

# Enrichment of genetic linkage maps and mapping QTLs specific to seed strength - hardness / softness - in guava (*Psidium guajava* L.)

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## ABSTRACT

The present research focuses mainly on molecular mining and morphological evaluation of guava genome within a full-sib population and, thereby, mapping of quantitative trait loci related to fruit quality traits, viz., seed strength (hardness/softness) and average fruit weight. Linkage maps were enriched for both parental lines, 'Kamsari' and 'Purple Local' using a set of 60 RAPD markers following the pseudo-testcross strategy on a panel of 94 progeny. A total of 480 scorable markers were identified, of which 131 were specific to 'kamsari' and 28 to 'Purple Local', segregating as test cross markers, and, 321 showing intercross pattern common to both. 'Kamsari' spanned a total length of 1959.1cM with average marker interval distance of 3.93cM, while 'Purple Local' spanned a length of 1537.9cM with average marker interval distance of 3.29cM, by forming 11 linkage groups. Estimated genome length observed was 93.02% and 92.77% in 'Kamsari' and 'Purple Local', respectively. Composite Interval Mapping (CIM) was computed at significance of 0.05 and LOD threshold greater than 3.0, which led to detection of one major QTL for the trait of average fruit weight, and, four QTLs for the trait of seed strength (hardness/softness). Of these, two were major and two minor QTLs. Our study provides molecular mapping information on marker-assisted selection for improvement of guava in a breeding program.

Key words: Composite interval mapping, guava, linkage map, pseudo-testcross, quantitative trait loci (QTL)

## **INTRODUCTION**

Guava (Psidium guajava L.), native to tropical America, is a perennial tree crop with heterozygous and heterogeneous genome comprising approximately 460 Mbp (Sara et al, 2012). It is a diploid with 2n=22 and belongs to the family Myrtaceae (Nakasone and Paull, 1998). Familiarly known as the Apple of the tropics / Poor man's apple, guava is one of the important and major fruit crops in India. It is a repository of nutrients, vitamins and antioxidants, and, has incredible medicinal and pharmaceutical properties (Shruthi et al, 2013). Guava acts as a dual-purpose fruit used as fresh fruit as well as after processing. Development of medium-sized fruits with high TSS, pink pulp and soft seeds is a major breeding objective in guava which requires basic understanding of the role of complex genomic regions controlling these traits, i.e., quantitative trait loci (QTL).

Genetic linkage maps provide ready means for localization and map-based cloning of genes, and provide the necessary infrastructure for marker assisted breeding. Besides, developing a linkage map with consistent molecular markers forms the basis for analysis of agronomically important traits. Construction of linkage maps in heterozygous species is most efficiently achieved using double pseudo-testcross mapping strategy (Grattapaglia and Sederoff, 1994). Guava genome exhibits a high degree of heterogeneity and heterozygosity (Chandra and Mishra, 2007), and, perennial nature of the crop complicates basic understanding of the genomic sites contributing to various economically important phenotypes.

Guava is still considered an orphan crop with reference to its exploration at the genomic and/or genetic level. Only limited number of reports are available on molecular profiling of the guava genome within a mapping population (Valdés-Infante et al, 2003; Rodriguez et al, 2007; Lepitre et al, 2010; Padmakar et al, 2015a, 2015b) or on its quantitative genetics (Valdes-Infante et al, 2003; Rodriguez et al, 2007; Ritter et al, 2010). Various markers have been used for molecular characterization in guava (Nimisha et al, 2013), of which, random amplified polymorphic DNA (RAPD) markers have been used for assessing molecular diversity (Prakash et al, 2002), studying genetic relatedness/diversity (Dahiya et al, 2002; Sharma et al, 2007; Ahmed et al, 2011; Pessanha et al, 2011), or determining phylogenetic relationships (Chen et al, 2007). Hence, in the present study, we report enrichment of the intra-specific linkage maps developed in guava using RAPD markers in a pseudo-testcross mapping configuration, and identification of fruit quality related QTLs. To our knowledge, this is the first report of a linkage map developed with SSR, SRAP and RAPD markers identifying major OTLs for the trait of seed strength (hardness/softness) in guava.

## MATERIAL AND METHODS

#### Plant material and DNA isolation

The mapping population comprised 94  $F_1$  progeny obtained from a cross between two cultivars, 'Kamsari' (2n = 2x = 24) and 'Purple Local' (2n = 2x = 24), maintained in the field germplasm bank at ICAR-Indian Institute of Horticultural Research, Bengaluru, India. Total genomic DNA was extracted from young leaves of the parent plants and  $F_1$  progeny, using modified CTAB-method (Kanupriya *et al*, 2011).

## Morphological and molecular characterization

Three traits, namely, seed strength (SS) - hardness/ softness, average fruit weight (FrWt) and total soluble solids (TSS) related to fruit quality, were assessed from a set of five fruits randomly selected per  $F_1$  progeny plant, as per Dinesh and Vasugi (2010). Descriptive statistics and Pearson correlation coefficient were computed using SPSS software.

