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IDENTIFYING SIMPLE SEQUENCE REPEAT (SSR) MARKER LINKED TO MITE TOLERANCE IN JUTE SPECIES

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Abstract

Mite infestation is one of the main factors restricting jute production in Bangladesh. The utilization of jute cultivars tolerant to mite attack can be one of the most economic strategies for expanding jute production. Among jute genotypes, *C. olitorius* O-7/95 has been reported to be most tolerant to mite attack. SSR or microsatellite markers for jute have been successfully used in distinguishing mite sensitive and tolerant jute varieties. To increase selection efficiency for mite tolerance we have used an F_2 population of O-7/95 and O-72 (sensitive to mite attack) to map a number of these microsatellite markers and to determine their linkage with mite tolerant trait. A preliminary genetic linkage map based on 35 F_2 populations, was constructed using software MAPMAKER/EXP (ver 3.0b). This map was based on ten SSR markers and a phenotypic marker (M-11) related to mite sensitivity. In this analysis mite resistance has been found to be linked with a SSR marker, M-66 at LOD threshold of 3. This marker has the potential of being useful in Marker Assisted Selection (MAS) in jute breeding programs for selection of lines resistant to mite.

Introduction

With the advent of molecular marker based techniques, the scope for characterization of genetic variability in plants presents immense opportunities. Marker-dense genetic maps contribute greatly to our understanding of evolutionary process, enable marker assisted-selection and mapping of agronomic traits and facilitate many aspects of crop improvement.

Many linkage maps in plants are based on F_2 population or recombinant inbred line (RIL) populations (Burr *et al.* 1988, Gardiner *et al.* 1993, Röder e*t al.* 1998, Davis *et al.* 1999, Temnykh *et al.* 2000). The aim of linkage mapping is to find out the approximate location of genes relative to genetic markers on a chromosome based on recombination frequencies observed in pedigrees or progeny populations (Clark and Wall 1996). Polymorphism between individuals in a population and their detection with genetic markers are essential elements of linkage mapping. Construction of a genetic linkage map requires selection of an appropriate population, defining markers or genes, calculation of pair-wise recombination frequencies, establishment of linkage groups, determination of gene or marker order and estimation of map distances (Staub *et al.* 1996).

A number of PCR-based marker technologies have expedited the construction of high-density linkage maps; facilitated genetic analysis and map based cloning (Yang *et al.* 2002, Peters *et al.* 2003). These techniques involve RFLP (McCouch *et al.* 1997), RAPD (Williams *et al.* 1990), AFLP (Vos *et al.* 1995), SSR (Tautz 1989).

PCR-based markers like the Simple Sequence Repeat (SSR) or microsatellite markers are robust, inexpensive to assay, easily shared among researchers, and readily accessible in public and private domains, making them a much more appropriate approach for developing countries. With

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access to a simple text file containing the sequences of the oligonucleotide primers for the PCR-based markers of interest, a breeder can rapidly and efficiently evaluate the germplasm under study.

Microsatellite based linkage maps have been constructed for a wide variety of species including man (Dib *et al.* 1996), mouse (Dietrich *et al.* 1996), a number of plant species, such as maize (Chin *et al.* 1996, Taramino and Tingey 1996), rice (Temnykh *et al.* 2000), wheat (Bryan *et al.* 1997, Röder e*t al.* 1998), potato (Milbourne *et al.* 1998) and soybean (Akkaya *et al.* 1995, Cregan *et al.* 1999). There are many reports on the identification of SSR markers associated with pest resistance (Fu *et al.* 2006, Shalini *et al.* 2007).

In Bangladesh, jute *(Corchorus* spp. 2*n* = 14; genome size = 1,250 Mb) suffers from losses both in quantity and quality due to attack of about 40 species of different kinds of insects and mite pests. Yellow mite (*Polyphagotarsonemus latus)* is the most destructive pest of both the cultivated species of jute (*C. capsularis* and *C. olitorius*) and its incidence is very high in the field during summer seasons especially from April to July when about 38% yield loss occur.

