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Research article

Genetic analyses of morphological traits, and phenotypic screening of tossa jute germplasm grown under salinity stress

M.M. Mukul

Breeding Division, Bangladesh Jute Research Institute, Manik Mia Avenue, Ministry of Agriculture, Dhaka-1207, Bangladesh

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ABSTRACT

Corchorus olitorius L. jute is a natural fiber crop that is very susceptible to salinity stress. Salinity in soil is a serious threat to tossa jute cultivation in salt-prone regions of Bangladesh. Development of salinity stress tolerant tossa jute variety is a crying need in Bangladesh. Hence, 22 tossa jute genotypes were investigated in an augmented design against $7.86-7.93 \text{ dSm}^{-1}$ salinity stress at coastal regions of Bangladesh during 2017-2019. Salt-tolerant jute genotypes were phenotypically screened out based on survivability, vegetative growth, plant height, base diameter, and fiber yield capacity. Among all genotypes, Acc. 4160 and Acc. 2311 showed good fiber yield (18.0 and 13.8 g plant-1, respectively) with the lowest mortality rate (5.67 and 8.0%, respectively). Acc. 1381, Acc. 2307, Acc. 1334, Acc. 1381 and the pre-released varieties, namely O-9897, BJRI Tossa Pat 5, JRO-524 (Green) were also found good for the aforesaid traits. Among the studied characters, high heritability in a broad sense, genetic advance (%) of mean and significant associations were found which contribute to fiber yield significantly. According to the Clustering and principal component analyses, the relatively salt-tolerant genotypes of cluster II (Acc. 4160, Acc. 2311, Acc. 2307, JRO-524 (Green), Acc. 4327, BJRI Tossa Pat 5, Acc. 1334 & Acc. 1381) could be used as breeding materials and sources of salt-tolerant genes to develop salt-tolerant tossa jute variety.

1. Introduction

Jute is a natural bast fiber crop next to cotton in respect of commercial importance. It is an important industrial bio-fiber crop and its fiber can be used as a good source of biopolymer which is alternative to environment pollutant polythene [1,27]. It is one of the natural fibers producing diploid annual crops having diploid (2n = 2x = 14) chromosome number belonging to the *Tiliaceae* family of *Corchorus* genus. Jute fiber is a bast fiber which is collected from the secondary phloem tissues (bark) of the stem [42]. The two cultivated species namely white jute (*Corchorus capsularis* L.; 338 Mb sized genome) and tossa jute (*C. olitorius* L; 445 Mb sized genome) of the *Tiliaceae* family yield commercial bast fiber. The major amount of world's supplies of jute is grown in India, Bangladesh, China, Pakistan, Thailand, Srilanka, Nepal, Brazil etc. [15,31].

Corchorus capsularis was originated from Indian subcontinent or Indo-Barma or South East Asia and *C. olitorius* from Tropical Africa [21]. Hence, *Capsularis* jute is adjusted with the environment of Bangladesh and grows well; but, *Olitorius* jute is very susceptible to diseases and pests due to less adjustable environment [27]. Chand [8] has opined that, jute consists of several cells and chemical compositions i.e. hemicellulose (12%), cellulose (64.4%), pectin (0.2%), lignin (11.8%), water soluble (1.1%), water (10%) and wax (0.5%). Jute is a good source of biopolymer to be used for industrial purposes [27]. The ecofriendly 'Sonali Bag' made from jute fiber in Bangladesh is an important alternative to environment polluting polythene, and other necessary products like tea bag, face mask,

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E-mail address: mukulbjribreeding@gmail.com.

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personal protection equipment (PPE), sanitary napkin etc. can be produced in large scale for commerce. Bangladesh is the first largest country for jute and jute goods export, which earns a significant amount of foreign exchange annually through exporting jute products [10]. Faridpur, Tangail, Jashore, Kushtia, Mymensingh, Dhaka, Sirajganj, Bogura, Rangpur and Jamalpur are the major jute growing districts in Bangladesh [14].

The dissolved salts present in water as well as in soil is known as salinity. It is an abiotic stress which is threat to jute cultivation in southern or coastal regions of Bangladesh [50]. Saline water contains the predominant ions Na⁺ (Sodium) and Cl⁻¹ (chloride), and the concentrations of magnesium, calcium, and sulfate ions are also substantial. The growth, development, and productivity of agricultural crops are restricted due to salinity stresses [3]. The more soil salinity is the most alarming among the biotic and abiotic stresses. Approximately, 45 million ha (\approx 20%) among the 230 million ha of global irrigated land is affected by salinity [24,25] and this situation is worsened by the global rise in sea level. According to Haque [13], an average of 1.02 million ha of farmland is affected by salinity in Bangladesh. According to the extent of salt and salinity classes like salt free (0-2 dS/m), very slightly saline (2-4 dS/m), slightly saline (4-8 dS/m), moderately saline (8-15 dS/m) and strongly saline (>15 dS/m), these areas were characterized as these areas were characterized as moderately to strongly saline (>15 dS/m) which is increasing rapidly in Bangladesh due to global climate change [39,46]. The southern districts (Satkhira, Khulna, Patuakhali, Chittagong, Barisal, Noakhali) of Bangladesh are highly salt affected [46,19]. More salt stress occurs in March-April, the peak on 15 January to February, and minimum salinity occurs at the onset of monsoon rain [13].

The demand of jute crop is gradually increased for its broad-spectrum use and eco-friendly nature [14,35]. *Olitorius* jute is more cultivated than *Capsularis* jute in Bangladesh due to good quality fiber. However, *C. olitorius* is more susceptible to biotic and abiotic stresses than *C. capsularis* [22].

Agriculture is a major sector of Bangladesh's economy and the coastal area of Bangladesh is suitable for growing of salt-tolerant rice. More than 30% of the cultivable land in Bangladesh is in the coastal area. Out of 2.86 million hectares of coastal and offshore lands about 1.056 million ha of arable lands are affected by varying degrees of salinity. Salinity tolerant, quality fiber producing and high yielding tossa jute variety development is very important in Bangladesh [29]. Tossa jute can't grow in a large saline affected area in Bangladesh. Salt tolerance improvement in tossa jute contribute to extend the jute cultivation in saline areas, and to reduce competition with food crops for arable land [13]. The salt stress highly affects the seed germination as well as growth and development of jute plants and showed adverse effects on its fiber yield. The ion imbalance and hyperosmotic stresses in plants



Figure 1. Schematic diagram of salt tolerance mechanism in jute plant.

resulting from high salinity affect the content and enzyme activities as well as alter plant metabolism.

