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Biochemical properties and mineral nutrient interrelationships in interspecific citrus hybrids for enhanced fruit quality

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Abstract
This study investigated the correlations between biochemical traits and mineral nutrients in interspecific citrus hybrids and their parental genotypes, pummelo (*Citrus maxima*) and sweet orange cv. Mosambi (*Citrus sinensis*), evaluated under subtropical conditions at the ICAR-Indian Agricultural Research Institute, New Delhi. Biochemical parameters such as total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, sugars, ascorbic acid, total phenols, total flavonoids, total carotenoids, lycopene, and antioxidant activity, along with mineral nutrients (P, Ca, Mg, Fe, Zn, Cu, Mn), were analyzed. Correlation analysis revealed non-reducing sugars correlated positively with Ca (r = 0.62, $p< 0.01$) and negatively with Mg (r = -0.48, $p< 0.05$), while total phenols correlated negatively with K (r = -0.46, $p< 0.05$) and Mg (r = -0.53, $p< 0.05$). These results provide insights into the interdependence of traits influencing fruit quality, aiding in the selection of hybrids with improved nutritional and biochemical profiles for citrus breeding programs.

Keywords: Biochemical traits, citrus, mineral nutrients, antioxidant activity, carotenoids, correlation analysis

Introduction
Citrus fruits are among the most economically important and nutritionally valuable horticultural crops worldwide, ranking 2nd in terms of international trade and human nutrition. The global annual production of citrus fruits is estimated at 124.24 million tons (FAO, 2020) [6], with nearly 40% processed into juice, (Turner and Burri, 2013) [21]. In India, citrus fruits occupy a prominent position, covering 1.07 million hectares and yielding approximately 14.7 million tons annually, and are third most important fruit crops after mango and banana (Anonymous, 2023) [1]. Rising consumer awareness of health benefits, improvements in economic status, and advancements in production and post-harvest technologies have significantly boosted the demand for citrus fruits in recent years (Lv *et al*., 2015) [9]. Citrus fruits are renowned for their refreshing flavor and their role as a rich source of bioactive compounds, including organic acids, carotenoids, flavonoids, and essential vitamins and minerals. These compounds provide citrus fruits with numerous health-promoting properties that have garnered considerable scientific and commercial interest (Paul and Saha, 2004; Okwu and Emenike, 2007) [14, 13]. Bioactive compounds in citrus fruits exhibit a wide range of pharmacological properties, such as antioxidant, anti-inflammatory, anti-cancerous, and cardiovascular protective effects (Kruger *et al*., 2014; Castell *et al*., 2014) [7, 3]. Additionally, they have shown potential in preventing age-related neurodegenerative diseases, reducing vascular fragility, and exhibiting antibacterial and antiviral activities (Marzocchella *et al*., 2011; Raghavan and Gurunathan, 2021) [11, 16]. Among the bioactive compounds, flavonoids are one of the most extensively studied groups. Citrus fruits are particularly rich in flavanones, which vary among citrus species. For example, naringin predominates in sour oranges and grapefruit, while hesperidin is abundant in sweet oranges, mandarins, and lemons (Peterson *et al*., 2006) [15].

These flavonoids, present in glycoside and aglycone forms, contribute significantly to the health benefits of citrus fruits. However, certain compounds, such as limonoids, pose challenges in citrus juice processing due to their contribution to delayed bitterness (Chandler and Kefford, 1966; Maier *et al*., 1969) [4, 10]. Apart from health promoting bioactive compounds citrus fruits are also important source of mineral nutrients (macro- as well as micro) supplementing the benefits to human health. Considering this, there is a growing interest in improving the mineral and bioactive compound content of fruit crops, including citrus (Czech *et al*., 2020) [5].

Despite the progress in citrus breeding there is a lack of indigenous high-yielding citrus varieties adapted to Indian agro-climatic conditions with improved nutritional and functional properties (Wu *et al*., 2018; Raveh *et al*., 2020) [22, 17]. To address these gaps, systematic citrus breeding efforts have been initiated at the ICAR-IARI, New Delhi, focusing on developing interspecific hybrids with improved nutraceutical properties and juice quality. The characterization of biochemical properties, including flavonoids, organic acids, and limonoids, along with mineral nutrient profiling, is crucial for identifying elite hybrids with enhanced health benefits and consumer acceptability. Understanding the correlations among these traits is vital for advancing breeding strategies aimed at developing nutritionally superior citrus varieties. This study aims to evaluate the association among the biochemical properties and mineral nutrient content in interspecific citrus hybrids and their parental genotypes.

