

From Forest Nursery Notes, Summer 2011

37. Boron in plants. Ozturk, M., Sakcali, S., Gucl, S., and Tombuloglu, H. Chapter 13 in Plant Adaptation and Phytoremediation, Part 2, p. 275-311. 2010.

- Wu L, Enberg M, Tanji KK (1993) Natural establishment and selenium accumulation of herbaceous plant species in soils with elevated concentrations of selenium and salinity under irrigation and tillage practices. *Ecotoxicol Environ Saf* 25(2):127-140
- Yensen NP, Hinchman RR, Negri MC, Mollock GN, Settle T, Keiffer CS, Carty DJ, Rodgers B, Martin R, Erickson R (2000) Using halophytes to manage oilfield saltwater: disposal by irrigation/evapotranspiration and remediation of spill. USDA Natural Resources Conservation Service
- Yordoan AY, Kruger H (1998) Notes on the cuticular ultrastructure of six xerophytes from Southern Africa. *South African J Bot* 64(1):82-85

Chapter 13 Boron and Plants

Munir Ozturk, Serdal Sakcali, Salih Gucel, and Huseyin Tombuloglu

Abstract Boron is found naturally in the earth's crust in the oxidized form as borax and colemanite, particularly in the oceans, sedimentary rocks, coal, shale, and some soils. It is never found in the elemental form in nature possessing a complex chemistry similar to that of silicon, with properties switching between metals and non-metals. Boron has become an important and strategic element in terms of developing technologies. It is released into the environment mainly through the weathering of rocks, volatilization from oceans, geothermal steam, burning of agricultural refuse and fuel wood, power generators (coal/oil combustion), glass industry, household use of boron-containing products (including soaps and detergents), borax mining and processing, leaching from treated wood and paper, chemical plants, and sewage/sludge disposal, but a major proportion originates from the weathering of rocks. Boron is regarded as an essential element for human beings, animals and plants. Boron occurs in soils at concentrations ranging from 10 to 300 mg kg⁻¹ depending on the type of soil, amount of organic matter, and amount of rainfall. The treatments lead to significant increases in the productivity of some plants but in certain cases a decrease is seen as the boron level increases with the boron content of irrigation water, in particular on the soils with

M. Ozturk (✉)

Botany Department, Ege University, 35100 Bornova, Izmir, Turkey
e-mail: munirozturk@gmail.com

S. Sakcali (✉)

Biology Department, Fatih University, Istanbul, Turkey
e-mail: sakcali@fatih.edu.tr

S. Gucel (✉)

Near East University, Institute of Environmental Sciences, Nicosia, Cyprus
e-mail: sgucel@yahoo.com; sgucel@hotmail.com

H. Tombuloglu (✉)

Biology Department, Fatih University, Istanbul, Turkey
e-mail: htombuloglu@fatih.edu.tr

Dedicated to Prof. Dr. Yusuf VARDAR (Ege University) and Prof. Dr. Hubert ZIEGLER (Munich Technical University) on their sad demise in 2009.

a heavy texture, high CaCO_3 and clay content. Lack of boron in plants results in necrosis but excess amounts are said to produce poisonous effects. Turkey produces more than 60% of the world's borax, with important boron reserves located in Susurluk, Bigadic and Sındirgi regions of Balıkesir, Kestelek-Bursa, Emet-Kütahya, the largest reserves occur in Kirka-Eskisehir. Therefore, there is a naturally occurring high level of boron in the ground waters in some of these areas due to the excess amounts of boron given out to the environment during washing and purification processes which result in the pollution of cultivated areas. An attempt will be made here to present an overview of the plant diversity on the boron contaminated soils in Turkey, effects of different concentrations of boron on the germination ability of some plants and possible candidates for phytomining of the soils showing boron toxicity symptoms.

Keywords Boron · Toxicity · Phytoremediation · Genotoxicity · *Polygonum*

Contents

| | |
|------------------------------------------------------------------|-----|
| 1 Introduction | 276 |
| 2 Boron Production and Usage | 277 |
| 3 Boron and Living Beings | 278 |
| 4 Boron and Plants | 278 |
| 4.1 Boron Tolerance, Deficiency and Toxicity in Plants | 280 |
| 4.2 Boron Uptake By Plants | 283 |
| 4.3 Molecular Basis of Boron Uptake and Transport | 284 |
| 4.4 Boron Remobilization | 286 |
| 5 Boron Pollution | 287 |
| 6 Phytoremediation | 291 |
| 7 Boron and Seed Germination | 293 |
| 8 Boron and Genotoxicity in Plants | 297 |
| 9 Conclusion | 301 |
| References | 305 |

1 Introduction

Elemental boron (B) is a member of Group IIIA of the periodic table, along with aluminum, gallium, indium, and thallium, differing distinctly in its chemical properties from aluminum but resembles silicon (Si), arsenic (As), and germanium (Ge) possessing a very complex chemistry (Cotton and Wilkinson 1988; Marschner 1995). Tanaka and Fujiwara (2008) have recorded it as a member of metalloid group of elements belonging to group V, because its characteristics lie between metals and non-metals (Marschner 1995), being a semiconductor rather than a metallic conductor.

It is extensively distributed in low concentrations throughout nature in the form of various inorganic borates constituting about 10 mg kg^{-1} of the Earth's crust, ranging

from 5 mg kg^{-1} in basalts to 100 mg kg^{-1} in shales (Woods 1994), and occurs in soils at concentrations ranging from 10 to 300 mg kg^{-1} (average 30 mg kg^{-1}), depending on the type of soil, amount of organic matter, and rainfall. Economic reserves of borate minerals are rare and are usually found in arid desert regions with a geological history of volcanic and/or hydrothermal activity (Mellor 1980). The majority of the boron occurs in the ocean, at an average concentration of about 4.5 mg L^{-1} (Weast et al. 1985), but is also released from anthropogenic (agricultural, industrial and domestic) sources to a lesser extent (Butterwick et al. 1989). Natural weathering of clay-rich sedimentary rocks, coal and shale on land surfaces accounts for a large proportion of the boron, mobilized into the soils and the aquatic environment, in the form of borates. Boron in soil solution is present as boric acid and easily leached out of the soil due to its high solubility (Shorrocks 1997; Yan et al. 2006). It is adsorbed onto the surfaces of soil particles, with the degree of adsorption depending on the type of soil, pH, salinity, organic matter content, iron and aluminum oxide content, iron-and aluminum-hydroxy content, and clay content (Kekeç 2008; Ayvaz 2002).

The availability of B in soil is limited in many regions in the world with a high rainfall and seasonal water availability. On the contrary, in the arid and semi-arid regions, ground water reaches the topsoil by capillary action and evaporates to leave solutes in soil. In regions with high-boron groundwater, boron concentration in topsoil reaches to a toxic level for plants and reduces crop yields. South Australia, Egypt, Iraq, Jordan, Libya, Morocco, Syria, Turkey, California, and Chile are regions/countries with boron toxicity problems in agricultural lands (Yau et al. 1995).

2 Boron Production and Usage

Borate minerals have been employed in a wide range of uses for many centuries, dating from at least the eighth century when they were used primarily as a flux for assaying and refining gold and silver as well as production of wall plaster and ceramics (Ayvaz 2002; Bayca et al. 2008; Batar et al. 2009). Their valuable properties and relative rarity has stimulated international trade in borates. Marco Polo claimed to have transported Chinese borate minerals from Tibet to Europe and Venice was the center for borate imports (Travis and Cocks 1984). It is widely used in the industry. A large number of minerals contain boric oxide, but five of them are the most important from a worldwide commercial standpoint. The most widely used commercial productions and materials of boron include borax-pentahydrate, borax, sodium perborates, colemanite, ulexite as well as boric acid. These are produced in a limited number of countries, dominated by the Turkey and United States, which together furnish about 90% of the world's borate supplies (Lyday 1993; Culver et al. 1994). The principal end usage for borate include insulation and textile-grade fiberglass, laundry bleach (sodium perborate), borosilicate glass, fire retardants, chemical fertilizers and herbicides (as a trace element), and enamel coating, frit and ceramic glazes, as well as several other applications (Etiproducts 2005; WHO 1998). Other

minor usage include cosmetics and pharmaceuticals (as a pH buffer), boron neutron capture therapy (for cancer treatment), and pesticides. The cancer treatment application which preferentially accumulates in tumor versus normal tissue, utilizes a boron compound made with ^{10}B isotope, (Barth and Soloway 1994).

3 Boron and Living Beings

The lowest lethal dose for humans exposed to boric acid is reported to lie around 640 mg kg^{-1} body weight by oral exposure, 8600 mg kg^{-1} body weight by dermal exposure, and 29 mg kg^{-1} body weight by intravenous injection (Stokinger 1981). After establishment of essentiality, understanding a role(s) of boron became the major task in boron biology, however, its essentiality in humans has not been established, although its beneficial effect has been reported. Boric acid and borax were widely used in medicine at the beginning of the century for therapeutic purposes, both locally as well as orally. Boric acid was used to treat various diseases, such as epilepsy and infectious diseases. Several case studies reviewed by Kliegel (1980) describe mild to severe responses to boron compounds. Linden et al (1986) have published a retrospective review of 364 cases of boric acid exposure. Vomiting, diarrhea and abdominal pain were the most common symptoms given by the 276 cases exposed.

Boron is also required by animals, including zebrafish, trout (Rowe and Eckhert 1999), and frogs (Fort et al. 1998). Its deprivation causes impaired growth, abnormal bone development, increase in urinary calcium excretion, and change of macromineral status in animals (Devirian and Volpe 2003), also affecting carbohydrate and mineral metabolism, energy consumption, and regulation of the activity of several enzymes; however, the molecular basis of boron function in animals is not well understood (Devirian and Volpe 2003). Excessive boron intake causes acute neurological effects, diarrhea, anorexia, weight loss, and testicular atrophy in mice, rats, and dogs. It also causes decrease in fetal body weight and increase in skeletal malformation and cardiovascular defects in pregnant female animals (Yazbeck et al. 2005; Pawa and Ali 2006). Several investigators have studied the effects of borates on bacteria, protozoa and algae. The effective concentrations for the bacterium *Pseudomonas putida* range widely (Schöberl and Huber 1988; Guhl 1996; Bringmann and Kuhn 1980). Nitrogen-fixing cyanobacteria require boron for proper functioning of the heterocyst cell wall (Bonilla et al. 1990). Mateo et al. (1986) concluded that boron is essential for nitrogen fixation in *Anabaena*.

4 Boron and Plants

Since the discovery of boron as an essential element for plants, evidence has been accumulating that boron is an essential element not only for vascular plants, but also for diatoms, cyanobacteria, and a number of species of marine algal flagellates (Marschner 1995). Initial phase of the studies was based on the symptoms of

boron deprived plants. It is considered to be involved in the metabolism of nucleic acids, carbohydrates and proteins, indole acetic acid, phenol, cell wall synthesis and structure, membrane integrity and function; however, molecular basis of these roles is mostly unknown (Marschner 1995; Goldbach et al. 2001). It is an essential micronutrient for higher plants, with interspecies differences in the levels required for optimum growth and plays an important role in some plant functions such as metabolic pathways, uptake of Ca^{2+} , sugar translocation, pollen germination, hormone action, root development, flower and fruit formation, normal growth and functioning of the apical meristem, water translocation from roots to the upper portions of the plant body and membrane structure and function (Abdulnour et al. 2000; Liu et al. 2000; Lou et al. 2001). Nobel (1981) studied the effect of several boron compounds on photosynthesis in submerged macrophytes, watermilfoil (*Myriophyllum alterniflorum*), buttercup (*Ranunculus penicillatus*) and waterweed (*Elodea canadensis*).

Early investigation of the effects of boric acid and borax on the field bean (*Vicia faba*) and other plants indicated the role of boron in plant nutrition (Ayvaz 2002). There is an overlap of the beneficial and injurious effects of boron between species; therefore, three broad categories of tolerance (sensitive, semi-tolerant, and tolerant) have been established (Ayvaz 2002). The sensitive species can tolerate 0.5 mg L^{-1} of boron but tolerant species can tolerate up to 4 mg L^{-1} (Batar et al. 2009). Plants in general use less than 5% of boron in the soils (Uygan and Çetin 2004). The tolerant plants endure a wide range of boron concentrations with little effect, and the sensitive plants exhibit a strong reaction to either too much or too little boron. Phytoremediation is the use of plants to make soil contaminants non-toxic and is one form of bioremediation. The term phytoremediation generally refers to phytostabilization and phytoextraction. In phytostabilization, soil amendments and plants are used to alter the chemical and physical state of the heavy metal contaminants in the soil. In phytoextraction, plants are used to remove contaminants from the soil and are then harvested for processing.

Boron is an essential element for higher plants. Many studies have shown that certain boron concentrations are necessary for biochemical, physiological and morphological development of plants. Our studies revealed that boron is an essential requirement for maize. The growth rate of radicle and genomic stability increased at 10 mg L^{-1} boron concentration. Similar findings have been reported by Kocacaliskan and Olcer (2006) and Konuk et al. (2007). Boron toxicity may limit crop productivity in boron rich agricultural soils. In dry seasons/conditions, boron supply to roots is reduced due to reduced mass flow from soil to the root (Shorrocks 1997).

In many countries, the absence of B in the soil causes deficiency problems in plants (Shorrocks 1997). However, in Turkey high levels more commonly end up in the toxicity (Ataslar et al. 1995). According to Ayvaz (2002) and Kekeç (2008) the symptoms of boron deficiency in plants include cessation of root and leaf growth, necrosis of leaf primordia and primary root tips, necrosis of stem and leaf phloem, bark splitting, retardation of enzyme reactions, reduced pollen germination, and even death. Normal growth will usually resume if boron is added to the growth

medium. A boron-deficient nutrient solution also inhibits mitosis in the root tip of the field bean. A 10 mg L^{-1} boron solution produces optimum cell division and elongation of the root tip; however, 50 mg L^{-1} boron causes a reduction in mitosis. The studied on the effects of boron deficiency and toxicity in *Pinus radiata* seedlings grown in water culture have revealed that profound changes occur in cell wall morphology, suggesting that boron is critical to cell wall expansion (Cakmak and Römheld 1997). It has been proposed that this structural, cross-linking function of boron is involved with the pectin fraction, which contains apiose and other hydroxylated fragments amenable to complexation by borate (Loomis and Durst 1992). Hu et al. (1996), studied the fourteen species of crop plants, and it was concluded that high pectin content requires more boron for forming cell walls or that pectin forms a tightly held boron complex that depletes boron availability for other critical functions, thereby increasing the overall demand for boron. Kobayashi et al. (1996) have isolated and characterized a rhamnogalacturonan II/borate complex from enzyme-digested cell wall pectin.