A set of 200 RAPD markers were used for screening parental lines, of which polymorphic informative markers were used for genotyping mapping population. PCR amplification was carried out in 25 $\mu$ l reaction mixture containing 50mM KCl, 1mM Tris-HCl (pH 8.8), 0.01% gelatin, 1.5mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.3 $\mu$ M primer, 100ng genomic DNA, and 0.5 units of Taq DNA polymerase (Bengaluru Genei, India). PCR was carried out on a Master Cycler Gradient (Eppendorf AG, Hamburg, Germany) thermal cycler, as per Padmakar *et al* (2015b). Amplification products were screened on 1.5% agarose gel for confirmation of the amplification. PCR was repeated thrice for checking reproducibility of the polymorphic markers identified.

The amplicons generated were scored in a binary format by assigning '1' for presence of a band and '0' for absence of the band. Each amplicon was named after the primer name used for amplification, along with a suffix indicating the respective allele size that was amplified. In the case of fragments heterozygous with only one of the parents considered as testcross markers, segregation ratio across the mapping population was tested against a 1:1 ratio, using chi-square ( $\chi^2$ ) test at a significance of p<0.05; while, those heterozygous in both the parents were considered as intercross markers and were tested against a 3:1 ratio.

#### Linkage map enrichment and QTL analysis

The data generated was used for enriching the parentspecific maps developed by our group following the protocol of Padmakar *et al* (2015a). A run test (Sokal and Rohlf, 1981) was performed using the Tseries package in R (Trapletti and Hornik, 2013) to determine randomness in distribution of the markers. Genome coverage was calculated by taking the average value of linkage map length estimated, using the method of Fishman *et al* (2001), and Method 4 of Chakravarti *et al* (1994). In the methodology of Fishman *et al* (2001), average spacing of the markers is doubled, and added to the length of each linkage group; whereas, Method 4 of Chakravarti *et al* (1994) expands each linkage group by (m+1)/(m-1), where m is the number of loci mapped.

Quantitative trait loci (QTL) detection was achieved using Windows QTL Cartographer software (Wang *et al*, 2010) employing composite interval mapping (CIM) method (Zeng 1994). The walking speed chosen for all QTL was 1.0cM. Additive effects of each QTL were estimated by the Bayesian test. A QTL was declared as significant at LOD value of 3.0.

Table 1.	Characteristics	of	trait	variation	in	the	guava	mapping
populati	on							

	FrWt (g)	SS (kg/cm <sup>2</sup> )	TSS (°B)
Mean ± SE	$272.9 \pm 5.60$	$11.69 \pm 0.22$	$9.41 \pm 0.10$
Min.	158.50	7.20	6.50
Max.	400.00	12.20	14.50
CV	19.90	19.00	10.50

FrWt – Average fruit weight; SS – Seed Strength (hardness/softness); TSS – Total Soluble Solids; CV – Coefficient of Variation

## **RESULTS AND DISCUSSION**

#### Morphological and molecular characterization

Adequate variation was available within the fruit quality trait evaluated (Table 1). The value for fruit weight (FrWt) ranged from 158.5g to 400g, with a mean of 272.9  $\pm$  5.60g. Similarly, this ranged from 6.5kg/cm<sup>2</sup> to 14.5kg/cm<sup>2</sup>, with a mean of 11.69  $\pm$  0.22kg/cm<sup>2</sup> and 7.2°B to 12.2°B, with a mean of 9.4  $\pm$  0.10°B, for the traits of seed strength (SS) and TSS, respectively. Coefficient of variation (CV) depended strongly on a particular trait under evaluation. CV values observed were 19.9, 19.0 and 10.5 for FrWt, SS and TSS, respectively. Positive correlation was observed between the traits of SS and FrWt, significant at  $\alpha$ =0.01, with Pearson coefficient value of r=0.40; but, a negative correlation was observed between the traits of SS and TSS, as well as FrWt and TSS at  $\alpha$ =0.01, with a value of r=0.06 and r=0.21, respectively.

Initial screening of 200 RAPD primers in the parental lines revealed 30% polymorphism. The 60 decamers (Table 2) were used for further genotyping the mapping population.

 Table 2. List of polymorphic RAPD markers used in the study

S. No.	RAPD primer	S. No.	RAPD primer
1	OPAG20	31	OPK10
2	OPAO4	32	OPK11
3	OPAO19	33	OPK17
4	OPAU2	34	OPK20
5	OPAZ11	35	OPM4
6	OPAZ14	36	OPN9
7	OPAZ15	37	OPN11
8	OPAZ16	38	OPN12
9	OPAZ18	39	OPN13
10	OPB7	40	OPN20
11	OPB19	41	OPO2
12	OPBA2	42	OPO9
13	OPBA6	43	OPO11
14	OPBA12	44	OPO12
15	OPBA13	45	OPO13
16	OPBA14	46	OPO14
17	OPBA16	47	OPO16
18	OPC2	48	OPO18
19	OPC3	49	OPP2
20	OPC8	50	OPP10
21	OPC13	51	OPP17
22	OPD8	52	OPP19
23	OPH15	53	OPQ1
24	OPK1	54	OPQ2
25	OPK2	55	OPQ3
26	OPK3	56	OPQ6
27	OPK4	57	OPQ18
28	OPK6	58	OPY1
29	OPK7	59	OPY3
30	OPK8	60	OPY9

A total of 480 scorable bands was produced, with an average of 8.00 bands per primer. Size of the amplified products ranged from 150bp to 3kb. Of the 480 bands scored, 159 (33.12%) were polymorphic and segregated as testcross markers, of which 131 markers were specific to 'Kamsari' and 28 to 'Purple Local'. The remaining 321 common fragments segregating in 3:1 ratio were treated as intercross markers. Finally, a set of 57 markers (11.87%) showing segregation distortion was identified and excluded from further mapping studies.

