Identification of suitable parents for the development of populations for mapping genomic regions controlling commercially favourable pomological traits in guava (*Psidium. guajava*)

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Abstract

Developing mapping populations to identify DNA markers linked to economically important traits in crop plants with no exception, requires identification of genotypes contrasting for target traits and for a larger number of DNA markers. Putative parents contrasting for pomological traits as well as at several SSR markers were identified from among the 52 guava accessions being maintained at Indian Institute of Horticultural Research (IIHR), Bengaluru, India. The pairs of accessions namely, Benaras and Seedless, Dhareedar and Seedless, Dhareedar and EC-147039, Abu Ishaqwala and Pati for fruit weight, Benaras and CIW1, Benaras and Surkha Chitti Neptuani, Sringeri Seedless and Surkha Chitti Neptuani for outer pulp thickness, Allahabad Safeda and CIW1, Hisar Safeda and Local 4, Hisar Safeda and CIW1, for total soluble solids and 9-35EC147036 and Florida Seedling, 9-35 EC-147036 and Arka Mridula, GR1 and Hisar Safeda, GR1and Florida Seedling for seed hardness were polymorphic at more number of SSR loci.

These contrasting pairs of accessions are suggested for use as parents in developing mapping populations which can be utilized to identify SSR markers linked to genomic regions controlling economically important pomological characters in guava. Additionally, ten different SSR markers that can be used to distinguish five best performing guava accessions with respect to fruit weight have been identified.

Keywords: Guava, SSR markers, Putative parents, ANOVA, Barcoding.

Introduction

The inheritance pattern of most of the economically important traits in crop plants is controlled by several genes, with large genotype × environment interaction[2]. The loci contributing to the expression of these traits are known as Quantitative Trait Loci (QTL). A prior knowledge on the location and mode of action of QTL's is a pre-requisite (among others) for their manipulation to improve crop productivity. The process of detection of chromosomal location and size effect estimation of QTL using DNA markers is called QTL mapping[5]. QTL mapping involves detection of statistical testing of significance of marker class traits means, in population derived from parents contrasting for target traits as well as for a large number of DNA markers. Thus, identification of genotypes most contrasting for target traits and for a large number of DNA markers is an initial step for QTL mapping.

Due to the lack of knowledge on DNA markers linked to various economically important traits in guava, marker assisted breeding is still in its infancy. SSR's are the markers of choice for genetic mapping and molecular breeding[10]. However, in many cases, parents contrasting for target traits might not be polymorphic for the DNA markers. Therefore, the present study aimed at identifying putative accessions contrasting for target traits and a set of SSR markers that can be used as parents for developing mapping populations in guava and to barcode the five best performing accessions with respect to fruit weight, utilizing the allelic data of 10 SSR markers.

Material and Methods

Plant material and experimental field design: The material consisted of a total number of 52 guava accessions, diverse for various pomological characters viz. Fruit weight (FW), Fruit length (FL), Sepal size (SS), Diameter calyx cavity (DCC), Stalk length (SL), Outer pulp thickness (OPT), Core diameter (CD), Total Soluble Solids (TSS), No. of seeds (NS), 100 Seed weight (100SW), Seed Hardness (SH) and Outer Pulp Thickness (OPT) (Table 1). These accessions are being maintained at the ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India which is located at 13º 58[1] latitude North and 78º longitude East and at an altitude of 890 meters above mean sea level. Soil is red sandy loam with pH of 5.2- 6.4. The accessions are being maintained in four replicates in randomized complete block design with a row spacing of 6x6 mts. The age of the plants ranged between 15- 18 years.

Sampling of plants and collection of data on pomological traits: The morphological data on pomological traits viz. FW, FL, SS, DCC , SL, OPT, CD,

TSS, NS, 100SW and SH were collected on five fruits, chosen randomly from each of the four replicates of each accession in the month of August for two successive years 2012 and 2013. All the traits related to weights were expressed in grams using weighing balance, lengths in centimeter by Vernier Callipers, seed hardness by seed hardness meter in kg/m^2, TSS was calculated in ^{0} Brix by using a hand refractometer and number of seeds was counted manually.

Assessment of SSR markers- based polymorphism among 55 accessions: DNA was extracted from freshly emerged leaves of all the 52 accessions with a slight modification to the CTAB method described by Kanupriya et al[4]. The quality of the genomic DNA was checked on 0.8% agarose gel followed by quantification by Gene Quant UV- Spectrophotometer (GE Health Care Bio-sciences Ltd, England). The 52 accessions were genotyped using a total of 220 guava specific SSR markers developed by Risterucci et al[7] and Risterucci et al[8]. From among these 135 polymorphic SSR markers were identified (Table 6). Of these, eighty markers were based on di-nucleotides repeat motifs. Amplification of the microsatellite priming regions of the 52 accessions was performed by a polymerase chain reaction with *Taq* DNA polymerase that supports the *in vitro* replication.

Amplification of the priming regions of the template DNA was performed by using PCR program[7] at 94^{o}C for 4mins; 30 cycles of 94^{o}C for 45sec, 55^{o}C for 1min, 72^{o}C for 1min and 8 mins final extension at 72^{o}C. A total volume of 20µl of PCR reaction mixture was prepared which constituted of 1X buffer 10mM Tris HCl of pH =8, 50mM KCl, 1.5mM $MgCl_2$), 0.5µM of each primer, 200µM of dNTPs, 0.5U of *Taq* DNA polymerase (Genei, Bangalore) and 50ng per tube6. The PCR products were mixed with Bromophenol blue dye and loaded on a 3% (3B Black Bio Biotech India Ltd) agarose gel mixed with ethidium Bromide, along with a 100bp ladder (Fermentas). The electrophoretic run was performed at 75V for 150-180 minutes. The Amplicons were visualized using a UV trans-illuminator and gels were documented by UVIPRO platinum gel documentation unit.

Scoring of the SSR marker data: Amplified size of the alleles was estimated using UVIpro Platinum software in comparison with a 100bp ladder. The missing alleles were repeated with the PCR carried out for second time to confirm if absence of amplification was a PCR borne error or due to the true absence of the locus. In some cases, PCR repetition resulted in successful amplification and in some samples no amplification.

Statistical Analysis

Assessment of *per se* performance of genotypes: The trait values averaged over five replications per accession per year (2012 and 2013) were used separately for statistical analysis. A highly significant 'p' value (p=0.00) almost all the quantitative traits indicated a non-cross over genotype × year interaction as well as statistical justification for the use of two year pooled data for analysis. Total variability among the 52 accessions was partitioned into components attributable to genotypes, years and genotype × interaction. Significance of the differences among all the 52 genotypes was assessed using the procedure followed by Fasoulas[3] (Table 2). The genotypes were arranged in the descending order of their mean values. The ranks were assigned from the highest mean to the lowest mean. If the mean values of a trait for two different accessions are similar, then they are numbered with the same rank. Differences between the mean values were calculated between the ranks serially until the mean difference reaches a value higher than CD, where

CD= $\sqrt{2 \times \text{mean sqaures due to pooled error from ANOVA}}$ × table't' value at p=0.05.

Identification of SSR markers and accessions contrasting for only four important quantitative traits namely FW, OPT, TSS, SH were analyzed further. The number of genotypes that have difference in mean values higher than the CD value were counted to assign m value to a particular genotype. Performance index (P) (Table 3) is calculated by P= 100m/ (n-1), where n is the number of accessions (52). Based on the averaged mean values of the four quantitative traits obtained in the two successive years, genotypes contrasting for the traits were identified.

