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SHORT COMMUNICATION

Identification of simple sequence repeat markers linked to lipoxygenase-1 gene in soybean

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Abstract Off-flavour generated in soy products is ascribed to soybean seed lipoxygenase-1, lipoxygenase-2 and lipoxygenase-3, controlled by single dominant genes *Lox1*, *Lox2* and *Lox3*, respectively. *Lox2* locus has already been mapped and reported to be tightly linked with *Lox1* locus. The objective of the present study was to map *Lox1* locus by investigating the SSR markers reported to be linked with *Lox2* locus and the neighbouring SSR markers in two mapping populations of 116 and 91 plants developed from LSb1 × PI408251 and JS335 × PI408251, respectively. Parental polymorphism was surveyed using SSR markers Sat_074, Satt522 reported to be linked with *Lox2* locus and the SSR markers in its proximity. $F_{2:3}$ seeds were used for assaying lipoxygenase-1 to identify the genotype of the F_2 individuals. SSR marker Satt656 was found to be tightly linked with *Lox1* locus at distance of 3.6 and 4.8 cM in the mapping population of LSb1 × PI408251 and JS335 × PI408251, respectively. SSR marker Satt656 can be useful for marker assisted selection for transferring recessive allele of lipoxygenase-1 in the background of high yielding soybean genotypes.

Keywords *Lox1* locus · Lipoxygenase-1 · Soybean · SSR markers

Abbreviations

SSR	Simple sequence repeat
MAS	Marker assisted selection
CTAB	Cetyl trimethyl ammonium bromide
LOD	Log of odds
USDA	United States Department of Agriculture

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One of the major constraints in utilization of soybean in food uses is the off-flavour developed in the soy products during processing. This is ascribed to the hexanal compounds which result from the catalytic oxidation of the polyunsaturated fatty acids, containing *cis, cis 1,4 penta diene* moiety, by the lipoxygenase isozymes viz. lipoxygenase-1, -2 and -3 in soybean seed. Presence of each of these isozymes is controlled by single dominant genes i.e. *Lox1*, *Lox2* and *Lox3*, while absence is ascribed to corresponding recessive alleles (*lox1, lox2, lox3*). Soy preparations made from lipoxygenases free genotypes are better accepted due to production of very low levels of hexanal compounds (Kobayashi et al. 1995). Heat inactivation of the lipoxygenases at industrial level not only incurs extra cost but also affects the solubility and functionality of proteins (Macleod and Ames 1988); therefore, development of lipoxygenases-free genotypes through genetic elimination is the key to get rid of the beany-flavour. In the plant breeding programme aiming for this purpose, conventional colorimetric method employed for identification of null lipoxygenase alleles necessitates fine grinding of the seed and hence can't be performed in early generations. SSR markers tightly linked to lipoxygenase loci can be used for MAS for development of lipoxygenases free soybean varieties. Foreground selection using SSR markers tightly linked with the lipoxygenase loci followed by background selection using SSR markers dispersed across the genome can expedite the recovery of gene pool of the recurrent variety in backcross breeding programme aimed at introgression of null lipoxygenase alleles in popular and high yielding soybean varieties.

Of the *Lox1*, *Lox2* and *Lox3* loci in soybean, *Lox2* locus has been found to be positioned at one end of linkage group F flanked by Sat_074 and Satt522 (Kim et al. 2004). *Lox1* and *Lox3* loci have not yet been mapped. However, *Lox1* and *Lox2* have been reported to be tightly linked (Kitamura et al.1984; Kitamura et al. 1985). Further, trisomic analysis performed by Xu et al. (2000) showed the presence of *Lox1*

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Table 1 Forward and reverse sequences of Satt657, Satt218, Satt522, AW756935, Sat_090, Satt656, Sat_417, Sat_074, Satt395 in the neighbouring region of *Lox1* locus used in the study

