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BLEACHING OF COCONUT COIR FIBRE AND RECYCLING COIR PITH INTO BIO-FERTILIZER

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ABSTRACT

The study investigated the value addition of coconut husk through the bleaching of coir fibre/yarn and the recycling of coir pith into biofertilizer. This work, conducted at the Regional Agricultural Research Station (RARS), Jashore in collaboration with Coco Fibre Mills Ltd, applied hydrogen peroxide and sodium silicate under alkaline conditions, with auxiliaries such as trisodium phosphate, Lissapol-D, soda ash, and sodium hydroxide. Results confirmed that maintaining a pH above 10 was critical for effective bleaching, with coir yarn showing superior whiteness and profitability. Economic analysis revealed the bleaching of coir yarn as a viable income-generating activity for coconut farmers. Coir pith was composted using Pleurotus mushroom spawn, lime, urea, and TSP, resulting in enhanced nutrient profiles (N, P, K, Ca, and Mg). Bioassay trials on tomato demonstrated enhanced growth and yield validating coir pith compost as a sustainable substitute for cowdung and chemical fertilizers. Findings highlighted that coconut husk valorization as a pathway to environmental sustainability in rural employment, and reduced dependence on synthetic inputs.

Keywords: Coconut fibre, Coir, Scouring, Softening, Textile.

INTRODUCTION

Bangladesh produces annually around 100 million coconuts, of which 70-80% are consumed either as fresh fruit at the mature stage or as immature fruits for drinking purposes. Be it fruit or drink, only 35% of the whole coconut is consumed. The remaining 65%, consisting of husk, shell, and water (liquid endosperm), is neglected or remains unutilized. Many coconut farmers burn or dispose of husks, which leads to the accumulation of both solid and liquid waste that pollutes the environment. However, these neglected, underutilized parts of the coconut can be processed into valuable products, contributing to environmental sustainability, economic development, and community livelihoods. The potential products that can be produced from coconut husk include coir (fibres), coir yarns, doormats, geo-textiles, exportable handicrafts, and biofertilizers. Coir fibres are short and hard, typically scratchy and rough to the touch, and difficult to weave. Bleaching treatment removes natural color, yield a permanent white color and enhance the sensory experience of coir products (van Dam, 2002). Bleaching treatment removes natural color, yields a permanent white color, and enhances the sensory experience of coir products (van Dam, 2002). Bleaching of coir/yarn is

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generally carried out using hydrogen peroxide (H_2O_2) and sodium silicate in alkaline conditions of pH 10.5-11.00 (Trotman, 1975). However, coir fiber possessing much higher lignin may change the pH conditions. Chemicals such as trisodium phosphate, Lisa Pól-D, and soda ash are used in the bleaching process as auxiliaries to maintain the required pH level of 10.5 to 11.00. Coir pith (coir dust), on the other hand, constitutes 70% of the coconut husks, is resistant to natural degradation, and remains unutilized for want of a viable technology for its economic utilization (Crawshaw, 2002; Nagaraja and Basavaiah, 2010). Aside from improper management, coir dust can cause polyphenol leaching and pollute the land and groundwater (Anderson, 1964). The challenge is to find another way to utilize coir dust that could increase the income of coconut farmers. Realizing the untapped potential of coconut husk, the study was undertaken to identify chemicals used for textile materials that can be suitably adopted for coir materials and the recycling of coir pith into organic fertilizers through a bio-conversion into bio-organic fertilizer (van Dam, 2002).

MATERIALS AND METHODS

The study was conducted in collaboration with a private coconut husk processing factory, the Coco Fibre Mills Limited (CFML), Chanchramorh, Jashore, from January to November 2010. The CFML was 5 km away from the Regional Agriculture Station (RARS), Bangladesh Agricultural Research Institute (BARI), and was equipped with specialized comb crushing decorticating machines, general-use decorticating machines, husk beating machines, and rope spinning machines. The CFML supplied the required fibre and coir dust for the study, while RARS provided technical and equipment support related to processing fibre, such as making kilns, chemical support, mushroom spawn, lime, pH meters, and labor. The proposed activities were conducted under two components:

I. Bleaching of coir fibre and coir yarn

II. Composing of coir dust into bio-fertilizer

Component: I

Chemicals used in bleaching recipes and their properties

a) Hydrogen peroxide (H_2O_2) - a strong bleaching agent and disinfectant. In acidic solutions, it is one of the most powerful oxidizers. In basic solutions, hydrogen peroxide reduces a variety of inorganic ions.

b) Sodium silicate (Na_2SiO_3) - available in aqueous solution and in solid form with the chemical formula. It is a white powder, readily soluble in water, producing an alkaline solution that is glassy, colorless, and stable in neutral and alkaline solutions. Anhydrous sodium silicate contains a chain polymeric anion that removes soluble and insoluble impurities found on the surface of fibers, including natural and adventitious impurities (Gerard et al., 2005).

c) Trisodium phosphate (Na_3PO_4): an inorganic compound with the chemical formula. It is a white, granular or crystalline solid, highly soluble in water, producing an alkaline solution. It is used as a cleaning agent, stain remover, and degreaser (Klaus, 2008).

d) Lissa Pól-D ($C_{34}H_{68}Na_2O_8S_2$): an anionic surfactant that acts as a wetting agent for wettable powder, having the ability to dissolve fat or lipid molecules in the cleaning action (Plenderleith, 1956).

e) Soda ash (Na_2CO_3): also known as sodium carbonate and washing soda, is a water-soluble sodium salt of carbonic acid. Sodium carbonate is well known domestically for its everyday use as a water softener, maintaining a high pH in the liquor (Larami, 1998). It removes soluble and insoluble impurities found on the surface of fibers, including natural and adventitious impurities such as oils, waxes, fats, vegetable matter, and dirt. (Na_2CO_3) improves the quality of water by removing carbonate, bicarbonate, and sulfate salts of calcium and magnesium.

f) Sodium Hydroxide (NaOH): eliminate impurities and remove the lignin ad hemicellulose layer on fiber surface (Senthamaraikannan and Kathiresan, 2018).

All these chemicals are essential for the textile industry and are frequently used as laboratory reagents in schools, colleges, and universities. Each of the reagents is available in the chemical shops of Bangladesh. A comparative study of the bleaching system led to the identification of various chemicals used for textile materials, which can be suitably adopted for coir materials. H_2O_2 and Na_2SiO_3 are the bleaching agents, while Na_3PO_4, $C_{34}H68Na_2O8S_2$, and Na_2CO_3 are auxiliaries that clean the substrates and maintain the required pH of the recipe during the bleaching process. Na_2SiO_3 in the recipe stabilizes H_2O_2 in alkaline conditions through premature degradation and increases its effectiveness. Since the rates of H_2O_2 (20 ml/l) and Na_2SiO_3 (5 g/l) for coir bleaching are well reported, the same have not been taken as variables (Ravindranath et al., 1997; van Dam, JEG, 1997). Our study was delimited to the effects of Na_3PO_4, $C_{34}H68Na_2O8S_2$, and Na_2CO_3 on the degree of whiteness of bleached coir in the recipes of bleaching agents H_2O_2 (20 ml/l) and Na_2SiO_3 (5 g/l). The combinations of auxiliary chemicals in the recipe types are stated in Table 1. Liquor rations were 1:12 for coir yarn and 1:20 for coir fibre (van Dam, 1997; Anto et al., 1997). Figure 1 illustrates the bleaching process, where coir and yarn were soaked in a bleaching solution and heated. The fibre/yarn turned white shortly after the water started to boil. The treated coir fibres and yarns were collected after cooling, washed, and dried in the sun. To investigate the effect of NaOH on softness, coir and coir yarn were soaked in a 30% solution of NaOH for 5, 10, 15, and 20 minutes, respectively, before treatment in brown recipes. The whiteness of the bleached fibre was rated from 1 to 10 through visual and manual evaluation. A score of 0 indicated no change in inherent color, and a score of 10 indicated the maximum whiteness. Similarly, the scoring of softness was done manually by holding the fibres in the fist and feeling the softness of the fibre. A score value of zero (0) indicated stiff fibre, while a score of 10 indicated very delicate softness of the fibre.

Table 1. Recipes for bleaching coir fibre and yarn using hydrogen peroxide and sodium silicate with auxiliary chemicals.

(Ravindranath et al., 1997)

Chemical used	Code			
	A	B	C	D
Hydrogen peroxide (ml/l)	20	20	20	20
Sodium silicate (g/l), remove insoluble impurities	5	5	5	5
Tri-sodium phosphate (g/l), remove stain and decreaser	2	2	-	-
Lissapol D (ml/l), dissolve fat and lipid	2	-	-	2
Soda Ash, remove soluble/insoluble impurities from the surface	-	2	-	2

Fig. 1: Heating process of coir fibre and yarn in hydrogen peroxide bleaching solution.

Component: II

Figure 2. A brick-built tank measuring 5 meters by 3 meters and 2 meters high was built in a shaded spot on the factory ground in February 2008 to dispose of and decompose coir pith into organic fertilizer.

Fig. 2. Brick-built tank constructed for disposal and decomposition of coir pith into organic fertilizer

The decomposing process was initiated as described by Prabhu and Thomas (2001) in the first week of March 2007 and continued until May 2007. One hundred kg (approximately) of coir pith was spread uniformly on the floor of the RCC tank. Over the well-spread coir pith, 10 bags of spawn of Pleurotus sajor-caju mushroom were spread uniformly. Another 100 kg of coir pith was spread over the inoculated layer of coir pith. Urea and Triple-phosphate (TSP), one kilogram of each, were sprayed uniformly over the layer of coir pith. The applied urea and TSP were covered with the next layer of 100 kg of coir pith. The process of sandwiching mushroom spawn and urea/TSP alternatively was repeated with hundred-kilogram layers of coir pith five times, as visualized in Figure 3 (A-D).

Fig. 3: Layering process of mushroom spawn, urea, TSP, lime, and coir pith during composting

Five kg of powdered lime was added before making the sandwich. The moisture content of the coir pith was maintained at around 200%. Around 500 kg of coir dust was used for the study. The dump was covered with black polyethylene. Water was added periodically to maintain the required moisture level. After 60 days, when the heap was reduced and turned blackish, emitted an earthy odor, and the waste particle size was reduced, the compost was harvested. Chemical analysis of the decomposed coir dust was performed in the laboratory of the Division of Soil Science, BARI, Gazipur, to estimate the nutritional status. The performance of the decomposed coir dust as organic manure against cow dung was evaluated using a bioassay technique on summer tomatoes during May-October 2007. Data on the growth and development of tomato plants were collected and summarized, and necessary transformations were done before analyzing the data. Means were separated by DMRT.

RESULT AND DISCUSSION

Part I: Bleaching fibre and coir yarn

On examination by visual and manual comparison, it was seen that coir fibre treated with recipes A, B, and D possessed greater whiteness than that obtained with recipe C (Figure 4-i). Variation in the degree of whiteness was similarly noticed in coir yarn when treated with recipes A, B, and D (Figure 4-ii). The observed variations might be associated with

the incorporated auxiliaries such as Na_3PO_4, $C_{34}H68Na_2O8S_2$, Na_2CO_3, and NaOH in the recipes (Table 1). The auxiliary chemicals, individually or in combination with others, maintained pH levels of 10.5 to 11.5, which are necessary for the ionic decomposition of H_2O_2 to nascent [O] for effective bleaching action of the recipes. Furthermore, the auxiliary chemicals are alkaline in nature and clean the fibre surface, removing insoluble stains. It is noted that recipe C incorporates no auxiliaries (Table 3), and the pH levels before and after treating the coir were 8.5 and 6.60, respectively. The lower pH levels of recipe C could not facilitate the decomposition of H_2O_2 to start bleaching. According to reports by Gerard et al. (2005), Klaus (2008), Plenderleith (1956), and Larami (1998), in alkaline conditions, H_2O_2 becomes unstable, releases nascent oxygen molecules [O], and breaks the chemical bonds of the color-producing agents of substrates. Venkatachalapathy et al. (1999) and Bhattarai et al. (2005) similarly reported that H_2O_2 in alkaline media loses the extra oxygen, becomes water (H_2O), and releases nascent oxygen [O], which desperately wants to bond to something or oxidize it. The rate of decomposition increases with a rise in temperature, concentration of H_2O_2, and pH value. The decomposition reactions are as follows:

$H_2O_2 \rightarrow HO_2^- + H^+$ ---------- Equation-I

$HO_2^- \rightarrow H_2O + [O^-]$ ----------Equation-II

Table 2: Comparative color outcomes of coir fibre and yarn treated with different bleaching recipes.

Recipes	Color of the product	
	Coir fibre	Coir yearn
A	Bright white	Bright white
B	Bright brown	Bright brown
C	Off white compared to that of A	Whited but dull
D	No change occurred	No change occurred

i) Whiteness of coir fibre when treated with recipes A, B, C and D

ii) Whiteness of coir yarn when treated with recipe A

Fig. 4: Effects of bleaching recipes on whiteness of coir fibre (i) and coir yarn (ii).

Coir Softening and color vision

The coir yarn, when treated with NaOH before being treated with the bleaching agent, showed a yellowish tint, differing from the appearance of being directly bleached with different concentrations of H_2O_2. Degrees of softness were determined on a scale of 1 to 10, where 1 indicated stiff and 10 very soft. Treating coir fibre with 5% NaOH starting from 5% with an interval of 5 resulted in a linear increase in softness score (Figure 6). Iyer and Iyer (1996) reported that the softness of coir was the result of the removal of lignin, hemicellulose and other soluble compounds on the fibre surface during alkali treatment. The reaction of sodium hydroxide with cellulose is as follows:

$$Cell - OH + NaOH \rightarrow Cell - O{-}Na+ + H_2O + [\text{surface impurities}].$$

However, Varma et al. (1994) reported that softening of coir with NaOH provoked a loss of fibre strength, indicating a trade-off between flexibility and durability. Anto et al. (1997) reported that bleached and softened coir yarn with NaOH solution exhibited a yellowish tint compared to bleach with unsoftened coir yarn, reinforcing the visual changes associated with alkali treatment. A statistical design of experiments would therefore be essential to optimize NaOH concentrations and treatment conditions, balancing softness, strength, and color characteristics for improved processing of coconut fibre.

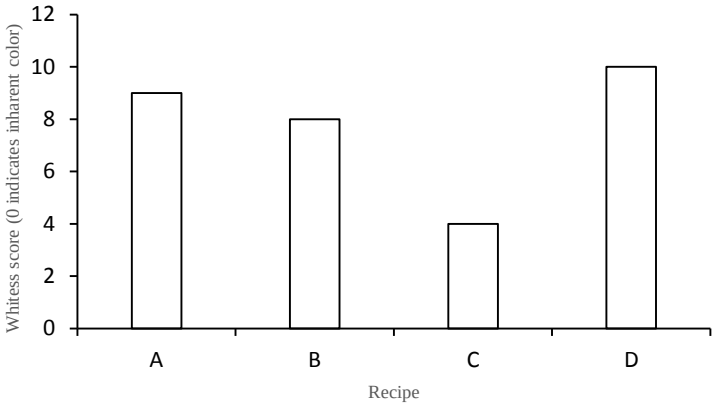


Fig. 5: Whiteness score of fibre treated by different recipes

Crawshaw (2002) similarly reported that coconut fibres are inherently yellowish-brown. When scouring and bleaching were carried out under alkaline conditions, the brown color was diminished, though it could sometimes result in a residual yellowish shade.

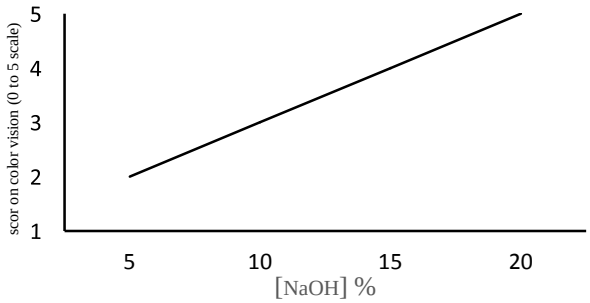


Fig. 6: Variation in color appearance of coir fibres treated with increasing concentrations of sodium hydroxide.

Table 3: pH levels of bleaching liquor before and after treatment under different recipe conditions.

Recipe types	Chemical contained in recipes	pH	
		Before treat	After
Bleached with Recipe A	TP and LD	12.00	11.5
Bleached with Recipe B	TP and SA	11.90	11.5
Bleached with Recipe C	TP alone	8.50	6.60
Bleached with Recipe D	LD & SA	11.8	11.7

TP= Trisodium phosphate, LD= Lisa Pól D, SA=Soda Ash

Economic analysis

Table 4: Cost of bleaching one kilogram of coir fibre and yarn, including chemical, labor, and fuel inputs

Cost item	Price (Tk)	Amount	1 kg yarn (12 litre)		1 kg coir (20 litre)	
			Required amount	Cost incurred by input (Tk)	Required amount	Cost incurred by input (Tk)
H_2O_2 (ml)	481.00/1000	8	96	46.18	160	76.96
NaSi (g/l)	600.00/1000	6	72	43.20	120	72
TS-P (g/l)	70.00/1000	2	24	1.68	40	2.8
Lissa Pól-D (ml)	100/1000	2	24	12.00	40	20
Coir/yarn (Tk)	30/kg	1	1	50.00	1	30.00
Labor	80/day	1	$1/30^{th}$	3.00	$1/30^{th}$	3.0
Fuel (wood)	30/40kg		4	3.00	4	3.00
	Total cost (Tk/kg)=		a)=	160.06		207.76
	Sale price (Tk)=		b)=	481.81		344.15
	Earning (Tk)=		c)=	321.75		136.63
	BCR=		(c/a)=	1.97		0.66

∗ In 2009, exchange rate of USD= Taka 68.83; labor price Tk 90/day; fire wood Taka 50.00 per 40 kg ; price of bleached coir in international market was SD- 7 and yarn was USD- 9)

∗ Estimated labor cost for processing per kg brown coir fibre amounted to Tk around Tk 3.00 at the rate of Tk 80/day (BBS 2008, Figure 7).

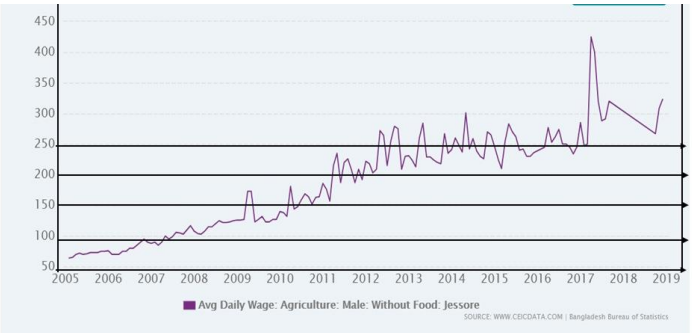
Costs and Returns

Costs

To estimate the cost of production, cost approach method was used. Labor, capital and interest of capital, and equipment cost were ignored because the fibre price included all costs. The variable costs, chemicals, fuel wood and labor cost (2 hours) were included for whitening process (Table 4). In the "Coco Fibre Mills", two workers used to bleached 30 kg of fibres daily. The labor cost for the experiment was determined based on the everyday factory output as stated above. Total cost for treatment of brown coir yarn /kg amounted to Tk 159.06 and coir to Tk 207.76. Labour expenses were calculated separately, as illustrated in Figure 7.

Returns

Gross return was obtained by adding the total value of the produce. Net income was estimated as the difference between the gross return and total cost of production. Thus, the average return for one kg of yarn is Tk 481.81 (USD 7.00). In other words, Tk 322.75 (481.81 - 159.06) was obtained as net returns for every kg of coir yarn. Further, for every Taka of investment in whitening coir yarn, about Tk 2.03 was obtained as returns, indicating its profitability. Similarly, for every Taka of investment in whitening fibre Tk 0.65 was obtained as returns, indicating its profitability (Table 3).



[Source: BBS 2018]

Fig. 7: Average daily wage of agricultural laborers in Jashore during 2007 (without food).

Income and employment opportunities from coconut will encourage more coconut plantations in the country, which will directly reduce environmental pollution by capturing free environmental carbon in its fruits. The technology also has the potential to reduce pressure on forests caused by the indiscriminate cutting down of trees. Leaves, floral parts, and fruit parts are eco-friendly recyclable fuels for the growers.

Part II: Composing of coir dust into bio-fertilizer

RESULT AND DISCUSSION

Nutrient content profile of coir pith

Chemical properties of fresh and decomposed coir dust were determined at the Soil Science Laboratory of Bangladesh Agricultural Research Institute (BARI), Gazipur. Data

in Table 4 and Table 5 reveal that supplying capacity of nitrogen (N), phosphorus (P) and potassium (K) and micro nutrients of coir pith after decomposition has improved. According to the report of Ravindranath et al., (1997), reported added lime weakened lignin structures and enhanced microbial population which broke down of cellulose and possibly speed up the decompose rate of coir dust. Salmones et al., (2005) and Yildiz et al, (2002) reported *Pleurotus mushroom* species consume lignocellulose from lignocellulos waste. Upon utilizing this property of *Pleurotus* it is used as an agent for degradation in coir pith composting.

Table 5: Nutrient composition (N, P, K, S, B) of undecomposed and decomposed coir pith.

Treatment	Appearance	pH	OM	Moisture (%)	N	P	K	S	B
Undecomposed	Brownish	6.7	6.05	60	0.12	0.12	0.07	0.039	0.004
Decomposed coir pith	Blackish	6.0	8.6	8.1	1.22	1.2	1.2	0.051	0.005

Table 6: Concentrations of metallic ions (Cu, Fe, Mn, Zn, Ca, Mg) in undecomposed and decomposed coir pith.

	Cu	Fe	Mn	Zn	Ca	Mg
Undecomposed	Trace	0.08	Trace	Trace	0.35	0.12
Decomposed coir pith	0.001	0.01	0.004	0.003	0.80	0.14

Bioassay of decomposed coir dust.

A field trial with six treatments and three replicates for each treatment (a total of 50 tomato plant polybags) was arranged in a Completely Randomized Design (CRD) (Table 7) with maintaining a spacing of 30 cm between rows, and 20 cm between polybags within rows. Tomatoes require nutrients such as N, P, K, Mg, Ca and S in large amounts. In trace levels, other elements like Fe, Cu, Zn, Mn, B, Mo, and Cl are required. Tomatoes are so frequently fertilized with N, P and K and infrequently with Ca and Mg. Tomato plants cannot grow properly or do not bear fruits, without these nutrients (Sainju et al., 2003). The treatments incorporated coco dust were compared with the treatments incorporated cow dung, recommended chemical fertilizers and control. The treatment combinations are as follows:

1) T_1= 10 t/ha coir pith + recommended dose of chemical fertilizers
2) T_2=20 t/ha coir pith + 50% recommended dose of chemical fertilizers
3) T_3=10 t/ha cowdung + recommended dose of chemical fertilizers
4) T_4=20 t/ha cowdung + 50% recommended dose of chemical fertilizers
5) T_5= recommended dose of chemical fertilizers
6) T_6= control

Data in Tables 6 and 7 revealed that tomato yield and growth parameters were significantly influenced by the decomposed coir pith. Hence, it would be possible to

increase tomato production in Bangladesh by improving the soil fertility by using decomposed coir pith as organic fertilizers. The results also implies that decomposed coir pith can also be used as substitute of any other organic fertilizers.

Table 7: Growth and development parameters of tomato plants influenced by coir pith and cow dung treatments.

Treatment	Plant height at 1st harvest	Days to 50% flowering	Flower/cluster	% fruit set in cluster	Days to 1st harvest
	(cm)	(day)	Nos		(day)
T_1 (10 CCD)	130c	26	6.56 b	57.45 (7.60) c	64
T_2 (20 CCD)	135a	26	6.67 ab	62.55 (7.98) b	64
T_3 (10t CWD)	133b	27	7.33 a	62.50 (8.19) b	65
T_4 (20 t CWD)	130c	27	7.33 a	69.86 (8.35)a	65
T_5 (recommended dose)	129c	26	6.77 b	55.13 (7.42) c	64
T_6 (control)	130c	27	6.56 b	50.09 (6.70) d	65
CV (%)	6.24	2.10	3.81	3.96	1.56

T_1= 10 t/ha coir pith + recommended dose of chemical fertilizers; T_2=20 t/ha coir pith + 50% recommended dose of chemical fertilizers; T_3=10 t/ha cowdung + recommended dose of chemical fertilizers; T_4=20 t/ha cowdung + 50% recommended dose of chemical fertilizers; T_5= recommended dose of chemical fertilizers; T_6= control

Table 8: Fruit characteristics and yield of tomato plants under coir pith, cow dung, chemical fertilizer, and control treatments.

Treatment	Individual fruit weight	Fruit size		Fruit/plant	Yield
		Length of fruit through the pole	Length of fruit through the equator)		
	(g)	(cm)	(cm)	(nos)	(t/ha)
T_1 (10 CCD)	53b	4.44 a	4.60	26 ab	26.98 b
T_2 (20 CC)	50c	4.49 a	4.55	29 a	31. 59 a
T_3 (10t CD)	54a	4.45 a	4.58	28 ab	30.12 ab
T_4 (20 t CD)	49d	4.45 a	4.44	26 ab	26.29 b
T_5 (recommended dose)	45e	3.20 b	4.49	21 bc	20.27 c
T_6 (control)	42f	3.14 b	4. 51	18 c	14.56 d
CV (%)	8.06	12.04	3.69	15.63	5.47

T_1= 10 t/ha coir pith + recommended dose of chemical fertilizers; T_2=20 t/ha coir pith + 50% recommended dose of chemical fertilizers; T_3=10 t/ha cowdung + recommended dose of chemical fertilizers; T_4=20 t/ha cowdung + 50% recommended dose of chemical fertilizers; T_5= recommended dose of chemical fertilizers; T_6= control

CONCLUSION

The study demonstrated that coir bleaching using hydrogen peroxide is effective only under alkaline condition (pH=≥10) and coir yearn provide higher economic return compared to fibre. Pre-treatment with NaOH solution improved softness and introduced a yellowish tint. This underscores the need for optimized treatment designs balancing whiteness index, softness and fibre strength. Recycling coir pith through mushroom-assisted composting significantly enhanced nutrient availability and improved tomato yield. This established coir pith compost as a viable alternative to cow dung and chemical fertilizers. These innovations offer dual benefits: (i) they create income through value-added coir products, and (ii) they help manage soil fertility sustainably by recycling organic materials. Adoption of this technology can reduce environmental pollution, promote coconut-based livelihoods, and contribute to national goals of sustainable agriculture and resource efficiency. Further, enhanced tomato yields from coir pith compost advance SDG 2, improving soil fertility, food security, and smallholder livelihoods through sustainable agriculture.

