140	0.2825	0.3122	0.3536	0.2834
CV (%)	1.29	2.33	2.66	2.54	2.64	2.46

Physiological parameters

Chlorophyll content

Combined treatment of boron and calcium showed the significant variation on chlorophyll content on leaves (Fig. 1). Chlorophyll content on leaves ranged from 40.20 to 50.80 mg cm^{-2}. Among the treatments the maximum chlorophyll content (50.80 mg cm^{-2}) on leaves was observed in B_3Ca_2 (2000 ppm boron and 5000 ppm calcium) treatment while minimum chlorophyll content (40.20 mg cm^{-2}) was in B_0Ca_0 (0ppm Boron and Calcium). B_1Ca_2 and B_2Ca_1 treatment showed the statistically similar. B_3Ca_2 treatment increased 26.37% chlorophyll content on leaves over B_0Ca_0.

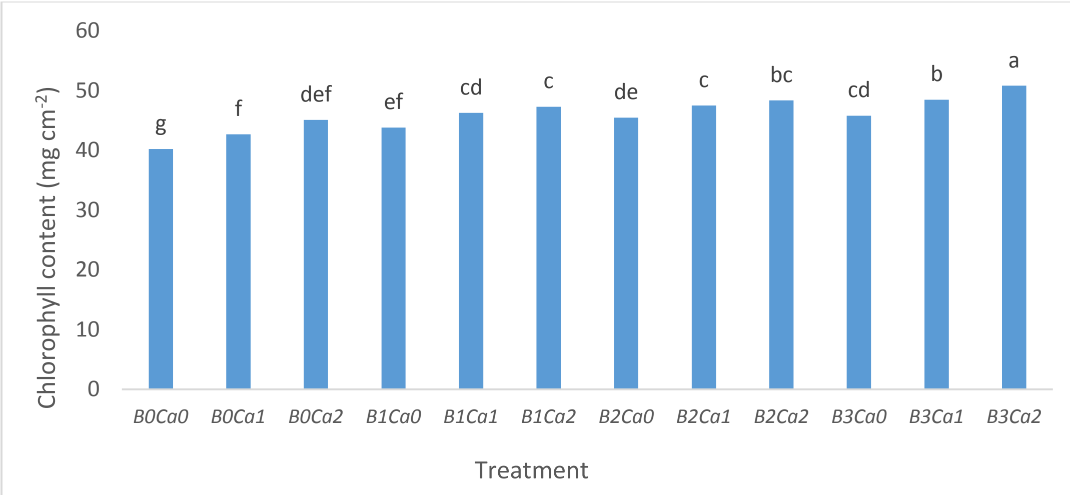

Fig. 1. Combined effects of boron and calcium treatments on chlorophyll content of onion leaves

Yield contributing characters

Umbels per plot

In case of combined treatments of boron and calcium showed a significant variation on numbers of umbel per plot (Fig. 2). Number of umbels per plot from combined treatment ranged from 62.33 to 68.03. B_2Ca_2 (boron 1000 ppm and calcium 5000 ppm) treatment showed the highest number of umbels per plot (68.03). The treatment B_1Ca_1, B_2Ca_1, B_3Ca_1 and B_3Ca_2 gave the statistically similar result. The lowest number of umbel per plot was recorded in B_0Ca_0 treatment (62.33). B_2Ca_2 treatment increased the umbel number per plot 9.14% over B_0Ca_0 treatment.

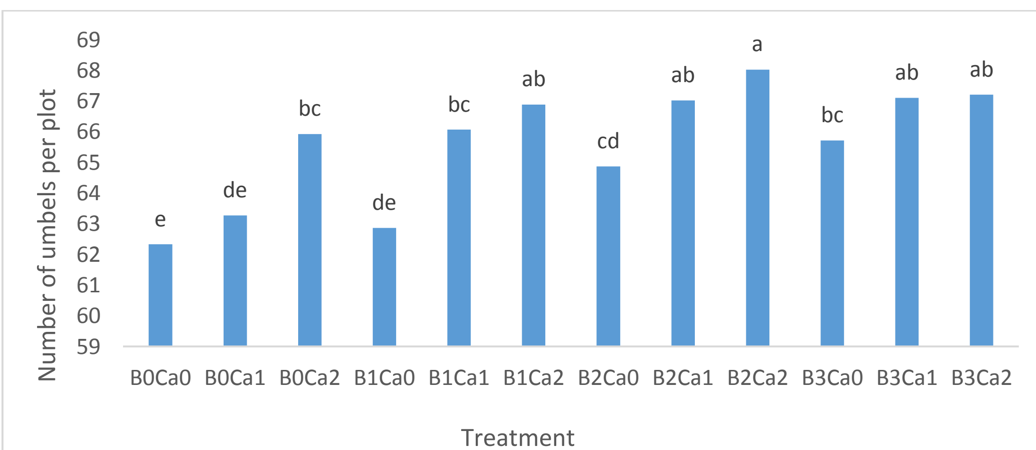

Fig. 2. Combined effects of boron and calcium on number of umbels per plot of onion

Flowers per umbel

The variation in the number of flowers per umbel was significant due to the combined effect of boron and calcium (Fig. 3). Number of flowers per umbel from combined treatment ranged from 149.03 to 169.77. Fig. 3 showed that B_2Ca_2 (boron 1000 ppm and calcium 5000 ppm) treatment produced the highest number of flowers per umbel (169.77) followed by B_3Ca_2 treatment (165.67). B_0Ca_0 gave the lower flowers per umbel (149.03) which was statistically similar with B_0Ca_1 treatment (150.77). B_2Ca_2 treatment increased the number of flowers per umbel 13.92% over B_0Ca_0 treatment.

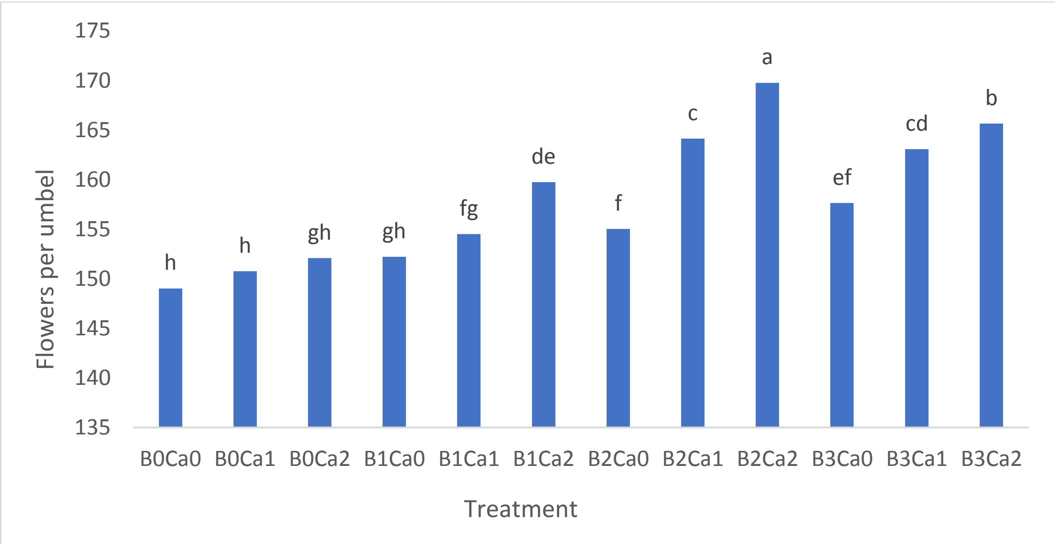

Fig. 3. Combined effects of boron and calcium treatments on flowers per umbel of onion

Fruits per umbel

Combined treatment of boron and calcium showed significant variation on fruits per umbel (Fig. 4). Number of fruits per umbel from combined treatment ranged from 110.33 to 157.67. B_2Ca_2 (boron 1000 ppm and calcium 5000 ppm) treatment produced the highest number of fruits per umbel (157.67). Second highest fruits per umbel (152.67) were found in followed by B_3Ca_2 treatment. Lower fruits per umbel (110.33) were recorded in B_0Ca_0 treatment. B_1Ca_1, B_0Ca_0 and B_3Ca_0 gave the statistically similar result on fruits per umbel. B_2Ca_2 treatment increased 42.91% fruits per umbel over B_0Ca_0 treatment.

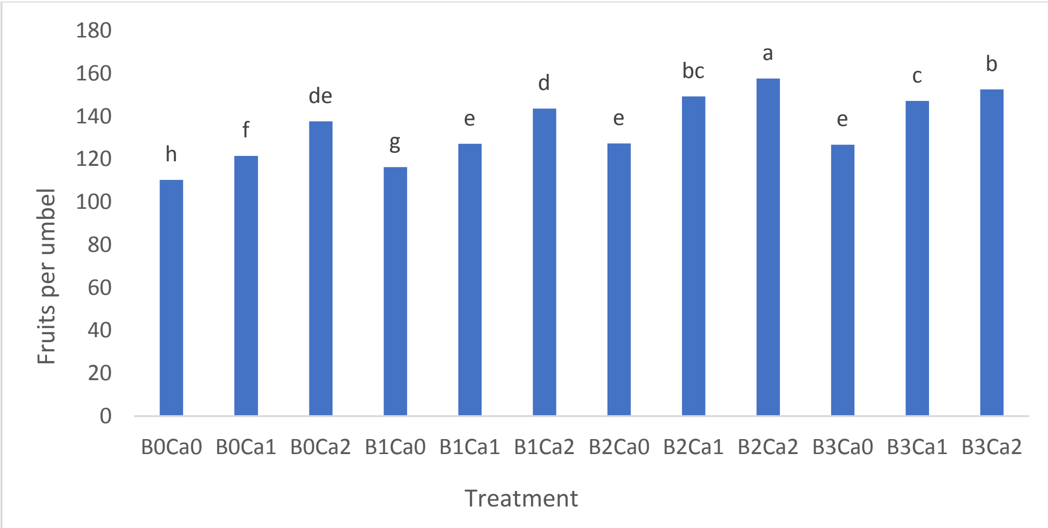

Fig. 4. Combined effects of boron and calcium on fruits per umbel of onion

Fruit set (%)

The variation in percentage of fruit set was found significant due to the combined application of boron and calcium (Fig. 5). Percentage of fruit set from combined treatment ranged from 74.03 to 92.93. The treatment B_2Ca_2 gave the highest percentage of fruit set (92.93 %). Also B_2Ca_1, B_3Ca_1 and B_3Ca_2 gave the statically similar result. Lowest percentage of fruit set (74.03%) was found in the B_0Ca_0 treatment. B_0Ca_0 and B_1Ca_0 showed the statistically similar result. Also B_1Ca_1 and B_2Ca_0 gave the statistically similar result.

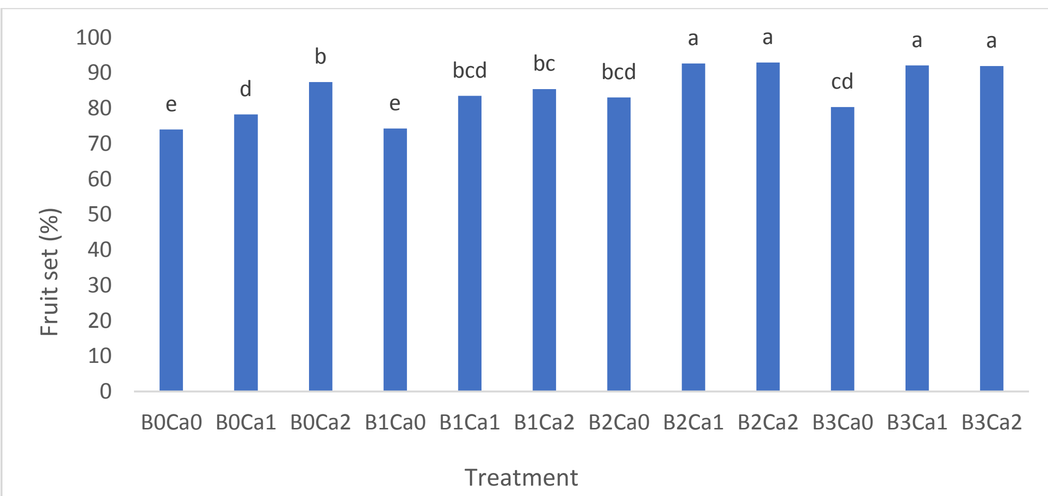

Fig. 5. Combined effects of boron and calcium treatments on fruit set (%) of onion

Seed yield per plant

Combined treatment of boron and calcium was significantly influence the seed yield per plant (Fig. 6). Seed yield per plant from combined treatment ranged from 1.95 to 3.14g. The treatment B_2Ca_2 gave the highest seed yield per plant (3.14 g) followed by B_3Ca_2 treatment. B_0Ca_1 and B_0Ca_2 gave the statistically similar result. Lowest seed yield per plant (1.95g) was found from B_0Ca_0 treatment. Application of B_2Ca_2 treatment increased 61.02% seed yield per plant over B_0Ca_0 treatment.

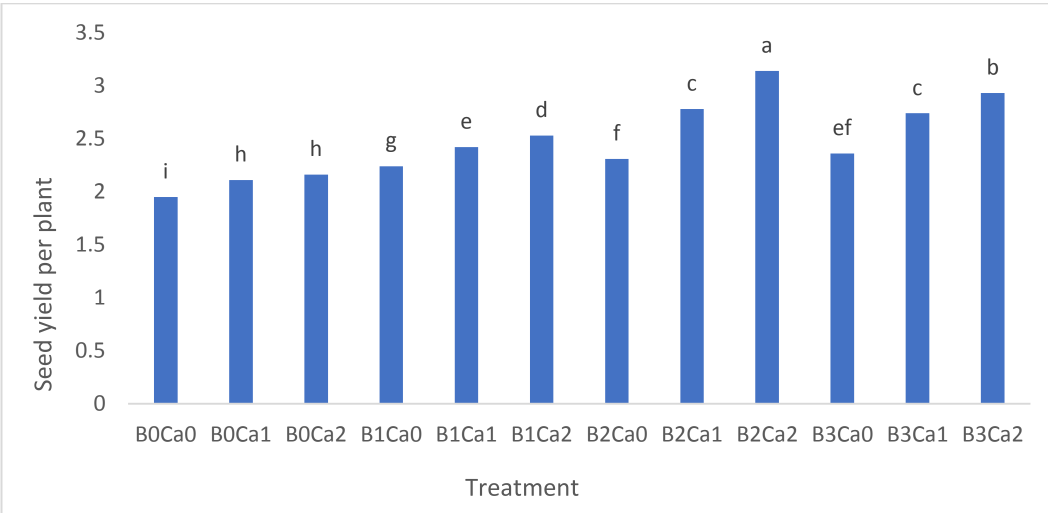

Fig. 6. Combined effects of boron and calcium on seed yield per plant of onion

1000-seed weight

Combined treatment of boron and calcium was significant on 1000-seed weight of onion (Fig. 7). Thousand seed weight from combined treatment ranged from 2.03 to 3.70g. Higher thousand seed weight (3.70 g) was observed in the treatment of B_2Ca_2 followed by B_2Ca_1 treatment. B_2Ca_1 and B_3Ca_2 treatment gave the statistically similar result. Lowest 1000-seed weight (2.03g) was found in B_0Ca_0 treatment. Application of B_2Ca_2 treatment increased 82.27% seed yield per plant over B_0Ca_0 treatment.

