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Construction of Genetic Map of Jute (*Corchorus olitorius* L.) Based on RAPD Markers

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Abstract

The first and preliminary genetic linkage map of the jute genome was constructed with RAPD markers using two parents (Variety O-9897 and Accession No. 1805) and their F_2 populations. Linkage analysis at a LOD (Log of odds base 10) score of 3.0 and a maximum distance 50 cM revealed 18 linkage groups. Among the 18 linkage groups, 15 contained single locus and the remaining three groups 16, 17 and 18 contained 2, 11 and 12 loci, respectively. The three multi locus linkage groups varying in length from 15.9 - 241.7 cM, snapped a total length of 463.7 cM with an average marker density of 19.6 cM between adjacent markers. The basic chromosome number of *Corchorus* spp. is seven (2n = 14), so in saturated map, seven linkage groups should have been obtained to represent the genome. But for linkage group analysis, the effort was very limited and the total number of loci (40) was also low.

Introduction

Jute is a dicotyledonous fibre yielding plant of the genus *Corchorus*, family Malvaceae former Tiliaceae (Barbara et al. 2003). Jute fibre is obtained from the bark of the two commercially important species, namely *Corchours capsularis* L. (White jute) and *C. olitorius* L. (Tossa jute). The yield and quality of *C. olitorius* jute is better than *C. capsularis* (Kundu 1968). The centre of origin of white jute is said to be Indo-Burma including South China, and Africa for that of Tossa (Kundu 1951).

Jute is basically self-pollinated and has 14 diploid chromosomes (2n = 14). Jute is a short-day plant. It needs long day light for vegetative growth and short-day for flowering (Anonymous 1990). As jute is a self-fertilized crop, its natural genetic variability is very narrow; this makes an obstacle to the plant breeders in the improvement of this crop. The molecular techniques could be a suitable

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alternative to improve the crop. Jute is a new crop in the field of molecular biology. Therefore, very little molecular information of jute or its related species is available at the Gene Bank, Bangladesh Jute Research Inst. (BJRI). So far very little efforts have been undertaken in the past to develop molecular markers to study its genetic variability (Hossain et al. 2002, 2003, Basu et al. 2004, Roy et al. 2006, Mir et al. 2008, Akter et al. 2008). So generation of information at the molecular level would be helpful for its improvement.

The present work was undertaken to develop a linkage or genetic map for Tossa jute. A genetic map is a linear map of the relative positions of genes along a chromosome. Distances are established by linkage analysis, which determines the frequency at which two gene loci become separated during chromosomal recombination. Genetic maps are based on the relative order and distances of genetic markers. The position of genes on a chromosome can be located via its linkage with these DNA markers. A genetic map can be built up by using enough linked pairs of markers lying at close intervals to each other (Paterson et al. 1991).

PCR-based marker technologies have expedited the construction of high-density linkage maps; facilitated genetic analysis and map based cloning (Yong et al. 2002). These techniques include RFLP (McCouch et al. 1988), RAPD (Williams et al. 1990), AFLP (Vos et al. 1995), and SSR (Tautz 1989) etc.

A mapping population is a prerequisite for the construction of a linkage map. Mapping population can be derived by selfing the F_1 to produce an F_2, which is then scored for segregation of the markers or backcross the F_1 to one of the parents and observing the segregation in the first backcross generation. It is better to use an F_2 population if this is possible, as more information can be gained from this than from a backcross population of comparable size (Winter and Kahl 1995). A mapping population of about 50 F_2 or backcross plants is sufficient for a fairly detailed map (Chawla 2002). Remnant seeds from previous crosses are also suitable for mapping populations. Recombination frequencies can also be estimated from doubled haploids (DH) derived from the pollen of F_1 plants. At first there will be many more linkage groups than the basic number of chromosomes, but the numbers will tend to converge as more markers are added (Chawla 2002).

As more markers were located on genetic maps, it became possible to detect a single genetic locus associated with the quantitative trait loci (QTL). Furthermore, the chromosomal location of the QTL was more precisely estimated, and their linkage relationships with other genes could be accurately determined (Paterson et al. 1991, Gregorio 1997).

The RAPD technique (Welsh and McClelland 1990, Williams et al. 1990) has widely been used in plants for the construction of genetic maps in species such as

Arabidopsis (Welsh and McClelland 1990), bananas (Faure et al. 1993) and slash pine (Nelson et al. 1993).

Materials and Methods

Two parents (cultivar O-9897 and accession no. 1805) were selected on the basis of low temperature tolerant- and sensitive traits. A cross was made between these two parents where cultivar O-9897 (sensitive) was the seed parent. The F_2 seeds were obtained by selfing F_1. A good number of segregating F_2 individuals (112) were raised by differentiating the low temperature tolerant (16°C) and sensitive in an Envoron Air Growth Cabinet.