High soil salinity decreases growth, photosynthetic activity, productivity, and causes nutrient imbalance in jute plants, resulting in osmotic and ion toxicity effects on cells, oxidative stress, and damages to cellular components, and metabolic disfunction [48]. Salts gradually accumulated in soil due to scanty rainfall, poor irrigation system, salt ingression, water contamination, and other environmental factors and causes salinity problem in the world [23]. Salt can be simply defined as an organic mineral that can dissolve in water. In this way, water-soluble salts are accumulated in soil to an extent that affects crop productivity, microbial community, and agricultural productions. In reality, a lot of salts that affect both surface water and groundwater often are a combination of sodium (NaCl), calcium (CaSO₄.2H₂O; CaCl₂.2H₂O), potassium (KCl), aluminum [Al₂ (SO₄)₃·18H₂O], Iron [(Fe₂ (SO₄)₃], magnesium (MgSO₄, chlorides (NaCl, CaCl₂.2H₂O; KCl), nitrates, sulfates, bicarbonates and carbonates. The saline water drained in soil and affects the movement of other nutrients. Both the saline water and soil reduce the cation exchange capacity (CEC), increase electrical conductivity (EC) of soil and ultimately restrict the availability of essential nutrients of the plants. Soil-water balance is affected by the accumulated excess salts and thus hinder plant growth and development.

The yield of crops, adaptability & nutrient availability in soil, and activity of soil microorganisms depend on the electric conductivity (EC) of growing media, and which influence on the soil processes including greenhouse gases (NO₂, CH₄, and CO₂) emission. Salinity affects jute plants at any age [40], and greatly hampers the plant growth and development [11,51]. Salt stress affects both water relation (osmotic stress) and ionic relations (ionic stress) leading to hampering the plant physiology [6]. At the onset of salt stress, it causes water stress in the plant body and ultimately inhibits the leaf expansion. In the later phase of exposure to salinity, the mounting of ions (Na⁺, Ca⁺⁺, Mg⁺⁺, Cl⁻) within the plant body is inflicted, whereby the uptake of nutrient(s) is hindered. The ionic stress results from salinity localized in the older leaves of the plant, causing premature senescence leading to reduced yield or death of the plant [43].

Some specific gene regulation pathways are coordinately linked for imparting the complex phenomena (salt-tolerance mechanism) in plant body.

There is always an expedition for auspicious stress-responsive genes that can temper plant physiology according to the salt stress, although a number of salt responsive genes have been reported from the salt loving plants [23]. Some transporters (*SOS1, SOD2, AVP1, NHX, SOS, AVP, HKT, Nha*), transcription factors (*NAC, ERF, DREB, ZIP, MYB, HARDY, CBF, ABP*), signaling molecules (*MKK, MPK, MAPK, CDPK, CIPK, SAPK, Galpha, Rab*), osmolytes (*CodA, Cox, beta, TPS, PDH, Prot, BADH, imt1, ostA, ostB*), helicases (*PDH*45,*MCM*6, *MH*1), proteins (*HVA1*), chaperons (*LEA, RcHSP, COR*), anti-oxidative enzymes (*APX, CAT, SOD, Gly*1,2) play an important role to activate the genes for salt stress tolerance in plant body (Fig. 1).

The responsive genes in plant body are triggered to activate the stress tolerance mechanisms through signaling of multiple proteins, up- or down-regulation of several genes, and, finally, the distinctive or collective effects of that genes [53]. Plants vary in their response to soil salinity. Salt tolerant plants are better able to adjust internally to the osmotic effects of high salt concentrations than salt-sensitive plants. Salt- tolerant plants are more able to absorb water from saline soils. Salt-sensitive plants have a limited ability to



Figure 2. (a) Jute germplasm grown, and (b) T-labeling of genotypes for morphological characterization at Binerpota, Satkhira. (c) Vegetative growth of Acc. 4160, Acc. 2311 and other salt-tolerant jute genotypes, and (d) Salinity recording using EC Meter at Kolapara, Patuakhali.

adjust and are injured at relatively low salt concentrations.

The wild species of jute are generally used as sources of genes for qualitative traits i.e. biotic (diseases, insects) & abiotic (salinity, drought, photo insensitivity, waterlog) stress tolerance, fiber quality (low lignin, high cellulose-hemicellulose contents), and but low yield potentials [7,30]. The jute genotypes identified for the desired stress tolerance would be used in hybridization program to accumulate the gene(s) for respective stress in another cultivars or genotypes of good yield but susceptible nature [20,5].

More arable land is required for food crop cultivation due to over population in Bangladesh. It is possible to increase the fiber production in Bangladesh by cultivating salt-tolerant tossa jute varieties in the saline areas. Low genetic variability among jute species is the main barrier for further improvement in jute varieties, hence serious concern has raised among the jute breeders in Bangladesh [28]. The analyses of population structure as well as genetic diversity are of great importance for different breeding approaches of jute crops [49]. Hence, 22 tossa jute genotypes were investigated and phenotypically screened to evaluate their responses to salt stress based on their survivability, fiber yield and yield attributing morphological characters in the salinized areas of Bangladesh.

2. Materials and methods

2.1. Experimental sites

The first set of the experiment was conducted in the saline-affected coastal area named Binerpota (Fig. 2. a-b) of Satkhira ($22^{\circ}44'59.7''N$, $89^{\circ}06'20.9''E$) in 2017-2018 and the second set was conducted in Pakhimara (Fig. 2. c-d) of Patuakhali ($21^{\circ}56'59.8''N$ 90°10'37.3''E) in 2018-2019 having an average 7.93 dSm⁻¹ and 7.86 dSm⁻¹ salinity levels, respectively to observe the effects of salinity as well as soil environment on tossa jute crops.