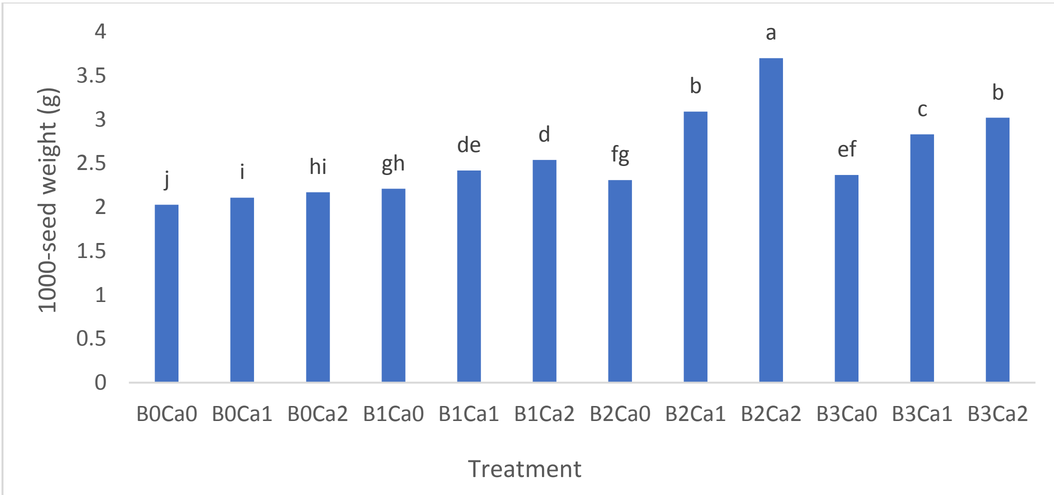

Fig. 7. Combined effects of boron and calcium on 1000-seed weight (g) of onion

Seed yield per hectare

Response of calcium and boron significantly influenced the per hectare seed yield of onion (Fig. 9). Seed yield per hectare from combined treatment ranged from 423.22 to 681.05 kg. Higher seed yield per hectare (681.05 kg) was recorded in B_2Ca_2 treatment followed by B_3Ca_2 treatment (645.67kg). B_2Ca_1 and B_3Ca_1 combined treatment gave the statistically similar result for seed yield per hectare. The lowest seed yield per hectare (423.22kg) was noticed in B_0Ca_0 treatment. Application of B_2Ca_2 treatment increased 60.92% seed yield per hectare over B_0Ca_0 treatment.

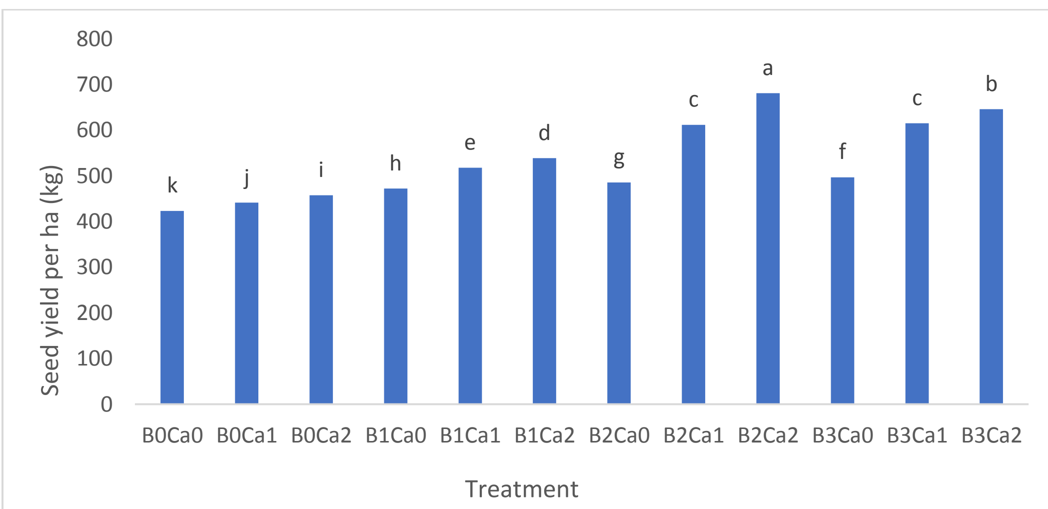

Fig. 8. Combined effects of boron and calcium treatments on seed yield per ha(kg) of onion

Seed quality parameters

Germination percentage

Combined application of calcium and boron significantly increased the germination percentage (Fig. 10). Germination percentage from combined treatment ranged from 96 to 71.67%. Germination percentage was highest in B_2Ca_2 (boron 1000 ppm and calcium 5000 ppm) where 96% germination was observed but 93.11% of germination from B_3Ca_2 treatment. B_2Ca_1 and B_3Ca_0 treatment gave the statistically similar result on germination percent. Lowest germination percentage (71.67%) was observed in B_0Ca_0 combined treatment. B_2Ca_2 treatment increased 33.94% of seed germination over B_0Ca_0 treatment.

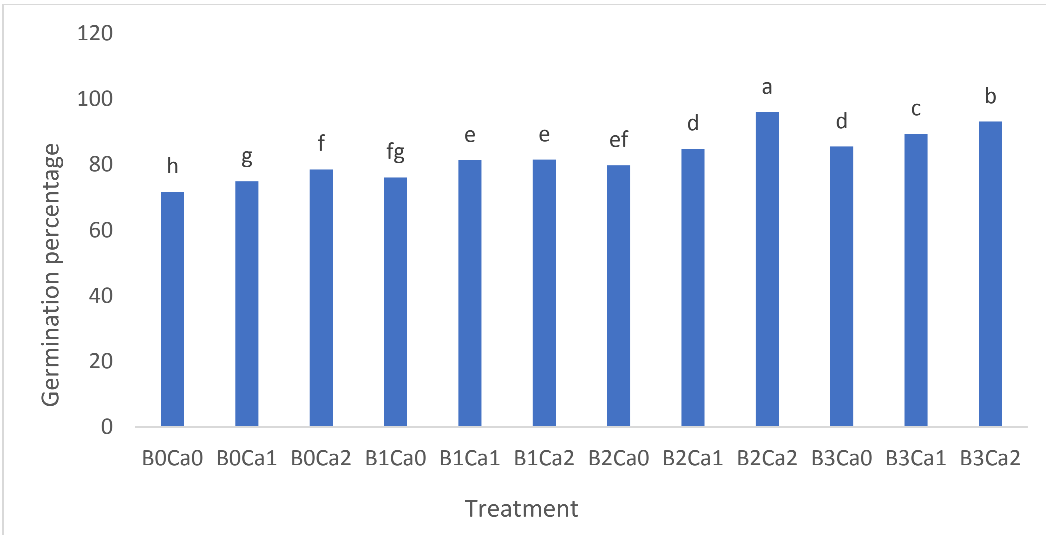

Fig. 9. Combined effects of boron and calcium ton germination percentage of onion seed

Normal seedling (%)

Variation was observed for normal seedling production due to combined application of boron and calcium (Fig. 11). Normal seedling percentage from combined treatment ranged from 92.69 to 53.67%. Among all the combined treatment B_2Ca_2 produced the highest normal seedling (92.69%) than other treatment where treatment B_3Ca_2 produced 89.32% normal seedling. B_0Ca_0 produced 53.67% of normal seedling which was the lowest percentage of normal seedling. B_2Ca_2 treatment increased 72.70% of normal seedling over B_0Ca_0 treatment.

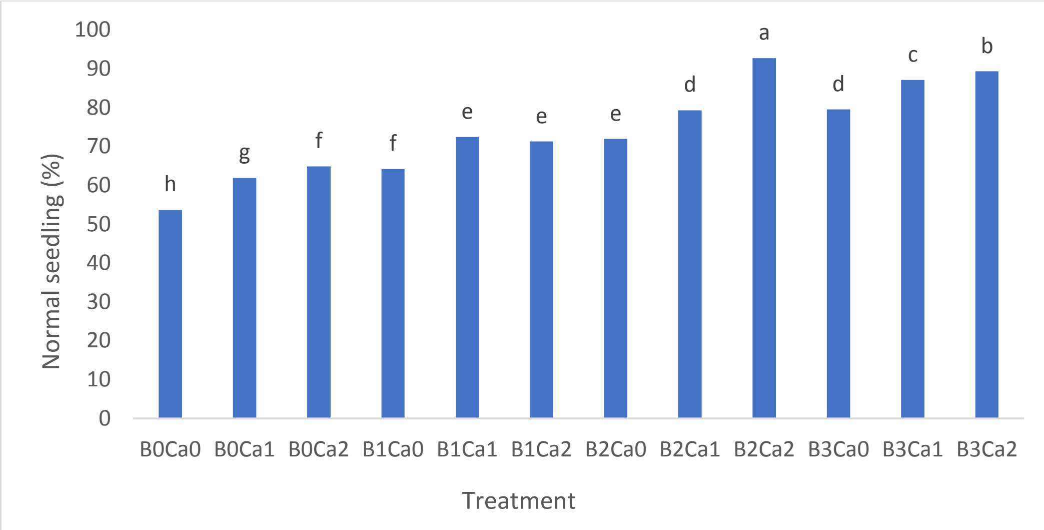

Fig. 10. Combined effects of boron and calcium on normal seedling (%) of onion

Abnormal seedling (%)

For various combined applications of boron and calcium significant variation was observed in abnormal seedling production (Fig. 12). Abnormal seedling percentage from the combined treatment ranged from 3.33 to 18%. Large number of abnormal seedling (18%) was found from B_0Ca_0 treatment. B_0Ca_1 and B_0Ca_2 treatment produced 15.56 and 15.33% of abnormal seedling, respectively which were statistically similar. Lower number of abnormal seedling (3.33%) was found from B_2Ca_2 treatment which reduced the abnormal seedling by 81.5% over B_0Ca_0 treatment.

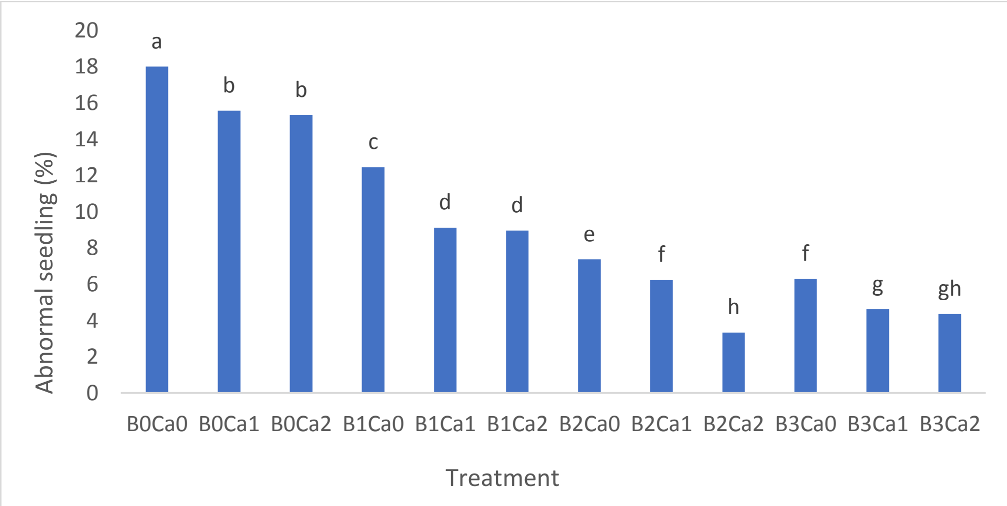

Fig. 11. Combined effects of boron and calcium on abnormal seedling (%) of onion

Conclusion

The results revealed that boron and calcium treatments had significant effect on crop growth parameters e.g. plant height and leaf number at different DAP. Different boron and calcium treatments had significant effect on the physiological parameters viz., chlorophyll content was highest in B_3Ca_2 treatment (50.80 mg cm^{-2}), and also yield and yield contributing characters viz., umbel per plot, flowers per umbel, fruits per umbel, fruit set (%), yield per plant, 1000-seed weight, yield per hectare. The highest germination percentage (96 %) was observed in B_2Ca_2 with normal seedling percentage (92.69 %) and reduced the abnormal seedling percentage (3.31%). These findings suggest that boron and calcium application effectively enhance onion growth, physiology, yield components, germination, and seedling quality.

Author's contribution

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Conflicts of Interest

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

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GROWTH AND YIELD OF MUSTARD AS INFLUENCED BY DIFFERENT COMBINED DOSES OF ZINC AND BORON

M. S. Reza[1*], S. Adhikary[2], M. K. A. Nadim[3] and M. E. Hossain[4]

[1]Soil Science Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh; [2]Plant Breeding Division, BINA, Gopalganj; [3]Biotechnology Division, BINA, Mymensingh; [4]Agroforestry, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur. Bangladesh.

Abstract

A field experiment was conducted at the research field of BINA substation, Gopalganj from November 2022 to February 2023 to evaluate the effect of different doses of micronutrients (Zn and B) on the growth and yield of mustard. The experiment comprised of eight (8) treatments viz. T_0= Control, T_1=100% NPKSZnB as Recommended dose of chemical fertilizer (RDCF), T_2= 100% NPKS, T_3= 100% NPKS+$Zn_{1.5}$$kgha^{-1}$+$B_{1.5}$$kgha^{-1}$, T_4= 100% NPKS+$Zn_{2.0}$$kgha^{-1}$+$B_{2.0}$$kgha^{-1}$, T_5= 100% NPKS+$Zn_{3.0}$$kgha^{-1}$+$B_{3.0}$$kgha^{-1}$, T_6=100% NPKS+$Zn_{2.0}$$kgha^{-1}$, T_7= 100% NPKS+$B_{2.0}$$kgha^{-1}$. The experiment was laid out in a randomized complete block design (RCBD) with three (3) replications. The results revealed that growth and yield were influenced significantly by the rate of 100% NPKS+$Zn_{2.0}$$kgha^{-1}$+$B_{2.0}$$kgha^{-1}$ application. The highest plant height (103.67 cm), primary branch/plant (6), secondary branch/plant (4.67), lowest days to flowering (35.67), days to maturity (83.00), plant population (728.67), siliqua length (7.30 cm), siliqua/plant (52.67), seeds/siliqua (39.00), 1000 seed weight (4.19 g) and yield (1.99 ton/ha) were obtained from 100% NPKS+$Zn_{2.0}$$kgha^{-1}$+$B_{2.0}$$kgha^{-1}$ treatment. The result showed that the growth and yield of mustard increased with increasing levels of Zn and B along with 100% NPKS fertilizers application. It may be concluded that the treatment T_4= 100% NPKS+$Zn_{2.0}$$kgha^{-1}$+$B_{2.0}$$kgha^{-1}$ would be suitable for mustard cultivation for getting higher yield and better performance.

Keywords: Zinc, Boron, Mustard, Yield.

Introduction

Mustard belongs to the family of Brassica ceae (Bayer, 2010). It plays an important role as a highly cultivated oilseed crop in Bangladesh, whereas mustard stood as the major oil-producing crop of both acreage and yield among the several oil crops cultivated in the country (BBS, 2019). Worldwide, 41.50% of mustard seed is produced just in Asia which occupies the first position in terms of percentage share of production followed by the USA (FAO, 2018). In Bangladesh, the demand for edible oil is increasing day by day and oilseeds are on an increasing trend (Alam, 2020) where the daily per capita recommended dietary allowance of oil is 6 gm per day for a diet with

[*] Corresponding author: selim.bsmrau48@gmail.com

2700 Kcal (BNNC, 1984). To meet this demand of edible oil, Bangladesh has to import edible oil and oil seeds which were worth USD 544 million in 2002-03 to USD 2371 million in 2018-19 (Bangladesh Bank, 2020). To meet up this demand, in Bangladesh mustard production is not in a favor position (Miah and Rashid, 2015) because of lack of short duration high-yielding varieties which can easily incorporate with cropping pattern, reduce land for oil crop production, improper cultural practices, insufficient nutrient management, soil nutrient depletions, imbalance used of fertilizers and so on. Mustard (*Brassica spp*. L.) is sensible to micronutrients (Zn and B) but in Bangladesh, there is a lack of awareness among farmers regarding the application of micronutrients in mustard fields. The decline in crop yield is due to either inadequate use of fertilizers or imbalanced fertilization practices (Roy *et al*., 2013; Haque *et al*., 2014; Rabbani *et al*., 2023). The growth of a plant can be retained if any of the micronutrients are absent in the soil, even if all other nutrients are available in sufficient quantities. The application of micronutrients is necessary to obtain desirable balance nutrition.

