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Construction of an Intraspecific Linkage Map of Jute

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Abstract: The first and very preliminary linkage map of the jute genome was constructed with eight ISSR (Inter Simple Sequence Repeat) markers and their different combinations using an F_2 population developed from a cross between jute cultivars O-9897 and Acc.1805 which differed in cold tolerance. Linkage analyses at a LOD [logarithm of the odds (to the base 10)] score of 3.0 and a maximum distance 50.0 revealed three linkage groups. The analysis revealed three linkage groups varying in length from 4.8 to 52.9 cM. The intraspecific map spanned a total length of 87.3 cM with an average marker density of 8.73 cM between adjacent markers. The utility of ISSR markers for mapping in jute was explored and the primer with a CTT repeat motif was found to be useful. ISSR primers were found to be 50% polymorphic between the parent cultivars.

Key words: Linkage map, ISSR, Jute, Polymorphism

INTRODUCTION

The development of a detailed genetic map, on which markers associated with desirable traits are identified, is a valuable tool to improve breeding efficiency. Genetic maps have been developed in almost all the agricultural crops (O'Brien, 1993). Maps developed from crosses between cultivars are the most useful for breeding applications as they identify polymorphic markers within the cultivated gene pool and are therefore more likely to be present in crosses involving other cultivated genotypes (Men_ndez *et al*., 1997). Genetic maps based on intraspecific crosses have also been recommended for the mapping of quantitative trait loci due to less segregation distortion (Havey and Muehlbauer, 1989). To date, no comprehensive jute map based on an intraspecific mapping population has been reported.

An advantage of DNA markers over morphological and isozyme markers is the high amount of polymorphism (Paterson *et al*., 1991) which can be generated to saturate linkage map. Inter-simple-sequence repeats (ISSR) have also been successfully used to map the genomes of wheat (Kojima *et al*., 1998) and chickpea (Santra *et al*., 2000; Winter *et al*., 2000). In jute, very little molecular work has been conducted and no real effort has been undertaken to develop molecular markers to study its genetic variability. Since a variety of approaches to develop molecular markers have been established in recent years, it is necessary to identify the most efficient and cost effective markers that can be used by practicing plant breeders for a particular plant. Among the different microsatellite based markers, ISSR markers have become available in several individual crops due to production of genomic libraries enriched for microsatellites. Since no such approach has been conducted on jute, conditions were needed to be optimized for efficient marker development.

An effort was made in our lab, to develop low temperature tolerance in cold sensitive jute. A cross was made between a cold sensitive popular jute variety O-9897 and a cold tolerant accession No. 1805 to develop cold tolerant DNA marker, which will be a basis for marker-aided selection. Cold tolerant accession was used as seed parent. The F_1 of this cross was harvested and tested for cold tolerance. The F_2s were produced by selfing F_1. A good number of segregating F_2 populations was raised by differentiating the cold tolerant (16°C) and sensitive in a phytotron (growth cabinet). The main objective of the present study was to develop a linkage map of the jute genome based on ISSR markers that could form the basis for elucidating the loci governing desirable traits that segregate in the F_2 populations.

MATERIALS AND METHODS

Plant material and DNA extraction: Twenty two F_2 plants, developed from an intraspecific cross between a cold sensitive popular jute variety O-9897 and a cold tolerant accession No. 1805, were used as the mapping population. Plants were grown in a growth cabinet phytotron. Genomic DNA was extracted from young leaves following the modified CTAB procedure of Taylor *et al*. (1995). The DNA was RNase-treated and subsequently quantified on agarose gel by comparison with standard lambda DNA markers.

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Table 1: ISSR primers used in the study

Primer name	Primer sequence
ISSR-TG	$C(TG)_8$
ISSR-AG	$T(AG)_8$
ISSR-TC	$AG(TC)_8$
ISSR-CAA	$T(CAA)_5$
ISSR-AAG	$CT(AAG)_6$
ISSR-CTT	$AG(CTT)_6$
ISSR-CCA	$G(CCA)_6$
ISSR-AT	$GC(AT)_9$

ISSR-Inter Simple Sequence Repeat, A-Adenine, T-Thymine, G-Guanine and C-Cytosine

ISSR analysis: Eight ISSR primers and three primer pairs were screened on the parents for polymorphism. The ISSR-PCR method (Zietkiewicz *et al*., 1994) was modified with a view to enhance the speed and sensitivity of detection of markers. We designed and synthesized eight 5' anchored primers (Table 1). Amplification was performed in 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 unit of Taq Polymerase, 4 μM primer with 50 ng of genomic DNA per 25 μL reaction. The thermal cycling profiles programmed to amplify the gene by Polymerase Chain Reaction (PCR) were as follows: Initial denaturation 94°C for 5 min; 35 cycles of amplification: 94°C for 1 min, ~50°C for 45 sec, 72°C for 2 min; final elongation 72°C for 5 min. For different primers different annealing temperatures were employed. For ISSR primers the optimum annealing temperature used was 50°C. The products were resolved on 2% agarose gel in TAE buffer and stained with ethidium bromide.

