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REMEDIATION OF LEAD TOXICITY BY EXOGENOUS APPLICATION OF SILICON IN RICE

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Abstract

Silicon (Si) application is reported to be very effective in mitigating different stresses; however, their impact on mitigating heavy metals, especially lead (Pb) toxicity has not been previously investigated. The objective of the current experiment was to assess the consequences of different silicon sources and rates on plant parameters of rice under Pb toxic soil condition. The study was set up at the net house of the Department of Soil Science of Patuakhali Science and Technology University during the monsoon 2024. A two factors completely randomized design with three replications was assigned to the study. Two levels of lead (0 and 100 mg Pb kg^{-1} soil as lead nitrate), and two sources of silicon (Ca silicate and Fumed silica), both at 50 and 100 mg Si kg^{-1} soil, were applied on BINA Dhan-23. The results revealed that Pb stress significantly ($p < 0.05$) reduced the shoot dry weight of rice (11.0 and 10.4 g pot^{-1} in Pb control and 100 mg Pb kg^{-1} soil treatment, respectively), whereas Si application improved the shoot dry weight. There was a strong antagonistic relation of Si with uptake of Pb by the plants. Increasing Si rates, the Pb content in shoot and root gradually decreased, with the lowest Pb contents recorded at 100 mg Si kg^{-1} soil treatment. Among the two Si sources, the fumed silica had a higher capacity to reduce Pb content by both shoot and root than calcium silicate. The Pb accumulation in the root was several folds higher than shoot. Although, 100 mg Si kg^{-1} soil was found promising to reduce Pb toxicity in plants; more dose of Si is needed to apply in future research to find out the effects of most dose of Si on the extent of reduction of Pb toxicity in plants.

Keywords: Heavy metal, Littoral zone, Root weight, Salt stress, Shoot weight.

Introduction

Lead (Pb) is the second most toxic heavy metal released into the environment, followed by arsenic (Rani *et al*., 2024). It has become an alarming contaminant in recent times due to many man-made actions like industrial chemicals use, vehicle generated toxin, emission of industrial wastages, use of contaminated water for irrigation purpose, activities of pesticide making industries or leather industries, use of metal fertilizer, chemical paints etc. (Gupta *et al*., 2024). After being discharged into the environment, Pb is assimilated by plants, causing harm to plants as well as human body through the food chain. As humans

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directly or indirectly rely on plants, there is great probability to develop poisonous effects of Pb in human body like mental disorder, neurological or cardiovascular diseases etc. (Naz *et al*., 2025). Children and aged persons are the most vulnerable to toxicity of Pb. Lead has no biological function in plants and it is not required for plants growth and development. However, Pb may enter in the plant roots through transporters or channels for other nutrients and interfere with different metabolic activities in plants or damaging plant cell (Busoms *et al*., 2021).

Rice has become firmly crucial food grain in today's world as basic food stuffs for billions and ensured safety of food, specifically among the people of South Asia (Binth., 2024). The dictate of rice production is expanding day by day due to global population growth, swift industrialization and per capita exhaustion. Nevertheless, the production is on a threat as the current nullify climate changes, dearth of safe water resources, salt stress, a confiscation of cultivable lands etc. (Jodder *et al*., 2016; Shila *et al*., 2016; Sikder *et al*., 2016; Haque 2018). Besides, in littoral zone especially in Bangladesh crop cultivation was greatly hampered by tidal water flow, salt stress, imbalanced fertilization (Haque *et al*., 2023a; 2023b; 2024a; 2025a; Haque and Haque 2023) and recent concern is heavy metal toxicity. Silicon is one of the most cost-effective ways to not only turn down heavy metal (e.g. Pb, Cd, As etc.) consumption by roots but also limit the translocation of heavy metal from root to shoot (Zhao *et al*., 2017).

Being a beneficial nutrient, the quantity of Si is ample in soil crust which contributes disease control as well as alleviates heavy metal stress (Swe *et al*., 2021; Datnoff and Rodrigues, 2005). Silicon plays a dynamic role in lessening numerous biotic and abiotic stresses thus accelerates growth of plants and enhances yield potential (Haque *et al*., 2024b). Silicon is a multifaceted constituent for plant nutrient which activates plant immune system under detrimental soil and climate state that compensates heavy metal toxicity eventually increases crop production of rice (Sume *et al*., 2023). Silicon application enhances chlorophyll content of leaf which ultimately improves vegetative growth of the plants (Sultana *et al*., 2021). Silicon lowers several stresses and metal toxicity mainly by preserving large quantities in different plant parts and strengthening the stiffness of plant cells (Yang *et al*., 2024; Akter *et al*., 2021). Surplus accretion of silicon in plants doesn't cause any harm in plants metabolism. In an earlier study we had tested silicic acid, sodium metasilicate and calcium silicate as the sources of Si on yield performance of rice, where all the sources had positive effect on rice yield but calcium silicate had the best (Haque *et al*., 2023c). In that experiment we could not include fumed silica source (SiO_2). Among different sources of Si, the particle size of fumed silica was the lowest and looks like very fine powder. We assumed that fumed silica might be more accessible for plant uptake due to its fineness.

Although several literatures have described the positive role of Si in improving plant growth and yield, and reducing biotic and abiotic stresses, but the specific role of Si in remediating Pb toxicity especially in the coastal soils of Bangladesh has not been investigated. More specifically the interaction effect between Si and Pb uptake by rice plant is not well understood. Different sources of Si may have differences on affecting uptake of Pb from the soil. We hypothesized that Si application will assist plants to improve biomass yield and reduce uptake of toxic metals. Keeping those in mind the experiment was

undertaken to find out the effects of different sources and rates of silicon to reduce the detrimental effect of Pb toxic in plants.

Materials and Methods

The experiment was carried out at the net house of the Department of Soil Science of Patuakhali Science and Technology University, Dumki upazila, Patuakhali district, Bangladesh throughout the monsoon season (August-October) of 2024. The research area was positioned between 22.4644°N latitude and 90.3849°E longitude at Bangladesh's south coastal region under AEZ 13 (Ganges tidal floodplains). The area was featured by low-lying land, frequent tidal flooding and many tidal rivers and creeks. The dominating crop in this area was transplanted Aman rice, often known as monsoon rice. Traditional tall rice varieties are typically grown by farmers.

Layout and treatments

The experiment was layed out in the completely randomized design with three replications. Two lead levels (0 and 100 mg Pb kg^{-1} soil) and five Si rates and sources (Si control, 50 mg Si kg^{-1} soil as Ca silicate and fumed silica, and 100 mg Si kg^{-1} soil as Ca silicate and fumed silica) were applied to BINA Dhan-23, a popular rice variety. The source of Pb was lead nitrate. The chemical composition of lead nitrate, calcium silicate and fumed silica were $Pb(NO_3)_2$, $CaSiO_3$ and SiO_2, respectively. The Bangladesh Institute of Nuclear Agriculture, located in Mymensingh, has released this rice variety (BINA Dhan-23).

Soil collection and pot preparation

Soil (0–15 cm deep) was collected from the farmer’s field of Sreerampur village of Dumki upazila which was very closer to the Patuakhali Science and Technology University main campus in August 2024. The collected soils were spread on a floor for drying. Firstly randomly selected 10 sub samples amounting around 1 kg soil were poised from the collected soils and mixed thoroughly to make a composite sample. Then the composite soil was air dried for a week in the laboratory and crushed with a wooden hammer. The prepared soil was stored and finally chemical and physical analysis of soil was done following the method described by Page et al. (1982). The soil was silty clay loam in texture, which had pH of 5.7 and EC (saturation paste extract) of 1.66 dS m^{-1}. Total nitrogen content was 1.0 g kg^{-1}, organic carbon 13.1 g kg^{-1}, Bray and Kurtz phosphorous 5.8 mg kg^{-1}, exchangeable potassium 0.27 cmol kg^{-1}, available sulphur 27.0 mg kg^{-1}, total Si content 30 % and total Pb content 11 mg kg^{-1} soil. In the drying floor total soil volume was broken down into small pieces. One kg soil was weighed and taken into a plastic pot. In the same way total thirty pots were prepared in the experiment.

Fertilizer application

In the experiment the rate of N, P, K, S and Zn were 120, 20, 100, 20 and 10 mg kg^{-1} soil, respectively. However, we have used double dose of those rate in the pot. N as urea was applied at three equal splits at 7, 20 and 35 days after transplanting of seedlings. The P, K, S and Zn were applied as basal dose. According to the layout and treatment of the experiment, the Si and Pb were also applied as a basal dose.

Transplanting and intercultural operations

Seeds of BINA Dhan-23 (Aman rice) were immersed under water for one day. Then the seeds were girdled into a gunny bag and stored in hot place. Within 4 days the seeds were perfectly germinated. The sprouted seeds were sown in the seedbed at 5th August 2024. Proper care was taken on the seed bed. Seedlings were uprooted at 7th September 2024 and transplanted 4 healthy seedlings per pot. Irrigation was done in the pots following wetting and drying method. Weeding was performed manually whenever necessary. Liquid insecticide (i.e. Virtako) was sprayed at every 7 days interval and granular insecticide was applied at 7 days after transplanting to avoid insect infestation in rice plants. Algal growth was manually removed. To provide similar environment the position of all the pots were rearranged weekly.

Harvesting

The crops were harvested at maximum vegetative growth phase in 27 October 2024. The plants were trimmed at ground level. Then the roots were also collected from the pot soil and cleaned gently with water. Both the root and shoot samples were dried under sun and weighted as sun dry basis. For chemical analysis the root and shoot samples were oven dried at 62° C, grinded and passed through 20 mesh sieves.

Data recording

To assess Si content, the complete root and shoot samples were cut up at 1 mm length and merged completely. A digestion tube was filled with one gram of the sample. Three inorganic acids were used to digest the plant samples: nitric acid (HNO_3), perchloric acid ($HClO_4$), and sulfuric acid (H_2SO_4) in 5:2:1 ratio (Yoshida *et al*., 1976). According to laboratory manual for physiological studies of rice, the Si content of the digested sample was estimated. Before harvesting, the third fully expanded leaf was collected and analyzed for chlorophyll determination (Coombs *et al*., 1985).

For determination of Pb in root and shoot sample 2g dried powdered sample was weighted in a conical flask.15 ml of HNO_3 was given in the flask for pre digestion overnight. Then 5-7 ml $HClO_4$ was added and digestion was completed using hot plate at 150 °C temperature until the sample became colorless. After cooling, the solution was diluted at 50 ml volume by using distilled water. Then the amount of Pb was measured from the sample using atomic absorption spectrophotometer. The amount of shoot and root Si or Pb uptake was assessed from their particular element content and biomass production. Other growth data were collected before harvesting.

Data analysis

The collected data were statistically analyzed by using a computer based software scheme named "Statistical Tool for Agricultural Research (STAR)". The least significant difference test at 95% confidence level was employed while estimating the mean separation value.

Results

Plant height and tiller production

There was no significant effect of Pb and Si and their interactions on plant height of rice (Table 1). Although not significant but Pb application at 100 mg kg^{-1} soil reduced plant height by 1.1 cm. Silicon application also showed a positive influence on plant height of rice. The single effect of Pb and Si had significant effect on number of tillers pot^{-1} but interaction effect was not significant. In Pb0 (control) treatment the number of tillers pot^{-1} was 10.3 which reduced to 9.1 in 100 mg Pb kg^{-1} soil treatment (Table 1). Silicon application significantly improved the number of tillers pot^{-1}. Among the Si rates 100 mg Si kg^{-1} soil treatment had better effect than 50 mg Si kg^{-1} soil treatment. The number of tillers pot^{-1} was significantly correlated with root and shoot dry weight, shoot Si uptake, and total chlorophyll content, and was negatively correlated with Pb content and Pb uptake parameters (Table 2).

Table 1. Single and interaction effects of lead and silicon on growth parameters of rice

Treatments	Plant height (cm)	Number of tillers pot^{-1}
Pb rates (mg Pb kg^{-1} soil)		
0	92.1	10.3 a
100	91.0	9.1 b
Significance level	ns	**
Standard error of means	0.92	0.33
Silicon rates (mg Si kg^{-1} soil)		
Si0	89.9	9.2 b
Ca silicate-Si50	91.2	9.5 ab
Fumed silica-Si50	92.9	9.2 b
Ca silicate-Si100.	91.0	10.3 a
Fumed silica-Si100	92.7	10.5 a
Significance level	ns	*
Standard error of means	1.46	0.52
Pb×Si interactions		
Pb0×Si0	90.1	9.7
Pb0×Ca silicate-Si50	91.2	9.7
Pb0×Fumed silica-Si50	94.4	9.7
Pb0×Ca silicate-Si100.	91.6	11.3
Pb0×Fumed silica-Si100	93.0	11.3
Pb100×Si0	89.7	8.7
Pb100×Ca silicate-Si50	91.3	9.3
Pb100×Fumed silica-Si50	91.3	8.7

Treatments	Plant height (cm)	Number of tillers pot^{-1}
Pb100×Ca silicate-Si100.	90.4	9.3
Pb100×Fumed silica-Si100	92.4	9.7
Significance level	ns	ns
Standard error of means	2.06	0.74
%CV	2.76	9.38

Different small letter in a column indicates that they are significantly different.*- significant at 5 % level, **- significant at 1 % level, ns- not significant, CV- Coefficient of variation

Table 2. Correlation matrix between different plant parameters of rice

6.3	Tillers pot^{-1}	Root dry weight	Shoot dry weight	Shoot Si content	Root Si content	Shoot Si uptake	Root Si uptake	Total Si uptake	Total chlorop hyll content	Shoot Pb content	Root Pb content	Shoot Pb uptake	Root Pb uptake
Root dry weight	0.36*												
Shoot dry wt.	0.48**	0.30ns											
Shoot Si content	0.46*	0.23ns	0.27ns										
Root Si content	0.31ns	0.59***	0.41*	0.24ns									
Shoot Si uptake	0.59***	0.32ns	0.88***	0.70***	0.42*								
Root Si uptake	0.34ns	0.80***	0.39*	0.25ns	0.95***	0.40*							
Total Si uptake	0.58***	0.62***	0.80***	0.61***	0.75***	0.89***	0.77***						
Total chl. Cont.	0.45*	0.40*	0.47**	0.32ns	0.51**	0.51**	0.51**	0.60***					
Shoot Pb cont.	-0.54**	-0.51**	-0.46**	-0.42*	-0.39*	-0.55**	-0.46*	-0.61***	-0.27ns				
Root Pb content	-0.55**	-0.56**	-0.55**	-0.42*	-0.44*	-0.62***	-0.51**	-0.68***	-0.35*	0.97***			
Shoot Pb uptake	-0.51**	-0.41*	-0.38*	-0.44*	-0.37*	-0.50**	-0.41*	-0.55**	-0.19ns	0.97***	0.92***		
Root Pb uptake	-0.54**	-0.50**	-0.53**	-0.43*	-0.41*	-0.61***	-0.47**	-0.65***	-0.31ns	0.98***	0.99***	0.94***	
Total Pb uptake	-0.53**	-0.46*	-0.45*	-0.44*	-0.40*	-0.55**	-0.44*	-0.60***	-0.25ns	0.99***	0.97***	0.99***	0.98***

Note: *, ** and *** indicates significant at 5, 1 and 0.1 % level respectively. ns- Not significant

Root parameters

Lead application had a significant effect to reduce root length of rice having 21.5 cm in Pb control treatment which reduced to 19.9 cm in 100 mg Pb kg^{-1}soil treatment (Table 3). The single effect of Si had no significant improvement on root length of rice. The interaction between Pb and Si was also not significant. Root dry weight was significantly varied by Pb and Si but not their interactions. In Pb control treatment the mean root dry weight was 1.91 g pot^{-1} but it reduced to 1.73 g pot^{-1} in 100 mg Pb kg^{-1} soil treatment (Table 3). The root dry weight was significantly improved by the application of Si in soil. The fumed silica at both 50 and 100 mg Si kg^{-1} soil treatment recorded higher root dry weight

than calcium silicate source. There were significant positive correlation of root dry weight with root Si content and uptake, and chlorophyll content, but was significant negative correlation with Pb content and uptake parameters (Table 2).

Table 3. Single and interaction effects of lead and silicon on root and shoot parameters of rice

Treatments	Root length (cm)	Root dry weight (g pot^{-1})	Shoot dry weight (g pot^{-1})
Pb rates (mg Pb kg^{-1} soil)			
0	21.5 a	1.91 A	11.0 A
100	19.9 b	1.73 B	10.4 B
Significance level	*	**	**
Standard error of means	0.72	0.005	0.20
Silicon rates (mg Si kg^{-1} soil)			
Si0	20.3	1.74 b	9.9 c
Ca silicate-Si50	20.5	1.79 ab	10.8 ab
Fumed silica-Si50	20.4	1.89 a	10.4 bc
Ca silicate-Si100.	21.8	1.78 ab	11.2 a
Fumed silica-Si100	20.7	1.91 a	11.4 a
Significance level	ns	*	**
Standard error of means	1.13	0.008	0.32
Pb×Si interactions			
Pb0×Si0	20.5	1.82	10.4
Pb0×Ca silicate-Si50	21.4	1.84	10.9
Pb0×Fumed silica-Si50	22.2	2.11	10.8
Pb0×Ca silicate-Si100.	22.4	1.86	11.4
Pb0×Fumed silica-Si100	21.0	1.92	11.6
Pb100×Si0	20.0	1.65	9.4
Pb100×Ca silicate-Si50	19.6	1.73	10.7
Pb100×Fumed silica-Si50	18.6	1.67	9.9
Pb100×Ca silicate-Si100.	21.2	1.69	11.0
Pb100×Fumed silica-Si100	20.4	1.91	11.2
Significance level	ns	ns	ns
Standard error of means	1.60	0.12	0.45
%CV	9.47	8.37	5.23

Different small letter in a column indicates that they are significantly different.*- significant at 5 % level, **- significant at 1 % level, , ns- not significant, CV- Coefficient of variation

Shoot dry weight

Both lead and different silicon rates and sources had significant influences on shoot dry weight of rice. Under without Pb stress condition the shoot dry weight was found 11.0

g pot^{-1} and it reduced to 10.4 g when 100 mg kg^{-1} soil Pb stress was imposed (Table 3). The Si control treatment had the lowest shoot dry weight (9.9 g pot^{-1}). Increasing Si rate progressively increased the shoot dry weight of rice. Over the Si sources 100 mg Si kg^{-1} soil treatment had significantly higher shoot production than 50 mg Si kg^{-1} soil treatment. Like other parameters, the interaction effect of Pb and Si was not significant. There were very strong correlation of shoot dry weight with shoot Si uptake and chlorophyll content of leaf; however, shoot dry weight significantly reduced by the increase of the Pb content and uptake by the roots and shoots (Table 2).

Silicon application ameliorates different growth and yield attributing parameters in rice like plant height, tillers number pot^{-1}, root length and root and shoot dry weight. Silicon application increased shoot dry weight by 5.0-15.2 % over different rates and sources of Si. The positive response of Si in rice was also reported by Mobaswera *et al*. (2023) in a pot experiment. Synchronization of several physiological and biochemical activities were the contributing factor for enhancing crop yield even under a number of stress condition (Asgher *et al*., 2024). Silicon application in rice soil relieves peroxidation and fatty acid deproteination occurs in plants, which fosters growth regulating variables (Greger *et al*., 2018).

In our experiment Pb application reduced tiller production by 11.7 %, root dry weight by 9.4 % and shoot dry weight by 5.5 %. However, different growth variables were progressive in silicon treatment even under Pb contaminated soil (Souri *et al*., 2023). Silicon activates the exudation of a number of organic acids (i.e. oxalic acid, acetic acid) and flavonoid phenolics in root zone (Fan *et al*., 2016). These organic acids and phenolic substances act as chelating agent of different heavy metals like Cd, Pb, Zn, Al etc. (Chandra and Keshavkant, 2021). Therefore, Si detoxifies the effects of heavy metal in plants root zone.

Shoot Si content and uptake

The Si content in rice shoot was significantly reduced by Pb application in soil. In Pb control and 100 mg Pb kg^{-1} soil treatment the Si content of shoot were 2.80 and 2.69 %, respectively (Table 4). Silicon application in the soil also improved the shoot Si content but the extent of improvement was not significant. The interaction effect on this parameter was also not significant. Single effect of both Pb and Si had significant effect on shoot Si uptake of rice, where Pb application reduced Si uptake, and Si application increased the Si uptake. The Pb control treatment recorded shoot Si uptake of 308 mg pot^{-1} and 100 mg Pb kg^{-1} soil treatment recorded shoot Si uptake of only 281 mg pot^{-1}. Among the Si rates 100 mg Si kg^{-1} soil treatment had higher effect than 50 mg Si kg^{-1} soil treatment. However, among two sources there was no significant difference on uptake of Si by rice shoot.

Table 4. Single and interaction effects of lead and silicon on silicon uptake parameters of rice

Treatments	Shoot Si content (%)	Shoot Si uptake (mg pot^{-1})	Total Si uptake (mg pot^{-1})
Pb rates (mg Pb kg^{-1} soil)			
0	2.80 a	308 a	387 a
100	2.69 b	281 b	343 b
Significance level	*	***	***
Standard error of means	0.04	6.15	7.08
Silicon rates (mg Si kg^{-1} soil)			
Si0	2.67	264 b	321 d
Ca silicate-Si50	2.77	298 a	355 c
Fumed silica-Si50	2.68	278 b	359 bc
Ca silicate-Si100.	2.84	317 a	381 b
Fumed silica-Si100	2.76	315 a	409 a
Significance level	ns	***	***
Standard error of means	0.06	9.73	11.1
Pb×Si interactions			
Pb0×Si0	2.68	278	338
Pb0×Ca silicate-Si50	2.80	304	366
Pb0×Fumed silica-Si50	2.75	298	401
Pb0×Ca silicate-Si100.	2.97	337	410
Pb0×Fumed silica-Si100	2.78	324	421
Pb100×Si0	2.66	250	304
Pb100×Ca silicate-Si50	2.74	293	345
Pb100×Fumed silica-Si50	2.61	258	318
Pb100×Ca silicate-Si100.	2.71	297	352
Pb100×Fumed silica-Si100	2.74	305	397
Significance level	ns	ns	ns
Standard error of means	0.09	13.7	15.8
%CV	4.34	5.72	5.31

Different small letter in a column indicates that they are significantly different.*- significant at 5 % level, ***- significant at 0.1 % level, , ns- not significant, CV- Coefficient of variation

Root Si content and uptake

The interaction of Pb and Si had significant effect on root Si content of rice (Fig. 1). Under Pb control condition the fumed silica source had significantly higher performance than calcium silicate source, unfortunately, there was no significant difference between 50 and 100 mg Si kg^{-1} soil rate (Fig. 1a). Under 100 mg Pb kg^{-1} soil condition only fumed silica source at 100 mg Si kg^{-1} soil treatment had significantly higher Si content than all other sources and rates of Si (Fig. 1a). The performance of different sources and rates of Si under Pb control and 100 mg Pb kg^{-1} soil condition on increasing root Si content has

been given in Fig 1b. From the figure it was found that fumed silica applied at 100 mg Si kg^{-1} soil rate recorded statistically similar root Si content in Pb control and 100 mg Pb kg^{-1} soil rate. The result indicates that although Pb application generally reduced the Si content of root but regarding fumed silica at 100 mg Si kg^{-1} rate the Pb toxicity could not significantly reduced the Si content of root (Fig. 1b)

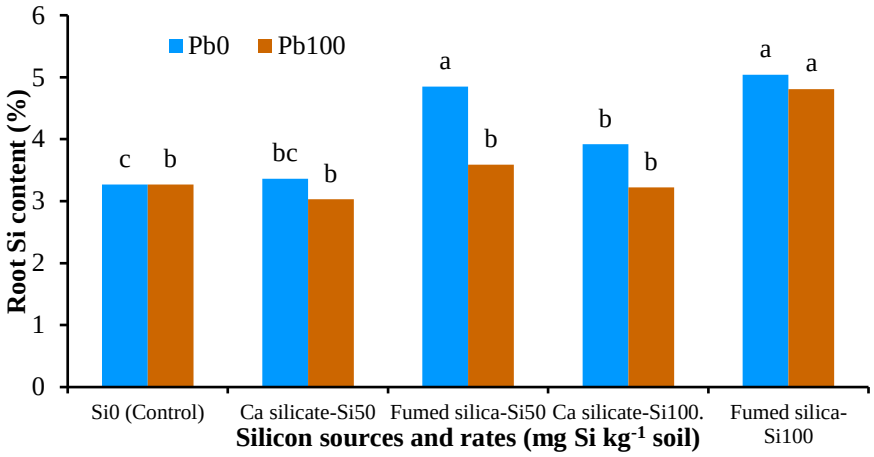

Fig. 1a

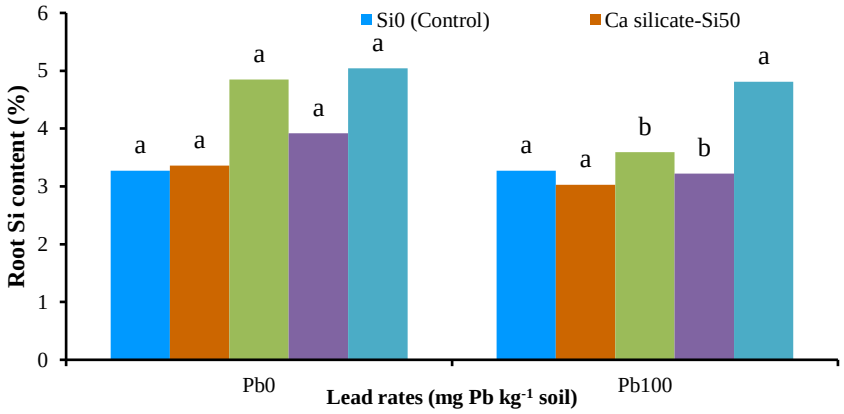

Fig. 1b

Fig. 1. Effect of lead×silicon interaction on root silicon content of rice a) Comparison of silicon at each level of lead, b) Comparison of lead at each level of silicon

Means with the same letter in same color bar are not significantly different
Significance level: Lead-***, Silicon-***, Lead:Silicon interaction-*;
Standard error: Lead-0.12, Silicon-0.19, Lead:Silicon interaction-0.27; CV (%)- 8.71

The root Si uptake was significantly affected by both single and interaction effect of Pb and Si (Fig. 2). Under Pb control condition only fumed silica source at both 50 and 100 mg Si kg^{-1} soil treatment had significantly higher Si uptake than other treatments (Fig.

2a). But at 100 mg Pb kg^{-1} soil applied condition only fumed silica source at 100 mg Si kg^{-1} soil treatment had significantly higher root Si uptake compare to calcium silicate source and 50 mg Si kg^{-1} soil rate. Again when fumed silica was applied at 100 mg Si kg^{-1} soil rate the Pb toxicity could not significantly reduce the root Si uptake of rice (Fig. 2b). The results indicated that fumed silica source at 100 mg Si kg^{-1} soil rate could reduce the detrimental effect of Pb in plants. There was an antagonistic correlation of root and shoot Si content and uptake with Pb content and uptake by root and shoot (Table 2). The results endorses that increasing Si content or uptake significantly reduced the Pb content or uptake, and vice versa.

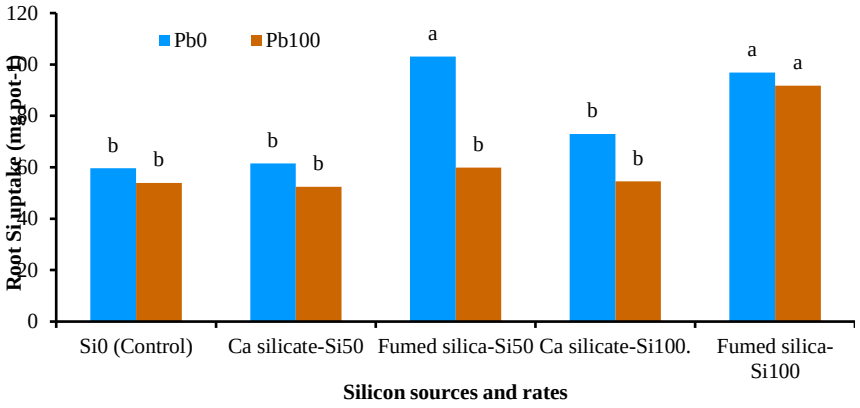

Fig. 2a

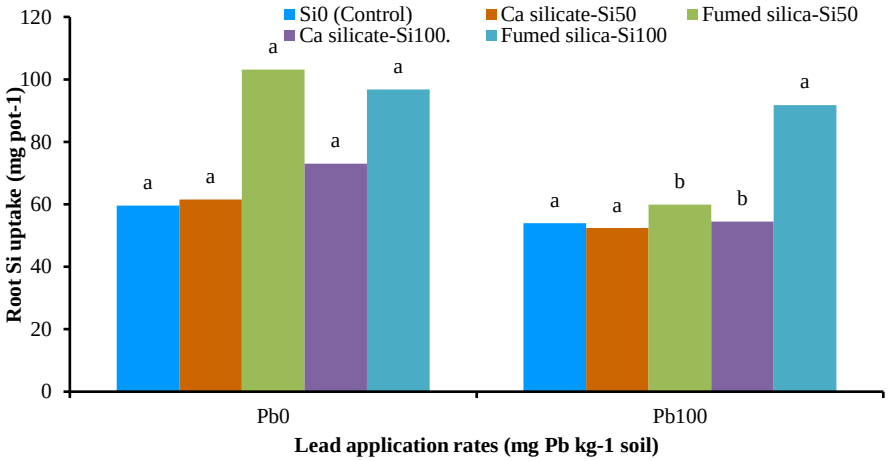

Fig. 2b

Fig. 2. Effect of lead:silicon interaction on root silicon uptake of rice a) Comparison of silicon at each level of lead, b) Comparison of lead at each level of silicon

Means with the same letter at same color bar are not significantly different
Significance level: Lead-***, Silicon-***, Lead:Silicon interaction-*;
Standard error: Lead-3.68, Silicon-5.82, Lead:Silicon interaction-8.23; CV (%)- 11.2

Total Si uptake (shoot plus root) was significantly affected only single effect of Pb and Si, but not their interactions (Table 4). Lead application reduced the Si uptake whereas Si application increased the Si uptake. Among the sources fumed silica at 100 mg Si kg^{-1} soil treatment had the highest Si uptake.

Silicon brings down lead (Pb) accumulation in plants cell by external and internal mechanisms. Externally Si rise soil pH which decline the availability of Pb by precipitating or chelating it into the soil. And internally higher Si accumulation in the shoot of rice plants persuades down-regulatory genes which restricts channels or regulatory pathway that may be associated with Pb uptake by roots of rice plants (Gong *et al*., 2023). In this way Si checks the toxic impact of Pb in rice plants. Again, silica form of silicon in soil assembled Pb silicate complexes precipitation that effectively reduced phyto-availability of toxic Pb in soil (Rachappanavar *et al*., 2024). Silicon also provokes antioxidant hormonal system and gene expression which diminishes heavy metal issues in soil (Bhat *et al*. 2019).

Shoot and root Pb content

Both shoot and root Pb content were significantly varied by single effect of Pb and Si, and their interactions. In Pb control condition the different sources and rates of Si had no significant difference on uptake of Pb by both shoot and root (Table 5). However, when Pb was applied to the soil at 100 mg Pb kg^{-1} soil the Pb content of both shoot and root were just jumped. Interestingly, among the Si sources and rates the Pb content was significantly different. The Si control against 100 mg Pb kg^{-1} soil treatment recorded highest Pb uptake of 10.65 and 193.6 mg kg^{-1} in shoot and root, respectively. There was a strong antagonistic relation of Si with Pb content of rice plants. Increasing Si rates the Pb content was gradually decreased having lowest Pb content were recorded at 100 mg Si kg^{-1} soil treatment. Among two Si sources, the fumed silica had higher capacity to reduce Pb content by both shoot and root. The Pb accumulation in root was several folds higher than the shoot.

Table 5. Interaction effects of lead and silicon on lead concentration of rice

Silicon sources and rates (mg Si kg^{-1} soil)	Lead levels (mg Pb kg^{-1} soil)	
	0 (Control)	100
Shoot lead content (mg kg^{-1})		
Si0 (Control)	2.68 Ba	10.65 Aa
Ca silicate-Si50	2.24 Ba	9.52 Ab
Fumed silica-Si50	2.51 Ba	8.65 Ac
Ca silicate-Si100.	2.52 Ba	8.68 Ac
Fumed silica-Si100	2.24 Ba	8.18 Ac
Significance level: Lead-***, Silicon-***, Lead:Silicon interaction-***; Standard error: Lead-0.12, Silicon-0.19, Lead:Silicon interaction-0.27; CV (%)- 5.82		
Root lead content (mg kg^{-1})		
Si0 (Control)	16.3 Ba	193.6 Aa

Silicon sources and rates (mg Si kg^{-1} soil)	Lead levels (mg Pb kg^{-1} soil)	
	0 (Control)	100
Ca silicate-Si50	11.2 Ba	158.4 Ab
Fumed silica-Si50	12.3 Ba	161.0 Ab
Ca silicate-Si100.	11.5 Ba	118.2 Ac
Fumed silica-Si100	13.7 Ba	97.6 Ad
Significance level: Lead-***, Silicon-***, Lead:Silicon interaction-***;		
Standard error: Lead-2.00, Silicon-3.16, Lead:Silicon interaction-4.47; CV (%)- 6.90		

Different capital letter in a row indicates that the Pb effects were significantly different. Similarly different small letter in a column indicates that the Si rates were significantly different. ***- significant at 0.1 % level, CV- Coefficient of variation

Shoot and root Pb uptake

The shoot, root and total Pb uptake was significantly varied by the interaction effect of Pb and Si application (Table 6). When Pb was not applied (Pb control treatment) all the Si sources and rates had statistically identical Pb uptake by shoot and or root. But when Pb was applied at 100 mg kg^{-1} soil rate, the Pb uptake trend was changed over the sources and rates of the Si. Every cases Si control treatment had highest Pb uptake. The lowest Pb uptake was found at 100 mg Si kg^{-1} soil treatment. Among the sources of Si the fumed silica had lower total Pb uptake than calcium silicate source (Table 6). The single effect of Pb and Si also had significant impact on these Pb uptake parameters. There was an antagonistic relation of Si application with uptake of Pb by root and shoot of rice. Lead application in the soil increased Pb uptake by the plants. Interestingly application of Si in that soil drastically reduced the Pb uptake by both root and shoot; and the reduction rate was much higher in the higher rates of Si application (100 mg Si kg^{-1} soil). The reason behind that might be Pb interfere the enzymatic reactions in rice plants which alter the mineral nutrient consumption, that eventually limits plants growth by altering photosynthesis and other morphological characteristics (Guo *et al*., 2018). Lead harmfulness prompts retard plant growth, brings down photosynthesis and limits the development of primary root also in banana and other fruits (Li *et al*., 2012). It also extremely damages the production of Maize; Si treatment lowers these problems to a great extent by different defense mechanism (Okant, and Kaya, 2019).

Table 6. Single and interaction effects of lead and silicon on lead uptake of rice

Silicon levels	Lead levels (mg Pb kg^{-1} soil)	
	0 (Control)	100
Shoot lead uptake (mg kg^{-1})		
Si0 (Control)	0.109 Ba	0.408 Aa
Ca silicate-Si50	0.090 Ba	0.405 Aa
Fumed silica-Si50	0.096 Ba	0.307 Ac
Ca silicate-Si100.	0.097 Ba	0.377 Ab
Fumed silica-Si100	0.093 Ba	0.355 Ab
Significance level: Lead-***, Silicon-ns, Lead:Silicon interaction-*; Standard error: Lead-0.014, Silicon-0.022, Lead:Silicon interaction-0.034; CV (%)- 10.9		
Root lead uptake (mg kg^{-1})		
Si0 (Control)	0.030 Ba	0.318 Aa
Ca silicate-Si50	0.021 Ba	0.275 Ab
Fumed silica-Si50	0.026 Ba	0.269 Ab
Ca silicate-Si100.	0.021 Ba	0.200 Ac
Fumed silica-Si100	0.026 Ba	0.186 Ac
Significance level: Lead-***, Silicon-***, Lead:Silicon interaction-***; Standard error: Lead-0.006, Silicon-0.010, Lead:Silicon interaction-0.014; CV (%)- 12.8		
Total lead uptake (mg pot^{-1})		
Si0 (Control)	0.139 Ba	0.726 Aa
Ca silicate-Si50	0.111 Ba	0.680 Aa
Fumed silica-Si50	0.122 Ba	0.576 Ab
Ca silicate-Si100	0.118 Ba	0.577 Ab
Fumed silica-Si100	0.119 Ba	0.541 Ab
Significance level: Lead-***, Silicon-*, Lead:Silicon interaction-*; Standard error: Lead-0.018, Silicon-0.029, Lead:Silicon interaction-0.042; CV (%)- 13.8		

Different capital letter in a row indicates that the Pb rates were significantly different. Similarly different small letter in a column indicates that the Si rates were significantly different. *- significant at 5 % level, ***- significant at 0.1 % level, ns- Not significant, CV- Coefficient of variation

Chlorophyll content

The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content were significantly influenced only by the application of Si. The lowest chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of 3.58, 1.03, 4.60 and 1.63 mg g^{-1} fresh leaf, respectively were noticed in Si control treatment, and all were progressively increased with increasing Si rates (Table 7). The 50 mg Si kg^{-1} soil treatment had higher chlorophyll content than Si control treatment. Every cases highest chlorophyll and carotenoid contents were found in 100 mg Si kg^{-1} soil treatment. Among the sources fumed silica had the higher

capacity to produce pigments of the leaves. Although the effect of Pb was not significant, but in value it reduces all the pigment granules. In agreement with our findings Aslam *et al*. (2021) reported that lead toxicity hampers physiological and metabolic actions including reduction of chlorophyll a and chlorophyll b levels, which reduce the photosynthetic capacity of rice leaves, consequently hinders plant growth and development. In our experiment Si application increased the chlorophyll content of rice which ultimately assists plant to reduce the toxicity of Pb.

Table 7. Single and interaction effects of lead and silicon on chlorophyll and carotenoid content of rice leaves

Treatments	Chlorophyll a content (mg g^{-1} fresh leaf)	Chlorophyll b content (mg g^{-1} fresh leaf)	Total chlorophyll content (mg g^{-1} fresh leaf)	Carotenoids content (mg g^{-1} fresh leaf)
Pb rates (mg Pb kg^{-1} soil)				
0	3.90	1.19	5.08	1.78
100	3.83	1.12	4.95	1.75
Significance level	ns	ns	ns	ns
Standard error of means	0.09	0.04	0.08	0.04
Silicon rates (mg Si kg^{-1} soil)				
Si0	3.58 c	1.03 b	4.60 c	1.63 c
Ca silicate-Si50	3.79 bc	1.16 ab	4.95 b	1.74 bc
Fumed silica-Si50	3.79 bc	1.15 ab	4.94 b	1.73 bc
Ca silicate-Si100.	4.03 ab	1.15 ab	5.17 ab	1.83 ab
Fumed silica-Si100	4.14 a	1.29 a	5.43 a	1.90 a
Significance level	*	*	***	**
Standard error of means	0.14	0.06	0.14	0.06
Pb×Si interactions				
Pb0×Si0	3.63	1.05	4.68	1.64
Pb0×Ca silicate-Si50	3.83	1.17	5.00	1.75
Pb0×Fumed silica-Si50	3.81	1.16	4.97	1.75
Pb0×Ca silicate-Si100.	4.07	1.25	5.32	1.86
Pb0×Fumed silica-Si100	4.14	1.30	5.45	1.91
Pb100×Si0	3.52	1.00	4.52	1.62
Pb100×Ca silicate-Si50	3.76	1.15	4.91	1.74
Pb100×Fumed silica-Si50	3.77	1.14	4.91	1.71
Pb100×Ca silicate-Si100.	3.98	1.05	5.03	1.80
Pb100×Fumed silica-Si100	4.13	1.27	5.40	1.89
Significance level	ns	ns	ns	ns
Standard error of means	0.21	0.09	0.19	0.09
%CV	6.70	10.3	4.84	6.61

Different small letter in a column indicates that they are significantly different.*- significant at 5 % level, **- significant at 1 % level, ***- significant at 0.1 % level, ns- not significant, CV- Coefficient of variation

Conclusion

Lead is considered toxic heavy metal for plants. Lead application at a rate of 100 mg kg^{-1} soil hinders plants growth by accumulation in plants cell and interferes with plants metabolism. All forms of silicon improved plant growth in Pb affected soil but among them fumed silica at a rate of l00 mg Si kg^{-1} soil rate was more potential in limiting Pb toxicity. Because of its delicate form, fumed silica can easily dissipate in soil and augment soil properties. It is readily assimilated by plants, nourishes plants health and makes plant more tolerant at unfavorable situations. Findings of the current research will assist policy makers, agriculturists, farmers and the ultimate consumers about production and consumption of safe foods. In future higher doses of both Si and Pb from the current study could be applied in rice using various types of soil to find out the dynamics of those elements in soil and plants.

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Author's contribution

The study conception, formulation of the research program, provision of materials, statistical data analysis, preparation of tables and graphs, manuscript editing, and research project funding were carried out by M A. Haque. Fieldwork, chemical analyses, and data collection were conducted by S. Pranto, S. Islam, P. S. Dhrubo, M. M. Soha, and F. N. Lamia. The initial draft of the manuscript was prepared by Samsunnahar Pranto. Md Lutfar Rahman performed the heavy metal analysis of soil and plant samples. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

The author has declared no conflict of interest.

