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SSR analysis for fruit and quality characters in infra-specific mapping population of melon

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ABSTRACT

Melon has variations in fruit trait that play an important role in consumer acceptance and breeding programms. These traits are controlled by single or multiple genes. Keeping this in view a mapping population ($F_{2:3}$) was developed between muskmelon (*Cucumis melo* var. Kashi Madhu) and snapmelon (*Cucumis melo* var. *momordica* accession B-159) having contrasting fruit traits. SSR (simple sequence repeat) markers selected from melon database were applied in F_2 population. Linkage analysis was performed using Mapmaker/Exp.3.0, multi point ordering at LOD = 3.0, to find possible allelic interactions between the loci and fruit traits. The result showed five QTLs for fruit characters, viz. fruit length, fruit weight, number of fruits per plant and ascorbic acid content was found to be significantly linked with markers CSWCT22A, CMMS1-7, CMCTN-86, CMBR-170b, CMBR-70, CMCT134b, CMBR73, CMGT-108, CMMS30-3 at marker interval from 56.5 cM to 153.3 cM with phenotypic variance of 5.9 to 69%, respectively. The study could be useful for the comparative genetics and increases utility of SSR markers across different populations and environment particularly Indian melons.

Key words: Fruit traits, Linkage analysis, Melon, SSR, QTL

Melon fruits are largely consumed as dessert fruit. Diverse wild and primitive melons are found in Indian sub continent are rich in genetic diversity (Dhillon *et al.* 2012) and has been the always a subject of molecular and genetic studies. Besides desserts, infra-specific crosses of melons can be used as salad and having good content of minerals (Pandey *et al.* 2010). Classical breeding pattern aided with marker techniques have been used to produce genetic linkage maps and to augment melon breeding (Baudracco-Arnas and Pitrat 1996, Gonzalo *et al.* 2005, Diaz *et al.* 2011). However, identification and mapping of yield and quality QTL (quantitative trait loci) is hindered by the use of different mapping population. The concurrent development of mapping population and use of molecular markers in melon gives an opportunity to determine the co-linearity among molecular maps. At present about 2000 melon SSRs are available in the public domain (Danin-Poleg *et al.* 2000, Chiba *et al.* 2003, Ritschel *et al.* 2004, Gonzalo *et al.* 2005, International Cucurbit Genomics Initiative database http://www.icugi.org/). The co- localization of highly reproducible, locus specific, co-dominant SSR markers is of particular value for the integration of genetic information from different populations, and will under pin much of the applied research in melon, including gene mapping, QTL analysis, and marker-assisted selection. In the present study, the marker linkage based on $F_{2:3}$ mapping design was completed and subsets of most informative markers were identified.

MATERIALS AND METHODS

The experiment was conducted in the year 2009 and 2010 at IIVR, Varanasi, India. $F_{2:3}$ populations were developed by crossing muskmelon (*Cucumis melo* var. Kashi Madhu and snapmelon (*C. melo* var. *momordica* accession 'B159' INGR-07044) (Pandey *et al*. 2008 and Kalloo *et al.* 2006). The muskmelon variety Kashi Madhu is characterized by andromonoecious flower, fruit medium size round in shape, salmon orange flesh, and have prominent green sutures on smooth scaly rind; ripe fruits have musky aroma with sweet and juicy flesh. While B159 (snapmelon) are monoecious, fast growing having early flowering , less sweet with thin rind fruits that burst at maturity. The F_1 between Kashi Madhu × B159 was subsequently self-pollinated to produce 239 F_2 progeny and $F_{2:3}$ families.

A total of 239 $F_{2:3}$ families along with their parents and F_1 hybrid were evaluated during summer season 2009 and 2010. Standard cultural practices were adopted to raise a healthy crop. Fifteen competitive plants of parental lines, F_1 and 30 plants per $F_{2:3}$ families were planted in RBD with row to row spacing of 60×45 cm in three replications. Phenotypic observations were taken for parents, F_1 and $F_{2:3}$ families for polar circumference of fruit (FL in cm),

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equatorial circumference of fruit (FD in cm), fruit weight (FW in kg), number of fruits/plant (NOF), yield/plant (YPP in kg) and total soluble solids (TSS in % in ^{o}Brix) fruit flesh pH (pH) and ascorbic acid content of fruit (AA in mg/100g) (Ranganna 1997).

