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PHYSIOLOGICAL AND NUTRITIONAL RESPONSES OF TWO DISTINCTIVE QUINCE (*CYDONIA OBLONGA* MILL.) ROOTSTOCKS TO BORON TOXICITY

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Abstract

The effects of excess boron (B) on some physiological and nutritional parameters of two distinctive quince (*Cydonia oblonga* Mill.) rootstocks were investigated. Throughout the world, B toxicity is a widely faced problem of soil in arid and semi-arid environments. In a greenhouse study, boron was applied at the rates of 0 and 40 mg kg^{-1} soil to quince A and quince C rootstocks. Toxicity of B differentially affected studied parameters and rootstocks. Boron toxicity increased B concentrations of both rootstocks however the increase was more pronounced in quince A rootstock. SPAD readings, (SPAD-meter, Minolta 502 Co Ltd., Japan) as a measure of chlorophyll decreased under B toxicity. Boron toxicity increased membrane permeability and anthocyanin in both rootstocks. Although, there is rootstocks difference, lipid peroxidation (MDA) and proline and TAA (non-enzymatic total antioxidant activity) increased in response to B toxicity. In general, quince C had lower MDA (Malondialdehyde) and TAA but lower level of proline as compared to quince A. Boron toxicity did not affect the concentrations of P, Ca, Zn and Cu however increased B and Mn concentrations. Magnesium (Mg), Mn and Fe concentrations of quince were found higher than that of quince C. Indicating a genotypic effect, quince A and quince C responded to B toxicity differentially.

Key words: Boron toxicity, Lipid peroxidation, Nutrient, Proline, Quince rootstock.

Introduction

Boron (B) is an essential plant micronutrient and involves in cell wall synthesis and structure, lignification, carbohydrate and phenol metabolism, and membrane integrity (Marschner, 1995; Brown *et al*., 2002, Goldbach & Wimmer, 2007). However the range of boron sufficiency and toxicity in plants is narrow and B can be toxic to plants easily when absorbed in excess. Excess soil boron may interrupt plant growth and development, resulting in decreased yield. Problems related to boron toxicity have been recognized in many areas across the world, including North and South America, Australia, West Asia, North Africa, Mediterranean and East Europe (Brdar-Jokanović *et al*., 2013).

Under excess B conditions, plants may accumulate B in cell walls and cytoplasm. This accumulated B disrupts the plant metabolism and results in the occurrence of B toxicity symptoms (Matoh, 1997) reduced plant growth. Excess B can be found in soil and ground water either naturally or soil B level can be enriched by fertilizer or irrigation water (Nable *et al*., 1997). Due to their poor drainage conditions, saline soils may also contain excess B (Grieve & Poss, 2000). Additionally, geothermal emissions or industrial activities, disposal site of fossil combustion residue and B containing waste materials can also cause excess B in soil and plants (Leyshon & James, 1993; Loppi *et al*., 2006; Pisani *et al*., 2009). Boron toxicity is a severe nutritional disorders and suppress the plant growth in excess B containing arid and semi-arid areas (Marschner, 1995). Despite the distinguishable agronomic importance, B toxicity mechanisms are not known precisely (Karabal *et al*., 2003) and remains an open question (Ruiz *et al*., 2003). As reported by Nable *et al*. (1997) the mechanisms of boron toxicity and salinity tolerance are similar, and generally sensitive plants accumulates more B in their tissue than tolerant plants. Some possible boron tolerance and resistance mechanisms are listed as reduced translocation, exclusion from roots and avoidance by shallow rooting (Nable *et al*., 1997). Structure and integrity of plant membrane has also been suggested as possible B toxicity mechanism by Karabal *et al*. (2003).

