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ORIGINAL PAPER

Anatomical features of pericarp and pedicel influencing fruit splitting in Daisy mandarin

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Abstract

Preharvest fruit splitting is a significant concern for Daisy mandarin cultivation due to its adverse effects on both fruit quality and yield. This study, which was conducted at Punjab Agricultural University, aimed to analyze the anatomical changes in the peel, pedicel and stem end of healthy and split fruits of Daisy mandarin. Light microscopy and Scanning Electron Microscopy were employed to examine the different sections of the peel, stem end and pedicel of the fruits. Significant differences were observed between the healthy and split fruits in terms of peel thickness, epidermal cell arrangement and vascular tissue organization. Split fruits, exhibited structural deformities in vascular bundles with limited functioning of the vascular bundles. Displaced xylem tissues in split fruits formed damaged cell masses in the pith and the parenchymatous cells lacked intercellular spaces. Additionally, pedicel vascular bundles in split fruits were often deformed and fused with adjacent bundles. Scanning Electron Microscopy analysis revealed a smooth and uniform epidermis with well-developed oil glands in healthy fruit peels, in contrast to disorganized and ruptured oil glands with empty spaces in split fruits. Thinner, coarser peels with larger oil glands and smaller epidermal thickness were found to be more susceptible to fruit splitting. Disorganized xylem tissue disrupted the flow of water and minerals to growing fruits, potentially due to irregular water transport and pulp expansion, leading to fruit splitting. This disruption of vascular tissues impaired the transport of water and nutrients to the albedo and flavedo, contributing to fruit splitting.

Keywords Citrus · Cracking · Daisy · Peel · Pedicel · Ultrastructure · Xylem tissues

Introduction

Mandarins are highly preferred in India due to their easy-to-peel nature and high juice content. Among them, Daisy mandarin (*Citrus reticulata* Blanco), a recently selected variety, has early maturation and high-quality, attractive coloured fruits [1]. This variety was introduced to extend the market period of mandarins in Punjab from November to February [2]. Daisy mandarin fruits ripen between 15 and 20th November, yielding medium to large-sized fruits with attractive dark orange peels. Mr Dowling Young of Young's Nursery in California (USA) named this cultivar Daisy tangerine, resulting from a cross between Fortune (*Clementine mandarin* × *Dancy mandarin)* and Fremont *(Clementine* × *Ponkan*) mandarins [3, 4].

Despite its promising attributes, Daisy mandarin faces significant developmental challenges, notably fruit splitting. This prevalent physiological disorder affects more than 60 per cent of global citrus production [5–7]. Fruit splitting, characterized by a meridian fissure starting at the stylar end, is influenced by a combination of cultural, genetic and environmental factors [8, 9]. Irregular climatic conditions, nutrient imbalances, uneven water supply and heavy crop loads are key contributors [10–12].

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Splitting mainly occurs under irregular growing conditions, such as water stress followed by sudden rainfall and uneven fertilizer application [13, 14]**.** This disorder renders fruits more susceptible to pathogen infections and water loss, making them unsuitable for commercial use. [7, 15, 16]. The mechanical properties of the peel and fruit growth dynamics play crucial roles in splitting and are important factors affecting the occurrence of fruit splitting [17–19]. The occurrence of fruit splitting is closely associated with the number of cell layers and the composition of the flavedo [20].

Studies on 'Newhall' and 'Bonanza' navel oranges revealed that varieties with a higher density of oil glands, like 'Newhall', exhibit less fruit splitting due to the ability of surrounding cells to resist greater pressure. Conversely, the split fruits show poor cell wall integrity and deformed layers [21]. Splitting mainly results from uncoordinated growth between the outer dermal and inner parenchyma tissues. During the rapid growth period, the inner parenchyma develops faster, while the epidermis develops slowly. The presence of interspace in cutin acts as a break for splitting. Rapid inner growth increases the internal turgor forces on the fruit surface and causes skin elasticity loss, which causes fruit cracking [22, 23]. Fruit splitting may occur as a result of disorganized rapid water absorption through the vascular system, which causes an uneven increase in fruit turgor pressure [24–26]. Fruit splitting is characterized by the separation of cells in albedo tissue, creating rind channels that weaken the flavedo [27–29]. A thicker peel correlates with lower splitting rates due to greater resistance to deformation. [30]. Considering the seriousness of fruit splitting in Daisy mandarin, which limits its production, the present research was conducted to unravel the anatomical variations associated with fruit splitting. Most of this research has involved fruit types like sweet cherry tomato, and lemon [26, 33], with less frequent studies on the relationship between Daisy mandarin fruit splitting and its anatomical structure. This study aims to bridge this gap in knowledge by exploring the potential link between the anatomical characteristics of Daisy mandarin peels and their susceptibility to splitting. By examining the peel's structure, this research will provide valuable insights into the mechanisms underlying fruit splitting in this specific citrus cultivar.

