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Pollen Morphology of Eight *Corchorus* spp. (Tiliaceae) and How Their Interrelationships Aid Efficient Breeding

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ABSTRACT

A comparative study of the pollen morphology of 8 *Corchorus* (jute) species (Tiliaceae; 2*n*=14) namely, *C. capsularis* L., *C. olitorius* L. (cultivated), *C. aestuans* L., *C. fascicularis* Lamk., *C. pseudocapsularis* L., *C. pseudoolitorius* I. and Z., *C. tridens* L. and *C. trilocularis* L. (wild) was performed based on light microscopy (acetolysis technique) and scanning electron microscopy. Pollen grains were found to have the following characteristics: prolate-subprolate; tricolporate, medium sized (24.96 ± 1.31 to 41.28 ± 1.74 µm); colpi long (22.41 ± 0.98 to 33.14 ± 1.12 µm), extending up to poles, rare often fused, linear and symmetrical or wide and asymmetrical; pore diameter varied from 0.7 to 6.0 µm; exine thick (2.0 to 3.8 µm), reticulate, reticulation not uniform in size, becoming smaller towards the colpi margin; lumen area ranging from 0.08-1.03 to 0.20-2.03 μm^2, mostly polygonal, rarely irregular; muri 0.28 to 0.50 µm thick. A key to the identification of the species has been prepared. Statistical methods (principal component analysis and cluster analysis by UPGMA) were employed taking into consideration 28 discrete variables, which revealed distinctiveness between *C. capsularis* and *C. olitorius* which is a hindrance to efficient breeding. However, relatedness among/between species was also studied that may be explored to enhance genetic diversity in *Corchorus* as well as to incorporate desirable trait(s) from wild to cultivated members. An unrooted phylogenetic tree suggested a divaricated mode of evolution of *Corchorus*.

Keywords: acetolysis technique, jute, key to identification, principal component analysis, SEM analysis, UPGMA, unrooted tree

INTRODUCTION

Corchorus (Family: Tiliaceae), including more than 170 species (Mahapatra and Saha 2008), are annual fibrous plants, distributed in warm regions of the world (Kundu 1951; Purseglove 1968). Based on species concentration, East Africa and South Africa are considered to be the centres of diversity (Kundu 1951; Edmonds 1990). *C. capsularis* L. (white jute) and *C. olitorius* L. (tossa jute; also known as busk okra or jute mallow in English) are cultivated members (2*n* = 14) and yield fibre (phloem fibre) from the bark of the stem which is commercialized. India contributes about 40% of world production (Karmakar *et al.* 2008). Apart from India, both species are cultivated in Bangladesh, Nepal, China, Indonesia, Thailand, Myanmar and South American countries (Mahapatra and Saha 2008). The cultivation of jute improves soil fertility by shedding leaves (on an average, 15 tonnes of green jute leaves per hectare: http://www.jute.org/planting.htm) in the field (International Jute Study Group: http://www.jute.org/ecology.htm). *C. olitorius* is also cultivated and consumed as a leafy vegetable in Nigeria (Ogunkanmi *et al.* 2010).

Wild *Corchorus* species, even though yielding poor amounts of fibre, are important genetic resources. *C. trilocularis* is the only flooding-tolerant genotype (Mahapatra and Saha 2008). Both *C. pseudocapsularis* and *C. pseudoolitorius* are resistant to most fungal diseases (Palve *et al.* 2004) while *C. aestuans*, *C. tridens* and *C. trilocularis* possess fine fibre quality (Mahapatra and Saha 2008). Therefore, characterization of jute species, including both cultivated and wild members, will be of paramount significance in ascertaining the interrelationship between or among themselves aiding efficient breeding and crop improvement.