According to Romero-Puertas *et al*. (2002) nutrient toxicity causes oxidative stress in plants that results in the generation (Mittler, 2002) and accumulation of reactive oxygen species (ROS) such as superoxide radical (O_2•−), hydroxyl radical (OH•−) and hydrogen peroxide (H_2O_2). Under toxic B conditions, ROS accumulation in apple (Molassiotis *et al*., 2006), citrus (Keles *et al*., 2004) and grapevine (Gunes *et al*., 2006) has been reported, but no information exists on quince plants. Oxidizing power of ROS damages biomolecules, such as lipids and proteins and ultimately leads to cell death (del Rio *et al*., 2003). Being a decomposition product of polyunsaturated fatty acids, malondialdehyde (MDA) has been utilized as a suitable biomarker for lipid peroxidation (Mittler, 2002). Besides antioxidant enzymes, a number of non-enzymatic antioxidant molecules (ascorbate, carotenoids, α-tocopherol, glutathione and total antioxidant activity that expressed as total equivalence of those molecules) have been produced by plants to avoid the detrimental effects of stress molecules (Dat *et al*., 2000). Anthocyanin accumulation can be induced by both biotic and abiotic stress (Li & Strid, 2005). Anthocyanin plays an important role in protection of plants against stress (Kong *et al*., 2003) and intermediates ROS scavenging (Yamasaki, 1997). Anthocyanin accumulation in plants under heavy metal toxicity is a non-specific plant response (Krupa *et al*., 1996). In addition to antioxidant production, plants also accumulate compatible solutes, such as proline which function as osmotic buffers in cytosol.

As a dwarfing pear rootstock in Europe, quince (*Cydonia oblonga* Mill.) widely used (Schuch *et al*., 2010). It is also used in medicinal purpose and food industries as preserves, marmalade and jellies (Ganopoulos *et al*., 2011). Turkey is the largest quince producer worldwide (Anon., 2012). Quince can be propagated easily by traditional and modern (tissue culture) methods (Morini & Sciutti, 1991) and induces early bearing and high yields (Sansavini, 2007).

Despite the fact that boron toxicity response of other plants studied broadly on field crops and vegetables but limitedly on fruit trees, to the best of our knowledge, less information exist concerning the B toxicity response of quince rootstocks. Because the management and amelioration of B toxic soil is difficult, rootstock can play a role in the tolerance of scion to B toxicity (Edelstein *et al*., 2005). In the present study, a greenhouse experiment was conducted to determine the response of two distinctive quince (*Cydonia oblonga* Mill.) rootstock to boron toxicity. The aim of the study was to determine the effects of boron toxicity by means of some physiological (chlorophyll, MDA, TAA, membrane permeability, proline, anthocyanin) and nutritional parameters (P, Ca, Mg, B, Cu, Zn, Mn, Fe) of quince rootstocks.

Material and Methods

The present study was used quince A and quince C rootstocks to test their response to B toxicity. The experiment was conducted under naturally lighted greenhouse conditions at Suleymen Demirel University using two distinctive quince (*Cydonia oblonga* Mill.) rootstocks (Quince A and Quince C) supplied by Department of Horticulture at the same university. In the study, one-year old Quince A and Quince C rootstocks were used. The uniform plants of both rootstocks were chosen and planted in plastic pots containing 14 kg soil on 25th July, 2012. Each pot contained one uniform plant in appearance. The soil used in this study was collected from 0-20 cm depth of a fallow wheat field on Research Farm, Suleyman Demirel University (30º 31' E, 37º 50' N) in April 2012. After transporting to greenhouse, the soil air-dried, mixed thoroughly and sieved. A portion of the soil sieved to <2 mm for physicochemical analysis. Soil physicochemical properties were determined according to Page (1982), procedures were as follows: texture was clay-loam (Bouyoucos, 1951), pH and EC (1/2.5 soil/water), total N (Kjeldahl), plant available P ($NaHCO_3$), $CaCO_3$ (Calcimeter), organic matter (wet digestion), exchangeable cations (ICP-OES after NH_4-Ac extraction), plant available Fe, Zn, Cu and Mn (ICP-OES after DTPA extraction) and B (azomethine-H). The soil used in the experiment was with a pH of 7.83, 0.40 dS m^{-1} salinity level, containing 79 g kg^{-1} $CaCO_3$. Total N and available P contents of soil were 0.49 g kg^{-1} and 7.50 mg kg^{-1}. Exchangeable K, Na, Ca and Mg concentrations of soil were 422, 19, 4366 and 502 mg kg^{-1}, and extractable Fe, Zn, Cu, Mn and B contents were 2.56, 0.23, 1.01, 5.40 and 0.50 mg kg^{-1}, respectively. After transplanting, the plants only watered for one-month till new shoots formed. After new shoot formation, the treatments initiated 30 days after transplanting (25th August, 2012). The treatments consisted of 0 mg B kg^{-1} (-B) and 40 mg B kg^{-1} (+B) soil as boric acid (H_3BO_3). As a basal fertilization, all pots received 100 mg kg^{-1} of N, P and K was applied as 19 + 19 + 19 + ME fertilizer. Basal fertilizer and B were applied with irrigation water. During the experiment, plants were watered at approximately 75% of field capacity by random weighing of pots.