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DEPICTING DNA MAKEUP OF WHEAT GENOTYPES USING SSR MARKERS AND THEIR ASSOCIATION WITH THERMOTOLERANCE

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Abstract

Wheat, a crop highly sensitive to high temperature, faces increased risks due to global warming. This study aimed to analyze DNA to identify wheat genotypes that respond well to heat and to uncover genetic similarities and differences among them. Fifteen wheat genotypes were planted on two dates, November 21 (Irrigated Timely Sowing, ITS) and December 21 (Irrigated Late Sowing, ILS), and analyzed with 13 SSR markers. A dendrogram divided the genotypes into two primary groups, each containing four sub-clusters. The greatest genetic distances were observed between BAW 1290 and BARI gom 28 (0.929), while the smallest genetic distance (0.000) was found between Nadi 2 and BAW 1147. These results suggest that genotypes in both Cluster I and Cluster II (Group A) display moderate tolerance to heat, as indicated by their Heat Susceptibility Index (HSI) for 1000-grain weight (TGW) and grain yield, with HSI values between 0.50 and 1.00, allowing them to perform under heat stress. In contrast, genotypes in Group B, especially those in Cluster III, showed the higher HSI values (1.04-1.23) and significant declines in TGW and yield under heat stress, indicating susceptibility. Similarly, Cluster IV genotypes exhibited heat sensitivity (HSI>1.00). Seven genotypes, specifically BARI gom33, BARI gom32, BARI gom31, BARI gom30, BARI gom29, BARI gom26, and BARI gom25 were identified as suitable for cultivation under heat stress. The findings from this and other studies on heat-tolerant genotypes are valuable for advancing heat-resilient breeding programs based on SSR marker selection.

Keywords: Genetic diversity, Heat stress, SSR marker, Spring wheat.

Introduction

Wheat, the world's staple crop, ranks second in Bangladesh after rice. Global warming threatens wheat production, as rising temperatures impact growth, yield, and food security (Kumer *et al.*, 2022). Abiotic stresses like heat, drought, salinity, and cold disrupt wheat's genetic integrity and metabolic functions. Climate change is increasing extreme weather events, further exacerbating these challenges (IPCC, 2023; CRP, 2024).

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In Bangladesh, the optimal wheat sowing period is from November 15 to 30, requiring day temperatures ≤25°C and night temperatures ≤16°C for proper growth (Hossain *et al.,* 2023; Alam *et al.,* 2014). However, delayed sowing leads to higher temperatures during grain filling, causing yield losses. Terminal heat stress, particularly above 30°C during reproductive development, significantly reduces yield and quality, affecting pollination and grain filling (Shenoda, *et al.,* 2021). Traditional breeding methods have prioritized yield under ideal conditions, leaving many cultivars susceptible to heat stress. Thus, developing heat-tolerant wheat varieties is crucial (Hossain *et al.,* 2023).

Breeding heat-tolerant wheat involves molecular and biochemical profiling, identifying resistance traits, and leveraging biotechnological advancements (Chaudhary *et al.,* 2020). In 2022-2023, Bangladesh produced 1.16 million metric tons of wheat across 0.31 million hectares, with heat stress threatening productivity (DAE, 2023; BBS, 2023). Wheat's large genome (~17 Gb) complicates breeding, but research has identified key genes, molecular markers, and QTLs associated with heat tolerance (Walkowiak *et al.,* 2020; Chaudhary *et al.,* 2020).

Advances in molecular breeding, including PCR-based markers and SSR markers, have enhanced wheat genetic studies. The Heat Stress Index (HSI) helps evaluate heat-tolerant genotypes based on agronomic performance (Shenoda *et al.,* 2021; Hossain *et al.,* 2023; Bhusal *et al.,* 2017). Bangladesh Wheat and Maize Research Institute (BWMRI) has released 38 wheat varieties, some potentially heat-tolerant, though their precise temperature tolerance remains unclear (BWMRI, 2024). This study aims to characterize these varieties, identify heat-responsive genotypes through genetic analysis, and enhance wheat's resilience to climate change.

Materials and Methods

Plant materials

A total of fifteen bread wheat genotypes (*Triticum aestivum* L.)- comprising thirteen established varieties and two advanced lines- were selected to assess their molecular diversity in terms of tolerance to terminal heat stress. These genotypes were evaluated using thirteen Simple Sequence Repeat (SSR) markers, which are highly effective in identifying genetic variability and molecular characteristics linked to heat tolerance traits. Each genotype was sourced from the Bangladesh Wheat and Maize Agricultural Research Institute (BWMRI), located in Nashipur, Dinajpur, Bangladesh. BWMRI's focus on the development and selection of heat-tolerant wheat varieties made it an ideal source for obtaining seeds of the genotypes used in this study. Table 1 provides a detailed summary of each genotype's pedigree, along with their specific attributes, such as growth habits, phenological traits, and any previously documented heat tolerance traits, thus setting the foundation for understanding their potential in high-temperature environments.

Table 1. List of fifteen wheat genotypes with their pedigrees

Variety	Pedigree	Year of release	Life cycle (d)	Salient features
BARI gom25	ZSH 12/HLB 19//2*NL297	2010	102-110	• This variety tolerates heat stress, and salinity 8.0-10 dS/m salinity at the seedling stage • Grain yield (3.9-5.2 t ha^{-1}) • It is resistant to leaf rust (LR) and *Bipolaris* leaf blight (BpLB) diseases
BARI gom26	ICTAL 123/3/RAWAL 87//VEE/HD 2285 BD(JO)9585-0JO-3JE-0JE-0JE-HRDI-RC5DI	2010	104-110	• Heat tolerance, and appropriate for late sowing • It is high yielding variety (4.5-5.2 t ha^{-1}) • This variety displays strong resistance to leaf rust (LR), tolerance to the aggressive Ug99 race of stem rust, and moderate resistance to brown leaf spot (BpLB). These traits enhance its value for cultivation in disease-prone areas, increasing its potential for higher yields and improved resilience in challenging growing conditions.
BARI gom27	WAXWING*2/VIVISTI CGSS01BOOO56T-099Y-099 M-099 M-099Y-099 M-14Y-0B	2012	105-110	• It is high yielding variety (3.6-5.5 t ha^{-1}) • Having resistant to LR and stem rust (Ug99 race), with moderate resistance to BpLB.
BARI gom28	CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3/2*VEE#10 CMSS95Y00624S-0100Y-0200 M-17Y-010 M-5Y-0 M	2012	102-108	• Having early maturing and heat tolerant attributes. • Gives good yield in the late seeding condition. • Grain yield (4.5-5.0 t ha^{-1}) • Having Resistant and moderately resistant to LR and BpLB, respectively
BARI gom29	SOURAV/7/KLAT/SOREN//PSN/3/BOW/4/VEE#5. 10/5/CNO 67/MFD//MON/3/SERI/6/NL297 BD(DI)112S-0DI-030DI-030DI-030DI-9DI	2014	105-110	• Having moderately tolerant attribute to high temperature • Grain yield (4.5-5.0 t ha^{-1}) • Having moderately resistant to BpLB. It is also resistant to LR and stem rust
BARI gom30	BAW 677/Bijoy BD(JA)1365S-0DI-15DI-3DI-HR12R3DI	2014	100-105	• This is a very high-yielding variety. Grain yield: 4.5-5.5 t ha^{-1} • Exhibits resistance to leaf rust and moderate resistance to brown leaf spot (BpLB) • It has heat-tolerant attributes and • very suitable for late sowing
BARI gom31	KAL/BB/YD/3/PASTOR CMSS99M00981S-0P0M-040SY-040 M-040SY-16 M-0ZTY-0 M	2017	104-109	• Early maturing, heat tolerance • Resistant to LR and tolerant to spot blotch • Yield:4.5-5.0 t ha^{-1}

Variety	Pedigree	Year of release	Life cycle (d)	Salient features
BARI gom32	SHATABDI/GOURAB BD(DI)1686S-0DI-1DI-0DI-0DI-3DI	2017	95-105	• This variety possesses early maturing, heat tolerant, and short stature attributes • Generally, shows resistant to LR and tolerant to spot blotch • It tolerates newly found wheat blast disease (only 10-12% infection observed in Jashore region) • Grain yield: 4.5-5.5 t ha^{-1}
BARI gom33	KACHU/SOLALA	2018	110-115	• Culm robust, succulent, firm, erect, and found lodging under high wind flow • Leave and stem deep green from seedling to anthesis stages. So, farmers prefer it too much • Resistant to blast disease, LR, and tolerant to terminal heat stress • Grain zinc enriched (50-55 ppm)
BWMRI gom1	BARI gom21 (Shatabdi)/BARI gom24	2019	100-104	• Short stature, tolerant to lodging. • Having the shortest life cycle of all released varieties and early maturing, escaping terminal heat stress • Tolerant to blast and rust diseases
BWMRI gom2	BARI gom26/BARI gom 25	2021	108-115	• Grain in amber color • Panicle tall in length, 45-48 grains/panicle, 1000-grain weight 45-50 g • This variety shows resistant attributes to LR and is tolerant to high-temperature • Grain yield: 4.5-5.8 t ha^{-1}
BWMRI gom3	Borlaug 100 ROELFS-F-2007/4/BOBWHITE/NEELKANT//CATBIRD/3/CATBIRD/5/FRET-2/TUKURU//FRET-2	2021	108-114	• Dwarf sized and almost no lodging attribute • Resistant to LR, BpLB, and blast disease • Yield: 4.0-4.5 t ha^{-1}
BAW 1290	BARI gom21/BL 3503	-	-	-
BAW 1147	OASIS/3*ANGRA//708	-	-	-
Nadi 2	-	-	-	-

Table 2. Characteristics of 13 linked SSR markers used in the characterization

SL No.	Marker	QTL for	Primers sequence Reverse (5'- 3')	Primers sequence Forward (5'- 3')	Chromosomal location	Annealing temp (°C)
1	gwm291	Leaf Curl	AATGGTATCTATTCCGACCCG	CATCCCTAGGCCACTCTGC	5A	60
2	Gwm325	HSI grain filling duration HSI kernel weight	TTTTTACGCGTCAACGACG	TTTCTTCTGTCGTTCTCTTCCC	6D	60
3	Xgwm294	HIS single kernel weight of the main spike	GCAGAGTGATCAATGCCAGA	GGATTGGAGTTAAGAGAGAACCG	2A	55
4	Gwm268	HSI kernel weight	TTATGTGATTGCGTACGTACCC	AGGGGATATGTTGTCACTCCA	1B	55
5	Xwmc407	Grain-filling duration	CATATTTCCAAATCCCCAACTC	GGTAATTCTAGGCTGACATATGCTC	2A	61
6	Xcfa2129	HIS single kernel weight of the main spike	ATCGCTCACTCACTATCGGG	GTTGCACGACCTACAAAGCA	1A, 1B, 1D	60
7	gwm11	Grain-filling duration	GTGAATTGTGTCTTGTATGCTTCC	GGATAGTCAGACAATTCTTGTG	1A, 1B	50
8	Xcfd43	Grain-filling duration	CCAAAAACATGGTTAAAGGGG	AACAAAAGTCGGTGCAGTCC	2D	60
9	Xgwm356	HSI single kernel weight of the main spike	CCAATCAGCCTGCAACAAC	AGCGTTCTTGGGAATTAGAGA	2A, 6A, 7A	55
10	Xbarc137	Waxiness	CCAGCCCCTCTACACATTTT	GGCCCATTTCCCACTTTCCA	1B	52
11	Gwm484	Waxiness	AGTTCCGGTCATGGCTAGG	ACATCGCTCTTCACAAACCC	2D	55
12	Gwm293	Grain-filling duration	TCGCCATCACTCGTTCAAG	TACTGGTTCACATTGGTGCG	5A	55
13	WMC527	HIS kernel weight of the main spike	GCTACAGAAAACCGGAGCCTAT	ACCCAAGATTGGTGGCAGAA	3A, 3B	61

Experimental site and sowing date

During the Rabi season (15 Oct to 15 Mar), the genotypes were evaluated at the BWMRI Regional Station (RS) research farm, Joydebpur, Gazipur, which is located in the agro-ecological zone 28 (Madhupur Tract) (FAO/UNDP, 1988). This region is

distinguished by intricate relief and soils that have grown on the Madhupur Clay. The field experiments were conducted followed by RCBD with three replications. The plot size was 4 feet × 3 feet. The sub-plots were fertilized @ 100-27-50-20-1-4.5- 5000kg ha^{-1} as N-P-K-S-B-Zn-Cowdung as the source of urea, TSP, MoP, Gypsum, Boric acid, and Zinc sulphate, respectively. TSP, MoP, Gypsum, Boric acid, Zinc sulphate, Cowdung and two-third of urea were applied as basal dose at last ploughed. Seeds were treated with Provax 200 WP@3g kg^{-1} seed, containing Carboxin and Thiram. Seeds @100 kg ha^{-1} were sown continuously in line. Seeds were sown on two dates viz. Nov 21 (ITS) and Dec 21 (ILS). The cultural practices were performed as the recommended guidelines of the BWMRI (2024). Laboratory experiments took place at the Biotechnology Division of the Bangladesh Agricultural Research Institute and BWMRI Regional Station Molecular Laboratory in Joydebpur, Gazipur.

Extraction of DNA and SSR analysis

Genomic DNA was isolated from fresh leaves of fifteen wheat genotypes using a modified CTAB method. From various chromosome sites, thirteen SSR markers (gwm291, Gwm325, Xgwm294, Gwm268, Xwmc407, Xcfa2129, gwm11, Xcfd43, Xgwm356, Xbarc137, Gwm484, Gwm293, WMC527) were chosen. Fresh leaves were grounded in a mortar, then placed in a 2 ml tube, 400 µl chloroform was added, and the mixture was heated for an hour at 65°C. The supernatant was moved to a fresh tube after centrifuging the sample for ten minutes at 4ºC at 12000 rpm. Following the addition and mixing of isopropyl alcohol, the samples were kept at -20°C for two hours. The DNA pellet was centrifuged one more, cleaned with 75% ethanol, allowed to air dry for a full day, and dissolved in 100µl of 1X TE buffer. Thirteen primer pairs were utilized for SSR analysis, and a spectrophotometer was used to verify the quality and concentration of the DNA. PCR parameters were adhered to Röder et al. (1998).

Heat susceptibility index (HSI)

The Heat Susceptibility Index (HSI) was used to assess the impact of heat stress on thousand-grain weight (TGW) and grain yield. The HSI was calculated using the formula provided by Paliwal et al. (2012).

$$HSI \text{ of } X = [(1 - X_{heat\ stress}/X_{control})/D]$$

where

X represents TGW and grain yield

$X_{heat\ stress}$ represents phenotypic values of individual genotypes for TGW and grain yield under late sowing.

Control represents phenotypic values of individual genotypes for TGW and grain yield under normal sowing conditions (control conditions).

$$D_{(stress\ intensity)} = (1 - Y_{heat\ stress}/Y_{control})$$

$$Y_{heat\ stress} = Mean \text{ of } X_{heat\ stress} \text{ of all genotypes}$$

$$Y_{control} = Mean \text{ of } X_{control} \text{ of all genotypes}$$

Statistical analysis

A bivariate 1-0 data matrix was created from scorable loci, assigning one locus to each band. Genetic distances between genotypes were quantified using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on shared alleles. The values were designed to estimate genetic diversity using PowerMarker software (Version 3.25), dendrograms, and polymorphism information content (PIC) (Botstein et al., 1980**)**.

Results

The thirteen SSR primers were used in this study (Table 2). The information provided in Table 2 included the primer sequences, their lengths, and any relevant details regarding their application. The primers' allele counts and sizes were presented in Table 3. Screening 15 genotypes with 13 SSR markers yielded 51 polymorphic alleles, averaging 3.92 per locus. The primers detected 2.0 to 8.0 alleles per genotype. Gwm293, with eight alleles, was the most polymorphic microsatellite marker, followed by Xgwm356 (having seven alleles) (Table 3; Fig. 1). Xcfd43 had the lowest alleles (two). The markers exhibited Polymorphism Information Content (PIC) values that varied significantly, ranging from a low of 0.33 for the marker Xwmc407 to a high of 0.83 for the marker Xgwm356. The average PIC value across all markers was calculated to be 0.57, indicating a moderate level of polymorphism within the selected markers. These values were presented in Table 3 and illustrated in Figure 1.

Table 3. Allele numbers, sizes, and PIC values for fifteen wheat genotypes for thirteen SSR markers.

Marker	Allele No	Allele size and range	Difference (bp)	Major Allele Frequency	Gene Diversity	Heterozygosity	PIC
gwm291	3	150-160	10	0.46	0.64	0.00	0.57
Gwm325	3	150-160	10	0.38	0.66	0.00	0.58
Xgwm294	4	50-120	70	0.50	0.65	1.00	0.59
Gwm268	3	180-285	105	0.67	0.48	0.11	0.40
Xwmc407	2	140-145	5	0.71	0.41	0.00	0.33
Xcfa2129	4	120-190	70	0.47	0.66	1.00	0.59
gwm11	3	200-210	10	0.71	0.44	0.00	0.39
Xcfd43	2	160-165	5	0.50	0.50	0.00	0.38
Xgwm356	7	185-230	45	0.20	0.57	0.80	0.83
Xbarc137	4	245-260	15	0.44	0.67	0.00	0.61
Gwm484	4	90-190	100	0.36	0.71	0.92	0.66
Gwm293	8	105-190	85	0.23	0.84	1.00	0.82
WMC527	4	345-450	105	0.40	0.70	0.00	0.65
Mean	3.92 (total 51)	-	-	0.46	0.63	0.37	0.57
Range	2.0-8.0	-	2.00 - 105	0.20 - 0.71	0.49-0.85	0.00 - 1.00	0.33-0.83

The primers Xgwm294, Xcfa2129, and Gwm293 exhibited the highest heterozygosity (He) values of 1.00, while the lowest values ranged from 0.33 for Xwmc407 to 0.83 for Xgwm356, resulting in an average PIC value of 0.57. Notably, the primers gwm291, gwm325, Xwmc407, gwm11, Xcfd43, and Xbarc137 all recorded He values of 0.0. Heterozygosity was widely used to assess genetic variation within populations (Table 3). For the microsatellites included in this investigation, PIC exhibited a strong positive connection with the number of alleles (Table 4). Table 4 displayed the highest amount of genetic diversity (0.85) found at locus Xgwm356 and the lowest level of genetic diversity (0.44) found at locus gwm11. Gene diversity was higher in markers that detected more significant alleles than in markers that detected fewer alleles. A dendrogram was created to illustrate the genetic distances between common alleles derived from 15 genotypes and 51 alleles (Fig. 2).

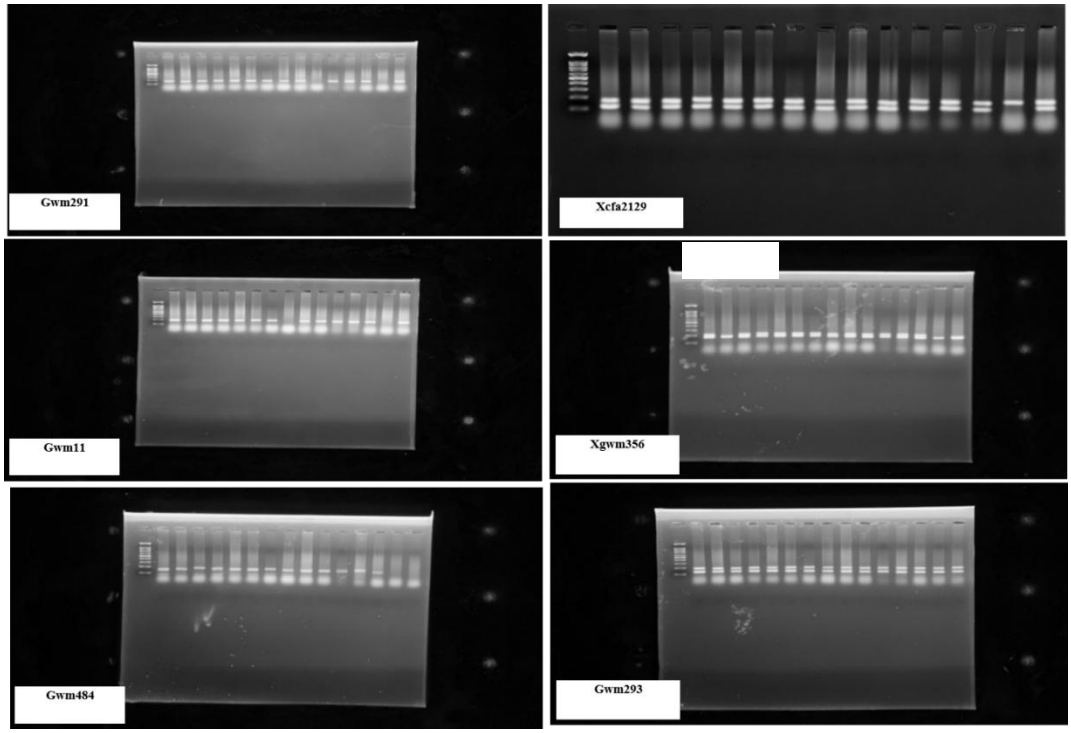

Fig. 1. Profiles of wheat genotypes were created using SSR markers with the primers Gwm291, Xcfa2129, Gwm11, Xgwm356, Gwm484, and Gwm2. In the picture (Left to right), the genotypes showed the bands: 1. BARI gom25, 2. BARI gom26, 3. BARI gom27, 4. BARI gom28, 5. BARI gom29, 6. BARI gom30, 7. BARI gom31, 8. BARI gom32, 9. BARI gom33, 10. BWMRI gom1, 11. BWMRI gom2, 12. BWMRI gom3, 13. BAW 1290, 14. BAW 1147, 15. NADI 2(CB -83), respectively.

Table 4. Genetic distance of fifteen wheat genotypes based on thirteen SSR markers

	BARI gom25	BARI gom26	BARI gom27	BARI gom28	BARI gom 29	BARI gom 30	BARI gom 31	BARI gom 32	BARI gom33	BAW 1147	BAW 1290	Nadi 2	BWMRI gom1	BWMRI gom 2	BWMRI gom 3
BARI gom25	0.000														
BARI gom26	0.150	0.000													
BARI gom27	0.545	0.500	0.000												
BARI gom28	0.583	0.500	0.458	0.000											
BARI gom29	0.583	0.600	0.591	0.250	0.000										
BARI gom30	0.667	0.700	0.591	0.333	0.083	0.000									
BARI gom31	0.708	0.650	0.727	0.542	0.292	0.292	0.000								
BARI gom32	0.714	0.786	0.500	0.714	0.571	0.571	0.571	0.000							
BARI gom33	0.714	0.786	0.571	0.786	0.500	0.500	0.429	0.143	0.000						
BAW 1147	0.750	0.750	0.667	0.875	0.875	0.875	0.875	0.667	0.833	0.000					
BAW 1290	0.714	0.714	0.714	0.929	0.786	0.786	0.786	0.500	0.583	0.333	0.000				
Nadi 2	0.800	0.800	0.800	0.900	0.700	0.700	0.700	0.400	0.500	0.000	0.200	0.000			
BWMRI gom1	0.714	0.786	0.500	0.786	0.500	0.500	0.286	0.333	0.167	0.875	0.700	0.625	0.000		
BWMRI gom2	0.700	0.813	0.778	1.000	0.800	0.800	0.600	0.417	0.333	0.875	0.500	0.500	0.167	0.000	
BWMRI gom3	0.800	0.889	0.667	0.900	0.700	0.700	0.600	0.417	0.333	0.625	0.214	0.300	0.333	0.333	0.000

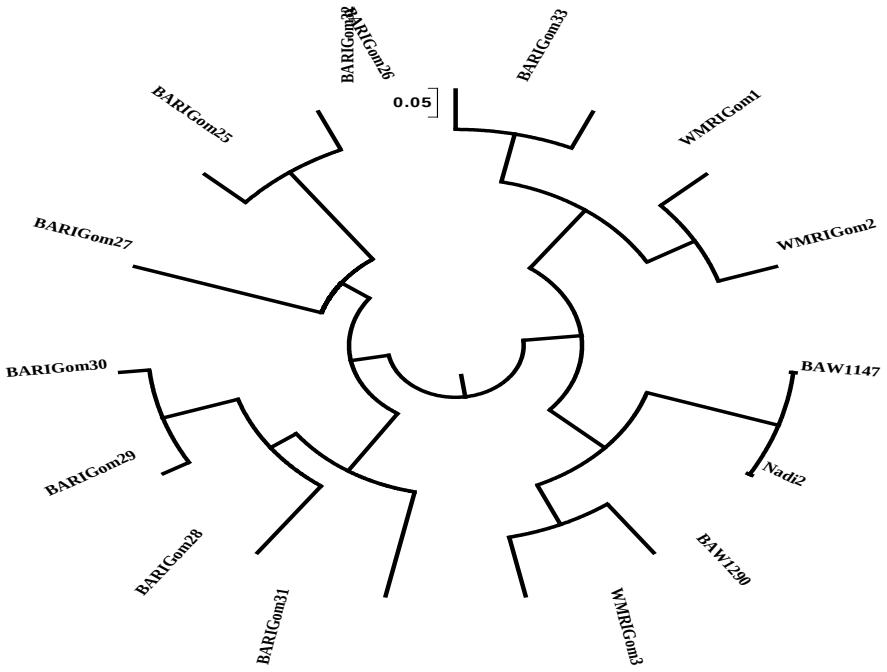

Fig. 2. The dendrogram, generated through UPGMA analysis, illustrates the genetic relationships among fifteen wheat genotypes, with the names of the genotypes listed at the ends of the branches.

Fifteen genotypes could easily be identified as heat tolerance or susceptible. The UPGMA cluster tree analysis drew the dendrogram with those fifteen genotypes using a distance coefficient of 0.050 (Fig. 2). Values ranging from 0.000 to 0.929 were obtained from the combined data for the 13 primers in pairwise comparisons of shared alleles and genetic distances across the variations (Table 4). Tables 5 and 6 display the mean TGW and grain yield HSI values, respectively. The analysis examined TGW and grain yield under ILS and ITS conditions, along with the mean percentage change in these parameters between the two conditions for each cluster member. The HSI, TGW, and grain yield under stress conditions, along with the percent decrease in TGW and grain yield from ILS to ITS, were key parameters that differentiated the two major groups identified in the cluster analysis as indicators of heat tolerance under field conditions (Tables 5, 6).

Table 5. Summary of wheat genotype clusters according to thousand grain weight (TGW)

Cluster	Genotypes	HSI	TGW		%TGW decrease	HSI	TGW		%TGW decrease
			ITS	ILS			ITS	ILS	
Group A									
Cluster I	BARI gom25	0.887	49.00	43.87	10.47	0.887	49.00	43.87	10.47
	BARI gom26	0.885	48.05	43.03	10.45				
	BARI gom27	0.870	42.85	38.45	10.27				
Cluster II	BARI gom28	0.772	45.00	40.90	9.11	0.772	45.00	40.90	9.11
	BARI gom29	0.892	42.35	37.89	10.53				
	BARI gom30	0.823	46.80	42.25	9.72				
	BARI gom31	0.909	42.75	38.16	10.74				
Group B									
Cluster III	BWMRI gom3	1.038	43.15	37.86	12.26	1.038	43.15	37.86	12.26
	BAW 1290	1.181	44.30	38.12	13.95				
	BAW 1147	1.218	45.05	38.57	14.38				
	Nadi 2	1.106	43.55	37.86	13.07				
Cluster IV	BARI gom32	1.169	48.55	41.85	13.80	1.169	48.55	41.85	13.80
	BARI gom33	1.061	48.85	42.73	12.53				
	BWMRI gom1	1.129	48.85	42.34	13.33				
	BWMRI gom2	1.043	49.30	43.23	12.31				

Table 6. Summary of wheat genotype clusters according to grain yield

Cluster	Genotypes	HSI	Yield		% Yield decrease	HSI	Yield		% Yield decrease
			ITS	ILS			ITS	ILS	
Group A									
Cluster I	BARI gom25	0.903	2.68	2.31	13.81	0.903	2.68	2.31	13.81
	BARI gom26	0.964	2.17	1.85	14.75				
	BARI gom27	0.960	2.52	2.15	14.68				
Cluster II	BARI gom28	0.742	2.38	2.11	11.34	0.742	2.38	2.11	11.34
	BARI gom29	0.833	2.59	2.26	12.74				
	BARI gom30	0.756	2.68	2.37	11.57				
	BARI gom31	0.930	2.67	2.29	14.23				
Group B									
Cluster III	BWMRI gom3	1.128	1.97	1.63	17.26	1.128	1.97	1.63	17.26
	BAW 1290	1.189	2.64	2.16	18.18				
	BAW 1147	1.253	2.66	2.15	19.17				
	Nadi 2	1.135	2.65	2.19	17.36				
Cluster IV	BARI gom32	1.050	2.49	2.09	16.06	1.050	2.49	2.09	16.06
	BARI gom33	1.004	2.54	2.15	15.35				
	BWMRI gom1	1.102	2.61	2.17	16.86				
	BWMRI gom2	1.056	2.60	2.18	16.15				

The HSI was evaluated for TGW and grain yield to identify heat-tolerant and heat-susceptible genotypes. Grain yield ranged from 0.74 to 1.25, while the HSI for TGW varied from 0.77 to 1.23. Data from this assessment revealed heat-tolerant genotypes, with heat-stress tolerance correlated with a low HSI (HSI<1) (Table 5). Genotypes were categorized according to their HSI values, which serve as an important indicator of their tolerance to heat stress. The classification is as follows: those with high heat tolerance were defined as having an HSI value of less than 0.50, indicating a strong ability to maintain performance under elevated temperatures. Genotypes that exhibited moderate heat tolerance were identified with HSI values ranging from 0.50 to 1.00, suggesting a more variable response to heat stress but still capable of reasonable yield performance. In contrast, genotypes classified as heat-sensitive displayed HSI values greater than 1.00, indicating a significant decline in performance under heat-stress conditions. This classification system is based on the framework established by Khanna-Chopra and Viswanathan (1999). Table 5 summarizes the mean HSI values for TGW under ITS and ILS conditions, the mean percentage change in TGW and grain yield between ILS and ITS, and the corresponding values for each cluster member. The three key parameters that differentiated the two major groups identified in the cluster analysis as indicators of heat tolerance under field conditions were the HSI, TGW, and grain yield under stress, along with the percent decrease in TGW and grain yield from ILS to ITS (Table 5).

Three genotypes, BARI gom25, BARI gom26, and BARI gom27, comprised Cluster I. Their respective HSI values for TGW and grain yield were more significant, falling between 0.885-0.887 and 0.903-0.964, respectively (Tables 5 and 6). The heat tolerance of these genotypes was moderate (HSI 0.50-1.00). Under ILS, the TGW (g) and grain yield/plot (kg) varied from 38.5 to 43.8 and 1.85 to 2.31, respectively. When comparing the grain yield under the ILS to the ITS, these genotypes' TGW dropped from 10.3 to 10.5%, and their grain yield dropped from 13.7 to 14.8% (Tables 5 and 6). The TGW and grain yield cluster means, and the corresponding declines in TGW and grain yield were 0.887, 0.903, 10.5%, and 13.8%, respectively. These genotypes closely matched group A's cluster II members (Tables 5 and 6). Cluster II was composed of four distinct genotypes: BARI gom28, BARI gom29, BARI gom30, and BARI gom31. These genotypes were grouped based on their genetic similarities and shared characteristics, which likely reflect their responses to environmental stresses such as heat. The inclusion of these specific genotypes in Cluster II indicates their potential for comparable performance traits, making them valuable for further studies and breeding programs aimed at enhancing heat tolerance in wheat. Their classification within this cluster allows researchers to explore the genetic diversity and agronomic traits associated with each genotype, facilitating the identification of candidates for cultivation in regions affected by elevated temperatures.

The ranges of the HSIs for TGW and grain yield, as well as the decreases in TGW and yield from ITS to those under ILS, were 0.772-0.903, 0.742-0.930, 9.11-10.7%, and 11.3-14.2%, respectively. These findings open up new avenues for understanding and improving heat tolerance in wheat. The losses in TGW and grain yield, as well as the cluster means of the HSI for these variables, were 0.772, 0.742, 9.11%, and 11.3%, respectively. Additionally, these genotypes have a moderate tolerance to heat (HSI 0.50-1.00). Eight genotypes, separated into two clusters (III and IV), comprised Group B. Four genotypes made up Cluster III were BWMRI gom3, BAW 1290, BAW 1147, and Nadi 2. For this cluster, the mean HSIs were 1.04-1.23, 1.135-1.253, 12.3-14.4%, and 17.3-19.2% for TGW, grain yield, and the proportional decreases in TGW and grain yield under ILS compared to ITS, respectively. According to the HSI, TGW and grain yield's cluster means and the decreased percentages of TGW and grain yield were 1.04, 1.13, 12.3%, and 17.3%, respectively. There was heat susceptibility in these genotypes (HSI>1.00). Cluster IV was made up of four genotypes: BARI gom32, BARI gom33, BWMRI gom1, and BWMRI gom2 For this cluster, the mean HSIs for TGW and grain yield were 1.06-1.17 and 1.004-1.1.102, respectively; the relative decreases in TGW and grain yield under ILS compared to ITS were 12.5-13.8% and 15.4-16.9%. Additionally, some genotypes showed heat sensitivity (HSI>1.00). The losses in TGW and grain yield, as well as the cluster means of the HSI for these variables, were 1.17, 1.05, 13.8%, and 16.1%, respectively.

Discussion

Genetic similarity information ensures long-term productivity gains during breeding operations, which prevents elite germplasm from becoming homogenous. Numerous unique genes are probably present in cultivars with different DNA profiles. Both phenotypic and molecular data made practical assessments of genetic variation and heat-tolerant genotypes possible. SSR markers provided helpful information for DNA

fingerprinting and genetic diversity estimation (Gupta *et al.*, 2022). They generated distinct bands for heat-tolerant genotypes, suggesting their potential use in enhancing heat tolerance. By integrating phenotypic and molecular data, wheat varieties with improved yields under abiotic stress could be developed (Haliloglu et al., 2022).

The 13 SSR markers produced 51 polymorphic bands, yielding an average PIC of 0.57. The number of bands per marker varied from 2 for Xcfd43 and Xwmc407 to 7 for Xgwm356, with an overall average of 3.92 bands. PIC values varied between 0.38 for the marker Xcfd43 and 0.83 for the marker Xgwm356, highlighting the informative nature of certain SSR markers. This variation in PIC values reflects the different levels of genetic diversity captured by these markers, with higher values, indicating a greater ability to distinguish between genotypes. Consequently, markers like Xgwm356 could provide more detailed insights into genetic relationships and variations among the wheat genotypes, making them valuable tools for studies aimed at understanding and enhancing traits such as heat tolerance. This outcome was similar to what Sharma *et al.* (2017) found. Variations in allele frequency may cause PIC value differences. Polymorphic bands revealed changes between genotypes, allowing researchers to analyze systematic links (Haliloglu *et al.*, 2022). Eleven SSR markers produced distinct bands for heat-tolerant genotypes; these markers may be utilized to indicate heat tolerance, but further testing in a range of populations is required. Table 3 indicates that distinct chromosomes host SSR markers associated with heat tolerance. The SSR markers utilized in this study demonstrated low heterozygosity, with an average value of 0.37 among the examined wheat genotypes, indicating limited genetic variation. Thirteen loci with PIC values exceeding 0.50 were identified as informative, as a PIC above 0.5 signifies high diversity, while a PIC below 0.25 indicates low diversity (Nagy *et al.*, 2012; Ramadugu et al., 2015). The mean PIC for the SSR markers was 0.57, with values ranging from 0.33 to 0.83 (Table 3). Thus, most primers provided valuable information, demonstrating that SSRs are effective markers for selecting terminal heat stress tolerance in molecular plant breeding. Mourad *et al.* (2020) suggested that evaluating the number of alleles at each locus alongside their PIC values is essential for objectively assessing genetic diversity in genotyping collections.

Each of the four clusters exhibited distinct traits. Cluster IV had the highest mean HSI value (>1.00) and the largest decrease in TGW under ILS (sown on December 21) compared to ITS (sown on November 21) (Table 5). In contrast, cluster III showed the highest mean HSI value and the most significant decline in grain yield under ILS relative to ITS (Table 6). Notably, BARI gom25 and BARI gom30 demonstrated greater genetic potential for yield under ILS, outperforming other genotypes in group A. BAW 1147 was categorized as heat-sensitive due to its higher HSI for grain yield (Table 6). According to Table 6, the genotypes in group A-BARI gom25, BARI gom26, BARI gom27, BARI gom28, BARI gom29, BARI gom30, and BARI gom31 were found to be well-suited for ILS. Similar findings were reported by Alam *et al.* (2014). Although few discrepancies were observed, most morphological data confirmed the molecular findings. For instance, Nadi 2 in group B showed a higher HSI (1.135) and a more notable decrease in mean grain production (17.4%) under ILS compared to ITS (Table 6). It should not be categorized as heat-sensitive because it had the highest grain yield in the ILS despite being in the heat-sensitive group. In contrast, BARI gom26 in group A had a higher HSI value (0.97) but the

most significant drop in mean grain output (14.8%) under ILS and the lowest grain yield within the heat-tolerant group. This was comparable to the grain yield of WMRI gom3 in group B, indicating that it should not be categorized as a terminal heat stress-tolerant group. These variations could be attributed to the regional diversity of heat stress, which affects plants differently depending on length and timing. Heat stress significantly affects yield, with genotype-environment interactions playing a vital role in yield expression. This field study, reflecting noticeable weather variations, aligns with the findings of Haliloglu *et al.* (2022). High temperatures above the optimal range (21.3±1.27°C) during the grain-filling stage adversely impact wheat output, underscoring the need for developing heat-resistant wheat varieties. It is essential to identify or develop genotypes capable of withstanding terminal heat stress or maturing early with minimal yield losses (Shenoda *et al.*, 2021). The molecular and genetic techniques utilized in this study, such as marker-assisted breeding and DNA polymorphism characterization, enabled the identification of both heat-tolerant and heat-sensitive wheat genotypes. The effective distinction of these genotypes through SSR markers contributes to the development of future heat-tolerant wheat varieties and supports the establishment of intellectual property.

Conclusion

SSR markers provided valuable information for DNA fingerprinting and genetic diversity estimates, producing distinct bands for heat-tolerant genotypes. The study assessed fifteen wheat genotypes, creating a dendrogram with a distance coefficient of 0.050 and dividing them into two main categories. The greatest genetic distances were observed between BAW 1290 and BARI gom28 (0.929), and the lowest genetic distance of 0.000 was noted between Nadi 2 and BAW 1147. Group A, which included seven genotypes from Clusters I and II BARI gom25, BARI gom26, BARI gom27, BARI gom28, BARI gom29, BARI gom30, and BARI gom31, was identified as suitable for cultivation under heat-stressed conditions. This study focused on analyzing DNA to identify both heat-tolerant and heat-sensitive wheat genotypes, providing valuable insights for future molecular breeding programs aimed at enhancing thermotolerance.

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Authors' contribution

MNA and AR conceptualized and designed the experiments. MFA conducted the trials. MNA supervised the projects. The manuscript was written by MNA and MFA. It was revised and edited by AR. All authors approved the final version.

Conflict of Interest

All authors affirm that there are no conflicts of interest regarding this research paper.

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MORPHOLOGY, DISEASE AND PEST INFESTATION VARIATIONS IN DIFFERENT BETEL VINE GENOTYPES

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Abstract

Betel vine is one of the important cash crops that gaining popularity in recent time in Bangladesh. In the varietal improvement program, collection and evaluation of the germplasm from different parts of the country is crucial. Thus, the present experiment was conducted to collect, characterize and evaluate betel vine genotypes at Spices Research Centre, Shibganj, Bogura during 2021-2022 and 2022-2023. A total of 30 genotypes of betel leaf were collected from different places for characterization and evaluation. The experimental plots were arranged in a Randomized Complete Block Design (RCBD) with three replications. Data were collected three times annually during leaf harvests, encompassed a range of morphological traits. Four genotypes (BL0027, BL0018, BL0024 and BL0030) were found promising in terms of leaf quality and less susceptible to disease and pest infestation. This study is important for betel vine breeding, offering insights into its variation and potential for tailored cultivation. Future research should explore the morphological and environmental factors that affect these traits to improve betel vine cultivation and management.

Keywords: Betel vine, Disease, Germplasm collections, Insect.

Introduction

Betel vine (*Piper betle* L) is an important cash crop in Bangladesh belonging to the family *Piperaceae*. Betel vine is basically consumed in South Asia and world widely known as betel quid or paan, in combination with areca nut or tobacco (Shah *et al.*, 2021). The betel vine is believed to have originated in Central or Eastern Malaysia (Chattapdayay and Maity, 1967). Betel vine is a perennial, dioecious, evergreen vine cultivated in tropical and subtropical regions for its leaves utilized as a chewing stimulant. It is a spreading vine, rooting readily where trailing stems touch the ground. The betel vine plant is a perennial creeper that remains green throughout the year. It is characterized by its glossy heart-shaped leaves and white catkin. The leaves are alternate, entire, 5 to 10 cm long and 3 to 6 cm across. The flowers are small, produced on

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pendulous spikes 4 to 8 cm long at the leaf nodes, the spikes lengthening up to 7 to 15 cm as the fruit matures. Betel vine have antioxidant (Rathee *et al.,* 2006), anticarcinogenic (Bhide *et al.,* 1991), hypolipidemic (Gramza and Korczak, 2005), and antibacterial properties (Gramza and Korczak, 2005; Nalina and Rahim, 2007; Bissa *et al.,* 2007; Ramji *et al.,* 2002). Betel vine and stem possess a strong, fragrant aroma. Chewing betel vine is believed to be a dietary source of calcium. Betel oil is utilized for various medicinal purposes. The stem climbs using numerous short adventitious roots (Hassan and Shahadat, 2005).

