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Morpho-biochemical, antioxidant and pigmentation in different genotypes of pummelo fruits (*Citrus maxima*) in Meghalaya

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ABSTRACT

Pummelo [*Citrus maxima* (Burm.) Merr.] is a potential commercial citrus due to its wide adaptability, economic, and health benefits. In spite of the vast genetic resources of citrus, the lack of systematic characterization has primarily caused significant gaps in understanding the valuable traits of pummelo, resulting in their sparing utilisation. The present study was carried out during 2021–2023 at ICAR Research Complex NEH Region, Umiam, Meghalaya to evaluate 14 genotypes of pummelo to identify the ideal genotypes and popularise the crop. Ten fully mature and healthy fruits from each genotype were collected randomly in different directions of the canopy with three replications. The results showed significant variations in fruit morphology, biochemical, antioxidant and pigmentation among different genotypes of pummelo. The highest fruit weight was found in RCCG-07 (900.3±57.5 g), pulp in RCCG-05 (74.0±2.2%), and juice content in RCCG-14 (49.5±2.8%). The maximum TSS (11.1±0.4%) and total carbohydrates (44.6±2.7 mg glucose/ml) were recorded in RCCG-07; vitamin C (97.6±2.9 mg/100 ml) and carotenoids (140.1±7.1 µg/100 ml) in RCGH-06. Furthermore, total phenolic content was obtained the maximum in RCCG-09, total flavonoid in RCCG-05, FRAP in RCCG-08, and pulp a* value in RCCG-06. The correlation indicated that ascorbic acid, carotenoids, and flavonoids contributed significantly to the antioxidant properties of this fruit. It was also found that the white-fleshed genotypes had higher fruit weight, pulp, and juice content, with fewer seeds. The quality attributes, including biochemical, antioxidant and pigmentation, were higher in red-fleshed genotypes; however, such traits may be associated with a higher seed number. Therefore, RCCG-05 (white-fleshed) and red-fleshed genotypes (RCCG-06 and RCCG-07) with higher fruit weight, pulp, and biochemical, antioxidant and pigmentation quality, can be ideal genotypes suitable for incorporation in breeding programmes, popularisation, and processing industries.

Keywords: *Citrus maxima ,* Colour, Functional attributes antioxidants, Genotypes, Pulp

The NEH region of India is part of the Himalaya and Indo-Myanmar biodiversity hotspot, indicating its rich crop genetic resources, particularly citrus, which is considered their place of origin (Malik *et al.* 2012). Among citrus species, pummelo [*Citrus maxima* (Burm.) Merr.] synonyms as *Citrus grandis* (L.) Osbeck, also referred to as the Chinese grapefruit, pomelo, or shaddock, showed wide diversity in the region. It is classified as either pigmented (or pink) or common (or white) pummelo. Based on the karyotype study, it is regarded as one of the true citrus species, along with *C. medica* and *C. reticulata* (Hynniewta *et al.* 2014). It is also the progenitor of modern citrus hybrids such as *C. paradisi* and tangelo. Pummelo had greater tolerance to insects and diseases (Singh *et al.* 2015). Furthermore, this fruit has the potential to thrive as an export commodity due to its rind attributes: a thin rind and easy peel are ideal for local consumption, and a thick rind is easy to handle and transport to distant markets. Recently, the demand for pummelo has surged, mostly in warm regions where other sweet citrus fruits are not suitable for growing. Authors observed that pummelo were growing mostly in the wild, semi-domestication , backyards, roadsides, etc with very few commercial orchards in Meghalaya. Genetic diversity is the basis for the conservation of plant genetic resources and their potential utilization. Furthermore, morphological traits are very important parameters in breeding strategies, being the first markers used for germplasm management (Paganova 2009). Traits like fruit weight, juice content, and seed behaviour significantly contribute to citrus diversity and can be used to differentiate the accessions and the identification of ideal genotypes for commercial purposes. However, the lack of systematic characterization in pummelo primarily causes significant gaps in understanding the valuable traits

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of pummelo, and therefore, the enormous genetic diversity has only been sparingly utilised in the improvement programmes. In view of these, a study was conducted to determine the morphological and biochemical variation among genotypes of pummelo to identify ideal genotypes for commercial uses and popularization of this crop.

MATERIALS AND METHODS

The present study was carried out during 2021–2023 at ICAR Research Complex NEH Region, Umiam (25°41'24.5"N, 91°55'08.9"E and 1010 m amsl), Meghalaya. Fourteen genotypes of pummelo of seedling origins and ages of 20–25 years were maintained in ICAR Research Centre NEH Region, Umiam, Meghalaya, under the recommended package of practices (Patel *et al.* 2008). Out of 14 genotypes, four were white-fleshed and 10 genotypes were red-fleshed (Fig. 1). Ten fully mature and healthy fruits from each genotype were collected randomly in different directions of the canopy, and the analysis was replicated thrice. The harvested fruits were thoroughly washed with tap water to remove adhering impurities. The harvested fruits were investigated for fruit morphological, biochemical, and functional properties. The soil of the experiment site consisted of clay loam to sandy clay loam with an acidic *p*H of 4.9. The nutrient status of the soil is SOC (1.77%), exchangeable Al^{3+} (148.6), Ca^{2+} (240.5), Mg^{2+} (120), K^{+} (66.7), Bray's P_2-P (1.2), and available B (0.9 mg kg). The climate of the experimental site is of the subtropical monsoon type. About 88.8% of the total rainfall (2230 mm) was occurring during May to October. The average minimum and maximum temperatures varied from 6.6˚C in January to 29.06˚C in August. Relative humidity ranges between 51.1% during winter and 90.13% during rainy season (Institute Agrometeorology Unit).

