Journal of Phytology 2011, 3(8): 53-61
ISSN: 2075-6240
www.scholarjournals.org

JOURNAL OF PHYTOLOGY

www.journal-phytology.com

Elucidation of Diversity among *Psidium* Species using Morphological and SPAR methods

Abin Mani*, Rachana Mishra and George Thomas

Allahabad Agriculture Institute –Deemed University, Allahabad, U.P., 211007, India
C.S.R.D., People's Group, Bhanpur, Bhopal, Madhya Pradesh, India, 462037

Article Info

Article History

Received : 19-06-2011
Revised : 01-08-2011
Accepted : 01-08-2011

*Corresponding Author

Tel : 08982000810
Fax : 07554005226

Email:
abinmani@gmail.com

Summary

Molecular marker assisted characterization is lacking in *Psidium* genus. This study focuses on the genetic variability among eleven *Psidium* species by employing morphological and SPAR (RAPD and ISSR) methods. Morphological characterization was done based on parameters such as size, shape, foliage characters and habit of the plant specimens. In SPAR methods, 16 RAPD and 31 ISSR primers were used to distinguish the genetic variability. 3 RAPD and 4 ISSR primers showed 100% polymorphism, while average polymorphism in both marker systems was 77% and 81.6% respectively and the cluster analysis showed a more or less similar pattern. Dendrograms revealed two main clusters, separating the genus, *F. sellowiana* and *Psidium* sp. with a genetic distance of 0.86.

Key Words: *Psidium* species, Morphological characterization, RAPD, ISSR.

Introduction

The genus *Psidium* belongs to the family Myrtaceae, which originated in tropical South America. Now it is naturalized in tropical and subtropical countries. In early 17th century, Portuguese introduced guava to India (Menzel, 1985) which spreaded throughout the tropical and sub tropical Asian countries. It is believed that most of the guava introductions to India were first made at Basti in Uttar Pradesh (Prakash *et al*., 2002). The genus *Psidium* comprises approximately 150 species of small trees and shrubs in which only 20 species produce edible fruits and the rest are wild with inferior quality of fruits. Most commonly cultivated is the common guava, (*Psidium guajava* L.) and the other cultivated species include the Cattely guava or Strawberry guava (*P. cattleianum* Sabine), the Brazilian guava (*P. guineense* Sw.) and Costa Rician guava or Chinese guava (*P. friedrichsthalianum* Ndz.). The other species of *Psidium* are utilized for regulation of vigour, bearing programme, fruit quality improvement and resistance to pest and diseases (Morton, 1987). The chromosomal number of this genus is 2n = 22 (Nakasone and Paull, 1998) and all these species might share a common gene pool (Prakash *et al*., 2002).

The SPAR (Single Primer Amplification Reaction) method offers a simple and economical means of genotype characterization. Many horticulturally important fruit crops including some guava varieties (Prakash *et al*., 2002; Dahiya *et al*., 2002; Rueda *et al*., 2006; Chen *et al*., 2007, Feria-Romero *et al.* 2009), its few species (Prakash *et al*., 2002; Sharma *et al*., 2007) and its related species *Feijoa sellowiana* (Dettori and Polambi, 2000) have been characterized using RAPD markers. The ISSRs help to identify closely related cultivars and to study evolutionary processes and phylogenetic studies (Prevost and Wilkinson, 1999; Raina *et al*., 2001). This marker system provides reproducible results that generate abundant polymorphism (Tsumura *et al*., 1996).

Lot of confusion exists within *Psidium* species, as the different literatures arrange them in different order and the synonyms used in the nomenclature makes it more confusing, therefore, molecular marker assessed identification of duplicates in the germplasm is essential for the maintenance, commercialization and conservation of this genus. The plant genetic resource is one of the most valuable assets available to the mankind, their protection and conservation is of great importance for the future generation. This study focuses on assessing the genetic variability among the *Psidium* species and establishment of the genetic relationships between them.

Material and Methods

Morphological characterization of plant samples

Two trees of each species, which received similar cultural treatments and matured trees, were selected for the field study and sample collection. Four uniform, healthy branches were tagged in all the four directions from each selected trees. The important parameters like height of the plant, mode of branching, stem form and shape; leaf arrangement, shape, size, colour and venation, the colour and morphology of the young shoots and leaves were compared within the samples as suggested by Melvilles (1960). Identification of the samples was done with the help of taxonomic literatures and herbarium and inter comparison was done within the samples, from different sites for clarification of species. The collected samples are listed in Table 1.

