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# Assessment of Genetic Diversity and Population Structure in Jute (*Corchorus* spp.) Using Simple Sequence Repeat (SSR) and Amplified Fragment Length Polymorphism (AFLP) Markers

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## ABSTRACT

The genetic diversity and population structure of 63 jute genotypes from *C. capsularis* L. and *C. olitorius* L. were investigated using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. The average polymorphism information content (PIC) value for the SSR and AFLP marker systems was 0.41 and 0.47, respectively. Primer MJM136 for SSR and primer combination E-AGG/M-CTA for AFLP showed the highest PIC values (0.51 and 0.50, respectively), indicating they were the most informative primers for the assessment of genetic diversity in jute and that SSR and AFLP markers are useful for distinguishing jute genotypes. The PIC value for *C. olitorius* was relatively higher (0.45) than for *C. capsularis* (0.43). Cluster analysis based on the unweighted pair group method of arithmetic means clearly classified the genotypes of the two jute species into two main clusters. The results from the analysis of molecular variance revealed 81% molecular variation between species but it was low (19%) within species. The most diverse genotypes were identified as IND4546, TAN4231 and BRA4794 in *C. olitorius* and CVL-1, BAN2596C and CHI4995C in *C. capsularis* and these could be used as the most diverse genetic material in future breeding programs for jute improvement.


**Keywords:** amplified fragment length polymorphism (AFLP), analysis of molecular variance (AMOVA), genetic diversity, jute, simple sequence repeat (SSR)

## INTRODUCTION

Jute (*Corchorus* spp.) is a natural bast fiber crop and has considerable commercial significance in the world after cotton, as it is a completely biodegradable, recyclable and environmentally friendly lingo-cellulose fiber (Kundu, 1951; Mir *et al*., 2008). The genus *Corchorus* consists of about 100 species (Saunders, 2001) of which *C. capsularis* L. and *C. olitorius* L. are commercially cultivated worldwide (Hossain *et al*., 2002). The centre of origin of *C. capsularis* is said to be Indo-Burma while for *C. olitorius*, it is Africa (Kundu, 1951).

In recent years, the average yield of jute in India and Bangladesh, the main producers in the world, has slightly increased from 1.93 $t.ha^{-1}$ in 2000–2001 to 2.35 $t.ha^{-1}$ in 2009–2010 (Bangladesh Bureau of Statistics, 2010; Statistical Year Book India, 2012) despite the expanding global demand for natural fibers because there has been little work on breeding new and superior varieties. In addition, the two cultivated species of jute are different in terms of growth habitat, disease

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and pest resistance, and characteristics related to fiber and seed yield (Kundu, 1951; Edmonds, 1990). For example, *C. olitorius* is relatively tolerant to diseases and pests, and produces a stronger fiber than *C. capsularis*, whereas *C. capsularis* is more tolerant to flood and drought than *C. olitorius* (Roy *et al.*, 2006). Combining the most desirable traits of the two species would be advantageous. Unfortunately, these two species are cross-incompatible possibly because of the presence of a strong sexual incompatibility barrier between them (Patel and Datta, 1960). However, if jute is to be genetically improved, the divergent genotypes within species must be identified.

In the past, the genetic diversity in jute has been studied but mainly using morpho-physiological traits. This method is slow and unreliable, and phenotypic identification based on morphological traits is subject to environmental variation (Palit *et al*., 1996; Cooke, 1999). This limitation can be overcome by the use of molecular markers that are not influenced by the environment and are useful for the evaluation of genetic diversity, germplasm collections and marker-assisted breeding (Mir *et al*., 2008; Banerjee *et al*., 2012).

