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# 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

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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 QTLs 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 25µl reaction mixture containing 50mM KCl, 1mM Tris-HCl (pH 8.8), 0.01% gelatin, 1.5mM $MgCl_2$, 0.2 mM of each dNTP, 0.3µM 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 parent-specific 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 population**

| | FrWt (g) | SS (kg/cm$^2$) | TSS (°B) |
|---|---|---|---|
| Mean ± SE | 272.9 ± 5.60 | 11.69 ± 0.22 | 9.41 ± 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 ± 5.60g. Similarly, this ranged from 6.5kg/cm$^2$ to 14.5kg/cm$^2$, with a mean of 11.69 ± 0.22kg/cm$^2$ and 7.2°B to 12.2°B, with a mean of 9.4 ± 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: Kamsari | | | Parent 2: Purple Local | | |
|---|---|---|---|---|---|---|
| LG[a] | K-LG | TM[b] | cM[c] | PL-LG | TM[b] | cM[c] |
| 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 |
| GC[d] | | 93.02% | | | 92.77% | |

[a]Linkage Group
[b]Total number of markers
[c]LG length in centiMorgans (cM)
[d]Genome Coverage (estimation of)

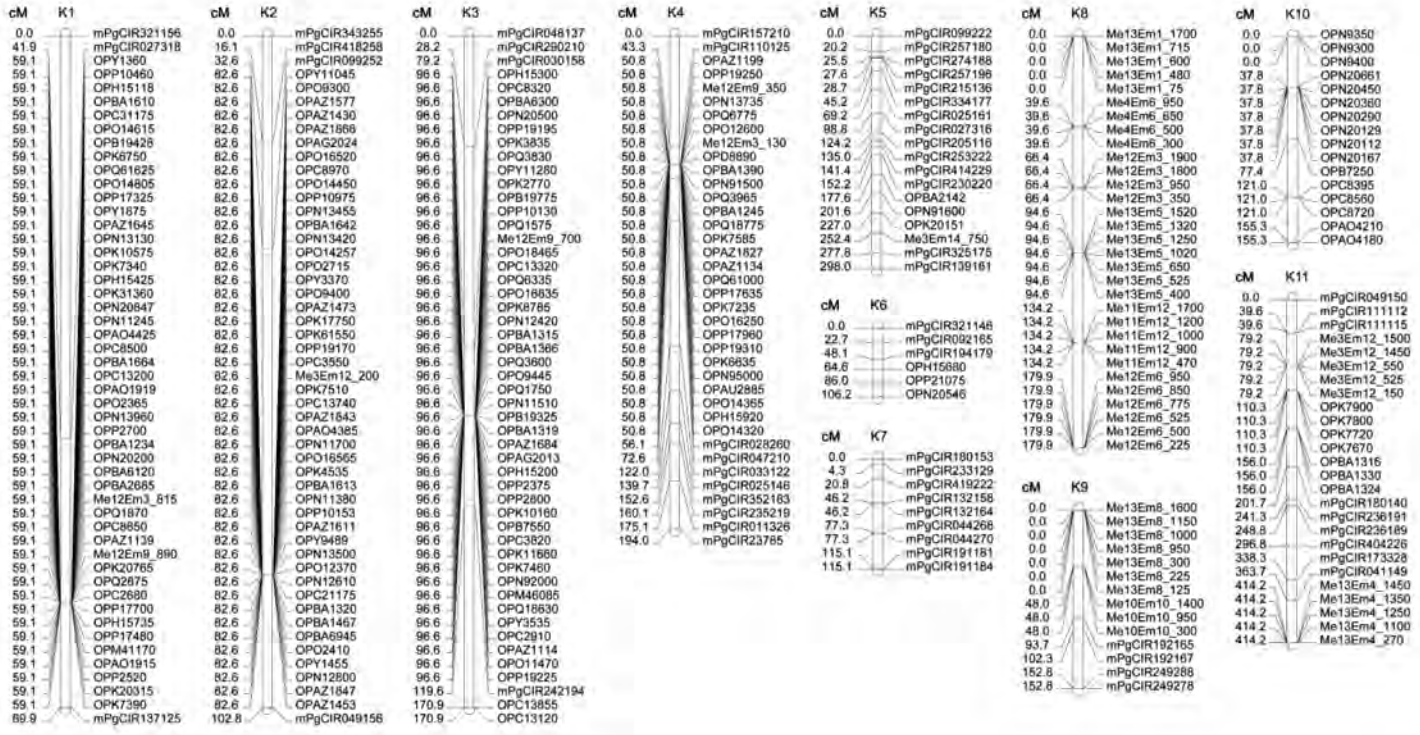


**Fig 1. Genetic linkage map of 'Kamsari': Map distances in centiMorgans (cM) are indicated to the left, and loci to the right, of each linkage group**

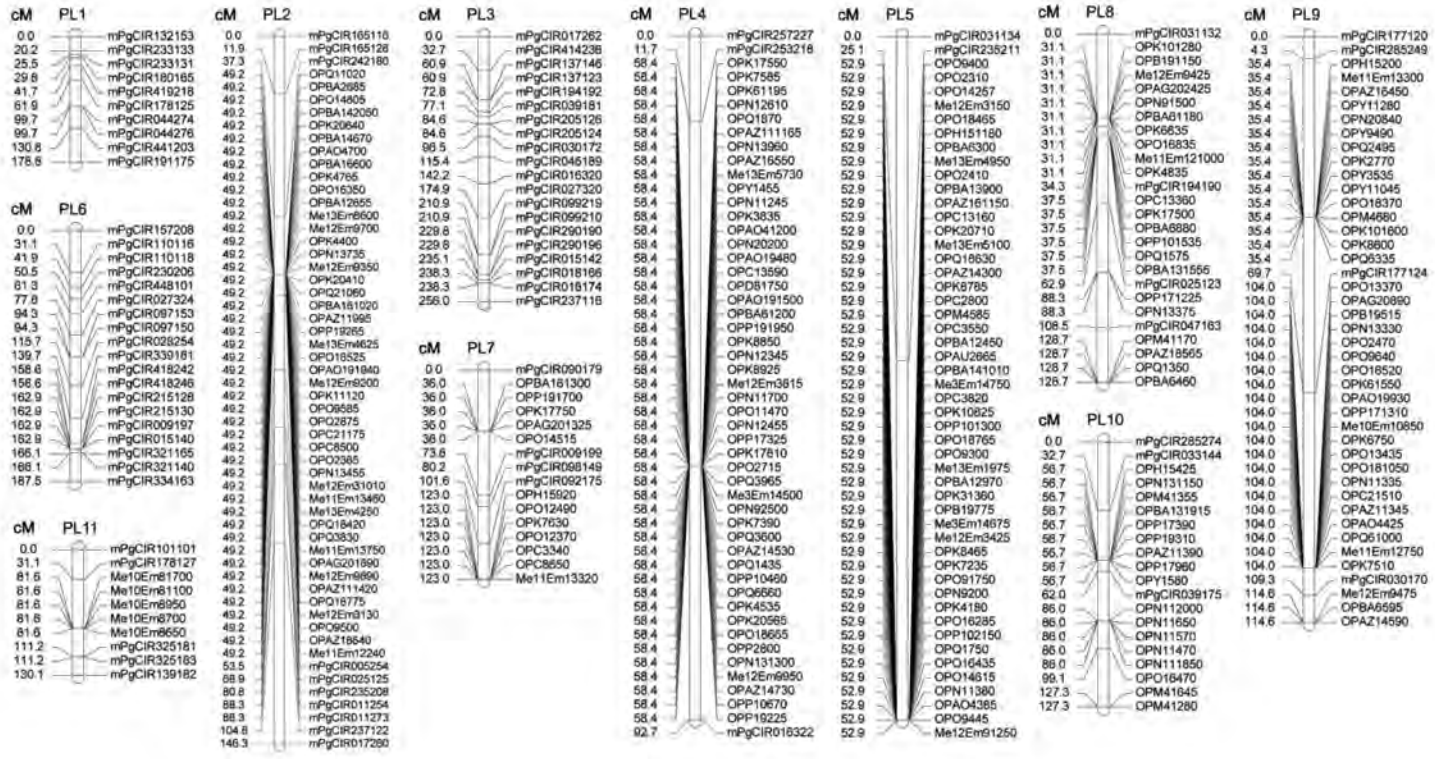


**Fig 2. Genetic linkage map of 'Purple Local': Map distances in centiMorgans (cM) are indicated to the left, and loci to the right, of each linkage group**

