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PHYSIOLOGICAL AND GENETIC BASIS
OF PLANT TOLERANCE TO EXCESS BORON

ABSTRACT: Boron (B) deficit as well as excess may significantly limit the organic
production in plants. In extreme cases they may kill the affected plants. Boron excess oc-
curs primarily in arid and semiarid regions, in saline soils or in consequence to human
action. Excessive boron concentrations retard plant growth and cause physiological and
morphological changes (chlorosis and necrosis) first of all in leaf tips and then in marginal
or intercostal parts of the lamina. Physiological mechanisms of plant tolerance to boron
excess have not been studied in sufficient detail. The predominant opinion holds that they
are based on restricted uptake and accumulation of boron in the root and aboveground plant
parts. Significant differences in boron excess tolerance have been observed not only be-
tween different crops but even between different genotypes of the same crop. This has ena-
bled the breeding of crop genotypes and crops adapted to growing on soils rich in available
boron and intensified the research on the inheritance of plant tolerance to high B concentra-
tion. Sources of tolerance to high B concentration have been found in many crops (wheat,
mustard, pea, lentil, eucalypt). Using different molecular techniques based on PCR (RAPD,
SRAP), plant parents and progenies have been analyzed in an attempt to map as precisely
as possible the position of B-tolerant genes. Small grains have been studied in greatest de-
dtail for inheritance of B tolerance. B tolerance in wheat is controlled by at least four addi-
tive genes, Bo1, Bo2, Bo3 and Bo4. Consequently, there exists a broad range of tolerance
levels. Studies of Arabidopsis have broadened our understanding of regulation mechanisms
of B transport from roots to above ground parts, allowing more direct genetic manipulations.

KEY WORDS: boron toxicity, mechanism of action, inheritance of tolerance, crop
plants

INTRODUCTION

Effect of a chemical element on vital processes and organic production in
plants depends primarily on its physiological role in plant growth and develop-
ment and its concentration in the environment. Optimum provision with essen-
tial elements is a prerequisite for normal growth and development of plants. If
an element’s concentration in nutritive medium is above the optimum but
below a toxic level, it may accumulate in plant tissues, i.e., there occurs a la-
tent excess of that element. If the element’s concentration in nutritive medium
exceeds the toxic level, disturbances in plant vital processes may affect plant condition and chemical composition, organic matter production and they may cause death of the affected plants.

Boron is a biogenous element for higher plant. Boron excess as well as shortage cause physiological and morphological changes in plants. The range between boron deficit and excess is quite narrow. Excess B in nutritive medium is a frequent limiting factor in crop production. Reasons for the occurrence of excess B may be lithogenic and pedogenetic processes or human activities. Symptoms of B toxic action on crop plants were observed and identified for the first time in the early 1930s. Signs of B excess have been registered in southern Australia, western Asia, northern Africa, Turkey (central Anatolia) and other parts of the world. Previously, scientific attention has been focused on B shortage. Presently, the attention is beginning to shift towards B excess, as the practical aspect of this problem gains importance.

SOURCES OF BORON

Boron is widely distributed in nature, but in exceedingly low concentrations. Its content in Earth’s crust is approximately 0.0003%. In nature it is exclusively bound to oxygen, most frequently in the form of polyborates such as borax, kernite or colemanite. Turmaline is the most important B-containing mineral (3 to 4%). Total B content in soil ranges from 20 to 200 mg kg⁻¹, most frequently from 30 to 40 mg kg⁻¹. A major portion of soil B is not available to plants. Of total B in soil solution, only 5 to 15% are available to plants, mostly in the form of boric acid (Gupta, 1979). B concentration in soil solution typically ranges from 0.01 to 5 mg L⁻¹ (Schilling, 2000). Soils in moderate climate regions also contain available B in the form of calcium borate. In arid regions, available Ca-, Na- and K-borate can be found in increased amounts. Soils developed from igneous rocks are typically poorer in B than those developed from sedimentary rocks. Average B proportion in arid and semiarid soils is higher than in humid soils. This is why B excess is more frequent in semiarid and arid regions than in humid ones. Because of facilitated B leaching in humid regions, their soils are typically poorer in B, especially sandy soils. Soils in littoral zones frequently have an increased B content because surf spray contains 4 to 5 mg B L⁻¹. In arid conditions, especially in soils subject to sodium accumulation, Na- and Ca-borate accumulate on soil surface. Besides, irrigation water in arid regions frequently has an increased B content. Also, soils developed from marine sediments have an increased B content. Toxic concentrations of B had been found in water extracts of most saline soils from the Vojvodina Province (Milković, 1968).