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BRUCELLOSIS IN SMALL RUMINANTS AND HUMAN IN BANGLADESH

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ABSTRACT

Brucellosis is a globally important zoonotic disease caused by *Brucella* species, leading to reproductive losses in animals and a range of non-specific febrile illnesses in humans. Despite its significance, the epidemiology of brucellosis in Bangladesh remains inadequately understood, particularly in small ruminants and high-risk human populations. This study investigated the seroprevalence and molecular detection of *Brucella* spp. in goats, sheep and humans in Mymensingh, Bangladesh. Blood samples were collected from 150 goats, 75 sheep and 182 humans. Serum was separated using standard procedures and stored at –20°C. All sera were screened using the Rose Bengal Plate Test (RBPT), followed by confirmation with indirect ELISA (I-ELISA). RBPT- and ELISA-positive goat samples were further subjected to DNA extraction, PCR amplification using nine MLSA loci, sequencing and phylogenetic analysis. Among goats, 4 of 150 sera (2.67%) reacted in RBPT and 3 (2.00%) were confirmed positive by ELISA. One of 75 sheep samples (1.33%) showed weak RBPT reactivity but was ELISA negative. In humans, 4 of 182 sera (2.20%) were positive in both RBPT and ELISA, with the highest seroprevalence observed among artificial inseminators (10.0%). PCR detected *Brucella abortus* in three goats sera, and multilocus phylogenetic analysis placed these isolates within the *B. abortus* clade with strong support. Overall findings indicate low-level circulation of *Brucella* infection in small ruminants and humans in the study area. The detection of *B. abortus* in goats underscores potential cross-species transmission. These results highlight the need for strengthened surveillance and a coordinated One Health intervention strategy to reduce zoonotic risk and prevent further spread.

Keywords: Brucellosis**,** Ruminants**,** Abortion, Human, One Health

INTRODUCTION

Brucellosis is a globally distributed zoonotic disease caused by bacteria of the genus Brucella, comprising several species with varied host specificity (Rajendhran, 2021). Small ruminants serve as important hosts for *B. melitensis*, a highly pathogenic species responsible for reproductive losses and significant public health impacts (Rossetti et al.,

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2022). Although controlled in many developed nations, brucellosis remains endemic in low and middle-income countries, including Bangladesh, where it continues to affect livestock productivity and human health. The first report of brucellosis in Bangladesh dates back to 1967, with subsequent studies confirming its presence across cattle, buffalo, goats and humans (Rahman et al., 2014). Seroprevalence in goats has been documented using RBPT, ELISA and related assays, with reported prevalence ranging from 1.56% to 3.24% in different regions of Bangladesh (Ahmed et al., 2018; Rahman et al., 2012a; Munsi et al., 2021). Transmission to humans occurs primarily through contact with infected animals or consumption of contaminated animal products (Makita et al., 2008; Lapaque et al., 2006), while human-to-human transmission is rare (Godfroid et al., 2005). Brucellosis poses occupational risks for veterinarians, farmers, abattoir workers and other animal handlers (Pappas et al., 2005). Clinical manifestations range from acute febrile illness to chronic complications involving musculoskeletal and organ systems (Franco et al., 2007).

Various serological and molecular diagnostic tests are used for detection (Godfroid et al., 2010; Saeed et al., 2019). Human studies in Bangladesh have reported prevalence ranging from 2.0% to 18.2% depending on occupational exposure (Rahman et al., 2012b; Rahman et al., 2016). Despite this, data on many high-risk groups remain limited, underscoring the need for updated epidemiological assessments. The present study contributes to understanding the burden of brucellosis in small ruminants and high-risk human populations and can support future control strategies in Bangladesh.

METHODOLOGY

The study was conducted at the Zoonotic Disease Diagnostic Laboratory, Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh. Small ruminant samples were collected from the BAU Goat Farm (BAU-GF) and the Trishal Sheep Farm, Mymensingh.

Human and Animal Sampling

A total of 182 human serum samples were collected from different upazilas of Mymensingh. The participants included animal workers (n = 82), animal owners (n = 40), village doctors treating animals (n = 30), artificial inseminators (n = 20), and butchers (n = 10). A structured questionnaire recorded demographic and exposure-related variables such as age, sex, disease history, duration and type of animal contact, and raw milk consumption.

Similarly, data from randomly selected small ruminants were obtained using a questionnaire. Animal-level variables included age, sex, breed and reproductive disorders, while farm-level variables recorded the flooring system (brick floor or bamboo-based macha system).

Collection of Blood and Serum Preparation

Approximately 5–7 ml of blood was collected aseptically from 75 sheep and 150 goats using sterile syringes (Figure 1). Samples were kept undisturbed at room temperature to facilitate clotting before overnight storage at 4°C. Serum was separated by centrifugation at 2,500 rpm for 10 minutes and stored at –20°C until analysis. Blood samples from 182 humans were processed similarly (Figure 2).

All sera were initially screened using the Rose Bengal Plate Test (RBPT). Samples positive on RBPT were further confirmed by indirect enzyme-linked immunosorbent assay (I-ELISA). Goat samples positive in both RBPT and ELISA were subjected to DNA extraction, PCR amplification, sequencing and phylogenetic analysis.

Rose Bengal Plate Test (RBPT)

RBPT was performed following the standard procedures of EURLB (2021) and WOAH (2023). For each test, 30 µL of serum and 30 µL of Rose Bengal antigen were mixed thoroughly on a test plate and rocked gently for 4 minutes. Visible agglutination was interpreted as a positive result (Figure 3).

Indirect ELISA (I-ELISA)

I-ELISA was conducted according to the manufacturer's protocol (Svanova Biotech AB, Sweden). Reagents were brought to room temperature (18–25°C). Serum samples (4 µL) and controls were added to antigen-coated wells containing 100 µL of dilution buffer. After incubation at 37°C for 1 hour and three washes with PBST, 100 µL of HRP conjugate was added and incubated for another hour. Plates were washed again, followed by addition of 100 µL substrate solution and a 10-minute incubation. The reaction was stopped by adding 50 µL stop solution. Optical density was measured at 450 nm within 15 minutes to avoid variation. Samples with percent positivity (PP) values ≥ 40 were considered positive.

The percent positivity values (PP) were calculated using the following formula.

$$PP = \frac{\text{Test sample or Neg C (OD)} \times 100}{\text{Positive control (OD)}}$$

The assay was calibrated against the OIE ELISA Standard sera and Standardized against the EU derivatives 64/432/EEC, Annex C. The PP value of > = 40 was considered as positive.

DNA extraction, PCR, sequencing and phylogenetic tree construction

Genomic DNA was extracted from goat serum samples that tested positive in both RBPT and I-ELISA using previously described methods (Rahman et al., 2020). PCR amplification was carried out using nine pairs of primers targeting the genes *gap* (glyceraldehyde-3-phosphate dehydrogenase), *aroA* (3-phosphoshikimate-1-carboxyvinyl transferase), *glk* (glucokinase), *dnak* (chaperone protein), *gyrB* (DNA gyrase subunit B), *trpE* (anthranilate synthase), *omp* (25 kDa outer-membrane protein), *cob* (cobyric acid synthase) and *int-hyp* (5′ upstream region of a hypothetical protein), following the MLSA-9 scheme (Rahman et al., 2022).

PCR products were visualized by gel electrophoresis using a gel documentation system. Amplicons of expected size were purified and submitted to a commercial sequencing facility for bidirectional sequencing. For phylogenetic comparison, a dataset of 483 reference sequences corresponding to the nine loci was retrieved from the Brucella PubMLST database.

Multiple sequence alignments of concatenated nucleotide sequences (nine loci) were performed using MAFFT (Multiple Alignment using Fast Fourier Transform). Phylogenetic reconstruction was conducted using the maximum-likelihood method implemented in RAxML. The resulting tree was visualized and annotated using TreeGraph software to determine the phylogenetic placement of Bangladeshi isolates relative to recognized *Brucella* species.

Statistical analysis

The chi-square test was employed to find out the significant relationship between the prevalence of brucellosis and the demographic variables such as age, sex, breed, pregnancy status, and flooring system in small ruminants by using SPSS version 17.0.

RESULTS AND DISCUSSION

Screening of brucellosis in goats, sheep and humans using RBPT and I-ELISA is summarized in Table 1. Among 150 goat sera, 4 (2.67%) were RBPT-positive, of which 3 (2.00%) were confirmed by ELISA. In sheep, only one of 75 samples (1.33%) showed weak RBPT reactivity but was ELISA-negative. Human sera showed 4 positives (2.20%) in both RBPT and ELISA. Three ELISA-positive goat samples were identified as Brucella abortus by PCR. Phylogenetic analysis of concatenated MLSA-9 loci confirmed clustering of these isolates within the B. abortus clade.

Overall seropositivity in goats (2.67% RBPT; 2.00% ELISA) aligns with previous low-to moderate prevalence reports in Bangladesh. Age-wise patterns indicated higher prevalence in animals >24 months, though differences were not statistically significant. Female goats showed slightly higher seropositivity than males, but without significant association. Pregnancy status also showed no significant effect, despite marginally higher prevalence in pregnant animals.

A notable finding was the markedly higher prevalence in goats with a history of abortion (RBPT 30%; ELISA 20%), supporting earlier evidence that reproductive disorders constitute major risk factors. Flooring systems showed no meaningful influence on seropositivity.

Human brucellosis prevalence was 2.20%. Higher rates occurred in individuals above 30 years (9.09%), males (2.5%), and artificial inseminators (10%). No positives were detected among animal workers, village doctors or butchers. Prevalence was higher among individuals with 10–20 years of animal contact (6.38%) and among those consuming raw milk (5.80%).

Taken together, the results confirm low-level circulation of *Brucella* spp. in small ruminants and humans in the study area, with evidence of *B. abortus* infection in goats. The findings highlight significant exposure in high-risk groups and reinforce the need for strengthened surveillance and a coordinated One Health-based control approach in Bangladesh.

Table 1: Screening of brucellosis in human, sheep and goats sera with Rose Bengal Plate Test and the degree of agglutination.

Sl. No.	Species	Total no. of animal examined	Total no. of RBPT Reactors		Degree of agglutination (Positive)			
			Positive	Negative	Positive	Week (+)	Moderate (++)	Strong (+++)
01	Sheep	75	1	74	1.33%	1	0	0
02	Goat	150	4	146	2.67%	1	0	3
04	Human	182	4	178	2.20%	0	4	0

Table 2: Age-wise sero-prevalence of brucellosis in goats of Bangladesh Agricultural University Goat farm.

Age (months)	Number of Sera samples tested	Number and percentage (%) of positive reactors in RBPT	Number and percentage (%) of positive reactors in-ELISA	Significance
6-12	30	0 (0.00)	0 (0.00)	NS
12-24	80	1 (1.25)	1 (1.25)	
>24	40	3 (3.75)	2 (5.00)	
Total	150	4 (2.67)	3 (2.00)	

NS=Not significant

Table 3: Sex-wise zero-prevalence of brucellosis in goats of Bangladesh Agricultural University Goat farm.

Sex	Number of Sera samples tested	Number and percentage (%) of positive reactors in RBPT	Number and percentage (%) of positive reactors in-ELISA	Significance
Male	50	1 (2.00)	0 (0.00)	NS
Female	100	3 (3.00)	3 (3.00)	
Total	150	4 (2.67)	3 (2.00)	

NS=Not significant

Table 4:. Sero-prevalence of brucellosis in non-pregnant and pregnant goats of Bangladesh Agricultural University Goat farm.

Pregnancy status	Number of Sera samples tested	Number and percentage (%) of positive reactors in RBPT	Number and percentage (%) of positive reactors in-ELISA	Significance
Non-pregnant	60	1 (1.67)	1 (1.67)	NS
Pregnant	90	3 (3.33)	2 (2.22)	
Total	150	4 (2.67)	3 (2.00)	

NS=Not significant

Table 5: Sero-prevalence of brucellosis with respect to reproductive disorders in goats of Bangladesh Agricultural University Goat farm.

Reproductive disorders	Number of sera samples tested	Number and percentage (%) of positive reactors in RBPT	Number and percentage (%) of positive reactors in-ELISA	Significances
Previous abortion	10	3 (30.00)	2 (20.00)	**
Retained placenta	5	0 (0.00)	0 (0.00)	
Failure to conceive	15	0 (0.00)	0 (0.00)	
No disorder	120	1 (0.84)	1 (0.84)	
Total	150	4 (2.67)	3 (2.00)	

** Significant at 1% level.

Table 6: Sero-prevalence of brucellosis in goats regarding the flooring system of Bangladesh Agricultural University Goat farm.

Flooring system	Number of sera samples tested	Number (%) of positive reactors in RBPT	Number (%) of positive reactors in-ELISA	Significances
Brick floor	100	3 (3.00)	2 (2.00)	NS
Macha System	50	1 (2.00)	1 (2.00)	
Total	150	4 (2.67)	3 (2.00)	

NS= Not significant

Table 7. Age wise distribution of human brucellosis.

Age of human	Number of sera samples collected and tested	Number & % of sera positive by RBT	Total No. & % of sera positive by RBT	Number &% of sera positive by I-ELISA	Total No. & % of sera positive by I-ELISA
Below 20 years	11	0 (0)	182 (2.20)	0 (0)	182 (2.20)
20-30 years	138	1 (0.72)		1 (0.72)	
Above 30 years	33	3 (9.09)		3 (9.09)	

Table 8. Gender wise distribution of human brucellosis

Gender of human	Number of sera samples collected and tested	Number & % of sera positive by RBT	Total No. & % of sera positive by RBT	Number & % of sera positive by I-ELISA	Total No. & % of sera positive by I-ELISA
Male	160	4 (2.5)	182 (2.20)	4 (2.5)	182 (2.20)
Female	22	0 (0)		0 (0)	

Table 9. Distribution of brucellosis among types of human contact.

Type of contact	Number of sera samples collected and tested	Number & % of sera positive by RBT	Total No. & % of sera positive by RBT	Number & % of sera positive by I-ELISA	Total No. & % of sera positive by I-ELISA
Animal workers	82	0 (0)	182 (2.20)	0 (0)	182 (2.20)
Animal owners	40	2 (5.0)		2 (5.0)	
Village doctors	30	0 (0)		0 (0)	
Artificial inseminators	20	2 (10.0)		2 (10.0)	
Butchers	10	0 (0)		0 (0)	

Table 10. Distribution of human brucellosis with duration of contact with animal.

Duration of contact	Number of sera samples collected and tested	Number & % of sera positive by RBT	Total No. & % of sera positive by RBT	Number & % of sera positive by I-ELISA	Total No. & % of sera positive by I-ELISA
Below 10 years	131	1 (0.76)	182 (2.20)	1 (0.76)	182 (2.20)
10-20 years	47	3 (6.38)		3 (6.38)	
Above 20 years	4	0 (0)		0 (0)	

Table 11: Distribution of human brucellosis with taking raw milk.

Taking raw milk	Number of sera samples collected and tested	Number & % of sera positive by RBT	Total No. & % of sera positive by RBT	Number & % of sera positive by I-ELISA	Total No. & % of sera positive by I-ELISA
Yes	69	4 (5.80)	182 (2.20)	4 (5.80)	182 (2.20)
No	113	0 (0)		0 (0))	

Fig. 1: Collection of blood from goats (left) and sheep (right) for sera sample.

Fig. 2: Collection of blood samples from radial vein of humans.

Fig. 3. Rose Bengal Plate Test-strong agglutination reaction (positive for brucellosis) in sample no. 8 and 10 (left) using Rose Bengal Plate Antigen (right).

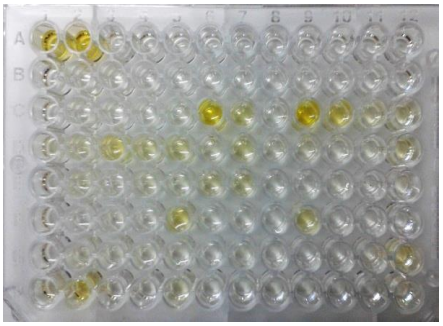

Fig. 4: I-ELISA reagents (upper and lower left) for detection of brucellosis in small ruminants-colored well indicates positive cases for brucellosis (lower right).

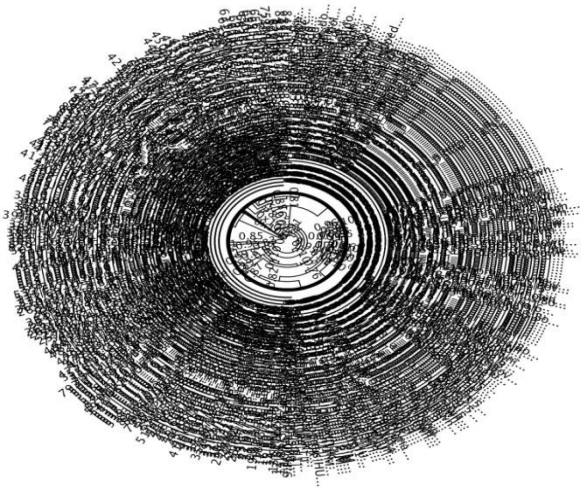

Fig. 5: A multi-locus analysis of concatenated data sets of dnak, glk, omp and int-hyp inferred the phylogenetic position of the sample 1 in *B. abortus* clade were recorded with well supported value.

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CONCLUSION

This study provides updated evidence on the occurrence of brucellosis in small ruminants and high-risk human populations in Mymensingh, Bangladesh. The overall seroprevalence was low in goats (2.0% ELISA), sheep (1.33% RBPT only) and humans (2.20%). However, the markedly higher prevalence among goats with a history of abortion, and among occupationally exposed groups—especially artificial inseminators—indicates ongoing transmission risks. Molecular detection and phylogenetic analysis confirmed the presence of Brucella abortus in goat sera, suggesting possible cross-species exposure within mixed farming systems. Although prevalence levels were modest, the findings underscore the potential for wider spread due to close human–animal interaction, consumption of raw milk and limited awareness. Strengthened surveillance, improved biosecurity and targeted awareness programs are essential. Adoption of a coordinated One Health strategy is recommended to interrupt transmission pathways and protect both livestock productivity and public health in Bangladesh.

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CONSERVATION AGRICULTURE AS A TOOL FOR IMPROVING CROP PRODUCTIVITY AND SOIL FERTILITY IN A RICE-WHEAT-MUNGBEAN CROPPING SYSTEM IN THE DROUGHT PRONE BARIND TRACT

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ABSTRACT

A seventeen years long term field experiment was conducted during 2005-2022 to study the productivity, soil fertility and N-use efficiency of intensified Rice Wheat (RW) systems including a pulse crop. System productivity, soil fertility and N use efficiency were evaluated under five N levels- 0, 40, 80, 100 and 120 % N of recommended dose, two straw retention (SR) (0 and 30%) and two tillage options permanent bed (PRB) and conventional tillage practice (CTP). Permanent beds with 30% straw retention produced the highest productivity for all three crops in the sequence. Within each N rate total system (rice-wheat-mungbean) productivity was higher with 30% SR on PRB and least in CTP with 0% SR. At 80% of recommended N rate, mean annual system productivity was 12.5 t/ha for PRB with 30% SR, 11.2 t/ha with PRB on 0% SR and 10.3 t/ha with CTP without straw. N uptake and use efficiency were increased with increasing N levels with bed planting up to 120% N application (120 kg N ha^{-1}) in wheat, both 100% (80 kg N ha^{-1}) in rice and (20 kg N ha^{-1}) in mungbean for all crops. System productivity in N unfertilized plots increased when straw was retained due to increased supply and uptake of N. The results suggest that N fertilizer rates can be reduced when 30% straw is retained both from rice and wheat & full residue retention from mungbean. Soil organic matter in surface layers of the PRB had been increased by 0.72% after twelve years (12 rice-wheat-mungbean crop cycles) with 30% SR. The combination of PRB with residues retained appears to be a very promising technology for sustainable intensification of RW systems in Bangladesh

Keywords: Conservation agriculture, Cropping system, Productivity, Fertility, Sustainability

INTRODUCTION

Land degradation and soil fertility decline are among the main causes of stagnation and fall of agricultural production in many tropical countries, including those with intensive irrigated cropping systems. Approximately 85% of the area under intensive rice-wheat (RW) sequential cropping is found in the Indo-Gangetic Plain (IGP) of South Asia in India, Pakistan, Nepal and Bangladesh (Timsina and Connor, 2001). Rice transplanted in flat fields are typically ponded for long periods or continuously flood irrigated from

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transplanting until about a week before harvest. The traditional practice of soil puddling for wetland rice destroys the soil physical structure that has implications for the subsequent upland crop **(**Hobbs *et al*., 2000**).** Wheat is then planted in these structurally disturbed soils, often after many tillage operations to prepare the seedbed or, Increasingly, with little soil disturbance using zero-till seed drills.

Growing crops on raised beds instead of on flat land facilitates more effective control of Irrigation and drainage. This may be particularly beneficial for non-rice crops grown in rotation with rice, allowing better rainwater management during the monsoon season for rice (Timsina and Connor, 2001) suggested that permanent raised beds might offer farmers further significant advantages such as increased opportunities for crop diversification, mechanical weeding and placement of fertilizers; relay cropping and inter-cropping; and reduced tillage and water saving.

There are also indications that crop yields from beds can be increased by using higher rates of N fertilizer and later irrigation because of the reduced risk of lodging (RWC-CIMMYT, 2003). Raised beds are increasingly used in many developed and developing countries in mechanized agriculture but have been introduced only recently in Bangladesh, with the aim of improving system productivity (Talukder et al. 2002). Inclusion of grain legumes as a third crop during the dry-wet transition period in the rice-wheat cropping system may be another option to increase cropping intensity and improving soil fertility, and, thus, enhancing productivity of the system. Although, heavy pre-monsoonal rain can occurs growing maize or mungbean crops during establishment or grain filling stage before T-Aman rice could be shown of water logging (Timsina and Connor, 2001; Quayyam et al., 2002).

In traditional crop establishment technique and temporary water logging at reproductive stage, inclusion of a grain legume like mungbean in rice-wheat cropping system very often faces problems. Bed planting may be a solution to this problem because raised beds not only facilitate irrigation but also drainage and therein lies their potential to increase the productivity of non-rice crops in the system. On the other hand, growing leguminous crops in a cropping system is beneficial not only for economic products but also for soil amelioration **(**Singh *et al.*, 1995). Raised bed systems can successfully grow of rice in northern Australia and high yielding irrigated wheat in Mexico (Sayre et al., 2005).

Crop residues are an important source of soil organic matter vital for the sustainability of agricultural ecosystems. About 25% of N and P, 50% of S and 75% of K absorbed by cereal crops is retained in crop residues, making them valuable nutrient sources (Singh et al., 2003). However, straw retention is not a common practice in the RW systems of Bangladesh, as is also the case elsewhere in south Asia. Wheat and rice straw are usually removed from fields for use as cattle feed and fuel purposes most of the country but crop residue for incorporation into the soil is beneficial for crops growing. An exception is in the north-western IGP, where most of the rice residues are burnt due to limited number of livestock. As a result, soil organic matter levels have declined in any cropping systems, and optimization of nutrient uptake and absorption efficiency has become one of the most important goals in crop production strategies. (Talukder *et al.*, 2004) reported that N use efficiency was the highest in permanent raised beds, giving higher crop yields than that in a conventional system. The concept of ‘Conservation Agriculture’ which is rooted in

giving a practical shape to a few scientifically proven basic guiding principles has globally emerged as a way to achieve sustainability goals. The basic guiding principles that can leverage a change from the conventional agricultural system includes:

- Developing and promoting a system of raising crops with minimum soil disturbance through operations involving direct seeding of crops in untilled soils.
- Keeping the soil surface covered by practices such as leaving and maintaining crop residues cover on the soil and /or growing cover crops.
- Adopting diversified crop sequencing, spatially and temporally

Farming practice based on these basic principles when adopted in an integrated fashion over a period of time contribute to sustainable increases in crop productivity, improving soil health, biodiversity and in reversing processes contributing to land and water degradation. Sufficient evidence has accumulated to show that CA practices have both the potential to mitigate GHG emissions from agriculture related activities and as adaptive strategies to cope increasingly with climate related changes. (Witt et al., 2000). Table below summarizes the way in which elements of CA approach can mitigate climate change and provide a way to adapt to new situations.

Conservation Agriculture constitutes an integrate approach to address multiple challenges facing the agriculture and environmental sectors- enhancing productivity in the face of acute and wide spread problems of resource degradation (soil erosion, declining water availability and quality, declining diversity) and increasingly stressed ecosystems and climate change. The approach enables addressing the immediate concerns of enhancing productivity, long-term sustainability, and food security concerns. It is well recognized that the problems of enhancing productivity which are immediately linked to resource degradation and climate change issues are region specific and prevailing problems. Limon-Ortega et al., (2000) observed that permanent beds with straw retention had the highest mean wheat grain yield (5.57 t/ha), N use efficiency (28.2 kg grain/kg of N supply) and total N uptake (133 kg/ha), with positive impacts on soil health.

Thus, crop residue management and raised beds, along with efficient N fertilization strategies, are likely to be key components of new farming practices that can increase and maintain yields from intensive RW systems in Bangladesh. In this paper, we report results of research undertaken in north-western Bangladesh with the objectives of:

- Evaluating system productivity of intensive multi-crop sequences under CA practices compared with conventionally tilled systems.
- Assessing the effect of mulching on crop performance and soil properties in CA
- Assessing the effect of N doses on crop productivity and estimating N uptake and N efficiency
- Determining the changes in soil properties over time.

MATERIALS AND METHODS

A rabi season wheat (*Triticum aestivum*)-spring mungbean (*Vigna radiata*)-monsoon rice (*Oryza sativa*) cropping pattern was implemented over 17 years, starting with wheat

sown in November 25, 2005, at the Regional Wheat Research Centre (RWRC), Shyampur, Rajshahi, Bangladesh (24°3'N, 88^{0}41E, 18 m above sea level). The site has a subtropical climate and is located in AEZ (Agro-Ecological Zone) 1 (Old Himalayan Piedmont Plains) on flood-free high land, with course-textured, highly permeable soil. The area receives 1,457 mm mean annual rainfall, about 97% of which occurs between May and September. Total rainfall was highest during the mungbean season and lowest in the wheat season in all years (Fig. 1). The temperature was fluctuation with uneven distribution of rainfall was increased from last 10 to 15 years (IPPC, 2007).

