Chapter III
MATERIALS AND METHODS

Three experiments on Tossa jute were conducted during the period from April 2016 to August 2018 at the Jute Agricultural Experimental Station (JAES), Manikgonj under Bangladesh Jute Research Institute. The list of experiments, with their duration and locations (Table 3.1) are given bellow:

3.1 List of the experiments

Experiment I. Evaluation and Character Association of Tossa Jute Germplasm

Experiment II. Combining Ability Analysis for Yield and Yield Contributing Characters in Tossa Jute

Experiment III. Selection in F_2 progenies to evolve desirable lines in advanced generation

Table 3.1 Durations and locations of experiments

Serial. No.	Experiment	Duration	Location
01.	Experiment I	2016 (April- August)	JAES, Manikgonj
02.	Experiment II	2017 (April - August)	JAES, Manikgonj
03.	Experiment III	2018 (April - August)	JAES, Manikgonj

3.2 General experimental features of the experimental sites

3.2.1 Jute Agricultural and Experimental Station, Manikgonj

The experimental field (JAES, BJRI) was situated in the tropical climate zone, characterized by heavy rainfall during the period of April to August and scanty rainfall during rest of the year (Appendix 1). The average temperatures were maximum 31.00 ^{0} C and minimum 20.64^{0}C in 2016, maximum 30.04^0 C and minimum 23.84^{0}C in 2017 and maximum 30.03^0 C and minimum 23.28^{0}C in 2018 respectively and Average rainfall was 108.26 in 2016, 162.42 in 2017 and 122.93 in 2018 (Appendix 1). The land was medium high with uniform topography and almost homogenous with respect to soil fertility. The site belonged to the Active Brahmaputra Jamuna Floodplain which is Agro-Ecological Zone (AEZ) No. 8. The land was not flooded and therefore flood was standed less than a month. Depth of flood ranged from 0.60-1.25 m. The soil was sandy and silty loam in texture, pH ranged from 6.00

to 6.65. The soil was deficit in all major nutrient elements (NPK) and also in organic matter. For experimentation with normal crop growth, amendment of soil with cowdung and NPK fertilizers were, therefore, necessary.

3.2.2 Land preparation: The experimental plot was at a lower elevation with high water holding capacity. The land was prepared thoroughly with 3-4 ploughing and cross ploughing followed by harrowing and laddering.

3.2.3 Fertilizer application: The crop was fertilized with recommended doses of cowdung (5t/ha) and fertilizers such as 100kg/ha Urea, 25kg/ha TSP and 45kg/ha MOP respectively (Hand notes on jute production, 2007). The whole amount of TSP, MOP and half of the amount of urea were applied at final land preparation. The remaining half urea was applied in two equal installments after first and final weeding.

3.2.4 Irrigation and drainage: After germination of seed, the field was irrigated properly and, necessary soil moisture maintained throughout the cropping period. A good drainage system was also ensured for immediate release of excess rainwater from the experimental field.

3.2.5 Intercultural operations: Necessary intercultural operations were performed for proper growth and development of the plants. Thinning and weeding were done simultaneously at the time of two top dressings of nitrogen. These practices were helpful to break the soil crust, to maintain uniform growth of plant population and to incorporate the nitrogen fertilizer (urea) into the soil, thus reducing the loss through denitrification. Proper control measures were taken against mite, nematode and stem rot of jute. The infested jute hairy caterpillars at larval and pupal stage were handpicked and destroyed.

3.2.6 Crop harvesting: The sample plants of individual plots were harvested for fibre yield at 110 days from the date of sowing, which was considered as the physiologically optimum age for quality fibre. The crop was cut at the base with the help of sickle and kept separately. The other details of materials and methods are described in the relevant of the specific experiments.

