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Genetic diversity assessment in Indian jute (*Corchorus* spp) cultivars using gene-targeted molecular markers

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ABSTRACT

During the last two decades a number of molecular marker techniques like RAPD, AFLP, ISSR etc. have been used in crop plants for various applications. With the genomics research gaining momentum, many novel gene-targeted markers which are derived from polymorphic sites within the genes have been developed that can be used for diversity analysis. In the present study, 31 jute cultivars belonging to two cultivated species *Corchours olitorius* and *C. capsularis* were evaluated using novel, genomics-information-related technique called Start Codon Targeted (SCoT) markers for the first time. Twenty-two SCoT primers resulted in 67.3% and 62.4% polymorphism in *C. olitorius* and *C. capsularis*, respectively. The extent of genetic diversity observed was comparable to earlier findings using other marker techniques. All the cultivars could be unequivocally differentiated from one another. Results on the pattern of diversity observed and the utility of these markers in jute improvement are discussed.

Key words: *Corchorus* spp, Gene-targeted markers, Genetic diversity, Molecular markers

Jute (*Corchorus* sp. 2n = 14) is an important fibre crop that dominates the packaging sector in India with a production of 10.37 million bales from 0.90 million ha of area in 2008–09 (http://www.dacnet.nic.in). Although raw jute occupies only 0.55% of the gross cropped area of the country yet it contributes significantly to the national exchequer. Indian export market share was around 30% of the global market and the foreign earnings from export of jute goods totaled 12 000 million during 2005–06 as against 2 330 million in early sixties. Around 80% of the total jute area in India is still confined to rainfed conditions and the crop is frequently exposed to both deficit and excess water stress during its growth stages. Besides, the jute crop is facing tough competition from synthetic fibers and production figures are showing declining trend from 2006–07 onwards, despite marginal increases in the area under cultivation. Therefore, for sustenance of its trade and for increased yield there is an urgent need for its genetic improvement.

Genetic gain in any crop is based on the available reservoir of genetic variability. A variety of DNA-based molecular marker techniques have been developed in higher plants during the last two decades for assessing this genetic diversity. The use of some of these markers such as random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), and amplified fragment length polymorphism (AFLP) has been very popular because these marker techniques generate relatively high number of DNA markers per unit assay and are technically simple. These marker techniques have been extensively used for genetic diversity assessment in many crop plants including jute (Akter *et al.* 2008, Basu *et al.* 2004, Mir *et al.* 2008, Roy *et al.* 2006, Das *et al*. 2011, Banerjee *et al*. 2012). Each of these multi-locus marker techniques represents genomic regions that are phenotypically neutral. In recent years, however, with the rapid growth of genomics research, it has become possible to scan functional regions of the genome and it has been emphasized to use gene-targeted instead of anonymous markers for assessing genetic diversity (Andersen and Lübberstedt 2003, Gupta and Rustgi 2004). These functional-region-targeted markers are derived from polymorphic sites within the genes targeting mostly exonic, intronic or promoter regions. Start Codon Targeted (SCoT) polymorphism is one such technique that is based on the short conserved region in plant genes surrounding the ATG translation start (or initiation) codon (Collard and Mackill 2009). In the present study we made use of SCoT markers for diversity analysis in jute (*Corchorus* sp.) cultivars. The main goal of the present study was to find the suitability of SCoT markers for assessing genetic relationships and to

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measure the extent of genetic diversity prevalent in a set of 31 jute cultivars belonging to two cultivated species. This is the first report on the use of SCoT markers for molecular characterization in jute.

MATERIALS AND METHODS

The experimental material for the present study consisted of 31 cultivars of jute belonging to two cultivated species namely *C. olitorius* and *C. capsularis*, popularly known as *tossa* jute and white jute, respectively. The list of these cultivars along with their pedigree, species to which they belong, year of release and breeding centre is provided in Table 1.

Total genomic DNA was extracted following CTAB method (Saghai-Maroof *et al.* 1984). DNA samples were fluorometerically quantified and were diluted in TE buffer to a working concentration of approximately 10ng/ml.

Thirtysix SCoT primers, as described by Collard and Mackill (2009) were used in the present study of which 22 resulting in good amplification are listed along with their characteristics in Table 2.