However, some varieties of jute show resistance toward mite. *C. olitorius* has two recommended varieties, cv. O-72, mite sensitive and cv. O-7/95 is mite tolerant. In this study, to establish linkages between molecular markers and mite resistance traits using an F_2 population raised from a cross between mite sensitive O-72 and tolerant O-7/95 was tried. Molecular Marker-Assisted Selection (MAS) for mite resistance in jute would be very convenient in terms of increasing breeding efficiency, and genetic maps would be a useful tool to implement this strategy. Recently a number of microsatellite markers (SSR) have been developed for jute (Mir *et al.* 2008, Akter *et al.* 2008*)*.

With some of the microsatellite markers at hand, the present work was undertaken to study them in a segregating F_2 population in order to (i) determine their linkage to mite resistance trait and their subsequent use in marker aided selection, and (ii) to develop a molecular framework map of jute.

Materials and Methods

Extraction of DNA: Seeds of jute cultivars, O-72 and O-7/95 and leaf tissues of the F_2 population were obtained from the Bangladesh Jute Research Institute (BJRI). For the isolation of total genomic DNA from the leaf tissues of F_2 as well as from the seeds of O-72 and O-7/95, CTAB method without phenol was chosen because this method gave good yields of total genomic DNA of high molecular weight (Delaporta *et al.* 1983). The genomic DNAs along with λ DNA were run on a 0.8% agarose gel which was stained by ethidium bromide solution and visualized using UV illumination and documented by Kodak Biodoc system. The DNAs were quantified by comparing with fluorescence of known concentration of λ DNA. The final DNA concentration of each template stock was adjusted to 50 ng/µl.

Microsatellite primers: Linkage analysis of 35 F_2 jute DNA samples was carried out by ten microsatellite primers (Table 1). The microsatellite enriched library was constructed commercially from Vizon Sci. Inc., BC Research Complex 3650, Westbrook Mall, Vancouver, British Columbia, Canada. Clones containing simple sequence repeats were sequenced and from these sequences the microsatellite sites were identified and SSR primers were designed. Primers were designed manually using the following standard rule: (i) 20-25 nucleotides in length, (ii) 40-60% GC content, and non-complementary 3´ nucleotides, (iii) the expected sizes of the amplification products to be between 115 and 300 bps and (iv) annealing temperatures to be between approximately 58-61°C with no greater than 3°C difference in T_m between primers of each pair.

PCR amplification: PCR amplification was conducted with 25 µl reaction mixtures containing 50 ng of jute genomic DNA, 10 µM of each primer pair, 10X PCR buffer, 50 mM $MgCl_2$, 2 mM

dNTPs, 0.2 unit Taq DNA polymerase using a GeneAmp® PCR system 9700 (Applied Biosystems). The amplification was programmed as follows: Preheating for 5 min at 95°C; 35 cycles, each for 30 s at 95°C (denaturation), 40 s at the annealing temperature of a particular primer pair, and 30 s at 72°C (extension) and a final extension at 72°C for 5 min, followed by cooling at 4°C for infinite period. For different primers different annealing temperatures were employed. For SSR primers the optimum annealing temperature used was between 55 and 62°C.

Table 1. List of SSR primers used, showing their T_m, GC content, product sizes and the standardized annealing temperatures.

Primer No.	Primer pair		T_m (°C)		GC %		Product size (bp)	Annealing temp.
	F	R	F	R	F	R		
51	HK-F-SSR101	HK-R-SSR102	58.9	57.7	33.3	40.9	200	58
54	HK-F-SSR107	HK-R-SSR108	58.4	62.4	55.0	39.1	211	59
56	HK-F-SSR111	HK-R-SSR112	60.4	58.4	45.0	50.0	115	58
59	HK-F-SSR117	HK-R-SSR118	61.2	61.0	43.5	52.2	306	60
63	HK-F-SSR125	HK-R-SSR126	60.8	60.8	45.5	40.9	133	60
64	HK-F-SSR127	HK-R-SSR128	58.9	59.4	40.9	37.5	192	59
66	HK-F-SSR131	HK-R-SSR132	62.7	60.8	45.5	30.8	136	61
69	HK-F-SSR137	HK-R-SSR138	62.7	60.4	50.0	43.5	146	61
74	HK-F-SSR147	HK-R-SSR148	58.9	59.2	39.1	36.4	N/A	59
79	HK-F-SSR157	HK-R-SSR158	59.4	58.7	42.9	36.4	210	58