Genomic DNA was extracted from young leaves of the parents, sensitive and tolerant F_2 plants following the method modified from Dellaporta et al. (1983) protocol. A total of 114 DNA samples including the two parents, 50 low temperature sensitive- and 62 tolerant F_2s were taken for marker development studies. The DNA was RNase-treated and subsequently quantified on 0.8% agarose gel by comparison with a known concentration of standard lambda (λ) DNA.

The F_2 individuals and their parents were evaluated with 40 RAPD primers (OPAB-01, 03, 05, 06, 08, 10, 12, 18, 20, OPG-03, 05, 06, 07, 08, 09, 11, 13, 15, 16, OPH-02, 03, 04, 05, 07, 10, 12, 13, 14, 15, OPQ-01, 05, 06, 07, 08, 16, 17, 20, OPE-17, OPN-02 and 13 from Operon Technologies, USA). The reaction mixture (25 µl) contained the following: 1X reaction buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 0.2 mM dNTPs, 2 mM $MgCl_2$, 60 ng primer, 1.0 unit of *Taq* DNA polymerase, and 25-40 ng genomic DNA. DNA was amplified in a thermal cycler (Eppendorf Mastercycler Gradient) that was programmed as follows: after preheating for 5 min at 95°C; 40 cycles of 1 min at 95°C (denaturation), 1 min at 40°C (annealing) and 2 min at 72°C (extension), and a final extension at 72°C for 7 min that was followed by cooling to 4°C. As RAPD is a flexible marker, the samples were amplified two or more times and only the common bands of the amplified products were scored as '1' or '0' for presence or absence of a particular band respectively. A typical gel picture is shown in Fig. 1. A genetic linkage map was constructed using MapMaker version 3.0 (Fig. 2). Linkage groups were established at a LOD score of 3.0 and a maximum distance 50 cM by two-point analysis using the 'group' command. The characteristics of the linkage group with more than 1 locus are presented in Table 1. The 18 linkage groups with their locus/loci number(s) are shown in Table 2.

Results and Discussion

The first and preliminary linkage map of the jute genome was constructed using software MapMaker version 3.0 (StatSoft 1994) considering 40 RAPD markers and their different combinations with an F_2 population developed from a cross

between O-9897 and accession no. 1805 as low temperature sensitive and tolerant, respectively. Linkage analysis at a LOD score of 3.0 and a maximum distance 50 cM revealed 18 linkage groups. Among the 18 linkage groups, 15 groups (groups 1 to 15) contained a single locus and the remaining three groups designated as linkage group-16, 17 and 18 contained 2, 11 and 12 loci, respectively (Fig. 2). The analysis revealed that the distance of 2 loci in linkage group-16 was 15.90 cM (Table 1). A total of 11 and 12 loci were mapped in a length of 241.70 and 206.10 cM with an average marker density of 24.17 and 18.73 cM between adjacent markers in the linkage group-17 and 18, respectively (Table 1). Marker density on an average obtained in the study (with 40 loci) seemed to be high. More intense markers (10.35 cM) were observed in the first RFLP map in rice constructed with 135 loci (McCouch et al. 1988).

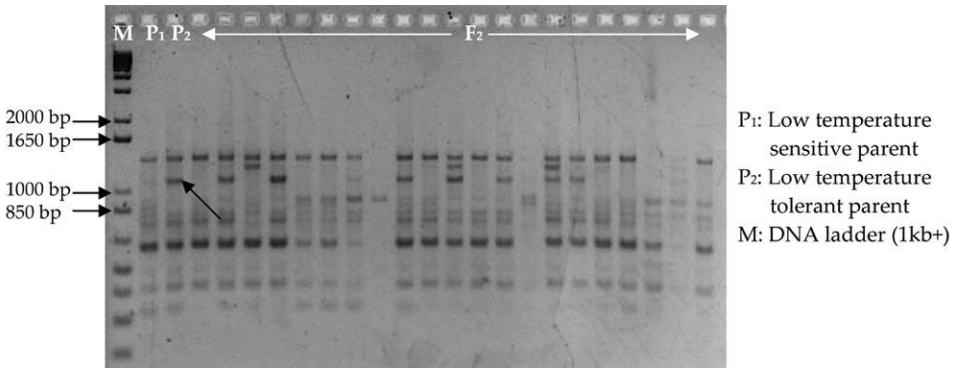

Fig. 1. Polymorphic band associated with low temperature tolerance amplified with RAPD primer OPG-05 (a model gel picture, arrow showing the polymorphic band).

Table 1. Characteristics of the intraspecific genetic linkage group with more than one locus of jute.

Linkage group	Length (cM)	Number of markers	Average distance between markers (cM)
Linkage group-16	15.90	2	15.90
Linkage group-17	241.70	11	24.17
Linkage group-18	206.10	12	18.73
Total 463.70		25 19.60	

Linkage group with a single locus: Linkage groups 1 to 15 are not mentioned in the table.

