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Segregation Analysis of SSR Markers in Safflower (*Carthamus Tinctorius* L.) for Oil Content

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ABSTRACT

In the present study, 176 $F_{2:3}$ population of the cross A-1 (low oil contentvariety, ~26%) x EC-755673-1(high oil content variety, ~38%) is genotyped with250polymorphic SSR markers.Out of which only a set of 30 (~12%) SSR have shown polymorphism among the parents.The mapping population was genotyped using 30 polymorphic markers. The Chi-square (c^2) test revealed that, out of 30 polymorphic markers genotyped, 25 markers followed the Mendelian ratio of 1:2:1 whereas the remaining 5 markers deviated from the expected ratio.The genotypic data of the SSRs developed in this study can be used for augmenting the linkage maps developed in the previous studies. Further the polymorphic markers identified in the present study were not mapped in the previous studies.

Keywords: *Chi-square, Genotyping, Mapping, Polymorphism and SSRs.*

Saffloweris a valuable oilseed crop with high-quality edible oil rich in polyunsaturated fatty acids (Linoleic acid, ~75%) or monounsaturated fatty acid (Oleic acid, ~75) which is good for human health.Currently, safflower is grown commercially in 15 countries including India, Mexico, U.S.A and Argentina. In India, it is grown in an area of 70,000 ha. with a production of 47,000 tonnes and productivity of 633 kg/ha. In India, Maharashtra accounts for 72% of area and 69% of production, followed by Karnataka,Telangana and Madhya Pradesh. Despite the economic importance, safflower growing areas are declining in India due to low productivity and profitability. Concerned efforts are required to improve the oil yield potential in safflower to make it attractive to the farmers.

The safflower breeding programmes mostly rely on conventional breeding approach, which has been successful; but has limitation to break the yield stagnation currently being experienced.Genetic enhancement of a quantitatively inherited trait like oil content requires simultaneous improvement of seed yield and oil content. However, in safflower, due to the genetic complexities and negative relationship between seed yield and oil content, it is a great difficulty to combine these two traits to achieve desirable oil yield. Furthermore, the direct selection for seed yield and seed oil content simultaneously is hampered due to genotype – environmental interactions and also low heritability (Ranga rao *et al.* 1977 and Golker *et al.* 2011). Therefore, new impetus is required to develop innovative breeding methods for improvement of the oil yield potential in safflower.

A good knowledge of genetic basis of oil content and related traits may help to decide a suitable strategy for development of high oil yielding cultivars in safflower.Safflower being an orphan crop with a large genome size of ~1.4Gb (Garnatje*et al.*, 2006), molecular breeding research is limited.Lack of a fine

genome map is a major drawback, hampering the genetic studies in safflower.However,only two SSR linkage maps have been published so far, using intraspecific (*C. tinctorius*x*C. tinctorius*) and interspecific (*C. tinctorius*x*C. oxyacantha*) crosses which only comprised of 116 and 160 SSR markers (Mayerhofer*et al.*, 2010) in safflower. A similar attempt was madeusing intraspecific (*C. tinctorius*x *C. tinctorius*) cross by Raju (2016) using SSR markers.In this context, an attempt was made in the present studyto saturate the linkage mapdeveloped in the previous studies using SSR markers.

MATERIAL AND METHODS

The present study was carried out at ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, Telangana, during *Rabi*, 2019. A set of 176 F_2 plants of the cross between A-1(Annegiri-1, low oil content variety, ~26%) and EC-755673-1, high oil content variety, ~38%) was used as the mapping population. The DNA was extracted from the fresh young leaves of F_2 plants for genotyping purpose using CTAB method (Murray and Thompson, 1980).

The extracted stock DNA is quantified using 0.8% agarose gel electrophoresis. About 3 µl of stock DNA sample was mixed with 1µl of bromophenol blue dye (6X dye from Genei) and run on 0.8% agarose gel along with ë DNA marker (100 ng from Genei) at a constant voltage of 60 V for about an hour. Then the gel was stained with Ethidium bromide and viewed under the UV transilluminatorand documented in gel documentation system (SYNGENE Gene flash, U.K.). Based on the quantification results, the stock DNA of all the samples were diluted to approximately 50 ng/µl of working samples with distilled water for SSR analysis.