Locus	Forward Sequence (5′➔3′)	Reverse Sequence(5′➔3′)
Satt657	GCGCATTTGGACTTTTACTTC	GCGACGATGTTAATTGGTAGAATC
Satt218	TCAATCAACAAAAACATAATTCTTC	ATTTGTGTTTTGTTTTAGCTCTCTA
Satt522	GCGAAACTGCCTAGGTTAAAA	TTAGGCGAAATCAACAAT
AW756935	GCGGCTGGTGATTGTGTAAT	GCGTAATATAGTTTTGTATTGAAAT
Sat_090	CTCGCTGCTACTGGTC	AAGAATGCGTTGGATTTA
Satt656	GCGTACTAAAAATGGCAATTATTTGTTG	GCGTGTTTCAGTATTTGGATAATAGAAT
Sat_417	GCGAATATGGCGTTGAAAATAGTGAT	GCGACCCAGATTCTGTGCTAAGA
Sat_074	GGGTGAGAAATACATGCAACTTACA	GGGCATCAAAATTGATATTAAATGTCTAA
Satt395	CGCGCTAGTTGAATGAATGT	GCGCATTGAGGAATTTTTTAT

gene on chromosome 13, which corresponded to linkage group F in the molecular linkage map given by Cregan et al. (1999). It was thought worthwhile to map *Lox1* locus by investigating the linkage of the SSR markers Sat_074 and Satt522, reported to be linked with *Lox2*, and the neighbouring SSR markers in two F_2 mapping populations: generated by crossing two lipoxygenase-1 positive (*Lox1Lox1*) Indian varieties viz. LSb1 and JS335 with PI408251 (*lox1lox1)* was procured from USDA. One hundred sixteen F_2 individuals for LSb1 × PI408251 and 91 F_2 individuals for JS335 × PI408251 constituted mapping population 1 and mapping population 2, respectively.

Genomic DNA was extracted from the finely ground leaf tissue of tender young leaves of F_2 plants by CTAB method (Doyle and Doyle 1990). Crude DNA obtained above was purified following phenol extraction and ethanol precipitation method, quantified spectrophotometrically and suitably dissolved in TE buffer (pH 8.0). The PCR reactions were performed in a Thermocycler model PTC100 and the reaction mixture (10 µl) contained 2 µl DNA (20 ng/µl), 1 µl PCR 10× buffer, 1.1 µl $MgCl_2$ (25 mM), 0.1 µl dNTPs (25 mM), 0.4 µl each forward and reverse SSR primers (30 ng/µl), 0.068 µl Taq DNA polymerase (3 U/µl), and 4.932 µl distilled water. Initially, DNA was denatured at 94 °C for 2 min. followed by 30 cycles, each cycle comprising of denaturation at 94 °C for 1 min, primer annealing at 50 °C for 2 min., primer elongation at 72 °C for 3 min. Finally, elongation was carried out at 72 °C for 10 min. PCR products so obtained were resolved on 3 % metaphore agarose gel stained with ethidium bromide using a 96-well horizontal gel electrophoresis unit (Atto Corporation) and analysed in a gel documentation unit (Syngene). Two SSR markers Sat_074 and Satt522 from LG F reported to be linked with *Lox2* and 7 neighbouring SSR markers from linkage group F were amplified for parental polymorphism survey. Subsequently, only polymorphic markers were amplified with the genomic DNAs of F_2 individuals. $F_{2:3}$ seeds harvested from two mapping populations were subjected to lipoxygenase-1 assay as given by Suda et al. (1995). Linkage of the polymorphic SSR markers with the *Lox1* locus was determined using MAPMAKER version 3.0 as given by Lander et al. (1987). Linkage was considered significant if the logarithm of odds (LOD) score was > 3.0. The Kosambi mapping function was used to convert recombination fraction to map distance among the markers and the *Lox1* gene (Kosambi 1944).

Segregation observed for presence and absence of lipoxygenase-1 activity in $F_{2:3}$ seeds for mapping population 1 (LSb1 × PI408251) and mapping population 2 (JS335 × PI408251) conform to a segregating ratio of 1:2:1, indicating thereby that the lipoxgenase-1 activity is controlled by single gene. For the mapping population 1, out of 116 F_2 individuals, 27 were lipoxygenase-1 positive (*Lox1Lox1*), 30 lipoxygenase-1 negative (*lox1lox1*) and 59 showed segregation (*Lox1lox1*) for lipoxygenase-1 activity. For mapping population 2, out of 91 F_2 individual plants, 25 were lipoxygenase-1 positive (*Lox1Lox1*), 20 were lipoxygenase-1 negative (*lox1lox1*) and 46 were seggregant (*Lox1lox1*) for lipoxygenase-1 activity.