Apart from all the nutrients, zinc is one of the major micronutrients that are essential for the plant growth which is transported to the plant root surface through diffusion (Maqsood *et al.*, 2009). If zinc is deficient in plants, it may last throughout the entire crop season (Asad and Rafique, 2000) and in severe cases, the plant appears to be stunted. Zinc is an important component of numerous enzymes that play a regulatory role in diverse metabolic processes and found to have a stimulating effect on pod and seed development, and oil synthesis in mustard seeds (Halim *et al.*, 2023). Moreover, it has been noticed to enhance the overall formation and oil synthesis in mustard not only for seed but also for stover production (Sultana *et al*., 2020). Zinc application has become necessary for improved crop yields ~~(Kutuk *et al*., 2000)~~ and $ZnSO_4$ application was recommended at the rate of 20 kg ha^{-1} for oilseeds including mustard (Mandal and Sinha, 2004). Reza *et al*. (2023) suggested that the application of zinc with recommended dose chemical fertilizers increased the mustard production.

Boron has an important role in the phenology of mustard production and in promoting the growth and productivity of crops (Karthikeyan and Shukla, 2011). As a result, the yield of mustard has been reduced when cultivated on low boron soils or where availability of boron is restricted under high soil pH, liming, and drought periods during the growth period. For this reason, boron fertilization is required to increase the crop production. Saha *et al*. (2003) stated that mustard responds positively to boron fertilization and a positive correlation between the mustard plant and the use of B fertilization (Jaiswal *et al.*, 2015). In Bangladesh, farmers are unaware of the recommended fertilizer rates where boron and zinc fertilizers are occasionally disregarded and many of them apply fertilizers in quantities that are inconsistent with recommended for mustard. The objective of this study was to know the effects of different doses of micronutrients (Zn and B) on the growth and yield of mustard.

Materials and Methods

The experiment was carried out at the research field of the Bangladesh Institute of Nuclear Agriculture (BINA), located in Gopalganj at coordinates of $23^0$11’57” N latitude and $89^0$76’05” E longitude, during the period from November 2022 to February

2023. The land was of clay loam to sandy clay as a part of the Gopalganj Khulna Beels (AEZ-14) and Arial Beels (AEZ-15) (UNDP and FAO, 1988) and pH ranges from 6.5 to 9.0.

Experimental treatments and design

The experimental treatments are (T_0= Control, T_1=100% NPKSZnB as Recommended dose of chemical fertilizer (RDCF), T_2= 100% NPKS, T_3= 100% NPKS+$Zn_{1.5}kgha^{-1}$+$B_{1.5}kgha^{-1}$, T_4= 100% NPKS+$Zn_{2.0}kgha^{-1}$+$B_{2.0}kgha^{-1}$, T_5= 100% NPKS+$Zn_{3.0}kgha^{-1}$+$B_{3.0}kgha^{-1}$, T_6=100% NPKS+$Zn_{2.0}kgha^{-1}$, T_7= 100% NPKS+$B_{2.0}kgha^{-1}$). The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications where the total number of experimental units was 24 (8 × 3). The size of each plot was 6 m^2 (3.0 m × 2.0 m). The distance between plot to plot and block to block was maintained at 0. 75m and 1 m, respectively.

Experimental material and land preparation

Binasarisha-9, a high-yielding variety of *Brassica napus* L. developed by the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh was used as a test crop. The experimental land was prepared by plowing and cross plowing followed by laddering with power tiller and crop debris of the previous season was removed from the field. The plot was fertilizers with urea (N), triple super phosphate (P), murite of potash (K) and gypsum (S) and the rate were 210 kg/ha, 185 kg/ha, 124 kg/ha, 136 kg/ha respectively. All the fertilizers and half of the urea were applied at the final land preparation and the rest half of the urea was top-dressed after 30 days after sowing (DAS). Zinc sulphate (36% Zn) and boric acid (17%) were applied as per treatments and the seed rate was 6 kg ha^{-1}. Seeds were sown manually in a continuous pattern in 25 cm apart rows on 9 November 2022 and after sowing, seeds were then covered with soil. Weeding was done as needed and thinning was done after 15 and 25 DAS. Care was taken to maintain a constant plant population in $plot^{-1}$ and there was no disease or insect was observed in the plots.

Harvesting and data collection

On February 1 to 10, 2023 when 90% of the siliqua were mature, the crop was harvested plot by plot. Data on growth and yield components were recorded randomly by selecting five plants from each plot and bound together into bundles which were then transported to the designated area for threshing. After that, the bundle was laid out on the threshing floor, the plants were dried in the sun, and after sundry, using bamboo sticks to beat the bundles to collect the seeds. After that, cleaning was done and yield was calculated on a hectare basis and documented each plot.

Statistical analysis

The recorded data were compiled and tabulated for statistical analysis. Analysis of variance was done following the RCBD with the help of the computer package R version 4.3.1. The LSD was used to determine the mean differences (Gomez and Gomez, 1984).

Result and Discussion

Plant height

The highest plant height (103.67 cm) was recorded in T_4 (100% NPKS+$Zn_{2.0kgha^{-1}}$+$B_{2.0kgha^{-1}}$) T_6 (Table 1) while the shortest plant height (74.33 cm) was observed T_0 (control). The height of plants showed a progressive rise in absorption as the concentration of zinc and boron. This finding aligns with the studies conducted by Reza *et al*. (2023) which have elucidated a significant impact of varying doses on plant height.

Flowering days

The results regarding days of flowering of mustard variety as influenced by different zinc and boron doses are presented in (Table 1). The analysis of variance indicated that the days to flowering of mustard were significantly influenced by zinc and boron doses. The highest days (50.67) were taken for flowering T_0 (control) whereas the shortest days (35) were taken T_5, T_4, T_6 and T_7 respectively. Balanced fertilizers along with zinc and boron fertilizers have a positive relation with flowering days (Bhagchand Kansotia *et al*., 2013).

Days to maturity

The highest days (94.67) were taken T_0 and the shorten days (83) were taken at T_4 (Table 1). Application of recommended doses along with zinc and boron for mustard cultivation will improve the maturity days (Sana *et al*., 2003).

Primary branches plant^{-1}

Branches plant^{-1} responded significantly to various doses of zinc and boron application (Table 1). The maximum (6) branch plant^{-1} was observed T_4 which was at par T_2, T_3, T, T_6, T_7 and minimum branch plant^{-1} was recorded T_0 (control). Zinc and boron had significantly increased the primary and secondary branches plant^{-1} (Hossain *et al.*, 2011; Rashid *et al.*, 2012).

Table 1. Effects of different doses zinc and boron on growth attributes of mustard

Treatments	Plant height (cm)	Days to flowering	Days to maturity	Primary branch plant^{-1} (no.)	Secondary branch plant^{-1} (no.)
T_0	74.33 e	50.67 a	94.67 a	4.00 b	1.33 d
T_1	88.33 c	42.00 b	88.33 b	5.67 ab	2.66 bcd
T_2	83.00 d	40.00 bc	88.67 b	4.67 ab	2.00 cd
T_3	92.00 c	39.00 bcd	86.00 c	5.33 ab	3.33 abc
T_4	103.67 a	35.67 d	83.00 d	6.00 a	4.67 a
T_5	98.33 b	35.00 d	85.33 c	4.33 ab	3.00 bc
T_6	101.00 ab	36.00 cd	86.00 c	4.67 ab	4.00 ab
T_7	99.33 b	36.00 cd	86.33 c	5.00 ab	3.33 abc
CV (%)	2.32	5.99	1.02	19.68	25.23

In a column figures having similar letter (s) do not differ significantly at 5% level of probability where (T_0= Control, T_1=100% NPKSZnB as Recommended dose chemical fertilizer (RDCF), T_2= 100% NPKS, T_3= 100% NPKS+$Zn_{1.5}$kgha^{-1}+$B_{1.5}$kgha^{-1}, T_4= 100% NPKS+$Zn_{2.0}$kgha^{-1}+$B_{2.0}$kgha^{-1}, T_5= 100% NPKS+$Zn_{3.0}$kgha^{-1}+$B_{3.0}$kgha^{-1}, T_6=100% NPKS+$Zn_{2.0}$kgha^{-1}, T_7= 100% NPKS+$B_{2.0}$kgha^{-1})

Secondary branch plant^{-1}

The highest secondary branches plant^{-1} (4.67) was observed T_4 and the lowest was recorded (1.33) at T_0 (control). Suitable doses of zinc and boron fertilizers significantly influenced plant height and branches per plant of mustard (Naser and Islam, 2001).

Plant population plot^{-1}

The plant population plot^{-1} varied significantly because of zinc and boron application with recommended doses of fertilizers (The maximum plant population plot^{-1} (728.67) was recorded at T_4 which was statistically significant from other treatments and the minimum plant population plot^{-1} (371.67) was observed at $T_{0.}$

Siliqua length (cm)

The varieties of rapeseed differed significantly in respect of siliqua length (Hossain *et al.* 1996). The effect of zinc and boron fertilizers significantly influenced the siliqua length **(Table 2).** The highest siliqua length (7.30) was observed at T_4 which was statistically significant from other treatments whereas the lowest siliqua length (3.13) was observed from T_0.

Siliqua plant^{-1}

The highest siliqua plant^{-1} was observed (52.67) from T_4 which was statistically significant from others and the minimum siliqua plant^{-1} was observed from T_0 (19). The number of silique plant-1 of mustard was significantly affected by different varieties (Mamun *et al.*, 2014).

Seeds siliqua^{-1}

Effects of zinc and boron significantly influenced the number of seeds siliqua^{-1} (Table 2). It was observed that the highest seed siliqua^{-1} was recorded (39) from T_4 which was statistically significant from others and the lowest number of seeds was recorded (19.33) from T_0 treatment. Significant variation in terms of the number of seed siliqua^{-1} among the varieties was observed due to reason difference in the genetic makeup of the variety, which is primarily influenced by heredity (Helal *et al.* 2016). Mandal and Sinha (2004) stated that the application of Zn and B fertilizers along with NPK fertilizers increased the mustard siliqua per plant, number of seeds per siliquae, and 1000-seed weight.

Table 2. Effects of different doses of micronutrients on yield and yield contributing characteristics

Treatments	Plant population/plot	Siliqua length (cm)	Siliqua/plant (no.)	Seeds/silique (no.)	1000 seed weight (g)
T_0	371.67 d	3.13 e	19.00 d	19.33 e	2.21 e
T_1	500.67 c	6.17 c	27.00 c	26.67 d	2.94 d
T_2	475.67 c	5.50 d	27.67 c	24.67 d	2.70 d
T_3	558.00 b	6.93 b	39.33 b	30.00 b	3.30 c

Treatments	Plant population/plot	Siliqua length (cm)	Siliqua/plant (no.)	Seeds/silique (no.)	1000 seed weight (g)
T_4	728.67 a	7.30 a	52.67 a	39.00 a	4.19 a
T_5	602.67 b	6.82 b	41.67 b	28.33 cd	3.27 c
T_6	589.33 b	6.94 b	41.33 b	31.67 bc	3.63 b
T_7	603.00 b	6.98 b	39.67 b	28.00 cd	3.94 a
CV (%)	5.84	2.64	6.29	8.76	5.04

In a column figures having similar letter (s) do not differ significantly at 5% level of probability where (T_0= Control, T_1=100% NPKSZnB as Recommended dose chemical fertilizer (RDCF), T_2= 100% NPKS, T_3= 100% NPKS+$Zn_{1.5}$$kgha^{-1}$+$B_{1.5}$$kgha^{-1}$, T_4= 100% NPKS+$Zn_{2.0}$$kgha^{-1}$+$B_{2.0}$$kgha^{-1}$, T_5= 100% NPKS+$Zn_{3.0}$$kgha^{-1}$+$B_{3.0}$$kgha^{-1}$, T_6=100% NPKS+$Zn_{2.0}$$kgha^{-1}$, T_7= 100% NPKS+$B_{2.0}$$kgha^{-1}$)

1000 seed weight (g)

There was a significant difference found among the treatments. The highest 1000 seed weight was (4.19) observed from T_4 which was statistically significant from all other treatments whereas the lowest 1000 seed weight (2.21) was observed from T_0 (Table 2). Mamun *et al.* (2014) stated that the performance of rapeseed and mustard varieties with the application of zinc and boron response quality and mature seeds which will ultimately affect different seed weight.

Yield (ton/ha)

The highest yield (1.99 ton/ha) was observed from T_4 which was statistically significant from others whereas the lowest yield (0.49 ton/ha) was observed from T_0 treatment. From the finding, the application of $ZnSO_4$ at the rate of 0.05% solution foliar spray at 25 and 40 days after sowing (DAS) resulted in a 9.02% increase in seed yield in mustard (Biswas *et al.*, 2010). There was a positive correlation between the rise in Zn and B content and the mean seed yield.

Application of Zn and B along with other micronutrients improved soil organic matter and resulted in increasing the mustard yield (Maqsood *et al.*, 2009). Effect of Zn and B application in addition to NPK fertilizers on production and with yield of mustard.

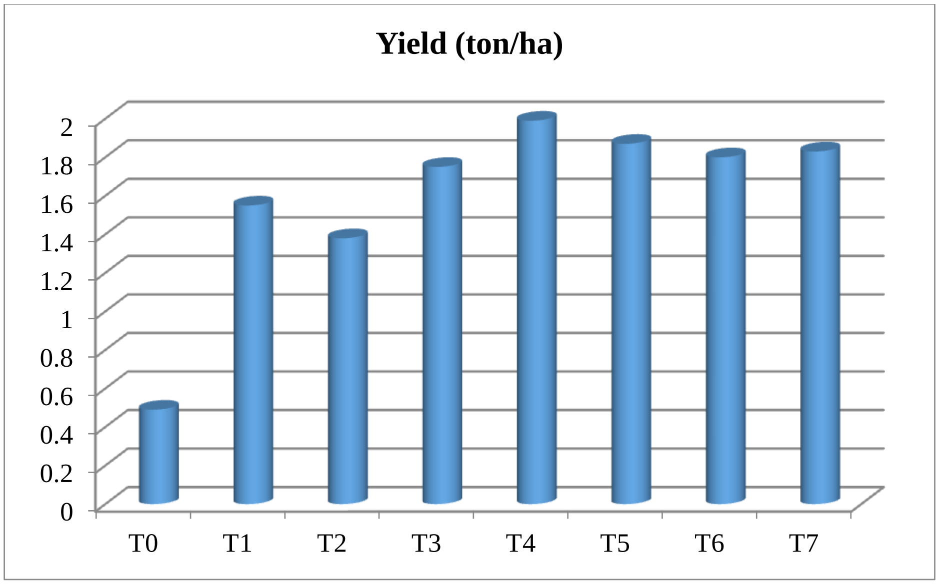
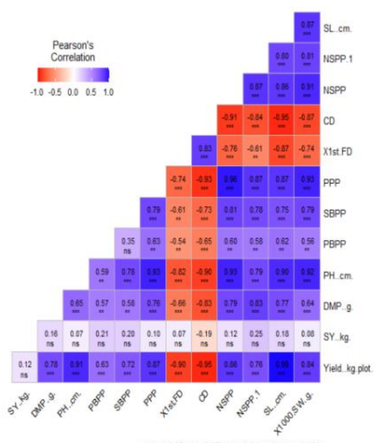


Fig. 1. The effect of different micronutrients (Zn and B) doses on yield of mustard



Legend:

SL=Siliqua length (cm)

NSPP[1]= Number of seeds per plant

NSPP=Number of siliqua per plant

CD= Crop duration (Days)

1st FD= Flowering day (1st)

PPP= Plant population per plot

SBPP= Secondary branches per plant

PBPP= Primary branches per plant

PH= Plant height (cm)

DMP=Dry matter per plot

SY= Straw yield

Yield= Yield

Fig. 2. Correlation among the growth and yield attributes of mustard

The correlation among the parameters (Fig. 2), there was a positive correlation among the yield, straw yield, dry matter, plant height, primary branch/plant, secondary branch/plant, plant population/plot, number of seeds per siliqua, number of siliqua/seeds, siliqua length but there was a negetive correlation between flowering days and maturity days. Jha *et al*. (2023) stated that a positive correlation between the rise of Zn and B content and the seed yield.