Materials and methods

The investigation of fruit splitting in Daisy mandarin was conducted at the Jallowal-Lesriwal, Jalandhar, Fruit Research Farm of the Department of Fruit Science and laboratories of the Department of Botany, Punjab Agricultural University, Ludhiana in 2021 and 2022. Samples of both split and healthy Daisy mandarin fruits were collected for subsequent anatomical analysis.

Light microscopy

For anatomical examinations, both split and healthy fresh Daisy mandarin fruit samples were collected. Hand sections of the fruit peel, stem end and pedicel of the fruits were meticulously cut by embedding the samples within the potato pith. These sections were stained using safranin-fast green combination techniques [31], following the specified procedure:

Staining procedure

Initially, the sections were immersed in a 2% aqueous solution of safranin (2%) for 5 min. After safranin staining, sections were washed with water and swiftly passed through an ascending series of alcohol. Counterstaining was achieved by briefly submerging the sections in a 2% solution of fast green followed by passage through alcohol, alcohol: xylene (1:1) and ultimately pure xylene. Subsequently, these sections were permanently mounted using DPX under a cover slip.

Microscopic examination

The prepared slides were then examined under a microscope equipped with a digital camera and computing imaging systems utilizing software equipped with NIS Elements F 3.0. Observations were made at magnifications of 4X and 10X. Measurements of tissues in the fruit peel, stem end and pedicel were recorded at various locations in each cross-section and the following parameters were investigated:

Observations recorded

Cross-sections of peels

a. Epidermal thickness
b. Size of oil glands

Cross-sections of the stem end

a. Number of vascular bundles
b. Arrangement of vascular bundles

Cross-sections of pedicels

a. Epidermal thickness
b. Size of vascular bundles
c. Arrangement of vascular bundles

Scanning *electron* microscopy (SEM)

For Scanning Electron Microscopy (SEM), peel samples from both healthy and split fruits were sectioned into small fragments measuring 0.5 to 1 cm^2. These fragments were fixed using a 2.5% glutaraldehyde solution (v/v), overnight at 4 °C. After draining the fixative, the samples were washed three times with 0.1 M Caco buffer, each lasting 15 min. Subsequently, the wash buffer was removed and a 1% solution of osmium tetraoxide was applied for 1–2 h at 4 °C. Following the osmium tetraoxide treatment, the samples were subjected to three additional washes with 0.1 M Caco buffer.

Next, a dehydration process was initiated using a series of ethanol solutions (30%, 50%, 70%, 80%, 90% and 100% v/v). Following dehydration, the samples were subjected to critical-point drying and subsequently placed in vacuum desiccators overnight for thorough drying. The dried samples were then coated with gold using a sputter coater process to enhance the surface conductivity.

SEM examination

The samples were examined using a scanning electron microscope located at the EMN lab, at Punjab Agricultural University, Ludhiana. During SEM analysis, the thickness and texture of the peel were studied, focusing on differences between healthy and split fruits.

Statistical analysis

The statistical analysis of the data was performed using Tukey's HSD test to determine statistically significant differences. Differences were considered significant at the level of $p < 0.05$. The analysis was conducted using the statistical analysis software SAS (version 9.3 for Windows).

Results

Peel cross-section

Epidermal thickness

The ultrastructure of peels from both split and healthy Daisy mandarin fruits were thoroughly examined (Fig. 1). There was a significant difference in epidermal thickness between the peels of healthy and split fruits (Table 1). The epidermal thickness was greater in the healthy peels (2.62 mm) compared to split fruits (2.18 mm). The peel of healthy fruit exhibited consistent and uniform epidermal thickness (Fig. 2).