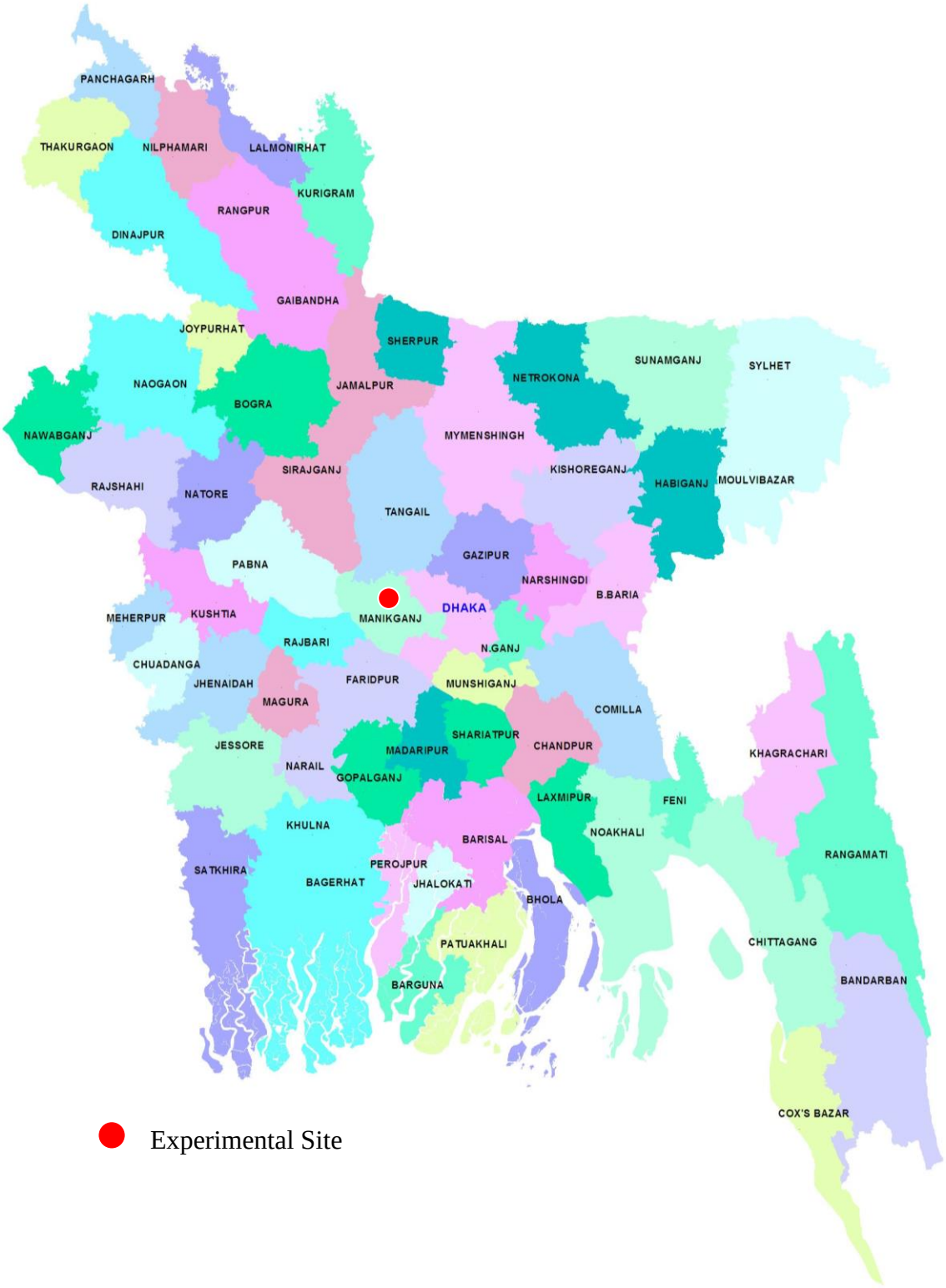

Fig. 3.1 Map of Bangladesh showing the experimental site

3.3 Experiment I. Evaluation and Character Association of Tossa Jute Germplasm

3.3.1 Materials and Methods

A total of fifty three genotypes consisting of 50 accessions and three cultivated varieties with different origin, were included for the study. The genetically pure and healthy seeds of those genotypes were collected from the Gene Bank of BJRI, Dhaka. The list of genotypes including their origin was given in Table 3.2.

The experiment was conducted during the period from April to August, 2016. The seeds of the experimental materials were sown in well-prepared land in randomized complete block design with three replications. The seeds were sown on 06 April, 2016 and each genotype was allotted in 3 rows of 3m long. The rows were 30 cm apart with planting space of 5-7 cm. The replications (blocks) were interspaced with 60 cm. The genotypes were assigned randomly to each plot within each block. The agronomic practices with fertilizer application were in accordance with standard recommendation.

3.3.2 Data recording and statistical analysis

The data on fibre yield and its attributes were recorded from five randomly selected plants of each genotype from each replication after harvest (110 days crop age). The details of data recording are provided as bellows:

1. **Plant height (m):** It was measured from the base to the tip of the main shoot of five randomly selected plants in meter and the means were computed.

2. **Base diameter (mm):** Average base diameter of five randomly selected plants was measured at the base of the stem in millimeter using slide calipers.
3. **Green bark thickness (mm):** The green bark thickness was measured in mm. It was done by subtracting from basal diameter to core diameter. Core diameter was measured by pulling the basal bark of the stem.