Separation and staining of PCR products: The amplified PCR products were subjected to electrophoresis in two gel systems, agarose gel (2%) and polyacrylamide gel (40% acrylamide/ bis-acrylamide, 10% ammonium per sulfate, 5X TBE buffer, Gel loading dye). The agarose gel electrophoresis was performed for confirmation of the PCR amplification. For obtaining better resolution of polymorphic bands, amplified PCR products were subjected to PAGE which was carried out in 1X TBE buffer at 100W for about 2 hours. After electrophoresis, the products were stained in ethidium bromide and distained in ddH_2O and then visualized and photographed under UV light using Kodak electrophoresis documentation and analysis system.

Scoring and analysis of the data: The amplified products were scored for further analysis. During scoring, only intense and clearly resolved amplification products that were reproducible in multiple runs were considered for linkage analysis. Polymorphisms were scored for length variation of bands on polyacrylamide gels. The size of the SSR markers varied from ~100 - 300 bp.

The PCR amplification products of F_2 plant DNA were scored as A, B, H and null (-) by comparing with PCR amplification products of parental DNA (O-72 and O-7/95). When the bands resembled the parental (O-7/95: mite tolerant plant) DNA, they were designated as "A" (homozygous). Similarly, the bands resembling the parental (O-72: mite sensitive plant) DNA were designated as "B" (homozygous). The bands, when resembled both the parental (O-7/95 and O-72) DNA, were designated as "H" (heterozygous). When no bands were obtained after PCR amplification with specific SSR primer from plant DNA, they were scored null (-). A phenotypic marker was scored as ‘A’ for mite tolerant plant and ‘B’ for mite sensitive plant. It is known that different pathogen races or insect biotypes based on host pathogen or host parasite interaction can

be used as a marker since the genetic constitution of an organism can affect its susceptibility to pathogens or parasites (Ribaut *et al.* 2001).

Linkage map construction: The linkage map of jute genome was constructed using software MAPMAKER version 3.0 (stat soft 1994) considering 10 SSR markers and one phenotypic marker with 35 individuals of an F_2 population. A logarithm of the odds ratio (LOD) score of 3.0 and a maximum distance of 50 cM was considered for linkage analysis. A raw data was prepared for construction of a linkage map. This raw format contained the polymorphic bands which were scored as A, B, H and null (-).

Results and Discussion

Genotype determination between mite sensitive and tolerant jute varieties in segregating F_2 population by comparing the two parents.

In the present investigation, 43 SSR markers were used for assessing variation between the two parents used in developing the mapping F_2 population. Of these 43 markers, 10 were found capable of distinguishing between the parents. These primers were subsequently used for genotype determination of the segregating F_2 population because they produced polymorphic bands between mite sensitive parent (O-72) and mite tolerant parent (O-7/95).

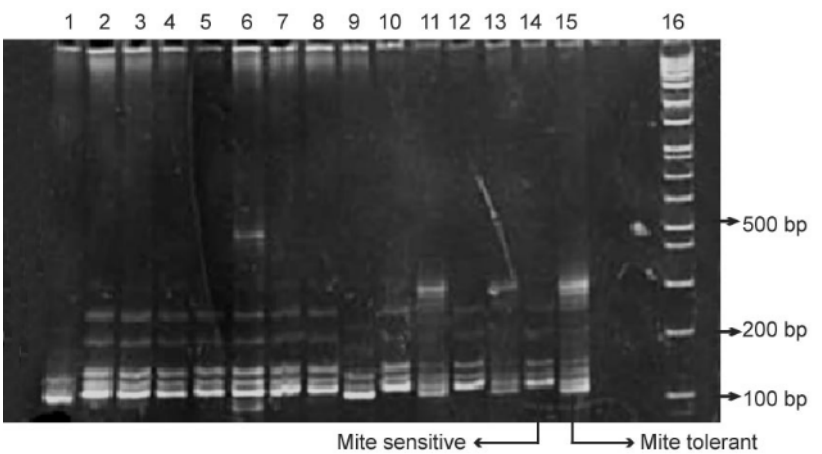

Fig. 1. A representative gel photograph used in scoring of F_2 population using SSR primer M 63.