Conclusion

In the present study, the results showed that the treatment T_4 (100% NPKS+$Zn_{2.0kgha^{-1}}$+$B_{2.0kgha^{-1}}$) gave the highest yield, the tallest plant, greatest number of branches plant^{-1}, number of total siliqua plant^{-1}, siliqua length, seed siliqua^{-1}. From the experimental findings, it may be concluded that the application of zinc and boron along with NPKS played a significant role in increasing the yield of mustard in Gopalganj region of Bangladesh.

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Author's contribution

M. S. Reza: Conceptualized and designed the experiment, conducted field trials, performed data collection and manuscript preparation. S. A.: Provided assistance in experiment implementation at the research field, contributed to data interpretation and reviewed the manuscript. M. K. A. N: Assisted with the application of methodologies, provided technical guidance during the experimental process and contributed to statistical analysis. M. E. H.: Supported in literature review and manuscript revision.

Conflicts of Interest

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

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MORPHO-PHYSIOLOGICAL ATTRIBUTES OF BLACKGRAM VARIETIES AS INFLUENCED BY PLANTING GEOMETRY

K. Kader[1*], M. Ahmed[1] , M. F. Karim[2] and A. K. M. R. Amin[1]

[1] Agronomy Division, Bangladesh Jute Research Institute (BJRI), Manik Mia Avenue, Dhaka; [2]Department of Agronomy, Sher-e- Bangla Agricultural University (SAU), Sher-e- Bangla Nagor, Dhaka. Bangladesh.

Abstract

The study was carried out at the research field of Sher-e-Bangla Agricultural University, Dhaka during March to June 2021 in Kharif-I season to evaluate the effect of varieties and different plant spacing of blackgram. The trial comprised of three varieties of blackgram viz., V_1=BINA mash1, V_2=BARI mash2, V_3=BARI mash4 and four different plant spacing viz., P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm). The experiment was laid out in a RCB design with three replications. The results revealed that the blackgram variety BARI mash4 produced the highest seed yield (1.83 t ha-1), whereas BINA mash1 produced the lowest seed yield (1.27 t ha^{-1}). In case of different plant spacing the seed yield ranges between (1.27 -1.83 t ha^{-1}) where maximum seed yield was recorded in spacing of (30 cm × 15 cm). When compared to other treatment combinations, the combination of variety BARI mash4 and 30 cm × 15 cm spacing had an impact on plant growth and yield-contributing characteristics; thus, the results indicated that this combination (30 cm × 15 cm) was suitable for blackgram's maximum growth, seed yield, and yield-contributing parameters.

Keywords: Blackgram, Harvest index, Planting geometry, Variety.

Introduction

Blackgram (*Vigna mungo*) is one of important pulse crop and protein sources for predominately vegetarian populations of our country. Among the pulses, blackgram is one of the most consumed pulses in our country and is the third most widely grown crop in terms of both total cultivated area and consumption (BBS, 2021). The area under pulse crop is 0.406 million hectare with a production of 0.322 million tone (BBS, 2005), where blackgram is cultivated in the area of 0.188 million ha with production of 9.5% of total pulse production (BBS. 2005). Reddy (1997) reported that the genotypic and phenotypic variation of blackgram were significant for branches/plant, 100-seed weight, pods/plant and grain yield/plant. Days to maturity, Clusters/plant, pods/cluster and seeds/pod also varied significantly due to genotypic variation. Plant density can have a major effect on

[*] Corresponding author: kamiliya747@gmail.com

the final yield of most of the legumes and the general response of yield to increasing population is well documented (Kumar *et al.,* 2013)

Several high yielding varieties, such as BARI mash2, and BARI mash4 were developed by the Bangladesh Agricultural Research Institute (BARI) (Islam *et al*., 2019) whereas Bangladesh Institute of Nuclear Agriculture (BINA) has developed one variety i.e., BINA mash1. Plant density can have a major effect on the final yield of most of the legumes. To get the maximum yield potential of blackgram during summer and rainy season, maintenance of optimum space made available to individual plant is of prime importance. The spacing requirement depends upon the growth behaviour of genotype (Amanullah *et al.,* 2016). Optimum spacing between rows is required to utilize efficiently the available production factors such as moisture, nutrients, sunlight and space which impact on seed yield (Amare and Gebremedhin, 2020). So, it is required to maintain spacing for obtaining higher yield (Veeramani, 2019).

Materials and Methods

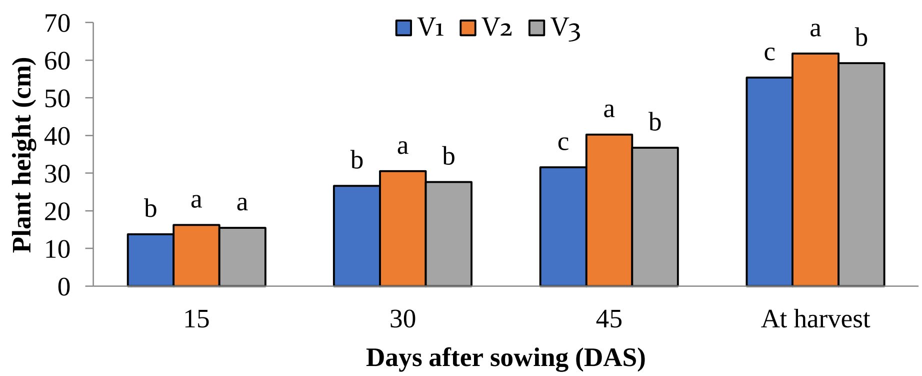
A field experiment was conducted in the field of Sher-e-Bangla Agricultural University (SAU) during the Kharif-1 season of 2021. The soil of the experimental plot belongs to the agro ecological zone of Madhupur Tract (AEZ-28). A soil sample from 0-15 cm depth was collected from experimental field. The experimental area was under the subtropical climate. Usually the rainfall was heavy during Kharif-1 season and scanty in Rabi season. The atmospheric temperature increased as the growing period proceeded towards Kharif season. The experiment was laid out in factorial RCB design with 3 replications. The unit plot size was 5.4 m^2 (2.7 m × 2 m). There were two factors: Factor A. Blackgram varieties (three): V_1= BINA mash1, V_2= BARI mash2, V_3= BARI mash4 and Factor B. Different planting methods (four), P_1= Broadcast, P_2= Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm). The fertilizers namely Urea, Triple Superphosphate (TSP), Muriate of Potash (MoP), Zinc Sulphate, and Boric acid were given as sources of nitrogen, phosphorus, potassium, zinc, and boron as per FRG, 2018. The seeds were sown in broadcast and in solid rows in the furrows having a depth of 2-3 cm from the soil surface. Row to row distance was 30 cm and plant to plant distance was according to the treatments. Before sowing, the seeds is treated with bavistin to control the seed borne disease. Thinning was done at 10 DAS. Pre sowing irrigation was done to maintain equal germination. After sowing two irrigations were applied during the life cycle. First irrigation and second irrigation were done at 15 DAS and 30 DAS respectively. The data were recorded from 15 DAS and continued until the end of recording of yield contributing characters of the crop after harvest. The heights of five plants were measured and expressed in cm. The leaves were separated from each sampled plant and counted and then averaged to express at per plant. Number of nodules plant^{-1} was counted from each selected plant sample at 45 DAS and at harvest respectively. For measuring the dry matter weight plant^{-1}, the parts of the plants were separated and then dried in oven at 60 °C for 72 hours and weight was taken carefully. The data collected on different parameters were statistically analyzed at 5% level of significance (Gomez and Gomez, 1984) to compare the mean differences among the treatments following DMRT method.

Results and Discussion

Results obtained from the study have been presented and discussed with a view to study the response of blackgram varieties as affected by different plant spacing. The results have been discussed, and possible interpretations are given under the following headings.

Effects of varieties on plant height at different growth stages

Plant height varied greatly depending on the variety at day after sowing (DAS). Experimental result revealed that at harvest, the highest plant height (16.27, 30.52, 40.27 and 61.74 cm) at 15, 30, 45 DAS and respectively was observed in V_2 treatment BARI mash2 (Fig. 1) which was statistically similar with V_3 (15.52 cm) treatment (BARI mash4) at 15 DAS. Whereas the lowest plant height at 15 DAS, 30 DAS, 45 DAS and at harvest respectively was observed in V_1 treatment (BARI mash1) which was statistically similar with V_3 (27.67 cm) treatment (BARI mash4) at 30 DAS.

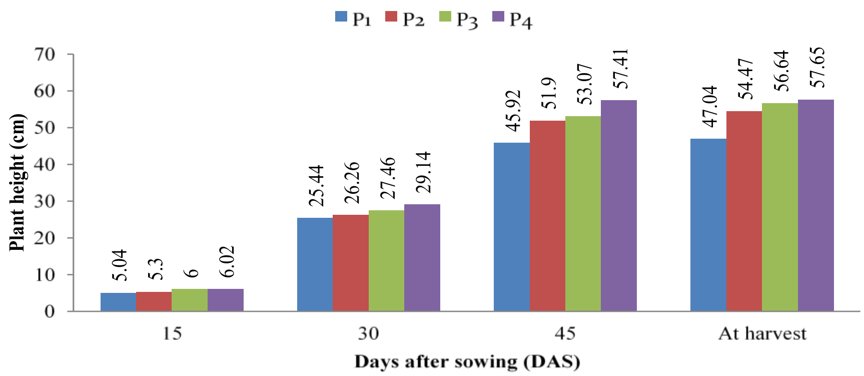


Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash4

Fig. 1. Effects of varieties on plant height at DAS

Effects of planting geometry

Significant variation in blackgram plant height was observed on different days after sowing because of different planting geometry (Fig. 2). Experimental results showed that the treatment P_1 (Broadcasting) had the lowest plant height 5.04, 25.44, 45.92 and 47.04 cm at 15 DAS, 30 DAS, 45 DAS and at harvest, respectively which was statistically similar with P_2 treatment (5.30 cm) at 15 DAS. The highest plant height 6.02, 29.14, 57.41 and 57.65 cm was observed in P_4 (30 cm × 10 cm) treatment at 15 DAS, 30 DAS, 45 DAS and at harvest, respectively which was statistically similar with P_3 treatment (6.00 and 56.64 cm) at 15 DAS and at harvest, respectively.



Here, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Fig. 2. Effects of plant spacing on plant height of blackgram at different DAS

At different times after sowing, the height of blackgram plants was significantly impacted by plant variety and spacing (Table 1). The V_2P_4 treatment interaction showed maximum plant height in V_3P_4 (19.00, 34.32, 46.56 and 68.60 cm) at 15 DAS, 30 DAS, 45 DAS and harvest respectively. While V_1P_1 treatment combination showed the lowest plant height (10.34, 20.13, 22.10 and 41.13 cm) 15, 30, 45 DAS and at harvest respectively which was statistically comparable to V_3P_1 and V_2P_1 respectively.

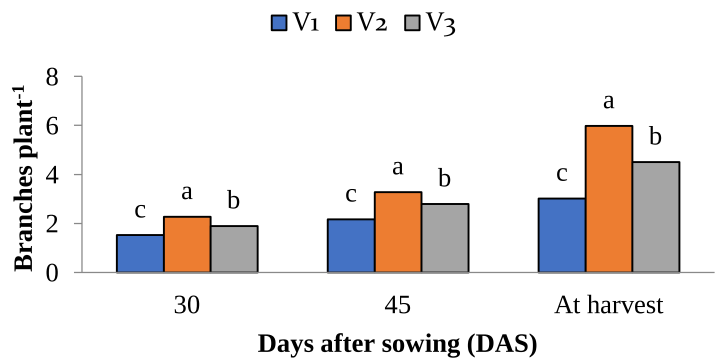
Table 1. Interaction effects of variety and planting geometry on plant height of blackgram at different DAS

Treatment combinations	Plant height (cm)			
	15 DAS	30 DAS	45 DAS	At harvest
V_1P_1	10.34 f	20.13 g	22.10 e	41.13 g
V_1P_2	13.43 de	26.34 e	31.60 cd	59.17 de
V_1P_3	15.13 cd	28.90 d	32.24 cd	58.78 de
V_1P_4	16.27 bc	31.19 c	40.20 b	62.49 cd
V_2P_1	13.33 de	27.57 de	34.17 c	44.60 g
V_2P_2	15.83 bc	27.52 de	34.72 c	63.83 bc
V_2P_3	16.32 bc	32.40 bc	42.45 b	67.40 ab
V_2P_4	19.60 a	34.60 a	49.73 a	71.12 a
V_3P_1	12.52 ef	22.44 f	30.07 d	53.57 f
V_3P_2	12.56 ef	22.60 f	31.30 cd	57.52 ef
V_3P_3	18.00 ab	31.32 c	38.98 b	57.11 ef
V_3P_4	19.00 a	34.32 ab	46.56 a	68.60 a
$LSD_{(0.05)}$	2.29	2.22	3.57	4.32
CV (%)	7.83	4.48	5.59	4.42

Here, V_1 = BINA mash1, V_2 = BARI mash2, V_3 = BARI mash4, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Effects of variety on branches plant^{-1}

The experimental results showed that, the V_2 (BARI mash3) treatment had the highest number of branches plant^{-1} (2.27, 3.28 and 5.98) at 30 DAS, 45 DAS and at harvest respectively. While the V_1 (BARI mash2) treatment, had the lowest number of branches plant^{-1} (1.52, 2.17 and 3.02) at 30 DAS, 45 DAS and at harvest respectively (Fig. 3).

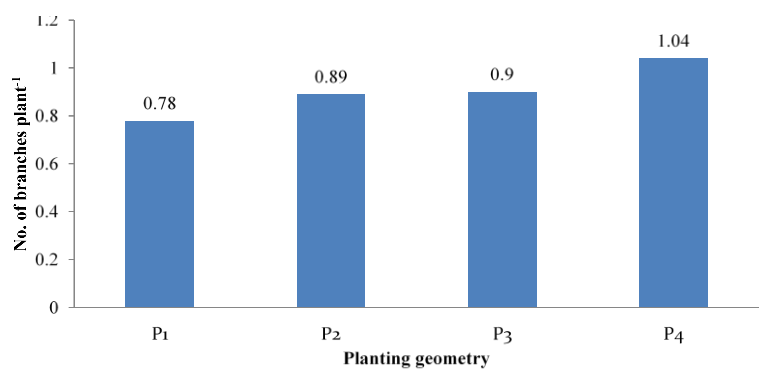


Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash4

Fig. 3. Effects of varieties on branches plant^{-1}of blackgram at different DAS

Effects of planting geometry on branches plant^{-1} of blackgram

Different planting geometry had shown significant effect in respect of number of branches plant^{-1} of blackgram at different days after sowing (DAS) (Fig. 4). According to the experimental results, the P_4 (30 cm × 10 cm) treatment had the highest number of branches plant^{-1} (1.04) at harvest (Fig. 4). While the lowest number of branches plant^{-1} (0.78) at harvest, was found in P_1 (broadcast) treatment.