Fruit morphology parameters: Fruit morphological parameters such as fruit weight, fruit length, fruit width, rind thickness, number of segments/fruits, and number of seeds/fruits were recorded. Fruit dimension (length and width) and rind thickness were measured using a digital Vernier calliper (Model: Insize Code 1108-150) and expressed in millimeters (mm). Fruit and total seed weight were determined using a precision electronic balance (Model: SMART Aqua series) and expressed in g.

The pulp recovery percentage was estimated using the following formula:

$$\text{Pulp recovery \%} = \frac{\text{Pulp weight (g)}}{\text{Fruit weight (g)}} \times 100$$

Determination of biochemical properties: The juice was extracted from the pulp by straining through a muslin cloth and used for the estimation of different biochemical and functional attributes. The total soluble solids (TSS) were measured in three positions (base, middle and apex) of the fruit area by direct readings of juice using a digital hand-held refractometer (HI 96801), with results expressed in °Brix. Titratable acidity (TA) was determined according to Nielsen (2017). Ascorbic acid was estimated according to Mehta *et al.* (2018). The prepared extract was measured at 515 nm in a UV-visible spectrophotometer (Shimadzu model UV-1720) and expressed in mg/100 ml juice.

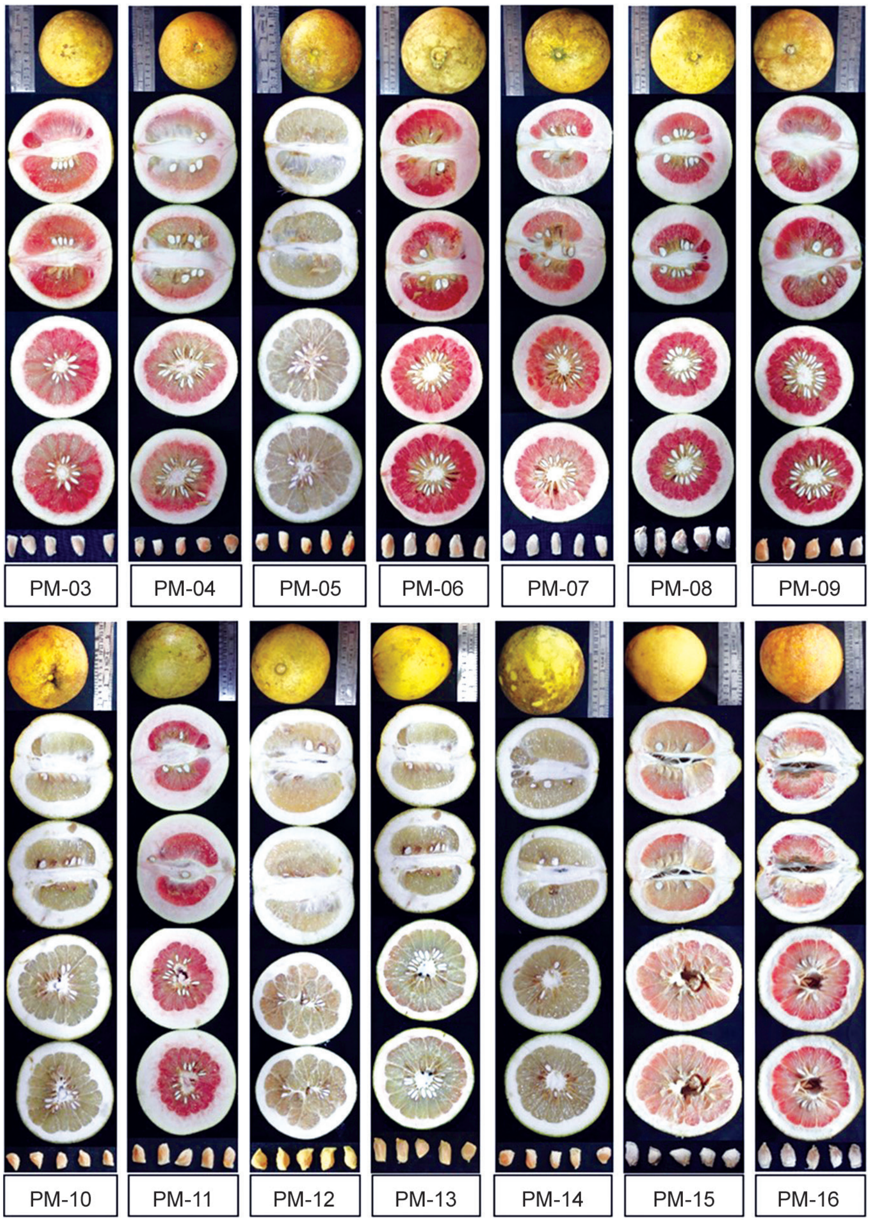

Fig. 1 Pictorial representation of fruits and pulp of different pummelo genotypes.