RESULTS AND DISCUSSION

Out of 250 SSRs analysed, only 30 SSRs (~12%) showed polymorphism between the parents. The polymorphic set of 30 SSR loci included 23 markers (SAFM series) (Unpublished), 5 markers (MCT-IIOR series) (Usha Kiran *et al.*, 2019), 1 marker (NGSAF series) (Ambreen *et al.*, 2015), 1 marker (CTDES series) (Yamini *et al.*, 2013). List of polymorphic SSR loci used for genotyping the mapping population is presented in the Table 1. Out of the 30 SSRs, the genotypic data of only 25 SSRs was considered for further analysis and the data of the remaining 5markers wereignored due to higher missing values, as higher missing data may lead to skewed segregation and finally would affect the linkage relationships.Thealleles were scored in the Mendelian pattern as 1 for A-1 allele, 2 for heterozygotes and 3 for EC-755673-1 allele.

Chi-square test was conducted usingthe genotyped data of 25 SSR loci to check whether the segregation of the markers in the F_2 population followed the Mendelian segregation pattern of 1:2:1 or not. The allelic segregation and the Chi-square values of all SSR markers is presented in the Table 2. The Chi-square values of below mentioned 20 markers followed the Mendelian segregation pattern of 1:2:1.

CTDES10, NGSAF217, SAFM6, SAFM37, SAFM38, SAFM45, SAFM62, SAFM118, SAFM149, SAFM189, SAFM192, SAFM193, SAFM220, SAFM 426, SAFM465, SAFM310, SAFM324, SAFM336, MCT-IIOR18, MCT-IIOR72.

Whereas, the remaining 5 (SAFM10, SAFM266, SAFM300,MCT-IIOR73 and MCT-IIOR89) markers deviated from the expected segregation ratio.Genotypic profiles oftwoSSR loci showing Mendelian segregation pattern are presented

Table 1. List of the SSR marker loci used for genotyping the mapping population (A-1 x EC-755673-1 - F_2) of safflower