Oil glands

In both healthy and split fruit peels, oil glands were located directly below the epidermal layer. The oil glands of healthy fruit peels were well-developed. The size of the oil glands was found to be significantly greater in the peel of the split fruit (788.52 mm^2), whereas it was lower in the peel of the healthy fruit (615.93 mm^2) (Table 1). However, in split fruit peels, the oil glands were deformed and appeared as empty spaces lacking any cellular arrangement

Table 1 Epidermal thickness and size of the oil glands of the peels of healthy and split Daisy mandarin fruits

Sr. No	Treatments	Epidermal thickness (mm)	Size of oil glands (mm^2)
1	Healthy	2.62^a	615.93^b
2	Split	2.18^b	788.52^a
	CD ($p < 0.05$)	0.043	10.57

Fig. 1 **A** and **B** Healthy and split fruits of Daisy mandarin

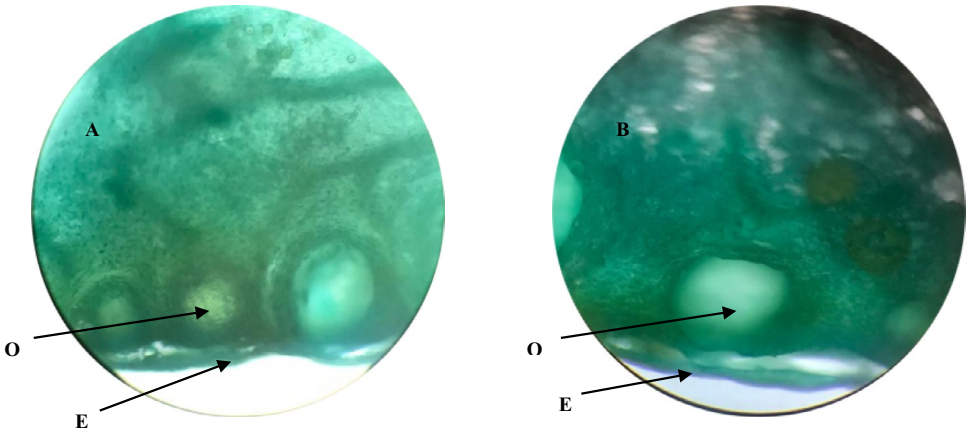

Fig. 2 Cross section of peel of Daisy mandarin fruit. **A** TS of healthy peel **B** TS of split peel (*TS* transverse section, *E* epidermis, *O* oil glands)

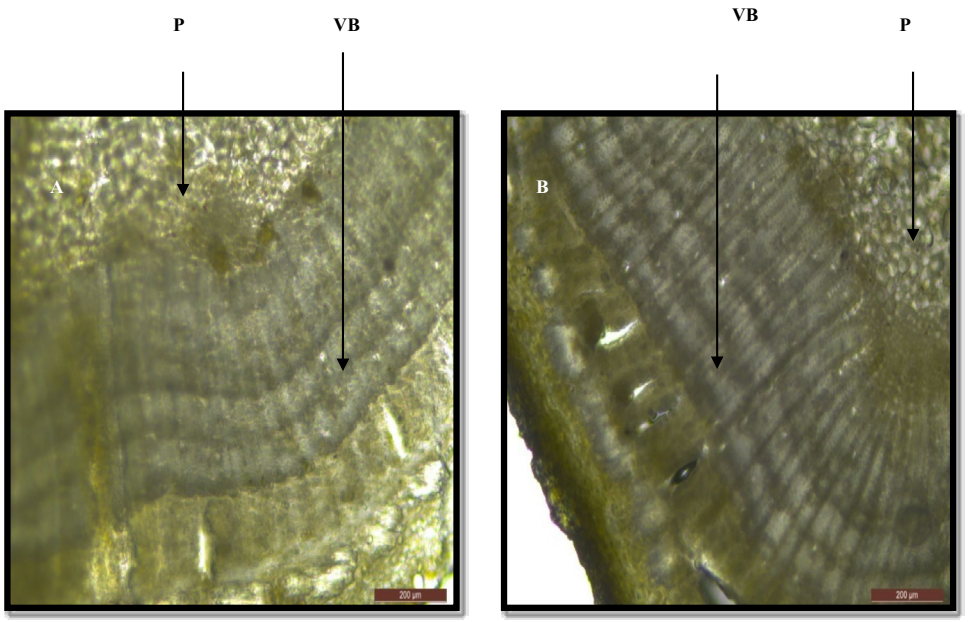

Fig. 3 Cross section of the stem end of Daisy mandarin fruit. **A** TS of split fruit stem, **B** TS of healthy fruit stem (*TS* transverse section, *P* = pith, *VB* = vascular bundles)

(Fig. 2). Compared with those of healthy fruits, the peels of split fruits had larger oil glands (Fig. 2).