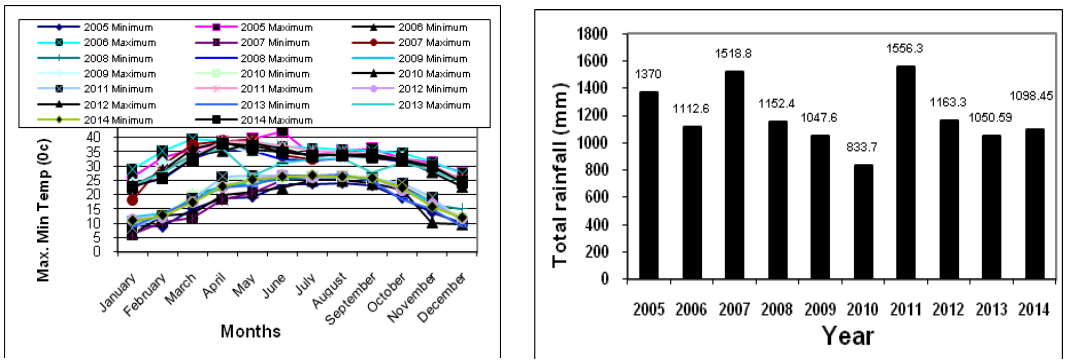

Fig. 1: Mean monthly rainfall (10 years, 2004-05 to 2014-15) and maximum and minimum temperatures at the RWRC, BARI, Rajshahi

The trial involved a three-crop rice-wheat-mungbean (RWM) annual rotation planted on raised beds (RB) and in cultivated flat systems. Rice was transplanted (25-day-old seedling per hill) with a hill-to-hill spacing of 15 cm and line-to-line spacing of 30 cm on RB in late July and manually harvested in late November. Wheat was sown at the nationally recommended seeding rate of 100 and 120 kg/ha for beds and conventional flat plots, respectively, in late November and harvested in late March. After harvest of wheat, mungbean was sown at the same time at the nationally recommended seeding rate of 35 kg/ha in early April and harvested in mid-July from bot for both beds and conventional layout.

The trial was originally established as a Permanent raised bed (PRB) experiment with two straw management practices in the main plots, 30% straw retention (SR) and 0% SR in sub plot, and five N rates in the sub sub-plots like 0, 40, 80, 100 and 120% of recommended N rates. The area of each subplot was 15 m^2 (5m x 3m). The experiment consisted of 20 subplots with four tillage/straw treatments (30% SR + PRB, 30% SR + CTP, 0% SR + PRB and 0% SR + Conventional tillage practices (CTP) and five N levels (0, 40, 80, 100 and 120% of recommended) with three replicates. After planting wheat or rice, straw from the preceding cereal crop was returned as mulch into the plot from which it had been removed at harvest. During harvesting, the rice and wheat straw were kept without chopping as standing way. The width of PRB was 60 cm (furrow to furrow) and the depth of the furrows on average was 15 cm. Two rows of wheat (var. BARI Gom 33) and rice (var. BRRI dhan71) with a spacing of 25 cm, were planted by hand sowing on the beds, two rows of rice on the beds, Mungbean (var. BARI Mungbean 6) was sown by bed former in the furrows between the beds. The mungbean was harvested about 60 days

after sowing (DAS). In CTP, wheat, rice and mungbean were planted in 20 cm, 25 x 15 cm (row x plant) and 30 cm rows, respectively. Basal doses of P (20, 22 and 26 kg/ha) as TSP (triple super phosphate), K (15, 35 and 33 kg/ha) as muriate of potash (MoP) and S (10, 11 and 20 kg/ha) as gypsum was applied to mungbean, rice and wheat, respectively. In rice the entire amounts of TSP, MoP and gypsum were broadcast before transplanting and mulching on both PRB and CTP. For CTP the fertilizer was broadcast before tillage as is the usual practice. The recommended rates of N (80 kg/ha for rice, 100 kg/ha for wheat and 20 kg/ha for mungbean) were applied as urea. For mungbean all N was applied before seeding. With CTP, N was broadcast, while on RB it was banded on top of the soil between two rows in three equal installments 15, 30 and 45 days after transplanting (DT). For wheat, two-thirds of the N was applied before seeding and the remaining one-third at crown root initiation (CRI) coinciding with the Zadoks growth stage 1.3 (Z1.3).

Sufficient irrigation water was applied to fill the furrows between the raised beds. The flat plots were flood irrigated. Wheat received three irrigations-at CRI (Z1.3), booting (Z4.0-4.9) and grain-filling stages (Z8.0-8.9). For rice, irrigation water was applied for CTP with approximately 4 cm of irrigation and then re-irrigation when the soil dried to near saturation, continuing this practice up to the grain filling stage of rice. For irrigation of rice in PRB, water was applied and maintained initially over the beds for 14 days after transplanting to ensure seedling establishment. Thereafter, water was added only to fill the furrows (no overflow to the RB top). Generally, both CTP and PRB plots were irrigated on the same days, but less water was needed to fill the furrows in the PRB plots than that for CTP. Mungbean received pre-sowing irrigation to enhance germination. Glyphosate [N-(phosphomethy1) glycine] @ 1.4 kg/ha a. i. was applied to all plots three days before planting for initial weed control. Additionally, for wheat weed management was done by the application of the herbicide Affinity @ 2 g/L of water after the first irrigation, and for rice, the herbicide Ronstar was applied @ 1ml/L of water 25 and 45 date of transplanting. No additional herbicide application was done even if there was an outbreak of weeds to ensure a uniform level of weed management for all treatments. Grain and straw yields were determined by 15 m^2 areas in each plot. Soil samples were dried in the oven at 105^0C for 72 hours and moisture determined by the digital soil moisture meter.

Grain and straw samples of the test crops were analyzed for N and K by standard analytical methods. After rice harvest, soil samples were collected from 0-30 cm depth from three points of each RB and furrow, and from three points of each CTP plot using a 6 cm diameter auger. The soil samples were mixed thoroughly and sub-samples were air dried and crushed to pass through a 2 mm sieve to remove roots and other debris. Nutrient contents in the Soil pH, organic C and nutrient contents were determined using standard analytical techniques.

Total system productivity

The total system productivity (TSP) was calculated for each treatment was calculated as the total annual productivity (or the annual total of economic yield of the individual crops) based on equivalent yields (1.35, 1.39 and 1.54, respectively, for rice, wheat and mungbean) as outlined by Tanaka (1983). TSP (rice-wheat-mungbean) = (rice grain yield*1.35) + (wheat grain yield*1.39) + (mungbean grain yield*1.54)

Estimation of N uptake

Nitrogen uptake by grain and straw was calculated by the following formula:

$$\text{N uptake by grain (kg ha}^{-1}) = \frac{\text{Total N (\%) in grain} \times \text{grain yield (kg ha}^{-1})}{100}$$

$$\text{N uptake by straw (kg ha}^{-1}) = \frac{\text{Total N (\%) in straw x straw yield (kg ha}^{-1})}{100}$$

Statistical analysis of data

The Microsoft Excel software was used for data compilation and analysis. The data were analyzed statistically using the computer software CROP STAT. ANOVA was done by the same method to use for determine the significant differences among treatment means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

System productivity (Rice, wheat and mungbean yield)

The yield of rice, wheat and mungbean was found significantly with the increase of residue retention. From the experiment, residue retention increased productivity rapidly, starting from the second crop cycle. System productivity increased by 10-12% with 30% straw retention in the permanent bed planting system over the conventional tillage practices (Fig.2). The system productivity of the rice-wheat-mungbean (R-W-M) were 12 t $ha^{-1}yr^{-1.}$ Figure 2 presents the system yields with different tillage options and straw retention (SR) levels. For all crops the highest system yields were achieved with PRB + 30% SR. Total system productivity under PRB consistently increased as SR increased from 0% to 30% but the yield differences between 0% and 30% SR were always significant from all three crops. System productivity also occurred low from 0% SR with CTP due to less performance of crop growth with conventional method. Yields tended to be lower in differences of residue retention for all crops. Similar observations were made by Sayre et al., (2005) in Mexico. We believe this is an important finding because, if applied in farmers' fields, farmers will quickly realize the benefits and they will be interested in adopting the technology.

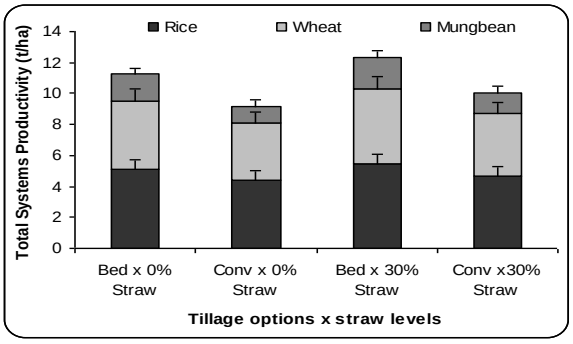

Fig. 2: Total system productivity as influenced by tillage and and straw retention level in a rice-wheat-mungbean system

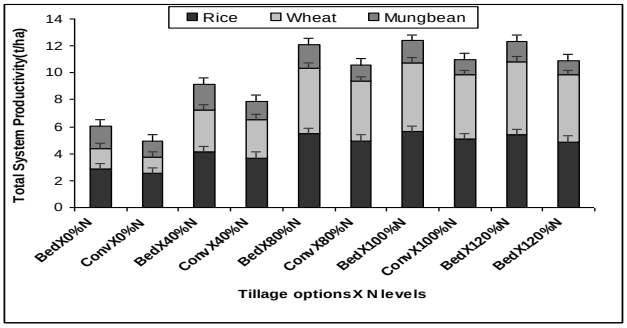

Fig. 3: Total system productivity as influenced by tillage and applied N level in a rice-wheat-mungbean system

In response to the applied N level, the productivity of wheat, mungbean and rice were significantly increased by 11% in rice, 14% in mungbean with increasing N level up to 100%, and by 16% in wheat with N level up to 120% of the recommended N level (Fig. 3). For all three crops the highest productivity was found from 120% N (120 kg N/ha) of wheat and 100% N both for rice and mungbean (80 kg N/ha for rice, 20 kg N/ha for mungbean) under PRB. Crop yields and productivity under PRB consistently increased as N rate increased but the differences between 0 and 120% N applied was differ in wheat.

The productivity with 0% N in CTP was significantly lower due to lower N uptake and N use efficiency. Yields tended to be lower between 0 to 100% nitrogen levels for all three crops with residue retention. Similar observations were made by Gupta et al., (2002) in India. Yields tended to be lower in differences between four N levels in wheat, at three N levels in rice and at two N levels in mungbean (Fig. 3). Averaged over the 7 years, PRB + 30% SR with 100% N gave a 17% increase in wheat yield over PRB + 0% SR at the same N rate (Fig. 3) but there was no significant mungbean yield increase with additional N with 30% SR. However, yield with PRB + 30% SR and 120% N was significantly higher than PRB + 0% SR with 100% N. Average rice yield with PRB + 30% SR with 50% N was significantly higher than that with 0% SR at the same N rate, and there was no further yield increase at higher N rates. Rice yield declined with 30% SR at the two highest N rates, mostly due to lodging.

The maximum average wheat grain yield was obtained on PRB from 120% N with 30% SR and after 5 years yield was similar with a lower N dose, i.e., 80 % N with 30% straw retention, which was 17% higher than that on PRB with 0% SR (Fig. 3). These yield increases with straw retention were probably due to moisture conservation by way of reduced soil evaporation, suppression of weeds and more efficient use of fertilizers. Limon-Ortega *et al*., (2000) reported that on permanent beds both wheat and maize yields increased when grown with higher rates of N fertilizer.

Wheat and rice yields were comparatively low under the CTP system with 0% residue retention due to lower N uptake and less fertility of the soil. Reduction in wheat yield in Bangladesh is mostly the result of the high temperature that can occur during the grain filling stage, especially for a late-sown crop. The introduction of PRB can help to increase wheat yield because of more nutrient uptake with more photosynthesis.

Nitrogen uptake and use efficiency

Total N uptake increased with increasing N level up to 120% and it was similar as 80% N for all crops. The total N uptake increased with PRB by 31% in rice, 25% in wheat and 19% in mungbean over that in CTP (Fig.4. Retention of straw resulted in increased N uptake in both N fertilized and zero N plots (Fig. 4). Nitrogen uptake was significantly ($P<0.5$) influenced by straw retention and N level. In PRB+30% SR plots, total N uptake by rice was maximum at 50-100% by rice, 80-120% by wheat and 50-100% by mungbean. In contrast, in both PRB and CTP without straw retention, there was a consistent trend for increasing N uptake up to 120% N rate in all three crops. Lauren et al., (2006) also observed that permanent beds with straw retention gave the highest average wheat grain yield (5057 t/ha), N use efficiency (28.2 kg grain/kg of N supply) and total N uptake (133 kg/ha).

There was a consistent trend for higher N uptake on PRB as the amount of SR increased from 0 to 100% across all three crops. Yadvinder-Singh *et al.* (2004) reported that N uptake was significantly higher in straw retained + green manure cultivated treatments than other treatments for wheat. System productivity is closely related to the availability of N and its uptake and utilization by crops. Nitrogen availability and its utilization are controlled by numerous abiotic and biotic factors in the soil-plant system. These are include cultivar, fertilizer input, weather, pests and its management of soil, crop residues, irrigation and drainage. (Kataki PK, 2001). Given the complexity of the RW cropping system associated with the pronounced anaerobic-aerobic cycles, an important question concerns how N use efficiency can be improved. Good N management and straw retention in a PRB system may allow this. Compared to other parts of the IGP, such as the Punjab, our Rajshahi site received far more spring and summer rainfall (1,415-1,962 mm from May to August during the 3 years) in the mungbean and rice seasons (Fig.1) and has a shallower water table (only 80-90 cm depth). The result is a much wetter upper soil profile that is favorable for rice growth. It also helps the decomposition of retained straw, resulting in a higher uptake of nutrients and more efficient use of water.

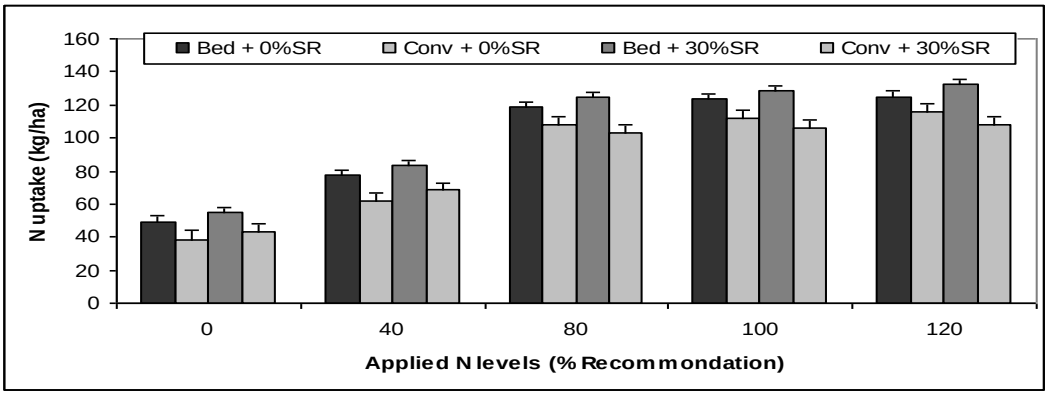

Fig. 4: Total nitrogen uptake under tillage options, straw and nitrogen levels in rice-wheat-mungbean systems

Soil organic matter (SOM)

After three years of R-W-M cropping (2019 to 2021), retention of straw from all three crops in the zero-till PRB system had increased SOM 0.22% (Table 3). While some of the increase may have been due to formation of the beds from topsoil, the organic C in soil actually increased as the rate of residue retention increased from 0-100%, indicating that straw retention influenced soil organic C on the beds. Govaerts et al., (2006) found similar finding from their experiments. Also, 30% SR under PRB increased P, K and Zn availability in soil (Table 3). At low N levels (0 and 50% of recommended doses) appeared to be a slightly decline in soil organic C. After three years of CTP without residues, soil organic C had decreased by a few percent at all N rates and there was a consistent trend for a large decline at lower N rate. The increase in soil organic C with 30% SR at 50-150% N was almost double that with 0% N, Kumar and Goh (2000) reported that, in the longer term, residues and untilled roots from crops can contribute to the formation of SOM. It seems clear that further increases in the productivity of the RW system will depend on improvement in soil fertility through proper management and use of crop residues and other agricultural wastes. After the ten RWM crop cycles, the soil color had darkened, presumably due to the build-up of organic matter in the topsoil

Table 3: Changes in soil chemical properties changes with 30% straw retention after three years of cropping (2019-2021)

Characteristics	Initial	Final	Difference
pH (1:2.5 in water)	8.3	8.0	- 0.3
Soil organic matter (%)	1.06	1.28	+ 0.22
Total N (%)	0.12	0.15	+ 0.03
Exch. K (ml eq/100g soil)	0.26	0.48	+ 0.22
Avail. P (mg / g soil)	24.5	52.5	+ 38.0
Avail. S (mg / g soil)	25.6	38.9	+ 13.3
Avail. Zn (mg /g soil)	0.84	0.97	+ 0.13
Avail. B (mg / g soil)	0.19	0.37	+ 0.18
Ca (ml eq/100g soil)	18.22	21.24	+ 3.02
Mg (ml eq/100g soil)	5.05	6.17	+ 1.12
Fe (mg /g soil)	76.4	63.5	- 12.9
Mn (mg /g soil)	22.9	12.7	- 10.2

CONCLUSION

Retention of 30% crop residues together with zero-till permanent bed soil systems offer an important soil restorative management strategy likely to have a long-term positive impact on soil quality and crop productivity in intensive rice-wheat-mungbean (RWM) cropping systems in Bangladesh. Lignified residual straw and roots added more organic matter and nutrients into the soils under PRB, resulting in increased nutrient uptake by the crops. Crop productivity on beds with 30% straw retention rose by about 15% for

rice, 19% for wheat and 25% for mungbean at 100% N rates over a three years cycle of the RWM cropping pattern compared with 0% SR+PRB at the same N rate. Compared with conventional tillage practice, crop productivity on PRB with 50% for rice, wheat and mungbean crops, respectively at 100% N rates over a three years cycle of the RWM cropping pattern. Yield in N unfertilized rice, wheat and mungbean increased when straw was retained appeared to be due to increased uptake of N. This increase in soil N supply led to a reduction in N use efficiency in the N-fertilized plots, suggesting that N fertilizer application rates can be reduced when straw is retained. Retention of crop residues as a mulch reduced moisture depletion and increased 0.78% SOM content over relatively long periods of time. Fertilizer use efficiency increased by implementing permanent bed management in addition to reducing weed and crop lodging problems. Permanent raised beds also helped ameliorate the adverse effects of tillage on soil structure, which lead to water logging under excess water conditions and hamper establishment, growth and development of most crops including mungbean. The use of PRB reduced the overall cost of production and long turnaround time. Thus, our results showed that PRB with straw retention can help to sustainable intensify RW systems under proper management to the farmer's field in Bangladesh.

ACKNOWLEDGEMENT

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CONSUMER CATEGORIZATION OF PREFERENCE FOR DIFFERENT COMMERCIAL BROILERS IN BANGLADESH

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ABSTRACT

This survey study was carried out to identify the consumer classes, reasons for their choices, and other associates related to the consumption of different body weights broilers. Initially, a KAP (Knowledge, Attitude, and Practice) based on survey was conducted in Kapasia, Gazipur; Bazitpur, Kishorgonj; Modhupur, Tangail, and Mymensingh Sadar on the 36 poultry farmers, 25 poultry dealers, 19 general consumers, 3 restaurant owners, 4 poultry professionals, 3 poultry industrialists, and 10 ready-to-eat meat processors. This survey shown that more than 50% of farmers bought day-old chicks (DOC) directly from the hatchery, while the rest chicks purchased from other sources. More than 70% farmers gave glucose or saline water to the DOC on arrival at the farm. The survey indicates a strong demand (39% or more) for broilers which weight are 1.25 to 1.35 kg and takes time approximately 25 days. This market segment of birds mainly serves fast-food catering such as grills, tandoori, and others. The second tier, accounting for 20% broilers aged 35-40 days is primarily used for family consumption and hotels. The survey reveals that over 45% of farmers sell their birds through middlemen and 11% is selling directly to restaurant owners. More than 50% people suggested that rearing period of broiler should be prolonged and meat quality should be improved with different dietary regimes. In conclusion, there is a steady increase in the demand of broilers weighing between 1.25-1.35 kg and above 2 kg.

Keywords: Commercial broiler**,** Consumer class, Consumer choice, Different body weights of broiler

INTRODUCTION

Broiler plays an important role in supplying protein for human consumption. Commercial poultry farming has emerged as a profitable business opportunity for entrepreneurs and small-scale farmers (Raha, 2000). A recent report published by OECD-FAO Agricultural Outlook (2024-33) claimed that poultry meat is expected to account for 41% of the total protein consumed from all meat sources, followed by pork, beef, and mutton, due to economic growth, urbanization, and the growth of the fast-food industry in low-income countries. Akter *et al*., (2023) reported that over the past few decades, consumption of

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chicken meat has grown substantially, driven by factors such as increased urbanization, rising disposable incomes, and a shift in dietary patterns.

In Bangladesh, there is a significant shift in consumer preferences from commercial broiler meats to local or cross-breed Sonali chickens. The Bangladesh Poultry Industries Central Council reported that, in July 2018, 80% of consumers in Dhaka were purchasing broiler chicken, whereas only 20% chosen for Sonali chickens. This figure fluctuates over the course of a year, indicating that 55% of consumers purchase broiler chickens, whereas the consumption of Sonali rises to 45% (Dhaka Tribune, 2019). This increased results from various speculations regarding farming practices and the quality of broiler meat. A prevalent misconception among the public is that broilers lack versatility in cooking with local methods and have a flavor that differs from traditional alternatives. Broiler chicken is predominantly appropriate for frying and various Western culinary techniques. Heating chicken at high temperatures for an extended duration results in excessive softness. Additionally, meat from broiler chickens is characterized by its softness and tenderness, lacking in flavor and being insipid. Consequently, there is a perception that individuals are consuming ingredients deemed unhealthy, including antibiotics, steroids, and heavy metals, to promote accelerated growth. This phenomenon has created a stigma surrounding broiler chicken, resulting in a stagnant market over the past few years.

Producers rear broilers for 28-30 days with high-nutrient diets, harvesting them at 1.5-1.8 kg, with less developed muscle fiber and fat deposition, which negatively affects the taste and texture of the meat and discourages many local consumers. According to Mishu & Rahman (2024), consumer preferences can be influenced by a wide range of variables such as price, quality, health concerns, and perceptions of product safety.

Despite the significant shift in consumer preferences and the growing demand for different broiler body weights, little research has been conducted on the factors influencing consumer choices and how these factors vary by body weight. Additionally, there is a lack of detailed knowledge regarding small-scale farmers' practices and attitudes, which directly impact the supply and marketing of broilers. This study addresses these gaps by focusing on consumer preference for broiler weight categories and small-scale farmers' knowledge, attitudes, and practices (KAP).

This study was carried out for the following objectives.

i) To identify the consumer classes, reasons for their choices, and other associates related to the consumption of different body weights of broilers,

ii) To identify small-scale broiler farmers' knowledge, attitude, and practice regarding farming and its related aspects, and

iii) To provide useful information to policymakers, researchers, extension workers, and farmers.

MATERIALS AND METHODS

Study period and location: The cross-sectional study was conducted from September to November 2023 on poultry farmers, dealers, general consumers, restaurant owners,

poultry professionals, poultry industrialists, and ready-to-eat meat processors in four districts: Kapasia, Gazipur; Bazitpur, Kishorgonj; Modhupur, Tangail, and Sadar, Mymensingh. The area is known for its high concentration of small-scale and commercial poultry farmers who rely on indigenous chicken breeds and broilers for their livelihoods and as a source of protein. The study was designed to be conducted over a period of two months, during which time a questionnaire was administered to farmers, dealers, general consumers, restaurant owners, poultry professionals, poultry industrialists, and ready-to-eat meat processors.

Knowledge, attitude, and practices study steps: We followed the subsequent steps to conduct the KAP study. Those steps included identifying the topic, selecting the target population and participants, preparing the KAP questions and answer options, data collection and management, and data analysis and interpretation.

Study design and sampling: In the survey, 100 people were selected for interview, where poultry farmers (N=36), dealers (N=25), general consumers (N=19), restaurant owners (N=3), poultry professionals (N=4), poultry industrialists (N=3), and ready-to-eat meat processors (N=10), and in four districts, Kapasia, Gazipur; Bazitpur, Kishorgonj; Modhupur, Tangail, and Sadar, Mymensingh. The study was planned to collect data on demographics, and to check the knowledge about farming practices in farming, and the attitude of farmers towards farming. We performed convenience sampling as it is affordable to gather data from a sizable on-hand population. In addition, it is argued that convenience sampling offers the opportunity to receive specific feedback from individual perspectives. Therefore, despite having fewer representative results over a random sample area, we employed the convenience sampling technique to collect information from the poultry farmers from the nearest physical locations of the study areas. The structured questionnaires were personally administered to the farmers through face-to-face interviews to collect the quantitative data in their domiciles. The survey questionnaires used in this study were checked for completeness and consistency before use.

Development of the KAP questionnaire: We used a KAP questionnaire, which comprised two main sections. Section one consisted of eight statements concerning the general demographic data and poultry farm enterprise information. Section two assessed the KAP of selected people toward broiler meat, farming, and management. This section consisted of three sub-sections. The second section consisted of 12 statements intended to evaluate farmers' knowledge about broiler farming, 10 statements to evaluate the farmers' key personal attitudes, and 12 statements to determine the practices at the farms. Some questions in three sub-sections consisted of two answer options: "yes" and "no." The "yes" answer was awarded one mark, and the "no" responses received zero marks. Some questions were designed with a five-point Likert scale ranging from "highly acquainted" to "not acquainted" to indicate the degree of acquaintance with the statement. Numerical scores 5, 4, 3, 2, and 1 were given to the categories "highly acquainted," "acquainted," "fairly acquainted," "poorly acquainted," and "not acquainted," respectively. We prepared the questionnaires in English.