Genetic diversity and relatedness have been assessed in different *C. capsularis* and *C. olitorius* germplasm accessions as well as in a few wild members using molecular markers (RAPD - Hossain *et al.* 2002; ISSR - Qi *et al.* 2003; SSR and AFLP - Basu *et al.* 2004; STMS, ISSR and RAPD - Roy *et al.* 2006; SSR - Huq *et al.* 2009). However, germplasm characterization involving the use of other parameters may widen the base for assessment. Pollen grains, associated with reproductive outcome and heredity, are significant in detailing morphological variations to delineate taxonomic relationships among plant taxa at different levels (Cooper *et al.* 2000; Banks *et al.* 2006). Keeping this scope in mind, the present investigation details the characterization of 8 species of *Corchorus* (cultivated: *C. capsularis* L. and *C. olitorius* L.; wild: *C. aestuans* L., *C. fascicularis* Lamk., *C. pseudocapsularis* L., *C. pseudoolitorius* I. and Z., *C. tridens* L. and *C. trilocularis* L. - all growing in India and possessing 2*n*=14 chromosomes – Maity and Datta 2009; Mandal and Datta 2011) based on pollen morphology (shape, size, colpi length, pore character and exine thickness and ornamentation) under light microscopy (using acetolysis technique) and scanning electron microscopy (SEM). The objective of the work was to obtain comparative pollen morphological data and to carry out statistical methods to ascertain relatedness among the studied species. An unrooted phylogenetic tree was constructed to describe the possible mode of evolution of the genus *Corchorus*. Furthermore, one application of the present work is the possible exploitation of desirable wild germplasm(s) into an efficient breeding programme with cultivated species, a major focus area of the jute industry.

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Original Research Paper

MATERIALS AND METHODS

Germplasm

Seeds of two cultivated (*C. capsularis* – JRC 321, *C. olitorius* – JRO 524) and six wild (*C. aestuans* – WCIJ 088, *C. fascicularis* – WCIJ 150, *C. pseudocapsularis* – CIM 036, *C. pseudoolitorius* – OIN 507, *C. tridens* – WCIJ 149 and *C. trilocularis* – KBA 222) species of *Corchorus* were obtained from CRIJAF (Central Research Institute for Jute and Allied Fibres), Nilganj, Kolkata, West Bengal, India in 2006. The 8 accessions were maintained in the experimental field plots of the Department of Botany, University of Kalyani (West Bengal plains, Nadia; latitude 22° 50′ to 24° 11′ N, longitude 88° 09′ to 88° 48′ E, elevation 48 feet above sea level, sandy loamy soil, organic carbon 0.76%, soil pH 6.85) for five successive years following selfing to attain homozygosity.

Pollen morphology

Pollen grains from fully open flowers were acetolysed following Erdtman (1952) and examined under a light microscope (10X × 40X). For the SEM study, the pollen grains of each species were placed into 70% ethanol (approximately 1000 to 7000 pollen grains, depending on the species, in 1.0 ml 70% ethanol and counted using an improved Neubauer hemocytometer) for 2 days in 2-ml micro centrifuge tubes (Tarsons, India) and cleaned in an ultrasonic (50/60 Hz, 80 W, 240 V) vibrator (Branson® Ultrasonics Corp., Danbury, Connecticut, USA) for 6 min. Pollen grains of each species were fixed on glass plates and then mounted on specimen stubs with double-sided adhesive tape and painted with silver. Pollen grains mounted on specimen stubs were placed on a revolving disc and coated with a 200-300 Å thick layer of gold in a vacuum evaporator (Polaron, East Sussex, UK) sputter coating system. The specimen stubs were then observed under SEM (Zeiss EVO® HD, Oberkochen, Germany) at 15 kV accelerating voltage at GSI (Geological Survey of India, Kolkata). On average, 30 pollen grains were analyzed for each species to assess their morphological parameters. Pollen shape and size were determined as per Erdtman (1952). Photomicrographs were taken from suitable preparations.

