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***RESEARCH ARTICLE***

# Morphological Note on Sweet Orange at Different Developmental Stages

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**ABSTRACT**

**Background:** *Citrus sinensis* (L. Osbeck) is commonly known as sweet orange and widely distributed as an excellent source of antioxidants and vitamin C, which play a pivotal role in strengthening the immune system. The peel of sweet orange is a major source of various bioactive compounds that are utilized in different medicines.
**Objective:** This study aimed to explore the anatomical changes occurring at different developmental stages of sweet orange fruit.
**Methodology:** Orange fruits at different developmental stages were collected from the Botanical Garden of the University of Agriculture, Faisalabad. For this purpose, 16 developmental stages of sweet orange were selected on the basis of their size, growth, and development. Data were obtained for stomatal density, number of cells, and cell size. Furthermore, the thickness of albedo and flavedo was also recorded at each developmental stage.
**Results:** Results revealed that fruit diameter, cell size, stomatal number, number of hesperidia, and the thickness of albedo and flavedo increased progressively with fruit development and maturation. The maximum increase in all the recorded parameters was noted at stage 16, followed by stage 15. Moreover, significant variations in anatomical structures were observed across different developmental stages.
**Conclusion:** This study highlights the growth dynamics and structural modifications of sweet orange across different developmental stages and explores the progressive enlargement of cell size, albedo, and flavedo thickness with developmental stages.

## INTRODUCTION

Citrus is one of the most popular fruits worldwide, grown in over 130 countries, including Brazil, China, and the USA (Ladaniya 2008; Spreen *et al*. 2020). It has major nutritional and economic importance (Liu *et al*. 2012) and plays a key role in the fresh juice market (Cuenca *et al*. 2018). In the early 20$^{th}$ century, the words citrus production surpassed 105 million metric tons per year (FAOSTAT 2019). However, biotic and abiotic stress hindered its growth during the last two decades (Febres *et al*. 2011; Luckstead and Devadoss 2021). These problems significantly affect fruit development and quality, leading to a yield penalty (Gong and Liu 2013; Gottwald 2007). Sweet orange (*Citrus*× *sinensis* (L.) Osbeck), widely regarded as a cornerstone of global agriculture, accounts for nearly half of total citrus production. The crop maintained an estimated yield of 47.4 million tons for 2023–2024, with major contributions from Brazil, the United States, and China (Gabash *et al*. 2023). Sweet orange grows well in subtropical and tropical regions, representing both natural adaptation and human cultivation, and holds immense economic value. True citrus species are characterized by distinct morphological traits such as pulp vesicles, which make them among the most advanced within this genus (Penjor *et al*. 2014).

Morphological and biochemical analyses have long played an important role in clarifying citrus phylogeny, but these methods are often limited by environmental variability (Martasari *et al*. 2013). Other studies have described phylogenetic relationships based on the origin of oil glands in citrus, which arise through schizogenous and lysigenous

processes (Thomson *et al*. 1976; Bosabalidis and Tsekos 1982; Turner *et al*. 1998). Sweet orange fruit formation occurs in three layers: the exocarp (flavedo), mesocarp (albedo), and endocarp. The falvedo layer consists of secretory cavities of volatile compounds that are an enriched source of monoterpenes, responsible for botanical and economic values (Bishnoi *et al*. 2025). Moreover, orange peel is a great source of bioactive compounds, including monosaccharides, pectin, minerals, fibers, polyphenols, and essential oils (Brezo-Borjan *et al*, 2023). The essential oil fraction is characterized by terpenoid compounds dominated by limonene. These are oxygenated derivatives and include ester forms, aldehyde, and alcohol (Senit *et al*. 2019). Polyphenolic compounds are another major group of biomoelcules that are present in orange peel, which includes flavonoids, phenolic acid, and their derivatives (Senit *et al*. 2023l; Rathod *et al*. 2023). The major carbohydrates include hemicellulose, cellulose, glucose, monosaccharides, disaccharides, and pectin (Brezo-Borjan *et al*, 2023). However, orange peel is often discarded as waste, but it can be utilized for the treatment of diseases (Grover et al. 2024; Odetayo *et al*. 2025). The present research was conducted to examine anatomical modifications at different growth stages of fruit development to explore its structural changes and growth patterns.