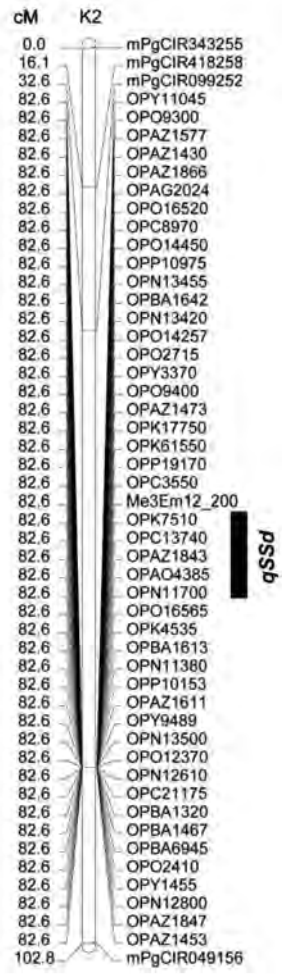


**Fig 3. QTLs mapped on linkage map of 'Kamsari'**

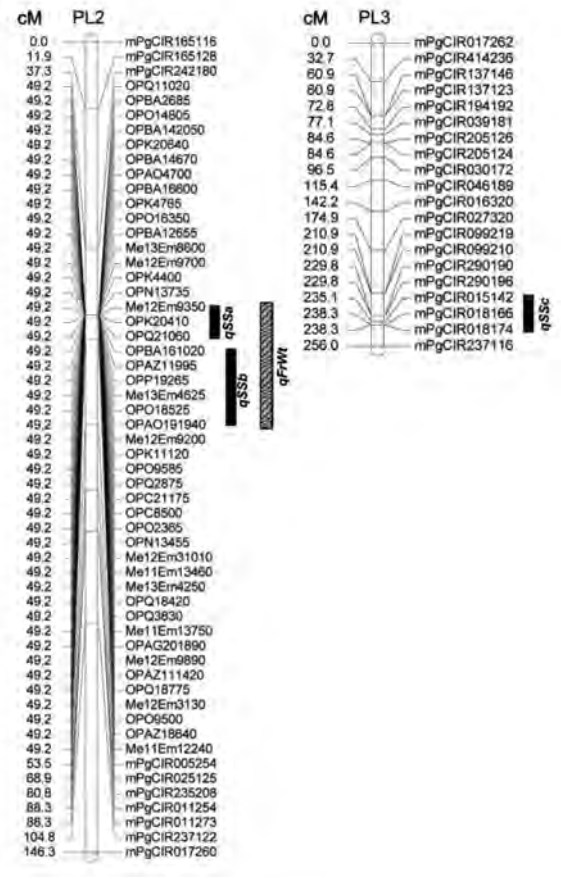


**Fig 4. QTLs mapped on linkage map of 'Purple Local'**

**Table 4. Summary of results of QTL analyses**

| Trait | QTL | Linkage Group | Marker Interval | QTL position (cM) | LR | Additive effect | $R^2$ |
|---|---|---|---|---|---|---|---|
| Average fruit weight | qFrWt | PL2 | OPQ1_1020 - mPgCIR025_125 | 49.21 | 29.47 | 63.07 | 26.3 |
| | | | | | | | Total $R^2$ 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 $R^2$ 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 medium-sized 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.

