Anatomical Development of Phi Thickening and the Casparian Strip in Loquat Roots

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The anatomical development of cell wall ingrowth (\textit{phi} thickening) in the cortical tissue adjacent to the endodermis and Casparian strip in endodermal cells was investigated in loquat roots. \textit{Phi} thickening initially appeared simultaneously approximately 10 mm from the root tip and expanded as the distance from the root tip increased. \textit{Phi} thickening was observed with increased age in several layers of cortical tissue. Then, the \textit{phi} thickening attained full size at approximately 30 mm from the root tip. When \textit{phi} thickening began around the cortex, the initiation of Casparian strip formation appeared as a dot in the endodermis. At the next stage, the Casparian strip appeared partly in several endodermal cell walls facing the phloem sectors before eventually appearing in all endodermal cells. \textit{Phi} thickening developed considerably before the completion of the Casparian strip in loquat roots. When the development of the Casparian strip finished in the endodermis, the cortical tissue with the \textit{phi} thickening shed from the endodermis. Upon separation of the cortex and endodermis, the pericycle layers increased laterally to a thickness of 2 to 3 cells and accumulated auto-fluorescent substances in their cell walls. The Casparian strip appeared further away from root tip in young and white roots than in the old and light brown roots. When roots were sampled from trees planted under drought stress conditions, \textit{phi} thickening was observed to have developed dramatically compared to normal conditions. The development of \textit{phi} thickenings of cortex in loquat roots under drought conditions may be regarded as a defense mechanism against water stress.

Key Words: Casparian strip, drought stress, loquat (\textit{Eriobotrya japonica} Lindl.), \textit{phi} thickening, root.

Introduction

Environmental stress is the major factor by which plants adapt to the fertility and soil water content limiting plant productivity; however, Rosaceae trees are tolerant to drought stress. Although a review revealed that many compounds accumulate under stress conditions, only a few reports have described physiologically and anatomically why Rosaceae trees can tolerate drought conditions (Cui et al., 2003, 2004). Knowledge of anatomical changes in the roots should contribute to our understanding of drought tolerance in Rosaceae trees. Recently, Nii et al. (2004) and Pan and Nii (2005) reappraised cell wall ingrowth (\textit{phi} thickening) in the cortical cells and Casparian strip of Rosaceae fruit roots. As a result, the extent of \textit{phi} thickening was pronounced in loquat (\textit{Eriobotrya}), moderate in apple (\textit{Malus}) and pear (\textit{Pyrus}), and slight in peach (\textit{Prunus}). However, \textit{phi} thickening in the cortex of the roots could not be observed in satsuma mandarin (\textit{Citrus}), grape (\textit{Vitis}), and persimmon (\textit{ Diospyros}) (unpublished data). They also reported that the appearance of \textit{phi} thickening was earlier than the anatomical development of the Casparian strip (Nii et al., 2004). With a poorly developed Casparian strip in young roots, \textit{phi} thickening of the cortical cell wall may serve as its substitute, although \textit{phi} thickening is considered to function primarily as supportive tissue (Weerdenburg and Peterson, 1983). To strengthen this hypothesis, more extensive knowledge of the anatomy of the cortex and its relation with the Casparian strip is needed.

While many reports have been published on \textit{phi} thickening, generally in Rosaceae fruit trees (Esau, 1943; Mackenzie, 1979; Nii et al., 2004; Pan and Nii, 2005; Peterson et al., 1981; Riedhart and Guard, 1957), relatively little has been published on the relationship between \textit{phi} thickening and the development of the Casparian strip in relation to aging of the root and environmental conditions, although the differentiation and development of the Casparian strip has been reported for several species of fruit trees (Esau, 1943; Mackenzie, 1979; Riedhart and Guard, 1957; Weerdenburg and Peterson, 1983). Particularly, the relationship between the Casparian strip and \textit{phi} thickening in relation to root development and aging should be investigated in order to understand the defense mechanism against drought stress in Rosaceae trees.

In the present study, we investigated the anatomy of loquat roots with respect to the distance from the root
tip by employing continuous sectioning of the same root to determine the following: (1) the time lag between the appearances of phi thickening in the cortex and the Casparian strip in the endodermis, and (2) changes of phi thickening brought about by drought stress.

Materials and Methods

Three-year-old ‘Mogi’ loquat (Eriobotrya japonica Lindl.) trees grafted on self-seedling rootstock were grown outdoors in containers (30 cm diameter) potted with sandy soil.