Betel vine grows well under shade with low light intensity, mild temperature (15 to 30ºC), high humidity with 2250 to 4750 mm annual rainfall (Rahman *et. al.,* 2015). In an area with lower rainfall, however, betel vine can be grown with frequent irrigation throughout the year. Traditionally, betel vine is cultivated under shade in a structure called a boroj, which is a small hut standing at approximately two meters in height. Betel vine is cultivated almost in all areas of Bangladesh, however, the districts like, Cox's Bazar, Kushtia, Chittagong, Greater Khulna, Greater Barisal, Greater Faridpur, Greater Rajshahi are notable for its production. There are about 100 varieties of betel leaf (paan) across the world of which 40 are encountered in India and 30 in West Bengal and Bangladesh (Guha, 2006). The total betel vine production in Bangladesh in 2022-2023 was estimated at 206994 M. tons, and total cultivated area was about 21850 hectares (BBS, 2023). But the acreage of betel vine is decreasing fast because of some physical and socioeconomic barriers like unavailability of credit facilities, uncontrolled marketing system and infestation of diseases and pests (Islam, 2005). The leaf is usually plucked throughout the year, but maximum production obtained during the months of July to October. In order to enhance the production of betel vine in the nation, it is crucial to identify superior genotypes or germplasm that can be utilized for the development of a high-yielding variety of the crop. Based on the aforementioned information, the current study was conducted to identify appropriate cultivars/germplasm that can contribute to improve the yield and quality of betel vine in Bangladesh (Rahman *et al.,* 2020).

There are lot of problems faced in betel vine cultivation. It is not a major crop in Bangladesh, so there is a scarcity of appropriate technology, lack of proper education and access to information for its cultivation. Significant yield losses have been attributed to insect pests (Hossain *et al.,* 2020). Recently, Rahman (2019) recorded 12 insect species that cause damage to betel vines. Of the various insect pests, *Aleurocanthus rugosa Singh* (Hemiptera: *Aleyrodidae*) is one of the significant foliage pests of betel vine recorded in Bangladesh and India locally known as 'blackfly' (Raut and Nandi, 1984; Raut and Bhattacharya, 1999; Jana, 2006; Rahman, 2019; Hossain *et al.,* 2020). The white and black fly, red mite, and mealy bug have been identified as the major constraints in increasing the leaf yield of betel vine (Jana, 2006).

Disease damage to the crop is one of several known limiting factors. The betel vine is highly susceptible to diseases, pests and some natural climates (Sayeeduzzaman, 1988). Disease is one of the most important constraints for betel leaf cultivation. Among the diseases of betel vine, leaf rot caused by *Phytophthora parasitica var. piperina* and foot and root rot of betel vine caused by *Sclerotium rolfsii Sacc*., are the most devastating diseases which decrease the production of betel vine to a great extent. In 2004, Sixty

percent betel vine damaged due to foot rot disease in 3 upazilla of Rajshahi (Islam, 2005). The crop is also infested by several insect-pests and diseases which is a major reason for reduced profit margin (Jana, 2017). Humid and moist shaded conditions are favorable for its growth and development. However, these humid and moist shaded conditions are also prone to root and foliage disease development of betel vine (Goswami *et al.*, 2002).

The precise evaluation of betel vine genotypes should be focused within its unique geographical context, signifying the importance of morphological traits as key indicators of the plant's adaptability and productivity. Bangladesh's varied climatic zones, ranging from the lush plains to the hilly terrain, provide a dynamic backdrop for the cultivation of betel vine, giving rise to a rich tapestry of genotypes with distinct morphological characteristics. To meet the increased demand for food over the years mostly research, technologies have focused mainly on annual crops rather than the perennial crops. But being the perennial crop, betel vine has a high demand and market value, especially for Asian people. So, forecasting betel vine production and prices will be truly beneficial for farmers, governments and agribusiness industries to understand its future potentialities and growth. The assessment of various betel vine genotypes in Bangladesh based on morphological characteristics is crucial for enhancing productivity, maintaining quality, and preserving traditional methods. This in-depth analysis aims to equip stakeholders with the necessary information to make well-informed choices and pave the way for a prosperous betel vine sector that not only sustains livelihoods but also honors the nation's cultural legacy. Considering this perspective, the present study was conducted to evaluate betel vine genotypes for identification of the potential genotype(s) in terms of leaf quality as well as less prone to disease and pest infestation.

Materials and Methods

Site and genotypes

The experiment was conducted at the Spices Research Centre, Shibganj, Bogura, spanning two consecutive growing seasons: 2021-2022 and 2022-2023. Thirty betel leaf genotypes collected from various regions across Bangladesh such as - BL0024, BL0025, BL0026, BL0027, BL0028, BL0029, BL0030, BL0031, BL0032, BL0034, BL0035 etc. were conserved and used for characterization and evaluation in this study (Supplementary Table S1). Throughout both seasons, we utilized BARI Pan-1, BARI Pan-2 and BARI Pan-3 were used as the reference or check variety to facilitate comparative analysis.

Experimental design

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

Observations recorded and statistical analysis

Data were meticulously recorded three times annually, specifically during the leaf harvest periods. The parameters under evaluation encompassed a range of morphological traits, including Internode length (IL), Internode diameter (ID), Peduncle length (PL), Peduncle diameter (PD), Leaf length (LL), Leaf width (LW) of the betel vine plants. In addition to these primary observations, also calculated some diseases and insect infestation data such as black fly (BF), Red spider mite (RSM), Foot rot (FR), Leaf rot (LR) and Leaf spot (LS). All other observations were averaged from the three data collection points within a year. These recorded data form the basis of subsequent analysis and findings. To analyze variance based on the extensive dataset obtained from these observations, employed the Rstudio platform which is widely recognized for its versatility and robust statistical analysis capabilities in statistical analysis (R Core Team, 2022).

Results and Discussion

The table 1 showes the summary performances of betel vine for different traits during 2021-2022 and 2022-2023 where leaf length ranges from 4 cm to 13.60 cm with an average length of 10.45 cm. Variance due to genotypes were significant for all the traits while variance due to G×Y were insignificant for all the traits except ID and PD. In case of leaf width, the range was between 6 cm to 11.80 cm with an average width of 8.71 cm. the lower mean values were found in case of insect and disease infestation for the studied genotypes of betel vine. The performance of individual betel vine genotypes is presented in table 2.

Table 1. Performances of betel leaf genotypes for different traits during 2021-2022 and 2022-2023

Traits	Min	Max	Mean	SD	SE	GV	CV	LSD	F (G)	F (G×Y)
IL	3.10	8.56	6.54	1.34	0.25	1.69	1.52	0.14	**	ns
ID	0.22	3.17	0.46	0.52	0.09	0.25	4.97	0.05	**	*
PL	3.20	10.60	6.64	1.90	0.35	3.40	1.16	0.14	**	ns
PD	0.20	2.35	0.43	0.50	0.09	0.27	3.49	0.04	**	*
LL	4.00	13.60	10.45	2.33	0.43	5.87	1.69	0.26	**	ns
LW	6.00	11.80	8.71	1.63	0.30	2.49	2.28	0.28	**	ns
BF	0.00	3.00	1.10	0.92	0.17	1.19	9.95	0.35	**	ns
RSM	0.00	3.00	2.00	1.05	0.19	1.09	9.08	0.46	**	ns
VR	0.00	2.00	1.03	0.85	0.16	0.84	7.15	0.25	**	ns
LR	0.00	3.00	1.63	1.03	0.19	1.27	7.49	0.33	**	ns
LS	0.00	10.00	3.80	1.92	0.35	3.87	3.45	0.26	**	ns

IL=Internode length; ID=Internode diameter; PL=Peduncle length; PD=Peduncle diameter; LL=Leaf length;LW=Leaf width; BF= Black fly; RSM=Red spider mite; FR=Foot rot; LR=Leaf rot; LS=Leaf spot; Min=Minimum; Max=Maximum; SD=Standard deviation; SE=Standard error; GV=Genotype variance; CV=Coefficient of variation; LSD=Least significant variation; F (G)=F-test for genotype; F (G×Y)=F-test for genotype × year.

Table 2. Performances of different betel leaf genotypes evaluated during 2021-22 and 2022-23

Name	IL	ID	PL	PD	LL	LW	BF	RSM	FR	LR	LS
BL0027	7	0.58	10.6	0.42	13.6	11.8	1	3	1	3	6
BARI pan3	7	0.55	9.5	0.35	13	11.12	0	2	2	2	3
BL001	6.16	0.44	6	0.22	11.1	8.5	2	2	2	2	3
BL003	5.76	0.4	5.96	0.2	11.2	9.5	1	2	2	1	2
BL0020	8.26	0.37	7.6	0.32	12.3	10.7	0	2	2	2	2
BL0021	7.36	0.37	6.24	0.232	12.3	10.5	0	3	1	1	2
BL0012	7.6	0.36	6.5	0.32	12.6	10.5	0	3	0	2	4
BL0022	6.4	0.34	5.6	0.22	11.54	8.9	2	3	0	0	10
BL0019	5.34	0.36	4.8	0.21	12.16	8.7	0	3	0	0	6
BL0018	6.7	0.3	5.92	0.28	13.37	8.54	0	0	0	1	1
BL0014	7.78	0.4	6.86	0.29	13.2	9.98	2	0	2	1	0
BL0016	5.8	0.36	6.8	0.26	12.12	10.8	0	1	2	2	2
BL0023	5.24	0.32	5.56	0.24	8	6.1	1	0	2	2	3
BL003	6.32	0.28	6.26	0.28	12.28	10.7	1	0	2	2	3
BL002	5.62	0.25	5.48	0.25	8.84	8.24	2	0	2	0	3
BL0011	4.76	0.26	3.2	0.24	7.9	6.15	1	3	2	3	3
BL0010	4.58	0.34	3.77	0.22	4	7.95	2	2	0	2	4
BL009	3.1	0.42	3.5	0.34	7.8	6.7	3	3	0	2	3
BL008	4.28	0.17	4.37	2.35	8.2	7.3	2	2	0	2	3
BL006	5.27	0.22	4.3	2.17	8.9	7.4	2	3	1	2	3
BL0024	8.2	0.38	8.4	0.34	11.8	9.8	2	2	1	3	4
BL005	8.56	0.34	6.2	0.3	9.04	7.8	1	3	2	2	3
BL004	7.48	0.32	6.4	0.32	9	8.06	0	2	1	3	5
BARI pan1	6.64	0.46	8.84	0.36	9.8	8.2	0	2	1	3	5
BARI pan2	6.8	0.44	8.76	0.38	9.4	7.6	0	2	0	3	5
BL0025	8.06	0.42	9.64	0.3	13.4	9.8	2	2	1	2	4
BL0028	6.76	0.34	8.14	0.33	8.46	6.2	1	2	1	1	5
BL0029	7.5	0.34	7.5	0.33	7.2	6	1	2	0	0	5
BL0030	7.96	0.36	8.7	0.34	10.9	9	2	3	0	0	6
BL0031	7.8	0.34	7.9	0.34	10	8.8	2	3	1	0	6

IL=Internode length; ID=Internode diameter; PL=Peduncle length; PD=Peduncle diameter; LL=Leaf length; LW=Leaf width; BF= Black fly; RSM=Red spider mite; FR=Foot rot; LR=Leaf rot; LS=Leaf spot.

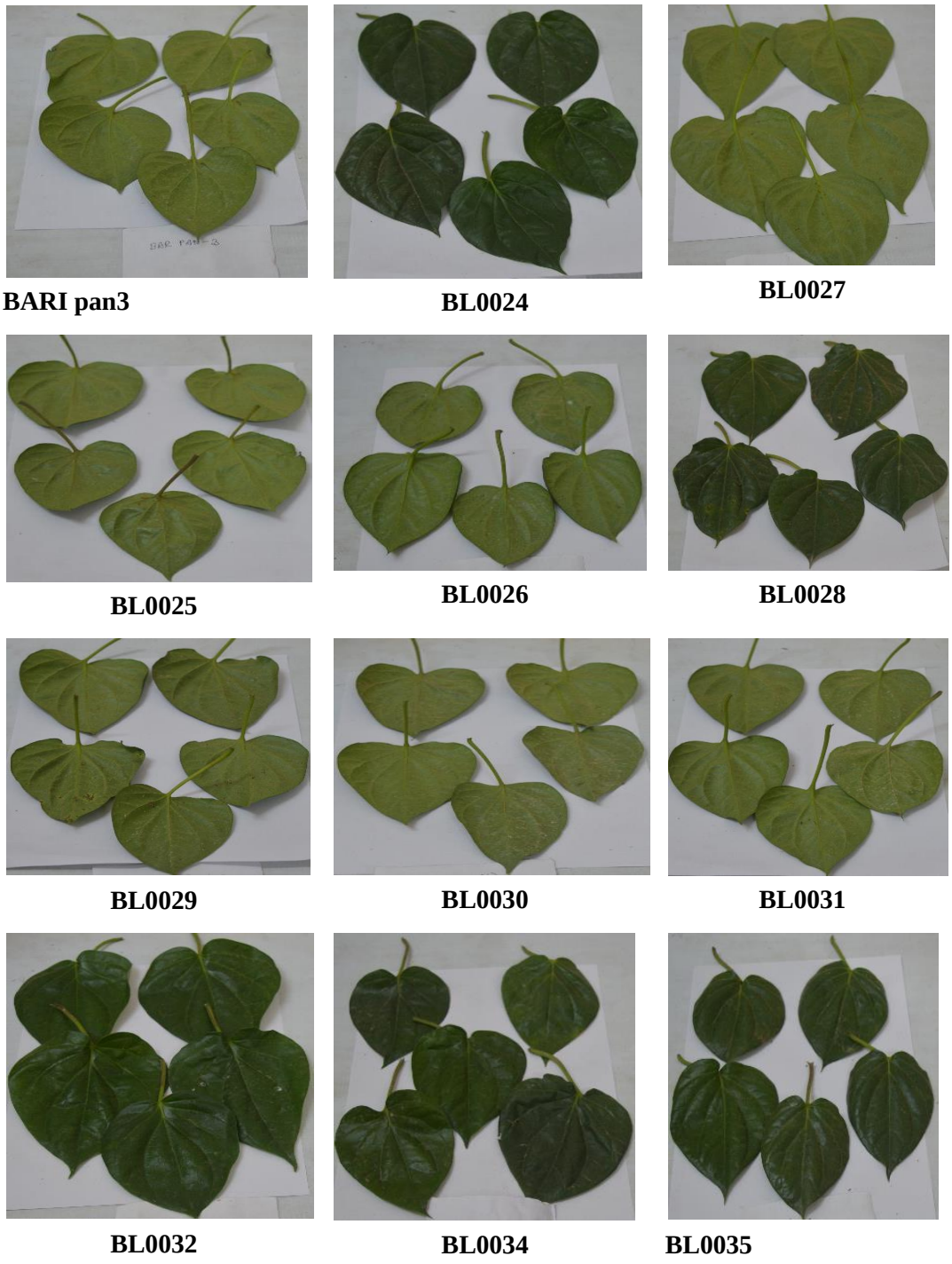

Fig. 1. Morphological variations of leaves in different betel vine genotypes.

The Individual performances of the betel vine genotypes were presented in table 2. The findings of table 2 are described below-

Vine growth

Among the evaluated betel vine genotypes, significant variation was observed in vine growth characteristics, particularly internode length and diameter (Table 2). The genotype BL005 exhibited the longest average internode length (8.56 cm), followed by BL0026 (8.26 cm) and BL0025 (8.06 cm). In contrast, BL009 demonstrated the shortest internode length at 3.10 cm, closely followed by BL008 (4.28 cm). These differences in internode length can influence the vine's structural development and overall growth potential. According to Rahman *et al.* (2020), a longer vine with shorter internodes is considered desirable as it can increase the number of leaves produced due to a higher number of nodes. Notably, BL0027 showed the thickest internode diameter (0.58 cm) combined with an internode length of 7 cm, indicating its potential for higher yield, as thicker vines are often associated with improved nutrient transport and productivity.

Peduncle growth

Peduncle growth, as indicated by peduncle diameter (PD) and length (PL), reflects structural robustness and support capacity. The length and diameter of the peduncle are vital for transporting nutrients and developing leaves (Alam *et al.,* 2023). Higher PD values (e.g., BL0027 at 0.42) indicate stronger, thicker peduncles suited for heavier inflorescences, while lower values (e.g., BL008 at 0.17) suggest a more delicate structure. Longer peduncles (e.g., BL0027 at 10.6) elevate inflorescences, potentially enhancing pollination and display, while shorter peduncles (e.g., BL0011 at 3.2) keep fruit closer to the plant. Varieties with high PD and PL (e.g., BL0027) exhibit robust, elevated growth, ideal for supporting larger clusters, whereas lower values suggest suitability for smaller inflorescences.

Leaf size

Leaf size is a crucial determinant of photosynthetic efficiency and overall yield in betel vine. BL0027 produced the largest leaves, with an average length of 13.60 cm, followed by BL0018 (13.37 cm), while the smallest leaves were produced by BL0010 (4 cm) and BL0023 (8 cm). In addition to leaf length, BL0027 also exhibited the widest leaves, measuring 11.80 cm, highlighting its superior photosynthetic capacity (Fig. 1). Previous studies by Pariari and Imam (2012) have indicated leaf widths ranging from 8.65 to 10.45 cm, which aligns with the present findings. Similarly, Rahaman *et al.* (1997) reported significant variation in leaf length among 27 betel leaf genotypes, ranging from 6.2 cm to 15.3 cm. Several other genotypes, including BL0018, BL0016, BL003, BL0024, BL0025, BL0014, and BARI Pan-3, were also found to produce relatively larger and wider leaves, contributing to higher photosynthetic potential and increased yield.

Pest infestation

The infestation of black fly, a common pest in betel vine cultivation, was recorded in varying degrees across the studied genotypes. BL009 had the highest mean

black fly population, with an average of three insects per vine, while other genotypes exhibited infestation levels ranging from 0 to 2 insects per vine, indicating minor damage overall (Table 2). Black fly infestation affects leaf quality by causing curling, discoloration, and reduced size of leaves, along with stunted plant growth. The damage also results in the development of sooty mold, which further diminishes the market value of the leaves. Hossain *et al.* (2020) reported higher black fly populations, ranging from 3.2 to 22.7 per vine, with peak infestations occurring in October and May. Red spider mite infestation was relatively low among the studied genotypes, with infestation levels ranging from 0 to 3 mites per vine. This pest colonizes the ventral surface of leaves under a protective web, sucking the sap and causing yellow blotches that eventually lead to desiccation. Severe infestations reduce leaf quality and market value. Hossain *et al.* (2020) reported higher red spider mite populations, reaching up to 16.1 per vine during peak seasons. The minimal infestation observed in this study suggests that the genotypes evaluated have some level of resistance to red spider mites.

Disease incidence

Foot rot, caused by *Phytophthora parasitica* and *Pythium vexans*, was observed at low levels, with incidences ranging from 0 to 2 plants per vine (Table 2). This disease, commonly referred to as wilt, leads to wilting, leaf drop, and eventual desiccation of the vine. The subterranean parts of the plant decay, reducing the overall productivity of the crop. The disease becomes most severe during or after the rainy season (Jana, 2017). Previous research by Haider *et al.* (2013) and Rahman *et al.* (2021) has highlighted the devastating impact of foot rot on betel leaf production, with *Sclerotium rolfsii* being identified as a major pathogen responsible for yield losses. Leaf rot, primarily caused by *Phytophthora parasitica var. piperina*, was another disease observed among the genotypes. The disease, prevalent during the rainy season, manifests as water-soaked lesions that quickly expand, leading to leaf decay. In the present study, the incidence of leaf rot ranged from 0 to 3 leaves per vine, indicating minor severity (Table-2). Jana (2017) described leaf rot as a serious threat to betel vine, particularly during the rainy season, when the pathogen is most active. Leaf spot disease, caused by *Fusarium semitectum*, along with other pathogens like *Colletotrichum capsici*, was moderately severe in the studied genotypes, with incidences ranging from 0 to 10 leaves per vine (Table-2). The highest disease incidence was recorded in BL0022, with an average of 10 leaves affected per vine. This disease, characterized by circular black lesions with a yellow halo, significantly reduces leaf quality and marketability. Severe infections can cause premature leaf drop, leading to yield losses. The findings of this study are consistent with earlier reports by Singh and Shanker (1971), Maiti and Sen (1979), Patra and Pradhan (2018), and Jana (2017), who described similar symptoms and impacts of leaf spot disease in betel vine.Therefore, the study highlighted the variability in growth, leaf size, pest infestation, and disease incidence among the betel vine genotypes evaluated. The genotypes BL0027 and BL005 exhibited superior growth and leaf size, while pest infestations and disease incidences were generally low across the genotypes, indicating their potential resilience and suitability for cultivation.

Conclusion

The results of this study revealed that significant variations in vine growth, leaf size as well as other characteristics among the studied betel vine genotypes. Considering various morphological traits, the genotypes BL0027, BL0018, BL0024, and BL0030 were found as promising among these studied genotypes. Moreover, minor infestation of black fly and red spider mite was observed in all of the studied genotypes. In case of disease incidence, foot rot and leaf rot took place at a lower rate, whereas leaf spot occurred at moderately severe rate referring devastating loss of betel leaf production. The genotype BL0027 consistently demonstrated superior vine growth, leaf characteristics, and ento-pathological attributes when compared to the other genotypes. Further research and breeding efforts could focus on enhancing these desirable traits to improve betel leaf productivity and quality.

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Author's contributions

Abu Jafor Mohammad Obaidullah: conceptualization, methodology, validation; M. A. Alam, M. R. Islam,: reviewing and editing; M. A. Alam: data analysis; A. J. M. Obaidullah, S. Naher: original draft preparation; A. J. M. Obaidullah, M. A. Rahman: reviewing and final editing; M. A. Alam, M. R. Islam, S. Naher: investigation, supervision; A. J. M. Obaidullah, M. A. Alam: software, S. Naher, R. Islam: methodology; M. A. Alam, M. A. Rahman: formal analysis. All authors reviewed the findings and accepted 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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IMPROVEMENT OF BIOCONTROL EFFICACY OF *Stenotrophomonas rhizophila* STRAIN pstu-hort-14 WITH SODIUM BICARBONATE AND LEMONGRASS EXTRACT TO CONTROL ANTHRACNOSE OF MANGO

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Abstract

The study was conducted at the Postharvest and Plant Biotechnology Laboratory, Department of Horticulture, Patuakhali Science and Technology University, Patuakhali, Bangladesh during the period from July to December 2017 to test the potential of sodium bicarbonate and lemongrass extract to increase the biocontrol efficacy of *Stenotrophomonas rhizophilla* strain PSTU-Hort-14. All treatments were arranged in a completely randomized design (CRD) with replications and repeated twice. This bacterium was found to be highly compatible with 20% lemongrass extract and 2% sodium bicarbonate (SBC) or mixture of both. Both of these have suppressive activity against *Colletotrichum gloeosporioides* of mango and could be used as enhancer for biocontrol efficacy of PSTU-Hort-14 during mango storage. The survival and proliferation of PSTU-Hort-14 in mango wounds and on fruit surfaces was not affected by lemongrass extract and SBC throughout the storage period. In addition, PSTU-Hort-14 strain was able to colonize and multiply on the surface of mango fruits. The combination of PSTU-Hort-14 with lemongrass extract and SBC was more effective in controlling the anthracnose disease than PSTU-Hort-14 alone or other treatments including fungicide Dipheniconazol® both in inoculated or naturally infected fruits stored at 12±1ºC and 90±5% RH for 18 and 14 days, respectively. However, this combination offered a greater control by reducing 98.2% of the disease over control in naturally infected fruits at the end of 14 days storage at 12±1ºC and 90±5% RH and six days post ripening at 28 ± 2ºC, which was superior to that found with Dipheniconazol® or other treatments tested.

Keywords: Anthracnose, Biocontrol efficacy, Lemongrass extract, Mango, Sodium bicarbonate.

Introduction

Postharvest pathogens caused significant losses in fruits and they are normally controlled by using synthetic fungicides. Recently, biological control has been developed

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as an alternative to synthetic fungicide treatment and considerable success has been achieved upon utilizing antagonistic microorganisms to control both pre harvest and postharvest diseases (Janisiewicz and Korsten, 2002). Alternatives to chemical control, biological controls often less effective than chemical fungicides especially for postharvest disease management. So, the efficacy of antagonists in controlling postharvest disease must be enhanced (Janisiewicz and Korsten, 2002). In order to enhance biocontrol ability of antagonists against postharvest fungal pathogens, certain strategies, such as supplemented with calcium salts, carbohydrates, amino acids and other nitrogen compounds with antagonists, have been proposed (Tian *et al*., 2001; EI-Ghaouth *et al*., 2000). Organic and inorganic salts are antimicrobial agents against a range of phytopathogenic fungi. Among them, sodium bicarbonate (SBC), commonly known as baking soda (Hadzic *et al*., 2019), which is generally regarded as safe (GRAS) by the United States Food and Drug Administration.

The potential of SBC for the enhancement of biocontrol ability of antagonists has been investigated in controlling postharvest decay. Liu *et al*. (2011) claimed that the performance of SBC combined with *Bacillus subtilis* 11 exhibited better control of pear ring rot disease caused by *Botryosphaeria berengeriana* than that of individual treatments. Smilanick *et al*. (2006) found that the effectiveness of sodium bicarbonate (SBC) and carbonate was improved significantly in the control of citrus green mold when the treatment was followed by the application of the biological control agent *Pseudomonas syringae* Esc 10. Similarly, SBC also increased the bio efficacy of different biocontrol agent to control different postharvest diseases, such as *Candida oleophila* (Lanza *et al*., 2004), and *Bacillus subtilis* (Obagwu and Korsten, 2003).

A novel approach to extend postharvest shelf life is the use of edible coatings of natural antimicrobial compounds is preferred since they pose minimal risk to health (Baldwin *et al*., 1995). Presently, wax coating is practiced for postharvest commodities. Wax coatings are mostly made of synthetic waxes and fatty acids, oils, shellac, emulsifier, plasticizers, antifoam agents, and surfactants (Baldwin, 1994). These coatings are somewhat effective in delaying ripening, but in general, do not prevent decay. Thus, combining lemongrass extract and sodium bicarbonate with *Stenotrophomonas rhizophila* strain PSTU-Hort-14 will make it possible to exploit the antifungal and eliciting properties of these chemicals and biological activity of this bacterium. Therefore, the present study was undertaken to select suitable enhancer (s) that increases the biocontrol efficacy of antagonistic bacteria to suppress the disease.

Materials and Methods

Culture and preparation of conidial suspension of *C. gloeosporioides*

Colletotrichum gloeosporioides, the causal organism of anthracnose of mango was collected and isolated from naturally infected mango fruits. The purified isolate of *C. gloeosporioides* was culture on potato dextrose agar (PDA) at 28 ± 2°C for seven days. The spores were harvested from the cultured and the concentration of conidia in the filtered suspension were adjusted to 5×10^5 conidia ml^{-1} with sterile distilled water using a haemacytometer (Obagwu and Korsten, 2003).

Experimental treatments

Five different concentration (1%, 1.5%, 2%, 2.5% & 3%) of SBC and four concentration (10%, 20%, 30% & 40%) of lemongrass extract were separately evaluated for their efficacy in controlling *C. gloeosporioides*. Both of the concentrations were also used to observe the compatibility with bacterial strain PSTU-Hort-14 (10^8 CFU mL^{-1}). In *in vivo* test four different combinations of bacterial strain PSTU-Hort-14, SBC and lemongrass extract were used..

Preparation of aqueous suspension of bacterial strain PSTU-Hort-14

Fourteen isolates were selected based on their antagonistic activity tested against *C. gloeosporioides* in *In-vitro*. In preparing aqueous antagonist suspension, isolates were grown on nutrient agar media (NA) at 28 ± 2°C for 24 hours. A loop of each culture were then transferred to 250 mL conical flask containing 50 mL of nutrient broth and incubated on a rotary shaker at 150 rpm for 72 hours at 31°C. To enumerate the colony forming units (CFU), cultures were serially diluted in sterile distilled water and plated on nutrient agar. The number of CFU were counted after 48h of incubation at 28 ± 2°C. At the time of use, the suspensions of PSTU-Hort-14 were adjusted to approximately 10^8 CFU ml^{-1} by spectrophotometer standard growth curve.

Preparation of sodium bicarbonate solutions

Solutions of SBC at concentrations of 0 (control, water only), 1, 1.5, 2, 2.5 and 3% (w/v) were used. Different concentrations of SBC were filtered through a 0.45µm pore filter before adding them to the autoclaved PDA. Desired concentration of lemon grass extract solutions were prepared by following Mehedi *et al.* (2020). Biocontrol activity of bacterial strain PSTU-Hort-14 enhanced with sodium bicarbonate and lemongrass extract on mango fruits pre inoculated with *C. gloeosporioides.*

Fruit inoculation and lesion measurement

A total of 108 mango fruits at color stage two (according to MAFC) were surface sterilized with 75% ethanol followed by rinsing with distilled water. Each of the fruit were wounded (3 mm deep and 5 mm diameter) with a sterilized cork borer. Two wounds were made at the mid area of fruit with six centimeters apart. Each of the wound were then inoculated with 50 µL conidial suspension of *C. gloeosporioides* (5×10^5 spores ml^{-1}) and held at 28 ± 2ºC for two hours (Gamagae *et al*., 2003). Each of the 12 inoculated fruits were then dipped for 15 min in i) aqueous suspension of bacterial strain PSTU-Hort-14 (10^8 CFU ml^{-1}); ii) 2% SBC solution and iii) 20% lemongrass extract solution.

In case of combination treatments (i) 12 inoculated fruits were initially dipped in 2% SBC solution for 15 minute and allowed to air dry for five minutes. Then the fruits were immersed in aqueous solution of bacterial strain PSTU-Hort-14 for 15 min. (ii) 12 inoculated fruits were initiallysoaked in bacterial suspension for 15 minute and allowed to air dry for five minutes. then immersed in 20% lemongrass extract solution for 15 min. (iii) 12 inoculated fruits were initially immersed in bacterial suspension for 15 minute and allowed to air dry for five minutes and then immersed in 20% lemongrass extract solution amended with 2% SBC solution for another 15 min. The control treatments consisted of a set of 12 inoculated fruits that were immersed either in sterilized

distilled water or commercial fungicide Difenoconazol® @ 0.50 ml l^{-1} acted as negative and positive controls, respectively. Fruits were allowed to air dry for five min after treatment. Each fruit were wrapped using 70 g offset paper and held at 12ºC and 90±5%RH for 18 days (Rahman *et al*., 2007). Data on lesion diameter were recorded on each alternate day starting from ten days after inoculation. The experiments were arranged with 12 replications and repeated twice.

Biocontrol activity on naturally infected fruits

Fruits selection and sterilization were done as in artificially inoculated fruits. Fruits were allowed to treat as in previously described (fruit inoculation and lesion measurement) without wounding the fruits. Treated fruits were packed and stored for 14 days. At completion of storage time, fruits were removed from storage and ripened with ethylene at room temperature (28 ± 2ºC). Ten mL/L 2% ethylene were placed in sealed polyethylene bag of fruits for 24 hours. Ethylene was then removed by opening the sealed polythene bag and the fruits were allowed to ripen at room temperature for another six days. Data on anthracnose incidence and severity were recorded everyday started on third day of post-storage, when disease symptoms began to appear in ripened fruits. Disease incidence and severity were calculated using formulae and scales (Uddin *et al.*, 2023).

$$\text{Disease incidence (DI) (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

$$\text{Disease Severity (DS)(\%)} = \frac{\Sigma\,(\text{Severity rating} \times \text{number of fruits in that rating})}{\text{Total number of fruits assessed} \times \text{highest scale} \times 100}$$

Experimental design and statistical analysis

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

Results

Effect of SBC on mycelia growth and spore germination of *C. gloeosporioides*

At 3% SBC treatment the complete inhibition of mycelial growth of *C. gloeosporioides* was found which is similar to 2% and 2.5% SBC treatment but significantly different from 1% and 1.5% SBC treatments (Plate 1). After seven days of incubation increase in concentrations of SBC significantly ($p \leq 0.05$) inhibited the radial growth of *C. gloeosporioides*. These results showed that SBC inhibited the growth of *C. gloeosporioides* . In control no inhibition was observed (Plate 1).

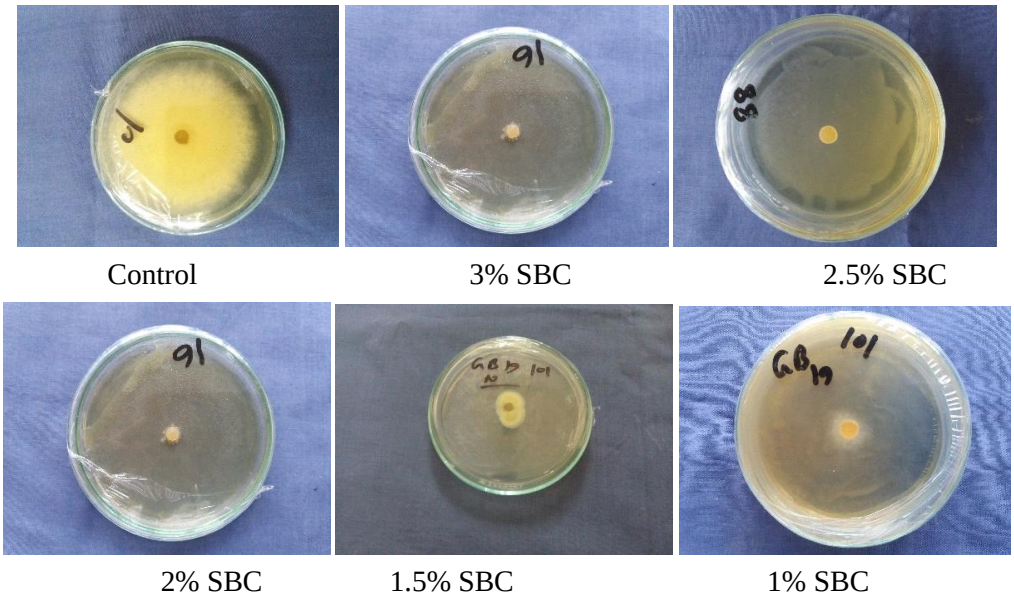


Fig. 1. Effect of different concentrations of SBC on the radial growth of C. gloeosporioides after seven days of incubation at 28 ± 2ºC

The spore germination of *C. gloeosporioides* was significantly lower on PDA plates amended with higher concentrations of SBC compared to control by microscopic observation. On PDA plates treated with concentrations of more than 2% SBC after seven hours of incubation no spore germination was observed. On the contrary, few spores were germinated in 1-1.5% SBC amended plates but numerous were found on the control plates. The results of 2 and 2.5% SBC similar with the results of 3% SBC treatment are shown in the Plate 1.

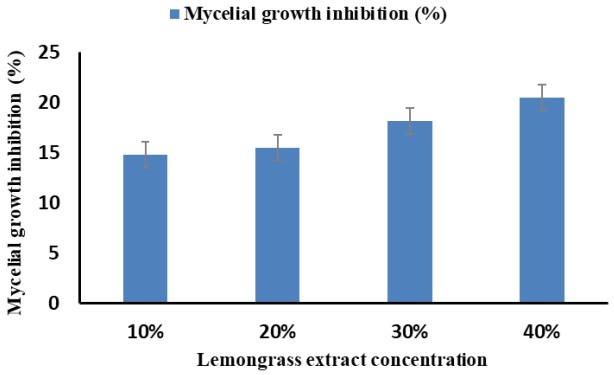


Fig. 2. Effect of different concentrations of lemongrass extract on the percentage inhibition of the radial growth of *C. gloeosporioides* after six days of incubation at 28 ± 2ºC. Each value is the mean of five replications. Means were separated by Least Significant Difference (LSD) test at 5% level of significance. Vertical bars represent standard error

Effect of lemongrass extract on mycelia growth and spore germination of *C. gloeosporioides*

At 40% lemon grass extract concentration treatment the inhibition of mycelial growth of *C. gloeosporioides* was recorded in 20.08% whereas in 30% lemon grass solution inhibited the 18.28% mycelial growth and 20% lemon grass extract solution controlled 15.8% mycelial growth which is statistically similar to 10% solution treatment (Fig. 1). With increasing concentrations of lemongrass extract the inhibition of spore germination of *C. gloeosporioides* are decreased. When PDA was amended with 10% of lemongrass extract, the highest inhibition of spore germination (20%) was recorded which was statistically similar with 20% lemongrass extract treatment (Table 1). However, after seven hours of incubation spore germination in control plates was found 100%. After 11 hours, 40% lemongrass extract amended plate showed 2.2% inhibition. After 13 hours of incubation, in all treatment did not show any inhibition on spore germination.. Germination of spores was not totally inhibited by lemongrass extract, only the rate of germination was reduced (Table 1).

Table 1. Effect of different concentrations of lemongrass extract on percentage inhibition of spore germination of *C. gloeosporioides* after 7, 9, and 11 hours of inoculation at 28 ± 2ºC

Time after incubation (hr)	% inhibition of spore germination				
	Control	10%	20%	30%	40%
7	0.00	19.00 a	18.00 a	15.20 a	12.60 a
9	0.00	10.00 b	8.60 b	7.60 b	5.40 b
11	0.00	4.80 c	4.00 c	3.00 c	2.20 c
13	0.00	0.00	0.00	0.00	0.00
LSD(0.05)	ND	1.57	1.68	2.01	1.54
Level of Significance	ND	*	*	*	*
CV%	ND	13.63	16.41	23.26	22.79

**Significant at 5% level of probability, ND= Analysis not done*

Effect of SBC and lemongrass extract on mango fruit colonization by *Stenotrophomonas rhizophila* (PSTU-Hort-14)

On the wounds and surfaces of mango fruit in the presence of lemongrass extract or lemongrass extract amended with SBC the population of the total *Stenotrophomonas rhizophila* (PSTU-Hort-14) was monitored. The concentration of *S. rhizophila* in wounds and on fruit surfaces were 5.44 $\log_{10}$ CFU per wound and 5.55 $\log_{10}$ CFU cm^{-2}, respectively immediately after dipping the fruits in the cell suspension of *S. rhizophila* (PSTU-Hort-14), which were statistically similar with other treatments (Table 2). The population of *S. rhizophila* in wounds markedly increased in all treatments after 18 days of storage at 12ºC and 90±5% RH. However, the population reached the maximum level

of 6.23, 6.67 and 6.77 $\log_{10}$ CFU per wound in wounds treated with *S. rhizophila* - lemongrass extract, the combination of *S. rhizophila* + lemongrass extract + SBC and *S. rhizophila* + SBC, respectively, which were not significantly different from each other (Table 2).

Table 2. Effect of lemongrass extract, SBC and their combination on the populations of *Stenotrophomonas rhizophila* (PSTU-Hort-14) in mango wounds and on surface during storage at 12±1°C and 90±5% RH for 18 days

Treatments	Wound population Log CFU cm^2		Surface population Log CFU cm^2	
	Immediate after treatment	At the end of storage	Immediate after treatment	At the end of storage
Bacteria alone	5.17 d	6.48 c	5.50 c	5.76 a
Bacteria + Lemongrass extract	5.67 a	6.23 d	5.74 a	6.55 c
Bacteria + SBC	5.35 c	6.77 a	5.46 d	6.37 d
Bacteria + Lemongrass extract + SBC	5.44 b	6.67 b	5.55 b	6.70 b
LSD $_{(0.05)}$	0.074	0.060	0.070	0.063
Level of Significance	*	*	*	*
CV%	1.02	0.69	0.94	0.74

**Significant at 5% level of probability*

Similarly, significantly higher population *S. rhizophila* was recorded on fruit surface (6.70 $\log_{10}$ CFU cm^{-2}) in combined treatment of *S. rhizophila* + Lemongrass extract + SBC at the end of the storage period (Table 2). This was statistically similar with *S. rhizophila* + lemongrass extract and *S. rhizophila* +SBC. Therefore, the survival and proliferation of *S. rhizophila* in mango wounds and fruit surface were not affected by lemongrass extract and SBC or its mixture. Moreover, it seemed that coating with lemongrass extract enhanced the multiplication of *S. rhizophila*.

Biocontrol activity of *S. rhizophila* (PSTU-Hort-14) enhanced with SBC and lemongrass extract on mango fruits pre-inoculated with *C. gloeosporioides*

The values of area under disease progress curves (AUDPC) derived that the ability of the treatments to delay the onset of disease symptoms as well as to reduce the disease severity was expressed as the percentage disease reduction. By AUDPC disease development on inoculated fruits was also evaluated. The highest AUDPC was recorded for the water-treated control fruit which was significantly different from other treatments at the end of storage period (Table 3). Whereas, the lowest AUDPC was recorded in pre-inoculated fruits treated with the combination of *S. rhizophila* +lemongrass extract +SBC

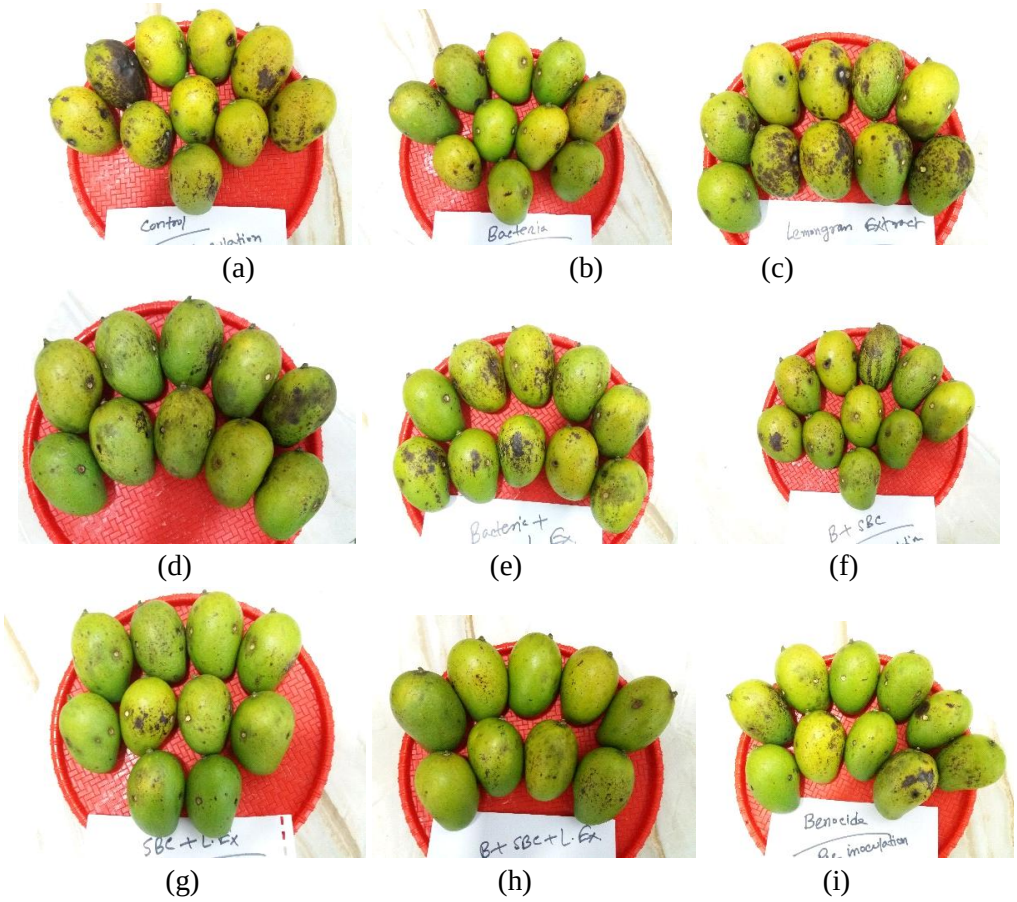
followed by the fungicide Dipheniconazol® treatment, which is statistically different from *S. rhizophila*+ SBC. In respect of AUDPC there was no statistical difference between the treatments of *S. rhizophila* suspension and *S. rhizophila*+ lemongrass extract.

