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Research Article

Molecular mapping of important agro-botanic traits in sesame

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Abstract
Sesame (*Sesamum indicum* L.) ((2n=26) is one of the most ancient oilseed crop of the world. The present study was undertaken to develop a molecular map of the important agro-botanic traits in sesame. Two sesame genotypes Chandana and TAC-89-309 that differ in respect of the important agro-botanic traits were crossed to study the inheritance of these traits. The F_2 population along with the F_1 and parents was evaluated under field conditions and observations were made on nine agro-botanic traits using International Board for Plant Genetic Resources (IBPGR) descriptor grading. For mapping trait related QTL, a genetic framework linkage map was constructed employing a mapping population of 120 F_2 individual plants and effective 60 RAPD polymorphic markers chosen based on the study of parental polymorphism (23.07 %). The linkage map places the 60 markers on nine linkage groups spanning a total length of 1547.16 cM at an average distance of 25.78 cM between markers. These nine linkage groups could be covering about 60% of the map length of the genome. Length of the linkage groups ranged between 58.8 (Linkage Group 8) to 423.8 cM (Linkage group 9). The segregation and normal distribution observed in respect of most of the traits in the F_2 population have been indicative that the mapping population had sufficient amount of genetic variability for mapping trait specific QTL. Seventeen QTL have been identified for the nine agro-botanic traits by single marker analysis. In all, 19 QTL have been identified by using QTL cartographer v 2.5. Of which, 7 and 12 QTL have been identified by Simple interval mapping and Composite interval mapping respectively. Two genomic regions - one on LG 1 and the other on LG 6 had more than one QTL. The marker interval between OPAE 15_{350} and OPD 6_{480} on the linkage group 1 has three QTL *viz*., leaf angle, capsule hair length and stem hairiness, while on LG 6 between the markers OPP 8_{380} and OPR 8_{980}, two QTL for basal leaf shape and capsule hair density are distributed. In all, nine tightly linked markers for nine different traits have been identified with marker-QTL distance of < 2.6 cM. Out of the 19 QTL detected, five explaining high phenotypic variation are promising. These include one QTL for corolla colour, two for capsule shape and one each for capsule hair density and number of nodes.

Key words:
Sesame, Agro-botanic traits, Genetics, Molecular Markers, QTLs

Introduction:

Sesame (*Sesamum indicum* L.) (2n=26)also known as sesamum, til, gingelly, simsin, gergelim etc. is the most ancient oilseed crop of the world. It is being cultivated in Asia for last 5000 years (Joshi, 1961 and Weiss, 1971). It is regarded as the 'Queen of Oilseeds' as the quality of oil is of high nutritional and therapeutic value combined with stability. Sesame is the sixth most important oilseed crop of the world, occupying an area of 6.6 m. ha, with a production of 3.15 m. tonnes and its average productivity being 460 kg/ha. India is the largest sesame growing country in the world with an area of 1.85 m.ha, producing 0.64 m. tones but productivity wise it is among the lowest with 345 kg/ha (CMIE, 2011). It is grown in marginal and sub marginal lands as rainfed crop.

Despite its shorter life cycle, suitability to different cropping systems and land types, adaptation to moisture stress and low input management conditions, sesame's contribution to the country's oilseed production is very minimal. The major reason for the dismal state of production is very low and inconsistent productivity of varieties *in vogue*. All past efforts to raise the genetic yield level by conventional recombination breeding could not improve the yield substantially because of the dependence of breeders on narrow cultivar gene pool for desired variability. Also, lack of basic information on genetics and breeding behaviour of traits of economic importance, especially complexly inherited traits is causing hindrance to the breeders in realization of higher yields. So far the knowledge of genetics of traits of economically important traits is very meagerl known.

Recent advances in cellular and molecular biology have provided a wide array of innovative techniques capable of finding solution to the problems encountered in conventional breeding/selection approaches. Molecular marker technology enables precision in selection/screening

at genotype level and in unfolding the hitherto hidden variability of breeding value. Hardly any serious attempt has been made to take advantage of such ingenious tools/techniques for improvement of sesame. Keeping in view the information gap of basic tools such as well defined molecular linkage maps for resorting to molecular assisted selection breeding approaches, the present study was undertaken to study the inheritance as well as molecular mapping of important agro-botanic traits in sesame.

Material and methods

Two sesame genotypes, Chandana (a high yielding variety released from Regional Agricultural Research Station, Jagtial, Acharya N.G. Ranga Agricultural University, India) and the accession TAC-89-309 (made available by Jawaharlal Nehru Krishi Vishwa Vidyalay, Jabalpur) with differential agro-botanic traits were chosen as parents to study the inheritance as well as mapping of the traits. At flowering, plants were selected and tagged for effecting crosses. Crossing was done between the parents to generate F_1 during *kharif*, 2009 at Seed Research and Technology Centre, Rajendranagar. The F_1 was selfed to produce F_2 during *rabi*, 2009-10. The F_2 population along with F_1 and parents was evaluated under field conditions at the College Farm, College of Agriculture, Rajendranagar, Hyderabad during *kharif*, 2010. Observations were made on nine agro-botanic traits (Table 1) using IBPGR descriptor grading.

Frequency distribution and correlations among character pairs in the F_2 generation were computed at $p < 0.05$ and $p < 0.01$ in Microsoft-Excel (2007) using trait mean. Test of significance among F_2 was done by chi-square method. χ^2 test was applied for testing the deviation of the observed segregation from theoretical segregation.

Leaf samples were collected from F_2 generation. DNA extraction from sesame was difficult due to presence of polyphenol and polysaccharides. These compounds have also been reported to cause difficulty in DNA purification in other plant species and inhibit enzyme action. Therefore, five different DNA extraction protocols were tried in the present study to obtain high quality and pure DNA and of them the method developed by Porebski *et al.* (1997) was found efficient and used. Estimation of quantity and quality of the DNA was done based on spectrophotometric measurement of UV absorbance at 260 nm and 280 nm.