## Linkage Map Enrichment

'Kamsari' parental map (Fig. 1) was enriched from 351 markers, leaving 53 unlinked, and grouped into 11 linkage groups (LG) spanning a length of 1951.9cM, with a mean of about 45.2 markers per LG. The LGs (Table 3) varied in genetic length from 69.9cM to 414.2cM, with a mean of 178.1cM. Average marker interval distance observed was 3.93cM ranging from 0.00cM to 50.5cM. Estimated genome length was 2,166.8cM, attributable to 90.41% of genome coverage and 2,048.3cM attributable to 95.64% of genome coverage, as per Fishman et al (2001) and Chakravarthi et al (1991), respectively. Thus, an average of the two methods resulted in genome coverage of 93.02%. In 'Purple Local', out of the 336 markers tested, 318 markers assembled into 11 LGs (Fig. 2) covering a total distance of 1537.9cM, with a mean of 42.4 markers per LG. The LGs (Table 3) varied in genetic length from 52.9cM to 256.0cM, with a mean of 139.8cM. Inter-marker separation ranged

#### Table 3. Characteristics of parent linkage maps

	Parent	1: Kamsa	Parent	Parent 2: Purple Local					
LG <sup>a</sup>	K-LG	$\mathbf{T}\mathbf{M}^{\mathrm{b}}$	cM <sup>c</sup>	PL-LG	$\mathrm{T}\mathrm{M}^{\mathrm{b}}$	cM <sup>c</sup>			
1	K1	101	69.9	PL1	10	178.8			
2	K2	101	102.8	PL2	101	146.3			
3	K3	101	170.9	PL3	20	256			
4	K4	75	194	PL4	101	92.7			
5	K5	18	298	PL5	101	52.9			
6	K6	6	106.2	PL6	19	187.5			
7	K7	9	115.1	PL7	16	123			
8	K8	31	179.9	PL8	26	128.7			
9	K9	14	152.8	PL9	43	114.6			
10	K10	16	155.3	PL10	20	127.3			
11	K11	26	414.2	PL11	10	130.1			
Total		498	1959.1		467	1537.9			
Min.		6	69.9		10	52.9			
Mean		45.2	178.1		42.4	139.8			
Max.		101	414.2		101	256			
$\mathrm{GC}^{d}$		93.0	02%		92	.77%			

<sup>a</sup>Linkage Group

<sup>b</sup>Total number of markers

<sup>c</sup>LG length in centiMorgans (cM)

<sup>d</sup>Genome Coverage (estimation of)