F_2 population: Lanes 1-13; Parent O-72 (mite sensitive): Lane 14; Parent O-7/95 (mite tolerant): Lane 15; 1 Kb+ Ladder: Lane 16.

Scoring and analysis of the data: Ten pairs of SSR primers were used to amplify 35 F_2 plant DNA. The SSR markers were scored for determining parental or recombinant phenotype of the F_2 population. Based on the similarity of desired band size of the PCR product by M-56 with resistant parent, 12 plants were scored as 'A' (Table 2). Five plants were scored as 'H' for the presence of bands similar to both resistant and sensitive parent. Twelve plants were scored as 'B' for the presence of band only similar to sensitive parent. The remaining six plants were scored as null (-) because of the ambiguity resulting from dissimilarity of band size with parents. Scoring ambiguous data affect mapping result more negatively rather than scoring simply as null.

Table 2. Scoring SSR markers for determining parental or recombinant phenotype of the F_2 population.

Plant No.	SSR M-51	SSR M-54	SSR M-56	SSR M-59	SSR M-63	SSR M-66	SSR M-69	SSR M-64	SSR M-79	SSR M-74	Phenotypic marker
1	A	A	A	B	A	A	-	B	A	A	B
2	A	B	B	B	B	B	B	B	B	A	B
***3**	**A**	**B**	**B**	**A**	**B**	**A**	**-**	**B**	**B**	**A**	**A**
4	A	B	B	B	B	B	-	A	B	A	B
5	A	B	A	A	B	B	A	B	B	A	B
6	B	B	A	B	B	B	A	B	B	B	B
7	B	B	A	A	B	B	A	A	B	B	B
8	A	B	B	B	B	B	A	B	B	B	B
***9**	**A**	**B**	**A**	**A**	**A**	**A**	**A**	**B**	**A**	**A**	**A**
10	A	B	B	-	B	B	B	B	B	A	B
***11**	**A**	**-**	**H**	**-**	**A**	**A**	**B**	**B**	**A**	**A**	**A**
12	B	B	B	-	B	A	-	-	B	B	B
13	A	B	B	-	H	B	B	-	A	B	B
14	A	A	A	A	A	A	A	A	B	B	B
***15**	**B**	**A**	**H**	**A**	**A**	**B**	**B**	**A**	**A**	**A**	**A**
***16**	**-**	**B**	**H**	**A**	**B**	**A**	**A**	**A**	**A**	**B**	**A**
17	A	A	H	B	B	A	A	A	A	B	B
18	-	-	-	-	-	A	B	A	B	B	B
19	-	A	B	B	A	B	B	B	A	A	B
20	-	A	A	A	A	A	A	B	B	A	B
***21**	**A**	**B**	**A**	**B**	**B**	**A**	**-**	**A**	**A**	**B**	**A**
22	B	B	A	A	B	B	B	A	B	B	B
23	-	A	-	-	-	A	B	A	-	B	B
24	A	-	H	A	B	B	A	A	H	B	B
***25**	**B**	**A**	**A**	**A**	**A**	**A**	**B**	**A**	**A**	**B**	**A**
26	A	-	-	-	-	B	-	B	A	-	B
27	B	-	-	-	-	B	B	A	A	-	B
28	B	-	B	-	A	B	-	-	-	-	B
29	A	A	A	-	A	B	B	B	B	-	B
30	A	A	B	-	-	B	A	A	A	-	B
31	A	A	B	-	B	B	A	A	-	-	B
32	B	A	B	-	B	B	-	A	B	A	B
33	B	-	-	H	A	B	B	B	B	A	B
34	A	-	-	-	B	B	B	-	B	-	B
35	B	-	A	H	B	-	A	-	-	-	B

* and the numbers and alphabets in bold face indicate the seven mite tolerant F_2 plants as per field data.

The segregation of individual markers was analyzed by chi-square test at 1% level of significance and the following result (Table 3) was obtained.

Table 3. Results of segregation test (Generation: F_2; No. of progenies: 35; No. of markers: 11).