PCR amplification was performed in a 10 µl volume of PCR reaction mix containing Taq buffer (10X) with Mg Cl_2 – 1.2 µl (1X), dNTP mix (2.5 mM each - 1mM (1.0 µl), Taq DNA polymerase (3U/µl) – 1 U (0.3 µl)(Bangalore Genei), 10-mer oiligo RAPD primer – 0.2 µM (1.0 µl), Genomic DNA (50 ng/µl) – 2.0 µl and Sterile distilled water – 4.5 µl. PCR amplification was carried out on thermal cycler (Eppendorf, or Applied Biosystems, USA) with Initial denaturation at 94^{o}C for 5 min followed by 45 cycles with denaturation at 94^{o}C for 1 min, primer annealing at 37^{o}C for 45 sec, extension at 72^{o}C for 45 sec and final extension of 72^{o}C for 8 min. Following amplification the samples were stored at 4^0 C for short periods and at -20^0 C for long duration. The amplified products were checked on ethidium bromide stained agarose gels (1.8 %) along with the marker 100 bp + 1.5 Kbp DNA ladder (Bangalore Genei,) and polymorphic primers were noted. The resolved PCR bands were documented using Bio-Rad Molecular Imager Gel Doc XR System. A primer was considered polymorphic, if it amplified a band in one parent and absent in the other.

A set of 160 operon RAPD primers (OPA, OPAE, OPC, OPD, OPH, OPM, OPP, OPR, OPS and OPT) were screened between the parents, Chandana and TAC-89-309. Each amplification product was considered as an RAPD marker. Based on the parental polymorphism, markers which clearly distinguished the parents were used to screen the individual plants of the mapping population (F_2).

Linkage map was constructed using the MAPMAKER/EXP version 3.0 (Lincoln *et al*., 1992) following Kosambi Mapping Function (Kosambi, 1944) and MapDisto software version 1.7b 132 (Lorieux, 2006). Linkage groups were determined using 'group' command with LOD (Logarithm of odds ratio) score of 3 and recombination fraction of 0.4. Order of the markers for each group was determined using 'order' and 'ripple' commands. Each of the scored traits (MAPMAKER result file) along with phenotypic means was subjected to QTL mapping. QTL were detected by interval mapping (IM), (Lander and Botstein, 1989) and composite interval mapping (CIM) procedure of Windows QTL Cartographer v. 2.5 software (Wang *et al*., 2007). The $\chi 2$ goodness of fit against 3:1 segregation ratio was estimated using Map Disto v. 1.3 software (Lorieux, 2006).

Results and Discussion

The segregating population (F_2) along with parents Chandana and TAC-89-309 and F_1 was evaluated for nine agro-botanic traits (Table 2) and the inheritance of these agro-botanic traits was studied. The study revealed wide variation for all the nine agro-botanic traits which broadly include leaf, stem, floral and capsule characters(Table 3).

Inheritance of important agro-botanic traits**:** Mixed leaf position is dominant over opposite position and

is governed by a single dominant gene as evident from the trait segregating in the ratio of 3 mixed: 1 opposite in F_2 population (Fig. 1). This finding is in agreement with that of Mohammad and Gupta (1941). In the case of leaf shape, 'entire' is dominant over 'lobed' and their segregation in the ratio of 15 normal (entire) to 1 lobed suggests that entire leaf shape was governed by duplicate dominant gene action (Fig. 2). The findings are in conformity with those of Langham (1945) who had reported entire leaf to be dominant over wrinkled leaf. Also, Murthy and Oropeza (1989), based on their study of the induced mutant trait narrow leaf in M_4 generation concluded that normal leaf was dominant over narrow leaf and was controlled by duplicate dominant gene action. In respect of leaf angle, horizontal leaf angle is dominant over acute leaf angle and they have been found to segregate in the ratio of 9 horizontal to 7 acute suggesting horizontal leaf angle to be controlled by two pairs of dominant genes showing complementary gene action. This is reported for the first time. Corolla colour varies widely in different combinations and shades. And, equally the genetics of it is quite complex and purple colour has been reported to be dominant over purple white and white in India (Ashri, 1995). In the present study, with purple segregants in F_2 appearing in a variety of shades judging it has been found very difficult. The trait appears to be much more complex than thought hitherto (Fig. 3). The problem could probably be more satisfactorily solved by studying it on chemical basis of anthocyanin inheritance as suggested and actually done by Lawrence and Price (1940) in Dahlia. Hairiness on various plants is yet another trait that shows great variation depending on environmental influence. The simple mode of inheritance governing the trait is in confirmation with Tan (1998) and Falusi (2000) who have reported hairiness to be governed by a single dominant gene. In the present study, profuse hairiness on capsule has been found to be dominant over sparse hairiness and controlled by single dominant gene whereas, medium hair length found to be dominant over short hair length but controlled by two pairs of genes exhibiting complementary gene action (9:7). Capsule shape appears to be simply inherited and governed by a single dominant gene with broad oblong capsule being dominant over narrow oblong (Fig. 4). On the main stem, number of nodes to first flower was recorded. In Chandana, number of nodes were five as against seven in TAC-89-309. F_1 had four nodes and F_2 with number of nodes varying from three to seven showed normal distribution and 39.1 % of it were transgressive segregants. The type and degree of stem hairiness, although a varietal characteristic, level of expression is subject to environmental influence. In agreement with earlier reports stem hairiness has been found in the present study to be controlled by a single dominant gene (Tan, 1998 and Falusi, 2000). Ability to select genotypes with desired degree of stem pubescence could be of value in breeding for tolerance to biotic/abiotic stresses as strong association has been reported between singly inherited stem pubescence and complexly inherited drought resistance (Weiss, 1971).

