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CHROMATOGRAPHY

Identification of Novel Interspecific Citrus Hybrids with High Hesperidin Content and Favorable Sensory Quality

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ABSTRACT

Citrus fruits are well known for their health-protecting compounds, with hesperidin being one of the key components of citrus juices that plays a crucial role in combating various human health issues. The study aimed to quantify hesperidin levels using the high-performance liquid chromatography (HPLC) method and assess the sensory quality of the juice from different interspecific citrus hybrids. Results revealed substantial variation in hesperidin content (0.63–194.68 mg L^{-1}) among the hybrids, along with differences in sensory attributes. Hybrid SCSH 8-18 exhibited the highest hesperidin content (194.68 mg/L), surpassing both male and female parents. Hybrids SCSH 3-6 and SCSH 7-7 exhibited higher levels of hesperidin content in addition to the superior sensory attributes. The genotypic coefficients of variance (GCV) and heritability were higher for the hesperidin content and sensory qualities among the studied citrus genotypes. The novel hybrid genotypes having enhanced hesperidin content and higher acceptability for sensory properties are potentially suitable for a trait-specific citrus improvement programme.

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Citrus hybrids; flavonoids; hesperidin; high-performance liquid chromatography (HPLC); sensory quality

Introduction

Globally, citrus fruits are among the most important fruit crops in terms of area and production. Among different *Citrus* species, orange (*Citrus sinensis*), tangerine (*Citrus reticulata*), limes (*Citrus aurantifolia*), lemons (*Citrus limon*), and grapefruit (*Citrus paradisi*) have attained commercial significance. These fruits are popular due to their distinct refreshing aroma and flavor, as well as their health-promoting properties. Citrus fruits contain substantial amounts of bioactive compounds, particularly in the peel and pulp, reported to various health issues, including antitumor, anti-inflammatory, anti-cancer, antiviral, and antimicrobial activities, as well as their role in combating

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cardiovascular and macular degeneration diseases (Chavda et al. 2025; Ma et al. 2020; Singh et al. 2025).

The flavonoids are the most important class of bioactive compounds present in citrus fruits. Flavonoids are secondary metabolites in plants, originating from the shikimic acid pathway and phenylpropanoid metabolic pathway, and contribute significantly to the taste and color of fruits (Wang et al. 2024). The flavonoid family comprises several metabolites, among which hesperidin is the most common in various *Citrus* species (Adham 2015; Rouseff, Martin, and Youtsey 1987). As an abundant and cost-effective dietary bioflavonoid, hesperidin offers numerous biological and medicinal benefits (Ma et al. 2020; Pandey and Khan 2021).

The beneficial effects of hesperidin have been extensively studied for their neuro-pharmacological and neuroprotective properties, with particular emphasis on their anti-depressive effects and their role in protecting against neurological impairments (Kim et al. 2019). Hesperidin has been reported to enhance microvascular circulation, exert potent antioxidant activity, improve venous tone, and demonstrate anti-inflammatory and analgesic properties. Additionally, it has been associated with lipid-lowering effects, facilitation of ulcer healing, and therapeutic use in managing hemorrhoids and chronic venous insufficiency (Adham 2015; Pandey and Khan 2021; Rouseff, Martin, and Youtsey 1987).

Given the significance of flavonoids in contributing to the health benefits of citrus fruits, the identification and quantification of these bioactive compounds play a crucial role across various scientific disciplines. Different citrus fruits have different amounts of hesperidin content in their juice, such as in *Citrus maxima* (0.12 g/100g) (Manikyam et al. 2022), *Citrus sinensis* (26.85 mg/L) (Büyüktuncel 2017), *Citrus limon* (101 mg/L) (Caristi et al. 2003), *Citrus unshiu* (25 mg/L), and *Citrus paradisi* (33 mg/L) (Abad-García et al. 2014). However, there is a need to improve existing citrus varieties and develop new cultivars with enhanced health-promoting properties, in addition to traditional desirable traits (Huang et al. 2024; Lu et al. 2023; Seminara et al. 2023; Wang et al. 2024). Bioactive compounds are becoming increasingly important in the breeding of functional and nutritious fruit crops, such as citrus. Nevertheless, the underlying factors, i.e., heritability, driving the selection and variation of these compounds in citrus are still not well understood (Liang et al. 2024). There is a growing demand for the development of novel citrus cultivars that not only exhibit superior taste and flavor profiles but also possess elevated concentrations of health-promoting bioactive compounds. Hybridization remains one of the most successful approaches for genetic improvement of perennial citrus fruits (Raveh et al. 2020; Seminara et al. 2023). These breeding targets are driven by increasing consumer preference for nutritionally enhanced and functionally beneficial foods.