Ninety days after transplanting (on 25th October, 2012), leaf chlorophyll contents were estimated indirectly and non-destructively with portable SPAD-meter (Minolta 502 Co Ltd., Japan) by reading 5-6 random leaves on each pot. For the fresh tissue used in assay, third fully matured leaves for the analysis from each pot were collected. To assess the membrane damage lipid peroxidation (MDA level) and membrane permeability of leaves were measured. For the measurement of MDA, the thiobarbituric acid (TBA) method that determines the MDA as an end product of lipid peroxidation was used (Hodges *et al*., 1999). Membrane permeability (EC %) was measured by using the electrical conductivity method for the leaf samples as described by Yan *et al*. (1996). Proline contents of leaves were estimated by the method of Bates *et al*. (1973). Anthocyanin contents of leaves were measured by using the method of Reay *et al*. (1998). The non-enzymatic total antioxidant activity (TAA) was estimated by the method of Prieto *et al*. (1999).

After completing the fresh matter analysis, all remaining recently expanded leaves were collected, washed once with tap water, twice with deionized water. They were then dried in a forced-air oven at 60 °C until constant weight and were ground (40 mesh sieve) for elemental analysis. Dried leaf samples (0.5 g) were dry-ashed in a muffle furnace at 500 °C for 6 h. The ash dissolved in 0.5 M HCl and B was determined by the azomethine-H method of Wolf (1971), P was determined by vanadomolibdate method, and Ca, Mg, Fe, Cu, Zn and Mn were determined by AAS (Analytik Jena) method (Kalra, 1998).

The 2 × 2 factorial (2 quince cultivars and 2 B treatments) experiment was conducted as complete randomized design with 4 replicates. The significance of treatments was determined with two-way ANOVA by using MINITAB 13. Where significance found, means of treatments were compared by Duncan's Multiple Range test at $p< 0.05$ level.

Results

Physiological responses of quince rootstocks to B toxicity: Boron treatments significantly affected the membrane permeability, chlorophyll and anthocyanin concentration of quince rootstocks (Table 1). Boron toxicity increased the anthocyanin concentration and membrane permeability but decreased the chlorophyll (SPAD readings) content of quince rootstocks. With the effects of B treatments, the increase in anthocyanin concentration and membrane permeability was almost the same and around 18%. The decrease in chlorophyll content was around 12%.

Table 1. Effects of boron toxicity on membrane permeability, chlorophyll (SPAD readings) and anthocyanin concentration of quince rootstocks.