Data management and analysis: Individual respondents' KAP answers were scored and transformed into a percentage. The whole analysis was performed with the help of the Microsoft Excel statistical tool pack 2013.

RESULTS AND DISCUSSION

Respondent Type:

The distribution of respondent types in this study is shown in Figure 1. Poultry producers made up the largest group, accounting for 36% of participation. They play a crucial role in advancing broiler farming and marketing by interacting with customers and other stakeholders both directly and indirectly. Poultry dealers, who make up 25% of the respondents, are essential middlemen who help get poultry goods from farmers to customers.

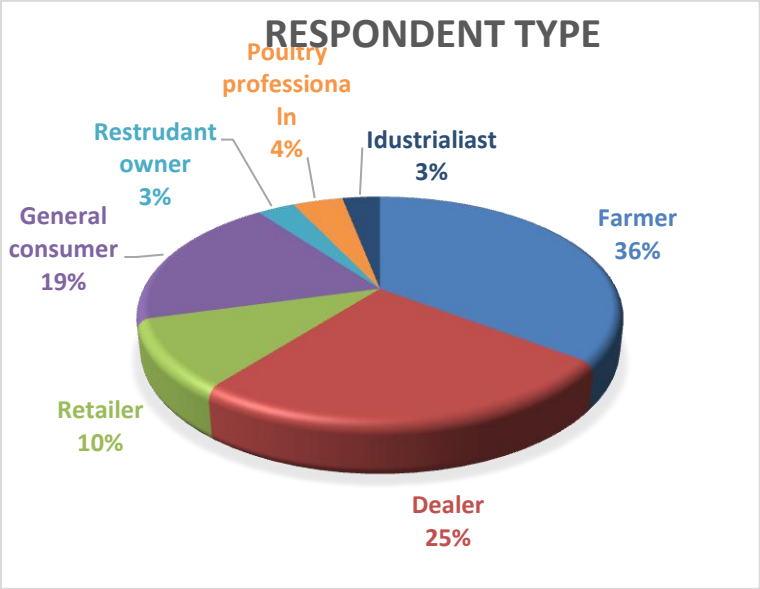

Fig. 1: Types of respondents for KAP analysis

19% of the sample consisted of general consumers, who are representative of the general public and offer crucial information about consumer preferences. 3% of the respondents were restaurant owners, who are significant actors in the marketing and consumption of poultry and regularly purchase large quantities of broilers. 4% of the respondents were poultry professionals who contributed their expertise to the study, bringing's specialized knowledge in poultry husbandry, feeding, and marketing.

To provide a more comprehensive understanding of the poultry industry and its multifaceted interactions across various stakeholders, poultry industrialists (3%) and ready-to-eat flesh processors (10%) were also incorporated. This diversity in respondent categories is consistent with the findings of Rahman et al. (2007), who highlighted the significance of incorporating a diverse range of stakeholders to comprehend the dynamics of poultry markets. Their research emphasized the influence of a variety of stakeholders, including producers and consumers, on the development and trends of the poultry industry. This method is crucial for the development of effective marketing strategies and industry enhancements, as it offers deeper insights into consumer preferences and market dynamics.

Demographic and farming-related information:

Table 1: Demographic profile and farm-related information of the respondents

Parameters	Variables	N (%)
Age	≤35 years	48
	36–45 years	32
	≥46 years	20
Level of education	Uneducated	12
	Primary	65
	Secondary	15
	Higher secondary	8
Experience in farming (Years)	3–5 years	32
	6–10 years	28
Farm size	≤1000 (Small scale)	19
	≥1000 (Large scale)	16

An overview of the study participants' demographic profile and farming-related information is given in Table 1. The majority of the responders (44 out of 48) were male, and nearly half (48%) were 35 years of age or younger. All of them were male, with almost one-third (32%) being between the ages of 36 and 45 and 20% being 46 or older. According to Rahman et al. (2007), this age distribution is consistent with larger trends in Bangladesh's poultry farming industry, where younger people are increasingly entering the field because of its perceived profitability and relatively low capital investment needs. The educational background of the respondents revealed that 12% were uneducated, primarily consisting of poultry farmers (5), dealers (2), and general consumers (5).

The remaining participants exhibited diverse educational backgrounds: 65% had finished primary education, 15% had attained secondary education, and 8% had completed higher secondary education. The poultry sector typically exhibits a relatively low level of formal education, with a greater emphasis placed on practical knowledge rather than formal schooling. Apart from this, it has been observed that poultry farmers, even those with minimal formal education, frequently exhibit significant proficiency in critical farming practices, such as feeding, disease management, and biosecurity (Hassan et al., 2021). In terms of farming experience, 32% of respondents reported having 3–5 years of poultry farming experience, whereas 28% indicated they had between 6–10 years of experience. This indicates a workforce that is comparatively youthful regarding hands-on poultry farming, yet is acquiring expertise in essential management areas. The variety in agricultural experience underscores the importance of experience in influencing farming methods and enhancing farm management. Previous studies indicate that farmers with more experience are likely to implement superior management techniques, resulting in enhanced productivity and efficiency on their farms (Hassan et al., 2021).

The respondents exhibited a range of farm sizes: 19% engaged in small-scale operations with fewer than 1000 birds, whereas 16% managed large-scale farms with over 1000 birds. The distribution illustrates the diverse characteristics of the poultry farming sector in Bangladesh, highlighting the interaction of both smallholder and larger-scale operations. The variation in farm size indicates that the poultry sector in the region is complex, presenting unique challenges and opportunities for various types of producers. The knowledge domain items with the corrected answers calculated are displayed in Table 2.

Table 2: Frequency of farmers' knowledge on broiler farming issues (n=100)

Statement	Options (%)	
	Yes	No
Training experience	41.4	58.6
Knowledge about different stages of broiler (starter, grower, and finisher)	66.7	33.3
Idea about safe broiler farming	50.5	49.5
Knowledge about the heat stress of broiler	80.8	19.2
Knowledge about the effect and management of heat stress	31.3	68.7
Knowledge about the vaccination of broiler	63.6	36.4

Frequency of farmers' knowledge on broiler farming

Table 2 shows respondents' basic and advanced broiler farming expertise. Most respondents (66.7%) understood broiler growth stages—starter, grower, and finisher—indicating an accurate understanding of broiler management. In common farming, these fundamentals are crucial. Specialized topics like heat stress management were less well-known. Only 31.3% of respondents knew how to control heat stress, a major influence on broiler productivity. Heat stress can impair feed efficiency, growth rates, and broiler mortality; hence, this knowledge gap is important. This lack of understanding underscores the need for more focused environmental stressor management training, especially in Bangladesh's hot and humid climate. Only 41.4% of respondents had official broiler farming training, showing that many farmers rely on informal knowledge. Lack of formal training may contribute to knowledge gaps, especially in advanced farm management areas like veterinary drug safety and biosecurity. Although 63.6% of respondents knew immunization protocols, they didn't know enough about antimicrobial stewardship. This is concerning because incorrect antibiotic usage and biosecurity can lead to antimicrobial resistance (AMR), which threatens poultry health and public safety. The findings correspond to Hassan et al. (2021), who found similar knowledge deficits in Bangladeshi commercial chicken production. Their study found that farmers know basic broiler management but undervalue disease prevention, heat stress management, and biosecurity. Such a lack of understanding may limit the implementation of optimal practices and threaten the region's poultry agricultural sustainability. Given these data, it is clear that while broiler farming basics are widely known, advanced farming approaches need better education. The low formal training among respondents suggests that

extension services and training programs should focus on heat stress management, biosecurity, and veterinary drug safety. These focused educational interventions could help farmers enhance production, minimize disease risks, and sustain their enterprises by bridging knowledge and practice.

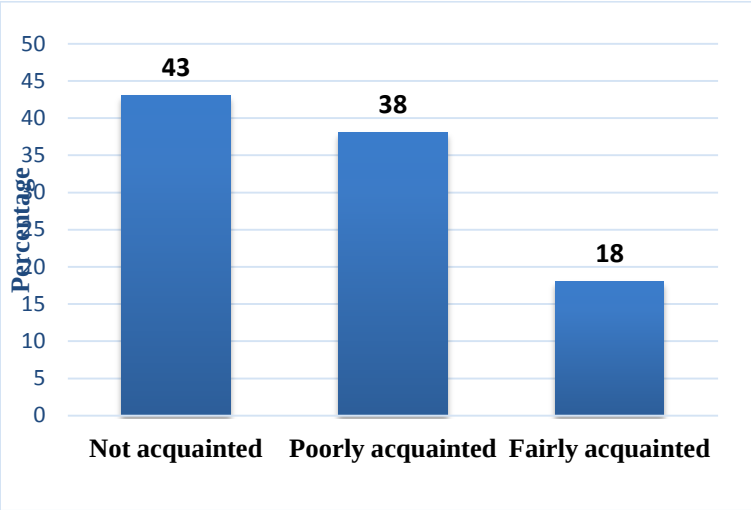

Fig. 2: Knowledge about the withdrawal period of antibiotics, steroids, and veterinary drugs

Figure 2 illustrates the participants' understanding of the withdrawal period associated with antibiotics, steroids, and veterinary medications. The findings indicate that a notable percentage of participants (43%) were "not acquainted" with the withdrawal period, highlighting a crucial deficiency in their comprehension of this vital component of poultry health management. The withdrawal period is essential, as it outlines the minimum duration necessary between the administration of veterinary drugs and the slaughter of poultry, ensuring that harmful residues are eliminated from the meat, thereby protecting public health. Furthermore, 38% of participants were classified as "poorly acquainted," indicating that although they might possess a basic awareness of the concept, their comprehension remains inadequate or lacking. Less than 18% of participants reported being "fairly acquainted" with the withdrawal period, underscoring the necessity for focused educational efforts in this domain. The absence of understanding in this area raises significant concerns, as the misuse of antibiotics and other veterinary medications may lead to the presence of antibiotic residues in poultry meat, thereby threatening consumer health. Moreover, insufficient understanding of withdrawal periods causes the escalating problem of antimicrobial resistance (AMR), which has emerged as a worldwide issue affecting both human and animal health.

The findings align with those presented by Hassan et al. (2021), indicating that numerous poultry farmers are unaware of the withdrawal periods for antibiotics and other veterinary drugs. This lack of awareness may result in potential breaches of food safety regulations and heightened risks related to antimicrobial resistance. A lack of understanding in this domain can weaken the efficacy of food safety protocols and facilitate the proliferation of resistant pathogens. Considering that a substantial number of respondents are either

unfamiliar or have limited knowledge regarding the withdrawal period, it is evident that there is a considerable necessity for education and training. Extension services and formal training programs need to emphasize the significance of understanding withdrawal periods and their contribution to the safety of poultry products. Furthermore, these initiatives must tackle the wider concern of antimicrobial resistance, providing farmers with knowledge about the correct application of veterinary medications and the enduring dangers linked to improper practices.

Enhancing farmers' comprehension of these practices is crucial not only for food safety but also for improving the overall health management of poultry, minimizing the spread of AMR, and securing the sustainability of the poultry sector. Policymakers, extension services, and other stakeholders in the poultry industry need to prioritize access to dependable information and training regarding drug management and withdrawal periods.

Table 3 provides a summary of the attitudes of respondents towards broiler farming. The majority of respondents agreed that farmers require scientific training for broiler farming, and 58.6% agreed with the statement that broiler farming should be improved with modern facilities. However, more than 50% of consumers prefer grading and are willing to pay for premium quality. The majority think that scientific facilities should be incorporated for stress management, and more than 55% respondents think that traditional practice should be improved, and broiler meat quality could be improved with different dietary regimes.

Table 3: Agreement of respondents' attitudes towards broiler farming (n=100).

Statement	Options (%)	
	Yes	No
Farmers' attitude towards the requirement of scientific training	70.7	29.3
Farmers' attitude towards broiler farming improvement with modern facilities	58.6	41.4
Consumer preference for grading	51.5	48.5
Consumer is willing to pay for premium quality	52.5	47.5
Respondents are thinking about prolonging the rearing period of broilers.	47.5	51.5
Respondents are thinking about the improvement of traditional farming practices.	58.6	41.4
Respondents are thinking about the improvement of broiler meat quality with different dietary regimes.	55.6	44.4
Respondents are thinking about incorporating scientific facilities for stress management.	60.6	37.4
Respondents are thinking about meat inspection by a meat expert.	42.4	57.6
Respondents' preference for the inspection laboratory	31.3	68.7

Hassan et al. (2021) reported that the majority of the farmers (70.7% of the total respondents) recognized the necessity for scientific education, and 58.6% said that contemporary facilities could enhance broiler farming. This shows a positive attitude toward knowledge-based agriculture, which is in line with worldwide trends that stress the importance of education and technology in improving productivity and sustainability (Rogers, 2003). According to Hassan et al. (2021), a general attitude observed was that most farmers believed that antimicrobials should be placed in a restricted place and accessed by the farmers or a specific person; only 18.1% of all the respondents mentioned the desirable response "No." A large number of people (58.6%) said that modern facilities may make broiler farming better. This is linked to global trends, which regard technological adoption as a critical factor in improving productivity and efficiency (Khumairoh et al., 2018). Nevertheless, the fact that 41.4% of respondents did not perceive the value of these enhancements suggests that there are substantial obstacles. These constraints are prevalent in agricultural systems and may encompass high initial investment costs, inadequate technical support, or limited access to credit. Consumers are divided in their preferences, with about equal numbers of people rejecting grading (48.5%) and premium payments (47.5%). This shows that price is still a big factor in buying decisions. There is a strong desire to improve traditional techniques (58.6%) and adopt dietary regimes to improve meat quality (55.6%). The highest level of support (60.6%) is for scientific facilities that help with stress management, which shows how important animal welfare is for production and quality. However, there is a lot of opposition to required meat inspection (57.6%) and laboratory use (68.7%) because people are worried about the costs and are aware of the threats to food safety. Also, most people (51.5%) are not thinking about prolonging the rearing period. This is aligned with the fast-growth economics of traditional broiler production, which makes alternatives like slow-growing breeds less tempting because they cost more (Henchion et al., 2014).

Table 4 provides a summary of the practices of respondents performed in broiler farming. The majority of the respondents do not check birds' abnormalities while receiving DOC or purchasing adult birds. The findings show that there wasn't a proper ante-mortem inspection, which is necessary to find abnormalities. About 96% have no lairage or slaughterhouse facilities. Another important fact is that meat or residue is not inspected by the majority of the respondents. The lack of such controls corresponds with earlier research by Rahman et al. (2020), which indicated that numerous small- and medium-sized slaughtering establishments in Bangladesh function without sufficient veterinarian oversight or laboratory assistance.

Table 4: Frequency of farmer practices on broiler farming issues (n=100)

Statement	Option	
	Yes	No
Birds abnormalities check	32.3	67.7
Lairage facilities	4	96
Meat inspection and residue tests	4	96

Hassan et al. (2021) also reported that some practices considered to be at risk for Antimicrobial resistance (AMR) were common between both groups. Self-medication was prevalent in similar proportions among layer and broiler farmers. A notable proportion (30.2% of all the farmers) of the respondents reported they had used antimicrobials by themselves. In terms of using antimicrobials during the brooding period, only 18.1% of all farmers reported the desirable response "No", which means the vast majority of the farmers (81.9%) followed this practice inappropriately. When asked, "Do you seek suggestions for using antimicrobials from a nonvet?", only 20.5% of the farmers said "No"—meaning the majority of them practiced as such.

Other considering points:

Knowledge about GAP (Good Agricultural Practice), GMP (Good Manufacturing Practice), and HACCP (Hazard analysis and critical control point):

Acquaintance of the respondents with GAP, GMP, and HACCP is very poor. More than 50% of the respondents are not acquainted with the term, only 38% of the respondents are poorly acquainted, and 9% of the respondents are fairly acquainted with the term.

In the KAP survey, we found that the cleanliness condition of 23% farms remains good. About 62% DOC (Day Old Chick) is purchased directly from the hatchery, and the rest from other ways or sources.

In the survey, about 74% respondents provided saline or glucose water after receiving DOC.

Figure 3 depicts that about 40% of farmers kept birds for 28–30 days because they achieved the targeted final body weight (1250g-1350g), which is in high demand on the market (Figure 4). The reasons for different rearing periods vary depending on the purpose of use. Some birds are directly marketed, while some are purchased by the restaurant's owner. Only 5% farmers keep them for 35-40 days. Adaszyńska-Skwirzyńska et al. (2025) studied that the maximum number of farmers keep birds for 40 days in Poland, which is different from our findings in Bangladesh. The reason is that the longer the rearing period, the tougher the meat will be, which is preferred by some consumers.

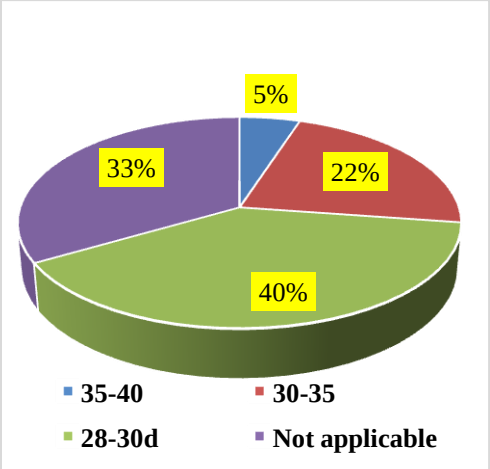

Fig. 3: The average number of days farmers keep birds

Figure 4 shows almost all categories of weight that are in the highest demand among consumers in Bangladesh. People are most interested in 1250g to 1350 g-weighted birds out of all the groups based on weight. The main reason for it is that about 55.8% of Bangladeshi families are nuclear (Khan et al., 2024); therefore, a chicken size of 1250 to 1350 g is ideal for these families because it meets their needs more effectively. The above 2 kg weight range is particularly convenient for portioning and menu creation, which restaurant and fast-food owners in Bangladesh choose. (Bostami et al., 2022).

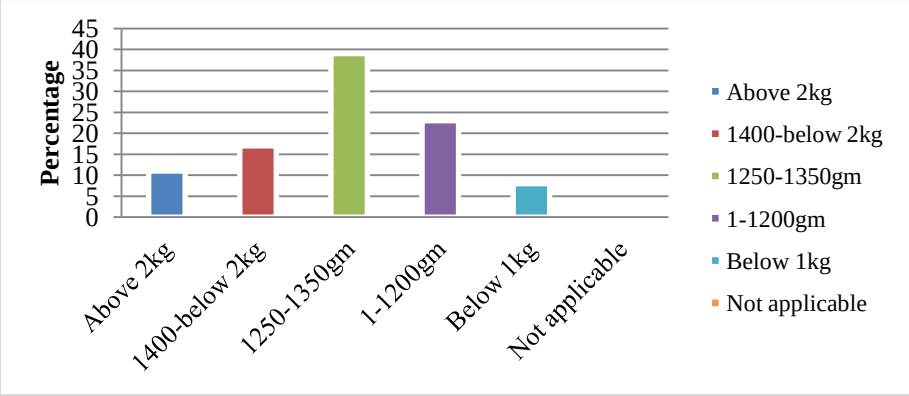

Fig. 4: The most popular weight categories among consumers

CONCLUSION

The overall KAP analysis indicates that consumers preferred the medium-sized broiler meat (1.25-1.35 kg) at 25 days of age. Similar weight interest was shown in the case of restaurant owners when they purchase a grill and a tandoori chicken cooking. However, they preferred the large-sized chicken above 2 kg for curry cooking purposes. On the other hand, farmers are more interested in selling medium-sized broiler chickens as they require less feed with less stress. This study provides an overview of how consumers, restaurant owners, and poultry farmers perceive the purchasing and selling of broiler chickens, respectively. This first-hand data about preference will help to improve the poultry industry of Bangladesh in developing effective marketing strategies and new products, and targeting consumer groups.

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FUNGI ASSOCIATED WITH WATERMELON SEED AND EVALUATION OF WATERMELON GERMPLASM AGAINST FUSARIUM WILT DISEASE

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ABSTRACT

This study evaluated seed health, *Fusarium* wilt resistance, and yield performance across 18 commercial and inbred watermelon genotypes to support sustainable production and enhancing the grower income. Seed health screening revealed high germination rates (mean 87.51%) with variable pathogen prevalence, notably *Fusarium oxysporum*, *Didymella bryoniae*, and *Acidovorax citrulli*. Microscopic analysis confirmed seed-borne fungal and bacterial contamination, underscoring the need for integrated sanitation protocols. Wilt resistance trials under controlled conditions identified several BARI breeding lines (G15–G18) and Black Giant as moderately resistant, with significantly lower disease incidence and severity. Yield assessments highlighted Sugar Emperor, Black Bull, and Dragon Beauty as top performers, while BARI lines demonstrated balanced resistance and productivity. Multi-location screening confirmed genotypic variation in disease expression, reinforcing the importance of adaptable cultivar selection. If future opportunities arise, activities will include molecular characterization, expanded field trials, and participatory validation to accelerate deployment of resilient cultivars. These findings offer a foundation for breeding programs targeting wilt-prone agro-ecological zones, contributing to climate-smart agriculture and improved livelihoods of the growers.

Keywords: Fusarium wilt, Fusarium oxysporum f.sp. Nivieum, germplasm evaluation, Seed health, Resistance

INTRODUCTION

Watermelon is the most widely consumed cucurbit globally, valued for its high lycopene, citrulline, and essential nutrient content (FAO, 2006; Reetu & Tomar, 2017). In Bangladesh, it is cultivated across 23,417 hectares, yielding over 550,000 metric tons annually (BBS, 2022). Its popularity as a summer fruit has made watermelon a lucrative crop for commercial farmers, especially in southern districts. However, seed-borne pathogens pose a major threat to watermelon production by reducing germination, weakening plant vigor, and triggering disease outbreaks in previously unaffected areas

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(Neergaard, 1977). Among these, *Fusarium oxysporum* f.sp. *niveum* (FON), the causal agent of Fusarium wilt (FW), is particularly destructive—capable of causing up to 100% crop loss under severe infection (Egel & Martyn, 2007; Booth, 1971). First reported in southern Bangladesh in 2018 (Alam et al., 2024), FON has since spread to major watermelon-producing regions, including Patuakhali, Noakhali, and Khulna (Alam et al., 2020). Management strategies include crop rotation, soil disinfection, chemical control, and host resistance (Martyn, 2014; Everts et al., 2014). This study aims to evaluate seed health and Fusarium wilt resistance in commercial and inbred watermelon lines to support sustainable production and improve grower income.

MATERIALS AND METHODS

Seed health status of commercial varieties of watermelon

Seeds of fourteen commercial watermelon varieties collected from seed markets and four inbred lines developed by the Bangladesh Agricultural Research Institute (BARI) were evaluated in the laboratory (Table 1). The blotter paper method was used for detecting various fungi. In this method, the growth of mycelial and fruiting bodies during incubation is an indicator of fungus. Seeds were placed in Petri dishes lined with three layers of water-soaked filter paper. The seeds were then sterilized using a 2% NaCl solution with available chlorine for 5 minutes, followed by rinsing in sterilized distilled water 3-4 times. They were then air-dried and subjected to the standard blotter method to assess the incidence of mycoflora and germination rate (Figure 1). Five seeds were added per dish; each replication contained four Petri dishes with three replications and was incubated for seven days at a temperature of 25±2°C, either under 12 hours of light or in darkness. After the incubation period, all Petri dishes were examined using a stereo and compound microscope to identify any fungi or bacteria present within the seeds (Abdullah and Atroshi, 2016).

Assessment of germplasm for Fusarium wilt in inoculated conditions

The study was conducted under artificially inoculated conditions in the pot house of the Plant Pathology Division, BARI, Gazipur, Bangladesh. NSWF001 isolates of FON were utilized to assess the germplasm. NSWF001 was cultured in a water-soaked mixture of ground corn, wheat bran, and grass pea seed coat (1:10:5, w/w) for 10 days. Thirty-five mycelial blocks (5 mm in diameter) from PDA cultures (7 days old) were used to inoculate 1 kg of the mixture, which was then incubated for 10 days at room temperature with a 12-hour photoperiod. To verify their pathogenicity, five seeds per pot were sown in 20-cm-diameter pots filled with soil mixed with 10 g of inoculum per kg of soil; each replication contained four pots and was repeated three times.

Table 1: Commercial seeds of hybrid varieties/inbreed lines of watermelon tested in the study

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

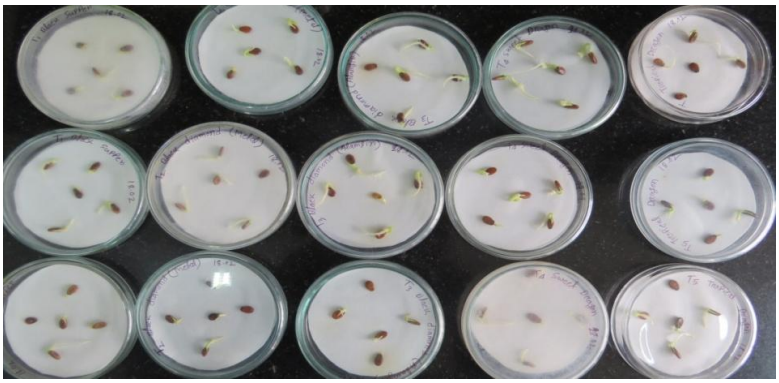

Fig. 1: Seed were placed on sterilized distilled water-soaked blotter and incubated at 25 ± 2^0c for 10 days.

Assessment of germplasm for Fusarium wilt in field conditions

A sick and natural nursery for disease screening was established in the experimental field of the Regional Horticultural Research Station (RHRS), BARI, Patuakhali, and the Plant

Pathology Division of BARI, Gazipur, aimed at identifying watermelon varieties resistant to wilt disease. Watermelon varieties/lines (Table 1) were sown, five seeds per hill; each treatment contained four hills with three replicates. Standard horticultural practices were employed to maintain the crop's health. Disease incidence was recorded as stated by Elmstrom and Hopkins (1981).

