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Boron toxicity in higher plants: an update
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Abstract:	Boron (B) is a unique micronutrient for plants given that the range of B concentration from its essentiality to toxicity is extremely narrow, and also because it occurs as an uncharged molecule (boric acid) which can pass lipid bilayers without any degree of controls, as occurs for other ionic nutrients. Boron frequently exceeds the plant's requirement in arid and semiarid environments due to a poor drainage, and in agricultural soils close to coastal areas due to intrusion of B-rich seawater in fresh aquifer or because of dispersion of seawater aerosol. Global releases of elemental B through weathering, volcanic and geothermal processes are also relevant in enriching B concentration in some areas. Considerable progress has been made in understanding how plants react to B toxicity and relevant efforts have been made to investigate: (I) B uptake and in planta partitioning, (II) physiological, biochemical, and molecular changes induced by B excess, with particular emphasis to the effects on the photosynthetic process, the B-triggered oxidative stress and responses of the antioxidant apparatus to B toxicity, and finally (III) mechanisms of B tolerance. Recent findings addressing the effects of B toxicity are reviewed here, intending to clarify the effect of B excess and to propose new perspectives aimed at driving future researches on the topic.
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Athens, March 27th, 2019

To the Editors-in-Chief of *Planta*

Prof. Dr. Dorothea Bartels and Anastasios Melis

Dear Editors,

Please find enclosed a manuscript submitted for possible publication in *Planta*. The manuscript is titled: "**Boron toxicity in higher plants: an update**".

The present manuscript reviews the most recent literature about morpho-anatomical, physiological, biochemical and molecular responses adopted by plants to cope with B excess in order to provide a comprehensive picture of changes in plant allometric trajectory and plant metabolism in response to B excess and to propose new perspectives aimed at driving future researches on the topic.

The present manuscript consists of unpublished work which is not under consideration for publication elsewhere. The authors have carefully read and are fully aware of the *Planta's* policies.

Best regards,

Ioannis E. Papadakis

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Boron toxicity in higher plants: an update

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Main conclusion **In this review, emphasis is given to the most recent updates about morpho-anatomical, physiological, biochemical and molecular responses adopted by plants to cope with B excess.**

Abstract

Boron (B) is a unique micronutrient for plants given that the range of B concentration from its essentiality to toxicity is extremely narrow, and also because it occurs as an uncharged molecule (boric acid) which can pass lipid bilayers without any degree of controls, as occurs for other ionic nutrients. Boron frequently exceeds the plant's requirement in arid and semiarid environments due to a poor drainage, and in agricultural soils close to coastal areas due to intrusion of B-rich seawater in fresh aquifer or because of dispersion of seawater aerosol. Global releases of elemental B

through weathering, volcanic and geothermal processes are also relevant in enriching B concentration in some areas. Considerable progress has been made in understanding how plants react to B toxicity and relevant efforts have been made to investigate: (I) B uptake and *in planta* partitioning, (II) physiological, biochemical, and molecular changes induced by B excess, with particular emphasis to the effects on the photosynthetic process, the B-triggered oxidative stress and responses of the antioxidant apparatus to B toxicity, and finally (III) mechanisms of B tolerance. Recent findings addressing the effects of B toxicity are reviewed here, intending to clarify the effect of B excess and to propose new perspectives aimed at driving future researches on the topic.

Keywords boric acid ☐ boron partitioning ☐ boron-polyol complexes ☐ boron tolerance ☐ boron transporter ☐ oxidative stress

Introduction

Boron (B) toxicity limits crop yield and quality in several agricultural areas worldwide, and frequently occurs naturally in alkaline and saline soils together with a low rainfall and very scarce leaching (Camacho- Cristóbal et al. 2008; Landi et al. 2012), in agricultural lands close to coastal area (Kabay et al. 2010) or in areas with persistent geothermal activities (Princi et al. 2016a). In addition, B-rich soils occur as a consequence of over fertilization and/or irrigation with B-enriched water (Reid 2010). Therefore, differently to other pollutants, anthropogenic activities only have a minor role in increasing B release in the environment.

Boron is essential for the stability of the cell wall due to the ability of B to bridge pectic polysaccharide rhamnogalacturonans, even though there is increasing evidence for a possible role of B in several alternative metabolic processes. B toxicity causes a reduction of plant growth, fruit yield and fruit quality, although the degree of tolerance can significantly differ at inter- (Keren and Bingham 1985; Papadakis et al. 2003; Landi et al. 2013b) and intraspecific level (Sotiropoulos et al. 2006; Cervilla et al. 2007; Ardic et al. 2009; Landi et al. 2013a; Landi et al. 2014; Pardossi et al. 2015; Wu et al. 2018). The effects of B toxicity are also dependent on the ability of a plant species to re-translocate B within the phloem. In species where B is relatively immobile the symptoms of toxicity are firstly detected in older tissues, whereas in other species,

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67 where B is re-mobilized in the phloem sap, visible symptoms appear principally in
68 actively growing tissues (Brown and Hu 1996).

69   Here, a 20-year research on the effects of B toxicity is been reviewed at the
70 physiological, biochemical and molecular level. Moreover, discussion on previous
71 milestones on the topic is made, with the attempt to derive general conclusions on the
72 effects of B toxicity in plants, and suggestions are presented for future research on
73 understanding basic B tolerance mechanisms and on selecting B-tolerant genotypes.

74

75 Chemical properties of boron and its distribution on Earth

76 Boron is the most electronegative element of Group III of the periodic table and this
77 semimetal is characterized by an electron deficiency in p-orbital ($1s^2$ $2s^2$ sp^1) which
78 determines the typical B behavior in chemical and biochemical processes (Kot 2009).
79 In its compounds B shows an oxidation state of +3. However, the first three ionization
80 energies of B are much too high to allow formation of compounds containing the B^{3+}
81 ion; thus, in all its compounds B is covalently bonded. For example, B-containing
82 compounds often behave as Lewis acids, readily bonding with electron-rich substances.
83 There are 13 known isotopes of boron; the shortest-lived isotope is ^{7}B, whereas in the
84 biosphere B has two naturally occurring and stable isotopes: 20% ^{10}B and 80% ^{11}B
85 (WHO 1998). There are over 200 naturally occurring B-containing minerals, but the
86 most commercially important and frequently traded minerals are: tincal
87 ($Na_2B_4O_7 \cdot 10H_2O$), colemanite [$CaB_3O_4(OH)_3 \cdot H_2O$], kernite ($Na_2B_4O_7 \cdot 4H_2O$), ulexite
88 ($NaCaB_5O_9 \cdot H_2O$), boric acid [$B(OH)_3$], and borate-derivated compounds. Boric acid is
89 a weak acid (pKa 9.2) and is the main B compound present at soil neutral pH (O'Neil
90 et al. 2004; Tanaka and Fujiwara 2008) and, in that condition, it exists as odorless,
91 colorless, translucent crystals or white granules or powder at ambient temperatures,
92 namely sassolite.

93   Ocean evaporation is the predominant source of B release in the biosphere (65-
94 85%), whereas natural chemical and mechanical weathering of sedimentary rocks
95 provides B compounds in soil and water (Princi et al. 2016a). However, the most
96 impactful source of highly concentrated B, with an average of 5–6 mg B l^{-1} (Kabay et
97 al. 2010), is certainly the seawater, whose intrusion occurs naturally in most coastal
98 aquifers, owing to the hydraulic connection between groundwater and seawater, thereby
99 increasing B concentration in irrigation water. Differently to other pollutants,
100 environmental B release that is directly or indirectly to attributable to human activities

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101 plays a minor role compared to the amplitude of the environmental B-enrichment

102 deriving from natural sources. List of anthropogenic sources based on their increased

103 contribution to B release worldwide are: agriculture, wood burning, power generation

104 from coal and oil, glass manufacture, use of borates/perborates, borate mining and

105 processing, leaching of treated wood/paper, and sewage disposal of B (HSDB 2003).

106   Boron availability in irrigation water, as well as in soil, represents an essential

107 factor for crop production, although concentration and availability of edaphic B

108 worldwide is extremely variable (Gupta et al. 1985). Deficiency of B in the soil is

109 mainly dependent on the prevalent chemical form of B, $B(OH)_3$ (boric acid), which is

110 easily leached out by rainwater due to its high solubility (Bolaños et al. 2004). In many

111 countries (including Japan, China, USA and Brazil) B concentration in soil is

112 insufficient for agricultural production and B is added as fertilizer (Gupta et al. 1985).

