土壌乾燥処理がヤマアンズ根の皮層細胞壁の内部成長（phiー肥厚）ならびに細胞核、デンプン粒の変動に及ぼす影響

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Anatomical Development of Cell Structure Including Phi-thickening, Nuclei Degradation and Starch Accumulation in Root Cells in Relation to Water Stress in Siberian Apricot (Prunus armeniaca L.) Trees

Yang Song*, Lihong Ye*, Shimao Cui** and Naosuke Nii*

Abstract The anatomical development of phi-thickening in the cortical tissue adjacent to the endodermis, degradation of nuclei and starch accumulation in the distal portion of roots in 3-year-old Siberian apricot seedlings were investigated in relation to water stress. The degree of water stress was shown by the water saturated deficiency (WSD) of the leaves and was graded into three levels: (a) unstressed, (b) moderate stress, and (c) severe stress plots. The roots under water stress, phi-thickening developed typically as compared with normal conditions. The development of phi-thickening in the cortex of Siberian apricot roots may be a defense mechanism against water stress. Water stress accelerated the degradation of nuclei and decreased starch accumulation in the distal portion of the roots. These features are important for understanding the response of the roots to soil water conditions.

Key words: Nuclear degradation, phi-thickening, root, Siberian apricot, starch

Introduction

Rosaceae trees are generally tolerant to drought, but the physiological and anatomical mechanism has not been examined in detail (Cui et al., 2003a, b; 2004). The anatomical changes in the distal portion including apical meristem of roots would elucidate the mechanism of drought tolerance in Rosaceae trees. Recently, Nii et al. (2004) and Pan et al. (2006) reappraised cell-wall ingrowth (phi-thickening) in the cortical cell of loquat roots. In loquat roots sampled from trees planted under stress conditions, phi-thickening developed dramatically compared with normal conditions (Pan et al., 2006). In addition, the phi-thickening differed with the Rosaceae fruit tree species (Pan and Nii, 2005). The extent of phi-thickening was pronounced in loquat (Eriobotrya), moderate in apple (Malus) and pear (Pyrus), and slight in peach (Prunus). We also reported that the appearance of phi-thickening was earlier than the anatomical development of the Casparian strip in loquat roots (Pan et al., 2006). In young loquat roots with a poorly developed Casparian strip, the phi-thickening of the cortical cell wall had already formed. From these features, phi-thickening is considered to serve as a substitute for the Casparian strip. Although many reports have been published on phi-
thickening in Rosaceae fruit trees (Esau, 1943; Mackenzie, 1979; Nii et al., 2004; Pan et al., 2006; Peterson et al., 1981; Riedhart and Guard, 1957), little has been published on the relationship between phi-thickening and environmental conditions.

In experiments on salt stress, nuclear degradation occurred in the apical meristem of root cells in barley (Katsuhara, 1997; Katsuhara and Kawasaki, 1996; Katsuhara and Shibaoka, 2000). Degradation of nuclei has been also reported to occur in the apical meristem of the root cells of citrus (Cui et al., 2002) and loquat (Cui et al. 2003a) trees under salt and water stresses. However, anatomical changes of nuclear degradation of Siberian apricot (Prunus) roots subjected to water stress have not been studied in detail. Starch content also decreased in the roots of loquat trees under water stress (Cui et al., 2003a). These changes could serve as good indicators of the response to drought stress. However, such observations are lacking for Siberian apricot trees subjected to water stress.

In the present study, we investigated the root anatomy of Siberian apricot in Rosaceae trees under water stress with respect to: (1) the appearances of Phi-thickening in the cortex adjacent to the endodermis and (2) the changes of nuclei and starch grains in the distal portion of the roots.

**Materials and Methods**

Three-year-old seedlings of Siberian apricot (Prunus armeniaca L.) were grown in containers (35 cm in diameter and 30 cm in depth) with sandy soil and grown in a greenhouse.

The effects of water stress (drought condition) on the appearance of phi-thickening, the features of nuclei and starch accumulation were observed in the distal portion of roots. Plants were grown under two different soil water conditions: (a) unstress (control) and (b) stress (no irrigation for 28 days). At 7 day intervals after the onset of the drought treatment until day 28, the degree of water stress was estimated from the water saturated deficiency (WSD) of the leaves. Six plants were used for each treatment.

For WSD measurement, five leaves at the middle of the current shoots from each plant were cut at the basal end of the petiole and the fresh weight of the leaves recorded immediately (field fresh weight), then they were weighed again 24 hr after their petioles were immersed in water under saturated moisture conditions (saturated fresh weight). The dry weight was determined after lyophilization (lyophilized dry weight). WSD was calculated, based on fresh weight and dry weight from the following equation (Cui et al., 2004).

\[
\text{WSD} = \left(\frac{\text{saturated fresh weight} - \text{field fresh weight}}{\text{saturated fresh weight} - \text{lyophilized dry weight}}\right) \times 100\%
\]

During the treatment, the new white roots for anatomical observations were sampled from unstressed (control) and stressed plants: moderate stress (m-plots, no irrigation for 14 days) and severe stress (s-plots, no irrigation for 28 days).

Roots were cut with a razor blade, fixed in 3% glutaraldehyde (0.1 M cacodylate buffer, pH 7.2), and stored at 4°C. They were then dehydrated through a graded ethanol series and finally embedded in Technovit 7100 (Kulzer, Germany). Transverse sections (1.5 μm) of the tissue were cut with an ultramicrotome with a glass knife. Phi-thickening was observed by fluorescent microscope (excitation wavelength: 365 nm) without staining. Anatomical features of nuclei and starch grains of roots were also observed after staining with 4′-6-diamidino-2-phenylindole (DAPI) or I−-KI under fluorescent microscope and light microscope, respectively.