Statistical analysis

1. Principal component analysis

Twenty eight discrete variables from pollen morphological parameters were considered and analyzed statistically. PCA (principal component analysis) is the simplest of the true eigen vector-based multivariate analyses and was used on the basis of the net merit of the species by taking together the scores of each character, as proposed by Jain (1982). Based on a correlation matrix, PCA as described by Dillon and Goldstein (1984) was performed to judge the factor score of each species caused by the two highest eigen values. Percentage variation explained by the two highest eigen values was calculated. Factor coordinates of variables were plotted using the software STATISTICA, version 7.1 (www.statsoft.com).

2. Cluster analysis

The pollen parameters were scored as present (1) or absent (0) in the 8 studied species and entered into a binary data matrix. Based on the results, a proximity matrix was generated for all possible pairs based on the Squared Euclidean Distance and used to construct a dendrogram by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using the software STATISTICA, version 7.1 (www.statsoft.com).

3. Construction of an unrooted tree

The pollen parameters of each species were used to construct an unrooted tree using the software DendroUPGMA (genomes.urv.cat/UPGMA/; Garcia-Vallve *et al.* 1999).

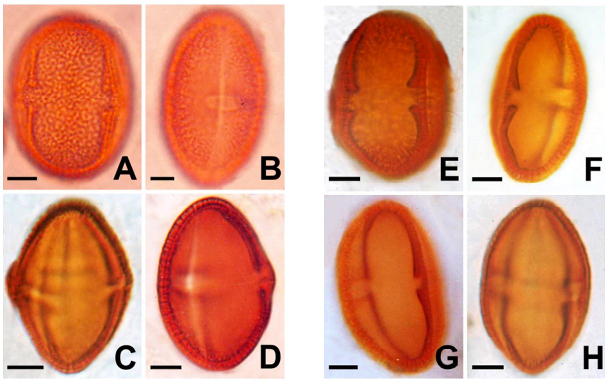

Fig. 1 Pollen morphology of *Corchorus* species under light microscopy using acetolysis technique. (**A**) *C. capsularis*; (**B**) *C. oiltorius*; (**C**) *C. aestuans*; (**D**) *C. fascicularis*; (**E**) *C. pseudocapsularis*; (**F**) *C. pseudo-olitorius*; (**G**) *C. tridens*; (**H**) *C. trilocularis*. Scale bar = 5 µm.

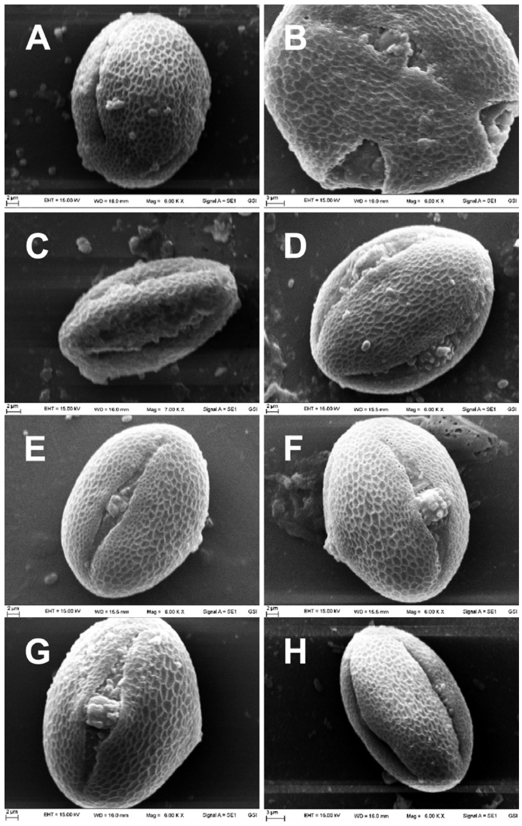

Fig. 2 Scanning electron micrographs of pollen grains of *Corchorus* species. (**A**) *C. capsularis*; (**B**) *C. oiltorius*; (**C**) *C. aestuans*; (**D**) *C. fascicularis*; (**E**) *C. pseudocapsularis*; (**F**) *C. pseudoolitorius*; (**G**) *C. tridens*; (**H**) *C. trilocularis*.