B availability to plants depends on a large number of factors (Kastori 1990). Soil organic matter has a particularly positive influence on B availability. The amount of available B in soil varies in the course of the year. B availability decreases in proportion to increase in pH, except in the case of saline soils. If a high pH is due to a high Na concentration, B availability to plants tends to increase.
Human activities may affect B content in soil. The problem of B excess intensifies in greenhouses used for production of vegetables and decorative plants. Use of irrigation water containing 0.5 to 1.0 mg⁻¹ of B may gradually lead to its excess (Nable and Paul, 1991). Plant species vary in reaction to B content in irrigation water. According to Nable et al. (1997), B content in irrigation water should range from 0.3 to 1 mg⁻¹, 1 to 2.1 mg⁻¹ and 2.1 to 4.0 mg⁻¹ for susceptible, medium tolerant and tolerant species, respectively. Irrigation with tap water containing an increased B content also may cause accumulation of excess B in plants. Tap water with B content of 2 mg⁻¹ or more is considered as unsuitable for irrigation. Ground waters also may have a high B content. Fertilization with urban compost (Purves and Mackenzie, 1974) or residues containing a large proportion of brown coal may lead to B excess because of a relatively high B content in brown coal. There was a case when toxic B action occurred after the application of manure that had been treated with boric acid to control insects and their larvae. Excess B may also occur in clay and enameled pots (Mitscherlich pots) used for laboratory growth trials. Barren soil with high B content removed from strip mines and used as landfill also may cause B toxicity in plants. Industrial and urban air pollutions, especially if they contain large amounts of ash, may contribute to B accumulation in soils and plants (Kozma and Tölgyesi, 1978). According to Romeg et al. (1977), risk of B accumulation in soils and plants is present in the vicinity of coal-fueled power plants because lignite contains up to 300 mg kg⁻¹ B, and its ash, which is an air pollutant, contains from 19 to 51 mg kg⁻¹ B. Conifers are particularly sensitive to air pollution. B accumulation in soil may result from systematic application of B fertilizers of B-enriched mineral fertilizers. According to Reisenauer et al. (1973) toxic B action may be expected to occur in field crops when B content extracted from soil with boiling water exceeds 5 mg kg⁻¹, and B shortage when B content goes below 1 mg kg⁻¹. Symptoms of B excess in sandy soil, loamy sand, loamy sand and clayey soils occur when B content extracted with boiling water exceeds 0.80 mg kg⁻¹, 1.00 mg kg⁻¹, 1.20 mg kg⁻¹ and 2.00 mg kg⁻¹ (Robertson et al., 1975). Considering the narrow range between optimum and toxic B concentrations, it is necessary to be careful when applying B fertilizers, especially to light soils.

**PHYSIOLOGICAL BASIS OF TOLERANCE**

Boron movement in plants is mostly associated with transpiration course, which explains why it accumulates in leaf tips and margins. According to Petrović and Kastori (1983), B concentration in leaves identified for excess B fell from 212 mg B kg⁻¹ DM at leaf margin to 45 mg B kg⁻¹ DM in the center of the leaf. Because of such distribution pattern, first signs of B excess occur on leaf tips and margins, in the form of a necrosis, first in mature and later in juvenile leaves. In the case of high B excess, symptoms also occur in intercostal parts of the lamina, first as brown spots which necrotize later on. Progression of symptoms of B excess depends also on B concentration in the
environment. If B accumulation in plants is gradual, chlorotic spots first occur on leaves which then become necrotic. If B accumulation is rapid, necrotic spots develop immediately. Also, various plant organs react differently to high B concentration. After Kluge (1990) symptoms of B excess on wheat leaves appear at lower B concentration than the reduction of grain yield. B content in wheat seeds may increase 20 times without negatively affecting germination rate and seedling growth (Nable and Paul1, 1990). In addition to leaf necrosis, excess B causes other morphological and physiological changes such as reduced plant height, impaired growth of aboveground plant parts (Paul1 et al., 1990) and roots (Huang and Graham, 1990).

Some plant species exhibit specific symptoms of B excess (Bergmann and Neubert, 1976). In wheat, the stem acquires a pink-red color. In tomato, there occurs the curling of top leaves. In grapevine, the edges of young leaves curl dorsally and pollen variability is reduced. In rice, numbers of spikes and grains per spike are reduced. Special care should be exercised when applying B in orchards. Apple fruits mature earlier and such fruits are susceptible to various physiological disorders. If B content in apple fruits is above 40 mg kg\(^{-1}\) DM, the fruits have shorter shelf life.