Simple correlations among important agro-botanic traits: Nature and strength of relationship between traits estimated by regressing phenotypic values of one trait on those of another trait gives an idea to breeders how selection of one trait affects the other. If some of the easily visible morphological traits follow strong association with complex traits like yield etc, the former could be a reliable marker for selection of the latter. The following trait pairs showed significant correlation coefficients. The trait leaf position exhibited significant and positive correlation with corolla colour (r=0.289**) and capsule shape (r=0.204**) (Table 4). Basal leaf shape followed negative and significant correlation (r= - 0.181*) with the trait capsule hair length, while, significant positive association was observed between leaf angle and capsule hair length (r= 0.174*). Whereas corolla colour and capsule shape followed significant positive correlation (r=0.334**). Correlation between capsule hair length and number of nodes to first flower (r= - 0.236**) was significant but negative. Interestingly, capsule hair density and stem hairiness were correlated with none of the traits studied. A highly positive and significant association of leaf position with corolla colour and capsule shape, leaf angle with capsule hair length and corolla colour with capsule shape may be due to either pleiotropic action of leaf arrangement gene or due to its close physical linkage with others. No significant correlation between capsule hair density and stem hairiness suggests that these traits might be controlled by independent genes. The findings might prove valuable, if they are found linked to complexly inherited traits of economic value such as yield and its major components, tolerance to abiotic stresses etc.

Parental polymorphism and linkage map construction: Out of 160 operon RAPD 10-mer primers screened between the parents, 260 repeatable amplified fragments were produced. Sixty markers were detected to be polymorphic between the parents amounting to a polymorphism percentage of 23.07%. The markers, which were polymorphic between the parents were used to screen the F_2 mapping population (Fig. 5).

High density genetic map with high levels of genome coverage is the foremost need for applications in plant breeding. In sesame, as yet no well developed classical linkage map using

morphological markers is available for use in crop improvement programmes. In the present study, tremendous segregation and normal distribution observed in respect of most of the traits in the F_2 population have been indicative that the mapping population had sufficient amount of genetic variability for mapping trait specific QTL. For mapping QTL, a genetic framework map was constructed employing a mapping population of 120 F_2 plants and 60 RAPD markers using MapDisto software version 1.7b 132 (Lorieux, 2006) at a minimum LOD threshold of 3.0 resulted in nine linkage groups. Linkage groupwise number of markers, map length and average marker interval are presented (Table 5). Length of the linkage groups ranged between 58.8 (Linkage group 8) and 423.8 cM (Linkage group 9) covering a distance of 1547.16 cM with average length being 25.78 cM between adjacent marker loci (Fig 6). Nine regions on the linkage groups 3, 4 and 9 however, showed large gaps exceeding 50 cM. The linkage groups 3, 6 and 9 had maximum number of 9 markers followed by the linkage groups 1, 5 and 7 with 6 markers on each. Linkage groups 2, 4 and 8 had five markers each. The genetic linkage map consisting of 35 linkage groups was constructed earlier with AFLP markers by Padmavathi *et al.*, (2003) using F_2 population measuring a total map length of 2500 cM length. The nine linkage groups accounting for 1547.6 cM in the present investigation could be covering about 60% of the map length of the genome. Variation in the number of linkage groups and their length found in the present study and by Padmavathi *et al.* (2003) suggest that they in a way correspond to the chromosome complement. Possibly the longest linkage groups may correspond with two pairs longest chromosomes designated as A and B by Mukherjee (1959). As against the expected 13 or 26 linkage groups representing 'n' number of chromosomes and double the number of chromosome arms, as many as 35 or so linkage groups reported by Padmavathi *et al.* (2003) could be due to genome regions remaining far apart without markers in between. Identification of more and more markers would help bring down the linkage groups not exceeding 13. Higher average genetic distance between markers observed in the present study could be due to two reasons *viz.*, (i) higher homology between DNA strands in the population and (ii) stretching effect of markers on chromosomes contributing to increased map length. Employment of small size mapping population and more number of markers showing distorted segregation are considered to be the probable reasons for the stretching effect (Subudhi and Huang, 1999). Being an exploratory mapping exercise, the level of resolution and genome coverage achieved of the present linkage map not may be adequate but indicative for at least for some of the chromosomes to detect QTL for agro-botanic traits.

Segregation distortion, which is the deviation from the expected Mendelian ratio (3:1, RAPD being dominant marker), was observed. Of the 60 markers used to screen the mapping population, 8 (13.33%) showed segregation distortion and they were found distributed randomly on nearly all the linkage groups. The skewed segregation of the marker loci may be due to physiological or genetic factors such as selective abortion of male or female gametes, or selective gametic mating (Xu *et al.*, 1995) or due to preferential selection at gametic or zygotic levels, chromosome rearrangement, incompatibility, relative pollen competition for fertilization, unequal crossover during meiosis, chromosome loss, zygotic embryo abortion, changes in genetic load and lethal alleles. The phenomenon was seen on all the linkage groups suggesting that it occurs at random and not confined to any specific part of the genome as opined by Xu *et al.* (1995).

Mapping of QTL for important agro-botanic traits: Phenotypical analysis of the agro-botanic traits although showed them to be governed by a few genes molecular analysis revealed QTL to govern almost all of them. Windows QTL cartographer version 2.5 software was used to identify QTL by simple interval mapping (SIM) and composite interval mapping (CIM). Phenotypic data of the nine agro-botanic traits and corresponding genotypic data generated using 60 polymorphic markers for 120 F_2 plants were used to develop a source file for identifying trait related QTL. The QTL identified by SIM and CIM methods were not always in agreement with each other. The relative contribution of a particular locus to a specific trait, QTL effect (A) and proportion of phenotypic variance explained (R^2) were determined for each of the QTL detected.