RESULTS

Pollen morphology

Pollen attributes analyzed under light microscope (**Fig. 1A-H**) and SEM (**Fig. 2A-H**) are presented in **Table 1**. Pollen grains in *Corchorus* spp. are prolate to subprolate; tricolporate; medium sized (24.96 × 13.65 µm to 41.28 × 32.71 µm); colpi are long, extending up to poles, rare often fused (*C. aestuans*), linear symmetrical (*C. capsularis*, *C. aestuans* and *C. trilocularis*) or wide and asymmetrical; pore diameter varies from 0.7 ± 0.18 µm (*C. trilocularis*) to 6.0 ± 0.32 µm (*C. tridens*); exine thick (2.0 ± 0.12 µm to 3.8 ± 0.16 µm), reticulate, reticulation not uniform in size, becoming smaller towards the colpi margin; lumen area ranges from 0.08-1.03 µm^2 to 0.20-2.03 µm^2, mostly polygonal and rarely irregular; muri 0.28 ± 0.06 µm to 0.50 ± 0.08 µm thick.

Table 1 Pollen attributes following light and scanning electron microscopy of *Corchorus* spp. (*N*=7).

Species	Mean polar axis (µm)	Mean equatorial diameter (µm)	Shape of pollen grains	Colpus length (µm)	Colpus shape	Pore diameter (µm)	Exine thickness (µm)	Muri diameter (µm)	Lumen shape	Lumen area (µm²)
C. capsularis (**Figs. 1A, 2A**)	27.33 ± 1.23	21.61 ± 1.34	Subprolate	22.41 ± 0.98	Linear and symmetrical	0.85 ± 0.20	3.00 ± 0.14	0.50 ± 0.08	Polygonal	0.08-1.70
C. olitorius (**Figs. 1B, 2B**)	41.28 ± 1.74	32.71 ± 1.66	Subprolate	33.14 ± 1.12	Wide and asymmetrical	5.60 ± 0.32	3.80 ± 0.16	0.37+0.06	Polygonal	0.23-1.61
C. aestuans (**Figs. 1C, 2C**)	24.96 ± 1.31	13.65 ± 1.06	Prolate	22.64 ± 0.88	Linear and symmetrical	0.80 ± 0.18	2.75 ± 0.10	0.40 ± 0.04	Irregular	0.10-0.62
C. fascicularis (**Figs. 1D, 2D**)	31.25 ± 3.24	23.52 ± 1.98	Prolate	28.88 ± 1.64	Wide and asymmetrical	4.60 ± 0.22	2.00 ± 0.30	0.40 ± 0.06	Polygonal	0.13-0.92
C. pseudocapsularis (**Figs. 1E, 2E**)	28.56 ± 1.67	21.12 ± 1.76	Prolate	23.82 ± 0.88	Wide and asymmetrical	3.70 ± 0.30	2.00 ± 0.28	0.32 ± 0.04	Polygonal	0.08-1.03
C. pseudoolitorius (**Figs. 1F, 2F**)	29.27 ± 1.17	22.04 ± 1.38	Subprolate	24.73 ± 1.00	Wide and asymmetrical	5.00 ± 0.34	2.70 ± 0.12	0.40 ± 0.04	Polygonal	0.20-1.54
C. tridens (**Figs. 1G, 2G**)	32.03 ± 3.28	24.06 ± 2.12	Prolate	28.22 ± 1.34	Wide and asymmetrical	6.00 ± 0.32	2.00 ± 0.12	0.40 ± 0.06	Polygonal	0.20-2.03
C. trilocularis (**Figs. 1H, 2H**)	28.56 ± 2.13	17.82 ± 1.68	Prolate	23.00 ± 1.08	Linear and symmetrical	0.70 ± 0.18	2.60 ± 0.20	0.28 ± 0.06	Irregular	0.10-0.60

Table 2 Similarity indices among different *Corchorus* species.

