Effects of excess and deficient boron and niacin on the ultrastructure of root cells in *Daucus carota* cv. Nantes

Hatice DEMİRAY*, Aylin EŞİZ DEREBÖYLU

Section of Biology, Department of Botany, Faculty of Science, Ege University, 35100 Bornova, İzmir, Turkey

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Abstract: The effects of excess and deficient boron and niacin on vascular tissues of carrot roots (*Daucus carota* L. cv. Nantes) were investigated in plants grown in medium both rich and poor in boron and also boron with niacin. Five media were investigated: control (MS medium), boron-deficient MS medium, MS medium with excess boron, niacin-deficient MS medium, MS medium with niacin excess, and MS medium with excess boron and niacin. In anatomical cross sections, lignification was seen in middle lamellar pectins in the tracheary cells of boron deficit grown carrot roots, while in the other applications including excess boron lignification was in the secondary walls. Number of xylem arches and tracheary lengths of root cells were different, but not significantly so. Scanning electron microscopic (SEM) sections of vessels from roots grown in media with excess boron and deficient boron revealed paramural bodies in the tracheary walls. Paramural bodies were found in the tracheary cell walls of both boron deficient and boron excess grown carrot roots. In root cells grown in media with excess and deficient boron, tracheary cells had amyloplasts. While the boron deficient medium grown carrot roots had amyloplasts scarcely, in boron excess grown root cells these amyloplasts filled the vessels densely.

Key words: *Daucus carota*, amyloplasts, excess boron

1. Introduction

Boron (B) is an essential microelement for higher plants and has important physiological functions in plant growth and development (Goldbach et al., 2007). During the past decade, B has been shown to be essential to the structure and function of plant cell walls and membranes, in addition to causing different effects on root elongation, carbohydrate metabolism, and pollen tube growth (O’Neill et al., 2004; Goldbach & Wimmer, 2007; Camacho-Cristóbal et al., 2008). B deficiency is frequently observed because the boric acid in soil is easily leached under high rainfall conditions, thus reducing the yield of many agricultural products, such as wheat (*Triticum aestivum* L.), barley (*Hordeum sativum* L.), and *Citrus* L. species (Jamjod et al., 2004; Yan et al., 2006; Tanaka & Fujiwara, 2007). In addition, considerable genotypic variations in response to B deficiency exist among many agricultural crops, or even cultivars within a species, as reported by Jamjod et al. (2004).

Due to the importance of B as an essential element for higher plants, B deficiency and B toxicity are a worldwide problem in food production because of their reducing crop quality and yields in soils, especially in arid areas (Nable et al., 1977). The primary effect of B deficiency is reduction of cell enlargement in growing tissues, which has been explained mainly by the structural role of B in the cell wall. Borate cross-linked (RG-II) rhamnogalacturonan II, a pectic polysaccharide, was shown to be essential for plant growth and B was found to be located predominantly in cell walls in association with rhamnogalacturonan II (O’Neill et al., 2004). In addition to B cross-linked RG-II pectins (Höfte, 2001), the pectin network of calcium cross-linked de-esterified homogalacturonan pectins (Matoh & Kobayashi, 1998; Jarvis, 1984) is also important for the regulation of the mechanical properties of cell walls (Li et al., 1997). Ricardo et al. (2004) suggested that the formation of di-pentose-borate complexes might have stabilised ribose/ribulose (besides other cyclic pentoses such as arabinose, xylose, and lyxose) in pre-biotic phases in interstellar dust or during the earth’s early history.

As a consequence of its essential role in growing tissues and inherent phloem mobility of B in most plant species, many species are also sensitive to high levels of B in soil and water and growth inhibition has been retarded as a result of excess B uptake in many agricultural regions. B uptake under conditions of adequate or excessive B concentration is the result of passive diffusion of undissociated boric acid (H₃BO₃) (Brown & Shelp, 1997; Hu & Brown, 1997).