113 Conversely, B excess preferentially occurs in arid or semiarid countries, such as South

114 Australia, Iraq, Egypt, Jordan, Libya, Morocco, Syria, Turkey, Chile, California (You

115 et al. 1995) and Italy (Pennisi et al. 2006), where the main reason of B accumulation in

116 topsoil is water evapotranspiration or, in coastal areas, the use of B-enriched water for

117 irrigation. Referring to Keren and Bingham (1985), the maximum permissible

118 concentrations of B in irrigation water range from 0.3-1.0 mg L^{-1} for sensitive plants

119 (i.e. avocado < apricot < peach < cherry < fig < apple < pear < bean < walnut), 1-2 mg

120 L^{-1} for semi-tolerant plants (sweet potato < pumpkin < oat < maize < wheat < barley <

121 olive < tomato < cotton < potato), and 2-4 mg L^{-1} for tolerant plants (carrot < lettuce <

122 onion < alfalfa < sugar beet < date palm < asparagus).

123

124 The role of boron in plants

125 The essential role of B in plants was established since the 20's in *Vicia faba* (Warington

126 1923), and more recently it has been hypothesized that it plays a key role in several

127 metabolic processes (Goldbach et al. 2001; O'Neill et al. 2004). Under physiological

128 conditions of plant cells, B exists as $B(OH)_3$ or tetrahydroxyl borate anion $B(OH)_4^-$

129 (Woods 1996; Fig. 1). However, the 98% of the total B exists in free form as $B(OH)_3$

130 or even higher (about 99.95%) at lower pH values (such instance 5.5 in apoplast) (for a

131 review refer to Woods 1996). Boric acid is a weak acid at cytoplasmic pH (about 7.0-

132 7.5) and, under physiological conditions, $B(OH)_3$ can freely pass lipid bilayers, in

133 contrast with other ionic nutrients. Under physiological pH, $B(OH)_3$ binds to molecules

134 with mono, di- and poly-hydroxyl groups, such as ribose, apiose, sorbitol and other

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polyalcohols (Ralston and Hunt 2001). Indeed, the first B-containing compound identified in the plant kingdom, which is stable under physiological conditions, is the pectic polysaccharide rhamnogalacturonan II (RGII), a molecule in which B cross-links two RGII monomers by a borate bridge providing stability to the cell-wall matrix (O'Neill et al. 1996; Fig. 2). Recently, Voxeur and Fry (2014) highlighted the role of B in plant cell membranes through the formation of complexes with the major components of lipid rafts, i.e. glycosyl inositol phosphoryl ceramides. Therefore, B contributes to the formation of GIPCs-B-RGII complexes, bridging the cell plasma membrane with cell wall (Wang et al. 2015). Boron can also form complexes with polyalcohols (e.g. sorbitol; Fig. 3) even when those moieties are incorporated into other compounds [i.e. NAD(P)H, ATP, nucleotides; Ralston and Hunt 2001]. Ralston and Hunt (2001) further pointed out a gradient of stability of B complexes, in order: apiose $>$ NAD$^+$ $>$ NADH $\cong$ 5'ATP $>$ 5'ADP $>$ 5'AMP $>$ adenosine $>$ 3'AMP $\cong$ 2'AMP $\cong$ cAMP $\cong$ adenine. B-NADH, B-NADPH, and B-NADP$^+$ affinity was also demonstrated *in vivo* (Reid et al. 2004). Furthermore, increasing pH typically stabilizes more *cis*-diols than *trans*-diols (Boeseken 1949). Phenolics and amino acids are other compounds that form complexes with B (Brown et al. 2002).

Many other roles have been proposed for B in plants, such as its influence in reproductive growth and development, stimulation of reproductive tissues, improvement of seed quality and its influence on the biosynthesis of some metabolic compounds, i.e. antioxidants and polyphenols (Brown et al. 2002; Goldbach and Wimmer 2007; Camacho-Cristobal et al. 2008; Chatzissavvidis and Therios 2011; Princi et al. 2016a; Camacho-Cristobal et al. 2018). Additionally, involvement in nucleic acid synthesis, phenolic metabolism, carbohydrate biosynthesis and translocation, indole-3-acetic acid oxidase, as well as pollen-tube growth and root elongation, have been recently reported, stressing the B critical role in plant development (González-Fontes et al. 2008; Shireen et al. 2018).

Besides the various examples illustrating the essential role of B in plants, a recent paper by Lewis (2019) argued against the essentiality of B because, from the author's point of view, an alternative interpretation of published evidence negates its compliance with one of the criteria for essentiality: the direct metabolic effect. Lewis (2019) proposed that B is, and always has been, potentially toxic for plants, and this feature needs to be nullified for normal growth, development and reproduction. The hypothesis was that B as well as phenolics (compounds considered toxic for cellular metabolism)

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169 are strictly related and plants have evolved the ability to mitigate adverse effects of both

170 B and phenolics by chemical (as organic complexes: *cis*-diols for B and lignin for

171 phenolics) and physical (into vacuoles/apoplast) sequestration. Therefore, formation of

172 B complexes in the cell wall is a detoxifying mechanism and not an evidence of B

173 essentiality. This recollects a milestone published by the same author 40 years ago

174 (Lewis 1980), in which it was proposed a unified explanation for the development of

175 vascular from prevascular plants, which encompasses B, phenolics and lignin

176 formation. Although this review focuses on B toxicity, we believe this hypothesis

177 should seriously be considered when designing strategies to test B essentiality.

178

Boron uptake and translocation

180 At common soil pH (5.5-7.5), B is found principally as undissociated $B(OH)_3$ and it is

181 widely accepted that plants take up B in that form from roots (Camacho-Cristobal et al.

182 2008). Until the late 90s, the high permeability of lipid bilayers to $B(OH)_3$ (Raven 1980)

183 was thought to be the exclusive mechanism for B passive diffusion across the plasma

184 membrane. The description of protein-based transportation channels (Dordas and

185 Brown 2001a; Dordas et al. 2000) and the identification of a B transporter in

186 *Arabidopsis thaliana* (Noguchi et al. 1997; Takano et al. 2002) changed the entire

187 scenery of B uptake mechanisms. B uptake is therefore considered to involve different

188 mechanisms depending on its availability: (I) passive diffusion across lipid bilayer, (II)

189 facilitated transport by major intrinsic protein (MIP) channel, and (III) high-affinity B

190 transporters (e.g. BOR); the latter in responses to low B supply (Tanaka and Fujiwara,

191 2008; Chatzissavvidis and Therios 2011). However, active B uptake was observed to

192 occur under sub-optimal B availability or even in condition of B starvation (Stangoulis

193 et al. 2001), whereas under conditions of adequate or excessive B supply the hypothesis

194 that B is principally passively absorbed by roots is the most widely accepted (Brown et

195 al. 2002; Tanaka and Fujiwara 2008). That process is attributed largely to the high

196 permeability of $B(OH)_3$ to lipid bilayers (Brown and Shelp 1997; Dordas et al. 2000).

197 Once absorbed by the roots, B is primarily translocated to mature leaves through

198 non-living xylem cells driven by transpiration, since mature leaves represent the sites

199 with the highest transpiration rate (Chatzissavvidis and Therios 2011). However, in

200 some plant species, including many important crop genera (e.g., *Pyrus*, *Malus*, *Prunus*,

201 *Allium* and *Brassica*), B has been found to be uniformly distributed within the whole

202 plant or even at a higher concentration in young tissues than in mature leaves (Brown

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203 and Hu 1996; Camacho-Cristobal et al. 2008). These results demonstrated, at least in
204 some plant species, the B ability to move along the phloem flux. Phloem translocation
205 does not follow the transpiration stream and it supplies the major proportion of nutrient
206 requirements to actively growing areas such as young leaves and fruit, organs that do
207 not readily transpire (Brown and Shelp 1997). *Pyrus*, *Malus*, *Prunus*, *Allium* and
208 *Brassica* commonly produce high amounts of sugar alcohols (i.e. mannitol and sorbitol)
209 which are used for the phloem translocation of photosynthates in place of sucrose
210 (Brown et al. 1999). Sorbitol and mannitol with their *cis*-hydroxyl groups can readily
211 bind to boric acid originating diol-B complexes (Reid et al. 2004) (Fig. 3). That bond
212 is likely to allow B to be transported through phloem where it is present as a stable
213 polyol-B complex with mannitol and sorbitol as ligands (Hu and Brown 1997).
214 Therefore, active B transport by specific carriers is not involved in condition of
215 adequate or luxury B supply, whereas some efflux transporters can be part of an
216 efficient tolerance mechanism adopted by plants under conditions of B excess (see
217 section: Roles of boron transporters and efflux pumps in B tolerance).