Lox2 and *Lox1* loci have been suggested to be tightly linked to each other in earlier studies (Kitamura 1984; Kitamura et al.

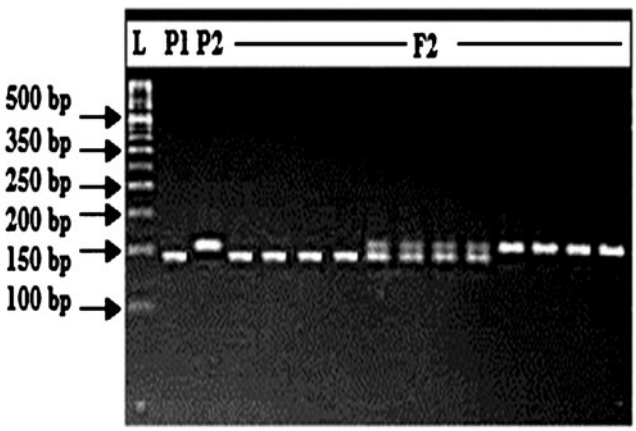

Fig. 1 Patterns of segregating DNA fragments using SSR marker Satt656 in parents and F_2 population of LSb1 × PI408251. P1 is LSb1 (*Lox1Lox1*) and P2 is PI408251 (*lox1lox1*). The lanes showing double fragments correspond to the heterozygotes

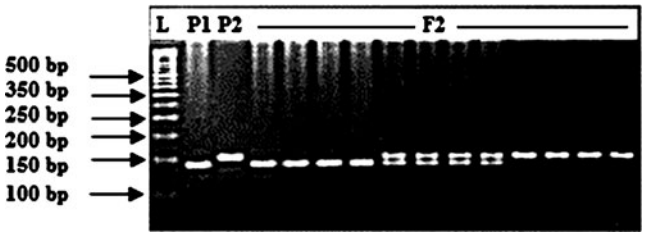

Fig. 2 Patterns of segregating DNA fragments using SSR marker Satt656 in parents and F_2 population of JS335 × PI408251. P1 is JS335 (*Lox1Lox1*) and P2 is PI408251 (*lox1lox1*). The lanes showing double fragments correspond to the heterozygotes

1985). Trisomic studies carried out by Xu et al. (2000) showed the location of *Lox1* on chromosome 13, which corresponded to linkage group F in the molecular linkage map given by Cregan et al. (1999). Based upon the linkage studies between *Lox2* locus and SSR markers, Kim et al. (2004) reported the position of *Lox2* gene at one end of linkage group F. The study showed SSR markers Satt522 and Sat_074 at 14.5 and 8.6 cM, respectively, from *Lox2* locus. Therefore, we tested the parental genotypes LSb1, JS335 and PI408251 for polymorphism using Sat_074 and Satt522 and 7 neighbouring SSR markers Satt657, Satt218, AW756935, Sat_090, Satt656, Sat_417, and Satt395. The forward and reverse primer sequences of these SSR markers are given in Table 1. Only Satt522, Sat_074, Satt656, Satt657, Sat_417 were found to be polymorphic for both the parental combinations i.e. LSb1 *vs* PI408251 and JS335 *vs* PI408251. All these polymorphic markers conformed to a normal genetic segregation ratio of 1:2:1 in both the mapping populations.