* Correspondence: haticedemus@yahoo.com

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were harvested. The experiments were performed 3 times and 10 seeds each. The jars were placed in a Fitotron® regulated to 16 h/8 h photoperiod and 25 ± 2 °C temperature conditioned. Water and sowed to culture jars of 200 mL, with 5 seeds in sterilisation, the seeds were washed with sterile distilled 31 mg/L boron and 2.5 mg/L niacin (5B+5N) added. In our experiments we used Nantes carrot (Daucus carota L.) seeds. The seeds were germinated in Murashige-Skoog (MS) (Murashige & Skoog, 1962) nutrient medium and in vitro conditions. Media were prepared as follows: without boron (0), with 6.2 mg/L boron and 0.5 mg/L niacin (control = MS), with 31 mg/L (5-fold) boron (5B), with 2.5 mg/L niacin (5-fold) (5N), without niacin (0N), and with 31 mg/L boron and 2.5 mg/L niacin (5B+5N) added.

Seeds were sterilised with hypochlorite solution (7.5%) by shaking for 20 min before sowing to the media. After sterilisation, the seeds were washed with sterile distilled water and sowed to culture jars of 200 mL, with 5 seeds in each. The jars were placed in a Fitotron® regulated to 16 h/8 h photoperiod and 25 ± 2 °C temperature conditioned. The experiments were performed 3 times and 10 seeds were used in every application. After 8 weeks the seedlings were harvested.

2.2. Anatomical investigations

Transverse hand sections, approximately 0.2 to 0.5 mm thick, were made with a razor blade from roots. Replicate sections were stained with Mirande’s reagent (a mixture of carmin alum and iodine green) (Deysson, 1954), iodine-green and Wiesner (characterises aldehyde groups of the cinnamic type) and Mäule solutions (to observe syringyl groups) (Monties, 1989), and 0.2% (w/v) iodine in 5% (w/v) KI (10 min) (Johannes et al., 2001) to reveal and confirm the location of lignification and starch containing amyloplasts.

In the anatomical longitudinal sections the lengths of tracheary cells were measured in all application groups. In the cross-sections of root cells the numbers of xylem arches in the central cylinder were counted.

Elongation zones of the roots of the seedlings in different applications were fixed in FAA [ethyl alcohol (50 cc): formaldehyde (10 cc): acetic acid (5 cc): distilled water (35 cc)], embedded, and sectioned (15 μm) with a rotary microtome. Anatomical cross and longitudinal sections were taken from them and photographed using an Olympus 50 Jena microscope and Leica DM 4000 B. In addition, specimens were taken for scanning electron microscopy (SEM) using a JSM-5200; they were covered with gold after being fixed with gluteraldehyde, cacodylate tampons, and osmium tetroxide and dehydrated with different alcohol and xylol series. These samples were processed using 2% glutaraldehyde, in a 0.2 M cacodylate buffer. After 2 h at 4 °C, these samples were washed in 0.1 M cacodylate buffer and dehydrated with sequences of acetone series (25%, 50%, 75%, 90%, and 100%). They were then dried to critical point, and covered with gold. The coated specimens were examined in a scanning electron microscope operating at 20 kV.

2.3. Statistical analysis

Data obtained for the lengths of vessel members and the number of xylem arches were analysed by applying the analysis of variance and the means were compared by the LSD test (Steel & Torrie, 1980).

3. Results

In our application groups the lengths of trachearies increased in MS (85.200 μm) < 5B (87.700 μm) < 5N (90.900 μm) < 0B (96.000 μm) < 5B/5N (110.500 μm), and finally in niacin deficit (0N) (113.000 μm) media, while the numbers of xylem arches in vascular cylinders were simultaneously reduced in 5N (12.83) > MS (9.83) > 0N (9) > 5B/5N (8.83) > 0B (8) > 5B (6.83) media (Tables 1, 2; Figure 1). There were no statistically significant differences between the groups.

Transverse sections from the roots of carrot (Daucus carota) in B-deficient medium stained with Wiesner and Mäule reaction showed bright red coloration in the middle lamellae of the cell walls of the trachearies. The colour was dark red in the secondary walls of tracheary cells of carrot roots grown in the other media except for the B-deficient medium in our experiment (Figure 1).

Carrot tracheary cell walls were stained by iodine green to determine the lignification in the walls (Mandolot et al., 2001). The red coloration of the middle lamellae of
the cell walls increased in the presence of lignified tissues in carrot tracheary cells grown in B-deficient medium, while the coloration was blue in excess B grown cell walls of trachearies because of the absence of lignified tissues (Figure 1). Our control group was the root cells of carrots grown in MS standard growth medium (Figure 1).

Table 1. Number of xylem arches in *Daucus carota*.

