J. Hortic. Sci.
Vol. 19(2), 2024

https://doi.org/10.24154/jhs.v19i2.2192

Original Research Paper

Characterization and evaluation of pummelo (*Citrus grandis* L.) half-sib population for economic traits

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ABSTRACT

The present investigation was carried out to identify potential progenies based on physico-chemical attributes among the half-sib progenies of pummelo. The fruit weight, diameter, length, and rind thickness were varied from 0.49-2.42 kg, 9.96-20.54 cm, 9.68-17.90 cm and 0.98-3.20 cm, respectively. Among the biochemical attributes, TSS, acidity, ascorbic acid and phenol content ranged from 6.46 to 11.48ºB, 0.57-3.56 %, 20.40 to 63.30 mg100 mL^{-1} and 32.20 to 181.44 mg 100mL^{-1} GAE, respectively. The progeny HS46-13 and HS48-12 were superior in terms of economic traits among the evaluated half-sib population of pummelo.

Keywords: Biochemical traits, bitterness, fruit quality, physical, rind thickness

INTRODUCTION

Pummelo (*Citrus grandis* L. Osbeck), often known as *shaddock* or *jabong*, is a natural, non-hybrid, mono-embryonic citrus species that originated in the Malayan and East Indian archipelago, belonging to the family Rutaceae (Verdi, 1988). Pummelo, along with mandarin and citron, is one of the ancestral species of the genus *Citrus* and is considered as the best source of female parents in the development of several citrus fruits, *viz*., sweet orange, grapefruit and tangelo.

Citrus juice holds immense health benefits due to its high antioxidant potency, mineral nutrients, phenolics, ascorbic acid, carotenoids, and flavonoids further escalating its wholesomeness (Singh et al., 2023). Although pummelo, nutritionally stands parallel to other citrus fruits, *viz.*, sweet oranges and mandarins, its commercial exploitation is less in India due to its thick rind, low TSS, high acidity, large number of seeds and bitterness as compared to other citrus fruits. The bitterness of the fruit becomes the major constraint of consumer acceptance which is attributed to flavonoids and limonoids (Puri et al., 1996).

The compound flavonoids are responsible for the immediate bitterness observed in the fruit, while, the direct consumption whereas, limonoid compounds are responsible for the bitterness caused in the juice, after its extraction from the fruits, called the delayed bitterness. Naringin (Naringenin-7-O-neohesperi-doside) and limonin being the predominant bitter principle in the fruit juice (Zaare et al., 2008).

Morphological study is an essential component for the assessment of diversity and classification of a population. Dozens of pummelo-derived cultivars, including grapefruits, tangelos, and hybrids, have been produced in recent years. The crop also represents a crucial gene pool for breeding new varieties of citrus fruits. Evaluating plant genetic diversity is a critical first step in every crop development strategy. Due to its complexity, yield is influenced by a variety of yield and yield-attributing factors, as well as by environment and polygenes. As a result, the genetic influences of the relevant genes and the impact of the environment combine to create the diversity in the collections for these features. Separating the observed variability into heritable and non-heritable components using the genetic advance, phenotypic advance, heritability, and genetic advance expressed as a per cent mean (GAM) measurement (Singh et al., 2023). With the wide availability of pummelo progenies, a preliminary study was carried out to screening the superior progenies based on physico-biochemical attributes and economic traits.