Among molecular markers, simple sequence repeat (SSR) has become established as an excellent tool for studying genetic diversity due to several desirable attributes including abundance, its multiallelic and codominant nature, high level of reproducibility and cross-species transferability (Gupta *et al*., 1996; Gupta and Varshney, 2000). An SSR marker has been shown to be almost twice as informative as an random-amplified polymorphic DNA (RAPD) marker and much more informative than restriction fragment length polymorphism in soybean (Powell *et al*., 1996). On the other hand, amplified fragment length polymorphism (AFLP) is a high multiplex polymerase chain reaction (PCR)-based dominant marker (Vos *et al.*, 1995). It has the potential to generate large numbers of polymorphic loci (Powell *et al.*, 1996). These two markers (SSR and AFLP) have been used for the study of genetic diversity in almost all crops including jute (Basu *et al*., 2004; Lukonge *et al*., 2007; Setotaw *et al*., 2010; Singh *et al*., 2010; Das *et al*., 2011; Banerjee *et al*., 2012). Although some genetic diversity studies have been carried out with molecular markers to evaluate the genetic variation in jute (Basu *et al*., 2004; Roy *et al*., 2006; Akter *et al*., 2008; Huq *et al*., 2009; Banerjee *et al*., 2012), very little information is available for the study of genetic diversity in jute by combining SSR and AFLP molecular markers. Under the above circumstances, the objectives of this research were: i) to determine the genetic diversity and population structure between and within the two cultivated species of jute, ii) to identify the most divergent genotype(s) of jute and iii) to evaluate the SSR and AFLP markers useful for distinguishing jute genotypes in this study.

## MATERIALS AND METHODS

### Plant materials

Sixty-three genotypes of the two cultivated species of jute were selected on the basis of diverse geographical location, which represented 10 countries on the three continents of Asia, Africa and America (Table 1). Two cultivars (BJC-83 and CVL-1) and 37 accessions of *C. capsularis* and one cultivar (O-9897) with 23 accessions of *C. olitorius* were evaluated (Ghosh *et al*. 2013). The materials were provided by the Bangladesh Jute Research Institute.

### DNA extraction

The DNA of each of the 63 genotypes listed in Table 1 was extracted from young leaves of plants aged 30 d grown in a glasshouse of the Department of Agronomy, Faculty of Agriculture, Bangkhen Campus, Kasetsart University, Thailand following the modified CTAB method (Haque *et al*., 2004). The quality and concentration of DNA were checked on 1% agarose gel and compared with standard lambda DNA (Fermentas; Burlington, Ontario Canada). All samples were

stored at -20 °C before analysis.

**PCR reaction and amplification for SSR analysis**

Nineteen SSR primers (Mir *et al.*, 2008, 2009) were used for screening eight genotypes of both species of jute. Of these, six SSR primers (Table 2) produced clear polymorphic bands in this study. PCR reactions were performed in 10 µL reaction volumes containing approximately 20 ng of jute template DNA, 1X PCR buffer [(750 mM Tris-HCl (pH 8.8 at 25°C), 200 mM $(NH_4)_2SO_4$ 0.1% (v/v)], 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 µM of each primer and 0.3 unit of *Taq* DNA polymerase (Fermentas; Burlington, Ontario Canada). The reaction mixture was subjected to PCR amplification in a T1 Thermocycler (Biometra; Goettingen, Germany) using a PCR program of: preheating for 2 min at 94 °C; 30 cycles, each for 30 s at 94 °C (denaturation), 30 s at the annealing temperature of a particular primer pair (ranges were 56–60 °C), and 30 s at 72 °C for 1 min (extension) and a final extension at 72 °C for 5 min followed by cooling at 16 °C for an indefinite time.

**PCR reaction and amplification for AFLP analysis**

AFLP analysis was performed following the method of Vos *et al*. (1995) with minor modifications. For each sample, the templates were prepared by digesting 250 ng DNA with the restriction enzyme combination *Eco*RI-*Mse*I and by ligating with T4 DNA ligase (Fermentas; Burlington, Ontario, Canada) the corresponding oligonucleotide adaptors in a total volume of 10 µL. The ligation products were diluted 10 times with deionized $H_2O$. Pre-selective PCR amplification with primers corresponding to adaptor core sequences (E+A and M+C) was performed in a 25 µL reaction containing 2.5 µL of AFLP template. PCR contained 1X PCR buffer [750 mM Tris-HCl (pH 8.8 at 25 °C), 200 mM $(NH_4)_2SO_4$ 0.1% (v/v)], 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM dNTP and 1U *Taq* DNA polymerase and was performed using the $T_1$ Thermocycler. The temperature profile for pre-amplification involved: 20 cycles: 30 s at 94 °C for DNA denaturation, 1 min at 56 °C for annealing, and 1 min at 72 °C for extension, and finally a hold for 5 min at 72 °C before storing the sample

