Research Article

Identification of SSR markers associated with seed coat permeability and electrolyte leaching in soybean

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ABSTRACT

Seed coat permeability and electrolyte leaching are the important traits that have been negatively associated with seed longevity in soybean. The objective of this study was to use SSR markers to identify genomic regions significantly associated with QTLs controlling seed coat permeability and electrolyte leaching in a segregating F_2 population derived from a cross of Birsa soya-1 x JS 71-05. Parental polymorphism survey using 145 SSR markers identified 21 polymorphic ones, which were used to genotype 153 F_2 individuals. Four independent markers (Satt434, Satt538, Satt281 and Satt598) were significantly (P=0.05) associated with seed coat permeability. One of these markers (Satt 281) also showed significant association with electrolyte leaching that partly supported the observed positive correlation (r = 0.425) between the two traits. Markers for seed coat permeability individually explained 3.9% to 4.5% of the total phenotypic variation, while the marker linked with electrolyte leaching explained 5.6% of the total variation. **[Physiol. Mol. Biol. Plants 2008; 14(3) : 173-177]** *E-mail : ikrps@yahoo.com*

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Soybean (*Glycine max* (L.) Merrill) is a global crop with many uses. The rapid expansion of the soybean acreage in India has led to an increasing demand for high quality seed. The wide occurrence of unfavorable weather during soybean harvest, results in poor quality soybean seed and further speedy deterioration during storage under Indian conditions. Planting of such seeds lead to poor emergence, hence, poor crop stand and reduced productivity of soybean crop. Seed coat permeability is one of the first steps in breaking dormancy and initiating germination of soybean seed. Dormancy and viability can be maintained for long periods of those accessions that show seed impermeability to water (Rolston**,** 1978). Many workers have also reported very high degree of positive association of seed quality with slow rate of water absorption in soybean (Chachalis and Smith, 20002; Kuo,1989). During water imbibition seed leaches out some solutes, which include sugars, amino acids, most importantly electrolytes. Slow leaching of electrolytes is positively associated with seed vigour. Electrolyte leaching test has been carried out in soybean for testing the vigour of the seeds (Oliveira *et al.,* 1984; Yaklish *et al.,* 1979). Hence a potential method of retarding deteriorative process in the field and storage may be the incorporation of some degree of delayed permeability and slow rate of electrolyte leaching traits into adapted soybean cultivars.

Availability of molecular marker technology has made possible the genetic dissection and characterization of many quantitatively inherited seed quality traits in soybean. Keim *et al.* (1990) utilized restriction fragment length polymorphisms (RFLPs) to identify several major quantitative trait loci (QTLs) in soybean influencing

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hard seededness in an interspecific soybean population. Diers *et al.* (1992) used the same interspecific soybean population to identify several molecular marker loci that were significantly associated with QTLs for seed protein and oil. Other workers utilized molecular markers to identify several genomic regions significantly associated with seed protein, oil, seed weight and sucrose content in different intraspecific soybean populations (Mansure *et al*., 1993; Lee *et al.,* 1996; Mian *et al.,* 1996; Maughan *et al.,* 1996; Brummer *et al.,* 1997). The objective of this study was to use SSR markers to identify quantitative trait loci associated with seed coat permeability and electrolyte leaching in an intraspecific F_2 soybean population.

MATERIALS AND METHODS

Plant material

The experimental material consisted of 153 individuals of F_2 population, developed from an intraspecific cross between adapted soybean cultivars Brisa soya-1 (black seeded with hard seed coat and good longevity) and JS 71-05 (yellow seeded with soft seed coat and low longevity). At maturity, each F_2 plant was harvested and threshed separately to obtain 153 F_2 derived F_3 (F_{2-3}) lines. Seeds from each of the 153 F_{2-3} lines were planted in single rows in a randomized complete block design with two replications at the research farms of National Research Centre for Soybean, Indore and G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, in *kharif* 2004. At maturity seed of each F_{2-3} lines was harvested and threshed in bulk and stored for 9 months in ambient condition.