Table 1 : The list of the accessions and their half-sibs evaluated

Parent	Sl. No.	Progenies	Sl. No.	Progenies	Sl. No.	Progenies	Sl. No.	Progenies	Total progenies
	1	HS32-1	2	HS32-2	3	HS32-3	4	HS32-8	
	5	HS32-9	6	HS33-3	7	HS33-4	8	HS34-7	
Acc.18	9	HS34-10	10	HS34-13	11	HS34-19	12	HS35-3	22
	13	HS35-4	14	HS35-11	15	HS35-12	16	HS36-2	
	17	HS36-4	18	HS36-5	19	HS36-6	20	HS36-10	
	21	HS37-2	22	HS37-4					
	23	HS38-2	24	HS38-3	25	HS38-4	26	HS38-5	
Acc.6	27	HS39-2	28	HS39-3	29	HS39-4	30	HS39-9	12
	31	HS39-10	32	HS39-11	33	HS39-13	34	HS39-14	
	35	HS40-2	36	HS40-5	37	HS40-6	38	HS40-7	
	39	HS40-16	40	HS41-5	41	HS41-6	42	HS41-7	
	43	HS41-8	44	HS41-10	45	HS41-11	46	HS42-1	
	47	HS42-2	48	HS42-4	49	HS42-8	50	HS42-9	
	51	HS42-11	52	HS42-12	53	HS42-13	54	HS42-15	
	55	HS42-16	56	HS42-18	57	HS42-19	58	HS43-1	
	59	HS43-5	60	HS43-6	61	HS43-7	62	HS43-9	
Acc.12	63	HS43-10	64	HS43-11	65	HS43-15	66	HS43-16	58
	67	HS44-1	68	HS44-4	69	HS44-6	70	HS44-8	
	71	HS44-9	72	HS44-12	73	HS44-15	74	HS44-17	
	75	HS45-1	76	HS45-9	77	HS45-10	78	HS45-14	
	79	HS45-18	80	HS46-5	81	HS46-6	82	HS46-10	
	83	HS46-12	84	HS46-13	85	HS46-14	86	HS47-3	
	87	HS47-4	88	HS47-6	89	HS48-10	90	HS48-12	
	91	HS49 -11	92	HS49-12					

MATERIALS AND METHODS

The present investigation was carried out on the well-established half sib progenies of pummelo accession 18, 6 and 12 maintained at ICAR-Indian Institute of Horticultural Research, Bengaluru, during 2020 to 2023. Accession 18 is characterized by the presence of sweet pulp with uniform deep pink colour and thick rind. Similarly, the Accession 6 has got the sweet and deep pink pulp having thick rind with higher number of seeds, while, Accession 12 had bitter light pink pulp with comparatively thin rind. The half-sib population were planted in August 2016 and was in the reproductive phase during the period of the present investigation. Based on their precocity, regular bearing yield and quality attributes, a total of 92 half-sibs were selected out of 245 for further studies (Table 1). The morphological parameters of plants and fruits were visualised and recorded based on the citrus descriptor (IPGRI, 1999).

The pulp's total soluble solids (TSS) were determined using a digital refractometer. The titrable acidity (citric acid equivalent) and ascorbic acid content were estimated through the titration method (AOAC, 2000). The Nelson-Somogyi method determined fruit juice's sugar content (Krishnaveni *et al.,* 1984). The total phenolic content of the fruit was estimated by a spectro-photometric method using Folin Ciocalteu Reagent (FCR), as suggested by Sigleton & Rossi (1965). The total flavonoid content of the fruit was determined through the spectrophotometric method at 510 nm, according to Chun *et al.* (2003). The organoleptic acceptability of fruit juice was evaluated for the major commercially valuable traits, *i.e.* the overall taste and bitterness based on a 9-point Hedonic scale varies from 9 = like extremely to 1=dislike extremely (Gaikwad *et al.,* 2015).

Based on the sensory evaluation, superior progenies were further evaluated for naringin and limonin content through LC-MS. The juice samples were prepared using the method Nishad *et al.* (2018) and Xi *et al.* (2014) suggested. The chromatographic peak corresponding to each compound was identified by comparing the retention time with the respective standards, and concentrations were measured using a calibration curve.

The quantitative data were analysed in a one-way ANOVA to determine differences between accessions using the SAS 9.3 version (SAS Institute Cary, NS, USA). The principal components analysis (PCA) was performed for economically important traits. Phenotypic and genotypic coefficients of variation (PCV and GCV), broad sense heritability and genetic advance (GA) were estimated for all the fruit quantitative traits using R Studio (2022.07.1-554, R Studio, PBC).