Genetic maps were constructed using genotyping data of Satt522, Sat_074, Satt656, Satt657 and Sat_417 and phenotyping data for *Lox1* locus for the mapping population 1 and mapping population 2 using MAPMAKER version 3.0 (Fig. 3). Satt656 was found to be linked with *Lox1* locus at 3.6 cM in mapping population 1 and at 4.8 cM in mapping population 2. Moreover, the orientation of Satt656 with respect to *Lox1* locus was same in both the mapping population. Representative gel of the amplification pattern obtained for Satt656 using genomic DNA of parents and F_2 plants (*Lox1Lox1*, *Lox1lox1* and *lox1lox1*) for the mapping population 1 and 2 is shown in Figs. 1 and 2, respectively. Sat_074 reported to be linked with *Lox2* locus in the earlier report (Kim et al. 2004), showed a genetic distance of 37.4 cM from *Lox1* locus in mapping population 2 but did not exhibit linkage with *Lox1* locus in mapping population 1. This may be because of mutation and structural rearrangements in this region of soybean genome. Similar results were observed in a separate study conducted by Kim et al. (2006) who reported Satt429 to be linked at distance of 5.1 cM with the *Ti* locus in the mapping population derived from Jinpumkong × C242, however no linkage was observed between this SSR marker and *Ti* locus in the other mapping population derived from Clark × C242. It is evident from the Fig. 3 that the order of the SSR markers mapped to the *Lox1* locus in our study was same as reported in the molecular linkage map given by USDA (2008). Further, the genetic distance observed between Satt657 and Satt522, Satt522 and Satt656, Satt656 and Sat_417, as indicated in the linkage maps of both the mapping populations (Fig. 3), was higher than the corresponding distance depicted in the molecular linkage map given by USDA (2008). However, according to high density linkage map given by Hwang et al. (2009) the genetic distance between Satt656 and Sat_417, and Sat_417 and Sat_074 was 2.9 cM and 11 cM respectively compared to 0.83 and 6.4 cM between the corresponding SSR markers shown in the molecular linkage map given by USDA (2008). Satt522 the SSR marker reported to be linked at a distance of 14.5 cM with *Lox2* locus in the earlier study (Kim et al. 2004) was found to be at 26 cM from *Lox1* in the mapping population 1 and at 35 cM in the mapping population 2.

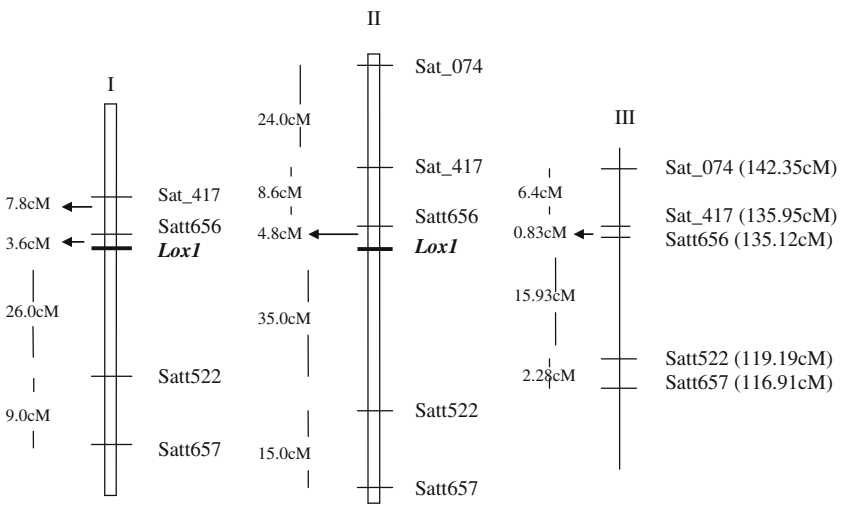

Fig. 3 Molecular linkage map of *Lox1* locus defined using population 1 (I) derived from the cross between LSb1 (*Lox1Lox1*) × PI408251 (*lox1lox1*) and population 2 (II) derived from the cross between JS335 (*Lox1Lox1*) × PI408251 (*lox1lox1*). Map was constructed using MAPMAKER version 3.0. III shows linkage map given by USDA 2008. (bldg6.arsusda.gov/cregan/soymap.html)

In conclusion, in contrast to the reported linkage of Sat_074 and Satt522 with *Lox2* locus, we did not find linkage of Sat_074 with *Lox1* locus in mapping population 1. Although, both these SSR markers were linked to *Lox1* locus in mapping population 2 but at significantly greater distance compared to their reported distance from the *Lox2*. However, SSR marker Satt656 in the proximity of Satt522 was found to be closely linked with *Lox1* locus in both the mapping populations and, therefore may be used for marker assisted selection for development of lipoxygenase-1 free soybean genotypes.

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