**Table 1** *C. capsularis* L. and *C. olitorius* L. genotypes used in this study.

| No. | *C. capsularis* | Status | Country/ Origin | No. | *C. capsularis* | Status | Country/ Origin | No. | *C. olitorius* | Status | Country/ Origin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | CVL-1 | C | Bangladesh | 26 | CHI/4937 | A | China | 40 | O-9897 | C | Bangladesh |
| 2 | BJC-83 | C | Bangladesh | 27 | CHI/4939 | A | China | 41 | KEN/3705 | A | Kenya |
| 3 | BAN/2596 | A | Bangladesh | 28 | CHI/4955 | A | China | 42 | KEN/3727 | A | Kenya |
| 4 | BAN/2749 | A | Bangladesh | 29 | CHI/4958 | A | China | 43 | KEN/3728 | A | Kenya |
| 5 | BAN/2753 | A | Bangladesh | 30 | CHI/4964 | A | China | 44 | KEN/3732 | A | Kenya |
| 6 | BAN/2761 | A | Bangladesh | 31 | CHI/4965 | A | China | 45 | KEN/3784 | A | Kenya |
| 7 | BAN/3465 | A | Bangladesh | 32 | CHI/4988 | A | China | 46 | KEN/3835 | A | Kenya |
| 8 | BAN/3466 | A | Bangladesh | 33 | CHI/4995 | A | China | 47 | TAN/4177 | A | Tanzania |
| 9 | BAN/3473 | A | Bangladesh | 34 | CHI/5000 | A | China | 48 | TAN/4178 | A | Tanzania |
| 10 | TAI/4374 | A | Taiwan | 35 | CHI/5002 | A | China | 49 | TAN/4189 | A | Tanzania |
| 11 | THA/4467 | A | Thailand | 36 | NEP/5062 | A | Nepal | 50 | TAN/4191 | A | Tanzania |
| 12 | THA/4520 | A | Thailand | 37 | NEP/5063 | A | Nepal | 51 | TAN/4231 | A | Tanzania |
| 13 | NEP/4588 | A | Nepal | 38 | NEP/5064 | A | Nepal | 52 | THA/4461 | A | Thailand |
| 14 | TAN/4617 | A | Tanzania | 39 | NEP/5065 | A | Nepal | 53 | THA/4466 | A | Thailand |
| 15 | BRA/4619 | A | Brazil | | | | | 54 | IND/4546 | A | Indonesia |
| 16 | BRA/4620 | A | Brazil | | | | | 55 | NEP/4566 | A | Nepal |
| 17 | BRA/4621 | A | Brazil | | | | | 56 | NEP/4574 | A | Nepal |
| 18 | THA/4683 | A | Thailand | | | | | 57 | NEP/4579 | A | Nepal |
| 19 | NEP/4701 | A | Nepal | | | | | 58 | NEP/4580 | A | Nepal |
| 20 | NEP/4705 | A | Nepal | | | | | 59 | CHI/4739 | A | China |
| 21 | NEP/4711 | A | Nepal | | | | | 60 | CHI/4740 | A | China |
| 22 | CHI/4724 | A | China | | | | | 61 | BRA/4792 | A | Brazil |
| 23 | CHI/4727 | A | China | | | | | 62 | BRA/4794 | A | Brazil |
| 24 | CHI /4731 | A | China | | | | | 63 | NIG/4796 | A | Niger |
| 25 | NEP/4879 | A | Nepal | | | | | | | | |

C and A indicate cultivar and accession, respectively; No. = Number

at 4 °C. Amplification products were diluted 10 times with deionized $H_2O$, and 5 µL were used for selective amplification in a total volume of 20 µL containing 1X PCR buffer [750 mM Tris-HCl (pH 8.8 at 25 °C), 200 mM $(NH_4)_2SO_4$ 0.1% (v/v)], 0.25 µM of each E-specific and M-specific primer extended by each of three selective nucleotides (Table 3), 0.2 mM dNTPs, and 1 U of *Taq* DNA polymerase. PCR was performed using a touchdown protocol with initial denaturation of a cycle of 30 s at 94 °C, annealing for 30 s at 65 °C for the first cycle, after which the temperature was lowered by 0.7 °C following each cycle until it reached 56 °C, with extension for 1 min at 72 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 56°C and 1 min at 72 °C.

**Electrophoresis and staining of PCR products**

After amplification, 10 µL of formamide loading dye (95% Formamide, 0.25% Bromophenol blue and 0.25% Xylene cyanol) was mixed with PCR products for both the markers. The amplification products were separated by electrophoresis in 6% denaturing polyacrylamide gels (Sequi-Gen1 GT Nucleic Acid Electrophoresis Cell, Bio-Rad) at 45 W constant powers for 2 h and visualized by silver staining as described by Benbouza *et al*. (2006). The band sizes were estimated by comparison with a 10 bp ladder (Invitrogen; Carlsbad, CA, USA).