RESULTS AND DISCUSSION

Tree morphological characterization

Different half-sibs showed a considerable diversity in the tree morphological traits. The trees height ranged from 3.80-5.70 m, with an average height of 5.07 m (Supplementary Table a). The tree HS48-10 was the tallest of all and the tree HS42-19 was observed as the most dwarf (3.80 m) individual. The tree spread from North to South varied from 3.40 m (HS48-12) to 6.50 m (HS35-12). The tree HS 32-9 showed the highest tree spread from East to West (7.1 m), while the tree HS36-6 showed the least canopy spread (2.7 m). The obtained results were comparable with the previous studies in different citrus fruits (Gaikwad et al., 2015; Kaur et al., 2019; Rohini et al., 2020). These variations can be ideally utilised for the identification of individual plants and they can visually distinguish the desirable plant and fruit characteristics.

Leaf morphological characterization

Supplementary Table b showed the variations recorded within the various leaf morphological characters. The leaves of the half-sib population were observed to be simple, brevipetiolate (petiole shorter than leaf lamina), with prominent and narrow obcordate wings. The leaf lamina length and width of these half-sibs ranged from 7.92 cm (HS43-6) to 14.12 cm (HS45-10) and 5.01 cm (HS38-5) to 9.16 cm (HS45-10), respectively, while the leaf thickness ranged from 4.1 mm (HS46-6) to 8.54 mm (HS41-11). The shape of the leaves was observed to be elliptic, ovate, obovate, lanceolate to orbicular with crenate, dentate, entire or sinuate leaf margin and with acuminate, acute or obtuse leaf apex. Narrow or broad petiolar wing width was observed with petiole wing shape varying from obcordate to obdeltate or obovate. Eighteen indigenous pummelo germplasm samples were morphologically characterised by Dubey et al. (2019), and observed obovate, elliptic to ovate leaf shapes. They observed that the leaf margin was crenate, entire or wavy, with a range of 8.19 to 9.49 cm for leaf length and 5.03 to 5.65 cm for leaf width. Similar results were also reported by Kaur et al. (2019).

Fruit morphological characterization

In the present study, all the fruits from the half-sib trees were evaluated for various fruit characters according to the IPGRI descriptors and a considerable amount of diversity was observed (Supplementary Table c and Table 2). The fruit shape included pyriform, spheroid, obloid, ellipsoid and oblique. The fruit weight, fruit diameter, fruit length ranged from 0.49 to 2.42 kg, 9.96 to 20.54 cm, and 8.62 to 17.9 cm respectively. The fruits produced by the tree HS40-16 weighed the heaviest with an average fruit weight of 2.42 kg and with 19.06 cm diameter and 16.4 cm fruit height. Highest fruit diameter was recorded on HS36-5 (20.54 cm), while, HS36-10 recorded the highest fruit length (17.9 cm). The tree HS48-12 produced smaller fruits with the lowest fruit weight, diameter and length recorded (0.49 kg, 9.96 cm and 9.68 cm). The external colour of the fruit varied *viz.,* green-yellow, light yellow, yellow, dark yellow to light orange with fruit base differing from necked, concave, to convex *etc.* The fruit apex was found in different forms like acute, rounded, truncate and depressed.

The rind thickness of the half sib population was ranged from 0.98 to 3.20 cm with mean, among which 47 trees produced white rind and 45 with pink rind (Supplementary Table d and Table 2). The HS48-12 not only produced the smallest fruits, but also recorded the thinnest rind of all (0.98 cm) while, HS49-11 showed thickest rind (3.2 cm). The number of segments per fruit ranged from 9.60 to 19.80 with 13 progenies showing uniform segments and the rest with irregular segments. The segment length ranged from 5.56 to 14.74 cm in HS40-16 (14.74 cm) and HS43-16 (14.72 cm) recorded longer segments. The fruit's central axis was found to vary from solid, hollow and semi hollow with round or irregular cross sections. The