Recent analytical work has provided robust approaches for flavanone quantification in citrus matrices. Chavda et al. (2025) developed an HPLC protocol for naringin standardization. The present study focuses on hesperidin, a different flavanone glycoside, which exhibits distinct chemical, sensory, and physiological relevance. Naringin, mainly associated with bitterness perception and hesperidin contribute to the flavor modulation, antioxidant potential, and nutraceutical quality of citrus juice. Moreover, from a trait-specific breeding perspective, quantifying hesperidin levels enables the identification of promising parents and hybrid genotypes as potential genetic resources for crop breeding programs.

Despite numerous studies quantifying flavonoids in citrus germplasm, few studies have been conducted on the heterotic performance and heritability of hesperidin content in interspecific citrus hybrids. The present study is among the first to integrate metabolite analysis with genetic estimates such as mid-parent and better-parent heterosis. This work provides new insights into the inheritance and variability of hesperidin content in citrus fruit juice. These findings advance current understanding of how hybridization influences functional metabolites in citrus and offer a breeding-oriented framework for developing flavor-rich, health-promoting cultivars.

Keeping in view, Pummelo (*Citrus maxima* [Burm. f.] Osbeck), Sweet orange (*Citrus sinensis* [L.] Osbeck), and Tangerine (*Citrus reticulata* [L.]) were utilized as parental lines for interspecific hybridization. The resulting new hybrids were evaluated in the present study for hesperidin content, sensory attributes and underlying heritability estimates to identify promising genotypes for the crop improvement program and commercial exploitation.

Materials and methods

Plant material

In the present investigation, 24 newly developed interspecific citrus hybrids and their four parental genotypes, namely, tangerine cv Dancy (*Citrus reticulata* Blanco), sweet orange cv. Mosambi (*Citrus sinensis* (L) Osbeck) and pummelo (*Citrus maxima* Merr.) genotype PS-8 and PS-13 were evaluated for hesperidin content and sensory properties. These new interspecific hybrids have been developed through the conventional method of emasculation, followed by manual crossing (pollination) and bagging at ICAR-IARI, New Delhi. The obtained new hybrids were established at $5\,m \times 5\,m$ spacing at the experimental orchards of the Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi (28°64′ N and 77°15′ E; 228 m elevation above the msl). The experimental site is characterized by a typical sub-tropical climate, with a dry, hot summer and cold winter, and rainfall mainly concentrated in July and August. The soil pH of the experimental site was 7.22 (1:2 soil to water ratio), with a sandy loam texture and medium organic matter content. The hybrid progenies were subjected to uniform management practices.

The mature fruits (15 fruits from each genotype) were harvested from the interspecific citrus hybrids and the parental genotypes growing at the experimental field of the ICAR-Indian Agricultural Research Institute (IARI), New Delhi (situated at 28°64′ N and 77°15′ E). The individual fruits were wiped and cut in half to extract the juice without crushing the seeds. The fruit juice from three biological replicates of each citrus genotype was extracted using a handheld citrus juice extractor. The juice samples were filtered and centrifuged (at 5000 rpm) to remove suspended particles and subjected to further analysis.

Sample preparation and high-performance liquid chromatography (HPLC) analysis

The 5.0 g juice sample was weighed into a 50 mL clean centrifuge tube, followed by the addition of 10.0 ± 0.1 mL of methanol (HPLC grade) and 10.0 mL of water (HPLC grade). The tubes were centrifuged (Hermle Z36HK, USA) for 10 min at 5000 rpm to ensure proper mixing and extraction of hesperidin in the miscible layer. The supernatant was collected and passed through a polytetrafluoroethylene (PTFE) 0.22 μm syringe filter. One milliliter of filtered solution was then transferred to HPLC glass vials. It was further diluted by adding 1.5 mL of methanol and 1.5 mL of water (HPLC grades). A stock solution of hesperidin 10 mg/10 ml (methanol) was prepared.

The samples were used for high-performance liquid chromatography (HPLC) analysis using a RP-18 column and acetonitrile: water (70:30) as the mobile phase with a flow rate of 1 mL per min. 20 μl of sample was injected into the instrument and detected at 284 nm. The hesperidin peak was detected at 3.0 min. Chromatograms of hesperidin content in the interspecific citrus hybrid are shown in Figure S1.