Recently, one of the primary functions of boron in higher plant has been reported at the molecular level. It cross-links pectins in cell walls, and this cross-linking is essential for normal expansion of leaves. Pectins, important components of plant cell wall, are complex polysaccharides, including homogalacturonans and rhamnogalacturonans I and II (RG-I and RG-II). It was demonstrated that the RG-II is cross-linked by a 1:2 borate-diol diester and forms the dimeric RG-II (Kobayashi et al. 1996). O'Neill et al. (2001, 2004) have demonstrated that the cross-link between RG-IIs formed by borate cis-diol ester bonds is essential for normal leaf expansion through analysis of the *mur1* mutant in *Arabidopsis thaliana*, which has abnormal sugar composition of RG-II. It is clear that this role of boron in cross-linking of pectin is among the number of roles of boron in plants.

4.1 Boron Tolerance, Deficiency and Toxicity in Plants

Boron is of great importance to plants. However, the amount needed is very little. The amount of boron useful for the growth of plants varies between 0.5 and 2.0 mg L^{-1} . Generally the soils containing less than 0.5 mg L^{-1} of boron are poor in terms of boron and boron deficiency symptoms can be observed in the plants. In the soil where the rate of boron is over 2.0 mg L^{-1} there is boron pollution and consequent decrease in production and defects in the products can be seen (Taiz and Zeiger 1991).

Many studies have shown that certain concentrations of boron are necessary for biochemical, physiological and morphological developments (Hale and Orcutt 1987). There is a very narrow range between boron deficiency and toxicity as more than 5.00 mg L^{-1} available boron can be toxic to many agronomic crops. Lack of boron often limits production of forage legumes (alfalfa, clover, trefoil) and some vegetable crops. The tolerant species are Alfalfa, Beet, Cotton, Grain, sorghum, Oat, Sugar beet and Tomato; moderately tolerant species being Barley, Cabbage, Celery, Corn, Squash, Sweet clover and Turnip, and moderately sensitive species are

Broccoli, Carrot, Cucumber, Pea, Pepper, Potato and Radish. The sensitive species are Avocado, Bean, Grape, Grapefruit, Lemon, Orange and Wheat. The growth of *Vicia faba* grown under a medium without boron supplementation is reduced, but a recovery occurs by supplying boron. It is toxic when present at higher concentrations. Thus, it is essential to maintain concentration of boron in media/soil within an appropriate range for maximum yields. In plant, symptom of boron deficiency occurs mainly in growing or expanding organs in the plant body.

Under boron deficient conditions, leaf expansion and root elongation are inhibited. Apical dominance, flower development, and fruit and seed sets are also inhibited under boron limitation. Thus, boron deficiency causes not only the reduction in crop yield, but also the decrease in the quality. According to Stavrianakou et al. (2006), besides inhibition of growth, boron deficiency causes a notable increase in the relative concentration of 'internal' leaf and root phenolic compounds of *Dittrichia viscosa* (Asteraceae). It does not have any negative effect on parameters related to photosynthesis (such as stomatal density, chlorophyll concentration, photosynthetic capacity and intrinsic photochemical efficiency of PS II). As boron is not efficiently remobilized, i.e., boron tends to stay in organs where it is first distributed, it is important to maintain continuous supply of boric acid for efficient agricultural production (Marschner 1995; Shorrocks 1997; Dell and Huang 1997).

In contrast to the deficiency symptoms, typical boron toxicity symptoms occur in the marginal region of mature leaves, and these portions become chlorotic or necrotic. Boron tends to accumulate in old leaves, especially at the margin of leaves. This is because boron is transported along the transpiration streams and accumulates at the end of transpiration stream. Excess boron also reduces crop yield reduction (Yau et al. 1995). Boron toxicity is an important disorder that can limit plant growth on soils of arid and semi arid environments throughout the world. Soil is generally the primary source of trace elements for plants. However, there are exceptions in which toxic concentrations of trace elements in plants, e.g., B, can be traced directly to water from certain wells, or indirectly to land application of drainage water and soil with high B availability (Kubata 1980). However, the adsorbed and solution phases of B in the soil influence potential B toxicity effects observed in the field (Cartwright et al. 1984; Shani and Hanks 1993); and sometimes lead to decreases in crop yields grown in different regions of the world (Cartwright et al. 1986). There is also a very narrow range between boron deficiency and toxicity as more than 5.00 mg L^{-1} available boron can be toxic to many agronomic crops (Nable et al. 1997). The initial symptom of boron toxicity in plants is chlorosis (yellowing) of the leaf tip, progressing along the leaf margin and into the blade. Necrosis of the chlorotic tissue occurs, followed by leaf abscission. Necrosis of the leaf tissue results in a loss of photosynthetic capacity, which reduces plant productivity (Lovatt and Dugger 1984). Pollen germination and pollen tube growth may also be inhibited (Versar Inc. 1975).

Several investigators have shown a direct relationship between the boron content in leaves (foliar) and the severity of the symptoms of toxicity. Gilliam and Watson (1981) conducted an experiment in which Anderson yews (*Taxus media*) were grown in soil at four boron concentrations (0.5 , 5.0 , 25.0 , or 50 mg kg^{-1}).

Symptoms of toxicity were observed when foliar boron accumulation reached concentrations ranging from 85 to 100 $\mu\text{g g}^{-1}$ of dry tissue. The observed symptoms included leaf tip yellowing, followed by necrosis and premature defoliation. Suppression of shoot and root growth was observed at 50 mg boron kg^{-1} soil. Shopova et al. (1981) found that concentrations of 16, 24, and 32 mg boron kg^{-1} soil resulted in a decline in plant development, yellowing of leaves, late flowering, reduction of mitotic frequency in root tip cells, and abnormalities during meiosis in the poppy (*Papaver somniferum*). Kluge and Podlesak (1985) found that symptoms due to boron excess begin to develop on the leaves (leaf tip necroses) of pot-grown spring barley (*Hordeum vulgare*) as soon as the boron content of the leaf tissue reaches 60–80 mg kg^{-1} dry weight. Gestring and Soltanpour (1987) grew alfalfa (*Medicago sativa*) in three soil types amended with sodium borate at rates of 0, 10, 20, and 40 mg boron kg^{-1} . Alfalfa yield was significantly reduced by boron application in both the sandy loam and loam soils; however, no yield reduction was observed in the silt loam soil. Soil extractable boron did not adequately assess boron toxicity, whereas plant boron levels were a more reliable index of toxicity. Sage et al. (1989) exposed the rare serpentine plant (*Streptanthus morrisonii*) to boron (0, 20, 60, 240, 650, 1200, or 2400 $\mu\text{mol L}^{-1}$) via watering. Plants showed mild to moderate toxicity symptoms (older leaves exhibiting chlorosis and necrosis) at boron concentrations of 240 and 650 $\mu\text{mol L}^{-1}$. Glaubig and Bingham (1985) reported significant linear relationships between both soil and leaf tissue boron concentrations and foliar damage in four tree species endemic to California (digger pine, *Pinus sabiniana*; California laurel, *Umbellularia californica*; madrone, *Arbutus menziesii*; bigleaf maple, *Acer macrophyllum*). Under experimental conditions, Shann and Adriano (1988) demonstrated that chronic foliar aerosol exposures of boron produced phytotoxicity in relation to boron accumulation in the leaves. The authors concluded that the visual damage (leaf tip necrosis) resulting from aerosol exposure was identical to that observed from root boron toxicity for all crops tested. Boron deficiencies in terrestrial plants have been reported in many countries. Boron deficiency is more likely to occur in light-textured, acid soil in humid regions, because of boron's susceptibility to leaching.

In general, there is a small range between deficiency and toxicity. However, considerable variation exists between species in their resistance to boron. Species sensitive to boron are known to include citrus, stone fruits, and nut trees; semi-tolerant species include tubers and cereals; and tolerant species include most vegetables. Toxicity due to excess boron is much less common in the environment than boron deficiency. Amongst a wide variety of plant species, the typical visible symptom of B toxicity is leaf burn-chlorotic and/or necrotic patches, often at the margins and tips of older leaves (Bennett 1993; Bergmann 1992). These symptoms reflect the distribution of B in most species, with B accumulating at the end of the transpiration stream. The chlorotic/necrotic patches have greatly elevated B concentrations compared with the surrounding leaf tissues and some species (e.g., barley) show characteristic patterns for different genotypes. In species in which B is phloem mobile (e.g., *Prunus*, *Malus*, *Pyrus*), in which B accumulates in developing sinks rather than at the end of the transpiration stream, the symptoms of toxicity are

fruit disorders (gummy nuts, internal necrosis), bark necrosis which appears to be due to death of the cambial tissues and stem die back (Brown and Hu 1996).

Although the lack of boron in the soil causes some problems in the plants, excess of boron also causes various physical and biochemical problems. These effects cause defects in the fruits and leaves of the plants (Hartmann 1981). According to researches done on the harmful effects of boron in the sunflower and bean fields the yield of sunflower is high at 0.5 mg L^{-1} (418 kg per 1000 m^2) but the yield decreases as the density increases. The yield decreases down to 306 kg per 1000 m^2 at 16 mg L^{-1} . As for the beans the yield is 180 kg per 1000 m^2 at 0.5 mg L^{-1} but goes down to 73 kg per 1000 m^2 at 16 mg L^{-1} (Şener and Özkara 1989).

Genetic variation in response to high concentrations of boron occurs at both the inter- and intra-specific levels. Boron tolerance of bread wheat (Paull et al. 1992), durum wheat (Jamjod 1996), barley (Jenkin 1993) and field pea (*Pisum sativum*) (Bagheri et al. 1996) is controlled by partially dominant nuclear genes. There have been many investigations on inter-specific variation, with each species or genus represented by a single variety (Maas 1987). All of these have identified a wide range in response to boron, either on the basis of plant growth, or the development of toxicity symptoms, or both. The tolerance to boron toxicity not only operates at the level of whole plants, it also operates at the organ and cellular level (Huang and Graham 1990). In recent studies, it has been reported that high pH can limit boron uptake (Baykut et al. 1987; Hu et al. 1996). The tolerance mechanism appears to be under the control of several major additive genes and specific chromosomal locations have been identified for the genes in some species (Nable and Paull 1991; Nable et al. 1997).

4.2 Boron Uptake By Plants

Boron exists in nature (at neutral pH) primarily as undissociated boric acid- $\text{B}(\text{OH})_3$ which is soluble in water and exists a small amount of borate anion, $\text{B}(\text{OH})_4^-$ (Bolanos et al. 2004). Plant takes up boron from soil in the form of boric acid (Brown and Shelp 1997). As a result of being a non charged molecule, boric acid is highly permeable to the lipid bilayers and hence, passage is proportionally dependent on the concentration gradient (Brown and Shelp 1997, Tanaka and Fujiwara 2008). In order to reach the aerial parts of the plant, B needs to load xylem and transported towards the upwards proportional with the transpiration rate. Finally, B accumulates into the destination point, mostly tips and margins of the mature leaves (Brown and Shelp 1997). Uptake is reduced when soil pH increases from 4 to 9 and increases by an increase in the light intensity; the rate of boron absorption rapidly increases at temperatures ranging from 10 to 30°C and is sharply reduced above 35°C (Ayvaz 2002).

Membranes are key players during the transport of the elements, solutes and water and possess ion transporters. Common traits of some elements are their low membrane permeability co-efficiencies that make their membrane transport more difficult. But some molecules such as boric acid which are moderately permeable

need a transporter. Recent studies showed that cells do not just need transporters for low permeability coefficient molecule, they also need transporters for solute, uncharged molecules and water even if, these molecules are permeable and require any energy to transport through the membrane (Alberts et al. 2002). Recent studies with artificial membrane and membranes isolated from different species have shown that the membrane permeability coefficient of boric acid is approximately 10^{-7} . According to this data, permeability of boric acid is much higher than tryptophan, glucose and Cl^- but much lower than glucose and urea. However, this value is changeable according to the type of the membrane, like lipid composition, intracellular pH.

4.3 Molecular Basis of Boron Uptake and Transport

Three mechanisms are known for across-membrane transport of boric acid: (1) passive diffusion across lipid bilayer (Dannel et al. 2000; Nuttall 2000; Dordas and Brown 2000; Frommer and von Wirén 2002; Kuchel et al. 2006 and Takano et al. 2002), (2) active transport by BOR transporter (Tanaka and Fujiwara 2008; Takano et al. 2008; Peres et al. 2002; Takano et al. 2002 and Frommer and von Wirén 2002), (3) facilitated transport by nodulin-like intrinsic protein (NIP) channel. All of these are involved in regulation of boron transport in plants.

The theory for boron uptake was that boric acid only entered in root apoplast (extracellular space) by **passive transport**. However, Nuttall (2000), Dordas et al. (2000) and Dordas and Brown (2000) showed that boron absorption can also occur by **facilitated diffusion**, through transmembrane channels- the aquaporins (Chrispeels et al. 1999). It was believed that boric acid does not require assistance of transporter called aquaporins (Benga et al. 1986; Frommer and von Wirén 2002; Kuchel et al. 2006). The findings of Agre and Kozono (2003) concluded that high permeable molecules/solutes (water, urea, glycerol etc.) can pass through the membrane with both passive diffusion and also channel-mediated transport as the membrane includes several transporters to make a rapid flux of molecules/solutes on two sides of the membrane by transporter proteins such as aquaporins (Fig. 13.1). The discovery of BOR1 (Takano et al. 2002), a boron transporter revealed that it is required for xylem loading. Takano et al. (2006) emphasized that the lower permeability of plant membranes imply the need of membrane proteins to satisfy a plant's demand of boron, especially under boron limitation.