<table>
<thead>
<tr>
<th>Application groups</th>
<th>Number of xylem arches</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>9.83</td>
</tr>
<tr>
<td>5B</td>
<td>6.83</td>
</tr>
<tr>
<td>5N</td>
<td>12.83</td>
</tr>
<tr>
<td>5B/5N</td>
<td>8.83</td>
</tr>
<tr>
<td>B deficient</td>
<td>8</td>
</tr>
<tr>
<td>N deficient</td>
<td>9</td>
</tr>
</tbody>
</table>

There are no significant differences between the groups (P < 0.05).

Table 2. Length of vessel members in *Daucus carota*.

<table>
<thead>
<tr>
<th>Application groups</th>
<th>Tracheary lengths (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>85.200 ± 1.564df</td>
</tr>
<tr>
<td>5B</td>
<td>87.700 ± 2.336df</td>
</tr>
<tr>
<td>5N</td>
<td>90.900 ± 2.039df</td>
</tr>
<tr>
<td>5B/5N</td>
<td>110.500 ± 2.912abcde</td>
</tr>
<tr>
<td>B deficient</td>
<td>96.000 ± 2.501df</td>
</tr>
<tr>
<td>N deficient</td>
<td>113.000 ± 1.956abcde</td>
</tr>
</tbody>
</table>

The difference between “a” and control (MS) group, “b” and 5B group, “c” and 5N group, “d” and 5B/5N group, “e” and boron deficient group, “f” and niacin deficient application group is statistically significant (P < 0.05).
In the root cells of carrots grown in the medium with excess B, the rod-shaped globular amyloplasts were layered in clusters in the middle of the tracheary cells (Figure 2), while no globular amyloplasts were seen in the vascular tissues of the root cells grown in the other media, except those with excess and deficient B. The formation of paramural bodies was observed in the cell walls of tracheary cells of root cells grown in deficient and excess B, besides the observation of different

**Figure 2.** Amyloplasts were seen as granules in the centre of the tracheary cells in the cross sections taken from carrot root cells grown in media with excess boron by SEM. a, b, c- Longitudinally fractured central cells with adhering globules, d, e, f, g, h, and i- Longitudinal sections of carrot root cells grown in boron deficient medium with microporate wall layer, f and g- Paramural bodies were seen in boron deficient root cells. j- No amyloplasts were seen in excess boron and excess niacin together.
accumulation types of amyloplast globules in tracheary cells. In the tracheary cells of carrot roots grown in B-deficient medium, amyloplasts were scarcely observed (Figure 2). Anatomical investigations using SEM of the tracheary cells of carrot roots grown in media with both excess and deficient B also revealed the presence of paramural bodies (Figure 2). While B deficiency reduced the amyloplast accumulations (Figure 2), amyloplast accumulations were never observed in carrot root cells grown in excess B with excess niacin together, only in medium with excess niacin and deficient niacin (Figure 2), in contrast with the B-deficient carrot cells (Figure 2).

4. Discussion
Cell walls of vascular tissues demonstrated different responses to B deficiency and toxicity. Middle lamellae tracheary cells were especially lignified because of the B deficiency. B deficiency affects the structure of middle lamellae by attending phenols that were precursors of lignin synthesis (Lewis, 1980; Pilbeam & Kirkby, 1983; Shkolnik, 1984; Downes et al., 1991). In addition, the formation of dimeric B-rhamnogalacturonan-2 (BRG-II) borate-ester crosslinking (Ishii & Matsunaga, 1996; Kobayashi et al., 1996; O’Neill et al., 1996) cannot occur. This crosslink forms a macromolecular complex that controls cellular growth (Fleischer et al., 1999) and the mechanical properties of primary cell walls (Ishii et al., 2001). Consequently, B deficiency leads to an accumulation of soluble carbohydrates, leaky membranes, and DNA, RNA, and lignin synthesis are inhibited, causing an accumulation of low molecular weight phenolics ( Çaşmak & Römheld, 1977).

