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Short communication

The characteristics of cork and hypodermis tissues and cracking in Asian pear (*Pyrus pyrifolia* cv. Mansoo)

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ABSTRACT

Mansoo is a russet pear cultivar cultivated in Korea and susceptible to cracking at maturity. Cracked regions were studied using light (LM), stereo (SM), and scanning electron microscopy (SEM) to compare the prevalence of this phenomenon in Mansoo and Niitaka cultivars. The initial cracking stage was observed as a short-length-shaped crack (SLSC) on the lenticel, followed by an enlargement and extension of the cracked region as a long-length-shaped crack (LLSC). Although the cracked region on the lenticel was discovered with the naked eye, cracking also occurred in the surrounding filling tissue region, which has a thin hypodermis because of a crevice of cork tissue. The cracking occurred in the intercellular space of parenchymal tissue and between stone cell clusters. Mansoo fruit has thinner, more irregular, and less numerous hypodermis layers than that of Niitaka. Stone cell clusters in the peripheral parenchyma were concentrated because they were larger and had a smaller distance between them in Mansoo cultivars than in Niitaka. These results demonstrate that the morphological and anatomical features observed in Mansoo fruit are cracking susceptibility factors.

1. Introduction

The Asian pear (*Pyrus pyrifolia)* is cultivated throughout most East Asian countries, including Japan, China, and Korea. The exocarp of the Asian pear is differentiated between green (cv. Nijisseki and Hwangkeumbae) and russet (Niitaka and Kosui) varieties. The russet pear exocarp consists of a cork layer and hypodermis, whereas the green pear exocarp comprises a cuticle, epidermis, and hypodermis (Wang et al., 2014). Commonly, Asian pears indicate a russet pear.

Niitaka pear is a dominant variety, which has 83% of cultivation area in Korea. So breeding and propagation of great and new pears are necessary. The Mansoo pear, which is an Asian pear variety cultivated in Korea, is of great quality. However, cracking at maturity occurs frequently and has been a source of commercial loss. Mansoo is susceptible to cracking but Niitaka is cracking-resistant cultivar. Cracking is a symptom of fruit surface fracture and is found in many fruit. Severe cracking can lead to commercial loss (Jansasithorn et al., 2014). Cracks may be differentiated into cuticle-, micro-, and flesh cracks by position and size (Ma et al., 2012; Opara et al., 2010).

In a previous study by our group, the cracking of Mansoo fruits was observed from maturity and was found to be closely related to light conditions (Choi et al., 2015). Little is known about those anatomical features that make them more susceptible to the incidence of cracking compared to other cultivars.

The exocarp varies in structure and feature depending on the fruit type. In some peach cultivars, trichomes attach to the outer epidermis (Cho et al., 2000), whereas many fruits, such as apples and pears, possess lenticels in the exocarp, which develop beneath stomata (Park and Park, 2000).

The exocarp is the outermost layer of pericarp (Rudall, 2007) and, generally, when mature, is composed of cuticle, epidermis, hypodermis, and parenchymal tissue (Kwon, 2014). Some exocarp consists of tannin cells (Park et al., 2003).

Exocarp features vary in color. Some fruits have an exocarp colored red, blue, or purple because of anthocyanins that occur in the vacuole (Clifford, 2000). The Asian pear at maturity has a unique exocarp color because chlorophyll contents decrease and cork layer is covered (Park et al., 2013).

The aim of the present study was to characterize cracking in Mansoo pears and determine the anatomical and morphological factors that influence the incidence of cracking. To that end,

Abbreviations: DAFB, days after full bloom; LLSC, long-length-shape crack; LM, light microscopy; SCIP, stone cell cluster in internal parenchyma; SCPP, stone cell cluster in parenchyma parenchyma; SEM, scanning electron microscopy; SLSC, short-length-shape crack; SM, stereo microscopy.

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Mansoo cracking was compared with the normal region of Mansoo and Niitaka pear fruits. For these purposes, exocarp tissues were observed using stereo microscopy, light microscopy and scanning electronic microscopy.

2. Materials and methods

2.1. Samples

Pear fruits (*Pyrus pyrifolia* cv. Mansoo and Niitaka) were used from an orchard of the Pear Research Institute (Naju, Korea) which is cultivated with Y-trellis training system. The fruits were collected at maturity, which were 170 DAFB in Niitaka and 200 in Mansoo. Ten cracked and non-cracked fruits were then classified for subsequent studies, respectively.

The anatomical features of the exocarp were compared in Mansoo and Niitaka varieties cultivated in the same orchard. The cracked region was compared with a normal region in Mansoo pears. Additionally, a normal region below the lenticel was compared with the exocarp using microscopy.

The surfaces of cracks and normal regions were observed using an SM (SZX-10, Olympus, Japan) and SEM (3000N, Hitachi, Japan). Anatomical aspects were observed using a LM (BX-51, Olympus, Japan).