The fruit extract for the estimation of reducing sugar and total carbohydrates was prepared according to Chow and Landhaussera (2004). The prepared extract was estimated for reducing sugar at the absorbance of 540 nm and expressed in mg glucose/ml juice (Miller 1959) . Total carbohydrate was determined at the absorption of 490 nm and expressed in mg glucose/ml juice (DuBois *et al.* 1956). The extraction of carotenoids was determined spectrophotometrically at 450 nm. Total carotenoid was calculated using the extinction coefficient of β-carotene in hexane (E1%=2505 100 m/L/g) and expressed as µg β-carotene/100 ml juice (Moo-Huchin *et al.* 2017).

Determination of antioxidant activity: The fruit extract for the determination of functional attributes and antioxidant activity, such as total phenolic content (TPC), total flavonoids (TFC), DPPH free radical scavenging capacity, and ferric reducing antioxidant power (FRAP) , was prepared as prescribed by Oyaizu (1986). The TPC was estimated using Folin-Ciocalteu procedure, measured at 743 nm and expressed as mg GAE/100 ml juice (Singleton and Rossi 1965). TFC was estimated using an aluminum chloride ($AlCl_3$) colourimetric assay at 510 nm and presented as mg QE/100 ml juice (Zhishen *et al.* 1999). The free radical scavenging activity of the fruit extracts was estimated with DPPH (1, 1-diphenyl-2- picrylhydrazyl) method measured at 517 nm, and the antioxidant activity of the extract was expressed as IC_{50} (the concentration of fruit sample required to decrease the absorption at 517 nm by 50%) (Shen *et al.* 2010). FRAP assay was determined according to Wetchakul *et al.* (2019). The absorbance of the coloured product (ferrous tripyridyl triazine complex) was checked at 593 nm and FRAP values were expressed as micromoles of $FeSO_4$ equivalents (mM$FeSO_4$E/100 ml juice).

Colour measurement: The colour of peel and pulp of mature fruits was estimated using a Colour Hunter metre (HunterLab Color Quest XE). The instrument was calibrated using the black and white tiles. The value was expressed as L* values indicated lightness (black, L* = 0 and white, L* = 100), a* values indicated redness-greenness (red, a* = 100 and green, a* = −100), b* values indicated yellowness-blueness (yellow, b* = 100 and blue, b* = −100). The observation was replicated thrice for each sample. Observations were taken at the base, middle, and apex of fruits at an equidistant space under the aperture of the colour meter.

Statistical analysis: The data were analyzed using IBM SPSS (Version 25.0) software, and the data were presented as mean ± standard deviation using one-way ANOVA (p<0.05) of Tukey's HSD (honestly significant difference) test. The relationship between morphology, biochemical and antioxidant activity was analysed using R Studio (version 4.3.1).

RESULTS AND DISCUSSION

Fruit morphological characteristics: The results showed a significant variation in fruit morphological characteristics among different genotypes of pummelo (p< 0.05, Table 1). The highest fruit weight was found in RCCG-07 (900.3±57.5 g), followed by RCCG-12 (817.7±217.2 g). The maximum fruit length was recorded in RCCG-06 (129.8±3.4 mm), fruit diameter in RCCG-12 (345.9±8.1 mm), and peel thickness in RCCG-11 (28.6±6.3 mm). The pulp content was obtained the maximum in RCCG-05 (75.8±4.5%), which was at par with RCCG-14 (74.0±2.2%), and the juice content was noted

