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Studies on crossing behaviour and hybridization in guava

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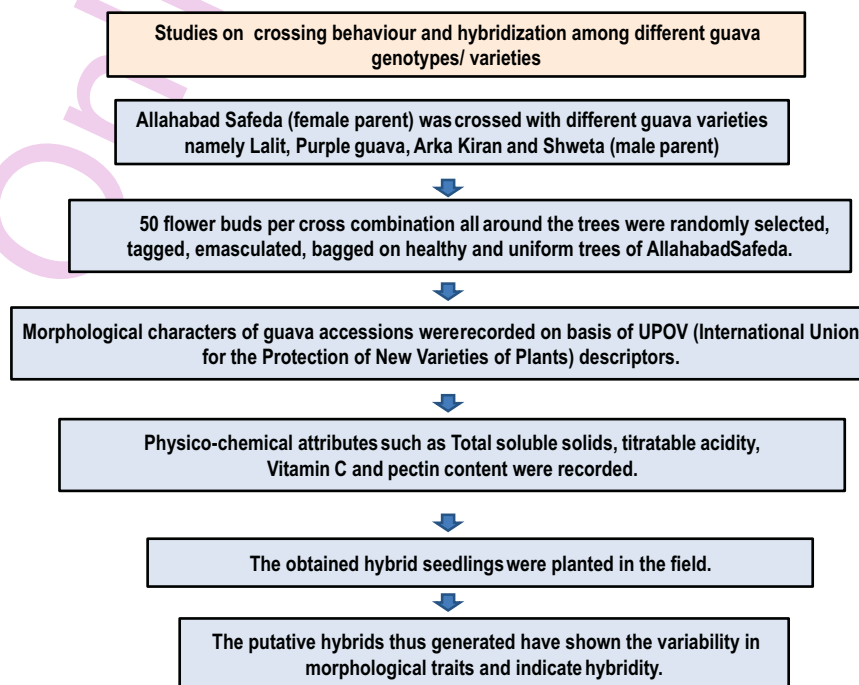
Abstract

Aim: The commercial guava cultivars though having high yield potential, lack the traits of commerce. In other words, development of high yielding guava variety having coloured skin and flesh, with low seed content/soft seeds and better shelf life is need of an hour.

Methodology: The present study on hybridization was undertaken using commercial varieties like Allahabad Safeda, Shweta and the varieties with potential traits like Arka Kiran, Purple guava and Lalit.

Results: Among different crosses, the maximum fruit length (51.25 mm), fruit width (48.70 mm) and fruit weight (71.84 g) were recorded in Allahabad Safeda x Shweta. The lowest number of seeds per fruit (171), minimum seed weight per fruit (1.070 g), seed hardness (13.86 Kg cm⁻²) and highest total soluble solids (7.45 °Brix) were recorded in Allahabad Safeda x Arka Kiran, whereas, the Allahabad Safeda x Purple guava fruits had significantly higher vitamin C (180.14 mg per 100 g fruit) content.

Interpretation: To conclude, the optimum time of crossing involving potential guava germplasm has been standardized. Sufficient number of hybrids as a result of different cross combinations have been obtained, which are likely to have potential traits.



Introduction

Guava fruit is commonly designated as 'apple of the tropics'. It belongs to family *Myrtaceae* which comprises about 150 genera and more than 5,650 species. Floral structure (Epigynous flower, with many stamens of various sizes), self-incompatibility, and heterozygous nature limit the scope of breeding programmes of guava. Germplasm across the genera and species are being used for hybridization studies. Distant hybridization involves crosses between different species of the same genus or different genera. Santos *et al.* (2012) observed that pink and red guava accessions have a greater beneficial potential and should be targeted for breeding programs. Being a cross-pollinated species, substantial variability is available in guava propagated through seeds.