Each reaction mixture (25 ml) for PCR amplification consisted of 1 × reaction buffer (10 mM Tris, *p*H 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatine), 0.5 U of *Taq* DNA polymerase and 0.24 mM of each deoxyribonucleotide triphosphates, 0.5 mM of primer and approximately 50ng of genomic DNA template. The PCR amplification conditions were as follows: initial extended step of denaturation at 94°C for 3 min., followed by 40 cycles of denaturation at 94°C for 1 min. primer annealing at 50°C for 1 min and elongation at 72°C for 1 min. The 40th cycle was followed by an extended primer extension step at 72°C for 5 min. PCR amplification was carried out in M J Research (Model PTC 200) thermal cycler. PCR products were mixed with 2.5 ml of gel loading dye (6 × dye: 0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol in water) and spun briefly in a microfuge before loading. The PCR products were electrophoresed on 1.2% agarose gel at 100 volts in 1 × TAE buffer. A 1 kb DNA

Table 1 Jute varieties used for SCoT markers in jute

Variety	Species	Pedigree	Year of release	Centre
JRO 632	*C. olitorius*	Pureline selection from an indigenous germplasm	1954	CRIJAF, Barrackpore
JRO 878	*C. olitorius*	JRO 620 × Sudan Green	1967	CRIJAF, Barrackpore
JRO 7835	*C. olitorius*	JRO 620 × Sudan Green	1971	CRIJAF, Barrackpore
JRO 524	*C. olitorius*	Sudan Green × JRO 632	1977	CRIJAF, Barrackpore
JRO 66	*C. olitorius*	Selection from a multiple cross	1998	CRIJAF, Barrackpore
JRO 8432	*C. olitorius*	IC 15901 × Tanganyika 1	1999	CRIJAF, Barrackpore
JRO 3690	*C. olitorius*	Tobaco leaf × Long internode	1985	CRIJAF, Barrackpore
TJ40	*C. olitorius*	Cross from a mutant of JRO 632	1983	BARC Trombay
JRO 128	*C. olitorius*	TJ-6 × Tanganyika-1	2002	CRIJAF, Barrackpore
KOM62	*C. olitorius*	Gamma ray mutant of JRO 878	1993	JRS, OUAT, Kendrapara
S19	*C. olitorius*	(JRO 620 × Sudan Green) × Tanganyika 1	2005	CRIJAF, Barrackpore
Bidhan Rupali	*C. olitorius*	X- ray mutant of JRO 632		BCKV, Kalyani
Sudan Green	*C. olitorius*	Introduction from Sudan	1956	CRIJAF, Barrackpore
Tanganyika1	*C. olitorius*	Introduction from Tanganyika	1978	CRIJAF, Barrackpore
JRO 36E	*C. olitorius*	Selection from Tanganyika 1	1981	CRIJAF, Barrackpore
Chinsurah Green	*C. olitorius*	Selection from local strain in Chinsurah	1915	CRIJAF, Barrackpore
JRO 2345	*C. olitorius*	Selection from KEN/SM/024C	2004	CRIJAF, Barrackpore
JRC 212	*C. capsularis*	Selection from an indigenous germplasm	1954	CRIJAF, Barrackpore
JRC 321	*C. capsularis*	Selection from an indigenous germplasm Hewati	1954	CRIJAF, Barrackpore
JRC 7447	*C. capsularis*	X-ray mutant of JRC 212	1971	CRIJAF, Barrackpore
JRC 4444	*C. capsularis*	X- ray mutant of JRC 212	1978	CRIJAF, Barrackpore
JRC 698	*C. capsularis*	Selection from a multiple cross	1999	CRIJAF, Barrackpore
Padma	*C. capsularis*	JRC 6165 × JRC 412	1983	CRIJAF, Barrackpore
UPC94	*C. capsularis*	JRC 321 × JRC 212	1983	JRS, NDUAT, Baharaich
KC1	*C. capsularis*	Gamma ray mutant of JRC 4444	1992	JRS, OUAT, Kendrapara
KTC1	*C. capsularis*	Selection from IC30730 collected from Tripura	1994	JRS, RAU, Katihar
JRC80	*C. capsularis*	Selection from CIN-114 × JRC 321	2005	CRIJAF, Barrackpore
Bidhan Pat 1	*C. capsularis*	Gamma ray mutant of D 154	2001	BCKV, Kalyani
Bidhan Pat 2	*C. capsularis*	Selection from D 154 × D18 (mutant)	2001	BCKV, Kalyani
Bidhan Pat 3	*C. capsularis*	Selection from D 154 × D18	2001	BCKV, Kalyani
D 154	*C. capsularis*	Selection from Kakya Bombai	1919	JRL, Dhaka