## MATERIALS AND METHODS

An experiment was conducted to study the anatomical changes at different developmental stages of the sweet orange. Samples were collected from the Botanical Garden of the University of Agriculture, Faisalabad. Sixteen orange stages were selected based on their growth stages, and the fruit diameter of each was measured using a vernier caliper. The samples were washed with water, dried, and coated with transparent nail polish on the fruit surface. Three replicates of similarly sized oranges were taken to minimize the experimental error. After drying, the nail polish layer was carefully peeled away. The replicas of stomata were placed on glass slides, examined under a microscope, and stomatal density was calculated. Cell size and the number of cells on the orange surface were also recorded. Finally, the thickness of the flavedo and albedo was measured at each developmental stage (Fig. 1).

## RESULTS

*Fruit diameter*

Graphical data indicated that fruit diameter increased progressively with developmental stages. The diameter at stage 16 was larger than at other stages. Overall, the results showed that fruit size increased with development, while non-significant differences were observed between stages 15 and 16. The trend of improvement for this attribute was; Stage-16 > Stage-15 > Stage-14 > Stage-13 > Stage-12 > Stage-11 > Stage-10 > Stage-9 > Stage-8 > Stage-7 > Stage-6 > Stage-5 > Stage-4 > Stage-3 > Stage-2 > Stage 1 (Fig. 2 A).

*Number of stomata*

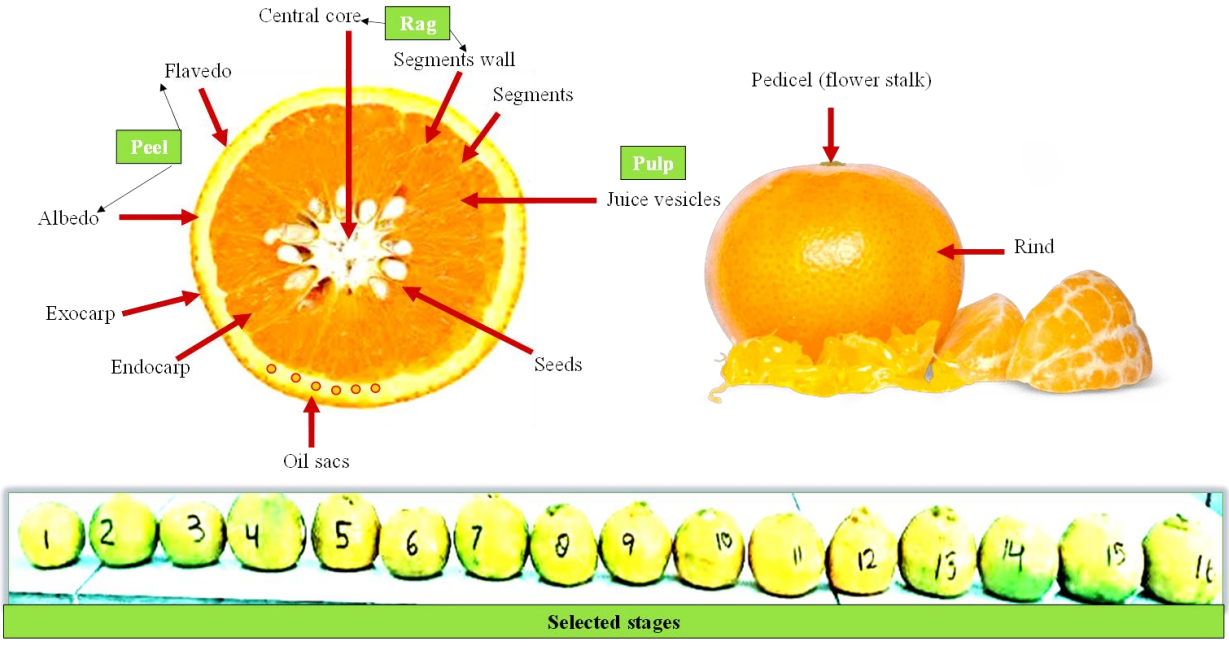


**Fig.1:** Depiction of different parts of the sweet orange peel and the selection of various developmental stages of citrus fruit