Variable	*C. capsularis*	*C. olitorius*	*C. aestuans*	*C. fascicularis*	*C. pseudocapsularis*	*C. pseudoolitorius*	*C. tridens*	*C. trilocularis*
C. capsularis	1.00							
C. olitorius	0.18	1.00						
C. aestuans	-0.04	-0.04	1.00					
C. fascicularis	0.13	0.13	0.26	1.00				
C. pseudocapsularis	0.35	0.02	**0.49**	**0.60**	1.00			
C. pseudoolitorius	**0.51**	0.18	0.31	**0.44**	**0.84**	1.00		
C. tridens	0.18	0.02	**0.49**	**0.76**	**0.84**	**0.67**	1.00	
C. trilocularis	0.18	-0.15	0.13	0.13	0.18	0.02	0.02	1.00

Values marked in bold font are significant and the level ranges from $P<0.05$ ($r<0.37$) to <0.001 ($r<0.58$); $N=28$.

Based on significant characteristic features, a key to the identification of the species was formulated and is presented in the key in **Box 1**.

Statistical analysis

1. Principal component analysis

The data sets were analyzed in order to find possible groupings between species. Based on a correlation matrix (**Table 2**), factor loadings (number of factors = 2) for the two largest eigen values were studied. Factor 1, corresponding to the largest eigen value (1.181287), accounts for about 59.064% of variance while the second factor, corresponding to a second eigen value (0.818713), accounts for approximately 40.94% of variance. The magnitude of the factor coordinates (variable – factor correlation) for the variables in the analysis and the supplementary variables (wild species) were graphically represented as a pie graph (**Fig. 3**). The pie graph provides a visual representation of the distribution of the supplementary variables (wild spp.) in relation to the current set of factors (active variables – cultivated species). The results indicate a close relationship among *C. pseudocapsularis*, *C. pseudoolitorius* and *C. tridens*. These three species are also related to *C. fascicularis* and *C. capsularis*. *C. aestuans* and *C. trilocularis* are represented in a different quarter of the circle. The location of the supplementary variables (wild species) indicates a close relatedness between/among species.

2. Cluster analysis

The dendrogram constructed by UPGMA (**Fig. 4**) revealed two major clusters, one of which comprises *C. olitorius* and *C. trilocularis* while the remaining species are in the other major cluster. Both clusters were sub-clustered. From the dendrogram it is evident that *C. pseudocapsularis*, *C. pseudoolitorius* and *C. tridens* are closely associated. These species are also related to *C. fascicularis*. *C. capsularis* and *C. aestuans* are grouped together and sub-clustered with *C. fascicularis*, which is in turn associated with *C. pseudocapsularis*, *C. pseudoolitorius* and *C. tridens*.

Box 1: Key

A. Pollen grains subprolate
- A.1. Pollen grains large in size (Polar axis: PA>40 µm)........*C. olitorius*.
- A.2. Pollen grains small in size (PA<30 µm)
  - A.2.1. Colpi linear and symmetrical........*C. capsularis*.
  - A.2.2. Colpi wide and asymmetrical........*C. pseudoolitorius*.

AA. Pollen grains prolate
- AA.1. Colpi linear and symmetrical, lumen shape irregular
  - AA.1.1. PA<25 µm, Equatorial diameter: ED<15 µm........*C. aestuans*.
  - AA.1.2. PA>25 µm, ED>15 µm........*C. trilocularis*.
- AA.2. Colpi wide and asymmetrical, lumen shape polygonal
  - AA.2.1. Colpi length small (23.82 ± 0.88 µm); muri diameter 0.32 ± 0.04 µm........*C. pseudocapsularis*.
  - AA.2.2. Colpi length large (>28.00 ± 1.64 µm); muri diameter 0.40 ± 0.04 µm
    - AA..2.2.1. Pore diameter small (4.6 ± 0.22 µm); lumen area ranges from 0.20 µm² to 0.92 µm²........*C. fascicularis*.
    - AA..2.2.2. Pore diameter large (6.00 ± 0.32 µm); lumen area ranges from 0.20 µm² to 0.92 µm²........*C. tridens*.