2.2. Plant materials

Table 1

Total 22 tossa jute genotypes were collected (Table 1) and investigated for salinity tolerance at field conditions. Plants were grown at farmer's fields where salinity level was found higher at sowing time and lower at growing to harvesting time of jute crops. No salinity tolerant tossa jute variety is available in Bangladesh till now. For this reason, no control variety or environment was used in this study. The tossa jute genotypes showing salt tolerance will be used as parent materials to develop high yielding salt tolerant variety.

2.3. Preparation of seed materials

Seeds were obtained from the Gene Bank, and the Breeding Division of BJRI, and exposed to sun dry conditions for 10 hours to break the dormancy. False seeds and impurities were discarded from the seeds, and then treated with Carbendazim@ 2g per Kg seeds to remove seed borne fungus. Seeds were packaged with a proper tag label, and ready for sowing.

2.4. Experimental design, seeding, and harvesting of jute

Fields were prepared by 3-4 times ploughing, harrowing, leveling, and fertilization with Urea (100 kg), TSP (50 kg), MoP (50 kg) per hectare area. During land preparation, organic fertilizers were supplied before 2-3 weeks of seed sowing. Urea was applied in two splits; half at land preparation and rest at 45 days after seeding. The experiments were laid down in an Augmented block design

Tuble I			
List of plant materials	with type and	source of	collection.

Genotype code	Genotype	Type of material; and Source of collection	Genotype code	Genotype	Type of material; and Source of collection
G1	Acc. 1509	Accession or germplasm; Gene Bank,	G ₁₂	Acc. 1336	Accession or germplasm; Gene Bank, BJRI
G ₂	Acc. 1381	BJRI	G ₁₃	Acc. 1345	
G ₃	Acc. 2307		G ₁₄	Acc. 1361	
G ₄	Acc. 2311		G ₁₅	Acc. 1379	
G ₅	Acc. 4160		G ₁₆	O-0419-3-1 (Pedigree)	Advanced breeding lines; Breeding
G ₆	Acc. 4327		G ₁₇	O-0419-3-2 (Pedigree)	Division, BJRI
G ₇	Acc. 1232		G ₁₈	O-043-7-9 (Pedigree)	
G ₈	Acc. 1298		G ₁₉	BJRI Tossa pat 5 (O- 795)	Pre-released variety; Breeding Division, BJRI
G9	Acc. 1306		G ₂₀	JRO-524 (Green)	
G ₁₀	Acc. 1318		G ₂₁	JRO-524 (Red)	Segregate (S); Breeding Division, BJRI
G ₁₁	Acc. 1334		G ₂₂	O-9897	Pre-released variety; Breeding Division, BJRI

keeping 3 lines of 3.0 m length and line to line 30 cm, plant to plant 7-8 cm distance for each genotype at both locations. The tested genotypes were not grown in replicates due to the large number of populations used for the preliminary selection against salt stresses. Plants were grown carefully following the required agronomic practices. Plants were observed carefully from seedling to maturity stage.

2.5. Field observation and data collection

The experiment was conducted in natural conditions. Electrical conductivity (EC) of soil indicates the extent of salinity (dS/m) in soil. Higher the EC value indicates the higher salinity of soil and lower crop growth. Salinity levels were recorded at 15 days intervals from the experimental plots using an EC meter (HI993310, Italy) (Fig. 2.d). Seed germination rate, plant mortality rate, number of plants per unit area were recorded; premature flowered plants were removed in time. At 120 days old, jute plants were matured and harvested for data collection. Plant population, plant height (m), girth or base diameter (mm), green weight (g plant⁻¹), dry fiber weight (g plant⁻¹), dry stick weight (g plant⁻¹), were recorded from randomly selected 20 plants after harvesting [34]. Plant survivability rate of each genotype was also recorded.

2.6. Statistical analyses

The collected data were assembled using Microsoft Excel program. The ANOVA (analysis of variability), LSD (least significant differences) among the jute genotypes, correlations among the morphological traits, mean square values of genotype (MS_G) and mean square values of error (MS_E) were calculated using the analysis software OriginPro 9.1 [36] and MS Excel program, and finally results were tabulated & presented in MS office program. LSD tests were checked at both p < 0.05 and p < 0.01 levels. Pearson correlation coefficients among all characters in all possible combinations were estimated and partitioned into genotypic and phenotypic associations. Genotypic and phenotypic variances of the trait(s) were calculated using the formulae (i & ii); and the covariance components (genotypic, phenotypic and environmental) between the two traits were estimated using the formulae (iii-vii).

GV or
$$\sigma_G^2 = \frac{MS_G - MS_E}{r}$$
 (i)

PV or
$$\sigma_{\rm P}^2 = \sigma_{\rm G}^2 + \sigma_{\rm E}^2$$
 (ii)

$$\sigma_{\rm E}^2 = \frac{\rm MS_{\rm E}}{\rm r}$$
(iii)

GCV (%) =
$$\frac{\sqrt{\sigma_{\rm G}^2}}{\overline{\rm X}} \times 100$$
 (iv)

$$PCV (\%) = \frac{\sqrt{\sigma_{P}^{2}}}{\overline{X}} \times 100$$
 (v)

ECV (%) =
$$\frac{\sqrt{\sigma_{\rm E}^2}}{\overline{\rm X}} \times 100$$
 (vi)

$$\overline{X}$$
 or $\overline{Y} = \frac{\Sigma x \text{ or } y \text{ variable (mean of replicates)}}{\text{Total number of observation (N)}}$ (vii)

Where, GV = Genotypic variance, PV = Phenotypic variance, $\sigma_E^2 = Error variance$, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, ECV = Environmental coefficient of variation, $MS_G = Genotypic mean square value$, $MS_E = Error mean square value$, r = No. of replication, $\sigma_E^2 = Environmental variance$ (Error mean square from the ANOVA), \overline{X} or $\overline{Y} = Mean$ value of the trait or variable X or Y being evaluated.