218

219 Physiological and biochemical responses to B excess
220 Excess B in soil results in growth impairment and alteration of plant metabolism,
221 causing progressive necrosis in leaves, stems and malformations of fruits (Reid et al.
222 2004). Beside damages to aerial parts of the plant (leaves, stems, buds and/or fruits), B
223 toxicity has been reported to affect root growth in major crops, such as wheat, barley,
224 maize (Choi et al. 2007; Aquea et al. 2012; Esim et al. 2013). The macroscopic side
225 effects of B excess are principally attributable to three main metabolic effects exerted
226 by B: (I) impairment of cell division and development by binding to ribose, both as the
227 free sugar and as a constituent of RNA, (II) interference with primary metabolism by
228 binding to ribose in ATP or NAD(P)H, and (III) reduction of the cytosolic pH, thereby
229 affecting protein conformation and biosynthesis (Reid et al. 2004). As a result, several
230 key physiological processes are strongly perturbed. In Table 1, we summarize the main
231 morpho-anatomical, physiological and biochemical responses to B excess observed in
232 different plants species.

233 The main morpho-anatomical changes promoted by B excess encompasses
234 modifications at root level, such as alteration of root meristems (Choi et al. 2007),
235 thickening of hypodermis and deposition of suberin in cortical cells (Ghanati et al.
236 2002), root lignification , which likely represent an attempt by the plant to reduce the

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237 intake of B and limit the toxicity to root apparatus. At stem level, B excess can promote
238 the development of cork and collenchyma cells with increased cell wall thickness in
239 loquat (Papadakis et al. 2018) and thickening in the cell wall of phloem cells in citrus
240 (Huang et al. 2014), mechanisms which reduce the amount of free B and represent a
241 further detoxifying mechanism by storing excessive B in tissues where it could be less
242 harmful. Addressing the effect in leaves, few reports have investigated the morpho-
243 anatomical changes that are provoked by B excess. In particular, there is no consensus
244 if B excess promotes the reduction (e.g. as observed in citrus leaves; Papadakis et al.
245 2004a, b) or the increment of leaf thickness (e.g. in loquat leaves; Papadakis et al. 2018)
246 and perhaps it could strongly depend on the species under investigation as well as on
247 the B doses applied by the experimental protocol. Conversely from morpho-anatomical
248 changes, the deleterious effect of B excess to several biochemical/physiological
249 processes which involved the leaves, first among equal, the photosynthetic processes,
250 are more consistently observed.

251   Photosynthesis is one of main metabolic processes impaired by B excess
252 (Sotiropoulos et al. 2002; Papadakis et al. 2004a, b; Han et al. 2009; Ardic et al. 2009;
253 Wang et al. 2011; Landi et al. 2013a, b; Papadakis et al. 2018) due to both stomata and
254 non-stomata limitations (Shah et al. 2017). Biochemical limitations principally
255 encompass: the decline of electron transport rate, reduced CO_2 use efficiency and
256 impairment of photosystem II (PSII) efficiency (Papadakis et al. 2004a, b; Landi et al.
257 2013a, b, c, 2014; Kaya et al. 2018) (for more details see Table 1). Papadakis et al.
258 (2014) demonstrated that B excess prevalently affects transpiration rate and detected
259 severe stomata limitations induced by B excess. These findings were in agreement with
260 other authors who observed, in association with the reduction of transpiration rate, a
261 severe increment of the abscisic acid metabolism (Macho-Rivero et al. 2017, 2018), the
262 major player in stomata closure, for example in case of reduced water availability.
263 However, some authors proposed that there is no evidence to support the hypothesis of
264 the generation of B toxicity effects in leaves due to osmotic stress induced by
265 accumulation of borate or boric acid (Reid et al. 2004). In some cases, B excess also
266 results in alterations of photosynthetic pigment content and its relative proportion, e.g.
267 chlorophyll *a*, *b* content and *a/b* ratio (Huang et al. 2014; Kayıhan et al. 2017) and
268 carotenoid (Sarafi et al. 2018). All these effects are not related to a particular target of
269 B toxicity at cellular level, but are rather the observed responses of the ability of B to
270 form complexes to molecules which are involved in different cellular processes. For

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271 example, the capacity to bind ATP and NADPH (Cervilla et al. 2009a) and limit the
272 availability of energy and reduce power, respectively, which are necessary for
273 carbohydrate synthesis in the light-independent reactions of the Calvin-Benson cycle.
274 Therefore, changes in sugar content and partitioning (Roessner et al. 2006; Papadakis
275 et al. 2018), as well as in carbon skeleton devoted to aminoacids, which are strictly
276 interlinked (Guo et al. 2014) can be observed as a response to B excess (Sang et al.
277 2015; Ayvaz et al. 2016). An imbalance of C/N ratio also promote phenomena of early
278 senescence (Lo Piccolo et al. 2018; Sotiras et al. 2019) and this explains the
279 enhancement of senescence-related hormones i.e. jasmonic acid and ethylene promoted
280 by B excess in barley and wheat (Öz et al. 2009; Kayıhan et al. 2017).

281   When the ability to process incident light by photosynthesis is compromised in
282 plants subjected to B excess, those plants are more prone to generate a surplus of
283 reactive oxygen species (ROS), with a consequently higher probability to incur in
284 oxidative stress (e.g. Molassiotis et al. 2006; Cervilla et al. 2007; Ardic et al. 2009;
285 Çatav et al. 2018; Simón-Grao et al. 2019b) and generation of cellular death and
286 necrotic areas over the leaf lamina (Landi et al. 2013b; Papadakis et al. 2018). Reid et
287 al. (2004) proposed that photo-oxidative stress is one of the main causes of the
288 inhibition of plant growth under B toxicity, rather than the effect of B on energy supply
289 or proteins biosynthesis. In agreement, Landi et al. (2013a, c, 2014) compared purple-
290 (anthocyanin-rich) and green-leafed (anthocyanin less) genotypes of sweet basil and
291 demonstrated that foliar anthocyanins photo-protect purple-leafed genotypes by
292 reducing supernumerary photons reaching the chloroplast and, in turn, ameliorating the
293 effect of B excess. Green genotypes of sweet basil where indeed more prone to photo-
294 inhibition and B-triggered oxidative stress. In view of the above, the enhancement of
295 the antioxidant apparatus is another typical response of plants when experiencing
296 conditions of B excess (Karabal et al. 2003; Gunes et al. 2006) (for more details, see
297 Table 1) and a "powerful" antioxidant apparatus could be the base of the higher
298 tolerance showed by some genotypes/species when compared to more sensitive ones
299 (see section: "Mechanisms involved in B tolerance).

300   The alterations in biochemical/physiological processes detailed above only
301 represent a part of the plant reactions that have been described against conditions of
302 excessive B supply (for more details see Table 1). Such a plethora of responses to B
303 excess are attributed to the unspecific effect of B toxicity at cellular level. In principle,
304 changes observed in several plant parameters are the results of a cascade of events

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305 promoted by the three main direct causes of B toxicity described at the beginning of the
306 section, which are connected to the ability of B to bind polyols and lower the cytosolic
307 pH.