Marker	N	Ratio	Chi-square value	P-value
M-51	30	19:0:11	30.09	0.0000 ***
M-54	26	12:0:14	21.86	0.0000 ***
M-56	29	12:5:12	11.34	0.0034 **
M-59	19	11:0:8	18.14	0.0001 ***
M-63	30	11:1:18	25.91	0.0000 ***
M-66	34	14:0:20	35.11	0.0000 ***
M-69	27	13:0:14	22.71	0.0000 ***
M-64	30	15:0:15	26.43	0.0000 ***
M-79	31	13:1:17	25.40	0.0000 ***
M-74	26	14:0:12	21.86	0.0000 ***
M-11	35	7:0:28	60.20	0.0000 ***

** and *** infer significance level, which differed at $p < 0.005$ and $p < 0.001$, respectively.

Low P-values indicate that markers have effectively segregated from F_1 in to F_2 population and it can be inferred at 1% significance level that two different bands for each marker is due to the presence of two different alleles and not for two different polymorphic state of a marker present in same DNA molecule in F_1 population.

Construction of linkage map: Segregation of 11 markers (10 SSR and 1 phenotypic marker) among 35 F_2 population was analyzed to construct a genetic linkage map. The distribution of the molecular markers on the different linkage groups was analyzed. Two linkage groups were observed at minimum of LOD score 3.00 and maximum of 50.0 cM distance. Group1 was constituted of markers M-54 and M-63 and distance between these two markers were 23.5 cM. Group2 constituted of markers M-66 and M-11 (phenotypic marker) and distance between these two markers was found to be 37.7 cM.

Group 1 = 2 5

Markers	Distance
2 M-54	23.5 cM
5 M-63	----------
	23.5 cM

Group 2 = 6 11

Markers	Distance
6 M-66	37.7 cM
11 M-11	----------
	37.7 cM

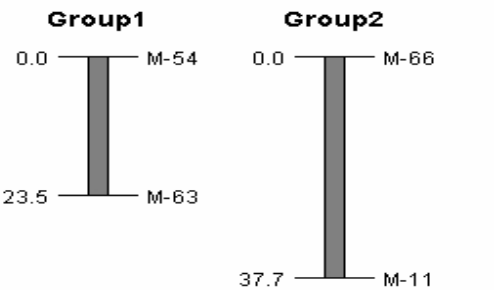

Fig. 2. Linkage map showing cM distance between markers.

The distances between the remaining markers were such that they could not be assigned to any linkage groups. When these remaining 7 markers were given as input to the mapmaker programme, the following order was observed among markers with the minimum total distance obtained as 479.5 cM with log-likelihood as -110.67:

Unlinked = 1 3 4 7 8 9 10

Markers	Distance
10 M-74	93.6 cM
1 M-51	76.4 cM
7 M-69	69.4 cM
3 M-56	52.5 cM
4 M-59	118.4 cM
8 M-64	69.3 cM
9 M-79	----------
	479.5 cM 7 markers log-likelihood = -110.67

The unique chromosome number of cultivar *Corchorus* species is seven (2n = 14); therefore, seven linkage groups were expected to represent the whole genome, since the number of linkage groups are the same as the unique chromosome number (Zickler *et al.* 1984). But, for linkage mapping, this effort was very limited and the total number of loci (ten) was also very low. By increasing the number of markers (~150 to 300) and by increasing the population size (more than 100), a marker dense saturated linkage map of jute genome can be obtained. The saturated map will help in future to identify the locations of genes responsible for mite resistance trait in jute by positional cloning. Most linkage maps have been constructed with far more markers than the number used in this study. A total of 769 markers were used to analyze the polymorphism between a soybean mosaic virus (SMV) resistant and sensitive soybean parents for linkage analysis and mapping of the SMV resistance gene (Fu *et al.* 2006). Among them 277 were polymorphic. As expected from the large number of markers used the average distance between markers was 11.8 cM, which is a very dense map. Twenty linkage groups were obtained and the numbers of markers in each group varied from 2 to 19.

It may be noted that in our study only markers which were polymorphic were used. Thus the 10 markers used were all polymorphic.

Genotypic variation in F_2 mite resistant plants: Molecular marker scores together with phenotypic data have been used to increase yield and resistance to crop pests (Johnson 2004, Shalini *et al.* 2007). Some important traits in cotton such as those linked to agronomic and fiber quality have been mapped using SSR markers which have facilitated the development of markers associated with Verticillium wilt resistance (Wang *et al.* 2008).