## REFERENCES

Ahmed, B., Mannan, M.A. and Hossain, S.A. 2011. Molecular characterization of guava (*Psidium guajava* L.) germplasm by RAPD analysis. *Int'l. J. Nat. Sci.,* **1**:62-67

Chakravarti, A., Lasher, L.K. and Reefer, J.E. 1991. A maximum-likelihood method for estimating genome length using genetic linkage data. *Genetics,* **128:**175-182

Chandra, R. and Mishra, M. 2007. Biotechnological interventions for improvement of guava (*Psidium guajava* L.). *Acta Hort.,* **735**:117-125

Chen, T.W., Ng, C.C., Wang, C.Y. and Shyu, Y.T. 2007. Molecular identification and analysis of *Psidium guajava* L. from indigenous tribes of Taiwan. *J. Food Drug Anal.,* **15**:82-88

Dahiya, K.K., Archak, S. and Karihaloo, J.L. 2002. DNA fingerprinting of guava (*Psidium guajava* L.) cultivars using RAPD markers. *Indian J. Pl. Genet. Res.,* **15**:112–115

Dinesh, M.R. and Vasugi, C. 2010. Phenotypic and genotypic variations in fruit characteristics of guava (*Psidium guajava*). *Indian J. Agril. Sci.,* **80**:998–999

Fishman, L., Kelly, A.J., Morgan, E. and Willis, J.H. 2001. A genetic map in the *Mimulus guttatus* species complex reveals transmission ratio distortion due to heterospecific interactions. *Genetics,* **159**:1701-1716

Grattapaglia, D. and Sederoff, R. 1994. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. *Genetics,* **137**:1121-1137

Kanupriya, C., Madhavi Latha, P., Aswath, C., Laxman, R., Padmakar, B., Vasugi, C. and Dinesh, M.R. 2011. Cultivar identification and genetic fingerprinting of guava (*Psidium guajava*) using microsatellite markers. *Int'l. J. Fruit Sci.,* **11**:184-196

Lepitre, V., Nansot, G., Grangeon, R., Pomies, V., Rivallan, R., Risterucci, A.M., Valdés- Infante, J., Rodríguez-Medina, N.N., Muth, J., Boike, J., Prüfer, D., Becker, D., Rohde, W., Ritter, E. and Billotte, N. 2010. The microsatellite (SSR)/AFLP reference linkage map of guava. *Acta Hort*., **849**:183-191

Li, G. and Quiros, C.F. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor. Appl. Genet.*, **103:**455-461

Lu, J.J., Zhao, H.Y., Suo, N.N., Wang, S., Shen, B., Wang, H.Z. and Liu, J.J. 2012. Genetic linkage maps of *Dendrobium moniliforme* and *D. officinale* based on EST-SSR, SRAP, ISSR and RAPD markers. *Sci. Hortic.*, **137:**1-10

Maliepaard, C., Jansen, J. and Van Ooijen, J.W. 1997. Linkage analysis in a full-sib family of an outbreeding plant species: overview and consequence for applications. *Genet. Res.,* **70**:235-250

Nakasone, H.Y. and Paull, R.E. 1998. Tropical Fruits: Crop production science in horticulture. CAB International, Wallingford, UK, pp 468

Nimisha, S., Kherwar, D., Ajay, K.M., Singh, B. and Usha, K. 2013. Molecular breeding to improve guava (*Psidium guajava* L.): Current status and future prospective. *Sci. Hortic.,* **164:**578-588

Padmakar, B., Kanupriya, C., Madhavi Latha, P., Prashant, K.S., Dinesh, M.R., Sailaja, D. and Aswath, C. 2015a. Development of SRAP and SSR marker based genetic linkage maps of guava (*Psidium guajava* L). *Sci. Hortic.*, **192**:158-165

Padmakar, B., Sailaja, D. and Aswath, C. 2015b. Molecular

exploration of guava (*Psidium guajava* L.) genome using SSR and RAPD markers: a step towards establishing linkage map. *J. Hortl. Sci.*, **10**:130-135

Pessanha, P.G.D.O., Viana, A.P., Júnior, A.T.D.A., Souza, R.M.D., Teixeira, M.C. and Pereira, M.G. 2011. Assessment of genetic diversity in access to *Psidium* spp. via RAPD markers. *Rev. Bras. Frutic.*, **33**:129-136

Prakash, D.P., Narayanaswamy, P. and Sondur, S.N. 2002. Analysis of molecular diversity in guava using RAPD markers. *J. Hort. Sci. Biotechnol.*, **77**:287–293

