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Development of SRAP and SSR marker-based genetic linkage maps of guava (*Psidium guajava* L.)

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ABSTRACT

In the present study genotyping of guava full-sib population with 94 F1 progenies, derived from a two-way pseudo-testcross strategy differing in fruit quality traits such as seed strength (hardness/softness), fruit weight, and TSS, was carried out using simple sequence repeat (SSR) markers and sequence-related amplified polymorphic (SRAP) primer combinations. In the case of SSR's high-throughput genotyping, through a M13-Tailed PCR principle, using a set of 160 SSR primer pairs revealed 64.3% parental polymorphism that generated 321 alleles during the mapping population survey. Twenty percent of parental polymorphism was revealed with SRAPs that generated 126 scorable markers. Two linkage maps were constructed for parents using maternal-specific 143 testcross marker loci and paternal-specific 127 testcross markers along with 60 intercross marker loci. At a minimum logarithm of the odds (LOD) score of 4.0 and a maximum map distance of 40 cM the maternal Kamsari map covered 2551.3 cM with an average marker interval distance of 13.21 cM having a total of 193 framework marker loci being ordered into 11 linkage groups. The paternal Purple Local map covered 2113.0 cM with an average marker interval distance of 12.07 cM having 175 framework marker loci being ordered into 11 linkage groups. The estimated genome coverage was of 87.32% in Kamsari and 83.74% in Purple Local. These genetic maps will play a pivotal role in identification of complex quantitative trait loci (QTLs) related to fruit quality. This is the first report of linkage maps in guava based on the combination of SSR along with SRAP markers.

1. Introduction

Guava (*Psidium guajava* L.), a diploid with $2n = 22$, belongs to the family Myrtaceae (Nakasone and Paull, 1998) and is native to the tropical America. In India it is a major fruit crop with an estimated area of 2,68,220 ha and production of 36,67,890 MT (NHB, 2014). Commonly known as Apple of Tropics or Poor Man's apple, it is a rich source of nutrients, vitamins, and minerals and is valued for its edaphoclimatic adaptability, hardiness to biotic and abiotic stresses, prolific bearing, and medicinal properties. Many pharmacological studies have demonstrated the ability of this plant as an antioxidant, hepatoprotective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory, and antinociceptive activities, supporting its traditional uses (Shruthi et al., 2013) and used as an effective remedy to treat and prevent diseases such as headache, cough (Khan and Ahmad, 1985; Jairaj et al., 1999), spasm, inflammatory, pyrexia, acute diarrhea (Jaiswal and Amin, 1992), colic, flatulence, and gastric pain (Lozoya et al., 2002). Guava is used as both fresh fruit and after processing into a variety of forms like puree, paste, jam, jelly, nectar, syrup, ice cream or juice. As far as the fresh fruit consumption is concerned consumers prefer medium-sized fruits with high total soluble solids (TSS), pink pulp, and soft seeds. Incorporation of these quantitative traits has therefore become a major breeding objective in this crop.

The molecular marker technology revolutionized the mode of plant breeding through the ability to perform genetic analysis of complex traits and to select for target regions through marker-assisted selection (MAS). SSR or microsatellite is a PCR (Polymerase Chain Reaction)-based molecular marker technique with many advantages, such as abundance, high polymorphism, codominant, and primer transferability. SSR marker is widely used

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in genetic map construction (Oliveira et al., 2008; Ogundiwin et al., 2009; Wang et al., 2010; Das et al., 2012; El Mannai et al., 2012; Pauly et al., 2012; Liu et al., 2013; Serba et al., 2013; Zhang et al., 2013). These SSR markers have been exploited for the improvement of the guava breeding program by involving in the molecular characterization and genetic diversity assessment of guava germplasm resources (Nimisha et al., 2013). SSR markers from guava were developed by Risterucci et al. (2005, 2010) and were applied in germplasm characterization and assessing the 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; Angelica et al., 2012). Additionally SSR markers were used for cultivar identification (Kanupriya et al., 2011), discrimination of wild guava species (Nogueira et al., 2012), and assessing the genetic homogeneity of guava plants derived from somatic embryogenesis (Rai et al., 2012). Thus developed guava SSR markers have also been even used across the Myrtaceae species (Briceno et al., 2010; Rai et al., 2013). SRAP is a PCR-based molecular marker described by Li and Quiros (2001), which aims for the amplification of ORFs (open reading frames). With the advantages of simplicity, high polymorphism, and primer generality, SRAP has already been used in genetic map construction (Gulsen et al., 2010; Xie et al., 2011; Lu et al., 2012; Liu et al., 2013; Zhang et al., 2013). These SRAP markers have not been used much in the guava breeding program and only one report (Youssef et al., 2013) is available on SRAP-based molecular characterization in comparison with that of morphological characterization of guava germplasm.