Traitwise QTL identified by single marker analysis: Single marker analysis using 60 polymorphic RAPD primers identified a total of 17 QTL for the nine agrobotanic traits (Table 6). Six (35.29 %) of these QTL had LOD score higher than 5.0. Two QTL were identified for leaf position on the LG 2 (*qLP 2.1*) and 9 (*qLP 9.1*) with LOD scores of 4.09 and 5.82 respectively. Two QTL *viz.*, *qBLS 1.1* and *qBLS 3.1* relating to basal leaf shape were identified on the LGs 1 and 3. Negative regression was observed (-0.265) for the QTL on LG 1. QTL *qLA 1.1* and *qLA 7.1* were identified for leaf angle on the LGs 1 and 7 respectively. The F (critic.) value of these two QTL was 10.235 and 4.927 respectively. Of three identified QTL *viz.*, *qCC 9.1*, *qCC 5.1* and *qCC 5.2* relating to corolla colour, two were distributed on the LG 5 and one

on 9. Only one QTL (*qCHD 6.1*) was identified for capsule hair density on the LG 6. For this QTL negative regression (-0.253) was observed. Two QTL viz., *qCHL 1.1* and *qCHL 6.1* effecting capsule hair length were distributed on the LGs 1 and 6 respectively. Two QTL distributed on the LGs 2 (*qND 2.1*) and 5 (*qND 5.1*) were found to effect number of nodes to first flower. The LOD score for the QTL were 6.219 and 4.844 respectively. Only one QTL (*qCS 3.1*) located on LG 3 was found to be effective on capsule shape. The QTL *qSH* 1.1 and *qSH* 7.1 located on the LGs 1 and 7 were identified to be related to stem hairiness.

Traitwise QTL identified by SIM and CIM: By SIM, seven QTL relating to nine agro-botanic traits were identified while by CIM, 12 QTL were identified. In all, 19 QTL were identified by these two methods as detailed below (Table 7). The 19 QTL found distributed on nine linkage groups amounted to on an average 2.11 QTL per trait and linkage group. Percent phenotypic variance explaining each of the trait related QTL ranged from 0.04 in leaf position to 20.09 in capsule hair density. Two QTL effecting leaf position were identified on the LGs 5 (*qLP 5.1*) and 9 (*qLP 9.1*) with the LOD score of 2.97 and 2.91 respectively. The two together contributed 7.77% phenotypic variance. One QTL *viz.*, *qBLS 6.1* located on the LG 6 was found to affect basal leaf shape. The TAC-89-309 allele showed negative effect on this QTL. QTL relating to agro-botanic traits has been identified as well in other crop species using different DNA markers. For instance, in *Vigna vexillata* using F_2 population of the intra-specific cross Tvnu 1443'× Tvnu 73 Ogundiwin *et al.* (2008) have identified a novel QTL for leaf shape with 70 RAPD markers. Two QTL (*qLA 1.1* and *qLA 1.2*) were identified for leaf angle on the LG 1. The phenotypic variance of these two QTL was 11.41 and 2.13 % respectively. Study by Wu *et al.* (1997) of F_2 population of the cross between *Populus trichocarpa* and *P. deltoids* of populus using RFLP, STS and RAPD markers reveals QTL on different linkage groups governing leaf angle at each of the four positions.

For corolla colour, two QTL *viz.*, *qCC 5.1* and *qCC 7.1* distributed on the LGs 5 and 7 respectively were found to govern this trait. The allele of TAC-89-309 contributed to one QTL while Chandana allele contributed to other QTL. Bradshaw *et al.* (1998) have studied F_2 plants of the cross between *Mimulus lewisii* and *M. cardinalis* species of monkey flower and identified one QTL for anthocyanin pigmentation and two QTL for carotenoid pigmentation, which imparts colour to corolla. Three QTL *viz.*, *qCHD* 6.1, *qCHD* 6.2 and *qCHD* 6.3 distributed on the LG 6 were found to govern capsule hair density. The three QTL together contributed 38.41% phenotypic variance. Only one QTL (*qCHL 1.1*) identified on the LG 1 was found to govern capsule hair length. One QTL (*qND2.1*) identified on the LG 2 was effecting number of nodes to first flower. The LOD score for the QTL was 3.16 and phenotypic variance of 15.43 %. The results are in agreement with those of Vaughan *et al.*, (2005) who based on their study of F_2 population of the cross between Primo, a marrowfat cultivar, and OSU442-15, a blue pea breeding line using RAPD, RFLP and AFLP report four novel QTL *viz., nff 1.1, nff 2.1, nff 3.1 and nff 4.1* to affect number of nodes. Suresh *et al.* (2003) from their study of F_2 population derived from the intervarietal cross between the varieties 'Sunrise' and UH356 of papaya have detected two QTL affecting node at first flowering. For capsule shape, three QTL were identified. Two of them (*qCS* 6.1 and *qCS* 6.2) were on the LG 6 and the third one (*qCS* 3.1) was on the LG 3. All the three QTL were influenced by the alleles from TAC-89-309 explaining a total phenotypic variance of 44.21 %. Only one QTL (*qSH* 1.1) located on the LG 1was found to govern stem hairiness. This was a common QTL in both SIM and CIM. The LOD value of the QTL was 3.16 in SIM and 3.21 in CIM. Frary *et al.* (2003) have reported novel QTL to govern for stem hairiness in brinjal as understood from the study of F_2 population of an interspecific cross.

Three QTL in all were common in SIM and CIM. One QTL each for the traits corolla colour, number of nodes to first flower and capsule shape was identified. For corolla colour, the QTL identified in both the methods was influenced by the allele of TAC-89-309. The allele of Chandana contributed to the QTL relating to capsule shape in both the methods. Out of the 19 QTL detected in all, five are promising with greater phenotypic variation when compared to other QTL. These include one QTL for corolla colour, two for capsule shape and one each for capsule hair density and number of nodes. They could be most rewarding in marker assisted breeding of complex economic traits having close linkage with them. Since the discriminatory ability of the analysis in the present study was limited by a population size of 120 F_2 plants, there is a chance of escape of QTL with small phenotypic effects. Apart from the population size, possible existence of closely linked QTL as suggested by Paterson *et al.* (1988) and high LOD threshold (3.0) used in the present investigation may also have contributed to underestimation of the number of QTL. Hence, the number of QTL reported represents the lower limit but mostly significant ones.