Inheritance and linkage analysis: Linkage analysis was performed using mapmaker version 3.0. Linkage groups were established at a LOD score of 3.0 and a maximum distance 50.0 by two-point analysis using the group command. The best marker order of the linkage group was identified using compare command.

RESULTS

Polymorphic markers for mapping: Eight ISSR primers (Table 1) and their different combinations were used on the segregating F_2 population of 22 progenies. The polymorphisms between the parents were detected and the progenies were scored. The screening of parents (O-9897 and Acc.1805) with ISSR primers revealed, 3 single primers and 2 primer pairs were polymorphic between the parents, which collectively produced 10 clear and reproducible markers for mapping (Fig. 1-3). Thus, a total of 10 segregating markers were used to construct the Jute linkage map. The size of the markers varied from approximately 500 bp to 1,400 bp.

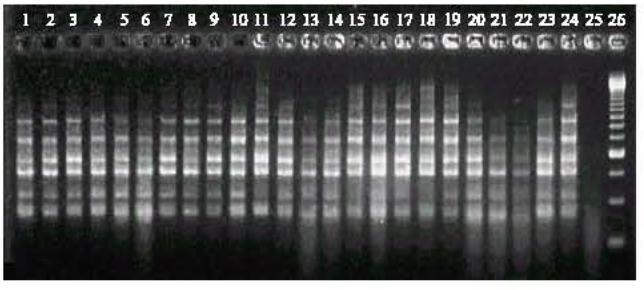

Fig. 1: ISSR banding pattern of cold tolerant and cold sensitive parent and their F_2 crossing population with the primer CTT. Lane 1-22: F_2 hybrids, Lane 23: O-9897, Lane 24: 1805, Lane 25: Control and Lane 26: 250 bp ladder

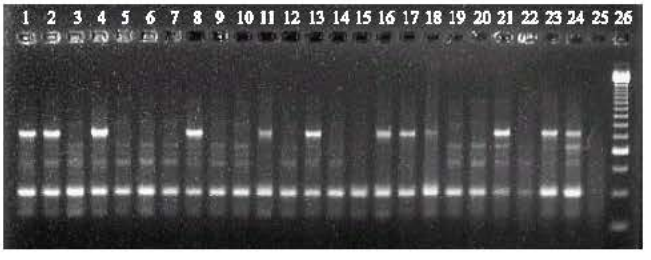

Fig. 2: ISSR banding pattern of cold tolerant and cold sensitive parent and their F_2 crossing population with the primer CAA. Lane 1-22: F_2 hybrids, Lane 23: O-9897, Lane 24: 1805, Lane 25: Control and Lane 26: 250 bp ladder

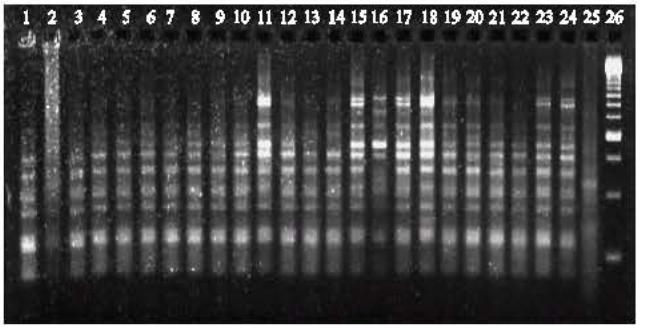

Fig. 3: ISSR banding pattern of cold tolerant and cold sensitive parent and their F_2 crossing population with the primer CAA + AG. Lane 1-22: F_2 hybrids, Lane 23: O-9897, Lane 24: 1805, Lane 25: Control and Lane 26: 250 bp ladder

Table 2: Characteristic of the intraspecific genetic linkage map of jute

Linkage group	Length (cM)	No. of markers	Average distance between markers (cM)
LG 1	4.8	2	4.8
LG 2	29.6	5	5.98
LG 3	52.9	3	26.4
Total	87.3	10	8.73

LG- Linkage Group, cM- Centi Morgan

Linkage mapping: Construction of Genetic Linkage map has been undertaken for Cold Tolerance trait of jute in cross between *C. olitorius* Acc.1805 (cold tolerant) vs. *C. olitorius* var. O-9897 (cold sensitive). The analysis revealed three linkage groups (Fig. 4) varying in length from 4.8 to 52.9 cM (Table 2). The map spanned a total length of 87.3 cM with an average marker density of 8.73 cM between adjacent markers.

DISCUSSION

Our immediate objective was to determine whether polymorphism obtained by ISSR was sufficient to establish a genetic linkage map of jute. Initial analyses were based on 8 different ~20 base-paired ISSR primers (Table 1) scored in 22 F_2 segregating populations and their parents to see whether they could generate suitable polymorphism to generate a map (Fig. 1-3).

Later we had tested different primer pairs and found that three primer pairs gave distinct and sharp bands. However, pair combinations of di- and tri- nucleotide motifs rendered a greater number of highly polymorphic bands. Best results of PCR amplification reactions were obtained with the poly (CTT) tri-nucleotide repeat anchored at the 5′end with the selective nucleotide AG (Fig. 1). Using this procedure varying numbers (4 to 11) of polymorphic bands was produced, depending on the SSR motif of the primer. When used alone, the poly CAA primer rendered only six bands (Fig. 2). The combination of the poly CAA with the AG motif (Fig. 3) produced the largest number of bands (11). All these bands were polymorphic. Some significant differences were observed between the numbers of bands produced by other primer combinations.