Rootstocks	Membrane permeability (%)		Chlorophyll, SPAD		Anthocyanin, mg 100 g^{-1} FW	
	-B	+B	-B	+B	-B	+B
Quince A	20.19	25.71	55.2	47.6	247.6	277.6
Quince C	23.40	26.07	58.6	53.5	267.2	325.7
Means	**21.79**	**25.89**	**56.9**	**50.5**	**257.4**	**301.6**
F values						
R	3.21^{ns}		4.11^{ns}		4.00^{ns}	
T	16.89^{**}		7.72^{*}		6.82^{*}	
R*T	2.04^{ns}		0.29^{ns}		0.71^{ns}	

*: p<0.05, **: p<0.01, ns: non-significant, R: Rootstocks, T: Treatments, R*T: Interaction, the values are means of four replicates

Table 2. Effects of boron toxicity on MDA (lipid peroxidation), TAA (non-enzymetic total antioxidant activity) and proline concentrations of quince rootstocks.

Parameters	Quince A		Quince C		F values
	-B	+B	-B	+B	
MDA, nmol g^{-1} FW	26.49 b	26.42 b	20.94 b	35.40 a	R: 1.11^{ns}, T: 19.73^{ns}, R*T: 20.13^{*}
TAA, mmol kg^{-1} FW	79.28 c	114.1 b	103.5 bc	178.5 a	R: 46.00^{**}, T: 70.82^{**}, R*T: 9.48^{**}
Proline, mmol kg^{-1} FW	0.194 c	0.672 a	0.201 c	0.525 b	R: 7.03^{*}, T: 110.35^{**}, R*T: 8.12^{*}

*: p<0.05, **: p<0.01, ns: non-significant, R: Rootstocks, T: Treatments, R*T: Interaction, means followed by the same letter do not differ significantly (Duncan multiple range test, p≤0.05). The values are means of four replicates.

Interaction effects of B toxicity and quince rootstocks on MDA (lipid peroxidation), TAA (non-enzymetic total antioxidant activity) and proline concentrations of quince rootstocks were found statistically significant (Table 2). The rootstock quince C had the highest level of MDA concentration although it had similar level of MDA under minus B (-B) condition to that of quince A rootstock meaning that rootstock A showed any changes in its MDA level under toxic B conditions. The non-enzymatic total antioxidant activities of both rootstocks were increased by the interactive effects of B toxicity and rootstocks. However, quince C rootstock had higher TAA under toxic B conditions than that of quince A rootstock. Under toxic B conditions, proline concentrations of both rootstocks increased and this increase was more pronounced in quince C rootstock.

Nutritional responses of quince rootstocks to B toxicity: No significant changes were observed in P, Ca, Cu and Zn concentrations both quince rootstocks (Table 3). The concentrations of P, Ca, Cu and Zn ranged between 1.25-1.56 g kg^{-1}, 16.92-18.73 g kg^{-1}, 6.63-830 mg kg^{-1} and 16.63-19.26 mg kg^{-1}, respectively. Boron toxicity increased the B concentrations of both quince rootstocks. Under B toxicity conditions, the increase in B concentration was significantly higher in the quince C rootstock than the quince A rootstock.

The effects of rootstocks on Mg, Mn and Fe concentrations of quince rootstocks were found significant (Table 4). Quince A rootstock showed significantly higher concentrations of Mg, Mn and Fe than that of quince C rootstock. Manganese (Mn) concentrations of rootstocks were also significantly affected by B treatments. Boron toxicity caused a significant increase in B concentrations of both rootstocks.

Discussion

As the pear rootstock, instead of pear, quince is generally chosen for its good growth performance, fruit production and quality (Sansavini, 2007). In this study, the effects of boron toxicity on some physiological and nutritional response of quince A and quince C rootstocks were investigated. Less information exist concerning the B toxicity response of quince rootstocks.