4. **Leaf length (cm**): The length of leaf was measured in cm.

5. **Leaf width (cm):** The width of leaf was measured in cm.

6. **Leaf angle (°):** Leaf angle (°) was taken manually from 6^{th} to 10^{th} leaves from the top of the plant of 5 randomly selected plants

Table 3.2 Sources and stem colours of Tossa jute genotypes used in this study

SL.NO.	Genotypes	Country/Dist. Name	Stem Pigmentation
01.	Acc.-1148	Faridpur, Bangladesh	Red
02.	Acc.-2381	Sirajgonj, Bangladesh	Light Red
03.	Acc.-2541	Bagerhat, Bangladesh	Green
04.	Acc.-2666	Jessore, Bangladesh	Green
05.	Acc.-2667	,,	Green
06.	Acc.-2670	,,	Green
07.	Acc.-3031	Jenaidha, Bangladesh	Green
08.	Acc.-3032	,,	Green
09.	Acc.-3033	,,	Green
10.	Acc.-3035	,,	Green
11.	Acc.-3038	,,	Green
12.	Acc.-3339	Rangamati, Bangladesh	Green
13.	Acc.-3340	,,	Green
14.	Acc.-3341	,,	Green
15.	Acc.-3342	,,	Green
16.	Acc.-3343	,,	Green
17.	Acc.-3423	Faridpur, Bangladesh	Green
18.	Acc.-3424	,,	Green
19.	Acc.-3425	,,	Green
20.	Acc.-3426	,,	Green
21.	Acc.-3429	,,	Green
22.	Acc.-3430	,,	Green
23.	Acc.-3432	,,	Green
24.	Acc.-3433	,,	Green
25.	Acc.-3435	,,	Green
26.	Acc.-3438	,,	Red
27.	Acc.-3439	,,	Green
28.	Acc.-3533	Madaripur, Bangladesh	Green
29.	Acc.-3535	Shoriotpur, Bangladesh	Green
30.	Acc.-3536	,,	Red
31.	Acc.-3538	Gopalgonj, Bangladesh	Green
32.	Acc.-3542	Shoriotpur, Bangladesh	Green
33.	Acc.-3543	,,	Green
34.	Acc.-3731	Kenya	Green
35.	Acc.-3733	Kenya	Green
36.	Acc.-3856	Chittagong, Bangladesh	Green
37.	Acc.-3857	Feni, Bangladesh	Green
38.	Acc.-3860	Chittagong, Bangladesh	Red
39.	Acc.-3985	Syria	Red

Table 3.2 Sources and stem colours of Tossa jute genotypes used in this study (cont'd)

SL.NO.	Genotypes	Country/Dist. Name	Stem Pigmentation
40.	Acc.-3986	Syria	Red
41.	Acc.-3987	Syria	Red
42.	Acc.-4674	Moudabag	Green
43.	Acc.-4756	Tanjania	Red
44.	Acc.-4785	Kenya	Red
45.	Acc.-4828	Kenya	Green
46.	Acc.-4848	Guinea	Red
47.	Acc.-4851	-	Red
48.	Acc.-4892	-	Red
49.	Acc.-5103	Tanjania	Red
50.	Acc.-5138	Ivorycost	Green
51.	O-9897	BJRI, Bangladesh	Green
52.	O-795	BJRI, Bangladesh	Red
53.	JRO-524	India	Green

7. **Petiole length (cm)**: The length of petiole was measured in cm.

8. **Green weight with leaves (kg):** Average green weight of plants from per m^2 area with leaves was recorded just after harvest.

9. **Green weight without leaves (kg):** Average green weight of previously selected plants without leaves was recorded after defoliation of leaves, which were seven days after harvest.

10. **Fibre yield (g):** Plants from per m^2 area were retted, extracted, dried and the mean weight of fibre were measured.

11. **Stick yield (g):** Weight of sun dried stick per m^2 area was measured after drying and the means were computed.

3.3.3 Statistical Analysis:

Different statistical tools were applied to analyze data using computer based software. Mean data of the characters were subjected to both univariate and multivariate analyses. Univariate analysis of the individual characters (analysis of variance) was done using Statistical Tool for Agricultural Research (STAR) software. The test of significance were done by F-test. Mean, range and coefficient of variance (CV) were also estimated using STAR software. Multivariate analysis was done using Genstat 5.13 and Microsoft Excel software through four techniques viz. Principal component analysis. Principal co-ordinate analysis, Cluster analysis and Canonical vector analysis as suggested by Anderson (1957).