Table 2 SCoT primers and their characteristics in jute cultivars

Primer	Sequence (5' to 3')	Size range	TB	PP	PIC
SCoT 09	CAACAATGGCTACCAGCA	200–1 080	5	80.0	0.46
SCoT 11	AAGCAATGGCTACCACCA	250–1 350	7	100.0	0.42
SCoT 14	ACGACATGGCGACCACGC	250–1 030	7	100.0	0.42
SCoT 15	ACGACATGGCGACCGCGA	500–2 000	6	100.0	0.32
SCoT 16	ACCATGGCTACCACCGAC	550–2 500	11	100.0	0.23
SCoT 17	ACCATGGCTACCACCGAG	270–2 000	9	100.0	0.43
SCoT 19	ACCATGGCTACCACCGGC	400–3 500	7	100.0	0.35
SCoT 21	ACGACATGGCGACCCACA	200–2 500	9	100.0	0.40
SCoT 22	AACCATGGCTACCACCAC	500–1 400	8	50.0	0.44
SCoT 23	CACCATGGCTACCACCAG	300–700	3	66.7	0.46
SCoT 24	CACCATGGCTACCACCAT	300–1 100	6	100.0	0.28
SCoT 25	ACCATGGCTACCACCGGG	780–1 500	5	80.0	0.29
SCoT 26	ACCATGGCTACCACCGTC	280–1 080	4	100.0	0.49
SCoT 27	ACCATGGCTACCACCGTG	500–2 000	7	85.7	0.30
SCoT 28	CCATGGCTACCACCGCCA	250–1 300	12	66.7	0.35
SCoT 29	CCATGGCTACCACCGGCC	500–1 400	5	60.0	0.46
SCoT 30	CCATGGCTACCACCGGCG	450–3 000	8	100.0	0.44
SCoT 31	CCATGGCTACCACCGCCT	350–1 600	10	100.0	0.39
SCoT 33	CCATGGCTACCACCGCAG	300–3 500	8	100.0	0.38
SCoT 34	ACCATGGCTACCACCGCA	600–2 100	7	57.1	0.45
SCoT 35	CATGGCTACCACCGGCCC	250–2 000	11	72.7	0.27
SCoT 36	GCAACAATGGCTACCACC	470–2 500	10	100.0	0.44

TB, Total number of bands; PB, total number of polymorphic bands; PP, per cent polymorphism; PIC, polymorphism information content

ladder was used as a molecular size standard (MBI Fermentas). The gels were stained with ethidium bromide and were photographed under UV light in gel documentation system.

For all the cultivars, bands on gels were scored as present (1) or absent (0). The binary SCoT data were subjected to produce a matrix of similarity values based on Jaccard's (1908) coefficient of similarity (J_{ij}) as follows:

$$J_{ij} = a/(n\text{-}d),$$

where, '*a'* is the number of SRAP bands present in both *i* and *j* genotypes, 'd' is the number of SCoT bands absent in both *i* and *j* and 'n' is the total number of SCoT bands. From the similarity matrix, average genetic similarity for cultivars within each species and between species was calculated. Genetic similarity value for a cultivar was calculated manually by taking average of its pair-wise Jaccard's similarity against all other cultivars. Similarity matrix was further subjected to UPGMA (Un-weighed Pair-Group Method with Arithmetic averages) clustering and genetic relationships were graphical represented in a dendrogram. Principal co-ordinate analysis (PCoA) analysis was done to verify the results of clustering and results were shown graphically. All these computations were carried out using computer software NTSYS-pc version 2.11v (Rohlf 2004). Polymorphism information content (PIC) was calculated as per Anderson *et al.* (1993).