Here, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Fig. 4. Effects of planting geometry on branches plant

Interaction effect of variety and plant spacing had significant variation of the number of branches plant^{-1} of blackgram at various days after sowing (Table 2). The treatment V_2P_4 combination, had the highest number of branches plant^{-1} (3.00, 6.30 and 12.30) at 30 DAS, 45 DAS and harvest respectively. While the lowest number of branches plant^{-1} of blackgram (1.33, 1.80 and 2.47) at 30 DAS, 45 DAS and at harvest respectively was found in V_1P_1 treatment combination which was statistically similar with V_1P_2 (1.47) treatment combination at 30 DAS and with V_1P_2 (2.60) and V_1P_3 (2.87) treatment combination and harvest, respectively.

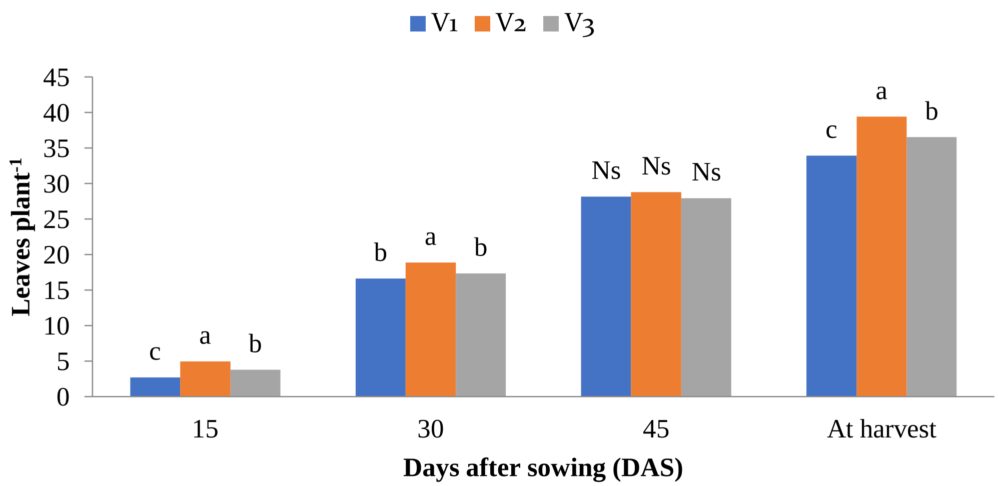
Table 2. Interaction effects of variety and planting geometry on number of branches plant^{-1}

Treatment combinations	Branches plant^{-1} (no)		
	30 DAS	45 DAS	At harvest
V_1P_1	1.33 h	1.80 g	2.47 g
V_1P_2	1.47 gh	2.14 ef	2.60 g
V_1P_3	1.53 g	2.27 e	2.87 fg
V_1P_4	1.74 ef	2.47 d	4.14 d
V_2P_1	1.63 e-g	2.07 f	2.86 g
V_2P_2	1.80 de	2.14 ef	3.46 e
V_2P_3	2.66 b	2.60 cd	5.30 c
V_2P_4	3.00 a	6.30 a	12.30 a
V_3P_1	1.60 fg	2.07 f	2.54 g
V_3P_2	1.74 ef	2.73 c	2.67 g
V_3P_3	1.94 d	2.60 cd	3.34 ef
V_3P_4	2.27 c	3.76 b	9.46 b
LSD $_{(0.05)}$	0.17	0.16	0.47
CV (%)	5.50	3.48	5.91

Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash4, P_1= Broadcast, P_2= Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Effects of variety on leaves plant^{-1}of blackgram at different DAS

The number of leaves plant^{-1} of blackgram at various days after sowing varied greatly depending on the varieties (Fig. 5). The V_2 (BARI Mash-4) treatment had the highest number of leaves plant^{-1} (4.96, 18.89, 28.79 and 39.41) at 15, 30, 45 DAS and at harvest respectively. While at 15 DAS, 30 DAS, 45 DAS and at harvest respectively the V_1 (BARI mash2) treatment had the lowest number of leaves plant^{-1}.

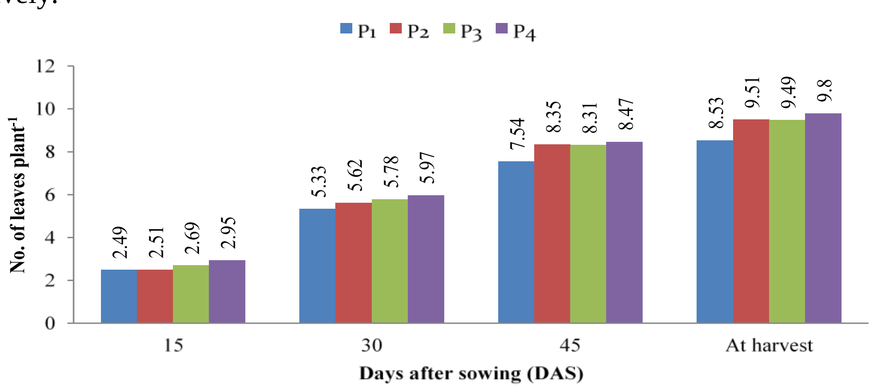


Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash4

Fig. 5. Effects of variety on leaves plant^{-1} of blackgram at different DAS

Effects of planting geometry on leaves plant^{-1} at different DAS

The number of leaves plant^{-1} of blackgram at different days after sowing varied significantly due to different planting geometry (Fig. 6). Experimental results showed that the P_4 (30 cm × 10 cm) treatment had the highest number of leaves plant^{-1} of 2.95, 5.97, 8.47 and 9.80 at 15 DAS, 30 DAS, 45 DAS and at harvest, respectively which was statistically similar with P_3 (8.31 and 9.49) and P_2 (8.35 and 9.51) at 45 DAS and harvest, respectively. On the other hand, the P_1 (Broadcast) treatment had the lowest number of leaves plant^{-1} of 2.49, 5.33, 7.54 and 8.53 at 15 DAS, 30 DAS, 45 DAS and at harvest, respectively.



Here, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Fig. 6. Effects of plant spacing on leaves plant^{-1} of blackgram at different DAS

Interaction effects of varieties and plant spacing

The V_2P_4 treatment combination had the highest number of leaves plant^{-1} (7.40, 22.40, 36.13 and 49.66) at 15, 30, 45 DAS and harvest respectively (Table 3) which was statistically comparable to V_3P_4 (48.27) treatment combination at harvest respectively.

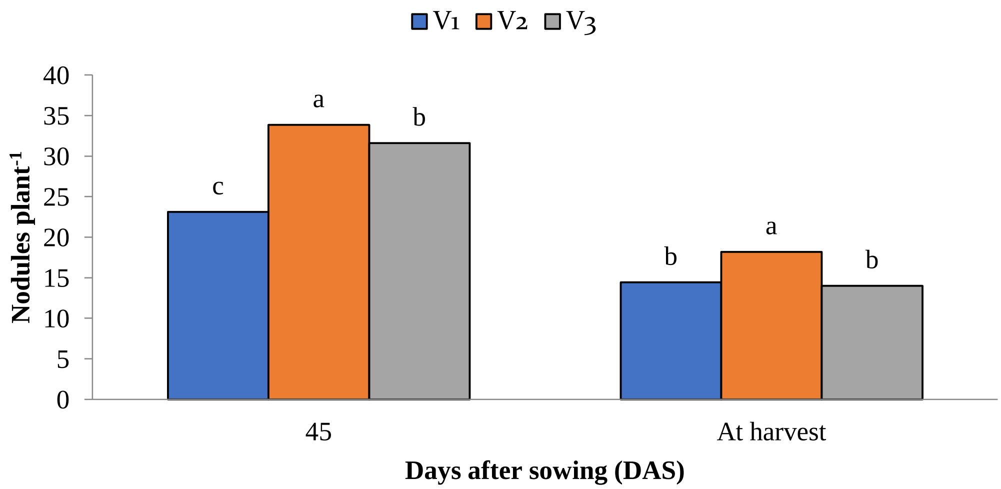
Table 3. Interaction effects of variety and planting geometry on number of leaves plant^{-1}of blackgram at different DAS

Treatment combinations	Leaves plant^{-1}			
	15 DAS	30 DAS	45 DAS	At harvest
V_1P_1	1.33 i	9.00 g	19.52 f	25.40 h
V_1P_2	1.86 h	18.00 de	29.00 c	31.87 f
V_1P_3	3.73 ef	19.27 b-d	31.94 b	34.20 e
V_1P_4	3.90 e	20.20 b	32.27 b	44.34 b
V_2P_1	3.00 g	16.60 e	22.20 e	29.50 g
V_2P_2	4.27 d	16.86 e	26.46 d	37.26 d
V_2P_3	5.20 b	19.73 bc	30.40 bc	41.20 c
V_2P_4	7.40 a	22.40 a	36.13 a	49.66 a
V_3P_1	1.80 h	14.40 f	23.93 e	28.67 g
V_3P_2	3.59 f	16.80 e	24.20 e	32.40 ef
V_3P_3	4.62 c	18.60 cd	31.47 b	36.74 d
V_3P_4	5.03 b	19.60 bc	32.20 b	48.27 a
LSD $_{(0.05)}$	0.26	1.59	2.21	2.25
CV (%)	4.08	5.18	4.56	3.25

Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash4, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Effects of varieties on nodules plant^{-1}

The findings of the experiment showed that the V_2 (BARI mash2) treatment had the highest number of nodules plant^{-1} (33.87 and 18.20 at 45 DAS and harvest, respectively). At harvest the lowest number of nodules plant^{-1} (14.00) was observed in V_3 treatment which was statistically comparable to V_1 (14.42) treatment. According to Singh *et al.* (2013), there was a substantial variation in the number of nodules per plant among different urd bean varieties.

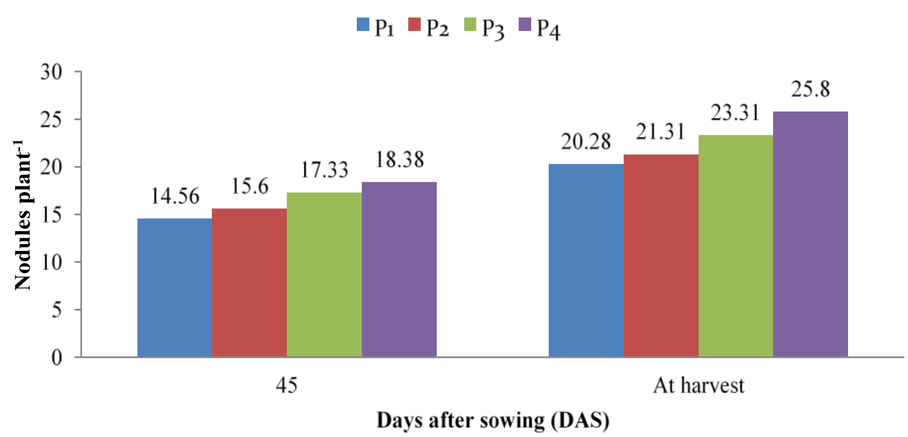


Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash4

Fig. 7. Effects of variety on nodules plant^{-1} of blackgram at different DAS

Effects of plant spacing

The P_4 treatment had the maximum number of nodules plant^{-1} (38.82 and 23.34) at 45 DAS and harvest respectively. Rasul *et al*. (2012) also reported that the highest nodules per plant (11.34) of blackgram was found with 30 cm row spacing, which was similar with present findings.



Here, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Fig. 8. Effects of plant spacing on nodules plant^{-1} of blackgram at different DAS

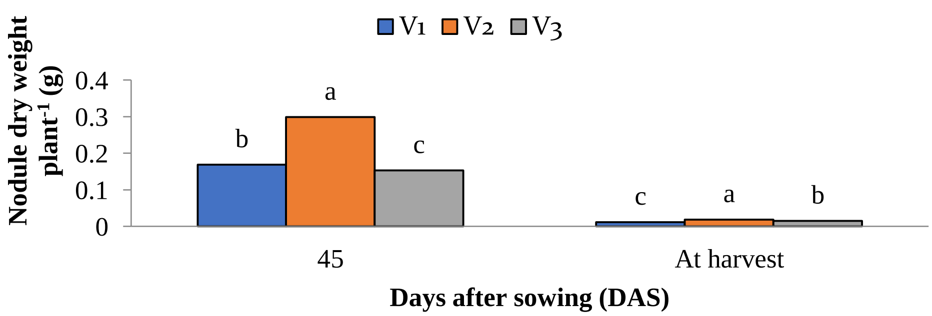
Combined effects of varieties and plant spacing

Combination of variety and plant spacing had shown significant difference in the number of nodule plant^{-1} of blackgram at various days after sowing (Table 4). The highest number of nodules plant^{-1} (40.12 and 25.34) at 45 DAS and harvest, respectively was found in the V_2P_4 treatment combination, which was statistically comparable with the V_3P_4 (38.34). While the lowest number of nodule plant^{-1} (10.0 and 4.34) at 45 DAS and at harvest, respectively was found in the V_1P_1 treatment combination.

Nodules dry weight plant^{-1} (g)

Effects of varieties

The results showed that the V_2 (BARI mash2) had the highest nodule dry weight plant^{-1} (0.299 and 0.019 g) at 45 DAS and at harvest, respectively (Fig. 9). However, at 45 DAS, the V_3 treatment had the lowest nodule dry weight plant^{-1} (0.153 g).

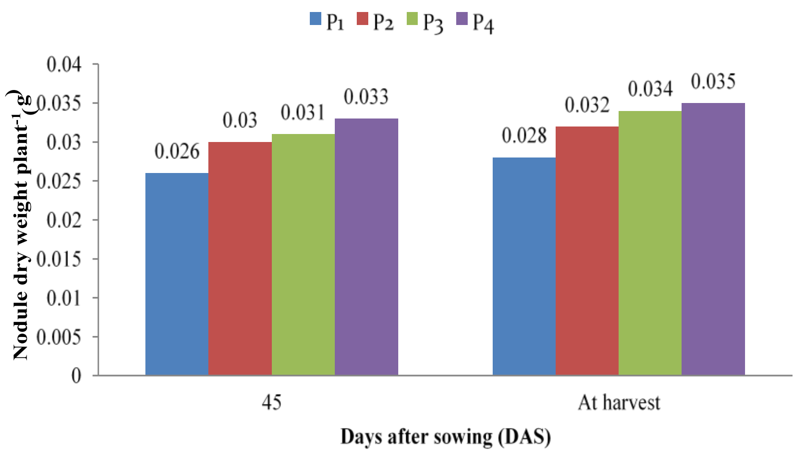


Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash4

Fig. 9. Effects of variety on nodules dry weight plant^{-1} of blackgram at different growth stages

Effects of plant spacing

The results showed that the P_4 treatment had the maximum nodules dry weight plant^{-1} (0.298 and 0.023g) at 45 DAS and harvest, respectively (Fig. 10). The result from the findings of Singh *et al.* (2009) revealed that the highest number and dry weight of nodules per plant were 30 cm × 10 cm spacing.