The high resistance of O-7/95 to mites makes it an ideal candidate for providing new genetic variation useful for improving disease resistance in sensitive varieties. A total of ten SSR markers were evaluated after PCR amplification of 35 F_2 plant DNA. Out of 35 F_2 plants, only seven plants were mite resistant (From field data; Source: BJRI). Analysis of the data (Table 2) showed genotypic variation in F_2. From Table 4, it was observed that none of the mite tolerant plants possessed 100% 'A' (tolerance) genotype.

From the data (Table 2) and from the linkage map (Fig. 2) it was observed that marker-66 showed linkage with a mite resistant trait. Out of seven resistant plants (Table 5), genotype of the six plants matched with the phenotype. But in one tolerant plant, sensitive-like genotype was observed. So, 85.71% of mite resistance genotypes among phenotypically resistant plants show linkage of mite resistant trait to M-66 marker. The remaining 14.29% of the characteristics, which cannot be explained can be inferred as rare crossing over or another copy of this same resistance

gene may be present in this plant which is not linked with marker 66 and this may impart resistance in this plant. It is known that disease resistance gene products do not act alone in controlling defense reactions (Hubert *et al.* 2001). It is reported that rare germinal unequal crossing-over can lead to recombinant gene formation and gene duplication (Jelesko *et al.* 1999).

Table 4. Genetic variation in mite resistant jute plants.

SSR marker	Number of segregating F_2 plants (Phenotype = Mite tolerant plant); Total mite tolerant plants = 7				% of **A** genotype in tolerant plants
	Genotype-A	Genotype-B	Genotype-H	Null (-)	
M-51	4	2	0	1	66.67
M-54	2	4	0	1	33.33
M-56	3	1	3	0	42.86
M-59	5	1	0	1	83.33
M-63	4	3	0	0	57.14
M-64	3	4	0	0	42.86
M-66	**6**	**1**	**0**	**0**	**85.71**
M-69	2	3	0	2	40.00
M-79	6	1	0	0	85.71
M-74	4	3	0	0	57.14

A = Mite tolerant trait; B = Mite sensitive trait; H - Heterozygous; Null (-) means no bands were obtained after PCR amplification.

Out of 27 mite sensitive F_2 plants (Table 5), eight plants showed genotype A (tolerance) for marker M-66. In these eight plants, a resistant trait linked to marker-66 was inherited together and in these plants, the intensity of mite infestation was very low. So, there is possibility that mite resistance is a polygenic trait and the presence of only one gene conferring resistance cannot prevent these plants completely from mite attack. Using SSR markers on coconut Shalini *et al.* (2007) have shown that the markers associated with mite resistance were located on different chromosomes indicating the presence of multiple QTLs for mite resistance. M-66 associated with the trait could be used in marker assisted selection for mite resistance in jute breeding or could be used in gene pyramiding.

Table 5. Use of marker M-66 in genotyping mite tolerant and mite sensitive jute plants.

Primer	For sensitive plant		For resistant plant	
	No. of plants	Genotype	No. of plants	Genotype
M-66	8	A	6	A
	19	B	1	B
	Total = 27		Total = 7	

However, in this study no QTLs for resistance segregating in the progeny were identified. Quantitative trait loci analysis requires the development of genetic maps with a large number of markers to provide full coverage of the genome. Therefore, the failure to obtain any QTL could possibly be for one or more of the following reasons: the markers did not cover the whole of the genome; there were unfavorable repulsion linkages between the QTLs and markers, or the gene effects were not large enough to be detected in an experiment of the size conducted. Bradshaw *et al.* (1998) has reported similar findings in their work on the identification of AFLP and SSR

markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisting selection.

Results of present work indicate that there are good prospects for detecting QTLs and using marker-assisted selection in a jute breeding programme, provided in future the population size is increased to over 250 and more SSR markers are used. In this study PCR-based markers have been successfully used in establishing linkage between some SSR markers and demonstrating linkage of one of these SSR markers to resistance to jute yellow mite, a major jute pest.