Active transport mechanism of boric acid to the xylem and then towards the aerial parts of the plants has been reviewed at length by Tanaka and Fujiwara (2008) and Takano et al. (2008). According to these investigators the xylem loading of boron is achieved by transporter proteins. The boron absorbed by apoplast first needs to enter the cell (symplast) to reach the xylem due to the Casparian band, an apoplast barrier in the endoderm. When these solutes enter the xylem, they return to the apoplast, since vase elements are made of dead cells. The process in which a nutrient leaves symplast and enters the xylem through an ion-efflux channel is called xylem loading (Peres et al. 2002). BOR1, characterized by Takano et al. (2002), is the first protein linked to boron transport in biological systems and is related to boron

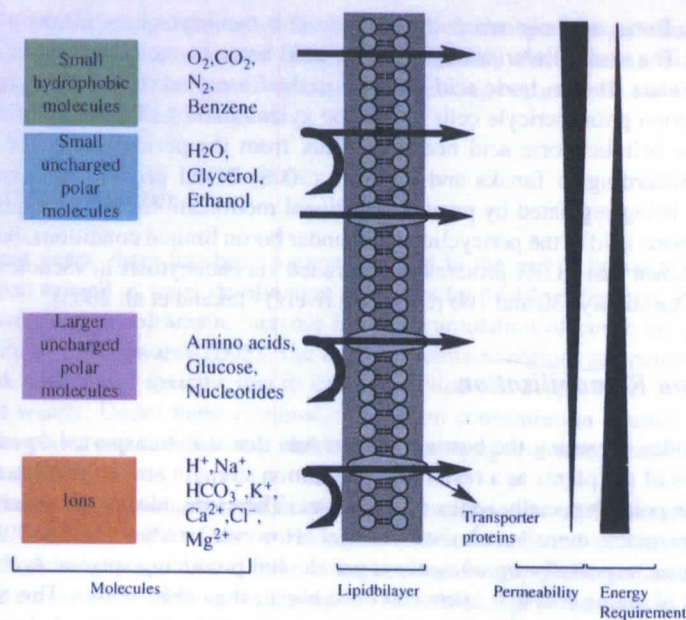


Fig. 13.1 Permeability of biological membranes that allow or prevent the passage of molecules/solutes according to their size, charge, chemical properties, concentration and pressure (Modified from: Alberts et al. 2004)

xylem loading. Among the ten BOR1 hypothetical transmembrane domains, Takano et al. (2002) found a difference of two amino acids in the second transmembrane domain of the putative protein expressed by *Arabidopsis* mutants which requires higher levels of boron. Frommer and von Wirén (2002) suggested that to maintain a boron transport to the xylem, xylem sap requires borate anions. The pH is 5.6 for xylem and 7.5 for cytosole, boric acid inside the cell is converted to borate anion in the cytoplasm because of high cytosolic pH. Therefore boron can easily pass through the membrane as a form of borate anion. Then these borate anions are reconverted in the xylem to boric acid.

Frommer and von Wirén (2002) also proposed three different ways that BOR1 could export borate into the xylem: the first mechanism is diffusion that depends on the concentration gradient for borate (uniport); second is related to borate/chloride exchange coupled to a chloride gradient established by X-QUAC anion channels; and the third one is coupled counter-transport (antiport) of borate with a proton. The proton is exported to the cell wall space by H^+ -ATPases inside which generates a negative membrane potential (Frommer and von Wirén 2002).

NIP5;1 is identified as a boric acid channel that resides on the plasma membrane and requires boric acid uptake under boron limitations for normal growth (Takano et al. 2006). Casparian strip has an active role during the boron transport. It blocks the passage of extracellular boric acid from endodermis to the pericycle. Under boron scarcity conditions, NIPs are translated and reside on the plasma membrane of epidermal, cortical and endodermal cells on root and import of boron into the cells

is limited. Boric acid can reach the pericycle and then xylem by means of these importers. The intracellular passage of boric acid between the cells is sustained by plasmodesmata. Hence, boric acid can pass to the Casparian strip and can reach to the destination point-pericycle cells before the xylem loading (Tanaka and Fujiwara 2008). The cellular boric acid needs to efflux from the pericycle cells for xylem loading. According to Tanaka and Fujiwara (2008) BOR1 proteins are expressed somehow, being regulated by posttranscriptional modifications. BOR1 exports the cytosolic boric acid to the pericyclic region under boron limited conditions, but studies have shown that BOR1 proteins are degraded via endocytosis in vacuoles under excess boron supply (30 and 100 μM respectively) (Takano et al. 2005).

4.4 Boron Remobilization

Common idea regarding the boron transport was that it is transported towards the upper parts of the plants as a result of transpiration strength and accumulates on its destination point especially edges of the leaves. Therefore, ideally the older leaves accumulate much more boron than younger. However, studies indicated that for some species, especially significantly sugar alcohol producing species, boron concentration of young leaves is estimated to be higher than older leaves. This stresses that boron can remobilize from the different portions of plants with the help of sugar alcohols especially species that commonly produce significant amount of sugar alcohols (mannitol and sorbitol). Brown et al. (1999) showed that this remobilization is highly related to the sorbitol synthesis. In the case of enhanced production of sorbitol synthase, transport is significantly increased. Tanaka and Fujiwara (2008) have suggested that boron can move along the flow of boron-binding sugar alcohol.

Recent metabolite study for boron toxicity tolerance in plants has shown that glucose level is increased in leaf at high boron exposure levels (1000 μM) compared to low (5 μM) (Roessner et al. 2006). Reid et al (2004) showed in boron intolerant plants, photosynthesis is suppressed by 23% at a high level of boron. Recently Unver et al. (2008) showed a possible role of photosystem II Protein D2 to regulate the boron toxicity in *Gypsophila perfoliata* by comparing the control and high boron exposed (500–1000 μM) leaves. DDRT-PCR results showed that one of the differentially expressed transcript had high level similarity (99% positive score) in the *Triticum aestivum* Photosystem II protein D2. qRT-PCR analysis showed that 500 and 1000 μM boron treated leaf samples showed 10 and 14 fold changes respectively compared to the control groups (30 μM). Thus boron tolerant plants probably tolerate the toxic effects of boron by remobilizing the excess boron between the leaves by forming sugar-boron complexes through phloem. By reverse reaction, deficiency-tolerant plants might tolerate the boron essentiality with the same mechanism and transportation with the same way as of sugar alcohols. However, non-sugar alcohol producing plants can transport boron preferentially to young tissues as observed in *Arabidopsis* (Noguchi et al. 2000), *Brasica napus* (Stangoulis et al. 2001), and *Helianthus annuus* (Matoh and Ochiai 2005) in case of the limited boron exposures (Tanaka and Fujiwara 2008). It is proposed that non-sugar alcohol producing plants have to activate different mechanism to translocate

boron into the young portions of the plants. Boron transporters and channels may be involved in this translocation (Noguchi et al. 2000). Also Tanaka and Fujiwara (2008) hypothesized that plants are capable of sensing boron levels and regulate the transport under limited conditions.

5 Boron Pollution

In recent years, there has been a great increase in the use of boron at the industrial level as well as water desalination processes for healthy irrigation. The mining processes lead to a dramatic increase in the accumulation of boron in agricultural soils (Parks and Edwards 2005). The arid and semiarid regions are potentially having risk with boron toxicity, due to capillary action and evaporation of boron rich ground waters. Under these circumstances boron concentration reaches to a toxic level for plants and reduces crop yields by polluting agricultural areas (Tanaka and Fujiwara 2008).

Turkey is the important producer of naturally occurring borax fertilizers (Norman 1998). More than 50% of the world boron reserves are found in Turkey (Roskill 1999; Kalafatoglu and Ors 2000). It has become an important and strategic element in terms of developing technologies (Kose et al. 2003; Oren et al. 2006). The proven reserves are 375 million tons, whereas possible reserves are 483 million tons. This is equivalent to the 72.2% of the world reserves (Bayca et al. 2008). These are found in Susurluk, Bigadiç, Sındırgı regions of Balıkesir (Fig. 13.2), Kestelek



Fig. 13.2 Satellite images of Boron mines in Bigadiç, Balıkesir (White spots indicate boron mines)

District of Bursa, Emet District of Kütahya and Kirka District of Eskişehir. The largest reserves are found in Emet, Bigadiç, Kirka and Mustafakemalpaşa Districts (72% of the world boron reserves). These are located in an area of $100 \times 200 \text{ km}^2$. Mines are situated alongside the drainage areas of Simav and M. Kemalpaşa rivers. During the mining processes, boron containing drainage waters, cause pollution of Simav Creek, which is used for the irrigation of nearly 40,000 ha of agricultural area in Balıkesir, Kepsut, Susurluk and Karacabey plains (Şener and Özkara 1989; Uygan and Çetin 2004). The boron carried by the Simav Creek is over 2 mg L^{-1} and threatens the fertile agricultural soils (Şener and Özkara 1989). Watery wastes from the mining areas in general contain 14–18% B_2O_3 which flows in to the collection ponds (Kose et al. 2003). A total of 60,000 tons of wastes are produced every year from the boron extraction mining areas (Batar et al. 2009). The boron concentration in the collection ponds is above the limits given by WHO (Oren et al. 2006). Some work has been done to purify these wastewaters (Kalafatoglu et al. 1997). Very few studies have been carried out on the soil-plant interactions in relation to boron in Turkey. Dündar and Çepel (1979) have reported harmful effects of boron on the leaves of some species in the forest vegetation around Emet (Kütahya) Borax Production Plant. Through the wastewaters of the river Simav the boron is spread to a wide area and causes boron pollution in agricultural soils of this area, rendering the soil infertile (Önel 1981).

Especially in the areas around the boron reserves in Turkey industrialization and urbanization have developed dramatically and this pollution can be seen intensively. The wastewater with a high boron content flowing into the rivers like Simav adversely affects the agricultural areas in the region (Şener and Özkara 1989). The washing waters, rich in boron which are released from boron mines are collected in the Çamköy Dam (Fig. 13.3). However, other waters rich in boron from inactive

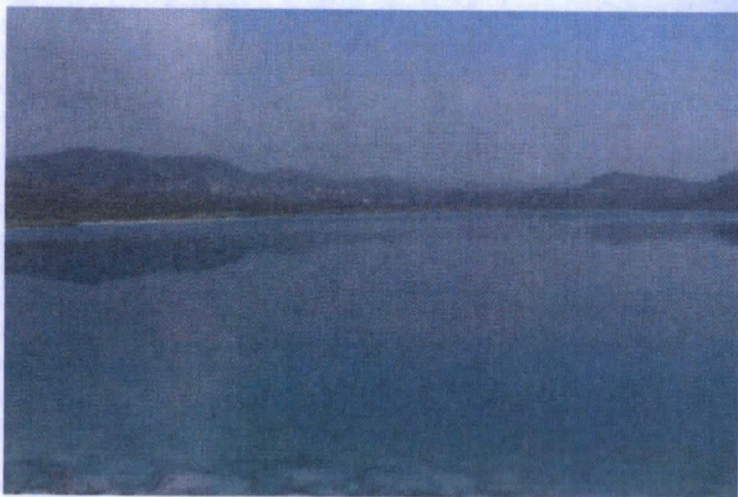


Fig. 13.3 The wastewater from the Boron mines flown into the Çamköy Collector Dam



Fig. 13.4 Boron mines which are not used but cause environmental pollution through rain and underground waters

and closed boron mines are flown into the river Simav which reach the agricultural areas through rain as well as underground waters (Fig. 13.4).

According to Uslu and Türkmen (1987) boron levels recommended for permanent usage should be up to 0.75 mg L^{-1} , and 2 mg L^{-1} for short term usage. The samples taken from Simav Creek and its environs in Bigadiç showed boron levels as 22.56 (open mine surface water); 22.85 (Çamköy Dam water); 23.07 (water taken after ore washing); 23.07 (water from collected pools); 11.35 (water from Simav River); 1.64 (water from the Simav River-500 m away from the mine); and 16.89 mg L^{-1} (open mine surface water). Soils associated with these reserves are high in boron and host a plant diversity with tolerance to high levels of boron.

The natural plant cover of the boron mining areas around Kirka-Eskişehir is represented by the taxa like (Türe and Bell 2004); *Gypsophila perfoliata* L. var. *perfoliata*. *Catapodium rigidum* (L.) C.E. Hubbard ex Dony subsp. *rigidum* var. *rigidum*; *Juniperus oxycedrus* L. subsp. *oxycedrus*; *Adonis flammea* Jacq.; *Glaucium leiocarpum* Boiss.; *Papaver rhoeas* L.; *Hypocoum imberbe* Sibth. & Sm.; *Alyssum pateri* Nyâr. subsp. *pateri*; *Reseda lutea* L. var. *lutea*; *Chenopodium album* L. subsp. *album* var. *album*; *Melilotus officinalis* (L.) Desr.; *Medicago sativa* L. subsp. *sativa*; *Potentilla recta* L.; *Carduus nutans* L. subsp. *nutans*; *Centaurea solstitialis* L. subsp. *solstitialis*; *Centaurea depressa* Bieb.; *Centaurea virgata* Lam.; *Tragopogon latifolius* Boiss. var. *angustifolius* Boiss.; *Convolvulus lineatus* L.; *Quercus trojana* P. B. Webb. T; *Galium verum* L. subsp. *verum*; *Allium atroviolaceum* Boiss.; *Aegilops cylindrica* Host.; *Aegilops triuncialis* L. subsp. *triuncialis*; *Hordeum distichon* L.; *Hordeum murinum* L. subsp. *leporinum* (Link) Arc. var. *leporinum*; *Chrysopogon gryllus* (L.) Trin.; *Stipa lessingiana* Trin. & Rupr.; *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe; *Neslia apiculata* Fisch.; *Matthiola longipetala* (Vent.) DC. subsp. *longipetala*; *Helianthemum canum* (L.)

Baumg.; *Polygala pruinosa* Boiss. subsp. *pruinosa*; *Dianthus crinitus* Sm. var. *crinitus*; *Paronychia carica* Chaudhri; *Hypericum avicularifolium* Jaib. & Spach. subsp. *depilatum*; *Linum hirsutum* L. subsp. *anatolicum* (Boiss.) Hayek var. *anatolicum*; *Haplophyllum thesioides* (Fisch. ex DC.) G. Don; *Genista aucheri* Boiss.; *Astragalus vulneraria* DC.; *Coronilla varia* L. subsp. *varia*; *Onobrychis gracilis* Besser; *Sanguisorba minor* Scop. subsp. *muricata* (Spach.) Briq.; *Sedum sartorianum* Boiss. subsp. *sartorianum*; *Eryngium campestre* L. var. *virens* Link.; *Morina persica* L.; *Scabiosa argentea* L.; *Anthemis tinctoria* L. var. *pallida* DC.; *Achillea wilhelmsii* C. Koch.; *Onopordum tauricum* Willd.; *Jurinea consanguinea* DC.; *Centaurea urvillei* DC. subsp. *stepposa* Wagenitz; *Leontodon asperrimus* (Willd.) J. Ball.; *Asyneuma limonifolium* (L.) Janchen subsp. *limonifolium*; *Asyneuma virgatum* (Labill.) Bornm. subsp. *virgatum*; *Onosma bracteosum* Hausskn. & Bornm.; *Anchusa officinalis* L.; *Anchusa stylosa* Bieb.; *Convolvulus compactus* Boiss.; *Convolvulus holosericeus* Bieb. subsp. *holosericeus*; *Lappula barbata* (Bieb.) Gürke; *Linaria corifolia* Desf.; *Orobancha alba* Stephan; *Acanthus hirsutus* Boiss.; *Globularia orientalis* L.; *Teucrium chamaedrys* L. subsp. *chamaedrys*; *Teucrium polium* L.; *Scutellaria orientalis* L. subsp. *pinnatifida* Edmonson; *Phlomis armeniaca* Willd.; *Marrubium parviflorum* Fisch. & Mey. subsp. *parviflorum*; *Sideritis montana* L. subsp. *montana*; *Stachys byzantina* C. Koch; *Thymus leucostomus* Hausskn. & Velen var. *argillaceus* Jalas; *Salvia sclarea* L.; *Salvia cryptantha* Montbret & Aucher ex Benth; *Acantholimon acerosum* (Willd.) Boiss. var. *acerosum*; *Plantago lanceolata* L.; *Euphorbia macroclada* Boiss.; *Quercus pubescens* Willd.; *Cruciata taurica* (Pallas ex Willd.) Ehrend.; *Asphodelina damascena* (Boiss.) Baker subsp. *damascena*; *Muscari neglectum* Guss.; *Koeleria cristata* (L.) Pers. and *Puccinella convoluta* (Homem.) P. Fourr.