Cross section of the stem end

In healthy fruits, vascular bundles were consistently observed, forming a well- organized circular pattern with phloem and xylem components properly aligned with their respective channels. However, in split fruits, structural deformities were evident in the arrangement of vascular bundles, and only a limited number of vascular bundles were observed in split fruits. Furthermore, in split fruits, xylem tissues were dislodged from the vascular bundles and shifted to the pith as a mass of damaged cells (Fig. 3).

Cross section of the pedicel

Ultrastructural analysis of a healthy pedicel revealed a consistent epidermal layer comprising oval cells covered by a cuticle. Within the cortical region, parenchymatous cells were organized without any intercellular spaces. In the pedicel of the split fruits, vascular bundles were deformed as they fused with adjacent bundles (Fig. 4).

Scanning *electron* microscopy

Scanning electron microscopy revealed that the peels of healthy fruits exhibited a uniform and smooth epidermis along with well-developed oil gland pits. The oil glands in

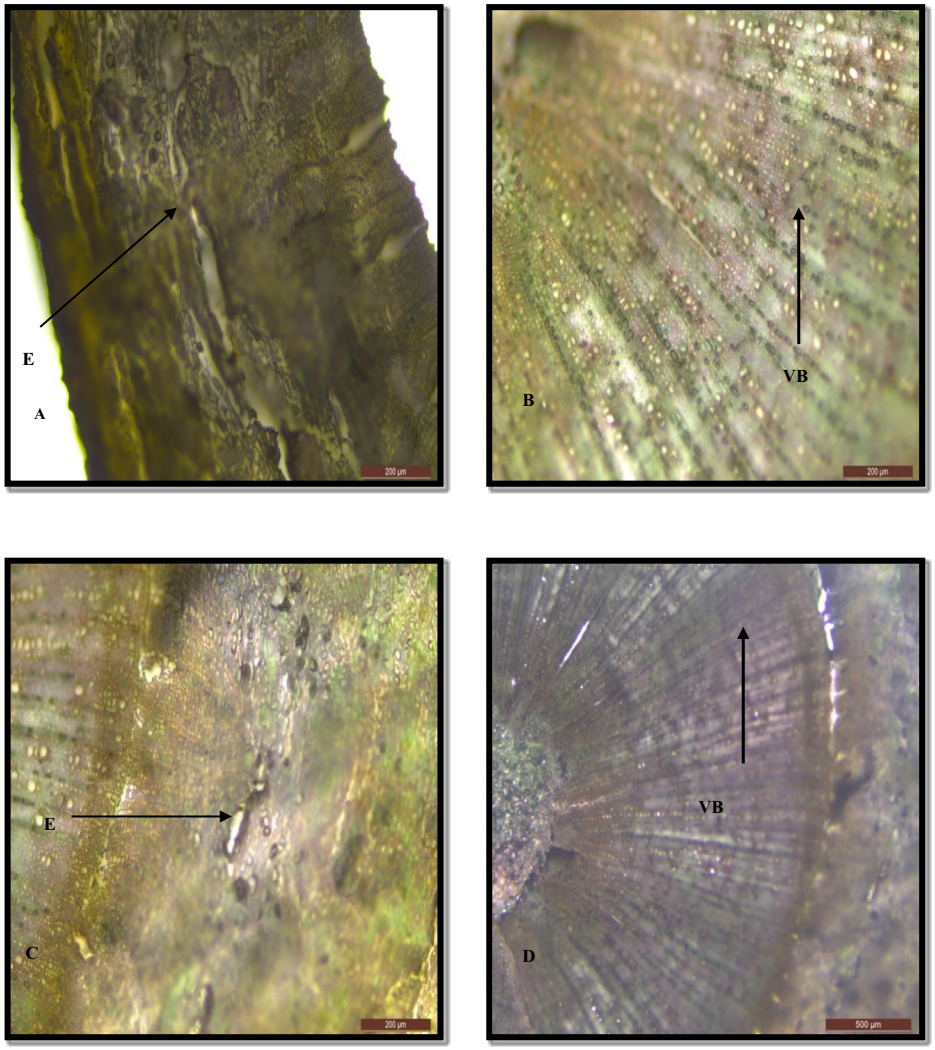

Fig. 4 Cross section of the pedicel of Daisy mandarin fruit. **A** and **B**: TS of the pedicel of healthy fruit, **C** and **D**: TS of the pedicel of split fruit (*TS* transverse section, *E* epidermis, *VB* vascular bundle)

healthy fruits were well developed. However, in the peel of the split fruits these oil glands appeared disorganized and ruptured and contained empty spaces (Figs. 5,6,7).