Table 1: List of *Psidium* species under study

Sample No.	Species
1	*Psidium araca*
2	*Psidium* sp. (unknown species – 'Bracilica')
3	*Psidium cattleianum* Sabine
4	*Psidium chinense* (Pink fleshed)
5	*Psidium chinense* (White fleshed)
6	*Psidium friedrichsthalianum*
7	*Feijoa sellowiana* (related genus of *Psidium*)
8	*Psidium guineense*
9	*Psidium guajava* (Safeda)
10	*Psidium guajava* (Sardar)
11	*Psidium guajava* (Gelli type)
12	*Psidium cattleianum* var. lucidium
13	*Psidium molle*
14	*Psidium polycarpon*
15	*Psidium cattleianum* var. longipes

Genomic DNA isolation

Young matured leaves were harvested from the orchard, washed free of dirt and mopped dried. The leaves were transported from the collection site in ice; samples were sealed in poly bags and labeled. In laboratory the leaves where de-ribbed and powdered rapidly in liquid nitrogen and either the DNA isolation procedures were immediately followed or the powdered tissue was stored in -80°C till further use. Genomic DNA was isolated using the modified protocol of Porebski *et al*. (1997). Five gram of liquid nitrogen powered leaf tissues were transferred into pre-warmed (65^{0}C) sterile DNA extraction buffer (3% CTAB, 100 Mm Tris pH 8.0, 20 Mm EDTA pH 8.0, 1.4 M NaCl) in the centrifuge tube, to it 2% PVP (Polyvinylpyrrolidone) and 1.5% 2-mercaptoethanol were added. The mixture was incubated for 1 hour with interval mixing of 15 minutes at 65^{0}C. After incubation, the homogenate was cooled to room temperature and equal volume of Chloroform: Isoamyl alcohol (24:1 v/v) solution was added and emulsified gently for 15 minutes. The homogenate was centrifuged at 15000 rpm for 20 min at room temperature and the supernatant was collected. This step was repeated at least thrice till it become a clear supernatant. To the aqueous phase, equal volume of ice cold propanol was added to the precipitated DNA. The mixture was stored overnight at -20^{0}C to accentuate the precipitation and laterspun at 8000 rpm for 20 min at 4^{0}C to pellet the DNA. The DNA pellet was washed with 70% ethanol and air dried. The dried pellet was dissolved in 500 µl of TE buffer (10 Mm Tris HCl pH 8.0, 1 Mm EDTA pH 8.0). The undissolved compounds were removed by incubating at 50^{0}C for 5 minutes followed by centrifugation at 7000 rpm for 5 min. The aqueous phase was transferred to a fresh tube. The isolated DNA was purified using the standard protocol (Sambrook *et al*., 1989) and stored in TE buffer at 4^{0}C for further experimentation.

SPAR analysis: Based on the pre-screening 16 RAPD primers (Table 2) were selected for RAPD (Williams *et al*., 1990). The amplification conditions with initial denaturation at 94°C for 4 min was continued with 45 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 1 min and extension at 72°C for 2 min, which was followed by a final extension at 72°C for 5 min., were performed with the final volume of 25 µl PCR reaction mixture (PCR buffer1x; dNTPs, 215 µM each; $MgCl_2$, 2 mM; Primer, 5 pmoles; *Taq* DNA polymerase, 1 U; DNA template, 25 ηg) overlaid with mineral oil. The amplified fragments were separated on 1.5% Agarose gel containing ethidium bromide (0.5µg per ml) at 70 V for 3-4 hours in 0.5X Tris Borate EDTA buffer. After completing the run, the gel was documented. The ISSR analysis was based on the method of Zietkiewicz *et al*. (1994), 31 UBC ISSR primers (Table 3) were used for amplification reactions with a final volume of 25 µl PCR reaction mixture (PCR buffer1x; dNTPs, 200 µM each; $MgCl_2$, 2 Mm; Primer, 0.2 µM; *Taq* DNA polymerase, 0.5 U, DNA template, 25 ηg), overlaid with mineral oil. The amplification was programmed for 35 cycles as denaturation at 94°C for 30 sec, annealing at 42°C for 30 sec, extension at 72°C for 2 min and a final extension at 72°C for 10 min. The annealing temperature was in the range of 30 to 65^{0}C depending on GC content and length of the primers. Amplicons were checked by separating on 2% metaphor agarose gel electrophoresis for 3 to 4 hour at 70V in 1x TBE running buffer. Finally the gel was stained with ethidium bromide (0.5 µg/ml), and documented.

All the PCR reactions were carried out in a DNA Thermocycler (Applied biosystems, U.S.A.) and repeated at least twice to check the reproducibility. Each PCR was conducted as an experiment, with controls (distilled water instead of template DNA) to test the purity and viability of reagents (Elrich *et al*., 1991).