**Statistical analysis**

The scoring of bands was marked as present (1) or absent (0) for each SSR and AFLP marker allele, and data were entered in a binary data matrix as discrete variables. Jaccard's coefficient of similarity was calculated, and a dendrogram was constructed using the unweighted pair group method of arithmetic means (UPGMA). The computer package NTSYS-PC, version 2.02 (Rohlf, 1998) was used for cluster analysis. Genetic diversity parameters, that is, the number of different alleles (Na), the number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He) and unbiased expected heterozygosity (UHe) were calculated using GenAlEx (Genetic Analysis in Excel) v.6.3 (Peakall and Smouse, 2006). GenAlEx is unique in that distance matrices can be generated for multiple marker types, including codominant, haploid and binary genetic data sets. Analysis of molecular variance (AMOVA) was performed using Nei's genetic distance matrix according to Nei (1972) in GenAlEx v.6.3. To visualize differences between populations, principal coordinate analysis (PCoA) was conducted using GenAlEx v.6.3. This multivariate approach was chosen to complement the cluster analysis information because cluster analysis is more sensitive with closely related individuals, whereas PCoA is more informative regarding distances among major groups (Hauser and Crovello, 1982). The polymorphism information content (PIC) was calculated according to the simplified formula of Anderson *et al*. (1993) as shown in Equation 1:

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} P_{ij}^2 \qquad (1)$$

where P*ij* is the frequency of the $j^{th}$ allele for the $i^{th}$ marker and is summed over n alleles.

## RESULTS AND DISCUSSION

**SSR analysis**

Out of the 19 pairs of SSR primers, only 6 pairs produced polymorphic bands and therefore, these 6 pairs were selected for use in the analysis of the genetic diversity of jute. The polymorphism was seen by SSR efficiently distinguishing all genotypes of jute. A total of 37 alleles were identified among the 63 jute genotypes with an average of 6.17 alleles per locus (Table 2), which was higher than that reported (4.61) by Akter *et al*. (2008) involving 23 SSR primers with 10 jute cultivars, but very close (6.33) to the finding of Huq *et al*. (2009) using 27 SSR primers with 16 jute genotypes of *C. olitorius* and *C. capsularis*. Differences in the average number of alleles per locus may arise from using different combinations

**Table 2** Simple sequence repeat (SSR) primers, number of alleles and polymorphism information content (PIC) at 6 SSR loci in 63 genotypes of jute.

| SSR primer | Number of alleles | PIC |
|---|---|---|
| J47 | 6 | 0.48 |
| MJM6 | 8 | 0.35 |
| MJM136 | 5 | 0.51 |
| MJM600 | 6 | 0.40 |
| MJM912 | 5 | 0.34 |
| MJM916 | 7 | 0.37 |
| Total | 37 | na |
| Average | 6.17 | 0.41 |

MJM = Meerut jute microsatellite; na = Not applicable.

of genotypes and different loci in the present study. The PIC value, a reflection of allelic diversity and frequency among the jute genotypes analyzed, ranged from 0.34 to 0.51, with an average of 0.41. The highest PIC value (0.51) was observed in the MJM136 locus, indicating that this was the most informative primer in this study.

**AFLP analysis**

Twelve selected AFLP primer combinations generated a total of 548 reproducible amplification bands across all jute genotypes, among which, 458 bands were polymorphic with an average of 38.16. The average number of polymorphic bands was higher than previously reported by Basu *et al*. (2004) in jute (30.5) and Das *et al*. (2011) in *C. capsularis* (32.3) and in *C. olitorius* (31.5). The highest (89.13%) and lowest (66.67%) percentages of polymorphism were produced by the primer combinations E-ACA/M-CTA and E-AAC/M-CAG with an average of 83.42%. The high average percentage polymorphism indicates significant genetic variation among populations of the two species. Average polymorphism values of 51.72% and 41.95% have been reported by Benor *et al*. (2012) in wild accessions and cultivars of jute, respectively. Moreover, a high percentage range of polymorphism (16–80%) in jute based on AFLP has also been reported by Basu *et al*. (2004).