308

Mechanisms involved in B tolerance

310 It is generally accepted that B tolerance encompasses the ability of plants to: (I) reduce
311 the uptake of B by the root apparatus (Nable et al. 1990; Papadakis 2016) and efflux B
312 from the roots when B concentration becomes excessive (Reid 2007; Sutton et al. 2007),
313 (II) stimulate the biosynthesis of B-chelating organic compounds such as polyalcohols
314 (Papadakis et al. 2018) and phenolics (Landi et al. 2015; Lewis 2019), (III) enhance the
315 antioxidant apparatus thus allowing the plant to counteract B-triggered oxidative stress
316 (Landi et al. 2012 and reference therein), and (IV) compartmentalize B into organelles
317 (e.g. vacuole; Wakuta et al. 2016) and sites (e.g. cell wall; Papadakis et al. 2018) where
318 it is less harmful for cellular processes. Below, we report in detail the main mechanisms
319 adopted by plant species to counteract the effects of B toxicity, most of which arise
320 from the comparison between species which have a different degree of sensitivity to B
321 excess.

322

Polyols, phenolics and B toxicity: which ameliorative role?

324 The significance of B for nutrition of higher plants has been under investigation from
325 the beginning of the previous century (Agulhon 1910), whereas B phloem mobility has
326 started to be investigated in the last decades of XX century (Hanson 1991; Picchioni et
327 al. 1995; Hu et al. 1997). Indeed, even though symptoms of B toxicity were previously
328 observed in tree fruit species such as apple and prunus (Hansen 1948; Woodbridge
329 1955), the agronomists at that time did not recognize that these symptoms were due to
330 B toxicity, but they were conversely mistaken for other diseases and physiological
331 disorders. Relative B phloem mobility influences plant responsiveness to foliar B
332 fertilization and sensitivity to fluctuations in ambient soil B concentration (Brown and
333 Shelp 1997). Knowledge of phloem B mobility is therefore essential not only for
334 efficient management of B nutrition but also for estimating where the symptoms of
335 possible B toxicity will appear in different plant species.

336   Efforts have been made in characterizing the relationship between B mobility and
337 the quality of sugar alcohols in the phloem, suggesting that complex formation of B
338 with specific types of sugar alcohols (polyalcohols) affects B uptake and re-

translocation. Work on three tobacco (*Nicotiana tabacum*) lines which were modified to differ in sorbitol production showed that the line that had increased sorbitol production was accompanied with increased B uptake and that B could be translocated from mature leaves to meristematic tissues (Bellaloui et al. 1999). Similar evidence about B- sorbitol complex formation in sorbitol-rich species and B translocation were detected in broccoli, celery (Tao et al. 1995; Hu et al. 1997), and in many fruit trees, such as apple, pear, peach, plum and cherry (Van Goor and Van Lune 1980; Hanson 1991; Picchioni et al. 1995; Hu et al. 1997). Moreover, matching results were presented in olive tree about mannitol-B complex formation (Perica et al. 2001).

Foliar B application to leaves in different developmental stages led to significant B export out of the treated leaves and enrichment in non-treated adjacent organs, including inflorescences and fruit. On the contrary, plant species which do not use polyalcohols as translocating compounds, such as citrus, fig, pistachio and walnut, had higher B concentrations in older than young leaves and floral organs (Brown and Hu 1996), demonstrating the positive correlation between polyalcohol levels and B uptake and translocation. The analytical determination of B concentrations in different parts of grafted orange (*C. sinensis*) plants, which exclusively use sucrose for the translocation of photosynthates, revealed the following order: old leaves>young leaves>bark of rootstock>root>stems of scion>stem of rootstock>wood of rootstock (Papadakis et al. 2004a). More recently, observations in soybean plants treated with exogenous applications of sorbitol or mannitol to test B uptake showed that, even though B uptake was increased, B distribution within the plant was not affected (Will et al. 2011). These results suggest that only the natural occurring B-sorbitol complexes can assist B re-translocation from older leaves to developing plant organs. In a recent study carried out with loquat (*Eriobotrya japonica*) seedlings, which are characterized by the presence of high sorbitol levels, B concentrations increased in the row: basal wood < basal leaves < root, basal bark, top wood < top bark < top leaves (Papadakis et al. 2018).

Under the condition of B excess, distinctive symptoms of B toxicity occur in mature-older leaves (chlorosis followed by necrosis in lamina tip and margins) of most species where sorbitol and/or mannitol are negligible (Fig. 4). Conversely, in plants species which produce sugar alcohol, in which B translocation occurs, the symptoms of B toxicity firstly appear in the meristem regions and fruits, while do not occur in mature leaves (Brown and Hu 1996). Therefore, in those species main symptoms of toxicity are fruit disorders (gummy nuts, internal necrosis), bark necrosis caused by

373 death of the cambial tissues, and stem die back (Brown and Hu 1996). Curiously, visible
374 symptoms of B toxicity do not appear in roots and B concentrations in these tissues
375 remain relatively low compared to those found in leaves, even when plants are
376 subjected to high levels of B supply (Nable 1988) (Fig. 5).

377 Recently, Papadakis et al. (2018) presented evidence related to the role of sugars
378 and polyols in loquat leaves when plants were subjected to B excess and revealed an
379 orchestrated defensive mechanism adopted by loquat plants to cope with B toxicity.
380 Specifically, a shift of sugar metabolism from the production of sucrose to that of
381 sorbitol and fructose was observed, which was associated to the thickening of the stem
382 bark due to noticeable acceleration of the secondary growth of the stem producing a
383 layer of cork cells and several layers of collenchyma cells with thicker cell walls. In
384 that way, both sorbitol and fructose, which have high affinity to form phloem mobile
385 B-complexes, favored accumulation of B principally to young tissues, therefore
386 sequestrating/immobilizing a relevant proportion of B in young leaves. This, in turn,
387 ameliorated the effect of B toxicity in mature leaves. The observed increase in the
388 number of collenchyma cells and the increase of the thickness of their cell wall in the
389 stems of high B-treated plants represented a further attempt by the plant to "store"
390 excessive B in tissues where B ions are less harmful. This ability to trap B has been
391 demonstrated also in species which do not produce sugar alcohol as main translocating
392 agent, e.g. castorbean (Eichert and Goldbach 2010), canola (Stangoulis et al. 2010) and
393 *Camelia sinensis* (Hajiboland et al. 2013). Hajiboland et al (2013) reported the presence
394 of glucose, fructose and sucrose in the phloem sap of *C. sinensis* and observed the re-
395 translocation of B to young leaves. This aspect is uncommon for those species which
396 do not produce polyols as translocating agents. Therefore, fructose and glucose
397 (characterized by low phloem mobility) have the ability bind B and trap it in the
398 phloem, whereas B-sucrose complexes, even though less stable than B-fructose and B-
399 glucose complexes, move to young tissues and diffuse to sink cells (Stangoulis et al.
400 2010). These results are in agreement with previous findings which reported similar B
401 re-translocation in Scots pine (mannitol- and myo-inositol- producing species) and
402 Norway spruce seedling, where sucrose was the only translocating sugar (Lehto et al.
403 2000). However, these experiments did not deal with B excess and the lower stability
404 of B-sugar complexes than those of B-polyol complexes make the contribution of this
405 detoxifying mechanism less relevant in species which do not translocate sugar alcohols.

13

406   According to the above, the possibility that a proportion of B is stored in phloem
407   sap as well as in young tissues might reduce the concentration of accumulated B in old
408   leaves can be considered as a detoxifying mechanism in polyol-producing species. On
409   the other side, one should consider that in some cases young leaves can be more
410   vulnerable than mature leaves to B toxicity, given that, for example, chloroplasts are
411   not fully functional and the photosynthetic apparatus, as well as the antioxidant
412   apparatus, are only partially developed. Theoretically, plant species in which B is
413   relatively immobile should be more tolerant to B excess due to their ability to preserve
414   actively-growing young tissues, even though the plant ameliorative effect can greatly
415   be dependent to B doses and even to species-specific traits. Fruits are also another
416   important sink to be considered when investigating effects of B toxicity, and the phloem
417   re-translocation of B poses serious concerns for fruit yield and quality in tree species
418   which use polyols as translocating compounds.