There are a large number of cultivars in India, but only a few with commercial importance such as 'Allahabad Safeda' and L-49 (Sardar) have been identified for commercial cultivation; however, this fact does not affect the existence of a large number of breeding programs. For example techniques such as interspecific crosses (Dinesh *et al.*, 2010a) and induced mutation have been used to bring variability in guava (Pommer, 2012). Inter-specific hybridization between *Psidium molle* and *P. guajava* has led to the development of hybrids (Negi *et al.*, 2007). Seedless varieties of guava have been found to be autotriploids ($2n = 33$); likewise, hybridization between *P. guajava* and *P. guineense* has also been reported (Landrum *et al.*, 1995). Elite commercial guava cultivars, round and pyriformed, were reciprocally crossed to develop hybrids and fruits after crossing were analyzed to explore metaxenial effects on fruit quality (Usman *et al.*, 2013). The present study on inter-varietal hybridization was thus planned involving varieties with coloured skin, flesh and less seed content.

Materials and Methods

Morphological characters of guava accessions were observed on the basis of UPOV (International Union for the Protection of New Varieties of Plants) descriptors (Rodriguez *et al.* 2010). Hybridization and crossing behaviour of guava cultivar Allahabad Safeda (female parent) was assessed with different varieties; Lalit, Purple Guava, Arka Kiran and Shweta (male parent) of guava *i.e.*, with coloured skin, coloured flesh and low/soft seeds. Randomly selected fifty flower buds per combination all around the trees were tagged, emasculated, bagged on healthy and uniform trees of Allahabad Safeda. For observing the time of anthesis, flower buds expected to open the next day were tagged on the previous evening and the number of fully opened flowers were noted at an interval of one hour (starting from 4:30 a.m. in the next morning). To determine the pollen germination, 0.08 % agar-solidified medium and 10 % sucrose solution were used and observed for pollen grains germination within 16-48 hrs. The number of fruits which had set on tagged

flowers were recorded after initial stage of fruit development and number of fruits retained on each tagged flower were counted a week before harvesting. The length and width of mature fruits were measured with the help of digital vernier caliper (Mitutoyo Inc., Japan). The weight of mature fruits, seed weight per fruit and hundred seed weight were recorded with a pan balance and expressed in grams. Extracted seeds from each fruit was counted manually and average was calculated and expressed as number of seeds per fruit. Seed size in each variety was observed visually and expressed under small, medium and bold categories. Seed hardness was measured with the help of hardness tester which showed the hardness of seeds in kg cm^{-2} , whereas, the colour of fruit and flesh was estimated visually. Total soluble solid content of juice was measured with the help of digital hand refractrometer. Acidity was estimated by titrating a known volume of juice against N/10 Sodium hydroxide (NaOH) using phenolphthalein as an indicator. Vitamin C content was measured with 2,6-dichlorophenol indophenol dye (AOAC, 2005). Pectin content as percent calcium pectate per 100 g pulp was estimated using gravimetric method. Seed germination percentage of different F_1 hybrids viz. Allahabad Safeda x Lalit, Allahabad Safeda x Purple Guava, Allahabad Safeda x Arka Kiran and Allahabad Safeda x Shweta were recorded. Young shoot characters were observed at the time of emergence, whereas mature leaf characters were observed during growing season. The colour of young twigs was observed visually and categorized as green and red. Similarly, presence or absence of anthocyanin colouration for fresh leaves were observed visually. The petiole length, leaf blade length and width of ten randomly selected mature leaves were measured with the help of digital vernier caliper (Mitutoyo Inc., Japan). The results obtained during both the years of study (2014-15) were subjected to analysis of variance using RBD design, and the treatment means were compared using least significant difference (LSD) values at a significance level of $P \leq 0.05$ using the procedures of Statistical Analysis System 9.3.