Microsatellite markers are used for genotyping in F_2 population. The SSR primers used in this study were selected from different sources (Katzir *et al*. 1996, Fazio *et al*. 2002, Chiba *et al.* 2003, ICUGI database). A total of 149 primers were screened with parental polymorphism and those showing results were selected for F_2 genotyping. Leaf samples were taken at the seedling stage and preserved at -80°C. Genomic DNA was isolated from the parents, F_1 and F_2 individuals (Murray and Thompson 1980). PCR reaction was performed using a 15 µl reaction volume which contained 2.5 µl 10×PCR buffer, 1.0 µl 25mM $MgCl_2$, 1.0 µl dNTPs, 1.0 µl/ each 5 µM primer (forward and reverse), 1.5 µl, 3ng genomic DNA, 0.5 µl (3unit/µl) *Taq* DNA polymerase (Fermentas, USA), and 6.5 µl distilled water. The thermo profile of SSR assay was as follows 94^{o} C for 4 min, 35 cycles of 94^{o} C for 1 min, annealing temperature was set from 45-60^{o}C for 1 min (according to the individual Tm value of the primers) and 72^{o}C for 1min, with final extension cycle of 10 min at 72^{o}C. After the completion of PCR amplification, the amplification products were resolved on 2.5% agarose gel (SFR grade, Ameresco), containing 0.05 µg/ml ethidium bromide in 1X TAE buffer at 65 volts X 30 mA for two and half hours, and visualized in gel documentation system in *Alpha Imager* (Alfa Innotech Corporation, and California). Scoring was done on the basis of co-dominant bands as (a) and (b) for the homozygous parents P_1, and P_2 and the (h) for heterozygous lines, respectively. For each locus the observed segregations were tested against the expected Mendelian ratio (1:2:1). Chi-square test was used to compute segregation distortion by bi-parents genotype and analyzed.

Mapmaker/EXP software version 3.0 was used to assign molecular markers to each linkage group (LG), and to calculate their order of probability and distance in each LG. The LGs were generated at a minimum logarithm of odds (LOD) threshold of 3.0. The Kosambi mapping function was employed to calculate genetic distances between markers at a recombination fraction of <0.3. Simple interval mapping and composite interval mapping was performed for all traits using Windows QTL Cartographer 2.0 (Wang *et al.* 2004). The threshold value of LOD for a presence of QTL was 2.5, as estimated by running 1000 permutation tests at a significance level of 0.05.

RESULTS AND DISCUSSION

Phenotype of fruit characters in $F_{2:3}$ populations

The phenotype data was showed continuous frequency distribution for all selected fruit traits FL, FD, FW, NOF, YPP, pH, TSS and AA over generation indicated polygenic inheritance. The quantitative inheritances over generations of the traits are similar to the previous reports on horticulture crops like melon (Georgelis *et al.* 2004). The distribution of FW, NOF, TSS and YPP was skewed while the distribution for other traits was normal. This kind of distribution pattern was reported for quantitative traits in melon (Sinclair *et al.* 2004, Blanca *et al.* 2012).

Polymorphism and segregation of SSR markers

Parental screenings of 149 SSR primers were completed, out of these 17 markers were genotyped in 239 F_3 lines with variable allelic size difference (Table 1). Segregation of all SSR loci adequately fitted into the 1:2:1 ratio ($P > 0.05$) as shown in Table 2. Co-dominant markers screened the observed level of heterozygous genotype frequency of both the parents homozygous alleles. Allelic contribution of polymorphic SSR markers was found 29.63% (homozygous parent Kashi Madhu), 23.64% (homozygous parent B159) and 46.72% (heterozygous both parents) in the population respectively. A single primer combination generated on average 1–2 polymorphic bands of 50-1000 base pairs and segregated in 1:2:1. A significant deficit of heterozygosity was detected at six loci (CMCTN86, CMBR70, CMBR42, CMBR2, CMCT134b and CMBR73). A significant (P<0.01) level of segregation distortion occurred at loci CMBR70, at two markers CMBR2 and CMBR73 (P<0.001) and with very high significance at marker CMBR42 (P<0.0001).

Linkage and QTL analysis

Linkage analysis of 17 SSR markers loci of muskmelon detected associations. It was observed that markers were arranged in large linkage group (LG), and the distribution of loci was even. The linkage group spanned 908.84 cM with an average marker interval of 53.46 cM. Few markers CMBR70, CMBR42, CMBR2 and CMBR73 in LG showed deviation from the expected ratio (P< 0.01) and clustered in a small area. A total of five QTLs were found in the linkage group for fruit length, fruit weight, number of fruit (2 QTL), and ascorbic acid in the $F_{2:3}$ populations derived from the infra-specific cross (Table 3). One QTL was found for FW at marker interval of 61.8 cM between SSR markers (CSWCT22A-CMMS1-7) at 11% phenotypic variance, one QTL was found for fruit length at marker interval 153.3 cM between flanking markers (CMCTN86-CMBR70) with 27.1% phenotypic variance. Two QTLs were determined for number of fruits at distance of 56.5 and 72.7 cM, between markers CMCT134b and CMBR73 and CMGT108 and CMMS 30-3 with 69.4% and 32% phenotypic variance. One QTL were detected for ascorbic acid between the flanking markers (CSWCT22A-CMMS1-7) at a distance of 102.5 cM with 5.9% phenotypic variance. Although there is scarcity of molecular markers based on the developed population, but it contains useful markers in identifying loci around genes of interest tagged with important markers CSWCT22A and CMMS1-7 showing QTL (qfw 5.1) for fruit weight. Marker CSWCT22A had been reported as flanking markers linked to QTL (fw 5.8) for fruit weight at linkage group 10 in RILs; USDA 846-1 × Top Mark (Cuevas *et al.* 2008).