Unlike in many crop species, very few researchers worked on development and use of molecular linkage maps for mapping/tagging of genes of interest and in introgressing them by marker assisted breeding (Zhang *et al*. (2013) and Zhang *et al.* (2013 a). The information generated through this effort would benefit the genetic research towards understanding the genome structure, location of genes of interest on chromosomal regions and their linkage with DNA markers. Also, this would be a starter for building in phases a high density molecular linkage map paving way for development of marker assisted breeding for traits of economic value, especially those which are complexly inherited like yield and its major components, tolerance to abiotic stresses etc.

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Table 1. Expression of agro-botanic traits and grading in parents and F_1 (Chandana x TAC-89-309)

Trait	Category	Grading
Leaf position	Opposite	1
	Alternate	2
	Mixed	3
Basal leaf shape	Entire	1
	Lobed	2
Leaf angle	Acute	3
	Horizontal	5
	Drooping	7
Exterior corolla colour	White	1
	White with violet/purple shading	2
	White with deep violet/purple border	3
	Violet	4
	Purple	5
Capsule hair density	Glabrous	0
	Sparse	3
	Profuse	7
Capsule hair length	Short	1
	Medium	2
	Long	3
Number of nodes to first flower	On main stem	
Capsule shape	Tapered	1
	Narrow oblong	2
	Broad oblong	3
	Square	4
Stem hairiness	Glabrous	0
	Sparse	3
	Hairy	7
	Very hairy	9

Table 2. Expression of agro-botanic traits in parents and F_1 (Chandana x TAC-89-309)

Trait	Chandana	TAC-89-309	F_1
Leaf position	Mixed (3)	Opposite (1)	Mixed (3)
Basal leaf shape	Lobed (2)	Entire (1)	Entire (1)
Leaf angle	Horizontal (5)	Acute (3)	Horizontal (5)
Corolla colour	White with deep violet shading (3)	White with light violet shading (1)	White with deep violet shading (3)
Capsule hair density	Profuse (7)	Sparse (3)	Profuse (7)
Capsule hair length	Medium (2)	Short (1)	Medium (2)
Number of nodes	Five	Seven	Four
Capsule shape	Broad oblong (3)	Narrow oblong (2)	Broad oblong (3)
Stem hairiness	Sparse (3)	Glabrous (0)	Sparse (3)

Table 3. Segregation pattern of agro-botanic traits in F_2

Character	Observed/Expected			χ^2	Ratio proposed
Leaf position		Mixed	Opposite		
	Observed	91	29	0.04	3:1
	Expected	90	30		
Basal leaf shape		Entire	Lobed		
	Observed	109	11	1.73	15:1
	Expected	112.5	7.5		
Leaf angle		Horizontal	Acute		
	Observed	66	54	0.07	9:7
	Expected	67.5	52.5		
Capsule hair density		Profuse	Sparse		
	Observed	88	32	0.17	3:1
	Expected	90	30		
Capsule hair length		Medium	Short		
	Observed	64	56	0.41	9:7
	Expected	67.5	52.5		
Capsule shape		Broad oblong	Narrow oblong		
	Observed	96	24	1.60	3:1
	Expected	90	30		
Stem hairiness		Sparse	Glabrous		
	Observed	87	33	0.40	3:1
	Expected	90	30		

Table 4.Simple correlations among important agro-botanic traits

Characters	Leaf position	Basal leaf shape	Leaf angle	Corolla colour	Capsule hair density	Capsule hair length	Number of nodes	Caspule shape
Basal leaf shape	0.044							
Leaf angle	0.076	0.171						
Corolla colour	0.289**	0.012	0.042					
Capsule hair density	0.012	-0.070	-0.091	0.103				
Capsule hair length	0.060	-0.181*	0.174*	0.111	0.035			
Number of nodes	0.040	0.038	0.072	-0.035	-0.045	-0.236**		
Caspule shape	0.204**	0.014	0.008	0.334**	0.028	-0.033	-0.019	
Stem hairiness	0.045	0.066	0.006	0.098	0.093	0.090	-0.073	0.065

*, ** significant at 0.05 and 0.01 level respectively

Table 5. Linkage group wise markers, map length and average marker interval

Linkage Group	No of markers	Map length (cM)	Average marker interval (cM)
1	6	82.19	13.69
2	5	119.32	23.86
3	9	380.28	42.25
4	5	165.00	33.00
5	6	97.86	16.31
6	9	127.66	14.18
7	6	92.07	15.34
8	5	58.8	11.76
9	9	423.80	47.08
Total	**60**	**1547.16**	**24.16**

Table 6. QTL relating to nine agro-botanic traits identified on different linkage groups (LG) by Single Marker Analysis