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cM	K1		cM	K2		cM	K3		cM	K4	L	cM	K5		сM	K8		cM	K10
0.0	100	mPaCIR321156	0.0	- 01	mPoCiR343255	0.0	100	mPoCiR048137	0.0	12	mPaCiR157210	0.0	- 72-	mPaCIR099222	0.0	1.6	Me13Em1 1700	0.0	OPN9350
41.9	211	mPoCIB027318	16.1		mPoCIR418258	28.2	210	mPpCIR290210	43.3	- 11	-mPaCIR110125	20.2		mPoCIR257180	0.0	- M I	Ma13Em1 715	0.0	OPN9300
59.1	111	OPY1360	32.6	2.110	mPqCIR099252	79.2	3111	mPaCIR030158	50.8	211	OPAZ1199	25.5	100	mPgCIR274188	0.0	11	Me13Em1 600	0.0	OPN9400
59.1		OPP10460	82.6	3111	OPY11045	96.6	2111	OPH15300	50.8	111	OPP19250	27.6	-0H0	mPoCIR257196	0.0	2011	Mo13Em1 480	37.8	OPN20661
59.1	3	OPH15118	82.6	T = T	OP09300	96.6	3/10/	OPC8320	50.8	311	Me12Em9 350	28.7	-21 V	mPoCIR215136	0.0	711	Mat3Em1 75	37.8	1 DPN20450
59.1		OP8A1610	82.6	1011	OPAZ1577	96.6	$1   1 \rangle$	OPBA6300	50.8	111	OPN13735	45.2	7411	mPnCIR334177	30.6	20	ModEm6 950	37.8	OPN20360
59.1	111	OPC31175	82.6	10 I /	OPAZ1430	96.6	1116	OPN20500	50.8		OP06775	69.2	7942	mPaCIR025161	39.6	2.11	ModEm6 650	37.8	OPN20207
59.1		OP014615	82.6	101/	OPAZ1668	96.6	11.0	OPP19195	50.8	31.1	OP012600	8.89	NII-	mPoCIR027316	39.6	34	MedEm6 500	37.8	OPN20129
59.1		OP819428	82.6	R4-7.	OPAG2024	96.6	111	OPK3835	50.8	31.3	Me12Em3 130	124.2	222	mPaCIR205116	39.6	501	MadEm6 300	97.8	OPN20112
59.1		OPK6750	82.6		OPO16520	96.6	1/71	OP03830	50.8	$X^{\dagger}$	OPDasso	135.0	24-80	mPaC1R259222	68.4	311	Me12Em3 1900	37.8	OPN20167
59.1		OPO61625	82.6	1010	OPC8970	96.6	1 1	OPV11280	50.8	эн	OPBA1390	141.4	74 1	mPoCIR414229	65 A	3.U	Ma12Em3 1800	77.4	OP87250
59 1		OP014805	82.6	1111	OP014450	96.6	111	OPK2770	50.8	211	OPN91500	152.2	211	mPaCIR230220	66 A	-201	Ma12Em3 950	121.0	OPCR305
59 1	111	OPP17325	82.6	1117	OPP10975	96.6	111	OPB19775	50.8	20.3	OP03965	177 6	2985	OPBA2142	65.4	547	Ma12Em3 350	121.0	OPC8560
59.1		OPV1875	82.6	1111	OPN13455	96.6	111	OPP10130	50.8		OP8A1245	201.6	7UN	OPN91600	94.6	211	Ma13Em5 1520	121 0	OPC8720
59.1		OPA71645	82.6	118	OP841642	96.6	111	OP01575	50.8	18	OP018775	227.0	STN	OPK20151	04 G	3.11	Ma13Em5 1320	155 9	OPAD4210
50 1		OPN13130	82.6	111	OPN13420	96.6	111	Me12Em9 700	50.8	10	OPK7585	252.4	Set.	Me3Em14 750	04 6	201	Ma13Em5 1250	155.3	OPACIALER
59.1		OPK10575	82.6	111	OP014257	96.6	111	00018465	50.8		OPA71827	277 8	- UL :	mPoCIR325175	04.6	- 14	Ma13Em5 1020	19910	
50 1		OPK7340	82.6		OP02715	96.6	1.1	OPC13320	50.8		OPA71134	208.0		mPaCIR139161	04.6	211	Mat3Em5 650		
59 1		OPH15425	82.6	111/	OPV3370	96.6		0206335	50.8	11	OP061000	640.0	- 02	un Benzins int	94 E	5/11	Ma13Em5 525	cM	K11
50 1		OPK31360	82.6	1	0203400	96.6	1.1	00016635	50.8		OPP17635				04.6	211	Ma13Em5 400		
50 1		OPN20847	82.6		OPA71473	96.6		OPK8785	50.8		OPK7235	cM	K6		134.2	211	Ma11Em12 1700	0.0	mPgCIR049150
59 1	34 H	OPN11245	82.6	111	OPK17750	96.6	10.0	OPN12420	50.8	8 I I	OPO16250			- Dimension in the	134.2	311	Mat1Em12 1200	38.0	mPgCIR111112
50 1	111	OPAO4425	82.6	111	OPK81550	96.6		OPBA1315	50.8	10	OPP17960	0.0		mPgCIR321148	414.2	24/1	MattEmt2 t000	-39.0	meguikiiiiis
