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Variation in fruit quality traits and health promoting compounds of citrus fruits grown in semi-arid region

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ABSTRACT

The present study was conducted to assess the variation in quality characteristics of nine commonly cultivated citrus genotypes grown in semi-arid region. Fruit weight varied from 130g ('Minneola') to 622g ('Pummelo Red Flesh') within different groups of citrus fruits. Juice content ranged from 31% ('Pummelo Red Flesh') to 54% ('Mosambi'). Pummelo fruits were larger in size than fruits of other citrus groups. TSS varied from 8°B ('Dancy') to 11°B ('Pummelo Red Flesh'), while ascorbic acid varied from 32 to 54 mg/100g. The maximum phenolic content was recorded in 'Pummelo White Flesh' fruits (44.66 mg GAE/100g) and the minimum was found in 'Minneola' (23.33 mg GAE/100g). The fruits of 'Pummelo Red Flesh' and 'Pummelo White Flesh' had highest flavonoids of 50.85 mg QE/100g and 48.10 mg QE/100g, respectively. The antioxidant activity was also high in the fruits of pummelo and grapefruit as compared to others. Principal Component Analysis revealed that TSS and acid content were the major quality attributes governing the citrus acceptability. Total flavonoid content and the *in vitro* FRAP antioxidant assay were the best to determine the functional quality of the fruits. Biplot analysis grouped the genotypes into two groups and the result was further verified by Agglomerative Hierarchical Clustering technique which too yielded similar results.

Key words: *Citrus* species, TSS: acid ratio, antioxidant activity, flavonoids, phenolic content, FRAP.

INTRODUCTION

Citrus fruits have been recognized as an important dietary component and are relished for their distinct aroma, delicious taste and abundant amount of nutrients. Consumption of citrus fruits is believed to ameliorate different diseases owing to the presence of bioactive compounds beneficial to human health (Zou *et al*., 16). The abundant presence of vitamin C (ascorbic acid) plays an important role in disease prevention (Dhuique-Mayer *et al*., 2). In addition, the fruits contain substantial amounts of vitamin B complex, carotenoids, flavonoids, antioxidant capacity and limonoids.

Genus citrus includes a large number of species such as sweet orange, mandarin, grapefruit, pummelo, tangelo, tangerine etc. Currently, the production of citrus in India is 12.54 million MT from an area of 1003 thousand ha (Anonymous, 1) that is still on an uprise. However, the research on appraising the quality of citrus fruits produced in the country, particularly with reference to bioactive compounds has not been properly carried out. Explorative studies on determining the bioactive potential of locally grown citrus fruits is needed to further use them for development of novel/elite species through breeding programs. Hence, in the present study quality characteristics of nine commonly cultivated citrus genotypes grown under semi-arid region were assessed with respect to their physico-chemical attributes and bioactive components.

MATERIALS AND METHODS

Nine citrus genotypes (sweet orange: 'Itaborai', 'Hamlin' and 'Mosambi'; grapefruit: 'Duncan' and 'Marsh Seedless'; pummelo: 'Pummelo Red Flesh' and 'Pummelo White Flesh'; tangelo: 'Minneola' and tangerine: 'Dancy') were procured from the main garden of Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi in 2019 during the months of October-November having typical sub-tropical hot and humid climate. Fruits were harvested at their optimum physiological maturity stage (TSS/acid ratio ≥ 12). All fruits were harvested from uniformly maintained healthy trees budded on *Jatti Khatti* (*C. jambhiri*).

Fruit dimensions such as length and width and peel thickness of 10 randomly selected fruits of each genotype were measured with vernier caliper (Miyototo, Japan). Average fruit weight was calculated by weighing ten randomly selected fruits using an electronic balance (Citizen, New Delhi). The juice was extracted by electronic citrus juicer (Sujata Citromatic, India) and juice content was calculated on volume by weight basis. Fruit firmness (Newton),

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recorded as the peak force was determined by texture analyzer (TA-XT2, Stable Micro Systems, Surrey UK) using 2 mm cylinder probe with a test speed of 5 mm/s and a distance of upto 10 mm.