The plant taxa recorded from Bigadiç, Balıkesir are (present study);

Pinus nigra Arn.; *Juniperus oxycedrus* L. ssp. *oxycedrus*; *Delfinium peregrinum*; *Amaranthus retroflexus* L.; *Chenopodium album* L. ssp. *album* var. *album*; *Polygonum lapathifolium* L.; *Polygonum aviculare* L.; *Polygonum equisetiforme* Sibth. & Sm; *Rumex Pulcher* L.; *Quercus ilex* L.; *Quercus pubescens* Willd.; *Silene otites*; *Lavatera punctata*; *Tamarix* sp.; *Sinapis arvensis* L.; *Neslia Apiculata* Fisch.; *Reseda lutea* L.; *Anagallis aquatica*; *Rosa canina* L.; *Malus sylvestris* miller ssp. *orientalis* (A. Uglitzkich) Browicz var. *orientalis*; *Crateagus monogyna* Jacq. ssp. *monogyna*; *Spartium junceum* L.; *Trifolium angustifolium* L. var. *angustifolium*; *Trifolium hybridum* L. var. *hybridum*; *Ononis spinosa*; *Lythrum salicaria* L.; *Pistacia terebinthus* L. ssp. *terebinthus*; *Pistacia vera*; *Ruta montana* (L.) L.; *Tribulus terrestris* L.; *Linum bienne* Miller; *Eryngium campestre* L. var. *visens*; *Eryngium creticum*; *Bupleurum odontites*; *Ammi visagna*; *Bupleurum tenuissimum*; *Papaver rhoeas* L.; *Olea Europea* L. var. *europaea*; *Phillyrea latifolia* L.; *Solanum nigrum* L. ssp. *nigrum*; *Convolvulus arvensis* L.; *Ballota nigra* ssp. *anatolica*; *Mentha spicata* ssp. *spicata*; *Stachys byzantina*; *Teucrium polii*; *Thymbra spicata*; *Plantago major* L.; *Plantago lanceolata* L.; *Rubia tinctorum* L.; *Paliurus spina-christi*; *Viscum album*; *Osyris alba*; *Scabiosa columbaria* L. ssp. *columbaria* var. *Columbaria*; *Dipsacus laciniata*; *Xanthium spinosum* L.; *Pallenis spinosa* (L.) Cass.; *Picnomon acarna* (L.) Cass.; *Carduus nutans* L.; *Centaurea solstitialis* L. ssp. *solstitialis*; *Centaurea iberica* Trev. ex Sprengel; *Centaurea virgata*; *Cardopatum*

corymbosum (L.) Pers.; *Echinops ritro* L.; *Scolymus hispanicus* L.; *Cichorium intybus* L.; *Picris altissima* Delile; *Helminthotheca echinoides* (L.) Holub; *Carthamus Lanatus*; *Xeranthemum annuum*; *Hordeum murium* L.; *Hordeum bulbosum* L.; *Lolium perenne* L.; *Dactylis glomerata* L.; *Cynosurus echinatus* L.; *Phragmites australis* (Cav.) Trin. ex Steudel; *Cynodon dactylon* (L.) Pers.; *Elymus elongatus* ssp. *eloggatus*; *Juncus conglomeratus*; *Cyperus longus* L.; *Draculus vulgaris*; *Ruscus aculeatus* L. var. *angustifolius* Boiss.; *Asparagus acutifolius* L.; *Asphodelus aestivus* Brot.; *Allium neapolitanum* Cyr. and *Tamus communis* L. ssp. *communis*.

The plant diversity of the areas shows variation depending upon the boron content of the soils. The soils with lower boron concentrations (0.1–2 mg kg⁻¹) show a rich species diversity (84 species), whereas those with higher levels (10 mg kg⁻¹) are poor in the plant cover (28 species). According to Babaoglu et al. (2004) only five species *Catapodium rigidum* ssp. *rigidum* var. *rigidum* and *Gypsophila perfoliata* var. *perfoliata* show resistance to boron levels in excess of the accepted toxic levels (35 mg kg⁻¹); these species are reported to flourish in the zone with highest boron concentration. Our investigations revealed that in Bigadiç, Balıkesir boron mining area *Polygonum equisetiforme* was tolerating high levels of boron.

6 Phytoremediation

Plants which uptake high levels of an element from the soil are called hyperaccumulators; these are now being closely investigated, both by molecular techniques and by soil/plant analyses, at the sites where they occur (Karenlampi et al. 2000). The term hyperaccumulator was first used in relation to plants containing more than 1000 µg g⁻¹ (0.1%) Ni in dry tissue (Jaffre et al. 1976; Brooks et al. 1977). A later publication (Baker and Brooks 1989) extended the use of the term to include plants containing more than 1% Zn or Mn, or more than 0.1% Cu, Co, Cr and Pb. The ability of *Thlaspi caerulescens* to accumulate Zn to more than 10,000 µg g⁻¹ (1%) in dry tissue has been known since the 1860s, but it has become apparent from more recent work that several species of this genus can also hyperaccumulate (Reeves and Brooks 1983; Reeves 1988) from metal-rich soils and can hyperaccumulate a wider variety of metals (including Cd, Mn and Co) from amended nutrient solutions (Baker et al. 1994). There has also been recent interest in high-Cd populations of *T. caerulescens* from mine soils (Robinson et al. 1998; Reeves et al. 2001). A recent study of hyperaccumulators for some metals (Zn, Cd, Pb, Ni, Cu, Se and Mn) has been published (Reeves and Baker 2000). This list did not include several other elements, such as B, As and Al. As accumulation by ferns has been studied by Ma et al. (2001), and also Kochian et al. (2002) reported a plant which accumulates 3000 mg kg⁻¹ Al, nevertheless there is not much information about boron accumulation in plants.

Recently, Gezgin et al. (2002) surveyed the boron content of 898 soil samples from 7 States in Turkey. These States include 3.5 million ha of cultivated land in Central Southern Anatolia. However, nearly 50% of soils in these areas contained low levels of available boron which can be corrected by external boron applications

in the form of borax or boric acid. However, another 18% of soils contain boron at more than the critical upper level for available soil born, which is considered to be 3 mg kg^{-1} (Keren and Bingham 1985) for most crops. These areas can be released from this abiotic stress by phytoremediation using boron accumulating species. Soil amendments by conventional techniques such as leaching or increasing pH by liming (Nable et al. 1997) for increased boron adsorption on soil seem not to suit Central Anatolian conditions due to its low annual rainfall and water shortages, and the high lime content of the soils. For this reason, boron accumulating species appear as a solution to this problem.

First hyperaccumulation studies of boron in Turkey were undertaken by Babaoglu et al. (2004) on different taxa of *Gypsophila sp.* commonly growing on the boron rich areas around Kirka, Eskisehir-Turkiye. *Gypsophila sphaerocephala* var. *sphaerocephala*, *G. perfoliata*, *Puccinellia ssp. distans* and *Elymus elongatus ssp. turcicus* species were found in the highest boron containing sections of the mine. Out of these species, *G. sphaerocephala* was able to accumulate extraordinarily high concentrations of boron (Babaoglu et al. 2004). The species were found growing successfully under high total (8900 mg kg^{-1}) and available (277 mg kg^{-1}) soil boron concentrations. *G. sphaerocephala* contained considerably higher boron concentrations in its above-ground parts ($2093 \pm 199 \text{ SD mg kg}^{-1}$, seeds; $3345 \pm 341 \text{ SD mg kg}^{-1}$, leaves), compared to the roots ($51 \pm 11 \text{ SD mg kg}^{-1}$) and organs of the other species.

We also determined a boron tolerant species during our studies undertaken during 2000–2003 namely; *Polygonum equisetiforme*, which showed luxuriant growth over boron mining areas in the Balikesir region. It appears to us as one of the candidates as for phytoremediation of boron contaminated soils. It is a perennial deciduous taxon, with procumbent to erect stems, up to 100 cm tall, and few flowering shoots bearing pink or white flowers and distributed in Canakkale, Istanbul, Izmir, Antalya, İcel and Gaziantep. Water samples were taken from waste water of the collecting dam as well as Simav creek near the mining area.

The samples were collected around the Etibor mining area of Bigadic, Balikesir, one of the richest boron mines in the world. Plant samples along with their representative soils (0–50 cm deep) were collected from the area. Samples of surface soils were collected from pits measuring $20 \times 20 \times 20 \text{ cm}$.

All samples were put into plastic bags and directly brought to the laboratory for analyses. The plant samples were carefully washed with water to remove any traces of soil, then oven-dried at 70°C for 48 h before measuring dry weights. Samples (0.5 g) of finely ground plant material were digested with concentrated HNO_3 in a microwave system (CEM). Boron in the extracts was analyzed by ICP–AES (Varian-Vista model) (Nyomora et al. 1997) in at least 4 plant samples with 3 replicates. The boron standard used was from Merck, Germany. The extractable boron concentrations in soil were determined according to the method of Cartwright et al. (1984) by extraction with 0.01 M mannitol plus 0.01 M CaCl_2 using a soil solution ratio of 1:5 and a shaking time of 16 h. Boron extracted was determined by ICP–AES (Bingham 1982). The results of boron content of the soils and plants from the sampling sites is presented in Table 13.1.

Table 13.1 Boron content of the soils and plants from the sampling sites

| Sampling Sites | Boron content (ppm) | | | |
|----------------|---------------------|--------|--------|---------|
| | No | Soil B | SD | Plant B |
| 1 | 6.84 | 0.56 | 150.22 | 2.52 |
| 2 | 6.80 | 0.38 | 112.26 | 1.81 |
| 3 | 6.91 | 1.05 | 156.44 | 3.14 |
| 4 | 6.96 | 0.95 | 155.29 | 2.52 |
| 5 | 6.91 | 0.35 | 144.54 | 1.96 |
| 6 | 6.95 | 0.46 | 150.36 | 4.13 |
| 7 | 6.87 | 0.39 | 146.89 | 2.69 |
| 8 | 6.78 | 0.45 | 147.99 | 3.41 |
| 9 | 6.84 | 0.78 | 151.53 | 2.48 |
| 10 | 6.79 | 0.95 | 160.15 | 1.82 |
| 11 | 6.85 | 0.16 | 154.64 | 2.74 |
| 12 | 6.81 | 1.05 | 156.02 | 2.61 |
| 13 | 1.39 | 0.12 | 146.36 | 1.94 |
| 14 | 6.81 | 0.35 | 146.24 | 1.30 |
| 15 | 6.81 | 0.42 | 153.14 | 2.28 |
| 16 | 1.48 | 0.08 | 145.35 | 1.28 |

7 Boron and Seed Germination

The studies undertaken by us on the germination behavior of bean, chickpea, maize, wheat, barley and tomato revealed that there is a significant difference ($p < 0.001$) between control and 1000 mg L^{-1} boron exposure of seeds. The growth rates and measurements of radicle and plumule lengths were calculated for all crop seedlings in response to different boron concentrations (control, 10, 50, 100, 250, 500, 750, 1000 mg L^{-1}) and hormones (10 mg L^{-1} GA_3 , IAA, ABA, KIN). After seven days of germination, bean root length was 8.6 cm in control. It decreased to 7.1 cm at 10 mg L^{-1} , and increased to 9.2 cm at 50 mg L^{-1} boron. However, the length of radicle decreased gradually to 1.05 cm at the concentrations above 50 mg L^{-1} . The length of plumule was 11.6 cm in control, but decreased gradually to 2.4 cm for increasing boron concentrations (Fig. 13.5).

The chickpea radicle length was 3.5 cm in control and decreased to 1.8 cm at 10 mg L^{-1} boron, but increased to 8.8 cm at 50, 100 mg L^{-1} . For other concentrations, the radicle length decreased gradually to 1.61 cm. The plumule length was 1.8 cm in control but increased to 3.4 cm at 10, 50, 100 mg L^{-1} boron and decreased gradually to 0.5 cm for other concentrations (Fig. 13.6).

The maize radicle length was 20 cm in control. It decreased to 7.5 cm at 10 mg L^{-1} boron, and increased to 13.7 cm at 50, 100 mg L^{-1} . For other concentrations, the radicle length decreased gradually to 2.1 cm. The plumule length was 7.5 cm in control and decreased gradually to 2.1 cm as the boron concentrations increased (Fig. 13.7).

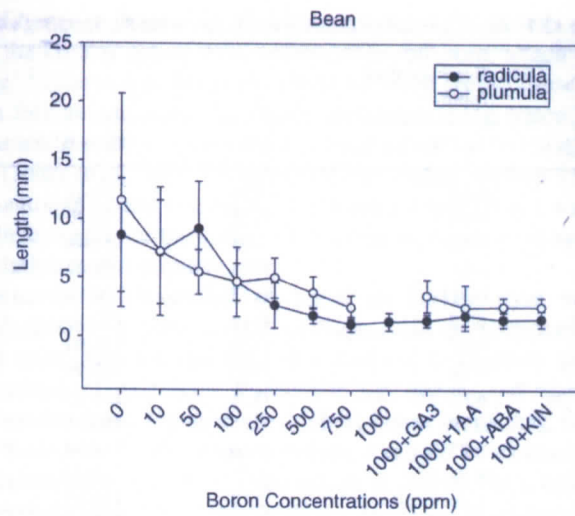


Fig. 13.5 Radicle and plumule length of bean seedlings under different boron concentrations and plant hormones

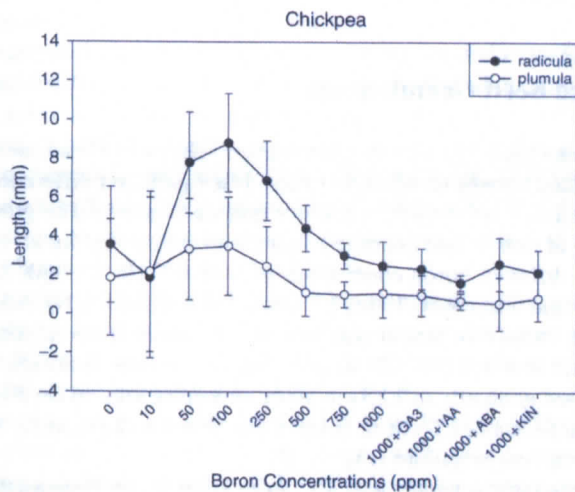


Fig. 13.6 Radicle and plumule length of chickpea seedlings under different boron concentrations and plant hormones

The wheat radicle length was 11 cm in control, increased to 13.2 cm at 10 mg L⁻¹ boron and decreased gradually to 1.4 cm at 50, 100, 250, 500, 750, 1000 mg L⁻¹ and GA₃, but increased to 6.7 cm under IAA, ABA and KIN exposures. The plumule length was 11.3 cm in control. It decreased gradually to 5.6 cm for different boron concentrations (Fig. 13.8).