Table 2: Detail information of RAPD primers

Primers	Amplicons	Number of Polymorphic bands	Percentage of polymorphism	Fragment size (bp)
OPA – 05	11	07	63.63	2500-300
OPA – 13	09	09	100	2500-700
OPB – 01	10	05	50	3000-400
OPB – 05	12	09	75	2500-400
OPB – 09	16	11	68.75	2200-400

OPB – 11	07	07	100	2000-900
OPB – 17	09	08	88.88	2000-600
OPC – 05	13	09	69.23	3000-400
OPD – 18	08	07	87.5	3000-300
OPE – 13	21	15	71.43	1200-600
OPF – 02	11	09	81.81	2500-400
OPG – 06	08	05	62.5	2500-500
OPH – 19	07	05	71.43	2000-600
OPJ – 01	09	07	77.78	2000-600
OPJ – 14	12	12	100	2500-400
OPJ – 20	11	09	81.81	2500-300

Table 3: Detail information of ISSR primers

Primers	Amplicons	Number of polymorphic bands	Percentage polymorphism	Fragment size
Dinucleotides				
UBC-807	13	07	53.85	3000-300
UBC-811	12	12	100	3000-500
UBC-812	07	07	100	3000-1200
UBC-813	10	09	90	3000-700
UBC-814	09	08	88.88	3000-700
UBC-815	10	06	60	3000-600
UBC-816	12	09	75	3000-500
UBC-817	04	03	75	2000-900
UBC-818	07	04	57.12	2000-700
UBC-822	13	09	69.23	3000-600
UBC-824	12	08	66.67	3000-450
UBC-825	09	09	100	2000-500
UBC-827	08	07	87.5	2500-600
UBC-829	07	05	71.43	3000-700
UBC-830	05	03	60	3000-400
UBC-834	10	08	80	3000-800
UBC-835	04	03	75	3000-1000
UBC-840	13	13	100	2500-350
UBC-842	11	10	90.91	2000-350
UBC-852	07	05	71.43	3000-700
UBC-887	10	07	70	3000-600
UBC-890	09	07	77.78	2000-200
UBC-891	12	10	83.33	3000-400
Trinucleotides				
UBC-864	13	10	76.92	3000-800
UBC-865	07	06	85.71	3500-1000
UBC-866	09	07	77.78	2000-500
UBC-867	10	09	90	3000-700
Tetranucleotides				
UBC 873	08	06	75	2500-800
UBC 878	10	07	70	3000-600
Pentanucleotide				
UBC-880	12	11	91.67	3000-400
UBC-881	11	09	81.82	2500-400

Analysis of PCR Data

Clear and well-marked bands were coded in a binary form by denoting '1' and '0' for presence and absence of bands respectively in each genotype and these data were used as input for further calculations. In order to describe the genetic diversity among the different species of the genus *Psidium*, RAPD and ISSR band data were used to estimate genetic distances, based on Jaccard's similarity coefficient (Jaccard, 1908). The Jaccard's similarity coefficients were calculated and used to construct dendrograms based on unweighted pair group method with arithmetic mean algorithm (UPGMA) using SAHN cluster analysis of NTSYS-pc version 2.0 (Rohlf, 1992).

Result and Discussion

Morphological characterization

P. araca and *P. cattleianum* var. lucidium are morphologically similar, in the leaf structure, texture, size, habitat and mode of branching. The colour of bark is slightly varies between them, meanwhile the leaf colour in the young stage is different, in case of *P. araca* it is light reddish but the *P. cattleianum* var. lucidium got light green young leaves. *P. araca* got ovate leaf shape whereas *P. cattleianum* var. lucidium has elliptical to oval oblong leaf shape. *P. araca* leaves are bigger in size compared to *P. cattleianum* var. lucidium (Bailey, 1941; Hirano and Nakasone 1969). It is a characteristic feature for both the species that the waxy and fleshy leaves which make them distinguishable from rest of the members in the genus *Psidium*. Flowering is not very common for both the species in Uttar Pradesh. *P. friedrichsthalianum* a tree in habit got little similarity with the above two species in leaf morphology. *P. friedrichsthalianum* too have waxy leaf but they are not fleshy, the leaf edge is pointed which are the most distinguishable characteristic of this species. The wood and the branching pattern are similar to *P. guajava. P. friedrichsthalianum* is a small tree of about 7-10 m height. The branches are slender and smooth. Leaves are oval or oblong/oval, smooth glossy above and pubescent below. Fruits are globose, small and sour (Bailey, 1941; Dinesh and Iyer, 2005). The petiole of *P. friedrichsthalianum* and *P. guajava* are the longest among the all *Psidium* species studied. 'Bracilica', the unknown species have similarity to *P. friedrichsthalianum* and *P. guajava* in their stem morphology but the 'Bracilica' possesses drooping branching pattern and is similar to *P. guajava*. There is no literature available to verify this species. *P. guajava* L. (common guava) is the most cultivated crop in the genus *Psidium*. In India, the common guava has two base lines viz., Allahabad safeda and Lucknow 49 (sardar). Morpholgicaly they look alike but sardar guava got wider leaf than Allahabad safeda. The fruit size is smaller in Allahabad safeda than in sardar. The fruit skin of Allahabad safeda is light green with yellowish colour when matured where as the fruit colour of sardar is green. Sardar guava used to have red spots in the fruit skin when ripe but the Allahabad safeda lacks it. Allahabad safeda has drooping branching pattern but sardar has a spreading habit with upright growth, grown generally irregular (Mathew and Shanker, 1963). Both the cultivar is naturally propagated through seedlings so the genetic purity is less. The selection is truly based on the basic morphology. Another cultivar of *P. guajava* viz., the 'Gelli type' guava differs in the morphological characters from Allahabad safeda and sardar. i.e., the 'Gelli type' guava possesses a thick dome shaped appearance. The leaves are dark green while the other cultivars have light/off green. The fruits of 'Gelli type' guava got orange flesh while the other cultivars have white to cream flesh. *P. polycarpon* is similar to Allahabad safeda and sardar. The major differences observed in *P. polycarpon* are the drooping habit of branches and the pear shaped fruits (Bailey, 1941; Hayes, 1953; Dinesh and Iyer, 2005). *P. chinense* is a bushy shrub with small leaves which differ from rest the species in its morphology they got thick dark green foliage. Leaf and fruit are small in size. The fruit of *P. chinense* is similar to fruits of *P. cattleianum. P. chinense* is a synonym of *P. littorale* Raddi (Snow, 2001). The morphological characteristics of all *Psidium* species under study are categorized in Table 4a and Table 4b and the comparison of leaf structure is shown in Figure 1.