Identification of parents contrasting for pomological traits: The high and low 'P' value of a particular accession was considered as a measure to identify the superiority and inferiority of a particular accession over the others used in the study. Accessions with three highest 'P' values and three lowest 'P' values for each of the quantitative traits were evaluated and contrasting combinations of accessions can be used as parents. Accessions that are high in performance (high 'P' value) for a particular trait were limited but low performing accessions (P=0) for various traits were higher in number (Table 4).

Identification of putative mapping population parents: The genotyping data of the 135 polymorphic SSR markers was used to identify the alleles differing among the genotypes contrasting for pomological traits. The total numbers of alleles were counted and *per cent* polymorphism was calculated as 'Kp/k' as described by Blair et al[1] using Microsoft Excel package; where 'kp' reflects the number of polymorphic markers and 'k' is the total number of SSR markers assayed. For all the 135 SSR markers, the number of scored alleles was counted to estimate the average dissimilarity coefficients as described by Sokal and Michener[9].

Barcoding the best five accessions: The five highly performing accessions were barcoded using a set of SSR markers with unique alleles which enabled the discrimination of the accessions.

Results and Discussion

Barcoding the best accessions: Among the 135 polymorphic SSR markers, five bi-allelic (mPgCIR31, mPgCIR38, mPgCIR44, mPgCIR48, mPgCIR340) and five tetra allelic ones (mPgCIR277, mPgCIR182, mPgCIR180, mPgCIR102, mPgCIR93) could differentiate the five best accessions (Fig. 1). At each of these 10 SSR markers, alleles unique to each of the five best accessions were identified.

Quantitative traits- based differences among the accessions: The 52 accessions differed significantly for the traits investigated (Table 2). For FW, the genotypes Abu Ishaqwala and Dhareedar significantly outperformed 98% of individuals followed by Benaras by 96% (Table 3). For OPT, Sringeri seedless and Dhareedar outperformed 98% of other genotypes followed by Benaras by 84%. The accessions Allahabad Safeda, Hisar Safeda and Local 1 were best performing over other genotypes by 100%, 92% and 90% respectively for TSS. For SH, the genotypes Lucknow-42 (100%), followed by Bangalore Local, Ceylon Guava, 9-35EC147036, Abu Ishaqwala, GR-1, Superior Sour Lucidum have shown superior performance by 88% respectively.

Based on morphological data, the genotypes like Seedless, Local 4, Sindh, G-1, Ceylon Guava, Pati, EC-147039, Red Flesh, EC-147037, Aneuploid 1, GR-1 for FW, Lalit, Smooth Green, Local 1, Portugal, Hisar Safeda, CIW1, Sindh, Ceylon Guava, EC-147039, Surkha Chitti Neptuani, Red Flesh, CIW4, Aneuploid 1, Aneuploid 2 for OPT, Local 4, CIW1, Bangalore Local for TSS, Hisar Safeda, Arka Amulya, Florida Seedling, Aneuploid 2, Arka Amulya, for SH have shown a lower *per se* performance, which can be used as one of the parents. SSR genotyping of a total number of 119 combinations of contrasting accessions (Table 7) exhibited higher per cent polymorphism, number of detected alleles and average dissimilarity coefficient.

Contrasting pairs of accessions, Benaras and Seedless, Dhareedar and Seedless, Dhareedar and EC-147037, Abu Ishaqwala and Pati for FW, Benaras and CIW1, Benaras and Surkha Chitti Neptuani, Sringeri Seedless and Surkha Chitti Neptuani for OPT, Allahabad Safeda and CIW1, Hisar Safeda and Local4, Hisar Safeda and CIW1, for TSS and 9-35EC147036 and Florida Seedling, 9-35 EC147036 and Arka Mridula, GR1 and Hisar Safeda, GR1 and Florida Seedling for SH (Table 5) were identified as putative parents for developing mapping populations to identify SSR markers linked to genomic traits controlling economically important fruit characters in guava like TSS, SH, FW, OPT respectively.

Conclusion

Identification of alleles unique to each of the five best performing accessions at 10 SSR loci helped to address intellectual property issues that might arise in future. The genotypes which were shortlisted to be used as putative parents can be used to effect the crosses to derive elite varieties which can be selected and exploited commercially.

Table 1
List of guava accessions used in the present study

S.N.	Accession	S.N.	Accession	S.N.	Accession
1	Lalit	19	KG Guava (Bijapur Guava	37	CIW4
2	Benaras	20	Florida Seedling	38	Aneuploid-1
3	Nasik	21	Sringeri Seedless	39	Aneuploid-2
4	Chittidar	22	Sindh	40	Local 2
5	Smooth Green	23	G-1	41	Thailand Guava
6	Allahabad Safeda	24	Ceylon Guava	42	Surka Chitti
7	Local1	25	9-35147036	43	GR1
8	Sardar Guava	26	Lucknow- 42	44	Local White
9	Portugal	27	Chakaiya Ruthamanagar	45	Bendror (EC538097)
10	Seedless	28	Dharwad	46	Arka Mridula
11	Mirzapur Seedling	29	Pati	47	Behat Coconut
12	G-6	30	Dhareedhar	48	Karela
13	Phillli Pink	31	Kamsari Red	49	Local 3
14	Hisar Safeda	32	EC-147039	50	Superior Sour Lucidum
15	Arka Amulya	33	Surka Chitti Neputani	51	White Flesh
16	Local 4	34	Red Flesh	52	Spear Acid
17	CIW1	35	Abu Ishaqwala		
18	Bangalore Local	36	EC-147037		

Table 2
Pooled analysis of variance for thirteen quantitative traits in Guava (*Psidium guajava*)

Source of variation		Season	Genotypes	Geontypes x season	Pooled error
Df		**1**	**51**	**51**	**408**
Fruit weight (g)	**MSS**	237593.30	9438.65	4457.01	288.75
	F ratio	822.83	32.69	15.44	
	P	0.00	0.00	0.00	
Fruit length (cms)	**MSS**	98.24	4.17	1.97	0.15
	F ratio	659.69	28.00	13.22	
	P	0.00	0.00	0.00	
Fruit width (cms)	**MSS**	43.68	3.02	1.36	0.11
	F ratio	403.07	27.87	12.53	
	P	0.00	0.00	0.00	
Sepal size (cms)	**MSS**	1.79	0.24	0.27	0.04
	F ratio	49.28	6.69	7.33	
	P	0.00	0.00	0.00	
Diameter calyx cavity (cms)	**MSS**	5.30	0.44	0.22	0.03
	F ratio	173.19	14.28	7.30	
	P	0.00	0.00	0.00	
Stalk length (cms)	**MSS**	0.55	2.07	1.02	0.23
	F ratio	2.37	8.95	4.42	
	P	0.12	0.00	0.00	
Outer pulp thickness (cms)	**MSS**	0.15	0.56	0.20	0.05
	F ratio	3.36	12.12	4.27	
	P	0.07	0.00	0.00	
Core diameter (cms)	**MSS**	26.03	1.75	0.54	0.12
	F ratio	219.95	14.83	4.53	
	P	0.00	0.00	0.00	

Table 3
Quantitative trait means of the 55 guava (*Psidium guajava*) accessions.