59.1		OPC8500	82.6		OPP19170	96.6	1.4	OPBA1366	50.8	1	000010310	22.7		mPgCIR092165	134.2	(3F)	MattEm12 900	19.2	Me3Em12_1500
50 1	3111/	OPBA1664	82.6	111	OPC3550	96.6		0203600	50.8	1/3	OPK6835	48.1	-++-	mPgCIR194179	134.2	011	Ma11Em12 470	19.2	Me3Em12_1450
59.1		OPC13200	82.6		Me3Em12 200	96.6		OP09445	50.8	111	OPN95000	64.0		OPHIBBIO	179.9	211	Ma12Em6 950	79.2	Me3Em12_550
59.1		OPAO1919	82.6		OP(7510	96.6		OP01750	50.8	1/U	OPA112885	60,0		OPP21075	179.0		Ma12Em8 850	19.2	Meacm12_020
59 1	111	0002365	82.6		OPC13740	96.6	3118	OPN11510	50.8	1/13	OP014365	106.2		OPN20546	170.0	1011	Ma12Em6 775	(9,2	Mesem12 150
59.1		OPN13960	82.6		OPA71543	96.6	218	OPB19325	50.8	10.0	OPH15920				170 0	2011	Ma12Em6 525	110.3	00000
59.1	11.11	OPP2700	82.6		OPAC4385	96.6	21 h	OPBA1319	50.8	101-3	OP014320		in		179.9	2011	Ma12Em6 500	110.3	OPK7800
59.1	111	OPRA1234	82.6		OPN11700	96.6	21.0	OPA71684	56.1	10.4	mPoCIP028260	CM	K/		170.0	- 4.1	Ma12Em6 225	110.3	0000000
59 1		OPN20200	82.6		OP018555	96.6		OPAG2013	72.6	10.7	mPaCIR047210	0.0	-01	mPaCIR180153	11.8(6	- 63	monacino_aco	110.3	00000000
59.1		OPRA6120	82.6		OPK4535	96.6		OPH15200	122.0	3/11	mPaCiR033122	4.3	ZAN	mPaCIR233129				150.0	OPEA1316
59.1		OPBA2685	82.6		OPBA1613	96.6		OPP2375	139.7	тH	mPoCIR025146	20.8	114	mPgCIR419222	684	KO		100.0	OPBA1330
59 1		Mat2Em1 A15	82.6		OPN11380	96.6	1.1	OPP2800	152.6	TA P	mPo(18352183	46.2		mPaCIR132158	CINI	140	and the second second second	156.0	UPBA1324
59.1		OP01870	82.6		OPP10153	96.6	101	OPK10160	160.1	2/11	mPoCiR235219	46.2	211	mPaCIR132164	0.0	$-\alpha$	Me13Em8_1600	201.7	A MPUCIATEUTAU
59.1		OPC8850	82.6		OPA71611	96.6	31.11	OP87550	175.1	711	mPoCIR011326	77.3	2144	mPgCIR044268	0.0	्रा	Me13Em8_1150	241.0	mPgCIR230191
59.1		OP471139	82.6		OPV9489	96.6	1111	OPC3820	104 0	98	mPoCIR23785	77.3	2113	mPnCIR044270	0.0	-11-1	Me13Em8_1000	290.0	mPgCIR236189
59.1	11	Me12Em9 890	82.6		OPN13500	96.6	<b>1</b> 0 IV	OPK11660	144.4	۰	- In gennen op	115.1	C.111	mPaCIR191181	0.0	-411	Me13Em8_950	290.8	mPgCIR404226
59.1		OPK20765	82.6	э.	OP012370	96.6	30 M	OPK7460				115.1	312	mPoCIR191184	0.0	314	Me13Em8_300	300.0	mPgCIR173328
59.1		OP02875	82.6	214	OPN12610	96.6	1114	OPN92000					111		0.0	244	Me13Em8_225	303.7	mPociR041149
59.1	24 B	OPC2680	82.6	3 N	OPC21175	96.6	10.13	OPM46085							0.0	-41	Me13Em8_125	414 2	Melaema 145
59.1	10	OPP17700	82.6	30.0	OPBA1320	96.6	1111	OP018630							48,0	-111	Me10Em10_1400	414.2	-MetdEm4_tabl
59.1	1	OPH15735	82.6	11	OP8A1467	96.6		OPY3535							48.0	-1.1-1	Me10Em10_950	414.2	Me13Em4_1250
59 1		OPP17480	82 6	1	OP8A6945	96.6	1	OPC2910							48.0	-78-1	_Me10Em10_300	414 2	MetaEm4_110E
59.1	111	OPM41170	82.6		OP02410	96.6	T	OPA71114							93.7	-701	mPgCIR192165	414.2	Meldema 270
59.1		OPA01915	82 F		OPY1455	96.6		OP011470							102.3	T	mPgCIR192167		
59.1		OPP2520	82.6		OPN12800	96.6		OPP19225							152.8	S 11	mPgCIR249288		
59.1	TIL	OPK20315	82.6		OPA71547	119.6	111	mPaCIR242194							152.8	1.24	- mPgCIR249278		
59 t	2.1.17	OPK7390	82.6	111	OPA71453	170.9	24.4	OPC13855											
69.9	200	- mPgCIR137125	102.8	78	-mPgCIR049156	170.9	20	- OPC13120											