P. cattleianum Sabine and *P. cattleianum* var. longipes are small tree or shrub with smooth bark. Leaves are obovate, elliptic and glabrous. Fruits are obovate to round with purplish red colour in the former and light yellow colour in the latter. Fruit skin is thin, with soft flesh and has numerous seeds. It has sweet flavour and good aroma. It is also known as strawberry guava because of the sweet aroma reminiscent of strawberry and lacks the muskiness of common guava (Dinesh and Iyer, 2005). The differences of the both the genotypes are in its habit and in the fruit colour. *P. cattleianum* Sabine have red fruit skin while the other genotype got yellow fruit skin. *P. cattleianum* var. longipes grows taller and it is a small tree whereas *P. cattleianum* Sabine is a shrub. *P. guineense* is known as brazilian guava. It is a shrub or small tree. It differs from *P. guajava* in having four angled branchlet, leaf structure and drooping branches (Bailey 1941; Harold *et al*., 1953; Seth, 1962). It has similarity with *P. cattleianum* Sabine and *P. cattleianum* var. longipes in leaf structure. It has a thick growth, highly branching habitat and wider leaves. *P. guineense* blooms but fruiting is not common in Uttar Pradesh. They got a thick flowering in branches. *P. molle* having morphological similarities with *P. cattleianum* Sabine, *P. cattleianum* var. longipes and *P. guineense.* It is a shrub or small tree with dome shaped appearance. The leaves are obovate and leathery. The lower surface of the leaves is reddish and velvety. Fruits are pale yellow in colour when fully ripe. Flesh is white with many seeds and has acidic flavour (Bailey, 1950; Uphof, 1957; Dinesh and Iyer, 2005). One related genus of *Psidium* viz., *Feijoa sellowiana* has morphological similarity to *P. guineense* and *P. cattleianum*. Recently the *Feijoa sellowiana* has been renamed *Acca sellowiana*, but most of the sources still use the older name. It is commonly known as pineapple guava. The leaves are rough with acute apex and stiff. It has a dome shaped appearance with drooping branches and touches the ground.