Before quantifying hesperidin content from juice samples, method validation was performed according to ICH guidelines. The calibration curve for hesperidin (≥97.0% HPLC) was constructed using five standard concentrations (0.5–100 μg mL^{-1}) with a linear relationship ($R^2 = 0.999$). The limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.12 and 0.40 μg mL^{-1}, respectively, based on signal-to-noise ratios of 3:1 and 10:1. Method recovery was assessed by spiking known amounts of standard hesperidin into citrus juice samples, resulting in recovery rates between 96.2% and 102.5%. Repeatability and reproducibility were confirmed by triplicate injections, with relative standard deviations (RSD) below 2%. The analytical standard of hesperidin and required solvents, viz., methanol, acetonitrile, and HPLC-grade water, were procured from Sigma Aldrich (Burlington, Massachusetts, United States).

Sensory evaluation

The organoleptic properties of citrus juice, including taste, aroma, and overall acceptability, were assessed on a 9-point hedonic scale (where one indicated "dislike extremely" and nine indicated "like extremely") as described by Peryam and Pilgrim (1957). The sensory evaluation of fruit juice was conducted by a panel of ten trained panelists (5 males and 5 females) aged 25–49 years, all of whom were familiar with citrus fruits and regularly involved in sensory studies. Before the evaluation, the panelists underwent orientation and training sessions to familiarize themselves with the evaluation procedure, sensory attributes, and use of the hedonic scale. For evaluation, 25 mL of equilibrated fresh juice from each genotype was presented in coded, odor-free plastic cups at 25 ± 1 °C under standardized lighting conditions in individual sensory booths to minimize distractions and bias. The samples were presented in a randomized order, and water and unsalted crackers were provided between samples for palate cleansing.

Statistical analysis

Statistical analyses were conducted to evaluate the significance and variability of hesperidin content and sensory attributes among the citrus hybrids and parental lines.

Duncan's Multiple Range Test (DMRT) was employed to determine statistically significant differences between sample means at a significance level of $p \leq 0.05$. Descriptive statistics, analysis of variance (ANOVA), and heritability estimates were performed using RStudio (RStudio, PBC, Version 2022.07.1-554) software. Genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), genetic advance per cent mean, and broad-sense heritability (H^2) were calculated based on the obtained results for new interspecific hybrids (Singh et al. 2023). Multivariate analysis through principal component analysis (PCA) was carried out, utilizing XLSTAT 2024 (Addinsoft; https://www.xlstat.com). The hierarchical clustering, heatmap and genetic component analysis were conducted to elucidate the structure and grouping of hybrids based on hesperidin accumulation and sensory parameters using TBtools-II (Version 2.1; https://github.com/CJ-Chen/TBtools).

Results and discussion

Hesperidin content and sensory properties of new citrus hybrids and parents

In contemporary society, the incidence of health-related risks is escalating due to increasing environmental pollution, imbalanced nutrition, and sedentary lifestyles. This study aims to develop novel interspecific citrus hybrids enriched with bioactive compounds, particularly hesperidin. Genotypes exhibiting an optimal balance of hesperidin content and favorable sensory attributes hold promise for juice production targeted at the consumer market. Conversely, genotypes with elevated hesperidin concentrations but lower sensory appeal may serve as valuable raw material for the pharmaceutical industry in the formulation of health-promoting products.

Cahyono et al. (2023) studied the optimization condition of a high-performance liquid chromatography (HPLC) method to determine hesperidin contents from various orange peel extracts. The standardized protocol was used to analyze the hesperidin content in the fruit juice of newly developed interspecific hybrids (Pummelo × sweet orange and Pummelo × tangerines) and their parental genotypes. The hesperidin content in the juice of the seed parent pummelo varied from 9.59 mg/L to 12.11 mg/L in PS-8 and PS-13, respectively (Table 1). Whereas the pollen parents, tangerine cv Daisy and sweet orange cv Mosambi, had hesperidin levels of 46.45 and 22.01 mg/L, respectively.

Among the new hybrids evaluated in the present study, SCSH 8-18 had the highest hesperidin content (194.68 mg/L), followed by hybrid SCSH 7-7 (52.82 mg/L), both exceeding the content of their male and female parents. In contrast, the lowest hesperidin levels were found in hybrid SCSH 4-13 (0.63 mg/L). Among hybrids from the pummelo (white) and mosambi cross, SCSH 7-7 had the highest hesperidin content (52.82 mg/L), while SCSH 4-13 had the lowest (0.63 mg/L) hesperidin content. For hybrids from the pummelo (red) and mosambi combination, SCSH 3-5 had the maximum hesperidin content (27.08 mg/L), and SCSH 3-10 had the minimum (2.62 mg/L). In the pummelo and tangerine cross, SCSH 8-18 had the highest hesperidin level (194.68 mg/L), and SCSH 8-19 had the lowest (1.87 mg/L) hesperidin content (the chromatograms given in Figure S1). Mainly, the variation for hesperidin content is attributed to transgressive segregation.