Fig 1. Genetic linkage map of 'Kamsari': Map distances in centiMorgans (cM) are indicated to the left, and loci to the right, of each linkage group

cM	PL1		cM	PL2		cM	PL3		cM	PL4		cM.	PL5		cM	PL8	cM	PL9	
0.0	-11	mPgCIR132153	0.0	-11-	mPgCIR 165118	0.0	-0-	mPgCIR017262	0.0	-0-	mPgCIR257227	0.0	-17	- mPgCIR031134	0.0	mPgCIR031132	0.0	-11-	-mPgCIR177120
20.2	100	mPgCIR233133	11.9	3112	mPgCIR165128	32.7	31.12	- mPgCIR414238	11.7	-0110	mPgCIR253218	25 1	311.	- mPgCIR235211	31.1	OPR101280	43	SUP	- mPgCIR285249
25.5	2110	- mPgCIR233131	40.2	CALLY.		60.9	-XXV	- mPgCIR137146	68.4	7111	- OPK17550	52.9	1111	- OPO9400	34.4	Ma12Em0425	35,4	3111	- OPH15200
29.0	_7HM	mPgCIR180165	49.2	311/	OPBA2685	009		mPgCIR13/123	00.4	1117	OPK/585	52.9	111	0002310	31.4	OP4G203425	35.4	111	Mettem13300
61.0	SUIX	mPgGIR419210	49.2	411	OP014805	77.1	28-1/	mPaCIR030181	58.4		OPN12610	52.9	3	Me12Em3150	31.1	- OPN91500	35.4	111	OPV11280
99.7	C/TA	mPrcIR044274	49.2	an	- OPBA142050	84.6		mPnC18205126	58.4	111	OP01870	52.0		OP018465	31.1	OPBA81180	35.4		CEN20840
99.7	기법의	-mPgCIR044276	49.2	4111	- OPK20640	84.6	211	- mPgCIR205124	58.4	111	OPAZ111165	52.9		OPH151180	31.1	- OPK6635	35.4	3111	OPY9490
130.8	2115	- mPgCIR441203	49.2	300	02404700	96.5	200	- mPoCIR030172	58.4	411	- OPN13960	52.9		- OPBA6300	311	OP016835	35.4	el 1 1	- OPQ2495
178.6	-14-	mPgCIR191175	49.2	30.0	OPEA16600	115.4	-	mPgCIR046189	58,4	- 1 I U	OPAZ16550	52.9	9111	Me13Em4950	31.1	Me11Em121000	35.4	-11	- OPK2770
			49.2	-11.11	-OPK4765	142.2	-	- mPgCIR016320	58.4	411.1	- Me13Em5730	52.9	-111/	- OPO2410	31.1	- OPK4835	35.4	-111	- OPY3535
			49.2	-11.10	OP016350	174.9	5.117	- mPgCIR027320	58.4	21.11	OPY1455	52.9	- N N	OPBA13900	34,3	-mPgCIR194190	35.4	-11	- OPY11045
cM	PL6		49.2	CN 11	OPBA12655	210.9	NUZ	mPgCIR099219	58,4		- OPN11245	52.9	- 117	OPAZ161150	37.5	OPC13300	35.4	-31.0	< OPO18370
0.0			49.2		MetaEmploud	210.9	-NH/	mPgCIR099210	53.4	1114	OPK3835	52.9	111	OPC13160	37.5	OPRASSO	35.4	16	- OPM4680
31.1	2110	mPgCIR110116	49.2	-11	OPK4400	228.8	3VU/	mPgCIR290190	59.4	31.14	OPA041200	52.9		Me125m5100	37.5	- OPP101535	35.4	2011	OPK101600
41.9	$\Delta 10$	mPnCIR110118	49.2	-18	OPN13735	235.1	_N+40	mPaCIR015142	58.4	3111	OPACIO480	52.9	311 I A	- OPO14630	37.5		35.4	SALL	- OP06335
50.5	344/	mPgCIR230206	49.2	-14	Me12Em9350	238.3	-34/	mPaCIR018166	58.4		OPC13590	52.9		-OPAZ14300	37.5	- OPBA131555	69.7	211	- mPoCIR177124
613	AN 16	- mPgCIR448101	49.2	- T K	OPK20410	238.3	2HK	-mPgCIR016174	58.4	- 11	OPD81750	52.9	- 11	OPK8785	62.9	-// mPgCIR025123	104.0	4111	- OPO13370
77.8	SHO	- mPgCIR027324	49 2	711	CIPI221060	256.0		- mPgCIR237118	58.4	400	-OPA0191500	52.9	- 1 I/i	- OPC2800	88.3	-/ OPP171225	104.0	111	- OPAG20890
94 3	-01173	-mPgCIR097153	46.2	-11	OPAZ11995		~		58,4	400	- OPBA61200	52.9	-111	- OPM4585	88,3	OPN13375	104.0	111	- OPB19515
94,3	-NHV	- mPgCIR097150	49.2	-11	- OPP19265				58.4	-101	OPP191950	52.9	2111	OPC3550	108.5	mPgCIR047163	104.0	4117	- OPN13330
115.7	-10	- mPgCIR028254	49.2		Me13Em/625	cM	PL7		58.4		OPK8850	52.9	3111	OPBA12450	128.7	-OPM411/0	104.0	-111/	- OPO2470
139.7	3/11//	- mPgCIR339181	49.2		- OPD18525	0.0	- 8		58.4	-	- OPN12345	52.9	7 H I	- OPAU2665	128.7	OPA216565	104.0		- OPO9640
100.0	311-1/1	mPgCIR418242	49.2	<b>10</b>	OPACIT91840	26.0		OPDA181300	P. 80		OPK8920	52.9		OPBA141010	126 7	OPBAGAGO	104.0	311/1	OP016520
162.0	3NT//	mPoCIR215128	492	38.8	OPK11120	36.0	217	OPP191700	58.4	311	OPN11700	52.9	C	OPC3820	160.1	Ci cristos	104.0	<b>1</b> H I	OPA01000
162.9	SW V/	- mPoCIR215130	49.2		OP09585	36.0	110	- OPK17750	58.4	311	OP011470	52.9	CH 11	OPK10825	- 14	and the literature	104.0	CI UI	- OPP171310
162.9	-WHW	- mPoCIR009197	492	444	OP02875	35.0	NK	- OPAG201325	58.4		OPN12455	52.9		- OPP101300	cM	PL10	104.0		-Me10Em10850
162.9	-14	mPgCIR015140	49.2	10.0	OPC21175	36.0	-11-	- OPO14515	58.4	- 18	- OPP17325	52.9	-	- OPO18765	0.0	mPoCIR285274	104.0	3111	- OPK6750
165.1	-16-	mPgCIR321165	49.2		00003385	73.6		-mPgCIR009199	58.4	-318	- OPK17810	52.9	9111	OPO9300	32.7	- mPgCIR033144	104.0	-111	- OPO13435
166.1	2012	mPgCIR321140	49.2	3111	OPN13455	80.2	AU.	- mPgCIR098149	58.4	-16	OPO2715	52.9	-	- Me13Em1975	56.7	-OPH15425	104.0	-111	- OPO181050
187.5		mPgCIR334163	49.2	-11.11	Me12Em31010	101.6	511//	mPgCIR092175	58.4	-71 N	- OPQ3965	52.9		OPBA12970	56.7	-\/- OPN131150	104.0	-111	OPN11335
			49.2	-11.0	-Mo11Em13460	1230	THE W	OPH15920	58.4	- 1	Me3Em14500	52.9		OPK31360	:56.7	OPM41355	104.0	-111	OPC21510
1.00	1.00		49.2	10.0	Me13Em4250	123.0		OPU12490	P.0C	-11	- OPN92500	52.9	C 11	- OP8197/5	58,7	OPBA131915	104.0	-11	OPA211345
CM	PL11		49.2	10.0	0003810	123.0	- WW	OP012370	58.4		0203500	52.0		- Me3cm14075	56.7	OPP17390	104.0	311	- OPA04425
0.0		mPgCIR101101	49.2	- 111	-Mo11Em13750	123.0	-111	-OPC3340	58.4	-11	- OPAZ14530	52.9		- OPK8465	55.7	-OPA711390	104.0	31.10	- Me11Em12750
31.1	3115	-mPgCIR178127	49.2	-10.00	OPAG201890	123.0	-N 12	- OPC8650	58.4	-11	- OPQ1435	52.9	C 11	OPK7235	56.7	OPP17960	104.0	<u>-1</u> k	- OPK7510
81.5	- CHY	Me10Em81700	49.2	-10.13	Me12Em9890	123.0	-11	-Me11Em13320	58.4	-111	OPP10460	52.9		OPO91750	56.7	-OPY1580	109.3	SIL	- mPgCIR030170
61.6	A 14	Ma10Em81100	49.2	CHA 14	OPA2111420		· · · ·		58.4	-1111	OPQ6560	52.9		OPN9200	62.0	mPgCIR039175	114.6	24	- Me12Em9475
81.6	-3110	-Me10Em8950	49.2	C1/1 19	Mat2Emat30				58.4	-1111	- OPK4535	52.9	-11	- OPK4180	86,0		114.6	-3112	OPBA6595
81.0	16	MetoEm8700	49.2		-OP09500				58.4	1111	OPK20565	52.9		OPO16285	86.0	OPN11650	114.6	-44	- OPAZ14590
111 2	110	mPaCiP325181	49.2	48.13	OPA218540				58.4	-111	- OPO18665	52.9		- OPP102150	86.0	OPN11570			
1112	212	- mPoCIR325183	49.2	111	-Me11Em12240				58.4		OP12000	52.0		OPO1/50	86.0	OPN11470			
130.1	-	- mPgCIR139182	63.5	d III	mPgCIR005254				58.4		Me12Em6950	52.9	CH 18	-OP014615	0.60	OP016470			
	~		80.8	<b>3</b> 111	mPgCIR025125				58.4		OPA214730	52.9	21.12	- OPN11380	127.3	OPM41645			
			88.3	911	mPgCIR011254				58.4	-1	- OPP10670	52.9	31	- OPA04385	127.3	OPM41280			
			88.3	-411	mPgCIR011273				58,4	1.140	OPP19225	52.9	-11	- OPO9445		C. In more			
			104.8	문민	mPgCIR237122				92.7	3.80	-mPgCIR016322	52.9	200	- Me12Em91250					
			146.3	1.1	mPgCIH017280						and the second se								