Phenotyping

The seed coat permeability was measured as amount of water absorbed per unit of seed weight and expressed as percent water absorbed. For this two replicates of 50 seeds harvested from 153 F_{2-3} lines were weighed first and then soaked in 100 ml distilled water for one hour. Seeds were taken out from water, excess water was removed with the help of tissue paper and weight was determined. The rate of seed coat permeability was calculated as percent water absorbed. For testing the electrolyte leaching, two weighed replicates of 50 seed each from F_{2-3} lines were soaked in 250ml-deionized water at 20^{o}C for 24 hrs. 250 ml of deionized water was used as control. After 24 h the leachates were stirred using a glass rod, poured into another beaker for measurement of conductivity and the mean of two replicates were expressed as µs/g seed.

SSR assay

DNA was isolated from the young leaves of 153 individuals of F_2 population as well as from the two parents using a method as described by Doyle and Doyle (1990). Parental polymorphism survey was carried out using 145 mapped SSR markers distributed on 20 different molecular linkage groups (MLG) of soybean genome (Cregan *et al.,* 1999). Genotyping of the F_2 population was carried out using the polymorphic markers. SSR amplification was carried out in a 10 ml reaction mixture consisting of 10x PCR assay buffer, 25mM $MgCl_2$, 200 mM of each dNTP, 0.5 units of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India), 12 ng (1.8 picomole) each of forward and reverse primers (Life Technologies, USA) and 25 ng template DNA. The PCR reaction was carried out in a peltier thermal cycler (M J Research, Model PTC-200) using following cycling parameters: initial denaturation at 94^{0}C for 5 min, followed by 33 cycles of 92^{0}C for 1min, 47°C for 1 min, 68°C for 2 min and finally a primer extension cycle of 7 min at 68°C. The amplification products were separated on 3% metaphor agarose gel containing 1.5% gel star (FMC Bio Products, Rockland, USA). Gels were run for 3h at 50V in 1x TBE buffer. DNA fragments were visualized under UV light and photographed using gel documentation system.

Statistical analysis

Trait means, normality, correlations and analysis of variance were determined and broad-sense heritability was calculated using variance component estimates. Segregation of each marker in the mapping population was tested for goodness of fit to an expected 1:2:1 ratio using the X^2 analysis. The linkage relationship among the markers was established using the computer package MAPMAKER/EXP 3.0 (Lander *et al*, 1987; Lincoln *et al.,* 1992). To test the significant associations between seed coat permeability as well as electrolyte leaching and marker loci, single factor analysis of variance was employed first as described by Edwards *et al.,* (1987) on each pair wise combination of the traits and marker locus using the computer package SYSTAT 6.0 (SPSS Inc. USA). Associations of marker loci with QTLs were considered significant when level of significance was ≤ 0.05. The coefficient of multiple determinations (R^2) from the single factor ANOVA for each significant marker locus was used as an indication of the proportion of total phenotypic variance explained (Edward *et al*., 1987).

RESULTS AND DISCUSSION

Mean, standard deviation and range for seed coat permeability and electrolyte leaching measured in the F_{2-3} line and parents at Indore and Pantnagar are presented in Table 1. The parents were significantly different ($P<0.01$) at both the locations. The transgressive segregation in either direction was observed for both the traits among the F_{2-3} lines at both the locations. The two traits were highly correlated ($r = 0.895$) at both the location. The genotype × location interaction was not significant. Broad sense heritability calculated from estimates of variance components were 83.9% and 84.6% for seed coat permeability and electrolyte leaching, respectively. For both traits, there was significant genotypic variation among F_{2-3} lines ($P<0.001$) and the frequency distribution was continuous, as typically observed for a quantitative trait (Figure 1). Due to absence of genotype x location interaction ($P <0.01$) QTL mapping was performed on entry means across locations.

