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Black guava (*Psidium guajava* L.): Morphological, biochemical and molecular characterization for pulp colour

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ABSTRACT

Guava is known as "Super fruit"; it is the third richest source of Vitamin C. In this study Black guava is characterized morphologically, biochemically and on molecular basis. Result revealed that Black guava had the maximum total anthocyan in content (9.663 mg/100g) compared to white and pink pulped counterparts. Pink pulped Hisar Surkha and Lalit genotypes contained both lycopene and anthocyanin while another pink pulped genotype, Punjab Pink, had only lycopene. Further, all the eight guava genotypes irrespective of their pulp colour had total carotenoids (0.203 mg/100g - 0.730 mg/100g). Based on our results, the Black guava had unique morphological features like leaf colour, shoot colour and fruit pulp colour. The pulp colour of Black guava is due to the presence of anthocyanins. In general, the pulp colour of guava is due to combinations of more than one pigment and the final colour depends on the relative proportion of those pigments and suggests that "pigments in proportions" explains the pulp colour in guava. Anthocyanin pathway study in Black guava revealed that the primers for DFR and CHS gene of anthocyanin pathway amplified in Punjab Pink and not in case of Black guava.

Key words: Anthocyanins, carotenoids; colour, guava, lycopene, trait

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the most important and commercially cultivated fruit crops from the Myrtle family. Guava fruit is the third richest source of vitamin C (299 mg/100g); it is also a rich source of vitamin A (0.46 mg/100g), calcium (17.8-30 mg/100g), iron (200-400 IU/ 100g) and phosphorus (0.30-0.70 mg/100g) while guava seeds are rich in omega-6, omega-3polyunsaturated fatty acids and dietary fiber (0.9-1.0 g/100g) (Kamath *et al.* 2008). The vitamin C content of guava is 2-5 times more than citrus (Singh, 2005). Although cultivated guava belongs to same species of a single genus it has considerable variations. Red/pink pulped (Anakpalli, Hafsi, Red fleshed, Banarasi Surkha, Lalit, Arka Kiran, Hisar Surkha), white pulped (Allahabad Safeda, Chittidar, Sardar, Smooth green, Nasik, Seedless, Shweta, Hisar Safeda), red peel coloured (Apple Colour) and both red peeled and pulped (Allahabad Surkha) (Dinesh and Vasugi 2010). White pulped guava has better anti-oxidant activity and are better source of vitamin C as compared to pink pulped guava. However, coloured guavas are considered more nutritious than yellow green guavas as they contain more pigments such as pro-vitamin A, carotenoid, polyphenols, and retinoid (Joseph and Priya 2011). Especially, pink pulped guavas are a good source of lycopene and contain even more lycopene than tomato (Bramley 2000). Thus, lycopene has shown to be responsible for pink coloured pulp of guava. Further, pink guava is suitable for fresh as well as processing purpose. In plants, lycopene synthesis takes place in plastids by enzymes that are nuclear encoded through 2-C-methyl-D-erythritol- 4-phosphate (MEP) pathway (Phillip *et al.* 2008). There are many pink pulped guava varieties across the world, namely, Ruby, Hong Kong Pink, Red Guava, Paluma, Beaumont, Fan Retief (Mitra *et al.* 2018). Mondragon *et al.* (2010) reported that Mexican guava had lycopene content ranging from 0.54 to 3.28 mg/100g. Indian guava germplasm is still richer in lycopene content with Hisar Surkha (14.691 mg/100g) containing maximum lycopene followed by Arka Kiran (7.45 mg/100 g), Punjab Pink (6.163 mg/100g) and for Lalit (6.067 mg/100g) (Dinesh and Vasugi 2010, Thakre *et al.* 2016). The available classifications pulp colour in guava do not include purple colour for peel (Ruehle 1948) and hence Black guava,

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.a purple peeled and pulped genotype, has been grouped along with the pink ones and were expected to contain more amount of lycopene as in to other pink pulped genotypes. Our preliminary studies indicated Black guava, (as per grey purple group as per Royal colour chart) had negligible amount of lycopene (0.16 mg/100g) as compared to pink pulped guava genotype. So the present work designed to characterize Black guava on morphological, biochemical and molecular basis to find out unique features which makes it very different from other guava genotypes.