Discussion

Fruit splitting

Citrus fruits are susceptible to a physiological disorder known as fruit splitting, a severe form of cracking that occurs during the fruit's maturation or cell enlargement stages [11, 21]. This physiological disorder is characterized by cracks in the exocarp and mesocarp of the fruit, resulting from mechanical rupture. The peel, acting as a barrier between the external environment and fruit tissue, protects the fruits from external injury while bearing internal pulp pressure during development. Consequently, both the stricture of the peel and its degree of ageing are critical factors in fruit cracking [32].

The anatomical structure of the fruits is a significant factor affecting the occurrence of fruit splitting. The previous studies investigated the anatomy of lemon peel, revealing significant variations in peel thickness [26]. They reported considerable differences between epidermal thickness, and vascular bundles arrangement between healthy and split fruits. Current research on fruit anatomy and splitting has mainly focused on the fruit peel, the size of oil glands, epidermis, subepidermal thickness, the arrangement of vascular bundles and the overall mechanical properties of the peel.

Anatomical characteristics of healthy and split fruits

The peel, also known as the flavedo, plays a vital role in protecting citrus fruits from external damage and internal pressure caused by the developing pulp [32]. The structure and integrity of the peel are critical factors influencing its

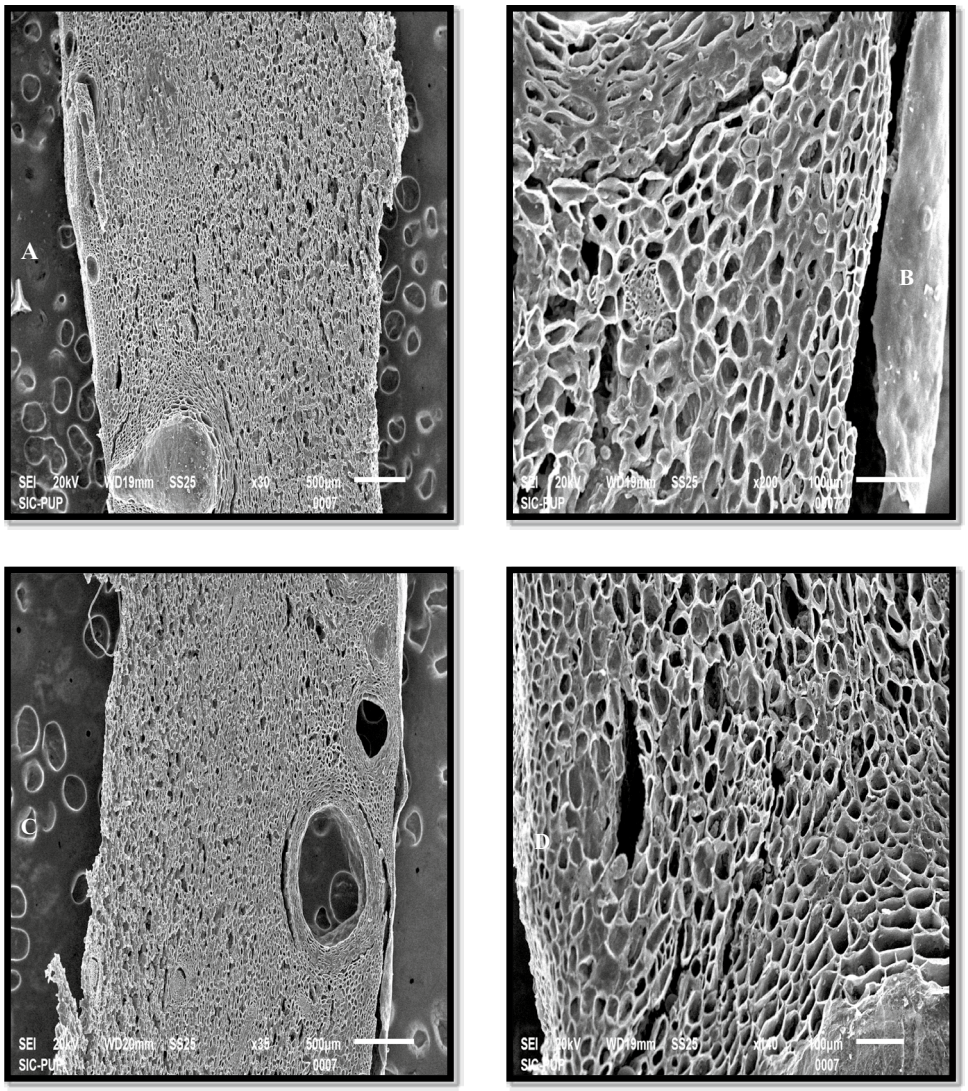

Fig. 5 Scanning electron microscopy of peel of Daisy mandarin fruit **A** and **B**: TS of peel of healthy fruit **C** and **D**: TS of peel of split fruit