B promotes sugar transport and stabilises cell walls. Therefore, in B excess grown roots lignification was observed in the primary and secondary walls of tracheary cells, whereas the middle lamellae were not lignified. An investigation on the ultrastructural effects of B deficiency and toxicity in castor bean (Ricinus communis) plants revealed thickening of the middle lamellae in B deficiency and a low level of starch granules in leaves (Herisson da Silva et al., 2008). It is known that vessel length distribution varies in different branching levels and under different environmental conditions (Makbul et al., 2011). More distal branches mostly have shorter vessels, and many trees produce longer vessels in spring than in summer (Zimmermann & Potter, 1982). Auxins and cytokinins play roles in vessel element differentiation and vascular patterning (Ye, 2002). The vessel termination chance might be the result of a hormone flux or gradient. The high-concentration IAA streams induce protoxylem vessels. Cytokinin (CK), synthesised in the root cap, promotes cytokinesis, vascular cambium sensitivity, vascular differentiation, and root apical dominance. Auxin (indole-3-acetic acid (IAA)), produced in young shoot organs, promotes root development and induces vascular differentiation. Both IAA and CK regulate root gravitropism (Aloni et al., 2006). B deficiency symptoms may be the result of supraoptimal endogenous levels of IAA. These high levels of IAA may inhibit cell division and lead to an induction of the IAA oxidase enzyme (Charles et al., 1977). For the reasons mentioned above, B excess induced fewer xylem arches compared with the other application groups while the niacin (nicotinic acid) excess induced the most, although the results were not statistically significant.

Of the water-soluble vitamins, nicotinic acid, precursor of NAD and NADP, participates in cellular redox reactions. A concentration of 32.4 μM nicotinic acid induced 76% embryogenesis (Barwale et al., 1986). It was concluded that reducing the concentration to half or quarter of the Murashige et al. (1974) medium, or removing the vitamins, did not have any significant effect on growth over 3 passages (each 4 weeks), except in the case of 1 cultivar requiring nicotinic acid (Soczek & Hempel, 1988). It has previously been shown that B deficiency inhibits growth of the plant apex, which consequently results in a relatively weak apical dominance, and a subsequent sprouting of lateral buds. Auxin and CKs are the 2 most important phytohormones involved in the regulation of apical dominance. In B-deficient plants, the levels of both auxin and CKs were reduced, and the export of auxin from the shoot apex was considerably decreased relative to plants well supplied with B (Wang et al., 2006).

Nicotinic acid seems to be related to the evolution process by participating in cellular redox reactions. Deficiency of this vitamin increased the flow of auxin to the apex, resulting in the highest length of tracheal cells in carrot roots. Recent studies have suggested that Palaeozoic hyperoxia enabled insect gigantism, and the subsequent hypoxia drove a reduction in animal size. This evolutionary hypothesis depends on the gas exchange in insects being almost exclusively determined by the tracheal system, providing a particularly suitable model to investigate possible limitations of oxygen delivery on size (Kaiser et al., 2007). A number of highly successful insect groups were found to be evolved in conjunction with flowering plants, a powerful illustration of coevolution (Carter, 2005).

Paramural bodies (lomasomes, plasmalemmasomes) originate when exocytotic plasma membrane invaginations contain additional internal membranous structures. These membrane-bound vesicles are usually involved in the deposition of cell wall components, especially calluses, often leading to the synthesis of irregularly thickened and folded cell walls or papillae (Siegfried, 1999). They may become more obvious in cases where the plasma membrane has slightly retracted from the cell wall, thus leaving a space where the normal discharge of
Golgi vesicles is somehow interrupted. In root cells of sunflower (*Helianthus annuus*), paramural bodies have been observed in response to B deficiency, adding new cell material (Hirsch & Torrey, 1980). Similar structures may also occur during the process of frost-hardening (Wisniewski & Ashworth, 1986).

Paramural bodies and amyloplast presence improved the severe effects of both B deficiency and excess in carrot root cells’ vascular system. Higher plant organs sense gravity primarily through the sedimentation of starch-root cells’ vascular system. Higher plant organs sense the severe effects of both B deficiency and excess in carrot (*Wisniewski & Ashworth*, 1986).

A greater amount of auxin accumulates in the bottom flank of the organ, where it inhibits cell elongation in the root and promotes it in the shoot. As a result of this growth differential, the root curves downward and the shoot curves upward (Masson et al., 2002). Therefore, amyloplast flow was more densely observed in the tracheal cells of excess B grown root cells than in B deficient ones. Moreover, paramural bodies and amyloplast movement were absent in the tracheary cells of carrot roots grown in excess B/excess N media and only excess niacin media since niacin eliminated the toxic effects of B with a metabolic disruption by binding to the ribose moieties of molecules such as adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (reduced form) (NADH), or nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) (Reid et al., 2004).

**References**


