



## Molecular exploration of guava (*Psidium guajava* L.) genome using SSR and RAPD markers: a step towards establishing linkage map

B. Padmakar<sup>1</sup>, D. Sailaja<sup>2</sup> and C. Aswath<sup>3\*</sup>

Division of Biotechnology  
ICAR-Indian Institute of Horticultural Research  
Hesaraghatta Lake Post, Bangalore - 560089, India  
\*E-mail: [aswathihr@gmail.com](mailto:aswathihr@gmail.com)

### ABSTRACT

In the present study, molecular evaluation of two guava mapping populations (MP), MPI comprising 94 F<sub>1</sub> progenies and MPIO comprising 46 F<sub>1</sub> progenies, was carried out using simple sequence repeat (SSR) and random amplified polymorphic DNA (RAPD) markers. A pseudo-test cross strategy was implemented where 'Kamsari' X 'Purple Local' and 'Purple Local' X 'Allahabad Safeda' were crossed, and, these showed variation in fruit quality traits such as seed-strength (hardness/softness), fruit weight, TSS and pulp color. A set of 30 RAPD markers was used for genotyping MPI while a set of 55 SSR markers was used for genotyping MPIO. In case of MPI, 30 RAPD markers generated 214 scorable markers, of which 80 markers were specific to 'Kamsari', 14 markers to 'Purple Local' and the remaining 120 were intercross markers. As for MPIO, 55 polymorphic SSR markers resulted in generation of 207 alleles (with a maximum of 4 alleles and a minimum of 3 alleles per locus), of which 108 alleles were specific to 'Purple Local' while 99 were specific to 'Allahabad Safeda'. Genotypic data thus generated can be further exploited for constructing genetic linkage maps and mapping complex QTLs governing fruit quality traits in guava.

**Key words:** Guava, genotyping, M13-tailed PCR, SSR, RAPD

### INTRODUCTION

Guava (*Psidium guajava* L.), belonging to the family Myrtaceae, is a diploid with 2n=22 and comprises approximately 430 Mbp genome. It is popularly known as the apple of the tropics or the poor man's apple. Guava is believed to be native to Mexico (Rios *et al.*, 1977) and is present throughout South America, Europe, Africa and Asia. Its edaphoclimatic adaptability, prolific bearing, hardness to biotic and abiotic stresses and medicinal properties impart a great value to this fruit crop. Besides, it is a rich source of nutrients, vitamins and minerals; it also has a pharmaceutical potential by way of having antioxidant, antimicrobial and antidiabetic properties (Shruthi *et al.*, 2013). A major traditional use of the fruit is that of an anti-diarrheal. Other uses reported include treatment of gastroenteritis, dysentery, stomach ailments, antibacterial colic pathogenic germs of the intestine (Gutierrez *et al.*, 2008). Guava, a dual-purpose fruit, is used as a fresh fruit and as also after processing into a variety of forms like puree, paste, jam, jelly, nectar, syrup, ice cream or juice.

SSR or microsatellite is a PCR (Polymerase Chain Reaction)- based molecular marker technique with advantages such as abundance, high polymorphism, co-dominance and primer transferability. SSR markers are widely used for constructing genetic maps (Oliveira *et al.*, 2008; Ogundiwin *et al.*, 2009; Wang *et al.*, 2010; Das *et al.*, 2012; Pauly *et al.*, 2012; Liu *et al.*, 2013; Serba *et al.*, 2013; Zhang *et al.*, 2013). SSR markers have also been utilized for molecular characterization and genetic diversity assessment of guava germplasm resources (Nimisha *et al.*, 2013). SSR markers in guava were developed by Risterucci *et al.* (2005, 2010) and were applied in germplasm characterization and for assessing existing genetic variability (Risterucci *et al.*, 2005; Valdés-Infante *et al.*, 2007; Viji *et al.*, 2010; Aranguren *et al.*, 2010; Santos *et al.*, 2011; Coser *et al.*, 2012; Noia *et al.*, 2012; José *et al.*, 2012; Angélica *et al.*, 2012). SSR markers have been earlier used for cultivar identification (Kanupriya *et al.*, 2011), discrimination of wild guava species (Nogueira *et al.*, 2012) and for assessing genetic homogeneity of guava plants derived from somatic embryogenesis (Rai *et al.*, 2012). Guava SSR markers so developed have also