419   Beside the ability of B to from complexes with sugars, B can bind to *cis*-
420   hydroxyl groups of different classes of polyphenols (Brown 2002; Lewis 2019). In
421   addition, many experimental works have reported a consistent accumulation of total
422   phenolic compounds in responses to B excess (e.g. Chamacho-Cristobal et al. 2002;
423   Landi et al. 2013a) suggesting a possible detoxifying mechanism exerted by phenolic
424   compounds through sequestration, immobilization and compartmentalization of
425   excessive B (Lewis 2019). Recently, Landi et al. (2013a, 2015) proposed that, besides
426   the photo-protective role of foliar anthocyanins in B-stressed sweet basil (see section:
427   "Physiological and biochemical responses to B excess"), anthocyanins may
428   additionally ameliorate the deleterious effect of B excess by forming B-anthocyanin
429   complexes and favoring the shuttle of such compounds into the vacuole, therefore
430   preventing B toxic effects to cytosolic processes. The ability of B to chelate to
431   cyanidine-3-glucoside has been proved *in vitro* (data not published) by the evaluation
432   of the bathochromic shift and the hypochromic effect which is caused by the formation
433   of B-anthocyanin adduct when boric acid is added to a cyanidine-3-glucoside solution.
434   UV–vis spectroscopy and colorimetric models for detecting anthocyanin-metal
435   complexes are described by (Fedenko et al. 2017).

436

437   **Roles of boron transporters and efflux pumps in B tolerance**
438   In nature, B concentration and availability in the soil can vary significantly, therefore
439   plants need to optimize B uptake, use and partitioning to prevent toxic effects of high

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440 cellular concentrations. From an agronomical point of view, a crucial role of rootstock

441 in horticultural crops cultivated with B-rich irrigation water was highlighted by

442 Papadakis (2016) with *Citrus* species, which are usually very sensitive to B toxicity.

443 The authors demonstrated that 'Clementine' mandarin and 'Navelina' orange plants

444 grafted on 'sour orange' rootstock are more sensitive to high B compared to those

445 grafted on 'Swingle citrumelo' due to lower B uptake, which resulted in decreased B

446 accumulation in leaves (Papadakis et al. 2004a, b). Recently, a proteomic approach

447 carried out in roots of *C. sinensis* and *C. grandis* revealed that under high B

448 concentration proteins involved in many cellular processes, including B transport and

449 accumulation, are differentially expressed in the two species and play a key role in

450 conferring B-tolerance to *C. sinensis* (Sang et al. 2017). Therefore, understanding the

451 intimal mechanism of B transport within plant and the mechanisms which allow tolerant

452 genotype to cope better with higher B concentration is of crucial importance to drive,

453 for example, future breeding programs aimed to select tolerant genotypes.

454 It has been established that plants take control of B distribution by channels under

455 conditions of low B availability, and by active efflux pumps in excess conditions

456 generating a concentration gradient across the plasma membrane lowering internal B

457 concentration (Reid 2014). To support the "efflux-hypothesis" under condition of B

458 excess, different anion channels have been recognized as responsible for the higher B

459 tolerance, such as BOR4 in *A. thaliana* (Aibara et al. 2018; Julkowska 2018), BOR1 in

460 barley (Sutton et al. 2007), and BOR2 in wheat and barley (Reid 2007). The first

461 identified B efflux pump was AtBOR1 in *A. thaliana* (Takano et al. 2002) and it was

462 found to be involved in boric acid/borate export from stellar cells to xylem when B

463 levels were adequate inside the cell (Takano et al. 2008). Expression analyses of

464 *AtBOR1* along with its paralog *AtBOR2* have shown transcript accumulation in mature

465 endodermal cells, root tip cells and epidermal cells (Miwa et al. 2013). The *Atbor1*

466 mutant lines exhibit impaired root to shoot B transport and growth deficiencies at low

467 concentrations, while at sufficient B concentrations the growth was not affected.

468 Noteworthy, *AtBOR1* is constitutively expressed and transcript levels are not changed

469 under B deficiency, whereas, when B levels are adequate or excessive, AtBOR1 protein

470 is subjected to post translational degradation leading to control B uptake (Takano et al.

471 2005). Recently, it was demonstrated in Arabidopsis that stabilization of BOR1 confers

472 tolerance to B excess by excluding B from the cytosol of shoot cells and highlighting

473 that this approach could be suitable for selection of B tolerant genotypes (Wakuta et al.

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474 2016). The characterization of *A. thaliana* B transporters revealed that some B efflux

475 transporters are tissues specific, for example AtBOR2 was shown to act as B efflux

476 transporter for radial B transport in roots (Miwa et al. 2013), while under condition of

477 B excess AtBOR4 was involved in B efflux from root epidermis to the soil (Miwa et al.

478 2007). It has been shown that high B concentrations induced a drastic downregulation

479 of *BOR4* leading to B toxicity tolerance (Miwa et al. 2014).

480   Another study in Arabidopsis showed that the *SHB1/HY1* gene, encoding for a

481 heme oxygenase (HO), is upregulated under condition of B excess. When *SHB1/HY1*

482 was overexpressed or HO inducer was applied, B accumulation in roots was reduced

483 thereby conferring B tolerance (Lv et al. 2017). An eco-TILLING analysis carried out

484 with a B tolerant and a B sensitive rice cultivar revealed that a single-base insertion

485 mutation in a gene encoding for a NAC-like transcription factor abolished its expression

486 and conferred tolerance against B toxicity (Ochiai et al. 2011).

487   Aquaporins (Major Intrinsic Proteins, MIPs) have been shown to not only

488 facilitate root water uptake and transportation (Chaumont et al. 2005), but also to be

489 involved in B tolerance. For example, when Arabidopsis plants are subjected to B toxic

490 levels, several root and shoot MIPs are down regulated, causing reduced cell-to-cell

491 water movement leading to a form of passive mechanism for prevention of excess B in

492 tissues (Macho-Rivero et al. 2018). In another study, overexpression of the tonoplast

493 aquaporin *AtTIP5;1* significantly increases B tolerance in Arabidopsis (Pang et al.

494 2010).

495   Plasma membrane Intrinsic Proteins (PIPs) have also been shown to be involved

496 in mediating B permeability, thereby modulating B movement and increasing B

497 tolerance. More recently, two representative rice PIPs, *OsPIP1;3* and *OsPIP2;6*, were

498 reported to function as influx and efflux bidirectional transporters and conferred an

499 enhanced B tolerance when overexpressed in Arabidopsis plants (Mosa et al. 2016).

500   After the investigation of B pumps in *A. thaliana*, several orthologous genes have

501 been reported as involved in B toxicity tolerance in other species, such as cereals. In

502 maize, *rte* mutant showed defects in vegetative and inflorescence development and

503 sterility (Chatterjee et al. 2014). Positional cloning revealed that *RTE* encodes an

504 orthologue of Arabidopsis *BOR1* membrane-localized B efflux transporter. More

505 recently *RTE2*, a paralogue of *RTE* has been also identified (Chatterjee et al. 2017).

506 Database analyses in rice identified 4 genes, *OsBOR1-4*, that share similarities with

507 Arabidopsis *AtBOR1* gene (Nakagawa et al. 2007). For example, OsBOR1 was found

16

508 to function as B efflux transporter, playing a critical role in B acquisition by roots and

509 translocation of B into shoots and its expression was regulated by B availability.

510 OsBOR4 was shown to have tissue-specific B transporter action and it is required for

511 normal pollen germination and pollen tube elongation (Tanaka et al. 2013).

512 Examination of wheat and barley B tolerant and sensitive cultivars demonstrated that

513 *TaBOR1-3* in wheat and *HvBOR1* and *HvBOR2* in barley, are homologous to

514 Arabidopsis *AtBOR1* and rice *OsBOR1* and act as B efflux transporters (Reid 2007;

515 Sutton et al. 2007; Leaungthitikanchana et al. 2013). Sutton et al. (2007) found that the

516 tolerant Sahara, a landrace of barley, had 4-time higher levels of transcript of *HvBOR1*.

517 In barley, downregulation of an aquaporin from the nodulin-26-like intrinsic protein

518 (NIP) subfamily, HvNIP2;1, resulted in limiting B uptake showing that is an important

519 determinant of B toxicity tolerance (Schnurbusch et al. 2010). PIP1 has been shown to

520 be involved in mediating B permeability, leading to B tolerance by redistributing

521 internally toxic B in maize (Dordas and Brown 2001b) and in barley (Fitzpatrick and

522 Reid 2009).

523 Active efflux pump transporters have also been identified in non-cereals: *AtBOR1*

524 homologues have been characterized in the citrus species *Citrus macrophylla* (Canon

525 et al. 2013), in grapevine (Pérez-Castro et al. 2012) and in rapeseed (Zhang et al. 2017).