Results and Discussion

Variability in guava varieties for morphological traits: The peak period of anthesis in guava varieties (Allahabad Safeda, Lalit, Arka Kiran, Purple Guava and Shweta) was observed from 6.30 to 8.30 a.m. during rainy season. Similarly, Dhaliwal *et al.* (2002) observed peak period of anthesis and dehiscence from 6.30 to 8.30 a.m. in Bangalore Seedling Selections (6/10, 6/12, 17/7), Sardar Seedling Selections (4/10, 4/12, 6/8), Hisar Surkha, Hisar Sufeda, Dharwad and Sardar. Pollen is an important vector of gene flow in plants particularly for out crossing species. The highest pollen germination of 87.33 % was observed in Allahabad Safeda followed by Lalit (85.30 %), Shweta (85.16 %), Purple Guava (83.59 %) and Arka Kiran (72.35 %). Allahabad Safeda, Shweta, Lalit and Purple Guava varieties had no significant difference between them but these were significantly different from Arka Kiran (Table 1). Likewise, germination of pollen tube

was tested (Coseret *et al.*, 2012) earlier in different guava varieties using staining techniques.

The maximum fruit set (61.22 %) was recorded in Allahabad Safeda x Lalit, followed by Allahabad Safeda x Shweta (55.56 %) and Allahabad Safeda x Purple Guava (54.16 %), while minimum fruit set (45.83 %) was observed in crossed between Allahabad Safeda x ArkaKiran. Dhaliwal *et al.* (2003) reported that the maximum fruit set percentage of 92.42 % was noted in Selection 4/12 variety and minimum of 68.09 % in Dharwad variety during winter season. Chatterjee *et al.* (1992) also observed 64.00 % fruit retention in variety Red Fleshed and 60.00 % in Sardar.

Among different crosses maximum fruit length was recorded in Allahabad Safeda x Shweta (51.25 mm) followed by Allahabad Safeda x Arka Kiran (49.34 mm), Allahabad Safeda x Lalit (48.15 mm) and Allahabad Safeda x Purple Guava (43.50 mm); however, maximum fruit width was observed in Allahabad Safeda x Shweta (48.70 mm) which was closely followed by Allahabad Safeda x Purple Guava (48.39 mm). Allahabad Safeda x Shweta and Allahabad Safeda x Purple Guava crosses had no

significant difference among them but both were significantly different from other crosses. Minimum fruit width of 45.70 mm was recorded in Allahabad Safeda x Lalit followed by Allahabad Safeda x Arka Kiran (46.66 mm).The data pertaining to fruit weight during rainy season crop of Allahabad Safeda x Shweta had significantly higher fruit weight (71.84 g) than remaining crosses (Table 2). It was closely followed by Allahabad Safeda x Arka Kiran (68.53 g), Allahabad Safeda x Lalit (66.24 g) and Allahabad Safeda x Purple Guava (64.32 g). Similarly, Aulakh (2005) also found that the average fruit weight was maximum in BarafKhana (110 g) and minimum in strawberry (68 g).

The fruit surface colour of various guava crosses; Allahabad Safeda x Lalit, Allahabad Safeda x Shweta, Allahabad Safeda x Purple Guava and Allahabad Safeda x Arka Kiran was light green colour at maturity during rainy season crop. The fruit flesh colour of Allahabad Safeda x Lalit, Allahabad Safeda x Shweta, Allahabad Safeda x Purple Guava and Allahabad Safeda x Arka Kiran was observed to be cream colour at maturity.

