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Full Length Research Paper

Relationship between peach endocarp cracking and specific vascular bundle development

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Vascular bundles play a very important role in fruit development and quality. Each set of bundles has its own specific function. In order to know the relationship between endocarp cracking and vascular bundles, we observed specific vascular bundle development during fruit growth. The results showed that embryo bundles and ventral bundles are closely related to pit-splitting. We found that pit-splitting was caused by the occurrence of both an over-expanded seed and a weakened endocarp. The rapid development of the seed was controlled by abnormally thickened embryo vascular bundles. The weakened endocarp was caused by an abnormal thickening of the ventral vascular bundles that formed two grooves on the endocarp of the abdominal suture, thus rendering the endocarp weak and fragile. Once the expansion force of the seed is too large, the endocarp will rupture due to a weak position of the grooves, a phenomenon known as pit-splitting. Our results demonstrate that the over-development of the embryo bundle and ventral bundles is the key cause of pit-splitting. We anticipate our assay to be a starting point for more studies examining the relationship between vascular bundles and fruit quality. For example, excessive nitrogen, water and thinning can cause pit-splitting; however, the manner in which these factors influence the development of the embryo vascular bundle and abdominal vascular bundle of the endocarp is unknown. The individual and combined effect of these factors on pit-splitting could be tested and related to other fruit quality problems.

Keywords: Peach fruit, embryo and ventral vascular bundles, excessive nitrogen, split-pit.

INTRODUCTION

The vascular bundle is the main channel for the transportation of moisture and nutrients into the fruit. This channel plays a very important role in fruit development and quality. We systematically studied vascular bundle types and their distribution in peaches in 2009 (Zhang et al. 2009) and divided the bundles into three types: (1) main bundles, i.e., embryo vascular bundles, endocarp vascular bundles, abdominal vascular bundles and dorsal vascular bundles; (2) branches of the vascular bundle, i.e., the secondary vascular bundles of ventral bundles, dorsal bundles and endocarp bundles; and (3) capillary bundles, i.e., all of the thinner vascular bundles that are mainly distributed throughout the mesocarp. Each group of bundles has its own specific function: embryo vascular bundles provide nutrients and moisture for seed development, whereas the bundles within the mesocarp, develop into a glandular cavity in the phloem position to accommodate the large demands for nutrients and water in rapidly growing fruit and enable the development of fruit flesh cells at later stages of fruit growth (Zhang et al. 2009). According to our observations, abnormal vascular bundles can lead to impaired fruit quality and sequelae, such as suture softening and endocarp cracking.

Our research has shown that suture softening is associated with anomalies in the development of the abdominal vascular bundle (Pan et al. 2009, Liu et al.

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Figure 1. Pit-splitting fruit appearance.A. Fruit malformation. B. Stem end crack, C. Mouldy interior after pit-split.

2006, Liu et al. 2010); however, the relationship between endocarp cracking (split-pit) and vascular bundles has not been studied. Cracked endocarps can result in shape deformity (Fig. 1a) or stem-end cracking (Fig. 1b), which allows air or rain to enter the fruit and causes the build-up of mould (Fig. 1c). Therefore, peach endocarp cracking is a serious quality defect. Sometimes the cracking rate can be as high as 50% (Ge et al. 2007), causing huge economic losses. Studies have shown that pit-splitting is mainly caused by excessive thinning (Nakano and Nakamura 2002) or the excessive application of nitrogen fertiliser (Malley and Proctor 2002). According to the rules of nutrition transportation, excessive nutrition is regulated by the vascular bundles. We found that all split fruits have an expanded seed volume and deep grooves in the ventral bundle of the endocarp; both of these events can lead to endocarp cracking. This study examined the effect of nitrogen on the development of the embryo vascular bundles and abdominal vascular bundles in the endocarp and the relationship between nitrogen and endocarp cracking. The goal of the study was to provide a theoretical basis for solving the problem of endocarp cracking in peach production.

MATERIALS AND METHODS

Plant material

Ten-year-old *Prunuspersica* (L.)Batsch cv. ‘Okubo’ trees were grown in a commercial orchard. Nitrogen was applied in the form of urea fertiliser at rates of 1.5 kg/tree (high N), 1 kg/tree (middle N), 0.5 kg/tree (low N), and 0 kg/tree (control) two weeks before the flowering stage and two weeks and four weeks after the flowering stage, respectively. All trees were regularly irrigated. Observation of the morphological structure of vascular bundles was performed in trees with different rates of N application. Observation of the endocarp hardening and seed development was performed in trees with low N application. Observation of the endocarp splitting pattern was performed in trees with high N application.

