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Inheritance studies and validation of hybridity in guava (*Psidium guajava*)

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ABSTRACT

The guava (*Psidium guajava* L.) having originated in tropical America and rich in nutrients is extensively cultivated in India. The varieties observed in India are of two types with white and pink pulp. Three white pulp varieties (Allahabad Safeda, Sardar Guava and Apple Colour) and two pink pulp varieties (Purple Local and Thailand) were utilized for crossing and 800 progenies were raised. The ANOVA showed that there is significant variability within the progenies for most of the traits in all the half-sib families. The genotypic variance was observed to be higher than the phenotypic variance for all the characters except TSS indicating non-additive gene action for TSS. Heritability (ns) was observed to be high for all the characteristics except TSS, indicating that heterosis can be exploited for TSS. The genotypic correlation coefficient was observed to be higher than the phenotypic correlation coefficient between pairs of characters, indicating that strong intrinsic correlations are reduced at the phenotypic level. The association of characteristics was observed to be negative between fruit weight and seed hardness, both at the phenotypic and genotypic level, indicating that selecting medium sized fruits would help in isolating progenies with moderately soft seeds. One of the important findings of this study is the co-heritability estimate, which was noticed to be high for all the pairs of characteristics indicates that selection for one character would help in the heritability of the other. Validation of hybridity was carried out using highly polymorphic SSR markers which were selected based on earlier screening at IIHR, Bengaluru. The SSR markers, SSR 220 and 185 gave clear reproducible bands, which clearly confirmed the hybridity of the progenies.

Key words: Correlation, Guava, Heritability, Hybrid, Inheritance, Markers, Traits, Variance

Guava (*Psidium guajava* L) is an important fruit crop of India, said to have originated from tropical America. Guava cultivation is becoming commercially viable as the demand for fresh fruit and processed products is increasing. Basically two types of varieties are noticed in guava, one having white pulp and another having pink pulp. The pink pulp varieties are known to be a good source of a carotenoid called lycopene. However, most of the pink pulp varieties are hard seeded with high acidity. In guava self-pollination has been recorded to an extent of about 80%. Natural cross-pollination reaches up to 35% in some cases in guava (Purseglove 1968). This coupled with heterozygosity has resulted in a large variability in the seedling population from which promising genotypes have been selected. Genetical studies conducted in guava have indicated that red pulp colour is dominant over white and this character is governed monogenically. Many cultivated red-fleshed varieties are heterozygous for this character and bold seeds were found to be dominant over soft seeds and this was also found to be determined monogenically (Subramanyam and Iyer 1993). Production of varieties having pink pulp colour with soft seeds is one of the main breeding objectives of the guava improvement programme. Subramanyam and Iyer (1992), observed linkage between pulpcolour and seed size when they raised a large number of F_1 seedlings by using the cultivars Allahabad Safeda, Red Flesh and Apple Colour. Choice of parents in breeding decides the performance progenies developed. The lack of morphological markers coupled with heterozygosity makes it difficult to decide about the parentage of the progenies and in understanding the inheritance pattern of quantitative characteristics, which is one of the pre-requisites for improvement. Hence, an experiment was undertaken by crossing varieties having pink pulp colour as well as white pulp coloured varieties and validation of the parentage of the progenies raised from the various combinations was carried out through molecular markers. Among the genetic markers microsatellites are being extensively used and downtrodded for the improvement of the guava breeding program by assessing the genetic diversity studies of the Guava germplasm (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 and Nimisha *et al*. 2013). Simple sequence repeats or SSR markers consists of tandem repeats, type of DNA- based that promises to be a reliable marker for

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validation of hybridity, as they high level of allelic diversity per locus, co-dominance and highly reliable . Thus, can be used for genotyping and genetic diversity in both plant and animals (Cholastova and Knotova 2012).

MATERIALS AND METHODS

The guava varieties Apple Colour, Purple Local, Sardar Guava, Allahabad Safeda, and Thailand Guava were utilized for the crossing programme. The varieties Apple Colour, Sardar Guava, Allahabad Safeda have white pulp, whereas the varieties Purple Local, Thailand Guava and Kamsari have pink pulp colour. Hybridization was carried out during 2009, field planting and progeny evaluation were carried out from 2010-14 and the fruit traits were observed for two cropping seasons. The number of crosses, fruit set and seed germination per cent are detailed in Table 1. In each of the combinations, 800 progenies were selected for the analysis. The progenies were evaluated for fruit characteristics, viz. fruit weight (g), fruit length and width (cm), TSS (°Brix) and seed hardness (kg/cm^2). The seed hardness was measured using seed hardness metre, where the pressure required to break the seed was measured by applying external force after placing the seed between two plain surfaces. The fruit characteristics were recorded for all the individual progenies of various combinations, in three replications consisting of five fruits per replication. The SSR primers were used for validating the parentage of the hybrids.