The highest total soluble solid (7.45 °Brix) content was obtained in Allahabad Safeda x Arka Kiran, which were significantly higher among the other evaluated crosses. Allahabad Safeda x Shweta had 7.42 °Brix total soluble solids and it was also significantly higher than all other crosses (Table 3). In rainy season, the lowest total soluble solids were recorded in Allahabad Safeda x Lalit (7.22 °Brix) followed by Allahabad Safeda x Purple Guava (7.32 °Brix) and the lowest titratable acidity was recorded in Allahabad Safeda x Arka Kiran (0.222 %), followed by Allahabad Safeda x Shweta (0.236 %) and the highest titratable acidity (0.283 %) was obtained in Allahabad Safeda x Purple Guava, which was significantly higher among the evaluated crosses. Allahabad Safeda x Lalit had 0.245 % titratable acidity and it was also significantly higher than all other crosses. The Allahabad Safeda x Purple Guava had significantly higher vitamin C (180.14 mg 100 g⁻¹ fruit) content than remaining crosses. Adrees *et al.* (2010) concluded that maximum vitamin C (220.4 mg 100g⁻¹) and total sugars (6.36%) were found in variety Hong Kong, TSS (11.87%) in Sufaida. It was closely followed by Allahabad Safeda x Lalit (165.77 mg 100 g⁻¹ fruit), Allahabad Safeda x Shweta (161.34 mg 100 g⁻¹ fruit). Anupa *et al.* (2012) also observed the fruit of apple colour (R4P3-32) had a TSS of 15.38 °B with moderate acidity (0.27%) and ascorbic acid (210.01 mg 100g⁻¹).

Guava fruit have a pectin content that is causative to strengthen the tissues (Kumar *et al.*, 2015). The pectin content ranged from 0.800 % in Allahabad Safeda x Shweta to 0.833 % in Allahabad Safeda x Arka Kiran. Allahabad Safeda x Shweta (0.800 %) and Allahabad Safeda x Purple Guava (0.803 %) crosses had no significant difference among them, but both were significantly different from other crosses (Table 3). The pectin percent, significantly decreased at maturity stages and fruit ripening in both seasons of study (Ahmed *et al.*, 2013).

Table 1: Pollen germination percentage among different guava varieties

Varieties	Pollen germination (%)
Allahabad Safeda	87.33 ± 0.70 ^a
Lalit	85.30 ± 0.60 ^a
Arka Kiran	72.35 ± 3.33 ^b
Purple Guava	83.59 ± 1.37 ^a
Shweta	85.16 ± 0.56 ^a
S.E. Mean	2.61
LSD (p ≤ 0.05)	6.02

Means with the same letter are not significantly different (LSD, p ≤ 0.05). Each value represents treatment mean of three replicates ± S.E.

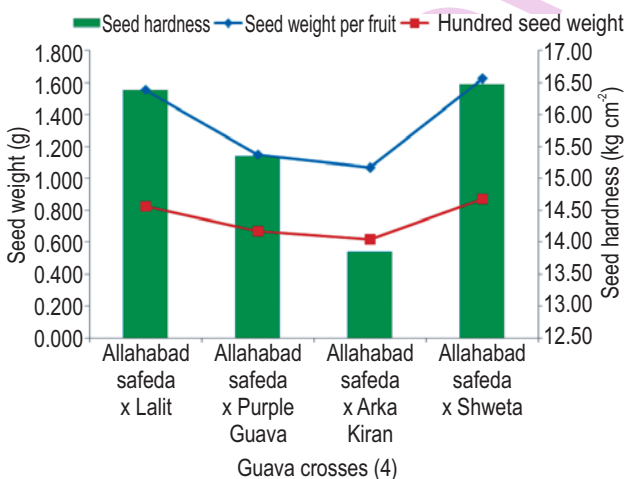


Fig. 1 : Comparative performance of seed hardness, seed weight per fruit and 100-seed weight in different guava combinations

Table 2: Fruit and seed characteristics of different guava cross combinations

Varieties	Fruit length (mm)	Fruit width (mm)	Fruit weight (g)	Seed per fruit (no.)
Allahabad Safeda x Lalit	48.15 ± 1.10 ^b	45.70 ± 0.28 ^c	66.24 ± 0.63 ^{bc}	190 ± 0.88 ^a
Allahabad Safeda x Purple Guava	43.50 ± 0.56 ^c	48.39 ± 0.20 ^a	64.32 ± 0.62 ^c	173 ± 0.67 ^c
Allahabad Safeda x Arka Kiran	49.34 ± 0.63 ^{ab}	46.66 ± 0.09 ^b	68.53 ± 0.54 ^b	171 ± 0.33 ^d
Allahabad Safeda x Shweta	51.25 ± 0.62 ^a	48.70 ± 0.07 ^a	71.84 ± 0.92 ^a	184 ± 0.58 ^b
S.E. Mean	1.22	0.25	0.96	0.45
LSD (p ≤ 0.05)	2.98	0.60	2.36	1.10

Means with same letter are not significantly different (LSD, p ≤ 0.05). Each value represents treatment mean of three replicates ± S.E.