The PIC values ranged from 0.44 (E-ACA/M-CTA) to 0.50 (E-AGG/M-CTA) with an average of 0.47 (Table 3). The PIC value provides an estimate of the discriminatory power of a marker. The highest PIC value (0.50) was observed in the primer combination E-AGG/M-CTA, which indicates it is the most informative primer combination to study genetic diversity among jute genotypes in future studies. This result shows its usefulness for germplasm identification in jute.

**Polymorphism information content of two jute species**

The PIC values of the two species of jute are presented in Table 4. The average PIC value of the two species was 0.44. The PIC value was relatively higher (0.45) in *C. olitorius* ($P_2$) than in *C. capsularis* ($P_1$) (0.43) indicating the allelic diversity and frequency was greater in $P_2$ compared to $P_1$ in the present study. Similar results were reported by Mir *et al*. (2008), who found that the PIC value in *C. olitorius* was relatively higher (0.24) compared to *C. capsularis* (0.23).

**Genetic differentiation in the two jute species**

A summary of the mean values of the genetic diversity parameters (Na, Ne, I, He and UHe) for each population and the combined total population are shown in Table 5. The values of

**Table 3** Total number of bands, proportion of polymorphic bands and polymorphism information content (PIC) for each amplified fragment length polymorphism primer pair used to evaluate genetic diversity in 63 genotypes of jute.

| Primer pair | Number of bands | Number of polymorphic bands | Polymorphic bands ( % ) | PIC |
|---|---|---|---|---|
| E-AAC / M-CTA | 52 | 45 | 86.54 | 0.47 |
| E-AAC / M-CAT | 62 | 51 | 82.26 | 0.49 |
| E-AAC / M-CAG | 36 | 24 | 66.67 | 0.45 |
| E-AAC / M-CAC | 50 | 38 | 76.00 | 0.49 |
| E-AAG / M-CAG | 51 | 43 | 84.31 | 0.48 |
| E-AAG / M-CAC | 52 | 43 | 82.69 | 0.49 |
| E-ACA / M-CTA | 46 | 41 | 89.13 | 0.44 |
| E-ACA / M-CAT | 54 | 48 | 88.89 | 0.48 |
| E-ACA / M-CAG | 30 | 26 | 86.67 | 0.46 |
| E-ACA / M-CAC | 33 | 29 | 87.88 | 0.46 |
| E-AGG / M-CTA | 34 | 28 | 82.35 | 0.50 |
| E-AGG / M-CAC | 48 | 42 | 87.50 | 0.45 |
| Total | 548 | 458 | na | na |
| Average | 45.67 | 38.16 | 83.42 | 0.47 |

Na = Not applicable.

**Table 4** Polymorphism information content (PIC) of two jute species in this study.

| Population | PIC |
|---|---|
| *C. capsularis* ($P_1$) | 0.43 |
| *C. olitorius* ($P_2$) | 0.45 |
| Average | 0.44 |

$P_1$ and $P_2$ indicate population 1 and 2, respectively.

**Table 5** Mean and standard error (SE) of different diversity parameters involving genotypes of *C. capsularis* (39) and *C. olitorius* (24) using simple sequence repeat and amplified fragment length polymorphism markers.

| Population | | N | Na | Ne | I | He | UHe |
|---|---|---|---|---|---|---|---|
| *C. capsularis* ($P_1$) | Mean | 37.843 | 1.036 | 1.147 | 0.154 | 0.095 | 0.097 |
| | SE | 0.084 | 0.041 | 0.011 | 0.010 | 0.007 | 0.007 |
| *C. olitorius* ($P_2$) | Mean | 23.242 | 1.171 | 1.240 | 0.219 | 0.143 | 0.146 |
| | SE | 0.054 | 0.040 | 0.015 | 0.012 | 0.008 | 0.008 |
| Total | Mean | 30.543 | 1.103 | 1.193 | 0.187 | 0.119 | 0.121 |
| | SE | 0.235 | 0.029 | 0.010 | 0.008 | 0.005 | 0.005 |

Na = Number of different alleles, Ne = Number of effective alleles, I = Shannon's information index, He = Expected heterozygosity and UHe = Unbiased expected heterozygosity.

all genetic diversity parameters were higher in *C. olitorius* ($P_2$) than in *C. capsularis* ($P_1$) suggesting there is a higher level of genetic diversity in *C. olitorius* genotypes than in *C. capsularis*. Roy *et al*. (2006) and Das *et al*. (2011) reported a higher level of gene diversity in *C. olitorius* than in *C. capsularis*, which is in accord with this finding. A higher genetic diversity may imply a higher level of natural outcrossing in *C. olitorius* compared to *C. capsularis*. Previously, Ghose and Gupta (1945) reported a relatively higher percentage of natural cross pollination within *C. olitorius* (8–12%) than within *C. capsularis* (3–4%).