Materials and Methods

The study was conducted on 8-10-year-old interspecific citrus hybrids comprising sixteen hybrid genotypes, and parental genotypes pummelo (*Citrus maxima* [Burm. f.] Osbeck) and sweet orange cv Mosambi (*Citrus sinensis* [L.] Osbeck) at ICAR-Indian Agricultural Research Institute, New Delhi, during 2020 and 2021. The hybrid seedlings were established at 5 m × 5 m spacing, while parental genotypes were budded on recommended rootstock. The experimental site is located in a typical subtropical climate (28º64' N, 77º15' E; 228 m above mean sea level), characterized by hot, dry summers and cold winters. The experimental orchard was managed uniformly with recommended irrigation and fertilization practices for citrus crops. Plants were irrigated at intervals of 5-7 days during summer and 10-15 days during winter. Fertilization included the application of 600:400:400 g/tree of N:P:K and 30 kg of farmyard manure (FYM) per tree. Fully mature fruits from these hybrids were harvested and the biochemical properties *viz*., total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, sugars content, ascorbic acid, total phenols, total flavonoids, total carotenoids, lycopene, and antioxidant activity (DPPH) were determined as per the standard methods (Nishad *et al*., 2018) [12]. The mineral nutrients (P, Ca, Mg, Fe, Zn, Cu and Mn) were determined in the fruit juice of citrus genotypes previously in Singh *et al.* (2023) [19].

The Pearson's correlation coefficient was computed using RStudio (v2022.07.1–554, R Studio, PBC) to quantify the strength and direction of the linear relationships between variables. The significance of the correlation coefficients was ascertained using p-values derived from Student's t-tests. A significance level of p<0.05, was used to determine statistically significant correlations, while p<0.01 and p<0.001 indicated highly significant and very highly significant correlations, respectively. To visualize the correlations among parameters, a heatmap was generated using the "ggplot2" package in RStudio. The heatmap employed a colour gradient to represent the strength and direction of correlations, with darker shades indicating stronger relationships.

Results and Discussion

This detailed correlation analysis helped identify key associations among biochemical traits and mineral nutrients, providing insights into the correlation among the important traits for citrus breeding programs.

The correlation analysis revealed several significant associations for the several traits (Fig 1.). The antioxidant activity showed a strong positive correlation with the total phenols, total carotenoids, and lycopene content suggesting that phenolics and carotenoids significantly contribute to the antioxidant potential of citrus fruit juice. This finding aligns with previous studies indicating that phenolics and carotenoids, being powerful antioxidants, play a crucial role in scavenging free radicals and enhancing the nutritional value of citrus fruits (Nishad *et al*., 2018) [12]. Lycopene showed a perfect correlation with total carotenoids (r = 1.00; p< 0.001), confirming that lycopene is a major component of the carotenoid profile in the citrus fruits (Nishad *et al*., 2018) [12]. This result highlights the potential of these hybrids as rich sources of carotenoids, particularly lycopene, which is known for its health benefits. Among the mineral nutrients a significant positive correlation (r = 0.71; p< 0.001) was noted between Zn and Mn concentrations. This relationship suggests co-regulation or shared uptake and transport mechanisms for these micronutrients in citrus fruits. Such interactions are crucial for enzymatic functions, as both Zn and Cu serve as cofactors for various enzymes involved in metabolic processes (Savic *et al*., 2015) [18]. Furthermore, a significant negative correlation (r = -0.53; p< 0.01) was observed between Ca and Mg. This inverse relationship may reflect antagonistic accumulation dynamics in the edible tissue of citrus fruits. Non-reducing sugars in the fruit juice had negative correlation with Mg content (r = -0.48; p< 0.05) and positive correlation with the Ca content in fruit juice (r = 0.62; p< 0.01). Total phenol content also showed a significant negative correlation with the K (r = -0.46; p< 0.05) and Mg content (r = -0.53; p< 0.05). Furthermore, the significance of the correlations was ascertained using Pearson's correlation coefficient, with p-values indicating the strength and reliability of the associations. Traits with p-values below 0.05 were considered significant, while those with p-values below 0.01 and 0.001 were categorized as highly significant. This rigorous statistical approach ensures the reliability of the findings and minimizes the likelihood of Type I errors.

The correlations observed in this study have practical implications for citrus breeding and nutrient management strategies. The strong positive associations between antioxidant activity, total carotenoids, and phenolic compounds suggest that selecting hybrids with higher carotenoid and phenolic content can enhance the antioxidant potential of citrus fruits. Similarly, the strong correlation between Zn and Mn highlights the inverse relationship for the content of micronutrient in these fruits (Czech *et al*., 2020) [5].