Table 2 : Descriptive statistics for different physico-chemical attributes of pummelo half-sib population

Traits	Range	Population Mean	CD	CV
Fruit weight (kg)	0.49- 2.42	1.28	0.31	19.73
Fruit diameter (cm)	9.96-20.54	15.16	1.74	9.24
Fruit length (cm)	9.68-17.90	14.04	1.64	9.37
Rind thickness (cm)	0.98-3.20	1.92	0.47	19.67
Number of segments	9.6 - 19.8	12.76	2.22	14.01
Fruit axis diameter (cm)	0.86- 5.34	2.66	0.73	21.99
Segment length (cm)	5.56- 14.74	10.58	1.90	14.39
TSS (ºB)	6.46- 11.48	8.87	0.86	7.75
Acidity (%)	0.57- 3.56	1.35	0.19	11.05
Ascorbic acid (mg100 mL^{1} juice)	20.40- 63.30	39.26	4.59	9.40
Reducing sugar (%)	0.84- 3.71	2.03	0.28	8.66
Total sugars (%)	2.83- 7.34	4.80	0.19	2.44
Total phenols (mg 100 mL^{-1} GAE)	32.20 – 181.44	115.50	9.29	4.99
Total flavonoids (mg catechin equivalents/100 g)	4.26- 21.66	13.28	0.96	4.49

diameter of their axis was found to range from 0.86 to 5.34 cm averaging to 2.66 cm. The pulp colour ranged from white, pink and deep pink with light and dark intensity, HS48-12 showed a unique orange pink colour pulp with light colour intensity. The results on the fruit morphological traits are in agreement with the previous studies carried out by Ahmed et al., (2019); Dubey et al. (2019) and Kaur et al. (2019); Roy et al. (2020). The morphological difference in morphological attributes of traits might be attributed to the unique genetic composition of individual genotypes, and further signifies the high heterozygosity of parental accession of citrus (Singh et al., 2023).

Fruit biochemical characterization

The wide variation observed in the biochemical composition of fruits offers broad scope in the developing of desirable hybrids through different breeding approaches (Supplementary Table e and Table 2). The total soluble solids (TSS) ranged from 6.46°B to 11.48°B with a population mean of 8.87°B and CV of 7.75. Higher TSS content was recorded on HS43-6 and the least in HS35-3. The titrable acidity of the half-sibs varied significantly, which ranged from 0.57 % in HS45-1 to 3.56 % in HS43-5 with a mean acidity of the population to be 1.35 %. The ascorbic acid content varied significantly among the progenies (20.40- 63.30 mg 100 mL^{-1} juice) with the CV of 9.40. The higher ascorbic acid content was observed in the juice of HS39-9 (63.3 mg 100 mL^{-1} juice) and lowest in HS41-5 (20.40 mg 100 mL^{-1} juice). The overall mean of the population for ascorbic acid was found to be 39.26 mg 100 ml^{-1} juice. The reducing sugars was found maximum on HS41-8 (3.71%) and least on HS42-18 (0.84%) with mean of 2.03%. The total sugars ranged 2.83 – 7.34 % with population mean of 4.80%. A higher total sugar was recorded on HS45-18 (7.34%) followed by HS48-12 (7.25%), while, a considerably lower total sugars value was recorded on HS46-14 (2.83%). The primary and secondary metabolites found in plants are phenols. The tissues are shielded by phenols against diseases and insect damage. The antioxidant and anti-hyper-lipidemic properties of edible fruits are thought to be mostly attributed to phenolic substances. As a result, the total amount of polyphenols in our diet is crucial for preventing various degenerative diseases like cancer, cardiovascular disease, immune system deterioration, cataract, etc. (Makynen et al., 2013). A wide variation was observed in the total phenolic content of the population in the current study, varying from 32.20-181.44 mg 100mL^{-1} GAE. The progeny HS42-9, HS42-8 and HS40-2 had higher phenolic content in the fruit juice. The total flavonoids showed significant variation among the progenies ranging 4.26-21.66 mg catechin equivalents 100g^{-1} (HS45-9 and HS39-10, respectively). The flavonoids are a group of compounds contributing to the total antioxidant

