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ARTICLE; AGRICULTURE AND ENVIRONMENTAL BIOTECHNOLOGY

Does excess boron affect hormone levels of potato cultivars?

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Potato crop production in Turkey ranks on the thirteenth place in the world. Toxicity is a problematic issue for some parts of the Turkish soils. Hence, it is very important to clarify the physiological responses of plants to toxic mineral stress. In this study, two different potato cultivars – Solanum tuberosum cv. Resy and Solanum tuberosum cv. Agria – were used as a study material. Excess boron was applied in two different concentrations (5 mmol/L and 12.5 mmol/L) 32 days after planting the tubers. Plants were harvested at the end of 15 days of excess boron application. Chlorophyll fluorescence (Fv/Fm) was measured. Shoot height and shoot – root fresh weight contents were determined. Analyses were carried out for the contents of the endogenous hormones indole-3-acetic acid (IAA) and abscisic acid (ABA) by using gas chromatography-mass spectrophotometry (GS-MS). According to the obtained data, plants’ shoot height did not change, whereas the shoot’s fresh weight decreased significantly with increasing of the boron concentrations in cv Resy, by applying 12.5 mmol/L boron. With 12.5 mmol/L boron, the photosynthesis was negatively affected in both cultivars. Boron application led to increased endogenous IAA and ABA content in both cultivars. As a result, cv. Resy showed more resistance to excess boron. Findings on the hormone metabolism and chlorophyll fluorescence in different cultivars will shed a light on understanding the physiological response to excess mineral stress.

Keywords: boron toxicity; Solanum tuberosum L.; chlorophyll fluorescence; IAA; ABA; GS-MS

Introduction

Boron is an essential micronutrient for plant growth and development and has a wide range of roles in the physiological processes in plants.1 Excessive boron in the soil or in the irrigation water occurs in Turkey.2 Abiotic mineral toxicity stress is one of the limiting factors for plant development and crop productivity.3 However, biochemical and physiological adaptation mechanisms allow plants to maintain their life cycle under adverse stress conditions.4 Phytohormones play a role in plants’ stress responses and adaptation.5 Hormonal regulation of growth and development under abiotic stress is a complex process with interactions of various hormones at the cellular level.6 Many of the adaptation mechanisms are related with the change of endogenous phytohormone levels, including indole-3-acetic acid (IAA) and abscisic acid (ABA).7

The essential major auxin IAA plays a role in the growth and development of the plant and takes place in the plant’s life cycle, including cell division, expansion, differentiation, lateral root formation, flowering and tropic responses.8 The possible role of boron on auxin or IAA metabolism was suggested as early as in 1940.9 The local cellular auxin levels and distribution may be regulated by changes in auxin transport in plants under different stresses.10

On the other hand, ABA plays a major role in signaling and adaptation to abiotic stresses, such as water, drought and salt stress.11 Especially in glycophytes, increased endogenous ABA content in plants’ cells and tissues is an adaptive mechanism for sodium (Na) stress.12 De Costa et al.13 proposed that the salt resistance of newly developed maize hybrids was determined by ABA. The ABA is also a hormone that regulates stomatal closure in plants, which causes a reduced water loss via transpiration.14 On the other hand, ABA limits the shoot growth15 and leaf area expansion16 whereas it stimulates the root growth.17 However, the role of ABA and IAA in boron stress remains unclear.

Chlorophyll fluorescence is a technique that measures photosystem II (PSII) activity. It is an indicator of how plants respond to environmental abiotic and biotic factors, by measuring the PSII sensitivity. Information, regarding the plant-based stress status, may be provided by screening the PSII activity. It has been used as one of the most common techniques for measuring stress in leaves.18

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problematic issue for some parts of the Turkish soils. Hence, it is very important to clarify the physiological responses of plants to toxic mineral stress and these processes should be clarified in more detail. In this paper, we examined whether excess boron leads to a possible significant change in IAA and ABA contents of two different potato cultivars. Our aim was to shed a light on the physiological responses of potato cultivars under excess boron stress.