S. No.	SSR Marker	Forward Sequence (5'-3')	Reverse Sequence (3'-5')	Reference
1	CTDES-10	ACGGGTAGATTTAAGGAAGG	ACAATCCAACAGAGATTTGC	Yamini *et al.* (2013)
2	SAFM-6	TGGAGTCCTTCTTCCATCGC	TGATGAGCAAGGCCAGGTAC	Jegadeeswaran*et al.* (unpublished)
3	SAFM-10	ACGATGATTTCCAAGGGCAC	AGAAGGCTCAAGTGGTCTCC	Jegadeeswaran*et al.* (unpublished)
4	SAFM-37	TCGTCTCCTGATCAACGACC	GGATGCCAGCACCAAGAATG	Jegadeeswaran*et al* . (unpublished)
5	SAFM-38	CCTTGCCTTGCCTACACAAC	ATACTCATCATCAACCACCATTG	Jegadeeswaran*et al* . (unpublished)
6	SAFM-45	TTGAAAGGCGGAATGTGCAG	ACCTAAGATGGCATTTAACCGC	Jegadeeswaran*et al* . (unpublished)
7	SAFM-62	TTAAGATGGGCCAGGTAGAC	AATGCCAGCTTGTGTGTTGC	Jegadeeswaran et al. (unpublished)
8	SAFM-86	CTTCCCTTCCCTCCCACAC	GCCATTGGAGGAAGCTCATG	Jegadeeswaran*et al* . (unpublished)
9	SAFM-118	TCGACTGAACCGTTGAATGC	GCACCCATCAATTATAACACCATC	Jegadeeswaran*et al.* (unpublished)
10	SAFM-149	CTTACGACTTCCCTCTCCCG	GCGAGTGATGTTGCTCTACG	Jegadeeswaran*et al.* (unpublished)
11	SAFM-189	CGAAGGCAGCAACAACATCC	ACTGTGATCCAAGGCAGACG	Jegadeeswaran*et al* . (unpublished)
12	SAFM-192	TCCAACAGAGATTTGCATCCAG	TATGGAGGCTGACTTCGAGC	Jegadeeswaran*et al* . (unpublished)
13	SAFM-193	TAGCGGTTCTCACGGTTCTC	GAGCTCTTGTGGAACGTGAC	Jegadeeswaran*et al.* (unpublished)
14	SAFM-220	AAATGTCCCTCGTGTTGCTG	GCCAAGGGAAACACGTAAAC	Jegadeeswaran*et al* . (unpublished)
15	SAFM-266	TGGGCAAACTCATCGCTTTC	ACGGAATATCGATGTTATTTGTCAG	Jegadeeswaran*et al.* (unpublished)
16	SAFM-299	GAAGAAGATTGGGTGTGGCG	TGCAGCAGACATGAAAGAAG	Jegadeeswaran*et al.* (unpublished)
17	SAFM-300	GGAAGGAGTCATCGGAGGTC	GGTCCCTCCTCACTAACTTCG	Jegadeeswaran*et al.* (unpublished)
18	SAFM-310	CCTGATGCAACGTTTCTGTAAC	TGCATGCTATGTCCACAAGG	Jegadeeswaran*et al* . (unpublished)
19	SAFM-312	CAACCGGTCAATCTCATCCC	GGCCAGATCGTCAAACCTTG	Jegadeeswaran*et al.* (unpublished)
20	SAFM-324	GATTTCAGATCGCGCTTCCC	GGCCCTCAATAGCACAACAC	Jegadeeswaran*et al.* (unpublished)
21	SAFM-336	CCACCCTCGCATGAAAGTTC	ACCATTCTACACCTGCACCC	Jegadeeswaran*et al* . (unpublished)
22	SAFM-339	GCGCAACCCACTAAACCTAC	CTTTGGCAGGCTCACAGATC	Jegadeeswaran*et al.* (unpublished)
23	SAFM-426	CGAGGAGGAGATGAGATCGTC	CTGGTTTACGTCCACACATCC	Jegadeeswaran*et al* . (unpublished)
24	SAFM-465	CCCTTGCTGTACCATTATTGTTC	TGGAGGCCACATACGACC	Jegadeeswaran*et al* . (unpublished)
25	NGSAF-217	ATGTGAGATAGGACATTGAGTTGG	AGCCAGATTTTGAGATTACCTACG	Ambreen *et al.* (2015)
26	MCT-IIOR-18	AACGTCCTCAGCTAACACGA	CCCCATCATGGCTCTCACTG	Usha Kiran (unpublished)
27	MCTIIOR-72	GTTCTCCCGTCTGCCAAAGA	CCTGTTTGCCATTCTCATTCCA	Usha Kiran (unpublished)
28	MCTIIOR-73	ACAAAAACAGGGGCCATACC	GTGGCAGTCGAAGGTCAGAA	Usha Kiran (unpublished)
29	MCTIIOR-89	AGTGGCGACAACCCAATCC	AGTCGAATCGAAGCTCACCT	Usha Kiran (unpublished)
30	MCTIIOR-100	GCTGGACCCAAGAAGGCAAT	ACGACCATGGAGAAGGAAACA	Usha Kiran (unpublished)