526 Poplar is known to be tolerant species to B toxicity and accumulation and it is an

527 excellent candidate for investigating B toxicity regulated genes. Genome-wide

528 transcriptome profiling in leaf and roots of black poplar identified many candidate

529 genes for B uptake, transport and detoxification (Yıldırım and Uylaş 2016).

530 Even though B transporters have been only identified and isolated in

531 angiosperms, a phylogenetic analysis on an evolutionary scale showed that lycophytes

532 and bryophytes have B transporters similar to those found in angiosperms (Wakuta et

533 al. 2015), suggesting that B efflux pump play a key role in B homeostasis and in

534 counteracting condition of B excess even at lower evolutionary levels in Planta

535 kingdom.

536

Antioxidant apparatus: an efficient "weapon" against B excess?

538 Plants, being sessile organisms, have evolved prompt, well-orchestrated and finely-

539 tuned mechanisms to counteract dynamic changes of environmental conditions. Among

540 others, the antioxidant machinery, composed by both enzymatic and non-enzymatic

541 antioxidants, is one of the most powerful "weapon" against a plethora of environmental

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cues which lead to increased level of oxidative stress. Non-enzymatic apparatus consists of different isoforms of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and also include those enzymes directly involved in Halliwell-Asada cycle: ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR). Ascorbate (ASA) and glutathione (GSH) are probably the best well-known antioxidant compounds, even though several other secondary metabolites have been proven to be efficient *in planta* ROS scavengers (Gill and Tuteja 2010).

Plants with high antioxidant defenses, either constitutive or induced, have been reported to usually have a higher tolerance to B toxicity. For example, Cervilla et al. (2007) observed in two cultivars of tomato treated with high B levels that B promoted a general enhancement of antioxidants. However, the cultivar 'Josefina' (more sensitive) has a less profound enhancement of ASA content and APX activity than 'Kosacco' (more tolerant). In addition, an increment of GSH was observed only in the tolerant 'Kosacco'. Enhancement of GSH under conditions of excessive B supply was also observed by other authors (e.g. in pear by Whang et al. 2011). The increment of APX (Eraslan et al. 2007b; Han et al. 2009; Landi et al. 2013a, b), CAT (Garcia et al. 2001; Lee 2006; Cervilla et al. 2007; Wang et al. 2011) and SOD activity (Garcia et al., 2001; Karabal et al. 2003; Gunes et al. 2006; Molassiotis et al. 2006; Sotiropolus et al. 2006; Cervilla et al. 2007; Eraslan et al. 2008; Ardic et al. 2009; Landi et al. 2013a, b; Kaya et al. 2019) has also been recorded as a consistent observation in several plant species under B toxicity, suggesting a relevant role of these enzymes in B tolerance. Despite such generalization based on strong evidence, other researches have shown that the activities of those enzymes are decreased, e.g. APX in lettuce by Eraslan et al. (2007b) or CAT in citrus leaves by Han et al. 2009, or insensitive to some excessive B doses (e.g. Cervilla et al. 2007).

The activity of GR, MDHAR and DHAR in plants suffering B toxicity have shown a less consistent trend. In some cases, it has been observed an enhancement of the enzymes involved in ASA regeneration (Karaba et al. 2003; Cervilla et al. 2007; López-Gómez et al. 2007; Han et al., 2009; Wang et al. 2011), whereas in other cases the activity of these enzymes was unrelated to B stress (Ardic et al. 2009; Wang et al. 2011).

The aforementioned observations suggest that the B dose and/or the species under investigation might be relevant to predict antioxidant reaction to B toxicity, and those

18

576 aspects should be seriously considered in the attempt to select B tolerant genotypes by

577 classical breeding, when genotypes are screened for the performance of their

578 antioxidant apparatus.

579

580 **Conclusions**

581 Global warming results in rising temperatures and changing precipitation patterns,

582 which are expected to increase desertification rates, thereby favoring conditions of

583 edaphic B excess in several areas worldwide. Reduction of fertile arable lands contrasts

584 to increasing food demand which is attributed to global raise of human population and,

585 therefore, there is an imperative need to identify in plants the significant traits

586 contributing in B tolerance without influencing crop yield and quality. Based on

587 updated literature, we provide a clear picture of the main morpho-anatomical,

588 physiological, biochemical and molecular responses of plants to B excess. Consistent

589 evidence demonstrates that enhancement of B efflux transporter, stimulation of the

590 biosynthesis B-chelating compounds (e.g. polyols and phenolics), and a "powerful"

591 antioxidant apparatus are key features by which tolerant species/genotypes cope the

592 effect of B toxicity. These features should be considered as key traits when performing

593 breeding programs aimed at generating B tolerant crops. However, future investigations

594 are necessary to improve knowledge of the tolerance mechanisms against B toxicity

595 and to provide efficient strategies to increase crop performances under conditions of

596 excess edaphic B.

597

598 *Author contribution statement* All authors listed have made a substantial, direct and

599 intellectual contribution to the work, and approved it for submission.

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601 **Conflict of interest** The author declares no conflict of interest.

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1044 **Table 1:** Effects of boron toxicity on different morpho-anatomical, physiological and biochemical traits

SPECIES	[B]	CULTURE SYSTEM	EFFECTS	REFERENCES
Arabidopsis thaliana (L.) Heynh.	3 mM	Agar medium	Induced double strand breaks and Condensin II alleviates DNA damage	Sakamoto et al. 2011
	5 mM	Agar plates	Reduced water and B uptake, triggering an osmotic stress response that inhibited root growth.	Aquea et al. 2012
	5 mM	Hydroponic	Leaded to an increase in root of the AtNCED3 (encodes a crucial enzyme for ABA biosynthesis) expression and leaf ABA content and, consequently, decreased the transpiration rate.	Macho-Rivero et al. 2017
	5 mM	Hydroponic	Reduced, through ABA burst and downregulated the expression of major PIP aquaporin gene, the transpiration rate and water transport from root to shoot.	Macho-Rivero et al. 2018
	3 mM	Agar medium	Induced leaf senescence differentially regulating JA and ETH metabolism.	Kayihan et al. 2019
Capsicum annuum L.	5 mg L^{-1}	Pot	Reduction of Mn and Zn concentrations in the leaves as well as increment of carbohydrates and carotenoids.	Sarafi et al. 2018
Cicer arietinum L.	0.05-6.4 mM	Hydroponic	Plant sensitivity to B was higher in the genotype with reduced capacity to activate the antioxidative system (total SOD, APX and CAT)	Ardıc et al. 2009
Citrus sp.	0.05-5 mg L^{-1}	Pot	Reduced Mn use efficiency preventing Mn to act effectively in its main metabolic sites or organs	Papadakis et al. 2003
	2.50 mg L^{-1}	Pot	Reduced leaf thickness (due to spongy parenchyma tissue reduction), chloroplast size, thylakoid density and incremented the relative volume of plastoglobuli. Moreover, Chl content and PSII efficiency were also affected	Papadakis et al. 2004a, b
	400 μM	Pot	Induced changes related to signal transduction, metabolism of carbohydrate, nucleic acid, energy, amino acid and lipid, protein, cytoskeleton and cell wall modification and cell transport	Guo et al. 2014
	400 μM	Pot	Affected photosynthesis and Chl content, altered the cell wall thickness in cortex and phloem cells, and induced plant cell death in leaf phloem tissue. Moreover, it induced exocytosis in the companion cells and the parenchyma cells. The tolerant species was characterized by a higher bound of B to the older leaves	Huang et al. 2014