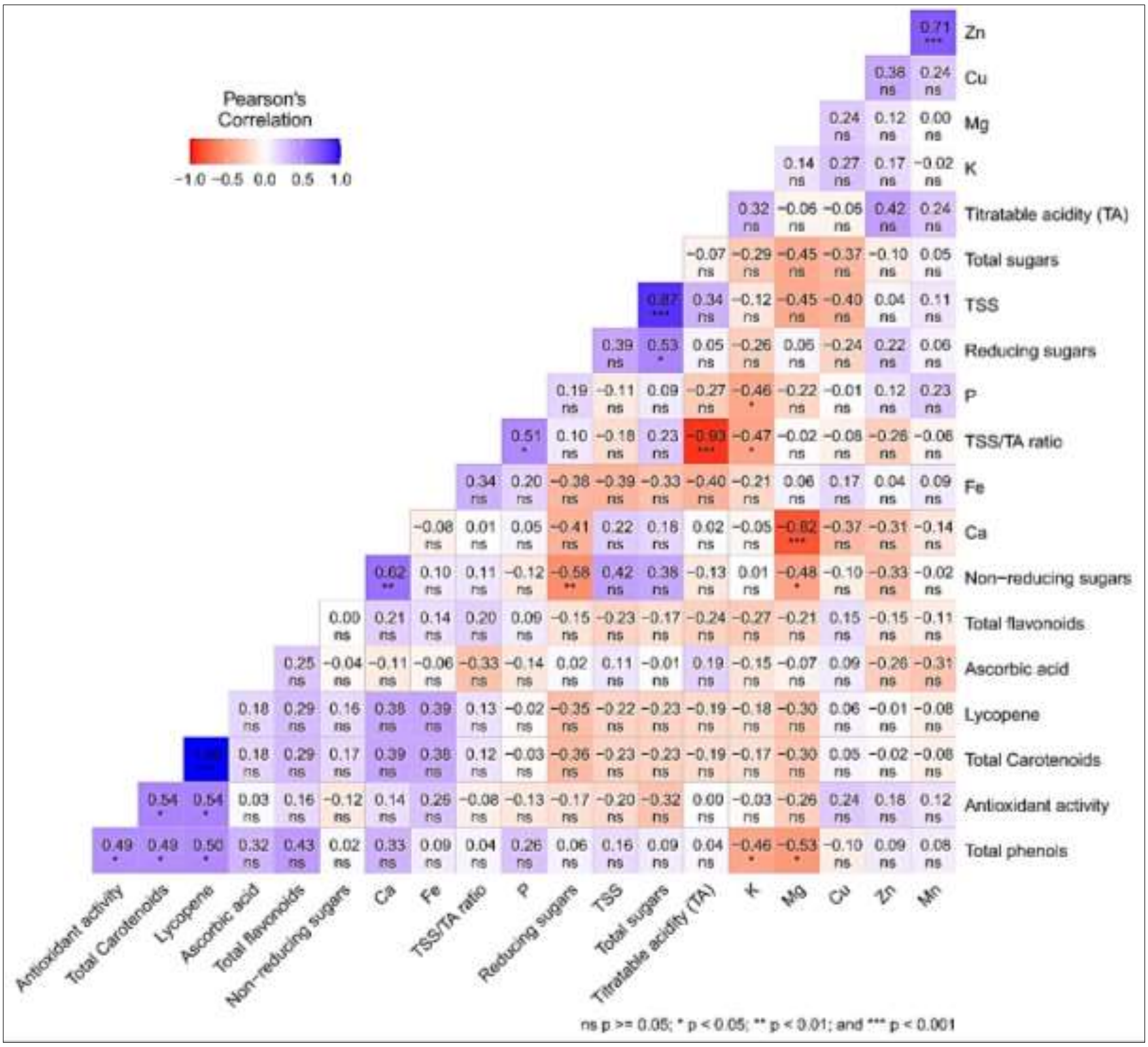

Fig 1: Pearson's correlation coefficients among the biochemical parameters and mineral nutrients in citrus fruit juice.

Several trait combinations, did not show significant correlations. These results indicate that these traits the complexity of biochemical and physiological interactions in citrus fruits, where multiple factors and pathways influence trait expression.

The present study revealed significant correlations among various biochemical traits and mineral nutrients content, providing valuable insights into the interrelationships that govern fruit quality and nutritional value. These findings align with earlier reports on different citrus species, which reported the correlation among the studied traits of fruit quality (Lado *et al.*, 2018; Tadeo *et al.*, 2020 and Anticona *et al.*, 2022) [8, 20, 2]. These studies similarly emphasized significant associations among physical characteristics and biochemical properties of citrus fruit juice. Analysing the correlations between these traits offers critical information for understanding the complex interactions influencing fruit quality. Such insights are particularly relevant for citrus breeding programs, where the identification of interrelated traits can streamline the selection process. By recognizing attributes that are positively or negatively associated, breeders can more effectively target combinations of traits that enhance fruit quality. This approach not only optimizes selection strategies but also facilitates the development of superior hybrids with desirable physical and biochemical characteristics, contributing to advancements in citrus crop improvement.

Conclusions

This study provides valuable insights into the relationships among biochemical and mineral nutrient traits in interspecific citrus hybrids. The significant correlations observed highlight the potential for selecting hybrids with superior nutritional and biochemical profiles. By recognizing attributes that are positively or negatively associated, breeders can more effectively target combinations of traits that enhance fruit quality. Future research should focus on exploring causal relationships and investigating the genetic basis of these associations to further enhance citrus breeding programs.

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