Fig. 13.7 Radicle and plumule length of maize seedlings under different boron concentrations and plant hormones

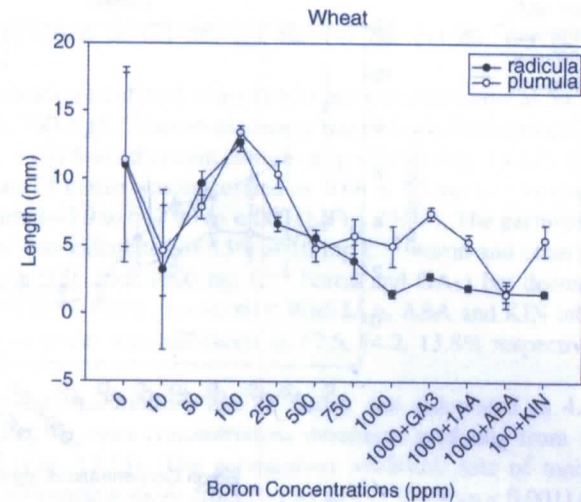
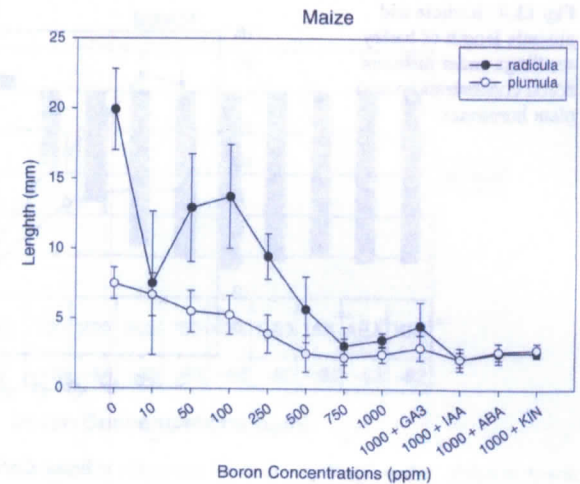


Fig. 13.8 Radicle and plumule length of wheat seedlings under different boron concentrations and plant hormones

The barley radicle length was 14.7 cm in control, increased to 15.4 cm at 10 mg L⁻¹ boron, but decreased gradually to 0.9 cm at other concentrations. The plumule length was 8.2 cm in control. It increased to 8.6 cm at 10 mg L⁻¹, but decreased gradually to 3.5 cm at 50, 100, 250, 500, 750 mg L⁻¹ of boron. It abruptly increased to 7.1 cm at GA₃, it decreased gradually to 1.3 cm under IAA, ABA exposures and abruptly increased to 3.72 cm with KIN (Fig. 13.9).

Fig. 13.9 Radicle and plumule length of barley seedlings under different boron concentrations and plant hormones

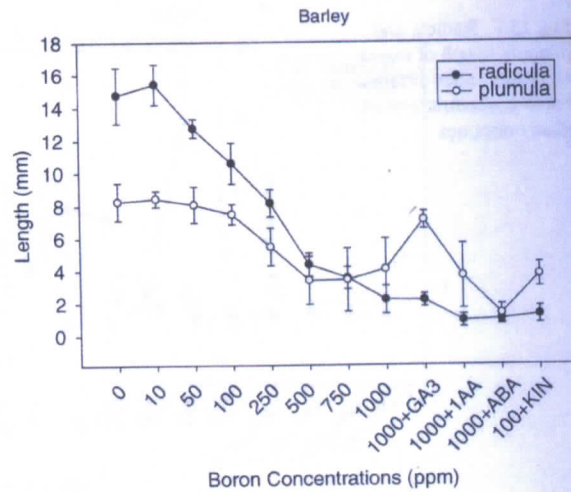
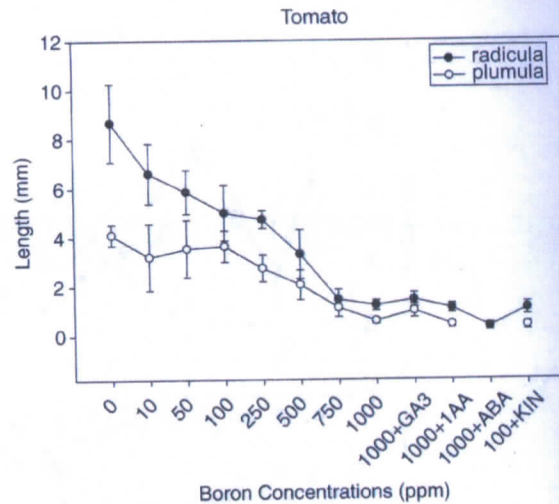


Fig. 13.10 Radicle and plumule length of tomato seedlings under different boron concentrations and plant hormones



The tomato radicle length was 8.7 cm in control. For the following concentrations it decreased gradually to 0.33 cm. The plumule length was 4.1 cm in control and decreased gradually to 0.3 cm under all concentrations (Fig. 13.10).

After seven days of varying amounts of boron and hormone applications, at 50 mg L⁻¹ germination inhibitory rate in beans was calculated as 7%, at other concentrations it decreased gradually from (-) 19 to (-) 86 (p < 0.001). A highly significant correlation was observed between boron concentrations and inhibitory rates. At 10 mg L⁻¹ GA₃, IAA, ABA and KIN applications the inhibitory

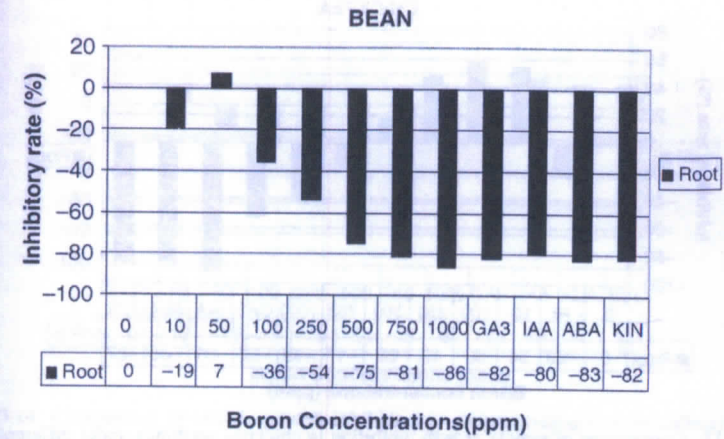


Fig. 13.11 Comparison of radicle growth inhibition in bean seedlings under different boron concentrations

rate was calculated as (-) 86, (-) 82, (-) 80, (-) 83, (-) 82% respectively (Fig. 13.11).

The germination inhibitory rate of chickpea was calculated as 54, 60, 49, 20% at 50, 100, 250, 500 mg L⁻¹ boron exposures respectively. It decreased gradually from (-) 23% to (-) 91% at other concentrations (p < 0.05) (Fig. 13.12). The germination inhibitory rate of maize was calculated as 10% at 50 mg L⁻¹ boron but decreased gradually from (-) 9 to (-) 90 (p < 0.001) (Fig. 13.13). The germination inhibitory rate of wheat was calculated as 13% at 10 mg L⁻¹ boron and other concentrations (50, 100, 250, 500, 750, 1000 mg L⁻¹ boron and GA₃) but decreased gradually from (-) 13% to (-) 87% (p < 0.001). With IAA, ABA and KIN inhibitory rate of germination in wheat was calculated as 17.5, 14.2, 13.8% respectively (p < 0.05) (Fig. 13.14).

The germination inhibitory rate of barley was calculated as 4.4% at 10 mg L⁻¹ boron and the other concentrations decreased gradually from 14 to (-) 94% (p < 0.001) (Fig. 13.15). The germination inhibitory rate of tomato decreased gradually at all concentrations from (-) 31 to (-) 100% (p < 0.001) (Fig. 13.16).

The results confirmed that boron is indeed an essential micronutrient element (at 10 and 50 mg L⁻¹ concentrations) but when it is in excess it is toxic for plants as (Kocacaliskan and Olcer 2006; Konuk et al. 2007). GA₃, IAA, ABA and KIN did not alleviate the boron induced growth inhibition effect significantly.

8 Boron and Genotoxicity in Plants

Plant mutagenicity bioassays have been in existence for many years. The plant bioassays are now well-established systems, used for screening and monitoring of

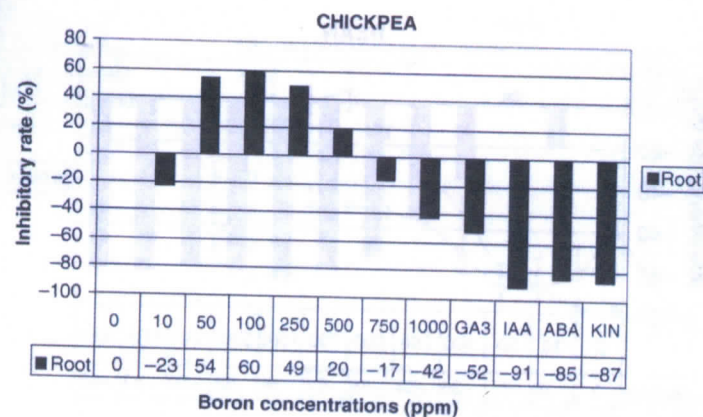


Fig. 13.12 Comparison of radicle growth inhibition in chickpea seedlings under different boron concentrations

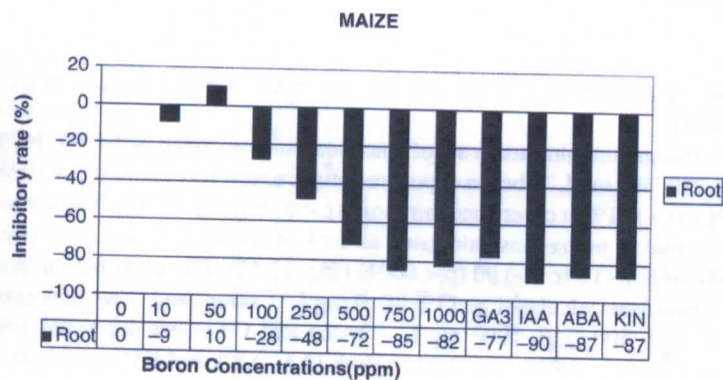


Fig. 13.13 Comparison of radicle growth inhibition in maize seedlings under different boron concentrations

environmental chemicals with mutagenic and carcinogenic potential (Knasmuller et al. 1998; Ma 1999). Genotoxicity of environmental exposures is hard to elucidate by one-way approaches, but requires multi-step methods, both deductive and inductive, at the same environmental design. Most higher plant bioassays are based on the detection of chromosomal aberrations, sister chromatid exchanges, and recently, on the analysis of DNA strand breaks. The cytogenetic tests analyze the frequency and type of chromosome aberrations in mitotic cells and the frequency of micronuclei in interphase cells (Uhl et al. 2003). Several studies have used the comet assay, micronucleus assay or chromosome aberration assay to measure the genotoxic effect of metals on plants (Steinkellner et al. 1999; Angelis et al. 2000). The advantages of measuring effects of genotoxic chemicals directly on DNA are mainly related to the sensitivity and short response time. The advances in molecular biology have led to

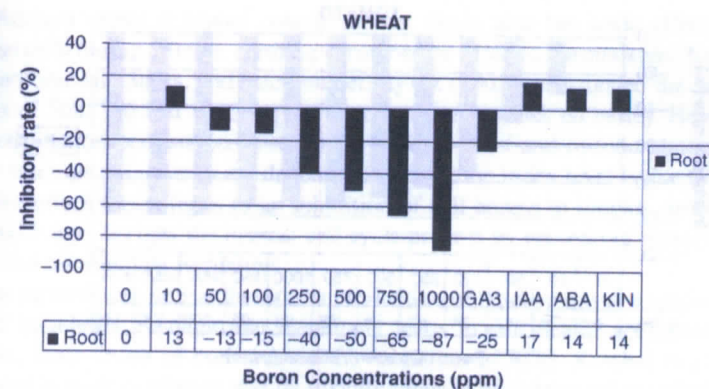


Fig. 13.14 Comparison of radicle growth inhibition in wheat seedlings under different boron concentrations

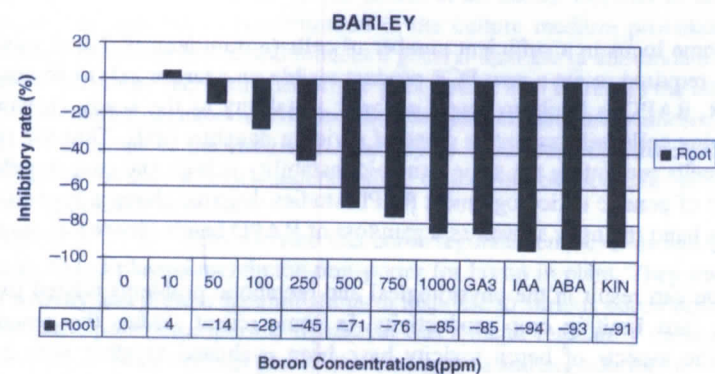


Fig. 13.15 Comparison of radicle growth inhibition in barley seedlings under different boron concentrations

the development of a number of selective and sensitive assays for DNA analysis in the field of genotoxicology. RAPD, developed by Williams et al. (1990) and Welsh and McClelland (1990), is a PCR-based technique that amplifies DNA fragments of genomic DNA with single short primers of arbitrary nucleotide sequence under low annealing conditions. This technique is used extensively for species classification, genetic mapping and phylogeny etc. In addition, their use in surveying genomic DNA for evidence of various types of DNA damage and mutation shows that RAPD may potentially form the basis of novel biomarker assays for the detection of DNA damage and mutational events in cells of bacteria, plants, invertebrate and vertebrate animals (Savva 1996; Savva 1998; Atienzar et al. 2000). RAPD assay has proved useful to detect genomic instability manifested such as point mutations, genetic and chromosomal rearrangements, deletion and insertions (Liu et al. 2005, 2007). Mutations can only be responsible for the appearance of new bands if they occur

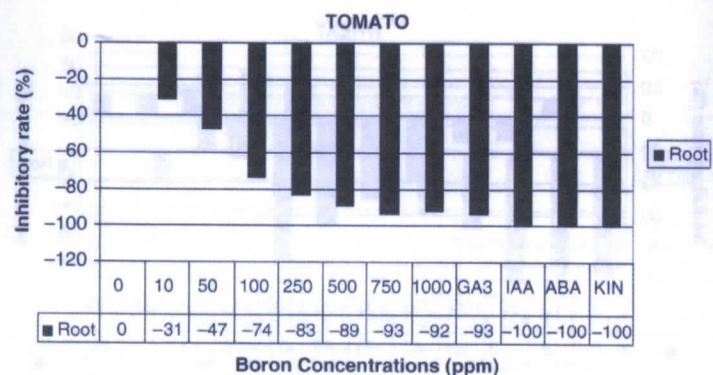


Fig. 13.16 Comparison of radicle growth inhibition in tomato seedlings under different boron concentrations

at the same locus in a sufficient number of cells (a minimum of 2% of mutations may be required to get a new PCR product visible on agarose gel) to be amplified by PCR. RAPD is likely to detect genomic instability as the newly growing and developing cells will produce a clone of dividing daughter cells. Thus the proportion of cells presenting the same genomic instability is high and easy to detect. In the field of genetic toxicology most RAPD studies describe changes such as differences in band intensity as well as a gain/loss of RAPD bands, defined as diagnostic RAPD.