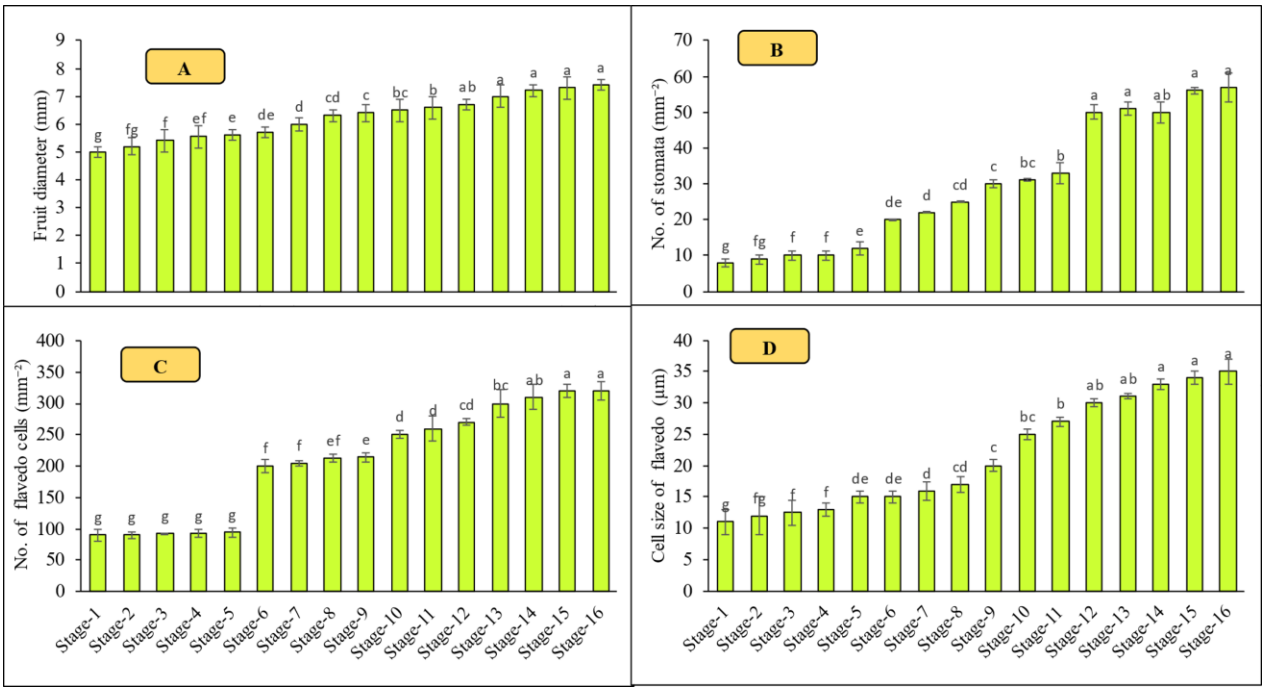


**Fig. 2:** Variations in the fruit diameter (A), number of stomata (B), number of flavedo cells (C), and cell size of flavedo (D) of sweet orange at different developmental stages

Results revealed that the number of stomata increased with developmental stages. The lowest stomatal count was recorded during the early stages; however, the number increased during fruit ripening. Stomatal density increased progressively with fruit development: stage-16 > stage-15 > stage-14 > stage-13 > stage-12 > stage-11 > Stage-10 > stage-9 > stage-8 > Stage-7 > stage-6 > stage-5 > stage-3 = stage-4 > stage-2 > stage-1 (Fig. 2B).

*Number of cells in flavedo*

Graphical data demonstrated that the number of cells in the flavedo layer increased with developmental stages. The highest cell count was observed at stage-16, followed by stage-15. However minimum count was recorded at stage-1 (Fig. 2C).

*Size of cells in flavedo*

The data revealed that the cell size of the flavedo layer increased progressively with developmental stages. Cells at stage-16 were larger compared with other stages. Overall, results confirmed an increase in cell size with fruit development, and non-significant differences observed were recorded between stages-15 and 16 (Fig. 2D).

*Thickness of flavedo*

Results revealed that the thickness of the flavedo increased with developmental stages. The flavedo at stage-16 was

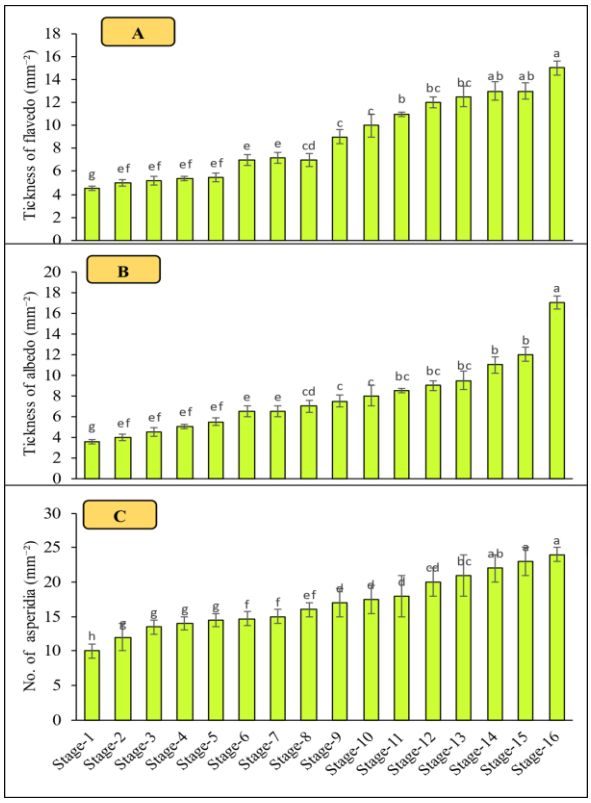


**Fig. 3:** Variations in the thickness of flavedo (A), thickness of albedo (B) and number of hesperidia (C), of sweet orange at different developmental stages