Table 1. Hesperidin content in interspecific citrus hybrids and their parents.

Number	Citrus genotypes	Hesperidin content (mg/L)
	Hybrids	
1.	SCSH 2-2	20.80 ± 1.33
2.	SCSH 2-3	41.86 ± 0.94
3.	SCSH 2-6	13.92 ± 1.04
4.	SCSH 3-5	27.08 ± 1.17
5.	SCSH 3-6	24.51 ± 1.77
6.	SCSH 3-9	5.75 ± 1.46
7.	SCSH 3-10	2.68 ± 1.15
8.	SCSH 4-13	0.63 ± 0.49
9.	SCSH 6-4	34.24 ± 1.04
10.	SCSH 6-6	29.32 ± 1.72
11.	SCSH 7-2	1.64 ± 1.79
12.	SCSH 7-3	2.29 ± 1.74
13.	SCSH 7-4	12.42 ± 1.16
14.	SCSH 7-7	52.82 ± 1.70
15.	SCSH 7-8	2.67 ± 1.54
16.	SCSH 8-4	27.12 ± 1.34
17.	SCSH 8-11	16.11 ± 1.64
18.	SCSH 8-15	13.12 ± 0.99
19.	SCSH 8-18	194.68 ± 0.77
20.	SCSH 8-19	1.87 ± 1.29
21.	SCSH 9-14	0.83 ± 0.52
22.	SCSH 11-16	18.34 ± 0.57
23.	SCSH 13-4	20.78 ± 1.71
24.	SCSH 13-6	13.15 ± 1.41
	Parental genotypes	
25.	Mosambi	22.01 ± 1.14
26.	PS-8	12.11 ± 1.66
27.	PS-13	9.59 ± 1.75
28.	Tangerine cv Daisy	46.45 ± 1.64
	LSD ($p \leq 0.05$)	0.032

Furthermore, the results of the sensory analysis of citrus fruits showed that in the parent varieties, the hedonic score varied between 7 (Tangerine cv Daisy) and 9 (Mosambi and PS-13). Whereas, among the hybrids, hedonic scores were minimum (3) for the hybrid SCSH 2-2 and SCSH 2-3. On the other hand, the highest hedonic score among new hybrids (9) was recorded for hybrid SCSH 3-10 and SCSH 7-2, with an average of 6. A total of six hybrids demonstrated high sensory scores (8 and 9), indicating strong potential for juice production (Table S1).

Hybrids with good hesperidin content having high palatability are important; therefore, the correlation study between hesperidin content and sensory attributes was carried out. Notably, the newly developed interspecific citrus scion hybrids SCSH 3-6 and SCSH 7-7 had the highest expression levels for both hesperidin content and sensory attributes among the studied genotypes, while remaining hybrids exhibited varying expression for the traits in the present study (Figure 1). Adham (2015) studied the quantification of hesperidin from different Citrus species, and variation was found both among the species and within the different tissues of the same species (i.e., peel and juice). In *Citrus sinensis*, the hesperidin content was found to be 19.5 mg/L in the peel and 20.6 mg/L in the juice. The hesperidin content in the peel of Citrus reticulata was observed to be 184.2 mg/L, whereas in the juice it was 14.8 mg/L. Furthermore, variations in flavone content, including hesperidin content, have also been observed in orange juices (Gámbaro et al. 2021; Lubinska-Szczygeł et al. 2018; Silva et al. 2014).