Trait/Marker	LG	QTL	LOD	Regression	F-crit	Fobs
Leaf position						
OPH 1_{1180}	2	*qLP2.1*	4.091	0.249	4.096	0.045
OPT 16_{220}	9	*qLP9.1*	5.824	0.312	5.869	0.017
Basal leaf shape						
OPAE 12_{570}	1	*qBLS1.1*	4.493	-0.265	4.502	0.036
OPM 2_{400}	3	*qBLS3.1*	4.158	0.257	4.160	0.044
Leaf angle						
OPAE 12_{570}	1	*qLA1.1*	9.982	0.369	10.235	0.002
OPA 8_{1400}	7	*qLA7.1*	4.909	-0.258	4.927	0.028
Corolla colour						
OPT 16_{220}	9	*qCC9.1*	4.661	0.250	4.673	0.033
OPA 9_{750}	5	*qCC5.1*	3.936	0.218	3.935	0.050
OPP 1_{1400}	5	*qCC5.2*	7.333	0.299	7.436	0.007
Capsule hair density						
OPR 8_{980}	6	*qCHD6.1*	3.975	-0.253	3.974	0.049
Capsule hair length						
OPAE 12_{570}	1	*qCHL1.1*	5.741	0.292	5.782	0.018
OPC 13_{800}	6	*qCHL6.1*	4.943	0.270	4.963	0.028
Number of nodes						
OPH 1_{1180}	2	*qND2.1*	6.219	0.294	6.277	0.014
OPP 1_{1400}	5	*qND5.1*	4.844	0.263	4.860	0.029
Capsule shape						
OPR 6_{1100}	3	*qCS3.1*	3.962	0.312	3.421	0.029
Stem hairiness						
OPAE 12_{570}	1	*qSH1.1*	5.419	0.254	5.451	0.021
OPA 8_{1400}	7	*qSH7.1*	4.662	-0.233	4.674	0.033

Table 7. QTL identified for important agro-botanic traits by SIM and CIM methods

Trait	QTL	Marker interval	Allelic effect	SIM			CIM		
				Additive	LOD	R^2	Additive	LOD	R^2
Leaf position	*qLP 5.1*	OPP 1_{1400}-OPS 15_{380}	TAC-89-309	-0.97	2.97	0.04	-	-	-
	qLP 9.1	OPT 4_{400}-OPT 16_{220}	Chandana	-	-	-	42.65	2.91	7.73
Basal leaf shape	*qBLS 6.1*	OPP 8_{380}-OPR 8_{980}	Chandana	-	-	-	-50.66	3.17	7.23
Leaf angle	*qLA 1.1*	OPAE 12_{570}-OPAE 15_{350}	Chandana	-	-	-	54.76	2.97	11.41
	qLA 1.2	OPAE 15_{350}- OPD 6_{480}	Chandana	20.59	3.38	2.13	-	-	-
Corolla colour	*qCC 5.1*	OPH 7_{1300}-OPP 1_{1400}	Chandana	-	-	-	47.57	2.72	11.47
	qCC 7.1	OPC 19_{1100}-OPM 10_{250}	TAC-89-309	-27.57	3.42	17.47	-32.12	3.12	10.68
Capsule hair density	*qCHD 6.1*	OPC 13_{800}-OPH 13_{850}	Chandana	-	-	-	47.13	2.51	8.21
	qCHD 6.2	OPP8_{380} –OPR 8_{980}	TAC-89-309	-	-	-	-87.45	3.73	20.09
	qCHD 6.3	OPR 8_{980}-OPS 6_{450}	TAC-89-309	-	-	-	-61.54	3.06	10.11
Capule hair length	*qCHL 1.1*	OPAE 15_{350}-OPD 6_{480}	Chandana	13.72	2.55	0.89	-	-	-
Number of nodes	*qND 2.1*	OPC 17_{550}-OPH 1_{1180}	Chandana	41.56	3.16	10.56	43.42	3.21	15.43
Capsule shape	*qCS 3.1*	OPR 6_{1100}-OPR 12_{450}	TAC-89-309	-55.23	3.02	18.87	-65.23	3.32	16.87
	qCS 6.1	OPA7_{600}-OPAE 11_{800}	TAC-89-309	-	-	-	-75.56	2.73	18.07
	qCS 6.2	OPAE 11_{800}-OPC 13_{800}	TAC-89-309	-	-	-	-45.87	2.74	7.27
Stem hairiness	*qSH 1.1*	OPAE 15_{350}-OPD 6_{480}	Chandana	18.08	2.67	1.92	-	-	-

Fig 1: Segregation for Leaf Position in F_2

Fig 2: Segregation for Leaf Shape in F_2

Fig 3: Corolla colour in Chandana, TAC-89-309, F_1 and F_2

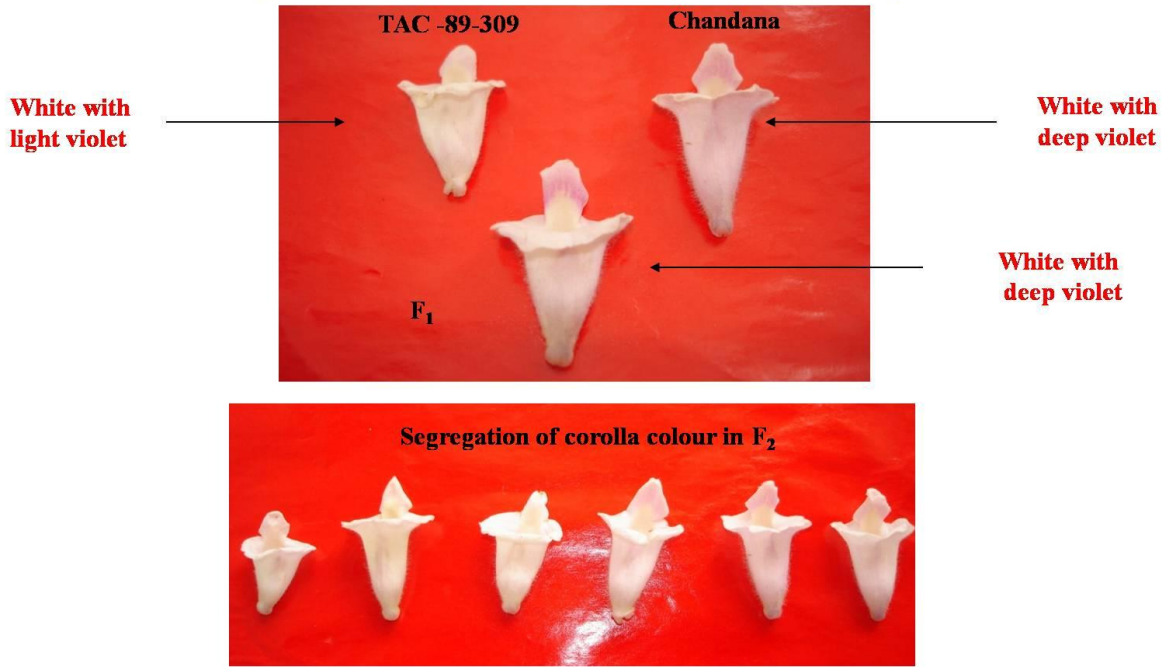
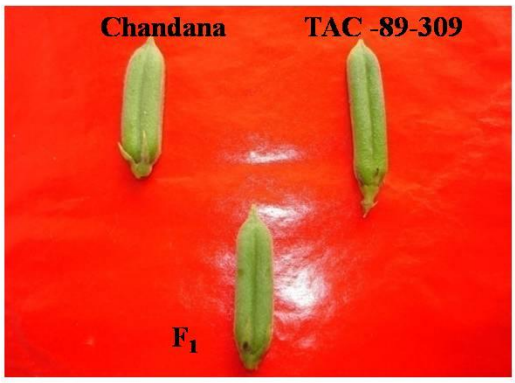