Boron can result in the physiological and metabolic problems related to genotoxicity thus limiting crop productivity. In some recent studies the genetic and epigenetic aspects of boron toxicity have been evaluated together with a reference to the mitotic index in some plant species where mitotic abnormalities have been recorded (Papadakis et al. 2004; Konuk et al. 2007). Konuk et al. (2007) has reported that boron inhibits mitosis in *Allium cepa* at doses of 100 mg L⁻¹ and above. However, according to Karabal et al. (2003) and Cervilla et al. (2007) although boron causes oxidative damage, but its genotoxic effect is still unclear. In some recent studies, leaf cupping, a specific visible symptom of boron toxicity in some species, has been suggested to result from inhibition of cell wall expansion, through disturbance of cell wall cross-links (Loomis and Durst 1992). The nutritional importance and toxic effects of boron on plant growth have been investigated at length in different maize cultivars (Goldberg et al. 2003). These studies revealed that in general boron tolerance of cultivars varied from high to low and boron concentrations of low tolerant cultivars were higher than those of high boron tolerant cultivars. A considerable genotypic variation in susceptibility to boron toxicity has been identified for agronomic species like wheat and barley (Nable and Paull 1991; Paull et al. 1992). Donghua et al. (2000) investigated the effects of boron ions on root growth and cell division of broadbean. The results indicated that boric acid has a stimulatory effect on root growth at concentrations of 10⁻⁶ and 10⁻³ M, and

an inhibitory effect at higher concentrations. Boric acid has toxic effects on the root tip cells during mitosis, forming chromosome bridges, chromosome fragments, chromosome stickiness, and micronuclei. Ayvaz (2002) investigated the genotoxic effects of 500, 750 and 1000 mg L⁻¹ boron concentrations on barley. He recorded the germination percentage, root length, mitotic index and mitotic abnormalities. These findings point out that a decrease in the mitotic index level is due to mitodepressive effect which leads to an inhibition of cell access to mitosis, stressing the fact that boron disrupts the normal cell cycle process by preventing biosynthesis of DNA and microtubule formation.

During oxidative stress, the excess production of reactive oxygen species (ROS) causes membrane damage that eventually leads to cell death. As in most ionic stresses, toxic levels of boron cause the formation of ROS. Karabal et al. (2003) observed in barley cultivars that its toxicity induced oxidative and membrane damage in leaves. Recently it has been reported in apple and grapevine that boron toxicity induces oxidative damage by lipid peroxidation and hydrogen peroxide accumulation (Molassiotis et al. 2006; Gunes et al. 2006). Cervilla et al. (2007) too found that high boron concentration in the culture medium provokes oxidative damage in tomato leaves and induces a general increase in antioxidant enzyme activity, in particular increasing ascorbate pool size. It also increases the activity of L-galactose dehydrogenase, an enzyme involved in ascorbate biosynthesis, and the activity of enzymes of the Halliwell-Asada cycle. This work therefore provides a starting point towards a better understanding of the role of ascorbate in the plant response against boron stress.

Takano et al. (2005) demonstrated that boron regulated endocytosis and degradation of BOR1, a plasma membrane transporter for boron in plant. They monitored BOR1 activity and protein accumulations in response to various boron doses. They found that the posttranscriptional regulation was a major regulatory mechanism in this connection. Their findings proved that endocytosis and degradation of BOR1 are regulated by B availability in order to avoid accumulation of toxic levels of boron in shoots under high-boron supply, while protecting the shoot from boron deficiency under limited boron supply.

9 Conclusion

In conclusion this overview on the interrelations of plants and boron stresses the following points; using plants for phytoremediation should possess (a) targeted metal(s) accumulating capability, preferably in aerial parts; (b) tolerance to the accumulated metal concentrations; (c) fast growth of the metal accumulating biomass; and (d) ease of cultivation and harvesting (Baker and Brooks 1989).

This study has also revealed that the boron concentrations in plants are 20 times more than in the soils around Bigadiç-Balikesir. *Polygonum equisetiforme* appears as a hyperaccumulator of boron. Its wide distribution in the region implies that it can be used for restoration of desertified agricultural lands. Biochemical and molecular studies on this plant will enlighten the mechanisms of growth of hyper-boron

accumulating species on boron rich soils. These findings can be used in the molecular and genetic studies in agricultural plants. This study stresses the fact that this plant can be used to evaluate the boron polluted agricultural soils irrigated by Simav stream which contains high boron levels. In this way more than 3 million ha of boron polluted soils can be again used for agricultural productivity. At the same time it can be used as a fertilizer in the boron poor soils.

Germination results indicate that some of the plants show sensitivity and some are tolerant. For example; in bean the inhibitory rate is (-) 19% at 10 mg L⁻¹ boron whereas it is (-) 86% at 1000 mg L⁻¹, indicating its sensitivity. In chickpea the inhibitory rate was (-) 23% at 10 mg L⁻¹ boron and (-) 42% at 1000 mg L⁻¹, depicting a high tolerance. Our data confirms the fact that maize is a semi-tolerant species. The inhibitory rate of maize is (-) 9% at 10 mg L⁻¹ boron but (-) 82% at 1000 mg L⁻¹. Barley has been reported as a semi tolerant species (Maas 1987) but in our studies it appears percent at 1000 mg L⁻¹. Wheat also has been recorded as a sensitive species but it was reasonably tolerant and growth rate was 13% at 10 mg L⁻¹ boron and (-) 87% at 1000 mg L⁻¹. Finally tomato was highly sensitive, the inhibitory rate was (-) 31% at 10 mg L⁻¹ boron and (-) 92% at 1000 mg L⁻¹ (Fig. 13.17). Bean and tomato are sensitive, maize is semi tolerant, chickpea, wheat and barley are tolerant species on the basis of germination results.

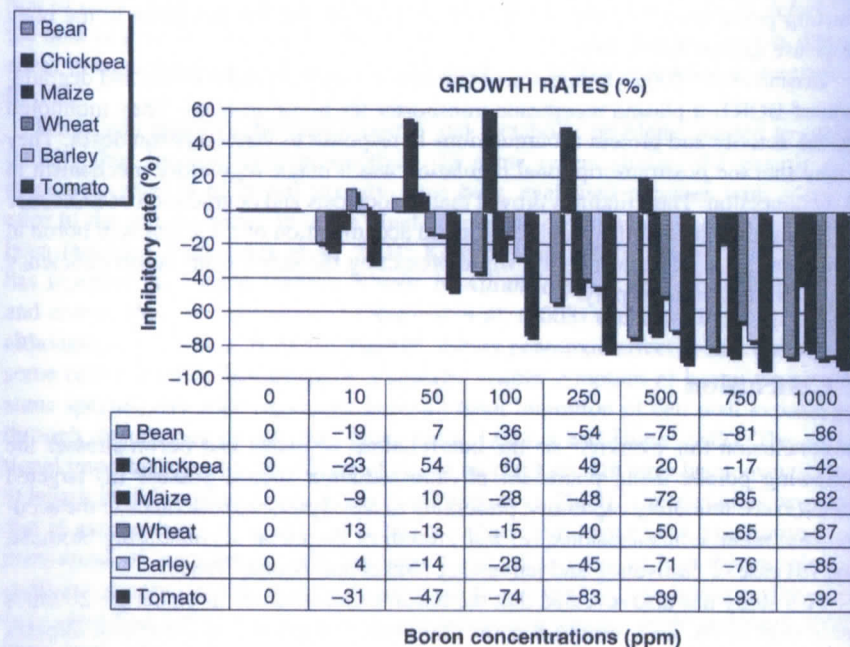


Fig. 13.17 Comparison of radicle growth inhibition in crop seedlings under different boron concentrations

Boron induced polymorphism is higher than many chemicals like mercury, chromium and zinc (Cenkci 2009). The RAPD-PCR method can be used as an investigational tool for boron induced genomic alterations. RAPD-PCR fingerprinting in conjugation with physiological parameters can be a powerful strategy for assessing boron exposure. OPA-08 primer is informative and may have great potential for detecting boron-induced specific genomic alterations, but the nature and amount of DNA impact in RAPD band can only be obtained by sequencing or probing (Atienzar and Jha 2006). Genomic targets of boron exposure should further be assessed with systematic sequencing to make RAPD-PCR assay a quantification method rather than a qualification method.

Changes in the boron-exposed maize genome observed in the present study is mainly variations in RAPD band intensity in the profiles. Short-term treatment with boron did not seem to induce many permanent genomic mutations or changes in oligonucleotide priming sites that would mainly produce new or result in lost RAPD bands. In this study the appearance of new PCR products was detected at 25 mg L⁻¹ and at 50 mg L⁻¹ respectively (Tables 13.2 and 13.3). Appearance of bands may be a result of the genomic instability related to DNA damage. These damages may be induced directly as seen in aflatoxins or indirectly as seen in oxidative stress (Risom et al. 2005). Many studies show that toxic levels of boron influence the excessive production of ROS in different plants (Cervilla et al. 2007; Ardic et al. 2009). Oxidative stress induces ROS production and may cause chromosomal aberrations and DNA damages (Martindale and Holbrook 2002; Risom et al. 2005). The potential for genotoxicity of boron comes either through the production of ROS via oxidative stress or toxicity determination parameters (Beddowes et al. 2003). The RAPD technique is promising for the detection of boron-induced DNA effects but requires further experimentation and validation. The first thing to evaluate should be the innate genetic variation of the organism and then the acquired and additional genotoxic factors.

Table 13.2 Permeability coefficient of boric acid on artificial and natural membranes, isolated from different species

| Permeability coefficient of Boric acid | Organism | Reference |
|----------------------------------------|----------------------------------------------------------------------------------------------------|--------------------------|
| $8 \times 10^{-6} \text{ cm s}^{-1}$ | Theoretical | Raven (1980) |
| $4.9 \times 10^{-6} \text{ cm s}^{-1}$ | Artificial liposome consisting of phosphatidylcoline | Dordas and Brown (2000) |
| $3.9 \times 10^{-7} \text{ cm s}^{-1}$ | Membranes isolated from Squash roots (<i>Cucurbita pepo</i>) – plasma membrane | Dordas et al. (2000) |
| $2.4 \times 10^{-8} \text{ cm s}^{-1}$ | Membranes isolated from Squash roots (<i>Cucurbita pepo</i>) – plasma membrane depleted vesicles | Dordas et al. (2000) |
| $4.4 \times 10^{-7} \text{ cm s}^{-1}$ | Plasma membrane of the giant internodal cells of charophyte alga <i>Chara coralline</i> | Stangoulis et al. (2001) |

Table 13.3 Boron transporter-like protein encoding genes identified in different species

| Organism | Genes | Locus identifier | Reference |
|----------------------------------|----------|------------------|------------------------------|
| <i>Rice (Oryza sativa)</i> | OsBor1 | Os12g37840 | |
| | OsBor2 | Os01g08040 | |
| | OsBor3 | Os01g08020 | |
| | OsBor4 | Os05g08430 | Takano et al. (2005) |
| | AtBOR1 | At2g47160 | |
| <i>Arabidopsis thaliana</i> | AtBOR2 | At3g62270 | |
| | AtBOR3 | At3g06450 | |
| | AtBOR4 | At1g15460 | |
| | AtBOR5 | At1g74810 | |
| | AtBOR6 | At5g25430 | |
| <i>Hordeum vulgare</i> | AtBOR7 | At4g32510 | |
| | AtNIP6;1 | At1g80760 | Tanaka and Fujiwara (2008) |
| | AtNIP5;1 | At4g10380 | Takano et al. (2006) |
| | HvBOR2- | LOC100127239 | Reid et al. (2004); |
| | BOT1 | | Sutton et al. (2007) |
| <i>Triticum aestivum</i> | TaBOR2 | ABX26206 | Zhao and Reithmeier (2001) |
| <i>Physcomitrella patens</i> | PpBOR1 | EDQ69077 | Shelp et al. (1998) |
| <i>Chlamydomonas reinhardtii</i> | PpBOR2 | EDQ75588 | Stangoulis et al. (2001) |
| | BOR1 | EDP05760 | Matoh and Ochiai (2005) |
| <i>Saccharomyces cerevisiae</i> | Atr1 | YML116W | Kaya et al. (2009) |
| <i>Citrus macrophylla</i> | BOR1 | EDN62551 | Takano et al. (2007) |
| <i>Homo sapiens</i> | Bor1 | EF581174 | Canon et al. (unpublished) |
| | NaBC1 | SLC4A11 | Frommer and von Wiren (2002) |

These results may suggest that short-term (1 week) boron treatment induces mainly DNA damage, which causes the specific RAPD band intensity to either increase or decrease. Although our results strongly suggest that boron-induced genomic DNA instability is reflected by the RAPD-PCR method, it is important to note the change of RAPD band patterns do not show a dose-dependent tendency to boron exposure. This might be explained with the short exposure time which may not be enough for the toxic effects to develop. The target tissue for the ultimate genotoxic effects of boron might not be the root tissue, that needs further work to clarify the target tissue of boron. Its concentrations in agricultural soils hardly exceed 1000 mg L^{-1} , however, the accumulation of boron in various plant species can even be above 2000 mg L^{-1} e.g., *Gypsophila sphaerocephala* (Babaoglu et al. 2004) accumulating in leaves. Further studies should focus on the correlation

between the accumulation of boron in indicator species and the target tissues of boron in comparison to genomic instability.