3.3.3.1 Estimation of Phenotypic and Genotypic Variance

Genotypic and phenotypic variances were estimated by Johnson *et al.* (1955). Genotypic variance (δ^2_g) were obtained by subtracting error mean sum of square from the genotype mean sum of square and dividing by the number of replications as shown below:

$$\delta^2_g = \frac{GMS - EMS}{r}$$

Where,

GMS = Genotypic mean sum of square

EMS = Error mean sum of square

r = Number of replication

The phenotypic variances (δ^2_p) were derived by adding genotypic variances (δ^2_g) as given by the following formula:

$$\delta^2_p = \delta^2_g + \delta^2_e$$

3.3.3.2 Estimation of Coefficient of range

Coefficient of range was calculated using following formula:

$$\text{Coefficient of range} = \frac{(H - L)}{(H + L)}$$

Where,

H = the highest value in a set of observation

L = the lowest value in a set of observation

3.3.3.3 Estimation of Genotypic and Phenotypic Coefficient of Variation

Genotypic and phenotypic coefficient of variations were estimated according to the formula given by Johnson *et al.* (1955).

$$\text{Genotypic Coefficient of Variation (GCV)} = \frac{\delta_g}{\bar{X}} \times 100$$

Where,

δ_g = Genotypic standard deviation

$\bar{X}$ = Grand mean

$$\text{Phenotypic Coefficient of Variation (PCV)} = \frac{\delta_p}{\bar{X}} \times 100$$

Where,

δ_p = Phenotypic standard deviation

$\bar{X}$ = Grand mean

3.3.3.4 Estimation of Heritability

Broad sense heritability was estimated by the formula suggested by Johnson *et al.* (1955).

$$\% \, h^2_b = \frac{\delta^2_g}{\delta^2_p} \times 100$$

Where,

h^2_b = Heritability in broad sense

δ^2_g = Genotypic variance

δ^2_p = Phenotypic variance

3.3.3.5 Estimation of Genetic Advance

The expected genetic advance for different characters under selection was estimated using the formula suggested by Johnson *et al.* (1955).

$$\text{Genetic Advance (GA)} = \frac{\delta^2_g}{\delta^2_p} \times K \times \delta_p$$

Where

K = Selection intensity, the value of which is 2.06 at 5% selection intensity

δp = Phenotypic standard deviation

δ^2_g = Genotypic variance

δ^2_p = Phenotypic variance

3.3.3.6 Estimation of Genetic Advance as Percentage of Mean

Genetic advance in percentage of mean was calculated from the formula given by Comstock and Robinson (1952).

$$\text{Genetic advance in percentage of mean} = \frac{\text{Genetic advance}}{\text{Grand mean}} \times 100$$

3.3.3.7 Genetic divergence analysis

Multivariate analysis is a potent tool in divulging the divergence among the genotypes based on multiple characters. Genetic divergence was estimated following Mahalanobis (1936) generalized distance (D^2) extended by Rao (1952). Tocher's method (Rao, 1952) was followed for determining the group constellations. Canonical analysis was also done according to Rao (1952) to confirm the results of cluster and D^2 analysis. Mean data for each character were subjected to multivariate analysis techniques for Principal component analysis (PCA), Principal co-ordinate analysis (PCoA), Canonical vector analysis (CVA) and Cluster analysis (CLSA) by computer using GENSTST 5.13 software program. Mahalanobis's (1936) D^2 – statistic analysis was used for assessing the genetic divergence among the test entries for a rational choice of potential parent in a breeding program.

The generalized distance between any two populations is given by formula, $D^2 = \sum\sum \lambda ij SiSj$

Where,

D^2 = Square of generalized distance

λij = Reciprocal of the common dispersal matrix

S^{ai} = (μi 1 - $\mu i2$)

S^{aj} = (μj 1 - $\mu j2$)

μ = General mean

Since the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized character means (Xs) to standardized uncorrelated variable (Ys) was done to simplify the computational procedure. The D^2 values were obtained as the corresponding uncorrelated (Ys) values of any two genotypes (Rao, 1952).

3.3.3.8 Principal component analysis (PCA)

The technique was used to examine the interrelationships among five quantitative characters. The principal components were computed from the correlation matrix obtained from sum of squares products matrix of the characters, and genotype scores obtained from the first component and the succeeding component with latent roots greater than unity. The latent roots are called 'Eigen values'. The component has the property of accounting for maximum variances. The PCA displays most of the original variability in a smaller number of dimensions, since it found linear combinations of a set of variates that maximized the variation contained within them, contributions of the different characters towards divergence are discussed from the latent vectors of the first two principle components.