Broad sense heritability in $h_{bs}^{2}(\%)$: Broad sense heritability were estimated for all traits using the formula (viii) as

$$h_{bs}^2(\%) = \frac{\sqrt{\sigma_G^2}}{\sqrt{\sigma_P^2}} \times 100$$
 (viii)

Genetic advance (GA), and genetic advance in percentage of mean (GAM): The expected GA for different characters under selection was calculated using the formula (ix) as

$$GA = \frac{\sqrt{\sigma_{G}^{2}}}{\sqrt{\sigma_{P}^{2}}} \times K \times \sigma_{P}$$
(ix)

Where: K = Selection differential (K= 2.06 at 5% intensity of selection); σ_P = Phenotypic standard deviation (STDEV.S) of the character.

Genetic advance in percentage of mean (GAM) was calculated as:

(

Where, \overline{x} = grand mean of the character.

 $GAM~(\%) = \frac{GA}{\overline{x}} \times 100$

Correlations at genotypic ($\mathbf{r}_{G_{XY}}$) **and phenotypic** ($\mathbf{r}_{P_{XY}}$) **levels:** The variance and covariance among the traits were estimated considering the variables X and Y using the formulae xi and xii; and Pearson correlations among all traits were estimated using the formula xiii; and the genotypic correlations ($\mathbf{r}_{G_{XY}}$) and phenotypic correlations ($\mathbf{r}_{P_{XY}}$) for all characters were obtained using these covariance components and the formulae (xiv-xv) as follows:

$$\sigma_x^2 = \frac{\sum_{i=1}^{N} (x_i - \mu)^2}{N}$$
(xi)

$$(\text{Cov}_{XY}) = \sum_{i=1,2,\dots,n}^{N} [(X_i - \overline{X}).(Y_i - \overline{Y})]/N - 1)$$
(xii)

$$(\mathbf{r}_{\mathrm{XY}}) = \frac{\mathbf{Cov}_{\mathrm{XY}}}{\sqrt{\sigma_{\mathrm{X}}^2 \times \sigma_{\mathrm{Y}}^2}}$$
(xiii)

$$(\mathbf{r}_{G_{XY}}) = \frac{\text{Cov}_{G}XY}{\sqrt{\sigma_{G}^{2}X \times \sigma_{G}^{2}Y}}$$
(xiv)

$$\mathbf{r}_{\mathbf{P}_{XY}}) = \frac{\mathbf{Cov_PXY}}{\sqrt{\sigma_P^2 \mathbf{X} \times \sigma_P^2 \mathbf{Y}}}$$
(xv)

Where, σ_x^2 = Variance (population) of variable X; Cov_{XY} = Covariance between variables X and Y; r_{XY} = Correlation between variables X and Y; $r_{G_{XY}}$ = Genotypic correlation between variables X and Y;

 $r_{P_{XY}}$ = Phenotypic correlation between variables X and Y; N = Number of observations in the variables X or Y.

Genetic divergence: Jute genotypes were grouped using Euclidean distances and Ward's method.

The transformed uncorrelated means of characters used to calculate the Mahalanobis' distance (D) values. The mean deviation, $Y_i^1 - Y_i^2$ with $i = 1, 2 \dots p$ was estimated and the D^2 value was calculated as the sum of squares of these deviations, that is, $\Sigma (Y_i^1 - Y_i^2)^2$. The D^2 values were estimated for all possible pairs of combinations between genotypes.

Clustering: According to Singh and Choudhary [45], the D^2 values of genotypes were arranged in order of relative distances from each other which was used for clustering of genotypes. Average intra & inter-cluster distances were calculated by the formulae (xvi, xvii) as follows.

Average intra-cluster,
$$D^2 = \frac{\sum D^2}{n}$$
 (xvi)
Average inter-cluster, $D^2 = \frac{\sum D^2 ij}{ni \times nj}$ (xvii)

Principle Component Analysis (PCA): Large datasets are difficult to interpret in the field of research activities. PCA reduces the difficulties of such datasets, makes it comprehensive along with minimizing information loss. It does so by creating new uncorrelated variables that successively maximize variance.

Finding such new variables and principal components, defined by the dataset at hand, and reduces to solving an eigenvalue/ eigenvector problem by PCA technique in the study [17]. D^2 statistics, hierarchical cluster analysis and PCA were carried out by using the statistical analysis software [36].

 Table 2

 Analysis of variance (Mean square values) for phenotypic characters of 22 jute genotypes.

Sources of variation	D.F.	PH (m)	BD (mm)	GWL (g $plant^{-1}$)	GWL_1 (g plant $^{-1}$)	FY (g $plant^{-1}$)	STY (g $plant^{-1}$)	MR (%)
Replication	2	0.0062	0.4314	43.44	259.70	0.3199	27.111	2.36
Genotype	21	0.15**	9.93**	6213.51**	5795.25**	27.29**	230.62**	1475.72**
Error	42	0.0015	0.3134	4.13	11.32	0.2280	2.066	8.00
CV (%)		1.39	3.89	1.05	1.92	4.79	4.52	7.26
'P' value		0.000	0.000	0.000	0.000	0.000	0.000	0.000
'F' value		96.91	31.67	1505.03	512.13	119.72	111.62	184.50

Note: D.F. = Degree of freedom; PH = Plant height; BD = Base diameter; GWL = Green weight with leaves; GWL_1 = Green weight without leaves; FY = Dry fiber yield; STY = Dry stick yield; MR = Mortality rate (%); ** Denotes highly significant at 0.01 probability level.

3. Results

3.1. Analysis of variances

ANOVA revealed significance differences (p < 0.01) among the jute genotypes for morphological traits like, plant height, stem diameter attributing the jute fiber yield (Table 2). The coefficient of variation (%) were found prominent for these important characters.

3.2. Phenotypic performance

The lowest error differences were found among all tossa jute genotypes for the studied morphological traits (Table 3). Jute genotypes were categorized based on LSD tests where Acc. 2311 was a good performer for plant height, fresh weight with leaves, and dry stick yield; Acc. 4160 for fresh weight without leaves and dry fiber yield and plant survivability rate; and Acc. 1298 was good for stem diameter. For the Anderson Darling Normality Tests among the genotypes, the 'A' square and 'P' values were described for plant height (0.69, 0.063), base diameter (0.54, 0.14), dry fiber yield (0.57, 0.121) and mortality rate (1.23; p < 0.05) of selected genotypes [Suppl. Figure S1 (i-iv)].