Accessions	**Fruit Weight (g)**				**Outer pulp thickness (cms)**				**Total soluble solids (^{0} Brix)**				**Seed hardness (kg/m$^{2)}$**			
	Mean	**Rank**	**m**	**P**	**Mean**	**Rank**	**m**	**P**	**Mean**	**Rank**	**m**	**P**	**Mean**	**Rank**	**m**	**P**
Lalit	118.3	25	20	39	0.93	29	0	0	5	15	22	43	10.8	34	7	14
Benaras	182.2	3	49	96	1.43	3	43	84	4.4	26	10	20	10.55	36	5	10
Nasik	95	38	4	8	1.27	9	34	67	4.9	18	22	43	11.8	26	15	29
Chittidar	127	17	25	49	1	24	3	6	5.42	9	28	55	13.15	12	33	65
Smooth Green	103.1	32	10	20	0.92	30	0	0	5.29	12	26	51	12.2	20	22	43
Local 1	107.1	29	11	22	0.92	30	0	0	6.265	3	46	90	11.9	24	16	31
Sardar Guava	124.3	20	24	47	1.02	22	4	8	5.775	6	36	71	12.6	19	25	49
Portugal	99.5	35	6	12	0.93	29	0	0	4.91	17	22	43	13.8	5	42	82

Seedless	83.5	45	0	0	1.38	6	39	76	5.54	8	30	59	12.95	14	28	55
Mirzapur Seedling	118.9	24	20	39	1.05	20	5	10	4.79	19	19	37	13.3	11	34	67
KG Guava(Bijapur Guava)	128.6	15	25	49	1.02	22	4	8	3.81	36	6	12	13.15	12	33	65
Hisar Safeda	99.5	35	6	12	0.82	36	0	0	6.48	2	47	92	9.4	42	0	0
Arka Amulya	140.9	6	33	65	1.18	13	23	45	3.13	41	3	6	9.95	41	1	2
Local 4	85.2	44	0	0	1.11	16	12	24	1.26	45	0	0	13.6	7	38	75
CIW1	99	36	6	12	0.87	33	0	0	1.15	46	0	0	11.2	31	10	20
Bangalore Local	96.4	37	5	10	1.09	17	10	20	1.36	44	0	0	14	4	45	88
Florida Seedling	93.3	39	3	6	1.07	18	8	16	5.41	10	27	53	10.35	38	2	4
Sringeri Seedless	121.9	22	22	43	1.82	2	50	98	4.785	20	19	37	13.475	9	37	73
Sindh	88	41	0	0	0.93	29	0	0	4.02	32	7	14	13.05	13	31	61
G-1	79.8	47	0	0	0.96	27	1	2	4.225	28	9	18	11.812	25	15	29
Ceylon Guava	85.3	43	0	0	0.86	34	0	0	3.96	34	7	14	14.1	2	45	88
9-35 EC147036	135.5	8	28	55	1.12	15	15	29	6.08	5	41	80	14	4	45	88
Lucknow- 42	133.3	10	27	53	1.3	8	36	71	4.09	30	8	16	20.6	1	51	100
Chakaiya Ruthmanagar	127.4	16	25	49	1.04	21	4	8	4.03	31	7	14	13.7	6	41	80
Dharwad	123.7	21	24	47	1.17	14	22	43	4.23	27	9	18	11.65	28	14	27
Pati	87.5	42	0	0	1	24	3	6	4.46	25	11	22	10.5	37	4	8
Dhareedhar	214.5	2	50	98	1.99	1	50	98	5.39	11	27	53	11.2	31	10	20
Accessions	**Fruit Weight (g)**				**Outer pulp thickness (cms)**				**Total soluble solids (^{0} Brix)**				**Seed hardness (kg/m$^{2)}$**			
	Mean	**Rank**	**m**	**P**	**Mean**	**Rank**	**m**	**P**	**Mean**	**Rank**	**m**	**P**	**Mean**	**Rank**	**m**	**P**
Kamsari	132.9	11	26	51	1.39	5	40	78	5.56	7	31	61	11.9	24	16	31
EC-147039	88.3	40	0	0	0.88	32	0	0	5.215	12	26	51	11	33	8	16
Surka Chitti Neputani	105.4	30	11	22	0.94	28	0	0	3.545	38	5	10	12.1	22	21	41
Red Flesh	81.2	46	0	0	0.91	31	0	0	6.11	4	42	82	13.8	5	42	82
Abu Ishakwala	222.2	1	50	98	1.36	7	38	75	5.56	7	31	61	14.05	3	45	88
EC-147037	75.5	50	0	0	0.96	27	1	2	4.03	31	7	14	10.7	35	6	12
G-6	102	33	8	16	0.988	26	1	2	4.912	17	22	43	12.75	18	25	49
PhilI Pink	127	17	25	49	1.05	20	5	10	5.06	13	23	45	13.45	10	37	73

CIW4	126.6	18	25	49	0.82	36	0	0	5.56	7	31	61	11.25	30	11	22
Aneuploid 1	77.9	48	0	0	0.94	28	0	0	2.91	42	3	6	11.6	29	14	27
Aneuploid -2	104.8	31	11	22	0.88	32	0	0	5.01	14	22	43	10.2	40	2	4
Local 2	137.6	7	30	59	1.23	10	29	57	4.715	21	18	35	11.05	32	9	18
Thailand Guava	134.5	9	28	55	1.21	11	28	55	4.93	16	22	43	13.5	8	38	75
Surka Chitti	130.2	13	25	49	1.06	19	7	14	3.96	34	7	14	12.9	15	26	51
GR1	76.3	49	0	0	0.83	35	0	0	4.18	29	9	18	14.05	3	45	88
Local White	108.6	28	12	24	0.77	37	0	0	4.47	24	11	22	11.75	27	14	27
Bendror (EC538097)	117.3	26	19	37	1.07	18	8	16	3.4	39	3	6	12.75	18	25	49
Arka Mridula	159.4	4	47	92	1.2	12	26	51	4	33	7	14	10.3	39	2	4
Behat Coconut	129.8	14	25	49	0.96	27	1	2	3.91	35	7	14	12.15	21	22	43
Karela	125.4	19	24	47	1.01	23	4	8	3.71	37	5	10	12.85	16	26	51
Local 3	120.8	23	22	43	0.99	25	1	2	4.6	22	14	27	11.95	23	17	33
Superior Sour Lucidum	111.4	27	14	27	1.23	10	29	57	4.5	23	11	22	14	4	45	88
White Flesh	100.9	34	8	16	0.98	26	1	2	3.21	40	3	6	13.15	12	33	65
Spear Acid	132.4	12	26	51	0.87	33	0	0	2.84	43	3	6	12.85	16	26	51
Allahabad Safeda	152	5	46	90	1.41	4	41	80	8.72	1	51	100	12.8	17	26	51
CD@5%	**14.9**				**0.18**				**0.63**				**3.73**			
S.E.diff	**7.6**				**0.1**				**7.6**				**1.9**			

Table 4
Guava accessions differing in pomological traits

Contrasting Parents based on morphological evaluation		
Trait	**High scoring accessions**	**Low scoring traits**
Fruit weight	Dhareedar, Abu Ishaqwala, Benaras	Seedless, Local 4, Sindh, G-1, Ceylon Guava, Pati, EC-147039, Red Flesh, EC-147037, Aneuploid1, GR-1
Outer pulp thickness	Dhareedar, Sringeri Seedless, Benaras	Lalit, Smooth Green, Local 1, Portugal, Hisar Safeda, CIW1, Sindh, Ceylon Guava, EC-147039, Surkha Chitti Neputani, Red Flesh, CIW4, Aneuploid 1, Aneuploid 2
Total soluble solids	Allahabad Safeda, Hisar Safeda, Local 1	Local 4, CIW1, Bangalore Local
Seed hardness	Lucknow 42, Bangalore Local, Ceylon Guava, 9-35EC147036, Abu Ishaqwala, GR-1, Superior Sour Lucidum	Hisar Safeda, Arka Amulya, Florida Seedling, Aneuploid 2, Arka Amulya

Table 5
Pairs of guava (*Psidium guajava*) accessions most contrasting for pomological traits and their polymorphism at SSR loci by microsatellite evaluation