Although it is not clearly revealed how B is involved in chlorophyll and photosynthesis metabolisms, there are reports on decreased chlorophyll level both under deficient and toxic B conditions (Papadakis *et al*., 2004a, b; Han *et al*., 2008, 2009). In kiwifruit (Sotiropoulos *et al*., 2002) and in apple (Mouhtaridou *et al*., 2004) under B toxicity, due to the decrease of mesophyll cell chloroplast volume and cell damage, the photosynthetic rate decreased this may explain the decrease of SPAD readings in the study under B toxicity. In response to B toxicity, increased anthocyanin concentrations indicates that plant possibly be suffering stress. Brosche & Strid (2003) reported that anthocyanin concentration can be increased by either biotic or abiotic stress in many plants. Landi *et al*. (2014) postulated that anthocyanin serve to mitigate effects of photoinhibitory stress in leaves once the photosynthetic machinery is compromised by B toxicity, and they showed that purple-leafed plants was more tolerant to B toxicity than green-leafed plants. There is evidence that anthocyanin may directly scavenge ROS (Neill & Gould, 2003).

In the present study, proline level increased in response to B toxicity in both quince rootstocks although the increase was more pronounced in quince A rootstock. Proline accumulation is a well-known strategy of plants under B toxicity (Karabal *et al*., 2003; Gunes *et al*., 2006, 2007), and this is linked to the scavenging of ROS (Xiong & Zhu, 2002). Peroxidation of membrane lipids induced by ROS reflects the stress induced damage (Jain *et al*., 2001).

Therefore, MDA level produced by peroxidation of membrane lipids is generally used as an indicator of oxidative damage. The fact that MDA level did not increase in quince A but increased in Quince rootstocks under B toxicity suggests that quince A has an efficient antioxidant system that copes well with the ROS (Gimeno *et al*., 2012). Also, Hossaın *et al.* (2015) stated that MDA content was increased with further application of B of leaf of wınter rapeseed. As the result of the peroxidation of membrane lipids, membrane permeability increased under B toxicity. Excess B mediated membrane damage, membrane permeability increase and lipid peroxidation have also been reported in onion (Inal & Tarakcioglu, 2001), tomato and cucumber (Alpaslan & Gunes, 2001), grapevine (Gunes *et al*., 2006), lettuce and spinach (Eraslan *et al*., 2007, 2008). Little information on B toxicity and non-enzymatic antioxidant activity is available in the literature. According to Mittler (2002) H_2O_2 produced under stress conditions can be detoxified by non-enzymatic antioxidants. Suppressed glutathione synthesis from cysteine in sunflower plants has been reported by Ruiz *et al*. (2003). In this study, boron toxicity increased TAA in both quince rootstocks. This is the indication of non-enzymatic antioxidant system stimulation in both quince rootstocks. Under B toxicity, non-enzymatic antioxidant power increase in apple rootstock has also been reported by Sotiropoulos *et al*. (2006).

Similar to the results of this study, Sotiropoulos *et al*. (2007) reported that P concentrations of two quince genotypes did not show statistically significant changes under B toxicity however they found that B toxicity decreased the Ca and Fe concentrations of quince rootstocks in contrast to the results of the present study. The unaffected nutrient concentrations (P, Ca, Cu and Zn) under B toxicity indicates that there was no competition between B and P, Ca, Cu and Zn absorption by either quince rootstocks (Edelstein *et al*., 2005). Gimeno *et al*. (2012) stated that there were no significant differences for the leaf nutrient concentrations of Verna lemon trees between the B-treated and control plants. Thus, in turn, Ca and K addition to growth media did not show antagonistic effect on B absorption (Brown & Hu, 1994). Phosphorus (P), Ca, Zn and Cu are all essential for plant growth. Phosphorus is needed for ATP and nucleic acids synthesis and protein phosphorylation. Calcium, being a crucial second messenger, plays a major role in plant response to stress. Zinc (Zn) and Cu being the metal components of antioxidative enzymes again play a major role in plants to response oxidative stress. Thus, not affected concentrations of P, Ca, Cu and Zn in two quince rootstocks might be involved in the response of rootstocks to B toxicity (Zhou *et al*., 2014). Sotiropoulos *et al*. (2007) reported decreased Mn but increased Zn concentrations under B toxicity in EMA quince genotype but no changes in BA 29 genotype. In contrast to this study, B toxicity caused increases in Mn but no changes in Zn concentrations in the present study. It is well known that plant growth condition, plant type and even cultivar or genotype play a significant role in nutrient absorption and accumulation (Sotiropoulos *et al*., 2007). Increasing B levels in nutrient solutions caused significant increases of B concentrations in two quince genotypes and genotypes showed different trends of B accumulations (Sotiropoulos *et al*., 2007). Mouhtaridou *et al*. (2004) reported increasing B, P, Ca and Mg whereas decreasing Fe, Mn and Zn concentrations under B toxic conditions in apple rootstock grown *in vitro*. Simón *et al*. (2013) stated that the general trend was an increase in leaf K and Mg concentrations by excess B. Applied B rate, plant transpiration and absorption or exclusion of B by plant roots are the important factors that affect the B concentrations in plants (Marschner, 1995; Edelstein *et al*., 2005) hence quince A rootstocks might be the excluder type when compared to quince C rootstock. Magnesium (Mg), Mn and Fe concentrations of quince A rootstock were generally higher than that of quince C rootstock. These results indicate a genotypic effect on Mg, Mn and Fe uptake by quince rootstocks (Sotiropoulos *et al*., 2002, 2007).