**Cluster analysis**

Cluster analysis based on the genetic similarity matrix derived from SSR and AFLP combined data divided the 63 genotypes from *C. olitorius* and *C. capsularis* into two distinct clusters (similarity value 0.03), which suggested that significant divergence existed in the DNA sequence between the two cultivated species of jute. This distinction could have been due to the different modes of evolution of the species together with natural self-pollination and the strong sexual incompatibility barrier existing between these two cultivated species of jute (Kundu, 1951; Patel and Datta, 1960). This pattern of clustering is in agreement with that previously obtained using molecular markers such as SSR and AFLP (Basu *et al*., 2004); sequence tagged microsatellite site, inter simple sequence repeat and RAPD (Roy *et al*., 2006) and SSR (Akter *et al*., 2008). Thus, this finding further strengthens the earlier concept that the two species originated from two different geographical locations; C. *capsularis* originated from the Indo-Burma region and *C. olitorius* from Africa (Kundu, 1951). Within the cultivated species, the 24 *C. olitorius* genotypes were subdivided into four subclusters (Figure 1). Subcluster Ia comprised five genotypes collected from Indonesia, Tanzania, Nigeria and Brazil. The Indonesian (IND4546) and Tanzanian (TAN4231) genotypes were the most divergent in this subgroup (similarity values 0.69 and 0.71, respectively). Subcluster Ib consisted of 14 genotypes from 6 countries–namely, Tanzania, Bangladesh, Nepal, China, Thailand and Kenya. Subcluster Ic was composed of four genotypes collected from Kenya and Thailand. However, one Kenyan genotype, KEN3705, formed a separate cluster (subcluster Id), quite distant from the others in *C. olitorius*. Cluster II was divided into five subgroups. Subgroup IIa comprised eight genotypes, all of which were collected from Bangladesh, including one cultivar (BJC-83), representing the same maternal origin. Subgroup IIb consisted of two genotypes from China. Subgroup IIc was composed of three genotypes, of which two came from China and the other from Nepal. Subgroup IId, a large subgroup, comprised 25 genotypes from China, Nepal, Brazil, Thailand, Tanzania and Taiwan, of which genotypes NEP4705C and BRA4621C clustered at a higher similarity value (0.97), indicating a close interrelationship between them. CVL-1, a Bangladeshi popular cultivar was distinctly isolated (similarity value 0.39) from the rest of the *C. capsularis* genotypes in subgroup IIe, indicating molecular differentiation, which might have occurred during selection after cross-hybridization.

The lack of discrimination between geographical origin and molecular differentiation in jute genotypes (except in subcluster IIa) may have been due to seed trade or germplasm exchange across boundaries. The results of Basu *et al*. (2004) and Roy *et al*. (2006) are in agreement with this finding, since they reported that genotypes of a particular location do not affect genetic diversity in jute.

**Principal coordinate analysis**

PCoA based on the genetic similarity matrix was used to visualize the genetic relationship of genotypes, which is mainly explained by the first two principal coordinates. The first and second principal coordinates explained 89.87%, and 4.01% molecular variation of the total, respectively. In the