Contrasting Parents	Trait	No. of polymorphic SSR loci					No of detected alleles	Per cent polymorphism	Average dissimilarity coefficient
		Total	Repeat Motiffs						
			Di (2)	Tri (3)	Tetra (4)	Complex			
Benaras and seedless	Fruit weight	103	60	0	1	3	131	76.2	0.29
Dhareedar and Seedless	Fruit weight	101	66	0	1	4	126	74.8	0.33
Dhareedar and EC-147039	Fruit weight	100	61	1	2	5	128	74.0	0.29
Abu Ishaqwala and Pati	Fruit weight	100	61	1	1	3	122	74.0	0.28
Benaras and CIW1	Outer pulp thickness	102	57	1	2	4	123	75.5	0.25
Benaras and Surka Chitti Neputani	Outer pulp thickness	104	59	1	2	3	127	77.0	0.25
Sringeri Seedless and Surka Chitti Neputani	Outer pulp thickness	104	57	1	2	4	125	77.0	0.26
Allahabad Safeda and CIW1	Total soluble solids	97	56	1	2	4	122	71.8	0.28
Hisar Safeda and Local 4	Total soluble solids	94	59	1	1	5	126	69.6	0.33
Hisar Safeda and CIW1	Total soluble solids	96	60	1	1	4	121	71.1	0.33
9-35Ec147036 and Florida Seedling	Seed hardness	102	64	0	2	4	121	75.5	0.28
9-35Ec147036 and Arka Mridula	Seed hardness	101	62	1	0	4	122	74.8	0.29
GR1 and Hisar Safeda	Seed hardness	101	63	1	1	5	125	74.8	0.30
GR1 and Florida Seedling	Seed hardness	110	64	1	0	5	132	81.4	0.24

SSR marker	Allele size (bp)	Accessions				
		Benaras	Dhareedar	Abu Ishaqwala	Arka Mridula	Allahabad Safeda
mPgCIR31	153	■		■	■	■
	175		■			
mPgCIR38	102	■				
	115		■	■	■	■
mPgCIR44	234				■	
	243	■	■	■		■
mPgCIR48	115	■	■	■	■	
	124					■
mPgCIR340	109			■		
	119	■	■	■	■	■
mPgCIR277	144					■
	154				■	
	164			■		
	176	■				
	191		■	■		
mPgCIR182	133	■				
	147				■	■
	174		■		■	
	185			■		
mPgCIR180	106			■		
	120		■			
	138	■			■	
	151	■				■
mPgCIR102	181	■		■		
	207		■	■	■	
	219					■
	255				■	
mPgCIR93	102	■				
	123		■		■	
	134			■		
	145					■
	157				■	

Solid cells indicate the presence of alleles

Figure 1: SSR marker alleles unique to the best five guava accessions at 10 loci

Table 6
List of polymorphic markers used for genotyping in Guava (*Psidium guajava*)

SSR marker	Forward and Reverse sequence	Repeat Motif and their number	Expected amplicon size (bp)	Annealing Temperature (^{0}C)
mPgCIR027	F: AGCACTTAGGGACAAATTCA R: CTCACTCTCCTCCATTCAAG	(GA)28	292	55
mPgCIR029	F: CTCGCTTCAATCTCCATCTA R: AGCGACACAGACTCTTCATT	$(GA)_8$ / $(GAA)_5$	162	50
mPgCIR031	F: TCTCACTGATGCAACTTTTC R: CCCATTTTCATCTCAAAGTC	$(GA)_{24}$	128	50
mPgCIR032	F: CGCCTTTCGTAAAAGAAGT R: TCATATACTCGGACAAAACG	$(GA)_{16}$	100	50
mPgCIR034	F: CTTTTAAGCACGTTTGACCT R: GCGTATTAACAGCTTGGAGA	$(GA)_{16}$	101	55
mPgCIR035	F: TTGACCTGGCATTAACAGA R: CATTGGGAAAGGGAAGAA	(GA)27	292	55
mPgCIR038	F: AGCCTGTTTTACGCCTTC R: CGGCTGCTCTATTGTTATTT	(GT)6	111	55
mPgCIR039	F: GCTCACCTTACTCATTCAGC R: CTGTTGCTAAGAGCTTTCGT	$(GA)_{17}$	155	50
mPgCIR040	F: TGAATCTCCAGTGTCTTATCG R: TGATTTCAACTGCGTATGTC	$(GTA)_6$	141	50
mPgCIR041	F: AAGTGGTGTCAGCAACTACC R: CTTAGTTTGACCGCTCCAGT	$(GA)_{13}$	136	50
mPgCIR042	F: CTCACCCAAAATCTACACAAG R: AAGGGACTGGACGATGTT	$(GA)_9$	107	50
mPgCIR044	F: TTCCAGGTCTATTGGATGTC R: GGGGACACAAAACTTCATTA	Unknown	243	55
mPgCIR046	F: ATAGAACGCCATGTTACCAA R: CAGGCTTATCTGTTACACCA	$(GA)_{36}$	159	56
mPgCIR048	F: TGCAGCTTCTTCAATGTTC R: AAAACTTGGCAACGTCAGT	(GA)20	112	55
mPgCIR089	F: TCGTCCAGAATCTTAAAAGC R: AACTAGCATGTGACCAGGAG	$(GA)_{16}$	274	50
mPgCIR091	F: GCGGTGGATTTGAATTTAG R: CCAAGTAACCCACAACAATA	$(GA)_{16}$/$(GGGA)_3$	125	56
SSR marker	**Forward and Reverse sequence**	**Repeat Motifs and their number**	**Expected amplicon size (bp)**	**Annealing temperature (^{0}C)**
mPgCIR092	F: GTAAGTTTTCCGCATCCTG R: ATATTGCACAGGGTGGTATC	$((GA)_{18}$/$(GT)_{14}$	151	50
mPgCIR093	F: GCATCATGTGTTTGAACGAT R: AAGTGTGCGTTCTCCATCT	Unknown	123	55
mPgCIR096	F: ACGCTGCAAACGATACTAAT R: AACTCACACGAGCACAGAG	(GA)12	131	55
mPgCIR098	F: CATCAACTTTCCAGGCATA R: CCATTCAGTCGGTTTGAC	$(GA)_{15}$	127	50
mPgCIR099	F: TCAAAGTCCAAAACTCATGC R: GGGATGGAGTAAAGATGAAA	$(GA)_{20}$/$(GAT)_{14}$	220	55
mPgCIR100	F: CTAGAAGTCGAAGAATGGAA R: TTTGTTAGTATCGGAGTCGAG	$(GA)_{15}$/$(GGAA)_3$	128	50
mPgCIR101	F: ATGGCTGTAAGAAGCAAAAG R: GAAGAAATGTAGGTGCGTTC	$(GT)_{10}$	110	55
mPgCIR102	F: AATTGGTGTAGCATCTGGA	(GA)8	176	55