Total Soluble Solids (TSS) was determined using a hand held refractometer (ATAGO make) while titratable acidity was estimated by titration with standard alkali and calculated in terms of per cent citric acid. The ascorbic acid content was determined by the method prescribed in Kumar *et al*. (5). The Folin-Ciocalteau method was used to determine the total phenolic content (Xu and Chang, 13). The aluminum chloride method of Nayak *et al*. (7) was used to determine the total flavonoid content.

Since, no single assay can determine the full antioxidant potential of any fruit, the total antioxidant capacity of citrus juice was analyzed by four different assays, namely, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), FRAP (ferric reducing antioxidant power), CUPRAC (cupric ion reducing antioxidant capacity) and ABTS (2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) (Sethi *et al*., 10; Nishad *et al*., 8).

Data were analyzed by two way analysis of variance (ANOVA) using SAS (r) Proprietary Software 9.4 (TS1M1). The ANOVA technique was followed by multiple comparison procedure to compare the significant difference between the cultivar means using t-test based on Least Significant Difference value. Principal Component Analysis (PCA) which is a multivariate statistical technique was used to determine the significant attributes that govern the quality and compositional profile of the citrus fruits. Based on PCA results, bi-plot analysis was carried out to see the impacts of different attributes on different citrus cultivars. The results were again verified by Agglomerative Hierarchical Clustering (AHC) techniques and the similarity between the cultivars was presented as a dendrogram. For PCA, bi-plot analysis and AHC, R software version 3.6.3 [(2020-02-29) Platform: x86_64-w64-mingw32/x64 (64-bit)] software was used.

RESULTS AND DISCUSSION

Physical characteristics such as fruit dimensions in terms of length and breadth govern the visual appeal of the fruits. Peel thickness further determines the per cent edible portion in the fruit. The data on fruit length, breadth and peel thickness of nine citrus cultivars are presented in Table 1. Tangelo cv. 'Minneola' had the least fruit dimensions in terms of width (5.5 cm) and length (6.03 cm). Maximum fruit length (9.87 cm) and fruit width (8.83 cm) was exhibited by fruits of 'Pummelo Red Flesh'. The variation observed in the citrus fruit shape and size is a function of genotypic variability. Significant variation in peel thickness was observed among the cultivars. Cultivar 'Dancy' showed the least peel thickness (1.6 mm) while

Table 1. Physical parameters of citrus genotypes grown under semi-arid region.

Genotype	Fruit length (cm)	Fruit width (cm)	Peel thickness (mm)
'Itaborai'	7.02^{cd}	6.43^{bc}	3.0^{bcd}
'Hamlin'	6.63^{d}	5.87^{c}	3.3^{bcd}
'Mosambi'	6.43^{d}	5.83^{c}	4.1^{bc}
'Dancy'	6.30^{d}	6.13^{c}	1.6^{d}
'Minneola'	6.03^{d}	5.50^{c}	2.0^{cd}
'Marsh Seedless'	8.33^{bc}	7.57^{ab}	5.0^{b}
'Duncan'	8.93^{ab}	8.33^{a}	4.8^{b}
'Pummelo White Flesh'	9.13^{ab}	8.33^{a}	8.1^{a}
'Pummelo Red Flesh'	9.87^{a}	8.83^{a}	8.1^{a}

Means with same superscript are not significantly different at $p \leq 0.05$.

'Pummelo White Flesh' and 'Pummelo Red Flesh' recorded the highest peel thickness of 8.1 mm (Table 1). Zambrano *et al*. (14) also found variation in the peel thickness of Valencia orange, which was due to genetic variation among the cultivars along with crop load, pollen influence etc. As shown in Fig. 1, fruits of 'Pummelo White Flesh' exhibited the maximum firmness (1880.08 N), while it was lowest (1160.87 N) for the fruits of 'Dancy'. Such variation might be due to the thickness and cell wall composition of rind of the different citrus genotypes (Muramatsu *et al*., 6).

Medium sized fruits usually hold a better appeal with the consumers. Fruit weight of different citrus cultivars was found to vary significantly (Fig. 1). 'Minneola' fruits weighed the least (130g) and maximum fruit weight was found in case of 'Pummelo Red Flesh' (622g). The fruit weight is directly correlated to the fruit size with bigger fruits, recording highest fruit weight (Shinde *et al*., 11). Citrus fruits are relished for their juiciness. Juice content is also an important attribute required in the beverage industry. In this study, a significant variation in juice content was observed amongst the citrus cultivars (Fig. 1). Sweet orange cultivar 'Mosambi' yielded the highest juice content (54.4%), while it was lowest in 'Pummelo Red Flesh' (31.18%). The variation in juice yield among the cultivars could be attributed to their genetic makeup as well as their pulp to peel ratio (Kashyap *et al*., 4).