In this study, during the whole storage period the highest lesion expansion rate was recorded for water-treated control fruits, which was statistically different than all treated fruits. The disease reduction over control is a good indicator that reflected the progress of disease over time. Significantly lower rate of lesion expansion was recorded in fruit treated with Dipheniconazol® followed by *S. rhizophila* +SBC treatment. The combination of *S. rhizophila* + lemongrass extractwas statistically similar with *S. rhizophila* treated fruits. However, anthracnose symptoms did not develop on inoculated fruits subjected to the combination of *Stenotrophomonas rhizophila* +lemongrass extract +SBC during the whole storage period. Thus, there was no lesion expansion for this treatment (Table 3).

Table 3. Effect of *Stenotrophomonas rhizophila*, lemongrass extract, SBC and their combinations on the reduction of anthracnose disease in pre-inoculated mango fruits stored at 12ºC for 18 days

Treatments	AUPDC (cm^2)	Lesion expansion rate (mm day^{-1})	Disease reduction over control (%)
Control	30.75 a	5.30	--
Lemongrass extract	25.36 b	3.01	20.16 h
SBC	15.78 c	3.45	40.21 g
SBC + Lemongrass extract	12.43 d	3.00	50.43 f
Bacteria	7.49 e	2.61	70.11 e
Bacteria + Lemongrass extract	4.42 f	2.50	71.60 d
Bacteria + SBC	3.70 g	1.71	89.15 c
Dipheniconazol® (0.5 mlL^{-1})	0.09	0.21	98.00 b
Bacteria + Lemongrass extract + SBC	0.00	0.00	100 a
$LSD_{(0.05)}$	0.089	--	0.720
Level of Significance	*	NS	*
CV%	0.38	133.23	0.57

*Significant at 5% level of probability, NS= Non significant



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Fig. 2. Effect of Stenotrophomonas rhizophila, sodium bicarbonate (SBC 2%) and Aloe vera (lemongrass extract 20%) and their combinations on the lesion diameter of anthracnose caused by C. gloeosporioides in pre-inoculated mango fruits stored at 12±1°C and 90±5% RH for 18 days. Two hours after inoculation, fruits were dipped in (a) sterilized distilled water (control), (b) cell suspension of Stenotrophomonas rhizophilan (1×108 CFU mL-1), (c) lemongrass extract (20%), (d) Sodium bicarbonate (SBC) solution (2%), (e) lemongrass extract (20%) + Stenotrophomonas rhizophila, (f) SBC (2%) + Stenotrophomonas rhizophila, (g) SBC (2%) + lemongrass extract (20%) (h) Stenotrophomonas rhizophila + 2% SBC amended with 20% lemongrass extract and (i) Dipheniconazol® (0.50 mlL-1). Dipping time in each solution/suspension was 15 min. Photographs on lesion development were taken after 14 days of inoculation.

This study showed that the combination of *S. rhizophila* +-lemongrass extract +SBC treatment was the most effective treatment in suppressing the anthracnose disease on pre-inoculated fruits followed by Dipheniconazol® in terms of disease reduction over the control. Pre-inoculated fruits treated by the combination of *S. rhizophila* +lemongrass extract +SBC resulted in complete control (100%) of the disease. The lowest disease reduction (20.16%) was recorded in lemongrass extract treatment compared to water-treated control (Plate 2).

Biocontrol activity on naturally infected fruits

Disease incidence

With ripening period the incidence of the disease gradually increased. The naturally infected fruits subjected to the combination of *S. rhizophila* +lemongrass extract +SBC showed significantly lowest anthracnose incidence than in fruits dipped in *S. rhizophila* suspension, lemongrass extract +SBC and Dipheniconazol®. At the third and fourth day of shelf period, water-treated control fruits showed anthracnose spots and the disease incidence increased to 65 and 100% after 14 days and 18 days of storage respectively (Figure 2). At the end of the ripening period the lowest disease incidence (12%) was recorded in fruits subjected to the combination of *S. rhizophila* +lemongrass extract +SBC treatment, followed by 2% SBC + *S. rhizophila* (50%). At the end of the post storage period disease incidence recorded in fruits treated with *S. rhizophila* suspension, Dipheniconazol® and lemongrass extract + SBC were 35, 20 and 35% after three days of ripening, and increased gradually to 70, 45 and 75%, respectively (Fig. 2).

The data of present study showed that the combination of *S. rhizophila* with lemongrass extract + SBC was not only effective in reducing the disease incidence but also delayed the onset of anthracnose infection. This treatment delayed the anthracnose appearance on fruits by four days compared to water-treated control fruits. Anthracnose incidence was only noticed after five days of ripening on a few fruits (10%), which did not increase until the end of the ripening period.

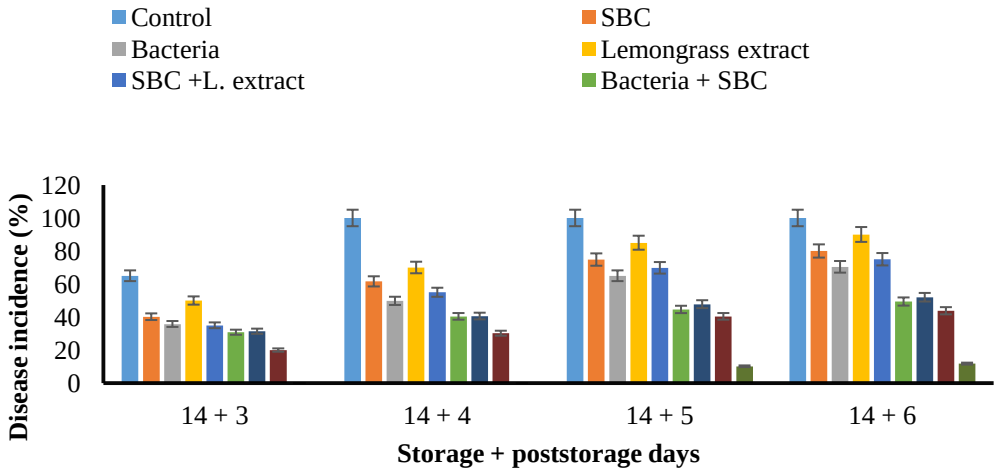


Fig. 2. Effect of *Stenotrophomonas rhizophila*, sodium bicarbonate (2% SBC) and lemongrass extract (20% lemongrass extract) and their combinations on the incidence of anthracnose in naturally infected mango fruits stored at 12ºC for 14 days and six days post storage under ambient temperature (28 ± 2ºC). Each value is the mean of twelve replications. Means were separated by Least Significant Difference (LSD) test at 5% level of significance. Vertical bars represent standard error.

Disease severity

In all fruit treatments, disease severity was significantly ($P \leq 0.05$) lower compared to water treated control fruits during 14 days of storage and six days post ripening at 28 ± 2°C (Figure 3).. The combination of *S. rhizophila* +lemongrass extract +SBC was the most effective treatment, which showed significantly lower anthracnose severity on naturally infected fruits than in fruits dipped in *S. rhizophila* suspension, lemongrass extract + SBC and Dipheniconazol®.

The combined treatment *S. rhizophila* + lemongrass extract + SBC reduced the disease severity with complete absence of symptoms until 14 days of storage at 12ºC and four days of post storage period. At the end of post storage period, disease severity was recorded as 2.15% for the fruits subjected to the combination of *S. rhizophila* + lemongrass extract + SBC. In contrast, at the end of post storage period anthracnose symptoms appeared in water-treated control fruits at the end of the 14 days storage and disease severity increased gradually reaching 84.88%.

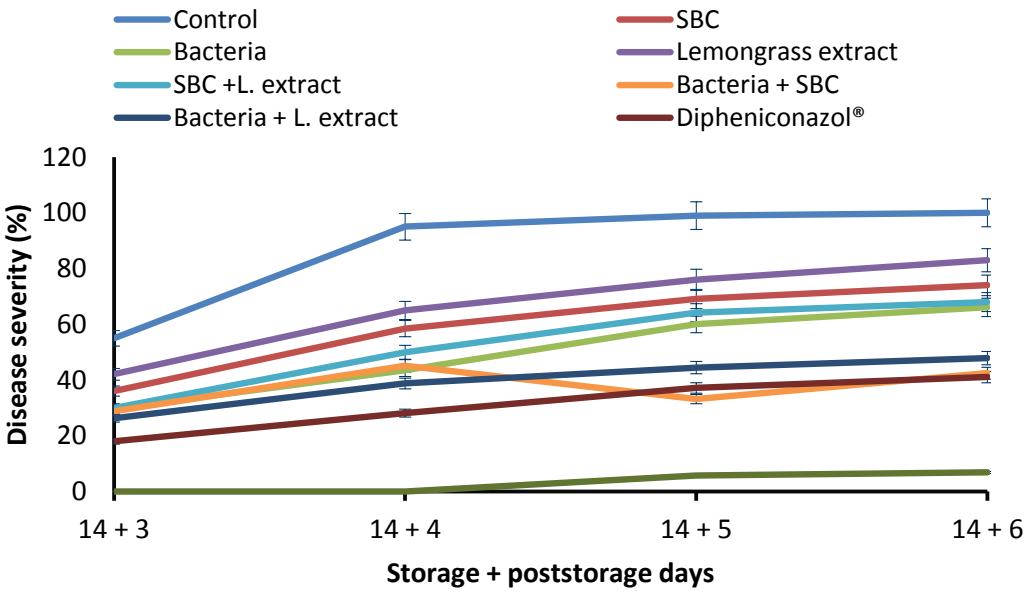


Fig. 3. Effect of *Stenotrophomonas rhizophila*, sodium bicarbonate (2% SBC) and lemongrass extract (20%) and their combinations on the severity of anthracnose in naturally infected mango fruits stored at 12ºC for 14 days and six days post storage under ambient temperature (28 ± 2ºC). Each value is the mean of eight replications. Means were separated by Least Significant Difference (LSD) test at 5% level of significance. Vertical bars represent standard error

From the AUDPC values derived that the efficacy of treatments to delay the onset of disease symptoms as well as to reduce the lesion expansion rate was expressed as percentage disease reduction. The AUDPC was also calculated to evaluate the treatment efficacy against disease progress over time. At the end of storage and post

storage period, the highest AUDPC was recorded in water-treated control fruits and the lowest value was found in fruits subjected to combination of *Stenotrophomonas rhizophila* +lemongrass extract + SBC. AUDPC of Dipheniconazol® was was statistically similar with the treatment combination of lemongrass extract-*S.s rhizophila* and SBC-*S. rhizophila*.

Table 4. Effect of *Stenotrophomonas rhizophila*, 2% sodium bicarbonate (SBC), lemongrass extract or their combinations on the reduction of anthracnose disease in naturally infected mango fruits stored at 12±1°C for 14 days and six days ripening under ambient temperature (28 ± 2°C)

Treatments	AUPDC (cm^2)	Lesion expansion rate (mm day^{-1})	Disease reduction over control (%)
Control	72.50 a	19.90 a	--
Lemongrass extract	51.30 b	13.73 b	30.19 h
SBC	44.54 c	11.50 c	40.30 g
SBC + Lemongrass extract	36.40 d	9.52 d	53.05 f
Bacteria	13.48 e	3.80 e	84.73 e
Dipheniconazol® (0.5 mL^{-1})	9.54 f	3.14 g	88.04 b
Bacteria + Lemongrass extract	9.14 g	2.85 h	85.52 d
Bacteria + SBC	10.27 h	3.26 f	85.71 c
Bacteria + Lemongrass extract + SBC	2.21 i	0.54 i	98.70 a
LSD(0.05)	0.730	0.509	1.879
Level of Significance	*	*	*
CV%	1.26	3.22	1.43

**Significant at 5% level of probability*

The rate of lesion expansion was significantly higher in water-treated control than other treatments and the lowest rate was recorded in fruits subjected to the combination of *S. rhizophila* + lemongrass extract + SBC (Table 3) almost similar trend was observed among the treatments on the lesion expansion rate in water-treated control fruits. The lesion expansion rate of Dipheniconazol®, lemongrass extract + *S. rhizophila*, SBC + *S. rhizophila* treatments were statistically similar.

In the present study results showed that the combination of *S. rhizophila* + lemongrass extract + SBC was the most effective treatment in controlling anthracnose disease in naturally infected mango fruits. In treated fruits disease severity was reduced by 98.70% at the end of 14 days storage and six days post storage period. The level of disease control obtained was superior to that obtained with Dipheniconazol® treatment. The lowest disease reduction was recorded in fruits treated with 30.19% lemongrass extract solution followed by SBC and SBC+lemongrass extract (Table 4).

Plate 3. Effect of *Stenotrophomonas rhizophila*, sodium bicarbonate (SBC 2%) lemongrass extract (20%) and their combinations on the incidence and severity of anthracnose in naturally infected mango fruits stored at 12±1°C and 90±5% RH for 14 days and six days under ambient temperature (28 ± 2ºC). (A) Sterilized distilled water (control), (B) cell suspension of *Stenotrophomonas rhizophila* (1×10^8 CFU mL^{-1}), (C) lemongrass extract (20%), (D) Sodium bicarbonate (SBC) solution (2%), (E) lemongrass extract (20%) + *Stenotrophomonas rhizophila*, (F) SBC (2%) + *Stenotrophomonas rhizophila*, (G) SBC (2%) + lemongrass extract (20%) (H) *Stenotrophomonas rhizophila* + 2% SBC amended with 20% lemongrass extract and (I) Dipheniconazol® (0.50 mlL^{-1}). Dipping time in each solution/suspension was 15 min. Photographs on anthracnose incidence and severity were taken after 14 days + 3 days under ambient temperature, when symptoms were developed on fruit surfaces

Discussion

Findings of this study showed that, postharvest application of *S. rhizophila*, SBC and lemongrass extract alone in artificially inoculated and naturally infected mangos resulted in a significant reduction of anthracnose severity compared to water-treated

fruits. Moreover, it was shown that *S. rhizophila* was effective as a postharvest dip treatment on wound invaders and in latent infection of mango caused by *C. gloeosporioides*. These findings are in agreement with Obagwu and Korsten (2003), who reported that combinations of SBC and *S. rhizophila* was very effective in controlling green and blue molds of citrus and superior to either treatment alone. Similarly, in postharvest treatment, *S. rhizophila* combined with 2% SBC significantly reduced the ring rot of pear (Liu *et al.*, 2011). However, the addition of 2% SBC amended with 20% lemongrass extract to *S. rhizophila* created friendly environment. This is supported, in part, by its higher performance on artificially inoculated and naturally infected mango. This combined treatment effectively reduced the postharvest anthracnose during storage by delaying the onset of infection and slowed down the infection process.

The combination of biocontrol activity of *S. rhizophila*, antifungal property of SBC, and improved micro environment by lemongrass extract resulted in improved consistency and efficacy of disease control. The observed preventive and curative activities of the combination of *S. rhizophila* + SBC + lemongrass extract showed the synergy between the antagonist and SBC-lemongrass extract. In addition, in artificially inoculated fruits, treatment with the combination showed to confer a level of disease control equivalent to the fungicide Dipheniconazol®. However, it was superior to Dipheniconazol® when applied on naturally infected mango. These findings are in agreement with Obagwu and Korsten (2003), who found that the level of disease control provided by *S. rhizophila*, when applied alone, was inferior to control provided by fungicide 'fungazil', but it was equivalent to fungicide 'fungazil' when *S. rhizophila* applied with 2% SBC. Similar trend of anthracnose control in mango was also achieved with the combined application of 2% sodium bicarbonate in wax formulation and *Candida oleophila* (Gamagae *et al*., 2004).

Performance of Dipheniconazol®, when applied on naturally infected mango, was lower compared to the combined treatment *S. rhizophila* + SBC + lemongrass extract. The lower performance of fungicides might be due to the quiescent infection of mango caused by *C. gloeosporioides*. Being a quiescent infection, anthracnose is difficult to control by postharvest treatment (Janisiewicz and Korsten, 2002; Yakoby *et al.*, 2001), even along with synthetic fungicides (Ippolito *et al.*, 2004). This is because the infecting hyphae are protected once the pathogen has penetrated the plant cuticle (Yakoby *et al.*, 2001).

The mechanism by which SBC, lemongrass extract and *S. rhizophila* (PSTU-Hort-14) integrated to control *C. gloeosporioides* is not fully understood. This might be happened by multi-layer protection of fruits created by lemongrass extract, SBC and *S. rhizophila* (PSTU-Hort-14). Firstly, lemongrass extract produces a bio-edible-coating, which reduce the transpiration and respiration and maintain the firmness of fruits (Mart´ınez-Romero *et al*., 2006). As a result, fruit remain fresher, which indicate a certain level of self-defense from pathogen. Secondly, SBC is fungistasis in nature (Davide *et al*., 2004), so it can give short period protection from fungal pathogen. Since, *S. rhizophila* (PSTU-Hort-14) isolated from mango surface so, it can be attached and multiplied on the surface of mango. So, whenever *C. gloeosporioides* germinate after the action of SBC, *S. rhizophila* (PSTU-Hort-14) will imposed further action on *C. gloeosporioides*.

Conclusion

Based on the experimental data, the biocontrol efficacy of *Stenotrophomonas rhizophila* strain PSTU-Hort-14 against mango anthracnose was significantly enhanced by the incorporation of 20% lemongrass extract supplemented with 2% sodium bicarbonate (SBC). This combined treatment demonstrated superior disease suppression compared to PSTU-Hort-14 alone or its combinations with either lemongrass extract or SBC individually. Notably, it outperformed even the chemical fungicide Difenoconazole® in controlling anthracnose. Given its enhanced efficacy and eco-friendly nature, the integrated use of *S. rhizophila* PSTU-Hort-14 with lemongrass extract and SBC presents a promising alternative to chemical fungicides. It is recommended that this biocontrol formulation be further evaluated under field conditions and developed as a sustainable management strategy for postharvest anthracnose in mango.

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Author's Contribution

M.J.U.: Conceptualization, methodology, investigation, data curation, formal analysis, resources, software, validation, visualization and writing – original draft. M.R & M.F.H.: Concept, planning, supervision, validation, resources, funding acquisition, project administration, and reviewed. M.N.H.M: Methodology, data curation, formal analysis, software, reviewed, revised and edited the content. U.R.T.: Methodology, investigation, data curation, statistical analysis. S.M.A.I: Methodology, validation, data curation, statistical analysis and writing – review & editing. All authors equally contributed to the preparation of the manuscript and have approved the final version for submission.

Conflicts of Interest

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

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INVITRO ALPHA AMYLASE INHIBITORY AND ANTI-INFLAMMATORY ACTIVITY OF THE LEAVES EXTRACT OF DENSEFLOWER KNOTWEED *(Persicaria glabra)*

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Abstract

The primary objective of this study was to examine the inhibitory effects of *Persicaria glabra* on α-amylase and its potential anti-inflammatory properties. The α-amylase inhibitory activity of leaves extracts from *P. glabra* was assessed using the 3,5-dinitrosalicylic acid (DNSA) methodology, while the anti-inflammatory activities were evaluated using the egg albumin denaturation method. The methanolic and aqueous extract of *P. glabra* exhibited strong inhibitory activity against α-amylase and demonstrated anti-inflammatory properties. The methanolic extract exhibited an IC50 value of 5.43 mg/mL for α-amylase inhibitory activity, while the aqueous extract had an IC50 value of 6.01 mg/mL. In terms of anti-inflammatory activity, the IC50 values for the methanolic and aqueous extracts were 128.04 µg/mL and 95.96 µg/mL, respectively. The phytochemicals have been found in *P. glabra* that demonstrated strong inhibition of α-amylase and possess anti-inflammatory properties, which could have significant implications in the fields of medical and veterinary science.

Keywords: Anti-inflammatory*, Persicaria glabra,* α-Amylase.

Introduction

Type 2 diabetes mellitus (T2DM) is a multidimensional chronic condition characterized by impaired functioning of pancreatic β cells and resistance to insulin, resulting in high blood sugar levels (DeFronzo, 2004). The disease remains a significant global health issue and economic burden as a result of contemporary lifestyle and heightened carbohydrate intake. The frequency may increase, leading to a substantial effect on the population of developing nations due to the lack of effective and affordable treatment options for DM.

In the presence of diabetes, untreated chronic hyperglycemia leads to an increase in the formation of reactive oxygen species (ROS) in both the mitochondria and non-mitochondrial components. This process enhances the activation of protein kinase C (PKC) iso forms, hexosamine pathway flux, polyol pathway flux, and advanced glycation end products (AGE) that contribute to oxidative damage caused by hyperglycemia (Moussa,

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2008). A current therapeutic objective in the treatment of type 2 DM is inhibiting α-glucosidase and α-amylase to reduce the absorption of glucose in the gut (Sim *et al.*, 2010). Alpha-amylase, also known as α-1,4-glucan-4-glucanohydrolases, is a vital enzyme generated by the pancreas and salivary gland. It is essential for intestinal mucosa breakdown of complex carbohydrates into oligosaccharides and disaccharides. Alpha-glucosidase digests these sugars into monosaccharides. Clinically utilized alpha-amylase and glucosidase inhibitors can cause hypoglycemia, diarrhea, gas, and colon bloating, limiting its effectiveness in diabetes management (Evans and Rushakoff, 2002). Hence, it is crucial to seek out supplementary and alternative treatments that have low negative implications and can be used in conjunction with the management of DM (Evans and Rushakoff, 2002). Inflammation is a complex physiological reaction of the body to damage, infection, or destruction, which is marked by increased temperature, redness, pain, swelling, and disrupted physiological processes. The process is initiated by the liberation of chemical mediators from damaged tissue and migratory cells (Sangeetha and Vidhya, 2016). Inflammation serves as the primary catalyst for the development of Type 2 Diabetes Mellitus (T2DM). This occurs due to the influence of the aforesaid risk factors and the persistent activation of pro-inflammatory cytokine pathways in the specific tissues affected by insulin-related processes, such as adipose tissue, muscle mass, and the liver(Pradhan *et al.*, 2001; Marques-Vidal *et al.*, 2012; Sakashita *et al.*, 2015; Tsalamandris *et al.*, 2019). Even a slight deviation in glucose levels is associated with inflammatory mechanisms and consequences connected to type 2 diabetes (T2D) (Löbner and Füchtenbusch, 2004). Inflammation has also been connected with additional illnesses related to type 2 diabetes mellitus (T2DM), including atherosclerosis and blood clotting, metabolic syndrome, heart failure, cardiometabolic diseases, renal diseases, and malignancies(Chen *et al.*, 2012; Sarvottam and Yadav, 2014; Adar *et al.*, 2015; Ellulu *et al.*, 2016). Modern medicine has benefited from natural products. Traditional medicine is being evaluated globally through study on plant species and their strong medicinal ingredients. The plant kingdom's diversity may yield novel anti-inflammatory compounds. Herbal medications are considered effective, affordable, and have less serious side effects (Chandra *et al.*, 2012). *P. glabra* is a member of the polygonaceae family. The Bengali name for this plant is Bihagni, also known as Sada Kukri. The aerial parts of the plant include several physiologically active terpenoids and flavones. The plant's leaves, flowers, stems, and seeds contain many significant components that can be utilized for different reasons (Runa *et al.*, 2019). The current investigation aimed to assess the potential *in vitro* anti-inflammatory impact of *P. glabra* extract on protein denaturation and determine the *invitro* α - amylase inhibitory activity.

Materials and Methods

Chemicals

All chemicals used were of analytical reagent grade. The compound used is 3,5-dinitro salicylic acid (DNSA), and the enzyme used is α-amylase obtained from Sigma, USA. The compound Dimethylsulfoxide (DMSO), and methanol were acquired from Sigma-Aldrich (Germany). The acquisition of diclofenac sodium and other chemicals were collected from HS Scientific, located at Hatkhola, Dhaka, Bangladesh. A freshly laid hen egg was purchased at a local market.

The study was conducted from October to December 2023, at the Department of Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh.

Collection of plant materials

Fresh leaves of *P. glabra* were collected from the Islamic University, Kushtia campus and were carefully rinsed and dried in a shady area at room temperature until their weight stabilized. Then, each dried leaf was ground into a fine powder using an electric grinder. The powder was sieved to make it uniform and fine. The powder was then packaged and stored in a dark, dry place for future use.

***P. glabra* plant** ***P. glabra* plant's dried leaves**

Fig. 1. *P. glabra* plant (left) and dried leaves (right)

Preparation of extracts

The leaf powder was immersed in methanol and distilled water for 24 hours, separately. Then strained through muslin cloth and collected in conical flask. Subsequently, the filtrates underwent a second filtration process using Whatman filter paper (No. 1) and were then subjected to vacuum concentration in a rotary evaporator at a temperature of 40 °C.

α-Amylase Inhibitory Assay

The α-amylase inhibition assay was conducted employing the 3,5-dinitrosalicylic acid (DNSA) technique (Wickramaratne, *et al*., 2016). *P. glabra* leaf extract was dissolved in a small amount of 10% DMSO and then in a buffer solution at pH 6.9 (Na_2HPO_4/NaH_2PO_4 at 0.02 M, NaCl at 0.006 M). This yielded values of test solution 0.5 to 15 mg/mL (w/v). A 2unit/ml α-amylase solution was mixed with an extract in a 200 µl volume. The mixture was incubated at 30 °C for 10 minutes. Then, 200 µl of 1% starch solution in water (w/v) was added to each tube and incubated for 3 minutes. To stop the reaction, add 200 µl of DNSA reagent, which contains 12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-dinitrosalicylic acid solution. The mixture was heated for 10 minutes at 85–90 °C in a water bath. The solution was diluted with 5 mL of distilled water after cooling to room temperature. The solution's absorbance was measured at 540 nm using a UV-Visible spectrophotometer (Hitachi U-

2900, Japan). A blank solution was produced by replacing plant extract with 200 µl of buffer, resulting in 100% enzyme activity. A control reaction using plant extract without enzyme solution at each concentration was also produced. A positive control sample was produced using Acarbose at doses from 100µg/ml to 5µg/ml. To calculate α-amylase inhibitory activity, the percentage of inhibition was calculated using the following equation: The % inhibition of α-amylase was compared to the extract concentration. The graph yielded IC50 values.

$$\%\ \alpha\ amylase\ inhibition\ = 100 \times \frac{Abs\ of\ control - Abs\ of\ Sample}{Abs\ of\ control}$$

Evaluation of in vitro anti-inflammatory activity

The *in-vitro* anti-inflammatory activity was studied using egg albumin denaturation method (Chandra *et al.*, 2012). The reaction mixture was prepared combining 0.2 mL of egg albumin from fresh hen egg, 2.8 mL of phosphate buffered saline (PBS) with a pH of 6.4, and 2 mL of extract at different concentrations to achieve final concentrations of 31.25, 62.50, 125, 250, 500/; and 1,000 µg/mL. A control was provided using an equivalent amount of double-distilled water. Subsequently, the mixtures were placed in an incubator (Froilabo) and incubated at a temperature of 37°C for 15 minutes. Following this, the mixtures were subjected to a heat treatment at a temperature of 70°C for 5 minutes. Following the chilling process, the absorbance of the sample was measured at a wavelength of 660 nm using a double beam spectrophotometer (Hitachi U-2900, Japan). The vehicle was used as a reference to account for any background absorbance. The reference drug, diclofenac sodium, was used at final concentrations of 78.125, 156.25, 312.5, 625, 1250, and 2500 µg/mL. It was processed in a similar manner to determine absorbance. The proportion of protein denaturation inhibition was determined using the following formula:

$$\%\ inhibition = 100 \times (At / Ac - 1)$$

Where, A_t = absorbance of test sample, A_c = absorbance of control

The IC50 value was calculated by generating a graph that plotted the percentage of inhibition in relation to the treatment concentration, compared to the control.

Result and Discussion

α-Amylase Inhibitory Assay

The potential of leaves extract of *P. glabra* to inhibit α-amylase activity was examined at six different concentrations (0.5, 1, 2, 5, 10, and 15 mg/mL). Individual dose-response calibration curves were developed for methanol and water extracts of *P. glabra* leaves. The percentage of α-amylase inhibition and the IC50 values were calculated based on the dose-response calibration curves for each extract. The α-amylase inhibitory activities of the leaf extracts were methanol (IC50 5.43 mg/mL; $R^2$0.910) > water (IC50 6.01 mg/mL; $R^2$0.9288). The standard positive control Acarbose showed an IC50 of 46.13 µg/mL (R^2 0.9796). The conducted α-amylase inhibitory investigations revealed that the extracts of *P. glabra* leaves exhibited substantial inhibitory properties. The IC50 value of methanol extracts was comparable to that of Acarbose, an extensively utilized and

commercially available anti-diabetic medication. These α-amylase inhibitors, also known as starch blockers, hinder or delay the absorption of starch into the body by primarily obstructing the hydrolysis of 1,4-glycosidic bonds in starch and other oligosaccharides, resulting in the prevention of their conversion into maltose, maltriose, and other simple sugars (Dineshkumar *et al*., 2010).The methanol extract likely contains polar molecules that exhibit α-amylase inhibitory activity. Further investigation and isolation of these pure active compounds is recommended.

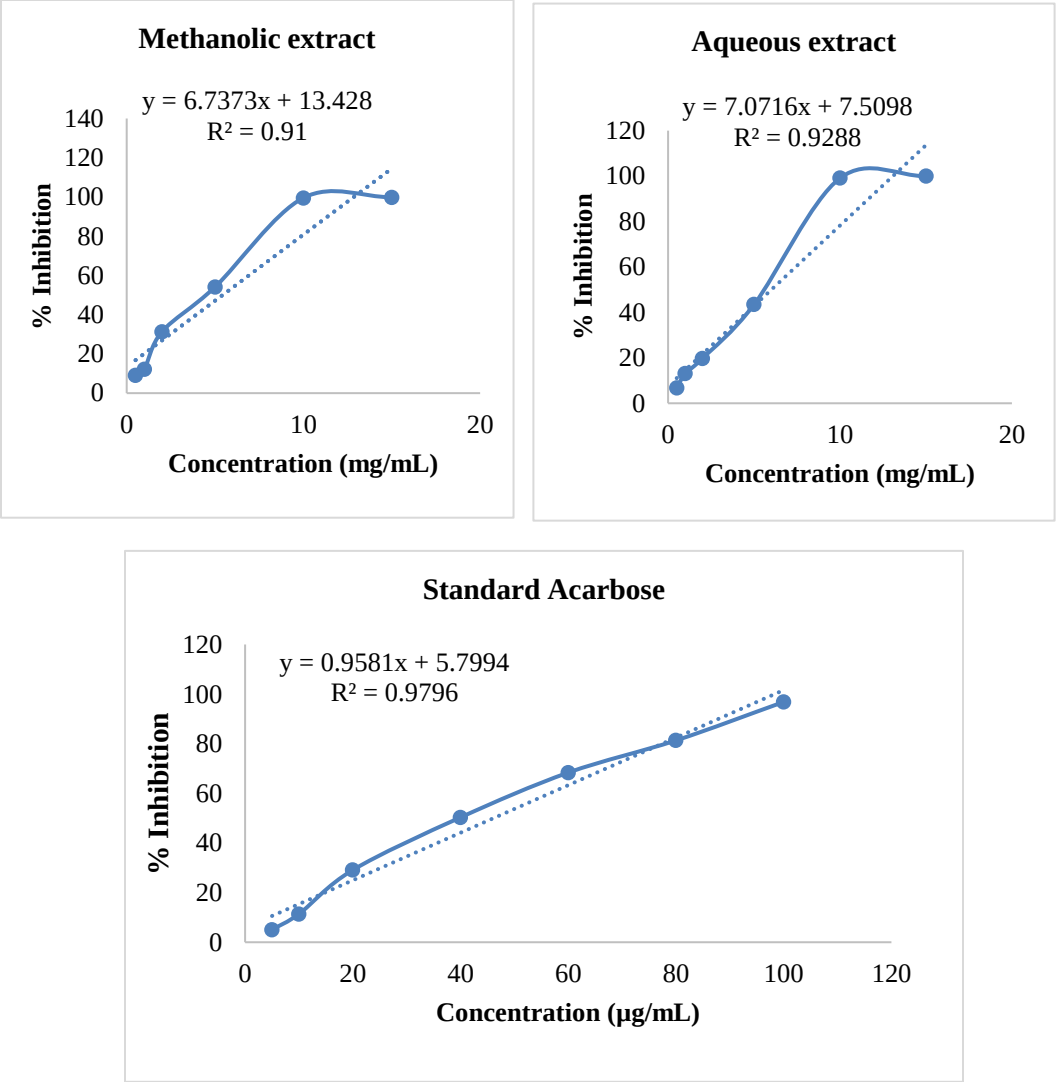

Fig. 2. Calibration curve for α-amylase inhibition assay

In vitro anti-inflammatory activity

The current study aimed to assess the *in-vitro* anti-inflammatory properties of the leaf extracts of *P. glabra* by examining its impact on the denaturation of egg albumin. The findings are succinctly presented in Table 1. The current results demonstrate that test samples effectively inhibit protein denaturation (specifically albumin) in a concentration-dependent manner, covering a range of 31.25 to 1,000 µg/mL. The reference drug utilized was diclofenac sodium, with a concentration range of 78.125 to 2,500 µg/mL. It showed suppression of protein denaturation that varied depending on the concentration. However, the effects of both extracts were shown to be slightly reduced compared to diclofenac sodium. This was additionally validated by comparing the IC50 values of the two. The IC50 value of methanolic extract and aqueous extract were 128.04 µg/mL and 95.96 µg/mL respectively, while the IC50 value of diclofenac sodium was 38.59 µg/mL. Denaturation of tissue proteins is an established factor that contributes to the development of inflammatory and arthritic disorders. The production of auto antigens in some arthritic disorders may result from the denaturation of proteins within the body (Opie, 1962; Umapathy *et al.*, 2010). The observed changes in absorbance of the test samples compared to the control samples suggest that extracts and the reference medication diclofenac sodium effectively prevented the denaturation of the protein albumin caused by heat (Jagtap *et al.*, 2011).

Table 1. Effect of leaves extract of *P. glabra* on protein denaturation

Concentration (µg/mL)	% Inhibition	
	Methanolic extract	Aqueous extract
31.25	16.208	14.679
62.5	20.795	30.428
125	39.144	53.67
250	106.42	129.36
500	221.1	282.26
1000	481.04	496.33

Conclusion

The study revealed that the leaf extracts of *P. glabra* demonstrate significant α-amylase inhibitory action, particularly in the crude methanolic extract. The leaves of *P. glabra* have the potential to be utilized into ayurvedic decoctions for the management and treatment of diabetes mellitus. From this study, it can also be inferred that *P. glabra* leaves extract has a significant anti-inflammatory impact, specifically against protein denaturation. Additional conclusive research is required to determine the specific mechanisms and components responsible for its antidiabetic and anti-inflammatory effects.

Author's contribution

M. M. Rahman executing, investigating and formal data analysis of the research, M. M. Rahman, Z. R. Moni and M. M. Rahman writing-review, editing the original manuscript.

Conflicts of Interest

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

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ESTIMATION OF BORO RICE AREA IN BANGLADESH USING SENTINEL-2 IMAGERY AND MACHINE LEARNING ALGORITHMS

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Abstract

Rice is the primary food crop in Bangladesh, and a significant portion of agricultural land is dedicated to its cultivation. Annually, approximately 36.87 million tons of rice are produced from 11.54 million hectares of land (BBS, 2021). Among the three rice seasons in Bangladesh, namely aus, aman, and boro, the winter rice boro production holds the highest percentage. Accurate delineation of boro rice-growing area is crucial for estimation of total production, which plays a vital role in policy planning and decision-making. Rice fields in Bangladesh are fragmented into smaller plots, emphasizing the importance of high-resolution, cloud-free satellite images for precise delineation of rice areas. In this context, a comparative study was conducted to estimate boro rice area in Bangladesh using high-resolution (10-meter) Sentinel-2 data, aiming to overcome the challenges posed by fragmented land. Machine Learning (ML) based supervised classification algorithms namely Decision Tree, k-Nearest Neighbor (k-NN), Random Forest (RF) and Support Vector Machine (SVM) were employed on Sentinel-2 images of the study area to identify rice fields. The study's findings are expected to contribute to the development of boro rice area estimation, predict yield and productivity system in Bangladesh, ultimately enhancing food security and the livelihood of farmers.

Keywords: Area Delineation, Boro rice, Machine learning , Sentinel-2 imagery, Yield forecasting.

Introduction

Over the past five years (2018-19, 2019-20, 2020-21, 2021-22, 2022-23), Bangladesh has consistently produced an average of 37.57 million tons of rice annually from an area of 11.59 million hectares (BBS, 2022). Among the different rice seasons in Bangladesh, boro rice production has emerged as the highest contributor, accounting for 53.3% of the total rice production, while aman and aus rice accounted for 38.9% and 7.8% respectively during the same period. Accurately delineating the boro rice area holds immense importance in national-level production planning as it enables the development of an accurate yield forecasting system and facilitates rice growth monitoring. Failure to achieve accurate estimations can lead to crop losses, unfair pricing, and other detrimental

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consequences for farmers. Additionally, policymakers face challenges in planning export/import activities and formulating appropriate farming strategies, resulting in significant economic losses for both farmers and the nation. To address these challenges, this study was conducted utilizing high-resolution (10-meter) Sentinel-2 satellite data to delineate boro rice cultivation areas. By leveraging Sentinel-2 optical satellite data and employing four machine learning algorithms, the study seeks to compare the performance of different algorithms in accurately delineating rice areas within the context of Bangladesh. Recent advancements in remote sensing technology, particularly satellite imagery, have provided valuable tools for monitoring and estimating crop areas. High-resolution and cloud-free images have shown great potential in accurately identifying and delineating agricultural areas. The Sentinel-2 satellite offers multi-spectral imagery with a spatial resolution of 10 meters, making it well-suited for detailed land cover classification. As the boro rice grows in winter season, therefore cloud free images are expected to be available during the season. Machine learning algorithms have also gained prominence in the field of remote sensing analysis. These algorithms, when trained with labeled satellite imagery, can effectively classify land cover types, including crops such as rice. By harnessing the capabilities of machine learning and utilizing Sentinel-2 imagery, it becomes possible to develop a reliable and efficient system for estimating the boro rice area in Bangladesh. Several studies have contributed to the field of rice area estimation using remote sensing technology (Setiyono *et al., 2018).* demonstrated the operational capability of a system developed by the RIICE project, conducting an extensive evaluation of its performance in key rice-growing areas across South and Southeast Asia (Nelson *et al.,* 2014). In Bangladesh, MODIS images were utilized to detect changes in rice cultivation, focusing on the phenological study of five districts: Pabna, Manikganj, Sherpur, Sylhet, and Gazipur (Setiyono *et al.*, 2018). The Bangladesh Space Research and Remote Sensing Organization (SPARRSO) has predominantly employed MODIS data to monitor aman and boro rice, with the satellite TERRA being utilized for this purpose (Rahman, H., 2014). Additionally, the area delineation under rice cultivation has been carried out using GIS-based crop suitability maps, as demonstrated (Hussain *et. al.*, 2012). It is important to note that the approach did not involve remote sensing technology, potentially leading to discrepancies between the calculated rice area from the suitability map and the actual area coverage.