The unique basic chromosome number of *Corchorus* spp. is seven (2n=14); so seven linkage groups were expected to represent the genome. But for linkage group analysis, this effort was very limited and the total number of loci (40) was also low. In the software (MapMaker version 3.0), it was indicated that, if the distance between two adjacent loci were more than 50 cM, it would be a split point. More markers are needed to be mapped to merge smaller linkage

Table 2. Eighteen-linkage group with their locus/loci.

Linkage group	Total locus	Locus number/Primer/Size (bp)			
1	1	3.OPQ-17/450			
2	1	8.OPAB-01/660			
3	1	10.OPAB-03/1650			
4	1	12.OPAB-01/1000			
5	1	14.OPAB-08/1350			
6	1	17.OPG-11/900			
7	1	26.OPH-10/850			
8	1	29.OPH-12/550			
9	1	30.OPH-17/1200			
10	1	32.OPQ-06/950			
11	1	33.OPQ-06/650			
12	1	37.OPQ-08/500			
13	1	38.OPQ-16/750			
14	1	39.OPQ-16/450			
15	1	40.OPQ-16/400			
16	2	1. OPQ-17/760	27. OPH-10/700		
17	11	23. OPAB-20/700	25. OPH-10/1000	15. OPAB-08/700	24. OPAB-20/570
		20. OPAB-12/1050	22. OPAB-20/950	13. OPG-07/100	28. OPH-10/670
		5. OPE-17/900	21. OPAB-20/1330	2. OPQ-17/700	
18	12	36. OPQ-08/1350	34. OPQ-06/600	7. OPG-05/1100	11. OPAB-01/1350
		9. OPAB-01/150	18. OPG-10/200	35. OPQ-06/500	31. OPH-17/750
		19. OPG-13/1050	16. OPAB-08/650	4. OPE-17/950	6. OPG-05/1650

groups to larger ones. In this way, the total of 40 loci of the 18 linkage groups may be in a single chromosome i.e. in a single linkage group in the saturated mapping. It was mentioned by Chawla (2002) that the total number of linkage groups should always be more than its real number till saturation. Due to the unavailability of co-dominant markers for jute, dominant markers (RAPD) were used for mapping. Dominant markers are unable to distinguish heterozygotes from homozygotes; however, they allow many polymorphic markers to be quickly identified, which had been useful for mapping genomes such as legume crops (Menndez et al. 1997, Eujayl et al. 1998, Laucou et al. 1998, Santra et al. 2000) and and for extending the existing linkage map of rye (Masojc et al. 2001). So, the present effort could be a base map of jute, which could be enriched by using more dominant and co-dominant markers.

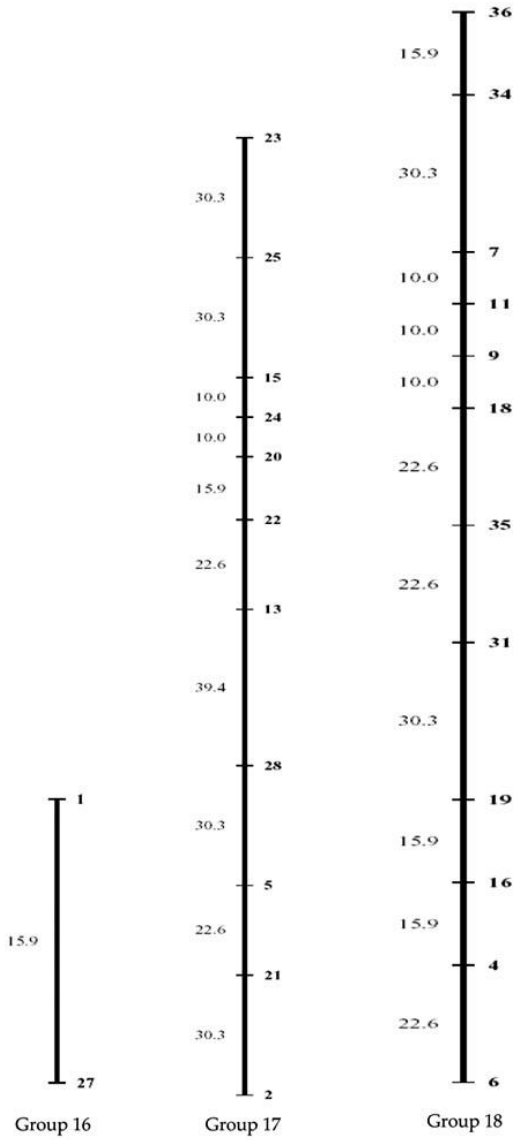

Fig. 2. Three linkage groups representing 25 RAPD loci developed from F_2.

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