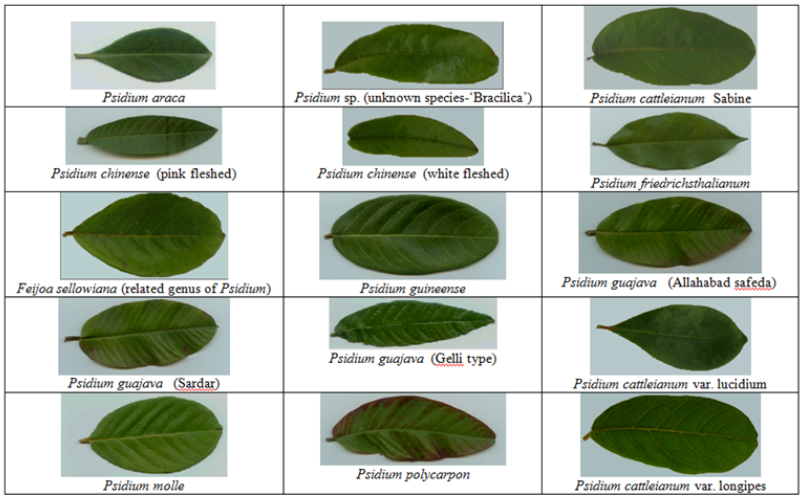

Figure 1: Leaves of different species of the genus *Psidium* and *Feijoa*

Table 4a: Morphological characteristics of different *Psidium* species

S. No	Species	Tree shape	Average height	Petiole		Leaf color		Leaf shape	Average size of leaf	
				Type	Length (cm)	Young stage	Mature stage		Length (cm)	Breadth (cm)
1	*Psidium araca*	Domed shaped bushy	3.25	Slightly grooved	0.86	Light reddish green	Dark green	Ovate	8.0	4.3
2	*Psidium* sp. (unknown species – 'Bracilica')	Domed shaped bushy	4.86	Slightly grooved	0.90	Light green	Light Polo green	Lanceolate	13.3	5.1
3	*P. cattleianum* Sabine	Domed shaped shrubby	4.8	Slightly grooved	0.85	Pony brown	Dark green	Ovate	7.4	3.4
4	*P. chinense* (Pink fleshed)	Domed shaped bushy	6.13	Adaxially grooved	0.65	Light green	Dark green	Oblong	4.2	1.5
5	*P. chinense* (White fleshed)	Domed shaped bushy	6.25	Adaxially grooved	0.67	Light green	Dark green	Oblong	4.7	1.5
6	*P. friedrichsthalianum*	Domed shaped bushy	4.87	Slightly grooved	0.94	Brown	Dark green	Lanceolate	8.4	3.4
7	*Feijoa sellowiana* (related species of *Psidium*)	Domed shaped bushy	7.9	Slightly grooved	0.88	Light green	Polo green	Ovate	6.8	6.2
8	*P. guineense*	Domed shaped bushy	4.8	Adaxially grooved	0.85	Light green	Polo green	Oblong to elliptical	11.5	5.3
9	*P. guajava* (Allahabad safeda)	Domed shaped spreading	6.51	Slightly grooved	0.94	Light green	Polo green	Elliptical to oval oblong	7.5	4.2
10	*P. guajava* (Sardar)	Domed shaped spreading	4.85	Slightly grooved	0.84	Light green	Polo green	Elliptical to oval oblong	6.3	3.9
11	*P. guajava* (Gelli type)	Domed shaped shrubby	3.2	Slightly grooved	0.79	Light green	dark green	Elliptical to oval oblong	5.4	2.7
12	*P. cattleianum* var. lucidium	Domed shaped		Slightly	0.92	Light reddish	Dark	Ovate	9.4	4.4

		bushy	3.0	grooved		green	green			
13	*P. molle*	Domed shaped bushy	6.5	Adaxially grooved	0.95	Dark green	Dull green	Ovate	13.2	7.4
14	*P. polycarpon*	Domed shaped spreading	5.8	Slightly grooved	0.65	Light green	Polo green	Elliptical to oval oblong	9.1	4.2
15	*P. cattleianum* var. longipes	Domed shaped shrubby	5.3	Slightly grooved	0.85	Light green	Polo green	Oblong to elliptical	11.9	5.5

Table 4b: Morphological characteristics of leaves of different *Psidium* species

S. No	Species	Apex	Margin	No. of veins/leaf	Texture	Phyllotaxy	Leaf surface	
							Dorsal	Ventral
1	*Psidium araca*	Acute	Entire	14-18	Coriaceous	Opposite decussate	Smooth, shiny surface with depressed veins	Smooth and prominent midrib
2	*Psidium* sp. (unknown species – 'Bracilica')	Acuminate	Entire	18-22	Glabrous	Opposite decussate	Smooth, depressed vein and mid rib	Rough with prominent midrib and veins
3	*P. cattleianum* Sabine	Mucronate	Entire	17-23	Glabrous	Opposite decussate	Smooth, depressed vein and mid rib	Rough with prominent midrib
4	*P. chinense* (Pink fleshed)	Acuminate	Entire	18-20	Glabrous	Opposite decussate	Smooth, depressed vein and mid rib	Puberulous with prominent veins
5	*P. chinense* (White fleshed)	Acuminate	Entire	18-19	Glabrous	Opposite decussate	Glabrous, depressed vein and mid rib	Puberulous with prominent veins
6	*P. friedrichsthalianum*	Acuminate	Entire	22-24	Glabrous	Opposite decussate	smooth, glossy, shiny, surface with oil glands	Pubescent with prominent midrib
7	*Feijoa sellowiana* (related species of *Psidium*)	Acute	Entire	23-26	Glabrous	Opposite decussate	Glabrous, depressed vein and mid rib	Rough with prominent midrib
8	*P. guineense*	Acute	Entire	18-19	Glabrous	Opposite decussate	Glabrous, depressed vein and mid rib	Rough with prominent midrib and veins