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

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

RESULTS AND DISCUSSION

Seed health status

Seed health screening across 18 watermelon germplasms revealed notable variation in germination rates and seed-borne pathogen prevalence (Table 3). Germination ranged from 80.45% (G3) to 95.88% (G4), with a mean of 87.51% and low variability (CV = 5.47%), indicating robust physiological seed quality. High germination is essential for crop establishment and is widely used as a proxy for seed vigor (ISTA, 2022). Microscopic observations (Figure 2, a–h and j–m) revealed the presence of creamy white mycelia along with macro- and microconidia characteristic of *Fusarium oxysporum*. Post-germination, various lesions developed on the seed surface due to infection by *F. oxysporum* f.sp. *niveum* and *Didymella bryoniae*. Panel (i) illustrates bacterial colonization, while panels (j–m) show fungal growth and fruiting bodies formed by *F. oxysporum* f.sp. *niveum*, *Alternaria* sp., *Aspergillus* sp., and *Penicillium* sp. These pathogens are associated with seed decay, damping-off, and seedling blight, highlighting the importance of seed health screening and sanitation measures.

In contrast, seed infection rates were more variable (CV = 28.89%), ranging from 11.98% (G4) to 35.11% (G6), with a mean of 26.46%. *Fusarium oxysporum* (mean 8.32%) and *Didymella bryoniae* (6.69%) were the most prevalent fungal pathogens, both linked to damping-off and seedling blight in cucurbits (Bruton, 1998; Anjorin & Mohammed, 2015). Other fungi, including *Aspergillus sp.*, *Penicillium sp.*, and *Alternaria sp.*, were detected at lower levels, though *Alternaria sp.* showed the highest relative variability (CV = 40.91%), often associated with poor storage conditions and mycotoxin production (Neergaard, 1977).

Bacterial contamination was present in all germplasms (mean 3.35%, CV = 40.96%), with *Acidovorax citrulli*—the causal agent of bacterial fruit blotch—recognized as a major seed-transmitted threat. It invades ovules via stigma pathways and persists in seed lots, leading to systemic infection and yield loss (Dutta et al., 2014; Rane, 1992). Microscopy studies confirm its rapid ingress via pollen tubes within 24 hours (Dutta et al., 2014).

Germplasms G4, G2, and G3 showed high germination and low infection, indicating promise for seed multiplication. In contrast, G5, G6, and G13 require targeted interventions. Fungicidal seed dressing (e.g., Seedplus® 30 WS) has shown efficacy under pathogen pressure (Anjorin & Mohammed, 2015). These findings support integrated seed health screening and sanitation protocols to strengthen cucurbit seed systems (FAO, 2010; Munkvold, 2009).

Table 3: Seed associated pathogens of commercial varieties watermelon and BARI advanced lines.

Variety/ Germplasm	% germination	% seed infection	F. oxysporum	D. bryoniae	Alternaria sp.	Aspergilus sp.	Penicilium sp.	Bacteria
G1	91.91	32.14	8.09	7.32	2.03	6.73	3.90	4.06
G2	93.17	17.69	4.99	5.76	1.03	2.20	2.13	1.57
G3	80.45	13.90	3.69	2.79	1.03	2.67	1.92	1.80
G4	95.88	11.98	3.74	2.36		2.70	2.26	0.92
G5	83.84	34.82	10.84	9.84	2.03	4.53	2.88	4.69
G6	83.35	35.11	9.36	9.03	2.03	5.50	3.77	5.41
G7	88.89	34.66	13.35	9.35	1.03	4.13	5.75	1.03
G8	82.74	21.46	4.76	3.86	2.07	4.00	3.52	3.25
G9	85.63	21.81	6.01	3.78	1.03	4.03	3.45	3.50
G10	90.14	26.91	9.09	6.09	1.03	3.97	2.58	4.16
G11	92.57	34.51	11.75	9.52	1.03	4.67	3.35	4.19
G12	81.17	33.33	11.80	8.36	1.03	3.27	3.16	5.72
G13	91.35	34.97	13.31	11.42	1.03	3.23	2.52	3.45
G14	93.31	30.94	10.58	8.36	1.03	4.03	2.69	4.25
G15	87.38	24.20	7.39	5.27	1.10	3.07	3.64	3.74
G16	85.97	24.22	8.09	6.98	1.03	3.10	2.14	2.87
G17	81.95	21.41	6.76	4.86	1.07	3.57	2.43	2.73
G18	85.43	22.24	6.16	5.39	1.10	4.03	2.52	3.04
Mean	87.51	26.46	8.32	6.69	1.27	3.86	3.03	3.35
Stdev	4.78	7.64	3.13	2.63	0.52	1.08	0.92	1.37
CV	5.47	28.89	37.65	39.35	40.91	27.96	30.19	40.96

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

Wilt disease incidence

Eighteen watermelon varieties and breeding lines were evaluated under pot house conditions at Plant Pathology Division, BARI Gazipur to assess their response to wilt disease (Table 4). Wilt incidence ranged from 18.48% to 47.18%, with a coefficient of variation (CV) of 5.87%, indicating moderate variability among genotypes. The lowest wilt incidence was recorded in *BARI line 04-21x144* (18.48%), followed closely by *BARI line 03-144x21* (19.20%) and *Black Giant* (19.68%), all classified as moderately resistant (MR). In contrast, *World Queen* exhibited the highest incidence (47.18%) and was the only variety rated as susceptible (S). The remaining genotypes showed moderate susceptibility (MS), with wilt incidence ranging from 26.33% to 45.82%. Statistical groupings (denoted by superscript letters) revealed significant differences among entries, with several BARI lines (G15–G18) consistently outperforming commercial varieties in disease resistance. These findings suggest that selected BARI lines and *Black Giant* may serve as promising genetic resources for breeding wilt-resistant cultivars and for deployment in wilt-prone production zones.

Yield Performance under fusarium wilt screening

Table 5 presents the yield-contributing traits of 18 watermelon genotypes evaluated under *Fusarium wilt* conditions at RHRC, Patuakhali. The results reveal substantial variation in fruit number, size, and overall productivity, underscoring the genetic diversity among the tested lines. *Sugar Emperor* (G14) emerged as the top performer, producing the highest fruit weight per plot (68.06 kg) and yield (48.85 t/ha), followed by *Black Bull* (G11), *Dragon Beauty* (G12), and *Sweet Green* (G13), all of which demonstrated strong commercial potential. *Thailand 2* (G10) recorded the largest individual fruit weight (6.43 kg) and longest fruit length (30.77 cm), while *BARI line 01-08x07* (G15) showed the highest fruit count per plant (3.71), indicating prolific fruiting capacity. Several BARI breeding lines (G15–G18) exhibited moderate yield performance and acceptable fruit traits, with *BARI line 03-144x21* and *BARI line 01-08x07* showing promise for integrated resistance and productivity. These findings are particularly relevant for breeding programs aiming to combine Fusarium wilt resistance with market-preferred traits. The observed variability offers a valuable foundation for selecting resilient, high-yielding cultivars suited to wilt-prone agroecological zones, contributing to sustainable watermelon production and improved farmer profitability.

Table 6 summarizes wilt incidence and severity across 18 watermelon genotypes evaluated at HRC Gazipur and RHRC Patuakhali. Disease incidence ranged from 10.28% to 43.38%, with severity from 9.27 to 42.33%, indicating significant genotypic variation. Four BARI lines (G15–G18) showed the lowest incidence and severity, with *BARI line 04-21x144* (G18) being the most resistant (10.28%, 9.27), followed by *BARI line 03-144x21* and *BARI line 01-08x07*. These lines are classified as moderately resistant (MR) and are promising for wilt-prone regions. In contrast, commercial varieties like *Tropical Dragon*, *Sweet Green*, and *World Queen* exhibited high susceptibility, with severity exceeding 30%. The significant t-test ($p = 0.02$) confirms meaningful differences among genotypes. Regional variation between Gazipur and Patuakhali may influence disease expression, reinforcing the need for multi-location screening. These findings support the selection of resistant, adaptable lines for breeding and sustainable watermelon production.

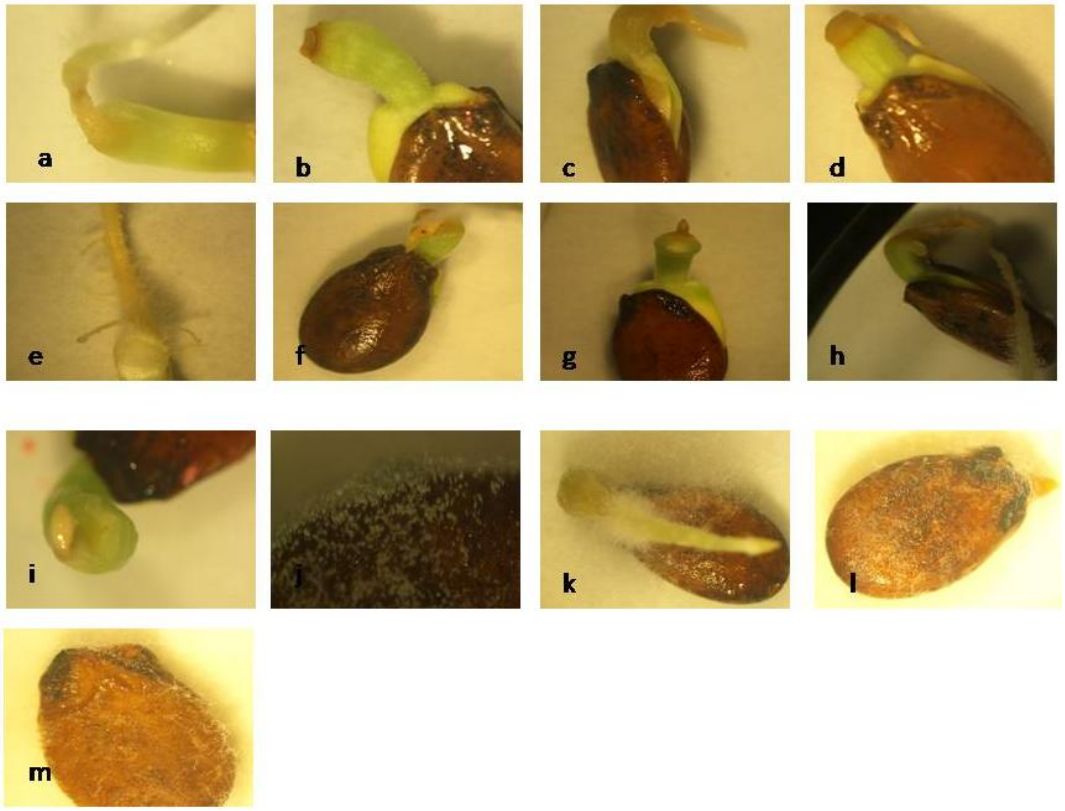

Fig. 2: Illustrating infection developed on seed in blotter test

Table 4: Incidence of wilt disease of watermelon in 18 varieties/lines Pot house, PPD, BARI Gazipur.

Variety/Germplasm	% Wilt incidence	Disease reaction
G1=Black Super	26.33e	MS
G2=Black Diamond (Metal Seed)	26.89e	MS
G3=Black Diamond (Alamgir Seed)	34.97d	MS
G4=Sweet Dragon	38.66c	MS
G5=Tropical Dragon	44.30ab	MS
G6=Big Family	43.88b	MS
G7=World Queen	47.18a	S
G8=Jumbo Jaguar	26.42e	MS
G9=Black Giant	19.68fg	MR
G10=Thailand 2	35.10d	MS
G11=Black Bull	43.27b	MS
G12=Dragon Beauty	42.82b	MS

Variety/Germplasm	% Wilt incidence	Disease reaction
G13=Sweet Green	45.82ab	MS
G14=Sugar Emperor	38.53c	MS
G15= BARI line 01-08x07	20.74fg	MR
G16= BARI line 02-07x08	22.16f	MR
G17= BARI line 03-144x21	19.20fg	MR
G18= BARI line 04-21x144	18.48g	MR
CV (%)	5.87	

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

Table 5: Yield contributing characters of watermelon 18 varieties/lines at RHRC research field, Patuakhali

Variety/Germplasm	NFPP	IFW(kg)	FL(CM)	FB(CM)	FWP(kg)	YTH
G1=Black Super	2.83b	4.15^{ef}	27.00^{bc}	18.23^{cd}	39.68fg	28.58
G2=Black Diamond (Metal Seed)	2.22ef	3.88^{f}	27.88^{bc}	20.87^{de}	32.28i	23.30
G3=Black Diamond (Alamgir Seed)	2.51b-d	4.74^{b-e}	26.41^{cd}	20.97^{de}	45.66d	32.85
G4=Sweet Dragon	2.70bc	4.54^{c-e}	18.78^{d}	21.30^{cd}	52.68c	37.87
G5=Tropical Dragon	2.55b-d	4.44^{d-f}	27.35^{bc}	21.27^{cd}	41.89ef	30.16
G6=Big Family	1.89ef	4.54^{c-e}	25.65^{d}	20.37^{de}	21.89j	**15.87**
G7=World Queen	2.82b	4.66^{b-e}	27.86^{bc}	20.57^{de}	42.62e	30.68
G8=Jumbo Jaguar	2.36b-e	4.71^{b-e}	**29.95^{a}**	20.97^{de}	51.41c	36.96
G9=Black Giant	2.13df	4.39^{d-f}	29.22^{ab}	20.87^{de}	33.99hi	24.52
G10=Thailand 2	1.87ef	**6.43^{a}**	**30.77^{a}**	**25.00^{a}**	45.56d	32.78
G11=Black Bull	2.87b	4.79^{b-e}	26.90^{cd}	22.50^{bc}	55.79b	40.09
G12=Dragon Beauty	2.53b-d	4.87^{b-d}	27.58^{bc}	21.33^{cd}	53.03c	38.12
G13=Sweet Green	2.57b-d	5.11^{b}	**30.30^{a}**	22.93^{bc}	52.79c	37.95
G14=Sugar Emperor	2.80b	5.29^{b}	27.77^{bc}	24.13^{ab}	68.06a	**48.85**
G15= BARI line 01-08x07	**3.71a**	**2.83^{g}**	18.93^{e}	15.20^{bc}	38.93g	28.04
G16= BARI line 02-07x08	**1.84f**	3.00g	18.52^{e}	17.83^{ef}	23.18j	16.80
G17= BARI line 03-144x21	2.60b-d	4.28^{d-f}	18.69^{e}	18.23^{ef}	39.69fg	28.59
G18= BARI line 04-21x144	2.74b	3.82^{d-f}	**16.72^{f}**	20.87^{de}	35.94h	25.91
CV (%)	2.13	8.77	4.16	4.58	3.66	28.58

NFPP= No of fruit per plant, IFW= Individual Fruit Weight, FL (cm.) = Fruit length (cm.), FB (cm.) = Fruit breath (cm.), FWP= Fruit weight per plot and YTH= Yield ton ha^{-1}

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Table 6: Differential Disease Response of Watermelon Varieties to Wilt Across Agro-Ecological Zones of Gazipur and Patuakhali

Variety/Germplasm	Potuakhali	Gazipur
G1=Black Super	16.87g	15.13h
G2=Black Diamond (Metal Seed)	18.40fg	16.87g
G3=Black Diamond (Alamgir Seed)	26.30e	25.05e
G4=Sweet Dragon	20.51f	18.73f
G5=Tropical Dragon	**43.38a**	34.18c
G6=Big Family	35.84c	33.83c
G7=World Queen	39.68b	37.42c
G8=Jumbo Jaguar	17.12g	16.34gh
G9=Black Giant	16.46g	16.34gh
G10=Thailand 2	25.64e	25.23e
G11=Black Bull	31.16d	33.39c
G12=Dragon Beauty	34.49c	32.61c
G13=Sweet Green	40.27b	42.33a
G14=Sugar Emperor	29.93d	28.56d
G15= BARI line 01-08x07	12.46hi	10.85ij
G16= BARI line 02-07x08	13.81h	12.17i
G17= BARI line 03-144x21	11.25i	10.25j
G18= BARI line 04-21x144	10.28i	9.27j
Mean=	24.66	23.25
CV (%) =	6.16	4.30
t-stat=	2.58	
t Critical one-tail=	1.74	
P(T<=t) two-tail=	0.02	

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

CONCLUSION

This study underscores the potential of selected watermelon lines for enhancing seed health, Fusarium wilt resistance, and yield. If further opportunities arise, future activities may included molecular characterization of resistance traits, expanded multi-location trials, and participatory breeding with farmers. These efforts would strengthen cultivar deployment, support resilient seed systems, and contribute to sustainable watermelon production and improved grower livelihoods.

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GENETIC DIVERSITY OF PLANTAIN GENOTYPES (*MUSA PARADISIACA* L.)

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ABSTRACT

Thirty plantain genotypes (*Musa paradisiaca* L.) were studied to assess the nature and magnitude of genetic divergence among them for fruit yield and yield contributing characters. The Experiment was conducted at the Horticulture Research Centre of the Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during July, 2009 to July, 2012. Based on the 15 characters, the genotypes were grouped into five clusters. The lower and higher inter-cluster distances revealed that the genotypes within the same cluster were closely related. The intra-cluster distance 0.566 was observed maximum in cluster II followed by 0.564 of cluster III. The minimum intra-cluster 0.430 was observed in cluster IV. The highest inter-cluster distance 13.679 was observed between cluster II and V while the minimum inter-cluster distance 4.602 was observed between cluster I and IV. Banana breeding program in Bangladesh involves maintenance of various genetic resources of banana, of which triploids constitutes the maximum share over diploids and tetraploids. The study revealed that pseudo-stem base girth, finger weight, finger length, finger girth, peel weight and yield had maximum contribution towards genetic divergence of 30 plantain genotypes (AAA). The results of diversity analysis revealed that crosses between genotypes of cluster III and cluster II might result in developing diploid and tetraploid hybrids with desired attributes. Improved tetraploid lines may then be hybridized with diploids to produce triploids for further valuation.

Keywords: Genetic diversity, Genetic base, Mahalanobi's distance (D^2), *Musa paradisiaca L.*

INTRODUCTION

Bananas and plantains, members of the Musaceae family, trace their origins to two wild species: *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome), along with their hybrids and polyploids (Simmonds, 1966). Today's edible cultivars are predominantly seedless triploid clones, classified into genome groups AAA, AAB, and ABB. The term "plantain" typically refers to *Musa paradisiaca L.*, a starchy fruit consumed after cooking. In Bangladesh, plantains are a vital year-round vegetable crop,

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bridging seasonal gaps in vegetable supply. Most cultivars grown locally are somatic selections from the Cavendish group, propagated vegetative and genetically uniform.

However, climate change poses a growing threat to plantain diversity and productivity (Perrier et al., 2011). Genetic variability is key to resilience under stress conditions (Ashry, 2013), yet data on the genetic diversity of banana and plantain in Bangladesh remains limited. Earlier breeding efforts focused on maintaining genetic resources and selecting superior clones (Akhter et al., 2009; Haque, 1988). To meet future challenges, enhancing production through heterosis is essential. Understanding genetic diversity among diploid lines could unlock additional gains through strategic hybridization (Groose et al., 1989). While classical breeding is constrained by male sterility and vegetative propagation, global approaches now favor crossing triploid females with diploid males to produce tetraploids, which are then backcrossed to generate improved triploids. This study aims to assess the genetic diversity of 30 plantain germplasms collected from diverse agro ecological zones of Bangladesh, now conserved at the Horticulture Research Centre (HRC), BARI field genebank.

Standard Operating Procedures (SOPs) for selecting genotypes of breeding technique

The identification of improved diploids that provide donor traits of interest and cultivated triploids with superior quality characteristics takes on a vital role in synthesizing superior secondary triploids. Crossing between male diploids (2x) and female triploids (3x), tetraploid progenies are obtained (4x). The plantain triploid genotypes under the study can be utilized to produce hybrids of mixed ploidy levels such as diploids (2x), triploids (3x) and tetraploids (4x) with desirable attributes. The breeding programs perform triploid (3*x*) crossing with improved diploid (2*x*) to develop tetraploid hybrids (4*x*) with desirable attributes. These 4*x* hybrids are very fertile and contain seeds. So secondary 3*x* sterile offspring with parthenocarpy fruit with additional favorable alleles are developed by crossing the tetraploids (3x) with improved diploids (2x). Following is a flowchart diagram of use of improved diploid and synthesized tetraploid gene pools to develop secondary triploids of bananas and plantains (Figure 3). Under this breeding scheme, the identification of improved diploids that provide donor traits of interest and cultivated triploids with superior quality characteristics takes on a vital role in synthesizing superior secondary triploids.

MATERIALS AND METHODS

The Experiment was carried out at the Fruit Research Field of Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during July, 2009 to July, 2012. Thirty genotypes of plantain were included in this study. Names of the districts from where germplasm collected are given in Table-1. We used GIS tools to delineate the district boundaries (Figure 1). Each germplasm was coded with a number started by 1 up to 30 prefixed by the abbreviation of district name and *MS* represent the genus “Musa” (Table 1).

Table 1: Collection sites with Genotype codes

Sl. No.	Collection site	Germplasm Code
1..	RARS, Jamalpur	MS Jm-001
2.	Gazipur	MS Gp-002
3.	RARS, Jamalpur	MS Jm-003J
4.	RARS, Jamalpur	MS Jm-004
5.	RARS, Jamalpur	MS Jm-005
6.	RARS, Jamalpur	MS Jm-006 (BARI Kola-2
7.	RARS, Jamalpur	MS Jm-007
8.	RARS, Jamalpur	MS Jm-008
9.	RARS, Jamalpur	MS Jm-009
10.	RARS, Jamalpur	MS Jm-010
11.	RARS, Rahmatpur, Barisal	MS Br-011
12.	Valuka, Mymensingh	MS My-012
13.	HRC, BARI, Joydebpur, Gazipur	MS Gp-013
14.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-014
15.	HRC, BARI, Joydebpur, Gazipur	MS Ga-015
16.	HRC, BARI, Joydebpur, Gazipur	MS Gp-016
17.	Salna, Gazipur	MS Gp-017
18.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-018
19.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-019
20.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-020
21.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-021
22.	Narsingdi	MS Nr-022
23.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-023
24.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-024
25.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-025
26.	OFRD, Bandarban	MS Bb-026
27.	ARS, Khagrachari, Rangamati	MS Rm-027
28.	HRC, BARI, Joydebpur, Gazipur	MS Gp-028
29.	HRC, BARI, Joydebpur, Gazipur	MS Gp-029
30.	ARS, Raikhali, Chandraghona, Rangamati	MS Rm-030

(Germplasm correspond to collection sites coded in Figure 1)

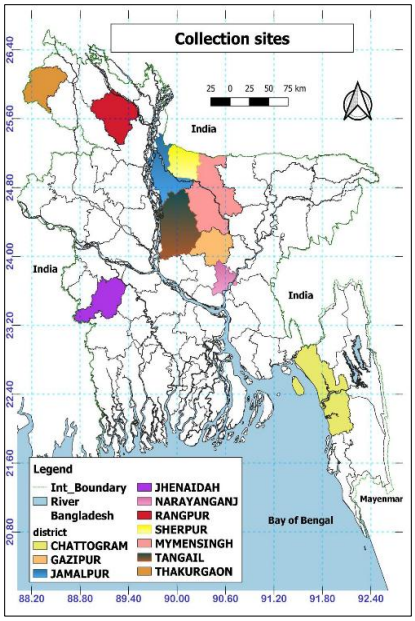

Fig. 1: Map of geographic location of germplasm collection (genotype codes correspond to those given in Table 1)

The experiment was laid out using RCB design with three replications. The unit plot size was 4x4m and four (04) plants were accommodated in a plot with a spacing of 2x2m. The land was fertilized with cow dung, N, P, K and S as recommended by BARI (2006). The intercultural operations (weeding, irrigation, insecticide spray etc.) were done as and when necessary. The data were collected from four (04) plants of each genotype. Data on days to 50% flowering, days to 1st harvest, days to last harvest, number of fruits/plant, weight of fruits/plant (kg), average fruit weight (g), total number of fruits/plot, plot yield (kg), fruit length at green stage (cm), fruit length at mature stage (cm), fruit diameter at green stage (cm), fruit diameter at mature stage (cm), plant height at 1st harvest (cm) and fruit yield (t/ha) were recorded and summarized. Genetic diversity was worked out following principal component analysis (PCA) as recommended by Rao (1964) and generalized distance (D2) analysis (Mahalanobi's 1936) extended by Rao (1952). Data were analyzed using the GENSTAT 5.5 computer program. Intra and inter-cluster distances were calculated followed by Singh and Chaudhury (1985).

RESULTS AND DISCUSSION

Mapping of collection site locations

The map of the germplasm collection's geographical location (Figure 1) reveals that the Gazipur, Jamalpur, Mymensingh, Sherpur, Tangail, and Narsingdi districts are neighboring and collectively represent a quadrat (Figure 1). The widening geographic distances between any two points in the quadrat contribute to the change in latitude (Wu J., 2000). According to Gao and Liu., (2018) and Brus and de Gruijter, (1997) the change in latitude is associated with the climatic factors, soil physical properties as well as

composition in local microenvironments of overall ecosystem processes. The climatic disparities among Jhenaidah, Rangpur, Thakurgoan, Chattogram, and the five northern districts are obvious due to large geographical scale (Harrison et al, 2017; Raduła et al., 2020). Wright et al., (2005) described that plant functional traits reflect the response and adaptation to the growth environment and thereby provide genetic resources that meet current and new challenges for farming in stressful environments. Out of 30 germplasms, 7 were collected from Gazipur, 9 from Jamalpur, 10 from Rangamati and 01 each from Bandarban, Barisal, Mymensingh and Narsingdi (Table 1). Areas that represent less germplasm imply that farmers in those areas are not interested in plantain cultivation. This may be because for them plantain is an unprofitable crop or does not fulfill their objectives.

Principal component analysis (PCA)

PCA summarizes the information content of large datasets into a smaller set of uncorrelated variables known as principal components (PC). These PCs are linear combinations of the original variables that have the maximum variance compared to other linear combinations. These components capture as much information from the original dataset as possible. The results revealed that the first three principal components accounted for 81.99% of the total variation. The rest of the components contributed 18.01 of the total variation (Table 2). The larger the variability captured, the larger the information retained from the original dataset.