**Results and Discussion**

The values of WSD of the leaves at day 0 increased proportionately with the duration of drought stress. The WSD of leaves was determined for 14 days, in m-plots, and 28 days, in s-plots, after the onset of the drought treatment (Fig. 1). The values of WSD in s- and m-plots were higher than those in the unstress plots. The WSD in unstress and m- and s-plots were 6.5, 24.7, and 41.8%, respectively.

The orientation of the turgid leaves before the drought treatment was more or less horizontal orientation to the shoots, whereas with increases in WSD during drought treatment, they gradually became very soft and flaccid. When the leaves had nearly attained their maximum WSD level, they began to turn yellowish. However, when the plants

![Fig. 1. Water saturated deficiency (WSD, %) of Siberian apricot leaves under drought stress for 28 days and unstress. m-plot: moderate stress (no irrigation for 14 days), s-plot: severe stress (no irrigation for 28 days).](image-url)
were watered to relieve their water stress; the leaf became hydrated, rigid, and gradually assumed a horizontal orientation. In the m-plots, almost all leaves recovered after watering, whereas numerous leaves in s-plots abscised but the tree did not die.

Since the rate of root elongation changes in response to the soil condition, root age cannot be determined simply by the distance from the root tip. For example, the lower the root elongation rate, the closer the position of the suberin lamellae including the Casparian strip. As aging of the roots advanced, the number of xylem vessels per pole increased typically. The xylem differentiation and development could be used to compare the development of the roots grown under different conditions, and root age can be determined by the number of xylem vessels per pole. The formation of phi-thickening was examined at a similar age (Fig. 2). The difference of phi-thickening in the cortex already appeared between treatments 14 days after treatment. Phi-thickening appeared earlier and was more extensive in the s- and m-plots than in the unstressed plot (Fig. 2A, B and C).

The appearance of phi-thickening in the cortex of the unstress roots was mostly detected in a single cell layer of the cortex (Fig. 2D and G). On the other hand, phi-thickening in the cortex of the roots under stress was mostly detected in two layers (Fig. 2H and I). Nii et al. (2004) and Pan et al. (2006) reappraised phi-thickening in the cortical cells and Casparian strip in Rosaceae fruit tree roots. They also reported that phi-thickening appeared earlier than suberin lamellae with Casparian strip in loquat roots. In young roots with a poorly developed Casparian strip, phi-thickening of the cortical cell wall may serve as a substitute for the Casparian strip, although phi-thickening has been considered to function primarily as supportive tissue (Weerdenburg and Peterson, 1983). In loquat roots sampled from trees planted under stress conditions, phi-thickening developed dramatically compared with normal condition (Pan et al., 2006). These

![Fig. 2. Relationship between the development of phi-thickening in the cortex of the Siberian apricot roots in relation to root age under three soil water conditions. Sections were examined by fluorescent microscope without staining. Left panels: unstress plots, central panels: m-plots, right panels: s-plots. Arrows indicate the thickening walls. The bar is 50 μm.](image-url)
findings indicate that phi-thickening is affected by water stress.

Phi-thickening may develop differently under different water stress conditions. Since the presence of lignified phi-thickening did not block the movement of apoplastic tracers (Peterson et al., 1981) and plasmalemma was not observed in the portion of phi-thickening (Haas et al., 1976), phi-thickening have not been considered being an apoplastic barrier. This opinion was accepted by Mackenzie (1979), who noted a high frequency of plasmodesmata in the outer tangential cell walls of the phi-layers. The mechanical function of phi-thickening remains controversial (Weerdenburg and Peterson, 1983). Although the function of phi-thickening is not clear (Guttenberg, 1968; Mackenzie, 1979; Peterson, 1992), the presence of phi-thickening was supposed to be common in the Rosaceae family, and to be a systematic characteristic (Gerrath et al., 2002). In agreement with Cui et al. (2002; 2003a) and Degenhardt and Gimmler (2000), the present findings indicate that phi-thickening formation is not constitutive, but, may at least partially, be a defense mechanism under drought conditions.

Water stress also induced nuclear degradation and a decrease in starch accumulation typically in Siberian apricot roots (Figs. 3 and 4). Nuclear degradation has been detected in roots of fruit trees under NaCl stress (Cui et al., 2002; 2003b). Cui et al. (2003a) also concluded that starch grains in the root apical region were also hydrolyzed to sugars in the loquat plants under water stress. In Siberian apricot plants under water stress, starch grains in the root apical region were also hydrolyzed to sugars. These results suggest that the photosynthetic activity is lowered under stress conditions, and starch may be used as a reserve source of sugars to maintain normal cell metabolism. The decrease in starch accumulation and nuclear degradation in the roots were observed typically under severe water stress. The anatomical features of phi-thickening, degradation of nuclei, and starch accumulation in the distal portion of roots differed within 7 days after stress treatment. These features could help us understand the response of the roots to soil water conditions.

Fig. 3. Photomicrographs of longitudinal sections of Siberian apricot root tips after staining with DAPI showing cell nuclei in response to drought stress. Arrows point to cell nucleus. Left panels: unstress plots, central panels: m-plots, right panels: s-plots. The bars are 100 μm (A, B, C) and 25 μm (a, b, c).
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References