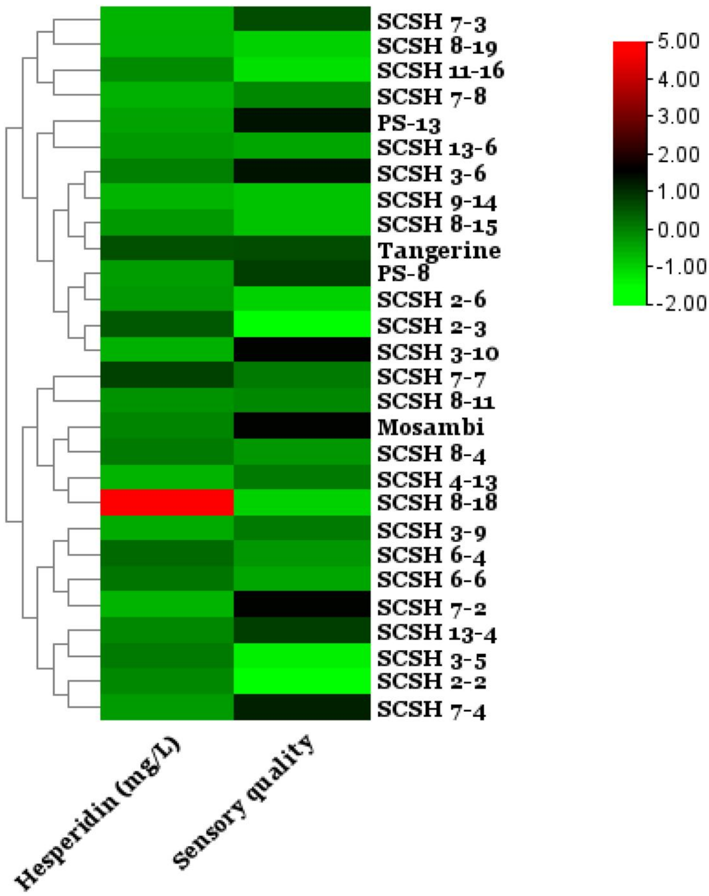

Figure 1. Clustered heat map depicting hesperidin content and sensory quality attributes across citrus hybrids and parental genotypes.

In the present study, hesperidin content was found in the range of 0.63–194.67 mg/L, indicating a considerable variation among the evaluated 24 interspecific citrus hybrids and parental genotypes. Additionally, six potential hybrids were identified with high sensory scores and consumer acceptance. Sensory attributes have a major impact on the overall likelihood of consumer purposes and market values. The present finding strongly correlated with the previous finding of sensory quality in different citrus fruits (Kumar et al. 2024; Lubinska-Szczygeł et al. 2020; Singh et al. 2025). The differences in studied parameters among the hybrids are attributed to the high heterozygosity of parental genotypes, which results in a unique genetic makeup for the hybrids and, consequently, metabolic differences in the newly developed genotypes (Huang et al. 2024; Lu et al. 2023; Seminara et al. 2023; Wang et al. 2024) (Figure 1).

ANOVA (analysis of variance) and heritability estimates for studied parameters

The ANOVA (analysis of variance) results indicated a highly significant variation ($p < 0.05$) among the studied citrus genotypes (24 new interspecific hybrids and parents) for both hesperidin content and sensory quality (Table S2). The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for hesperidin content were 152.14% and 152.17%, respectively, indicating minimal environmental influence. For sensory attributes, GCV and PCV were recorded at 29.58% and 30.85%, respectively (Table S2), suggesting substantial genetic variability. Notably, broad-sense heritability (H^2b) was very high for hesperidin content (99.9%) and sensory attributes (97.14%), as shown in Table S3. The small difference between GCV and PCV suggests limited environmental influence on these traits. The high magnitude of genotypic coefficient of variation and broad-sense heritability, both exceeding 90%, further supports the predominance of genetic control. These findings imply that the traits are primarily governed by additive gene action, thereby making them suitable candidates for effective selection and genetic improvement in breeding programs (Singh et al. 2023).

Expression of mid-parent and Better-parent heterosis in interspecific citrus crosses

Interspecific hybridization can result in a phenomenon known as heterosis, where the resulting hybrids exhibit enhanced fertility, increased longevity, and greater fruit production. In addition to their functional and health-related benefits, these newly developed fruits also possess distinct organoleptic characteristics (Lubinska-Szczygeł et al. 2018). The mid-parent (MPH) and better-parent heterosis (BPH) were computed for different crosses for two traits, hesperidin content and sensory values, which showed significant mean squares between crosses.

For the cross combination of PS-13 (White Pummelo) × Sweet orange, MPH ranged from −99.30% to 258.59% and BPH ranged from −99.52% to 144.50% for hesperidin content. For the same cross combination, MPH for sensory values ranged from −62.26% to 1.89% and BPH ranged from −62.96% to 0%. The other cross combination, PS-8 (Red Pummelo) × Sweet orange, MPH value ranged from −89.77% to 64.86% and BPH ranged from −92.34% to 23.44% for hesperidin content, as well as the MPH value for sensory values ranged from −56.00% to 8.00% and BPH ranged from −59.26% to 0%. In a cross combination of PS-13× Tangerine, the MPH ranged from −98.47% to 641.10% and the BPH ranged from −25.27% to 333.55% for hesperidin content. For sensory values, the MPH ranged from −58.33% to −25.00% and the BPH ranged from −54.54% to −18.18%.