Here, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Fig. 10. Effects of plant spacing on nodules dry weight plant^{-1} at different growth stages

Interaction effects of varieties and plant spacing

Due to the combined effects of plant spacing and variety at different times after planting, the nodule dry weight plant^{-1} of blackgram varied (Table 4). The V_2P_4 treatment combination had the highest nodule dry weight plant^{-1} (0.497 and 0.034 g) at 45 DAS and at harvest. However, at 45 DAS and harvest, respectively, the V_1P_1 treatment combination had the lowest nodule dry weight plant^{-1} (0.098 and 0.007 g).

Table 4. Interaction effects of varieties and plant spacing on number of nodules and nodules dry weight plant^{-1}of blackgram at different DAS

Treatment combinations	Nodule plant^{-1}(no)		Nodules dry weight plant^{-1}(g)	
	45 DAS	At harvest	45 DAS	At harvest
V_1P_1	10.00 h	4.34 g	0.098 e	0.007 f
V_1P_2	19.12 g	12.67 d	0.179 c	0.013 d
V_1P_3	25.34 ef	19.00 c	0.199 c	0.014 d
V_1P_4	38.00 ab	21.67 b	0.199 c	0.014 d
V_2P_1	27.00 e	10.34 e	0.150 d	0.009 e
V_2P_2	34.00 d	12.00 d	0.150 d	0.010 e
V_2P_3	34.34 cd	25.12 a	0.398 b	0.021 b
V_2P_4	40.12 a	25.34 a	0.497 a	0.034 a
V_3P_1	23.67 f	5.00 g	0.130 d	0.009 e
V_3P_2	27.67 e	8.67 f	0.133 d	0.014 d
V_3P_3	36.67 bc	19.34 c	0.149 d	0.016 c

Treatment combinations	Nodule plant^{-1}(no)		Nodules dry weight plant^{-1}(g)	
	45 DAS	At harvest	45 DAS	At harvest
V_3P_4	38.34 ab	23.00 b	0.199 c	0.022 b
$LSD_{(0.05)}$	2.62	1.58	0.02	0.001
CV(%)	4.98	5.60	5.32	5.99

Here, V_1= BINA mash1, V_2= BARI mash2 and V_3= BARI mash4, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Table 5. Interaction effects of variety and planting geometry on number of pods plant^{-1}, pod length (cm), seeds pod^{-1} and 1000-seed weight (g) of blackgram

Treatment Combinations	No. of pods plant^{-1}	Pod length (cm)	Seeds pod^{-1}	1000-seed weight (g)
V_1P_1	15.40 g	5.18 d	7.06 g	38.26 f
V_1P_2	18.73 f	6.87 bc	9.67 f	44.30 e
V_1P_3	20.27 ce	7.05 bc	10.13 f	45.13 de
V_1P_4	20.53 ce	7.09 b	11.27 de	46.03 de
V_2P_1	19.60 ef	6.49 c	11.47 cd	45.57 de
V_2P_2	19.73 df	6.51 c	10.46 ef	46.20 de
V_2P_3	21.40 ac	6.49 c	12.13 bc	47.30 cd
V_2P_4	22.07 ab	7.09 b	12.20 bc	48.83 bc
V_3P_1	20.13 ce	6.51 c	10.20 f	47.57 cd
V_3P_2	20.93 bd	6.77 bc	11.53 bd	50.90 a
V_3P_3	22.00 ab	7.26 ab	12.33 b	51.67 a
V_3P_4	22.40 a	7.82 a	16.87 a	52.90 a
LSD (0.05)	1.31	0.57	0.85	2.49
CV (%)	3.29	4.93	4.00	3.33

Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash4. Here, P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm

Table 6. Effects of variety and planting geometry on seed yield t ha^{-1}, stover yield t ha^{-1}, biological yield t ha^{-1} and harvest index (%) of blackgram

Treatment Combinations	Seed yield (t ha^{-1})	Stover yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest index (%)
V_1P_1	1.27 j	2.39 f-h	3.66 h	34.67 c
V_1P_2	1.31 ij	2.44 ef	3.75 gh	34.93 c
V_1P_3	1.35 hi	2.46 df	3.81 fg	35.43 c
V_1P_4	1.40gh	2.53 bd	3.93 de	35.64 c

Treatment Combinations	Seed yield ($t\ ha^{-1}$)	Stover yield ($t\ ha^{-1}$)	Biological yield ($t\ ha^{-1}$)	Harvest index (%)
V_2P_1	1.43 fg	2.32 h	3.75 gh	38.13 b
V_2P_2	1.47ef	2.36 gh	3.83 eg	38.38 b
V_2P_3	1.50 de	2.39 fh	3.89 df	38.56 b
V_2P_4	1.54 d	2.43 fg	3.97 d	38.79 b
V_3P_1	1.70 c	2.51 ce	4.21 c	40.38 a
V_3P_2	1.74 bc	2.56 ac	4.30 bc	40.47 a
V_3P_3	1.78 ab	2.59 ab	4.37 ab	40.73 a
V_3P_4	1.83 a	2.63 a	4.46 a	41.03 a
LSD (0.05)	0.06	0.07	0.11	1.00
CV (%)	3.13	2.18	3.45	2.27

Here, V_1 = BINA mash1, V_2 = BARI mash2 and V_3 = BARI mash 4. P_1= Broadcast, P_2=Paired row (15 cm × 10 cm), P_3= Square planting (20 cm × 20 cm) and P_4= Row sowing (30 cm × 15 cm)

Conclusion

The study showed that the maximum seed yield (1.83 $t\ ha^{-1}$) was achieved when BARI mash4 (V_3) was cultivated with 30 cm × 15 cm spacing (P_4), which had an impact on plant growth and yield-contributing traits. Thus, growing BARI mash4 and spacing plants 30 cm by 15 cm (V_3P_4) are suggested as the optimal crop management techniques for optimizing blackgram yield.

Author's contribution

K. Kader: Conceptualized the research and designed the experiments; conducted data analysis and created visualizations; performed the experiments; wrote the paper. M. Ahmed: Analyzed and interpreted the data. A. K. M. R. Amin and M. F. Karim: Supervised the entire experiment and revised the manuscript.

Conflicts of Interest

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

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INFLUENCE OF PLANTING METHOD AND LEAF CLIPPING ON THE YIELD PERFORMANCE OF WHITE MAIZE

M. R. Bepary[1*], M. J. Ullah[1], M. H. Mahmud[2], M. Hassan[1] and M. D. Hossain[1]

[1]Department of Agronomy, Sher-e-Bangla Agricultural University (SAU), Dhaka; [2]Project Implementation Unit (PIU), Bangladesh Agricultural Research Council (BARC), Farmgate. Dhaka. Bangladesh.

Abstract

An experiment was conducted at the experimental field of Sher-e-Bangla Agricultural University, Dhaka during January to June 2018 to evaluate the influence of planting method and leaf clipping on the yield performance of white maize. The experiment was laid out in a split plot design with three replications. The experiment comprised of two factors. Factor A: Planting method - 2 types [P_1 = Sowing, P_2 = Transplanting] and Factor B: Leaf clipping - 4 levels [C_1 = no leaf clipping, C_2 = all leaf clipping, C_3 = clipping of four leaves above cob, C_4 = clipping of four leaves below cob]. White Maize Yungnuo 3000 was used for the experiment. Significant difference was observed on growth, yield and yield contributing parameters. In the case of planting method, all growth and yield attributes were showed better performance in treatment P_1 than treatment P_2. In the case of leaf clipping, C_1 treatment performed best on the plant height (89.31, 168.32 and 217.21 cm, respectively) at different days after planting, leaf length (41.87 cm), cob length (19.77 cm), cob breadth (18.42 cm), number of row cob^{-1} (14.67), number of grain row^{-1} (34.65), grain yield (8.69 t ha^{-1}), 100 seed weight (28.47 g), oven dried shell weight (17.87 g) and oven dried chaff weight (10.88 g), whereas poor performance was found in C_2. In case of interaction, P_1C_1 treatment gave the highest performance in all aspect of growth and yield parameters and lowest found from P_2C_2.

Keywords: Leaf clipping, Maize, Planting method, Yield.

Introduction

Maize (*Zea mays* L.) belongs to the family Poaceae and it is the third important cereal crop of the World after wheat and rice. It is grown extensively in temperate, subtropical and tropical regions of the world. Maize is used as a staple food for human consumption and feed for livestock. Maize is produced primarily as an energy source crop, but specialized versions for protein, oil, wax, sweet corn and popcorn are also available (Akbar *et al*., 2016). Maize (*Zea mays* L.) is an important cereal crop over the world, is now well-fitted in diversified cropping systems in the Indo-Gangetic Plains (Gathala *et al*., 2015).

[*]Corresponding author: h.mahmud193@gmail.com

Maize is one of the most important food crop in the world and, together with rice and wheat, provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (FAOSTAT 2016). Like many other parts in the world, market demand for maize in South Asia and Bangladesh has significantly increased in the last decade and the trend has been especially remarkable in Bangladesh, where cultivated land area with maize jumped from 0.05 M ha in (2000) to > 0.33 M ha in 2021 (FAOSTAT, 2021).

Maize currently grown in Bangladesh is of yellow type and are used in the feed industry. White maize covers only 12% of the total acreage of the world which is mostly used as human food (FAO-CIMMYT, 1997). With the advanced breeding approaches worldwide, recent reports demonstrate that the yield productivity of white maize is almost at par with those of the yellow ones. Inappropriate planting method caused reduction in germination, growth and development, ear size and increased susceptibility to diseases and lodging (Bakht *et al*., 2011). It is suggested to improve planting techniques for suitable seed bed preparation in order to ensure optimum plant population (Buttar *et al*., 2006). Maize biometrical parameters are significantly affected by planting methods. Rasheed *et al.* (2003) observed increased leaf area, leaf area index, crop growth rate and net assimilation rate in different planting methods. Khaliliaqdam *et al*. (2012) found that mutual shading, particularly at high population density, reduces number of grains per cob. After anthesis, the staminate inflorescence, the tassel may have very little or no effect on grain filling (Leakey *et al*., 2006).

Very few or no research finding are available in our country on leaf clipping in white maize field. So, there is a wide scope to conduct research activities on the efficacy of leaf clipping in white maize and to relate with varietal performance of white maize. Considering the above facts, the study was under taken to evaluate the influence of planting methods and leaf clipping on the yield performance of white maize.

Materials and Methods

The experiment was conducted at Sher-e-Bangla Agricultural University Farm, Dhaka during the period from 04 January 2018 to 28 June 2018. The geographical location of the site is 90°33′E longitude and 23°77′N latitude which belongs to "The Modhupur Tract", AEZ-28.

Experimental design

The experiment was laid in split plot design with three replications (block). Each replication was first divided into 8 subplots where treatment combinations were assigned. Thus the total number of unit plots was 8×3=24. The size of the individual plot was 3 m x 1.4 m. The inter plot spacing was 0.20 m and inters block spacing was 0.60 m. The following treatments were included in this experiment: Factor A: Planting method - 2 types, P_1 = Sowing, P_2 = Transplanting and Factor B: Leaf clipping - 4 levels, C_1 = No leaf clipping, C_2 = All leaf clipping, C_3 = Clipping of four leaves above cob, C_4 = Clipping of four leaves below cob

Crop management

Seeds of white maize variety Yungnuo 3000 were collected from Personal communication, Prof. Dr. Md. Jaffor Ullah, Department of Agronomy, SAU, Dhaka. Land prepared with recommended fertilizer dose of well rotten cowdung manure and chemical fertilizers were mixed with the soil of each unit plot. For each treatment; dry, clean and homogenous air dried seeds were used. Seeds were treated with Provax 200FF @ 0.3% of seed weight. Some seeds were planted in lines each having a line to line distance of 60 cm and plant to plant distance of 20 cm having 3 seeds hole-1 under direct sowing in the well prepared plot. The seedlings were raised in seedbed. The plot was kept ready through tractor drawn cultivator for preparing seedbeds. The seeds were sown in line keeping the 20 cm apart and covered with soil. The seedlings (4 weeks of age) were transplanted keeping the row to row distance of 60 cm and plant to plant 20 cm in each plot and frequent irrigation was given to survive the seedlings.

Intercultural operations

Weeding were done to keep the plots free from weeds, easy aeration of soil and to conserve soil moisture, which ultimately ensured better growth and development. The excess plants were thinned out from all of the plots at 35 days after sowing (DAS) for maintaining optimum population of the experimental plots. First irrigation was given on 20 days after sowing. Second irrigation was given on 40 days after sowing. Third irrigation was given on 70 days after sowing and fourth irrigation was given on 90 days after sowing. Plant protection measures were taken as and when necessary.

Data collection

Crops were harvested when 90% of the cob became golden in color. Grains were separated from the ears. Grain and stovers thus collected were dried in the sun for a couple of days. Dried grain and stovers of each plot were weighed and subsequently converted into t ha^{-1} weight.

Plant height (cm) and leaf length (cm) were recorded at 40 DAS, 80 DAS and at harvest as the average of 5 plants selected at random from the inner rows of each plot and then it converted to whole plot. Randomly selected 10 cobs were considered for taking data of cob length (cm) and cob diameter (cm) using slide calipers. The number of rows and the number of grains of five cobs was counted at each of the five randomly selected plants in each plot and then averaged. Grains obtained from each plot were sun-dried and weighed of the respective plot was recorded carefully and converted to t ha-1. One hundred (100) seeds from 5 cobs were counted randomly from each plot and then weighed. Shells and Chaff were collected from 5 kernels of each plot; dried in an oven at 600 C for 72 hours and then weighed.

Statistical analysis

The data were compiled and tabulated in proper form and were subjected to statistical analysis. Analysis of variance was done following the computer package MSTAT-C program developed by Russel (1986). The mean differences among the treatments were adjusted by Least Significant Difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

Results and Discussion

Plant height (cm)

Significant variation was observed on plant height at 40, 80 DAP and at harvest due to differences in planting method, leaf clipping and its interaction (Table 1 and 2). The results revealed that at 40, 80 DAP and at harvest, the treatment P_1 produced the tallest plant (85.43, 163.56 and 212.34 cm, respectively) and the treatment P_2 produced the shortest plant (82.87, 152.59 and 202.68 cm, respectively). This result is supported by Maiti and Sen (2003) who reported that delay in transplanting reduced plant height and other biomass related factors (number of tillers produced, leaf area index). At 40 DAP, the highest plant height (89.31 cm) was recorded from C_1 and the lowest (74.12 cm) from C_2. At 80 DAP, the highest plant height (168.32 cm) was recorded from C_1 and the lowest (135.87 cm) from C_2. At harvest, the highest plant height (217.21 cm) was recorded from C_1 and the lowest (172.29 cm) from C_2. At 40 DAP, the highest plant height (91.38 cm) was recorded from P_1C_1 and the lowest (74.21 cm) from P_2C_2. At 80 DAP, the highest plant height (172.34 cm) from P_1C_1 and the lowest (142.87 cm) from P_2C_2. At harvest, the highest plant height (219.36 cm) was recorded from P_1C_1 and the lowest (181.66 cm) from P_2C_2.