Table 3. Effects of boron toxicity on P, Ca, Cu and Zn concentrations of quince rootstocks.

Parameters	Quince A		Quince C		F values
	-B	+B	-B	+B	
P, g kg^{-1}	1.56	1.51	1.41	1.25	R: 2.50^{ns}, T: 0.66^{ns}, R*T: 0.19^{ns}
Ca, g kg^{-1}	18.73	16.92	18.31	17.17	R: 0.01^{ns}, T: 1.92^{ns}, R*T: 0.10^{ns}
Cu, mg kg^{-1}	6.63	7.93	7.85	8.30	R: 2.67^{ns}, T: 3.19^{ns}, R*T: 0.75^{ns}
Zn, mg kg^{-1}	18.11	18.63	19.26	16.63	R: 0.10^{ns}, T: 0.61^{ns}, R*T: 1.35^{ns}
B, mg kg^{-1}	31.56 c	78.62 b	41.49 c	105.79 a	R: 43.85^{**}, T: 453.74^{**}, R*T: 16.97^{**}

**: p<0.01, ns: non-significant, R: Rootstocks, T: Treatments, R*T: Interaction, means followed by the same letter do not differ significantly (Duncan multiple range test, p≤0.05). The values are means of four replicates

Table 4. Effects of boron toxicity on Mg, Mn, and Fe concentrations of quince rootstocks.

Rootstocks	Mg, g kg^{-1}		Means	Mn, mg kg^{-1}		Means	Fe, mg kg^{-1}		Means
	-B	+B		-B	+B		-B	+B	
Quince A	4.44	3.97	**4.20**	60.88	71.95	**66.42**	111.33	116.23	**113.78**
Quince C	3.04	3.42	**3.23**	58.48	64.83	**61.66**	97.28	98.67	**97.98**
Means				**59.67**	**68.39**				
F values									
R	11.77^{**}			9.39^{*}			17.29^{**}		
T	0.03^{ns}			31.43^{**}			0.69^{ns}		
R*T	2.21^{ns}			2.31^{ns}			0.21^{ns}		

*: p<0.05, **: p<0.01, ns: non-significant, R: Rootstocks, T: Treatments, R*T: Interaction, the values are means of four replicates

In conclusion, the results of the present study showed that the two quince rootstocks exposed to B toxicity revealed a rootstocks dependent response, indicating a genotyping effect. The lack of knowledge on quince tolerance to B toxicity suggest that the valuable findings on quince A and quince C rootstocks represent a valuable contribution to pear industry, especially for the pear cultivating regions where B toxicity is prevalent. Quince A rootstock accumulated less B, MDA, proline and TAA under B toxicity. These indicate that quince A is more tolerant to B toxicity than quince C rootstock. Thus, it can be concluded that on B toxic soil quince A rootstock can be more tolerant than quince C rootstock.

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