Table 3: Physico-chemical attributes of different guava cross combinations

Varieties	TSS (°Brix)	Acidity (%)	Vitamin C (mg 100 g ⁻¹ fruit)	Pectin content (%)
Allahabad Safeda x Lalit	7.22 ± 0.007 ^d	0.245 ± 0.008 ^b	165.77 ± 2.43 ^b	0.817 ± 0.020 ^{ab}
Allahabad Safeda x Purple Guava	7.32 ± 0.006 ^c	0.283 ± 0.006 ^a	180.14 ± 6.34 ^a	0.803 ± 0.023 ^b
Allahabad Safeda x Arka Kiran	7.45 ± 0.007 ^a	0.222 ± 0.009 ^d	153.30 ± 7.84 ^c	0.833 ± 0.029 ^a
Allahabad Safeda x Shweta	7.42 ± 0.005 ^b	0.236 ± 0.001 ^c	161.34 ± 1.68 ^{bc}	0.800 ± 0.032 ^b
S.E. Mean	0.004	0.001	4.29	0.008
LSD (p ≤ 0.05)	0.009	0.004	10.51	0.019

Means with same letter are not significantly different (LSD, p ≤ 0.05). Each value represents treatment mean of three replicates ± S.E.

Table 4: Vegetative characters of different F₁ hybrids of guava

Hybrids	Colour of young twig	Anthocyanin colour of young leaf	Shape of leaf	Leaf base	Leaf apex
Allahabad Safeda x Lalit	Green	Absent	Elliptical	Obtuse	Obtuse
Allahabad Safeda x Purple Guava	Red	Present	Oblong	Round	Obtuse
Allahabad Safeda x Arka Kiran	Red	Present	Obovate	Obtuse	Round
Allahabad Safeda x Shweta	Red	Present	Obovate	Obtuse	Round

Table 5: Leaf characteristics of different F₁ hybrids of guava

Hybrids	Leaf length (mm)	Leaf width (mm)	Length to width ratio	Petiole length (mm)
Allahabad Safeda x Lalit	113.33 ^b	53.18 ^b	2.085 ^b	2.71 ^c
Allahabad Safeda x Purple Guava	130.79 ^a	64.25 ^a	2.101 ^b	3.95 ^b
Allahabad Safeda x Arka Kiran	139.74 ^a	62.29 ^a	2.186 ^a	4.86 ^a
Allahabad Safeda x Shweta	118.89 ^b	60.50 ^a	2.008 ^c	3.79 ^b
S.E. Mean	26.647	11.158	0.0001	0.012
LSD (p ≤ 0.05)	10.31	6.67	0.02	0.22

Means with same letter are not significantly different (LSD, p ≤ 0.05)

Fruit quality in guava depends upon seed content of fruit and generally guava contains higher seed content when compared to other fruit crops. Breeding new varieties with low seed content is one of the major objectives in guava breeding, so in the present investigation, the data regarding seed number per fruit of different guava crosses revealed that the highest number of seeds per fruit (190) was obtained in Allahabad Safeda x Lalit, and Allahabad Safeda x Shweta had 184 seeds per fruit and was significantly higher than all other crosses (Table 2). In this season, the lowest number of seeds per fruit was recorded in Allahabad

Safeda x Arka Kiran (171) followed by Allahabad Safeda x Purple Guava (173). Marak *et al.* (2007) was also found that out of ten seedlings, A.C.Seln.6/10 noticed, the fruits of this selection had less number of seeds (142 seeds) and on the basis of fruit quality attributes A.C. Seln 6/10 was found to be the best for less seeded commercial cultivation. Variation in seed weight per fruit ranged from 1.070 g in Allahabad Safeda x Arka Kiran to 1.628 g in Allahabad Safeda x Shweta. Significantly higher seed weight per fruit was recorded in Allahabad Safeda x Shweta (1.628 g), which was found at par with Allahabad Safeda x Lalit (1.558 g).