MATERIALS AND METHODS

This study was carried out at the guava germplasm block, Todapur orchard, Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi. The experimental site is situated at the altitude of 228 MSL and 280 40º N 770 13º E. The climatic condition of area is sub-tropical with alluvial soil which was slightly alkaline. The soil texture was clayey loam with low organic matter content. The experiment was conducted during 2020-21. Eight guava genotypes, comprising of four coloured pulp genotypes, Black guava , Hisar Surkha, Punjab Pink, Lalit, and four white pulped genotypes, Allahabad Safeda, Pant Prabhat, Shweta and Trichy-1formed the materials of the present study. The guava orchard had the trees planted at a distance of 6m x 3m and was managed with standard cultural practices. Physical parameters of fruits, namely, fruit weight, fruit length, width, core diameter, pulp thickness were recorded and fruit length to width ratio was also calculated.These eight guava genotypes were characterized morphologically as per UPOV guidelines (1983). Lycopene was extracted using hexane:ethanol:acetone (2:1:1) (v/v) mixture following the method of Gordon and Diane (2007); Godwin *et al.* (2015) with some slight modifications. Anthocyanin was extracted by using acidified methanol following the method of Paul *et al.* (2018) with slight modifications. For estimation of total carotenoid content, two gram of crushed fruit was taken and 20 ml acetone was added. This solution was kept overnight. Hexane: water (15ml:10ml) was added into that solution to separate the hexane layer with a separating funnel. The total volume of pigmented hexane layer was taken out by filtration with anhydrous sodium sulphate. Part of this layer was taken in a cuvette and carotenoid content was measured at 450 nm in spectrophotometer.

Carotenoid content (mg/100g) = O.D. (450) ×volume of separated×3.8×100/ 2×1000

One-way analysis of variance (ANOVA) was performed to test the significant difference among various genotypes on various physical and biochemical fruit attributes using PROG GLM of SAS software package, version 9.4 (SAS Institute, Cary, North Carolina, USA). Further, Tukey's Honest Significant Difference (HSD) test was used to identify the pair-wise significant differences among the genotypes which were significant, as per ANOVA. Least significant difference (lsd) or Critical Difference (C.D.) for each attributes was also worked out. Correlation analysis was also performed among various attributes using PROCCORR of SAS. Statistical significance was assessed at P≤0.05level.DNA was isolated from young healthy leaves of Black guava and Punjab Pink by using the modified CTAB method (Doyle and Doyle 1990). DNA Purification was done by RNAase (10mg/ml) and Chloroform: Isoamyl alcohol (CI) mixture (24:1). DNA quantification was done by using Nanodrop (ND8000, Thermoscientific) and quality of DNA was checked by 0.8% agarose gel along with diluted uncut lambda DNA as standard. Finally part of the DNA samples was diluted with appropriate amount of TE buffer to yield a working concentration of 25 ng /µl and stored at 4° C.

RESULTS AND DISCUSSION

Morphological characterization

As far as tree characters are concerned, Black guava belonged to separate class for attitude of branches, young shoot: colour of stem, and fully developed shoot: thicknesses of stem. Attitude of branches was erect in Black guava. Whereas remaining seven guava genotypes Hisar Surkha, Punjab Pink, Lalit, Pant Prabhat, Shweta, Trichy-1, Allahabad Safeda had spreading growth habit. The attitude of branches is a very important trait as it decides many plant features and cultural practices like planting distance, mechanical strength of the branches, pruning method, the incidence of disease-pest and colour development on fruit. This trait is related to crotch angle of branches

which is genetically governed (Petersen and Krost 2013). Young shoot colour of Black guava was dark red whereas it was green in the rest of seven guava genotypes. This is very important trait from breeding perspective as it can be act as a morphological marker in F_1s.Thickness of stem of fully developed shoot was medium in case of Black guava. It was thin in Hisar Surkha Punjab Pink, Lalit, Shweta and thick in case of Pant Prabhat, Trichy-1 and Allahabad Safeda.