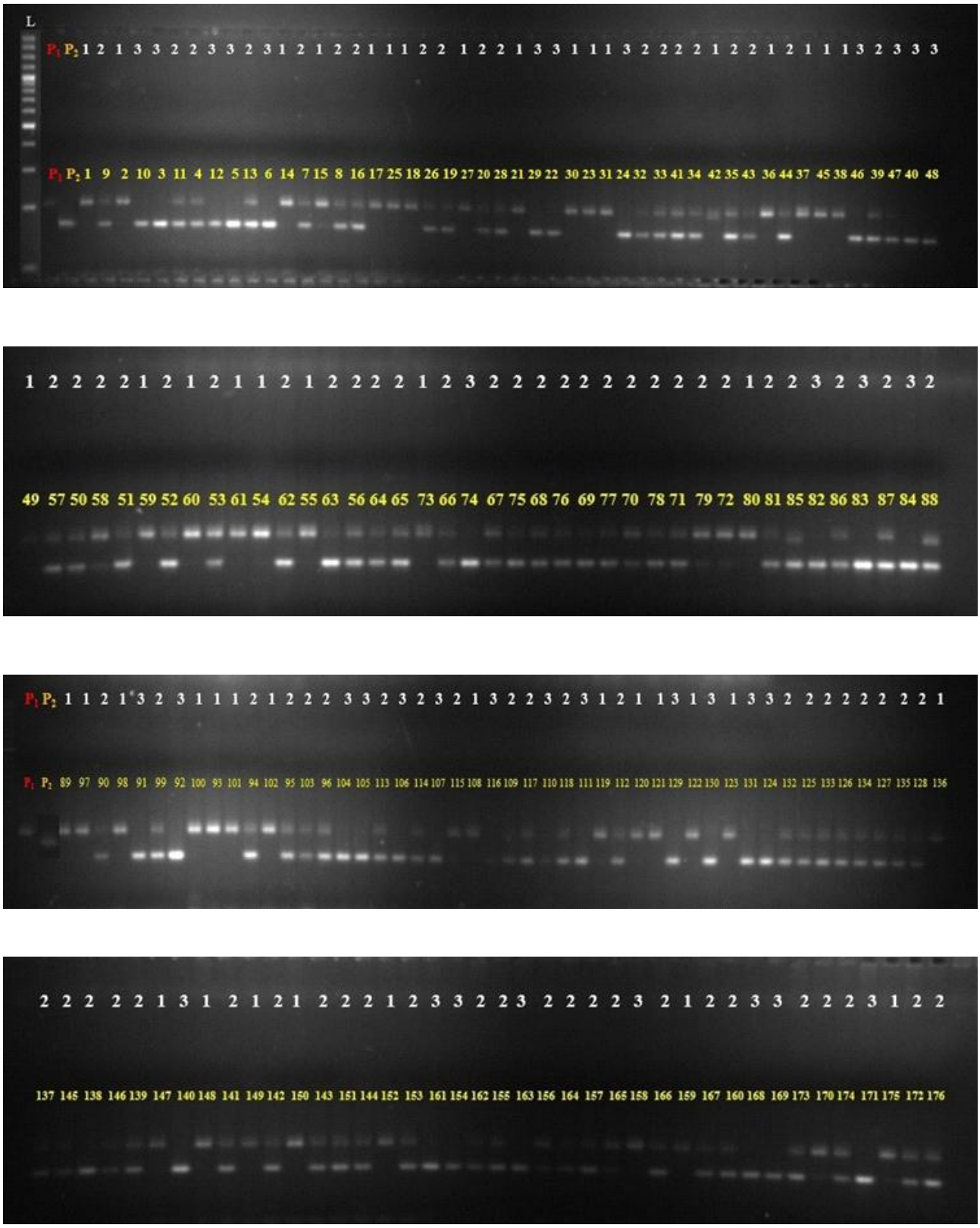

Figure 1. Segregation of SSR marker MCTIIOR 18 in the F_2 population of the cross A-1 x EC-755673-1

L = 100bp Ladder
P1 = A-1
P2 = EC-755673-1
1 = Parent-1 allele
2 = Heterozygote
3 = Parent-2 allele