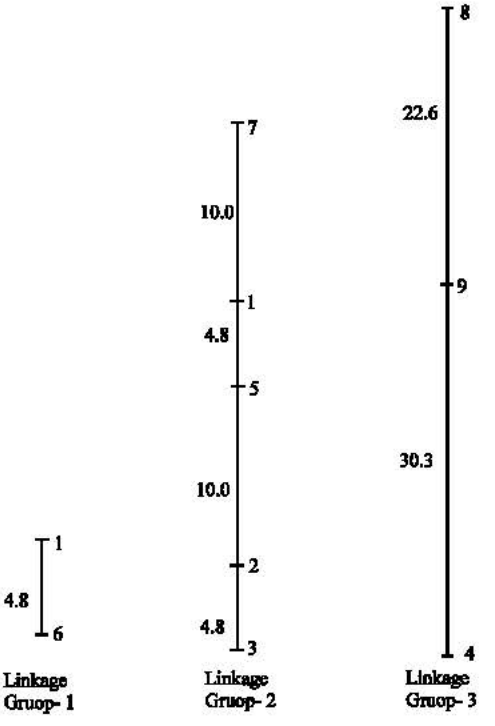

Fig. 4: Three linkage groups generated by the ISSR primers

The linkage map constructed from the F_2 population from the cross between jute cultivars O-9897 and Acc. 1805 is the first report of a linkage map of jute (Fig. 4) in which an intraspecific mapping population within the cultivated gene pool has been used. ISSR primers were 50% polymorphic between the parent cultivars.

The map spanned 87.3 cM with 10 markers and a density of 8.73 cM between adjacent markers, which is relatively small compared to the genome size of jute genome, the map length detected in the present study

therefore could not represent the entire jute genome. In the map, no unlinked marker was detected; the absence of unlinked markers in the map was most probably due to the use of an intraspecific cross as there is more homology among chromosomes of the same species.

Linkage analysis at a LOD score of 3.0 and a maximum distance 50 produced three linkage groups (Table 2), which were not equal to the haploid chromosome number of jute (n = 7). More markers may need to be mapped to merge smaller linkage groups to larger ones.

Due to the unavailability of co-dominant markers for jute, dominant markers (ISSR) were used for mapping. Dominant markers are unable to distinguish heterozygotes from homozygotes; however, they allow many polymorphic markers to be quickly identified, which is useful for mapping the genome. They have been used to construct linkage maps in various legume crops (Men_ndez *et al*., 1997; Eujayl *et al*., 1998; Laucou *et al*., 1998; Santra *et al*., 2000) and also to extend the existing linkage map of rye (Masojc´ *et al*., 2001).

In order for ISSR to be successful, pairs of simple sequence repeat must occur within a short distance (in base pairs) that can be amplified by PCR reaction, producing bands that are resolvable on standard gels (Zietkiewicz *et al*., 1994). In this study, four dinucleotide and four trinucleotide motifs amplified a total of 84 ISSR markers. The PCR amplification, performed with ISSR markers to assess the level of polymorphism among the F_2 segregating populations and the parents, revealed a high percentage of polymorphism.

From all ISSR primers tested for amplification, few produced well-defined and reproducible bands when used alone in PCR reactions. Out of 8 primers screened 7 gave sharp and distinct bands but the primer that contained AT repeats produced no amplification products even though several annealing temperatures and $MgCl_2$ concentrations were assessed. This was surprising since AT repeats were previously reported to be the most abundant repeat type in plant genomes (Wang *et al*., 1994). Similarly, $(AT)_n$ primers did not produce distinct bands in wheat (Nagaoka *et al*.,1997) and lentil (Rubeena *et al*., 2003).

Only those fragments that could be reproduced in a second experiment were considered. Weak and spurious bands were not included in the analyses. In the present study the DNA profile or fingerprint of each jute variety studied here was recorded. The data was also scored on the presence or absence of the amplified fragments.

Then we had tried to identify the primer that gave polymorphic markers between parents and identified three primers and two primer pairs, which collectively gave 10 markers. These markers were used to construct a linkage map in jute. The primer CTT gave the highest number of polymorphic markers (Fig. 1). Of the ISSR primers that did produce markers for mapping, three were tri-nucleotide repeats CAA, CTT, CCA and two were primer pairs.

The present study explored the utility of ISSR markers in mapping of the lentil genome and this lentil map will be useful in locating the gene loci governing desirable traits such as cold tolerance that may segregate in the population and for their marker-assisted selection. The markers can also be integrated for developing a more saturated genetic linkage map of lentil.

Further studies with more number of primers are highly recommended to generate map that spanned the entire length of the jute genome, which will help in the breeding programs for evolving cold tolerant varieties. This will fit in the three cropping pattern needed urgently for the betterment of jute cultivation in Bangladesh.

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