<sup>1</sup>Current address: Centre for Biotechnology, Jawaharlal Nehru Technological University, Hyderabad, Telangana, India

<sup>2</sup>Department of Biotechnology, Gokaraju Rangaraju Institute of Engineering & Technology, Hyderabad, Telangana, India

<sup>3</sup>Division of Ornamental Crops, ICAR-Indian Institute of Horticultural Research, Bangalore, Karnataka, India

been used across different species of Myrtaceae (Briceno *et al.*, 2010; Rai *et al.*, 2013). Additionally RAPD markers have been considered as the markers of choice for crops like guava that lack sufficient genomic resources. Here, RAPD markers were used with an objective of linkage-map enrichment by reducing inter-marker distance and substituting missing linkage between the mapped SSR and SRAP markers in guava linkage-maps (Padmakar *et al.*, 2015).

A strategy frequently employed for mapping F<sup>1</sup> populations in tree species and in perennials is the two-way pseudo-test cross mapping strategy (Grattapaglia and Sederoff, 1994) because it has been found to be efficient for mapping several heterozygous species. Guava, being a perennial, exhibits a high degree of heterogeneity and heterozygosity (Chandra and Mishra, 2007) which makes the underlying molecular mechanisms for phenotypic expression of various economically important traits difficult to understand. Therefore, linkage maps of progenies segregating for important economic traits such as fruit quality and yield, are required to be developed. In guava, only a few reports (Valdés-Infante *et al.*, 2003; Rodriguez *et al.*, 2007; Lepitre *et al.*, 2010) are available on construction of molecular linkage-maps. Here we report development of intra-specific linkage maps in guava using SSR markers, along with genotyping of the mapping population by RAPD markers. This could be used for further enrichment of the available linkage-maps in guava.

## MATERIAL AND METHODS

### Plant material

Three cultivars of *Psidium guajava* maintained in the field germplasm bank at Indian Institute of Horticultural Research, Bangalore, India, namely 'Kamsari', 'Purple Local' and 'Allahabad Safeda' were used as the parental lines for developing mapping populations. A hybridization programme was undertaken by crossing the varieties 'Kamsari' and 'Purple Local' (Dinesh and Vasugi, 2010) for developing mapping population I (MPI), and 'Purple Local' X 'Allahabad Safeda' for mapping population II (MPII) so as to develop hybrids suitable for table purpose as well as for processing. The mapping population developed was evaluated morphologically for traits like seed- strength (hardness/softness), fruit weight, TSS and pulp color.

### DNA extraction

In the case of MPI, a set of 94 F<sup>1</sup> progenies was shortlisted (based on morphological data on seed-strength

and fruit weight, as these two traits showed positive correlation) whereas, in the case of MPII, a set of 46 F<sup>1</sup> progenies was shortlisted (based on pulp color) for molecular characterization. Genomic DNA was extracted from mature, healthy leaf material using the modified CTAB method of Kanupriya *et al.* (2011). Quality of the DNA extracted was determined on 0.8% agarose gel electrophoresis and quantified using GeneQuant UV-spectrophotometer (GE Healthcare Biosciences Ltd. U.K.)

### Molecular characterization

In the case of MPII, SSR-based genotyping of Padmakar *et al.* (2015) was employed using 160 SSR primers (Risterucci *et al.*, 2010) to characterize parental lines along with their mapping population.

Genotyping of MPI-specific parental lines was done using a set of 200 RAPD markers (Table 1). PCR amplification was performed in 25 $\mu$ l reaction mixture containing 50mM KCl, 1mM Tris-HCl (pH 8.8), 0.01% gelatin, 1.5mM MgCl<sub>2</sub>, 0.2mM each of dNTP, 0.3 $\mu$ M primer, 100ng genomic DNA and 0.5 units of Taq DNA polymerase (Bangalore Genei, India). PCR was performed on a Master Cycler Gradient (Eppendorf AG, Hamburg, Germany) thermal cycler, with the following temperature profile: initial denaturation at 94°C for 3 min, 40 cycles of 2s at 94°C, 2s at 35°C, and 1 min at 72°C, and a final extension at 72°C for 10 min. Amplification products were screened on 1.5% agarose gel for confirmation of amplification. PCR was performed thrice for checking the reproducibility of the polymorphic markers identified.