Heterosis has been widely recognized as a key mechanism for improving fruit quality traits in horticultural crops. Felföldi et al. (2021) evaluated heterotic effects in tomato, focusing on physico-chemical, nutritional, and sensory attributes. Their study revealed substantial heterosis and heterobeltiosis in the commercial hybrids AS 300 F1 and AS 400 F1 with marked fluctuations in trait expression relative to their parental means. These findings provided valuable insights into the inheritance of quality-related traits and facilitated the chemical and organoleptic profiling of parental lines and hybrids for use in breeding programs. Further, Singh et al. (2023) studied the heterosis effect for nutritional qualities in interspecific citrus hybrids, and some of the hybrids exhibited mid-parent heterosis for Cu content in the fruit juice. Similarly, Lubinska-Szczygeł et al. (2020) observed notable variation in the physical, sensory, and volatile compound (monoterpene) profiles of the new citrus hybrid Jaffa compared to its parental genotypes, highlighting the influence of heterosis in determining fruit quality attributes.

The results of the present study are consistent with these previous findings and further substantiate the role of heterosis in determining both nutritional and sensory characteristics in interspecific citrus hybrids. The observed variation in trait expression across hybrids, surpassing mid-parent or better-parent values, indicates transgressive segregation due to heterozygous parents in perennial fruit crops (Singh et al. 2023). This also underscores the potential of exploiting heterotic effects to develop superior citrus genotypes with enhanced bioactive and market-preferred qualities.

Multivariate characterization of citrus hybrids based on hesperidin and sensory traits

The principal component analysis (PCA) revealed two components, PC1 and PC2, which explain 37.4% and 62.6% of the variation, respectively, collectively representing 100.00% of the total variability. The PCA biplot suggests that hesperidin content and sensory attributes have equal contributions in PC1 and PC2. The PCA analysis was able to distinguish the studied genotypes based on the hesperidin level and sensory qualities, indicating the potential use of employing the multivariate analysis in the study.

As illustrated in Figure 2, hybrids exhibiting elevated hesperidin content, viz., SCSH 8-18, SCSH 7-7, and SCSH 6-4, were positioned in the upper right quadrant of the principal component analysis (PCA) biplot adjacent to the parental genotype tangerine cv Daisy. In contrast, genotypes with lower hesperidin levels, including SCSH 3-10, SCSH 7-2, and SCSH 7-4, clustered in the upper left quadrant along with seed parent PS-13 and PS-8. The biplot further revealed that hybrids with superior sensory scores, viz., SCSH 3-10, SCSH 7-2, SCSH 3-6, and SCSH 7-4, were also situated in the upper left quadrant in addition to the parental genotypes Mosambi and PS-13. Conversely, genotypes with the lowest sensory scores were predominantly located in the lower right quadrant. These spatial distributions suggest a negative association between hesperidin content and sensory quality, indicating that these are contrasting traits among the evaluated genotypes. The observed variation underscores the potential for a trait-specific targeted breeding program. Notably, the inclusion of tangerine cv Daisy as a parental line contributed to enhanced hesperidin accumulation in hybrids, particularly in combinations with white pummelo, as crosses between white pummelo and tangerine showed elevated hesperidin content in their juices, thereby demonstrating its utility for improving nutraceutical quality in citrus breeding programs.

Principal component analysis (PCA) and hierarchical clustering revealed two promising hybrids, namely, SCSH 3-6 and SCSH 7-7, exhibiting both high sensory quality and optimal levels of hesperidin. The PCA distinctly separated genotypes based on variations in hesperidin content and sensory attributes, effectively highlighting the contrasting nature of these traits. Based on these analyses, hybrids combining high sensory appeal with moderate-to-high hesperidin content were considered more desirable than those excelling in only one of the traits. This selection preference aligns with the observed negative and non-significant correlation between hesperidin concentration and sensory quality, suggesting that a balance of both traits is critical for dual-purpose utilization in fresh consumption and health-based applications.