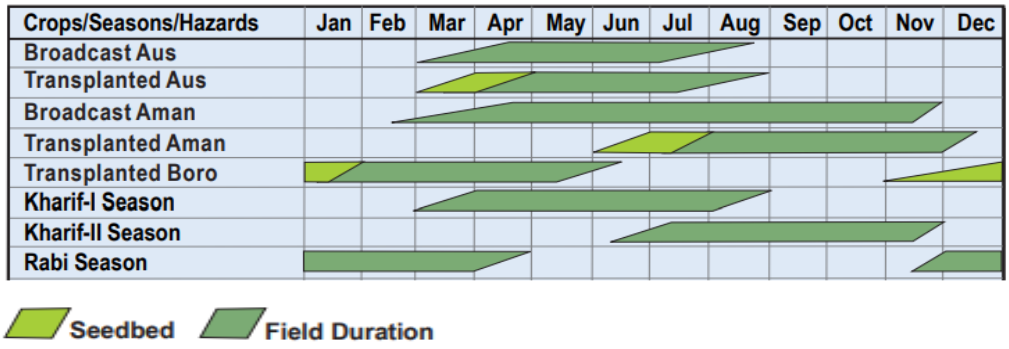
Nelson *et al.(*2014) combined moderate-resolution (100-meter resolution with a revisiting time of 16 to 24 days) time series data from Synthetic Aperture Radar (SAR) and MODIS optical sources (with a resolution of 500 meters) for rice crop characterization in Bangladesh. SAR and MODIS were employed for rice area mapping and seasonal monitoring within the delineated area, respectively, to overcome spatio-temporal challenges and establish an operational monitoring system. Singha *et al.(* 2019) conducted paddy rice mapping in Bangladesh and Northeast India using Sentinel-1 data and Random Forest (RF) classification. However, their study lacked a comparison with other supervised classification methods. In a similar vein (Rudiyanto *et al.*, 2019) developed an automated near-real-time mapping and monitoring system for rice extent, cropping pattern, and growth stages using Sentinel-1 time series in Southeast Asia, utilizing the Google Earth Engine (GEE) platform. They integrated temporal Sentinel-1 data with phenological parameters in GEE and employed K-means clustering, Hierarchical Classification Analysis

(HCA), and visual image interpretation to generate rice extent, cropping patterns, and spatiotemporal distribution of growth stages. The study automated the classification process using multiple machine learning algorithms and compared the results. In this study, we employed supervised classification algorithms such as Classification And Regression Tree (CART), k-Nearest Neighbor (k-NN), Random Forest (RF) and Support Vector Machine (SVM) for boro rice area delineation which are popular for image classification. By utilizing Sentinel-2 imagery and using these algorithms, we aim to contribute to the understanding of their performance in accurately estimating rice areas in Bangladesh. The successful implementation of an efficient boro rice area estimation system will have significant implications for policy planning, allocation of agricultural resources, and ensuring food security in Bangladesh. The objective of this study is to assess the potential of Sentinel-2 imagery and machine learning algorithms in more accurately estimating the boro rice areas in Bangladesh. By leveraging high-resolution satellite imagery and implementing supervised classification algorithms, the research aims to address the challenges posed by fragmented rice fields. The outcomes of this study will not only provide valuable insights into the extent of boro rice cultivation but also contribute to the development of in-season yield forecasting and end-of-season yield estimation systems.

Rice scenario in Bangladesh

There are three distinct rice seasons in Bangladesh known as aus, aman, and boro, each with its unique characteristics. Aman is considered a wet season, while boro and aus are categorized as dry seasons due to their overlapping cultivation (Fig. 1). The following is a summary of the three rice seasons based on the information provided by Maki *et al.*, 2017. Aus rice, classified as a kharif-I crop, is predominantly direct-seeded during the months of March and April, with harvesting taking place in July and August. The cultivars in this group mature irregularly and are not influenced by photoperiod changes. Although typically rain-fed and dry-seeded, Aus rice can also be transplanted when there is sufficient rainfall and irrigation available. Farmers opt for Aus as a short-duration drought-resistant crop during the pre-monsoon period of March to May. Transplanted Aus rice seedlings are raised in March and April, with harvesting occurring in July and August. Typically, the crop is transplanted using seedlings that are 20-30 days old. Local varieties are commonly preferred during the Aus season, but recently, farmers have started cultivating modern short-duration varieties as well. Aman, categorized as a kharif-II crop, is sown during the rainy season (July-August) and harvested in November and December. Aman rice can either be directly broadcasted or transplanted. In Bangladesh, broadcast aman rice is also known as deep water rice (DWR). Traditionally, it is directly seeded from March to May, which is the pre-monsoon period, in land that is deeply flooded (1-4 meters). However, due to changes in cropping patterns, transplantation is also carried out between May and June. In both cases, once the crop is established, the plants grow as the floodwater rises from June to September. Aman rice is sensitive to photoperiod changes and is harvested after the floodwater recedes, typically in November and December. Transplanted aman rice refers to traditional photoperiod-sensitive rice varieties that are transplanted in July and August, with harvesting taking place in November and December. Recently, photoperiod-insensitive varieties have become available in Bangladesh, and farmers have started cultivating them during the transplanted aman season. On land with shallow flooding

depth, aman rice is transplanted using shorter duration varieties. Boro, classified as a rabi crop, is transplanted during the winter season and harvested in summer. With the widespread use of groundwater irrigation, this group of photoperiod-insensitive irrigated rice can now be grown on all types of land. Boro rice is mainly transplanted in January and February, with harvesting taking place in May and June. Previously, farmers used to grow boro rice in very low-lying lands that were unsuitable for cultivating any crop during the monsoon season. After the floodwaters recede, boro rice is transplanted in November and harvested in April and May.



Source: *Hussain 2017*

Fig. 1. Rice calendar with cropping season in Bangladesh

Materials and Methods

Satellite image

This study utilizes the optical data from the European earth observation satellite Sentinel-2, which is accessed through the Google Earth Engine (GEE) platform. First, a Region of Interest (RoI) is selected to cover the entire country of Bangladesh. Then a stack of Sentinel-2 (COPERNICUS/S2_SR) images is created for boro rice, and spatio-temporal image filtering is performed on the stack based on the rice calendar. Consequently, time series Sentinel-2 images are used for boro rice during December 2020 to March 2021 and the field data collection took place during that time. The winter season in tropical regions like Bangladesh, when boro rice is grown, typically has fewer clouds, making Sentinel-2 data suitable for identifying boro rice areas. However the Sentinel image filtered out with the cloud above 20%.

Field/signature data

Field data collection is crucial for rice area classification using machine learning models. Sample locations for data collection are selected based on the boro rice-growing areas. Handheld GPS devices are used to capture point (latitude, longitude) and polygon data for rice, other crops, settlements, water bodies, etc., during the collection of signature data. Boro rice signatures are collected between December 2020 and March 2021. Four machine learning algorithms are applied separately for boro rice area estimation.

ML algorithm in GEE

In GEE, the CART, k-NN, Random Forest and SVM models are represented by

the smileCart(), smileKNN(), smileRandomForest() and libsvm() functions, respectively, which are used to calculate the boro rice area. The field datasets are divided into 80% for model training and 20% for validation (accuracy assessment). The training and validation datasets ensure a proportionate representation of each land cover category. The accuracy levels of different algorithms are compared, and the most accurate output is selected as the final boro rice area. The accuracy is obtained from confusion matrix. At the same time, the area of boro cultivation generated by Machine Learning classification is compared with BBS data.

Methodology of Boro rice area delineation

The methodology for boro rice area delineation is illustrated in the diagram in Fig.2

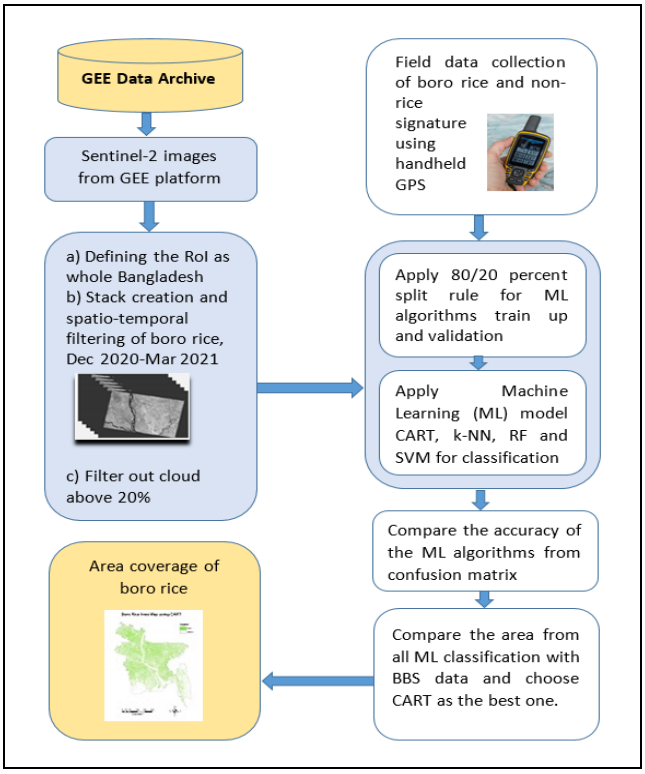


Fig. 2. Methodology of boro rice area delineation

Data collection

Quantitative and qualitative data were collected from in-situ rice fields, which served as the rice signature for the study. Questionnaires were developed for data collection and they were pretested in various locations during the boro season, and necessary adjustments were made based on the results of the experimental survey. To capture the location data, handheld GPS devices were used for each sample. The questionnaire comprised 29 parameters, including GPS coordinates, on which the survey was conducted. For field data collection, GPS locations were recorded from all directions around the plot to create polygon data. Point data were collected from inside the field. Additionally, photos

of the fields were captured using a digital camera. The farmer or, in some cases, the landowner was interviewed to gather relevant information about the crop or rice. This information included details about the farmer, crop or rice variety, seedling and planting dates, irrigation practices, fertilizer usage, pesticide application, yield from the previous year, and other related information.Sample locations for boro rice fields were selected based on the rice production areas throughout Bangladesh. Data on rice production at the district and Upazila level were obtained from the annual yearbook of agricultural statistics published by the Bangladesh Bureau of Statistics (BBS). The survey for boro rice was conducted from December 2020 to March 2021, covering a total of 606 locations across Bangladesh, including 205 rice signatures and 401 non-rice signatures. The non-rice signatures included other crops, settlements, rivers, water bodies, brick fields, and so on. The table below provides further information on the sample data collected for boro rice:

Table 1. Field survey information

Rice	Survey extent	Survey duration	No. of rice sample	No. of non-rice sample (other crop, settlement, rivers, water bodies etc.)	Total no. of sample
Boro	All over Bangladesh	December 2020 to March 2021	205	401	606

Data processing

The field data collected during boro seasons are compiled into an Excel sheet. Some conversions of measurement units, such as land size, fertilizer and pesticide application quantities, etc., are necessary to maintain a consistent format for the data. A desk validation process is conducted to verify the captured data, and any missing data are filled in by contacting the farmers via mobile phone in most cases. The validated data is then saved as a CSV file, which serves as the attribute file.The survey locations are shown in Fig. 3. Simultaneously, the GPX file from the handheld GPS devices is downloaded and imported into Google Earth Pro to verify the survey locations. Polygons are drawn using the GPS points and digital photos for most of the fields. The file is exported in KML format and opened in QGIS, where it is converted into a shapefile. The shapefile is then joined with the attribute file (CSV) to merge the land types and other parameters. Non-rice land types are generalized into a single class called 'non-rice'. In the shapefile, there are two columns named 'id' and 'feature_name', where the value of id equals '0' represents 'non-rice' and '1' represents ‘rice’ features. The generalized shapefile is imported into the Google Earth Engine (GEE) asset, where it is used as the signature data in the machine learning (ML) model.

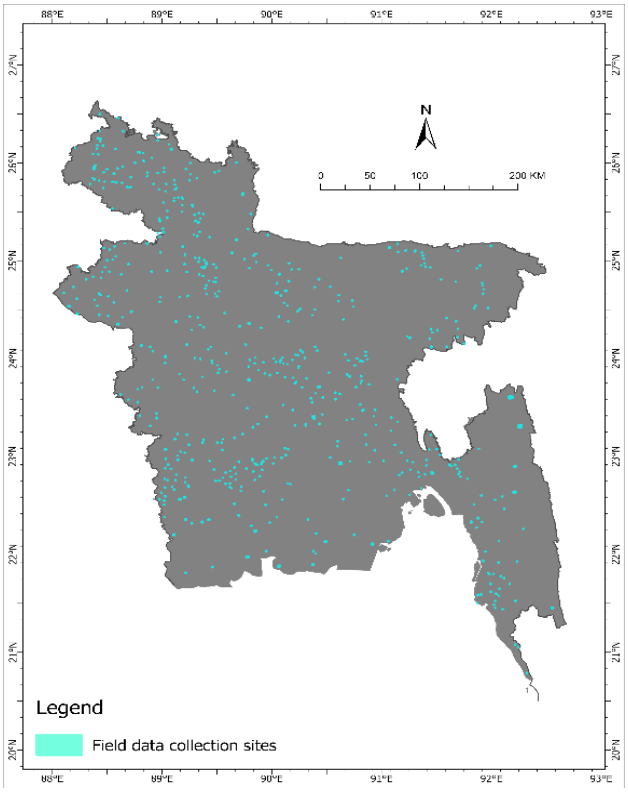


Fig. 3. Sample location of boro rice data collection (606 signatures)

Results and Discussion

The delineation of boro rice areas has been conducted using Sentinel-2 images, followed by the methodology discussed in the previous section. Four supervised machine learning algorithms CART, k-NN, RF and SVM have been employed and the resulting rice area is depicted in Fig. 4. The estimated area of Boro rice, derived using the CART, k-NN, RF, and SVM algorithms, along with the corresponding data from the Bangladesh Bureau of Statistics (BBS), is presented in Table 2. The overall classification accuracies for Boro rice using CART, k-NN, RF, and SVM were 96%, 97%, 97%, and 85%, respectively. These results indicate that all algorithms, except SVM, achieved high accuracy in identifying rice cultivation areas. Among them, k-NN and RF demonstrated the highest accuracy (97%) for Boro rice area classification. According to Table 2, the total rice area estimated by the CART algorithm is 4,199,579 hectares, which shows the smallest deviation (12%) from the BBS-reported area of 4,786,621 hectares. Therefore, the map generated using the CART algorithm is considered the best representation of the Boro rice area for the 2020-21 season.

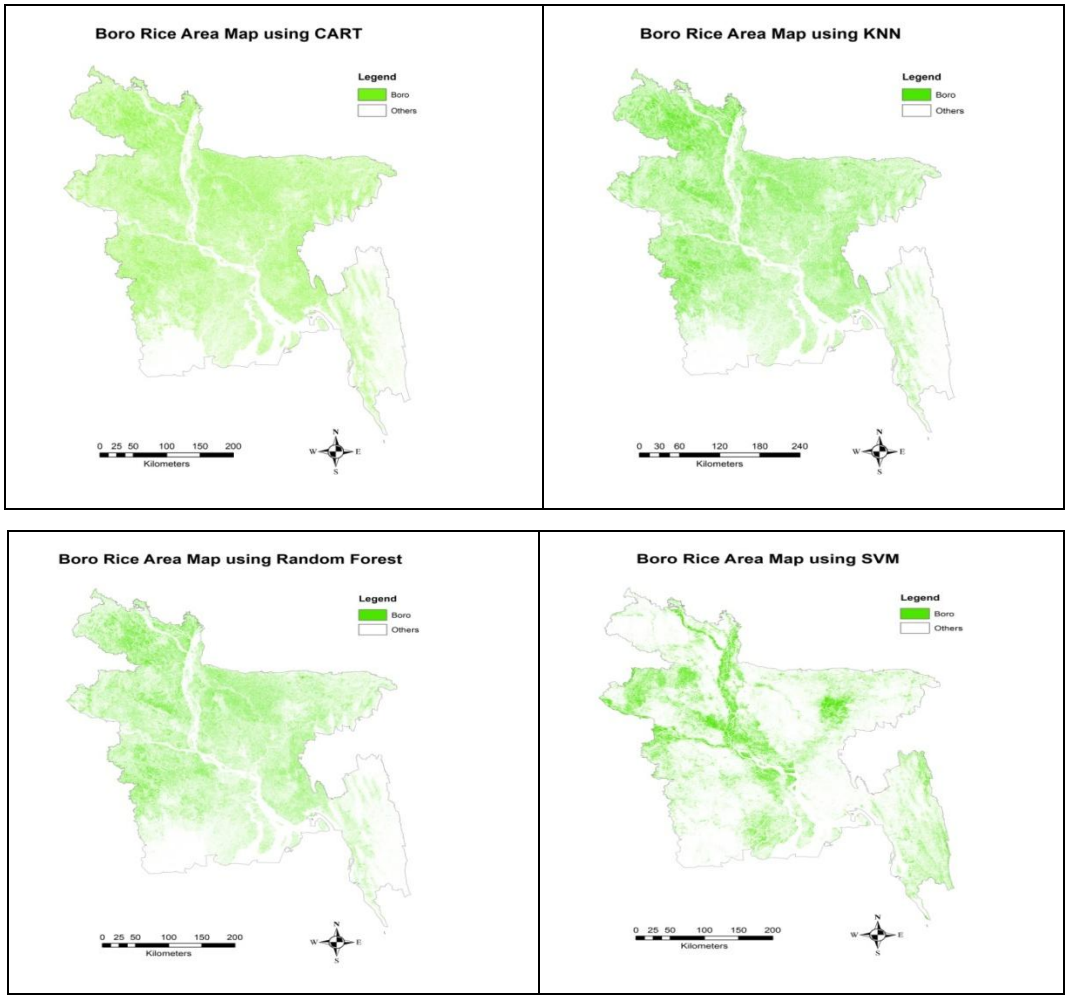


Fig. 4. Boro rice area generated by CART, k-NN, RF and SVM algorithm

Table 2. Boro rice area generated by CART, k-NN, RF, SVM and BBS 2021

Sl.	Classification Algorithm	Pixel count (10m x 10m)	Area in Sq. Meter	Area in Sq. km	Area in ha	Classification Accuracy of Algorithm (%)	Deviaton from BBS (%)
1	CART	419957945	41995794500	41996	4,199,579	96	12
2	k-NN	384058603	38405860300	38406	3,840,586	97	20
3	RF	295960922	29596092200	29596	2,959,609	97	38
4	SVM	240296058	24029605800	24030	2,402,961	85	50
BBS Statistics (2020-21)					4,786,621	-	-

In this study, all classes except for rice have been generalized into a single class called 'non-rice'. This approach contributes to improving the overall accuracy of the classification algorithms. Based on the conducted work following the proposed methodology, the CART algorithm is deemed more effective for the delineation of boro rice areas in Bangladesh using Sentinel-2 imagery. Bangladesh has mainly three rice growing seasons namely aus, aman and boro. Among them boro rice is grown on irrigated condition in Bangladesh and is vulnerable to flash flood damages thereby reducing rice yields (Hossain *et al.,* 2021). Previously, several efforts have been made to enumerate rice area using differnt field-based sampling globally (Xiao and He, 2021) but these methods required high cost and is also time-consuming. Scientist also applied RS based system such as optical and synthetic aperture radar (SAR) datasets for observing and mapping rice crops (Zhao *et al.,* 2021). RS based system can be divided into two broad groups, one is machine learning (Blickensdörfer *et al.,* 2022) and other is phenology-based threshold delineation (Xiao and He, 2021). Sentinel-1 and Sentinel-2 (S1, S2) were extensively used to map rice and quantify rice crop loss in Bangladesh (Islam *et al.,* 2022). Banngladeshi scientist also applied algorithms based single images (Alam *et al.,* 2019) as well as time-series imagery (Aziz *et al.,* 2023) to mapped the rice areas in Bangladesh.

This study delineated boro rice cultivation areas across Bangladesh using a limited number of signature points (606), which may slightly affect the accuracy of the method. However, the estimated Boro rice area deviates by only 12% from the BBS report, making it the most reliable model given that no such comprehensive effort has been undertaken in Bangladesh using Google Earth Engine (GEE) and Sentinel data at no cost (utilizing free 10m × 10m Sentinel-2 data from the GEE platform). In contrast, RF and SVM significantly underestimated the rice area.Generalizing all non-rice land types into a single ‘non-rice’ class improved classification performance by minimizing spectral. Despite using a modest sample size (606 points), the nationwide application demonstrated that high-resolution imagery combined with ML can provide accurate, cost-effective, and scalable rice area estimations. Furthermore, the successful application of Sentinel-2 imagery and the CART algorithm in this study provides a replicable framework for similar crop mapping initiatives in other regions, particularly where land fragmentation presents a challenge to conventional survey-based approaches. The integration of high-resolution satellite data with advanced classification models thus represents a transformative step forward in agricultural monitoring and food systems planning in Bangladesh.These findings support the integration of remote sensing and ML for crop monitoring, policy planning, and food security strategies, and establish a baseline for future enhancements using multi-temporal or multi-sensor data.

Conclusion

Accurate rice area mapping is critically important for yield forecasting, a task that can be both labor-intensive and error-prone when done manually. Satellite image-based delineation of rice areas provides a valuable alternative and is increasingly recognized as an effective approach for both present and future agricultural monitoring. Given the fragmented nature of agricultural land in Bangladesh, high-resolution satellite imagery offers enhanced capabilities for rice monitoring and yield prediction. In this study, rice area

maps covering 4,199,579 hectares were generated using 10-meter resolution Sentinel-2 imagery. The machine learning algorithm CART (Classification and Regression Trees) was found to be the most effective in generating these results. These maps, when combined with data on climate, soil conditions, crop genetics, and crop management practices, can be integrated into crop models to improve the accuracy of yield and production forecasts. In-season yield forecasting systems that utilize satellite imagery can deliver vital insights to a range of stakeholders, including farmers and policy planners.

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Author's contributions

Both authors contributed equally to the manuscript. They jointly conducted the literature review, analysis, and content organization. The first author prepared the final draft, which was reviewed and approved by the second author before submission.

Conflicts of Interest

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

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LABORATORY AND FIELD SCREENING OF ALLELOPATHIC POTENTIAL BREAD WHEAT (*Triticum aestivum* L.) VARIETIES IN BANGLADESH

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Abstract

Minimizing the use of herbicides for eco-friendly weed management in wheat (*Triticum aestivum* L.) has become increasingly necessary. A series of experiments were conducted to screen the potential allelopathic wheat varieties of Bangladesh. In the laboratory these studies used radish (*Raphanus sativus* L.) and lettuce (*Lactuca sativa* L.) as model receiver plants, along with lambsquaters (*Chenopodium album* L.), and slender amaranth (*Amaranthus viridis* L.) as test weeds, for the initial allelopathic activity screenings of 13 wheat varieties of Bangladesh. In the laboratory studies, the wheat var. BARI gom21 produced the highest inhibition effect on C. album roots and also reduced the speed of germination of seeds of *R. sativus*, *L. sativa*, *C. album*, and *A. viridis*. BARI gom21 also significantly affected the coefficient of the velocity of germination of *A. viridis*. The focus of field studies was on 11 wheat varieties, which had previously been screened in the laboratory. These eleven wheat varieties were selected and cultivated in the field using standard cultural practices, but with no additional weed control. The field studies showed that wheat var. BARI gom21 had the lowest weed infestation with maximum weed control efficiency. In addition, the var. BARI gom21 were free of many weed species, including *C. album* and *A. viridis*. Therefore, BARI gom21 was the most weed suppressive variety among the tested varieties.

Keywords: Allelopathy, *Amaranthus viridis***,** Bread wheat, *Chenopodium album*, Weed suppression.

Introduction

Weeds are generally defined as, any undesirable plants that compete for water, nutrients, space, and light with crops, limiting crop growth and productivity. Depending on the environmental settings and type of cropping practices, weeds can cause about 45-95% yield losses (Masum *et al*., 2016). Among many factors, the yield losses due to weed infestations primarily depend on the types of weeds, density of infestations, weed emergence time relative to the crop, and interference duration. In 2020-21, the total production of wheat in Bangladesh is 12.34 million metric tons in an area of 3.40 million

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hectares (BBS, 2021). However, weeds are a major threat to wheat grain yield and cause about 18.6% losses (Gharde *et al*., 2018). Some of the major weeds of the wheat fields in Bangladesh are Bermuda grass (*Cynodon dactylon* L.), goose grass (*Eleusine indica*), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], purple nutsedge (*Cyperus rotundus* L.), lambsquarters (*Chenopodium album* L.), wood sorrel (*Oxalis acetosella* L.), ground cherry (*Physalis heterophylla* Nees), hairy vetch [*Vicia hirsuta* (L.) Gray], diamond flower (*Hedyotis brachypoda*) and chickweed (*Stellaria media* L.) (Hossain *et al*., 2010). Their effective control is needed to achieve optimum and sustainable grain production in wheat.

Since mechanical weed control is costly; farmers in Bangladesh often use herbicides for weed control, often alone, or in combination with other methods. However, the excessive use of herbicides can lead to herbicide-resistant weeds and herbicide persistence in soil and water, which can lead to contamination (Ofosu *et al.,* 2023). Alternative weed management approaches, such as allelopathy, can be 'eco-friendly' novel tools for weed control. Allelopathy is generally defined as, the biochemical interaction among all plants, by which a plant may cause any direct or indirect, harmful, or beneficial effects to another plant through the release of allelochemicals. Allelochemicals are mainly secondary metabolites or waste products of primary metabolic pathways in the environment (Masum *et al.,* 2019). Allelopathic crops can be used to control associated weeds, and exploiting allelopathic crops is an important part of integrated weed management in recent years. Recent studies have already explored the allelopathy of several cereal crops such as rye, sorghum, canola, mustard, rice as well as durum wheat, and barley (Scavo and Mauromicale, 2021).

Wheat also has strong allelopathic potential against weeds. Allelochemicals from wheat straw, root exudates, notably, phenolic acids (p-hydroxybenzoic, ferulic, syringic, *vanillic*, *p-coumaric*), benzoxazinoids, as well as phenoxazinones, flavonoids and short-chain fatty acids, have potential to be exploited for weed control (Hussain *et al.,* 2022). Wheat genotypes to possess significant allelopathic potential against common weeds, such as canary grass (Phalaris minor), and wild oat (*Avena fatua* L.) (Mardani *et al.,* 2014). Recent studies show that wheat can suppress weeds in both laboratory and field conditions. Compounds in wheat root exudates, like phenolic acids and flavonoids, significantly inhibit key weeds such as *Bromus japonicus* and *Chenopodium album* (Younesabadi *et al.,* 2019; Hussain *et al.,* 2022). However, the exploration of the allelopathic potential of bread wheat varieties has not been adequately studied in Bangladesh. Thus, research to characterize the allelopathic potential among Bangladeshi wheat varieties appears an important forward step in developing allelopathy-based sustainable weed management systems in wheat. The primary objective of this study was to evaluate the allelopathic potential of major wheat varieties in Bangladesh against common weeds through both laboratory experiments and field trials.

Materials and Methods

The present research was conducted in the central laboratory and agronomic fields of the Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh (23°46′17″N latitude and 90°22′31″E longitudes) during the period September 2018 to March 2019. Thirteen Bangladeshi wheat varieties released by Bangladesh Agricultural Institute (BARI)

were collected and used in the laboratory experiments. Common weeds - *Chenopodium album* and *Amaranthus viridis* were also collected from the fields of Sher-e-Bangla Agricultural University and used as receiver plants. Radish (*Raphanus sativus*) and lettuce (*Lactuca sativa*) were used as model plants for bioassay.

Donor receiver bioassay

In the laboratory screening, the donor-receiver bioassay technique was used to select some possible allelopathic varieties as described by Wu *et al*. (2000a) and Kato-Noguchi *et al*. (2002). Wheat seeds were moistened by filter papers in Petri dishes (9 cm) and germinated in a growth chamber for two days under a 12/12 h dark/light period. Uniformly germinating wheat seedlings were transferred to Petri dishes (ten wheat seedlings per Petri dish) that contained a filter paper moistened with 2.5 mL of 1 mM phosphate buffer (pH) and grown for an additional 48 h. Ten seeds of *R. sativus* and *L. sativa* were also placed into the filter paper, containing the growing wheat seedlings. In the case of *C. album* and *A. viridis*, the seeds were pre-germinated by soaking in distilled water for 36 h, transferred into a Petri dish containing a sheet of moistened filter paper, as described above, and then incubated in dark at 25°C for 48 h. Finally, the germinating seeds were placed into the filter paper with the growing wheat seedlings. Wheat, as well as the 'receiver' weed species, were then allowed to grow for 48 h before growth measurements. The shoot (hypocotyls and/or coleoptiles) and root lengths of *R. sativus*, *L. sativa*, *C. album*, and *A. viridis* were measured. Along with the experimental treatments, control plants were established by treating and incubating the receiver species by the same procedure as above, in absence of wheat seedlings. Each experimental unit contained ten donor (wheat) seedlings and ten receiver seedlings (*R. sativus*, *L. sativa*, *C. album*, and *A. viridis*)

The experimental design for bioassay was a completely randomized design (CRD) with four replications.

Percentage inhibition was determined by the following formula (Lin *et al*., 2004).

$$Inhibition\ (\%) = \frac{\text{Control plant length} - \text{Plant length infested with wheat}}{\text{Control plant length}} \times 100$$

The speed of germination was calculated by the following formula given by Gairola *et al.* (2011).

Speed of germination = $N_1/D_1+N_2/D_2+N_3/D_3+\ldots\ldots\ldots N_n/D_d$

Where, N = number of germinated seeds, D= number of days.

The coefficient of the rate of germination of the receiver plant was measured by the following formula (Al-Mudaris, 1998).

$$(CRG) = \frac{(N1+N2+\ldots\ldots+Nn)}{(N1T1)+(N2T2)+\ldots\ldots(NnTt)} \times 100$$

Where, N_1= Number of germinated seeds on time T_1, N_2= Number of germinated seeds on time T_2, and Nn= Number of germinated seeds on time Tn

Field experiment

Eleven wheat varieties were selected based on both allelopathic and non-allelopathic from the laboratory test for the field study. The seed of wheat varieties was broadcasted in the respective plots. Irrigation and fertilizer were applied at recommended times and doses. Weeds of each plot were allowed to grow, collected after the critical period of weed competition, and sundried overnight, and then oven-dried for 48 hrs. at a temperature of 60°C to determine the dry weight. Randomized Complete Block Design was followed in the experiment with three replications.

Weed control efficiency was calculated by using the formula suggested by Mani *et al*. (1973).

$$WCE = \frac{\mathrm{DWC}-\mathrm{DWT}}{\mathrm{DWC}} \times 100$$

Where DWC = dry weight of weeds from control plots (weedy plots) and DWT = dry weight of weeds in treated plots.

Statistical analysis

The analysis of variance (ANOVA) and least significance difference (LSD) was performed with the Statistix 10 software package and the LSD test used a 5% level of significance.

Results and Discussion

Laboratory study (Donor-Receiver Bioassay)

In the donor receiver bioassay test significant differences in growth inhibition were observed on test plants due to different wheat varieties (Table 1). In the short-term co-cultivation of wheat varieties with test species and weeds, the highest level of inhibition caused by the BARI gom21 variety resulted in 81% root growth inhibition of *C. album*. On the other hand, the case of shoot of *C. album* BARI gom27 showed maximum inhibition (49%). BARI gom21 (52%) resulted in the highest inhibitory effect on *R. sativus* root but the shoot growth was restricted by BARI gom25 (67%). While BARI gom30 (48% inhibition in root) gave a stimulating effect on *R. sativus*. BARI gom30 (46%) and BARI gom21 (42%) showed over 40% growth inhibition on *A. viridis* root. The highest level of shoot inhibition in *A. viridis* was caused by BARI gom25 (53%). BARI gom26 (65%) and BARI gom 21 (57%) demonstrated the highest growth inhibition of *L. sativa* roots. Interestingly, some wheat varieties such as BARI gom29 (-4% inhibition) and BARI gom31 (-10% inhibition) stimulated the root growth of *L. sativa*. The highest (24%) shoot inhibition of *L. sativa* was observed from BARI gom28. Significant differences in growth inhibition were observed among wheat varieties in an equal compartment agar method bioassay test on little seed canary grass (*Phalaris minor*) (Kashif *et al*., 2015).

Table 1. The Allelopathic potential of wheat varieties on selected weed species in donor-receiver bioassay under laboratory conditions

Variety	Inhibition (%)							
	C. album		*R. sativus*		*A. viridis*		*L. sativa*	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
BARI gom21	81a	32.94c	52.13a	61.14b	42.48b	41.50c	56.65b	6.71d
BARI gom22	30.5f	33.88c	32.31b	52.65de	34.29d	32.42e	47.27c	3.45fg
BARI gom23	46.98d	36.18c	-29.55j	49.56ef	24.35f	40.10c	8.60h	12.22c
BARI gom24	37.44e	24.1d	9.14e	59.65bc	23.2f	26.97f	38.66e	2.21g
BARI gom25	64.20b	33.12c	26.10c	67.40a	14.22h	52.60a	44.13d	12.97c
BARI gom26	30.81f	43.62b	4.14f	56.18cd	-30.48j	23.36g	65.23a	4.82ef
BARI gom27	46.05d	49.24a	18.39d	46.78fg	18.43g	33.85de	3.91i	12.81c
BARI gom28	56.61c	22.83d	-1.92g	55.06d	31.48e	11.19h	17.84g	23.59a
BARI gom29	34.75ef	21.37d	-25.58i	47.54fg	39.03c	45.31b	-4.18j	7.27d
BARI gom30	49.32d	32.24c	-48.20k	52.56de	45.51a	45.88b	48.54c	14.77b
BARI gom31	49.32g	35.68c	32.54b	45.43g	12.76h	36.26d	-9.97k	6.24de
BARI gom32	45.87d	34.39c	32.46b	59.70bc	18.60g	2.05i	9.18h	6.29de
BARI gom33	46.14d	36.34c	-21.07h	36.41h	-8.03i	41.74c	35.45f	6.63fg
LSD $_{(0.05)}$	5.47	2.26	2.76	4.07	1.82	2.93	3.08	1.58
CV (%)	8.42	8.88	30.97	5.36	6.21	6.16	7.75	12.24

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at a 0.05 level of probability by LSD test

Speed of germination of the receiver plant

The calculated indices provided in Tables 2 & 3 showed different wheat varieties significantly affected the germination speed of the receiver plants. BARI Gom 21 reduced the speed of germination (5.35, 4, 4.32, and 5.83) against *R. sativus*, *L. sativa*, *C. album* and *A. viridis*. BARI gom21 also reduced the coefficient of velocity of germination of *A. viridis* (1.92). Masum *et al.* (2016) observed significant differences in germination parameters among rice varieties, noting that certain allelochemicals delayed the germination of *Echinochloa crus-galli* and significantly affected the germination index, speed, and coefficient of germination rate. However, in all receiver species, inhibitions on root growth were greater than those on shoot growth. Previous studies also reported greater inhibition of root growth than shoot growth by an allelopathic crop (Olofsdotter and Navarez, 1996). Allelopathic inhibition of annual ryegrass ranging from 3 to 100% was also reported (Wu *et al*., 2003a; Wu *et al.,* 2003b).

Table 2. The effect of allelopathic wheat varieties on the speed of germination of different receiver species in donor-receiver bioassay under laboratory conditions

Variety	Speed of germination			
	C. album	*R. sativus*	*A. viridis*	*L. sativa*
BARI gom21	5.35i	4.00j	4.32g	4.83i
BARI gom22	6.00f	4.83h	5.83a	5.66e
BARI gom23	5.50hi	5.50f	5.67b	6.66a
BARI gom24	6.83e	6.00c	5.67b	6.50b
BARI gom25	5.67gh	7.00a	5.83a	5.66e
BARI gom26	5.83fg	5.83d	5.17d	5.33g
BARI gom27	5.75g	5.67e	5.83a	5.99d
BARI gom28	6.83e	4.67i	4.83e	5.49f
BARI gom29	6.00f	5.16g	5.83a	6.50b
BARI gom30	7.83c	5.83d	5.33c	5.66e
BARI gom31	8.49a	6.17b	4.66f	6.33
BARI gom32	8.18b	5.67e	5.67b	5.50f
BARI gom33	7.66d	5.67e	5.83a	5.17h
LSD $_{(0.05)}$	0.17	0.09	0.05	0.07
CV (%)	1.83	1.17	0.71	0.91

In a column means having a similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at a 0.05 level of probability by LSD test

Table 3. The coefficient velocity of germination of different receiver species in donor-receiver bioassay under laboratory condition

Variety	The coefficient velocity of germination			
	C. album	*R. sativus*	*A. viridis*	*L. sativa*
BARI gom21	2.22e	2.03e	1.92e	1.97f
BARI gom22	2.19f	2.03e	2.03d	2.15b
BARI gom23	2.02i	1.96f	2.16b	‘2.19a
BARI gom24	2.25d	2.39a	2.08c	2.14c
BARI gom25	1.98j	2.34b	2.08c	1.97f
BARI gom26	2.02i	2.15c	1.93e	1.97f
BARI gom27	2.08h	1.96f	2.03d	2.08d
BARI gom28	2.25d	1.92g	1.93e	2.03e
BARI gom29	2.14g	2.08d	2.08c	2.14c
BARI gom30	2.37b	2.03e	2.03d	2.13c

Variety	The coefficient velocity of germination			
	C. album	*R. sativus*	*A. viridis*	*L. sativa*
BARI gom31	2.52a	2.03e	2.08c	2.08d
BARI gom32	2.37b	2.08d	2.08c	2.03e
BARI gom33	2.31c	1.96f	2.21a	1.93g
LSD $_{(0.05)}$	0.013	0.029	0.015	0.013
CV (%)	0.41	1.00	0.52	0.46

In a column means having a similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at a 0.05 level of probability by LSD test

From Fig. 2, it is also observed that across all the wheat varieties, *C. album* (39%) was the most inhibited when grown with wheat seedlings, followed by *R. sativus* (30%), *A. viridis* (27%), and *L. sativa* (18%). Based on donor-receiver bioassay results, the maximum average inhibition on test plants and weeds was from BARI Gom 21 (47%) followed by BARI gom25 (39%) and BARI gom22 (33%) (Fig. 3).

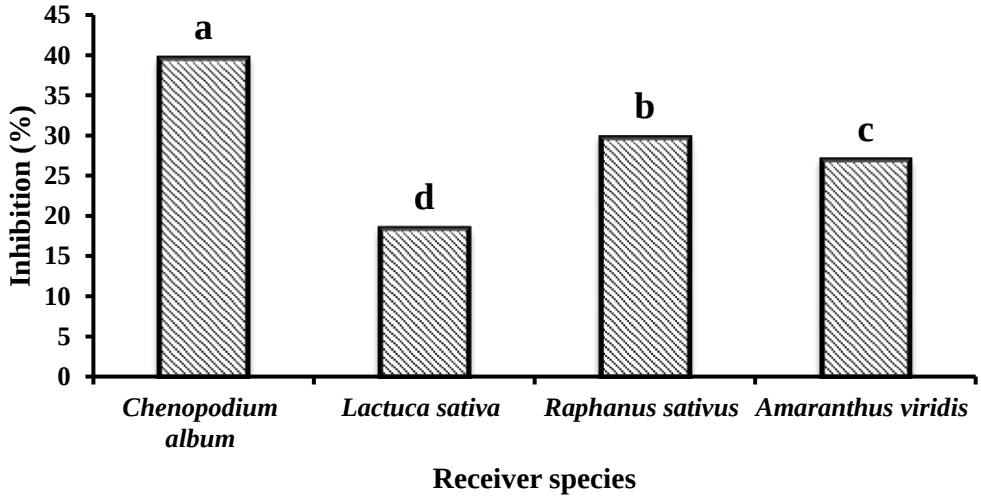

Fig. 2. Average inhibition on receiver species due to infestation with irrespective of wheat varieties.

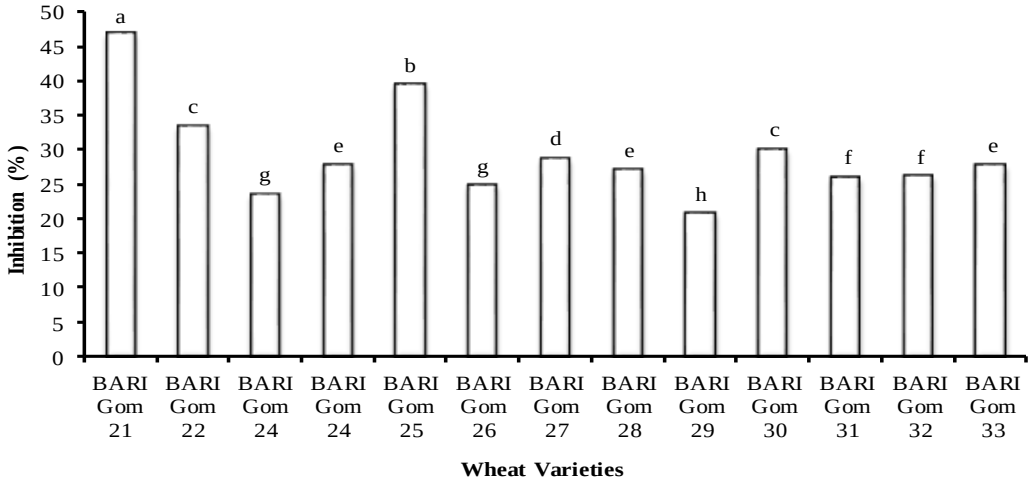

Fig. 3. Average inhibition irrespective of receiver species by tested wheat varieties from donor-receiver bioassay test

Field experiment

Weed density in no weeding plots at 15, 25, and 35 days after broadcasting were more or less similar for different wheat varieties (Table 4). BARI gom21 reduced the weed density (14.66 m^{-2}, 28.33 $m^{-2,}$ and 33.33 m^{-2}) at 15, 25, and 35 DAS. BARI gom21 thus reduced weed biomass (12.02g m^{-2}) showing the maximum weed control efficiency (86%) in the field test followed by BARI gom23 (84), BARI gom29 (81), BARI gom28 (41) (Table 5). In the field experiment, BARI gom21 raised plot was less infested by weed and showed the lowest (12.02 g m^{-2}) weed dry matter (Table 5) followed by BARI gom23, BARI gom29, and BARI gom28 raised plots showed 14.65, 16.33 and 17.17 g m^{-2}, respectively as statistically similar. On the contrary, the highest weed dry matter was recorded in BARI gom22 (29.44 gm^{-2}) followed by BARI gom24 (28.20 g m^{-2}). A similar result was recorded by Hossain *et al.* (2010).

