

# Chapter 24

## Micronutrient Toxicity and Deficiency



Peter Langridge

**Abstract** Micronutrients are essential for plant growth although required in only very small amounts. There are eight micronutrients needed for healthy growth of wheat: chlorine, iron, boron, manganese, zinc, copper, nickel and molybdenum. Several factors will influence the availability of micronutrients, including levels in the soil, and mobility or availability. Zinc deficiency is the most significant problem globally followed by boron, molybdenum, copper, manganese and iron. Deficiency is usually addressed through application of nutrients to seeds, or through foliar sprays when symptoms develop. There is considerable genetic variation in the efficiency of micronutrient uptake in wheat, but this is not a major selection target for breeding programs given the agronomic solutions. However, for some micronutrients, the concentrations in the soil can be very high and result in toxicity. Of the micronutrients, the narrowest range between deficiency and toxicity is for boron and toxicity is a significant problem in some regions. Although not a micronutrient, aluminium toxicity is also a major factor limiting yield in many areas, usually associated with a low soil pH. Agronomic solutions for boron and aluminium toxicity are difficult and expensive. Consequently, genetic approaches have dominated the strategies for addressing toxicity and good sources of tolerance are available.

**Keywords** Micronutrients · Boron · Aluminium · Deficiency · Toxicity

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## 24.1 Learning Objectives

- Recognizing the symptoms and possible causes of micronutrient deficiency or toxicity.
- An understanding of the agronomic or genetic strategies that can be used to correct the problems of micronutrient deficiency.
- Ability to decide when agronomic or genetic interventions may be needed.

## 24.2 Introduction

Seventeen elements have been identified as essential for healthy plant growth and development. These are usually grouped as major or macro-nutrients and micronutrients based on the amount required by the plants. The major elements and the concentrations (mmol/kg) needed for normal growth are: carbon (C, 40,000), oxygen (O, 30,000), hydrogen (H, 60,000), nitrogen (N, 1000), phosphorus (P, 60), potassium (K, 250), calcium (Ca, 125), magnesium (Mg, 80) and sulphur (S, 30). The demand for micronutrients is much lower reflecting their role in specific biological processes rather than as major building blocks for plant organs: chlorine (Cl, 3.0), iron (Fe, 2.0), boron (B, 2.0), manganese (Mn, 1.0), zinc (Zn, 0.3), copper (Cu, 0.1), nickel (Ni, 0.05) and molybdenum (Mo, 0.001). Several studies indicate that silicon (Si) may be beneficial, but not essential, for wheat production. Other elements, particularly heavy metals such as cobalt (Co), required by legumes, and cadmium (Cd) can be taken up by wheat plants and deposited in the grain and, although they may have little effect on plant growth, they are highly undesirable for human consumption.

Although micronutrients are required in only very small amounts, their absence can have highly adverse effects on healthy growth and, consequently, on yield. In extreme case, the plants will not survive since these nutrients are essential. Low levels of micronutrients in grain will also reduce their nutritive value for humans.

The availability of nutrients for plants can be highly variable and dynamic and is influenced by a range of inputs including fertilizers, pollutants and the chemistry of the soil, in addition to losses through leaching, erosion and removal (harvesting) of plant material. Weathering and solubilisation of rock, soil and organic matter can all lead to the input of metal ions. A dynamic equilibrium will develop between pools of nutrients and the soil solution. This is influenced by the rate of replenishment of ions. The replenishment is also referred to as the capacity factor for a particular soil and the ion activity in the soil solution is called the intensity. The interactions between the capacity and intensity are strongly influenced by the soil pH and soil structure.

In addition to affecting the availability of micronutrients, extremes of soil pH can also lead to nutrient toxicities. Highly acidic soils can lead to Al and Mn toxicity and deficiency in Mo, while alkaline soils will often show B toxicity and Fe, Zn and Mn deficiency. For all micronutrients, there is a range of concentration in the soil

that is ideal for growth; too little will limit growth, while too much can result in toxicity. The major toxicity problems for wheat production, apart from salinity, are due to aluminium, which is not a required micronutrient for wheat growth, and boron, which has the narrowest range of concentration for optimal growth of all micronutrients.