In the first investigation, the process of differentiation and development of phi thickening and the Casparian strip in the root was observed. New root samples were collected for observation in April, 2005. Transverse sections were cut by hand 5 mm from the root tip up to the basal portion of the root. The degree of development of the Casparian strip and phi thickening in relation to root aging were examined by fluorescent microscopy (excitation wavelength: 365 nm) in tissues without staining.

In the second investigation, two roots were collected to observe the appearance of phi thickening and the Casparian strip in April, 2005; one root was younger and still-creamy white up to a distance of 30 mm from the tip, while the other was older and light brown for almost its entire length. The length of both roots was approximately 130 mm. Subsequently, serial transverse sections, commencing 10 mm from the root tip, were cut at 10 mm intervals by hand for the remaining 130 mm length of the root. The site and development of the Casparian strip and phi thickening were examined by fluorescence microscopy in tissues without staining.

In the third investigation, the effects of water stress (under drought conditions) on the appearance of phi thickening were observed. Plants were grown under unstressed (control) and severely stressed conditions. Each treatment consisted of 10 plants. Just before beginning drought stress treatment, the plants were removed from the pots and the new white roots were almost cut off. Then, the plants were replanted in the same pot. All plants were transferred into a greenhouse to induce a rapid and drastic water stress. The degree of drought stress was judged from leaf orientation and color, as described by Cui et al. (2003). When plants reached the level of severe stress, they were watered from the bottom of the container. Plants suffered from similar stress conditions three times from August 28 to the middle of October. At the end of treatment, the new white roots were sampled from control and stressed plants for anatomical observations. Transverse sections of the roots were cut at two regions; at 10–15 mm and at more than 20 mm from the root tip. The area occupied by phi thickening and the number of cell layers that had developed phi thickening were classified into 4 grades (Fig. 1 and Table 1):

- phi thickening appeared in only the first cell layer of the cortex (+),
- phi thickening appeared in the first to second layers (++),
- phi thickening appeared in approximately the first to third layers (+++),
- phi thickening appeared in the first to more than the fourth layers (+++).

Results and Discussion

Phi thickening and the appearance of the Casparian strip were not observed in sections at 5 mm from the root tip (Fig. 2A). At 10 mm from the root tip, phi thickening appeared in the first to second layers of the cortex adjacent the endodermis, but the Casparian strip only appeared as a dot in the middle of the radial wall.

![Fig. 1](image-url) The degree of phi thickening classified into four levels in loquat roots. A: common level, appeared in only the first cell layer of the cortex (+), B: moderate level, appeared in the first to second layers (++), C: thicker level, appeared in approximately the first to third layers (+++), D: thickest level, appeared in the first to more than the fourth layers (+++). Arrows indicate phi thickening. The bar is 150 µm (A–D).

![Table 1](image-url) The development of phi thickening in loquat roots under drought stress conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Position behind root tip</th>
<th>Degree of phi thickening</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Stress</td>
<td>1.0–1.5 cm</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Control</td>
<td>1.0–1.5 cm</td>
<td>26 (57)</td>
</tr>
<tr>
<td>More than 2.0 cm</td>
<td>14 (33)</td>
<td>26 (60)</td>
</tr>
</tbody>
</table>

* +, ++, +++: phi thickening appeared in one layer, two layers, three layers, and more than four layers of the cortex, respectively.

† Number of roots used for measurement (percentage).
of the endodermis (Fig. 2B). In a distant portion from the root tip, the Casparian strip appeared partly in the endodermis (Fig. 2C, D), and was gradually enclosed in the endodermis (Fig. 2E). When the cortical tissue shed, the number of pericycle layers increased, appearing as several layers inside the endodermal cells at the final stage and accumulating auto-fluorescent compounds (Fig. 2F).