	R: GCCTACCATGAACAGAGAAA			
mPgCIR104	F:ATTCCCGTGGATTATGTATC R: ACAACCATTTTCTCCTCATC	(GGTT)3	120	50
mPgCIR108	F: AGGACCTCACAGAAGTTCAC R: CGCTGTTTACACTGTCGTT	(GA)13 / (GGAG)3	156	50
mPgCIR109	F: AATTTCCACAGATCACAAGG R: GGCATCTCCATCAAATACAT	$(GA)_{11}$	110	50
mPgCIR110	F:GCCCCATTCTAAGAGACAAT R: GAATGAAACCAGGTGTAGCA	Unknown	118	55
mPgCIR111	F: CAACCTCGTTTGAGTCTTCT R: AACATCATTGGGACCATTC	$(GA)_{19}$	115	50
mPgCIR133	F: CGATCTTGGAATGTAAGAGG R: TGGATTTGCAGGTTCTATCT	(GA)12	148	55
mPgCIR139	F: ATAATCCCCTCCCATAACTA R: CCAACTCAACATGAGAAGC	$(GT)_9/(GA)_9$	207	54
mPgCIR142	F: GTCGTGGTTTCCCAAAATA R: TACTGACTTCCCACACTCTTG	(GA)13	100	55
mPgCIR146	F: CGGATATTAGCAGGAGAAAA R: AATGTTTGACGACTCGAAAG	Unknown	110	55
SSR marker	**Forward and Reverse sequence**	**Repeat Motifs and their number**	**Expected amplicon size (bp)**	**Annealing temperature (^{0}C)**
mPgCIR147	F: ACTGACATCTCTGACCATAGC R: GATTGCCATAGGAACTGAAA	(GA)17	115	55
mPgCIR149	F:CTTCGTGGAAGAGGATGAC R: AATATAAACATCGCCACAGG	(GA)20	186	55
mPgCIR150	F: CCTAGTGACTCGAAGCAATC R: TTGAGCCCTAGCATAGACAG	$(GA)_{15}$	108	50
mPgCIR152	F: TTCGCAAGCATCTCAAGT R: CCATAAGTTTGGGTGTCAA	(GA)20	151	55
mPgCIR153	F:GCCTCTGGTAAATCTGTTGA R: ACATACGGATCAAGTCCAAA	$(GA)_{20}$	115	50
mPgCIR154	F: CTTCAGCTACAGCCTTTCC R: GGAGAAAGCAGAAATTCCA	(GA)24	138	55
mPgCIR157	F: AACCACCAAACCATACACC R: CGACCAACCCTACATTCTG	(GA)21/(GGAA)3	209	50
mPgCIR159	F: GGTTAAGAGGCTTCAGTTCA R: CAGCAAGGACAGGTTAAGAG	(GA)19	102	55
mPgCIR160	F: TGGCTATAAGAATGGGAGAT R: GACGAGCTTAGCCTCTGAAT	$(GA)_{10}$	116	50
mPgCIR161	F: TCTCAAGGACCAACAAGAAG R: AGGACTTAGCTTGGGTTTTC	$(GA)_{15}/(GA)_6$	246	55
mPgCIR163	F: TCTTTGCACATCAAACTCG R: CATGGTATCAATAGGTCAAGC	$(GT)_9/(ATAT)_3$	168	55
mPgCIR165	F: TAAGGGATTCATTTCCGAGT R: CTGGTGTGACGATGACTTTT	Unknown	124	55
mPgCIR166	F: CTTTCCCATCAAAACGTAAG R: CCAATTCATGCACTTAGACA	$(GA)_{11}$	155	50
mPgCIR169	F: TTCAGGCAGATCGTGTTACT R: GTGCCTAACCTACACCCTAA	(GA)9	102	55
mPgCIR171	F: TGACTTGCTCACCTAGATTGT R: TCGATGGGAGATCAGAAGT	$(GA)_{31}$	82	50
mPgCIR172	F: CACCCTTAACTTCTGCTTTG R: GTCTCTATTTCCCTCCGTTC	(GA)15	123	55
mPgCIR173	F: CGTGTTCTTTACATCCGTCT	(GA)24 / (GA)6	300	56

	R: TACCAGCAACACCAATGC			
SSR marker	**Forward and Reverse sequence**	**Repeat Motifs and their number**	**Expected amplicon size (bp)**	**Annealing temperature (^{0}C)**
mPgCIR174	F: GCCACTGTGTAAGAGGATTG R: ATTGTGGGAGATTGGAGAC	(GA)20 / (GA)9 / (GGTC)3	261	56
mPgCIR175	F: GCATTATGTGCCAAGCAA R: TGCCAAGGTGTAATGTTGTA	$(GA)_{16}$	104	56
mPgCIR176	F: TTCTCAATGATAGGTTACGG R: ATGACTATTCTCCACCAGAT	Unknown	151	55
mPgCIR179	F: GGGTCTCGACTAAAGAAGGA R: CCTCCATTTGCATCAACTTT	$(GA)_{16}$	147	54
mPgCIR180	F: CATGGATTCAACTCTTGTCG R: CTACATTGGAAGCAGAATGG	(GT)9	119	55
mPgCIR182	F:GAGGAAGAAACCCGAAGTTA R: GGTAGAAAGATCGGAAAGAC	(GA)13/(GGAA)3 / (CGAGAA)3	181	55
mPgCIR184	F: AAGCTACAATCGACGAAAAC R: CACTATTAGCGAACCTGCAT	(GA)23 / (GAGG)3	221	55
mPgCIR185	F: AACGCATCTGGCATTGAT R: CCTTGGTCTCCCTCTTACTC	(GA)18	117	55
mPgCIR190	F: GAAGATTGGGAACCTAAGAA R: AGAAATGGGTTGTGGAGAG	(GA)27	124	55
mPgCIR191	F: GACCCTCCCACTTATATTTTG R: AAGCTGACATAACAGTCGAA	(GA)25	210	55
mPgCIR192	F: ACGCTAACTATCGAAATGCT R: ACTACGCACTTGATGGAGAT	$(GA)_{23}$	153	50
mPgCIR193	F: GAACGTGGGTTACATACCAT R: ATCACCGTCCTCCTAAATCT	(GA)15	122	55
mPgCIR194	F: GCAGAGAATCGAAGCACTA R: GCAAGCACAGGTTCTACTTT	$(GA)_{20}$	172	55
mPgCIR195	F: AGCCGTAGACATAAGTTTCAG R: GCCCTTATCAAGTCCATGT	(GT)8	134	55
mPgCIR197	F: CACCCAACTCTATACCCAAC R: GTAGCTCACCAAGCTGAAAC	(GA)9	108	55
mPgCIR200	F: CCTTGCTTTGGTGAGGTC R: GCTAATTCAGTCCTTCCAACT	$(GA)_6$	178	55
mPgCIR201	F: TTTGCCTTCGAGCTTCTACT R: ACAATTTCGTGGGCTCGT	$(GA)_{19}$	133	55
SSR marker	**Forward and Reverse sequence**	**Repeat Motifs and their number**	**Expected amplicon size (bp)**	**Annealing temperature (^{0}C)**
mPgCIR203	F: ATGAAGGCATTACCTAAGAC R: ACCCTATTAACCCTTAGCAA	(GT)10	126	55
mPgCIR205	F: ACCTCTCCAGCTCTACACG R: GAGGTTGTCGAAGGTTGAT	(GA)11	101	55
mPgCIR206	F: GAAGTTTCAAAGTAACAGCAC R: AGAATGAGTCCATGCTCAAA	(GA)8 / (GT)11	181	55
mPgCIR207	F: CAAGATTTGCCTCAAGAAAC R: AACTAAATAGCCTGCTGGTG	(GA)37	136	55
mPgCIR209	F: CTAAAGCCACATCCAGCA R: CTAACATTTGCCTTCTACAGC	$(GA)_{15}$	139	55
mPgCIR212	F: CACTGTGCTTGAGTGAATGA R: GGCCTCCCTTTGTAAACT	$(GA)_{23}$	129	55