Allahabad Safeda x Arka Kiran (1.070 g) and Allahabad Safeda x Purple Guava (1.150 g) crosses had no significant difference among them but both were significantly different from other crosses. The highest range of 100-seed weight (0.874 g) was obtained in Allahabad Safeda x Shweta, which was significantly higher among the evaluated crosses. Allahabad Safeda x Lalit had 0.827 g 100-seed weight and it was also significantly higher than all other crosses. In this season, the lowest weight of 100-seed weight was recorded in Allahabad Safeda x Arka Kiran (0.616 g). Babu *et al.* (2007) was also studied the seediness was least pronounced in case of Selection-11 (210.40 mg 100 g⁻¹) and Selection-7 (192.50 mg 100 g⁻¹) showed increased content of ascorbic acid.

The seed hardness governs fruit quality in guava, as soft seed is chosen over hard seeds. Breeding for soft seeded cultivars is one of the main objectives of guava breeding programmes and its association with other fruit characters. This trait during rainy season crop; Allahabad Safeda x Shweta has significantly higher seed hardness (16.48 kg cm⁻²) than remaining crosses (Fig. 1). It was closely followed by Allahabad Safeda x Lalit (16.39 kg cm⁻²), Allahabad Safeda x Purple Guava (15.35 kg cm⁻²) and Allahabad Safeda x Arka Kiran (13.86 kg cm⁻²). The seed size of Allahabad Safeda x Lalit and Allahabad Safeda x Shweta were bold at maturity.

A total of 398 F₁ hybrids; Allahabad Safeda x Lalit, Allahabad Safeda x Purple Guava, Allahabad Safeda x Arka Kiran and Allahabad Safeda x Shweta of guava were developed involving one female (Allahabad Safeda) and four male parents (Lalit, Purple Guava, Arka Kiran and Shweta) with the objective to develop high yielding, better keeping quality, coloured skin and flesh and low/soft seeded fruits. The seed germination percentage and morphological parameters (only leaf) were recorded in different F₁ hybrids to distinguish among themselves.

High heterozygosity and numerous cross pollination are resulted in the existing variability in seedling populations from which promising varieties have been selected (Dinesh *et al.*, 2010b). Seed germination is an estimate of the viability of a population of seeds; the highest seed germination percentage (67.58 %) was observed in Allahabad Safeda x Purple Guava hybrid, followed by Allahabad Safeda x Arka Kiran (53.34 %) and Allahabad Safeda x Shweta (49.34 %). However, the lowest seed germination percentage was (34.37 %) recorded in Allahabad Safeda x Lalit hybrid.

Variation in young twig colour was observed among different hybrids which varied from green to red. Red coloured young twigs were found in cross combination viz; Allahabad Safeda x Purple Guava, Allahabad Safeda x Arka Kiran and Allahabad Safeda x Shweta. Anthocyanin colouration of young leaf was also present in these hybrids, whereas green coloured twigs were found in Allahabad Safeda x Lalit and the young leaves

in this hybrid were also green due to absence of anthocyanin colouration (Table 4). Intensity of anthocyanin colouration in young leaves was observed to be strong in hybrids Allahabad Safeda x Purple Guava, Allahabad Safeda x Shweta and of weaker intensity in Allahabad Safeda x Arka Kiran. Variation in leaf shape was observed among the obtained hybrids. Leaves were categorized according to different leaf shapes as elliptical in Allahabad Safeda x Lalit with obtuse leaf base and apex. Obovate leaves were observed in Allahabad Safeda x Arka Kiran and Allahabad Safeda x Shweta with obtuse leaf base and round leaf apex. However, oblong leaf shape was observed in Allahabad Safeda x Purple Guava with round leaf base and obtuse leaf apex.