3.3.3.9 Principal co-ordinate analysis (PCO)

Principal co-ordinate analysis was used to calculate the inter genotype distance, which gave the minimum distance between each pair of the N points using similarity matrix through the use of all dimensions of P (Digby *et al.*, 1989).

3.3.3.10 Cluster analysis

Cluster analysis was performed by D^2 analysis (originally proposed outlined by Mahalanobis, 1928 and 1936, and extended by Rao, 1952), which divided the genotypes based on the data set into more or less homogeneous groups. Here D^2 was the sum of squares of differences between any two populations for each of the uncorrelated variables, obtained by transforming correlated variables through pivotal condensation method.

3.3.3.11 Canonical Vector Analysis

In this method, vectors or canonical roots were calculated to represent the genotypes in the graphical form. Using canonical vector analysis, a linear combination of original variability that maximize the ratio in between groups to within groups variation could be found out and thereby given functions of the original variability that could be used to discriminate between groups. Therefore, in this analysis a series of orthogonal transformations were done sequentially for maximizing the ratio among the groups to within group variations. The canonical varieties were based on the roots and vectors of W-B, where W is the pooled within group covariance matrix and B was among the groups covariance matrix.

3.3.3.12 Computation of Average Intra-cluster Distances

The average intra-cluster distance for each cluster was calculated by taking possible D^2 values within the members of a cluster obtained from the Principal Coordinate Analysis (PCO) after the cluster were formed. The formula used to measure the inter-cluster distance $=\sum D^2/n$, where $\sum D^2$ is the sum of distances between all possible combinations (n) of the genotypes included in a cluster. The square root of the average D^2 values represents the distance (D) within cluster.

3.3.3.13 Computation of Average Inter-cluster Distances

The procedure for calculating inter-cluster distance was first to measure the distance between cluster I and II, between I and III, between I and IV, between II and III, between II and IV and so on. The clusters were taken one by one and their distances from other clusters were calculated.

The inter cluster distance were calculated by the formulae described by Singh and Chaudhary (1985).

$$\text{Square of inter cluster distance} = \frac{\sum Di^2}{ninj}$$

Where,

$\sum Di^2$ = The sum of distances between all possible combinations (ninj) of the entries included in the cluster study

ni = Number of entries in cluster i

nj = Number of entries in cluster j

3.3.3.14 Cluster Diagram

Cluster diagram was drawn using D^2 values between and within clusters i.e. the intra-and inter-cluster distances. It provided a brief idea of the pattern of diversity among the genotypes and relationships between different genotypes included in a cluster.

3.3.3.15 Estimation of correlation

The genotypic and phenotypic correlations were estimated by the formula suggested by Miller *et al*. (1958).

Genotypic correlation $r_{gxy} = Cov\ g_{xy}/\sqrt{(\delta^2 g_x\ \delta^2 g_y)}$

Where,

$Cov\ g_{xy}$ = Genotypic covariance between the trait x and trait y

$\delta^2 g_x$ = Genotypic variance of the trait x

$\delta^2 g_y$ = Genotypic variance of the trait y

Similarly

Phenotypic correlation $r_{pxy} = Cov\ p_{xy}/\sqrt{(\delta^2 p_x\ \delta^2 p_y)}$

Where,

$Cov\ p_{xy}$ = Phenotypic covariance between the trait x and trait y

$\delta^2 p_x$ = Phenotypic variance of the trait x

$\delta^2 p_y$ = Phenotypic variance of the trait y

3.3.3.16 Path co-efficient analysis

The cause and effect relationship between yields and its components characters, were studied through path coefficient analysis. Path coefficient analysis was done according to the procedure stated by Singh and Choudhury (1985).

Assuming ten independent (x_1, x_2…….x_{10}) and one dependent variable x_y. Path coefficient analysis was performed.