RESULTS AND DISCUSSION

Band statistics and level of polymorphism

A total of 165 markers were observed in 31 cultivars belonging to two species of jute. Fiftytwo of these markers were specific to either *C. olitorius* or *C. capsulari* and another set of 28 markers was monomorphic between the two species. Of the 52 species-specific markers, 38 were specific to *C. olitorius* and 14 to *C. capsularis* and were recorded from 20 and 8 SCoT primers, respectively. The percent polymorphism in *C. olitorius* and *C. capsularis* was found to be 67.3% and 62.4%, respectively; however 87% polymorphism was recorded based on 165 markers common to both the species. Five primers, namely, SCoT 15, SCoT 19, SCoT 24, SCoT 27 and SCoT 33 in *C. olitorius* and three primers, namely, SCoT 16, SCoT 21 and SCoT-30 in *C. capsularis* produced all polymorphic amplicons. A representative profile of 31 jute cultivars amplified using primer SCoT 31 is shown in Fig 1.

As expected, the number of primers producing polymorphic bands was more when markers from both the species were taken into consideration rather than within either *C. olitorius* or *C. capsularis.* In the latter case 13

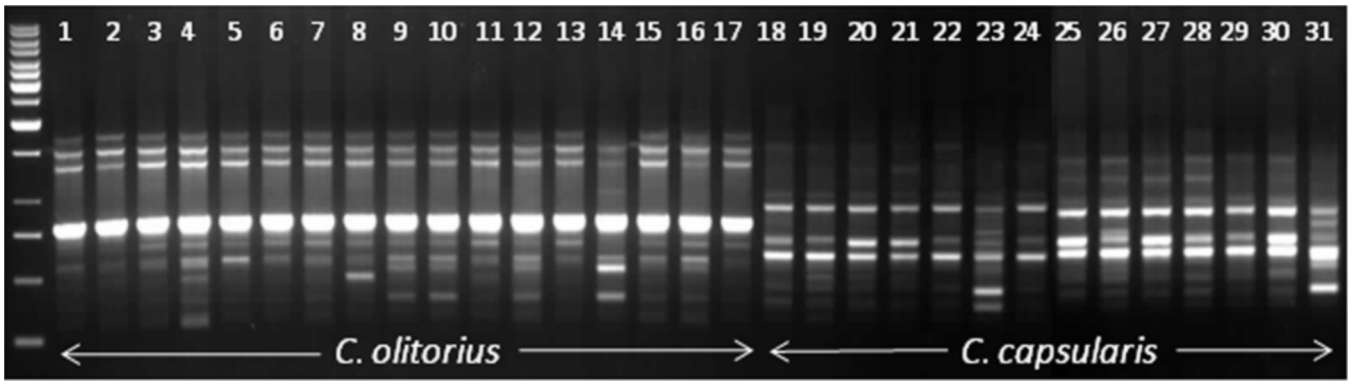

Fig 1 PCR amplification of 31 jute cultivars using primer SCoT 31. Numbers 1–31 on top represent cultivar numbers as depicted in Table 1. Left-most lane is 1 kb DNA size standard (MBI Fermentas)

primers (59%) were found to produce all polymorphic bands. Based on parameters such as per cent polymorphism and species-specific markers discussed above, it can be generalized that variability in *C. olitorius* cultivars is slightly more compared to the cultivars *C. capsularis*. However, the sample size of the two species included in the present study needs to be borne in mind while having serious consideration of this generalization. Das *et al.* (2011) using AFLP markers