Leaf blade length ranged from a minimum value 113.33 mm in Allahabad Safeda x Lalit to a maximum value of 139.74 mm in Allahabad Safeda x Arka Kiran which was at par with Allahabad Safeda x Purple Guava with the value 130.79 mm. Hybrids Allahabad Safeda x Lalit and Allahabad Safeda x Shweta were at par among each other and Allahabad Safeda x Lalit had least value for leaf length (Table 5). (Singh *et al.*, 2016) also observed a significant variation in leaf length among different guava varieties ranging from 101.89 mm in Lalit to 151.27 mm in Punjab Pink, while, maximum leaf blade width was recorded in L-49 variety (65.54 mm). Statistically significant variation for leaf blade width values were observed among tested hybrids. Highest for leaf blade width (64.25 mm) was observed in Allahabad Safeda x Purple Guava, which was statistically at par with Allahabad Safeda x Arka Kiran and Allahabad Safeda x Shweta with the values 62.29 and 60.50 mm, respectively whereas least average value for the same trait was recorded in Allahabad Safeda x Lalit having a value of 53.18 mm. In different hybrids, variation in leaf length to width ratio was observed varying from a minimum value of 2.008 in Allahabad Safeda x Shweta to the tune of 2.186 in Allahabad Safeda x Arka Kiran and these were significantly different from each other. Allahabad Safeda x Lalit (2.085) was found to be statistically at par (2.101) with Allahabad Safeda x Purple Guava.

Petiole length among different hybrids also varied significantly with maximum (4.86 mm) value recorded in Allahabad Safeda x Arka Kiran and minimum (2.71 mm) in Allahabad Safeda x Lalit. With respect to petiole length, hybrid Allahabad Safeda x Arka Kiran differed significantly from all the hybrids and was followed by Allahabad Safeda x Purple Guava and Allahabad Safeda x Shweta with the values 3.95 and 3.79 mm, respectively and these hybrids were statistically at par among each other. Among all the hybrids, green midrib colour on lower surface of leaf was observed in Allahabad Safeda x Lalit and Allahabad Safeda x Arka Kiran. Red midrib colour on lower surface of leaf was observed in Allahabad Safeda x Purple Guava and Allahabad Safeda x Shweta. Likewise, Boora *et al.* (2012) obtained a potential hybrid Punjab Pink a cross between Portugal



(A) Allahabad Safeda x Arka Kiran



(B) Allahabad Safeda x Purple Guava



(C) Allahabad Safeda x Lalit



(D) Allahabad Safeda x Shweta

Fig. 2 : Colour of young twig and anthocyanin pigmentation of young leaf in different guava F1 hybrids

XL-49 = F₁ X Apple Colour. Patel *et al.* (2011) also made nineteen crosses, the seedlings of fourteen crosses were well established in the field and six hybrids flowered and fruits were harvested and evaluated for fruit characteristics. The fruit of hybrid "Anand Selection (Red) x Exotica" was large in size, had pleasant flavour and high TSS with soft seeds.

To conclude, the commercial guava variety Allahabad Safeda was crossed with potential varieties having traits of commerce such as coloured skin/flesh and with soft/low seed number. The potential varieties included Lalit, Purple Guava, Arka Kiran and Shewta were used as male parents. The obtained hybrid seedlings have been planted in the field. The putative hybrids, thus generated have shown variability in morphological traits and indicate hybridity. Thus in present study, appropriate hybridization methodology has been work out to obtain guava hybrids. The coloured hybrids are likely to be the outcome of the present study which would help in boosting guava cultivation in the northern region of country.

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