Analysis of chemical composition of fruits is important as it signifies the palatability, consumer acceptance and nutritional value. The citrus genotypes were analyzed for their TSS, titratable acidity, ascorbic acid, total phenolic and flavonoid contents. TSS is an important attribute governing the taste of the fruit or the extracted juice. As shown in Table 2, the TSS

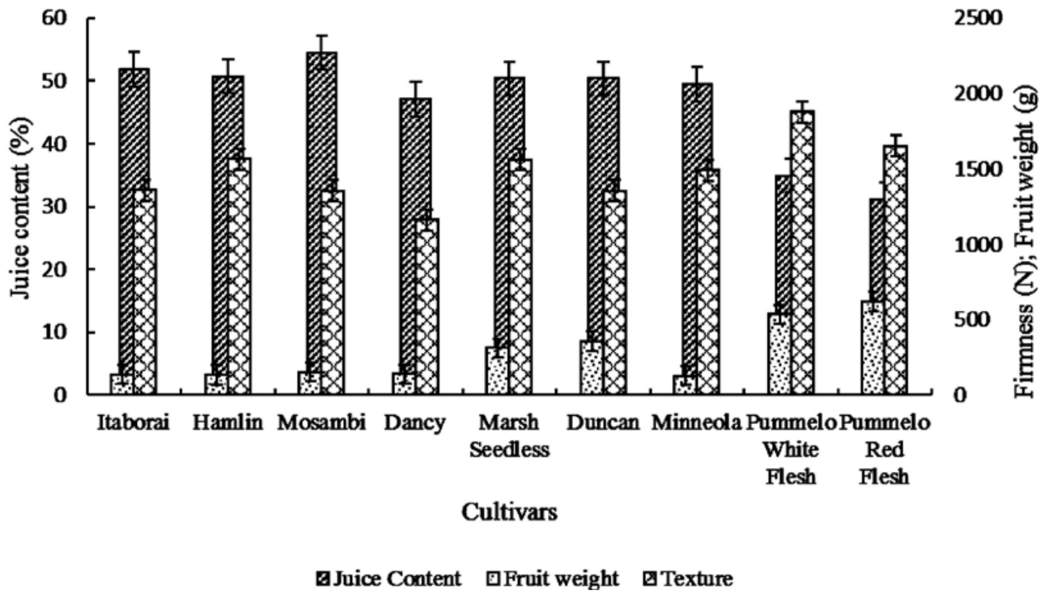

Fig. 1. Firmness, fruit weight, Juice content of citrus fruits of selected genotypes grown under semi-arid region (n = 3, vertical bars represent standard deviation).

varied widely amongst the citrus genotypes. 'Pummelo Red Flesh' (10.73°B) recorded the highest TSS while tangerine cv. 'Dancy' (8.1°B) showed the least. The titratable acidity was also found to differ significantly ($p \leq 0.05$) for the nine citrus genotypes and ranged from 0.37% ('Dancy') to 0.88% ('Pummelo White Flesh'). TSS governs the sweetness of citrus fruits while acidity imparts the sourness. TSS: acid ratio is responsible for the overall flavor profile of the fruits and varied from 12.04 ('Pummelo White Flesh') to 21.75 ('Mosambi'). The ratio is used as a maturity index for citrus fruits and should be ≥12 on harvest (Ramful *et al.*, 9).

Citrus fruits are well known for their ascorbic acid or vitamin C content, the major bioactive compounds, that impart high antioxidant potential to the fruits and contributes in maintaining good health. Maximum ascorbic acid was recorded in fruits of 'Pummelo Red Flesh' (54.45 mg/100g) while the least was in sweet orange cv. 'Itaborai' and tangelo cv. 'Minneola' (32.27 mg/100g). Phenolic compounds present in the fruits also impart antioxidant activity. The total phenolic content varied significantly from 23.33 mg GAE/100g ('Minneola') to 44.66 mg GAE/100g ('Pummelo White Flesh'). 'Pummelo Red Flesh' (50.85 mg QE/100g) recorded the highest flavonoid content, a class of functional phenolic compounds, while tangelo cv. 'Minneola' (33.44 mg QE/100g) exhibited the least values. Our study revealed that tangelo cv. 'Minneola' possessed the least values

Table 2. Biochemical attributes of the different citrus genotypes grown in semi-arid region.

