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Observation on the Viability of Root Apex Cells Treated with Different pH and Aluminum in Highbush Blueberry (*Vaccinium corymbosum* L.) and Maruba kaido (*Malus prunifolia* Borkh. var. Ringo) Cultured *in vitro*

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Summary

The viable root apices of highbush blueberry and apple rootstock were observed by staining with fluorescein diacetate (FDA) and propidium iodide (PI) to assess their pH and aluminum (Al) tolerance. In the pH treatment, roots were dipped in phosphate buffer solution ranging in pH from 3.5 to 6.0 for 2 to 6 hours. About 15 to 40 % of the blueberry root epidermis in all treatments except pH 6.0 for 2 hours were damaged, but the rest of the roots remained healthy. The apple rootstock 'Maruba kaido' suffered more damage at pH 3.5 than did the blueberry. In the Al treatment, blueberry roots dipped in an acetate buffer solution without Al, the root tip or the whole root was damaged; but roots treated with 1 mM Al retained its viability similar to the untreated root; almost all roots treated with 10 mM Al were injured. 'Maruba kaido' exhibited a tolerance to 1 mM Al.

Observation of root tissue by staining with FDA and PI is a simple technique that allows visible differentiation between viable and injured cells. Furthermore, because it is a nondestructive method, it is very useful for determining root viability.

Key Words: acidophilic character, Al tolerance, FDA-PI staining, root viability.

Introduction

The blueberry (*Vaccinium* spp.) is known as an acidophilic plant which grows vigorously in soil with a pH of 4.0-5.2 (Eck, 1988). Soil pH is one of the most important factors affecting blueberry bush growth and berry production. Consequently, various studies have examined the relationship of soil or culture solution pH (Cummings et al., 1981; Austin et al., 1986; Katakura and Yokomizo, 1995), with nitrogen forms (Spiers,1978; Sugiyama et al.,1989), and metals, such as Fe and Mn (Brown and Draper,1980; Korcak,1988). Additionally, soil pH of many orchards in Japan has been become 5.5 or higher by fertilizing the calcium containing chemicals; the growth of blueberry has been inferior when planted in areas with high soil pH. To assess the response of the blueberry root to soil pH is an important problem for blueberry culture.

Generally, the growth of crops is inhibited in acidic soil by the presence of H^+ ions, by a phosphate deficiency, or by Mn and Al toxicity. Because Al^{3+} ion increase in soil with a pH of 5.0 or lower, Al toxicity is considered to be the most growth-limiting factor associated with soil pH levels of 5.0 or lower (Wright, 1989). Therefore, acid-tolerant or acidophilic crops must have an ability or mechanism to tolerate Al. Although the blueberry is known as an acidophilic plant, few reports (Peterson et al., 1987) have addressed it; thus, a profile of its acidophilic characteristics has not been fully established.

We observed the viability of root apex cells of the highbush blueberry and an apple rootstock for comparison. We exposed roots different pH and Al concentrations and then stained them with fluorescein diacetate and propidium iodide to assess the damage. The ability of the highbush blueberry to tolerate differing pH and Al is discussed.

Materials and Methods

Induction of root from subcultured shoot

An *in vitro* subcultured shoot of the highbush blueberry (*Vaccinium Corymbosum* L.) 'Berkeley' and an apple rootstock 'Maruba kaido' (*Malus prunifolia* Borkh. var. Ringo) were used. The shoot was cut off at a point 1-2 cm from its tip, dipped in 100 $mg \cdot l^{-1}$ indole butyric acid for one minute, then clipped to a silicon gum disk with a diameter of 10 mm and a gap along its radius. The disk was floated in distilled water in a test tube. Within two to three weeks after floating, a root

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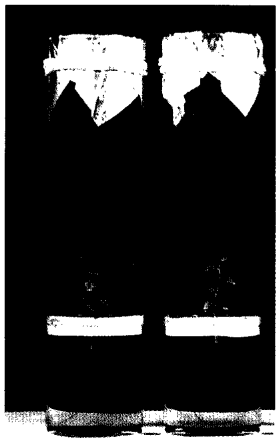

Fig. 1.