Observation of the endocarp hardening and seed development

All fruits were labelled at anthesis. Endocarp hardening and seed development were observed with the anatomic method at one-week intervals from 20 days to 82 days after anthesis. The status of 30 fruits was observed and photographed, and the pit-splitting time was recorded.

Observation of the morphological structure of vascular bundles

To observe the morphological structure of vascular bundles, small blocks of flesh, including the ventral vascular bundles and small blocks of endocarp with embryo vascular bundles, were taken from fruits of different N treatments and fixed with FAA solution 10 d, 20 d, 30 d and 40 d after blooming. Fixed samples were embedded in paraffin and sectioned with a microtome. The sections were stained with safranin/fast green and examined with a DMBA 450 microscope (Motic China Group Co., Ltd, Beijing, China)(Pan et al. 2008).

RESULTS

The endocarp hardening synchronised with the seed /cotyledon development

Peach fruit development consists of four phases: rapid growth, slow growth (endocarp hardening phase), cell enlargement and ripening (Zanchin et al. 1994).The endocarp hardening phase is when the pit split occurs (Davis 1941). We found that endocarp hardening was a process of lignin deposition that was progressive and synchronised with seed development. When cotyledon development was complete (approximately 68 d after full bloom), the endocarp lignification was complete as well (Fig. 2). Endocarp cracking occurred during this process; cracks and callus were visible 75 d after full bloom (Fig. 2i). Pit-splitting, therefore, started after (not during) endocarp hardening (Davis 1941).

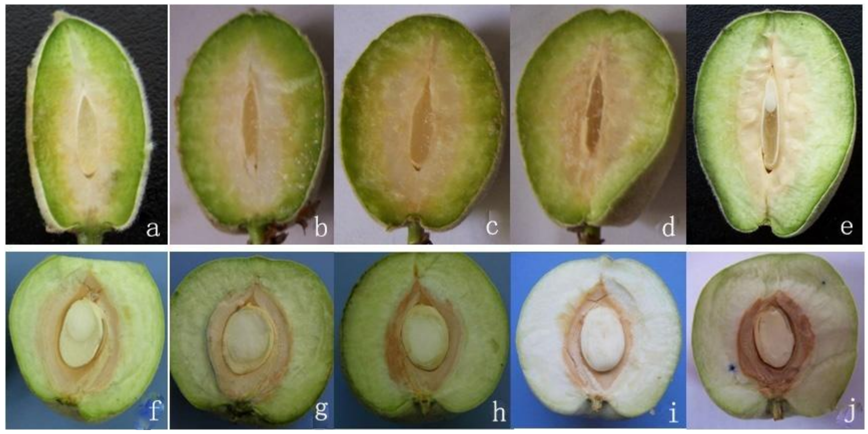

Figure 2. Peach endocarp hardening process .a,b,c,d,e,f,g,h,i and j refer to20 d, 27 d, 34 d, 41 d, 48 d, 54 d, 61 d, 68 d, 75 d and 82 d after full bloom, respectively.

Table 1. Comparison of seed status.

Fruit with	Seed				
	Thickness /cm	Diameter /cm	Length /cm	Weight /g	Water content/%
Split	0.786a	1.403a	2.144a	1.044 A	90.0 a
Not split	0.718b	1.389a	2.047b	0.853 B	82.5 b

Different lowercase letters in columns indicate significant differences at p = 0.05; uppercase letter indicate significant differences at p = 0.01