Genotype	TSS(°B)	Titratable acidity (%)	Ascorbic acid content (mg/100 g)	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg QE/100 g)
'Itaborai'	8.90^{abc}	0.53^{bc}	32.27^{c}	34.33^{bc}	41.01^{abc}
'Hamlin'	9.27^{abc}	0.50^{bc}	34.28^{bc}	38.33^{ab}	44.00^{abc}
'Mosambi'	9.57^{abc}	0.44^{b}	42.35^{abc}	35.66^{bc}	45.58^{ab}
'Dancy'	8.10^{c}	0.37^{c}	34.28^{bc}	30.00^{bc}	34.58^{bc}
'Minneola'	8.33^{bc}	0.45^{b}	32.27^{c}	23.33^{c}	33.44^{c}
'Marsh Seedless'	10.13^{ab}	0.83^{ab}	38.32^{abc}	39.33^{a}	49.46^{a}
'Duncan'	10.00^{ab}	0.81^{ab}	48.40^{abc}	36.00^{ab}	47.33^{a}
'Pummelo White Flesh'	10.60^{a}	0.88^{a}	50.42^{ab}	44.66^{a}	48.10^{a}
'Pummelo Red Flesh'	10.73^{a}	0.85^{a}	54.45^{a}	40.00^{a}	50.85^{a}

Means with same superscript are not significantly different at $p \leq 0.05$.

for all the measured functional attributes. Pummelos were observed to be the reservoir of these bioactive compounds. The observations are in accordance with the findings of Wang *et al*. (12) who also reported that pummelo groups are abundant in bioactive compounds such as flavonoids and polyphenols in comparison to other citrus groups.

Apart from their typical flavour, the citrus fruits are an abundant source of antioxidants that promote human health. Since, no single antioxidant assay can fully describe the antioxidant potential of the fruits, four different assays, namely, DPPH, FRAP, ABTS and CUPRAC were used by using different chromogenic redox reagents. As presented in Table 3, irrespective of any antioxidant assay used, the antioxidant activity in different species from maximum to minimum followed the pattern pummelo>grapefruit>sweet orange>tangerine>tangelo. Delineation was due to the higher phenolics and flavonoid content in pummelo and grapefruit that directly correlate with the total antioxidant content (Ghasemi *et al.*, 3). A wide range in total antioxidant capacity (1.52-3.06 µmol TE/ml) was observed for the nine citrus genotypes studied through the FRAP assay. ABTS assay, although varying non-significantly among the citrus genotypes, yielded the highest total antioxidant activity values with an average value of 8.80 µmol TE/ml while the CUPRAC protocol recorded the lowest (2.03 µmol TE/ml). The present data is corroborated with the earlier findings of Zhang *et al.* (15) and Nishad *et al.* (8). Further, all four methods of antioxidant activity evaluation were reliable and quantified the antioxidant activity with their own working principle. Major contributor for the determination of total antioxidant activity can be established by conducting principal component analysis (PCA).

PCA, a multivariate statistical technique, was conducted to determine the significant attributes that govern the quality and compositional profile of the citrus fruits grown under semi-arid region. Two PCA together explain 83.69% variations. First PCA alone explains 77.09%, variations in data while second PCA explains 6.60% variation. As evident from Fig. 2A, important characters having significant impact on the quality of citrus fruits (above the red line) are TSS, FRAP antioxidant assay, acidity, flavonoids, CUPRAC and DPPH in descending order. TSS and acid content were by far the major quality attributes governing the citrus sensory acceptability. Flavonoids recorded a better impact on quality along with the FRAP assay to determine the total antioxidant activity. Recently, Sethi *et al*. (10) have also reported maximum contribution of flavonoids to the total antioxidant activity with *in vitro* FRAP assay as the best protocol to determine antioxidant activity in apple fruit extracts. From the biplot (Fig. 2B), it is clear that two clusters can be formed with cluster 1 ('Itaborai', 'Hamlin', 'Mosambi', 'Minneola' and 'Dancy') showing the most variability contributed from juice content. For group 2 (including 'Duncan', 'Marsh Seedless', 'Pummelo Red Flesh' and 'Pummelo White Flesh') the major contributors for quality determination are TSS, presence of phenolics, including flavonoids and the FRAP antioxidant assay.