Out of 145 SSR markers, 21 showed polymorphism and thus used for genotypic analysis of 153 F_2 individuals along with parents (Figure 2). The segregation pattern of each polymorphic SSR marker was tested to an expected ratio of 1:2:1 using X^2 test. Seventeen (Satt513, 193, 194, 598, 233, 285, 175, 434, 281, 389, 545, 554, 354, 431, 600, 538 and Sat_127) out of the 21 markers showed normal Mendelian segregation and rest four (Satt209, 135, Sat_099 and Sat_070) deviated from the expected 1:2:1 ratio thereby showing distortion**.** The genotypic data generated by polymorphic

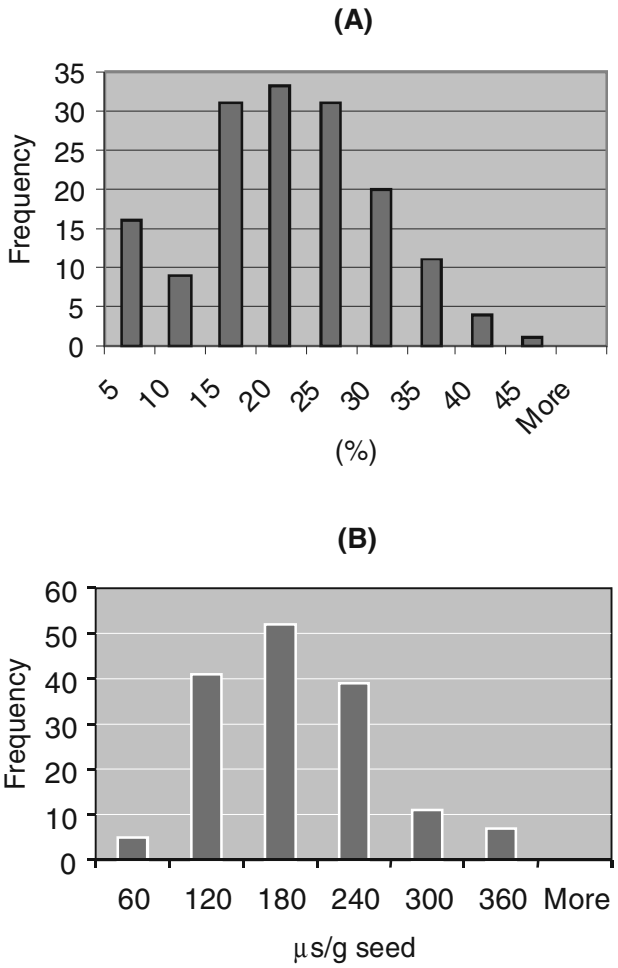

Fig. 1. Frequency distribution of the 153 F_{2-3} lines for seed coat permeability (A) and electrolyte leaching (B) in soybean.

Table 1. Means, range and parental values for seed coat permeability and electrolyte leaching in soybean grown at Indore and Pantnagar, 2004.

	F_{2-3} families				Birsa soya-1		JS 71-05	
Location	**n**	**mean±SD**	**min.**	**max.**	**n**	**mean±SD**	**n**	**mean±SD**
Seed coat permeability								
Indore	153	19.1±9.7	0.8	43.3	10	10.6±0.2	10	39.8±0.3
Pantnagar	153	21.1±9.1	1.7	40.7	10	11.5±0.1	10	38.6±0.2
Mean		20.1				11.05		39.2
Electrolyte leaching								
Indore	153	168.1±76.6	28.6	423.9	10	149.4±0.5	10	317.9±1.0
Pantnagar	153	156.1±78.1	23.8	354.0	10	147.7±0.6	10	305.7±0.9
Mean		162.1				148.6		311.8

Table 2. Putative independent SSR loci associated with variation in seed coat permeability and electrolyte leaching in soybean

Trait	SSR locus	Linkage group and its position in cM	Pr>F	R^2 %	Number of individuals in each marker locus genotypic class★			Marker locus genotypic class means	
					mm	ms	ss	Birsa soya-1	JS 71-05
Seed coat permeability	Satt538	A2 159.63	0.032	4.5	42	68	43	17.58	18.38
	Satt281	C2 40.30	0.049	4.1	37	73	43	16.19	20.91
	Satt598	E 34.20	0.039	4.2	36	81	36	18.11	19.60
	Satt434	H 105.74	0.046	3.9	32	77	44	17.76	17.97
Electrolyte leaching	Satt281	C2 40.30	0.014	5.6	37	73	43	157.1	171.5