Table 1 Characteristics of informative SSR (microsatellite) markers used in linkage analysis

Marker	Core motif	Sequence(5'-3')	T_a(°)	Allelic size (bp*)
CMCTN50	(TC)16(TA)12	TCTACTTCCATGAATCCATC	49	134
		TAGAATGGTTAGGAAACCCT		
CMCTN86	(CT)21	GTGACAGTTATCAAGGATGC	54.7	184
		AAGGGAATGCATGTGGAC		
CMCT170b	(CT)8	ATTGCCCAACTAAACTAAACC	53	160
		CACAACACAATATCATCCTTG		
CMBR70	(GA)38	GGGGAGGCACTCATTTTCTC	56	190
		CCTCATTTTTCCAAGGATGC		
CMBR42	(CT)30	AACCAACTTCCATTCCCCTC	54	136
		GAGGCACTCATTTTCTCTGAATG		
CSWCT22A	(CT)9	GGGAGTATCGAAACAAAAGC	50	334
		TTCTGATCAACGACGAAGTAA		
CMATN101	(GT)8	GCTTGTCTTTGTGTGTTTGC	51	342
		GAGAACAAGACTCCTTAATCC		
CMMS 1-7	(GT)12	CAAAAGACAAGGAGACGAAGACACC	54	152
		AGACAACTGGTCGTACACACACAGT		
CMBR54	(GA)23(TA)2(GT)2	TCTGAAACCATTGCTTATGGTAA	58	128
		TCACAATCTCTCCCTACCCAA		
CMBR2	(AG)22	TGCAAATATTGTGAAGGCGA	60	114
		AATCCCCACTTGTTGGTTTG		
CMCTN35	(CT)10	CCAATAATGTAATCGTCTTGG	49	186
		GTTCCAAACTTTCTACCAATCA		
CMCT134b	(TA)2(CT)8(AT)7	GCTCCTCCTTAACTCTATAC	50	123
		GCATTATTACCCATGTACGAG		
CMCTN71	(CT)11	TCAATTTTTGCCAAACAAGC	48.5	160
		CAAGGACACAGATTTAATAC		
CMBR73	(CT)15	TCAATTTTTGCAAAACAAGC	50	280
		CAAGGACACATATTTAATAC		
CMTC47	(TC)9(CT)6	GCATAAAAGAATTTGCAGAC	46	168
		AGAATTGAGAAGAGATAGAG		
CMGT108	(GT)9N65(CT)7	CTCCTTCAAACATTGTGTGTG	47	187
		GAGATAGGTATAGTATAGGGG		
CMMS 30-3	(GA)16	TTCCCACCAGCCCAACGGACACACT	58	271
		GAGATACAGAAACGACGACTAACCT		

*bp base pairs

Phenotype of developed $F_{2:3}$ populations clearly showed the recombination of both parental alleles at multiple loci for different fruit traits. The pattern of transgressive segregation in melon breeding has been reported earlier for different fruit characters like fruit length, fruit width, fruit weight and fruit number (Zalapa *et al*. 2006, Monforte *et al.* 2004). To breed cultivars with these important traits has been difficult, because the expression is greatly affected by the genetic background and environmental factors. Only 12.0% polymorphism that had 2-6 alleles were detected for SSR markers. Others have reported similar results of SSR markers used in *Cucumis* species (Danin Poleg *et al.* 2002). Conservation of SSR locus is of great importance while comparing with different mapping populations. The chi square test results showed direction of distortion towards 'Kashi Madhu' (*C. melo*.) for few SSR loci (Table 2). This kind of skewed segregation is reported mostly in those populations which are derived from inter-specific or inter-generic crosses (Bonierbale *et al.* 1994). Wang *et al.* (1997) reported skewed segregation in backcross population derived from two muskmelon cultivars. Hashizume *et al*. (1996) also reported this type of RAPD marker segregation in watermelon. An extreme example of all markers displaying the preferential distorted segregation favoring the female parent in a F_2 cotton population has been demonstrated by Li *et al*. (2007).