S. No	Species	Apex	Margin	No. of veins/leaf	Texture	Phyllotaxy	Leaf surface	
							Dorsal	Ventral
9	*P. guajava* (Allahabad Safeda)	Obtuse	Entire	28-30	Glabrous	Opposite decussate	Glabrous, depressed vein and mid rib	Puberulous with prominent veins
10	*P. guajava* (Sardar)	Obtuse	Entire	27-29	Glabrous	Opposite decussate	Glabrous, depressed vein and mid rib	Puberulous with prominent veins
11	*P. guajava* (Gelli type)	Obtuse	Entire	26-29	Glabrous	Opposite decussate	smooth, depressed vein and mid rib	Puberulous with prominent veins
12	*P. cattleianum* var. lucidium	Acute	Entire	16-19	Coriaceous	Opposite decussate	Smooth, shiny surface with depressed veins	Smooth and prominent midrib
13	*P. molle*	Mucronate	Entire	15-19	Glabrous	Opposite	Glabrous, depressed	Rough with

						decussate	vein and mid rib	prominent midrib and veins
14	*P. polycarpon*	Obtuse	Entire	30-32	Glabrous	Opposite decussate	smooth, depressed vein and mid rib	Puberulous with prominent veins
15	*P. cattleianum* var. longipes	Mucronate	Entire	17-21	Glabrous	Opposite decussate	Glabrous, depressed vein and mid rib	Rough with prominent midrib and veins

Evaluation of RAPD primers

The amplification products of 16 RAPD primers with the 15 samples were 174 scorable bands, out of which 134 were polymorphic (Table 2). The size of amplification products were ranged from 3000 to 300 bp. The number of amplified fragments varied from 7 to 21 with an average of 14 fragments per primer and the size ranged from 300 to 3000 bp of 77%. The present investigation shows more or less similarity with the works of Dahiya *et al*. (2002) on 9 RAPD primers producing 133 bands ranging from 300 bp to 3000bp in size with 74.7% polymorphic bands, Prakash *et al*. (2002) with 8 informative RAPD primers and produced 93 amplified polymorphic fragments of size ranged from 100 to 3000 bp with an average of 11.2 fragment per primer. Chen *et al.* (2007) analyzed *Psidium guajava* L. from indigenous tribes of Taiwan using 4 RAPD primers as suggested by Prakash *et al*. (2002) amplified 82 polymorphic RAPD patterns. RAPD analysis among *Psidium* species by Sharma *et al*. (2007) scored 347 bands as polymorphic accounting to 92.29 % polymorphism. It was stated that this high level of polymorphism is due to the fact that guava possess a mating system of out group. The present study reveals 100 % polymorphism with the RAPD primers viz., OPA-13, OPB-11 and OPJ-14. Dahiya *et al* . (2002) reported 100% polymorphism in OPA-13.

The grouping based on the genetic diversity values resulted in the formation of two clusters i.e., Cluster 1 with 14 *Psidium* species and Cluster 2 with one related genus of *Psidium* viz., *Feijoa sellowiana* (Figure 2). The average similarity index value observed between Cluster-1 and Cluster-2 was 0.48, indicating that *F. sellowiana* is genetically differs from the genus *Psidium*. The cluster 1 was again differentiated into 2 sub clusters 1a and 1b. The sub cluster 1a contains all the *Psidium* species expect the unknown genotype 'Bracilica' which was out grouped as 1b. The average similarity index observed in the RAPD dendrogram ranges from 0.94 to 0.48.

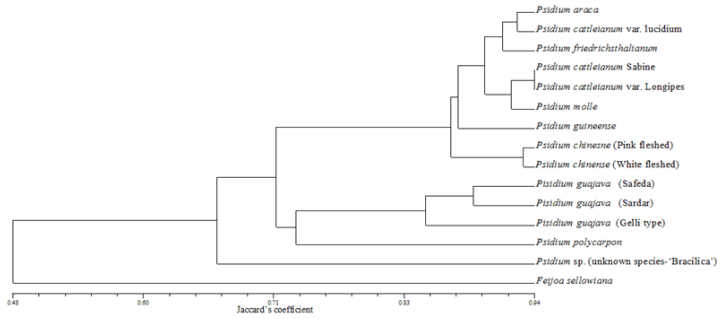

Figure 2: Dendrogram based on genetic similarities among different species of *Psidium* using RAPD primers

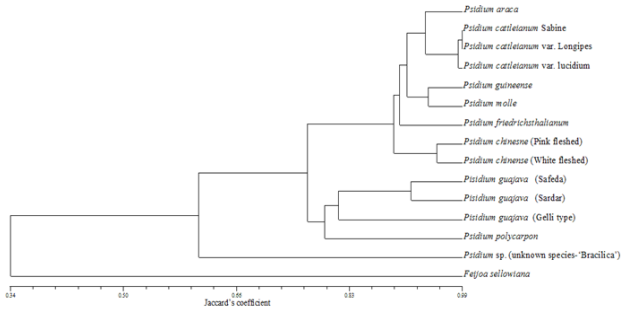

Figure 3: Dendrogram based on genetic similarities among different *Psidium* species using ISSR primers