susceptibility to splitting [34]. The thickness and hardness of the fruit peel serve as crucial parameters for assessing its strength, directly influencing citrus fruit peel splitting. Thicker peels are less likely to stretch and deform thus exhibiting greater resistance to splitting [35]. Studies have shown that cultivars with thicker peels have a lower incidence of splitting compared to those with thinner peels [21, 36]. Splitting damage progresses through distinct stages [37]. Initially, the peel appears normal. During the critical period, visible signs emerge, including a brown stripe, cuticle fractures, and crack development. Oil glands may also begin to deform at this stage [26, 38]. In the later stages, extensive surface damage makes it difficult to distinguish between the flavedo and albedo tissue layers. Research suggests that there is a potential link between oil glands health and spitting susceptibility. Studies have reported that split fruits tend to have more malformed or larger oil glands compared to healthy fruits [26, 38]. Alternations in the mechanical properties of the peel, including disordered subepidermal cells and oil glands, are associated with fruit splitting [20, 21]. These damaged tissues exhibit disorganized cytoplasm, leading to a loss of cellular osmoregulatory capacity and cellular lipids. Similar observations have been made in tomatoes, where cracking-resistant fruits have more tightly packed subepidermal cells compared to susceptible fruits [33]. The shape and arrangement of these cells may also influence splitting susceptibility.

Fruit growth in many crops depends on the accumulation of water, assimilates, and ions transported through the vascular tissue of the pedicel towards the fruit [26, 39]. Pedicels are crucial for yield, as the growth of fruits depends on the uptake of water, nutrients and assimilates transported from the other parts of the plant to the fruit through the xylem and phloem tissues of the pedicel [40, 41]. In healthy citrus fruits, the vascular bundles in the pedicel are well-organized