Morphological changes of plants are not a reliable indicator of B excess since toxic concentrations of other elements may cause similar symptoms. To reliably establish B status in plants, it is necessary to determine first B concentration in plants and then in the nutritive substrate. Here it should be kept in mind that the total B content in soil does not provide a reliable information of B availability to plants.

The investigations conducted so far seem to suggest that plant tolerance to toxic concentrations of B is based on several mechanisms. Nable (1988) stated that sensitivity to toxic B concentrations in wheat cultivars depends primarily on tissue capacity to release B. Paul1 et al. (1992) pointed out the importance of B release via roots, slow B uptake and limited B translocation to the aboveground parts, singling out the intensity of B uptake as the most important factor. According to Chantachume et al. (1995) wheat genotypes tolerant to B excess have a longer root system than sensitive genotypes. Root length could thus be used as a criterion in breeding for plant tolerance to excess B. In barley, reduced B uptake is based on genetically controlled slow passive B transport through the plasmatic membrane of root cells, and not on the anatomy of the root or transpiration intensity (Nable and Paul1, 1991). As sugars form complexes with B, high sugar concentration may reduce B toxicity (Yokota and Konishi, 1990). While some authors claim that plant species capable of effective B bonding in the cell wall have improved tolerance to high B concentrations, others hold the opinion that B bonding in the cell wall does not play an important role in B detoxication (Daniel et al., 1998). It is the prevailing opinion that the mechanism of plant tolerance to high B concentrations is based on a restricted uptake of B and thus on its lower accumulation in roots and aboveground parts.

A high B concentration in the cytosol causes disturbances in plant metabolism, also manifested through B complexing with NAD\(^{+}\) or rRNA or through a specific inhibition of ureide metabolism (Lukaszewski et al., 1992). Si-
milarities exist between mechanisms of plant tolerance to high concentrations of salt and high concentrations of B (Nable et al., 1997).

Unfavorable effects of B excess in soil can be mitigated to a certain measure by calcium application, use of irrigation water low in B or the application of 90 do 100 kg N ha⁻¹, in the form of lime-ammonium saltpeter. It was observed that satisfactory soil provision with K may lessen the harmful effects of excess B (Shalaby and Kádár, 1984).

Tab. 1 — Limit values of high and toxic B concentrations in some crops (cit. Bergmann and Neubert, 1976)*