Fig. 1. A root development from shoots cultured *in vitro*.

Fig. 2. Changes in the viability of root apex cells of blueberry (A) and apple rootstock (B) exposed to varying pH. Green and yellow cells are viable, and red cells are damaged.

Fig. 3. Changes in the viability of root apex cells of blueberry (A) and apple rootstock (B) exposed to aluminum. Green and yellow cells are viable, and red cells are damaged.

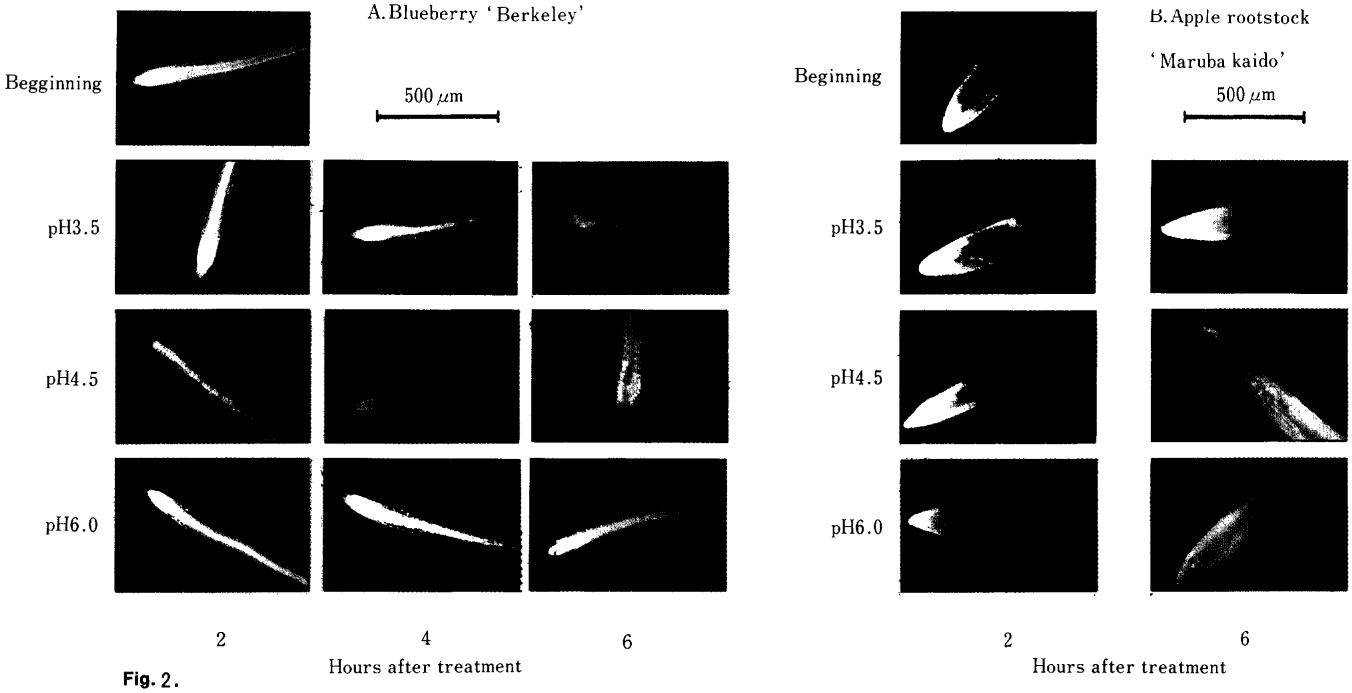

Fig. 2.