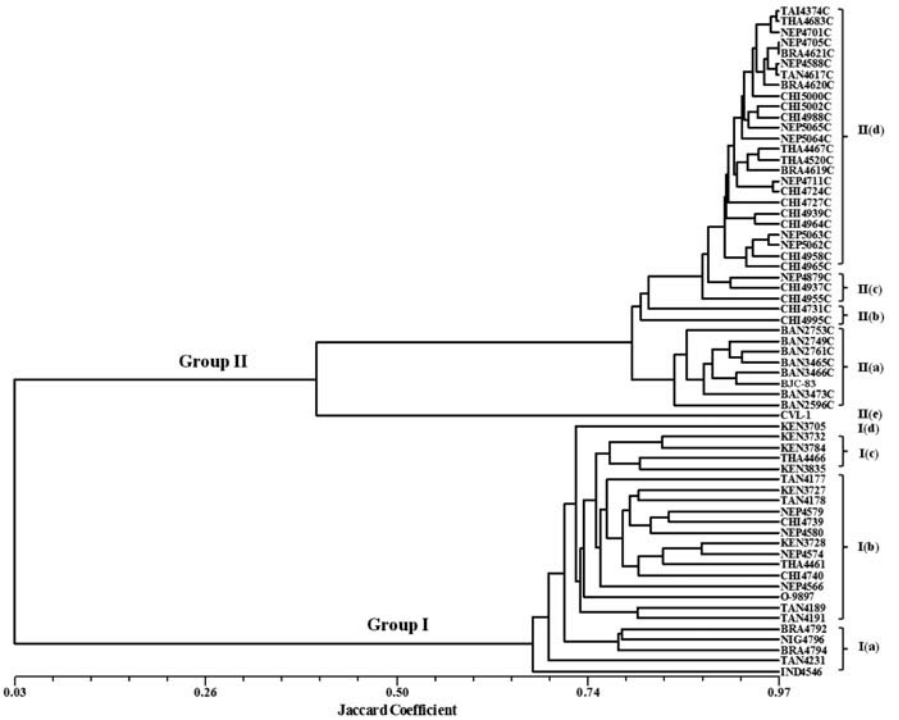


**Figure 1** Dendrogram of 63 jute genotypes produced by clustering using unweighted pair group method of arithmetic means based on the genetic similarity matrix derived from combined data of single sequence repeat and amplified fragment length polymorphism markers. Genotypes that contain a capital letter C on the right side indicates *C. capsularis* except for BJC-83 and CVL-1.

analysis, the genotypes of the two species of jute separated and formed two distinct groups (Figure 2a). The genotypes of *C. olitorius* were placed to the left, whereas the genotypes of *C. capsularis* were placed to the right of the two dimensional plane. Most of the genotypes of each species were placed very close to each other suggesting similarity among them. Only a few genotypes of each species, such as CVL-1 in *C. capsularis*, were scattered to some extent, which indicated greater diversity. The grouping of the genotypes of the two jute species was in general agreement with the cluster analysis in this study. A similar grouping pattern of the two cultivated species of jute was previously observed by Basu *et al*. (2004) using 8 SSR primers and 10 combinations of AFLP primers with 49 jute genotypes and by Banerjee *et al*. (2012) involving 172 SSR primers with 292 jute genotypes.

**Analysis of molecular variance**

Analysis of molecular variance (AMOVA) was chosen for partitioning the total molecular variation present at the inter- and intra-specific levels. The AMOVA results (Table 6) showed that most of the molecular variance (81%) was distributed between species and not within species (19%). This was possibly due to self-pollinating behavior, a strong sexual incompatibility barrier at the interspecific level and high selection pressure during domestication. Similar findings were reported by Banerjee *et al*. (2012) in jute, who observed that 63% molecular variance existed between species (*C. capsularis* and *C. olitorius*)