Based on the similarities in characteristics of the predominantly cultivated citrus genotypes taken in this study, AHC was carried out. It also yielded similar results dividing the nine genotypes into two distinct groups, with each group members having similar characteristics. The same has been represented as a dendogram as given in Fig. 3. 'Pummelo White Flesh' (PWF) and 'Pummelo Red Flesh' (PRF) and grapefruit cv. 'Duncan' and 'Marsh Seedless' (MS) were clustered under one group. Similarly, all sweet orange cultivars, tangelo and tangerine were clustered together in the second group. For group 1, the most important contributors to quality are TSS, *in vitro* FRAP assay, acidity, flavonoids, CUPRAC and DPPH. Major impact is due to 'Pummelo White Flesh' and grapefruit cv. 'Duncan'. Similarly, for group 2,

Table 3. Variation in total antioxidant activity of citrus genotypes as studied by different assays.

Genotype	DPPH (µmol TE/ml)	CUPRAC (µmol TE/ml)	FRAP (µmol TE/ml)	ABTS (µmol TE/ml)
'Itaborai'	1.73^{b}	1.08^{b}	2.17^{cd}	8.39^{a}
'Hamlin'	2.11^{ab}	1.49^{b}	2.27^{bcd}	8.62^{a}
'Mosambi'	2.05^{ab}	1.24^{b}	1.83^{de}	7.77^{a}
'Dancy'	2.03^{ab}	1.62^{b}	1.52^{e}	8.80^{a}
'Minneola'	2.26^{ab}	1.38^{b}	2.26^{bcd}	7.81^{a}
'Marsh Seedless'	3.18^{ab}	2.86^{a}	2.57^{bc}	9.38^{a}
'Duncan'	2.88^{ab}	2.59^{a}	2.67^{ab}	9.12^{a}
'Pummelo White Flesh'	3.43^{a}	2.92^{a}	3.06^{a}	9.68^{a}
'Pummelo Red Flesh'	2.91^{ab}	3.14^{a}	3.02^{a}	9.69^{a}

Means with same superscript are not significantly different at $p \leq 0.05$.