Black guava also showed considerable variation with respect to leaf characters. It had dense pubescence on lower side of young leaf, whereas the other guava genotypes Punjab Pink, Allahabad Safeda, Hisar Surkha, Lalit, Shweta, Pant Prabhat, Trichy-1 had sparse pubescence on lower side of young leaf. Black guava had reddish coloured midrib on lower side. Pubescence on lower side of fully developed leaf was also dense whereas, in the remaining seven guava genotypes it was sparse. Pubescence play a very important role in harvesting of dew water, this results in the decrease in water potential gradient between leaf interior and exterior and finally reducing water loss by evaporation (Konard *et al.* 2014). It helps the plant to survive better under water stress condition. It is a very important trait in the context to water stress and climate change. Leaf colour of fully developed leaf of Black guava was completely different then the classes mentioned and belonged to grayed purple group; other guava genotypes belonged to the class green. This is a morphological marker for identification of Black guava and can also be acted as morphological marker for F_1s whereas other genotypes have green leaf colour. Owing to this character, Black guava can be identified from a distance. Fruit juiciness in Black guava was medium whereas the other guava genotypes were juicy.

The fruit skin (peel) and flesh (pulp) colour of Black guava is completely different than the other seven guava genotypes (Table 1). The pulp colour of Black guava was belonged to greyed orange group (186B), which was completely unique as compared to other seven guava genotypes. Hisar Surkha was belonged to red group (38C), Lalit belonged to red group (52C) and Punjab Pink belonged to red group (37A). These three guava genotypes were basically categorised under pink pulped guava genotypes. Under white pulped category, Pant Prabhat, Allahabad Safeda, Trichy-1 and Shweta belonged to yellow white group with sub class 158A, 158A-145C, 158C and 158C respectively. For remaining leaf and fruit characters, Black guava did not belonged to separate class. However, Indian guavas are broadly categorized into four classes, red/pink pulp, white pulp, red peel with white pulp and coloured peel as well as pulp wherein colour refers to only red or pink colour (Dinesh and Vasugi 2010). Again in UPOV guidelines (1987) the colour of flesh is categorized into cream (1), white (2), pale pink (3), pink (4), dark pink (5), orange pink (6) orange (7) while fruit skin is categorized into pale-yellow-greenish (1), pale yellow (2), dark yellow (3), orange (4), orange green (5), dark green (6) and red (7). Thus, none of these classifications mention purple colour for defining either the peel or pulp except that of Ruehle (1948) wherein purple is mentioned for the pulp but not peel. Hence, one of the experimental materials, Black guava could not be classified properly according to the available classification for guava. It is unique with respect to leaf, shoot and fruit colour.

Table 1: Peel and pulp colour of eight guava genotypes showing variation in peel and pulp colour

Genotype	Peel colour	Pulp colour
Black Guava	Greyed orange group (174A)	Greyed orange group (186B)
HisarSurkha	Yellow orange group (18B)	Red Group (38C)
Pant Prabaht	Yellow green group (154B)	Yellow white group (158A)
Allahabad Safeda	Yellow green group (145B)	Yellow white group (158A) Yellow green group (145C)
Trichy-1	Yellow green group (140C) Yellow group (8A)	Yellow white group (158C)
Shweta	Yellow green group N144	Yellow white group (158C)
Lalit	Red group (53B)	Red group (52C)
Punjab Pink	Green Yellow group(1B)	Red Group(37A)