Fig 4: Capsule Hair Density and Capsule Shape in Chandana, TAC-89-309, F_1 and F_2



Capsule hair density

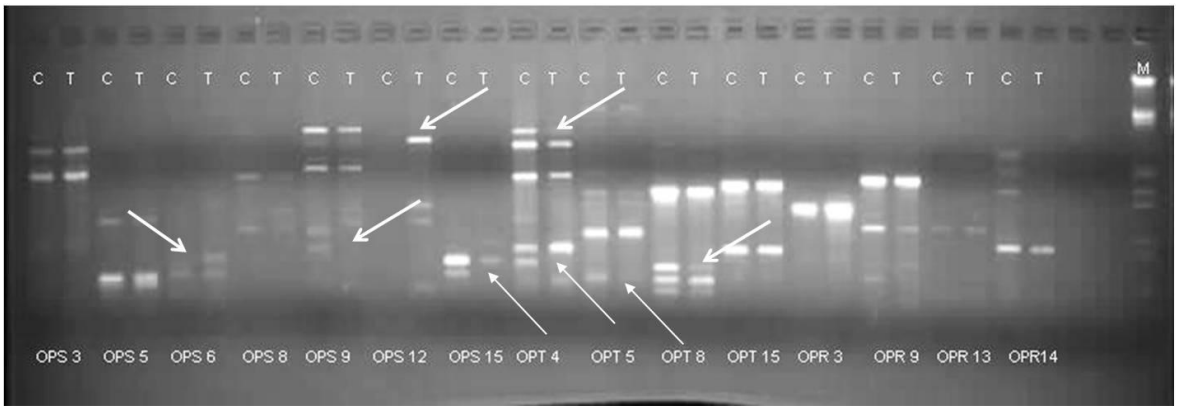
Chandana	– Profuse
TAC-89-309	– Sparse
Chandana x TAC-89-309	– Profuse

Capsule shape

Chandana	– Broad oblong
TAC-89-309	– Narrow oblong
Chandana x TAC-89-309	– Broad oblong

Segregation of capsule hair density and capsule shape in the F_2

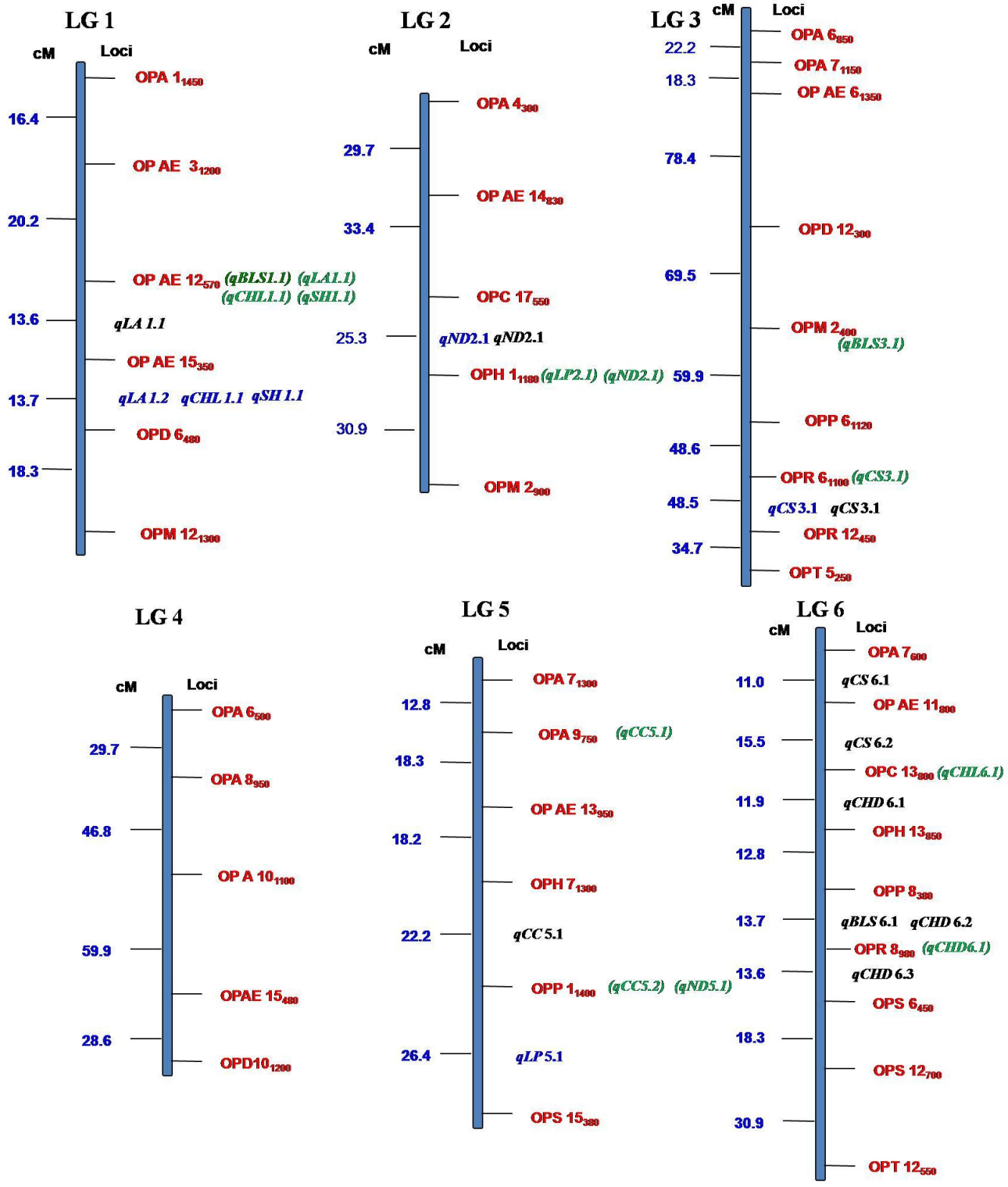
Fig 5: Gel picture showing polymorphism between parents (Chandana and TAC-89-309)