3.3. Distribution of variabilities for the morphological characters

In the box and whisker plots, the studied seven morphological characters were distributed for 154 cases where fresh weight of plants without leaves and fresh weight of plants with leaves showed prominent distribution of variabilities followed by plant mortality rate, dry stick yield, dry fiber yield, girth of stem and height of jute plants (Suppl. Figure S2).

3.4. Analysis of EWMA control charts

Analysis of Exponentially Weighted Moving Average (EWMA) for the studied morphological characters revealed subgroup case number= 22, subgroup size = 6, lambda (λ) weight = 0.15, sigma limit = 70.691, and E (R bar) = 191.15, upper control limit (UCL) = 89.50, lower control limit (LCL) = 43.869, process mean (expected value of the population of measurements) of central line or value of μ was 66.685 (Suppl. Figure S3).

 Table 3

 Statistical analyses for means, range, S.E., Std. Dev. and LSD of morphological traits of 22 genotypes.

Genotype code	Genotypes	PH (m)	BD (mm)	GWL (g plant ⁻¹)	GWL ₁ (g plant ⁻¹)	FY (g plant ^{-1})	STY (g plant $^{-1}$)	MR (%)
G1	Acc. 1509	2.9 ^{d-g}	15.57 ^{cd}	215.4 ^{gh}	199.0 ^d	10.0 ^f	34.8 ^{ef}	35.0 ^d
G ₂	Acc. 1381	2.9 ^d	16.4 abc	218.6 ^g	214.6 ^c	12.0 ^d	40.0 ^c	16.0 ^{cd}
G ₃	Acc. 2307	3.1 ^b	15.4 ^{de}	214.4 ^h	196.6 ^d	11.0 ^e	39.4 ^c	19.7 ^{cd}
G ₄	Acc. 2311	3.2 ^a	16.6 ^{ab}	262.8 ^a	245.8 ^a	13.8 ^b	51.4 ^a	8.0 ^{ab}
G ₅	Acc. 4160	3.0 ^c	15.8 bcd	261.8 ^a	246.4 ^a	18.0 ^a	40.4 ^c	5.7 ^a
G ₆	Acc. 4327	3.0 ^c	15.4 ^{de}	224.2 ^f	213.2 ^c	12.2 ^d	38.2 ^{cd}	13.7 ^{cd}
G ₇	Acc. 1232	2.7 ^j	13.0 ^g	150.0 ⁿ	133.8 ^{jk}	6.0 ¹	25.8 ^j	68.7 ¹
G ₈	Acc. 1298	2.9 efg	17.0 ^a	228.6 ^e	211.4 ^c	9.2 ^{gh}	37.0 ^{de}	42.0 ^e
G ₉	Acc. 1306	2.8 ^{hi}	15.4 ^{de}	166.8 ^k	159.6 ^g	8.8 ^{gh}	31.0 ^{gh}	48.7 ^f
G ₁₀	Acc. 1318	2.8 ⁱ	12.8 ^{gh}	151.0 ⁿ	139.0 ^j	7.8 ⁱ	24.0 ^{jk}	57.3 ^{hi}
G ₁₁	Acc. 1334	2.8 ^{gh}	14.6 ^{ef}	213.6 ^h	198.4 ^d	13.2 ^{bc}	22.8 ^k	12.7 ^{bc}
G ₁₂	Acc. 1336	2.9 ^{de}	15.0 def	234.6 ^d	226.4 ^{bb}	7.6 ^{ij}	33.2 ^{fg}	55.3 ^g
G ₁₃	Acc. 1345	2.5 ^k	12.6 ^{ghi}	135.8 °	124.0 ¹	6.6 ^{kl}	22.8 ^k	60.7 ^{ij}
G14	Acc. 1361	2.9 ^{de}	14.4 ^f	193.2 ⁱ	176.4 ^e	8.8 ^{gh}	34.0 ^f	54.3 ^g
G ₁₅	Acc. 1379	2.9 ^{e-h}	11.8 ⁱ	152.0 ⁿ	130.0 ^k	8.6 ^h	25.2 ^j	57.0 ^h
G ₁₆	O-0419-3-1	2.3 ¹	10.8 ^j	119.0 ^q	100.0 ⁿ	7.0 ^{jk}	19.4 ¹	61.3 ^{jk}
G ₁₇	O-0419-3-2	2.6 ^k	12.8 ^{gh}	157.4 ^m	134.4 ^{jk}	7.2 ^{ijk}	22.6 ^k	60.0 ^{ij}
G ₁₈	O-043-7-9	2.8 ^{fgh}	12.0 ^{hi}	162.0 ¹	147.0 ⁱ	9.4 ^{fg}	29.0 ^{hi}	35.3 ^d
G ₁₉	BJRI Tossa pat 5	2.9 ^{def}	15.8 bcd	255.2 ^b	170.0 ^f	13.0 ^c	44.2 ^b	12.7 ^{bc}
G ₂₀	JRO-524	3.0 ^c	16.9 ^a	247.0 ^c	215.0 ^c	13.4 ^{bc}	38.2 ^{cd}	11.3^{bc}
	(Green)							
G ₂₁	JRO-524 (Red)	2.4 ¹	13.0 ^g	132.0 ^p	112.8 ^m	6.8 ^k	18.0 ¹	64.3 ^{kl}
G ₂₂	O-9897	2.9 ^{fgh}	13.3 ^g	172.1 ^j	153.5 ^h	9.0 ^{gh}	28.2 ⁱ	57.3 ^{ij}
Maximum		3.22	17.0	262.8	246.4	18.0	51.4	68.67
Range (MaxMi	nimum)	0.88	6.2	143.8	146.4	12.0	33.4	63.00
Mean \pm S.E.		$2.83\pm$	$14.38\pm$	$193.98{\pm}~1.66$	$174.88{\pm}\ 2.75$	$9.97{\pm}~0.39$	$31.80{\pm}~1.17$	$38.96\pm$
		0.03	0.46					2.31
St. Dev.		0.22	1.82	45.51	43.95	3.02	8.77	22.18
LSD (0.05)		0.07**	0.92**	3.35**	5.54**	0.79**	2.37**	4.66**

Note: S.E. = Standard Error, LSD = Least Significant Difference, ** Denotes significant at 0.01 probability level.