mPgCIR213	F: CAGGAGGAACAAAACTGAAG R: TTACGCTTATTGGACAGGAC	(GA)20	222	55
mPgCIR216	F: GATGGAACTGCAATGTATGA R: AGACCTGCTCTGACTTGTGA	$(CGTA)_3$	131	55
mPgCIR218	F: CTGTTGCCTCAGATCGTAAT R: CAATGCAAAGCCATGATAGT	(GA)24	155	55
mPgCIR220	F: AGAGCAGTGGTTGCTATTTT R: CCCATCTCTTACTTTTCTTGTG	$(GT)_8/(GA)_{20}$	218	55
mPgCIR221	F: CTAAGCCTGAAGTCCCAAAT R: CCTCTTCTAAAGGCAACGAC	(GA)11 / (GA)8 / (GA)8	100	55
mPgCIR222	F: CAGAATCAGACATAGTTAGAGC R: CTGAAGACATCAACATGGAA	(GA)15 / (GT)13	166	55
mPgCIR224	F: GAATAAGACCAAATGGCACA R: GTTCCACACCCACATTCTAT	(GA)27	209	55
mPgCIR228	F: CAGAACAAAGAAGAGGATCTG R: TGGATCAGTAGAATCGTTTG	$(GT)_{14}/(GA)_{18}$	153	54
mPgCIR230	F: CACATTTGCTCCTGATTTTC R: GCTCTTCAACGACCATCTT	(GA)18	198	55
mPgCIR231	F: CTCCAAGAAAATGGAAAGG R: TGAAAACACCAAACAGCAC	(GT)9 / (GA)12	177	55
mPgCIR234	F:TTGGCTTGTCAACTACTGG R: GCATCTCTTTCATTGGTGAT	$(GT)_{11}$	159	50
SSR marker	**Forward and Reverse sequence**	**Repeat Motifs and their number**	**Expected amplicon size (bp)**	**Annealing temperature (^{0}C)**
mPgCIR235	F: TCTTCTGGACAACAAATTCC R: GATTTAAGGCAAAGGATCG	$(GA)_{28}/(GAGT)_4$	204	55
mPgCIR236	F: ACTCATATTCCGTTTGCATC R: GAATTAACGACGAGTTCCAC	Unknown	164	55
mPgCIR240	F: CGAATGTCCAAGATTCAGTT R: CCTTCTTCATCTCAGCCTTT	(GA)22	201	55
mPgCIR241	F: AGATGCTAGTCTGTATCCTGAA R: GAGAAAGGTTGGTATGGTGT	(GA)8	208	55
mPgCIR242	F: TTAAGGTGGGACCAAGAAG R: GACGTATCGGATCAAGTTTC	$(GA)_{12}$	178	55
mPgCIR243	F: ACAGCAGGACACAAAGGA R: GCTCTGAGGTGGTTTTCAT	$(GA)_{29}$	174	55
mPgCIR245	F: CCAGACAAAATTCCAACG R: AAATAGCCTCTCCAATCACA	$(GA)_{11}(GGTA)_3$	198	55
mPgCIR246	F: GAATTACAAATGCCTTGTCC R: GCTCTAAAGTGCAACCAAAG	Unknown	132	55
mPgCIR248	F: CTGATATTGCCTGGAAGAAG R: TAATTGAGCAAGAACCCTCA	(GA)23	230	55
mPgCIR249	F: TTTGTCTGGTCGTCCTAGTT R: CTTCAGTCCATCAGCAAAAT	$(GA)_{21}/(GAA)_5$	259	55
mPgCIR250	F: AACTCGAATGGCTTCTGG R: CTCGCGGTATTGAATGGT	Unknown	185	55
mPgCIR257	F: CGACTCATTTTCTGGTCTGT R: CAACCACCTTCATCAATTTC	Unknown	203	55
mPgCIR261	F: GTTGCCTGGTTACTTAATACCT R: TGCTGACGTATGAGTTCAAT	Unknown	291	55
mPgCIR262	F: TTGTTGGAGTCAAGCTACTG R: AGTTGTCGGTCAAGGAAGT	Unknown	234	55
mPgCIR263	F: ACGGTAGGCCACTATACAT R: CACTGGCATAGAGAACATAAG	Unknown	200	55

mPgCIR265	F: CTACAGCGAATTTCTCGATT R: GTCAACGGATCAATGTGG	Unknown	144	55
mPgCIR277	F: AGCCGATTATGATTACCTGA R: CGATTCACTCCCTCATTACT	$(TG)_{11}(GT)_9$	173	50
SSR marker	**Forward and Reverse sequence**	**Repeat Motifs and their number**	**Expected amplicon size (bp)**	**Annealing temperature (^{0}C)**
mPgCIR284	F: AACCCTTTCGGGGTCAAG R: GATCCGATTGCGGAAGAG	$(AC)_{10}$	90	55
mPgCIR288	F: AAAGCAGAAAAGGGGTAAAC R: GGAACCAGCACAGACATATT	$(CT)_{19}$	199	50
mPgCIR298	F: GCGTATGTCATTCCATGTG R: CAGGAATATGATGCTGGAAG	Unknown	198	55
mPgCIR316	F: GCTTCATATTACAAACCTTGG R: GATCTAACTGACTTGCCAAAA	$(GA)_{24}$	232	50
mPgCIR317	F:CAATAGCCACTAACTATGACATCT R: AGCCAAAATCGTTCCATC	$(CT)_7$ $(CA)_8$	143	55
mPgCIR321	F: TTTTGGCCTGGGAATATAG R: TAAAACGAAAGCAGAAAACC	Unknown	129	55
mPgCIR325	F: AAACGCTCGAATCAGTTG R: CCAAGAAACACAGGGATTAC	Unknown	172	55
mPgCIR326	F: AGAACAAGACACGAGAAGAG R: AAAATCTACGCACAAACC	$(CT)_{16}$	116	50
mPgCIR334	F: GGATCTAACCGACCTTTCTT R: AAACAGGACTGAGTTTCGAG	$(AG)_{25}$	190	55
mPgCIR339	F: CCGAAGACGAGGAGATTA R: TTAAGTGGAAAATCACAGTTG	Unknown	160	55
mPgCIR340	F: ACCGACCGTGATACCTTC R: GGGAAGAGAGATAAGAAGTAGA	Unknown	137	55
mPgCIR344	F: ACTTTTGGGGTGCTTAACAG R: ACCTGATGCAGAATAAATGC	Unknown	222	55
mPgCIR345	F: CTGGGAGACTTTTCAAGG R: GAGTCCGATGTTGATGAAG	$(TC)_{20}(CT)_{15}$	231	55
mPgCIR373	F: GGCGTAATGCAAGTAAAAGT R: CCACGTTGTTTTCCACAC	Unknown	129	55
mPgCIR379	F: ACGATCTTAAAGCAAAACCA R: CCGCAAGTCAGAATCAGTAT	Unknown	190	55
mPgCIR380	F: TATGAGGCCAGAAAAGATTG R: AGGATTTACGCTTCCCTACT	Unknown	132	55
mPgCIR384	F: GGAGACTTTGTCATCGAAA R: TTCCTTCATGGGTAAGTGAT	$(GT)_8$	217	55
SSR marker	**Forward and Reverse sequence**	**Repeat Motifs and their number**	**Expected amplicon size (bp)**	**Annealing temperature (^{0}C)**
mPgCIR385	F: GTGGTCAGACACGAAGTGA R: ATGAGAGCATCGGTAATTTG	Unknown	185	55
mPgCIR386	F: ACCCTCGAATCATAAGAACA R: ACTTACGCTCTCCTCACTA	Unknown	189	55
mPgCIR389	F: TTTCTCCGTTGGTTTATGAC R: ACGTGGATGTTTTACACCTG	Unknown	201	55
mPgCIR391	F: TAGACCAAAACTAGGCAGGA R: TTTGGAAGCTGTAGGAGAAA	Unknown	110	55
mPgCIR392	F: AACTGTTTCTCTGGTTTGATG	$(AC)_{10}$	107	55