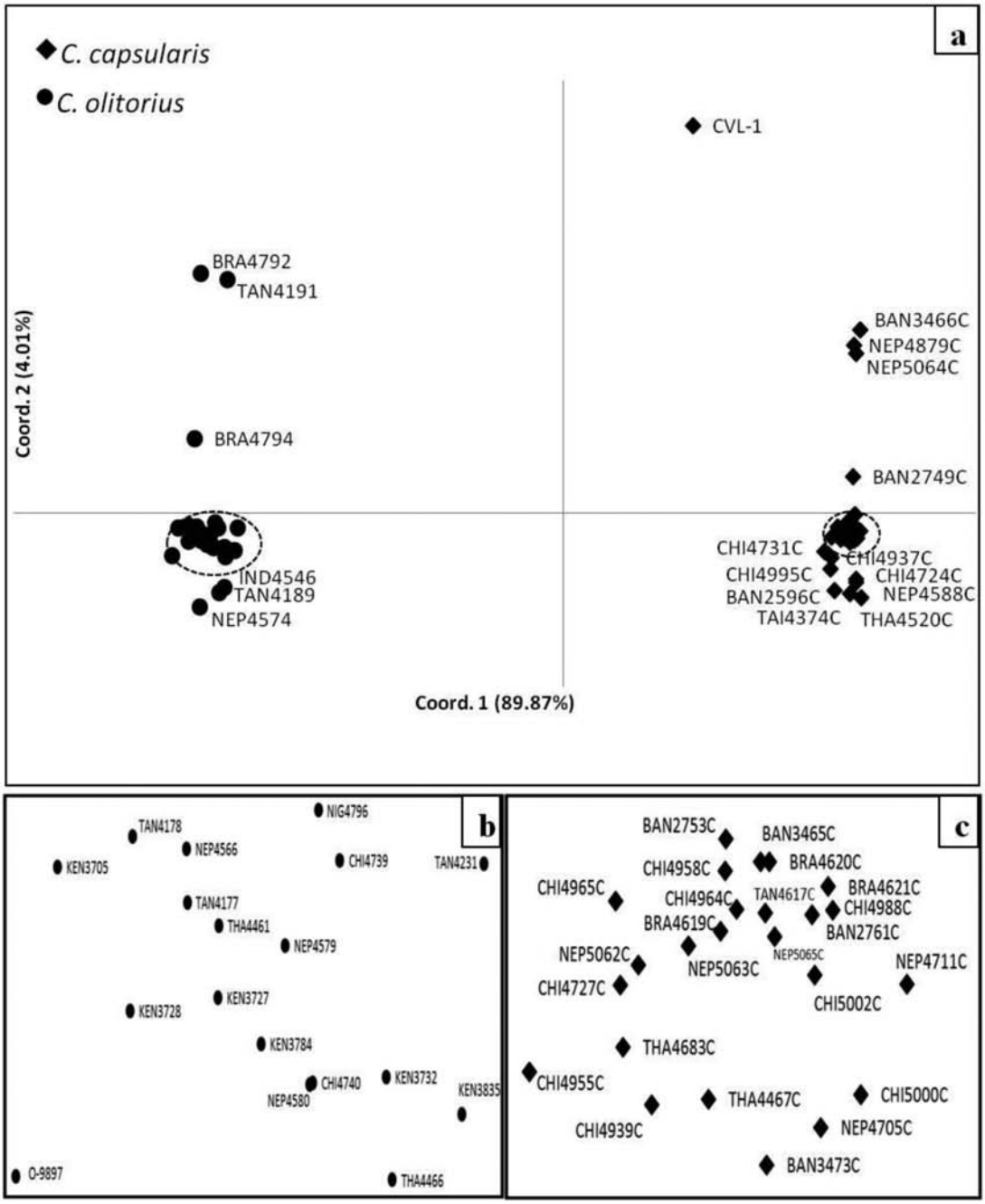


**Figure 2** Principal coordinate analysis of 63 jute genotypes based on the combined data from single sequence repeat and amplified fragment length polymorphism markers. The details of genotypes are presented in Table 1: (a) grouping results by principal coordinate analysis of the two species; (b) and (c) overlapped areas of the genotypes of *C. olitorius* and *C. capsularis*, respectively, in the circled areas of (a).

**Table 6** Analysis of molecular variance of the jute genotypes using single sequence repeat and amplified fragment length polymorphism markers to determine genetic diversity.

| Source | df | SS | MS | Estimated variance | % | *P* value |
|---|---|---|---|---|---|---|
| Between species | 1 | 4430.592 | 4430.592 | 147.904 | 81 | 0.001 |
| Within species | 61 | 2178.837 | 35.719 | 35.719 | 19 | 0.001 |
| Total | 62 | 6609.429 | | 183.623 | 100 | |
| Stat | Value | *P*(rand >=data) | | | | |
| ΦPT | 0.805 | 0.001 | | | | |

Df = Degrees of freedom; SS = Sum of squares; MS = Mean square; ΦPT = PhiPT.

and only 37% within species. Permutation tests suggested that the overall PhiPT (ΦPT) was significantly different from the null distribution (ΦPT = 0.805, P = 0.001), which indicated the existence of significant differences among genotypes.

## CONCLUSION

The molecular variation between species was high but was low within species. Therefore, to broaden the genetic variability at the intraspecific level, it is essential to undertake intraspecific cross-hybridization programs involving divergent genotypes of jute. Moreover, advanced technologies, such as somatic hybridization, chromosome doubling, embryo rescue and genetic transformation could be used to overcome the sexual incompatibility barrier between the two cultivated species of jute. In this study, the most divergent genotypes—namely, IND4546, TAN4231 and BRA4794 in *C. olitorius* and CVL-1, BAN2596C and CHI4995C in *C. capsularis*—were identified in cluster analysis, which could be used in hybridization programs for improvement of jute.

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