Construction of a linkage map with consistent molecular markers forms the preliminary point for the analysis of agronomically important traits. The regular strategy employed for mapping F1 populations in tree species and perennials is the two-way pseudo-testcross mapping strategy (Grattapaglia and Sederoff, 1994) because it was efficient for mapping several heterozygous species. Guava, being perennial, exhibits a high degree of heterogeneity and heterozygosity (Chandra and Mishra, 2007), which makes the underlying molecular mechanism controlling the phenotypic expression of various economically important traits difficult to understand. Hence linkage maps of progenies segregating for important economic traits such as fruit quality and yield are required to be developed. In guava only few reports (Valdés-Infante et al., 2003; Rodriguez et al., 2007; Lepitre et al., 2010) are available on the construction of molecular linkage maps. In the present study, we report the development of intra-specific linkage maps in guava using SSR and SRAP markers in a pseudo-testcross mapping configuration aiming at providing the technical support for further studies involving identification of fruit quality-related QTLs and MAS. To our knowledge, this is the first report of linkage map with SRAPs along with SSRs in guava.

2. Material and methods

2.1. Plant material

Two cultivars of guava maintained in field germplasm bank at Indian Institute of Horticultural Research, Bangalore, India, namely Kamsari (hard-seeded with pink pulp, good TSS, medium-size fruits, green skin) and Purple Local (soft-seeded with pink pulp, small-sized fruits, acidic, purple skin color) were used as parental lines for developing the mapping population.

2.2. Development and morphological characterization of mapping population

A hybridization program was taken up at the Indian Institute of Horticultural Research, Bangalore, using the varieties 'Kamsari' and 'Purple Local' to develop hybrids suitable for both table as well as for processing, which would have fruits with a uniform shape, size, good color, firm and thick pulp, good aroma, soft seeds, high TSS and high pectin, and long shelf-life. A progeny population of 513 full-sibs from the above cross was planted in the field during 2007 with a spacing of 2×1.5 m using the trench planting method. The developed mapping population was evaluated morphologically for traits seed strength (hardness/softness), fruit weight and TSS was carried out under guava improvement program and reported by Dinesh and Vasugi (2010).

2.3. DNA extraction

A set of 94 F1 progenies were shortlisted based on the evaluated morphological data of seed strength and fruit weight as these two traits have shown positive correlations and used for molecular characterization. Genomic DNA was extracted from mature and healthy leaf material by using the modified CTAB method described by Kanupriya et al. (2011). The integrity of isolated DNA was determined by electrophoresis in 0.8% agarose gels. Purified DNA was quantified using a GeneQuant UV-spectrophotometer (GE Health Care Bio-sciences Ltd, U.K.) and diluted for further genotyping study.

2.3.1. SSR-based genotyping

A set of 160 SSR primers (Risterucci et al., 2010) were used for high throughput genotyping by employing the M13 Tailed PCR technique (Schuelke, 2000) to characterize the parental lines along with their mapping population. The primers were modified by adding a tail of M13 sequence i.e. 'GTAAACGACGGCCAGT' (17mer) at their 5′ end for all the forward primers and a pig tail of 'CTTGTTT' sequence at the 5′ end of all the reverse primers. Additionally four M13 sequences (17mers) were further labeled with four different dyes namely FAM, PET, NED, and VIC at their 5′ end and used as probes. PCR amplification was carried out in 20 μl reaction containing 16 mM $(NH4)_2SO_4$, 67 mM Tris–HCl pH 8.8, 0.01% Tween-20, 1.5 mM $MgCl_2$, 0.16 mM each dNTP, 0.2 μM forward primer, 0.4 μM reverse primer, 0.4 μM M13 probe, 40 ng genomic DNA, and 0.5 unit of Taq DNA polymerase (Bangalore Genei, India). PCR was carried out on a Master Cycler Gradient (Eppendorf AG, Hamburg, Germany) thermal cycler with the following temperature profile: initial denaturation at 94 °C for 5 min, 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C, and a final extension at 72 °C for 5 min. Amplification products were initially screened on 3% agarose gel for confirmation of the amplification. In order to reduce the cost of genotyping, samples were multiplexed up to 4-fold by combining four PCR products, labeled with different fluorophores (6-FAM, VIC, NED, and PET) into a single sample. These multiplexed samples were mixed with formamide and internal standard (GeneScan™-500 LIZ size standard; Applied Biosystems) then denatured for 5 min at 95 °C, loaded onto the automatic 96-capillary ABI 3730xl DNA Analyzer. Electrophoretic separation and signal detection were carried out with default module settings for generating the raw data.

2.3.2. SRAP-based genotyping

A set of 70 SRAP primer combinations (Li and Quiros, 2001) was used for initial screening of parents. The polymorphic primer combinations identified were further employed in genotyping of mapping population. PCR amplification was carried out in 25 μl reaction containing 16 mM $(NH4)_2SO_4$, 67 mM Tris–HCl pH 8.8, 0.01% Tween-20, 3.5 mM $MgCl_2$, 0.32 mM each dNTP, 1 μM forward primer, 1 μM reverse primer, 100 ng genomic DNA, and 1.5 unit of Taq DNA polymerase (Bangalore Genei, India). PCR was carried out on a Master Cycler Gradient (Eppendorf AG, Hamburg, Germany) thermal cycler with the temperature profile stated by Li and Quiros