3.5. Correlation matrices

The correlations at genetic and phenotypic levels are important selection criteria for parental selection in a plant breeding approach; it is a crucial step for a plant breeder to select a parent with desired characters. Highly significant correlations were found among all morphological traits (p < 0.01). The plant mortality rate showed negative relations with other characters indicating growth and development as well as an increase in fiber yield with the decrease in plant mortality rate (Suppl. Figure S4). The correlation coefficients among all traits were partitioned into genotypic and phenotypic relations based on the error variance and covariance matrices of morphological traits, where all traits recorded highly significant associations. At both genotypic and phenotypic levels, significant relations were found among all morphological characters where the plant mortality rate gave negative relations with other characters. The basal diameter of the plants showed highly strong associations with the fresh weight of plants with green leaves and plants without green leaves; similar results were found between fresh weight of plants with green leaves and stick yield; plant mortality rate recorded highly strong negative relations with fiber yield (Table 4).

3.6. Genetic parameters

Genotypic variance (GV), phenotypic variance (PV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and environmental coefficient of variation (ECV), heritability in broad sense (h_{a}^{2}) , Genetic advance (GA) of plants, Genetic advance in percentage of mean (GAM) were estimated for seven agronomical traits (Table 5). The highest value for GV, PV, Hb, GA and the lowest ECV were recorded for the fresh weight of plants weight with green leaves. Plant height showed the lowest value for GV, PV, GCV, PCV, GA, GAM. Plant mortality rate recorded higher percentage of GCV, PCV, ECV, and GAM). The seven phenotypic characters gave higher values for the broad sense heritability (>98%) (Table 5).

3.7. Euclidean clustering

All genotypes of tossa jute were clustered into four major clusters at the genetic distance of 25.0 by the Euclidean distance and Ward linkage method, which indicate the notable genetic divergence present among all genotypes for major important phenotypic traits (Table 6, Fig. 3). In the Dendrogram, each cluster contains the jute genotypes having almost similar divergence for morphological characters.

3.8. Cluster centroids

The experimental jute genotypes of cluster II showed higher results followed by cluster I for these traits except plant height (Table 7). The good genotypes having better performance of desired traits like higher plant height, girth of stem, maximum dry fiber yield, and lower plant mortality rate were found in Cluster II followed by Clusters I, III and IV. The genotypes of Clusters II, IV showed higher records for plant height, base diameter and fiber yield than their cluster grand centroids (2.83 m, 14.38 mm, 9.97 g plant⁻¹ resp.); and genotypes of Cluster II and I showed similar performance for plant survivability (%) than their Grand centroids (61.05%). The genotypes occupied in Cluster II significantly and then Cluster I and III would be considered as diverged as breeding parents to develop the new high yielding tossa jute varieties.

3.9. Intra and inter-cluster distance (D^2)

Among four clusters, Cluster II and III showed maximum inter-cluster distance (51.09); cluster I and II showed medium distance (28.09) and cluster III and IV showed minimum distance (7.01). The intra-cluster distances for cluster I, II, III and IV were recorded 5.55, 3.64, 2.99 and 1.72, respectively (Fig. 4). Maximum inter-cluster distances were found than intra-cluster distances which

Analysis of genotypic correlation (r_g) and phenotypic correlation (r_p) coefficients among all morphological traits.										
Characters	Correlations	РН	BD	GWL	GWL1	FY	STY			
BD	r _g	0.742**								
	r _p	0.725								
GWL	r _g	0.837**	0.901**							
	rp	0.831**	0.886**							
GWL ₁	r _g	0.850**	0.889**	0.939**						
	rp	0.844**	0.874**	0.938**						
FY	r _g	0.715**	0.664**	0.825**	0.773**					
	rp	0.707**	0.678**	0.822**	0.769**					
STY	r _o	0.870**	0.847**	0.889**	0.829**	0.719				
	r _p	0.860**	0.829**	0.885**	0.824**	0.712				
MR	r _o	-0.745^{**}	-0.736^{**}	-0.838^{**}	-0.781^{**}	-0.943^{**}	-0.769^{**}			
	rp	-0.738^{**}	-0.722^{**}	-0.836^{**}	-0.781^{**}	-0.936**	-0.763^{**}			

Table 4

Denotes statistically significant at 0.01 probability level.

Table 5

Estimation of variance, covariance, heritability and genetic advance for morphological characters.

Characters	GV	PV	GCV (%)	PCV (%)	ECV (%)	Hb (%)	GA	GAM (%)
РН	0.05	0.05	7.82	7.87	1.58	99.33	0.46	16.10
BD	3.21	3.31	12.45	12.65	3.89	98.41	3.69	25.65
GWL	2069.79	2071.17	23.45	23.46	1.05	99.97	93.72	48.31
GWL ₁	1927.98	1931.75	25.11	25.13	1.92	99.90	90.45	51.72
FY	9.02	9.10	30.12	30.25	4.79	99.58	6.19	62.06
STY	76.18	76.87	27.45	27.57	4.52	99.55	17.98	56.54
MR	489.24	491.91	56.79	56.94	7.26	99.73	45.56	116.98

Note: GV-Genotypic variance, PV-Phenotypic variance, GCV-Genotypic co-variance, PCV-Phenotypic co-variance, ECV-Environmental co-variance, Hb-Heritability in broad sense, GA-Genetic advance, GAM-Genetic advance in percentage of mean.

Table 6

Euclidean Clustering of 22 tossa jute genotypes for plant height, base diameter, fiber yield, and survivability rate.