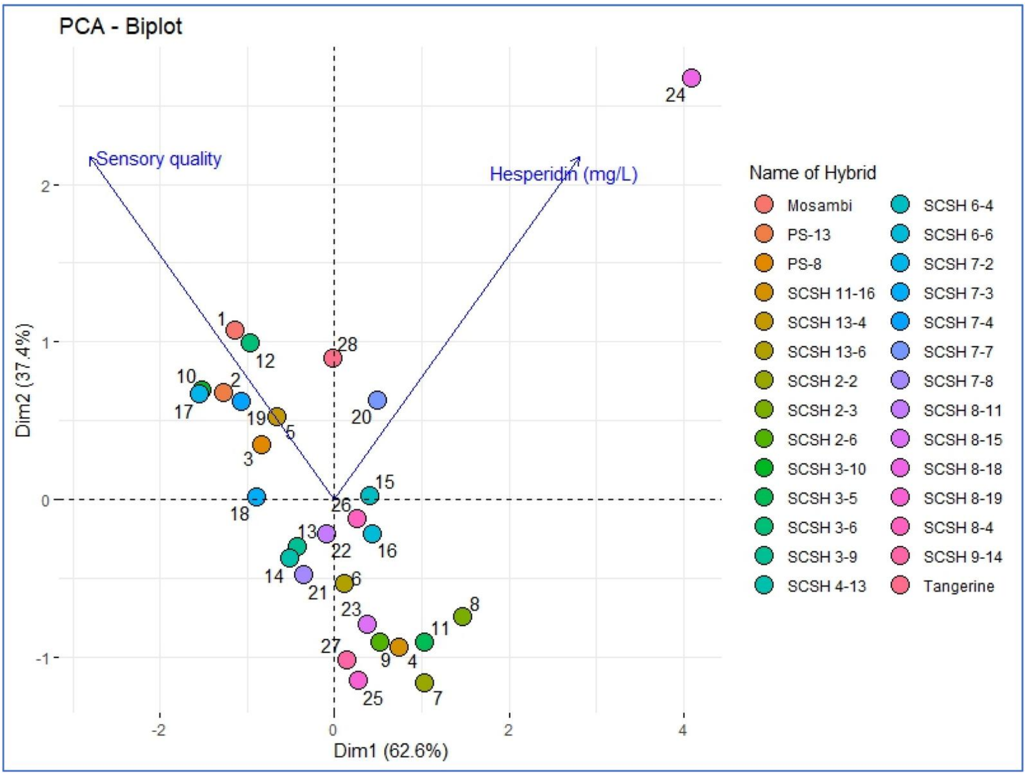

Figure 2. Principal component analysis (PCA) biplot showing 24 different hybrids and parental genotypes, with two major components, sensory evaluation and hesperidin content.

Comparable approaches have been employed in other crops to assess biochemical and quality trait diversity. Researchers have investigated metabolite diversity and fruit quality traits in blueberry, revealing heritability estimates ranging from 20% to 90%, which underscored the substantial genetic contribution to trait expression (Mengist et al. 2020). Furthermore, in kola (*Cola nitida*), researchers reported significant genotypic variability for bioactive compounds, supporting the scope for genetic improvement of nutraceutical traits (Nyadanu et al. 2020). Similarly, Mishra et al. (2022) characterized phenotypic diversity in red-fleshed guava and effectively utilized PCA and cluster analysis to interpret complex trait datasets (Delgado et al. 2013; King et al. 2012). These studies collectively validate the application of multivariate analysis as a powerful tool for identifying superior genotypes based on composite trait performance.

Hierarchical cluster analysis of studied citrus genotypes

To complement and validate the results obtained from principal component analysis (PCA), hierarchical clustering (HC) was performed using the combined dataset of 24 interspecific citrus hybrids and four parental genotypes. The HC analysis grouped the studied citrus genotypes into four distinct clusters, as illustrated in Figure 3. Cluster I comprised genotypes predominantly characterized by higher hesperidin content, including SCSH 7-8, SCSH 8-4, SCSH 7-4, SCSH 13-6, SCSH 6-6, and SCSH 7-3, along with

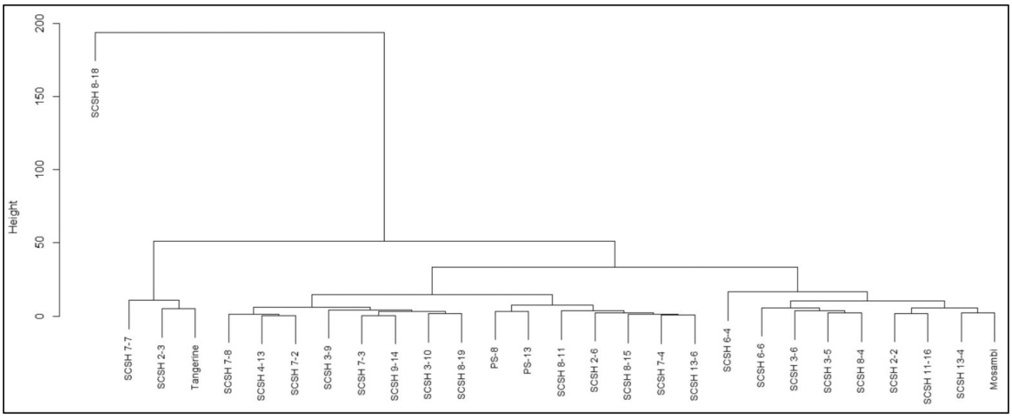

Figure 3. Hierarchical clustering of studied citrus genotypes showing distinct clusters associated with variations in hesperidin accumulation and sensory performance.