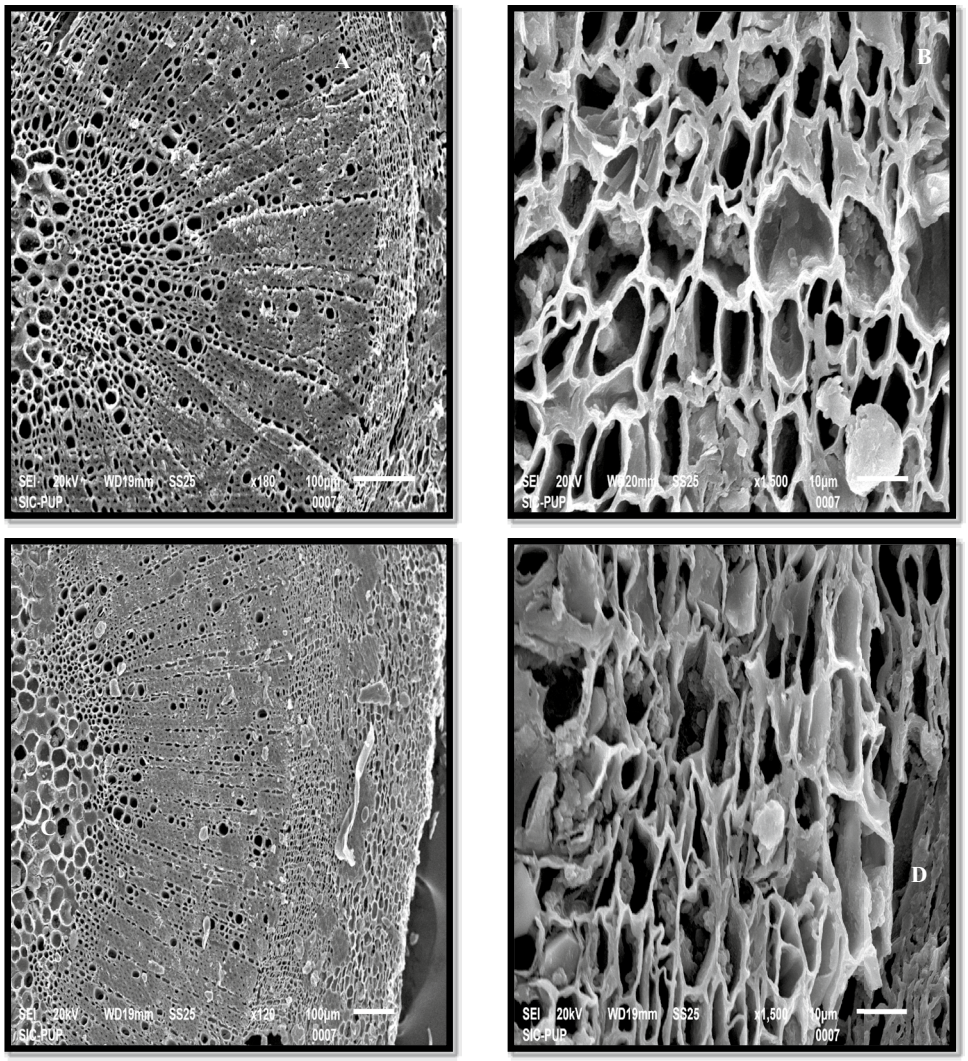

Fig. 6 Scanning electron microscopy of stem end of Daisy mandarin fruit

in a ring-like pattern for efficiently transporting water, nutrients, and photosynthates between the fruit and the rest of the plant. However, excessive internal pressure leading to fruit splitting disrupts this organization. The vascular bundles become twisted, compressed, or irregularly spaced, accompanied by a thinner epidermis. This weakening is attributed to environmental stress, nutrient deficiencies, and physiological stress [42].

Splitting often initiates from the inner peel along the path of these vascular bundles. The disruption weakens the peel due to reduced epidermal thickness and deformed vascular structures. Uneven and rapid water uptake through the compromised vascular systems is suggested as a potential cause. This can lead to unequal fruit turgor pressure, a major contributing factor to splitting [24–26].

Sudden water influx, especially after dry periods, is particularly damaging. The rapid expansion of fruit cells caused by this influx can lead to epidermal rupture and internal structural deformation, including damage to the vascular bundles. Uneven cell division and expansion during rapid growth further cause this problem, resulting in a weak epidermis and deformation of vascular bundles.

Consequently, studying vascular tissues is for understanding the mechanism involved in the transport of water, minerals and assimilates from the pedicel to the fruits and within the peel. The uptake of water and its movement in small citrus plants bearing fruits indicated that significant water flow occurs within the xylem elements, which serve as the major part of the transpiration stream. It was reported that fruit growth was limited by the rigidity of pericarp cell walls [43]. It was suggested that water uptake via the phloem could increase the pressure within the fruit. The restoration of water by xylem tissues decreases the susceptibility of plants to water stress [40] and potentially reduces their susceptibility to cracking [44].