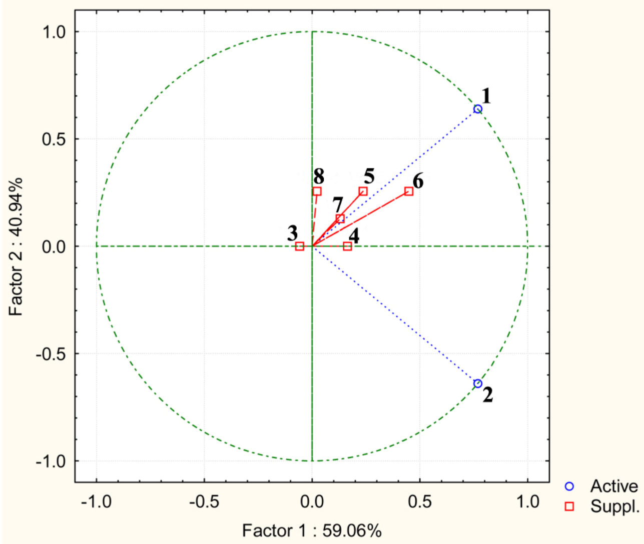

Fig. 3 Pie diagram showing projection of the variables on the factor-plane (1×2). Active variables: (1) *C. capsularis*; (2) *C. olitorius*; Supplementary: (3) *C. aestuans*; (4) *C. fascicularis*; (5) *C. pseudocapsularis*; (6) *C. pseudoolitorius*; (7) *C. tridens*; (8) *C. trilocularis*. Factors 1 and 2 show total variance.

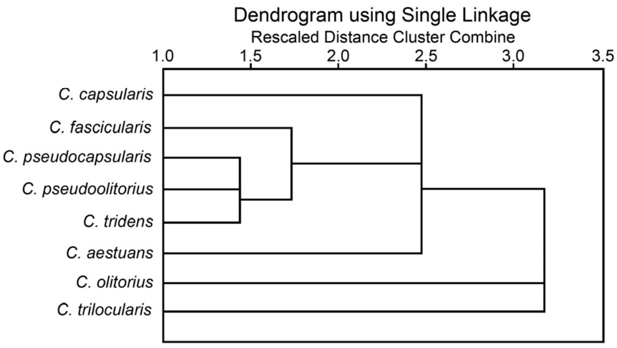

Fig. 4 Dendogram showing clustering of 8 *Corchorus* spp. following UPGMA.

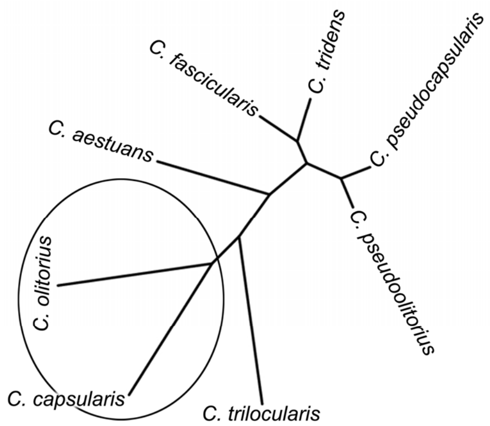

Fig. 5 An unrooted phylogenetic tree.