Acknowledgement We would like to thank Dr. Muaviz AYZAZ for his help by allowing us to go through his M.S. Thesis on boron interactions submitted to the Ege University, Institute of Sciences, Bornova, Izmir, Turkey.

References

- Abdulnour JE, Donnelly DJ, Barthakur NN (2000) The effect of boron on calcium uptake and growth in micropropagated potato plantlets. *Potato Res* 43(3):287–295
- Agre P, Kozono D (2003) Aquaporin water channels: molecular mechanisms for human diseases. *FEBS Lett* 555(1):72–78
- Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002) *Molecular biology of the cell*. Garland Science, New York
- Alberts B, Bray D, Hopkin K, Johnson A, Lewis J, Ralf M, Roberts K, Walter P (2004) *Essential Cell Biology*, 2nd edn. Garland Science/Taylor & Francis Group, USA
- Angelis KJ, McGuffie M, Menke M (2000) Adaption to alkylation damage in DNA measured by the comet assay. *Environ Mol Mutagen* 36:146–150
- Ardic M, Sekmen AH, Turkan I, Tokur S, Ozdemir F (2009) The effects of boron toxicity on root antioxidant systems of two chickpea (*Cicer arietinum* L.) cultivars. *Plant Soil* 314:99–108
- Ataslar E, Potoglu I, Tokur S, (1995) Eskisehir Hamidiye'de Yayilis Gösteren Bazi Bitkilerde Bor Degisimi. I. Spil Fen Bilimleri Kongresi, September 4–5. Celal Bayar Üniversitesi, Fen-Edebiyat Fakültesi, Manisa
- Atienzar FA, Jha AN (2006) The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mut Res* 613:76–102
- Atienzar FA, Cordi B, Donkin ME, Evenden AJ, Jha AN, Depledge MH (2000) Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, *Palmaria palmate*. *Aquat Toxicol* 50:1–12
- Ayvaz M (2002) Bazi Arpa Çeşitlerinde Borun Büyüme ve Gelişme Üzerine Etkileri, Yüksek Lisans Tezi, Biyoloji Anabilim Dalı, S. 21–32
- Babaoglu M, Gezgin S, Topal A, Sade B, Dural H (2004) *Gypsophila sphaerocephala* Fenzl ex Tchihat: A boron hyperaccumulator plant species that may phytoremediate soils with toxic B levels. *Turkish J. Bot* 28:273–278
- Bagheri A, Paull JG, Rathjen AJ (1996) Genetics of tolerance to high concentrations of soil boron in peas (*Pisum sativum* L.). *Euphytica* 87:69–75
- Baker AJM, Brooks RR (1989). Terrestrial higher plants which hyperaccumulate metallic elements: a review of their distribution, ecology and phytochemistry. *Biorecovery* 1:81–126
- Baker AJM, Reeves RD, Hajar ASM (1994). Heavy metal accumulation and tolerance in British populations of metallophyte *Thlaspi caerulescens* J. & C.Presl Brassicaceae. *New Phytol* 127:61–68
- Barth RF, Soloway AH (1994) Boron neutron capture therapy of primary and metastatic brain tumors. *Mol Chem Neuropathol* 21(2–3):139–154
- Batar T, Koksall NS, Yersel SA (2009) Production and characterization of wall plaster with borax and paper wastes and perlite additives. *Ekoloji* 18(72):45–53
- Bayca SU, Batar T, Sayin E, Solak O, Kahraman B (2008) The influence of coal ash and tincal (boron mineral) additions on the physical properties and microstructures of ceramic bodies. *J Ceramic Process Res* 9(2):118–120
- Baykut B, Aydin A, Baykut S (1987) Çevre Sorunlari ve Korunma. Güryay Matbaacilik Tic. Ltd. Sti. Istanbul

- Beddowes EJ, Faux SP, Chipman JK (2003) Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress. *Toxicology* 187 (2-3):101-115
- Benga GH, Popescu O, Borza V, Pop VI, Muresan A, Mocsy I, Brain A, Wrigglesworth J (1986) Water permeability of human erythrocytes. identification of membrane proteins involved in water transport. *Eur J Cell Biol* 4:252-262
- Bennett WF (1993) Nutrient deficiencies and toxicities in crop plants. APS Press, St Paul, MN, USA
- Bergmann W (1992) Colour atlas: nutritional disorders of plants. Gustav Fischer, New York
- Bingham FT (1982) Boron. In: Page AL (ed) Methods of soil analysis, part 2: chemical and mineralogical properties, Amer Soc Argon, Madison
- Bolanos L, Lukaszewski K, Bonilla I, Blevins D (2004) Why boron? *Plant Physiol Biochem* 42:907-912
- Bonilla I, Garcia-Gomez M, Mates P (1990) Boron requirements in cyanobacteria: Possible role in the early evolution of photosynthetic organisms. *Plant Physiol* 94:1554-1560
- Bringmann G, Kuhn R (1980) Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. *Water Res* 14:231-241
- Brooks RR, Lee J, Reeves RD, Jaffre T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J Geochem Explor* 7:49-57
- Brown PH, Hu H (1996) Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol-rich species. *Ann Bot* 77:497-505
- Brown PH, Shelp BT (1997) Boron mobility in plants. *Plant Soil* 193:85-101
- Brown PH, Bellaloui N, Hu H, Dandekar A (1999) Transgenically enhanced sorbitol synthesis facilitates phloem boron transport and increases tolerance of tobacco to boron deficiency. *Plant Physiol* 119:17-20
- Butterwick L, De Oude N, Raymond K (1989) Safety assessment of boron in aquatic and terrestrial environments. *Ecotoxicol Environ Saf* 17:339-371
- Cakmak I, Römheld V (1997) Boron deficiency-induced impairments of cellular functions in plants. In: Dell B, Brown P, Bell R (ed) Boron in soil and plants: Reviews; Kluwer Academic Publishers, Dordrecht
- Canon P, Rodriguez A, Arce-Johnson P Molecular cloning and expression analysis of putative boron transporter Bor1 from citrus. <http://www.ncbi.nlm.nih.gov/nuccore/147886438>. Accessed: 31 August 2009
- Cartwright B, Zarcinas BA, Mayfield AH (1984) Toxic concentrations of B in a red-brown earth at Gladstone, South Australia. *Aust J Soil Res* 22:261-272
- Cartwright B, Zarcinas BA, Spouncer LA (1986) Boron toxicity in South Australian barley crops. *Aust J Agric Res* 37:351-359
- Canekci S, Yildiz M, Ciğerci IH, Konuk M, Bozdağ A (2009) Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings. *Chemosphere* 76(7):900-906
- Cervilla LM, Blasco B, Rios JJ, Romero L, Ruiz JM (2007) Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to Boron toxicity. *Ann Bot* 100:747-756
- Chrispeels MJ, Crawford NM, Schroeder JI (1999) Proteins for transport of water and mineral nutrients across the membranes of plant cells. *Plant Cell* 11:661-675
- Cotton FA, Wilkinson G (1988) Advanced inorganic chemistry, 5th edn. Wiley, New York
- Culver BD, Smith RG, Brotherton RJ, Strong PL, Gray TM (1994) Boron. In: Clayton GD, Clayton FE (ed) Patty's industrial hygiene and toxicology. Wiley, New York
- Dannel F, Pfeffer H, Romheld V (2000) Characterization of root boron pools, boron uptake and boron translocation in sunflower using the stable isotopes ^{10}B and ^{11}B . *Aust J Plant Physiol* 27:397-405
- Dell B, Huang L (1997) Physiological response of plants to low boron. *Plant Soil* 193:103-120
- Devirian TA, Volpe SL (2003) The physiological effects of dietary boron. *Crit Rev Food Sci Nutr* 43:219-231

- Donghua L, Wusheng J, Lingxin Z, Lufang L (2000) Effects of boron ions on root growth and cell division of broadbean (*Vicia faba* L.). *Israel J Plant Sci* 48:47-51
- Dordas C, Brown PH (2000) Permeability of boric acid across lipid bilayers and factors affecting it. *J Membr Biol* 175:95-105
- Dordas C, Chrispeels MJ, Brown PH (2000) Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. *Plant Physiol* 124:1349-1361
- Dündar M, Çepel N (1979) Emet Yöresindeki Boraks Maden İşletmeciliğinin Çevredeki Orman Vegetasyonu Üzerine Yaptığı Zararlı Etkiler. Çevre Sorunları-Vejetasyon İlişkileri Sempozyumu, Tübitak Yayınları, Ankara
- Etiproducts (2005) http://www.etimaden.gov.tr/en_a_img/Annual_Report_2005.pdf
- Fort DJ, Propst TL, Stover EL, Strong PL, Murray FJ (1998) Adverse reproductive and developmental effects in *Xenopus* from insufficient boron. *Biol Trace Elem Res* 66:237-259
- Frommer WB, von Wirén N (2002) Plant biology: ping-pong with boron. *Nature* 420(6913):282-283
- Gestring WD, Soltanpour PN (1987) Comparison of soil tests for assessing boron toxicity to alfalfa. *Soil Sci Soc Am J* 51:1214-1219
- Gezgin S, Dursun N, Hamurcu M, Harmankaya M, Önder M, Sade B, Topal A, Soylu S, Akgün N, Yorgancılar M, Ceyhan E, Çiftçi N, Acar B, Gültekin I, Isik Y, Şeker C, Babaoglu M (2002) Determination of boron contents of soils in central Anatolian cultivated lands and its relations between soil and water characteristics. In: Goldbach HE, Brown PH, Rerkasem B, Thellier M, Wimmer MA, Bell RW (ed) Boron in plant and animal nutrition. Kluwer Academic/Plenum Publishers, New York
- Gilliam CH, Watson ME (1981) Boron accumulation in *Taxus media*. *Hort Sci* 16:340-341
- Glaubig BA, Bingham FL (1985) Boron toxicity characteristics of four northern California endemic tree species. *J Environ Qual* 14:72-77
- Goldbach HE, Yu Q, Wingender R, Schultz M, Wimmer M, Findeklee P, Baluska F (2001) Rapid responses of roots to boron deprivation. *J Plant Nutr Soil Sci* 164:173-181
- Goldberg S, Shouse PJ, Lesch SM, Grieve CM, Poss JA, Foster HS, Suarez DL (2003) Effect of high boron application on boron content and growth of melons. *Plant Soil* 256:403-411
- Guhl W (1996) Ecological aspects of boron. *SÖFW J* 118(18/92):1159-1168 (in German)
- Gunes A, Inal A, Alpaslan M, Eraslan F, Bağcı EG, Cicek N (2006) Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J Plant Physiol* 164(6):728-736
- Hale MG, Orcutt DM (1987) The physiology of plants under stress. Wiley, New York
- Hartmann TH (1981) Plant science, growth, development and utilization of cultivated plants. Prentice Hall Inc., Englewood Cliffs
- Hu H, Brown PH, Labavitch JM (1996) Species variability in boron requirement is correlated with cell wall pectin. *J Exp Bot* 295:227-232
- Huang C, Graham RD (1990) Resistance of wheat genotypes to boron toxicity is expressed at the cellular level. *Plant Soil* 126:295-300
- Jaffre T, Brooks RR, Lee J, Reeves RD (1976) *Sebertia acuminata*: a hyperaccumulator of nickel from New Caledonia. *Science* 193:579-580
- Jamjod S (1996) Genetics of boron tolerance in durum wheat. Ph.D. Thesis, The University of Adelaide, South Australia
- Jenkin MJ (1993) The genetics of boron tolerance in barley. Ph D Thesis, The University of Adelaide, South Australia
- Kalafatoglu E, Ors N, Sener S, Yuzer H, Erbil AC (1997) Bor bileşikleri içeren atık suların arıtılması. TUBITAK Marmara Araştırma Merkezi, Gebze-Kocaeli
- Kalafatoglu LE, Ors SN (2000) Yüzyılda bor teknolojileri ve uygulamaları, Kritek, TUBITAK-Marmara Araştırma Merkezi, Gebze-Kocaeli
- Karabal E, Yuçel M, Oktem HA (2003) Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. *Plant Sci* 164:925-933