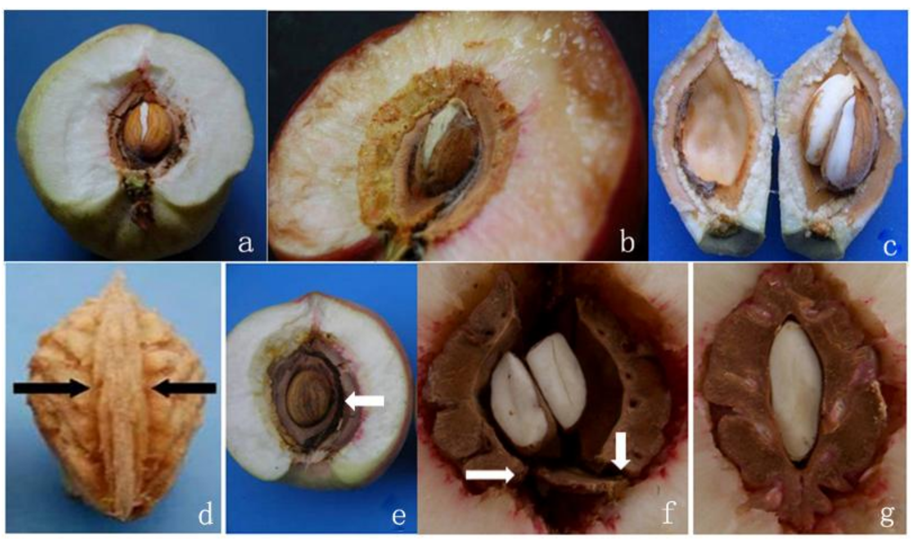

Figure 3. Internal forces and ventral vascular constriction groove causing the pit-split.a, Split core with burst seed coat. b. Split core with seed germination; c. Split core with double seeds. d, Arrows indicate a shell with two longitudinal ventral suture grooves. e. Arrows indicate cracking along the ventral vascular constriction groove; f, Cracking at the weak point. g, Intact peach, endocarp with shallow vascular constriction groove and the solid shell.

The mechanism of endocarp cracking

Pit-splitting is an event that requires a very large force inside the peach. Our results show that the force causing endocarp splitting originated from inside of the pit; indeed, all of the seeds were fully expanded in cracked

Table 2. Investigation of the rate of splitting in fruit with double seeds.

Fruit type	Fruit number	Split fruit number	Intact fruit number	Split fruit%
Fruit with double seeds	30	21	9	70
Fruit with single seed	30	7	23	23

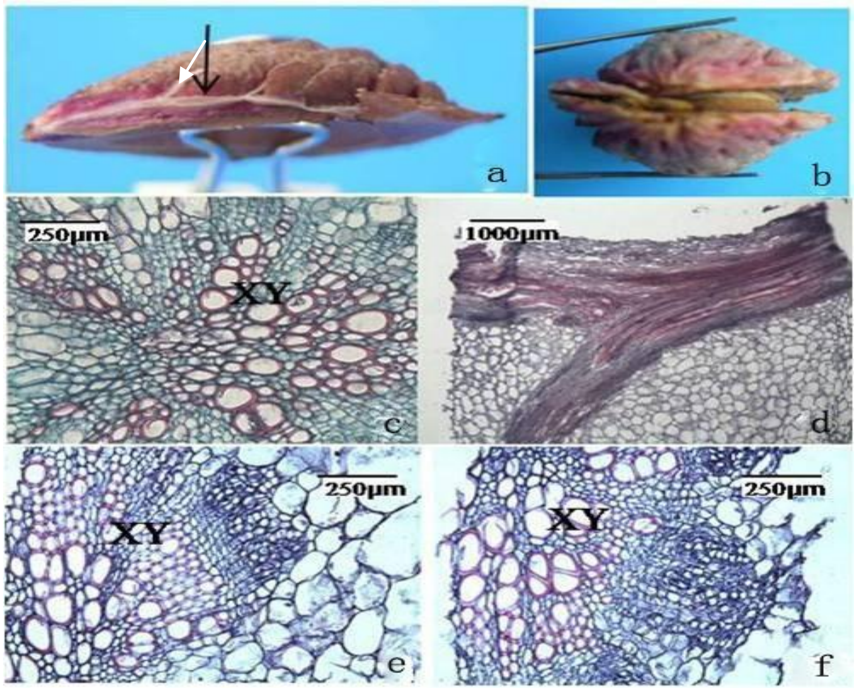

Figure 4. Ventral vascular anatomical drawings.a, Arrows indicate the ventral vascular bundle; b, Arrows indicate the vascular constricted groove on the abdominal suture of the endocarp. c,d, Cross-sectional slice and longitudinal slice of the ventral vascular bundles. e, Cross-section of the ventral vascular bundle in a split pit. f, Cross-section of the ventral vascular bundle in an intact pit.

shells, and some seeds had grown so large (Table 1) that their coats had burst (Fig. 3a). Other seeds had even germinated in the fruit (Fig. 3b). Peaches with double seeds were much more likely to crack, and indeed, 2/3 of these peaches split（Fig. 3c, Table 2）.