Evaluation of ISSR primers

31 ISSR primers amplified a total of 294 bands in size ranged from 3000 bp to 300 bp and out of which 81.63% bands were polymorphic (Table 3). These primers showed variation in the percentage of polymorphism ranged from 100 to 57. The primers viz., UBC 811, 812, 825 and 840 revealed the highest polymorphism (100%) while primer UBC 818 exhibited the lowest polymorphism (57.12%). UBC 807, 822, 840 and 864 showed the highest number of amplicons (13) and UBC 817 and UBC 835 showed the lowest amplicon number (04). The dendrogram clearly indicates two major Clusters (i.e., Cluster-1 with 14 *Psidium* species and Cluster-2 with one related genus of *Psidium* viz., *Feijoa sellowiana*) (Figure 3). A similarity index of 0.34 was observed for *Feijoa sellowiana* indicating that this species is an out group to the genus *Psidium*. The average similarity index observed in the ISSR dendrogram ranges from 0.99 to 0.34.

SPAR markers in diversity assessment

The 16 RAPD primers yielded an average of 10.88 bands per primer while 31 ISSR primers yielded an average of 9.48 bands per primer. The average number of polymorphic bands per primer was higher in case of RAPD (8.38) as compared to that in ISSR (7.74). The average value of band informativeness considering all RAPD primers together and ISSR primers together was higher for RAPD, i.e., 0.86 (RAPD) and 0.79 (ISSR). The polymorphic ISSR primers had higher Rp (3.8) than that of polymorphic RAPD primers (3.3). ISSR marker obtained also a higher value (5.8) of Maker Index (MI) than that of RAPD primers (4.4), indicating greater potential of ISSR markers. The distance matrices obtained using Dice's coefficient analysis was compared using correlation analysis showed a positive correlation (r = 0.95).

Conclusion

The use of Taxonomical classification and SPAR markers in the identification and characterization of different *Psidium* species would be of considerable help to the future guava breeding program particularly in choosing the potential parental genotypes for crosses and to optimize germplasm management to maximize the diversity. The development of disease resistance traits, especially the wilt disease is one of the focused areas in guava research. These markers could be employed for detecting many of the horticulturally important traits including disease resistance, fruit bearing, high nutritional values, fruit quality, etc and would increase the efficiency and precision of breeding.

Acknowledgements

The authors gratefully acknowledge the financial assistance provided by the Department of Biotechnology (Government of India), New Delhi. We also greatly appreciate to Horticultural Experiment and Training Center, Basti, India, for their support and help in collection and identification the samples.

Reference

Bailey, L. H. (1941). A concise dictionary of gardening and general horticulture and cultivated plants in North America. Hortus Second. The Mac Millan Co. New York, 604.

Bailey, L. H. (1950). The standard encyclopedia of horticulture. The Mac Millan Co. New York, 3: 2847-2849.

Balasaravanan, T., Chezhian, P., Kamalakannan, R., Ghose, M., Yasodha, R., Varghese, M., Gurumurthi, K. (2005). Determination of inter and intra species genetic relationships among six *Eucalyptus* species based on Inter Simple Sequence Repeat (ISSR). *Tree Physiology* 25: 1295-1302.

Chen, T., Ng, C., Wang, C., Shyu, Y. (2007). Molecular Identification and Analysis of *Psidium guajava* L from Indigenous Tribes of Taiwan. *Journal of Food and Drug Analysis* 15 (1): 82-88.

Dahiya, K. K., Sunil, A., Karihaloo, J. K. (2002). DNA fingerprinting of guava (*Psidium guajava* L.) cultivars using RAPD markers. *Indian Journal of Plant Genetic Resource* 15(2):112-115.

Dass, H. C., Prakash, D. (1981). Phylogenetic affinities in *Psidium* spp. as studied by flavonoid patterns. *National Symposium on Tropical and Sub-tropical fruit crops,* Bangalore, India.

Dettori, M. T., Palombi, M. A. (2000). Identification of *Feijoa sellowi*ana Berg. accessions by RAPD markers. *Scientia Horticulturae.* 86: 279-290.

Dinesh, M. R., Iyer, C. P. A. (2005). Significant research achievements in Guava-improvement and future needs. 1st IGS, India. 7-16

Elrich, H. A., Gelfand, D., Sninsky, Y. Y. (1991). Recent advances in the polymerase chain reaction. *Science*, 252: 1643-1650.

Feria-Romero IA, Astudillo-Dela HV, Chavez-soto MA, Rivera-arce E, Lopez M, Serrano H and Lozoya X (2009) RAPD markers associated with quercetin accumulation in Psidium guajava. Bio Plant 53:125–128

Grattapaglia, D., Bradshaw, H. (1994). Nuclear DNA content of commercially important *Eucalyptus* species and hybrids. *Canadian Journal of Forest Research,* 24: 1074-1078.