Fig 2. Genetic linkage map of 'Purple Local': Map distances in centiMorgans (cM) are indicated to the left, and loci to the right, of each linkage group



Fig 3. QTLs mapped on linkage map of 'Kamsari'

Table 4 Summary of results of OTL analyses

Fig 4. QTLs mapped on linkage map of 'Purple Local'

Trait	QTL	Linkage	Marker	QTL	LR	Additive	$\mathbb{R}^2$
		Group	Interval	position (cM)		effect	
Average fruit weight	qFrWt	PL2	OPQ1_1020 - mPgCIR025_125	49.21	29.47	63.07	26.3
-							Total R <sup>2</sup> 26.3
Seed strength (hardness/ softness)	qSSa	PL2	OPQ1_1020 - mPgCIR005_254	51.21	174.38	4.1	43.6
	qSSb	PL2	mPgCIR005_254 - mPgCIR025_125	64.51	200.15	4.1	43.6
	qSSc	PL3	mPgCIR290_190 - mPgCIR018_166	234.81	30.36	0.83	2.3
	qSSd	K2	mPgCIR099_252 - OPY1_1045	61.61	46.16	1.11	3.6
							Total R <sup>2</sup> 93.2

from 0.0cM to 50.5cM, with a mean marker interval distance of 3.29cM. The genome length computed was 1,705.7cM, attributable to 90.16% of genome coverage and 1,612.1cM attributable to 95.39% of genome coverage, as per Fishman *et al* (2001) and Chakravarthi *et al* (1991), respectively. The average of these two methods resulted in a genome coverage of 92.77%.

## **Mapping QTLs**

A total of five putative QTLs was detected, with each one explaining between 2% and 43% of the phenotypic variance (Table 4). Four seed-strength QTLs (Fig. 3, 4), namely, qSSa, qSSb, qSSc and qSSd, were identified and mapped to the LGs K2, PL2-1, PL2-2 and PL3. All showed a positive additive effect and accounted for, respectively, 3.6%, 43.6%, 43.6% and 2.3% of phenotypic variance. Similarly, one QTL for average fruit weight (Fig. 3), namely qFrWt, was identified in 'Purple Local' and was mapped to the LG PL2, and contributed to 26.3% of phenotypic variance and exhibiting a positive effect.