Table 4. Weed density in different wheat varieties raised plots on different days after sowing (DAS)

Wheat variety	Weed density (no. m^{-2})		
	15 DAS	25 DAS	35 DAS
BARI gom21	14.66f	28.33d	35.33d
BARI gom22	17.33ef	33.00b-d	59.33b
BARI gom23	22.33c-f	29.33d	35.33d
BARI gom24	20.66d-f	32.66b-d	54.00bc
BARI gom25	19.66d-f	35.00b-d	44.66cd
BARI gom26	27.33b-d	39.00b	53.33bc
BARI gom27	23..33c-d	3066d	44.33cd

Wheat variety	Weed density (no. m^{-2})		
	15 DAS	25 DAS	35 DAS
BARI gom28	32.33b	28.66d	46.33cd
BARI gom29	28.66bc	31.00cd	37.66d
BARI gom30	22.33c-f	30.33d	54.33bc
BARI gom31	21.66c-f	38.00bc	51.66bc
Weedy plot	41.66a	62.66a	91.33a
LSD $_{(0.05)}$	7.93	7.05	11.28
CV (%)	19.26	11.94	13.16

In a column means having a similar letter (s) are statistically similar and those having dissimilar letter(s) differ significantly at a 0.05 level of probability by LSD test

Relative weed density (%)

The weed species found in the experimental field were *Chenopodium album, Cynodon dactylon, Eleusine indica, Echinochloa colona, Solanum carolinense, Raphanus raphanistruma, Lindernia procumbens, Vicia sativa, Amaranthus viridis, Argemone mexicana, Corchorus acutangulus, Portulaca olerace, Nicotiana plumbaginifolia, Physalis heterophylla, Alternanthera philoxeroides, Heliotropium indicum,* and *Cyperus rotundus.* Of these, many species were under the family Poaceae, some species under Solanaceae and Amaranthaceae. Other species are Compositae, Cyperaceae, Chenopodiaceae, Brassicaceae, Boraginaceae, Portulacaceae. When classified based on habit, 30% of weeds were under grass, 65% under shrubs, and 5% under sedge. Similar findings were also reported by Hossain *et al.* (2010). The relative density of some major weed species was found in wheat plots during the experiment is shown in (Table 5). Interestingly some major weed species of wheat including *C. album and A. viridis* were not found in BARI gom21 and BARI gom30 raised plots.

Table 5. Relative weed density of different weeds in different plots of wheat and the weedy plot

Wheat Variety	Relative Weed Density (%)						
	C. album	*C. dactylon*	*E. indica*	*S. carolinense*	*L. procumbens*	*A. viridis*	*E. colona*
BARI gom21	0.00e	12.24a	31.11f	2.85bc	2.85bc	9.44b-d	3.78bc
BARI gom22	7.17b-d	12.29a	52.27a	2.01c	0.49c	5.59e	2.60c
BARI gom23	9.46ab	11.51ab	32.48ef	4.25a-c	7.88a	8.66c-e	5.72ab
BARI gom24	5.55d	12.38a	50.98ab	3.89a-c	4.63a-c	8.07c-e	3.04c
BARI gom25	10.61a	12.23a	38.32c-f	4.52a-c	6.08ab	13.47a	3.75bc
BARI gom26	5.95cd	10.05ab	50.26ab	3.58a-c	4.8sa-c	8.80b-e	2.83c
BARI gom27	7.57a-d	9.76ab	34.41d-f	4.46a-c	4.46a-c	11.95ab	2.98c
BARI gom28	0.00e	10.40ab	32.51ef	2.83bc	3.84a-c	13.11a	6.63a
BARI gom29	5.39d	12.37a	41.63b-e	6.16a	5.24ab	9.70bc	2.67c

Wheat Variety	Relative Weed Density (%)						
	C. album	*C. dactylon*	*E. indica*	*S. carolinense*	*L. procumbens*	*A. viridis*	*E. colona*
BARI gom30	9.18a-c	8.53ab	42.29b-d	4.91ab	3.77a-c	6.11e	3.61c
BARI gom31	8.99a-c	7.13b	39.65c-f	3.24bc	4.63abc	6.42de	3.19c
Weedy plot	5.32d	12.59a	45.96a-c	3.66abc	5.90ab	6.08e	5.84a
LSD $_{(0.05)}$	3.36	4.78	9.50	2.59	4.53	3.24	2.05
CV (%)	31.69	25.72	13.69	39.59	58.76	21.40	31.13

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability by LSD

Weed control efficiency (%)

In the field experiment, the raised plot of BARI gom21 was less infested by weeds and showed the lowest weed dry matter at 12.02 gm^{-2} (Table 6). This was followed by the raised plots of BARI gom23, BARI gom29, and BARI gom28, which showed 14.65, 16.33, and 17.17 gm^{-2}, respectively, and were statistically similar. In contrast, the highest weed dry matter was recorded in BARI gom22 at 29.44 gm^{-2} and BARI gom24 at 28.20 gm^{-2}. BARI gom 21 showed the maximum weed control efficiency (86%) followed by BARI gom23 (84%), BARI gom28 (81%), and BARI gom29 (81%) during the field experiment (Table 6).

Table 6. Above-ground dry matter weight of weed and weed control efficiency

Wheat Variety	Weed dry matter weight (g m^{-2})	Weed control efficiency (%)
BARI gom21	12.02g	86.47a
BARI gom22	29.44b	66.80e
BARI gom23	14.65fg	83.67ab
BARI gom24	28.20bc	68.08e
BARI gom25	22.32d	74.55d
BARI gom26	17.17d-g	80.59a-c
BARI gom27	16.43e-g	81.32a-c
BARI gom28	18.23 d-f	79.30b-d
BARI gom29	22.77cd	74.52d
BARI gom30	19.87d-f	77.58cd
BARI gom31	20.93de	76.89cd
Weedy plot	88.84a	
LSD $_{0.05}$	5.63	5.92
CV (%)	12.85	4.50

In a column means having a similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at a 0.05 level of probability by LSD test

The present work identifiesthe Bangladesh wheat variety BARI gom21 as allelopathic. The in vitro bioassay results were also successfully verified by comparing with field performance in terms of weed control, and successfully distinguished allelopathic effects from competition in crop-weed interference. This elite allelopathic wheat genotype could be used in breeding efforts to improve weed suppression traits in commercial varieties.

Conclusion

The present research suggested that 'BARI gom21' showed the most allelopathic performance to suppress weeds in both *in vitro* and field conditions out of 11 Bangladeshi bread wheat varieties. Therefore, this research beneficial for the resource-poor farmers of Bangladesh as well as for the researchers who work on the development of environmentally friendly sustainable weed management options. This information is important for organic farmers who have to control weeds without the use of herbicides. However, additional research is necessary to isolate and identify allelochemical(s) from the Bangladesh wheat variety 'BARI gom21' as bioherbicide by which significance in nature of allelochemicals could be found for attributing the constant need for new chemistries and new target sites. Moreover, this wheat variety could be developed by breeding and by adopting other agronomic practices for obtaining optimum yield performance and tolerance to weeds.

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Conflicts of Interest

The authors affirm that there are no conflicts of interest related to the publication of this manuscript.

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EFFECT OF HARVEST MATURITY AND VARIETY ON YIELD AND NUTRITIONAL QUALITY OF SQUASH IN BANGLADESH

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Abstract

Squash (*Cucurbita pepo* L.) yield can vary significantly depending on harvesting date. To overcome this issue, a field experiment was conducted at the Vegetable Research field of Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh, during October 2019 to March 2020 to find out the effect of variety and harvesting date on yield and yield attributes of squash. The experiment comprised two sets of treatments viz., (A) Variety: (i) BARI squash1 (V_1) and (ii) Kajol-F_1 (V_2) and (B) Harvesting date: (i) 5 days after fruit setting (H_1); (ii) 10 days after fruit setting (H_2); (iii) 15 days after fruit setting (H_3); (iv) 20 days after fruit setting (H_4) and (v) 25 days after fruit setting (H_5) in a Randomized Complete Block Design (RCBD) with three replications. The results showed that the variety BARI squash1 showed significantly the highest number of fruit plant^{-1}, fruit length, yield and vitamin C content, five days after fruit setting showed significantly the highest fruit plant^{-1} and vitamin C content but the highest yield plant^{-1} and yield ha^{-1} were found from 20 days after fruit setting. The interaction between variety and harvesting date was significant for yield and quality parameters except moisture (%). Based on the study results it is concluded that maximum yield plant^{-1} (4350 g) and yield (43.30 t ha^{-1}) were recorded from BARI squash1 with 20 days after fruit setting, but higher TSS and vitamin C was found in BARI squash1 where crop harvested 5 days after fruit setting. Variety BARI squash1 harvesting at 20 days after fruit setting (V_1H_4) is sutable for higher yield and Variety BARI squash1 harvesting at 5 days after fruit setting (V_1H_1) is sutable for higher nutritional quality of squash in Bangladesh.

Keywords: Harvesting date, Nutritional value, Squash, Variety, Yield.

Introduction

Squash (*Cucurbita pepo* L.) is a type of vegetable in the cucurbit family, known as Cucurbitaceae. It is commonly known as Zucchini. It is also known as marrow, courgette, baby marrow, summer squash, bush squash etc. It is widely cultivated in the world. Short

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days, low temperature, high relative humidity and bright sunshine are ideal for squash cultivation. This crop is relatively new in Bangladesh, but it is becoming popularity day by day. The health benefits of summer squash, including its bioactive compounds and nutritional composition are widely recognized (Tadros *et al.*, 2023; Díaz *et al*., 2020). Additionally, the fruit of the summer squash is rich in fiber and vitamins and contains moderate amounts of mineral salts (Abdein, 2016).

Harvesting time of any vegetable crop is one of the most important factors which affect nutritional quality as well as total yield. Generally, squash is harvested during the growing season, while the skin is still tender and the fruit relatively small, mostly consumed at immature stage for culinary purposes before seeds begin to enlarge and harden. The whole tender fruit is edible, without discarding seeds and seed cavity tissues. It has soft seeds and thin, edible skin, and tender flesh with high water content (Herbst, 2001). Squash can be consumed at different stages of fruit development. Those picked up in advanced phases of development or in full maturity, when receive the diameter of about 20 cm, and a weight of 1.5-2.0 kg, can be directly supplied to the fresh market or after cold storage (Gajewski and Grzeszczuk, 2005). Small size fruits with diameter 3-6 cm, harvested at the time before the skin begin to harden and do not need to remove it (Gajewski and Grzeszczuk, 2005). However, such immature fruit has limited storage life. At later development stages of squash, there is an increase in yield, but squash at early stages of maturity contain high content of nutrients and health promoting compounds, such as protein, ash, crude fiber, phenolics and flavonoids (Magda *et al.*, 2015). Squash cultivation has a great opportunity and a promising vegetable in Bangladesh. Keeping in view, of growing importance of this crop in Bangladesh, the present studies have been undertaken to find out the optimum harvesting date and variety on yield and quality of squash for Bangladesh.

Materials and Methods

Research Location

The research work was conducted at Vegetable Research field of Horticulture Research Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh during the period of October 2019 to March 2020. The experimental site is located at 24.0° N latitude and 95.25° E longitude, respectively at an elevation of 8.4 meters from the sea level (Anon, 1995). Topsoil was sandy clay loam in texture having pH around 6.0. The selected plot was medium high land. Plenty of sunshine and moderately low temperature prevails during experimental period. The weather data during the study period are presented in Table 1.

Experimental design

The experiment consisted of two factors. Factor A: Variety (2) *viz*. (i) BARI squash1 and (ii) Kajol-F_1 and Factor B: Harvesting date (5): (i) 5 days after fruit setting (H_1); (ii) 10 days after fruit setting (H_2); (iii) 15 days after fruit setting (H_3); (iv) 20 days after fruit setting (H_4) and (v) 25 days after fruit setting (H_5). The experiment was conducted in a randomized complete block design (RCBD) with three replications. The layout of the experiment was prepared for distributing the combination of variety and

different harvesting dates. The 10 treatment combinations of the trail were assigned at arbitrary into 30 plots. The size of each unit plot 3m × 2m). The distance between replication to replication 1.0m and plot to plot distance was 0.5m.

Table 1. Monthly mean weather data during the crop growing periods at BARI, Gazipur

Year	Month	Temperature (°C)			Relative humidity (%)		Sunshine (hr./day)	Total rainfall (mm)
		Maximum	Minimum	Average	9 am	2 pm		
2019	July	32.95	26.76	29.86	84.94	78.52	3.85	360.2
	August	33.78	27.22	30.50	83.23	74.32	7.07	169.8
	September	33.29	26.16	29.73	85.53	76.43	4.68	210.2
	October	32.24	24.36	28.30	82.42	69.65	5.83	231.6
	November	31.13	20.26	25.70	81.97	61.93	6.47	22
	December	24.84	14.62	19.73	84.71	64.81	4.69	3.6
2020	January	24.36	13.61	18.985	88.68	64.16	4.25	28.2
	February	28.00	14.52	21.26	77.31	47.97	6.15	0.8
	March	32.70	19.63	26.165	66.94	45.55	8.06	16.6

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Raising of seedlings and crop management

The seeds of BARI squash1 were collected from Olericulture Division, Horticulture Research Centre (HRC), BARI, Gazipur and another squash variety Kajol-F_1 were collected from "Mollika Seed Company" 145, Siddique Bazar, Dhaka. Squash seeds were sown on 14 October 2019 in polybags at net house of Olericulture Division of BARI. Seeds were sown in polybags which were filled with loose friable, dead roots free, sandy loam soil previously mixed with well rotten cowdung. Eighteen days old seedlings were transplanted in the experimental plots. One fourth of cowdung (20 t ha^{-1}) and all of S (18.6 kg ha^{-1}), Zn (10.3 kg ha^{-1}) and B (1.2 kg ha^{-1}), ½ P (84 kg ha^{-1}) and 1/3rd K (90 kg ha^{-1}) were to be applied, respectively during final land preparation. Cowdung @ 10 kg, P @ 30 g, K @ 30 g and Mg @ 5 g were to be applied each pit in 7-10 days before planting. N @ 14 g was to be top dressed each pit at 4 split applications and K @ 15g to be applied 10-15 days after planting according to Projukti Hatboi (BARI, 2019). Healthy and uniform sized 18 days old seedlings were taken from the net house and were transplanted in the main field on 1 November, 2019. Plants were spaced at 1m × 1m spacing. The seedlings were watered after transplanting. The insects were controlled successfully by spraying Malathion 57 EC @ 2 ml/L water. The insecticide was sprayed fortnightly from a week after transplanting to a week before first harvesting. Squash fruits were harvested according to treatment. The harvested squashes of each plot collected separately, tagged and taken to laboratory for data collection.

Data collection and analysis method

The following data were collected from the experiment.

Fruit plant^{-1}

Total number of fruit was counted from each plant of each plot from first harvest to last harvest and average number of fruit was calculated and termed as number of fruits per plant.

Individual fruit weight

From first harvest to last harvest total fruit number was counted and total fruit weight was measured from each plant of each plot to determine single fruit weight.

Fruit length

The length of the fruit was measured with a meter scale in centimeter from the neck of the fruit to the bottom of the fruit. It was measured from each plot and their average was calculated in centimeters.

Fruit diameter

The diameter of individual fruit was measured in several directions from five selected fruits with slide calipers and the average of all directions was finally recorded and expressed in centimeter.

Yield plant^{-1}

Weight of matured fruits harvested from each picking in the tagged plants in each replication was recorded till final harvest and total yield of fruits per plant computed in kilogram.

Yield ha^{-1}

After collection of fruit per plot, it was converted to ton per hectare by the following formula:

$$\text{Yield (ton ha}^{-1}) = \frac{\text{Fruit yield plot}^{-1}\text{ (kg)} \times 10000\ \text{m}^2}{\text{Plot size (m}^2) \times 1000\ \text{kg}}$$

Moisture (%)

Squash slice (10g) was taken in porcelain crucible and placed in an oven and heat at 80 ºC for 72 hours and until constant weight was obtained. The crucible with the sample was then transferred to a desiccator containing anhydrous calcium chloride and kept there for about 10 minutes for cooling are final weight was taken. Percent moisture content was calculated using following formula-

$$\%\ \text{moisture content} = \frac{I - F}{I} \times 100$$

Where,

I = Initial weight of slice,

F= Final weight of dry matter

TSS

Total Soluble Solids (TSS) content was determined by a refractometer by placing of drop of pulp on its prism. TSS obtained from direct reading of the refractometer.

Vitamin C content

The reagent used for the estimation vitamin C were as follows-

a) Metaphoporic acid solution (3%)

b) Standard ascorbic solution

c) Dye solution

For estimation of vitamin C were as follows

Five ml of Standard ascorbic solution was taken in conical flask and 5 ml metaphoporic acid (HPO_3) was added to it and shaken.

A micro burette was filled with dye solution then the mixed solution was titrated with dye using phenolphthalein as indicator solution to a pinked coloured end point, which persisted at least for 15 seconds. Dye factor was calculated using the following formula-

$$\textit{Dye factor} = \frac{0.5}{\textit{Titre}}$$

Preparation of sample

10 g of sample was taken and transferred to 250 ml volumetric flask and the volume was made up to the mark with metaphosphoric acid.

Titration

Five ml of metaphosphoric acid extracted sample was taken in an aliquot and titrated with standard dye solution, using phenolphthalein as indicator to a pink coloured end point which persisted for at least for 15 seconds.

Vitamin C content was calculated using the following formula-

$$\text{Vitamin C content (mg/100g sample)} = \frac{T \times D \times V_1}{V_2 \times W} \times 100$$

Where,

T= Titre

D= Dye factor

V_1= Volume made up

V_2= Volume of extract taken for estimation

W= Weight of sample taken for estimation

Firmness

Fruit firmness of squash fruit was measured (Rahman *et al.*, 2014) using a Digital Firmness Tester (DFT 14, Agro-Technologie, France) equipped with 5 mm diameter stainless probe. The tester was checked before use. The plunger of the tester was moved in and out about 10 times to ensure that it was running smoothly. Firmness was reported in kilogram-force cm^{-2} (kg-f-cm^{-2}). Measurements were taken at three different places of each fruit and mean was calculated.

Statistical analysis of data

The recorded quantitative data were analyzed statistically by using MSTAT-C a computer-based program to find out the variation among different treatments, treatment combinations and their interactions. Treatment means were compared by Duncan Multiple Range Test (DMRT).

Results and Discussion

Effect of variety

The result presented in Table 2 showed that significant effect of variety on fruit $plant^{-1}$, individual fruit weight, fruit length, fruit diameter, yield $plant^{-1}$ and yield ha^{-1}. The maximum number of fruits $plant^{-1}$ (5.07), longest fruit (43.87 cm), yield $plant^{-1}$ (3328 g) and yield (33.05 t ha^{-1}) was obtained from V_1 (BARI squash1). The highest individual fruit weight (969.7 g) and fruit diameter (7.63 cm) was obtained from V_2 (Kajol-F_1).

This result under the present study might be due to high genetic variability. Similar results were also observed by Wetzel and Stone (2019) and Esho and Saeed (2017) in squash, and they found significant variation on fruit yield per plant among different varieties of squash.

Table 2. The yield contributing parameters and yield of squash as influenced by different varieties

Variety	Yield contributing parameters and yield					
	Fruit $plant^{-1}$ (no.)	Individual fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Yield $plant^{-1}$ (g)	Yield (t ha^{-1})
V_1	5.07 a	907.3 b	43.87 a	6.85 b	3328 a	33.05 a
V_2	4.78 b	969.7 a	33.77 b	7.63 a	3240 b	32.79 b
LSD (0.05)	0.103	5.711	3.063	0.272	10.76	0.123
CV (%)	7.99	3.85	4.96	4.9	2.70	2.03

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. V_1 = BARI squash1 and V_2 = Kajol-F_1

The effect of variety did not show significant variation on moisture content, total soluble solid (TSS) and firmness of squash but vitamin C content showed significant effect (Table 3). However, results indicated that the highest moisture content (95.64%) and firmness (2.05 kg-f-cm^{-2}) was recorded in V_2 (Kajol-F_1) variety whereas the highest (5.81%) total soluble solid (TSS) was recorded from V_1 (BARI squash1).

The highest vitamin C content (16.34 mg/100 g) was found in the V_1 (BARI squash1) whereas the lowest percentage of vitamin C content (12.71 mg/100 g) was observed in the variety V_2 (Kajol-F_1). The BARI squash1 and Kajol-F_1 cultivars differ significantly in their vitamin C content, which is probably due to inherent genetic variations. High genetic heterogeneity in the biosynthetic pathways and regulatory mechanisms influencing vitamin-C accumulation in the fruit is reflected in these variations.

Table 3. Quality parameters of squash as influenced by different varieties

Variety	Quality parameters and firmness			
	Moisture (%)	TSS (%)	Vitamin C Content (mg/100g)	Firmness (kg-f-cm^{-2})
V_1	94.82	5.81	16.34 a	1.93
V_2	95.64	4.57	12.71 b	2.05
LSD (0.05)	NS	NS	1.079	NS
CV (%)	0.75	1.50	4.49	5.37

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. V_1 = BARI Squash-1 and V_2 = Kajol-F_1

Effect of harvesting date

The result presented in Table 4 showed that significant effect of harvesting date on fruit plant^{-1}, individual fruit weight, fruit length, fruit diameter, yield plant-^{1} and yield ha^{-1}. The maximum number of fruit plant^{-1} (10.49) was found from H_1 (5 days after fruit setting) whereas the minimum number of fruits plant^{-1} (1.88) was found from H_5 (25 days after fruit setting). The highest individual fruit weight (1710 g), longest fruit (53.0 cm) and highest fruit diameter (11.1 cm) were obtained from H_5 (25 days after fruit setting). The maximum yield plant^{-1} (4315 g) and yield ha^{-1} (42.97 t) were found from H_4 (20 days after fruit setting).

The lowest individual fruit weight (194.2 g), shortest fruit (21.5 cm), lowest fruit diameter (4.03 cm), minimum yield plant^{-1} (2010 g) and yield ha^{-1} (20.48 t) were found from H_1 (5 days after fruit setting).

Table 4. Yield contributing parameters and yield of squash as influenced by different harvesting dates

Harvesting date	Yield contributing parameters and yield					
	Fruit plant^{-1} (no.)	Individual fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Yield plant^{-1} (g)	Yield (t ha^{-1})
H_1	10.49 a	194.2 e	21.50 e	4.03 e	2010 e	20.48 e
H_2	5.01 b	595.0 d	32.33 d	5.68 d	3085 d	30.92 d
H_3	3.94 c	920.8 c	39.58 c	6.91 c	3700 b	37.23 b
H_4	3.33 d	1273.0 b	47.67 b	8.47 b	4315 a	42.97 a
H_5	1.88 e	1710.0 a	53.00 a	11.10 a	3310 c	32.98 c
LSD (0.05)	0.478	29.80	2.336	0.430	10.95	0.812
CV (%)	7.99	3.85	4.96	4.9	2.70	2.03

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRTH_1 = 5 days after fruit setting, H_2 = 10 days after fruit setting, H_3 = 15 days after fruit setting, H_4 = 20 days after fruit setting, H_5 = 25 days after fruit setting

The effect of harvesting date did not show significant variation on moisture content and total soluble solid (TSS) but vitamin C content and firmness of squash showed significant effect (Table 5). However, results indicated that the moisture content ranges from 94.37-95.70% and TSS ranges from 4.47%-5.93% from all treatments. The highest vitamin C content (25.95 mg/100 g) was recorded in the H_1 (5 days after fruit setting) and the lowest vitamin C content (5.75 mg/100 g) was found in the H_5 (5 days after fruit setting). The highest firmness (2.95 kg-f-cm^{-2}) was found in H_5 (25 days after fruit setting) and the lowest firmness (1.2 kg-f-cm^{-2}) was found in H_1 (5 days after fruit setting). Vitamin C is heat-sensitive and easily oxidized. Vitamin C is degraded by late harvesting because it is exposed to high temperatures or oxygen.

Table 5. Quality parameters of squash as influenced by different harvesting date

Harvesting date	Quality parameters and firmness			
	Moisture (%)	TSS (%)	Vitamin C Content (mg/100g)	Firmness (kg-f-cm^{-2})
H_1	95.70	5.93	25.95 a	1.20 e
H_2	95.55	5.53	18.98 b	1.47 d
H_3	95.44	5.21	12.47 c	1.85 c
H_4	95.10	4.82	9.48 d	2.48 b
H_5	94.37	4.47	5.75 e	2.95 a
LSD (0.05)	NS	NS	0.791	0.127
CV (%)	0.75	1.50	4.49	5.37

NS = Non-significant.

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. H_1 = 5 days after fruit setting, H_2 = 10 days after fruit setting, H_3 = 15 days after fruit setting, H_4 = 20 days after fruit setting, H_5 = 25 days after fruit setting

Interaction effect of variety and harvesting date

Interaction effect of variety and harvesting date showed significant effect on yield parameters and yield (Table 6). The maximum number of fruits plant^{-1} (10.97) was found from V_1H_1 whereas the minimum number of fruits plant^{-1} (1.78) was found from V_2H_5. The highest individual fruit weight (1827 g) was observed from V_2H_5 and the lowest individual fruit weight (191.7 g) and (196.7 g) were observed from V_2H_1 and V_1H_1 treatments. The longest fruit (59.67 cm) was produced from V_1H_5 and the shortest fruit (22.83 cm) and (20.17 cm) were produced from V_1H_1 and V_2H_1. The highest fruit diameter (11.57 cm) was found from V_2H_5 and the lowest fruit diameter (4.0 cm and 4.07 cm) were observed from V_1H_1 and V_2H_1. The highest yield plant^{-1} (4350 g) and yield ha^{-1} (43.3 t) were obtained found from V_1H_4 and the lowest yield plant^{-1} (1970 g) and yield ha^{-1} (19.93 t) were obtained from V_2H_1.

Variety BARI squash1 harvesting at 20 days after fruit setting (V_1H_4) is sutable for higher yield and Variety BARI squash1 harvesting at 5 days after fruit setting (V_1H_1) is sutable for higher nutritional quality of squash in Bangladesh.

Table 6. Yield contributing parameters and yield of squash as influenced by different variety and harvesting dates

Treatments	Yield contributing parameters and yield					
	Fruit plant^{-1} (no.)	Individual fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Yield plant^{-1} (g)	Yield (t ha^{-1})
V_1H_1	10.97 a	196.7 h	22.83 g	4.00 g	2050 i	21.03 f
V_1H_2	5.03 c	601.7 g	36.67 e	5.47 ef	3120 g	31.43 de
V_1H_3	3.98 d	898.3 f	45.50 c	6.47 e	3750 c	37.07 b
V_1H_4	3.42 de	1247.0 d	54.67 b	7.67 d	4350 a	43.30 a
V_1H_5	1.97 f	1593.0 b	59.67 a	10.63 b	3370 e	32.40 d
V_2H_1	10.02 b	191.7 h	20.17 g	4.07 g	1970 j	19.93 f
V_2H_2	4.98 c	588.3 g	28.00 f	5.90 e	3050 h	30.40 e
V_2H_3	3.90 de	943.3 e	33.67 e	7.37 d	3650 d	37.40 b
V_2H_4	3.23 e	1298.0 c	40.67 d	9.27 c	4280 b	42.63 a
V_2H_5	1.78 f	1827.0 a	46.33 c	11.57 a	3250 f	33.57 c
LSD (0.05)	0.675	42.14	3.304	0.609	15.33	1.148
CV (%)	7.99	3.85	4.96	4.9	2.70	2.03

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. V_1 = BARI squash1, V_2 = Kajol-F_1, H_1 = 5 days after fruit setting, H_2 = 10 days after fruit setting, H_3 = 15 days after fruit setting, H_4 = 20 days after fruit setting, H_5 = 25 days after fruit setting

Table 7 presented the interaction effect of variety and harvesting date had significant effect on total soluble solid (TSS), vitamin C content and firmness.

Table 7. Quality parameters of squash as influenced by different varieties and harvesting dates

Treatments	Quality parameters and firmness			
	Moisture (%)	TSS (%)	Vitamin C Content (mg/100g)	Firmness (kg-f-cm^{-2})
V_1H_1	95.20	6.37 a	29.33 a	1.20 f
V_1H_2	95.13	6.17 a	20.47 c	1.43 e
V_1H_3	95.07	5.92 b	14.13 e	1.80 d
V_1H_4	94.80	5.43 c	11.75 f	2.30 c
V_1H_5	93.90	5.17 d	6.03 h	2.90 a
V_2H_1	96.20	5.50 c	22.57 b	1.20 f
V_2H_2	95.97	4.90 e	17.50 d	1.50 e
V_2H_3	95.80	4.50 f	10.80 f	1.90 d
V_2H_4	95.40	4.20 g	7.20 g	2.67 b
V_2H_5	94.83	3.77 h	5.47 h	3.00 a
LSD (0.05)	NS	0.217	1.118	0.118
CV (%)	0.75	1.50	4.49	5.37

NS = Non-significant. In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. V_1 = BARI squash1, V_2 = Kajol-F_1

H_1 = 5 days after fruit setting, H_2 = 10 days after fruit setting, H_3 = 15 days after fruit setting, H_4 = 20 days after fruit setting, H_5 = 25 days after fruit setting

Moisture (%) did not differ significantly. The highest total soluble solid (6.37% and 6.17%) were recorded from V_1H_1 and V_1H_2 and maximum vitamin C content (29.33 mg/100 g) were recorded from V_1H_1 and the lowest TSS (3.77%) and vitamin C content (5.47 mg/100 g) were recorded from V_2H_5. The highest firmness (3.0 kg-f-cm^{-2}) was found in V_2H_5 and the lowest firmness (1.2 kg-f-cm^{-2}) was found in V_1H_1 and V_2H_1.

Conclusion

Based on the findings, it can be concluded that yield, quality and yield contributing characters of squash can be effectively manipulated by different harvesting date and variety. Fruit harvesting 5 days after fruit setting produces the higher number of fruits plant^{-1} and vitamin C content. Among the tested varieties, BARI squash1 consistently outperformed Kajol-F_1 in terms of both yield and quality. Therefore, for optimal squash production, BARI Squash-1 is recommended, with harvesting schedules tailored according to the desired priority early harvest for nutrition, or later harvest for yield. However, further experimentation need to be executed in different agro-ecological zones with more varieties.

Author's contribution

The study conception, formulation of the research program, provision of materials, statistical data analysis, preparation of tables and graphs, manuscript editing, and research project funding were carried out by M A. Hossen and M. N. Islam. Fieldwork, chemical analyses, and data collection were conducted by M. A. Hosesn, S. Akther and H. M. Rashid. The initial draft of the manuscript was prepared by M. Al-Amin. A. A. Sabuz performed the postharvest test and analysis. All authors reviewed and approved the final version of the manuscript.

Conflicts of Interest

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

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PRODUCTION, NUTRITIONAL EVALUATION OF JACKFRUIT JAM AND DEVELOPMENT OF NEW PRODUCT BY USING JACKFRUIT JAM

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Abstract

To produce and characterize jackfruit (*Artocarpus heterophyllus*) jam by the extraction of pulps from collected jackfruits was conducted June to December in 2019 at Department of Applied Chemistry and Chemical Technology, Chittagong Veterinary and Animal Sciences University, Khulshi-4225, Chattogram, Bangladesh. Pre-prepared lemon juice, ascorbic acid, pectin, color, citric acid, potassium metabisulfite, and sugar were added to the pulp. After heating and stirring, the mixture reached a 69% brix sugar content and appropriate consistency. Cake making used wheat flour, xanthan gum, caster sugar, salt and other food grade ingredients. Freshly made jam was injected into the cake to make a jackfruit jam cake. Protein (3.80%), carbohydrate (17.83%), ash (0.60%), crude fat (1.90%), crude fiber (0.95%), moisture content (74.89%), pH (5.20), vitamin C (6.23 mg/100g), and total acidity (0.07 g/100g) were found in fresh jackfruit. On the other hand, protein was 3.40% and carbohydrate 59.97% in prepared jackfruit jam. The crude fat content was 1.97% and the ash 0.45%. Jam moisture was 33.40% and crude fiber was 0.80%. The jam also had 13 mg of vitamin C per 100g. The jam's pH was 3.80 and its total acidity 0.43 g per 100g. A sensory and general acceptance test was performed on 10 untrained individuals. The tester used a 7-point hedonic scale to compare the jam cake to a commercial cake. Jackfruit and commercial mango jam differed in color, taste, and sweetness, according to testing. There was significant variation in hedonic scores between the four groups ($p<0.05$). This study reveals that jackfruit has high sensory qualities, making it appropriate for jams and other processed foods and increasing its market value.

Keyword: Cake, Jackfruit, Jam, Mineral, Proximate composition.

Introduction

The jackfruit (*Artocarpus heterophyllusLam.*) is the largest tree fruit belongs to Moraceae family. It may weigh up to 50 kg and be 60–90 cm long. There exist two primary types of jackfruit, the first type of jackfruit is small, fibrous, squishy, and mushy, with carpels that taste sweet like raw oysters. The second type is crisp and crunchy taste lacks sweetness. Despite its difficult digestion, this non-leguminous plant's seeds are edible (Siddappa, 1957). The seed's white aril encloses a thin brown spermoderm and a succulent

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white cotyledon. The cotyledons of the jackfruit exhibit a notable abundance of carbohydrate and protein content (Singh et al., 1991). Among the fruits cultivated in Bangladesh, the jackfruit has the third position in terms of area under cultivation and the second position in terms of output. A total land area of 17981.8 acres is dedicated to the cultivation of jackfruit, resulting in an annual production of 145316.82 M. tons in the nation (BBS, 2023). Jackfruit contains vitamin A, C, thiamin, riboflavin, calcium, potassium, iron, sodium, zinc, and niacin. Jackfruit has 94 calories per 100g. (Mukprasirt and Sajjaanantakul, 2004) The potassium content of jackfruit is 303 mg per 100 g. Jackfruit is a good source of vitamin C, an antioxidant and maintains oral health (Jagtap *et al.*, 2010). The processing of jackfruit into a pureed form allows for its utilization in several applications, including baby food, juice, jam, jelly, and as the basis for cordials (Susanta K. Roy and G. D. Joshi, 1995). The ripe perianth of the jackfruit yields around 2 MJ of energy per kg. The seeds of the jackfruit include a significant quantity of starch.(Singh *et al.*, 1991)Mature jackfruits are used in vegetable-based curries and salads. Ripe fruits may be eaten raw or cooked with creamy coconut milk to make a delicious dessert. Jackfruits are used to make desserts, fruit-rolls, marmalades, and ice cream. Freeze-drying, vacuum-frying, and cryogenic processing are new ways to preserve jackfruit-derived products. Several jackfruit tree components are used in medicine, and its wood is valued in the timber industry (Susanta K. Roy and G. D. Joshi, 1995).The bulbs (except the seeds) provide high sugar, moderate carotene, and vitamin C (Bhatia et al., 1955). Due to limited postharvest skill in harvesting, shipping, and storage, both quality and quantity of jackfruit, especially during glut season (June-July), is squandered every year. Therefore, postharvest technology must be used to enhance shelf life. The pulp and juice of a single fruit, including the whole fruit, are used to make jam. Boiling and gelling fruit and packing it for long-term storage is called "fruit preserves". These preserves are used in toast spreads, fillings, and jellies. Good jam is smooth and free of fruit pieces. It has a vivid color, delicious taste, and a semi-gelled consistency that spreads easily without extra liquid (NC *et al.*, 2014). The presence of pectin in jackfruit renders it a suitable candidate for the production of jam. As such the objective is to create a novel food product utilizing jackfruit jam that is well-suited for large-scale manufacturing.

Materials and Methods

The study was conducted from June to December in 2019 at Department of Applied Chemistry and Chemical Technology, Chittagong Veterinary and Animal Sciences University, Khulshi-4225, Chattogram, Bangladesh. The process of product development and subsequent laboratory analysis was conducted at the Department of Applied Chemistry and Chemical Technology and Food Processing and Engineering CVASU, Chittagong, Bangladesh as well as Dept. Of Applied Nutrition and Food Technology, IU, Kushtia-7003, Bangladesh. The entire study was divided into three major categories: production of jackfruit jam, nutritional evaluation of jackfruit jam and jackfruit, to develop a new variety food product by using jackfruit jam and shelf-life observation. Three fresh ripe jackfruits (*Artocarpus heterophyllus Lam*) were purchased from Reazuddin Bazar, New Market, Chattogram in June 2019. Sugar, Flour, Whole Egg, Oil, Lemons, Plastic Basin, Muslin Cloth commercial mango jam and Sieves were all purchased from Khulshi Mart, West Khulshi, Chattogram. Jam bottles were purchased

from RFL plastic, Pahartoli, Chattogram. Cake Gel (Finagel),Cake Mix(Foster Clark's), Glycerin(Sigma-Aldrich), Sorbitol (Spicy World), Vanilla Flavor(Foster Clark's), SAPP, SBC(Foster Clark's), Liquid Glucose, Potassium Sorbet (Purix), Citric Acid (LD Carlson), Ascorbic Acid (Scharlab), Pectin & Color were purchased from HS Scientific, Hatkhola, Dhaka.

Crude protein, moisture content, dietary fiber, crude fat, ash content, carbohydrate, minerals content, vitamin C, pH, total titratable acidity were determined of jackfruit and jackfruit jam. A new variety food product was developed by using jackfruit jam. In this study jackfruit jam cake was developed and prepared jack-fruit jam was observed the shelf life about three months. The study employed a Complete Randomized Design (CRD) using the Statistical Package for the Social Sciences (SPSS) software.

Jam preparation

The fresh and ripe jackfruits were thoroughly washed with tap water to remove dirt. Then, the jackfruits were sliced diagonally to separate the new bulbs from the seeds and other unwanted parts. Jackfruit bulbs, weighing 4.128 kg, were boiled in 3 liters of water for 10 minutes. This technique softened the mixture for simpler homogeneity. The mixture was blended with a fruit grinder after boiling. The mixture was sieved with a 2mm mesh sieve. After quantifying jackfruit pulp, it weighed 7 kg. Pulp was mixed with 3.769 kg sugar and 10 ml lemon juice. Lemon juice added acidity to lower the pH and boost pectin in the jam. The following equation determined how much sugar was needed in the fruit pulp to make jam:

$$Sugar\ to\ added = \frac{TSS(Final) - TSS(Pulp)}{100} \times W$$

Where, TSS (final) - is the preferred sugar concentration for jam production, which is 69%; TSS (pulp) - pulp sugar level and W- utilized pulp weight (in grams).

The juice combination underwent the process of cooking on a gas cooker until the brix level reached 69^0. In the cooking time 15 gram citric acid, 15 gram pectin, 5 gram ascorbic acid, 0.5 gram egg yellow color and 5 gram potassium metabisulfite was added for the production of jackfruit jam. Subsequently, the heated jam was carefully transferred into sterilized containers, securely sealed with lids, and allowed to cool naturally to ambient temperature (Molla *et al.*, 2011).

Cake preparation

Before making the cake, Custer Sugar 26.01%, Common salt 0.193%, and Whole egg 14.86% were combined in the mixer for 10 minutes at high speed. Xanthan gum 0.119%, liquid glucose 1.13%, potassium sorbet 0.595%, cake mix 1.486%, cake gel 0.446%, glycerin 1.486%, sorbitol 0.743%, vanilla flavor 0.193%, and cold water 7.40% were added to the mixer and mixed for 10 minutes at high speed. Wheat flour 31.51% (Gluten 8-9%), Sodium acid phosphate 0.312%, Sodium bicarbonate 0.104%, and Palm oil 13.38% were added to the mixer and mixed for 1 minute at medium speed. The specific gravity ranged from 0.55 to 0.65. For 30 minutes, the tunnel oven cooked the cake batter at 180 degrees Celsius. After baking, cake chilled for 20 minutes. Finally, cake was taken

from cup and kept at room temperature. The injector filled jackfruit jam into the cake after preparation. Then jackfruit jam cake was packaged.

Determination of Crude Protein (CP): The macro Kjeldahl method, per AOAC 920.87, was used to measure crude protein in fresh jackfruit and jackfruit jam. 1 g of each sample and a blank were put in a 100 mL Kjeldahl digestion tube. 2 g of Kjeldahl catalyst and five ml of conc. sulphuric acid were added to each tube. The materials were digested to generate a blue solution. The digestion process was prolonged to release nitrogen from the heterocyclic ring. After chilling the digest, 20 ml of distilled water was added to dissolve it. The diluted solution was macro-distilled using the Kjeltec^{TM}8200 Auto Distillation Unit (2012). To increase ammonia release, 50 ml of 40% sodium hydroxide solution was added to the digest. Ammonia was extracted by steam distillation and collected in a 50 ml flask with 4% boric acid. Using bromocresol green and methyl red as indicators, the distillate was titrated with 0.1520 N HCl.

The nitrogen content was calculated using the formula:

$$\%\ Nitrogen = \frac{(Titre\ blank)\ in\ ml \times Conc.of\ acid\ N/mol}{Weight\ of\ sample\ (gm)} \times 100$$

The following calculation uses factor 6.25, the nitrogen percentage, to calculate plant protein content:

$$\%\ CP = \%N \times Gbdups\ (6.25)$$

Determination of Moisture Content: Method number 925.09 from the AOAC (1995) standards measured the moisture content of fresh jackfruit and jam.(AOAC, 1995) Each sample was weighed in the crucible at around 2 g. The specimen was equally dispersed in the crucible and dried in a 105ºC oven for 48 hours. After that, the specimen was carefully placed in a desiccator to cool. Crucibles were reweighed. The formula below calculated the percentage of moisture content:

$$\%\ Moisture\ Content = \frac{W1-W2}{W1} \times 100$$

Whereby;

W_1 is the sample's original weight in gram before drying, and W_2 is its final weight.

Determination of Dietary Fiber: The dietary fiber content of fresh jackfruit and jackfruit jam was measured using method 920.86. 1 g of each sample was extracted to determine crude fiber content using the Fibertec^{TM}1020 FOSS model 2012. After 30 minutes with dilute H_2SO_4 (0.125M), the samples were digested with hot water. After 30 minutes of digestion with diluted alkali (0.125M KOH), the leftover product was rinsed three times with hot water. The leftover material was desiccated in an oven for 5 hours, cooled, and weighed. The leftover material was incinerated at 525°C for 2 hours in a muffle furnace. After incineration, the material cooled and was weighed again. The equation was used to calculate fiber content:

$$\%\ Crude\ Fibre = \frac{W1(g)-W2(g)}{W1(g)} \times 100$$

Where;

W1 and W2 are the sample residue's weights (g) before and after incineration.

Additionally, the dry sample for determination (W) is weighed (g).