Table 3 : The classification of pummelo progenies based on sensory evaluation given in Hedonic scale

Score	Half-sibs	No. of half-sibs
9	HS46-13, HS48-12	2
8	HS38-4, HS44-9, HS44-15, HS45-14, HS45-18, HS46-12, HS48-10	7
7	HS32-3, HS33-3, HS34-8, HS37-2, HS39-14, HS40-7, HS40-16, HS41-7, HS43-6, HS43-15, HS44-1	11
6	HS36-6, HS39-4, HS39-10, HS39-13, HS41-11, HS43-7, HS47-3, HS47-6, HS49-11, HS49-12	10
5	HS32-1, HS32-2, HS32-9, HS35-12, HS36-5, HS36-10, HS39-2, HS40-6, HS41-5, HS41-6, HS42-1, HS42-2, HS42-8, HS42-9, HS42-11, HS42-13, HS42-19, HS43-9, HS43-11, HS46-5, HS46-6	21
4	HS33-4, HS34-13, HS35-4, HS38-3, HS39-9, HS41-8, HS42-15, HS42-16, HS42-18, HS43-5, HS44-12, HS45-1, HS45-9, HS46-14	14
3	HS32-8, HS34-7, HS37-4, HS39-11, HS40-2, HS41-10, HS42-12, HS43-1, HS43-10, HS43-16, HS44-4, HS44-6, HS44-8, HS44-17, HS46-10, HS47-4	16
2	HS34-19, HS35-3, HS35-11, HS36-2, HS36-4, HS38-2, HS38-5, HS40-5, HS42-3, HS45-10	10
1	HS39-3	1

capacity of citrus fruits. The similar variations in the biochemical composition of pummelo fruits have also been reported by Kumar et al. (2015), Nishad et al. (2018) and Gaikwad et al. (2019). The variation observed for biochemical attributes among the half-sib progenies in the present study might be the result of genetic background and influence of parental genetic potential (Verma et al., 2019 and Singh et al., 2023)

Fruit sensory evaluation

The sensory evaluation can estimate the vital attribute of fruit quality acceptance. Sensory evaluation for 92 half-sibs and their classification based on organoleptic acceptability are presented in Table 3. Out of all 92 half-sibs evaluated, fruits of HS46-13 and HS 48-12 were very sweet with no or very little immediate bitterness and, hence scored 9. The progenies such as HS38-4, HS44-9, HS44-15, HS45-14, HS45-18, HS46-12 and HS48-10 possess a sweet pulp with a slight sourness and/ or bitterness. The progeny HS43-6 and HS43-5 had lower organoleptic acceptability scores, which is due to higher bitterness and acidity despite having higher TSS. Similar findings were observed by Gaikwad et al. (2015, 2019).

Determination of bitterness

Seven progenies, namely, HS37-2, HS38-4, HS39-3, HS39-13, HS44-15, HS46-13 and HS48-12, were subjected to determine naringin and limonin using LCMS (Table 4). It was observed that HS48-12 recorded least amount of naringin content (89.52 µg/

Table 4 : Naringin and limonin content in the juice of selected half-sib progeny

Name	Limonin (µg/g)	Naringin (µg/g)	Sensory scoring
HS37-2	32.03	252.55	7
HS38-4	10.79	207.86	8
HS39-3	153.41	336.86	1
HS39-13	18.88	246.55	6
HS44-15	40.78	248.55	8
HS46-13	66.36	154.82	9
HS48-12	65.04	89.52	9