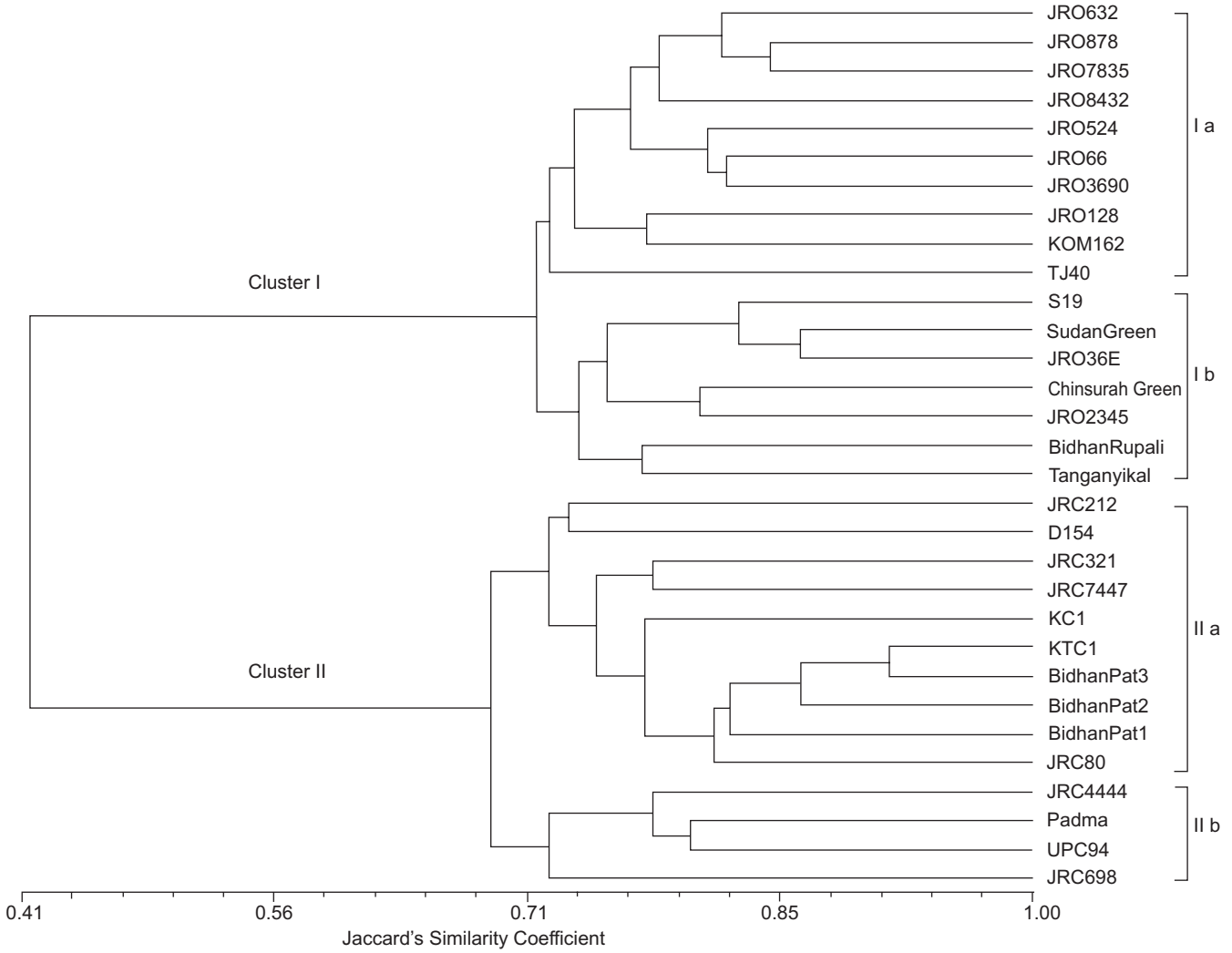

Fig 2 UPGMA clustering-based dendrogram depicting genetic relationships among 31 jute cultivars