3. Unrooted tree

Two different domains (**Fig. 5**) of origin are notable among the studied species. One of the domains, comprising *C. capsularis* and *C. olitorius*, has been encircled while the rest of the species lie in a separate domain. The two domains contain four variants: 1) *C. aestuans*, 2) *C. trilocularis*, 3) *C. fascicularis* and *C. tridens*, 4) *C. pseudocapsularis* and *C. pseudoolitorius*. *C. fascicularis* and *C. tridens*, and *C. pseudocapsularis* and *C. pseudoolitorius* are sub-variants. This tree suggests that *C. pseudocapsularis* and *C. pseudoolitorius* are more closely related to each other than to *C. fascicularis* and *C. tridens* or *C. capsularis* and *C. olitorius*.

DISCUSSION

Statistical analysis (PCA and cluster analysis by UPGMA) of pollen grains morphological parameters revealed distinctiveness between *C. capsularis* and *C. olitorius*. A correlation matrix revealed a non-significant ($P>0.05$) relationship between both species. These result are in agreement with the grouping or clustering observed in earlier reports using molecular markers (Hossain *et al.* 2002; Basu *et al.* 2004; Roy *et al.* 2006; Huq *et al.* 2009), karyomorphological (Maity and Datta 2009) and morpho-anatomical and biochemical (Maity *et al.* 2010) attributes. Present observations on pollen morphology revealed distinctiveness between the cultivated members which is a hindrance to efficient breeding; however, different attributes taken together may provide better insight on the aspect.

PCA and cluster analysis (by UPGMA) almost corroborate each other and therefore the assessment of relatedness among species seems to be meaningful. A close relationship was observed among *C. pseudocapsularis*, *C. pseudoolitorius*, *C. tridens* and *C. fascicularis*, all of which seem to be associated with *C. capsularis* and *C. aestuans*. In the dendrogram (**Fig. 4**), even though *C. olitorius* and *C. trilocularis* formed a separate cluster, they were still sub-clustered to all the other studied species. The wild *Corchorus* species are reportedly more primitive than cultivated species (Kundu 1951; Mahapatra and Saha 2008; Maity *et al.* 2010). Tao *et al.* (2012) analyzed 96 jute germplasms using SRAP (sequence-related amplified polymorphism) and ISSR (inter-simple sequence repeat) primers: the relative basic location of *Corchorus* spp. in the dendrogram as well as divergent time suggest that wild species are primitive to cultivated members.

The unrooted phylogenetic tree (**Fig. 5**) developed from pollen characteristics revealed two domains of origin, thereby indicating that evolution of the genus may possibly be of a divaricated type. One domain comprises both cultivated species while the other domain incorporates wild species. This result is rather different from observations made earlier using molecular markers in which *C. olitorius* was reported to be of African origin while *C. capsularis* possibly evolved in the Indo-Burma region from progenitor species (Roy *et al.* 2006; Tao *et al.* 2012). However, the centre of diversity and origin of jute species were reported earlier to be East Africa and South Africa based on species concentration (Kundu 1951; Edmonds 1990). Therefore, a critical assessment of different parameters, taken together, may provide better insight regarding agreement on the origin and migration of the cultivated species of jute. Patel and Datta (1958) reported a divergent mode of evolution of the genus *Corchorus* based on their study on pollen fertility and pollen grain sizes in different germplasms of *C. capsularis* and *C. olitorius*, and four wild species. Datta *et al.* (1966, 1975) also corroborated that finding showing the divaricated origin of *Corchorus* based on karyomorphological data. Roy *et al.* (2006) characterized jute accessions, including cultivated and wild species, using RAPD (random amplification of polymorphic DNA) and ISSR primers and suggested a polyphyletic mode of origin of the genus as was proposed earlier by Kundu (1951).

CONCLUSIONS

In this study, an analysis of pollen parameters using statistical methods revealed relatedness among the studied species, thereby offering the possibility of widening the gene pool and enhancing genetic diversity in *Corchorus* following a breeding endeavour. Further, the results also provided ample scope to explore desirable wild germplasm(s) in an efficient breeding programme with cultivated species to improve qualitative trait(s). Moreover, our results indicate the significance of pollen parameters in ascertaining relatedness and diversity among plant taxa.

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