Materials and methods

Plant materials and growth conditions

The two potato cultivars (Solanum tuberosum cv. Resy and Solanum tuberosum cv. Agria) were provided by Aegean Agricultural Research Institute (AARI). Potato tubers were grown in a greenhouse in quartz sand (0.1 mm–0.6 mm, SP-Minerals Oy, Nilsiän kvartsi, Finland). The chemical composition of the quartz sand was SiO₂ (99.4%), Al₂O₃ (0.2%), Fe₂O₃ (0.02%) and K₂O (0.04%). These were subjected to excess boron, are varying. Our results showed the following modifications: 2 ng 13C₆-IAA and 20 ng d₆-ABA were used as internal standards in each sample. For the IAA analysis, the samples were dried, silylated with 8 μL N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and diluted with 16 μL water free pyridine. After this, a gas chromatography-mass spectrometry (GC-MS) analysis was performed on a Trace-DSQ (Thermo) in the single ion monitoring mode on a ZB-35 capillary GC column (35% phenyl and 65% methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm) with splitless injection and 230 °C injector temperature. The column was held at 40 °C for 1 min after injection, then heated by 15 °C min⁻¹ to 250 °C, held for 4 min, heated by 20 °C min⁻¹ to 300 °C final temperature (kept for 3 min) with helium, as a carrier gas (1 mL min⁻¹ flow). The pytohormones’ results were calculated as ng g⁻¹ fresh weight (FW).

Chlorophyll fluorescence and growth parameter determinations

The chlorophyll fluorescence was measured with a FMS-2 pulse modulated fluorometer (Hansatech, UK) in the morning. The minimal fluorescence (Fo) was determined by a weak modulated light and a 0.7 s saturating light of 20,000 μmol m⁻² s⁻¹ was used on the dark-adapted leaves (30 min) to determine the maximal fluorescence (Fm) and variable fluorescence (Fv), calculated as Fm−Fo. The maximal quantum efficiency of PSII (Fv/Fm) was determined. Plants were sampled randomly from each treatment group (control, 5 and 12.5 mmol/L boron applied groups) at the harvest day with at least three replicates. The height of the shoots (cm) was measured and shoot and root fresh weights (g) were recorded.

Statistical analysis

The experimental results were expressed as mean ± standard error (SE). Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Dunnett’s test with SPSS version 12.0. The value of p < 0.05 was considered to be statistically significant.

Results and discussion

It is widely known that hormonal metabolism may change in plants, subjected to biotic and abiotic stresses.[22] In our study, increased boron concentrations led to increased endogenous IAA content in both cv. Resy and cv. Agria, as compared to the control (Table 1). The increased IAA content was statistically significant in both cultivars at 5 mmol/L and 12.5 mmol/L, as compared with their controls. IAA content has also been shown to increase in two different barley cultivars, subjected to excess boron.[22] Lambert et al. [23] have suggested that boron fertilization leads to decreased IAA oxidase activity in plant roots and, therefore, to increased IAA content.[22] The rise of the IAA content leads to movement of carbohydrates into the roots and, by this, to enhancement of mycorrhizal fungal infections. On the other hand, Triticum durum Desf., grown under boron deficiency, had an increased IAA content and it tended to decrease with the increasing of the boron concentrations.[24] In sunflower (Helianthus annuus L.), the IAA content decreased under boron stress, as compared to the control plants.[22,25] The data in the literature about the endogenous IAA contents in plants, subjected to excess boron, are varying. Our results showed
that excess boron application led to increased endogenous IAA contents in both cultivars, which was in line with a research done on two different barley cultivars.[22]

In our study, increased boron concentrations led to a significant ABA content increase in both cv. Resy and cv. Agria (Table 1). Some earlier studies have also shown that increased boron concentrations led to increased ABA contents.[22] Similarly, in a study with carrot (Daucus carota L.) root callus under boron stress, the ABA content also increased.[26] It is presumed that ABA might bind to an apoplastic locus of ABA perception site, inducing an internal calcium-based signal transduction cascade, causing stomatal closure.[27,28] This is a well-known cellular response to abiotic stress conditions.[7] A limited transpiration rate under toxicity might be beneficial for the reduced uptake of boron ions into the tissues.