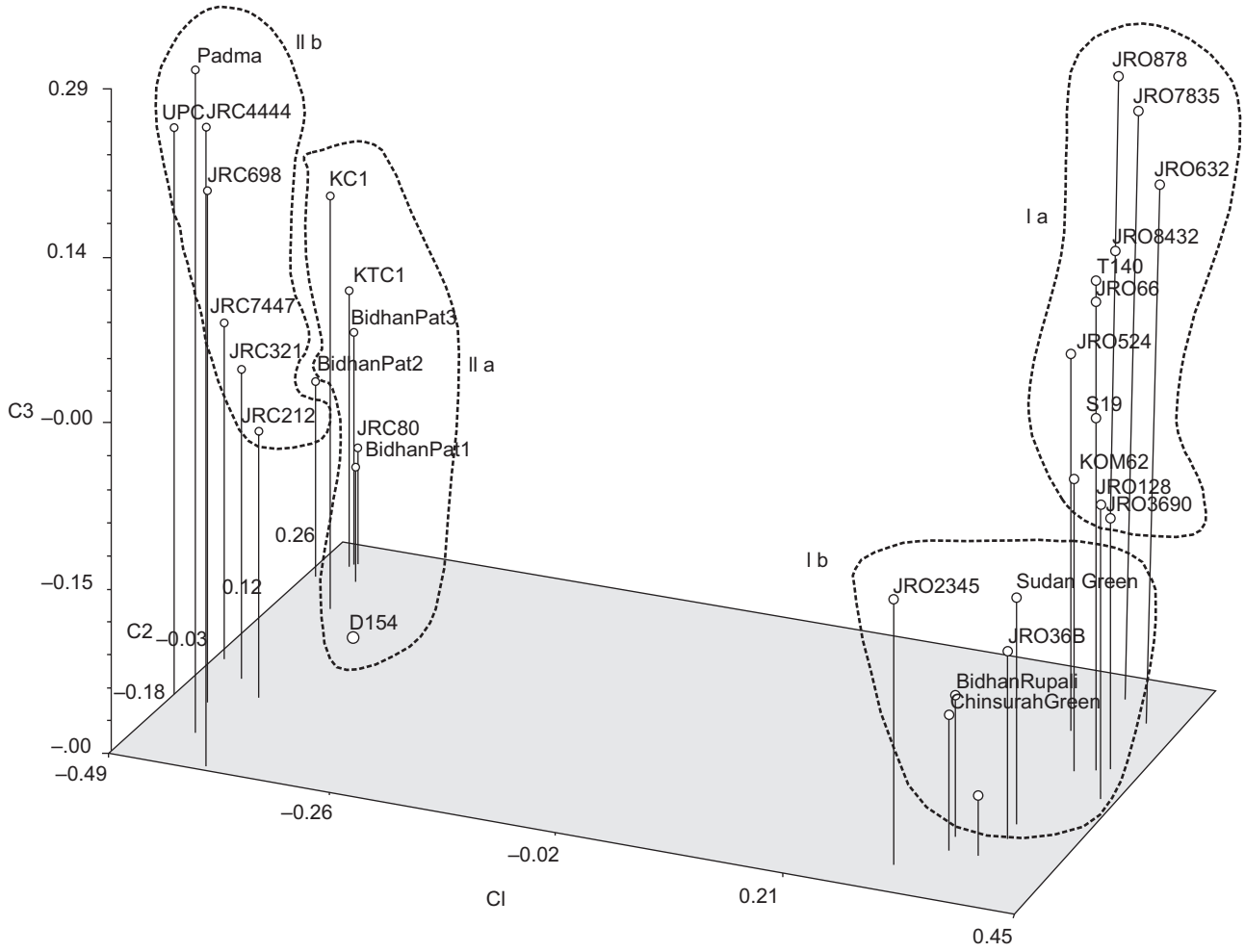

Fig 3 Principal co-ordinate analysis of 31 jute cultivars using SCoT markers

also showed higher level of polymorphism in *C. olitorius* as compared to *C. capsularis*.

Cluster analysis

UPGMA cluster analysis at 41% genetic similarity grouped all cultivars into two clusters namely Cluster I and Cluster II representative of *C. olitorius* and *C. capsularis* species, respectively (Fig 2).

Further, *C. olitorius* cultivars could be grouped into two sub-clusters Ia and Ib having 10 and 7 cultivars, respectively. Similarly, *C. capsularis* cultivars could also be grouped into two sub-clusters IIa and IIb having 10 and 4 cultivars, respectively. The magnitude of genetic similarity among *C. olitorius* and *C. capsularis* cultivars was found to be 64% and 73%, respectively. However, average similarity among 31 cultivars representing both species was found to be 59%. All the cultivars could be unequivocally differentiated from one another using SCoT markers. Mir *et al.* (2008) using genomic microsatellite markers for genetic diversity in jute observed 45% inter-specific diversity which was slightly higher than what we observed. This could be due to different set of the genetic material used for investigation in the two studies. However, the magnitude of intra-specific similarity in their study was 76% in *C. olitorius* and 77% in *C. capsularis* which was higher than observed using SCoT markers in the present study.