The relationship between them can be represented as follows:

$r_1y = r_{11}p_1y + r_{12}p_2y + r_{13}p_3y + r_{14}p_4y + r_{15}p_5y + r_{16}p_6y + r_{17}p_7y + r_{18}p_8y + r_{19}p_9y + r_{110}p_{10}y$

$r_2y = r_{21}p_1y + r_{22}p_2y + r_{23}p_3y + r_{24}p_4y + r_{25}p_5y + r_{26}p_6y + r_{27}p_7y + r_{28}p_8y + r_{29}p_9y + r_{210}p_{10}y$

$r_3y = r_{31}p_1y + r_{32}p_2y + r_{33}p_3y + r_{34}p_4y + r_{35}p_5y + r_{36}p_6y + r_{37}p_7y + r_{38}p_8y + r_{39}p_9y + r_{310}p_{10}y$

:

:

$r_{10}y = r_{101}p_1y + r_{102}p_2y + r_{103}p_3y + r_{104}p_4y + r_{105}p_5y + r_{106}p_6y + r_{107}p_7y + r_{108}p_8y + r_{109}p_9y + r_{1010}p_{10}y$

Where,

$p_1y, p_2y.....p_{10}y$ = Path coefficient of the variable $x_1, x_2.....x_{10}$ on variable x_y respectively

$r_1y, r_2y.....r_{10}y$ = Correlation coefficient of the variable $x_1, x_2.....x_{10}$ on variable x_y respectively.

The indirect effect of a particular character through other character was worked out by multiplying direct path and particular correlation coefficient between those characters, respectively.

Residual effect was estimated as follows:

Residual effect (R) = $[1-(r_1yp_1y + r_2yp_2y + ... r_{10}yp_{10}y)^{1/2}$

3.4 Selection of parents

Eight parents from five different clusters were selected for combining ability analysis. Selection was done with different morpho-physiological traits; details in Table 3.3 comprising three cultivated varieties (var. O-9897, var. O-795 and JRO -524) and five breeding accessions (Acc.-2381, Acc.-3423, Acc.-3438, Acc.-3533 and Acc.-3860) were used as parent materials (Table 3.3; Plate 3.1).

3.4.1 Raising of parental genotypes and diallel crossing

Eight parental lines were crossed in all combination (including reciprocal) to generate diallel population. The F_1 derived from diallel crosses were employed for genetic studies on various aspects. To initiate crossing program seeds of the parental genotypes were sown in three dates in 15 days interval 10 August, 25 august and 10 September; in 2016. This was to synchronize flowering and to obtain maximum number of flower buds for emasculation and

crossing. The cultural practices including application of fertilizers were in accordance with usual recommendations.

Floral buds of each of the female parents which were ready to open in the next morning were emasculated in the afternoon and covered with thin cellophane paper bag and the adjoining buds were removed. The emasculated floral buds were dusted in the next morning with freshly collected pollen as per crossing schedule. The pollinated buds were properly tagged and covered with bags. After 1-2 days of fruit set, the bags were removed allowing the capsules to grow normally. Mature hybrid seeds were collected for each cross from the tagged pods. They were sun dried and stored separately in proper bags with proper labeling.

Three lines were grown in the field. Among the lines two were parental lines and one hybrid/F_1 line was in between of two parental lines. The line of parental identical plants were discarded, heterozygous plants as like as in mid parents or better performer than parents were the true hybrids. These activities were done in February-March/2017.

3.5 Experiment II. Combining Ability Analysis for Yield and Yield Contributing Characters in Tossa Jute

3.5.1 Materials and procedure

On the basis of morpho-physiological characterization (Experiment I) eight parents were selected as materials for hybridization. Photographs of eight parents along with their short descriptions are presented in Plate 3.1 and 3.2.

3.5.2 Experimental design

The experiment consisted of sixty four entries including 8 parents and fifty six F_1s (with reciprocal). The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications at Jute Agriculture Experimental Station, BJRI, Jagir, Manikgonj. Seeds were sown in 3 rows of 2m length on 6th April 2017. After plant establishment, spacing was maintained at 30 × 7 cm by thinning out weak and thinner plants. The replications (blocks) were interspaced with 1m. The recommended standard cropping practices were followed throughout the cropping season.

3.5.3 Observations and data recording

The observations were made on five randomly selected plants for each entry over each replication and the mean was computed for 11 morphological characters viz. plant height (m), base diameter (mm), green bark thickness (mm), leaf length (cm), leaf width (cm), leaf angle

(^{0}), petiole length (cm), green weight (g)with leaves/plant, green weight (g) without leaves /plant, fibre yield (g) /plant and stick yield (g)/plant. Descriptions of these characters are presented in Chapter 3 Section 3.3.2.

3.5.4 Biometrical and genetic analysis

Data were analyzed by Plant Breeding Tools software for analysis of mean performance and standard error. All the quantitative data taken were subjected to ANOVA. The total variance of each character was partitioned into block, genotype and error differences. The differences within the classes of effects were tested by F-test. The analysis of variance for combining ability of the diallel crosses was performed following Griffing's (1956) method-1 (Parents and both set of F_1s), model-1 (Fixed effects model), as worked out by Singh and Chaudhary (1985).