Table 1 Fruit morphological characteristics in different genotypes of pummelo

Genotypes	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Peel thickness (mm)	Pulp (%)	Segments per fruit	Fruit axis diameter (mm)	Juice content (ml/100 g pulp)
RCCG-03	490.4±56.8^{cd}	106.3±7.4^{de}	103.1±8.6^{a}	14.6±0.6^{d}	53.8±2.0^{cd}	15.0±2.2^{ab}	20.0±2.1^{bcd}	30.8±2.3^{def}
RCCG-04	597.4±98.3b^{cd}	108.6±5.7^{cde}	116.8±7.7^{a}	13.7±1.5^{d}	62.0±7.5^{abc}	16.4±1.3^{ab}	20.7±4.4^{bcd}	28.7±2.5^{ef}
RCCG-05	599.0±82.0^{bcd}	107.3±13.0^{de}	108.2±11.9^{a}	13.4±2.1^{d}	75.8±4.5^{a}	13.0±1.6^{b}	16.1±3.6^{d}	35.7±1.7^{cde}
RCCG-06	760.4±57.2^{ab}	129.8±3.4^{a}	129.8±5.6^{a}	23.4±3.3^{ab}	53.7±6.7^{cd}	13.0±1.0^{b}	23.1±4.5^{a-d}	37.0±2.0^{cd}
RCCG-07	900.3±57.5^{a}	119.3±1.7^{a-d}	132.6±3.4^{a}	12.9±2.4^{d}	57.0±5.9^{c}	16.2±1.6^{ab}	20.6±2.2^{bcd}	31.7±5.4^{c-f}
RCCG-08	745.3±193.8^{ab}	117±13.6^{a-d}	123.8±11.1^{a}	16.1±2.7^{cd}	66.4±8.2^{abc}	16.0±2.0^{ab}	24.9±3.3^{abc}	37.7±1.6^{bcd}
RCCG-09	420.6±55.7^{d}	95.6±4.4^{e}	100.7±6.7^{a}	14.5±1.4^{d}	57.1±7.4^{c}	15.2±1.1^{ab}	18.1±2.3^{cd}	34.0±1.7^{c-f}
RCCG-10	776.7±127^{ab}	117.9±6.9^{a-d}	120.2±5.7^{a}	14.6±2.3^{d}	68.2±8^{abc}	16.0±1.9^{ab}	20.1±3.4^{bcd}	49.0±3.6^{a}
RCCG-11	738.5±217.2^{ab}	125.3±7.9^{ab}	135.2±8.1^{a}	28.6±6.3^{a}	38.8±14.9^{d}	18.0±1.6^{a}	29.7±4.0^{a}	27.7±2.2^{f}
RCCG-12	817.7±217.2^{ab}	111.8±7.9^{b-e}	345.9±8.1^{a}	14.3±6.3^{d}	59.8±14.9^{bc}	16.0±1.6^{ab}	23.2±4.0^{a-d}	48.3±2.2^{a}
RCCG-13	730.6±38.4^{ab}	124.8±3.0^{abc}	122.7±3.8^{a}	14.8±1.1^{d}	68.2±1.7^{abc}	15.0±0.7^{ab}	23.1±1.4^{a-d}	45.2±5.3^{ab}
RCCG-14	595.4±114.5^{bcd}	112.6±8.4^{bcd}	112.4±9.7^{a}	15.4±1.3^{cd}	74.0±2.2^{ab}	14.0±1.6^{b}	22.4±0.5^{bcd}	49.5±2.8^{a}
RCCG-15	713.4±46.3^{abc}	117.0±5.1^{a-d}	121.0±3.5^{a}	17.0±1.1^{cd}	62.3±5.0^{abc}	13.6±1.1^{b}	24.6±0.7^{abc}	39.6±7.5^{bc}
RCCG-16	686.3±53.2^{abc}	107.0±7.8^{de}	118.0±4.8^{a}	21.0±1.1^{bc}	61.2±3.3^{abc}	16.0±2.3^{ab}	27.0±1.9^{ab}	32.7±4.1^{c-f}

Values given are mean (n = 15) with ±SE followed by different letters on each column indicate significant difference from each other according to Tukey's test (p<0.05).

as the highest in RCCG-14 (49.5±2.8%), which was at par with RCCG-10 (49.0±3.6%) and RCCG-12. The variations among the genotypes might be due to individual genetic effects and their interaction with the environment. Self-incompatibility and sexual recombination were previously shown to be the main reasons for considerable variability among the pummelo genotypes (Cameron and Soost 1961). It was also observed the white-fleshed genotypes had higher fruit weight (697.2 g) by 2.8%, pulp content (69.5%) by 19.6%, and juice content (45.6%) by 32.2% as compared to red-fleshed genotypes. The presence of variation underlines the significance of morphological characterization for improvement through selection and subsequent research employing biotechnological and biochemical tools. Furthermore, the fruit weight in our studies was of medium size (420.6±55.7–900.3±57.5 g), with juice (28.7±2.5–49.5±2.8 ml/100 g pulp) and pulp content (38.8±14.9–75.8±4.5%) as compared to those earlier reported by Nandi *et al.* (2019), Mahardika *et al.* (2017), and Nishad *et al.* (2018). It is well known that genotypes with medium fruit weight, high juice and pulp content had high preference among consumers, thus an important trait for commercial cultivars of pummelo.

Seed characteristics: The results indicated a significant variation in the seed characteristics of pummelo genotypes ($p<0.05$, Table 2). The lowest seed number was noted in RCCG-14 (14.8±3.1/fruit), which was followed by RCCG-05. Similarly, RCCG-14 had the maximum seed weight (5.1±1.9 g/fruit). The maximum seed length was recorded in RCCG-08 (20.6±0.9 mm), and seed thickness in RCCH-05 (5.9±0.4 mm). Our finding showed that the number of seeds per fruit was 52.2% lower in white-fleshed as compared to red-fleshed; however, the seed weight was 8.5% higher than in red-fleshed (0.393 g/seed). Consumer preference for seedless fruit has increased and is emerging as an essential attribute for fresh markets and processing. Therefore, seedless or less-seeded pummelo genotypes are ideal for commercial production due to the convenience of consumption as fresh fruit and industrial uses. In our case, significantly reduced seed genotypes such as RCCG-14 (14.8±3.1 seeds/fruit) and RCCG-05 (22.0±17.5 seeds/fruit) could be potential cultivars and parents in breeding programmes.