Determination of Crude Fat: Fresh jackfruit and jackfruit jam crude fat content was measured using Soxtec System AOAC (1995) method 920.65, which entailed ether extraction.(AOAC, 1995) About 6 g of pre-dried materials were measured and placed in an extraction thimble. Thimbles were wrapped in fat-free cotton and placed in the Soxtec device's midsection. After drying and weighing the cups, 60 ml of petroleum ether petroleum spirit (40-60ºC) was added. It took around an hour to remove the cups in the Soxtec extractor. After extraction, the fat extract cups were dried in a 105oC oven for 30 minutes. After cooling in desiccators for 30 minutes, the cups were weighed. Crude fat % was calculated using an equation:

$$\% \, Crude \, Fat = \frac{\boldsymbol{Weight \, of \, Crude \, Fat(gm)}}{\boldsymbol{Weight \, of \, Dry \, Sample(gm)}} \times \mathbf{100}$$

Determination of Ash Content: Ash content was measured in fresh jackfruit and jackfruit jam samples using a muffle furnace according to AOAC standard method 923.03.(AOAC, 1995) About 5 g of each sample were placed in triplicate in a pre-weighed crucible and dried in a 105ºC oven for 48 hours. The dried samples were weighed and heated in a muffle furnace at 550º Celsius for 3 hours to generate a white or grey ash residue. After desiccating the samples to ambient temperature, their weights were assessed. Ash % was calculated using this equation:

$$\% \, Ash \, Content = \frac{\boldsymbol{Weight \, of \, Ash(gm)}}{\boldsymbol{Weight \, of \, Sample(gm)}} \times \mathbf{100}$$

Determination of Carbohydrate (CHO): According to the Association of Official Analytical Chemists (AOAC, 1995), fresh jackfruit and jam have different carbohydrate content percentages.(AOAC, 1995) This equation shows the formula:

%CHO = 100 -(%Protein+%Moisture+%Crude fat %+Crude fiber+% Ash content)

Determination of Minerals Content: The Unicam 919 Atomic Absorption Spectrophotometer was used to assess fresh jackfruit and jam for minerals, under AOAC (1995) Official technique number 968.08.(AOAC, 1995) The test parts were dried and ashed at 450°C, rising by 50°C each hour. Association of Official Analytical Chemists (AOAC, 1995) method was used to analyze mineralized ash. The ash sample was dissolved in 20 ml of 1N hydrochloric acid (HCl) and heated at 70°C for 5 minutes. The solute was then accurately transferred to a 100-ml volumetric flask and filled with distilled water to capacity. Calcium, sodium, iron, zinc, and potassium were measured using the Atomic Absorption Spectrophotometer technique in technique 968.08. After preparation, the samples were taken to the Bangladesh Council of Scientific and Industrial Research (BCSIR) lab in Dhaka. Absorbance was measured for sample and reference solutions. A standard curve graphic showed the connection between absorbance and standard solution concentration to calculate mineral content in unknown materials. Following the equation, the concentrations were represented.

$$\% \, Mineral \, Content \, mg/100gm = \frac{\boldsymbol{R \times Extract \, volume \, (l)}}{\boldsymbol{S \, (kg)}} \times \mathbf{100}$$

Where,

R = The mineral concentration in ppm or mg/Kg calculated using a linear regression formula, D.F = Dilution Factor and S = sample weight (Kg)

Determination of itamin C

Vitamin C concentration in fresh jackfruit bulb and jam was measured using the 2, 6-Dichlorophenol indophenols (DCIP) sodium salt, following method 967.21.This experiment used a phosphoric acid/acetic acid solution to ensure the right acidity (pH 1-3) and prevent acid oxidation. Two 5g samples of pulverized jackfruit and jam were put in 250ml Erlenmeyer flasks. Orthophosphoric acid (50 mL) was added to the extraction procedure to lower pH and remove proteins. After filtering, the extracted samples were titrated with standardized Dichlorophenol indophenols until they became pink, indicating reduction. Dichlorophenol indophenol volume was used to calculate vitamin C levels in samples using a particular calculation:

$$Mg\ of\ Ascorbic\ Acid = (X\text{-}B) \times (V/Y) \times (F/E)$$

Where,

X=titrate value, B= blank, V = initial assay solution volume, Y=volume of sample aliquot titrated. F=Amount of ascorbic acid equivalent to 1.0 milliliter of indophenols (mg) and E = number of ml assayed.

Determination of Total Titratable Acidity: Total titratable acidity (TTA) was determined in jackfruit pulp and jam using the AOAC (1995) method 942.15 and 920.49 standard procedures.(AOAC, 1995) 0.1 M NaOH standard solution was titrated against a 5 ml sample of diluted jackfruit pulp and jam diluted to 250 ml with hot water. AOAC (2000) recommended titrating with 0.3 ml of phenolphthalein indicator per 100 ml of solution until a pink end point lasted 30 seconds. The following equation calculated reported acidity in milliliters of 0.1N NaOH per 100 ml:

$$Total\ Titratable\ Acidity\ gm/100gm = \frac{\boldsymbol{Titrate\ Volume \times N}}{\boldsymbol{Sample\ Weight}} \times \mathbf{100}$$

Where, N represents the normality of the alkali solution used.

Determination of pH: The pH of a solution is defined as the logarithm, to the base 10, of the reciprocal of the hydrogen ion activity in the solution (McClements & Decker, 2009). P^H is the most important parameter for food product quality and safety control. The pH measurement of aqueous solutions was conducted using a glass electrode included in a pH meter (HI2210).

Sensory Evaluation for Jackfruit Jam and Jam Cake: Developed jam and control jam as well as developed jam cake and commercial cake as control cake samples were subjected to sensory evaluation using a 7-point hedonic scale ranging (1= dislike very much, 2=Dislike moderately, 3=Dislike slightly, 4=Neither like nor-dislike, 5=Like slightly, 6=Like moderately, 7=Like very much). Ten consumer panelist members were selected randomly where commercial mango jam was used as a control. The jam samples were evaluated for color, texture, flavor, taste, spreadability, sweetness, and overall acceptability. In contrast, cake evaluations included appearance, taste, texture, mouth feel,

sweetness, flavor, and overall acceptance.

Yeast and mold count of Jackfruit Jam: Yeast and mold (CFU) were counted initially and at the end of the storage period using standard plate count (SPC) method. Potato Dextrose Agar Media was used by pour plate method (Rana et al., 2021).

ANOVA was performed to investigate the mean hedonic score of overall acceptability among the developed jam cake, commercial cake, jackfruit jam and commercial mango jam. Independent t- test also performed to investigate the mean hedonic score of all sensory attributes separately between jackfruit jam and commercial mango jam as well as jackfruit jam cake and commercial cake by IBM SPSS windows version 21.

Results and Discussion

Proximate composition of Jackfruit and Jackfruit Jam Table 1 shows the comparison of Jackfruit and jam. The raw jackfruit has 17.83% carbohydrate, whereas the jam had 59.97%. Since processed carbs have less moisture than unprocessed ones. This study was found a little resemblance to another previous research, which found 13.92, 30.90, and 48.48% for fresh jackfruit, jackfruit jam, and pineapple jam (Eke-Ejiofor & Owuno, 2013). Sugar contributes to jams' high carbohydrate content. Fresh jackfruit has 74.89% moisture, whereas jam has 33.40%. The jam had the lowest moisture level. Sugar and heat during jam-making evaporate moisture, resulting in a moisture difference between processed and untreated jackfruit. Eke-Ejiofor and Owuno was found 24.60% moisture in jackfruit jam, 23.29% in pineapple jam, and 73.60% in fresh jackfruit. According to the Food and Agriculture Organization (FAO) and World Food Programme (WFP), jam made from stone fruits including apricots, peaches, and others had 29.6% moisture in 1970. The investigation was found that fresh jackfruit had 3.80% protein and jackfruit jam 3.40%. Fresh jackfruit has 1.90% fat, whereas jam had 1.97%. Fresh jackfruit contained slightly more fat than strawberry, blueberry, and grape jams, which had 0.01% to 0.03% fat (Naeem *et al.,* 2017). This implies jackfruit jam is low in fat also found that both jam and fresh jackfruit samples, which is good for weight loss and health. The ash percentage in jackfruit jam was 0.45%, whereas fresh jackfruit was 0.60%. This investigation confirms Eke-Ejiofor and Owuno (2013) findings on jackfruit jam (0.27%) and fresh jackfruit (0.43%) (Eke-Ejiofor and Owuno, 2013). Haque *et al.* (2009) also found ash amounts in fresh fruits between 0.053% to 0.902%.Fresh jackfruit has 0.95% crude fiber. Fresh jackfruit and jam had pH readings of 5.20 and 3.80, respectively. Fresh jackfruit, jackfruit jam, and pineapple jam have pH values of 5.57, 3.36, and 3.35, respectively, according to previous findings.(J, 2013) Jam pH is crucial to gel consistency. Low food pH inhibits microbiological growth. Fresh jackfruit has 0.07 g/100g of titratable acidity, whereas jam has 0.43. The addition of lemon juice and citric acid during jam-making increased its acidity. High acidity in processed foods indicates long storage. Acidity is also important for jam equilibrium. The research showed 23.70% TSS in fresh jackfruit which was 69.0% higher in processed jackfruit jam. Fresh jackfruit has a brix value of 23%, whereas jam had 40%.(Eke-Ejiofor, 2013)The vitamin C level of fresh jackfruit was 6.23 mg per 100 grams, whereas jackfruit jam was 13.00 mg. The combination of lemon juice and ascorbic acid during jam preparation was expected to increase vitamin C content. Goswami *et al.* (2011) was found vitamin C values of 8.18- 4.57 mg/100g in fresh jackfruit from different varieties. The

Jackfruit jam included calcium (29.57 mg/100g), sodium (6.98 mg/100g), potassium (256.72 mg/100g), zinc (0.21 mg/100g), and iron (0.31 mg/100g). The difference in mineral contents between fresh jackfruit and jam may be due to processing.

Table 1. Proximate Nutrient Composition of Jackfruit and Jackfruit Jam per 100 gm

Nutrients	Jackfruit	Jackfruit jam
Moisture (gm)	74.89	33.40
Crude protein (gm)	3.8	3.40
Crude fiber (gm)	0.95	0.80
Ash content (gm)	0.60	0.45
Crude fat (gm)	1.90	1.97
Carbohydrate (gm)	17.83	59.97
TSS (gm)	23.70	69.00
Total Titratable Acidity (gm)	0.07	0.43
P^H	5.20	3.80
Vitamin C (mg)	6.23	13.00
Calcium (mg)	30.87	29.57
Sodium (mg)	8.34	6.98
Iron (mg)	0.54	0.31
Zinc (mg)	0.32	0.21
Potassium (mg)	259.14	256.72

Sensory Evaluation for Jackfruit Jam and Jam-Cake: One-way ANOVA was performed to investigate the mean hedonic score of overall acceptability between groups. There was a statistically significant difference at the $p<0.05$ level in hedonic scores for the four groups: $F(3, 36) = 6.62$, $p=0.001$. Post-hoc comparisons using the Tukey HSD indicated that the mean hedonic score for jackfruit jam (M=5.9, SD=0.73), Commercial mango jam (M=5.0, SD=0.66), jackfruit jam-cake (M=6.1, SD=0.73) and commercial cake (M=5.2, SD=0.42). Independent t- test was used to investigate the statistically significant difference of mean hedonic scores of various sensory attributes between groups, individually. The mean differences of color $t(18)=3$, $p=0.001$; aroma $t(18)=2.27$, $p=0.035$; taste $t(18)=2.4$, $p=0.025$were found significantly high in developed jackfruit jam than the commercial mango jam but spreadability $t(18)=1.34$, $p=.19$; sweetness $t(18)=1.1$, $p=0.27$; and texture $t(18)=1.15$, $p=0.26$were not significantly different between jack-fruit jam and commercial mango-jam as well as the mean value of appearance $t(18)=2.3$, $p=0.03$; aroma $t(18)=2.1$, $p=0.04$; taste $t(18)=2.7$, $p=.01$; were also found significantly high in Jackfruit jam-cake than the commercial cake, on the other hand mouthful $t(18)=1.34$, $p=0.19$; sweetness $t(18)=1.3$, $p=0.19$;and texture $t(18)=1.15$, $p=0.26$were not significantly different between developed jam-cake and commercial cake. There was also no statistically significant difference in the mean hedonic scores of freshly prepared jam, and both ambient storage as well as refrigerator stored jam at the $p<0.05$ level. (Table-2).

Table 2. Sensory evaluation of Jackfruit Jam during storage

Particulars	Freshly prepared (0 day) (Mean±SD)	During storage (240 days)	
		Ambient Temp. (Mean±SD)	Refrigerated Temp. (Mean±SD)
Color	5.8±0.42	5.1±0.56	5.7±0.67
Aroma	5.9±0.73	5.8±0.63	5.9±0.73
Taste	6.1±0.56	5.5±0.52	5.7±0.82
Spreadability	5.1±0.56	5.2±0.42	5.1±0.73
Sweetness	5.7±0.67	5.4±0.51	5.8±0.78
Texture	5.1±0.56	5.3±0.48	5.2±0.63
Overall acceptability	5.6±0.51	5.3±0.48	5.5±0.52

Yeast and mold count of Jackfruit Jam: Developed jam was packaged using glass jar and stored in both ambient and refrigerated temperature for shelf-life evaluation. No growth of yeast and molds was observed at the beginning and end of 240 days in ambient as well as refrigerated temperature (Table -3).

Table 3. Yeast and mold count of Jackfruit Jam

Yeast and mold count (CFU/mL) in jackfruit jam			
Ambient temperature		Refrigerated temperature	
0 days	240 days	0 days	240 days
0	0	0	0

Conclusions

Jam is a popular food product in ready-to-eat foods. Based on the findings of the study, it can be inferred that jackfruit possesses a substantial amount of essential nutrients, encompassing both macro and micronutrients, even after undergoing processing into jam. The nutritional composition of Jam is of considerable importance for human growth, since it contains essential components such as total soluble solids and ascorbic acid content. The products were observed to maintain stability during storage at both ambient temperature and refrigeration for a duration of 8 months, as shown by the lack of significant changes in physico-chemical, sensory, and microbiological indices. The potential economic viability of using jackfruit jam into cake recipes is worthful. This is an opportunity to investigate the potential for creating additional value-added food items as a means of preserving the fruit during periods of low production and mitigating post-harvest losses. This study also reveals that jackfruit has high sensory qualities, making it appropriate for jams and other processed foods and increasing its market value. Therefore, this research endeavor has the potential to establish a novel domain for fortifying the food industry, therefore addressing the issues of unemployment and malnutrition in Bangladesh.

Author's contribution

M. M. Rahman and S. M. F. JINNAH executing and investigating of the research, M. M. Rahman and S.A. Rahman writing-review, editing and formal data analysis the original manuscript.

Conflicts of Interest

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

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EFFECTS OF URBANIZATION ON HOMESTEAD SIZE AND TREE SPECIES DIVERSITY AMONG FARMERS IN CUMILLA DISTRICT

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Abstract

Modernization plays a crucial role in socio-economic development globally and in Bangladesh. Agroforestry practices are increasingly adopted to support sustainable livelihoods. This study examines the impact of urbanization on homestead size and tree species diversity in Debidwar Upazila, Cumilla district. Data were collected through structured interviews with 90 randomly selected farmers from six villages between January and June 2018. Results indicate that most respondents in traditional and semi-modern areas were middle-aged with secondary education, while modern areas had more individuals with education beyond the secondary level. Family sizes were mostly medium (4–6 members), and income levels were moderate across all areas. Agriculture remained the primary occupation in traditional and semi-modern zones, whereas service sector jobs dominated in modern areas. Homestead sizes were generally medium in traditional and semi-modern areas but smaller in modern areas. Traditional agroforestry systems supported greater tree species diversity (18 species) compared to modern areas (11 species). Common tree species included mango (630), coconut (311), betel nut (240), and jackfruit (206). Key challenges such as transportation issues, unemployment, and economic difficulties were prevalent. Urbanization showed significant positive correlations with education, occupation, income, homestead size, tree species, and local problems, but no significant correlation with age or family size. The study concludes that urbanization has led to a notable reduction in homestead size and tree species diversity in the region.

Keywords: Agroforestry, Homestead size, Socio-economic development, Tree species diversity, Urbanization.

Introduction

Homesteads serve as small-scale farms, producing fruits, vegetables, and livestock, ensuring food security for rural households (Miah and Hussain 2010). Homestead is an age-old and traditional land use system with protection and production

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functions, contributing particularly to the food and nutrition security of smallholders (Vieira *et al.,* 2012). Trees grown in homesteads provide timber, bamboo, and firewood, fulfilling a large portion of the national demand. Homesteads also act as biodiversity hotspots, preserving native plant species and supporting ecological balance (Nath *et al*., 2014). Homesteads integrate multi-strata farming systems, combining trees, crops, and livestock to maximize land use (CGIAR, 2023). They support organic farming, reducing reliance on chemical fertilizers and pesticides.

Bangladesh is a densely populated agricultural-based country with about 68% of people living in rural areas (BBS, 2022). As of 2025, the population of Bangladesh is estimated to be 175.7 million with a population density of 1,350 people per square kilometer. Though the population is increasing every year, the population growth rate has been gradually declining over the years. Due to the rapid growth of population and the urbanization of rural areas, farm size has declined. Land fragmentation and declining farm size are critical problems that smallholder farmers are facing in maintaining traditional farming practices (Headey *et al.,* 2014).

In 2022, Bangladesh had approximately 32.07 million homesteads, covering roughly 0.27 million hectares of land (BBS, 2022). In Bangladesh, homesteads represent a well-established land use system where natural forests cover less than 10% of homestead gardens which are maintained by at least 20 million households, and represent one possible strategy for the conservation of biodiversity (Roy *et al*., 2012). Urbanization is defined as the demographic process whereby an increasing share of the national population lives within urban settlements (Potts, 2012).

Debidwar Upazila in the Cumila district is not the most urbanized. Rather, in the Cumilla district, Brahmanpara Upazila is recognized as one of the upazilas in the process of significant urbanization. Moreover, the environment of the upazila is at threat due to the establishment of a huge number of modern residential areas and different kinds of business structures such as garments, poultry, leather, shops, etc. The area of modern homesteads is becoming smaller to smaller day by day with no or little vegetation that affects livelihood as well as biodiversity (Ruba and Talucder, 2023). Homestead plays a vital role in the existence of rural people, providing them with food, fuel, fodder, timber, fish, and shelter. Homestead production is the most significant system of livelihood in rural areas. Its management affects the production, consumption, sale, and repurchase of field crops, livestock, fish, fruits, fuels, etc. The rural economy thus depends on the productivity of the natural resources, which is intimately linked with the biodiversity in the ecosystem (Khan *et al.,* 2020). Considering the situation mentioned above, the study was conducted to fulfill the following objectives to find out the change of homestead size due to urbanization and to assess the change of tree species due to urbanization.

Materials and Methods

Experimental site and Population sample

The study was conducted in three villages of Debidwar Upazila, Cumilla: Baniapara (Debidwar Sadar Union), Bankot-Padmokot (Dakshin Gunaighar Union), and Barashalghar-Choto Shalghar (Bara Shalghar Union), comprising a total of 350 farm

households. Within this Upazila, three samples Modern, Semi-modern, and Traditional were randomly selected under Sadar Upazila for data collection. A random sample of 90 households was selected, with an additional 15 households reserved as substitutes to ensure complete data collection. A detailed population structure and sample are presented in Table 1. Households were categorized into Modern, Semi-modern, and Traditional settlement types based on observable infrastructural, economic, and social characteristics, guided by local administrative insights and field observations.

Debidwar Upazila was purposively selected for this study due to its dynamic and transitional nature in terms of rural-urban transformation. Situated in the rapidly developing Cumilla District, Debidwar exhibits diverse settlement patterns and is undergoing notable infrastructural expansion and socio-economic change. This diversity makes it an ideal microcosm for examining urbanization trends in peri-urban and rural Bangladesh. The Upazila's mix of traditional villages, semi-modernizing communities, and emerging modern settlements provides a unique opportunity to study the continuum of urbanization and its implications on household livelihoods, land use, and service accessibility. Therefore, the area offers both representativeness and relevance for understanding broader patterns of rural transformation across similar regions in the country.

Table 1. Distribution of population and sample of the selected villages

Upazila	Union	Village	Population (Families)	Sample size	Reserved list
Debidwar	Debidwar Sador	Baniapara, Debidwar	132	30	5
	Dakshin gunaighar	Bankot, Padmokot	108	30	5
	Bara shalghar	Barashalghar, Choto Shalghar	110	30	5
Total			350	90	15

Variables and their measurement techniques

In a descriptive social study, variable selection and measurement are important. This study included both independent variables (e.g., age, education, family size, occupation, income from homestead, tree species, and problems caused by urbanization) and one dependent variable (effect of urbanization on homestead size). Independent variables are those presumed to influence or predict change, while the dependent variable reflects the observed outcome affected by variations in the independent variables.

Measurement of independent variables

Age: The respondents were classified into three categories: young (up to 35 yr), middle (36-50 yr), and old age (above 50 yr). Education: The education of respondents was classified under illiterate (0), sign only (0.5), Primary education (1-5), Secondary education (6-10), higher secondary (10-12), and above secondary (> 10). Family size: The family size

of the respondents was classified into three categories- small (up to 3), medium (4 to 6), and large family (> 6), respectively. Occupation: The occupation was classified as 1, 2, and 3 as agriculture, business, and service, respectively. Homestead farm size: Based on their farm size, the farmers were classified into three categories, followed as landless/marginal (up to 0.08), Small (0.09 - 0.14), Medium (0.15 - 0.20), and Large (>0.21), respectively. Income from homestead: Farmers were categorized into three groups based on their annual family income from homestead agroforestry: low income (up to 20,000 BDT), medium income (21,000 to 40,000 BDT), and high income (above 40,000 BDT). Tree species: Tree species were measured by counting the total species from each homestead, and total tree abundance was also measured from each homestead. Problems: Problems faced by the respondents from the scoring of some selected problems. Seven problems were selected, and scoring was given 2 marks for each problem, and the total was counted for each individual.

Measurement of the dependent variable

The dependent variable in this study was the effect of urbanization on homestead size.

Data collection and compilation

Primary data were collected by the researcher from January 15 to June 15, 2018, through face-to-face interviews conducted at respondents' homes and farms. Before each interview, the study's purpose was clearly explained to ensure accurate and honest responses. The relationship was established to reduce hesitation. The Sub-Assistant Agricultural Officer (SAAO) of DAE assisted in organizing respondent appointments. Collected data were coded, standardized, and transferred to a master sheet for tabulation and analysis in line with the study objectives. Although the data were collected seven years ago (January–June 2018), they remain relevant for the research objectives, which focus on assessing rural–urban transformation and associated socio-economic dynamics. Urbanization processes and rural livelihood transitions tend to evolve gradually, and the patterns observed during the study period still reflect key structural characteristics of the area. Thus, the 2018 dataset offers a credible and valuable empirical foundation for understanding ongoing developmental trajectories and informing policy discussions in similar rural and peri-urban contexts in Bangladesh.

Statistical analysis

The data collected were analyzed, coded, transferred from the interview schedule to a master sheet, summarized, categorized, and entered into a database using Microsoft Excel 2019. The data were analyzed using SPSS (Version 21.0) which was used to perform all statistical analyses. To explore the effect of urbanization on homestead size and tree species performed by the respondents and their selected characteristics, Pearson's Product-Moment Correlation Co-efficient (r) was used (Ray and Mondal, 2004).

Results and Discussion

Age

The age of the respondents varied from 25 to 61 years, the average being 41.03 years with a standard deviation of 11.88 for the traditional areas by farmers. Again, for semi-modern area farmers, the age of the respondents varied from 24 to 60 years, the average being 40.47 years with a standard deviation of 9.66. In terms of modern area practices by farmers, the age of the respondents varied from 26 to 58 years, the average being 39.67 years with a standard deviation of 9.57 (Table 2). Young respondents (≤35 years) were more common in semi-modern and modern areas (36.67%) than in the traditional area (26.67%), with the lowest average age in the modern area (39.67 years). Middle-aged respondents (36–50 years) were the largest group, especially in the traditional area (53.33%). Older respondents (>50 years) were few, the highest in traditional (20.00%) and lowest in semi-modern areas (13.33%). Dutta *et al*., (2023) reported that age is an important factor regarding knowledge because age has a significant positive correlation with the cultivation of lemon production.

Education

The education level of the respondents ranged from 0-15 for each of the homestead categories (traditional, semi-modern, and modern) following the year of schooling. The average education score of the respondents was 5.93, 5.45, and 7.48 with a standard deviation of 4.20, 4.34, and 4.89 for the traditional, semi-modern, and modern areas, respectively (Table 2). Illiteracy is highest in the semi-modern area (13.33%) and absent in the traditional area. Average schooling years are lower in the semi-modern (5.45) than in the modern area (7.48). Signature-only respondents are more common in semi-modern (16.67%) and modern (10.00%) areas. Primary education is highest in the traditional area (26.67%), while secondary education dominates in traditional and semi-modern areas (40.00%). Higher education is most prevalent in the modern area (33.33%). Urbanization improves access to basic education for all. Expanding education systems in urban areas is easier and costs less than in rural areas. There is a positive relationship between urbanization and education school enrollment at both primary and secondary levels increases with urbanization (Arouri *et al.,* 2014).

Table 2. Distribution of the farmers according to their age (year), education (schooling), family size (members), and homestead size (ha)

Categories	Basis of category	Number of respondents											
		Traditional area (N=30)				Semi-modern area (N=30)				Modern area (N=30)			
Age (year)													
Categories	Basis of category	No.	%	Avg.	SD	No.	%	Avg.	SD	No.	%	Avg.	SD
Young	Up to 35	8	26.67			11	36.67			11	36.67		
Middle	36 - 50	16	53.33	41.03	11.88	15	50.00	40.47	9.66	14	46.67	39.67	9.57
Old	> 50	6	20.00			4	13.33			5	16.67		
Total		30	100			30	100			30	100		

Categories	Basis of category	Number of respondents											
		Traditional area (N=30)				Semi-modern area (N=30)				Modern area (N=30)			
Education (schooling)													
Categories	Basis of category	No.	%	Avg.	SD	No.	%	Avg.	SD	No.	%	Avg.	SD
Illiterate	0	3	10.00			4	13.33			3	10.00		
Can sign only	0.5	4	13.33			5	16.67			3	10.00		
Primary	1-5	8	26.67	5.93	4.2	7	23.33	5.45	4.34	6	20.00	7.48	4.89
Secondary	6-10	12	40.00			12	40.00			8	26.67		
Above secondary	> 10	3	10.00			2	6.67			10	33.33		
Total		30	100			30	100.00			30	100		
Family size (member)													
Categories	Basis of category	No.	%	Avg.	SD	No.	%	Avg.	SD	No.	%	Avg.	SD
Small	Up to 3	7	23.33			9	30.00			7	23.33		
Medium	4 to 6	18	60.00	5.13	2.06	15	50.00	5.10	2.26	16	53.33	5.33	2.08
Large	> 6	5	16.67			6	20.00			7	23.33		
Total		30	100			30	100			30	100		
Homestead size (ha)													
Categories	Basis of category	No.	%	Avg.	SD	No.	%	Avg.	SD	No.	%	Avg.	SD
Landless/ marginal	Up to 0.08	5	16.67			3	10.00			6	20.00		
Small	0.09 - 0.14	8	26.67	0.18	0.12	11	36.67	0.17	0.12	13	43.33	0.15	0.11
Medium	0.15 - 0.20	12	40.00			12	40.00			8	26.67		
Large	> 0.21	5	16.67			4	13.33			3	10.00		
Total		30	100			30	100			30	100		

Family Size

The average number of family members was 5.13, 5.10, and 5.33 for the traditional, semi-modern, and modern areas, respectively with standard deviations of 2.06, 2.26, and 2.08, respectively (Table 2). Small households (up to 3 members) comprise 23.33% of respondents in both traditional and modern areas, and 30.00% in semi-modern areas, with average household sizes around 5 members. Medium-sized households (4–6 members) are the most common, accounting for 60.00% in the traditional area, 50.00% in the semi-modern, and 53.33% in the modern area, with consistent average sizes across regions. Large households (>6 members) are more frequent in semi-modern (20.00%) and modern (23.33%) areas compared to traditional (16.67%), indicating a trend toward larger household sizes in more developed settings (Table 2). Average homestead sizes were traditional, semi-modern, and modern. Landless/marginal farmers were most common in the modern area (20.00%), while small landholders dominated there (43.33%), reflecting fragmentation. Medium landholders prevailed in traditional and semi-modern areas

(40.00%), and large landholders were highest in the traditional area (16.67%), declining with modernization. Plant communities respond sensitively to urban sprawl and are therefore considered indicators for human-induced changes in habitats and landscapes (Vakhlamova, 2015). Islam *et al.,* (2017) also found a significant reduction in tree species due to urbanization.

Occupation

The occupations of the farmers in the study area varied distinctly. Based on their occupation, they are classified as agriculture, business, and service which were calculated with given scores of 1, 2, and 3, respectively. Based on scoring, the average occupation score was 1.40, 1.63, and 1.73 with standard deviations of 0.62, 0.76, and 0.78, respectively in respect of the traditional, semi-modern, and modern areas, respectively (Table 3). Agricultural engagement is highest in the traditional area (66.67%) and lowest in the modern area (20.00%), showing reduced reliance on farming with modernization. Business involvement rises across areas, peaking in the modern area (33.33%). Service-based employment increases significantly, from 6.67% (traditional) to 46.67% (modern), indicating a shift toward non-agricultural livelihoods. Arouri *et al.,* (2014) similarly found that urbanization drives economic transformation in Africa, with industrial employment rising from 6.1% in less urbanized areas to 26.1% in highly urbanized regions.

Table 3. Distribution of farmers according to their occupation

Categories	Number of respondents											
	Traditional area (N=30)				Semi-modern area (N=30)				Modern area (N=30)			
	No.	%	Avg.	SD	No.	%	Avg.	SD	No.	%	Avg.	SD
Agriculture	20	66.67	1.40	0.62	16	53.33	1.63	0.76	6	20.00	1.73	0.78
Business	8	26.67			9	30.00			10	33.33		
Service	2	6.67			5	16.67			14	46.67		
Total	30	100			30	100			30	100		

Household annual income

The average household annual family income from the homestead of the respondents was 28.43, 27.37, and 32.63 thousand takas with standard deviations of 10.84, 11.72, and 13.84, respectively under traditional, semi-modern, and modern areas, respectively (Table 4). Medium-income households dominate across all areas, highest in traditional (63.33%) and semi-modern (60.00%), but decline in modern areas (50.00%). Average income peaks in modern areas (32,630 Tk.), with higher income variability. High-income respondents are most common in modern areas (33.33%), indicating rising income levels with urbanization. Low-income prevalence is highest in semi-modern areas (23.33%). Modern urbanization is driven by higher productivity from the industrial and service sectors. Pull factors such as better job and income opportunities attract people from rural to urban areas (Hossain, 2001).

Table 4. Distribution of farmers regarding annual family income from the

homestead

Category	Basis of category ('000' Tk.)	Number of respondents											
		Traditional area (N=30)				Semi-modern area (N=30)				Modern area (N=30)			
		No.	%	Avg.	SD	No.	%	Avg.	SD	No.	%	Avg.	SD
Low	Up to 20	5	16.67			7	23.33			5	16.67		
Medium	21-40	19	63.33	28.43	10.84	18	60.00	27.37	11.72	15	50.00	32.63	13.84
High	> 40	6	20.00			5	16.67			10	33.33		
Total		30	100			30	100			30	100		

Abundance and changes of tree species due to urbanization

Homesteads of selected study areas are composed of multiple tree species. A total of 18 plant species and 13 families were recorded from the set of 90 homesteads surveyed. The names of species with family, their abundance in homesteads, and their percentage of abundance were arranged (Table 5). Tree species diversity and abundance decline with modernization. Traditional areas recorded the highest diversity (18 species, avg. 12.07) and abundance (806 trees, avg. 26.87), followed by semi-modern (14 species, 686 trees) and modern areas (11 species, 547 trees). Urbanization leads to a notable reduction in both tree diversity and abundance. Plant communities are sensitive indicators of urban sprawl and human-induced habitat changes (Vakhlamova, 2015).

Table 5. An abundance of dominant tree species according to urbanization

Categories	Tree species and abundance									
	Traditional area (N=30)			Semi-modern area (N=30)			Modern area (N=30)			Total
	No.	% of total	Avg.	No.	% of total	Avg.	No.	% of total	Avg.	
Tree species	18	100.00	12.07	14	77.78	10.23	11	61.11	8.37	18
Total abundance	806	39.53	26.87	686	33.64	22.87	547	26.83	18.23	2039

Changes in homestead size due to urbanization

Under modern areas, 90% of the respondents agreed that changes occurred due to urbanization whereas 73.33% of respondents under semi-modern areas observed their changes due to urbanization (Table 6). Positive perceptions of urbanization increase with modernization, rising from 6.67% in traditional areas to 73.33% in semi-modern and 90.00% in modern areas. Conversely, negative responses decline from 93.33% to 26.67% and 10.00%, respectively, indicating greater acceptance of urbanization in more developed areas.

Table 6. Changes in homestead size due to urbanization

Categories	Number of respondents					
	Traditional area (N=30)		Semi-modern area (N=30)		Modern area (N=30)	
	Number	%	Number	%	Number	%
Yes	2	6.67	22	73.33	27	90.00
No	28	93.33	8	26.67	3	10.00
Total	30	100.00	30	100.00	30	100.00

Reduction of homestead tree species

In modern areas, all the respondents (100%) agreed that the reduction of homestead tree species in their homesteads occurred due to urbanization whereas 80% of respondents in semi-modern areas observed their tree species reduction due to urbanization (Table 7). Tree species reduction due to urbanization was not observed in traditional areas (0% Yes), but affirmative responses rose to 80% in semi-modern and 100% in modern areas. Negative responses dropped from 100% in traditional to 20% in semi-modern and 0% in modern areas, showing a clear shift toward acknowledging tree loss with modernization.

Table 7. Reduction of homestead tree species due to urbanization

Categories	Number of respondents					
	Traditional area (N=30)		Semi-modern area (N=30)		Modern area (N=30)	
	Number	%	Number	%	Number	%
Yes	0	0.00	24	80.00	30	100.00
No	30	100.00	6	20.00	0	0.00
Total	30	100.00	30	100.00	30	100.00

Problems faced by the respondents

Eight major urbanization-related problems were identified in the study: income decline (P1), household issues (P2), economic challenges (P3), overcrowding and pollution (P4), unemployment (P5), poor health and disease (P6), higher urban crime rates (P7), and transportation problems (P8) (Table 8). Results showed that unemployment was the most reported problem in traditional (83.33%) and modern areas (73.33%), while transportation issues dominated in semi-modern areas (53.33%). Overcrowding and pollution were the least reported in traditional areas (13.33%) but increased with modernization. Economic problems and perceived income decline decreased from traditional to modern areas, indicating improved stability. Poor health issues declined from 30% to 16.67%, whereas urban crime peaked in semi-modern areas (46.67%). Transportation problems dropped significantly from traditional (76.67%) to modern areas (26.67%). Overall, unemployment and transportation were the primary concerns across the study areas. This aligns with studies showing that unemployment often persists in transitioning rural communities due

to limited industrial diversification and skill mismatches (Mohammed and Hashim, 2024).

Table 8. Problems faced by the respondents due to urbanization

Categories	Number of respondents											
	Traditional area (N=30)				Semi-modern area (N=30)				Modern area (N=30)			
	Yes	%	No	%	Yes	%	No	%	Yes	%	No	%
Income decreased (P_1)	18	60.00	12	40.00	11	36.67	19	63.33	9	30.00	21	70.00
Facing household problems (P_2)	16	53.33	14	46.67	10	33.33	20	66.67	11	36.67	19	63.33
Economic problems (P_3)	12	40.00	18	60.00	12	40.00	18	60.00	7	23.33	23	76.67
Overcrowding and pollution problems (P_4)	4	13.33	26	86.67	8	26.67	22	73.33	9	30.00	21	70.00
Unemployment problems (P_5)	25	83.33	5	16.67	8	26.67	22	73.33	22	73.33	8	26.67
Poor health and disease problems (P_6)	9	30.00	21	70.00	7	23.33	23	76.67	5	16.67	25	83.33
Urban crime more than traditional (P_7)	8	26.67	22	73.33	14	46.67	16	53.33	12	40.00	18	60.00
Transportation problems (P_8)	23	76.67	7	23.33	16	53.33	14	46.67	8	26.67	22	73.33

Changes in tree species due to urbanization

Table 9 shows that 100% of respondents in the modern area reported changes in tree species related to timber, fruits, and vegetables due to urbanization, while 83.33% and 73.33% noted changes in medicinal/ornamental plants and spices, respectively. In contrast, respondents in traditional areas reported no changes across all plant categories. Semi-modern areas exhibited intermediate responses, with most respondents observing changes. The presence of fuel and fodder trees, timber, fruits, and vegetables was confirmed by all modern-area respondents, whereas semi-modern areas showed moderate presence (26.67%), and traditional areas reported none. This indicates a marked increase in plant species diversity and utilization with urbanization and modernization.

Table 9. Changes in tree species due to urbanization

Categories	Number of respondents											
	Traditional area (N=30)				Semi-modern area (N=30)				Modern area (N=30)			
	Yes	%	No	%	Yes	%	No	%	Yes	%	No	%
Fuel and fodder tree	0	0	30	100	7	23.33	23	76.67	30	100.00	0	0.00
Timber	0	0	30	100	8	26.67	22	73.33	30	100.00	0	0.00
Fruits	0	0	30	100	8	26.67	22	73.33	30	100.00	0	0.00
Vegetables	0	0	30	100	9	30.00	21	70.00	30	100.00	0	0.00
Medicinal or ornamental	0	0	30	100	11	36.67	19	63.33	25	83.33	5	16.67
Spices	0	0	30	100	13	43.33	17	56.67	22	73.33	8	26.67

Relationship between the selected characteristics of the respondents and the effect of urbanization

Correlation coefficients (R-values) in this study (Table 10) were interpreted as follows: 0.00–0.19 indicated very low (negligible) correlation; 0.20–0.39 low (weak) correlation; 0.40–0.69 moderate correlation; 0.70–0.89 high (strong) correlation; and 0.90–1.00 very high (near-perfect) correlation. This classification provides a clear framework to evaluate the strength and direction of relationships between variables, facilitating informed analysis and interpretation of the data. Moderate to high correlations suggest meaningful associations that can guide further causal inference or prediction (Janse *et al*., 2021).

Table 10. The meaning of R-value

R-value	Meaning
0.00 to 0.19	A very low correlation
0.20 to 0.39	A low correlation
0.40 to 0.69	A moderate correlation
0.70 to 0.89	A high correlation
0.90 to 1.00	A very high correlation

Source: Cohen and Holliday (1982)

Pearson's Product-Moment Correlation Coefficient (r) was used to examine relationships between variables, with significance tested at the 5% and 1% levels (df = 88). Results (Table 11) revealed significant correlations between the effects of urbanization and variables such as education, homestead size, income from the homestead, tree species, and problems, some showing high to very high correlations. In contrast, age, family size, and occupation exhibited weak or non-significant correlations. These findings indicate that education, income, and homestead size significantly influence urbanization impacts, while demographic factors like age and family size have minimal effects.

Table 11. Co-efficient of correlation showing the relationship between selected characteristics of the respondents and the effect of urbanization

Dependent variable	Independent variable	Computed value of "r"	Tabulated value of "r" with 88 degrees of freedom	
			at 0.05 level	at 0.01 level
Effect of urbanization	Age	0.064^{NS}	0.205	0.267
	Education	0.405**		
	Family size	0.041^{NS}		
	Homestead size	-0.642**		
	Occupation	0.206*		
	Income from homestead	0.832**		

Dependent variable	Independent variable	Computed value of "r"	Tabulated value of "r" with 88 degrees of freedom	
			at 0.05 level	at 0.01 level
	Tree species	-0.713**		
	Problems	0.207*		

Note: NS=Correlation is not significant, *=Correlation is significant at the 0.05 level (2-tailed), **=Correlation is significant at the 0.01 level (2-tailed)

Relationship between the age of the respondents and the effect of urbanization

The correlation between respondents' age and the effect of urbanization was positive but very weak (r = 0.061), as shown in Table 11. Since this value was below the critical R-value (0.205) at the 5% significance level (df = 88), the relationship was not statistically significant. Thus, age had no meaningful influence on the perceived effects of urbanization.

Relationship between educational qualification and the effect of urbanization

A significant positive correlation was found between respondents' education and the effect of urbanization (r = 0.405), as shown in Table 11. This value exceeded the critical R-value (0.267) at the 1% significance level (df = 88), leading to the rejection of the null hypothesis. The result suggests that higher education is associated with greater awareness and better management of urbanization impacts, likely due to improved knowledge and experience. A significant positive relationship between education and urbanization (r = 0.405) highlights that better-educated individuals may possess greater awareness and adaptive capacity to manage urban impacts, consistent with findings by Chowdhury *et al*., (2022), who emphasized the role of education in enhancing rural livelihood strategies.

Relationship between the family size of the respondents and the effect of urbanization

As shown in Table 11, a very weak positive correlation was observed between family size and the effect of urbanization (r = 0.041), which was below the critical value (0.205) at the 5% significance level (df = 88). The relationship was not statistically significant, indicating that family size had no meaningful influence on the perceived effects of urbanization.

Relationship between the homestead size of the respondents and the effect of urbanization

Table 11 shows a significant negative correlation between the variables (r = -0.642), exceeding the critical value (0.267) at the 1% significance level (df = 88). The null hypothesis was rejected, indicating a strong inverse relationship between the variables.

Relationship between the occupation of the respondents and the effect of urbanization

As shown in Table 11, a positive correlation was found between respondents' occupation and the effect of urbanization (r = 0.206), slightly exceeding the critical value

(0.205) at the 5% significance level (df = 88). The null hypothesis was rejected, indicating a statistically significant relationship between occupation and urbanization effects.