This analysis partitioned the variation due to genotypic differences into general combining ability (GCA), specific combining ability (SCA) effects and reciprocal effects. The GCA measured the average performance of parents in hybrid combinations, whereas SCA referred to those instanced in which the performances of a hybrid was relatively better or worse than would be expected on the basis of the average performances of the parents involved. GCA represented additive gene effects while SCA represents non-additive gene effects. The reciprocal crosses were made to assess the cytoplasmic gene effect on the expression in F_1s. The mathematical model for the analysis of combining ability in model-1, method-1 is assumed to be:

$$Y_{ij} = m + g_i + g_j + s_{ij} + r_{ij} + 1/bc \sum \sum e_{ijkl}$$

Where,

i, j = 1……..n

k = 1……..b

l = 1……c

b = number of blocks

c = number of observation in each plot

m = population mean

Y_{ij} = mean of i × j th parent over k and l

g_i = general combining ability (gca) effect of i^{th} parent

g_j = gca effect of j^{th} parent

s_{ij} = specific combining ability (sca) effect such that $s_{ij} = s_{ji}$

Table 3.3 Qualitative Characteristics of Eight Selected Parents of Tossa jute

Sl. No.	Accessions/ Varieties	Origin	Important characteristics
01.	O-9897	O-5 × BZ-5	Plant full green, leaves ovate lanceolate, full green bud, yellow flower, fruit indehiscence in nature, seed bluish green in colour, less photosensitive and late maturing.
02.	O-795	Uganda red × O-4	Plant reddish, leaves ovate lanceolate, yellow flower with reddish sepal, fruit indehiscence in nature, seed bluish green in colour, less photosensitive and late maturing
03.	JRO-524	Indian origin	Plant full green, leaves lanceolate, full green bud, yellow flower, fruit indehiscence and sticky in nature, seed bluish green in colour, less photosensitive and late maturing.
04.	Acc.-2381	Local collection	Plant reddish, leaves ovate lanceolate, yellow flower with reddish sepal, seed bluish green in colour.
05.	Acc.-3423	Local collection	Plant full green, leaves ovate lanceolate, full green bud, yellow flower and seed bluish green in colour.
06.	Acc.-3438	Local collection	Plant reddish, leaves ovate lanceolate, yellow flower with reddish sepal, seed bluish green in colour.
07.	Acc.-3533	Local collection	Plant full green, leaves ovate lanceolate, full green bud, yellow flower and seed bluish green in colour.
08.	Acc.-3860	Local collection	Plant reddish, leaves ovate lanceolate, yellow flower with reddish sepal, seed bluish green in colour.

 O-9897 (Ovate lanceolate leaf, stem green)	 O-795 (Ovate lanceolate leaf, stem reddish)	 JRO-524(Lanceolate leaf, stem green)	 Acc.-2381 (Ovate lanceolate leaf, stem reddish)
 Acc.-3423 (Ovate lanceolate leaf, stem green)	 Acc.-3438 (Ovate lanceolate leaf, stem reddish)	 Acc.-3533 (Ovate lanceolate leaf, stem green)	 Acc.-3860 (Ovate lanceolate leaf, stem reddish)

Plate 3.1 Morphological feature of eight parental genotypes used in diallel crosses

 O-9897 (Full green bud, yellow flower)	 O-795 (Yellow flower with reddish sepal)	 JRO-524 (Full green bud, yellow flower)	 Acc.-2381 (Yellow flower with reddish sepal)
 Acc.-3423 (Full green bud, yellow flower)	 Acc.-3438 (Yellow flower with reddish sepal)	 Acc.-3533 (Full green bud, yellow flower)	 Acc.-3860 (Yellow flower with reddish sepal)

Plate 3.2 Floral morphology of eight parental genotypes used in diallel crosses

a. Pollination

b. Bagging after pollination

c. Crossing block

d. Mature pod

Plate 3.3 Photograph showing different crossing activities in the experimental field

r_{ij} = reciprocal effect such that $r_{ij} = r_{ji}$

e_{ijkl} = environmental effect particular to the ijklth individual observation the restriction imposed are- $\sum gi = 0$, $\sum (sij + sii) = 0$ (for each i and $\sum (sij + sii) = 0$ (for each i)

Analysis of variance for combining ability in method 1 (model 1):