We dissected and observed the fruits and found that pit-splitting was related to shell morphology. We observed two main vascular channels on either side of the suture line of endocarps (Zhang et al. 2009). These two channels constricted the shell with two longitudinal grooves (Fig. 3d). The grooves in the split shells were broader and deeper than in the intact shells, rendering the side structure thin and fragile (Fig. 3e,f and Fig. 4b) and resulting in the cracking of the endocarp.

Endocarp cracking is related to the ventral vascular bundles

We observed the structure of ventral vascular bundles with the paraffin method in both pit-splitted fruit and fruit with intact pits. The ventral vascular bundle had more secondary xylem cells and was much thicker in pit-splitted fruit (Fig. 4e) than in intact fruit (Fig. 4f). More organic and inorganic materials can typically be transported into the ventral suture region of the fruit via fully developed ventral bundles; however, in this case, the fruit fleshy cells were not yet sufficiently enlarged, resulting in the accumulation of nutrients and the thickening of the ventral vascular bundle. At this time, the endocarp had not yet hardened; therefore, a deep groove could be formed (Fig. 4a,b).

Endocarp cracking is related to the embryo vascular bundle

Seeds generally obtain nutrients and water through a specific vascular bundle supply, i.e., the embryo vascular bundle directly connected with the fruit stalk and embryo;

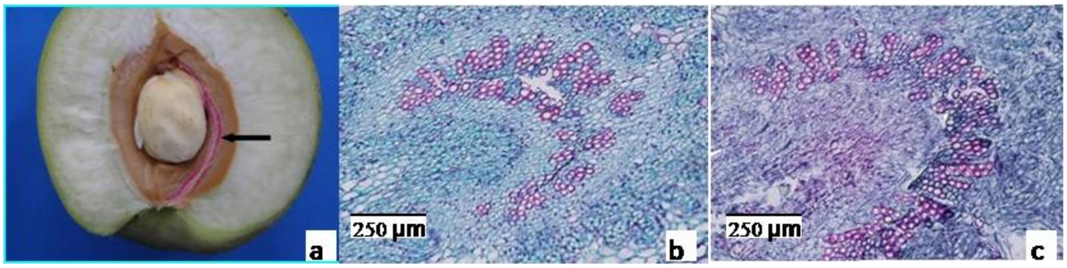

Figure 5. Embryo vascular anatomical drawings .a, Arrows indicate the embryo vascular bundle. b, Cross-section of the embryo vascular bundle with no pit-splitting at 40 d after blooming. c, Cross-section of the embryo vascular bundle with a split pit 40 d after blooming.

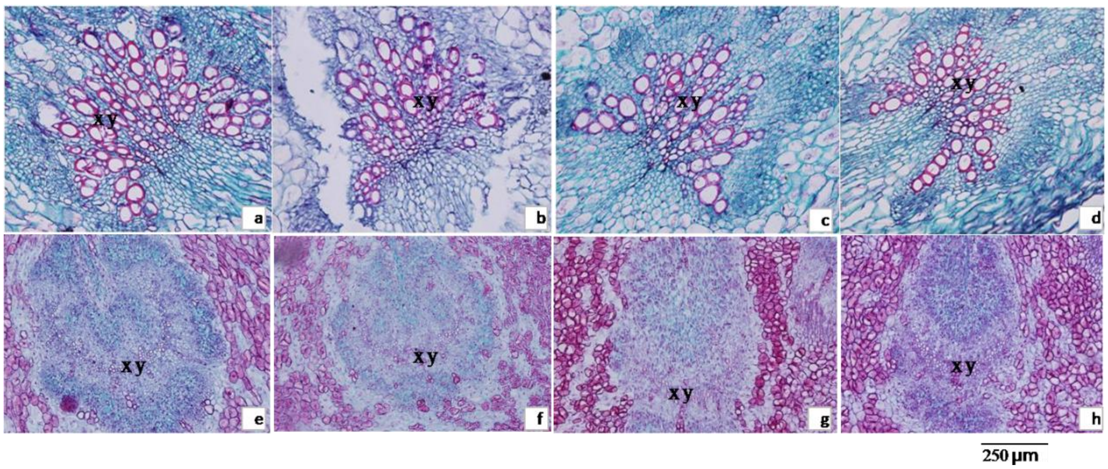

Fig. 6. Effects of nitrogen supply on abdominal and embryo vascular bundle development 48 d and 20 d after blooming, respectively. XY: Xylem. a,b,c,d: Ventral vascular bundle with high, middle and low levels of N and no N, respectively. e,f,g,h: Embryo vascular bundle with high, middle and low levels of N and no N, respectively.

the development of the bundle therefore directly affects the growth and development of the seed. A larger seed with thicker vascular bundles is able to obtain more nutrients and moisture. We observed the structure of the embryo vascular bundle with the paraffin method in both pit-splitted fruit and fruit with intact pits. The embryo vascular bundle had more xylem cells and was much thicker in pit-splitted fruit than in fruit with intact pits (Fig. 5).