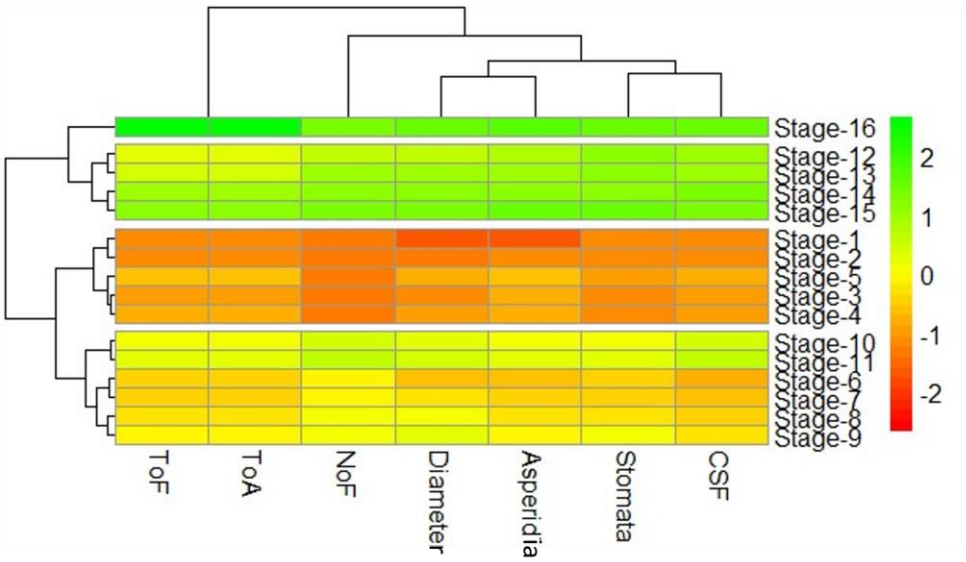


**Fig. 4:** The heatmap matrix on the variations in the thickness of flavedo; ToF, thickness of albedo (ToA), diameter of fruit; diameter, number of hesperidia, number of stomata; stomata and cell size of flavedo of sweet orange at different developmental stages

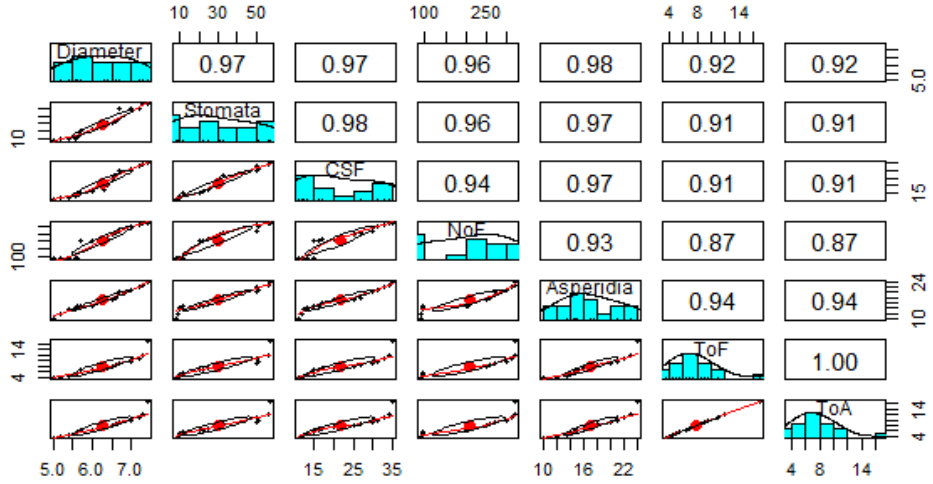


**Fig. 5:** Pearson correlation matrix on the variations in the thickness of flavedo; ToF, thickness of albedo (ToA), diameter of fruit; diameter, number of hesperidia, number of stomata; stomata and cell size of flavedo of sweet orange at different developmental stages

thicker compared with other stages. However slight reduction was observed at stage-15 as compared to satge-14, and again at stage-16 maximum length was recorded (Fig. 3A).