Risterucci, A.M., Duval, M.F., Rohde, W. and Billotte, N. 2005. Isolation and characterization of microsatellite loci from *Psidium guajava* L. *Mol. Ecol. Notes*, **5**:745-748

Risterucci, A.M., Nansot, G., Grangeon, R., Lepitre, V., de Reeper, A., Argout, X., Ruiz, M. and Billotte, N. 2010. Development of guava microsatellite (SSR) markers using the SAT software. *Acta Hort.*, **849**:113-119

Ritter, E., Rodríguez-Medina, N.N., Velásquez, B., Rivero, D., Rodríguez, J.A., Martínez, F. and Valdés-Infante, J. 2010. QTL (Quantitative Trait Loci) analysis in guava. *Acta Hort.*, **849**:193-202

Rodriguez, N., Valdés-Infante, J., Becker, D., Velazquez, B., Gonzalez, G., Sourd, D., Rodriguez, J., Billotte, N., Risterucci, A.M., Ritter, E. and Rohde, W. 2007. Characterization of guava accessions by SSR markers, extension of the molecular linkage map, and mapping of QTLs for vegetative and reproductive characters. *Acta Hort.*, **735**:201-215

Sharma, A.S., Sehrawat, S.K., Singhrot, R.S. and Boora, K.S. 2007. Assessment of genetic diversity and relationship among *Psidium* spp. through RAPD analysis. *Acta Hort.*, **735:**71-78

Shruthi, S.D., Roshan, A., Timilsina, S.S. and Sunita, S. 2013. A review on the medicinal plant *Psidium guajava* Linn. (Myrtaceae). *J. Drug Deliv. Therapeutics*, **3**:162-168

Sokal, R.R. and Rohlf, F.J. 1981. Biometry: the Principles and Practice of Statistics in Biological Research, W.H. Freeman and Company, San Francisco, USA

Tanksley, S.D., Ganal, M.W., Prince, J.P., Devincente, M.C., Bonierbale, M.W., Broun, P., Fulton, T.M., Giovanonni, J.J., Grandillo, S., Martin, G.B., Messeguer, R., Miller, J.C., Miller, L., Paterson, A.H., Pineda, O., Roder, M.S., Wing, R.A., Wu, W. and Young, N.D. 1992. High density molecular linkage maps of tomato and potato genomes: biological interferences and practical applications. *Genetics*, **132**:1141–1160

Trapletti, A. and Hornik, K. 2007. Tseries: time series analysis and computational finance. *R package version 0.10-11*

Valdés-Infante, D., Becker, D., Rodríguez, N., Velázquez, B., González, G., Sourd, D., Rodríguez, J., Ritter, E. and Rohde, W. 2003. Molecular characterization of Cuban accessions of guava (*Psidium guajava* L.), establishment of a first molecular linkage map and mapping of QTLs for vegetative characters. *J. Genet. Breed.*, **57**:349-358

Wang, S., Basten, C.J. and Zeng, Z.B. 2010. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, USA.

Wen, M., Wang, H., Xia, Z., Zou, M., Lu, C. and Wang, W. 2010. Development of EST-SSR and genomic-SSR markers to assess genetic diversity in *Jatropha curcas* L. *BMC Res. Notes*, **3**:42, doi: 10.1186/1756-0500-3-42.

Xie, W., Zhang, X., Cai, H., Huang, L., Peng, Y. and Ma, X. 2011. Genetic maps of SSR and SRAP markers in diploid orchard grass (*Dactylis glomerata* L.) using the pseudo-testcross strategy. *Genome*, **54**:212-221

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. *Genetics*, **136:**1457-1468

Zhang, F., Chen, S., Chen, F., Fang, W., Chen, Y. and Li, F. 2011. SRAP-based mapping and QTL detection for inflorescence-related traits in chrysanthemum (*Dendranthema morifolium). Mol. Breeding*, **27**:11-23

Zhang, R., Wu, J., Li, X., Awais Khan, M., Chen, H., Korban, S.S. and Zhang, S. 2013. An AFLP, SRAP, and SSR genetic linkage map and identification of QTLs for fruit traits in pear (*Pyrus* L.). *Pl. Mol. Biol. Rep.*, **31**:678-687

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