Biochemical characterization

Among the eight guava genotypes considered in the present study, four were coloured and four were white pulped. Maximum lycopene content (8.798 mg/100g) was recorded in Hisar Surkha followed by Lalit (4.594 mg/100g) and Punjab Pink (3.334 mg/100g) which were significantly different from one another (Table 2). Black guava contained very little amount of lycopene i.e. 0.517 mg/100g while the white pulped guava genotypes had negligible amount of lycopene ranging from 0.05 to 0.069 mg/100g. It is very interesting to note that maximum total anthocyanin content (9.663 mg/100g) was found in Black guava which had minimum lycopene content among the coloured group. Additionally, another coloured variety, Hisar Surkha with pink pulp, also had appreciable amount of anthocyanin (7.863 mg/100g) though it was less than that of Black guava. However, another pink pulped variety, Punjab Pink had minimum and negligible amount of anthocyanin (0.108 mg/100g). All the white pulped guava genotypes, Allahabad Safeda, Shweta, and Trichy-1 had negligible amount of total anthocyanins ranging from 0.135-0.548 mg/100g except Pant Prabhat (3.086 mg/100g). Irrespective of pulp colour, total caroenoids were present in all eight guava genotypes in a comparatively smaller range of 0.203 mg/100g to 0.730 mg/100g. However, as in the case of anthocyanin, except Pant Prabhat (0.573 mg/100g), remaining three white pulped guava genotypes Allahabad Safeda (0.203 mg/100g), Shweta (0.213 mg/100g), Trichy-1 (0.277 mg/100g) recorded significantly lower values as compared to the coloured guava genotypes. Black guava (0.730 mg/100g) followed by Lalit (0.683 mg/100g) recorded maximum total carotenoids. Other pink pulped genotypes also had appreciable amount of carotenoids.

The trend of presence and absence of these three pigments also explains many aspects of pulp colour in guava. As discussed above, our analysis revealed that total anthocyanins were higher in Black guava and it is solely responsible for its purplish pulp colour. But, Hisar Surkha which has pink pulp with maximum lycopene content also contained appreciable quantity of anthocyanins (second highest after Black guava). Similarly, Lalit also contained lycopene and some amount of anthocyanins. It means that anthocyanins also contribute to the pink pulp colour of Hisar Surkha and Lalit, besides lycopene, though these two genotypes differed in their pigment proportion. Thus, these two pink pulped guava genotypes have functional lycopene and anthocyanin pathway. Only in case of Punjab Pink, lycopene alone is contributing for its pink pulp since the total anthocyanin content is negligible. In chrysanthemum cv. “Kastelli” also, carotenoids and antocyanins contributed to its orange red colour. It contained higher level of carotenoids and a moderate level of anthocyanins (Park *et al.* 2015).

Table 2: Biochemical fruit parameters of guava genotypes showing variation in pulp colour

S. No.	Genotype	Lycopene content (mg/100g)	Total anthocyanins (mg/100g)	Total carotenoids (mg/100g)
1.	Black guava	0.517^{d}	9.663^{a}	0.730^{a}
2.	HisarSurkha	8.798^{a}	7.863^{b}	0.547^{b}
3.	Punjab Pink	3.334^{c}	0.108^{f}	0.467^{c}
4.	Lalit	4.594^{b}	2.192^{d}	0.683^{a}
5.	Pant Prabhat	0.050^{d}	3.086^{c}	0.573^{b}
6.	Shweta	0.078^{d}	0.210^{ef}	0.213^{de}
7.	Trichy-1	0.087^{d}	0.135^{ef}	0.277^{d}
8.	Allahabad Safeda	0.069^{d}	0.548^{e}	0.203^{e}
	SEm±	0.20	0.09	0.01
	LSD (P=0.05)	0.59	0.26	0.04
	C.V.	15.48	5.02	5.04

Molecular characterization

The PCR amplification indicates that primers for *DFR* gene, D4 and D5 amplified in Punjab Pink but did not amplify in case of Black guava. Similarly, in case of primers for *CHS* gene C1a, C1 and C3 amplified in Punjab Pink but not in case of Black guava. Sequencing of

the amplified products has been performed for their further utilization in future studies. The sequences submitted to NCBI (National centre for Biotechnology Information) and they have accession number OM337779 (partial sequence of gene DFR in guava), OM337780 (partial sequence of gene *CHS* in guava) and OM337781 (partial sequence of gene *CHS* in guava). The primers for *DFR* and *CHS* gene amplified in Punjab Pink and not in case of Black guava. This may be due the fact that both genes belong to multigene family. Second reason could be that the gene responsible for anthocyanins in Black guava is structurally different than the other guava genotypes.

In conclusion, Black guava is very different as compared to other seven guava genotypes. It is having unique leaf, shoot and fruit colour. The pulp colour of Black guava is due to the pigment anthocyanins. It is having different anthocyanin genes regulating its pulp colour as compared to other guava genotypes. It is having exclusive scope of utilization as parent for incorporation of pulp colour in the progeny with additional benefits of morphological markers as leaf and shoot colour.

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