<table>
<thead>
<tr>
<th>Crop</th>
<th>Growth stage at the time of sampling</th>
<th>Plant organ</th>
<th>Limit value (mg kg⁻¹ in dry matter)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
<td>Toxic</td>
</tr>
<tr>
<td>Maize</td>
<td>Start of flowering</td>
<td>Leaf next to the ear</td>
<td>25—35</td>
<td>&gt; 35</td>
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<td></td>
<td></td>
<td></td>
<td>*Jones, 1967</td>
<td></td>
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<tr>
<td>Wheat</td>
<td>Tillering</td>
<td>Entire plant</td>
<td>31—100</td>
<td>&gt; 100</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>*Finck, 1968</td>
<td></td>
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<tr>
<td>Potato</td>
<td>Start of flower</td>
<td>Basal and medium leaves</td>
<td>53—100</td>
<td>&gt; 140</td>
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<td></td>
<td></td>
<td></td>
<td>*Wrazidlo, 1973</td>
<td></td>
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<tr>
<td>Sugar beet</td>
<td>June/July</td>
<td>Lamina from rosette center</td>
<td>201—800</td>
<td>&gt; 800</td>
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<td></td>
<td></td>
<td></td>
<td>*Neubert et al., 1970</td>
<td></td>
</tr>
<tr>
<td>Bean</td>
<td>Start of flowering</td>
<td>Lamina of fully developed top leaf</td>
<td>&gt; 150</td>
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<td>*Chapman, 1967</td>
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<tr>
<td>Soybean</td>
<td>Before the start of pod forming</td>
<td>Fully developed top leaf</td>
<td>56—80</td>
<td>&gt; 80</td>
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<td></td>
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<td></td>
<td>*Jones, 1967</td>
<td></td>
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<tr>
<td>Alfalfa</td>
<td>1st cut before flower</td>
<td>Entire aboveground part</td>
<td>53—99</td>
<td>&gt; 99</td>
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<td></td>
<td></td>
<td></td>
<td>*Gupta, 1972</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>Before harvest</td>
<td>Entire aboveground part</td>
<td>41—60</td>
<td>&gt; 60</td>
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<td></td>
<td></td>
<td></td>
<td>*Roorda van Eysinga et al. 1971</td>
<td></td>
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<tr>
<td>Sour cherry</td>
<td>July/August</td>
<td>Leaf</td>
<td>54</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>*Neubert et al., 1970</td>
<td></td>
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<tr>
<td>Grapevine</td>
<td>Flower/maturity</td>
<td>Leaf opposite to the grape bunch</td>
<td>40</td>
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<td></td>
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<td>*Levy, 1968</td>
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<tr>
<td>Maize</td>
<td>—</td>
<td>Leaves</td>
<td>—</td>
<td>100</td>
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<td></td>
<td>El-Sheikh et al., 1971</td>
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<tr>
<td>Cucumber</td>
<td>—</td>
<td>Leaves</td>
<td>—</td>
<td>400</td>
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<td></td>
<td></td>
<td></td>
<td>El-Sheikh et al., 1971</td>
<td></td>
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<tr>
<td>Squash</td>
<td>—</td>
<td>Leaves</td>
<td>—</td>
<td>1000</td>
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<td></td>
<td></td>
<td>El-Sheikh et al., 1971</td>
<td></td>
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<tr>
<td>Wheat</td>
<td>—</td>
<td>Leaves</td>
<td>—</td>
<td>100—270</td>
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<td></td>
<td></td>
<td></td>
<td>Paul et al. 1988</td>
<td></td>
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<tr>
<td>Snap bean</td>
<td>—</td>
<td>Leaves</td>
<td>—</td>
<td>100</td>
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<td></td>
<td></td>
<td></td>
<td>François, 1989</td>
<td></td>
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<tr>
<td>Cow pea</td>
<td>—</td>
<td>Leaves</td>
<td>—</td>
<td>330</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>François, 1989</td>
<td></td>
</tr>
<tr>
<td>Picea sp.</td>
<td>—</td>
<td>Needles</td>
<td>—</td>
<td>960</td>
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<td></td>
<td></td>
<td></td>
<td>Judel, 1977</td>
<td></td>
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<tr>
<td>Grasses</td>
<td>—</td>
<td>—</td>
<td>270—520</td>
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<td>Judel, 1977</td>
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The only correct approach to increasing yields of crops grown on B-rich soils is the development of plant genotypes tolerant to B excess (Kraljević-Balalić et al., 2003). Recent physiological and genetic studies have contributed significantly to the understanding of the role of genetic variability in plant response to high B concentrations. Also, these studies facilitated the development of genotypes adapted to growing on soils high in B. Most of the crops found to possess large variability regarding the tolerance to B excess have the same tolerance mechanism — reduced B uptake.

Genetic variation for tolerance to B toxicity exists in a number of crops, including wheat and barley (Cartwright et al., 1987; Moody et al., 1988; Paul et al., 1988; Yau 2002, Torun et al., 2006), lentil (Yau and Erskine 2000), field pea and other forage crops (Paul et al., 1992). Huang and Graham (1990) stated that distinct and consistent differences among wheat genotypes in response to B toxicity, at both organ and cellular levels, could serve as a basis for breeding. The genotypes Evropa 90 (YUG), Peking 11 (CHI) and Kalayan Sona (IND), with lowest mean values of B concentration in leaves at heading stage, were appropriate sources for B tolerant germplasm. They may serve as B tolerant donors in hybridizations. Hexaploid wheat genotypes had, on average, higher B concentration in leaves at heading stage than tetraploid genotypes (Kraljević-Balalić et al., 2002).

Largest advances in the study of inheritance of tolerance to excess B have been registered in cereals. In wheat, this trait has been found to be coded for by at least four major genes, Bo1, Bo2, Bo3 and Bo4. As these genes exhibit an additive action, there exists a broad array of tolerance levels. The occurrence of transgressive segregation in some crosses indicates that more than four genes may be involved in the control of this trait (Paul et al., 1993, Campbell et al., 1994). The wheat chromosomes that carry these genes are labeled as 4A, 7B, 7D and 7EB, which confirms the earlier hypothesis that the group 7 of homologous chromosomes play an important role in the control of tolerance to high B concentration. It is also probable that an allele on the chromosome 4D contributes to the increased sensitivity to B (Chantachum et al., 1994). As the wheat genotypes tolerant to B excess originate from various regions (Kraljević-Balalić et al., 2004) and as the transgressive segregants were found in the progenies of tolerant parents, it may be assumed that chromosomes other than those in groups 4 and 7 may carry genes that control the tolerance to excess B. A tolerant genotype, which outyielded sensitive ones by 50% when grown on B-rich soils, had been obtained by backcrossing the gene Bo1 from a moderately tolerant genotype into a sensitive genotype which, on the other hand, was well adapted to other agroecological conditions (Campbell et al., 1994).