The linkage analysis explained the marker trait association of fruit characters along with two reference QTLs for fruit length and fruit weight as earlier reported by Zalapa *et al.* (2008). The fruit length QTL (*fl 5.2*) within

Table 2 Chi square test for goodness of fit for SSR markers

SSR Markers	Observed(1:2:1)	Expected(1:2:1)	Total (n)	X^2	P value	Missing
CMCTN50	67 108 46	55:111:55	221	4.10	0.1284	18
CMCTN86	67 98 53	54.5:109:54.5	218	4.02	0.1341	21
CMCT170b	70 113 54	59.2:118.5:59.2	237	2.67	0.2630	2
CMBR70	76 99 53	57:114:57	228	8.59	0.1365*	11
CMBR42	79 95 43	54.2:108.5:54.2	217	15.30	0.0004***	22
CSWCT22-A	65 111 47	55.7:111.5:55.7	223	2.91	0.2333	16
CMATN101	68 116 52	59:118:59	236	2.24	0.3267	3
CMMS 1-7	72 109 56	59.2:118.5:59.2	237	3.68	0.1585	2
CMBR54	58 101 55	53.5:107:53.5	214	0.76	0.6848	25
CMBR2	77 99 43	54.7:109.5:54.7	219	12.57	0.0018**	20
CMCTN35	57110 58	56.25:112.5:56.25	225	0.12	0.9417	14
CMCT134b	61 99 69	57.2:114.5:57.2	229	4.76	0.0927	10
CMBR73	74 92 65	57.7:115.5:57.2	231	10.26	0.0059**	8
CMCTN71	55 106 56	54.2:108.5:54.2	217	0.12	0.9396	22
CMTC47	68 112 54	58.5:117:58.5	234	2.10	0.3494	5
CMGT108	68 109 56	58.2:116.5:58.2	233	2.10	0.3325	6

*, **,*** significance at the level >0.5

Table 3 Positions of QTL (cM.) in linkage group (LG) along with their associated logarithm of odds (LOD), percentage of phenotypic variation (R^2), and additive effect in a F_2/F_3 population derived from a cross between Kashi Madhu x B-159

QTLs*	Trait	Marker	Marker Interval (cM.)	LOD	Additive	Dominance	R^2 (%)
f w5.1	Fruit weight (FW)	CSWCT22A-CMMS1-7	61.8	20.23	33.96	-4.83	11.0
fl5.2	Fruit length (FL)	CMCTN86-CMBR70	153.3	1.78	29.78	0.55	27.1
fn5.3	Number of fruits (NOF)	CMCT134b – CMBR73	56.5	8.52	3.12	-0.27	69.4
fn5.4	Number of fruits (NOF)	CMGT108- CMMS 30-3	72.7	3.89	3.13	-0.10	32.0
aa5.6	Ascorbic acid (As. A)	CMBR42-CMMS1-7	102.5	15.37	5.79	0.45	5.9

*QTL designated by the abbreviated trait name (*fl5.1*), linkage group number and QTL number.

marker CMCT 170b and CMCTN-86 was responsible for more than 27.1% of phenotypic variance in the population. This locus was significant and has been validated in the present study from previous reports (Fang *et al.* 2009). The position of fruit shape QTL is prominent than other traits and it is also consistent among other mapping experiments (Zalapa *et al.* 2008, Fernandez-Silva *et al.* 2010). The minor difference in QTLs among different experiments with similar markers might be partially explained by trait heritability differences. In this study QTL detected for ascorbic acid between flaking markers CMBR42-CMMS1-7 is important as melons are high source of Vitamin-C. Reported markers for this trait are RAPD markers with 14 and 12% phenotypic variance (Sinclair *et al.* 2004). Other reports on flesh pH and organic acid metabolites of QTL show that these markers are valuable source of candidate genes (Cohen *et al*. 2012).

The present experiment resolute genetic linkage of Indian melon, using a developed ($F_{2:3}$) population derived from an infra-specific cross. This population is of high relevance to the gene pool, which could be used by melon breeders and could reduce the frequency of skewed segregation and thereby increasing the mapping precision. However, due to the low level of polymorphism an extensive genotyping effort is required. Additionally the $F_{2:3}$ lines are advanced by selfing for development of recombinant inbred lines (RILs) and eventually the order of assigned markers will be determined in pure progenies providing more precise locus nearest to the gene of interest. The developed population includes parental genotypes from the broad and important horticultural group (*cantalupensis* and *momordica*) providing the future utility of the marker linkage and allelic association in a broad range of cultivars and experimental crosses of melon germplasm. The selection and specificity of markers helps to customize mapping and molecular breeding applications. The positioning of economically important QTL in the study and the standardization of trait nomenclature will facilitate comparative QTL analyses among populations of different origins and will provide deeper insights into the genetic

control of the diverse phenotypic variability observable in melons.

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