The effect of nitrogen on embryo and ventral vascular bundles and pit-splitting

Previous experiments have shown that nitrogen can promote vascular xylem development (Pan et al. 2005) and increase the number of split-pits (Claypool 1972, O' Malley and Proctor 2002). To understand why ventral vascular bundles become thicker with splitting, we ascertained the influence of nitrogen on ventral and embryo vascular bundle development anatomically and surveyed the number of fruits with pit-splitting as ripening occurred. The results are shown in Fig. 6. High nitrogen supply obviously promoted the development of both ventral and embryo vascular bundles (include the xylem area), while plants supplied with low levels of nitrogen and controls did not differ substantially during the period 18-48 d after anthesis. Splitting fruit number also increased with increasing N supply (Fig. 7).

DISCUSSION

Our results show that splitting is caused by two events, namely, an excessively expanded seed exerting a mechanical tension force outwards and a weakened endocarp with an excessively developed ventral vascular bundle. Whether the endocarp cracks or not also depends on its firmness, which, in turn, depends on the thickness of the vascular constriction groove along both sides of the suture line. This thickness also depends on the size of the main vascular bundles before the period of endocarp hardening. This is the first swelling period of fruit growth, and both the peel and kernel develop rapidly. Because the vascular network and cells in the mesocarp

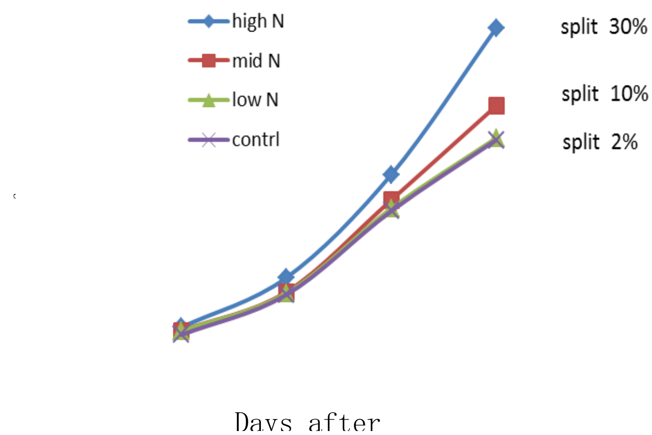

Fig. 7. The effect of nitrogen supply on the xylem area of the ventral vascular bundle and the number of fruits with pit-splitting.

were still not fully developed (Zhang et al. 2009), "sink" absorbing was limited. If the sap pressure exceeds normal levels, the turgor inside the fruit becomes large. Due to superfluous moisture and nutrition, the main vascular bundles from the fruit stalk (including the ventral vascular tissue and embryo bundle) expanded and thickened. At that time, the deposition of lignin in the endocarp had begun (Yang et al. 2009), but the endocarp had not yet hardened. Therefore, the thickened bundles deeply constricted the grooves on the suture line of the endocarp, making the endocarp thin and fragile. In addition, excessively expanded seeds are related to the overdevelopment of the embryo vascular bundle. Therefore, if the turgor pressure is reduced (e.g., reducing water, nitrogen supply and excessive thinning) inside the fruit before the endocarp has hardened, the vascular bundle would not become abnormally thick. Therefore, water, nitrogen, and excessive thinning, while required for promoting fruit growth, are also directly related to pit-splitting (Claypool 1972, Nakano and Nakamura 2002, O'Malley and Proctor 2002) and increase the pressure turgor inside of the fruit. To prevent pit-splitting, we propose the following recommendations. (1) For easily cracked cultivars, fruit thinning should not occur early and should not be excessive. (2) During the period of development of the young fruit, excessive irrigation should be avoided, and during the rainy season, thorough drainage is necessary. (3) Nitrogenous fertiliser should not be excessively supplied at the early stage of fruit development.

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