Table 2: Eigen values and percent of variance and cumulative variance yield and yield attribute traits of 15 characters in plantain

Principal component (PC)	Yield and yield attribute traits	Eigen value	% of total variation accounted for	Cumulative percent
1.	Days to harvesting	2.7419	46.77	46.77
2.	Hands/ bunch	1.6224	27.67	74.44
3.	Fingers/bunch	0.4422	7.54	81.99
4.	Finger weight (g)	0.2635	4.49	86.47
5.	Finger length (cm)	0.2140	3.65	90.12
6.	Finger girth (cm)	0.1845	3.15	93.27
7.	Peel weight (g)	0.1006	1.72	94.99
8.	%EP	0.0852	1.45	96.44
9.	% TSS	0.0696	1.19	97.63
10.	Yield (t/ha)	0.0466	0.79	98.42
11.	Leaves at shooting	0.0335	0.57	98.99
12.	Leaf length (cm)	0.0285	0.49	99.48
13.	Leaf breadth (cm)	0.0156	0.27	99.75
14.	Pseudo-stem height (m)	0.0147	0.25	100.00
15.	Base girth (cm)	0.0001	0.00	100.00

Principal components 1, 2 and 3 had 46.77%, 27.67% and 7.54% variability among the genotypes for evaluated traits respectively. Eigenvalue associated with each PC decreased gradually and stopped at 0.0001 (Table 2). The first PC was more related to days to harvesting, leaf at shooting, leaf length and leaf breadth (Table 3). This result also indicated that the major variance of 15 characters can be explained by two principal components namely Z1 and Z2.

Table 3: Latent vectors for 15 principal component characters of plantain genotypes

Sl. No.	Characters	Eigen vector (eigen value)	
		Z_1 (46.77%)	Z_2 (27.67%)
1.	Days to harvesting	0.0337	-0.4461
2.	Hands/ bunch	-0.2181	-0.4346
3.	Fingers/bunch	-0.1733	-0.4899
4.	Finger weight (g)	-0.4635	0.1959
5.	Finger length (cm)	-0.3864	0.1257
6.	Finger girth (cm)	-0.3762	0.0370
7.	Peel weight (g)	-0.4485	0.1585
8.	%EP	-0.0674	0.1869
9.	% TSS	-0.0625	0.2109
10.	Yield (t/ha)	-0.3954	-0.3206
11.	Leaves at shooting	0.0942	0.1294
12.	Leaf length (cm)	0.0211	0.1114
13.	Leaf breadth (cm)	0.1025	-0.0985
14.	Pseudostem height (m)	-0.0115	0.0013
15.	Base girth (cm)	-0.1723	0.2654

In the second PC the finger weight, finger length, peel weight, %EP. %TSS, leaf at shooting and base girth were the more related traits (Table 3). These traits acquired the maximum variation among each PC.

The output of PCA was described by cluster analysis. All the tested genotypes were clustered into 5 morphologically distinct clusters (Table 4). As stated in Table 4 and Table 5, cluster V is composed of the largest number of genotypes 9 but intra-cluster distance was not necessarily the highest. The highest intra-cluster distance was recorded in cluster II containing 4 genotypes followed by an intra-cluster distance in cluster III containing 6 genotypes. The intra-cluster distances of cluster V were 0.475 and composed of 9 genotypes. The least intra-cluster distance 0.430 was observed in cluster IV consisting of 5 genotypes. This least intra-cluster distance indicated that the genotypes in this cluster were more homogenous than the genotypes consisting of the other clusters. The lower intra-cluster and higher intra-cluster values suggested that the population groups were homogenous within and heterogeneous between clusters. The intra-cluster

distances in all the clusters were less than inter-cluster distances which revealed that the genotypes within the same cluster were closely related.

Table 4: Distribution of 30 plantain genotypes in 5 clusters

Cluster	No. o genotypes	Genotypes
I	6	4,5,10,11,12,17
II	4	3,6,7,9
III	6	8,13,14,15,21,24
IV	5	1,2,16,22,28
V	9	18,19,20,23,25,26,27,29,30

The maximum value of inter-cluster distance indicated that the genotypes belonging to the cluster II and V were far diverged. Similarly, the higher inter cluster values between cluster II and III, cluster II and IV indicated the genotypes belonging to each pair of clusters were far diverged. The lower inter cluster distances between the cluster I and IV, cluster IV and V, and cluster III and V indicated that they were less diverged suggesting a close relationship between the genotypes of these three clusters (Table 5). In the current study, it was noted that the genotypes in clusters II and V showed significant divergence, with a similar trend observed between clusters II and III. Crosses between cluster II and V or II and III are expected to create new, improved traits due to high heterosis (Mian and Bahl, 1989).

Table 5: Intra (bold) and inter cluster distances of 30 plantain genotypes

Cluster	I	II	III	IV	V
I	0.446	8.336	6.446	4.602	7.828
II		0.566	13.402	9.149	13.679
III			0.564	7.236	6.280
IV				0.430	5.462
V					0.475

Non-hierarchical clustering

Non-hierarchical clustering using covariance matrices among 30 genotypes was grouped into 5 clusters (Table 6 and Figure 2). The clustering pattern obtained coincided with the apparent grouping patterns performed by PCA. So, the results obtained through PCA were confirmed by non-hierarchical clustering. Two out of five clusters revealed an association between accessions and geographical regions. For instance, all accessions in Cluster II were collected from Jamalpur and accessions in Cluster V were from Rangamati. The lack of clear division among the accessions based on their geographical origin shows an enlargement of genetic diversity. The results of this study agree with others in showing that geographical separation did not generally result in greater genetic distance. This may be because plantain has been introduced into many countries, and materials from widely separate locations have been exchanged. Thus, our collection holds diverse germplasm

within it that can be used as sources of useful traits for plantain improvement as described in sesame by Kim *et al.* (2002). To some extent, the results were consistent with those of Zhang *et al*. (2011), Wei et al. (1994), and Zhao and Liu (2005), who showed no obvious regularity clustering in different types of cultivars and cultivars with different origins, which could be ascribed to the frequent exchanges of sesame cultivars and limited breeding parents in China. Pandey *et al.* (2015) have found that genotypes belonging to the same geographical area did not always occupy the same cluster.

Table 6: Distribution and place of collection of 30 plantain genotypes in 5 clusters

Cluster	No. of Members	Genotypes	Source/ place of collection
I	6	MS-004 (MPJa-1)	RHRC, BARI, Jamalpur
		MS-005 (MPJa-7)	RHRC, BARI, Jamalpur
		MS-010 (MPJa Local-1)	RHRC, BARI, Jamalpur
		MS-011 (MPBar-01)	HRC, BARI, Rahmatpur, Barisal
		MS-012 (MPG-024)	HRC, BARI, Joydebpur, Gazipur
		MS-017 (MP BSMRAU-04)	BSMRAU, Salna, Gazipur
II	4	MS-003 (MPJa-18)	RHRC, BARI, Jamalpur
		MS-006 (MP BARI Kola-2)	RHRC, BARI, Jamalpur
		MS-007 (MPJa Local-2)	RHRC, BARI, Jamalpur
		MS-009 (MPJa-6)	RHRC, BARI, Jamalpur
III	6	MS-008 (MPJa-24)	RHRC, BARI, Jamalpur
		MS-013 (MPG-007)	HRC, BARI, Joydebpur, Gazipur
		MS-014 (MPRA-010)	ARS, Raikhali, Chandraghona, Rangamati
		MS-015(MPG-006)	BARI, Joydebpur, Gazipur
		MS-021(MPRA-012)	ARS, Raikhali, Chandraghona, Rangamati
		MS-024 (MPRA-006)	ARS, Raikhali, Chandraghona, Rangamati
IV	5	MS-001 (MP ISD-02)	RHRC, BARI, Jamalpur
		MS-002 (MPRA Ja-001)	RHRC, BARI, Jamalpur
		MS-016 (MPG-005)	BARI, Joydebpur, Gazipur
		MS-022 (MPNar-01)	Narsingdi
		MS-028 (MPG-002)	BARI, Joydebpur, Gazipur
V	9	MS-018 (MPRA-008)	ARS, Raikhali, Chandraghona, Rangamati
		MS-019 (MPRA-002)	ARS, Raikhali, Chandraghona, Rangamati
		MS-020 (MPRA-003)	ARS, Raikhali, Chandraghona, Rangamati
		MS-023 (MPRA-007)	ARS, Raikhali, Chandraghona, Rangamati
		MS-025 (MPRA-004)	ARS, Raikhali, Chandraghona, Rangamati
		MS-026 (MPBAN-010)	ARS, Bandarban
		MS-027(MPRA-011)	ARS, Raikhali, Chandraghona, Rangamati
		MS-029(MPG-003)	BARI, Joydebpur, Gazipur
		MS-030 (MPRA-003)	ARS, Raikhali, Chandraghona, Rangamati

The two-dimensional scatter plot diagram (Z1 - Z2) consisted of four quadrants that illustrate the distribution of 30 plantain genotypes according to their morphological characters using component I as the c-axis and component II as γ-axis (Figure. 2). The scatter diagram indicated that there were 5 major genotype groups, different from each other. Considering the magnitude of genetic distance and, the magnitude of cluster means of different characters, genotypes from cluster II could be selected for days to harvesting, hands/branch, finger/branch, leaf breadth and pseudo-stem height. Genotypes under cluster I could be selected for finger girth and % EP. Genotypes under cluster III could be selected for fruit weight, leaf length, finger girth, peel weight, % edible portion, yield and girth of pseudo stem (Table 7).

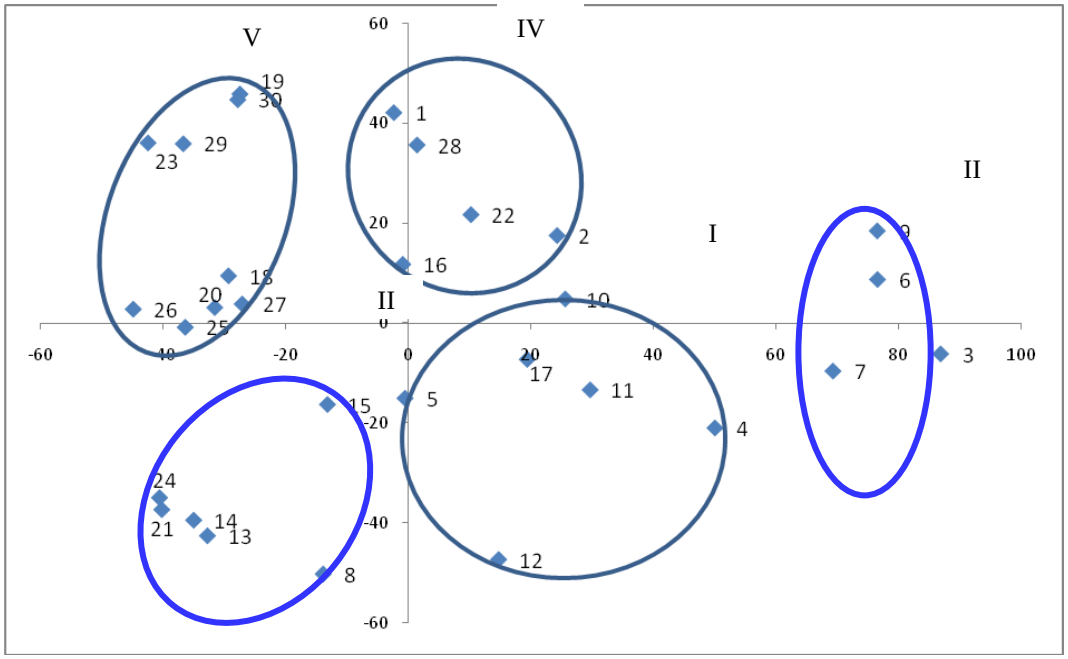

Fig. 2: Scatter distribution of 30 genotypes in plantain based on their principal component scores.

Table 7: Cluster mean values for different characters of plantain genotypes

Characters	Cluster				
	I	II	III	IV	V
Days to harvesting	405.12	447.75	361.55	376.00	340.54
Hands/ bunch	6.05	6.67	6.22	5.52	6.00
Fingers/bunch	69.22	83.75	72.28	63.02	68.54
Fruit wt.(g)	160.67	125.55	192.88	128.48	146.06
Leaf length (cm)	15.83	13.95	17.80	13.24	14.46
Finger girth (cm)	14.63	13.68	15.40	13.48	13.71

Characters	Cluster				
	I	II	III	IV	V
Peel weight (g)	65.45	53.15	79.02	52.94	58.54
%EP	59.35	57.45	59.13	58.68	59.87
% TSS	5.40	5.02	5.43	5.52	5.43
Yield (t/ha)	28.77	30.05	34.13	24.94	23.89
Number of leaves at shooting	12.78	12.25	12.27	12.00	12.51
Leaf length (cm)	223.90	217.57	228.93	225.32	238.63
Leaf breadth (cm)	59.33	60.78	57.52	59.34	61.81
Pseudostem height (m)	2.87	2.97	2.98	2.94	3.07
Base girth (cm)	62.28	64.42	70.83	65.62	68.11

Contribution of characters towards divergence of the genotypes

In vector I (Z_1), the other important characters responsible for genetic divergence in the major axis of differentiation were days to harvest, leaves at shooting, leaf length and leaf breadth having positive vector values. While in vector II (Z_2) finger weight, finger length, finger girth, peel weight, %Edible Portion, %TSS, leaves at shooting, leaf length, pseudo-stem height and base girth having positive vector values was important. The values of vector I and vector II revealed that both the vectors had positive values for the leaves at shooting and length of leaves. Negative values in both the vectors were present in hands per bunch, fingers per bunch and yield ton per hectare indicated that these characters had lowest contribution to the total divergence. (Table 8) Consequently, these results supported the hypothesis that this cluster possessed certain characteristics that made it more productive (Jagadeb *et al.*, 1991).

Table 8: Principal components for yield and yield attributing traits of evaluated plantain genotypes

Sl. No.	Characters	Component	
		I	II
1.	Days to harvesting	0.0337	-0.4461
2.	Hands/ bunch	-0.2181	-0.4346
3.	Fingers/bunch	-0.1733	-0.4899
4.	Finger weight (g)	-0.4635	0.1959
5.	Finger length (cm)	-0.3864	0.1257
6.	Finger girth (cm)	-0.3762	0.0370
7.	Peel weight (g)	-0.4485	0.1585
8.	%Edible Portion	-0.0674	0.1869
9.	% Total Soluble Solids (TSS)	-0.0625	0.2109
10.	Yield (t/ha)	-0.3954	-0.3206

Sl. No.	Characters	Component	
		I	II
11.	Leaves at shooting	0.0942	0.1294
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Standard Operating Procedures (SOPs) for selecting genotypes of breeding technique

The identification of improved diploids that provide donor traits of interest and cultivated triploids with superior quality characteristics takes on a vital role in synthesizing superior secondary triploids. Crossing between male diploids (2x) and female triploids (3x), tetraploid progenies are obtained (4x). The plantain triploid genotypes under the study can be utilized to produce hybrids of mixed ploidy levels such as diploids (2x), triploids (3x) and tetraploids (4x) with desirable attributes. The breeding programs perform triploid (3*x*) crossing with improved diploid (2*x*) to develop tetraploid hybrids (4*x*) with desirable attributes. These 4*x* hybrids are very fertile and contain seeds. So secondary 3*x* sterile offspring with parthenocarpy fruit with additional favorable alleles are developed by crossing the tetraploids (3x) with improved diploids (2x). Following is a flowchart diagram of use of improved diploid and synthesized tetraploid gene pools to develop secondary triploids of bananas and plantains (Figure 3). Under this breeding scheme, the identification of improved diploids that provide donor traits of interest and cultivated triploids with superior quality characteristics takes on a vital role in synthesizing superior secondary triploids.

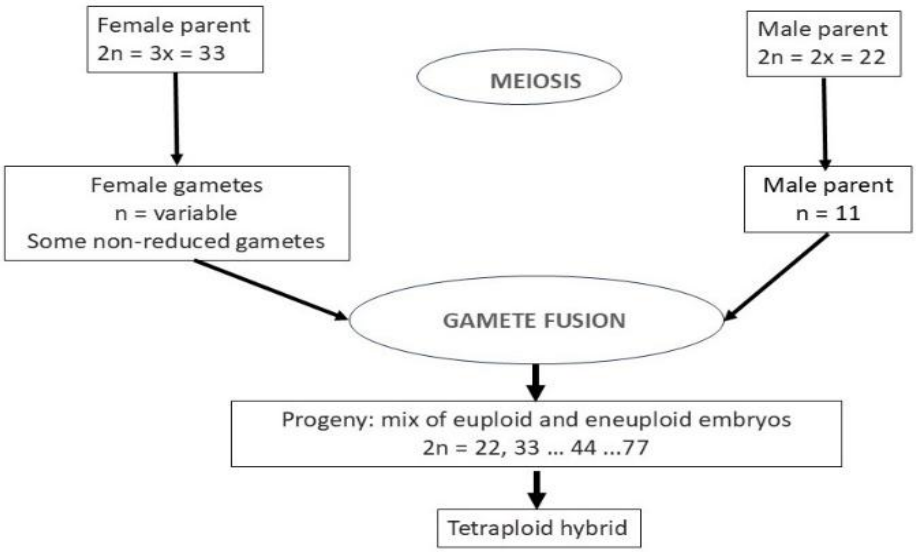

Fig. 3: Scheme for creating tetraploid hybrids from triploid and diploid parents.

CONCLUSION

Bananas and plantains are difficult crops to breed because they are highly sterile and therefore, do not produce seeds. The breeding program of plantain in Bangladesh involves maintenance of various genetic resources of triploids for selection of new varieties through field evaluation. Viable embryos could be produced from sterile triploid by making hybridizations with pollens of selected diploid banana. Recovery of seedless secondary triploid progenies relies on the diploid or tetraploid used as pollen source. The choice of the diploid parent is therefore obviously crucial and breeding efforts should be based on the improvement of the diploid male parent. Genetic knowledge of this piece of work might contribute to develop sound *Musa* breeding approaches in Bangladesh.

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IMPACTS OF HUMAN INTERVENTIONS ON THE FISH SPAWNING HABITAT IN THE RIVER HALDA OF SOUTH-EASTERN BANGLADESH

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ABSTRACT

The River Halda in southeastern Bangladesh is globally unique as the only natural spawning site for Indian Major Carps (IMC) such as *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala*. Its ecological health underpins freshwater fisheries, cultural traditions, and local livelihoods. However, this vital habitat faces mounting threats from human activities and hydrological alterations. In response, the Ministry of Fisheries and Livestock (MoFL) formed a twelve-member technical committee consisted of relevant ministries and agencies to evaluate the river's current conditions as the important fish spawning habitat. The committee conducted field visits to key locations in the River Halda and its surroundings for identifying constructions and practices that hinders its natural spawning functions. Major threats includes rubber dams, concrete installations, and water treatment facilities that obstruct the flow of the river. Encroachment, sand bars, unauthorized dredging, and unregulated fishing further degrading the ecosystems. Additionally, loop cutting and sluice gate operations on feeder canals are intensifying siltation, reshaping the riverbed, and eliminating critical spawning pockets. These disruptions are also contributing to rising salinity and declining water quality. The committee's findings, based on direct observation and consultations with fishers, traders, and local officials, underscore urgent concerns for both ecological sustainability and community well-being.

Keywords: Fish spawning, River Halda**,** Ecological sustainability, Hydrological alterations, Anthropogenic activities

INTRODUCTION

Bangladesh is a riverine nation with 761 rivers, yet nearly 200 are now considered ecologically dead and another 100 flow only during the monsoon season. Among the few remaining active rivers, the Halda in southeastern Bangladesh stands out as the country's only natural breeding ground for Indian major carps (IMC) such as *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala*. The River Halda, once renowned for its uninterrupted natural breeding grounds, has long supported the collection of fertilized carp eggs by

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local fishermen (Fig. 1). However, escalating anthropogenic pressures-including infrastructure development, unregulated encroachment, indiscriminate dredging, and hydrological alterations-have severely disrupted the river's ecological functions, degraded fish habitats, and reduced spawning success (Rahman *et al*., 2012). A prominent example is the construction of a rubber dam at Bhujpur in Chattogram, which has altered the river's natural flow regime, impeded sediment transport, and obstructed migratory routes for spawning fish (Fig. 2). As a result, the Halda has lost much of its natural breeding grounds, threatening the sustainability of its unique aquatic biodiversity.

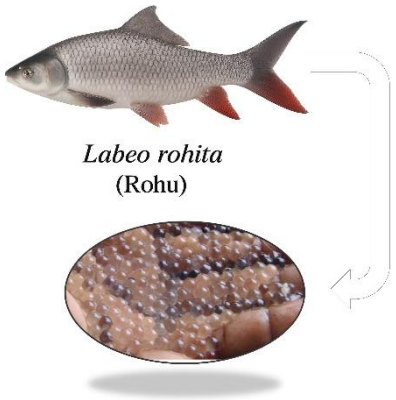

Fig. 1: Rohu (*Labeo rohita*) and its egg collected from the River Halda, Chattogram

This paper examines the nature and extent of these threats and highlights national efforts to restore the River Halda's ecological integrity, biodiversity, and community-based fisheries.

Fig. 2: Bangladesh map showing position of the River Halda and a rubber dam on it

SOURCES OF DATA AND INFORMATION

This review draws upon three primary sources of evidence such as field observations, stockholder consultations and reviewing of secondary literature. Field observations were conducted to assess hydraulic structures, sand extraction sites, and pollution sources

affecting the River Halda. Stakeholder consultations with local fishers and dependent communities provided insights into spawning success and socio-economic reliance. Reviewing of secondary literature contextualized ecological changes and highlight challenges for biodiversity conservation, fisheries sustainability, and watershed governance, providing a comprehensive understanding of human impacts.

Management Interventions and Gaps in Research on Spawn Production in the River Halda

Despite many years of ecological research, significant uncertainties remain regarding declining egg production, brood stocks of IMC, and long-term fishery impacts in the River Halda. In response, the Department of Fisheries (DoF) has implemented several management strategies to safeguard spawning ecology. Traditional mud-pit hatching has been substituted with controlled incubation, which lowers egg mortality rates and specialized hatcheries now protect spawn. Reducing egg mortality; purpose-built hatcheries now safeguard spawn; and identification cards. Issuing of identification cards help manage egg collectors (Azadi *et al.*, 2003). These interventions mark progress toward sustainable resource management. However, unresolved gaps in hydrological alterations, sediment dynamics, and cumulative impacts of dams and water abstraction demand integrated, basin-wise research to safeguard the Halda's ecological integrity and sustain its long-term role in national aquaculture.

Spawning Ecology and Carp Reproduction in the River Halda

The Halda River is renowned as a vital natural habitat for IMC, serving as a crucial breeding ground that has attracted extensive scientific research over the years. Its unique ecological characteristics and spawning behaviors have been the focus of numerous studies aimed at understanding the environmental and biological factors that facilitate successful natural spawning. Initial investigations by researchers such as Ahmad (1948, 1955), Ali *et al*. (1974), Azadi (1979, 1983, 1985a), and Tsai *et al*. (1981) established foundational knowledge regarding the limnological features of the river, including water quality, flow dynamics, and sediment composition. These studies provided critical insights into the reproductive biology of IMC, emphasizing the importance of environmental cues and traditional egg collection practices. Their work laid the groundwork for understanding the biological rhythms, environmental, meteorological and hydrological conditions necessary for successful spawning. Subsequent research by Patra and Azadi (1984, 1985a, 1985b, 1987), Rahman (2005), Alam (2004), Rahman *et al*. (2012), and Rahman & Akhter (2015) expanded this knowledge base by examining the complex interactions between hydrological variables and spawning success. These studies highlighted the significance of water flow regimes, sediment transport mechanisms, and habitat integrity, all of which are increasingly vulnerable to anthropogenic influences such as dam construction, pollution, and land use changes. The research highlighted the necessity of maintaining ecological balance to support sustainable carp populations.

Regional Perspectives and Comparative Studies

Research from neighboring regions, including India and Bangladesh, has contributed to a broader understanding of carp spawning dynamics across diverse river systems. Early

landmark studies by Khan (1924, 1942), Hora (1945), Mookerjee (1945), Majumder (1940), Job & Ganapati (1947), Khanna (1958), Alikunhi *et al*. (1964), Gopalakrishnan *et al.* (1966), Karamchandani *et al.* (1967), Ghosh & Rao (1969), and Dubey (1969) have enriched the collective knowledge regarding reproductive cycles, spawning habitats, and fisheries management practices in different ecological contexts. Together, these studies emphasize the critical role of hydrological cues, sediment dynamics, and ecological stability in ensuring the spawning success of Indian Major Carp. Such knowledge establishes the empirical basis for protecting the unique spawning ecology of the River Halda to maintain biodiversity, support local livelihoods, and ensure the long-term viability of carp populations in the region.

Declining Ichthyo-diversity and Spawn Production in the River Halda

The Halda River was once renowned for its rich biodiversity and recognized as South Asia's only natural spawning ground for Indian major carps. Historical accounts by Rainboth (1978) recorded an impressive 99 species of finfish and 12 species of prawn within the Halda-Ichhamati system. Today, however, recent surveys reveal a concerning ecological decline driven by human activities and environmental changes. For example, the Bangladesh Fisheries Research Institute (BFRI) documented just 72 finfish species, two prawn species, and a single dolphin (Rahman *et al.*, 2007). A separate assessment by the University of Chittagong (Arshad-ul-Alam, 2004) found 65 finfish species, six prawn species, a few crabs, and one dolphin. Nonetheless, Rahman *et al*. (2012) in another study reported 76 fish species, which might indicate some recovery or better survey methods.

In response to these concerns, a fish sanctuary was established between Maduna Ghat and Nazirhat Bridge to safeguard critical habitats. Yet new infrastructure projects present fresh challenges. A second WASA water treatment plant (WTP) is under construction at Maduna Ghat, designed to extract 270 million liters per day from depths of 7.6 to 9.2 meters only 6.5 kilometers upstream from the Karnaphuli confluence. Another WTP at Pomra, Rangunia, with similar capacity, is expected to further reduce river flow, particularly in the light of declining discharge from the Kaptai dam (Rahman *et al*., 2012). Collectively, these developments threaten to disrupt fish migration routes, diminish spawning success, and destabilize the river's ecological balance. To mitigate these emerging threats, researchers advocate basin-wise water governance, seasonal restrictions on water abstraction, and the preservation of ecological flow regimes. These measures are critical not only for conserving the Halda's biodiversity but also for sustaining its ecological functions and supporting the fisheries and livelihoods that depend on the river.