	R: ACGAAACGTCCTTGTTCTTA			
mPgCIR395	F: TCCATGCAAACAGAGTGTAA R: GACATTGATGAACAGCGTTT	Unknown	208	55
mPgCIR396	F: GTAGACCAAAGAACGAAGCA R: GAAATAGTATGCTCCCCAGAT	Unknown	197	55
mPgCIR398	F: TGGAGACAGAAGCTCACC R: AAGAACACACACCCACCA	Unknown	210	55
mPgCIR399	F: AAAAGCTCATCCTTGTTGC **R:** CCTCTCATTTTCTCCTCCA	$(TC)_7(TC)_{11}(TC)_{10}(CA)_{14}(CA)_8$	122	55
mPgCIR406	F: ACATTTGATGTCAGAAGGA R: CCAATACAAGCTCAAAAACC	$(AG)_{19}$	196	50
mPgCIR422	F: CCGAAAACACAAGGGTTC R: AGGTTCAAATGGTTTGTGG	$(GA)_{10}$	226	55
mPgCIR429	F: CGAGACTAAAATGAAATCACC R: TTCAACTTTCCAGATCCCTAC	Unknown	217	55
mPgCIR433	F: ATAAATAGCGCCACACTCTC R: CGCAAATCTGTCAAGAGG	Unknown	138	55
mPgCIR434	F: GCCGTCATATCTCAATC R: GGAGGATTTCACTCATTTT	Unknown	140	55
mPgCIR437	F: ACAACAGTTCTGATCCCAAA R: CTCGGAGACACAGAGGTCTA	$(AC)_{10}$	153	55
mPgCIR441	F: TAGGTATGGTTTGAAAGCTC R: GTCTTTCTGCAAATATCCAT	Unknown	179	55
mPgCIR454	F: CCTTTTGTCCGTAGCTTTT R: TCCTTCTTGTTCACATTTGTT	$(AC)_9(CACACA)_3$	184	50

Table 7
SSR marker assay based polymorphism between morphologically contrasting pairs of genotypes in guava (*Psidium guajava*)

Contrasting Parents	**Quantitative trait**	**No of polymorphic SSR loci**					**No of detected alleles**	**Per cent polymorphism**	**Average dissimilarity coefficient**
		Total	Repeat Motiffs						
			Di (2)	Tri (3)	Tetra (4)	Complex			
Benaras and Seedless	Fruit weight	103	60	0	1	3	131	76.2	0.29
Benaras and Local 4	Fruit weight	91	51	0	2	5	120	67.4	0.34
Benaras and Sindh	Fruit weight	89	49	0	2	5	119	65.9	0.33
Benaras and G-1	Fruit weight	96	54	0	2	4	122	71.1	0.28
Benaras and Ceylon Guva	Fruit weight	93	57	0	2	4	125	68.8	0.29
Benaras and Pati	Fruit weight	87	51	1	2	1	117	64.4	0.39
Benaras and EC-147039	Fruit weight	87	56	1	1	1	114	64.4	0.42
Benaras and Red Flesh	Fruit weight	69	42	0	2	1	91	51.1	0.50
Benaras and EC147037	Fruit weight	81	53	0	1	4	113	60.0	0.27
Benaras and Aneuploid 1	Fruit weight	97	56	0	1	2	130	71.8	0.29
Benaras and GR1	Fruit weight	95	54	1	1	4	123	70.3	0.30
Dhareedar and Seedless	Fruit weight	101	66	0	1	4	126	74.8	0.33
Dhareedar and Local 4	Fruit weight	73	45	1	0	2	102	54.0	0.51
Dhareedar and Sindh	Fruit weight	84	50	0	0	3	106	62.2	0.40
Dhareedar and G-1	Fruit weight	85	49	1	1	3	113	62.9	0.38
Dhareedar and Ceylon Guava	Fruit weight	81	50	0	0	4	107	60.0	0.45
Dhareedar and Pati	Fruit weight	82	49	1	1	5	114	60.7	0.43
Dhareedar and EC-147039	Fruit weight	100	61	1	2	5	128	74.0	0.29

Dhareedar and Red Flesh	Fruit weight	90	55	1	1	5	112	66.6	0.35
Dhareedar and EC-147037	Fruit weight	75	49	0	2	2	89	55.5	0.36
Dhareedar and Aneuploid 1	Fruit weight	91	56	0	2	4	122	67.4	0.39
Dhareedar and GR1	Fruit weight	65	40	1	2	3	87	48.1	0.50
Abu Ishaqwala and Seedless	Fruit weight	95	61	0	1	4	126	70.3	0.34
Abu Ishaqwala and Local 4	Fruit weight	88	56	1	0	5	123	65.1	0.38
Abu Ishaqwala and Sindh	Fruit weight	78	47	0	0	3	108	57.7	0.37
Abu Ishaqwala and G-1	Fruit weight	84	51	1	1	4	112	62.2	0.38
Abu Ishaqwala and Ceylon Guava	Fruit weight	78	51	0	2	3	114	57.7	0.44
Abu Ishaqwala and Pati	Fruit weight	100	61	1	1	3	122	74.0	0.28
Abu Ishaqwala and EC-147039	Fruit weight	97	62	0	2	3	130	71.8	0.31
Abu Ishaqwala and Red Flesh	Fruit weight	93	56	1	1	3	121	68.8	0.33
Abu Ishaqwala and EC-147037	Fruit weight	80	52	0	2	4	114	59.2	0.34
Abu Ishaqwala and Aneuploid1	Fruit weight	97	58	1	2	5	128	71.8	0.30
Abu Ishaqwala and GR1	Fruit weight	90	52	1	2	6	128	66.6	0.36
Benaras and Lalit	Outer pulp thickness	60	31	0	1	2	95	44.4	0.56
Benaras and Smooth Green	Outer pulp thickness	33	21	0	0	2	60	25.0	0.77
Benaras and Local 1	Outer pulp thickness	98	53	0	2	5	125	72.5	0.25
Benaras and Portugal	Outer pulp thickness	77	45	0	1	1	108	57.0	0.46
Benaras and Hisar Safeda	Outer pulp thickness	81	48	1	2	2	98	60.0	0.50
Benaras and CIW1	Outer pulp thickness	102	57	1	2	4	123	75.5	0.25
Benaras and Sindh	Outer pulp thickness	88	49	0	2	5	119	65.1	0.33
Benaras and Ceylon Guava	Outer pulp thickness	94	58	0	2	4	126	69.6	0.29
Benaras and EC147039	Outer pulp thickness	85	54	1	1	1	114	62.9	0.42
Benaras and Surka Chitti Neputani	Outer pulp thickness	104	59	1	2	3	127	77.0	0.25
Benaras and Red Flesh	Outer pulp thickness	70	42	0	2	1	93	51.8	0.50
Benaras and CIW4	Outer pulp thickness	94	53	1	1	4	122	69.6	0.30
Benaras and Aneuploid 1	Outer pulp thickness	98	57	0	1	2	130	72.5	0.29
Benaras and Aneuploid 2	Outer pulp thickness	97	59	0	2	2	123	71.8	0.26
Sringeri Seedless and Lalit	Outer pulp thickness	67	39	1	0	3	100	49.6	0.53
Sringeri Seedless and Smooth Green	Outer pulp thickness	63	39	1	1	2	91	46.6	0.54
Sringeri Seedless and	Outer pulp	90	52	0	1	4	120	66.6	0.33