- Karenlampi S, Schat H, Vangronsveld J, Verkleij JAC, Van der Lelie D, Mergeay M, Tervahauta AI (2000) Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environ Poll* 107:225–231
- Kaya A, Karakaya HC, Fomenko DE, Gladyshev VN, Koc A (2009) Identification of a novel system for Boron transport: *Atr1* is a main boron exporter in yeast. *Mol Cell Biol* 29:3665–3674
- Kekeç G (2008) Effect of Boron pollution on some crops germination, dertermination of modifications in genetic structure by using RAPD method and reducing this effect by using growth hormones. Fatih University, Istanbul
- Keren R, Bingham FT (1985) Boron in water, soils, and plants. *Adv Soil Sci* 1:229–276
- Kliegel W (1980) Boron in biology, medicine and pharmacy: physiological effects and use of boron compounds. Springer-Verlag, Berlin (in German)
- Kluge R, Podlesak W (1985) Plant critical levels for the evaluation of boron toxicity in spring barley (*Hordeum vulgare* L.). *Plant Soil* 83:381–388
- Knasmüller S, Gottmann E, Steinkellner H, Fomin A, Pickl C, Paschke A, God R, Kundi M (1998) Detection of genotoxic effects of heavy metals contaminated soils with plant bioassays. *Mutat Res* 420:37–48
- Kobayashi M, Matoh T, Azuma J (1996) Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. *Plant Physiol* 110:1017–1020
- Kocacaliskan I, Olcer H (2006) Excess boron reduces polyphenol oxidase activities in embryo and endosperm of maize seed during germination. *Z. Naturforsch* 62:111–115
- Kochian LV, Pence NS, Letham DLD, Pineros MA, Magalhaes JV, Hoekenga OA, Garvin DF (2002) Mechanisms of metal resistance in plants: aluminum and heavy metals. *Plant Soil* 247:109–119
- Konuk M, Liman R, Cigerci İH (2007) Determination of genotoxic effect of boron on *Allium cepa* root meristematic cells. *Pak J Bot* 39:73–79
- Kose H, Batar T, Kahraman B (2003) Dünya bor stratejisi ve borun Türkiye için önemi. EGIAD Yayınları, İzmir
- Kubata J (1980) Regional distribution of trace element problems in North America. In: Davies B (ed) *Applied soil trace elements*. Wiley, London
- Kuchel H, Landridge P, Mosionek L, Williams K, Jefferies SP (2006) The genetic control of milling yield dough rheology and baking quality of wheat. *Theor Appl Genet* 112:1487–1495
- Linden CH, Hall AH, Kulig KW, Rumack BH (1986) Acute ingestion of boric acid. *J Toxicol Clin Toxicol* 24:269–279
- Liu D, Jiang W, Zhang L, Li L (2000) Effects of boron ions on root growth and cell division of broad bean (*Vicia faba* L.). *Israel J Plant Sci* 48: 47–51
- Liu W, Li P, Qi X, Zhou Q, Sun T, Yang Y (2005) DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. *Chemosphere* 61:158–167
- Liu W, Yang Y, Zhou Q, Xie L, Li P, Sun T (2007) Impact assessment of cadmium contamination on rice (*Oryza sativa* L.) seedlings at molecular and population levels using multiple biomarkers. *Chemosphere* 67:1155–1163
- Loomis WD, Durst RW (1992) Chemistry and biology of boron. *Biofactors* 3:229–239
- Lou Y, Yang Y, Xu J (2001) Effect of boron fertilization on B uptake and utilization by oilseed rape (*Brassica napus* L.) under different soil moisture regimes. *J Appl Ecol* 12(3): 478–480
- Lovatt CJ, Dugger WM (1984) Boron. biochemistry of the essential ultratrace elements. Plenum Press, New York
- Lyday P (1993) Boron. Department of the Interior, Washington
- Ma TH (1999) The role of plant systems for the detection of environmental mutagens and carcinogens. *Mutat Res* 437:97–100
- Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED (2001) A fern that accumulates arsenic. *Nature* 409:579
- Maas EV (1987) Salt tolerance of plants. In: Christie IBR (ed) *Handbook of plant science in agriculture*. CRC Press, Boca Raton

- Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, San Diego
- Martindale JL, Holbrook NJ (2002) Cellular response to oxidative stress: signaling for suicide and survival. *J Cell Physiol* 192(1):1–15
- Mateo P, Bonilla I, Fernandez-Valiente E, Sanchez-Maeso E (1986) Essentiality of boron for dinitrogen fixation in *Anabaena* sp. PCC 7119. *Plant Physiol* 81:17–21
- Matoh T, Ochiai K (2005) Distribution and partitioning of newly taken-up boron in sunflower. *Plant Soil* 278:351–360
- Mellor JW (1980) Mellor's comprehensive treatise on inorganic and theoretical chemistry. Longman, London, New York
- Molassiotis A, Tanou G, Diamantidis G, Patakas A, Therios I (2006) Effects of 4-month Fe deficiency exposure on Fe reduction mechanism, photosynthetic gas exchange, chlorophyll fluorescence and antioxidant defense in two peach rootstocks differing in Fe deficiency tolerance. *J Plant Physiol* 163(2):176–85
- Nable RO, Paull JG (1991) Mechanism of genetics of tolerance to boron toxicity in plants. *Curr Top Plant Biochem Physiol* 10:257–273
- Nable RO, Banuelos GS, Paull JG (1997) Boron toxicity. *Plant Soil* 198:181–198
- Nobel W (1981) The effect of boron on submerged soft-water macrophytes. *Angew Bot* 55:501–514
- Noguchi K, Dannel F, Pfeffer H, Romheld V, Hayashi H, Fujiwara T (2000) Defect in root-shoot translocation of boron in Arabidopsis thaliana mutant bor1-1. *J Plant Physiol* 156:751–755
- Norman JC (1998) Boron. *Mining Eng* 50:28–30
- Nuttall CY (2000) Boron tolerance & uptake in higher plants. PhD Thesis, Department of Plant Sciences & Gonville and Caius College, University of Cambridge
- Nyomora AMS, Sah RN, Brown PH, Miller RO (1997) Boron determination in biological materials by inductively coupled plasma atomic emission and mass spectrometry: effects of sample dissolution methods. *Fresenius' J Anal Chem* 357:1185–1191
- O'Neill MA, Eberhard S, Albersheim P, Darvill AG (2001) Requirement of borate cross-linking of cell wall rhamnogalacturonan II for Arabidopsis. *Growth Sci* 294:846–849
- O'Neill MA, Ishii T, Albersheim P, Darvill AG (2004) Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annu Rev Plant Biol* 55:109–139
- Önel A (1981) Pollution of some water and soil resources in Marmara region by boron, arsenic and coal and the precautions to be taken. The ministry of Energy and Natural Resources, DSI, Turkey
- Oren Y, Linder C, Daltrophe N, Mirsky Y, Skorcka J, Kedem O (2006) Boron removal from desalinated seawater and brackish water by improved electro dialysis. *Desalination* 199:52–54
- Parks JL, Edwards M (2005) Boron in the environment. *Crit Rev Environ Sci Technol* 35:81–114
- Paull JG, Nable RO, Rathjen AJ (1992) Physiological and genetic control of the tolerance of wheat to high concentrations of boron and implications for plant breeding. *Plant Soil* 146: 251–260
- Papadakis I, Dimassi K, Bosabalidis A, Therios I, Patakas A, Giannakoula A (2004) Boron toxicity in 'Clementine' mandarin plants grafted on two rootstocks. *Plant Sci* 166:539–547
- Pawa S, Ali S (2006) Boron ameliorates fulminant hepatic failure by counteracting the changes associated with the oxidative stress. *Chem Biol Interact* 25:160(2):89–98
- Peres JM, Bureau F, Neuville D, Arhan P, Bougle D (2002) Inhibition of zinc absorption by iron depends on their ratio. *J Trace Elem Med Biol* 15(4):237–241
- Raven JA (1980) Short and long-distance transport of boric acid in plants. *New Phytol* 84:231–249
- Reeves RD (1988) Nickel and zinc accumulation by species of *Thlaspi* L., *Cochlearia* L., and other genera of the Brassicaceae. *Taxon* 37:309–318
- Reeves RD, Brooks RR (1983) European species of *Thlaspi* L. (Cruciferae) as indicators of nickel and zinc. *J Geochem Explor* 18:275–283
- Reeves RD, Baker AJM (2000) Metal accumulating plants. In: Raskin I, Ensley B (eds) *Phytoremediation of toxic metals: using plants to clean up the environment*. Wiley, New York

- Reeves RD, Schwartz C, Morel LM, Edmondson J (2001) Distribution and metal-accumulating behavior of *Thlaspi caerulescens* and associated metallophytes in France. *Int J Phytoremed* 3:145–172
- Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham RD (2004) A critical analysis of the causes of boron toxicity in plants. *Plant Cell Environ* 27:1405–1414
- Risom L, Møller P, Loft S (2005) Oxidative stress-induced DNA damage by particulate air pollution. *Mutat Res* 592:119–137
- Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PEH (1998) The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. *Plant Soil* 203:47–56
- Roessner U, Patterson JH, Forbes MG, Fincher GB, Langridge P, Bacic A (2006) An investigation of Boron toxicity in barley using metabolomics: Environmental stress and adaptation to stress. *Plant Physiol* 142:1087–1101
- Roskill (1999) The economics of Boron. Roskill Information Services Ltd., London
- Rowe RI, Eckhart CD (1999) Boron is required for zebrafish embryogenesis. *J Exp Bot* 202:1649–1654
- Sage RF, Ustin SL, Manning SJ (1989) Boron toxicity in the rare serpentine plant. *Streptanthus morrisonii*. *Environ Pollut* 61:77–93
- Savva D (1996) DNA fingerprinting as a biomarker assay in ecotoxicology. *Toxicol Ecotoxicol News Rev* 3:110–114
- Savva D (1998) Use of DNA fingerprinting to detect genotoxic effects. *Ecotoxicol Environ Safety* 41:103–106
- Schöberl P, Huber L (1988) Ecologically relevant data of non-tenside compounds in detergents and cleaners. *Tenside Deterg* 25:99–107 (in German)
- Shani Y, Hanks RJ (1993) Model of integrated effects of boron, inert salt, and water flow on crop yield. *Agron J* 85:713–717
- Shann JR, Adriano DC (1988) The chronic exposure of selected crop species to boron aerosols. *Environ Exp Bot* 28:289–299
- Shelp BJ, Kithaka AM, Vanderpool RA, Cauwenberghe ORW, Spiers GA (1998) Xylem to phloem transfer of boron in broccoli and lupin during early reproductive growth. *Physiol Plant* 104(3):533–540
- Shopova M, Petrovska D, Musalevski A, Najcevska C, Sekovski Z (1981) Cytogenetical and morphological effect of general toxicity caused with different concentrations of boron. *Mutat Res* 85:229
- Shorrocks VM (1997) The occurrence and correction of boron deficiency. *Plant Soil* 193:121–148
- Stangoulis JCR, Reid RJ, Brown PH, Graham RD (2001) Kinetic analysis of boron transport in *Chara*. *Planta* 213:142–146
- Stavrianakou S, Liakopoulos G, Karabourniotis G (2006) Boron deficiency effects on growth, photosynthesis and relative concentrations of phenolics of *Dittrichia viscosa* (Asteraceae). *Environ Exp Bot* 56:293–300
- Steinkellner H, Kassie F, Knasmüller S (1999) Tradescantia-micronucleus assay for the assessment of the clastogenicity of Austrian water. *Mutat Res* 426:113–116
- Stokinger HE (1981) Boron. In: Clayton GD, Clayton FE (ed) *Patty's industrial hygiene and toxicology*, volume 2B: Toxicology, 3rd edn. Wiley, New York
- Sutton T, Baumann U, Hayes J, Collins NC, Shi BJ, Schnurbusch T, Hay A, Mayo G, Pallotta M, Tester M, Langridge P (2007) Boron-toxicity tolerance in barley arising from efflux transporter amplification. *Science* 318(5855):1446–1449
- Şener S, Özkara M (1989) The boron pollution of Simav creek waters and its effects on the soils and agricultural crops of Balıkesir region, *Plants and Pollutants in Developing Countries*, Ege University, Bornova, İzmir
- Taiz L, Zeiger E (1991) *Plant physiology*. The Benjamin/Cummings Publishing Company Inc., Redwood City
- Takano J, Noguchi K, Yasumori M, Kobayashi M, Gajdos Z, Miwa K, Hayashi H, Yoneyama T, Fujiwara T (2002) Arabidopsis boron transporter for xylem loading. *Nature* 21:420(6913):337–340
- Takano J, Miwa K, Yuan L, von Wiren N, Fujiwara T (2005) Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. *PNAS* 102:12276–12281
- Takano J, Wada M, Ludewig U, Schaaf G, von Wiren N, Fujiwara T (2006) The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *Plant Cell* 18(6):1498–1509
- Takano J, Kobayashi M, Noda Y, Fujiwara T (2007) *Saccharomyces cerevisiae* Bor1p is a boron exporter and a key determinant of boron tolerance. *FEMS Microbiol Letts* 267:230–235
- Takano J, Miwa K, Fujiwara T (2008) Boron transport mechanisms: collaboration of channels and transporters. *Trends Plant Sci* 13:451–457
- Tanaka M, Fujiwara T (2008) Physiological roles and transport mechanisms of boron: perspectives from plants. *Pflügers Arch Eur J Physiol* 456:671–677
- Travis NJ, Cocks EJ (1984) *The tincal trail—a history of borax*. Harraps Ltd., London
- Türe C, Bell WR (2004) Plant distribution and its relationship to extractable boron in naturally-occurring high boron soils in Turkey. *Israel J Plant Sci* 52:125–132
- Uhl M, Plewa M, Majer BJ, Knasmüller S (2003) Basic principles of genetic toxicology with an emphasis on plant bioassays. In: Maluszynska J, Plewa M (ed) *Bioassays in plant cells for improvement of ecosystem and human health*. Katowice: Wydawnictwo Uniwersytetu Śląskiego
- Unver T, Bozkurt O, Akkaya MH (2008) Identification of differentially expressed transcripts from leaves of the boron tolerant plant *Gypsophila perfoliata* L. *Plant Cell Rep* 27:1411–1422
- Uslu O, Türkmen A (1987) Su Kirliliği ve Kontrolü. T.C. Başbakanlık Çevre Gen Müd Yay Eğt Dizisi 1, Türkiye
- Uygan D, Çetin Ö (2004) Bor'un Tarımsal ve Çevresel Etkileri: Seydisuyu su toplama havzası. In: II. Uluslararası Bor Sempozyumu, Eskişehir, Türkiye, 527–540
- Versar Inc. (1975) Preliminary investigation of effects on the environment of boron, indium, nickel, selenium, tin, vanadium and their compounds. Boron, Versar, Inc. Springfield, Virginia
- Weast RC, Astle MJ Beyer WH (1985) *CRC handbook of chemistry and physics*. CRC Press, Inc., Boca Raton
- Welsh J, McClelland M (1990) Genomic fingerprinting using arbitrarily primed PCR and a matrix of pairwise combinations of primers. *Nucleic Acid Res* 18:7213–7218
- WHO (1998) Boron. In: *Environmental Health Criteria Monograph* 204. World Health Organization, ICPCS, Geneva
- Williams J, Kubelik AR, Livak KJ (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res* 18:6531–6535
- Woods WG (1994) An introduction to boron: history, sources, users and chemistry. *Environ Health Perspect* 102:5–11
- Yan X, Wu P, Ling H, Xu G, Xu F, Hang Q (2006) Plant nutrionomics in China: an overview. *Ann Bot* 98(3):473–482
- Yau SK, Nachit MM, Hamblin J, Ryan J (1995) Phenotypic variation in boron-toxicity tolerance at seedling stage in durum wheat (*Triticum durum*). *Euphatica* 83:185–191
- Yazbeck C, Kloppmann W, Cottier R, Sahuquillo J, Debote G, Huel G (2005) Health impact evaluation of boron in drinking water: a geographical risk assessment in northern France. *Environ Geochem Health* 27:419–427
- Yu Q, Wingender R, Schulz M, Baluska F, Goldbach HE (2001) Short-term boron deprivation induces increased levels of cytoskeletal proteins in *Arabidopsis* roots. *Plant Biol* 3:335–40
- Zhao R, Reinhart AF, Reithmeier (2001) Expression and characterization of the anion transporter homologue YNL275w in *Saccharomyces cerevisiae*. *J Physiol Cell Physiol* 281:C33–C45