Table 1. Effects of planting method and leaf clipping on plant height, leaf length and leaf breadth at different days after sowing and transplanting of white maize

Treatments	Plant height (cm)			Leaf length (cm)	Leaf breadth (cm)
	40 DAP	80 DAP	At harvest		
Effect of planting method					
P1	85.43a	163.56a	212.34a	39.87a	5.17
P2	82.87b	152.59b	202.68b	36.44b	4.67
LSD (0.05)	0.68	0.46	0.65	0.53	0.57
CV (%)	2.39	6.40	4.47	2.76	2.38
Effect of leaf clipping					
C1	89.31 a	168.32 a	217.21 a	41.87 a	5.83
C2	74.12 d	135.87 d	172.29 d	33.82 c	4.93
C3	79.51 c	154.89 c	180.44 c	37.93 b	5.09
C4	84.37 b	162.38 b	191.56 b	38.76 b	5.36
LSD (0.05)	0.68	0.64	0.67	1.13	0.96
CV (%)	3.86	5.95	5.74	3.58	4.81

P_1 = Sowing, P_2 = Transplanting, C_1 = No leaf Clipping, C_2 = All leaf clipping, C_3 = Clipping of four leaves above cob, C_4 = Clipping of four leaves below cob

Leaf length (cm)

Leaf length showed significant variation due to differences in planting method, leaf clipping and treatment interaction (Table 1 and 2). The results revealed that, the treatment P_1 produced the highest leaf length (39.87 cm) and P_2 produced the lowest (36.44 cm). The findings of Liu *et al.* (2020) who stated that leaf clipping at early season significantly decreased the stem and leaf length. The treatment C_1 produced the highest leaf length (41.87 cm) and C_2 produced the lowest (33.82 cm). The highest leaf length (42.19 cm) was recorded from P_1C_1 which was statistically similar with P_1C_4 (40.95 cm) and the lowest (31.14 cm) from P_2C_2.

Leaf breadth (cm)

Non-significant variation was observed on leaf breadth due to differences in planting method and leaf clipping (Table 1), but significant at interaction of treatment (Table 2). The highest leaf breadth (6.08cm) was recorded from P_1C_1 which was statistically similar with P_1C_4 (5.81 cm) and the lowest (4.08 cm) from P_2C_2.

Table 2. Interaction effect of planting method and leaf clipping on plant height, leaf length and leaf breadth at different days after sowing and transplanting of white maize

Treatments	Plant height (cm)			Leaf length (cm)	Leaf breadth (cm)
	40 DAS	80 DAS	At harvest		
P_1C_1	91.38 a	172.34 a	219.36 a	42.19 a	6.08 a
P_1C_2	80.11 d	161.25 d	202.39 c	37.12 b	4.91 c
P_1C_3	83.62 c	162.59 c	202.87 c	39.02 b	5.33 b
P_1C_4	86.74 b	167.84 b	209.55 b	40.95 a	5.81 a
P_2C_1	85.97 b	152.20 e	190.88 d	36.86 c	4.82 c
P_2C_2	74.21 f	142.87 g	181.66 f	31.14 e	4.08 e
P_2C_3	77.29 e	147.66 f	184.74 e	34.11 d	4.51 d
P_2C_4	82.92 c	151.83 e	187.21 d	36.22 c	4.66 c
$LSD_{(0.05)}$	0.72	0.58	0.63	1.92	0.37
CV (%)	2.58	5.22	3.67	3.10	4.26

P_1 = Sowing, P_2 = Transplanting, C_1 = No leaf Clipping, C_2 = All leaf clipping, C_3 = Clipping of four leaves above cob, C_4 = Clipping of four leaves below cob

Cob length (cm)

Significant variation was found on cob length due to differences in planting method, leaf clipping and its combination treatment (Table 3 and 4). The treatment P_1 produced the highest cob length (17.63 cm) and P_2 produced the lowest (14.08 cm). The treatment C_1 produced the highest cob length (19.77 cm) and C_2 produced the lowest

(13.28 cm). The highest cob length (20.57 cm) was recorded from P_1C_1 which was statistically similar with P_1C_4 (19.91 cm) and the lowest (12.19 cm) from P_2C_2.

Cob breadth (cm)

Variation was found significantly on cob breath due to differences in planting method, leaf clipping and its combination treatment (Table 3 and 4). The treatment P_1 produced the highest cob breadth (12.45 cm) and P_2 produced the lowest (10.79 cm). The treatment C_1 produced the highest cob breadth (18.42 cm) and C_2 produced the lowest (11.91 cm). The highest cob breadth (18.79 cm) was recorded from P_1C_1 and the lowest (10.44 cm) from P_2C_2. Dry-matter production has been closely related to photosynthesis capacity, especially post-silking dry matter accumulation (Ma, *et al.,* 2010).

Number of cob bearing node

Significant variation was observed on number of cob bearing node due to differences in planting method, leaf clipping and interaction treatment (Table 3 and 4). The treatment P_1 produced the highest number of cob bearing node (7.67) and P_2 produced the lowest (5.33). The treatment C_1 produced the highest number of cob bearing node (9.33) which was statistically similar with C_4 (8.93) and C_2 produced the lowest (6.33). The highest number of cob bearing node (10.67) was recorded from P_1C_1 which was statistically similar with P_1C_4 (9.93) and the lowest (5.33) from P_2C_2. Number of cob bearing of maize depends on the variety. Similar result found by Mtyobile (2021).

Table 3. Effect of planting method and leaf clipping on cob length, cob breadth, number of cob bearing node and number of row cob^{-1} of white maize

Treatments	Cob length (cm)	Cob breadth (cm)	Number of cob bearing node	Number of row cob^{-1}
		Effect of planting method		
P1	17.63 a	12.45 a	7.67 a	13.67 a
P2	14.08 b	10.79 b	5.33 b	10.33 b
LSD (0.05)	0.88	0.67	0.73	0.92
CV (%)	4.45	3.59	6.26	4.16
		Effect of leaf clipping		
C1	19.77 a	18.42 a	9.33 a	14.67 a
C2	13.28 c	11.91 c	6.33 c	11.33 c
C3	17.22 b	15.48 b	7.67 b	12.57 b
C4	17.67 b	16.34 b	8.93 a	13.33 b
LSD (0.05)	0.74	0.96	0.46	0.89
CV (%)	3.58	4.82	3.11	5.59

P_1 = Sowing, P_2 = Transplanting, C_1 = No leaf Clipping, C_2 = All leaf clipping, C3 = Clipping of four leaves above cob, C_4 = Clipping of four leaves below cob

Number of row cob^{-1}

Significant variation was observed on number of row cob^{-1} due to differences in planting method, leaf clipping and interaction effect (Table 3 and 4). The treatment P_1 produced the highest number of row cob^{-1} (13.67) and P_2 produced the lowest (10.33). The treatment C_1 produced the highest number of row cob^{-1} (14.67) and C_2 produced the lowest (13.33). The highest number of row cob^{-1} (16.33) was recorded from P_1C_1 and the lowest (10.67) from P_2C_2.

Table 4. Interaction effect of planting method and leaf clipping on cob length, cob breadth, number of cob bearing node and number of row cob^{-1} of white maize

Treatments	Cob length (cm)	Cob breadth (cm)	Number of cob bearing node	Number of row cob^{-1}
P_1C_1	20.57 a	18.79 a	10.67 a	16.33 a
P_1C_2	18.15 b	15.72 c	8.79 b	15.33 b
P_1C_3	18.67 b	16.96 b	9.33 b	14.67 c
P_1C_4	19.91 a	17.31 b	9.93 a	15.67 b
P_2C_1	15.24 c	13.23 d	8.67 b	14.34 c
P_2C_2	12.19 e	10.44 f	5.33 e	10.67 e
P_2C_3	13.48 d	11.08 e	6.67 d	13.33 d
P_2C_4	14.69 c	13.06 d	7.63 c	13.67 d
$LSD_{(0.05)}$	0.75	0.42	0.87	0.53
CV (%)	6.54	4.29	3.66	5.41

P_1 = Sowing, P_2 = Transplanting, C_1 = No leaf Clipping, C_2 = All leaf clipping, C_3 = Clipping of four leaves above cob, C_4 = Clipping of four leaves below cob

Number of grain row^{-1}

Significant variation was found on number of grain row^{-1} due to differences in planting method, leaf clipping and interaction (Table 5 and 6). The treatment P_1 produced the highest number of grain row^{-1} (26.61) and P_2 produced the lowest (23.87) due to delay in transplanting resulted in decreasing grain yield due to delayed seeding might be associated with the significantly lower number of productive tillers per meter, less number of filled grains per panicle and low 1000 grain weight (Baloch *et al*. (2006). The treatment C_1 produced the highest number of grain row^{-1} (34.65) and C_2 produced the lowest (29.44). The highest number of grain row^{-1} (36.89) was recorded from P_1C_1 and the lowest (24.37) from P_2C_2.

Table 5. Effect of planting method and leaf clipping on cob length, cob breadth, number of cob bearing node and number of row cob^{-1} of white maize

Treatments	Number of grain row^{-1}	Grain yield (t ha^{-1})	100 seed weight (g)	Shell weight (g)	Chaff weight (g)
		Effect of planting method			
P1	26.61a	7.46a	21.58a	15.72a	7.29a
P2	23.87b	6.49b	19.03b	13.37b	5.57b
LSD (0.05)	0.88	0.67	0.73	0.92	0.85
CV (%)	4.45	3.59	6.26	4.16	5.62
		Effect of leaf clipping			
C1	34.65a	8.69a	28.47a	17.87a	10.88a
C2	29.44d	6.07d	23.89bc	14.32c	5.79c
C3	31.22c	7.26	25.20b	15.57b	7.12b
C4	32.80b	7.95	26.35a	16.91a	8.62a
LSD (0.05)	0.74	0.66	2.13	0.96	1.27
CV (%)	4.85	4.61	4.21	5.35	5.90

P_1 = Sowing, P_2 = Transplanting, C_1 = No leaf Clipping, C_2 = All leaf clipping, C_3 = Clipping of four leaves above cob, C_4 = Clipping of four leaves below cob

Grain yield (t ha^{-1})

Planting method, leaf clipping and treatment interaction had showed significant variation on grain yield (Table 5 and 6). The treatment P_1 produced the highest grain yield (7.46 t ha^{-1}) and P_2 produced the lowest (6.49 t ha^{-1}). The treatment C_1 produced the highest grain yield (8.69 t ha^{-1}) and C_2 produced the lowest (6.07 t ha^{-1}). This result is supported by Cheema *et al.* (2010) who reported that maize leaf clipping caused the seed yield reduction because of the seed number decrease. The highest grain yield (8.70 t ha^{-1}) was recorded from P_1C_1 and the lowest (4.92 t ha^{-1}) from P_2C_2. Reduction in yield with defoliation treatment in maize was reported by Gaias *et al.* (2017) and Heidari (2017).

100 seed weight (g)

Significant variation was found on 100 seed weight due to differences in planting method, leaf clipping and its interaction (Table 5 and 6). The treatment P_1 produced the highest 100 seed weight (21.58 g) and P_2 produced the lowest (19.03 g). The treatment C_1 produced the highest 100 seed weight (28.47 g) which was statistically similar with C_4 (26.35 g) and C_2 produced the lowest (23.89 g). This results are in conformity with Ahmadi *et al.* (2009) who reported that clipping significantly affect remobilization of grain yield and 1000-grain weight. The highest 100 seed weight (29.45 g) was recorded from P_1C_1 and the lowest (20.37 g) from P_2C_2.

Shell weight (g)

Significant variation was found on oven dried shell weight due to differences in planting method, leaf clipping and treatment interaction (Table 5 and 6). The results revealed that, P_1 produced the highest oven dried shell weight (15.72 g) and P_2 produced the lowest (13.37 g). The treatment C_1 produced the highest oven dried shell weight (17.87 g) which was statistically similar with C_4 (16.91 g) and C_2 produced the lowest (14.32 g). The highest oven dried shell weight (18.11 g) was recorded from P_1C_1 which was statistically similar with P_1C_4 (17.32 g) and the lowest (13.39 g) from P_2C_2.

Table 6. Interaction effect of planting method and leaf clipping on number of grain row^{-1}, grain yield and 100 seed weight of white maize

Treatments	Number of grain row^{-1}	Grain yield (t ha^{-1})	100 seed weight (g)	Shell weight (g)	Chaff weight (g)
P_1C_1	36.89 a	8.70 a	29.45 a	18.11 a	11.05 a
P_1C_2	31.66 c	6.86 d	25.38 c	15.72 b	6.65 d
P_1C_3	33.85 b	7.46 c	27.12 b	16.36 b	7.87 c
P_1C_4	34.28 b	8.16 b	27.66 b	17.32 a	9.22 b
P_2C_1	29.72 d	6.81 d	25.19 c	15.29 c	7.43 c
P_2C_2	24.37 f	4.92 g	20.37 e	13.39 e	5.61 e
P_2C_3	26.19 e	5.51 f	22.96 d	13.76 d	6.22 d
P_2C_4	29.41 d	6.19 e	23.57 d	14.64 c	6.51 d
$LSD_{(0.05)}$	0.56	0.48	0.64	0.83	0.65
CV (%)	4.97	6.61	2.43	5.39	4.12

P_1 = Sowing, P_2 = Transplanting, C_1 = No leaf Clipping, C_2 = All leaf clipping, C_3 = Clipping of four leaves above cob, C_4 = Clipping of four leaves below cob

Chaff weight (g)

Significant variation was observed on oven dried chaff weight due to differences in planting method, leaf clipping and their treatment combination (Table 5 and 6). The results revealed that, the treatment P_1 produced the highest oven dried chaff weight (7.29 g) and P_2 produced the lowest (5.57 g). The treatment C_1 produced the highest oven dried chaff weight (10.88 g) which was statistically similar with C_4 (8.62 g) and C_2 produced the lowest (5.79 g). The highest oven dried chaff weight (11.05 g) was recorded from P_1C_1 and the lowest (5.61 g) from P_2C_2.

Conclusion

The experiment was conducted to study the effect of planting methods and leaf clipping on yield performance of white maize. Considering the above results, it may be concluded that leaf clipping adversely affects all the yield related attributes.

Sowing showed better result in all aspects than transplanting. No leaf clipping produced better yield and yield contributing attributes than all leaf clipped. Seed sowing with no leaf clipping showed the best performance in terms of yield of white maize. However, further experimentation need to be executed in different agro-ecological zones with more varieties.

Author's contribution

M. R. Bepary performed the experiment, gathered, analyzed and interpreted the data and drafted the manuscript. M. J. Ullah developed the research idea, objectives, methodology and guided and oversighted throughout the research process. M. H. Mahmud reviewed, revised and edited the content. M. Hassan and M. D. Hossain helped in performing research, gathering and analyzing the data.

Conflicts of Interest

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

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GENETIC DIVERSITY ANALYSIS AND CHARACTER ASSOCIATION IN YIELD AND YIELD CONTRIBUTING TRAITS OF MUNGBEAN (*Vigna radiata* L.)

M. Rahman*, M. S. Hossain and N. Zeba

Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka. Bangladesh.

Abstract

An experiment was carried out at Sher-e-Bangla Agricultural University in *Kharif-1* season with 33 genotypes of mungbean (*Vigna radiata* L.) to identify genetically diverse genotypes for yield contributing characters. The genotypes were evaluated for eight yield and yield contributing characters using a Randomized Complete Block Design (RCBD) with three replications. High heritability coupled with a high genetic gain was observed in days to 50% flowering, plant height, pod length, 100 seed weight, and seed yield per plant which indicated the effect of additive genes. Selection of these traits might cause the probability of simultaneous improvement of mungbean. The genotypes were grouped into four clusters based on the D^2-value where cluster I was the largest and comprised of 13 genotypes followed by clusters III and IV with 6 genotypes in each. The highest inter-cluster distance was recorded between clusters II and IV (10.425) and the intra-cluster distance in cluster II (2.45). The lowest inter-cluster distance was found between clusters I and III (3.19). Principle component analysis revealed first four components contributed 88.77% towards genetic diversity in mungbean. Considering the magnitude of cluster means and agronomic performance the genotypes of cluster II and the genotypes of cluster IV might be suggested as parents for future hybridization programs.