★mm - homozygous P1 (Birsa soya-1), ms - heterozygous, ss - homozygous P2 (JS 71-05)

markers were used for linkage analysis, but none of them were found linked to each other. Of the 21 markers tested, four (Satt538, Satt281, Satt598 and Satt434) showed significant association ($P \leq 0.05$) with seed coat permeability. One of these (Satt281) was also associated with electrolyte leaching (Table 2). These 4 markers are located on four different linkage groups namely, A2, C2, E and H respectively, as reported by Cregen *et al.,* (1999). Earlier RFLP markers associated with seed traits of soybean were reported from the linkage group of A2 and E of soybean genome (Mansure *et al.,* 1983; Maughan *et al.*, 2000). Individually, these markers explained 3.9% (Satt434) to 4.5% (Satt538) of the total phenotypic variation for seed coat permeability. The SSR marker, Satt281, associated with electrolyte leaching explained 5.6% of the total variation for this trait in the present population. Birsa soya-1 alleles contributed to slow rate of seed coat permeability at the four independent loci and at one locus for electrolyte leaching of the progenies (Table 1 & 2). As transgression was also observed for these two traits in the population (Fig. 1) of this cross it would be possible to identify progeny with slow rate of seed coat permeability and electrolyte leaching than JS 71-05 with better seed

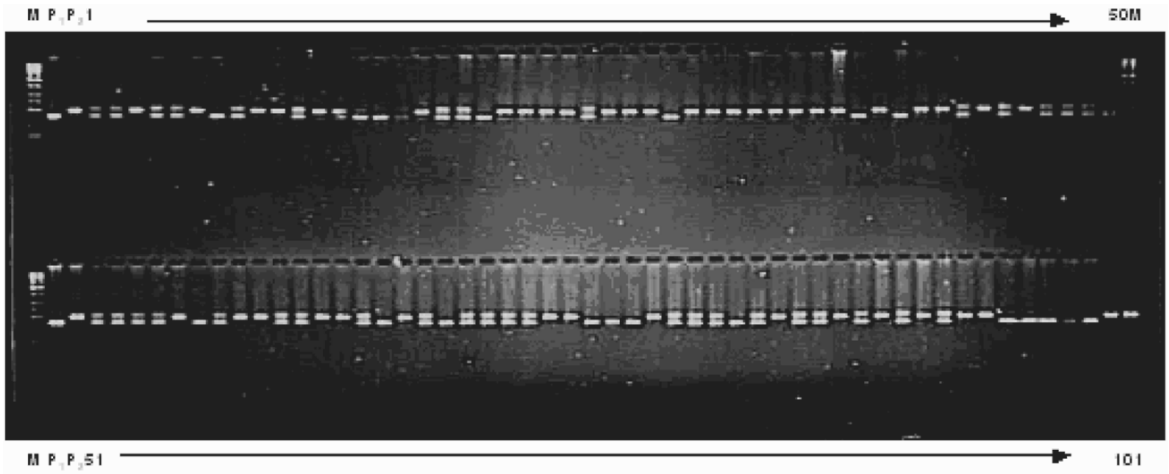

Fig. 2. A representative gel photograph of 101 out of 153 single plants showing segregation of a SSR marker Satt434 in the F_2 population. M=Molecular wt marker 50bp, P_1 = Birsa soya-1, P_2 = JS 71-05, 1-101 = single F_2 individual plant.

viability. The Birsa soya-1 allele at the marker locus Satt281 contributed to slow rate of seed coat permeability as well as to electrolyte leaching of the progeny, which might provide some genetic basis for the correlation between these two traits.

This is the first report on the identification of SSR markers related to seed coat permeability and electrolyte leaching in soybean. This study demonstrates the utility of using molecular markers to study quantitative traits related to seed viability in this important oil seed crop. A limited number of polymorphic markers used in this study did not allow complete Mendelisation of the two traits. Therefore, there is a need to apply more number of polymorphic markers well distributed on different linkage groups for identifying additional QTLs, particularly the ones having major effect on trait expression, and their effective utilization in marker aided selection.

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