The validation of hybrids was carried out by extracting the genomic DNA using CTAB method proposed by Lodhi *et al*. (1994). A total reaction mixture of 20µl was prepared by adding 15mM complete buffer (from which 2.0µl was taken), 2.5 µl of forward and reverse primer, 4.0 µl of DNTPs, Taq DNA polymerase of 0.2 µl along with 4.85 µl of water and 3 µl of template DNA. PCR conditions were optimized for informative and reproducible finger print profile. DNA amplification was done as per the PCR program of Risterucci *et al.* (2005). The SSRs employed in this study was accessed from Chaitanya *et al.* 2014 (Table 2), amplified PCR products were separated on 3% agarose gel loaded with 100bp ladder (Fermentas). The gels were stained with ethidium bromide and were photographed using UVIPRO platinum gel documentation unit. Molecular weight analysis of the amplified alleles was made in comparison with a 100 bp ladder loaded along with the samples by using UVITEC platinum ID software (ver.12, Cambridge, UK).

Table 1 Details of cross combination, fruit set and seed germination per cent

Cross combination	Flowers crossed (number)	Fruit set (number)	Fruit set* (%)	Seed germination (%)*
Apple colour × Purple local	25	13	52.0	42.8
Purple local ×Apple colour	25	6	24.0	49.6
Purple local × Sardar Guava	20	11	55.0	75.8
Purple local × Allahabad Safeda	25	8	32.0	68.4
Sardar Guava × Purple local	48	10	20.8	52.6
Allahabad Safeda × Purple local	35	15	42.85	48.2
Thailand × Purple local	28	5	17.85	25.8

*Data not analyzed statistically.

Table 2 List of microsatellites employed for the study

Locus ID	Forward sequence (5'-3') Reverse sequence (3'-5')
mgPgCIR-182	F:GAGGAAGAAACCCGAAGTTA R: GGTAGAAAGATCGGAAAGAC
mgPgCIR -185	F: AACGCATCTGGCATTGAT R: CCTTGGTCTCCCTCTTACTC
mgPgCIR -206	F: GAAGTTTCAAAGTAACAGCAC R: AGAATGAGTCCATGCTCAAA
mgPgCIR -220	F: AGAGCAGTGGTTGCTATTTT R: CCCATCTCTTACTTTTCTTGTG
mgPgCIR -236	F: ACTCATATTCCGTTTGCATC R: GAATTAACGACGAGTTCCAC
mgPgCIR -277	F: AGCACTTAGGGACAAATTCA R: CTCACTCTCCTCCATTCAAG

Genetical parameters

The parameters, viz. genotypic variance, phenotypic variance, heritability (ns), phenotypic and genotypic coefficient of variance was estimated utilizing 800 progenies with 100 progenies for each of the cross combination. The PCV and GCV were calculated using the formulae as per Singh and Chaudhury (1977) where PCV is the ratio of square root of phenotypic variance to the mean of that sample, expressed in percent.

The analysis of variance was carried out for 800 progenies with three replications. The phenotypic and genotypic covariance and heritability estimates and variance were calculated

RESULTS AND DISCUSSION

Guava is one of those fruit crops wherein more than 80% self-pollination is recorded. Due to the lack of morphological markers and the presence of heterozygosity, validating the parentage of the hybrids is difficult. Validation of the parentage of a hybrid population helps in the interpretation of inheritance and further helps in the choice of parents to derive at good recombinants. The fruit weight was observed to be least among the parents in the variety Apple Colour (106.0g); maximum fruit weight was noticed in the variety Sardar (205.0g). The TSS was observed to be maximum in the variety Purple Local (9.2°Brix), among the parents seed hardness was noticed to be least in the variety Apple Colour (Table 3). The ANOVA showed that there is significant variability within the progenies for most of the traits in all

Table 3 Mean value of different fruit characteristics for parents

Parents	Crosses	Fruit length (cm)	Fruit width (cm)	TSS (°Brix)	Seed hardness (kg/cm^2)
Apple Colour	106.00	5.12	5.88	8.80	8.20
Purple Local	118.00	5.28	5.90	9.20	10.70
Sardar Guava	205.00	5.72	6.08	8.22	12.80
Allahabad Safeda	195.00	6.00	7.42	7.90	12.30
Thailand guava	167.00	6.90	6.50	8.60	13.00
CD (P=0.05)	48.90	1.17	0.94	0.80	1.62
CV	23.06	15.04	11.11	7.02	10.67

the half-sib families (Table 4). The genotypic variance was observed to be higher than the phenotypic variance for all the characters except TSS (Table 5). This indicated that the environment influences the expression of this particular

Table 4 Analysis of Phenotypic covariance, genotypic covariance, phenotypic correlation, genotypic correlation, coheritability among five characteristics

Character-istics	Pheno-typic co-variance	Genotyp-ic covari-ance	Pheno-typic cor-relation	Genotyp-ic cor-relation	Coherita-bility
1×2	79.08	62.34	0.90	1.00	78.83
1×3	86.35	70.50	0.92	0.98	81.65
1×4	41.17	38.17	0.45	0.96	92.73
1×5	-14.39	-17.41	-0.1	-0.18	121.04
2×3	1.72	1.33	0.93	0.96	76.99
2×4	0.73	0.72	0.4	0.94	97.79
2×5	-0.04	-0.13	-0.014	-0.07	309.5
3×4	0.8	0.78	0.41	0.88	97.61
3×5	-0.63	-0.63	-0.21	-0.30	99.12
4×5	-0.17	-0.17	-0.05	-0.14	102.43