Harold, M., Toy, L. R., Wolf, H. S. (1953). Miscellaneous tropical and subtropical florida fruits. Extn. Service. Gainesville, Florida. 91-94.

Hayes, W. B. (1953). Fruit Growing in India, Kitabistan, Allahabad.

Hirano, R. T., Nakasone, H. Y. (1969). Chromosome number of ten species and clones in genus *Psidium*. *Journal of the American Society for Horticultural Science,* 94: 83-86.

Jaccard, P. (1908). Etude comparative dela distribution florale dans une portion des Alpes et des jura. *Bulletin de la Societe Vaudoise des Sciences Naturelles,* 37: 547-579.

Jaiswal, V. S., Amin, M. N. (1992). Guava and Jacckfruit. In: Biotechnology of perennial fruit crops. (eds. F.A. Hamerschag and R.E. Litz). CAB International and Cambridge University Press, Cambridge, U.K. pp. 421-431.

Mathew, I. P., Shanker, G. (1963). Pomological description of important Guava varieties of U.P. *Allahabad Farmer* 37 (6): 27-32.

Melvilles, R. (1960). A metrical study of leaf shape in hybrid. I. the leaf shape of some F1 Hybrids and their parents. *Kew Bull*. 14: 88-102.

Menzel, C. M. (1985). Guava: an exotic fruit with potential in Queensland. *Queensland Agricultural Journal,* 111: 93-98.

Morton, J. F. (1987). In: Fruits of warm climates. (eds. J.F. Morton, F.L. Miami) Guava. pp 356–363.

Nakasone, H. Y., Paull, R. E. (1998). Tropical Fruits. CAB International, Wallingford, UK.

Pathak, R. K., Ojha, C. M. (1993). Genetic resources of guava. In: Advances in Horticulture (Vol III). (eds. K.L. Chadha and O.P. Pareek). Malhotra Publishing House, New Delhi, India pp 143-147.

Porebski, S. L., Bailey. G., Baum, R. B. (1997). Modification of CTAB DNA extraction protocol for plant containing high polysaccharides and polyphenol components. *Plant Molecular Biology Reporter,* 5: 8-15.

Prakash, D. H., Narayanaswamy, P., Sondur, S. N. (2002). Analysis of molecular diversity in guava using RAPD markers. *The Journal of Horticultural Science and Biotechnology,* 77(3): 287-293.

Prevost, A., Wilkinson M. J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Appied Genetics,* 98: 107-112.

Raina, S. N., Rani, V., Kojima, T., Ogihara, Y., Singh, K. P., Devarumath, R. M. (2001). RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationship in peanut (*Arachis hypogaea*) cultivars and wild species. *Genome* 44: 763-772.

Risterucci, A. M., Duval, M. F., Rohde, W., Billotte, N. (2005). Isolation and characterization of microsatellite loci from *Psidium guajava* L. *Molecular Ecology Notes* 5 (4): 745-748.

Rodriguez, N. N., Valdes-Infante, J., Becker, D., Velazquez, B., Gonzalez, G., Sourd, D., Rodriguez, J., Billotte, N., Risterucci, A. M., Ritter, E., 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 Horticulturae* 735: 201-215.

Rohlf, F. J. (1992). NTSYSpc. Numerical Taxonomy and Multivariate Analysis System, Version 2.0. State University of New York, Stony Brook, N.Y.

Rueda, A., Palacio, J. D., Munoz, J. E., Saavedra, R., Baravo, E. (2006). Caracterizacion molecular del banco de germoplasma de guayaba *Psidium* spp. Del centro de investigacion corpoica-palmira. *Fitotecnia Colombiana* 6 (2): 26-32.

Sambrook, J., Fritsch, E. F., Maniatis, T. (1989). In: Molecular Coloning - A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Seth, J. N. (1962). Floral biological studies in *Psidium.* II. Anthesis and dehiscence of anther, pollen studies, stigma receptivity fertilization and fruit setting. *Horticulture* (*Advanced*), 6: 110-136.

Sharma, A. S. Sehrawat, S. K., Singhrot, R. S., Boora, K. S. (2007). Assessment of genetic diversity and diversity relation ship among *Psidium* spp. through RAPD analysis. *Acta Horticulturae* 735: 71-77.

Tsumura, Y., Ohba, K., Strauss, S. H. (1996). Diversity and inheritance of Inter-Simple Sequence Repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and Sugi (*Cryptomeria japonica*). *Theoretical and Applied Genetics* 92: 40-45.

Uphof, J. C. (1957). Dictionary of economic plants. Weinhain (Brthsstrase) Publishing Co. Codicote, Wheldon and Weslay Ltd. pp. 298.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* 18: 6531-6565.

Zietkiewicz, E., Rafalski, A., Labuda, D. (1994). Genome fingerprinting by Simple Sequence Repeat (SSR) anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.