### Data analysis

In the case of SSR markers, raw data generated from the Genetic Analyzer was analyzed and compiled using Peak Scanner V1.0 software (Applied Biosystems, USA) for detection of alleles. Allelic profile of the SSR markers within a mapping population and their segregation pattern was identified by comparison with parental profiles. Allelic data so generated was then converted into the binary format, with 1 and 0 indicating presence and absence of the corresponding allele, respectively, within that population. As for RAPD markers, polymorphic markers were scored in

**Table 1. List of RAPD decamers used in the present study**

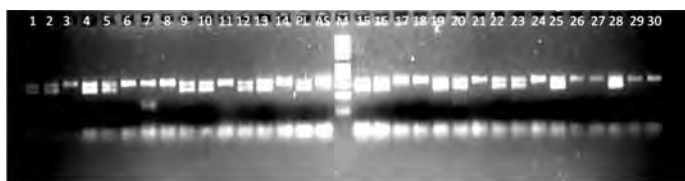
S. No.	RAPD Primer
1	OPA1 – OPA20
2	OPB1 – OPB20
3	OPC1 – OPC20
4	OPH1 – OPH20
5	OPN1 – OPN20

the binary format by assigning '1' for presence of the band and '0' for absence of the band. Genetic linkage maps were constructed for the parental lines 'Purple Local' and 'Allahabad Safeda' of MPII using segregation data generated for 46 mapping-population progeny, as per Padmakar *et al* (2015).

## RESULTS AND DISCUSSION

Guava is even now considered an orphan crop with respect to availability of genomic resources, as, the present day genomic technologies have still to be applied to this crop. Till date, only few reports are available on molecular characterization of germplasm and mapping populations in guava. Generating saturated genetic maps and functional markers in guava has become a major challenge which, in turn, plays a vital role in improvement of guava inbreeding programs. Hence, the present study focussed on application of biotechnological tools alongside genetics tools such as genotyping and linkage mapping. High-quality genomic DNA was extracted from a total of 94 progenies of MPI and 94 progenies of MPII along with their respective parental lines

Genotyping by SSR markers is a proven technique used in several areas of plant genetics research and development. In the case of MPII, only 120 primers (out of 160 SSR primer pairs screened) successfully amplified both the parental lines. The parental polymorphism rate observed between 'Purple Local' and 'Allahabad Safeda' was 45.83%, using a set of 55 polymorphic SSR primer pairs. Initial confirmation was done on 3% agarose gel (Fig. 1); and then, 4 primers were multiplexed into one tube and processed for gene scan analysis. High-throughput genotyping of the mapping population resulted in identification of 207 alleles, with a minimum of three alleles and a maximum of four alleles per primer. Of these, 108 alleles (52.17%) were found to be specific to 'Purple Local' and 99 alleles (47.83%) specific to 'Allahabad Safeda'. Markers showing segregation distortion were not considered for linkage analysis because these affect the detection power of QTL when QTL and SDMs, or loci, are closely linked.



**Fig. 1.** Agarose gel (3%) showing amplification pattern of SSR marker among mapping population II and parental lines 'Purple Local' (PL) and 'Allahabad Safeda' (AS)

Similar omission of segregation-distorted markers in map construction was reported earlier (Grattapaglia and Sederoff, 1994; Hackett *et al*, 2000; Jones *et al*, 2003; Grando *et al*, 2003; Sudarshini *et al*, 2014) especially in the case of perennial tree crops where pseudo-test cross strategy was employed.