Cluster	Number of observations	Genotypes or observations	Distance within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
I	4 (18.18%)	Acc. 1509, O-043-7-9, Acc. 1298, Acc. 1306	139.87	5.55	8.44
II	8 (36.36%)	Acc. 1381, Acc. 2307, Acc. 4327, Acc. 1334, BJRI	167.99	3.64	8.24
		tossa pat 5, JRO-524 (Green), Acc. 2311, Acc. 4160			
III	5 (22.73%)	Acc. 1232, JRO-524 (Red), Acc. 1345, O-0419-3-2, O-	55.52	2.99	5.74
		0419-3-1			
IV	5 (22.73%)	Acc. 1318, O-9897, Acc. 1379, Acc. 1336, Acc. 1361	15.46	1.72	2.20



Figure 3. Cluster Dendrogram showing similarity matrices among 22 tossa jute genotypes for plant height, base diameter, fiber yield, plant survivability rate (%).

Table 7

Cluster	centroids for	iute plai	nt height.	base diameter.	fiber vi	eld, and	plant survivability (%).
					/-		

Cluster	Plant height (m)	Plant base diameter (mm)	Fibre yield (g $plant^{-1}$)	Plant survivability (%)	Score	Rank
Ι	2.84 (3)	14.99 (2)	9.35 (2)	59.75 (2)	9	2
II	3.01 (1)	15.86 (1)	13.32 (1)	87.54 (1)	4	1
III	2.49 (4)	12.44 (4)	6.72 (4)	37.00 (2)	14	4
IV	2.86 (2)	13.46 (3)	8.36 (3)	43.73 (3)	11	3
Grand centroids	2.83	14.38	9.97	61.05	-	-

indicate the greater diversity among the jute genotypes of different clusters. The genotypes of the same cluster showed more genetic similarities than the genotypes of another cluster.

3.10. Principal component analysis (PCA)

PCA was done for the genetic diversity present among the jute genotypes for their phenotypic traits. The eigenvalues, variabilities, cumulative variabilities, eigenvectors, and scree plot were analyzed by PCA (Table 8; Fig. 5). The more the eigenvalues the more genetic variabilities among the genotypes but the variation in cumulative variability was vice-versa (Table 8). PC1 and PC2, the first two principal components were accounted for the total 91.71% of the cumulative variation. The PC1 having an Eigenvalue of less than 1.0 accounted for 81.29% of the total variation while the other components PC2, PC3 and PC4 recorded 10.43, 6.79 and 1.50% variations, respectively. For the first two principal components, plant height and base diameter showed positive Eigenvector values while the jute fiber yield and plant mortality rate showed negative values (Table 8). Here, the positive eigenvectors indicate the possibility of genetic improvement in plant height and base diameter leading to jute fiber yield. The first two PCs showed maximum variation while the others showed the lowest variations for morphological traits in the scree plot analysis of PCA (Fig. 5). The score plot of observations categorized the 22 genotypes into eight groups in the box plot based on genetic variability for the height of plant, basal diameter, yield of dry fiber, and plant survivability rate among them (Fig. 6). The genotypes (G2, G3, G4, G20) having maximum variability for the height of plant, basal diameter, yield of dry fiber were found in Group I and cluster II. The jute genotypes of group III (G₅) gave higher variability for plant survivability. The genotypes of groups VII and VIII showed negligible variability for the studied traits than the genotypes of groups IV, V, VI. The genotypes of groups I, II, III, IV, V would be concluded as better in respect of variability for the height of plant, basal diameter, yield of fiber, and plant survivability rate (Fig. 3 & 6). The Biplot analyses revealed the highest variability in jute genotypes for plant height (B) and base diameter (C) than fiber yield (D) and plant survivability rate leading to fiber yield content (Fig. 7).

4. Discussions

Jute fiber is directly attributed by the height of plant, basal diameter, fresh weight of plant, and plant survivability rate under salinity stress conditions. The higher extent of significance level for the morphological traits indicates more variability among the experimental plant genotypes, which would be the criteria for parent selection in jute breeding program [2,32].

The height of jute plant and basal diameter play a vital role to increase the yield of phloem fiber and jute stick [4,5]. In these studies, the Acc. 2311, Acc. 2307, Acc. 4160, Acc. 4327 showed higher plant height; the Acc. 1298, Acc. 2311, Acc. 1381, Acc. 4160 gave higher girth of stem compared to pre-released varieties. The Acc. 2311, Acc. 4160, Acc. 2307, Acc. 1381 gave a higher yield component; Acc. 4160 and Acc. 2311 gave less mortality rate which is a positive and good effect to increase the fiber yield. These genotypes having good morphological traits would be used as breeding materials to develop new and high yielding tossa jute varieties in Bangladesh. Similar findings were described by Mukul et al. [32] in tossa jute.

According to the Box and Whisker plot analysis, the variables with lower confidence intervals like plant height, basal diameter,



Figure 4. Intra and inter-cluster distances among four clusters of 22 jute genotypes for plant height, base diameter, fiber yield, plant survivability rate (%).

Table 8

Eigenvalues of the correlation matrix and extracted Eigenvectors for plant height, base diameter, fiber yield, and survivability rate.

Eigenval	ues of the correla	tion matrix		Extracted Eigenvectors			
PCs	Eigenvalue	Variance (%)	Cumulative variance (%)	Variables	Coefficients of PC1	Coefficients of PC2	
1	3.25	81.29	81.29	Plant height (m)	0.49	0.40	
2	0.42	10.43	91.71	Base diameter (mm)	0.48	0.62	
3	0.27	6.79	98.50	Fiber yield (g plant $^{-1}$)	0.51	-0.54	
4	0.06	1.50	100.0	Plant survivability rate (%)	0.52	-0.41	



Figure 5. PCA Scree plot of Eigen analysis for plant height, base diameter, fiber yield, plant survivability rate (%) of 22 jute genotypes.



Score Plot of Observations (Axes Component 1 and Component 2: 91.72%)

Figure 6. Two-dimensional score plot of PCA showing relationships among 22 tossa jute genotypes.

yield component, and rate of plant mortality would be used as criteria for parent selection. The analysis of EMWA chart for the studied morphological characters revealed that there is no centre mean value in the outside of UCL and LCL. These variables would be analyzed for morphological diversity to develop the new tossa jute plant varieties in future [9].