Declining Ecological Integrity and Spawn Productivity in the River Halda

The ecological health of River Halda has deteriorated significantly over the past several decades, largely due to hydro-morphological changes. In the 1950s, the river reached depths of 10 to 20 meters (Patra & Azadi, 1984), but widespread siltation has since led to substantial shallowing. Its width still varies between 35 and 210 meters (Azadi, 1979; Tsai *et al.*, 1981), although the reduction in depth has significantly modified key habitat conditions. Historically, the microbenthic richness of the riverbed and the high levels of primary productivity in the River Halda supported a wide array of bottom-dwelling fish

and complex trophic interactions, as documented by Azadi in 1979 and by Patra and Azadi in studies conducted between 1984 and 1987. In recent decades, the river's capacity to support natural spawning has declined sharply. Ahmed (1955) estimated spawn production of major carps at 4,000 to 5,000 kg in the 1950s, but by 2022 the Department of Fisheries reported a decline to just 129 kg, despite of occasional fluctuations. This dramatic reduction signals a deeper ecological imbalance and underscores the urgency of implementing integrated conservation and restoration strategies.

Anthropogenic Alterations and Habitat Degradation: Impacts on Major Carp Spawning

The Halda River holds a rare ecological distinction as the only tidal river in the World where Indian major carps spawn naturally. However, the ecological distinctiveness of the River Halda progressively undermined by the extensive anthropogenic disturbances. Processes such as erosion, siltation, the emergence of submerged sandbars, and declining of channel depth have collectively degraded the habitats particularly the grazing, spawning, and nursery zones essential for fish reproduction. The installation of sluice gates across 12 key feeder canals, including Sarta, Boalia, Chankhali, Sonai, and Kagtia, has further disrupted migratory pathways, impeding the natural spawning cycles of these species (Rahman *et al.*, 2012). For example, historical river engineering efforts between 1905 and 1910 introduced loop cutting at 11 locations, straightening the River Halda and eliminating the natural bends where Indian Major Carps traditionally released their eggs (Ahmad, 1948; 1955; Tsai *et al.*, 1981). This intervention shortened the river's length from 123 km to 98 km (Rahman *et al.*, 2007), accelerating the downstream drift of fertilized eggs into the saline waters of the River Karnaphuli where hatching is no longer viable due to elevated salinity levels (Rahman *et al.*, 2012). Despite these ecological setbacks, local communities continue to demonstrate remarkable resilience. Fishermen still collect fertilized eggs and hatch them in small earthen ponds or *Kua* along the riverbanks, where fry typically emerge within 18 to 22 hours (Tsai *et al.*, 1981). These traditional practices reflect deep-rooted knowledge and adaptability. At the same times they also underscore the urgent need for targeted habitat restoration and improved hydrological governance to ensure the long-term viability of carp spawning in the River Halda.

Hydro-Geographical Features and Tributary Dynamics of the River Halda

The Halda River originates from the Pata Chhara stream in the Badnatali Hill Ranges of Ramgarh Upazila of Khagrachhari District. From its source, the river flows southwest through Manikchhari, Fatikchhari, Hathazari, Raozan, and Chandgaon Thana of Chattogram Metropolitan City before merging with the River Karnaphuli (Rashid, 1991) (Fig. 3). Its course bends strategically to avoid elevated terrain, forming a fertile basin that supports both ecological diversity and agricultural productivity. Spanning approximately 98 kilometers, the Halda is navigable by large vessels up to Nazirhat (29 km), while smaller country boats can travel an additional 16 to 24 kilometers upstream to Narayanhat. One of its most dynamic tributaries, the River Dhurung, originates in the Pakshmimura ranges and runs nearly parallel to the Halda through Fatikchhari Upazila, joining it at Purba Dhalai about 48 kilometers downstream (Rashid, 1991). The river

system is further sustained by 19 major canals and numerous hilly streams (*Chhara*) from the eastern Chittagong Hill Tracts, which irrigate the basin as far as Kalurghat.

Figure 3 illustrates a detailed hydro-geographical map of the River Halda and its tributaries. The map highlights the freshwater nature of the rivers, the absence of salinity intrusion, and their strategic link to the Bay of Bengal. These hydro-geographical attributes collectively play a critical role in sustaining the natural spawning of Indian Major Carps. Despite these favorable conditions, Rahman *et al.* (2012) emphasize that both anthropogenic interventions and natural disturbances have significantly degraded spawning habitats, threatening the ecological integrity River Halda and its unique contribution to inland fisheries of Bangladesh.

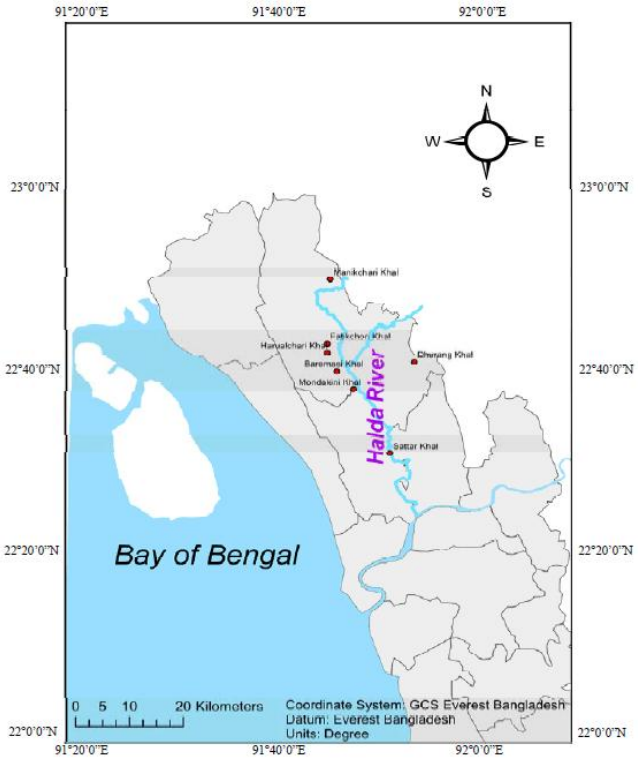

Fig. 3: Hydro-Geographical Features and Tributary Network of the River Halda

Human interventions in the River Halda catchment area

Rubber dam

Engineers install inflatable rubber dams, also known as Inflatable Flexible Membrane Dams (IFMDs), across riverbeds, channels, or weir crests to temporarily elevate upstream water levels. They typically fill these dams with water, air, or a combination of both, enabling multiple functions such as irrigation, flood control, aquifer recharge, salinity prevention, and facilitating fish passage when deflated, as noted by Mehta *et al.* 2015. While these structures aid irrigation, they have disrupted ecological connectivity. In the

River Halda basin, two inflatable rubber dams have been constructed to support agricultural irrigation. Rahman and Akhter (2015) report that the first inflatable rubber dam, built in 2011 at Koiya Ghat, Bhuzpur, spans approximately 55 meters in length and 4.5 meters in height, with a rubber membrane thickness of 12 mm. They further note that a second dam was installed in 2014 on the Harwalchhari Canal, also in Bhuzpur, measuring 30 meters in length and 4.5 meters in height. The rubber dam obstructs fish migration into the canal, preventing SIS and IMC from accessing critical breeding habitats.

This barrier effect poses a significant threat to the reproductive success and population sustainability of native fish species, underscoring the need for ecologically sensitive water infrastructure planning in the Halda basin.

Concrete structure (dam)

Rahman *et al* (2012) document that the River Dhurong, a vital tributary and freshwater contributor to the River Halda, underwent significant modification in 1964 through the construction of a concrete dam at Sundarpur, Bhuzpur, designed to support irrigation in surrounding agricultural zones. Measuring approximately 30 meters in length and 2.1 meters in height, the structure includes a boat pass and functions as a flat barrier to elevate upstream water levels. However, its ecological and geomorphological consequences have been profound. Severe bank erosion near the dam indicates destabilization of the river's natural flow regime, while the obstruction of fish migration routes has critically affected IMC and SIS that depend on the River Dhurong for grazing, feeding, and spawning. Local observations and prior studies suggest the dam has become functionally obsolete, failing to deliver its intended irrigation benefits while disrupting ecological connectivity. The blockage of migratory pathways has impaired the reproductive cycles of the Halda spawning fishes, reinforcing broader concerns about the adverse effects of rigid hydraulic infrastructure on riverine biodiversity and fish recruitment. The Sundarpur dam exemplifies the urgent need for integrated water resource management that reconciles agricultural utility with ecological sustainability. Retrofitting with fish-friendly designs or partial removal may be essential to restore habitat connectivity and support of the unique spawning ecology of the River Halda.

Water treatment plants

WASA Chattogram established a Water Treatment Plant (WTP) at Mohara in 1987, withdrawing 90 MLD from a depth of 7.0 m, located just 1 km upstream of the Karnaphuli confluence. Industrial abstraction by Asian Paper Mill at Nandirhat and agricultural withdrawals by BWDB at Sattarghat soon expanding to irrigate 20 sq km have further reduced river discharge. To conserve aquatic biodiversity, a fish sanctuary was declared between Maduna Ghat and Nazirhat Bridge. Despite this, a second WTP is under construction at Maduna Ghat, designed to extract 270 MLD from depths of 7.6-9.2 m, only 6.5 km from the Karnaphuli confluence. Additionally, a third WTP at Pomra, Rangunia on the River Karnaphuli, with similar capacity, is expected to critically lower flow, especially as Kaptai dam releases have declined (Rahman *et al.*, 2012). The reduced flow impairs fish migration, disrupts spawning period, spawning time, spawning success and degrades riverine ecosystems.

Illegal encroachment dredging and fishing

Rahman *et al.* (2012) report that unregulated sand dredging continues at multiple sites along the River Halda, driven by commercial demand. They further note extensive extraction to fill newly designated residential zones such as Ononno Abashik Ltd. Both manual and mechanical dredgers are in use, with mechanical operations causing greater ecological harm by creating uneven riverbeds and generating disruptive noise and vibrations. These disturbances displace brood fish from spawning grounds, significantly reducing spawning success of Indian Major Carps. In parallel, illegal fishing using various nets and traps occurs year-round, targeting both broodstocks and juveniles. Previous studies have linked such activities to declining fish populations and disrupted spawning cycles, underscoring the urgent need for enforcement and habitat protection to preserve the Halda's unique fishery.

Table 1: Impacts of straightening the River Halda by loop cutting

Place of loop cutting	Year of cutting	Length of the loop (km)	Problems occurred	Impact on spawning
1. Saidder char	1905-1910	1.80	Hwc, E*	S, Ns*
2. Masua ghona	1928	2.50	Hwc, E	S, Ns
3. Barighona	1948	8.30	Hwc, E	S, Ns
4. Abdullahpur	1948	1.10	Hwc, E	S, Ns
5. Seepatoli	1948	-	Hwc, E	S, Ns
6. Bhuzpur	1961-1962	0.80	Hwc, E	S, Ns
7. Ongkuri ghona	1964	2.00	Hwc, E	S, Ns
8. Haruwalchhari	1974	1.75	Hwc, E	S, Ns
9. Sundarpur	1974	1.00	Hwc, E	S, Ns
10. Sonair char	1988-1989	2.00	Hwc, E	S, Ns
11. Kagotiar ghona (A part has been straightened)	2002	2.00	Medium current, erosion	Pss

*Hwc= High water current, E= Erosion, S= Silted, Ns= No spawning, Pss= Poor spawning success

Loop cutting

Since 1905, eleven natural bends of the River Halda have been straightened through loop cutting, with the most recent at Kagotiar Ghona partially modified in 2002 (Rahman & Akhter, 2015). These bends serve as critical spawning microhabitats for Indian Major Carps, which avoid straight river stretches for spawning (Majumder, 1940). The loss of these habitats has led to a sharp decline in spawning frequency and egg volume (Fig. 4). Additionally, loop cutting has shortened the river by 25 km, causing fertilized eggs to drift rapidly into the River Karnaphuli before collection, reducing hatchery success (Patra & Azadi, 1985a, b). These findings highlight the ecological consequences of river channel modification.

Sluice gates on feeder canals

The River Halda's network of tributaries historically supported free fish movement, enabling access to secure and biologically suitable habitats. However, between 1975-76 and 1982-83, the Bangladesh Water Development Board (BWDB) constructed 12 sluice gates and navigation locks, effectively blocking key tributaries (Ali, 2003). These structures have long remained non-functional, severely restricting fish migration and habitat selection. As a result, brood fish are confined, and spawning success of IMC has declined markedly (Rahman *et al.*, 2012). Prior studies emphasize the need to restore connectivity to support ecological resilience, water quality, reproductive viability and spawning success.

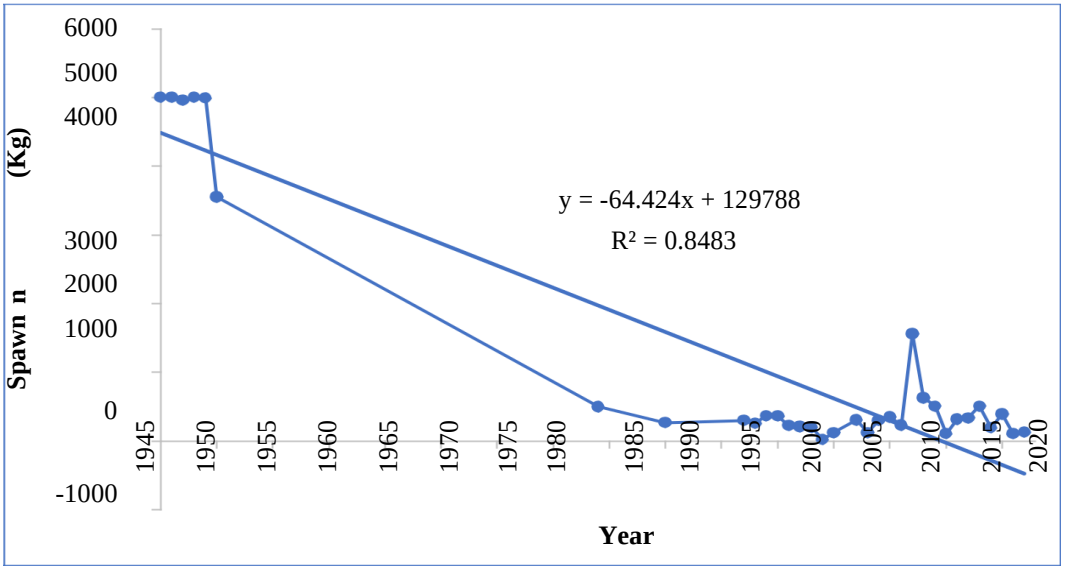

Fig. 4: Spawn production (1945-2022) from the River Halda

Aquatic life, including fish, crustaceans and invertebrates, has vanished from this segment. Further downstream, a 2.1 m high concrete dam constructed at Sundarpur across the River Dhurong has similarly disrupted water flow. During the dry season (December-March), the River Dhurong fails to contribute water to the River Halda due to insufficient input from its hilly catchment. As a result, the stretch between the Dhurong's mouth and Nazirhat Bridge is approaching ecological dormancy. Local observations confirm that brood fish historically migrated up to the Dhurong's mouth during the spawning season (Rahman & Akhter, 2015). The cumulative impact of these barriers underscores the urgent need for restoring flow connectivity to preserve the Halda's spawning ecology and biodiversity. Historical records show poor spawning success of IMC in 1990, 2003, 2004, 2013, and 2022 (Fig. 4), correlating with reduced flow conditions.

Low water discharge from the Lake Kaptai

Water discharge from the Kaptai Dam into the River Karnaphuli is directly dependent on the water reserve in Lake Kaptai, which in turn relies on rainfall within its catchment area. However, inflow from key feeder canals and *Chharas* has declined due to upstream diversions and damming in the hilly regions of a neighboring country, reducing overall lake recharge. Originally designed to store up to 6,476 million m^3 of water at a depth of 33.0 m, Lake Kaptai must maintain a minimum depth of 21 m to operate turbines 1-3, and 23 m for turbines 4-5. To run turbines 1-3, at least 16.0 million m^3 of water per day is required, supported by a dead storage of 903 million m^3. This discharge generates a water flow of approximately 184 m^3/sec in the River Karnaphuli. Spillway discharge is only possible when lake depth exceeds 33.0 m. During the dry season (December-March), limited rainfall and reduced inflow restrict operations to one or two turbines, resulting in minimal downstream discharge. Consequently, the River Karnaphuli receives only the base flow of 16.0 million m^3/day, which is insufficient to sustain ecological balance. These findings highlight the vulnerability of the river's hydrology and the need for transboundary water cooperation and seasonal flow management to ensure sustainable discharge from the Lake Kaptai.

Water pollution

The River Halda is increasingly polluted by waste discharge from brick fields, poultry farms, and open latrines (Rahman *et al.*, 2012). Untreated effluents from Asiatic Paper Mills at Nandirhat and the Peaking Power Plant at Hathazari enter nearby canals that flow directly into the River Halda. Similarly, the River Karnaphuli serves as a dumping ground for industrial, municipal, and domestic waste, severely contaminating its estuary and adjacent Bay of Bengal waters. These observations highlight the vulnerability of ecosystem destruction due to water pollution and the need for application of industrial rules to ensure sustainable quality discharge from the industries.

Available Act for Conservation and Management of Fish and Fisheries

Legislations are available for protection and conservation of fish and other aquatic organisms both for inland and marine waters of Bangladesh (Rahman & Akhter 2015). The Indian Fisheries Act, 1897 (Act IV of 1897) introduced by the then British Government has been amended and repealed by the then Pakistan Government in 1950 (Rahman & Akhter 2015). The Indian Fisheries Act, 1897 with few amendments was passed by the Assembly of the then East Bengal Government on 3rd March 1950, which is called Fish Act, 1950 and was published as "Dacca Gazette Extraordinary" on 18th May 1950 by the then Governor-General. It was again amended in 1982, 1985, 2002, 2007 and 2010 to incorporate some new laws and rules and to clarify some articles.

SUGGESTIONS AND RECOMMENDATIONS

To conserve the River Halda only natural spawning ground for Indian Major Carps, the rubber dam at Koiya Ghat should be urgently removed, and the Harwalchhari dam height reduced to restore ecological flow. Water abstraction by Mohara and Maduna Ghat plants must be reviewed, and alternative sources explored. Saline intrusion should be prevented by increasing flow and protecting upstream catchments. Illegal dredging, encroachment,

pollution, and loop cutting must be stopped through strict enforcement of environmental laws. Effective coordination among the relevant ministries and agencies is vital for sustainable management and restoration of the River Halda ecosystem.

CONCLUSION

Safeguarding the unique spawning habitat of the River Halda is essential. It supports carp fisheries and contributes to national food security of Bangladesh. To reverse ecological degradation, priority must be given to dam removal, pollution control, and habitat restoration. Effective coordination among agencies is also required. These actions are critical to ensure the long-term sustainability of this irreplaceable river ecosystem.

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MORPHOLOGICAL CHARACTERIZATION AND PERFORMANCE EVALUATION OF THIRTEEN JACKFRUIT GERMPLASMS IN SOUTH-EASTERN BANGLADESH

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ABSTRACT

The study was carried out at the Regional Agricultural Research Station, Hathazari, Chattogram during 2022-23. To find out the best jackfruit germplasms, thirteen plants had been selected for the experiment. The analysis of the jackfruit germplasm clusters reveals that distinct trait profiles with Cluster II is achieving the highest yield (279.94 kg/tree) but fruits size are smaller which making it suitable for high-density planting. Cluster I combines high yield (248.72 kg/tree) with bigger fruits size and better quality, that indicating the strong market potential. Cluster III, while yielding the least (54.53 kg/tree), offers the highest edible portion, highlighting its pulp efficiency. Variability in bulb characteristics among 13 germplasms shows significant genotypic influence, with bulb count and weight varying widely, suggesting potential for processing and fresh consumption. Seed traits also exhibit considerable diversity, with implications for breeding programs aimed at enhancing pulp yield and reducing seed bulk. Principal component analysis (PCA) indicates that the first three components explain nearly 80% of the variance, emphasizing the importance of traits like fruit weight and seed weight in breeding strategies. Overall, promising germplasms would be used for improving fruit quality and yield in future through breeding. Lines AH Hat-16, AH Hat-15, AH Hat-05, and AH Hat-01 were found to be superior.

Keywords: Jackfruit, Germplasm, Variability, Cluster, Biplot

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* L.), a member of the Moraceae family, is widely cultivated across Bangladesh and other tropical regions. As the national fruit of Bangladesh, it holds nutritional, economic, and cultural significance-often referred to as "the poor man's food" due to its affordability and accessibility. In 2022–23, Bangladesh produced approximately 10.62 lakh metric tons of jackfruit, with 2.09 lakh metric tons harvested from 30.69 thousand hectares of garden land (BBS, 2024).

Bangladesh is recognized as a secondary center of jackfruit diversity (Arora, 1998), with three primary types—"Khaja" (firm pulp), "Gala" (soft pulp), and "Do rasa" (intermediate)—exhibiting wide morphological and agronomic variation (Haque, 1993).

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The fruit is rich in carbohydrates, carotene, and vitamin C, and is increasingly used in processed forms such as chips, jams, and beverages. Jackfruit's monoecious nature and high heterozygosity, driven by cross-pollination and seed propagation, contribute to substantial phenotypic diversity. This variability offers opportunities for selecting elite genotypes with superior yield, quality, and stress tolerance. Characterizing germplasm through morphological traits is a prerequisite for biochemical and molecular studies. Multivariate techniques such as principal component analysis (PCA) and cluster analysis are effective tools for identifying promising genotypes for breeding and varietal development (Mohammadi and Prasanna, 2003). This study evaluates the performance and diversity of thirteen jackfruit germplasm in southeastern Bangladesh to identify superior accessions for future varietal release and sustainable cultivation.

MATERIALS & METHODS

During 2022–2023, the experiment was carried out using the jackfruit germplasm grown at RARS, Hathazari, Chattogram located between 22°24' and 22°38' north latitudes and in between 91°41' and 91°54' east longitudes. In 2010, 337 jackfruit germplasm were collected from different places in the southeast coastal region such as Chattogram and Chattogram Hill Tracts, and planted at RARS, Hathazari, Chattogram. Out of the said 337 germplasm, 13 superior germplasm, viz AH Hat-01, AH Hat-03, AH Hat-04, AH Hat-05, AH Hat-06, AH Hat-07, AH Hat-08, AH Hat-09, AH Hat-10, AH Hat-11, AH Hat-14, AH Hat-15, AH Hat-16 were finally selected for this study to identify the superior ones and also select as a variety.

The selected germplasm recorded ages of almost 15 years. The jackfruit plants were fertilized with cow dung or compost 30 kg, urea 1.3 kg, triple super phosphate (TSP) 600g, muriate of potash (MoP) 1.5 kg and gypsum 500 g. Total manures and chemical fertilizers were applied twice: first doses were applied in 20th September, 2021 and second doses were applied in 4th April, 2022. Intercultural operations were done regularly by weeding, irrigation and spraying of Pesticides. As the jackfruit plants cannot tolerate standing water, so adequate drainage systems were ensured during the rainy season. For analysis, two to five fruits were randomly selected from each plant. The fully mature harvested fruits were brought to the laboratory where fruit characters were recorded following the jackfruit descriptor (IPGRI, 2000). Total 33 characters were recorded. Using the Microsoft Office Excel package, the collected data were analyzed to determine the maximum, minimum, mean, standard deviation (SD), standard error of mean (SE), and coefficient of variation (CV). The cluster analysis, principal component analysis (PCA), and Biplot analysis were done using the software R 4.3.1 (Yan and Frégeau-Reid, 2018).

RESULTS AND DISCUSSION

***Flowering and Harvesting Dynamics*:** As shown in Table 4, the onset of male and female inflorescence varied across thirteen jackfruit germplasm, with male flowers emerging from December 25 (AH Hat-08) to February 3 (AH Hat-16), and female inflorescences from January 7 to February 19. These differences suggest genotypic variation in floral phenology, influencing pollination and fruit set timing, as also noted by

Ali *et al*. (2015). Most germplasm matured between June 13–18, except AH Hat-16, which was harvested on July 17, indicating extended fruit development possibly linked to genotype or environmental responsiveness (Ali *et al*., 2015).

Table 1: Flowering, harvesting and yield characteristics of jackfruit germplasm

Germplasm	Date of male inflorescence appearance	Date of female inflorescence appearance	Date of harvest	No. of fruits/tree	Yield (kg/tree)
AH Hat-01	02/01/2023	07/01/2023	14/06/2023	15	224.70
AH Hat-03	05/01/2023	30/01/2023	18/06/2023	43	190.92
AH Hat-04	28/01/2023	13/02/2023	16/06/2023	3	46.08
AH Hat-05	04/01/2023	08/01/2023	14/06/2023	48	418.56
AH Hat-06	10/01/2023	13/01/2023	16/06/2023	6	49.92
AH Hat-07	27/12/2022	07/01/2023	14/06/2023	43	248.97
AH Hat-08	25/12/2022	25/01/2023	16/06/2023	4	39.76
AH Hat-09	04/02/2023	25/01/2023	16/06/2023	12	181.92
AH Hat-10	03/01/2023	04/02/2023	13/06/2023	1	8.50
AH Hat-11	10/01/2023	12/02/2023	13/06/2023	6	76.32
AH Hat-14	10/01/2023	26/01/2023	18/06/2023	20	128.40
AH Hat-15	02/01/2023	15/01/2023	13/06/2023	36	511.92
AH Hat-16	03/02/2023	19/02/2023	17/07/2023	67	261.30
Max.	-	-	-	67	511.92
Min.	-	-	-	1	8.50
Mean	-	-	-	23.38	183.71
SD	-	-	-	21.49	146.19
SE(±)	-	-	-	5.96	42.19
CV (%)	-	-	-	91.92	79.57

***Fruit characters and yield*:** Substantial variation was observed in fruit number and yield per tree among the germplasm (Table 1). AH Hat-16 produced the highest number of fruits (67), while AH Hat-10 yielded only one fruit. However, the highest yield (511.92 kg/tree) was recorded in AH Hat-15, indicating that fruit size and weight also significantly contributed to overall productivity. The mean fruit count per tree was 23.38, with a standard deviation (SD) of 21.49, and a coefficient of variation (CV) of 91.92%, highlighting considerable variability in fruit-bearing capacity. Similarly, yield per tree averaged 183.71 kg, with an SD of 146.19 and CV of 79.57%, suggesting strong genotypic influence on yield potential. The high CV in fruit number may partly reflect sampling effects, as individual tree performance can be influenced by localized environmental conditions, or management history. The occurrence of a single fruit in AH Hat-10, while potentially genotypic, may also represent an outlier in the absence of replication. Multi-season evaluations are recommended to validate trait stability. Hiremata *et al*. (2022) reported such high CVs are not uncommon in fruit crops; for

instance, CVs exceeding 100% for fruit yield traits in muskmelon hybrids, underscoring the role of genetic diversity and environmental interactions.