However, further efforts are required to identify more closely linked markers for the purpose of application to MAS. Molecular marker maps, the necessary framework for any MAS programme, have been constructed for many agriculturally important species by using molecular markers (Moose and Mumm 2008). By using molecular markers the knowledge gained about genetic relationships among germplasm sources may guide choice of parents for production of hybrids or improved populations (Collard and Mackill 2008). This technology can significantly improve the efficiency of jute varietal development programs and reduce time and costs substantially thus effectively doubling the capacity of research programs, such as marker assisted breeding for resistance to jute yellow mite.

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References

Akkaya, M.S., A.A. Bhagwat and P.B. Cregan. 1995. Length polymorphisms of simple sequence repeat DNA in soybean. Genetics **132**: 1131-1139.

Akter, J., M.S. Islam, A.A. Sajib, N. Ashraf, S. Haque and H. Khan. 2008. Microsatellite markers for determining genetic identities and genetic diversity among jute cultivars. Australian J. Crop Sci. **1**(3): 97-107.

Bangladesh Jute Research Institute. 1990. Effect of intensity of yellow mite infestation on yield of O-4 jute under field conditions. Annual report 1990. Bangladesh Jute Res. Inst., Dhaka, Bangladesh.

Bradshaw, J.E., C.A. Hackett, R.C. Meyer, D. Milbourne, J.W. McNicol, M.S. Phillips and R.Waugh. 1998. Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection. Theor. Appl. Genet. **97**: 202-210.

Bryan, G.J., A.J. Collins, P. Stephenson, A. Orry, J.B. Smith and M.D. Gale. 1997. Isolation and characterization of microsatellites from hexaploid bread wheat. Theor. Appl. Genet. **94**: 557-563.

Burr, B., F.A. Burr, K.H. Thompson, M.C. Albertson and C.W. Stuber. 1988. Gene mapping with recombinant inbreds in maize. Genetics **118**: 519-526.

Chin, E.C.L., M.L. Senior, H. Shu and J.S.C. Smith. 1996. Maize simple repetitive DNA sequences: Abundance and allele variation. Genome **39**: 866-873.

Clark M.S. and W.J. Wall. 1996. Chromosomes: The Complex Code. Chapman and Hall, New York.

Collard B.C.Y. and D.J. Mackill. 2008. Marker assisted selection: An approach for precision plant breeding in the twenty-first century. Philos. Trans. R. Soc. London Ser. B. Biol. Sci. **363**: 557-572.

Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. Van-Toai, D.G. Lohnes, J. Chung and J.E. Specht. 1999. An integrated genetic linkage map of the soybean genome. Crop Sci. **39**: 1464-1490.

Davis, G.L., M.D. McMullen, C. Baysdorfer, T. Musket, D. Grant, M. Staebell, G. Xu, M. Polacco, L. Koster, K. Houchins, S. Chao and E.H. Coe, Jr. 1999. A maize map standard with sequenced core markers, grass genome reference points, and 932 ESTs in a 1736-locus map. Genetics **152**: 1137-1172.

Delaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation. Version II. Plant Mol. Biol. Rep. **4**: 19-21.

Dib, C., S. Faure, C. Fizames, D. Samson, N. Drouot, A. Vignal, P. Millaseau, S. Marc, J. Hazan, E. Seboun, M. Lathrop, G. Gabor, J. Moissette and J. Weissenbach. 1996. A comprehensive genetic linkage map of the human genome based on 5264 microsatellites. Nature **380**: 152-154.

Dietrich, W.F., J. Miller, R. Steen, M.A. Merchant, D. Damronboles, Z. Husai, R. Dredge, M.J. Daly, K.A. Ingalls, T.J. O'Connor, C.A. Evans, M.M. DeAngelis, D.M. Levinson, L. Kruglyak, N. Goodman, N.G. Copelang, N.A. Jenkins, T.L. Hawkins, L. Stein, D.C. Page and E.S. Lander. 1996. A comprehensive genetic map of the mouse genome. Nature **380**: 149-152.

Fu, S., Y. Zhan, H. Zhi, J. Gai and D. Yu. September. 2006. Mapping of SMV resistance gene Rsc-7 by SSR markers in soybean. Genetica **128**(1-3): 63-69.

Gardiner, J.M., E.H. Coe, S. Melia-Hancock, D.A. Hoisington and S. Chao. 1993. Development of a Core RFLP Map in maize using an immortalized F_2 population. Genetics **134**(3): 917–930.