Relationship between income from the homestead of the respondents and the effect of urbanization

Table 11 reveals a strong positive correlation between homestead income and the effect of urbanization (r = 0.832), surpassing the critical value (0.267) at the 1% significance level (df = 88). The null hypothesis was rejected, indicating a highly significant relationship suggesting that higher homestead income is strongly associated with greater urbanization impact.

Relationship between the tree species of the respondents and the effect of urbanization

Table 11 shows a significant negative correlation between tree species and the effect of urbanization (r = -0.713), exceeding the critical value (0.267) at the 1% significance level (df = 88). The null hypothesis was rejected, indicating that tree species significantly declined with increasing urbanization. Tree species and homestead size revealed strong negative correlations with urbanization (r = -0.713 and -0.642, respectively), indicating a decline in vegetation and land availability due to urban encroachment. This supports previous findings by Chen, (2020), who documented biodiversity loss in rapidly urbanizing regions of Bangladesh.

Relationship between problems of the respondents and the effect of urbanization

As presented in Table 11, a positive correlation was found between respondents' problems and the effect of urbanization (r = 0.207), slightly exceeding the critical value (0.205) at the 5% significance level (df = 88). The null hypothesis was rejected, indicating a statistically significant relationship—urbanization was associated with increased problems faced by respondents. The positive correlation between respondents' reported problems and urbanization (r = 0.207) suggests increased socio-environmental stress, consistent with James, (2024), who linked urban sprawl to heightened livelihood challenges.

Conclusion

The study concludes that urbanization has a significant impact on reducing homestead size and tree abundance in the selected areas. Key socio-economic factors such as education, occupation, income, and perceived challenges showed strong correlations with these changes, while age, family size, and number of tree species had no significant influence. Traditional and semi-modern areas were largely dependent on agriculture, whereas modern areas exhibited a shift toward employment in the service sector. Across all settlement types, unemployment and inadequate transportation emerged as major challenges in the study areas.

Author’s contributions

SUBM was responsible for data collection and preparation of the initial draft of

the manuscript. NN and MGJH contributed to the enhancement of the analysis and interpretation of the results and discussion. LM improved the reference of this manuscript. MRA and MSAT contributed significantly to the overall improvement and refinement of the manuscript. AUK provided substantial input in revising and improving the draft manuscript. All authors reviewed and approved the manuscript.

Conflicts of Interest

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

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CLIMATE CHANGE AND AGRICULTURE IN BANGLADESH: IMPACTS AND ADAPTATION STRATEGIES

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Abstract

The interrelationship between climate change and agriculture has been widely studied in Bangladesh. Bangladesh is predominantly an agricultural nation where farming is highly dependent on natural conditions such as temperature, rainfall, soil quality, and water availability. However, Bangladesh frequently faces extreme climatic events, including droughts and floods, which are further exacerbated by global climate change, threatening agricultural productivity and food security. This study employs a bibliometric approach to critically analyze existing academic literature on climate change and agricultural adaptation in Bangladesh. The primary objectives of the study are to (1) assess the impact of climate change on Bangladesh agriculture and (2) evaluate the effectiveness of current adaptation strategies. Data were sourced from Scopus, yielding 1,052 relevant documents, including 765 scholarly articles. VOSviewer software was used to map bibliometric connections, highlight key authors, and extract insights from journals and other sources. The study revealed that there are inconsistencies between agricultural policies and climate change adaptation strategies. To bridge this gap, the study recommends policy improvements, including targeted subsidies and pricing mechanisms that encourage farmers to adopt location-specific adaptation strategies, enhancing climate resilience in agriculture.

Keywords: Adaptation, Agricultural extension, Rainfall, Rural, Technologies.

Introduction

Climate change is an undeniable reality, with historical evidence indicating its ongoing presence for thousands of years (Kotir, 2011; Mercuri, 2025). For instance, the Sahara Desert was once tropical 6,000–7,000 years ago (Brown *et al.,* 2018). Today, observable signs of climate change include rising temperatures, shifting rainfall patterns, frequent extreme weather events, the melting Arctic ice cap, and consequent sea level rise. Changes in the distribution of birds and plants, altered migration patterns, and earlier leaf falls in deciduous trees further underscore this transformation (Navarro-Velez and Dhondt, 2025).

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Reports from the Intergovernmental Panel on Climate Change (IPCC) warn of severe social, environmental, and economic hazards. The IPCC has confirmed that global warming is real, accelerating, and nearing a tipping point beyond human control. Findings indicate rising ocean and air temperatures, widespread ice cap melting, and intermediate sea level increases worldwide (Siegert *et al.,* 2020).

Climate change profoundly impacts the environment, biodiversity, human health, and food systems. Water cycles and supply sources are shifting, exacerbating water stress, extreme temperatures, and unpredictable weather conditions, all of which challenge agriculture (Blanc *et al.,* 2017; Jun *et al.,* 2011). The adverse effects on farming are particularly concerning, as agriculture depends heavily on climatic stability. Temperature fluctuations, daylight variations, and inconsistent rainfall patterns pose significant challenges to farmers worldwide. Rapid climate shifts have intensified droughts and floods, jeopardizing food security by damaging crops, displacing farmers, and degrading land. Rising water temperatures disrupt aquatic ecosystems, affecting fish populations and food sources in many countries (Dasgupta *et al.,* 2017; Pham *et al.,* 2019).

Climate models predict substantial declines in crop yields due to global warming. Under the RCP8.5 scenario, winter wheat yields could drop by up to 21%, winter barley by 17.3%, and spring barley by 33.6% (Gammans *et al.,* 2017). A mere 1°C rise in global air and water temperatures may reduce cereal production by 5-10% (Hatfield and Maher, 2009). Extreme heat exceeding 30°C damages plants, while temperatures above 37°C compromise seed storage quality (Wahid *et al.,* 2007). Precipitation anomalies further threaten food production, as 80% of the world's cropped areas rely on rain-fed agriculture (Ghose *et al.,* 2021).

Bangladesh is particularly vulnerable, ranking eighth on the Climate Risk Index 2020 for weather extremes (Eckstein *et al., 2020*). Predictions suggest global sea temperatures will rise by 1.2-3.2°C by 2100, accompanied by a sea level increase of up to 1.3 meters between 2090 and 2099 (Schickhoff *et al.,* 2022; Mojid, *et al*., 2025). Even a modest 0.3-meter rise would increase coastal flood-risk areas by 15.3%, deepening inundation by 22.7% (Karim and Mimura, 2008).

Climate change also drives intranational migration. The World Bank projects that by 2050, 13.3 million people in Bangladesh may be displaced due to climate change (Podesta, 2019). Agricultural productivity is particularly threatened, with significant declines in staple crop yields across all three rice-growing seasons (Sarker *et al.,* 2014). One model forecasts that a 5.32°C temperature rise by 2100 would reduce potato, rice, and wheat yields by 38.6%, 67.8%, and 47.6%, respectively.

Salinity intrusion exacerbates Bangladesh's agricultural challenges, increasing from 83.3 million hectares in 1973 to 105.6 million hectares in 2009 (Islam *et al.,* 2021). Coastal salinity has damaged crop, fish, and livestock production (Alam *et al.,* 2022). More than 35 million people live in Bangladesh's 19 coastal districts, constituting 32% of the country's land area (Rabbani & Huq, 2011). Extreme weather events occur frequently—at least one disaster strikes the nation every three years (Rahman *et al.,* 2021).

As a developing nation reliant on agriculture, Bangladesh's food security is deeply intertwined with its farming sector. Agriculture contributes 13.82% of GDP (Talekar *et al.,*

2020) and employs 37.75% of the population (BER, 2017). Thus, urgent research is needed to assess climate change's effects on Bangladeshi agriculture and evaluate existing adaptation strategies. Although studies on this subject exist, a bibliometric analysis of climate change's impact on agriculture in Bangladesh remains unexplored. This study aims to fill that gap by reviewing the current agricultural status, climate change impacts, and the effectiveness of adaptation efforts.

Materials and Methods

The primary objective of this research is to conduct a thorough analysis of existing data to gain a deeper understanding of (i) how climate change is affecting agriculture in Bangladesh and (ii) the effectiveness of current adaptation strategies.

This study relies on data sourced from the Scopus database. The initial search terms—("climate change" AND "impact" AND "agriculture" AND "Bangladesh")—were used to ensure relevant materials were identified. To enhance the study's scientific rigor, extraneous materials were excluded, and selected references underwent verification. Additionally, supplementary data were obtained from news reports and publicly accessible websites.

A bibliometric approach was employed to compile literature on climate change's impact on Bangladeshi agriculture from the Scopus database, using TITLE-ABS-KEY ("climate change" AND "impact" AND "agriculture" AND "Bangladesh"). This search covered 34 years (1988–2022), yielding 1,052 documents, of which 765 were scholarly articles. The highest number of publications occurred in 2021, with 170 papers.

Bibliometric analysis requires comprehensive literature reviews and rigorous methodologies to ensure high-quality information and reliable outputs (Inamdar *et al.*, 2020; Souza & Bueno, 2022). Accordingly, VOSviewer software was utilized to map bibliometric sources, identify leading authors, and extract refined insights from academic journals (Nobanee *et al.*, 2021; Yu *et al.*, 2022).

Bibliometric methods offer several advantages. First, data-driven analyses provide greater credibility than subjective assessments, as traditional reviews often rely on critical written summaries. Second, bibliometric approaches facilitate broader overviews of research landscapes (Li *et al.*, 2021). To achieve research objectives and maintain bibliometric standards, an evaluation review was conducted to ensure logical consistency in mapping sustainability and risk dimensions. Microsoft Excel was employed for data analysis, complemented by VOSviewer for visual mapping.

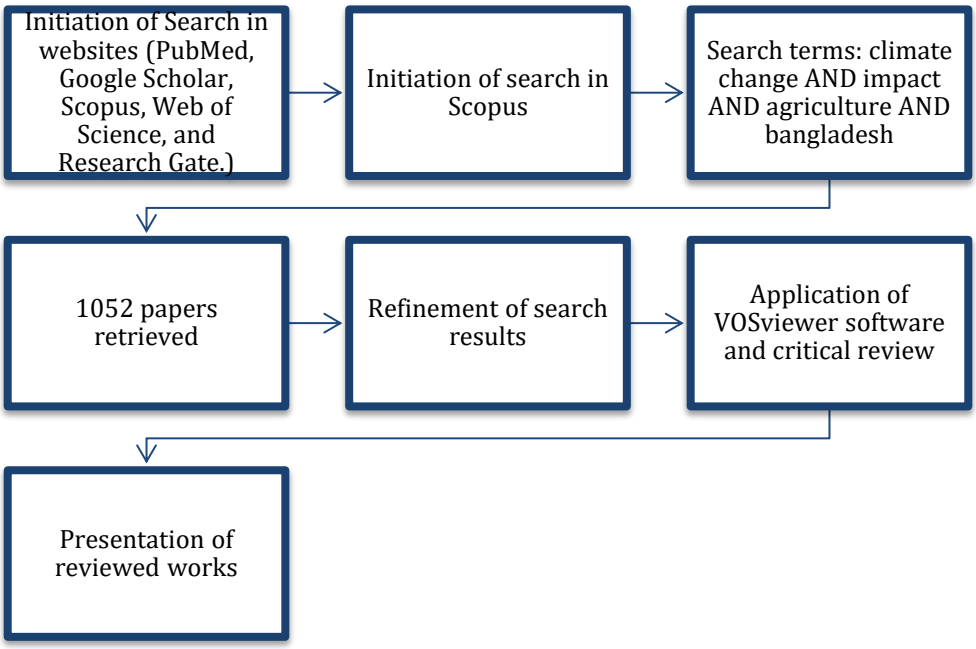

Fig. 1. Search and review process.

Results and Discussions

Climate change and agriculture in Bangladesh

A total of 5,200 keywords were used in the complete counting co-occurrence analysis. Figure 2 presents the network map illustrating the connections among keywords in the analyzed articles. The analysis identified eight distinct groupings representing various research perspectives on climate change and agriculture.

Each node in the figure corresponds to a keyword, with node size reflecting keyword frequency. The number of connecting lines indicates the extent of co-occurrence, while line thickness represents the strength of keyword associations (Tan *et al.*, 2021). Larger nodes with thicker connecting lines signify higher keyword co-occurrence and stronger interconnections.

The top 20 most frequently co-occurring keywords were Climate Change, Bangladesh, Agriculture, Adaptation, Food Security, Rice, Adaptive Management, Climate Effect, Vulnerability, Human, Coastal Zone, Crop Production, Drought, Salinity, Rain, Agricultural Robots, Floods, and Livelihood. Each of these keywords appeared more than 23 times, with a minimum link strength of 97.

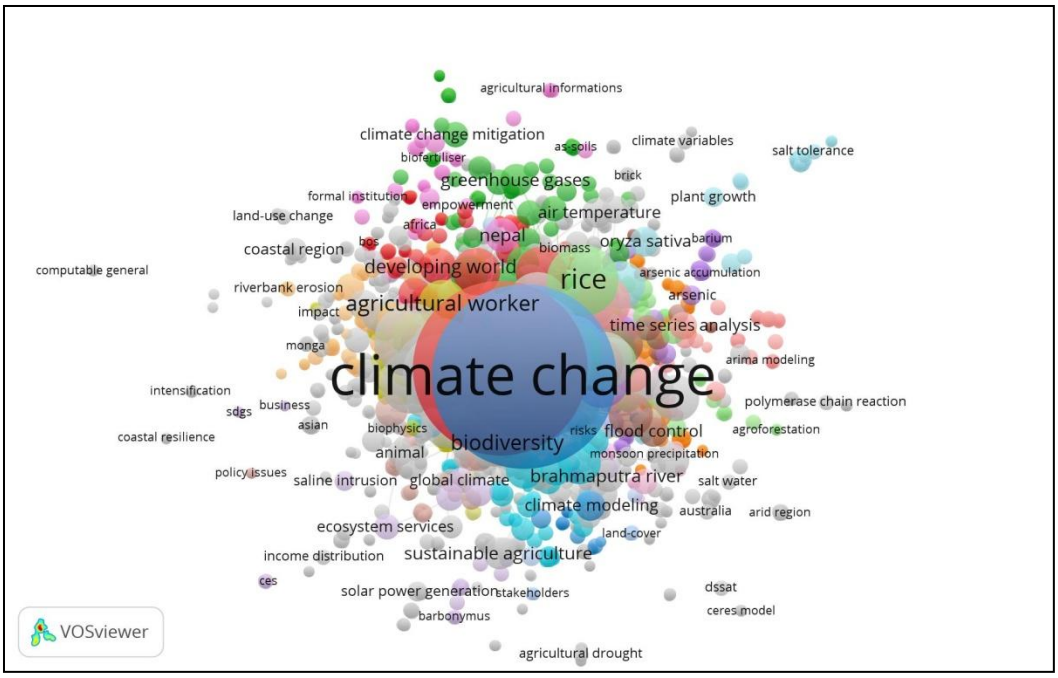

Fig. 2. VOSviewer co-occurrence network visualisation mapping of most common terms (minimum six occurrences) on the "Bangladesh agriculture-climate change" study.

Table 1. Hotspot areas of work on the topics

Keyword	Occurrences	Total Link Strength
Climate Change	253	891
Bangladesh	227	869
Agriculture	152	620
Article	48	295
Adaptation	43	188
Food Security	41	177
Rice	39	225
Adaptive Management	34	180
Climate Effect	31	146
Vulnerability	30	144
Human	28	174
Coastal Zone	27	145
Crop Production	27	162
Drought	27	151

Keyword	Occurrences	Total Link Strength
Salinity	26	150
Rain	25	157
India	24	128
Agricultural Robots	23	104
Floods	23	108
Livelihood	23	97

Fig. 3 presents the top nineteen authors contributing to research on Bangladesh's agriculture-climate change nexus from 1988 to 2022, ranked by citation frequency. The five most frequently cited authors were Gopal, B.; Monirul Qader Mirza, M.; Shahid, S.; Alam, G.M.M.; and Miyan, M.A., collectively amassing over 765 cited papers during this period.

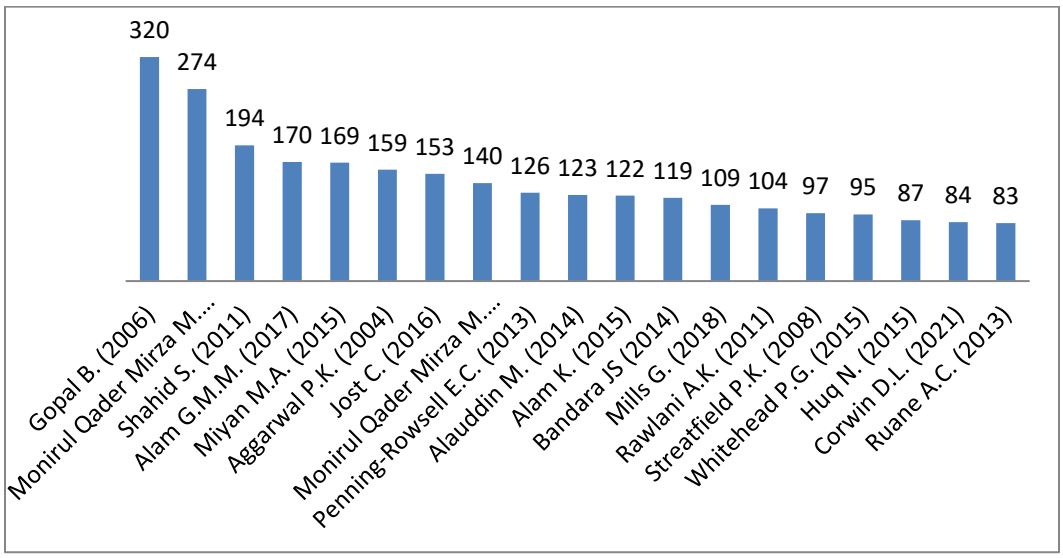

Fig. 3. From 1988 to 2022, the top 19 writers cited by authors in agriculture-climate change nexus in Bangladesh.

Bangladesh's climate change outlook

Bangladesh is highly vulnerable to climate change due to its flat, low-lying, deltaic geography (Ayers *et al.*, 2014), combined with high population density, poverty levels, and reliance on agriculture. Frequent natural disasters, inadequate infrastructure, and a population density of 1,368 people per sq. km (Bangladesh Population 2023, n.d.) further amplify its exposure, risk, and vulnerability. Additionally, large portions of the country endure annual cyclones, floods, and erosion, creating significant socioeconomic and environmental challenges that hinder national progress.

As climate change intensifies, extreme weather events—such as heavy rainfall, rising sea levels, and tropical cyclones—will become more frequent, threatening agriculture, water security, food systems, human health, and housing. For instance, Bangladesh's sea level is projected to rise by 0.30 meters by 2050, potentially displacing up to 900,000 people, and by 0.74 meters by 2100, putting 2.1 million people at risk of displacement (Davis *et al.,* 2018).

Bangladesh is already experiencing shifts in weather patterns due to global warming, with hotter summers, unpredictable monsoons, and reduced dry-season precipitation. These seasonal disruptions result in excessive rainfall during wet months, leading to flooding and landslides, while dry spells cause severe drought. Consequently, Bangladeshi farmers increasingly face a double crisis—crop failure due to drought followed by flooding losses of remaining crops.

Climate variability occurs when a region's climatic variables deviate from long-term averages (Beck *et al.,* 2007). This means annual fluctuations in temperature and precipitation, with some years recording below-average rainfall while others experience excess precipitation. For instance, Rajshahi, in northern Bangladesh, receives an annual rainfall of 1,494 mm (Dey *et al.,* 2011). Significant climate vulnerability exists in central and western coastal regions, the northwestern highlands, and the major river basins, identified as climate change hotspots (Das *et al.,* 2020).

Variations in temperature

Between 1976 and 2019, Bangladesh's average temperature increased by 0.5°C (Roy *et al.,* 2020), although this rise has not been uniform across regions. In the eastern parts of the country, maximum temperatures increased by 0.9°C, while the central regions, including Dhaka, Chattogram, and Sylhet divisions, recorded a 0.5°C increase during the same period (Abedin and Khan, 2022).

Additionally, summers are lengthening, winters are warming, and monsoons are becoming more unpredictable. Projections indicate that Bangladesh's annual average temperature will rise between 1.0°C and 1.5°C by 2050, even if global mitigation strategies, such as the Paris Climate Agreement, are implemented (Brown *et al.,* 2018).

Changing precipitation patterns

Rainfall patterns, both global and regional, are shifting due to climate change. Bangladesh remains one of the most flood-prone countries in the world, with droughts emerging as a growing concern in the northern regions. Understanding precipitation trends is crucial for disaster prevention, agriculture, and water resource management.

Bangladesh's average annual rainfall is 2,488 mm, varying from 1,527 mm in the west to 4,197 mm in the east, with a west-to-east rainfall gradient of 7 mm/km (Shahid, 2012). More than 89% of annual precipitation occurs between May and October (Shahid, 2012), highlighting the country's seasonal rainfall distribution.

Most regions experience a humid climate, with the northeast classified as a wet zone and the central-west region as sub-humid. The coefficient of variation in annual rainfall suggests moderate fluctuations, with deviations ranging between +408 mm and -586 mm over the past 50 years (Kaur *et al*., 2021).

Variations in humidity and wind

Humidity levels in northern Bangladesh show a long-term increasing trend. During the study period, the average annual maximum humidity was 216.75%, and the average minimum was 71.92%.

From 1960 to 2017, average annual precipitation increased, with March recording the lowest relative humidity of 57% in Dinajpur. The driest months in eastern Bangladesh are January through March, with Brahmanbaria registering the lowest monthly average humidity of 58.5% in March (Mahmud and Chowdhury, 2021). Between June and September, relative humidity remains consistently above 80%, reaching 78.1% in Cox's Bazar and 70.5% in Pabna.

Wind patterns in Bangladesh vary seasonally. Summer winds predominantly flow from the south, southwest, or southeast, with speeds ranging between 8–16 km/h, compared to winter winds averaging 3–6 km/h. Atmospheric pressure also fluctuates, with January's average pressure at 1,020 millibars, dropping to 1,005 millibars between March and September

Present status of agriculture in Bangladesh

Agriculture remains a cornerstone of Bangladesh's economy, covering more than 70% of the country's land area and employing nearly half of its population. In 2021, the sector contributed 11.6% to GDP growth (Bangladesh Population 2023, n.d.).

Rice and jute are the country's dominant agricultural products, though maize and vegetable cultivation has gained traction in recent years (Ali *et al.,* 2019). The expansion of irrigation networks has encouraged wheat farmers to shift toward maize production, particularly for poultry feed. In northeastern Bangladesh, tea is the primary agricultural product. Bangladesh's abundant land and water resources enable rice harvesting three times a year. The country ranks third in cultivated rice exports and fifth in farmed fish exports (Golub *et al.,* 2014). From 2000 to 2008–09, cereal production surged from 32.89 million metric tons to 45.50 million metric tons, marking a 38% increase over 12 years (Krishi Diary, 2022). The most substantial growth was recorded in maize (775%), pulses (375%), and vegetables (578%)

Table 2. Comparison of changes in production and growth of crops

Crops	Production (million metric tons)		Growth (%)
	2008-09	2020-21	
Rice (husked)	31.31	38.6	23
Wheat	0.84	1.23	45
Maize	0.73	5.66	775
Potato	5.26	10.61	101
Pulses	0.19	0.93	375
Oilseeds	0.66	1.19	81
Vegetables	2.90	19.91	578

Krishi Diary, 2022

Agriculture remains Bangladesh's most vital economic sector but faces escalating challenges, including loss of arable land, population expansion, climate change, poor management methods (inadequate fertilizer, water, outbreaks of pests & diseases), lack of quality seeds, inadequate credit assistance to farmers, unjust product pricing, and insufficient research. Between 1983 and 1996, Bangladesh lost 1 million hectares of farmland. Bangladesh's current arable land is only 0.04 hectares per capita, which will be around 0.033 (Kanak Pervez *et al.,* 2017). The amount of land is inadequate to ensure food security in this country. In addition, climate change will worsen the lives of the rural poor in Bangladesh and exacerbate food insecurity.

Additionally, climate change disproportionately impacts the rural poor, exacerbating food insecurity. Rising temperatures, erratic weather patterns, and extreme climatic events further strain agricultural productivity, making sustainable farming increasingly difficult.

Bangladesh's agriculture and climate change

Changes in temperature and precipitation patterns are significantly impacting agriculture in Bangladesh. A 4°C increase in average temperature is projected to reduce rice and wheat production by 28% and 68%, respectively (Impact of Climate Change on Agricultural | The Financial Express, n.d.). Additionally, droughts and delayed monsoons have altered the rain-fed Aman rice season, potentially causing rice and wheat yields to decline by 8% and 32% by 2050 due to insufficient rainfall (Islam *et al.,* 2013).

A 10°C rise in maximum temperature during the vegetative, reproductive, and ripening stages reduced Aman rice yields by 2.94, 53.06, and 17.28 tons, respectively (Islam *et al.,* 2021). Similarly, a 1mm increase in precipitation during these growth stages led to yield reductions of 0.036, 0.230, and 0.292 tons (Kaczan & Orgill-Meyer, 2020).

The Bangladesh Agriculture Development Corporation (BADC) currently provides only 56% irrigation coverage, leaving farmers vulnerable to erratic rainfall. Climate change has also intensified floods and flash floods. In March 2017, flash floods devastated crops in the hoar (lowland) regions, impacting 0.14 million hectares of cropland and 423,000 farmers (DAE, 2018).

Rising sea levels are accelerating saltwater intrusion into coastal farmland. Bangladesh's coastline experiences a 3mm annual sea level rise, contributing to a 27% increase in salinity intrusion between 1973 and 2009. Coastal seawater contamination has affected 1.1 million hectares of land, reducing freshwater availability and damaging soil fertility (SRDI, 2010; Pervez *et al.,* 2020).

The northwestern region of Bangladesh frequently experiences droughts, with broadcast rice suffering 40% drought-related damage. The Kharif season (mid-March to mid-October) sees drought damage across 2.32 million hectares of T. Aman rice fields, while the Rabi season (mid-October to mid-March) affects over 1.2 million hectares of farmland (Dey *et al.,* 2011).

Climate change adaptation and research gaps

Bangladesh lacked a formal climate change adaptation strategy until 2010, when several key policies were introduced to mitigate vulnerability. However, due to the absence of global consensus on climate change mitigation and the long-term inertia of climatic systems, adaptation remains essential for protecting agriculture in climate-vulnerable nations like Bangladesh (Groom, 2012).

Despite contributing minimal global carbon emissions, Bangladesh faces significant risks from climate change and must focus on adaptation strategies rather than mitigation. The country has several agricultural adaptation options, including:

- Mixed-crop livestock farming systems
- Adjusting planting and harvesting schedules
- Developing drought-resistant cultivars
- Introducing high-yield, water-sensitive crops
- Modifying irrigation methods and crop selection
- Diversifying animal farming (Bradshaw *et al.*, 2004).

Bangladesh's Vision 2021 emphasizes environmental protection through strategies such as planned foreign migration, disaster resilience in vulnerable regions, pollution reduction, and sustainable waste management. Safeguarding forests, waterways, and rivers is critical for building climate resilience. Initiatives like strengthening flood-prone communities in Gaibandha, developing drought and flood adaptation strategies, and implementing LACC programs in arid areas are key components of this transition.

Current adaptation techniques

Bangladeshi farmers are increasingly adopting climate-resilient crop varieties to mitigate the effects of climate change (Moniruzzaman, 2015). Many are shifting from Aman rice, which relies heavily on rainfall, to Boro rice, which is more irrigation-dependent. Integrated agriculture, combining livestock, fish, and crop farming, has gained popularity as a strategy to optimize resource use and enhance productivity. Rice-fish farming, in particular, improves efficiency and food security by outperforming monoculture systems in terms of resource utilization, diversity, yield quality, and overall output (Ahmed & Garnett, 2011).

Cage aquaculture has emerged as an alternative fishing method, particularly useful after floods or storms. Traditional aquaculture farms often suffer perimeter damage during extreme weather events, disrupting fish growth. In response, biofloc fish farming is becoming increasingly popular among small and marginal farmers, particularly in urban areas. Additionally, raising ducks in waterlogged or flooded regions provides a viable solution for utilizing submerged land efficiently.

Adapting agriculture to climate change requires short-, medium-, and long-term planning that relies on accurate, localized projections and adaptable strategies. Policymakers should integrate migration policies into development, environmental planning, and climate resilience efforts to reduce risks and enhance mobility. Indigenous,

family-based, and community-driven adaptation techniques remain essential for fostering resilience.

The gap in research

Most available studies rely on historical climate patterns to predict future trends, focusing on outcome-based vulnerability assessments. However, limited research has explored how agricultural workers in Bangladesh personally perceive climate change. A perception-based approach could provide critical insights into farmers' adaptability and inform strategic investments in short- and long-term climate resilience.

While some studies examine climate impacts on agriculture and fisheries, few address vulnerability dynamics in relation to climate change and market globalization. Additionally, minority groups, women, and youth remain underrepresented in discussions on climate adaptation policies and their socio-economic consequences.

Further localized, system-based research could better inform adaptation decisions for Bangladesh's agricultural sector. Understanding how adaptation-related information is developed, exchanged, and implemented across different levels—including formal and informal networks—could enhance horizontal and vertical collaboration in climate resilience efforts (Davies *et al.*, 2019).

Conclusion

Bangladesh's agriculture, highly dependent on natural conditions such as weather, soil, and water flow, faces annual disruptions from climate-related disasters. Local communities have developed and implemented various adaptive strategies, often without formal theoretical guidance, to cope with these changes. However, planning, policy development, and adaptation strategy execution remain in their early stages. The Government has established institutional frameworks and allocated funding to mitigate climate risks, but further efforts are needed.

Research indicates that future temperature and precipitation fluctuations will significantly impact crop yields in Bangladesh, necessitating the adoption of climate-resistant or climate-insensitive crops to maintain production levels. Crop switching could play a vital role in stabilizing food security amid climate challenges.

To support climate adaptation in agriculture, the distribution system for agricultural inputs—such as seeds and fertilizer—should incentivize climate-resilient farming practices. Policies should favor farmers who select crops suited to their respective agro-ecological zones while reducing support for those who do not.

Additionally, the Government should continue subsidizing fertilizer, fuel, and irrigation but restructure agricultural subsidies to encourage climate-responsive farming decisions. A pricing structure for farming inputs that promotes climate-resilient crop choices would be a practical way to integrate climate change policy into agricultural policy, ensuring the sector remains viable despite environmental challenges.

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Author's contribution

Both authors contributed equally to the preparation of the manuscript. The search initiation, retrieval, refinement of search results, application of VOSviewer software for critical review, and final presentation of reviewed works were collaboratively conducted by the first and second authors simultaneously. The second author prepared the final copy, which was approved by the first author for submission.

Conflict of Interest

The authors affirm that there are no conflicts of interest related to the publication of this manuscript

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Short Communication

ASSESSMENT OF GENETIC DIVERSITY IN MUNGBEAN (*Vigna radiata* L.)

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Abstract

An experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh to analyze the genetic diversity of fourteen mungbean varieties through Mahalanobis D^2 statistic and principal component analysis for nine quantitative traits related to yield. Based on the results of this study the varieties were grouped into four clusters by diversity (D^2) analysis where cluster IV comprised six varieties, cluster I had four varieties and cluster II and III had 2 varieties in each. The maximum inter-varietal distance was observed between G_1 and G_6 (1.288). Genetic diversity in mungbean can be explained 89.82% by the first four components, according to principal component analysis. G_1 (BARI mung1), G_2 (BARI mung2), G_6 (BARI mung6), G_7 (BARI mung7) and G_{10}(BINA moog-5) were shown to have potential for further hybridization in breeding programs, based on group distance and other agronomic performance measures.

Keywords: Cluster analysis, Genetic diversity, Mungbean, Principle component analysis.

Introduction

Mungbean (*Vigna radiata* L.) is a very common and important pulse crop belongs to the family Leguminosae sub family Papilionaceae, grown principally in the tropical and subtropical parts of the world (Bangar *et al*. 2018). Mungbean is rich in protein (24%) and sprouted mungbean is rich in calcium, iron and vitamin C (Mwangi *et al.,* 2021). Moreover, it is a short durated, well adapted, requires less water to grow and also a non-photosensitive crop (Anita *et al.,* 2025). India, Bangladesh, Pakistan, Sri Lanka, Philippines, Thailand are the main mungbean growing countries of the world (Gayacharan *et al.* 2022). In Bangladesh the production is increasing from 25000 mt to 42000 mt in the last decade (BBS, 2023). However, in spite of having various good nutritious qualities its worldwide production is 0.5 t ha^{-1} but the predictable yield potential is 2.5 to 3.0 t ha^{-1} (Gayacharan *et al,.* 2020). So there is a wide yield gap between the demand and production. Considering this factor there is a great opportunity for the breeder to improve the yield of mungbean.

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For successful breeding program genetic diversity is an essential requirement (Tabasum *et al.,* 2020). Analysis of genetic diversity is the fundamental means to detect the most diverse parents which are helpful to search out the higher transgressive segregants or heterotic hybrids (Islam *et al.,* 2020). The crosses between the genetically distant parents are capable to produce high heterotic hybrids (Falconer, 1960; Moll *et al.,* 1962; Ramanujam *et al.,* 1974; Ghaderi *et al*., 1989; Mian and Bhal, 1989). Since Mungbean is a self-pollinated crop its genetic base is narrow. So, yield improvement in mungbean from the existing germplasm is not easier. Identification of the elite mungbean germplasm will be helpful to beat the current obstruct. As such the present study was analyzed the degree and nature of genetic diversity among fourteen mungbean varieties to find out the potential parents for developing novel mungbean varieties.

Materials and Methods

Plant materials, experimental location and design

In this investigation 14 mungbean varieties were used as the plant materials, collected from three national institutes viz. Pulses Research Centre of Bangladesh Agricultural Research Institute (BARI), Gazipur Agricultural University (GAU), Salna, Gazipur, Bangladesh Institute of Nuclear Agriculture (BINA), Lalmonirhat and Barisal district (Table 1).

Table 1. List of 14 varieties of mungbean along with their sources

Sl. No.	Local name	Mark	Sources
1	BARI mung1	G_1	Pulses Research Centre, BARI
2	BARI mung2	G_2	Pulses Research Centre, BARI
3	BARI mung3	G_3	Pulses Research Centre, BARI
4	BARI mung4	G_4	Pulses Research Centre, BARI
5	BARI mung5	G_5	Pulses Research Centre, BARI
6	BARI mung6	G_6	Pulses Research Centre, BARI
7	BARI mung7	G_7	Pulses Research Centre, BARI
8	BARI mung8	G_8	Pulses Research Centre, BARI
9	BAU mung1	G_9	Department of Agronomy, GAU
10	BINA mung5	G_{10}	Plant Breeding Division, BINA
11	BINA mung8	G_{11}	Plant Breeding Division, BINA
12	BINA mung9	G_{12}	Plant Breeding Division, BINA
13	Chaitamung	G_{13}	Lalmonirhat
14	Sonamung	G_{14}	Barisal

The field investigation was carried out at the experimental plot of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The field was located at 90º22′ E longitude and 23º41′ N latitude, belongs to the Agro-ecological zone of the Modhupur Tract, AEZ-28. Randomized complete block design (RCBD) with three replications were followed for conducting the field experiment. The plot size was 2.5 m with single line. The distance between row to row was 30 cm and plant to plant was 10 cm. All the agronomic practices such as thinning, fertilizer application, irrigation, weeding, etc., were followed for mungbean cultivation as per the recommendation of Bangladesh Agricultural Research Institute (BARI).

Morphological parameter observation and analysis

Data were recorded on nine yield contributing characters viz. Plant height , number of leaves/plant, number of branches/plant, number of pods/plant, number of pods cluster/plant, pod length, number of seeds/pod, 1000 seed weight (g). The analyses in respect of nine different morphological traits of 14 mungbean varieties were analyzed by multivariate analyses using the GENSTAT 5.13 software program. The varieties were grouped into clusters based on Mahalanobis's D^2 statistics and canonical variate analysis.

Results and Discussion

Divergence analysis is usually performed to identify the different individual varieties for hybridization purposes. The genetic diversity analysis and clustering of the varieties were studied based on the traits studied which presented below.

Non-hierarchical clustering

By following Tocher's method (Rao, 1952) the fourteen varieties were grouped into four clusters based on the relative magnitude of Mahalanobis D^2 values (Table 2). Cluster II and Cluster III consists of two varieties, which found as the smallest cluster. Cluster IV obtained as the largest cluster composed of six varieties. Finally, cluster I composed of four varieties (Table 2). These results confirmed the clustering pattern of the varieties obtained through principal component analysis. Cluster mean values of nine different characters of 14 mungbean varieties are presented in Table 3. The clustering pattern obtained coincided with the apparent grouping patterns performed by PCA. It is clear that the results obtained through PCA were supported by non-hierarchical clustering. Joshi *et al.* (2022) also studied the genetic divergence of mungbean with thirty varieties and reported about twelve clusters. Tiwari *et al.* (2022), Zhang *et al*. (2024) also reported the same results while studying about thirty six and three hundred two mungbean varietiesrespectively that corroborate with the present findings.

Table 2. Distribution of 14 mungbean varieties into four different clusters

Cluster number	Number of varieties	Percent (%)	Name of varieties
I	4	28.57	G1 , G2 , G3 , G4
II	2	14.29	G6 , G7
III	2	14.29	G5 ,G10
IV	6	42.86	G8 , G9 , G11, G12, G13, G14

Table 3. Cluster mean values of nine different characters of 14 mungbean varieties

Characters	I	II	III	IV
Plant height (cm)	52.08	43.49	47.25	42.15
No. of leaves/plant	9.53	13.11	13.05	11.16
No. of branches/plant	3.08	3.77	3.39	3.22
No. of pods/plant	10.14	18.33	11.41	13.5
No. of pod clusters/plant	5.23	5.72	6.18	5.57
Pod length/plant (cm)	8.04	10.45	10.02	8.27
No. of seeds/pod	9.25	10.83	12.5	10.44
Wt. of 1000 seeds (g)	28.58	50.84	39.83	29.11
Seed yield/plant (g)	3.23	5.05	5.1	4.24

Principal component analysis

Principal components were computed from the correlation matrix and varieties scores obtained from first components and succeeding components with latent roots greater than the unity. Eigen values corresponding nine principal component axes and percentage of total variation accounting for them obtained from the principal component analysis are presented in Table 4. It represents that the cumulative eigen values of four principal components accounted for 89.82% of the total variation among the varieties. The 1st principal component accounted for 57.45% of the total variation, the second, third and fourth components accounted for 14.09%, 10.03% and 8.25% of the total percent of variation, respectively (Table 4). For selection of the best varieties these component characters will be more helpful. Mwangi *et al.* (2021) studied the genetic divergence among seven mungbean varieties and found that first three components contributed highest (83.4%) of overall variation. Similar results were also reported by Gayacharan *et al*. (2020) and they found first five principle components (PCs) explained 91.4% of the total variation. Tiwari *et al.* (2022) also studied about mungbean results were alike.

Table 4. Eigen values, percentage of variation and cumulative percentage in respect of nine axes in 14 varieties of mungbean

Principal component axes	Eigen value	Percent variation	Cumulative % of percent variation
I	5.17	57.45	57.45
II	1.268	14.09	71.54
III	0.903	10.03	81.57
IV	0.742	8.25	89.82
V	0.517	5.74	95.56
VI	0.184	2.05	97.61
VII	0.117	1.3	98.91
VIII	0.067	0.74	99.65
IX	0.032	0.35	100

Principal coordinate analysis

Principal coordinate analysis (PCO) was performed on auxiliary principal component analysis. This analysis helps in estimating distances (D^2) for all combinations between pairs of varieties. The highest inter varietal distance was observed between the varieties G1 and G_6 (1.288). The second highest value observed between the varieties G_2 and G_6 (1.270). The tenth highest pair distance was observed between varieties G_2 and G_{11} (1.150). The lowest distance was observed between the varieties G_1 and G_2 (0.150). The second lowest observed between varieties G_{13} and G_{14} (0.185). The tenth lowest distance was observed between the varieties G_8 and G_{12} (0.394). The difference between the highest and the lowest inter-genotypic distance indicated the prevalence of variability among the 14 mungbean varieties (Table 5).

Table 5. Ten of each lower and higher inter varietal distances (D^2) between pairs of mungbean varieties

Highest 10 inter genotypic distances				Lowest 10 inter genotypic distances			
Sl	Varieties	Varieties	Values	Sl	Varieties	Varieties	Values
1	G1	G6	1.288	1	G1	G2	0.150
2	G2	G6	1.270	2	G13	G14	0.185
3	G1	G7	1.254	3	G3	G4	0.261
4	G6	G13	1.250	4	G6	G7	0.268
5	G2	G7	1.229	5	G2	G13	0.346
6	G7	G13	1.209	6	G3	G9	0.359
7	G10	G13	1.186	7	G4	G9	0.359
8	G1	G10	1.171	8	G11	G12	0.380
9	G2	G10	1.154	9	G5	G9	0.390
10	G2	G11	1.150	10	G8	G12	0.394

Conclusion

Selection of genetically divergent varieties is an essential step for the hybridization program. So, the varieties would be selected based on specific objectives. A higher heterosis could be produced from the crosses between genetically distant parents. Considering the magnitude of genetic distance and agronomic performance, the varieties G_1, G_2, G_6, and G_7 from cluster I and cluster II would be suitable for selecting the best combinations that carry the desirable characteristics by the breeders.

Author's contributions

S. S designed, conceived, and executed the experiment and drafted the manuscript; B.N.S, S.P, S.I and S.N.B edited and reviewed the manuscript; MAS supervised the experiment, analyzed the data, and edited and reviewed the manuscript.

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Conflict of Interest

All authors affirm that there are no conflicts of interest regarding this research paper.

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