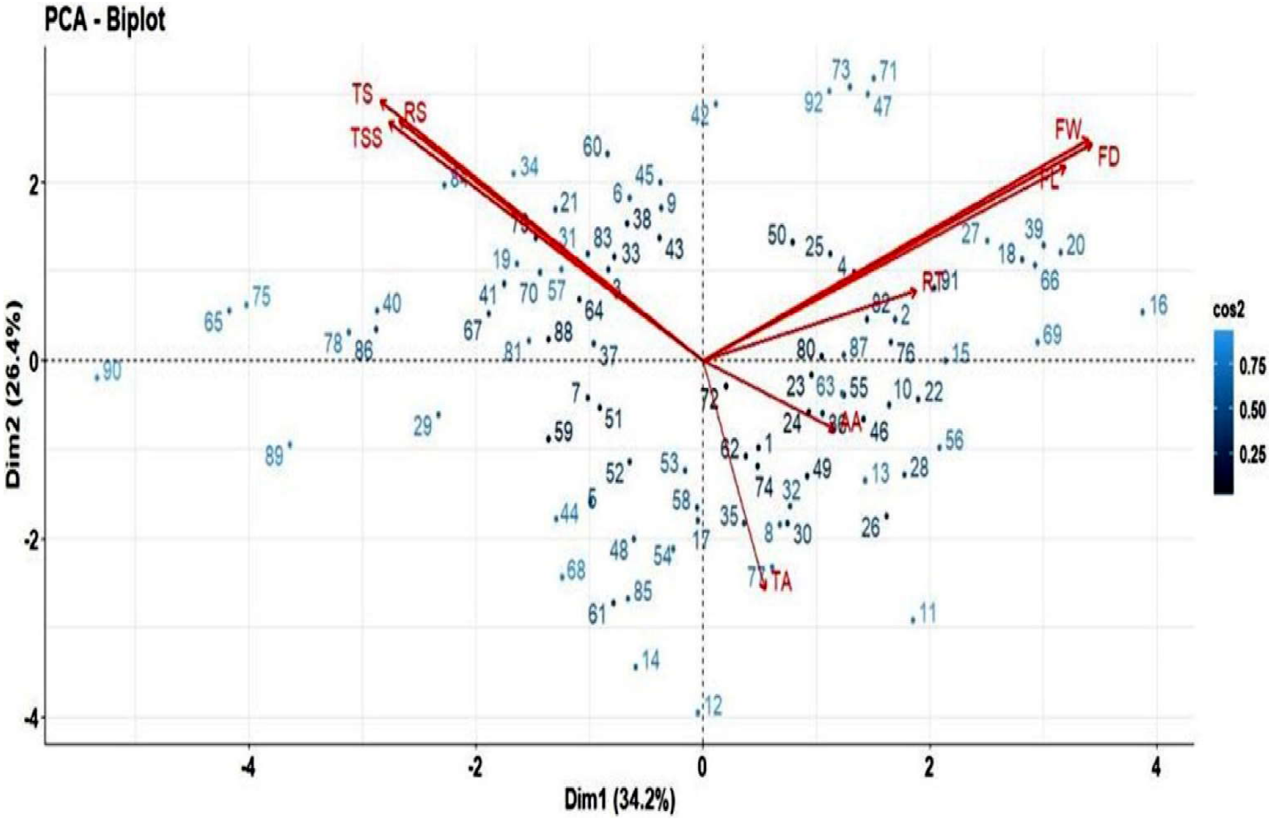

Fig. 1: PCA biplot showing 92 half sibs and variables along the two major principal components. FW= fruit weight, FD= fruit diameter, FL= fruit length, RT= rind thickness, TSS= total soluble solids, TA= titratable acidity, AA= ascorbic acid, RS= reducing sugars and TS= total sugars

g) and moderate limonin content (65.04 µg/g), while HS38-4 recorded least limonin content (10.79) accumulation (Table 4). The highest limonin (153.41 µg/g) combined with highest naringin contents (336.86 µg/g) were recorded in HS39-3. Similar range of these compounds were also reported (Sandhya Nishad et al., 2018; Rani et al., 2019; Zhao et al., 2021).