Independent linkage maps were constructed for each parent using double pseudo-test cross mapping strategy (Grattapaglia and Sederoff, 1994) for 'Purple Local' and 'Allahabad Safeda' of MPII only. A set of six and five linkage groups (Table 2) were observed in 'Purple Local' and 'Allahabad Safeda' respectively. Maternal 'Purple Local' map had a total of 49 marker loci distributed over six linkage groups (Fig. 2), with 13 markers remaining unlinked. This maternal framework map covered 746.5cM of the total map distance. Number of markers per linkage group ranged from three to 15. Linkage distance spanned by individual linkage groups ranged from 47.5cM (PL6) to 199.6cM (PL1). The linkage groups had an average length of 124.4cM and average marker interval length of 15.2cM. Paternal 'Allahabad Safeda' map had 44 markers distributed over five linkage groups (Fig. 3), with nine markers remaining unassigned. This paternal framework map covered 580.9cM of the total map distance, with a minimum of three and a maximum of 15 markers ordered into five linkage groups. Linkage distance spanned by individual linkage groups ranged from 23.3cM (AS5) to 245.4cM (AS4). Average length of the linkage groups was 116.2cM and average spacing was 13.2cM. As the number of markers mapped was low, the number of linkage groups observed was not equivalent to the haploid number of chromosomes. Similar study on development of partial linkage maps using just SSR markers has been reported in jute (Das *et al*, 2012). These maps can be further enriched with more markers and, thereby, used for mapping putative QTLs.

**Table 2.** Characteristics of parental linkage maps

LG <sup>a</sup>	P1: Purple Local			P2: Allahabad Safeda		
	PL-LG	TM <sup>b</sup>	cM <sup>c</sup>	AS-LG	TM <sup>b</sup>	cM <sup>c</sup>
1	PL1	15	199.6	AS1	7	53.1
2	PL2	8	118.1	AS2	11	130.0
3	PL3	10	128.7	AS3	8	129.1
4	PL4	8	113.0	AS4	15	245.4
5	PL5	5	139.6	AS5	3	23.3
6	PL6	3	47.5			
Total		49	746.5		44	580.9
Min.		3	47.5		3	23.3
Mean		8.1	124.4		8.8	116.2
Max.		15	199.6		15	245.4

<sup>a</sup>Linkage Group, <sup>b</sup>Total markers, <sup>c</sup>LG length (in centimorgan)