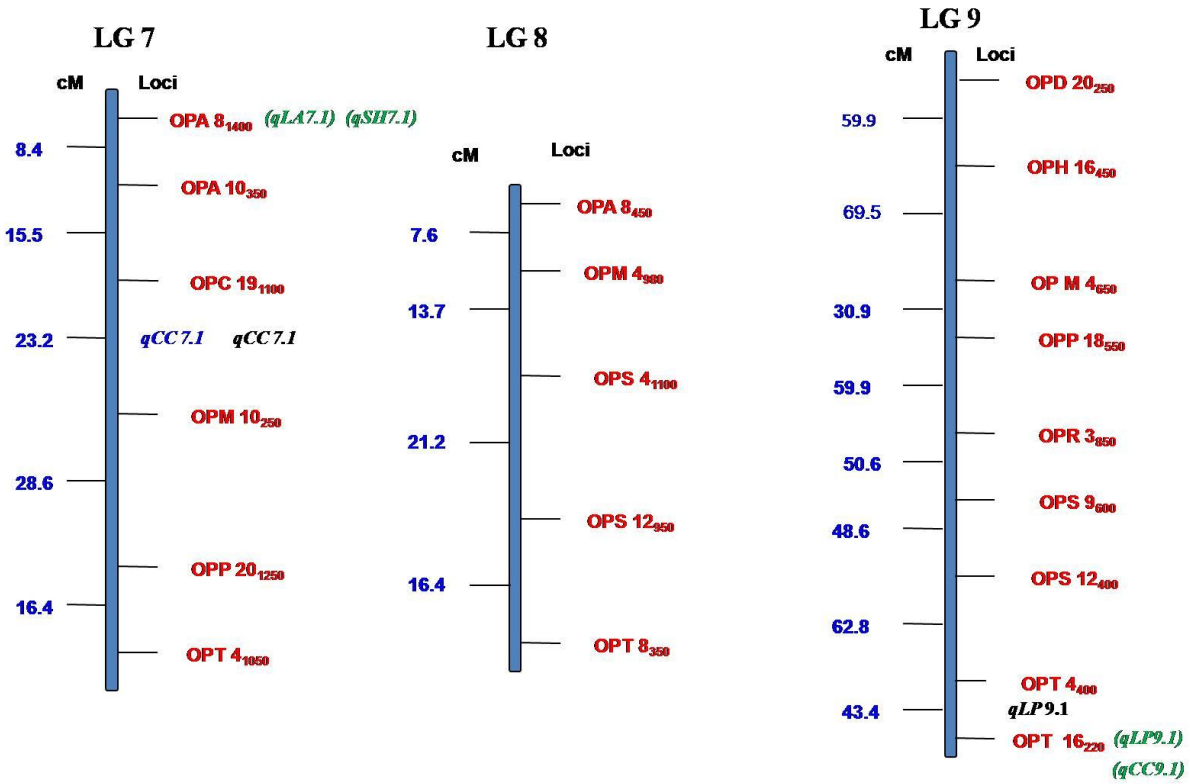


C - Chandana
T - TAC-89-309
M - 100 bp + 1.5 K bp DNA ladder (Bangalore Genei)

Total Primers – 15
Polymorphic primers – OPS 6, OPS 9, OPS 12, OPS 15, OPT 4, OPT 5 and OPT 8

Fig 6: Assignment of QTL for nine agro-botanic traits in F_2 population of the cross Chandana x TAC-89-309




LG 7
cM
Loci
OPA 8 1400 (qLA7.1) (qSH7.1)
8.4
OPA 10 350
15.5
OPC 19 1100
23.2
qCC 7.1
qCC 7.1
OPM 10 250
28.6
OPP 20 1250
16.4
OPT 4 1050
LG 8
cM
Loci
OPA 8 450
7.6
OPM 4 900
13.7
OPS 4 1100
21.2
OPS 12 950
16.4
OPT 8 350
LG 9
cM
Loci
OPD 20 250
59.9
OPH 16 450
69.5
OP M 4 650
30.9
OPP 18 550
59.9
OPR 3 850
50.6
OPS 9 600
48.6
OPS 12 400
62.8
OPT 4 400
qLP9.1
43.4
OPT 16 220 (qLP9.1)
(qCC9.1)