Using generation mean analysis, Kraljević-Balalić et al. (2004) found that the mode of inheritance of B concentration in wheat leaves in the F1 and F2 generations was intermediate, dominant or superdominant, depending on cross combination. B concentration was under the control of genes with additive, dominant and epistatic effects (a x a, a x d, d x d, respectively).
QTL analyses of barley showed that a major toxicity tolerance locus was located on the second arm of the chromosome 4H, while a moderate tolerance locus was on the chromosome 6H (Jefferies et al., 1999). To obtain reliable PCR markers for fine mapping of tolerance to excess B in all plant species, Schnurbusch et al. (2005) used the EST sequences from barley and wheat and genomic sequences identified in collinear regions of rice (Oryza sativa L., chromosomes 2, 3 and 6). These authors believe that the search through these EST data bases, together with the screening of a BAC library deriving from the lines tolerant to excess B, will enable a map-based isolation of such genes in barley and wheat, to determine ultimately the molecular mechanisms that ensure the tolerance to B excess in cereals.

Kaur et al. (2004) studied the genetic divergence of different canola genotypes (Brassica rapa) in hydroponic trials, field trials and by the method of molecular markers. The tolerant genotypes had significantly lower B contents than the sensitive ones, which confirmed that the tolerance mechanism is based on the capacity to avoid the uptake of high amounts of B. Analyses of genetic divergence by SRAP (Sequence Related Amplified Polymorphism) revealed that sufficient variability existed between the tolerant and sensitive genotypes for genome mapping of the B tolerance trait. The SRAP technique specifically targets coding sequences and results in the screening of co-dominant markers (Li and Quiros, 2001).

B tolerance exists also in some lentil genotypes (Lens culinaris), as reflected in their high yields when grown on soils rich in B. Application of these genotypes in breeding programs, particularly back crossing, produces significant results, especially when B tolerance is combined with tolerance to salts and diseases (Hobson et al., 2004). According to Bagheri et al. (1995), RAPD markers may be successfully used to characterize pea genotypes for genetic divergence regarding B tolerance.

Extensive studies have been conducted on arabidopsis (Arabidopsis) with the aim of identifying genes and proteins capable of providing tolerance to excess B. Five different types of genes have been found which, when expressed in yeast (a model eucariotic organism), exhibit tolerance to excess B (Fujiwara and Nozawa, 2005). An arabidopsis protein BOR1 has been found to transport B into the xylem. The amount of this protein regulates B transport from roots to aboveground parts. Under conditions of B shortage in the medium, it intensifies B transport to aboveground parts and under conditions of B excess it prevents excessive B accumulation in aboveground parts and toxic effects. It has been found that post-transcriptional mechanisms play a key role in the regulation of amounts of BOR1 (Takanó et al., 2005).

REFERENCES


ФИЗИОЛОШКЕ И ГЕНЕТСКЕ ОСНОВЕ ТОЛЕРАНТНОСТИ БИЉАКА ПРЕМА СУВИШКУ БОРА

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Резиме

Како недостатак тако и сувишак бора (В) може зна чајно да смањи органску продукцију биљака, а у експесним случајевима може да доведе и до њиховог утицања. Сувишак бора се пре свега јавља у аридним и сеамиридним пределима, на заслањеним земљиштима или као последица активности човека. Експесне кон центрације бора смањују раст биљака и изазивају физиолошке и морфолошке промене (хлорозу и некрозу) пре свега вршног и рубних или интеркосталних делова лице. Физиолошки механизми толерантности биљака према сувишку бора нису довољно познати. Преовладава мишљење да се они заснивају на рестрикцији усвајања и време накупљања В у корену и надземном делу биљака. Утврђено је да постоје значајне разлике у толерантности не само врста него и генотипова према сувишку В што омогућава да се путем оплетењивања створе генотипови гајених биљака подесни за гајење на земљиштима са повећаним садржајем присутачног В. С тим у вези интензивирају се истраживања у вези са наследи вањем толерантности биљака према сувишку В. Утврђено је да код великих броја биљних врста (нпр. пшеница, слацца, грашак, сочиво, еукалпитус) постоје извори толерантности према сувишку В. Употребом различитих молекулярних техника које се заснивају на PCR-у (RAPD, SRAP) за анализе родитељских компонената и потомства насталих код житарица. Утврђено је да код пшенице толерантност према сувишку В условају најмање четири гена, Boh, B02, B03 и B04 који делују адитивно, тако да постоји читав спектар нивоа толерантности. Прочувавања на арабидопису довела су до бољег разумевања механизма регулације транспорта В из корена у надземне органи, што отвара могућност директнијих генетичких манипулација.