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	400 μM	Pot	Decreased the protein biosynthesis and increased the abundances of protein species involved in antioxidation and detoxification, cell transport and proteolysis	Sang et al. 2015
	10-100 μM	Hydroponic	Reduced the photosynthetic and transpiration rate, Chl and carotenoid content, stomatal conductance, leaf gas exchange and intercellular CO_2. Moreover, B reduced SOD, POD, CAT, APX and stimulated lipid peroxidation and middle lamella thickness	Shah et al. 2017
	400 μM	Hydroponic	Sensitivity/tolerance to B toxicity is dependent by changes in cell wall compositions and structure alterations in response to treatments	Wu et al. 2018
	0.25-10 mg L^{-1}	Pot	Tolerant citrus rootstocks were low B-sensitive because of their efficient antioxidant system based on a high activity of CAT, which restricts the accumulation of MDA in leaves.	Simón-Grao et al. 2019a
	5 mg L^{-1}, 10 mg L^{-1}	Pot	Mycorrhization attenuated B toxicity reducing MDA and B translocation and accumulation in leaves	Simón-Grao et al. 2019b
Cucurbita pepo L. *Cucumis sativus* L.	18-1,800 μM	Pot	Induced oxidative load and decreased of Chl a/b ratio, actual PSII quantum efficiency, photosynthetic rate, stomatal conductance, and transpiration. B generally stimulated the antioxidant enzymes APX, CAT and SOD and the lead to increment of oxidized form of ascorbate and glutathione	Landi et al. 2013b
Eriobotrya japonica (Thunb.) Lindl.	400 μM	Hydroponic	Negatively affected the transpiration rate prior to the photosynthetic rate. Photosynthesis reduction, at the initial stages of B toxicity, was due to non-stomatal factors	Papadakis et al. 2014
	400 μM	Pot	Promoted the increase in the spongy layer of top leave and the development of cork and collenchyma cells with increased cell wall thickness. Moreover, it induced the accumulation of sorbitol (B-complexing polyol) in top leaves and stem bark. Both strategies could be considered as an attempt to detoxify B excess	Papadakis et al. 2018
Glycine max (L.) Merr.	5 mM	Hydroponic	B-induced root growth reduction was a result of hypodermis formation and the progressive deposition of suberin in cortical cell walls driven by an increase of PAL and SPOX, increase of lignin and wall bound phenols	Ghanati et al. 2005
	0.25 mM	Pot	Increased the level of Zn in leaves and decreased the levels of Ca in leaf tissues while increasing levels of Ca in seed suggesting that B had a role in Ca translocation	Pawlowski et al. 2019

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			B promoted accumulation of H_2O_2, MDA, electrolyte leakage and	
Holgarium vulgar	5 mM, 10 mM	Hydroponic	enhance APX activity in shoot, whereas in root a significant increases in total SOD, CAT and decrease in GR activities was observed	Karabal et al. 2003
Hordeum vulgare L.	0,1,5,20,50,100 mM	Hydroponic	Inhibited enzyme activity, either by binding to the enzyme itself, or by complexing substrates	Reid et al. 2004
	100 mM	Hydroponic	Reduced respiration by 60% and photosynthesis by 23%.	Reid et al. 2004
	5 mM, 100 mM	Hydroponic	Negatively affected membrane transport of amino acids but not protein synthesis	Reid et al. 2004
	0.2 mM, 1 mM	Hydroponic	Tolerant genotypes are particularly rich in putrescine. Moreover, the resistant genotype was characterized by a dramatically increase in 6-kestose that is intermediate for fructan biosynthesis, which are sugar polymers that play a pivotal role in stress responses in grasses	Roessner et al. 2006
	15 μM, 5000 μM	Hydroponic	Altered root meristem reducing root cells number, increasing their length and width and decreasing the content in reducing sugar levels	Choi et al. 2007
	5 mM, 10 mM	Hydroponic	Induced JA-related genes	Öz et al. 2009
Lactuca sativa L.	300 mM	Hydroponic	Induced changes SOD and APX, H_2O_2 but not in CAT activity	Eraslan et al. 2007b
Malus domestica Borkh.	0.1-6.0 mM	*In-vitro* culture	Induced oxidative stress and alterations in the antioxidant and nucleolytic enzymes	Molassiotis et al. 2006
	0.01-0.3%	Liquid culture	Inhibited pollen germination and tube growth decreasing $[Ca^{2+}]$ and inducing the disappearance of the $[Ca^{2+}]$ gradient, which are critical for pollen tube polar growth. B altered the actin filaments and influenced the accumulation and distribution of callose, de-esterified pectins, esterified pectins, and arabinogalactan proteins in pollen tubes	Fang et al. 2016
Musa acuminate Colla	400 μM	Hydroponic	Affected the nutrient status in banana plants increasing potassium and manganese content in leaves and pseudostems and decreasing calcium and magnesium content in leaves	Karantzi et al. 2016
Nicotiana tabacum L.	10 mM, 20 mM	Cell culture	Induced an increase of suberin and lignin levels as well as a stiffening of the cell wall matrix. Lignosuberization was accompanied by the increase of the enzymes PAL, TAL, POD and PPO	Ghanati et al. 2002
Ocimum basilicum L.	2 mg L^{-1}, 20 mg L^{-1}	Hydroponic	Negatively affected growth and photosynthesis in both sensitive and tolerant cultivars. Tolerant cultivars were characterized by a higher constitutive content of glutathione, ascorbic acid and foliar anthocyanins. Latter compounds play an important role in photoprotection	Landi et al. 2013a
	0.2 mg L^{-1}, 20 mg L^{-1}	Hydroponic	Induced oxidative stress and altered the photosynthetic machinery more in green-leafed than in purple-leafed cultivars	Landi et al. 2013c

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	1.8 mM	Pot	B toxicity was mitigated in species rich in foliar anthocyanins, which protect leaf mesophyll from photo-oxidative stress when chloroplast function is compromised by B excess.	Landi et al. 2014
	0.25-25 mg L^{-1}	Hydroponic	Boron concentrations in root and leaf tissues were comparable in 'Tigullio' and 'Red Rubin' or even higher in the purple cultivar, though 'Red Rubin' was less damaged. B compartmentalization and the high phenolic content was thought to contribute to the B tolerance of 'Red Rubin'	Pardossi et al. 2015
Portulaca oleracea L.	4-40 mg Kg^{-1}	Pot	Caused a significant increase in shoot K, Mg, and B contents and enhanced CAT, APX activity, proline accumulation, MDA and H_2O_2 content	Samet and Çıkılı 2018
Solanum lycopersicum L.	0.5 mM, 2 mM	Hydroponic	Caused oxidative damage, increased antioxidant enzyme activity LGALDH, the activity of enzymes of the Halliwell–Asada cycle and ascorbate pool size	Cervilla et al. 2007
	0.5 mM, 2.0 mM	Hydroponic	Caused a reduction in NO_3^- uptake and an increase in NH_4^+ assimilation. Moreover, It bounds to NADPH and ATP disturbing their functions in plant metabolic processes	Cervilla et al. 2009a
	0.50 mM, 2.0 mM	Hydroponic	Increased the H_2O_2, leakage of K^+, its passive efflux and induced higher lignin deposition in roots. Root lignification was connected to the increase of the PPO, GPOX and soluble SPOX activity in roots. Anyway, it was proven that root growth was not reduced by lignification	Cervilla et al. 2009b
	0.5 mM, 2 mM	Pot	Increased level of anthocyanin and flavonoid in tomato	Cervilla et al. 2012
	320 μM, 640 μM	Hydroponic	Inhibited net nitrate uptake and decrease nitrate transporter transcripts	Princi et al. 2016b
Solanum tuberosum L.	0.5-50 mM	Pot	Oxidative stress associated with high B treatments is a secondary effect of B toxicity, which arises from metabolic changes caused by the interference of B with major metabolites	Ayvaz et al. 2016
Triticum aestivum L.	5000 μM	Hydroponic	Induced JA- and ETH-related genes as well as genes associated with cell wall modification	Kayıhan et al. 2017
	5 mM	Hydroponic	Caused a significant decrease in leaf water potential and an increase in Chl a/b ratio	Kayıhan et al. 2017
	3-12 mM	Hydroponic	Leaded to an increase in lipid peroxidation and H_2O_2 followed by genotoxic effects	Çatav et al. 2018
Solanum lycopersicum L. *Capsicum annuum* L.	0.5-50 mg kg^{-1}	Pot	Induced an increase in membrane permeability, proline accumulation, nitrate reductase activity as well as N, P, K, Mg, and S concentrations	Eraslan et al. 2007a