2.2. LM preparation

For light microscopic observation, segments of exocarp were collected and immersed in bottles with 1% glutaraldehyde, followed by 2.5% osmium tetroxide in sodium phosphate buffer. The solution was then changed in a graded ethanol series (40, 60, 80, 90, 95, and 100%) for dehydration. Finally the samples were embedded in epon resin and were polymerized by incubation for three days. Sections of 1.5 μm were produced using ultramicrotome (UltraCut, Leica, Germany) and mounted on glass slides. The sections were stained with periodic acid Schiff (PAS) staining.

2.3. SEM preparation

Fragments of fruit with exocarp were sampled and treated with same procedure described above for cell fixation and dehydration. The fragments were immersed in isoamyl acetate (Sigma–Aldrich, USA) and placed in a critical point dryer (HCP-2, Hitachi, Japan). After coating with 10 nm in an ion-sputter (E-1010, Hitachi, Japan), the samples were observed at an accelerating voltage of 20 kV and a working distance of 11.6 mm.

2.4. Staining stone cells with HCl-phloroglucinol (Wiesner reaction)

The fruits were cross-sectioned with razor blade. The specimen was dried to cover with wiper. Then lignin, which is consisted of stone cell, was stained to pour a few drops of 2% phloroglucinol solution in 20% ethanol with 20% HCl. The distribution of stone cell clusters was observed with SM.

2.5. Statistical analysis

Data were analyzed statistically by *t*-test using the SPSS 14.0 software (Microsoft, US)

3. Results and discussion

3.1. Morphological characteristics of cork tissue and cracked region using SM

Superficial cracks were found evenly across the Mansoo pear fruit surface. These cracks did not penetrate deeply into the flesh. The edge of the cracks turned black, which received attention (Fig. 1A). The cracks had irregular lengths, varying in direction, shape, and length from 0.1 cm to over 1 cm. Two types of cracking occurred at maturity in Mansoo fruit: SLSC and LLSC. Most SLSCs were located on lenticels. The LLSCs extended from the SLSCs and enlarged along cork tissue crevices (Fig. 1B and C).

The cork tissue crevice, which covered the fruit surface, was observed in both Mansoo and Niitaka pears (Fig. 1D and E). The crevices had clearer shapes and narrow distance between crevices in Mansoo compared to Niitaka pears. Unfortunately, it was impossible to demonstrate a cause for differences of cork shape each cultivar in this study.

This study suggested that process of the cracking on the Mansoo fruit surface that initiation on the lenticel followed by extension through the crevice. The development process of cracking was not determined. In our previous study, cracking in Mansoo was observed on only ripen fruit and in case of fruits with paper bagging but few without paper bagging (Choi et al., 2015). It was suggested that the light condition such as fruit shading was an environmental cause of cracking in Mansoo. Further study is necessary to investigate an environmental condition and dynamic developmental process of cracking in Mansoo.

It has been reported that the lenticel is easy to physiological disorders in some species and cultivars. Lenticel damage, which appeared as diffuse browning around the lenticel, occurred in an avocado cultivar (Curry et al., 2008; Everett et al., 2008). Lenticel breakdown, which has a symptom of round pitting centered on the lenticel, has been found frequently in the 'Gala' apple cultivar (Curry et al., 2008; Everett et al., 2008). Little was reported that physiological disorder on lenticel in Asian pears. Therefore, it was demonstrated that Mansoo was a susceptible cultivar to physiological disorder, especially cracking, on lenticel among Asian pears.

3.2. SEM observation of cracked region

Morphological features observed by SEM showed that cells were regular and round in lenticel but varied and pointed in the outermost tissue (Fig. 2). These differences indicated that the filling tissue was newly formed inside stomata whereas cork tissue received tension with fruit growth. Because of their features, we were able to distinguish them from other tissues. As shown in Fig. 3F, the lenticel was found to be covered with cork tissue by LM. Therefore, the filling tissue was also covered with cork tissue. The filling tissue was observed among the outer features via SEM but not in cross section using LM.

The Asian pear in maturity has cork tissue at the outermost cork and hypodermis (Park et al., 2013). Filling tissue appears on the outside of the lenticel (Mauseth, 1988). Nevertheless, few studies have observed the filling tissue was consisted fruit lenticel. This study demonstrated that filling tissue was distinguishable from the cork tissue around the lenticel by using SEM in both Mansoo and Niitaka fruit.

Fruit surface crevices were formed by the division of some cork tissues and the exposure of internal tissues through the crevices in both cultivars. However, the size and shape of the crevices in the cork tissue varied with the cultivar. The area of the exposed tissue was larger in the cork tissue surrounding the lenticel than the prevalent cork tissue of the fruit skin. Mansoo crevices were also larger than Niitaka in the cork tissue surrounding a lenticel