Genetic diversity

Pairwise comparison among *C. olitorius* cultivars showed that cultivars JRO36E and Sudan Green had maximum similarity to each other (86%), followed by JRO878 and JRO 7835 (85%) and JRO 36E and S19 (84%), whereas cultivars Tangayanika 1 and JRO878 were the most distinct ones from one another having 60% similarity which was followed by cultivars Tanganyika 1 and TJ40 (61%). Pairwise estimates of genetic similarity among the *C. capsularis* cultivars revealed that cultivars KTC 1 and Bidhanpat 3 were having the highest similarity to one another (91%) which was followed by cultivars KTC 1 and Bidhanpat 2 (87%) and Bidhanpat 3 and Bidhanpat 2 (86%). The least pairwise

similarity (or the highest dissimilarity) in *C. capsularis* cultivars was found between JRC698 and D154 (58%), followed by JRC698 and Bidhanpat 1 (59%). A look at Fig 2 also illustrates these relationships. Principle co-ordinate analysis was also carried out among jute cultivars and their genetic relationships are presented graphically in Fig 3.

The first three co-ordinates explained a total of 52% of genetic variability and were 39%, 7% and 5%, respectively. The robustness of the clustering in Fig 2 tested by 10 000 bootstraps, gave a very high confidence in support of observed clustering pattern as all nodes were having 100% bootstrap values (figures not presented).

Very high genetic similarity, or in other words low diversity, in *C. capsularis* (range 89–99%) and *C. olitorius* (range 86 – 99%) has been observed by Basu *et al.* (2004) using 305 amplicons generated from 10 AFLP primer-pair combinations. In contrast, the extent of genetic diversity in our study in *C. capsularis* (60 – 86%) and in *C. olitorius* (58–91%) is considerably higher indicating that SCoT markers target more variable regions of the genome compared to AFLP markers. Additionally, the genetic architecture of the material employed in the study of Basu *et al.* (2004) was quite diverse compared to the material in our study since we have included only cultivars which are supposed to be genetically less diverse than the germplasm used in their study. In both these studies only four genotypes were common. Roy *et al.* (2006) using pooled data from three different kind of markers, namely, STMS, ISSR and RAPD markers observed 39% inter-specific genetic similarity, whereas very high genetic similarity in the cultivars of *C. capsularis* and *C. olitorius* (98% and 90%, respectively) was observed. Nearly 50% of the cultivars of their study were common to the cultivars used in our study. All of these studies using various kinds of markers that target different genomic regions of the jute genome point towards narrow genetic base of each of the *C. capsularis* and *C. olitorius* species. Earlier workers using various other kinds of markers have also reported that the genetic base of *C. capsularis* and *C. olitorius* species is quite narrow (Roy *et al.* 2006, Akter *et al.* 2008 and Mir *et al.* 2008).

The study revealed that the new kind of gene-targeted markers which are offshoots of genomics research are useful for diversity analysis and molecular characterization of jute cultivars and can be extended to other crop plants. In order to anticipate further genetic gains, jute breeders and geneticists need to focus their attention in broadening the genetic base of the two cultivated species to avoid losses due to unforeseen vagaries of biotic or abiotic stresses. Large repertoire of jute accessions conserved in the National Genebank at NBPGR, New Delhi (1 286 accessions of *C. olitorius* and 1 316 of *C. capsularis*) can be screened for divergent accessions and exploited in breeding programs for making further strides in jute improvement. Further, as suggested by Basu *et al.* (2004), there is need to break sexual barrier for inter-specific hybridization for unlocking usable genetic variability for genetic improvement of these species. Isolation of targeted genes of interest and their incorporation into the jute genome through genetic engineering and marker-assisted selection would be an alternative strategy to improve jute production. The identified makers in this study, along with other kind of markers, would be a step in this direction once these are mapped and tagged to traits of interest.

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