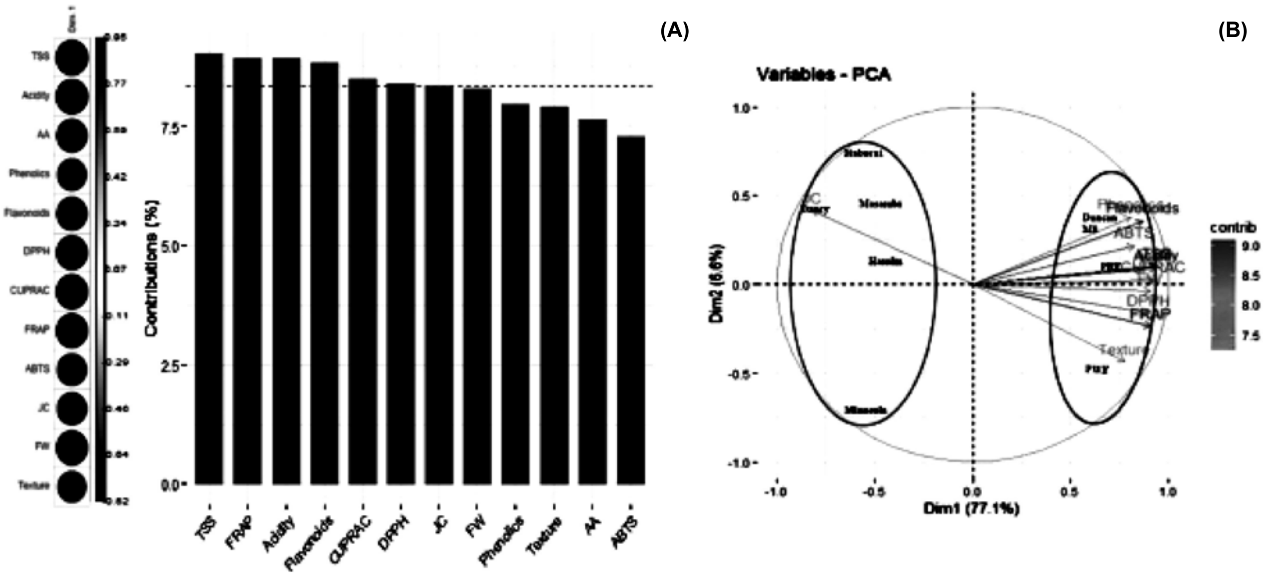

Fig. 2. Principal component analysis (A) and biplot (B) of different quality attributes.

the most important contributions are recorded due to juice content with cv. 'Minneola' and 'Itaborai' showing maximum impact.

Comprehensive study of fruit quality traits and health promoting compounds of the nine citrus genotypes studied reflects significant variations indicating their suitability for different applications. Principal Component Analysis (PCA) revealed that TSS and acidity were the predominant factors governing the acceptability of different citrus genotypes. Sweet orange genotypes possessing maximum juice content are the best suited for table purpose or the beverage industry. All citrus genotypes were abundant in health promoting metabolites namely ascorbic acid, flavonoids and phenolics. The antioxidant capacity of fruit tissues was correlated with total phenolic content and flavonoid

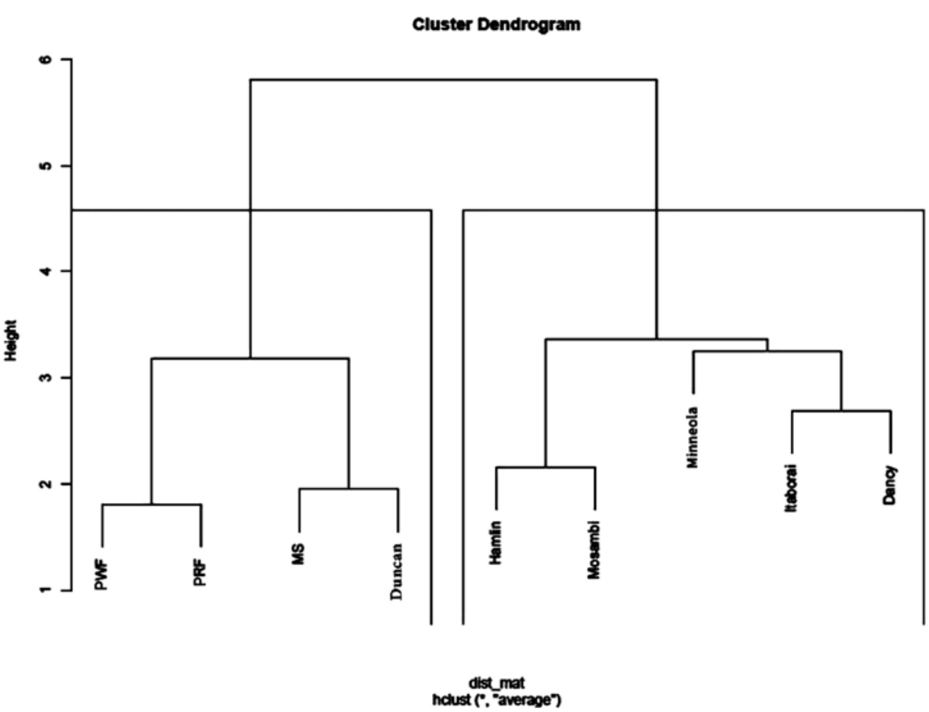

Fig. 3. Agglomerative Hierarchical Clustering of selected citrus genotypes.

content. Pummelo and grapefruit germplasm (with low juice yields and thick peel) were the promising source of phyto-chemicals and thus are suitable in food ingredient industry for extraction of bioactives. Biplot and AHC divided the nine genotypes into two groups having similar characteristics and clustered the pummelo and grapefruit genotypes in one group majorly based on their high phenolic and flavonoid content.

DECLARATION

The authors declare no conflict of interest.

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