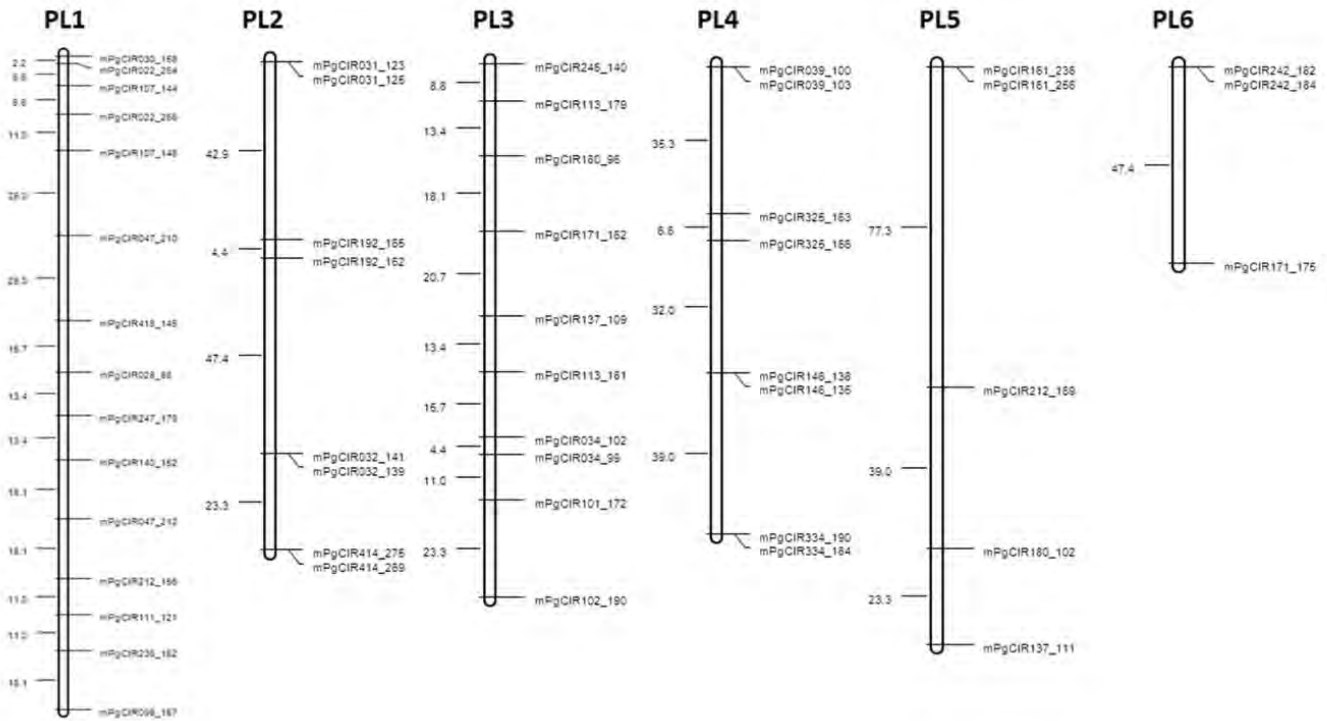


Fig. 2. Genetic linkage map of the parent 'Purple Local'; Map distances in centimorgans (cM) are indicated to the left, and loci to the right of each linkage group

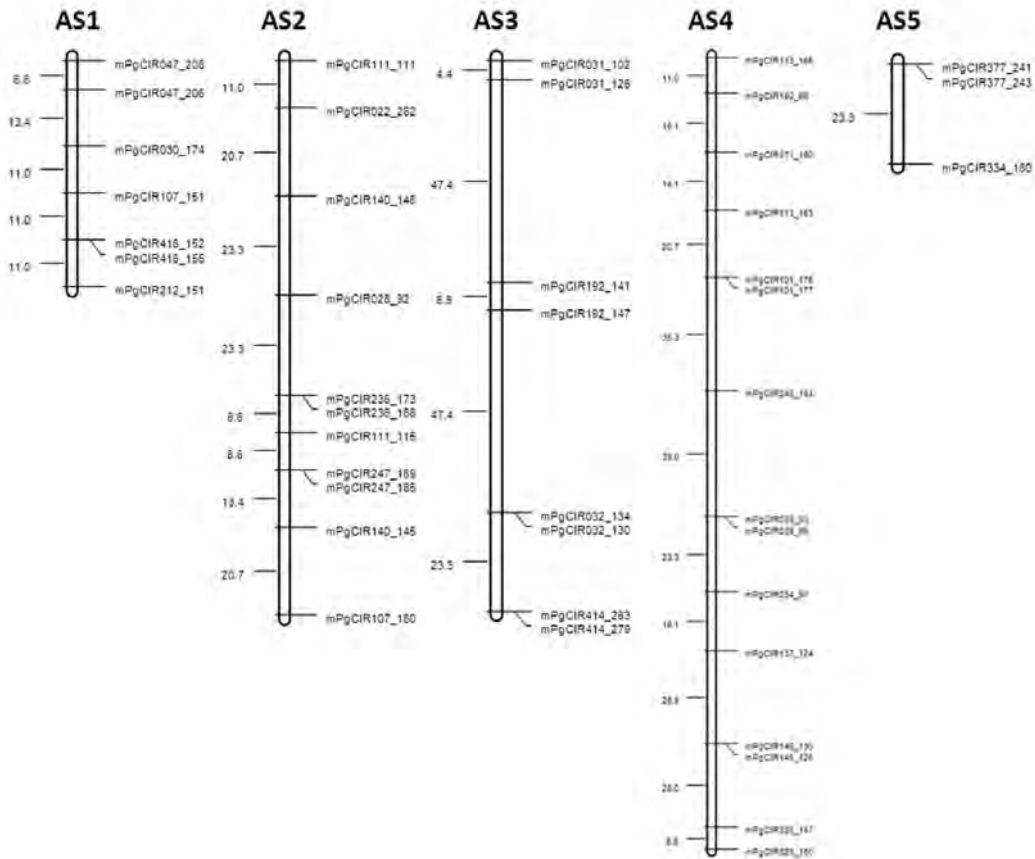
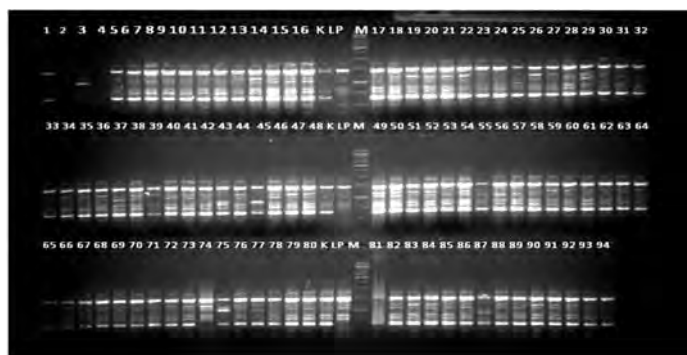