In the second investigation, the sequential appearance of phi thickening and the Casparian strip were clearly observed in two different root ages judged by root color, younger roots, and older ones. Younger roots lacked lateral roots until 80 mm from the root tip, and the tip was still white for several centimeters. Conversely, older roots were slightly brown, and lateral roots appeared at 50–100 mm from the root tip. Actually, xylem developed in a more distal portion in older roots (Fig. 3E, F). Phi thickening was observed in both ages of roots 10 mm from the root tip (Fig. 3A, E). The Casparian strip appeared in a more basal portion along the younger root compared to the older root. The endodermal Casparian strip of younger roots 10 mm from the root tip could be detected as a dot under the fluorescence microscope (Fig. 3A). In contrast, the Casparian strip in older roots occupied a small region in the endodermal cell walls that faced the phloem sectors (Fig. 3E). The development of the Casparian strip 30 mm from the root tip of younger roots was similar to 10 mm from the root tip of the older roots (Fig. 3B, E). The Casparian strip appeared as lines approximately 50 mm from the root tip. The Casparian strip was observed to expand until it occupied the entire radial wall in the endodermis, but endodermal cells opposite a xylem pole still had only a weak Casparian strip, even in fully mature primary root regions (Fig. 3C, G). The endodermal cells opposite the xylem constitute so-called 'passage cells' which generally remain unsuberized (Wilcox, 1962). The present study revealed that phi thickening appeared approximately 10 mm from the root tip in both younger and older roots. The anatomical features of the Casparian strip were similar to those of other plants reported by Reinhardt and Rost (1995) and Wilcox (1962). In growing roots, phi thickening developed considerably before the appearance of the Casparian strip in the endodermis, as described by Wilcox (1962). In apple roots, however, Mackenzie (1979) found that differentiation of the Casparian strip, lignified thickening in the anticlinal walls of all the endodermis and phi layer cells, occurs 4 to 5 mm from the root tip.

When we observed loquat roots grown under drought stress, phi thickening developed dramatically compared to normal conditions (Table 1). The appearance of Phi thickening in the cortex of the roots planted under non-drought stress conditions was mostly detected in one or
two cell layers of the cortex. On the other hand, \textit{phi} thickening in the cortex of the roots planted under drought conditions was mostly detected in two to three layers. Furthermore, \textit{phi} thickening in the stressed roots appeared beyond the fourth layers. These findings indicate that \textit{phi} thickening is affected by drought conditions. This work is the first to describe changes in \textit{phi} thickening during drought stress in fruit tree roots. In the present experiment, the difference of the Casparian strip between unstressed and stressed conditions was not significant. Peterson et al. (1981) indicated that \textit{phi} thickening does not function as a barrier to the apoplastic transport of relatively small molecules. They also reported the difficulty of determining whether \textit{phi} thickening regulates the rate of water and solute transport through cell walls. At present, the only well-defined role of \textit{phi} thickening is mechanical support for the root (Peterson et al., 1981). Mackenzie (1979) suggested that \textit{phi} thickening in the apple has a primarily supportive function and that the role of \textit{phi} thickening in permeability should be determined conclusively. In the present study, the enlargement and distribution of \textit{phi} thickening developed in loquat roots under water stress conditions. The appearance of \textit{phi} thickening was earlier than the development of the Casparian strip. This time lag suggests that \textit{phi} thickening of the cortex in loquat roots is a defense mechanism against water stress under drought conditions. We need to conduct more studies on the morphology and function of \textit{phi} thickening in the cortex in order to fully elucidate drought tolerance in loquat trees.

\textbf{Literature Cited}


ビワ根の成長にともなう皮層細胞壁の内部成長とカスパリー線の発達

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ビワ根の内皮側にみられる皮層細胞の内部成長 (phi 肥厚) と内皮のカスパリー線の形成過程を検討した。phi 肥厚は根端から約 10 mm の部位から出現し、30 mm 付近ではほぼ最大に達した。内皮側の皮層の第 1 層目に出現した Phi 肥厚は中心柱を取り囲むようにほぼ一斉にみられ始め、根の齢が進むとともに第 2 〜 3 層目に出現した。Phi 肥厚が内皮を囲むようにリング状に発達した段階でも、カスパリー線の発達はその初期段階であり、内皮の細胞壁に点状に観察された。カスパリー線の発達は師部に面した部位の細胞壁から出現し、根の齢が進むにつれて、すべての内皮にみられるようになった。phi 肥厚とカスパリー線の形成過程の時間的な差異からみて、ビワ根ではカスパリー線の発達する前に phi 肥厚が観察された。カスパリー線が内皮全体に発現する段階になると、phi 肥厚をともなった皮層組織と内皮との間に離脱帯が形成され、次第に皮層が脱落した。根の齢が進むにつれて、内皮の細胞層数が同心円方向に増加し、スベリンの蓄積と考えられる自家蛍光が観察できるようになった。カスパリー線の形成は褐色根においては白色根よりも根の先端に近い部位から発達した。phi 肥厚は土壌を乾燥させて生育させた根において、乾燥させていない根の範囲に比べて顕著であった。ビワ根にとって phi 肥厚の増大は土壌乾燥に対する防御機構の一つと考えられる。