*Thickness of albedo*

Results indicated that the thickness of the albedo increased with developmental stages. The albedo at stage-16 was thicker compared with other stages. While minimum readings were recorded at stage-1. Overall, results showed that albedo thickness increased with fruit maturation. Intriguingly, abrupt increase in thickness was recorded at stage-16 (Fig. 3B).

*Number of hesperidia*

Data showed that the number of hesperidia increased with developmental stages. The lowest count was recorded at stage-1 compared with other stages, while the maximum count was observed at stage-16 (Fig. 3C).

*Heatmap and Pearson correlation*

The heatmap matrix showed a strong linear relationship of cell size of flavedo, number of stomata, hesperidia, diameter of fruit, no of cells in flavedo layer, thickness of flavedo, and

albedo with stages 12, 13, 14, and 15, while an opposite relation was recorded at stages 1, 2, 3, 4, and 5 (Fig. 4). Moreover, a non-significant relationship was observed at stages 6 to 10. Pearson correlation showed a strong positive relationship with all the studied parameters (Fig. 5).

## DISCUSSION

Citrus fruits rank among the top fruits not only in total production but also in economic value. Among them, oranges, specifically sweet oranges, are among the most widely cultivated citrus fruits in the world. The orange peel consists of a thin outer layer known as the flavedo and the thicker inner layer known as the albedo (Afifi *et al*. 2023). The flavedo is comprised of the carotenoids responsible for the typical fruit color (Kato *et al.* 2004), and vesicles (minute sacs/cavities) filled with peel oil. This peel oil is responsible for the fresh smell of the fruit. The white spongy inner albedo, on the other hand, is composed of various substances like flavonoids, d-limonene, limon, and pectin (Nieto *et al*. 2021). This experiment was conducted to explore anatomical changes at different developmental stages of the sweet orange. Samples were collected from the Botanical Garden of the University of Agriculture, Faisalabad. About 16 oranges were selected based on growth stages, and the fruit diameter of each orange was recorded using a vernier caliper. Graphical data indicated that flavedo cell size increased with developmental stages, so cells at stage-16 were larger in size compared to other stages (Fig. 2A–D). The observations of Rafiei and Rajaei (2007) also support these results. Cell number increased progressively with fruit development, reaching its maximum at stage-16, though variations between stages-15 and 16 were statistically non-significant. The number of stomata showed the same pattern, being minimal during initial development and rising significantly with ripening. Fruit diameter grew uniformly with stages of development, reaching the maximum value with stage-16, and showing no difference between stages-15 and 16. Flavedo thickness also rose steadily, and fruits with stage-16 had higher thickness compared to previous stages. Similarly, albedo thickness continued to increase with fruit development, and stage-16 fruits showed the maximum values (Fig. 3A–C).

Increase in albedo thickness with fruit ripening is reported (Oikeh *et al*. 2013). Our data showed that the number of hesperidia increased with developmental stages (Fig. 3). The lowest number of hesperidia was observed at stage 1 compared with other stages. The heatmap matrix revealed a strong linear relationship of cell size of flavedo, number of stomata, hesperidia, diameter of fruit, no of cells in flavedo layer, thickness of flavedo, and albedo with stages 12, 13, 14, and 15, while an opposite relation was recorded at stages 1, 2, 3, 4, and 5 (Fig. 4), indicating that maximum size and length were achieved at the end of the fruit maturity. Moreover, a non-significant relationship was observed at stages 6 to 10. Pearson correlation showed a strong positive relationship with all the studied parameters (Fig. 5). Overall, the results affirmed that a progressive enlargement of cellular structures and tissue layers is a typical aspect of sweet orange fruit development and might be linked with the accretion of different bioactive compounds in this tissue.

## CONCLUSION

This study provides sufficient evidence that the anatomical features of sweet orange progressively transform during fruit maturation. It might be an adaptive strategy or an accretion and storage of bioactive compounds with the passage of time. The overall developmental trend indicated a persistent increase in tissue thickness that might be due to cell expansion and cell division. Maximum increase in flavedo, albedo thicknesses were noted between stages 15 and 16. These findings confirm structural changes in sweet orange peel with ripening. However, further studies on the composition of these layers at different growth stages are crucial, given the importance of medicinal and therapeutic uses.

## AUTHOR CONTRIBUTIONS

All authors contributed equally to the conception, design, and preparation of this manuscript.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

## ETHICS APPROVAL

Not applicable

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