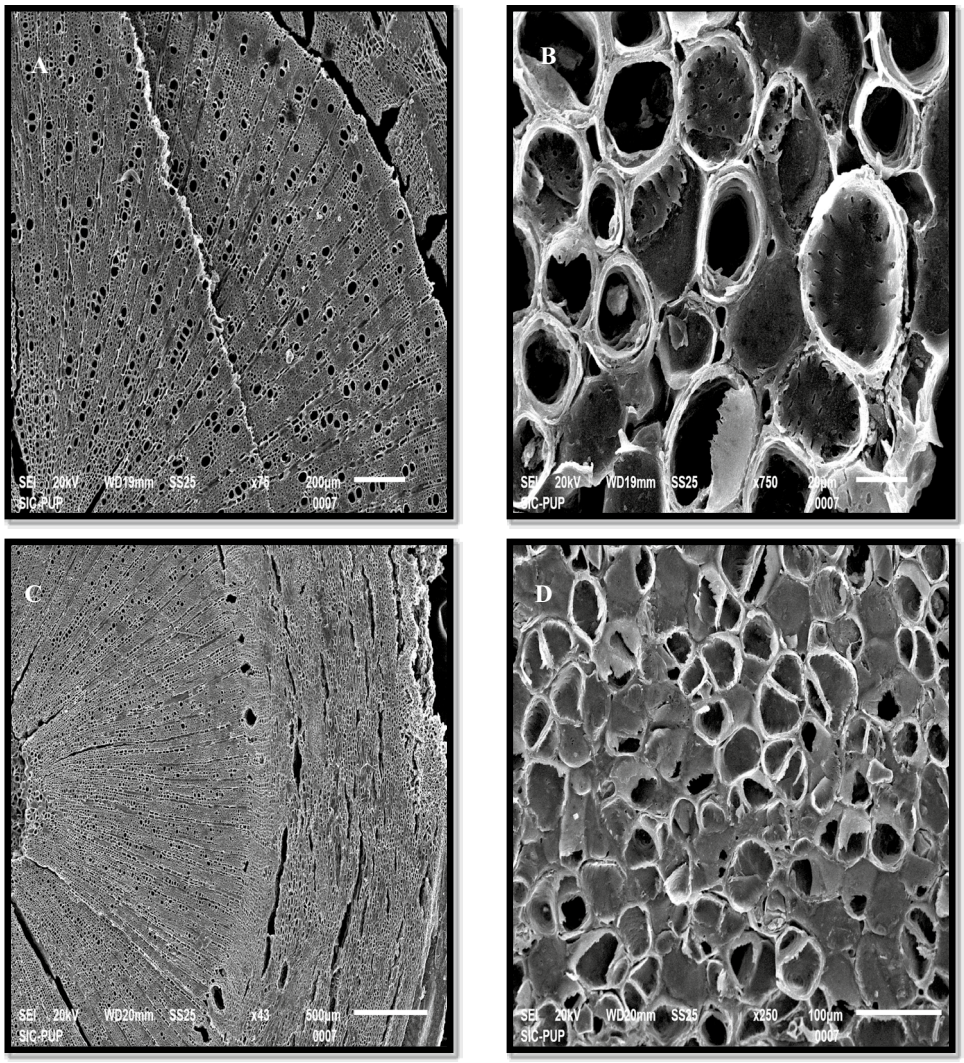

Fig. 7 Scanning electron microscopy of the pedicel of Daisy mandarin fruit

Scanning *electron* microscopy analysis

Scanning electron microscopy (SEM) analysis revealed significant differences in between healthy and split fruits of Daisy mandarin. Split fruits exhibited a ruptured, disorganized, and rougher peel texture compared to the smooth and uniform peel of healthy fruits. This observation is consistent with findings from previous studies, which indicate that the rind of citrus fruits, comprising the flavedo and albedo, exhibits structural variations among different cultivars (Hardiyanto et al., 2022). Supporting these observations, Kaur et al. and Agusti et al. reported similar findings in lemon and 'Navelate' oranges. Their studies suggest that rind breakdown initiates in the deeper flavedo and outer albedo cell layers. In affected fruits, these cells appeared twisted and compressed, forming a layer of collapsed cells between the healthy, intact cells of flavedo and albedo. This progression eventually leads to the oxidation of the albedo and, subsequently, the epidermis, where oxidized tissue manifests as dark-brown areas. Collectively, these SEM studies reveal the critical role of rind structural integrity in maintaining fruit health. Disruptions in the peel's architecture, as observed in split Daisy mandarin fruits, can lead to significant deterioration, evident from the the visual differences compared to healthy fruits.

Conclusions

In conclusion, this study highlights the significant anatomical differences between healthy and split Daisy mandarin fruits, specifically in the peel, pedicel and stem end. Split fruits exhibited reduced peel thickness and ruptured, malformed oil glands compared to healthy fruits. Additionally,

split fruits show structural deformities in vascular bundles, displaced xylem tissue and ruptured oil glands, which increase susceptibility to fruit splitting. Disorganization of vascular tissues in the pedicel results in disturbed nutrient and water translocation, leading to an imbalance that causes fruit splitting. Understanding these anatomical changes is crucial for developing effective strategies to reduce fruit splitting and enhance both the fruit quality and yield in Daisy mandarin.

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Author contributions Komalpreet Kaur performed the experiment, analyzed the data and wrote the manuscript, Monika Gupta designed the experiment and supervised the project, H S Rattanpal, Nirmaljit Kaur and T S Chahal supervised the project; T S Chahal provided the planting material; Monika Gupta and Nirmaljit Kaur revised the manuscript.

Data availability The data will be made available by the corresponding author upon request.

Declarations

Conflict of interest The authors declare that they have no competing interests that could have appeared to influence the work reported in this paper.

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