Species	Concentration	Method	Effect	Reference
Triticum aestivum L. *Triticum durum* L.	0.2 mM	Pot	Thiourea-mediated endogenous nitric oxide production significantly improved B tolerance of wheat plants	Kaya et al. 2019
Triticum aestivum L. *Hordeum vulgare* L.	10 mM	Hydroponic	Decreased nitrate reductase activity and increased activity of glutamate dehydrogenase in roots and leaves	Mahboobi et al. 2002
Triticum aestivum L. *Hordeum vulgare* L.	10 mM	Petri dishes	B toxicity is higher in root tips than in mature root sections.	Reid et al. 2004
Vicia faba L.	10^{-3} and 10^{-2} M	Petri dishes	Negatively influences the cell cycle in root tip causing mitosis aberrations and micronuclei formation	Liu et al. 2000
Vitis vinifera L.	10-30 mg kg^{-1}	Pot	Induced the antioxidant enzymes (SOD and CAT) activity to protect the membrane functions from ROS injuries	Gunes et al. 2006
Zea mays L.	5-125 mg L^{-1}	Petri dishes	Caused chromosomal aberration and genotoxic effects on maize	Sakcali et al. 2015
	0.05 mM, 2 mM	Hydroponic	Reduced the efficiency of PS II (F_v/F_m), and leaf relative water content. B enhanced electrolyte leakage, H_2O_2, free proline, lipid peroxidation and the activities of peroxidase, SOD and CAT	Kaya et al. 2018
Viewpoint			B is not an essential element but a toxic element with which plants have evolved to cope with. Plants have developed constitutive biochemical mechanisms in order to maintain a homeostatic balance between the proportion of B absorbed and the amounts of neutralizing agents synthesized (e.g. polyphenols). The lack of B in the soil medium and the continuous production of free neutralizing agents in plant will cause the accumulation of the latter and the development of toxicity effects, which might be erroneously interpreted as deficiency symptoms	Lewis 2019

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1046   Abbreviations: ABA (abscisic acid); APX (ascorbate peroxidase); B (boron); CAT (catalase); Chl (chlorophyll); ETH (ethylene); GPOX (guaiacol peroxidase); GR (glutathione
1047   reductase); JA (jasmonate); LGALDH (L-galactose dehydrogenase); MDA (malondialdehyde); PAL (phenylalanine ammonia-lyase); PIP (plasma membrane intrinsic proteins);
1048   POD (peroxidase); PPO (polyphenol oxidase); PSII (photosystem II); ROS (reactive oxygen species); SOD (superoxide dismutase); SPOX (syringaldazine peroxidase); TAL
1049   (tyrosine ammonia-lyase)

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Table 2: Genes involved in boron transport and tolerance mechanisms in different plant species

SPECIES	[B]	CULTURE SYSTEM	GENE	FUNCTIONAL ROLE	REFERENCES
Arabidopsis thaliana (L.) Heynh.	0.3-100 μM	Hydroponic	*BOR1*	Efflux pump receptor; boric acid/borate export from stellar cells to xylem when B levels are adequate inside the cell	Takano et al. 2002, 2008
	0.3 μM	Agar plates	*BOR2*	Paralog of *BOR1*; B efflux transporter for radial B transport in roots	Miwa et al. 2013
	3 μM	Agar plates/ Hydroponic	*BOR4*	Anion channel receptor; B transportation from root epidermis to the soil	Miwa et al. 2007
	2 mM	Agar plates	*SHB1/HY1*	Heme oxygenase; reduces B accumulation in roots when overexpressed	Lv et al. 2017
	10 μM / 5 mM	Hydroponic	*TIP5;1*	Tonoplast aquaporin; passive regulation of B excess by cell-to-cell water movement	Macho-Rivero et al. 2018
Hordeum vulgare L.	2 mM	Hydroponic	*BOR1*	B efflux transporter	Sutton et al. 2007
	11.7 μM	Hydroponic	*BOR2*	B efflux transporter	Reid 2007
	-	Hydroponic	*NIP2;1*	Aquaporin from the nodulin-26-like intrinsic protein subfamily	Schnurbusch et al. 2010
	5 mM	Hydroponic	*PIP1*	Aquaglyceroporin; redistributes internally toxic B leading to B tolerance	Fitzpatrick et al. 2009
Oryza sativa L.	0.03 μM	Hydroponic	*BOR1-4*	B efflux transporter; B acquisition by roots and translocation of B into shoots	Nakagawa et al. 2007
	-	Pot	*BOR4*	B efflux transporter; Tissue specific B transporter required for pollen germination and pollen tube elongation	Tanaka et al. 2013
	0.5 gm^{-3}/ 60 gm^{-3}	Hydroponic	*NAC-like*	Transcription factor; reduced expression confers tolerance against B toxicity	Ochiai et al. 2011
	5 mM	Hydroponic	*PIP1;3, PIP2;6*	Plasma membrane Intrinsic Proteins; influx and efflux bidirectional transporters	Mosa et al. 2016
		Pot/ Agar plates	*RTE*	*AtBOR1* orthologue; membrane-localized B efflux transporter	Chatterjee et al. 2014
	200 μM	Pot	*RTE2*	*RTE* paralog; membrane-localized B efflux transporter	Chaterjee et al. 2017
Triticum aestivum L.	18 nM/ 18 μM/ 1 mM	Hydroponic	*BOR1-3*	B efflux transporter	Leaungthitikanchana et al. 2013

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Species	Concentration	Growth system	Gene	Function	Reference
Zea mays L.	50 μM	Hydroponic	*PIP1*	Aquaglyceroporin; redistributes internally toxic B leading to B tolerance	Dordas and Brown 2001b
Citrus macrophylla L.	23 / 460 μM	Agar	*BOR1*	B efflux transporter	Canon et al. 2013
Vitis vinifera L.	60 to 70 mg L^{-1}	Field	*BOR1*	B efflux transporter	Pérez-Castro et al. 2012
Brassica napus L.	0.25 μM / 25 μM	Hydroponic	*BOR1*	B efflux transporter	Zhang et al. 2017

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Figure captions

Fig. 1 Chemical structure of boric acid (a), borate anion (b) and their esters (c and d)

Fig. 2 Phloem B complex with sorbitol

Fig. 3 Site of B attachment in plant cell wall B rhamnogalacturonan II complex

Fig. 4 Symptoms of B toxicity in various plant species where B is relatively immobile via phloem tissue: citrus (a, mandarin; b, sweet orange; c, sour orange; d, Swingle citrumelo; e, lemon), banana (f, g), sweet basil (h, green; i, purple), squash (j), and cucumber (k)

Fig. 5 Symptoms of B toxicity in various plant species where B is relatively mobile via phloem tissue: loquat (a, plant; b, top leaves; c, d, e stem-middle to upper part), and apple (f, plant; g, h top leaves; i, middle leaves; j, root under normal B (right) or high B (left); k, l and m, stem-middle to upper part)
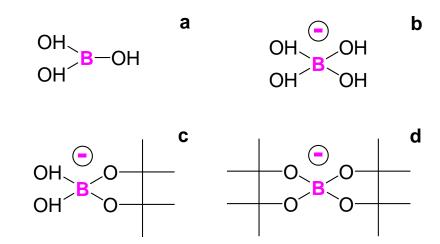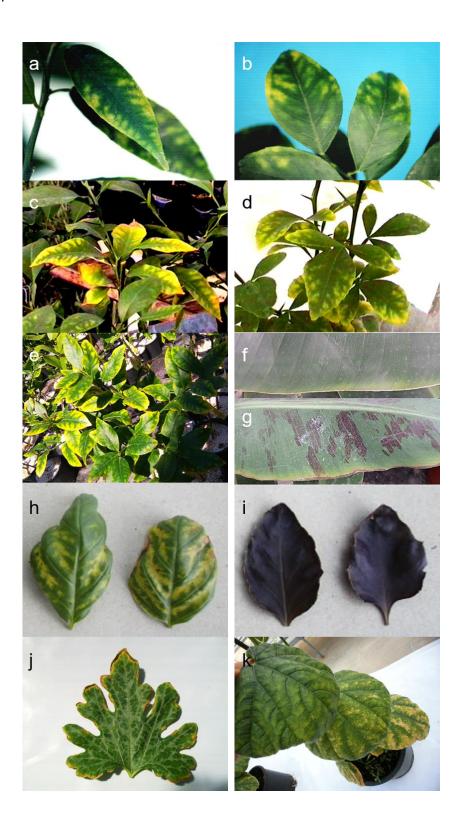
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5