## Morphological and molecular characterization

As reported earlier by our group (Dinesh and Vasugi, 2010), a hybridization program in guava was initiated at ICAR-Indian Institute of Horticultural Research, Bengaluru, with a primary goal of developing hybrids suitable for both table purpose and processing, having fruits of uniform shape, size, good color, firm and thick pulp, good aroma, soft seeds, high TSS, high pectin and a long shelf-life. Varieties selected as parents were 'Kamsari' of mediumsized fruits, pink pulp, TSS of 9.8°B, less seed-bearing portion, strong flavour with hard seeds, and, 'Purple Local' of dark purple skin, dull-pink pulp, with soft seeds. Fruit characteristics evaluated (FrWt, SS and TSS) showed significant amount of variation within the mapping population comprising 94 progeny. Molecular exploration of guava is still in its infancy owing to a lack of availability of sufficient genomic resources. Only a few reports are available on development and application of molecular markers for characterizing guava genome. We have reported genotyping and mapping of guava genome using SSR and SRAP markers in a previous study (Padmakar et al, 2015a), and have majorly focused on enriching the maps developed with RAPD markers in the present work. Since SSR markers available from guava (Risterucci et al, 2005, 2010) and SRAP primer combinations (Li and Quiros, 2001) have been already used, RAPD markers were used here for further characterization.

## Linkage mapping and QTL mapping

Construction of linkage maps in highly heterozygous species and perennial crops like guava is complicated because each parent is heterozygous, and linkage phase of the marker alleles is usually unknown (Maliepaard *et al*, 1997). However, in out-crossing species, linkage maps have been developed by a strategy known as double or two-way pseudo-testcross-mapping (Grattapaglia and Sederoff, 1994), where the  $F_1$  population is considered as the mapping population, and this has proved efficient in mapping several heterozygous species (Xie *et al*, 2011; Lu *et al*, 2012; Sudarshini *et al*, 2014; Padmakar *et al*, 2015a, 2015b). In our present study a similar technique was employed for parent-specific linkage map enrichment of both the parents.

Significant amount of difference was observed on the total distance spanned, average marker interval distance, as also the genome coverage estimated in both parental lines. In 'Kamsari', the total length of linkage map decreased from 2,553.7cM to 1959.1cM along with a reduction in average marker interval distance from 17.5cM to 3.93cM. In addition, the estimated genome coverage increased from 87.32% to 93.02%. Similarly, with 'Purple Local' the reduction observed was from 2,115.9cM to 1537.9cM, and 15.9cM to 3.29cM for the total length of linkage map and average marker interval distance, respectively, with increase in estimated genome coverage from 83.74% to 92.77%.

Marker loci showed some tendency to cluster, especially the SRAP markers. Some LGs consisted of more loci than the others. This could be due to a lack of marker polymorphism between mapping parents on some chromosomes, and/or, these might be sites on the genome representing suppressed recombination. Similar clustering was reported earlier too (Zhang *et al*, 2011; Zhang *et al*, 2013). Decamers used in the present study played the key role of missing links in the mapped SSR and SRAP markers. Thus, enriched maps were further exploited for mapping the complex QTLs governing fruit quality traits such as seed strength (SS), average fruit weight (FrWt) and total soluble solids (TSS).

Studies on understanding quantitative genetics in guava are scanty due to the complexity involved in generating mapping populations, long juvenile period of the crop, lack of adequate genomic resources, and the highly heterozygous nature of guava. Till date, only three studies are reported, that too from the same group (Valdes-Infante et al, 2003; Rodriguez et al, 2007; Ritter et al, 2010) on mapping of QTLs in guava. In our present study, two separate QTL analyses were performed with 'Kamsari' map (K1–K11, Fig. 1) and 'Purple Local' map (PL1–PL11, Fig. 2). We mapped five QTLs, acting on two fruit quality traits and distributed over 11 LGs (Table 4; Figs. 3, 4). Of the four seed-strength QTLs, two were responsible for a major proportion of phenotypic variance. Similarly, QTL identified for FrWt contributed a significant proportion of variance in trait. Besides, qFrWt and qSSa have been mapped very closely on LG PL2, but it is unclear whether this reflects existence of two independent loci, or that, a single locus is acting pleiotropically on these two traits. No significant QTLs were identified for the trait of TSS. This could be due to sampling bias in a mapping population based on correlation studies on the traits of SS and FrWt, as reported

earlier (Padmakar *et al*, 2015a). Detection (of major fruit quality QTLs, being spanned by the markers OPQ1\_1020 mPgCIR005\_254; mPgCIR005\_254 - mPgCIR025\_125; mPgCIR290\_190 - mPgCIR018\_166 and mPgCIR099\_252 - OPY1\_1045) is encouraging for the prospect of applying marker-assisted breeding in improving guava to develop elite varieties with medium-sized fruits with high TSS, pink pulp and soft seeds, considered to be major breeding objectives in this crop.

To the best of our knowledge, this is the first report on SSR, SRAP and RAPD-based linkage mapping and fruit quality related QTL identification in guava. Application potential of this map in the future for guava improvement is highlighted here. Due to a lack of anchor markers between the two maps at present, additional markers (especially, more co-dominant ones) can be used to integrate the two frameworks into a single, saturated map which may be exploited for further studies on gene tagging, MAS breeding and comparative genomics.

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# **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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