Hulbert, S.H., C.A. Webb, S.M. Smith and Q. Sun. 2001. Resistance gene complexes: Evolution and utilization. Annu. Rev. Phytopathol. **39**: 285–312.

Jelesko, J.G., R. Harpe, M. Furuya and W. Gruissem. 1999. Rare germinal unequal crossing-over leading to recombinant gene formation and gene duplication in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **96**: 10302-10307.

Johnson G.R. 2004. Marker assisted selection. *In:* Plant Breeding Reviews, Long Term Selection: Maize, J Janick, Ed., **24**: 293-309. John Wiley, Hoboken, NJ.

McCouch, S.R., X. Chen, O. Panaud, S. Temnykh, Y. Xu, Y.G. Cho, N. Huang, T. Ishii and M. Blair. 1997. Micro-satellite marker development, mapping and applications in rice genetics and breeding. Plant Mol. Biol. **35**: 88-89.

Milbourne, D., R.C. Meyer, A.J. Collins, L.D. Ramsay, C. Gebhardt and R. Waugh. 1998. Isolation, characterization and mapping of simple sequence repeat loci in potato. Mol. Gen. Genet. **259**: 233-243.

Mir, R.R., S. Rustgi, S. Sharma, R Singh, A. Goyal, J. Kumar, A. Gaur, A.K. Tyagi, H. Khan, M.K. Sinha, H.S. Balyan and P.K. Gupta. 2008. A preliminary genetic analysis of fibre traits and the use of new genomic SSRs for genetic diversity in jute. Euphytica. **161**: 413-427.

Moose, S.P. and R.H. Mumm. 2008. Molecular Plant Breeding as the Foundation for 21st Century Crop Improvement. Plant Physiology **147**: 969-977.

Peters J.L., F. Cnudde and T. Gerats. 2003. Forward genetics and map-based cloning approaches. Trends in Plant Sci. **8**: 484-491.

Ribaut, J.-M. 2001. Genetic basis of physiological traits. *In:* Application of Physiology in Wheat Breeding, CIMMYT, Mexico.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy and M.W. Ganal 1998. A microsatellite map of wheat. Genetics **149**: 2007-2023.

Staub, J.E., F.C. Serquen and M. Gupta. 1996. Genetic markers, map construction, and their application in plant breeding. HortScience **31**: 729-741.

Shalini, K.V., S. Manjunatha, P. Lebrun, A. Berger, L. Baudouin, N. Pirany, R..M. Ranganath and D T. Prasad. 2007. Identification of molecular markers associated with mite resistance in coconut (*Cocos nucifera* L.). Genome **50**(1):35-42.

Taramino, G. and S. Tingey. 1996. Simple sequence repeats for germplasm analysis and mapping in maize. Genome **39**: 277-287.

Tautz, D., M. Trick and G.A. Dover. 1986. Cryptic simplicity in DNA is a major source of genetic variation. Nature **322**: 652-656.

Temnykh, S., W.D. Park, N. Ayres, S. Cartinhour, N. Hauck, L. Lipovich, Y.G. Cho, T. Ishii, and S.R. McCouch. 2000. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. **100**: 697-712.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman and M. Kuiper. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407-4414.

Williams, J.G.K., A.K. Kubelik, K.J. Livac, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitary primers are useful as genetic markers. Nucl. Acids Res. **18**: 6531-6535.

Wang H.M, Z.X. Lin., X.L. Zhang, W. Chen, X.P. Guo, Y.C. Nie and Y.H. Li. 2008. Mapping and Quantitative Trait Loci Analysis of Verticillium Wilt Resistance Genes in Cotton. Journal of Integrative Plant Biology **50**(2): 174–182.

Yang H., M. Shankar, B.J. Buirchell, M.W. Sweetingham and P.M.C. Smith. 2002. Development of molecular markers tagging disease resistance to *Diaporthe toxica* in narrow-leafed lupin (*Lupinus angustifolius*). Theo. and Appl. Genet. **105**: 265-270.

Zickler, D., G. Leblon, V. Haedens, A. Collard and P. Thuriaux. 1984. Linkage group-chromosome correlations in Sordaria macrospora: Chromosome identification by three dimensional reconstruction of their synaptonemal complex. Current Genetics **8**(1): 57-67.

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