The observed variability in flowering time, fruit count, and yield underscores the genetic diversity within the evaluated germplasm. Genotypes with early flowering, high fruit count, and superior yield such as AH Hat-05, AH Hat-07, AH Hat-15, and AH Hat-16 should be prioritized for further evaluation in breeding programs aimed at improving jackfruit productivity and adaptability in southeastern Bangladesh.

Fruit characteristics: Table 2 highlights substantial variability in fruit traits across 13 jackfruit germplasm. Individual fruit weights ranged from 3.90 to 15.36 kg (mean: 9.88 kg), with a high coefficient of variation (CV %) of 42.38, indicating strong genotypic diversity. Fruit length and breadth averaged 39.73 cm and 23.21 cm, respectively, with breadth showing greater uniformity (CV = 3.87%) than length (CV = 24.31%). The current results agree with the findings of Bhatia et al. (1955), who reported that the range of jackfruit lengths varied from 20.32 to 91.44 cm and widths varied from 15.24 to 50.80 cm.

Rind weight varied from 1.72 to 6.93 kg (mean: 3.85 kg), contributing significantly to total fruit mass. Present results are close to the findings of Mondal and Rahim (2015), who reported 0.94 to 4.17 kg rind weight in jackfruit. Rachis weight ranged widely (140–1520 g), with the highest variability (CV = 76.78%), suggesting differences in fruit attachment and biomass allocation. Rind color ranged from greenish yellow to deep brown, with no consistent association with fruit size.

Genotypes such as AH Hat-01, AH Hat-04, AH Hat-09, and AH Hat-15 exhibited superior fruit weight and biomass traits, making them promising candidates for selection. Overall, the observed diversity offers valuable scope for breeding programs targeting fruit yield, processing efficiency, and market appeal.

Rind thickness ranged from 1.2 to 2.6 cm (mean: 1.6 cm), with moderate variability (CV = 26.6%). Greater rind thickness causes a reduction in the edible portion (Akter and Rahman, 2018). Stalk length and diameter showed greater diversity (CV = 41.24% and 13.46%, respectively), influencing fruit attachment and handling. Most germplasms exhibited depressed or flattened stalk attachment, while AH Hat-08, AH Hat-09, and AH Hat-16 showed inflated types. Total soluble solids (TSS), a measure of sweetness, varied from 13.5% to 24.5% (mean: 18.68%), with AH Hat-11 and AH Hat-03 showing the highest values. This outcome aligns with the findings of Mondal and Rahim (2015), who recorded the total soluble solids (TSS) of jackfruit ranging from 16.10% to 30.00%.

The edible portion varied between 38.22% and 76.90%, with an average of 54.19%, demonstrating significant variations in pulp yield (Manjula et al., 2022). This variation is influenced by the genetic characteristics of the plant and the size of the fruit (Rai, 2003). AH Hat-04 and AH Hat-05 were superior in edible recovery. The flake-to-fruit ratio ranged from 0.21 to 0.75, with AH Hat-01 showing the highest flake density. Pulp texture varied from firm to soft and fibrous, with soft types (AH Hat-09, AH Hat-15) potentially preferred for fresh consumption. These traits are critical for selecting germplasm suited to consumer preference and processing potential.

Table 2: Fruit characteristics of jackfruit germplasm

Germplasm	Individual fruit weight (kg)	Fruit length (cm)	Fruit breadth (cm)	Fruit rind color	Weight of rind (kg)	Weight of rachis (g)
AH Hat-01	14.98	59.7	29.6	Brownish green	6.79	1520
AH Hat-03	4.44	32.2	18.7	Greenish yellow	2.23	270
AH Hat-04	15.36	43.2	27.6	Brown	3.14	400
AH Hat-05	8.72	33.7	20.9	Greenish yellow	2.2	450
AH Hat-06	8.32	40.55	21.45	Brown	3.58	360
AH Hat-07	5.79	30.2	20.6	Yellowish green	3.06	150
AH Hat-08	9.94	32.2	23.05	Deep brown	3.07	300
AH Hat-09	15.16	51.2	28.5	Yellowish Brown	6.93	500
AH Hat-10	8.50	37	24.5	brown	3.93	320
AH Hat-11	12.72	44	24.3	Greenish brown	4.46	830
AH Hat-14	6.42	32.4	22.4	Yellowish Brown	3.65	310
AH Hat-15	14.22	51.4	24.2	Deep brown	5.29	840
AH Hat-16	3.90	28.75	16	Yellowish green	1.72	140
Max.	15.36	59.7	29.6	-	6.93	1520
Min.	3.90	28.75	16	-	1.72	140
Mean	9.88	39.73	23.21	-	3.85	491.5
SD	4.18	9.66	3.87	-	1.64	377.4
SE(±)	1.16	2.67	1.07	-	0.45	104.6
CV (%)	42.38	24.31	3.87	-	42.62	76.78

Table 2: Fruit characteristics of jackfruit germplasms (contd.)

Germplasm	Rind thickness (cm)	Stalk length (cm)	Stalk diameter (cm)	Stalk attachment	TSS (%)	Edible portion (%)	Flake/fruit ratio	Texture of Pulp
AH Hat-01	1.8	4.9	9.5	Depressed	17.5	44.52	0.75	Firm
AH Hat-03	1.2	4.3	6.7	Flattened	22.5	43.64	0.57	Fibrous
AH Hat-04	1.3	8.6	9.9	Flattened	16.6	76.90	0.53	Firm
AH Hat-05	1.4	3.7	9.1	Depressed	15.5	69.61	0.56	Firm
AH Hat-06	1.9	4.6	9.4	Depressed	15.5	52.59	0.54	Firm
AH Hat-07	1.9	1.8	9.4	Flattened	13.5	44.55	0.21	Firm
AH Hat-08	1.6	8.1	8.9	Inflated	16.75	66.00	0.32	Firm
AH Hat-09	2.1	5.6	9.1	Inflated	20.25	50.98	0.47	Soft
AH Hat-10	1.4	6.8	8.1	Depressed	19.5	50.00	0.47	Firm
AH Hat-11	1.3	4.9	12.3	Depressed	24.5	58.41	0.39	Fibrous
AH Hat-14	2.6	4.8	8.9	Depressed	18.5	38.22	0.54	Firm
AH Hat-15	1.2	4.2	9.2	Depressed	21.5	56.86	0.4	Soft

Germplasm	Rind thickness (cm)	Stalk length (cm)	Stalk diameter (cm)	Stalk attachment	TSS (%)	Edible portion (%)	Flake/fruit ratio	Texture of Pulp
AH Hat-16	1.2	1.8	8.6	Inflated	20.75	52.30	0.5	Firm
Max.	2.6	8.1	12.3	-	24.5	76.90	0.75	-
Min.	1.2	1.8	6.7	-	13.5	38.22	0.21	-
Mean	1.6	4.93	9.16	-	18.68	54.19	0.48	-
SD	0.42	2.03	1.23	-	3.16	11.19	0.13	
SE(±)	0.11	0.56	0.43	-	0.87	3.10	0.03	-
CV (%)	26.6	41.24	13.46	-	16.95	20.66	27.56	

Bulb characteristics: Table 3 summarizes the bulb characteristics of 13 jackfruit germplasms, highlighting substantial variability in both quantitative and qualitative traits. The number of bulbs per fruit ranged from 30 (AH Hat-07) to 418 (AH Hat-15), with a high coefficient of variation (CV 62.57%), indicating strong genotypic influence. Bulb length and breadth averaged 6.78 cm and 3.46 cm, respectively, with AH Hat-04 exhibiting the longest bulbs (10.26 cm) and AH Hat-07 the broadest (5.36 cm). The average length and width of the bulbs were 6.78 cm and 3.46 cm, respectively, with AH Hat-04 displaying the longest bulbs at 10.26 cm and AH Hat-07 showing the widest at 5.36 cm. Our results align closely with those reported by Mannan et al. (2006). According to Akter and Rahman (2018), the maximum bulb width was 3.02 cm, and the minimum was 1.58 cm among 23 genotypes. Observations by Rai (2003) indicated that bulb width ranged from 2.5 cm to 5.0 cm in different jackfruit genotypes.

Single bulb weight varied widely (6–51 g), with AH Hat-01 and AH Hat-04 producing the heaviest bulbs, contributing to the highest total bulb weight per fruit (9.41 kg and 7.43 kg, respectively). In contrast, AH Hat-03 and AH Hat-07 had significantly lower bulb mass, suggesting limited pulp yield. The CV for single bulb weight (48.73%) and bulb breadth (30.83%) further underscores the morphological diversity among germplasms.

Bulb color ranged from light yellow to deep yellow and whitish yellow, with shape types including rectangular, twisted, cordate, irregular, and obovate. Notably, AH Hat-15 combined the highest bulb count with an obovate shape and moderate weight, suggesting potential for processing and fresh consumption. These findings are critical for selecting germplasms with superior pulp yield, desirable bulb morphology, and market-preferred traits for breeding and commercialization.

Table 3: Bulb characteristics of jackfruit germplasm

Germplasm	Number of bulbs/fruit	Bulb length (cm)	Bulb breadth (cm)	Single bulb weight (g)	Weight of bulbs/fruit (kg)	Bulb color	Bulb shape
AH Hat-01	195.00	9.13	4.40	51.00	9.41	Light yellow	Twisted
AH Hat-03	119.00	4.56	1.76	6.00	1.70	Yellow	Rectangular
AH Hat-04	146.00	10.26	3.60	50.00	7.43	Light yellow	Rectangular
AH Hat-05	183.00	6.40	2.76	22.00	4.17	Yellow	Twisted
AH Hat-06	134.00	6.40	3.56	28.00	3.88	Light yellow	Rectangular
AH Hat-07	30.00	7.53	5.36	43.00	1.02	Light yellow	Twisted
AH Hat-08	92.00	6.36	3.46	29.00	2.69	Light yellow	Cordate
AH Hat-09	165.00	6.43	4.90	37.00	6.10	Deep yellow	Rectangular
AH Hat-10	146.00	6.70	2.60	27.00	3.38	Whitish yellow	Rectangular
AH Hat-11	256.00	5.80	2.60	14.00	3.73	Light yellow	Rectangular
AH Hat-14	78.00	6.70	4.53	39.00	3.05	Light yellow	Irregular
AH Hat-15	418.00	6.40	2.46	10.00	4.43	Yellow	Obovate
AH Hat-16	76.00	5.50	3.10	26.00	1.97	Deep yellow	Obovate
Max.	418.00	10.26	5.36	51.00	9.41	-	-
Min.	30.00	4.56	1.76	6.00	1.02	-	-
Mean	156.76	6.78	3.46	29.00	4.07	-	-
SD	98.09	1.48	1.06	0.14	2.36	-	-
SE(±)	27.20	0.41	0.29	0.03	57.98	-	-
CV (%)	62.57	21.89	30.83	48.73	0.65	-	-

***Seed characteristics*:** Table 4 outlines seed traits of 13 jackfruit germplasms, showing wide variation in seed count, size, and weight. Seed number per fruit ranged from 30 (AH Hat-07) to 416 (AH Hat-15), with a high coefficient of variation (CV = 62.56%), indicating strong genetic diversity. Seed length and breadth averaged 2.98 cm and 1.72 cm, respectively, with moderate variability. Single seed weight ranged from 3 g to 7 g (mean: 5 g), with AH Hat-09 producing the heaviest seeds and AH Hat-15 the lightest. Total seed weight per fruit varied from 170 g to 1820 g, with AH Hat-01 and AH Hat-15 showing the highest values, reflecting differences in seed density and fruit size. Seed shapes included irregular, ellipsoid, elongate, reniform, and oblong, with irregular forms being the most common. The results are almost similar to the findings of Mannan et al. (2006).

These variations are important for breeding programs aiming to enhance edible pulp yield and reduce seed bulk. Germplasms with fewer, smaller seeds such as AH Hat-07 and AH Hat-16 may be preferable for fresh consumption and processing. The observed diversity offers valuable selection criteria for improving fruit quality, consumer appeal, and post-harvest efficiency in jackfruit cultivation.

Table 4: Seed characteristics of jackfruit germplasms

Germplasm	Number of seeds/fruit	Seed length (cm)	Seed breadth (cm)	Single seed weight (g)	Weight of seeds/fruit (g)	Seed shape
AH Hat-01	195	3.4	2.13	6.00	1820	Irregular
AH Hat-03	119	2.8	1.46	4.00	840	Irregular
AH Hat-04	146	3.63	2.06	5.00	740	Irregular
AH Hat-05	183	2.8	1.6	4.00	720	Elongate
AH Hat-06	134	3.2	2.16	5.00	610	Irregular
AH Hat-07	30	3.36	1.96	6.00	170	Ellipsoid
AH Hat-08	90	2.83	1.9	5.00	480	Irregular
AH Hat-09	165	3.06	2.13	7.00	1150	Ellipsoid
AH Hat-10	146	2.4	1.6	4.00	620	Irregular
AH Hat-11	256	3.46	1.6	5.00	1280	Irregular
AH Hat-14	78	2.8	1.5	5.00	430	Reniform
AH Hat-15	416	2.83	1.16	3.00	1330	Oblong
AH Hat-16	75	2.2	1.1	6.00	450	Irregular
Max.	416	3.63	2.16	7.00	1820	-
Min.	30	2.2	1.1	3.00	170	-
Mean:	156.38	2.98	1.72	5.00	818.46	-
SD:	97.83	0.41	0.37	1.08	457.21	-
SE(±)	27.13	0.11	0.10	0.29	126.80	
CV (%)	62.56	14.07	21.18	21.60	55.86	-

DIVERSITY ANALYSIS

***Silhouette plot*:** One graphical technique employed to evaluate the quality of clusters is the silhouette plot (Fig.1). The silhouette values reflect the cohesiveness and dispersion of the clusters. The mean of the silhouette values can be utilized to ascertain the number of clusters present in the dataset. This mean falls within the range of [-1, 1]. A higher silhouette value increases our confidence in the accuracy of its label. Therefore, a high mean silhouette value across all points signifies effective clustering.

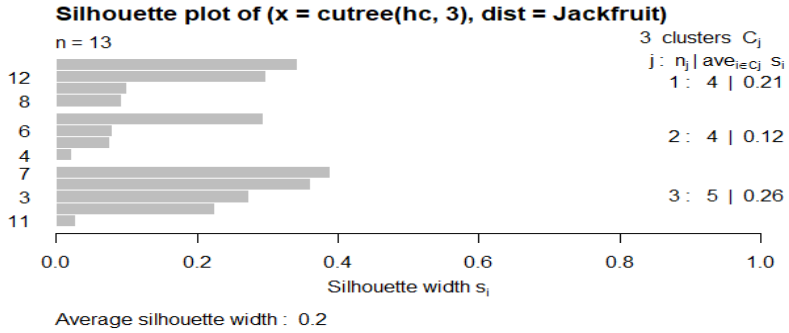

Fig. 1: Graphically shows the quality of the cluster in silhouette plot

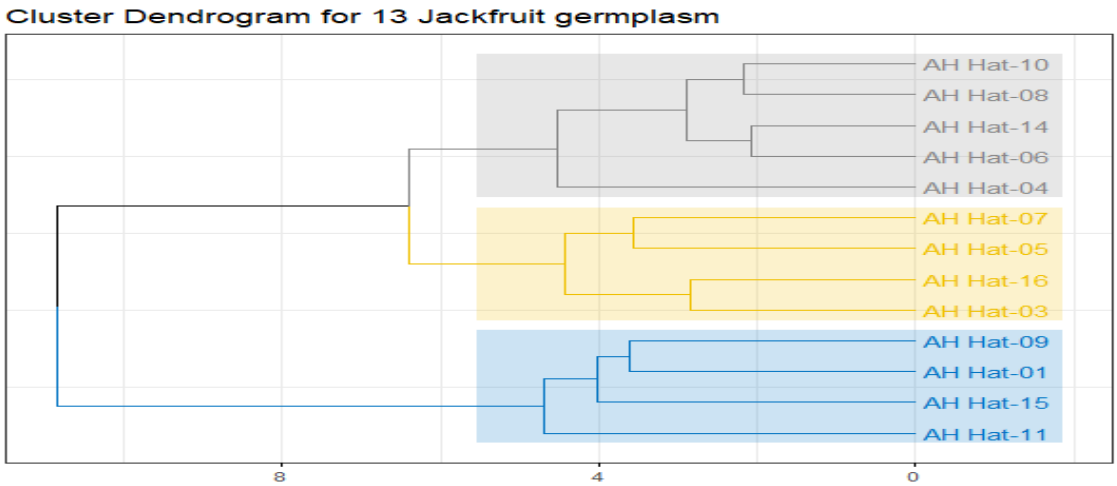

Fig. 2: Dendrogram illustrating thirteen germplasm of jackfruit based on quantitative characters

Among the multivariate techniques, cluster analysis has proven to be particularly useful in selecting genotypes for a breeding program that meets a plant breeder's objectives. This study used Ward's Hierarchical Clustering method. The studied germplasm was separated into three unique groups based on twelve quantitative characters among the thirteen germplasm (Table 5). This type of result was shown in Kavya, *et al.* (2019). The dendrogram was used to represent the clustering pattern (Fig. 2). Cluster III had the most germplasm, consisting of 5 germplasm, followed by clusters I (4) and II (4).

***Cluster mean*:** Table 6 highlights distinct trait profiles among three jackfruit germplasm clusters. Cluster II recorded the highest yield (279.94 kg/tree) with smaller fruits and fewer seeds, suggesting suitability for high-density planting. Cluster I combined high yield (248.72 kg) with the largest fruit size (14.27 kg), longest fruit (51.58 cm), and highest TSS (20.94%), indicating superior quality and market potential. Cluster III had the lowest yield (54.53 kg) but the highest edible portion (56.74%), reflecting pulp efficiency. Seed traits varied: Cluster I had the broadest and heaviest seed load, Cluster III had the longest seeds, and Cluster II had the lowest seed burden, favoring consumer preference. Overall, Cluster I is ideal for fresh consumption and processing, Cluster II for intensive cultivation, and Cluster III for pulp recovery. These findings support targeted selection and breeding strategies. Similar results were shown in Kavya *et al*. (2019), reinforcing the observed trait-based clustering.

Table 6: Different characters cluster mean values of jackfruit germplasm

Trait	I	II	III
Yield per tree (Ypt) (kg)	248.72	279.94	54.53
Individual fruit weight (IFW) (kg)	14.27	5.7125	9.71
Fruit Length (FL) (cm)	51.575	31.2125	37.07
Fruit Breadth (FB) (cm)	26.65	19.05	23.8
Weight of rind (WRI) (kg)	5.87	2.30	3.47

Trait	I	II	III
Weight of rachis (WRA) (g)	0.92	0.25	0.34
TSS (%)	20.94	18.06	17.37
Edible portion (EP) %	52.69	52.52	56.74
Seed length (SL) (cm)	4.9	2.9	6.58
Seed breadth (SD) (cm)	10.03	8.45	9.04
Number of seeds/fruit (NSPF)	280.5	122.5	110.4
Weight of seeds/fruit (g) (WSPF)	1.40	0.55	0.58

Principal component analysis (PCA)

The scree plot shows that the first three principal components explain nearly 80% of the total variance, indicating their suitability for trait-based clustering. This pattern of dominant early components was similarly observed in germplasm studies by Morelos-Flores *et al*. (2022), supporting effective dimensionality reduction through PCA.

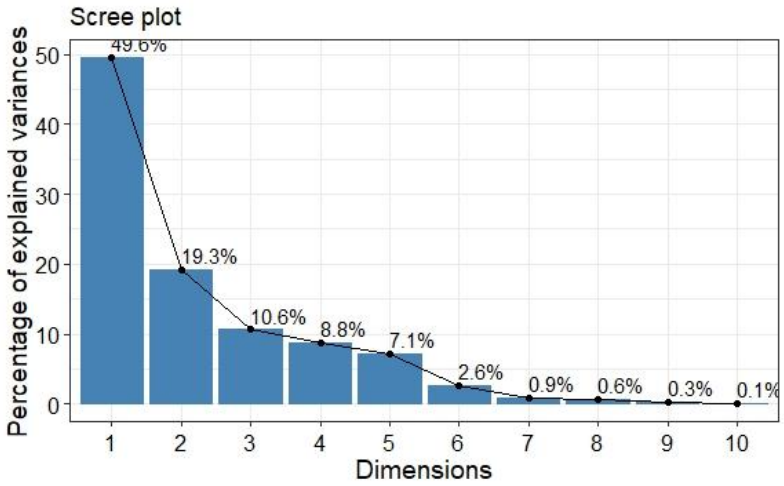

Fig. 3: Scree Plot of Principal Component Analysis (PCA) for Jackfruit Germplasm Traits.

Fig. 4 highlights the dominant contributions of AH-HH-01, AH-HH-15, and AH-HH-16 to Dim-1 and Dim-2. Ten germplasm individuals exceeded the 5% threshold, with AH-HH-10 notably involved, indicating their key roles in trait variation and potential for targeted selection.

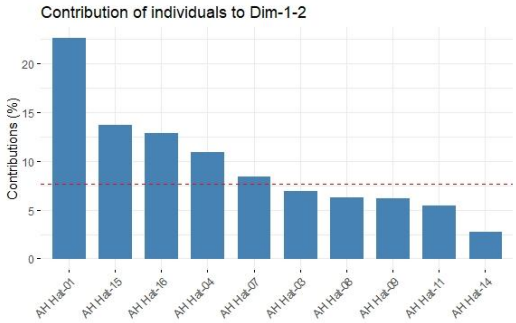

Fig. 4: Contribution of individuals to Principal Components.

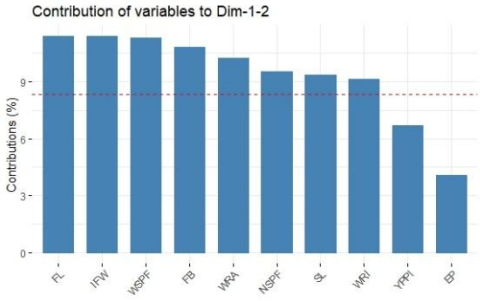

Fig. 5: Contribution of Variables to Principal Components (Dim-1 and Dim-2).

Fig. 5 reveals that fruit length (FL), individual fruit weight (IFW), weight of seed/fruit (WSPF) and fruit breadth (FB) are the most influential traits, each surpassing the reference threshold and contributing significantly to Dim-1 and Dim-2. These variables play a central role in germplasm differentiation and phenotypic variation. The findings align with Chandrashekar *et al*. (2018) and Singh *et al*. (2018), reinforcing their relevance in jackfruit trait selection and improvement strategies.

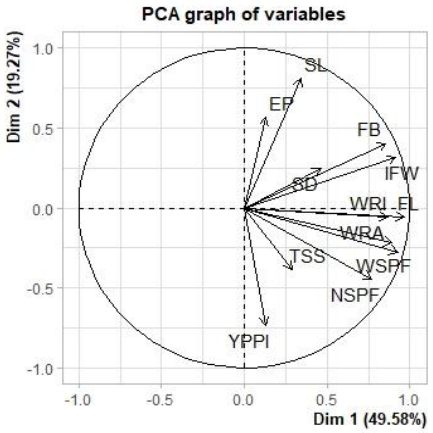

Fig. 6: PCA graph showing Variables status of first two component.

The first principal components explain the variability around 49.58% and it captured the majority of the variability (Fig.6). In this case, the first two components captured the majority of the variability which was 68.85%. Another way of interpreting the plot is that PC1 is positively correlated with the variables Edible portion, Seed breadth, Fruit breadth, and Individual fruit weight; and PC1 is negatively correlated with the weight of seed per fruit and number of seeds per fruit that show obtuse angle. PC2 is highly negatively correlated with Yield per plant and TSS. A similar type of result is reported by Morelos-Flores *et al.* (2022).

Biplot analysis

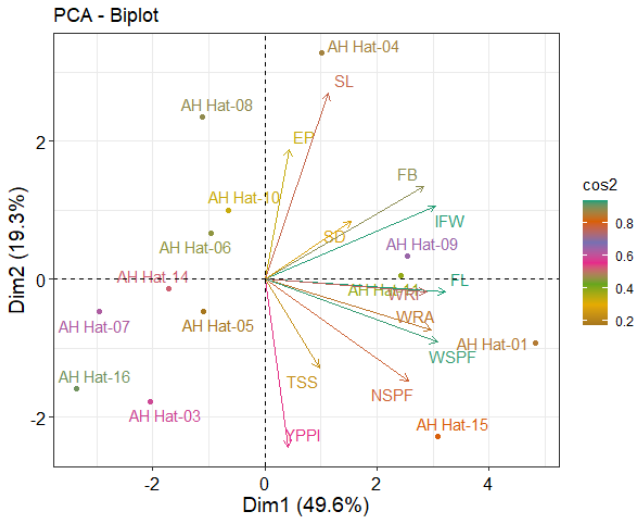

Fig. 7: The plot of Biplot based on the PC1 and PC2 values for Jackfruit germplasm.

Biplot of the first two principal components from a PCA of the jackfruit plant data are shown in Fig. 7. Each germplasm is represented by a code, with colors corresponding to the four sites and showing diverse clustering. The red vectors point in the directions in which variables increased most strongly. PC1 explains about 49.6% and PC2 explains about 19.3% of the variability. Here those variable arrows are closer to each other indicating the high correlation. Biplot indicated that from the origin of trait vector length was high, the variation of the traits across genotypes was high or well-represented here YPPl and SL vector was high across genotypes. In contrast, SD and TSS vector length was small and which was not well represented of genotypes. Between traits and genotypes an acute angle indicates that the genotype was above -average such as AH Hat-09 acute angle among IFW, FB, and also obtuse angle was below the average of the traits from different genotypes. Between vectors of two traits the cosine of the angle smaller than 90^0 indicated a positive correlation was shown in SD, FB, IFW, SL, and EP whereas an angle greater than 90^0 indicates a negative correlation such as YPPl among Sl, EP and so on.

CONCLUSION

The analysis of jackfruit germplasm reveals significant variability in yield, fruit quality, and seed traits, with specific clusters demonstrating distinct advantages for cultivation and processing. Notably, germplasms such as AH Hat-16 and AH Hat-15 exhibit superior characteristics, making them ideal candidates for breeding programs aimed at enhancing fruit quality and yield. These findings underscore the importance of targeted selection in optimizing jackfruit cultivation for both market appeal and agricultural efficiency.

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