Principal component analysis

The principal component analysis was performed for better visualization of the relationship of the half-sibs with their morphological and biochemical traits. PCA was performed using the mean fruit quality traits of 92 pummelo half-sibs. Considering a minimum threshold eigen value of one, the three PCs were considered, explaining 73.70% of the total variation among the studied pummelo half-sib progenies. PC1 contributed 35.87% of the total variation, followed by PC2 (23.16%). The traits such as fruit weight, diameter, length, rind thickness and titrable acidity were the dominant variables in PC1, thereby causing more significant variability among the genotypes under study. Fruit weight, diameter and length were observed with significantly high positive loadings in PC1. In PC2, reducing sugars, total sugars and TSS had significantly contributed to high negative loadings. PC1 and PC2 collectively explained 59.03% of the total variation in economically important traits among the pummelo half-sibs (Fig. 1).

Data presented in Table 5 showed the estimates of variability components and heritability. Among 14 traits. The PCV exceeded GCV for all the traits, indicating some environmental influence on phenotypic expression. However, the small differences between GCV and PCV suggest that environmental impact on variability was minimal (Nitin et al., 2024). Citrus species, known for their high heterozygosity, exhibit substantial genotypic and phenotypic variability in their F_1 progeny. In this study, traits such as total sugars (99%), total phenols (98%), total flavonoids (98%), reducing sugars (96%), acidity (92%), and ascorbic acid (88%) demonstrated high heritability (e” 80%). High GCV combined with high heritability suggests that selection would be effective for these

Table 5 : Heritability estimates of different physico-chemical parameters of half-sibs

Traits	GCV	PCV	H^2	GA	GAM
Fruit weight	31.74	37.38	72	0.71	55.51
Fruit diameter	13.26	16.16	67	3.40	22.42
Fruit length	12.21	15.40	63	2.80	19.96
Rind thickness	22.47	29.86	57	0.67	34.82
Number of segments	12.32	18.66	44	2.14	16.77
Fruit axis diameter	31.10	38.09	67	1.39	52.30
Segment length	14.78	20.62	51	2.31	21.81
TSS	10.79	13.28	66	1.60	18.06
Acidity	37.07	38.68	92	0.99	73.20
Ascorbic acid	25.65	27.32	88	19.48	49.62
Reducing sugar	42.23	43.10	96	1.73	85.21
Total sugars	26.29	26.40	99	2.59	53.92
Total phenols	34.94	35.29	98	82.29	71.25
Total flavonoids	29.64	29.98	98	8.02	60.37

traits, whereas, low heritability indicates that environmental factors might obscure genotypic effects, making selection less practical (Singh et al., 2023).

Traits with both high H^2 and high GAM are particularly valuable for selection processes, as they are primarily controlled by additive genetic effects and are less influenced by environmental factors (Nitin et al., 2024). Consequently, traits such as reducing sugars, acidity, and total phenols, which also showed the highest GCV and PCV, are identified as the most promising for effective selection.

CONCLUSION

A high range of variability has been observed among the half-sib progeny of pummelo for different economic traits. Out of 245 half-sibs evaluated initially, 92 progenies were selected for regular bearing, precocity and fruit quality attributes of which 2 half-sibs (HS48-12 and HS46-13) were found to be economically promising The fruits of HS48-12 produced very thin rind of 0.98 cm with sweet pulp having no/least bitterness. The average fruit weight ranged from 450-600 g, which is a beneficial trait as the consumers most prefer it. Similarly, the HS46-13 has least bitterness and dark pink pulp with low acidity (0.60%). Hence, these two promising pummelo progenies can be used as breeding stock/new variety for commercial cultivation.

ACKNOWLEDGEMENT

The authors acknowledged the ICAR-Indian Agricultural Research Institute (IARI), New Delhi, for providing financial support to this study.

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(***Received : 5.9.2024; Revised : 20.12.2024; Accepted : 24.12.2024)***