Source	d.f	Sum of squares	Mean squares
gca	n-1	$1/2n \sum (Yi. + Y.j)^2 - 2/n^2 Y^2..$	Mg
sca	n(n-1)/2	$½ \sum\sum Y_{ij} (Y_{ij} + Y_{ji}) - 1/2n \sum (Y_{.j} + Y_{i.})^2 + 1/n^2 Y^{2..}$	Ms
reciprocal	n(n-1)/2	$½ \sum\sum (Y_{ij} - Y_{ji})^2$	Mr
error	(r-1) (t-1)		Me

Where,

gca = general combining ability

sca = specific combining ability

n = number of parents

r = number of replications

t = number of treatments

$Y_{i.}$ = row total of any array

Y_{ii} = mean value of ith parent

Y.. = sum of individual values of parents and their crosses

Y_{ij} = progeny mean values in the diallel table

$Y_{.j}$ = column total of an array

Y_{jj} = mean value of the jth parent

M e = MS (error) from preliminary ANOVA/r (no. of replications)

Test of gca effects: F [(n-1), (r-1) (t-1)] = Mg /Me

Test of sca effects: F [n (n-1)/2, (r-1) (t-1)] = Ms/Me

Test of reciprocal effects: F [n (n-1)/2, (r-1) (t-1)] = Mr/Me

Estimation of GCA, SCA and reciprocal effects:

The GCA, SCA and reciprocal effect of each characters were estimated as follows-

$g_i = 1/2n\ (Y_i. + Y_{.i}) - 1/n^2\ Y. .$

$s_{ij} = ½\ (Y_{ij} + Y_{ji}) - 1/2n\ (Y_i. + Y_{.i} + Y_j. + Y_{ij}) + 1/n^2\ Y. .$

$r_{ij} = ½\ (Y_{ij} - Y_{ji})$

Standard Error (SE)

To test the accuracy of g_i and s_{ij} and the significance of differences between any two g_is, s_{ij}s, or r_{ij}s the SE and SEd were calculated according to following formula:

$SE\ (g_i) = \{(n - 1)/2n^2\}\ \delta^2e$

$SE\ (s_{ij}) = \{(n - 1)^2/n^2\}/\ \delta^2e$

$SE\ (r_{ij}) = ½\ \delta^2e$

$SE\ (g_i - g_j) = (1/n)\ \delta^2e$

$SE\ (s_{ij} - s_{ik}) = \{(n - 1)/n\}\ \delta^2e$

$SE\ (s_{ij} - s_{kl}) = \{(n - 2)/n\}\ \delta^2e$

$SE\ (r_{ij} - r_{kl}) = \delta^2e$

Significance level:

Each GCA and SCA effects estimate was subjected to‘t’ test to know the significance as follows-

$t = (g_i\text{-}0)/SE\ (g_i)$, $t = (s_{ij} - 0)/\ SE\ (s_{ij})$ and $t = (r_{ij} - 0)/\ SE\ (r_{ij})$

The‘t’ value obtained was tested against tabulated ‘t’ value at 5% and 1% probability levels for respective degrees of freedom.

3.5.5 Raising of materials (F_2 seeds) for experiment III

Ten cross combinations were selected on the basis of mean values of fibre weight and positively significance GCA and SCA estimated in Experiment II. The cuttings of selected crosses were planted in 26 august 2017 at Jute Agriculture Experimental Station, BJRI, Jagir, Manikgonj. The recommended standard cropping practices were followed throughout the cropping season. The seeds were harvested in December 2017 and preserved in proper way for the materials of next experiment.

3.6 Experiment III. Selection in F_2 progenies to evolve desirable lines in advanced generation

3.6.1 Materials and procedure

The experiment consisted of ten F_2s. On the basis of mean values of fibre weight and positively significance GCA and SCA estimated in Experiment II were basis of selection of ten segregants from F_2 generation of Tossa jute for the study. The experiment was laid out without replications at Jute Agriculture Experimental Station, BJRI, Jagir, Manikgonj. Seeds were sown in 10 rows of 3m length on April 2018. The recommended standard cropping practices were followed throughout the cropping season to have a good harvest.

3.6.2 Observations and data recording

The observations were made on three hundred plants for each segregant. The mean, variance and range were computed for six morphological characters viz. plant height (m), base diameter (mm), green bark thickness (mm), green weight without leaves (g)/plants, fibre yield (g) /plants and stick yield (g)/plants. Descriptions of these characters are presented in Chapter 3 Section 3.3.2.

On the basis of plant height, base diameter, green weight without leaves and fibre weight best thirty plants (10% of 300 plants) were selected. Then “t” test was done between the base population (300 plants) and selected population (30 plants) derivatives of selected ten crosses to estimate progress from selection. Pedigree record of 30 selected plants from each cross were recorded following row to plant selection method.