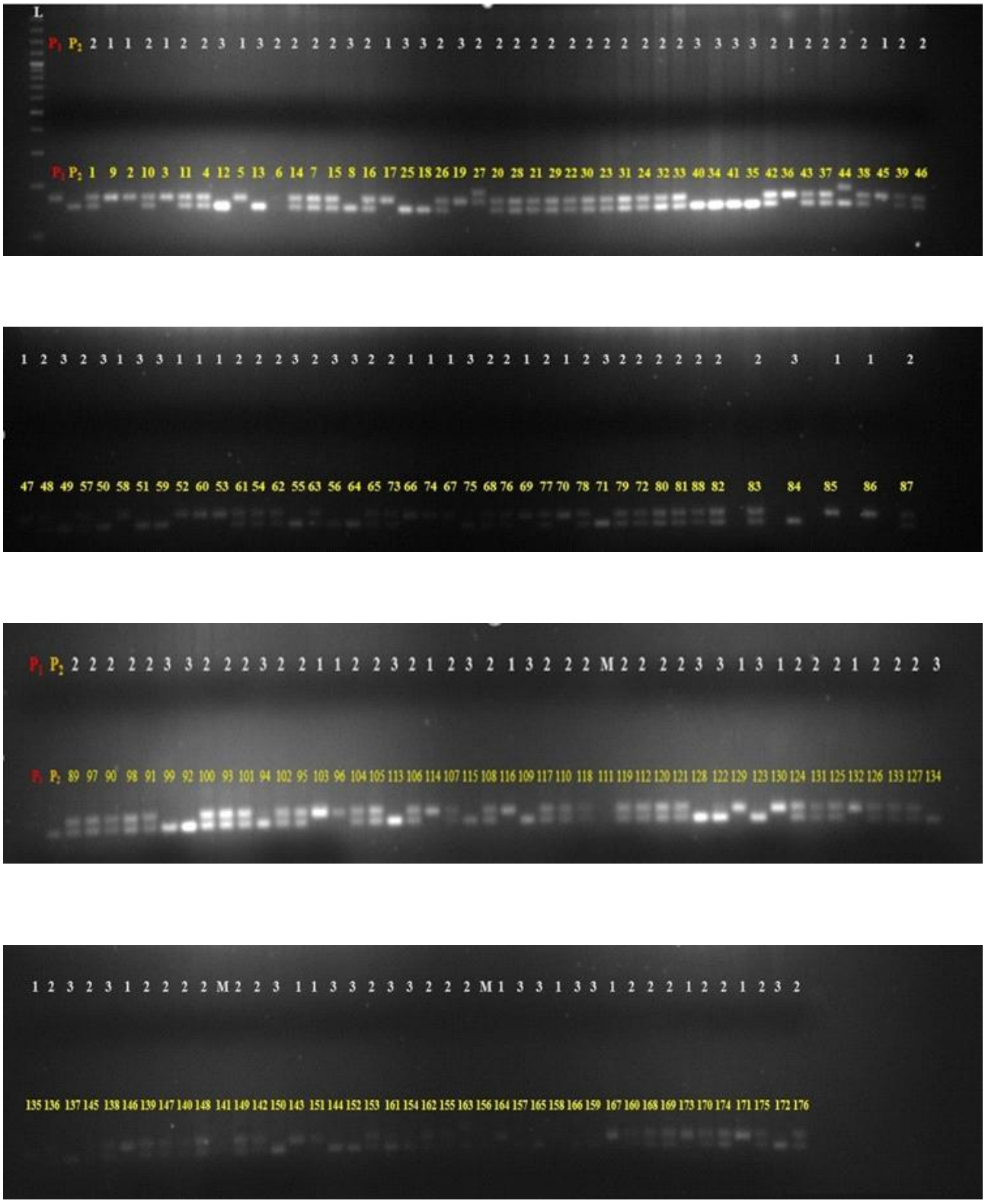

Figure 2. Segregation of SSR marker SAFM149 in the F_2 population of the cross A-1 x EC-755673-1

L = 100bp Ladder
P1 = A-1
P2 = EC-755673-1

1 = Parent-1 allele
2 = Heterozygote
3 = Parent-2 allele

Table 2. Mendelian segregation of SSR markers in F_2 population of the cross, A-1 x EC-755673-1

S. No.	SSR Locus Name	A-1 Allele	Heterozygotes	EC-755673-1 Allele	Chi-square value	Significance
1	SAFM37	53	76	44	3.57	NS
2	SAFM465	40	86	44	0.65	NS
3	SAFM220	35	76	52	4.4	NS
4	CTDES10	49	80	42	1.14	NS
5	SAFM192	44	86	44	0.02	NS
6	SAFM426	38	74	45	1.35	NS
7	SAFM193	42	88	41	0.16	NS
8	SAFM189	50	69	41	4.46	NS
9	SAFM118	40	88	39	0.26	NS
10	SAFM6	44	88	39	0.34	NS
11	MCTIIOR89	29	105	36	13.52*	S
12	SAFM149	36	96	39	2.03	NS
13	SAFM38	33	93	41	4.19	NS
14	SAFM300	32	106	30	12.91*	S
15	SAFM266	18	109	47	23.98*	S
16	MCTIIOR73	48	77	44	20.96*	S
17	SAFM339	45	76	34	1.42	NS
18	SAFM310	47	72	46	2.75	NS
19	SAFM62	43	81	48	0.89	NS
20	MCTIIOR72	28	100	47	1.86	NS
21	MCTIIOR18	48	91	37	1.49	NS
22	NGSAF217	46	90	31	4.42	NS
23	SAFM10	30	75	58	10.75*	S
24	SAFM324	34	81	55	5.14	NS
25	SAFM336	33	87	50	2.58	NS

in the Figures1,2.The polymorphic markers identified in the present study were not mapped in the previous studies. Hence the markers can be used for augmenting the previously generated linkage maps.

CONCLUSION

The Chi-square test results have shown that 20 markers followed the Mendelian segregation pattern of 1:2:1 and the remaining 5 markers significantly deviated from the expected ratio. Due to less number of polymorphic markersstudied, the genotypic data alone may not be usefulfor further analysis. But the genotypic data generated in the present study could be used for genome map construction for further identification of QTLs for any trait of our interest based on the phenotypic data.

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