Local 1	thickness								
Sringeri Seedless and Portugal	Outer pulp thickness	84	52	1	0	3	115	62.2	0.47
Sringeri Seedless and Hisar Safeda	Outer pulp thickness	65	39	1	1	3	98	48.1	0.60
Sringeri Seedless and CIW1	Outer pulp thickness	94	61	1	2	4	121	69.6	0.31
Sringeri Seedless and Sindh	Outer pulp thickness	90	51	0	2	4	119	66.6	0.34
Sringeri Seedless and Ceylon Guava	Outer pulp thickness	92	53	0	2	5	121	68.1	0.33
Sringeri Seedless and EC147039	Outer pulp thickness	68	43	0	0	2	100	50.3	0.55
Sringeri Seedless and Surka Chitti Neputani	Outer pulp thickness	104	57	1	2	4	125	77.0	0.26
Sringeri Seedless and Red Flesh	Outer pulp thickness	51	27	1	1	4	83	37.7	0.67
Sringeri Seedless and CIW4	Outer pulp thickness	95	54	1	0	5	117	70.0	0.36
Sringeri Seedless and Aneuploid 1	Outer pulp thickness	96	55	0	1	4	124	71.1	0.31
Sringeri Seedless and Aneuploid 2	Outer pulp thickness	95	61	0	2	3	120	70.3	0.29
Dhareedar and Lalit	Outer pulp thickness	95	53	1	2	4	120	70.3	0.30
Dhareedar and Smooth Green	Outer pulp thickness	88	48	1	2	5	119	65.1	0.32
Dhareedar and Local 1	Outer pulp thickness	87	53	0	1	2	113	64.4	0.39
Dhareedar and Portugal	Outer pulp thickness	82	62	1	2	5	61	60.7	0.26
Dhareedar and Hisar Safeda	Outer pulp thickness	87	54	1	1	5	115	64.4	0.38
Dhareedar and CIW1	Outer pulp thickness	72	44	0	0	2	96	53.3	0.49
Dhareedar and Sindh	Outer pulp thickness	82	50	0	0	3	117	60.7	0.40
Dhareedar and Ceylon Guava	Outer pulp thickness	78	49	0	0	4	105	57.7	0.45
Dhareedar and EC147039	Outer pulp thickness	98	61	1	2	5	127	72.5	0.29
Dhareedar and Surka Chitti Neputani	Outer pulp thickness	91	56	0	1	4	115	67.4	0.36
Dhareedar and Red Flesh	Outer pulp thickness	88	53	1	1	5	110	65.1	0.35
Dhareedar and CIW4	Outer pulp thickness	56	31	1	2	3	84	41.4	0.60
Dhareedar and Aneuploid 1	Outer pulp thickness	86	53	0	2	4	122	63.7	0.39
Dhareedar and Aneuploid 2	Outer pulp thickness	73	42	0	0	3	100	54.0	0.48
Allahabad Safeda and Local 4	Total soluble solids	90	52	0	2	5	120	66.6	0.27
Allahabad Safeda and CIW1	Total soluble solids	97	56	1	2	4	122	71.8	0.28
Allahabad Safeda and Bangalore Local	Total soluble solids	90	49	0	1	4	112	66.6	0.33

Hisar Safeda and Local 4	Total soluble solids	94	59	1	1	5	126	69.6	0.33
Hisar Safeda and CIW1	Total soluble solids	96	60	1	1	4	121	71.1	0.33
Hisar Safeda and Bangalore Local	Total soluble solids	86	48	1	2	4	110	63.7	0.40
Local 1 and Local 4	Total soluble solids	84	53	0	1	3	115	62.2	0.41
Local 1 and CIW1	Total soluble solids	85	54	0	1	2	107	62.9	0.38
Local 1 and Bangalore Local	Total soluble solids	79	50	0	2	4	106	58.5	0.44
Lucknow 42 and Hisar Safeda	Seed hardness	86	52	1	1	3	115	63.7	0.38
Lucknow 42 and Arka Amulya	Seed hardness	83	48	0	0	5	101	61.4	0.40
Lucknow 42 and Florida Seedling	Seed hardness	89	53	1	2	3	117	65.9	0.34
Lucknow 42 and Aneuploid 2	Seed hardness	91	56	0	0	3	113	67.4	0.35
Lucknow 42 and Arka Mridula	Seed hardness	85	50	1	0	5	108	62.9	0.36
Bangalore Local and Hisar Safeda	Seed hardness	86	48	1	2	4	110	63.7	0.40
Bangalore Local and Arka Amulya	Seed hardness	91	58	0	2	5	117	67.4	0.35
Bangalore Local and Florida Seedling	Seed hardness	96	56	1	1	3	115	71.1	0.29
Bangalore Local and Aneuploid 2	Seed hardness	98	55	0	2	4	128	72.5	0.29
Bangalore Local and Arka Mridula	Seed hardness	94	54	0	2	5	123	69.6	0.29
Ceylon Guava and Hisar Safeda	Seed hardness	90	56	0	1	4	121	66.6	0.37
Ceylon Guava and Arka Amulya	Seed hardness	60	33	0	0	4	86	44.4	0.61
Ceylon Guava and Florida Seedling	Seed hardness	99	61	0	2	5	125	73.3	0.28
Ceylon Guava and Aneuploid 2	Seed hardness	91	56	0	0	3	118	67.4	0.37
Ceylon Guava and Arka Mridula	Seed hardness	63	37	0	0	5	95	46.6	0.58
9-35EC147036 and Hisar Safeda	Seed hardness	93	54	1	1	4	112	68.8	0.39
9-35EC147036 and Arka Amulya	Seed hardness	87	59	0	0	3	109	64.4	0.39
9-35EC147036 and Florida Seedling	Seed hardness	102	64	0	2	4	121	75.5	0.28
9-35EC147036 and Aneuploid 2	Seed hardness	87	58	0	0	0	118	64.4	0.41
9-35EC147036 and Arka Mridula	Seed hardness	101	62	1	0	4	122	74.8	0.29
Abu Ishaqwala and Hisar Safeda	Seed hardness	87	53	1	1	3	115	64.4	0.40
Abu Ishaqwala and Arka Amulya	Seed hardness	83	53	0	0	4	115	61.4	0.41

Abu Ishaqwala and Florida Seedling	Seed hardness	95	56	0	2	4	122	70.3	0.33
Abu Ishaqwala and Aneuploid 2	Seed hardness	100	61	0	0	4	122	74.0	0.31
Abu Ishaqwala and Arka Mridula	Seed hardness	81	49	1	0	5	114	60.0	0.42
GR1 and Hisar Safeda	Seed hardness	101	63	1	1	5	125	74.8	0.30
GR1 and Arka Amulya	Seed hardness	78	47	0	2	3	106	57.7	0.46
GR1 and Florida Seedling	Seed hardness	110	64	1	0	5	132	81.4	0.24
GR1 and Aneuploid 2	Seed hardness	87	52	0	2	3	111	64.4	0.41
GR1 and Arka Mridula	Seed hardness	76	44	1	2	4	105	56.2	0.48
Superior Sour Lucidum and Hisar Safeda	Seed hardness	60	35	1	0	2	90	44.4	0.63
Superior Sour Lucidum and Arka Amulya	Seed hardness	94	59	0	1	3	122	69.6	0.34
Superior Sour Lucidum and Florida Seedling	Seed hardness	85	50	1	1	4	116	62.9	0.43
Superior Sour Lucidum and Aneuploid 2	Seed hardness	94	63	0	1	5	125	69.6	0.31
Superior Sour Lucidum and Arka Mridula	Seed hardness	92	58	1	1	3	123	68.1	0.33

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