Biochemical characteristics: The results showed significant differences in the biochemical characteristics among the genotypes of pummelo ($p<0.05$, Table 3). The highest TSS was exhibited in genotype RCCG-07 (11.1±0.4%), titratable acidity in RCCG-10 (3.8±0.2%), total carbohydrates in RCCG-07 (44.6±2.7 mg glucose/ml), and reducing sugar in RCCG-05 (17.1±3.0 mg glucose/ml). It was found that the red-fleshed genotypes had higher biochemical attributes, including TSS (9.81%) and total carbohydrates (36.31 mg glucose/ml) by 4.3% and 5.9%, respectively. The variation in biochemical traits among the genotypes might be due to the unique genetic constitution of the genotype (Rymbai *et al.* 2023). Biochemical attributes such as juice content, TSS and TA are important parameters for the selection of elite genotypes of citrus trees. Similarly, TSS and acidity are considered the two key elements determining the quality attributes of the fruits, the sweetness and taste, and thus serve as indicators of the fruit maturity indices and processing appropriateness (Rymbai *et al.* 2023). The TSS and TA in our study were comparable with those earlier reported (Mahardika *et al.* 2017, Nishad *et al.* 2018, Nandi *et al.* 2019). Therefore, genotypes (RCGH-06 and RCCH-07) with high TSS and low acidity can be considered suitable for fresh consumption as well as processing and value addition.

Table 2 Seed characteristics in different genotypes of pummelo

Genotypes	Number of seeds/ fruit	Seed weight/fruit (g)	Seed length (mm)	Seed diameter (mm)	Seed thickness (mm)
RCCG-03	124.0±9.9^{ab}	50.0±5.0^{ab}	14.0±1.1^{b-e}	6.4±0.4^{de}	3.0±0.2^{f}
RCCG-04	127.2±20.2^{a}	44.7±9.8^{b}	15.4±0.7^{bcd}	5.7±1.0^{e}	2.7±0.6^{f}
RCCG-05	22.0±17.5^{gh}	5.6±4.1^{e}	15.2±0.6^{bcd}	10.2±1.^{6}a	5.9±0.4^{a}
RCCG-06	88.8±29.7^{b-e}	31.0±1.1^{cd}	13.3±1.5^{de}	6.7±2.2^{de}	3.2±0.7^{ef}
RCCG-07	128.6±14.^{3}a	61.7±2.^{2}a	16.5±1.6^{b}	7.6±1.5^{cde}	2.9±0.6^{f}
RCCG-08	104.0±6.4^{abc}	37.7±3.8^{bc}	20.6±0.9^{a}	9.9±0.6^{ab}	5.5±0.2^{ab}
RCCG-09	97.0±11.4^{a-d}	27.7±2.6^{cd}	12.3±1.^{7}e	6.6±0.4^{de}	3.1±0.6^{ef}
RCCG-10	86.6±25^{c-f}	27.4±13.6^{cd}	13.7±1.5^{cde}	7.5±1.4^{cde}	3.2±0.6^{ef}
RCCG-11	101.0±30.6^{abc}	21.8±11.0^{d}	16.2±0.6^{bc}	8.5±0.3^{a-d}	4.7±0.3^{bcd}
RCCG-12	56.8±30.6^{efg}	20.8±11.0^{d}	13.1±0.6^{de}	7.5±0.3^{cde}	3.7±0.3^{def}
RCCG-13	51.2±2.8^{fg}	19.7±0.7^{d}	12.8±1.2^{de}	7.6±0.6^{b-e}	4.8±0.4^{a-d}
RCCG-14	14.8±3.1^{h}	5.1±1.9^{e}	15.2±0.6^{bcd}	9.1±1.3^{abc}	5.3±0.5^{abc}
RCCG-15	62.8±6.9^{def}	27.2±3.8^{cd}	15.3±1.2^{bcd}	8.3±0.2^{a-d}	4.2±0.4^{cde}
RCCG-16	58.2±5.0^{efg}	24.0±2.5^{d}	13.0±0.8^{de}	8.0±0.2^{a-e}	3.7±0.2^{def}

Values given are mean (n = 15) with ±SE followed by different letters on each column indicate significant difference from each other according to Tukey's test ($p<0.05$).