Fig. 3. Genetic linkage map of the parent 'Allahabad Safeda'; Map distances in centimorgans (cM) are indicated to the left, and loci to the right of each linkage group



**Fig. 4.** Agarose gel (1.5%) showing amplification pattern of RAPD markers among mapping population I and parental lines 'Kamsari' (K) and 'Purple Local' (PL)

In the case of MPI, 100 RAPD primers were initially screened in parental lines that revealed 30% polymorphism. These 30 decamers were used further for genotyping the mapping population. A total of 214 scorable bands were produced, with an average of nine bands per primer. Size of the amplified products ranged from 250bp to 2kb (Fig. 4) Of the 214 bands scored, 94 (43.92%) were polymorphic and segregated as test cross markers, of which 80 markers were specific to 'Kamsari' and 14 markers were specific to 'Purple Local'. The remaining 120 common fragments that segregated in 3:1 ratio were treated as intercross markers. RAPD data thus generated can be further used for enriching the available genetic maps of 'Kamsari' and 'Purple Local' reported by our group (Padmakar *et al*, 2015).

Linkage maps thus constructed will be further used for enrichment and, thereby, utilized as reference maps for identifying complex Quantitative Trait loci (QTLs) associated with fruit quality traits in guava. This would also help in identifying trait specific marker(s) for executing marker assisted selection (MAS)-based guava breeding programs.

## ACKNOWLEDGEMENT

We thank Department of Biotechnology (DBT), New Delhi, India, for providing financial support.

## REFERENCES

Aranguren, Y., Valecillos, C. and Fermin, G. 2010. Assessment of the variability of Venezuelan guava landraces by microsatellites. *Acta Hort.*, **849**:147-154

Briceno, A., Aranguren, Y. and Fermin, G. 2010. Assessment of guava-derived SSR markers for the molecular characterization of Myrtaceae from different ecosystems in Venezuela. IInd International Symposium on Guava and other Myrtaceae. *Acta Hort.*, **849**:139-146

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

Coser, M.S., Fontes, M.P.M. and Ferreira, F.S.M. 2012. Pollen viability of guava genotypes from different locations. In: 3<sup>rd</sup> International Symposium on Guava and Other Myrtaceae, Petrolina, PE, Brazil. Session III: Myrtaceae Plant Breeding and Post-Harvest Management

Das, M., Banerjee, S., Dhariwal, R., Vyas, S., Mir, R.R., Topdar, N., Kundu, A., Khurana, J.P., Tyagi, A.K., Sarkar, D., Sinha, M.K., Balyan, H.S. and Gupta, P.K. 2012. Development of SSR markers and construction of a linkage map in jute. *J. Genet.*, **91**:21-31

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

Grando, M.S., Bellin, D., Edwards, K.J., Pozzi, C., Stefanini, M. and Velasco, R. 2003. Molecular linkage maps of *Vitis vinifera* L. and *Vitis riparia* Mchx. *Theor. Appl. Genet.*, **106**:1213-1224

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

Gutierrez, R.M.P., Mitchell, S. and Solis, R.V. 2008. *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacology*, **117**:1-27

Kanupriya, 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. Fr. Sci.*, **11**:184-196