Keywords: Genetic advance, Genetic variation, Heritability, Yield contributing traits.

Introduction

Mungbean (*Vigna radiata* L.) is one of the most important pulse crops belonging to the Papilionoideae subfamily of the Fabaceae and has a diploid chromosome number of 2n = 2x = 22 and is widely grown in Bangladesh but production is lower. As it is a short-duration crop, it can fit in as a cash crop between major cropping seasons. It is grown three times in a year. About 60-65% of the total mungbean is grown under the Boro rice- mungbean-Aus rice (rainfed) cropping pattern. Mungbean contains 51% carbohydrate, 24-26% protein, 4% mineral, and 3% vitamins (Afzal *et al.*, 2008). Besides providing protein in the diet, mungbean has the remarkable quality of helping the symbiotic root rhizobia to fix atmospheric nitrogen and hence enrich soil fertility (Anjum

* Corresponding author: emma.marzan@gmail.com

et al., 2006). It is covering 109000 acres with an average yield of 41000 MT (BBS, 2022). The major area of mungbean is replaced by cereals (Abedin *et al*., 1991). There are a few constraints such as several biotic and abiotic stresses, low yield, rice cultivation all year round mainly in winter (*Boro*) season which limits pulse cultivation, limited study was done on mungbean and the area covered by this crop is not satisfactory. Only a few varieties of mungbean have been developed by the Pulses Research Centre, BARI, and disseminated with the package of management technologies to the farmers for cultivation. Therefore, to improve the yield and quality of mungbean several activities like building up diverse germplasm, selection, and evaluation of genotype from germplasm, etc. should be used in the improvement program. Genetic diversity is the basic element for an efficient choice of parents for the variety development program. Genetic diversity determines the inherent potential of a cross for heterosis and the frequency of desirable recombinants in advanced generations. Therefore, the present study was conducted to study the genetic diversity for finding suitable parental groups with better performances for future breeding programs.

Materials and Methods

The experiment was carried out at the experimental field of Sher-e-Bangla Agricultural University. The experimental materials of the study consisted of 33 genotypes of mungbean (Table 1) collected from the Plant Genetic Resources Centre (PGRC) of Bangladesh Agricultural Research Institute (BARI). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications with a distance of plant to plant 15cm, line to line 30 cm (Krishi Projukti Hatboi, 2014), and plot to plot 2.5 m. From land preparation to all necessary intercultural operations were properly done during the cropping period (*Kharif-1*: March-June, 2017) for better growth of plants for better yield. Thinning was done 25 days after sowing and the first weeding was done during thinning and the second one after about two months of sowing. Application of fertilizers at recommended doses (Urea: 40-50 kg/ha, TSP: 80-85 kg/ha, MP: 30-35 kg/ha) by BARI (Krishi Projukti Hatboi, 2014) was done properly. Harvesting of mungbean pods was done three times after the maturity stage. The data were recorded on five selected plants from each replication of a genotype on the following characters: i. days to 50% flowering ii. plant height (cm) iii. number of branches per plant iv. number pod per plant v. pod length (cm) vi. seed per pod vii. 100 seed weight (g) viii. yield per plant (g). The data were analyzed following principal component analysis (PCA), principal coordinate analysis (PCO), cluster analysis (CA) canonical vector analysis (CVA), and Duncan‘s multiple range test (DMRT). Intra and inter-cluster distances were calculated by the method of Singh and Chaudhury (1985).In RCBD MSTAT-C was used to analyze variance and mean performance. GENSTAT 5.13 was used for multivariate analysis.

Table 1. Genotypes of mungbean

Sl.No.	Designation	Accessions	Sl. No.	Designation	Accessions
1	G1	BD-6874	18	G18	BD-6917
2	G2	BD-6875	19	G19	BD-6918
3	G3	BD-6876	20	G20	BD-6920
4	G4	BD-6878	21	G21	BD-6923
5	G5	BD-6879	22	G22	BD-6924
6	G6	BD-6896	23	G23	BD-6925
7	G7	BD-6898	24	G24	BD-6926
8	G8	BD-9835	25	G25	BD-6927
9	G9	BD-9837	26	G26	BD-6928
10	G10	BD-6900	27	G27	BD-6929
11	G11	BD-6901	28	G28	BD-6932
12	G12	BD-6902	29	G29	BD- 6933
13	G13	BD-6903	30	G30	BD-6934
14	G14	BD-6904	31	G31	BD-6935
15	G15	BD-6906	32	G32	BD-6936
16	G16	BD-6907	33	G33	BD-6937
17	G17	BD-6909			

Results and Discussion

The present experiment was conducted to study the genetic variability, character association, and genetic diversity among 33 genotypes of mungbean. Variance, heritability, and genetic advance were measured by DMRT test where days to 50% flowering showed high heritability (95.95%) with high genetic advance (6.71) (Table 2). Reddy *et al*. (2004) reported a result similar to this experiment. High heritability for hundred seed weight in mungbean was similar to Sarwar *et al*. (2004). In the case of the number of pods, 100g-seed weight, and seed yield showed high variation. Moderate variation was found in plant height, number of branches, and lower in pod length. Fetemeh *et al*. (2012) also performed a similar study in mungbean using D2 statistic.

Table 2. Estimation of genetic parameters in eight characters of 33 mungbean genotypes

Parameters	Range	Mean	Mean sum of square (MS)	σ^2p	σ^2g	σ^2 e	PCV (%)	GCV (%)	ECV (%)	Heritability (%)	Genetic advance (5%)	Genetic advance (% mean)
Days to 50% flowering	7.67-19.33	14.99	33.61**	11.52	11.05	0.47	22.64	22.18	4.56	95.95	6.71	44.75
Plant height (cm)	33.20-72.87	49.32	253.09**	99.93	76.58	23.35	20.27	17.74	9.80	76.63	15.78	31.99
No. of branches per plant	1.74-3.61	2.41	0.83**	0.46	0.19	0.27	28.14	17.90	21.72	40.43	0.57	23.44
Pods per plant	6.00-22.40	11.15	49.11**	20.71	14.20	6.51	40.80	33.78	22.88	68.56	6.43	57.62
Pod length (cm)	5.42-8.98	6.43	2.24**	0.85	0.69	0.16	14.35	12.95	6.19	81.42	1.55	24.07
Seeds per pod	8.11-12.80	10.14	0.78**	1.48	0.70	0.78	11.99	8.22	8.73	47.01	1.18	11.61
100 seed weight (g)	2.01-7.30	3.11	3.18**	1.16	1.01	0.16	34.64	32.23	12.68	86.59	1.92	61.78
Seed yield per plant (g)	1.52-4.34	2.70	1.49**	0.66	0.42	0.25	30.20	23.89	18.46	62.61	1.05	38.94

σ^2p = Phenotypic variance, σ^2g = Genotypic variance and σ^2 e = Environmental variance, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation

The principal component analysis (PCA) showed eigenvalues and percent of variation with respect to eight component characters in 33 genotypes of mungbean (Table 3). The result indicated that the first four eigenvalues for four principal coordination axes of genotypes accounted for 88.77% variation shown in Table 3. The first principal component accounted for 47.42 % of the total variation. Thirty-three genotypes were grouped into four different clusters to non-hierarchical clustering (Table 4) where cluster I had the highest number of genotypes and it was 13; clusters II, III, and IV had 8, 6, and 6 genotypes respectively.

Table 3. Eigenvalues and percent variation of eight characters of 33 genotypes of mungbean

Principal component axes	Eigenvalues	Percent variation	Cumulative % of Percent variation
I	3.320	47.42	47.42
II	1.449	20.69	68.11
III	0.814	11.63	79.74
IV	0.632	9.03	88.77
V	0.352	5.00	93.78
VI	0.234	3.35	97.14
VII	0.200	2.80	99.94
VIII	0.156	0.06	100.00

Table 4. Distribution of 33 genotypes in different clusters

Cluster no.	Genotypes	No. of populations
I	3, 5, 7, 10, 15, 19, 20, 21, 23, 27, 29, 31, 33	13
II	1, 2, 9, 12, 13, 14, 16, 17	8
III	4, 6, 11, 18, 26, 32	6
IV	8, 22, 24, 25, 28, 30	6
	Total	33

Genotypes of cluster II earned the highest cluster mean value (Table 5) for plant height (63.09), number of branches per plant (2.80), and seeds per pod (11.01) (Table 5). The genotypes included in cluster III had the highest mean value for days to 50% flowering (16.11), pods per plant (13.66), and seed yield per plant (3.18). Genotypes in Cluster IV produced the maximum cluster mean for pod length (6.90) and 100 seed weight (3.81). Konda *et al.* (2007) experiment relevant to this experiment.

Table 5. Cluster means for eight yield and yield-related characters in 33 mungbean genotypes

Characters	I	II	III	IV
Days to 50% flowering	15.77	13.13	16.11	14.67
Plant height (cm)	45.11	63.09	51.07	38.35
No. of branches per plant	2.47	2.80	2.27	1.91
Pods per plant (no.)	10.38	13.42	13.66	7.30
Pod length (cm)	6.40	6.36	6.14	6.90
Seeds per pod (no.)	9.79	11.01	10.31	9.58
100 seed weight (g)	3.18	2.80	2.69	3.81
Seed yield per plant (g)	2.48	2.97	3.18	2.33

Canonical Variate Analysis (CVA) was done to compute the inter-cluster distances. The intra and inter-cluster distance (D2) values are shown in Table 6. In this experiment, the inter-cluster distances were higher than the intra-cluster distances thus indicating broader genetic diversity among the genotypes of different groups. The highest inter-cluster distance was observed between clusters II and IV (10.425), followed by between clusters I and II (7.450). In contrast, the lowest inter-cluster distance was observed between clusters I and III (3.199). On the other hand, the maximum intra-cluster distance was found in cluster II (2.45) which contained only 8 genotypes while the minimum distance was found in cluster IV (1.73) which comprises 6 genotypes (Table 6). However, the maximum inter-cluster distance was observed between clusters II and IV (10.425) indicating genotypes from these two clusters if involved in hybridization may produce high heterosis and a wide spectrum of segregating populations.

The latent vectors (1 and 2) obtained from principal component analysis (PCA) (Table 7) showed positive values in both the vectors in case of days to 50% flowering (0.3436, 0.0356), plant height (0.3877, 0.0148), number of branches per plant (0.1007, 2.040) and pod length (0.4361, 0.8728) indicated major role of these traits in the genetic diversity.

Table 6. Intra (Bold) and inter-cluster distances (D2) for 33 genotypes

Cluster	I	II	III	IV
I	2.21	7.450	3.199	3.261
II		2.45	5.110	10.425
III			1.87	5.892
IV				1.73

Table 7. Relative contributions of the eight characters of 33 varieties to the total divergence

Principal component Accessions	Principal Component	
	Vector-1	Vector-2
Days to 50% flowering	0.3426	0.0356
Plant height (cm)	0.3877	0.0148
No. of branches per plant	0.1007	2.0940
Pods per plant	0.1420	-0.0428
Pod length (cm)	0.4361	0.8728
Seeds per pod	-0.0156	0.4476
100 seed weight (g)	-0.1844	0.0929
Seed yield per plant (g)	-0.1133	-1.5368

The higher the inter-class distance and intra-cluster distance indicated the higher the diversity present among the genotypes between and within clusters, respectively. On the other hand, the lower inter and intra-cluster distances represent the lower diversity present among the genotypes between and within clusters, respectively. The genotypes present in the distant clusters would be used as parents in hybridization programs for gaining a wide spectrum of variation among the segregation generation.

Conclusion

The present experiment was undertaken to obtain a set of genotypes as parent materials with a wide range of diversity based on yield contributing characters for future breeding. Under the consideration of the variance, coefficient of variation, heritability, degree of magnitude of genetic distance, contribution of different traits towards the divergence, magnitude of cluster mean values for different characters and their performance, genotypes from cluster II for minimum days to 50% flowering, maximum number of pods per plant, maximum number of seeds per pod, highest plant height and genotypes from cluster IV for maximum pod length and 100 seed weight could be selected for further breeding program. So crossing between genotypes from cluster II and cluster IV can produce remarkable desired results in hybridization.

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Conflicts of Interest

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

Author contributions

M. R. : Conceptualization, methodology, conduction of field research, supervision, data collection, data analysis, studying relevant scientific papers, initial drafting of manuscripts, reviewing and editing in writing, ensuring clarity and coherence and funding acquisition. M. S. H. : Conceptualization, methodology, supervision, investigation, and funding acquisition. N. Z. : supervision, funding acquisition.

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Title: The title should be precise with the fewest words possible and no abbreviation.

Running title: A short title of less than 50 characters, to be used as a running head at the top of the page, should be provided.

Abstract: The abstract should be concise and clear. It should be in one paragraph and structured with background, objectives, methods, key findings, and conclusion. At the end of Abstract, maximum five keywords should be written in alphabetical order with the first letter in upper case. Keywords preferably should not contain any word which is already present in the Title.

Divide your article into clearly defined and numbered sections. Subsections should be numbered as 2.1 (then 2.1.1, 2.1.2, ...), 2.2, etc. (Abstract is not included in section numbering). Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction: State the objectives of the work and provide background information including relevant literature which demonstrates the need for a new study. This section could be of 3-5 paragraphs in length.

Materials and Methods: State the materials and methods that used in the study. Only new methods and any modifications to existing methods should be described in detail, and the methods that are published should be summarized, and indicated by a reference. Statistical design with replications of each experiment needs to be mentioned.

Results and Discussion: The text should be clear, concise and simply stated. Statistically significant results from each table or illustration should be stated in the text. The text should be consistent with the data in tables and figures. Results should be interpreted and compared with others, but not just repetition of results. Avoid extensive citations and discussion of published literature

Tables and Figures: Tables and figures should be placed at appropriate places of the manuscript. Figures should be black and white or colored with high resolution and adequate contrast.

Conclusion: This section should focus on the key results by concise and precise statements. It should be related to the objectives. Any recommendation and future research could be stated in this section.

Acknowledgements: It should be kept as minimum as possible including funding source and individuals who have provided help in carrying out the research.

References: References are listed chronologically by the author and year system without numbering; all entries in this list must correspond to references in the text. In the text, the names of 2 co-authors are linked by 'and'; for 3 or more, the first author's name is followed by *'et al.,*. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication. The reference list should be prepared alphabetically in the style as examples below.

Journal article

Alam, M. K., Bell, R. W., Haque, M. E., Islam, M. A., and Kader, M. A. 2020. Soil nitrogen storage and availability to crops are increased by conservation agriculture practices in rice-based cropping systems in the Eastern Gangetic Plains. *Field Crops Res.* 250:1-14.

Book

De Datta, S. K. 1981. Principles and Practices of Rice Production. John Wiley & Sons, New York, USA.

Book chapter

M. Jahiruddin. 2019. Natural Resource Management in South Asia. In: R. B. Shrestha, S. M. Bokhtiar, R. Khetarpal, Y. M. Thapa (Eds.), Agricultural Policy and Program Framework: Priority Areas for Research & Development in South Asia, Chapter 16, pp 347-357. SAARC Agriculture Centre, BARC Complex, Dhaka.

Conference proceedings

Islam, A. K. M. S., Haque, M. E., Hossain, M. M., Saleque, M. A., and Bell, R. W. 2010. Water and fuel saving technologies: non-puddled bed and strip tillage for wet season rice cultivation in Bangladesh. In: Gilkes, R. J., Prakongkep, N. (Eds.), Proceedings of the 19th World Congress of Soil Science, Soil Solutions for a Changing World. 1-6 August 2010, Brisbane, Australia. Published on DVD, pp.169–172.

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