For unstressed leaves, Fv/Fm values were highly consistent with ~0.83 and correlate to the maximal yield of photosynthesis.[29] Any type of stress that results in inactivation of PSII lowers the Fv/Fm value.[30] In cv. Agria, Fv/Fm decreased drastically after treatment with 12.5 mmol/L boron, indicating that photosynthesis was negatively affected (Table 1). In cv. Resy, treatment with 5 mmol/L boron did not cause a change in the Fv/Fm value, whereas application of 12.5 mmol/L boron led to a decrease in the Fv/Fm values, as compared to the control. The cv. Resy was more tolerant and the Fv/Fm value started to decline after application of 12.5 mmol/L boron. Excess boron did not affect shoots’ height, whereas shoot and root fresh weight decreased with increasing of the boron concentration (Table 2). Shoot fresh weight was the most affected parameter in cv. Resy, which showed a significant decrease at 12.5 mmol/L excess boron application. A decrease was also observed in the cv. Agria fresh weight at 5 and 12.5 mmol/L excess boron application, as compared to the control. Boron toxicity affected roots’ fresh weight negatively in both of the cultivars at 5 mmol/L and 12.5 mmol/L excess boron concentrations. No significant shoot height differences were observed in both cultivars throughout all the excess boron treatments. Plant species, such as Lycopersicon esculentum,[31] Cucumis melo,[32] Malus domestica Borkh. [33] and Artemisia annua,[34] subjected to excessive boron concentrations, have shown that their growth and fresh biomass decreased. The biomass decrease followed the pattern of chlorophyll fluorescence and our results showed that in high boron concentrations, shoot fresh weight was negatively affected. Similar results have been found earlier in tomato plants by Ferro et al. [35] and in sweet basil.[36] A combination of excess boron and salinity, applied to raspberries,[37] led to a decreased chlorophyll content and fluorescence, while in grapefruit [38] only the chlorophyll fluorescence decreased.

Overall, our results that the two different potato cultivars varied in their hormone levels in response to the

Table 1. Effect of different excess boron concentrations on indole-3-acetic acid content, abscisic acid content and chlorophyll fluorescence values.

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<tr>
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<tbody>
<tr>
<td>Control</td>
<td>5.47 ± 0.67</td>
<td>10.94 ± 1.29</td>
<td>4.40 ± 0.32</td>
<td>14.99 ± 1.09</td>
<td>3.58 ± 0.33</td>
<td>9.16 ± 0.74</td>
</tr>
<tr>
<td>5 mmol/L</td>
<td>113.32 ± 10.45</td>
<td>362.17 ± 135.23</td>
<td>261.74 ± 21.86</td>
<td>341.94 ± 22.46</td>
<td>12.33 ± 1.49</td>
<td>16.62 ± 1.35</td>
</tr>
<tr>
<td>12.5 mmol/L</td>
<td>173.88 ± 16.39</td>
<td>373.34 ± 15.23</td>
<td>261.74 ± 21.86</td>
<td>341.94 ± 22.46</td>
<td>12.33 ± 1.49</td>
<td>16.62 ± 1.35</td>
</tr>
</tbody>
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Note: "significant differences at P < 0.05 level, based on the one-way analysis of variance (ANOVA) followed by Dunnett’s test; Values in parentheses indicate percent of control; standard error of mean (±); fresh weight (FW); variable fluorescence (Fv); maximal fluorescence (Fm)."
application of different concentrations of excess boron, contribute to the better understanding of the possible roles of boron on hormone metabolism. Future work on excess boron application will be mainly on determination of the diverse physiological parameters in different plant species.

Conclusions
We assessed the physiological responses of two potato cultivars to excess boron by hormone, chlorophyll fluorescence and growth parameters determination. In our study, we showed that boron application in two different potato cultivars led to increased endogenous IAA and ABA contents. In the light of these findings, we believe that our results may contribute to understanding the physiological responses of plants to excess boron and the roles of excess boron in excess boron physiology.

Disclosure statement
No potential conflict of interest was reported by the authors.

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