Khan, M.L.H. and Ahmad, J. 1985. A pharmacognostic study of *Psidium guajava* L. *Int'l. J. Crude Drug Res.*, **23**:95-103

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

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

Nogueira, M.A., Guilhen, H.S.J., Mangaravite, E., Ferreira, A. and Ferreira, F.S.M. 2012. Discriminant analysis of wild guava tree by morphological descriptors and

- microsatellites. In: Proceedings of 3rd International Symposium on Guava and other Myrtaceae, Petrolina, PE, Brazil. Session IV: Myrtaceae Biotechnologies and Genomics
- Noia, L.R., Coser, S.M., Guilhen, J.H.S., Ferreira, A. and Ferreira, M.F.S. 2012. Genetic distance of guava genotypes from different altitudes by microsatellites. In: Proceedings of 3rd International Symposium on Guava and other Myrtaceae, Petrolina, PE, Brazil. Session IV: Myrtaceae Biotechnologies and Genomics
- Ogundiwin, E., Peace, C.P., Gradziel, T.M., Parfitt, D.E., Bliss, F.A. and Crisosto, C.H. 2009. A fruit quality gene map of *Prunus*. *BMC Genomics*, **10**:587, doi:10.1186/1471-2164-10-587
- Oliveira, E.J., Vieir, M.L.C., Garcia, A.A.F., Munhoz, C.F., Margarido, G.R., Consoli, L., Matta, F.P., Moraes, M.C., Zucchi, M.I. and Fungaro, M.H.P. 2008. An integrated molecular map of yellow passion fruit based on simultaneous maximum-likelihood estimation of linkage and linkage phases. *J. Am. Soc. Hort. Sci.*, **133**:35-41
- Padmakar, B., Kanupriya, C., Madhavi Latha, P., Prashant, K.S., Dinesh, M.R., Sailaja, D. and Aswath, C. 2015. Development of SRAP and SSR marker based genetic linkage maps of guava (*Psidium guajava* L.). *Sci. Hortic.*, **192**:158-165
- Pauly, L., Flajoulot, S., Garon, J., Julier, B., Béguier, V. and Barre, P. 2012. Detection of favorable alleles for plant height and crown rust tolerance in three connected populations of perennial ryegrass (*Lolium perenne* L.). *Theor. Appl. Genet.*, **124**:1139-1153
- Rai, K.M., Phulwaria, M., Gupta, K.M., Shekhawat, N.S. and Jaiswal, U. 2012. Genetic homogeneity of guava plants derived from somatic embryogenesis using SSR and ISSR markers. *Plant Cell Tiss. Organ. Cult.*, **111**:259-264
- Rai, M.K., Phulwaria, M. and Shekhawat, N.S. 2013. Transferability of simple sequence repeat (SSR) markers developed in guava (*Psidium guajava* L.) to four Myrtaceae species. *Mol. Biol. Rep.*, **40**:5067-5071, <http://dx.doi.org/10.1007/s11033-013-2608-1>
- 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
- 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
- Santos, C.A.F., Correa, L.C. and Costa, S.R. 2011. Genetic divergence among *Psidium* accession based on biochemical and agronomic variables. *Crop Breed. Appl. Biotechnol.*, **11**:149-156
- Serba, D., Wu, L., Daverdin, G., Bahri, B.A., Wang, X., Kilian, A., et al. 2013. Linkage maps of lowland and upland tetraploid switchgrass ecotypes. *BioEnergy Res.*, **6**:953-965
- 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
- Sudarshini, K.V., Padmakar, B., Manjunath, S.P., Lakshman, R. and Aswath, C. 2014. The genetic linkage maps of *Anthurium* species based on RAPD, ISSR and SRAP markers. *Sci. Hortic.*, **178**:132-137
- 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
- Viji, G., Harris, D.L., Yadav, A.K. and Zee, F.T. 2010. Use of microsatellite markers to characterize genetic diversity of selected accessions of guava (*Psidium guajava* L.) in the United States. *Acta. Hort.*, **859**:169-176
- 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. <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>
- 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.) *Plant Mol. Biol. Repr.*, **31**:678-687

(MS Received 19 September 2015, Revised 22 November 2015, Accepted 29 November 2015)