parental genotype tangerine cv Daisy. Cluster II primarily consisted of genotypes with superior sensory attributes, such as SCSH 3-10, SCSH 7-2, SCSH 3-6, and SCSH 13-4, in addition to the parental genotypes, sweet orange cv Mosambi and pummelo (PS-13), having low hesperidin content. Notably, some genotypes in this cluster, such as PS-13 and Mosambi, exhibited comparatively lower hesperidin content. Clusters III and IV included genotypes with intermediate values for both traits. Cluster IV, in particular, contained genotypes with the lowest sensory scores. These clustering patterns closely mirrored those observed in the PCA, reinforcing the inverse relationship between hesperidin content and sensory acceptability.

Heatmap visualization further highlighted genotype-specific expression profiles, with hybrids such as SCSH 7-4 and SCSH 7-3 demonstrating consistently high levels for both hesperidin concentration and sensory quality. In contrast, other hybrids displayed varying degrees of expression, suggesting the presence of distinct biochemical and sensory trait combinations. Collectively, these results confirm that, in most cases, elevated hesperidin levels are associated with reduced sensory appeal, underscoring the importance of identifying genotypes that balance both nutritional and consumer-preferred traits. Furthermore, the observed negative association between hesperidin concentration and sensory quality has important implications for citrus breeding (Huang et al. 2024; Liang et al. 2024; Lu et al. 2023; Wang et al. 2024). While high hesperidin levels enhance the nutraceutical value of citrus juice, excessive accumulation may contribute to bitterness, potentially reducing consumer acceptability in fresh juice products. Therefore, hybrids exhibiting moderate hesperidin levels combined with superior sensory scores identified in the study are optimal candidates for fresh consumption and juice-based markets. The observed transgressive segregation for hesperidin content further indicates the potential for developing novel hybrids with tailored metabolite composition (Liang et al. 2024). Such hybrids can serve as valuable germplasm for enhancing specific traits in future breeding cycles. Further, the hybrids with very high hesperidin concentrations (e.g., SCSH 8-18) may be more suitable for nutraceutical extraction, where bitterness is not a limiting factor and high bioactive yield is advantageous.

Conclusions

The present study demonstrated significant variations for hesperidin content across new interspecific citrus hybrids. Some of the hybrids exhibited hesperidin content in both higher and lower amounts than their parental genotypes, attributed to transgressive segregation and high heterozygosity of parents. Hybrid genotype SCSH 8-18 (Pummelo × Tangerine) was identified as the richest source of hesperidin content (194.68 mg/L). Furthermore, among the hybrids evaluated from two cross combinations, i.e., Pummelo × Tangerine and Pummelo × Sweet Orange, the inclusion of tangerine as a male parent showed superiority for enhanced hesperidin content in fruit juice.

Additionally, sensory attributes varied among the hybrids, enabling the selection of superior hybrids such as SCSH 3-6 and SCSH 7-7 that balanced high hesperidin levels with desirable taste and flavor. The heritability estimates provide valuable insight for trait-specific crop improvement programs. Thus, interspecific hybridization offers a promising approach for developing new genotypes rich in bioactive compounds. The novel hybrid genotypes identified with enhanced hesperidin content and higher acceptability for sensory properties also possess potential as parents for a targeted citrus improvement programme. Furthermore, the present study uniquely integrates interspecific hybridization with targeted quantification of hesperidin to assess genetic variation and heterotic patterns in novel citrus hybrids. By combining biochemical and sensory evaluations with heterosis estimates, this study provides new insights into how hybridization influences metabolite accumulation and flavor traits. The findings highlight hesperidin as a potential biochemical marker for selection in citrus improvement programs.

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Informed consent

This study did not involve any experiments on human or animal subjects requiring ethical approval. Sensory evaluation of citrus juice was conducted following the guidelines set by the International Organization for Standardization (ISO), (ISO 13299:2016) for sensory analysis. A trained panel of ten participants evaluated attributes such as appearance, aroma, taste, and texture. All participants provided informed consent prior to participation, and their identities have been kept confidential in accordance with ethical research practices.

Authors' contributions

Conceptualization: NSh; Methodology: DC, AK, CL; Formal analysis and investigation: DC, NJ, HV, NS; Writing—original draft preparation: DC, NS; Writing—review and editing: RMS, AKD, GPM, NS, SS, AK, and SKS; Funding acquisition- NSh; Resources: VSR; Supervision: NSh and RMS.

Disclosure statement

The authors have no relevant financial or non-financial interests to disclose.

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