Antioxidant activity: The result showed a significant difference among genotypes of pummelo for antioxidant properties ($p<0.05$, Table 3). Ascorbic acid was found to be maximum in RCCG-06 (97.6±2.9 mg/100 ml), followed by RCCG-05. Total carotenoids was recorded as the highest in RCCG-06 (140.1±3.2 µg/100 ml), which was on par with RCCG-16. The highest TPC was recorded in RCCG-09 (131.5±9.0 mg GAE/100 ml), TFC in RCCG-11 (81.1±11.0 mg QE/100 ml and FRAP in RCCG-08 (261.1±6.6 mM $FeSO_4$E/100 ml). Furthermore, the antioxidant properties of red-fleshed were greater than white-fleshed genotypes for ascorbic acid (88.44 mg/100 ml), carotenoids (97.0 µg/100 ml), TPC (85.97 mg GAE/100 ml), flavonoids (41.44 mg QE/100 ml), and FRAP (181.6 mM $FeSO_4$E/100 ml) by 1.2%, 100%, 7.3%, 27.0%, and 24.5%, respectively. The variation among the genotypes might be due to genetic influence (Rymbai *et al.* 2023). Our finding demonstrated that these genotypes have a higher ascorbic acid content than those earlier reported in pummelo (Mahardika *et al.* 2017, Nandi *et al.* 2019). Ascorbic acid is considered the most significant antioxidant vitamin; nevertheless, humans are unable to synthesise it due to a lack of the gulonolactone oxidase enzyme. While a shortage of dietary ascorbate causes scurvy and clinical syndrome (Paciolla *et al.* 2019). For this reason, it is crucial to add foods high in ascorbic acid to the diet. Furthermore, a positive correlation of ascorbic acid with TSS (0.292*) and reducing sugar (0.346**) might be due to complex and recurrent interactions between organic acids and sugars, which could be related to the synthesis process of ascorbic acid from glucose (Kustermann *et al.* 1998). Our study showed that carotenoids were detected only in the pink-fleshed pummelo genotypes; however, the white-fleshed genotypes such as RCCG-05, RCCG-10, RCCG-12, and RCCG-14 recorded no value for carotenoid content. This could be due to the minimal level of carotenoids in white-fleshed pummelo, either at traceable or non-detectable levels (Yan *et al.* 2018). A considerable carotenoid content in fruit is a key sign of their superior quality and nutritional value. These fruits also contained higher levels of TFC and TPC than those pummelo earlier reported, while DPPH IC50 values were lower than those earlier reported (30.8–65.4) (Nishad *et al.* 2018, Deb *et al.* 2024). Our study also showed a positive correlation of antioxidants (FRAP) and a negative correlation of DPPH with TSS, ascorbic acid, carotenoids, TPC and flavonoids (Fig. 2). It was earlier reported that the accumulation of total sugar, ascorbic acid, carotenoids, phenolics, and flavonoids in the fruit are primarily responsible for their higher antioxidant activity (Rymbai *et al.* 2022). Therefore, ingesting pummelo fruits can help humans comply with their nutritional requirements and provide protection against the occurrence of a number of degenerative disorders.

Pigmentation: Fruit pigmentation is a key consideration for marketing, consumer appeal, and quality standards, especially for fruit specified for dessert purposes. The myriad colours in citrus fruits were primarily due to variations in the composition of pigment compounds and their content (Wei *et al.* 2017, Lado *et al.* 2019). The primary pigments found in the fruits of citrus are carotenoids (Lado *et al.* 2019), which was corroborated by our finding that total carotenoids had a significant positive correlation with pulp a* (0.740) (Fig. 2). In addition to imparting colour to the fruits, carotenoids also

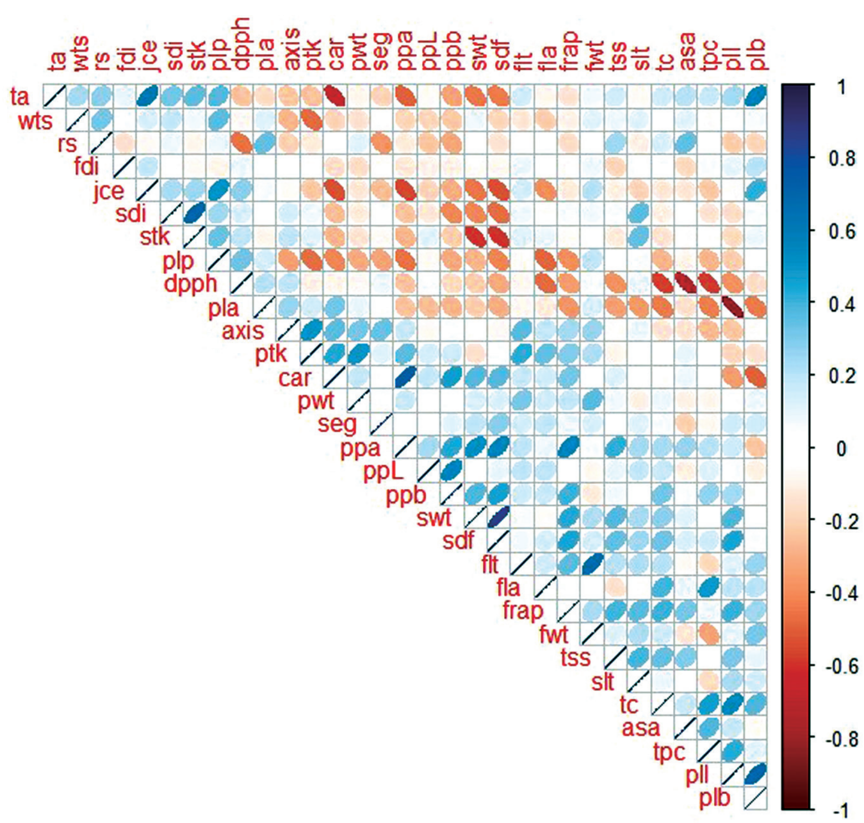

Fig. 2 Relationship among fruit morpho-biochemicals, antioxidants and pigmentations of pummelo.