The plant mortality rate showed a negative relation with other traits depicting the importance of plant survivability under salt stress conditions [3]. The height of plant, basal diameter, fresh weight of plants, dry fiber yield, and plant mortality rate showing significant correlation with each other would be used as breeding tools for jute crop improvement [38,32].

The genetic parameters like PCV, GCV, broad sense heritability, and genetic advance for the studied characters as well as the correlation coefficient among the traits give more detailed information on yield attributes and thus, the breeders commonly used these



Figure 7. Two-dimensional biplot of PCA showing relationships among 22 tossa jute genotypes and four variables (Note: B= Plant height, C= Basal diameter, D= Fibre yield, E= Plant survivability rate (%)).

techniques to determine yield and yield contributing characters [41,31,26,32].

Fresh weight of jute plants with green leaves recorded higher GV, PV, hbs, and GA; and plant mortality rate gave higher GCV, PCV, ECV and GAM. Plant height recorded lower σ_G^2 , σ_P^2 , GCV, PCV, GA and GAM; basal diameter gave the lowest heritability in broad sense (98.41%) and the lowest ECV was found for the fresh weight of jute plants with green leaves indicating the lower environmental effects on plant weight with green leaves. Generally, the environment influenced highly the quantitative characters of plants. Plant height gave lower GCV (<10%) and basal diameter gave medium PCV (10-20%), lower GA, GAM values among all the traits depicting the lower possibility of genetic improvement; while the other characters accounted for higher (>20%) GCV, PCV, GA, and GAM indicating a higher possibility of genetic advancement of jute crops [37]. The traits showing higher PCV than GCV can contribute the genotypic effects for phenotypic expression. Due to lower environmental effects, all traits except plant mortality rate gave lower ECV values (< 0.05). Plant mortality rate is affected by the environmental factor salinity stress resulting in an enhanced ECV value. Estimates of h_{DS}^2 ranged from 98.41% for basal diameter to 99.97% for fresh weight of jute plants with leaves. According to Singh [44], heritability in broad sense were classified as high (>80%), moderately high (60-79%), medium (40-59%) and low (<40%). All the studied traits showed high heritability in broad sense and it was also supported by the findings of Sreelathakumary and Rajamory [47]. High heritability indicates the lower environmental effects on the phenotypic expression of the genotypes, and it is very easy to select for such characters due to the high additive effects on jute genotypes. Good extent of genetic advance would be expected from the selection based on such characters having high GCV, PCV coupled with high h_{Ds}^2 . Hamdi et al. [12] opined that, high heritability coupled with higher GA is more useful for genetic improvement through selection and thus prediction of the expected genetic gain from one selection cycle of plant genotype. Estimate of GA for ultimate fiber yield per plant was 6.19 g indicating that, whenever we select the best 5% high yielding jute genotypes as parents, the mean fiber yield $plant^{-1}$ of the offspring could be improved for 6.19 g that means, genotypic value of the new population for fiber yield would be improved from 9.97 g to 16.16 g.

In the same way, plant height will be improved from 2.83 m to 3.29 m; basal diameter for 14.38 mm to 18.07 mm; stick yield 31.80 g to 49.78 g plant⁻¹ and plant mortality rate will be reduced from 38.96% to 6.60%. According to Johnson et al. [16], the GAM of plant height was classified as moderate (10–20%), whereas other characters showed higher (>20%). All the characters recorded moderate to higher GAM (%) where the lowest value of GAM (16.10%) was found in plant height and the highest GAM (116.96%) was recorded in mortality rate of jute plants under salinity stress. High h_{bs}^2 along with high GAM is usually more helpful in predicting the genetic gain under selection than heritability alone

Jute plant height exhibited high heritability with moderate GAM and the other morphological traits exhibited high heritability with high GAM reflecting the presence of additive gene action for the expression of these traits fixable for the next generations, and selection in the next population for such traits. Sreelathakumary and Rajamory [47] were also reported such results for the genetic variability in local Chili pepper.

In cluster analysis, relatively salt-tolerant genotypes were clustered into II followed by I and IV. JRO-524 (Green), BJRI Tossa pat 5 (O-795) were found in cluster II; the advanced lines O-0419-3-2, O-0419-3-1 and segregating line JRO-524 (Red) in cluster III and O-043-7-9 in cluster I. The genotypes of the cluster having higher variability would be selected for the development of salinity tolerant tossa jute with good fiber yield through diversified breeding approaches [33]. In PCA, 91.71% cumulative variation was found in the first two PCs among the total variation. The jute genotype(s) of the clusters having more genetic distance would be selected as parents for breeding programs to develop new high yielding tossa jute varieties. Genotypes of the same cluster showed more genetic compatibility. Clustering Dendrogram and PCA score plot clearly indicated that the superior genotypes, i.e., Acc. 4160, Acc. 2311, Acc. 1381, Acc. 2307, JRO-524 (Green), Acc. 4327, BJRI Tossa Pat 5, Acc. 1334, Acc. 1381 having good fiber yield and salinity tolerance

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were found in cluster II, and groups I, II, III which were more distant from the other clusters. The first two PCs showed more variability in the scree plot, and the genotypes were grouped into eight groups in PCA score plot based on their variability. Therefore, these tossa jute genotypes or germplasm having more diversity for desired morphological traits as well as salinity tolerance would be used as breeding material to develop salinity tolerant tossa jute for the coastal areas of Bangladesh [52,18].

5. Conclusions

The tossa jute genotypes were sensitive to salinity for survivability and fiber yield capacity. The 22 jute genotypes showed significant differences for yield attributing morphological traits. Among all genotypes, Acc. 4160 & Acc. 2311 showed relatively more salt tolerance, lower mortality or higher survivability rate, and good fiber yield under salinity stresses in fields at both Patuakhali and Satkhira regions. Acc. 2307, JRO-524 (Green), Acc. 4327, BJRI Tossa pat 5 (O-795), Acc. 1334 and Acc.1381 also performed well in respect of survivability and fiber yield content at both regions. Therefore, these eight genotypes among all germplasm could be used as breeding materials to develop high-yielding salt-tolerant tossa jute varieties through hybridization and thus contribute to the national economy of country.

Author contribution statement

MD. MIA MUKUL, Masters: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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