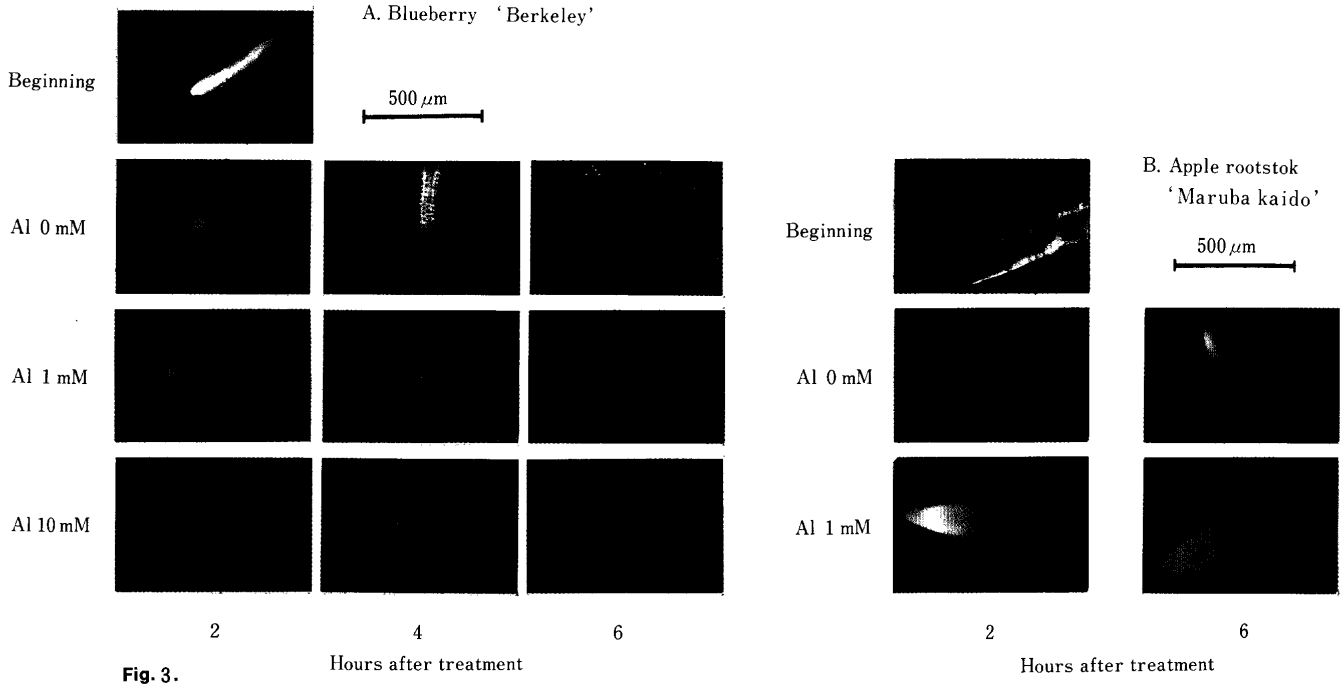

Fig. 3.

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pHおよびAl処理したハイブッシュブルーベリー (*Vaccinium corymbosum* L.) およびマルバカイドウ (*Malus prunifolia* Borkh.) の根端細胞活性の観察

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摘　要

ハイブッシュブルーベリー'バークレイ'およびリンゴ台木'マルバカイドウ'の *in vitro* 培養シュートから発生させた根のpHおよびアルミニウム耐性を，フルオロセイン2酢酸(FDA)—ヨウ化プロピジウム(PI)の2重染色法で観察した．pH3.5から6.0のリン酸緩衝液で2~6時間浸漬処理したpH処理の結果，ブルーベリー根についてはpH6.0の2時間処理以外の処理では約15~40%の根が表皮細胞に障害を受けたが，半数以上の根は活性を維持した．マルバカイドウ根ではpH3.5処理で障害を受けた根が多かった．Al処理において，ブルーベリー根はAl無添加処理(酢酸緩衝液のみ)で根端部または根全体に障害を受けていた．しかし，1 mM Al処理では処理前の根と同程度の活性を示した．一方，10 mM Al処理ではすべての根が全体に障害を受けた．マルバカイドウは1 mM Alに対して活性を示した．FDA-PI法による根細胞の観察は，非破壊的に活性部位と障害部位を識別できることから，根活性の診断に有効な手法であることが認められた．