Positive correlations are shown in blue and negative ones in red. The intensity and the size of the ellipse are proportional to the correlation coefficient. Ta, Titratable acidity; wts, Seed weight; rs, Reducing sugar; fdi, Fruit diameter; jce, Juice content; sdi, Seed diameter; stk, Seed thickness; plp, Pulp; dpph-1, 1-diphenyl-2- picrylhydrazyl; pla, Peel a*; Axis, Fruit axis; ptk, Peel thickness; car, Total carotenoids; pwt, Peel weight; seg, Segment/fruit; ppa, Pulp a*; ppL, Pulp L*; swt, Seed weight/fruit; sdf, Seed diameter/fruit; flt, Fruit length; fla, Total flavonoids; frap, Ferric reducing antioxidant power; fwt, Fruit weight; tss, Total soluble solids; slt, Seed length; tc, Total carbohydrates; asa, Ascorbic acid; tpc, Total phenolic compounds; pll, Peel L*; plb, Pulp b.

Table 3 Biochemical and antioxidant properties in different genotypes of pummelo

Genotypes	Total soluble solids (%)	Titratable acidity (%)	Vitamin C (mg/100 ml juice)	Carotenoids content (µg/100 ml juice)	Total phenolic compounds (mg GAE/100 ml juice)	Flavonoids (mg QE/100 ml juice)	DPPH IC_{50} sample (mg/ml)	FRAP (mM $FeSO_4$ E/100 ml juice)	Total carbohydrates (mg Glucose/ ml juice)	Reducing sugar (mg Glucose/ ml juice)
RCCG-03	9.3±0.3^{c-f}	1.7±0.2^{cd}	93.5±19.4^{ab}	67.5±1.4^{e}	95.1±4.2^{bcd}	36.3±1.7^{cde}	23.0±2.7^{de}	217.6±23.8^{bc}	37.2±4.6^{a-d}	13.0±2.3^{b-e}
RCCG-04	10.1±0.5^{a-e}	1.7±0.2^{cd}	77.8±7.8^{b}	92.0±2.7^{c}	76.0±9.3^{def}	36.1±3.0^{cde}	36.3±5.6^{ab}	90.6±6.0^{g}	30.5±8.0^{bcd}	12.2±3.5^{cde}
RCCG-05	9.5±1.1^{b-f}	3.6±1.^{1}a	95.3±5.3^{ab}	-	106.2±17.1^{b}	48.5±16.8^{bc}	23.0±2.3^{de}	98.4±3.9^{g}	36.4±1.2^{a-d}	17.1±3.0^{a}
RCCG-06	10.5±0.2^{a-d}	2.4±0.3^{bc}	97.6±2.^{9}a	140.1±3.2^{a}	98.6±7.2^{b}	33.0±3.2^{def}	21.0±1.0^{e}	232.9±11.0^{b}	39.7±2.9^{ab}	14.0±0.9^{a-e}
RCCG-07	11.1±0.^{4}a	1.7±0.1^{cd}	90.5±7.0^{ab}	77.7±5.4^{d}	77.3±2.7^{c-f}	37.7±5.4^{b-e}	23.1±0.8^{de}	209.7±12.4^{c}	44.6±2.7^{a}	15.3±0.6^{abc}
RCCG-08	11.0±0.5^{ab}	2.5±0.9^{bc}	89.4±32.4^{ab}	85.6±3.9^{cd}	76.4±19.7^{def}	23.5±9.6^{efg}	33.4±15.4^{a-d}	261.1±6.6^{a}	36.7±5.0^{a-d}	10.5±1.9^{e}
RCCG-09	9.5±1.1^{a-f}	1.8±0.3^{cd}	96.2±4.2^{ab}	84±2.4^{d}	131.5±9.0^{a}	46.3±3.3^{bcd}	24.9±3.6^{cde}	154.1±8.4^{def}	38.2±9.3^{abc}	11.9±0.7^{cde}
RCCG-10	10.7±1.8^{abc}	3.8±0.^{2}a	94.9±4.6^{ab}	-	62.8±7.7^{fg}	27.7±8.3^{ef}	24.8±6.7^{cde}	176.4±11^{d}	37.1±9.8^{a-d}	14.7±2.3^{a-d}
RCCG-11	9.4±0.7^{c-f}	1.9±0.1^{cd}	85.1±3.2^{ab}	85.6±2.3^{cd}	96.5±2.7^{bc}	81.1±11.0^{a}	24.4±0.6^{cde}	202.1±8.5^{c}	40.3±1.0^{ab}	11.4±0.6^{de}
RCCG-12	7.9±0.^{7}f	3.1±0.1^{ab}	75.9±3.^{2}b	-	94.0±2.7^{bcd}	34.3±11.0^{c-f}	34.2±0.6^{abc}	139.3±8.5^{f}	35.2±1.0^{a-d}	11.4±0.6^{de}
RCCG-13	9.2±0.2^{c-f}	3.1±0.1^{ab}	77.0±3.4^{b}	78.0±3.4^{d}	86.5±3.3^{b-e}	52.1±3.3^{b}	29.7±1.1^{b-e}	151.4±5.0^{ef}	41.4±1.1^{a}	12.1±0.2^{cde}
RCCG-14	9.5±0.2^{b-f}	2.6±0.2^{bc}	81.3±7.1^{ab}	-	55.8±3.7^{g}	10.5±0.9^{g}	42.3±1.0^{a}	134.2±4.3^{f}	28.0±1.3^{cd}	11.5±1.0^{de}
RCCG-15	9.1±0.2^{def}	2.0±0.2^{cd}	86.0±2.8^{ab}	119.4±6.7^{b}	70.5±8.7^{efg}	48.4±2.0^{bc}	30.4±0.6^{b-e}	162.8±4.9^{de}	27.2±1.1^{d}	15.9±0.9^{ab}
RCCG-16	8.8±0.3^{ef}	1.3±0.^{0}d	84.3±2.1^{ab}	140.1±7.1^{a}	51.1±4.2^{g}	20.0±1.4^{fg}	36.4±2.1^{ab}	133.7±12.6^{f}	27.2±1.5^{d}	14.3±0.5^{a-d}