Table 5 Estimation of genotype variance, phenotypic variance, heritability, PCV and GCV

Characteristics	Genotypic Variance	Phenotypic Variance	Herita-bility (ns)	PCV (%)	GCV (%)
Fruit weight (g)	3253.47	2029.19	72.80	54.54	0.12
Fruit length (cm)	1.19	0.83	68.95	23.15	0.05
Fruit width (cm)	1.59	0.79	80.18	23.22	0.05
TSS (°Brix)	0.49	1.53	25.71	12.41	0.02
Seed hardness (kg/cm^2)	2.82	2.52	60.95	17.66	0.03

trait, which suggests non-additive gene action. Hence, it is difficult to make selection based on TSS. However, other traits like fruit weight can be utilized for making selection. Heritability (ns) was observed to be high for all the characteristics except TSS, which also goes to show that this character can be improved by hybridization. The genotypic correlation coefficient was observed to be higher than the phenotypic correlation coefficient between pairs of characters, indicating that strong intrinsic correlations are reduced at the phenotypic level. This happens when genes governing the two traits are similar but the environmental factors pertaining to the expression have a small effect. This indicates the relative stability of the genotypes. The association of characteristics was observed to be negative between fruit weight and seed hardness, both at the phenotypic and genotypic level, which indicates that it is the quantity of pulp that determines the shape and size. It can also be concluded that selecting medium sized fruits would help in isolating progenies with moderately soft seeds. One of the important findings of this study is the co-heritability estimate, which was noticed to be high for all the pairs of characteristics indicating that selection for one character would help in the heritability of the other (Table 6). Reducing the seed hardness is one of the main objectives in the Guava-breeding programme. Variability for seed hardness was observed in the progenies of the half-sib families. Selection of soft seeded progenies is a

Table 6 Analysis of variance for fruit characteristics in half-sib analysis of guava

Cross combination	Treatment (MSS)					Error (MSS)				
	Fruit wt (g)	Fruit length (cm)	Fruit width (cm)	TSS (°Brix)	Seed hardness (kg/cm2)	Fruit wt (g)	Fruit length (cm)	Fruit width (cm)	TSS (°Brix)	Seed hardness (kg/ cm2)
Apple Colour × Purple Local	4366.69	2.80*	2.06*	4.67	2.77	6057.22	1.11	0.97	9.92	4.37
Sardar × Purple Local	3050.67*	1.12*	0.80*	4.44	5.48	1933.24	0.81	0.52	4.10	1.97
Purple Local × Apple Colour	5179.92*	1.49*	1.10*	5.01*	5.32	3076.36	0.83	0.58	3.46	2.06
Purple Local × Sardar	5235.19*	2.05*	1.51*	3.09*	4.53*	1588.38	0.44	0.39	1.71	2.02
Allahabad Safeda × Purple Local	3427.71*	0.95*	0.75*	3.03*	6.23*	2119.79	0.71	0.41	1.57	3.23
Thailand × Purple Local	2827.61*	1.25*	0.94*	3.03*	6.44*	2067.72	0.72	0.55	1.56	3.23
Purple Local × Allahabad Safeda	2774.84	1.41	1.04	3.75	1.04	2023.76	0.60	0.60	2.33	2.23

*Significant.

distinct possibility. There is a strong linkage between seed hardness and pink pulp colour in guava (Subramanyam and Iyer 1982). Dinesh and Yadav (1998) carried out half-sib analysis in the progenies of the variety Apple Colour. They observed that the genotypic variance was lower than the phenotypic variance and heritability to be moderately high for all the characters implying that selection can be practiced for the improvement of fruit characteristics. In this study also the inference is same, although the half-sib progenies are different.

Guava is being utilized both for table purpose and processing, production of pink pulp guava with soft seeds is of prime importance. To break the linkage, one of the methods would be to raise large number of progenies and go in for selection of progenies with desirable traits.

DNA recovery varied from 550 ng/µl to 960.25 ng/µl. The ratio of DNA to protein ranged from 1.62-1.8. Validation of hybridity using SSR markers was carried out for these progenies. A total of six SSR primers were used. Among the SSR markers, SSR 220 and 185 gave clear reproducible bands, which clearly confirmed the hybridity of the progenies. While the remaining primers produced banding pattern which were common to both the parents and were present in the entire set of hybrid progenies too. Manoj *et al*. (2012) observed that the high levels of crossgenera transferability of guava SSRs may be applicable for the analysis of intra- and inter specific genetic diversity of target species study. The slow moving banding patterns seen in this study could not be taken for analysis as these represent conserved sequences of guava. In crops where clearcut morphological markers are not there, validation through SSRs can be used for validation, which in perennial crops shortens the period of breeding.

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