Values given are mean (n = 15) with ±SE followed by different letters on each column indicate significant difference from each other according to Tukey's test (p<0.05). -, not detected.

play a variety of roles; capturing and discharging energy during photosynthesis, serving as precursors to ABA and strigolactones, and providing nutritional and health benefits through their pro-vitamin A and antioxidant capabilities (Rodriguez-Concepcion *et al.* 2018, Lado *et al.* 2019). The fruits of pummelo have appealing pigments, as evidenced by a significant variation in the value of L*, a*, and b* in the pulp (p<0.05, Supplementary Table 1). The pulp a* value was recorded as the highest in RCCG-06 (14.6±1.1), followed by RCCG-08 (9.0±4.1) and RCCG-07. However, the minimum pulp a* value was recorded in RCCG-12 (-1.3±0.4). Our findings indicated that such genotypes (RCCG-12) with a negative a* value are light green in colour. The pulp b* value was recorded as the highest in RCCG-13 (12.5±0.7), which was on par with RCCG-03 (12.4±2.2). The pulp a* value (6.34) and pulp b* value (9.76) were greater in red-fleshed by 115.7% and 34.5%, respectively, as compared to white-fleshed genotypes. The colour variation in fruit pulp among the genotypes was due to a genetic effect. It is well established that the L* value is a suitable indicator of darkening that arises either due to increasing pigment concentrations or oxidative browning reactions (Keshek *et al.* 2019). It is widely recognized that the L* value is an effective value to identify darkening in fruits resulting either from oxidative browning reactions or rising pigment levels (Keshek *et al.* 2019). Additionally, the higher values of a* and b* improve the decorative appearance of the product for better consumer preferences. In this case, the consumer's preference and visual appearance were further enhanced by the greater a* value (7.1–14.6), which had a decorative effect with pink flesh in genotypes (RCCG-06, RCCG-08, RCCG-07 and RCCG-04). Therefore, pummelo fruits can be an excellent option as a viable source of natural edible colour in food processing.

Correlation: A significant relationship between pigmentation and other important traits in pummelo was noted (Fig. 2). Fruit weight had a significant positive correlation with the fruit axis (0.250*) and pulp b* value (0.303*); however, it showed a significant negative correlation with TPC (-0.327*). The pulp percentage showed a significant positive correlation with juice content (0.500**), TA (0.379**), and DPPH (0.325**), but showed a negative correlation with the majority of the traits. Similarly, total carotenoids had a significant correlation with FRAP (0.318**) and pulp a* (0.740). TPC had a significant positive correlation with TFC (0.485**) and total carbohydrates (0.462**). In addition, TFC also showed a significant negative relationship with DPPH (-0.472**), suggesting that total flavonoids (TFC) and total carotenoids (car) are vital for the antioxidant activity of pummelo. Our research corresponds with that of Chandra *et al.* (2014), who found that flavonoids (TFC) provided 32% of the antioxidant activity in fruit crops. Ascorbic acid had a significant positive correlation with TSS (0.292*), TPC (0.382**), FRAP (0.310**), reducing sugar (0.346**), and pulp a* (0.259*), but had a negative correlation with DPPH (-0.714**), suggesting that ascorbic acid is one of the mechanisms influencing antioxidant qualities. The lower DPPH IC_{50} values designate a higher amount of antioxidant activity. Furthermore, the trait with pink flesh was also found to have higher biochemical and antioxidant properties. Therefore, selection on the basis of medium fruit weight, high ascorbic acid content, and red flesh colour might enhance the quality and preference attributes of pummelo.

Our findings concluded that a wide variation in morphological, biochemical and antioxidant properties was observed among genotypes of pummelo. The white-fleshed genotypes had higher yield attributes, including fruit weight, pulp content, and juice content, with less seed weight. While red-fleshed genotypes had higher quality attributes for biochemical, antioxidant and pigmentation of fruits than white flesh; however, such traits may be associated with a higher seed number. Therefore, potential genotypes such as RCCG-05, a white-fleshed genotype, had higher pulp content and was less seeded with high-quality attributes; and red-fleshed genotypes (RCCG-06 and RCCG-07) had higher fruit weight with better biochemical, antioxidants and pigmentation quality and can be potential genotypes for the selection of cultivars in breeding programmes and utilization in the processing industry.

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