### 24.3 Deficiency

Micronutrient deficiencies can lead to a wide range of alterations in normal plant growth and development. Visual symptoms (Table 24.1) are usually only apparent under extreme deficiency, but mild deficiencies can result in substantial reductions in grain yield. Given the variable role of these elements, the symptoms of deficiency also vary greatly (Table 24.1). There are good images available on the internet for the symptoms of micronutrient deficiencies (see Exercise 24.9.1). The nutritional

**Table 24.1** Micronutrients required for healthy plant growth, their role in plant metabolism and symptoms associated with deficiencies

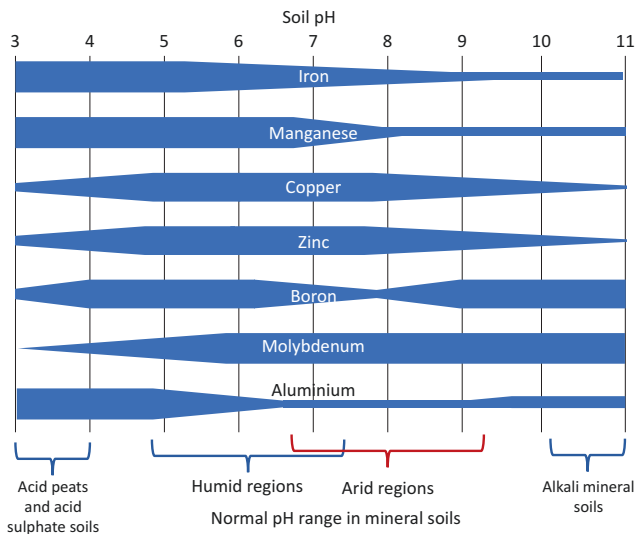
Micronutrient	Pathway	Enzymes	Symptoms
Copper	Electron transport	Ascorbic acid oxidase, tyrosinase, monoamine oxidase, uricase, cytochrome oxidase, phenolase, laccase, and plastocyanin	Unlignified cell walls, permanent wilting and limp leaves
Chlorine	Photosynthetic reactions		Poor germination, chlorosis and necrotic lesions
Manganese	Respiration	Some dehydrogenases, decarboxylases, kinases, oxidases and peroxidases	Reduced sugar and cellulose content, increased drought sensitivity, reduced fertility
Nickel	Unclear	Urease and hydrogenases	Impeded use of nitrogenous fertilisers
Molybdenum	Nitrogen use	Nitrogenase, nitrogen reductase	Nitrogen deficiency, chlorosis and necrosis on leaf margins. Leaves become pale and malformed.
Boron	Cell division, growth and membrane function	Synthesis of uracil, cell wall structure	Problems related to cell wall formation including reduced shoot and root growth, infertility
Zinc	Electron transport and auxin biosynthesis	Alcohol dehydrogenase, glutamic dehydrogenase, and carbonic anhydrase	Intervinal chlorosis, and necrosis particularly in older leaves

Based on information from [4]

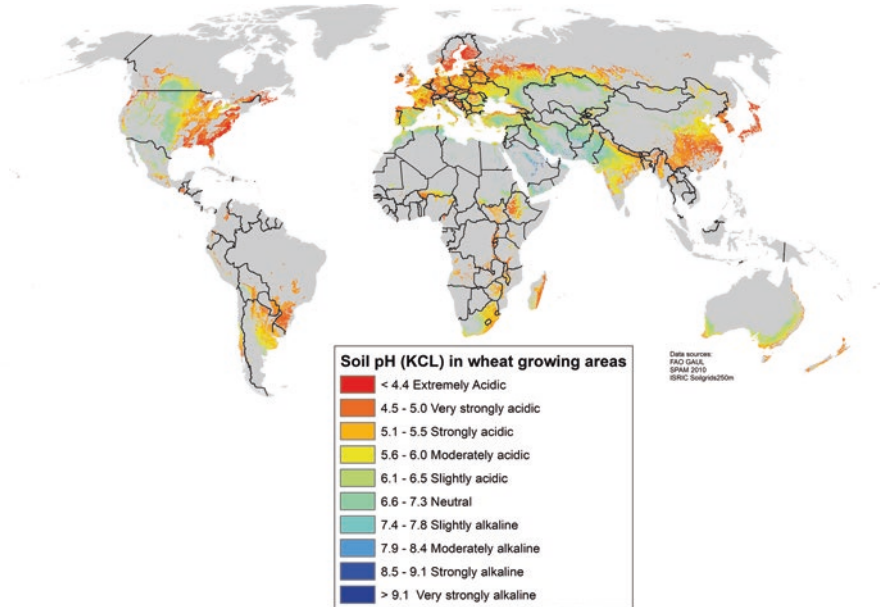
status of the plant will also affect its susceptibility to disease; in some cases, decreasing and in other, increasing disease susceptibility [1]. For example, Mn plays an important role in lignin and phenol biosynthesis and Mn application has been used to control a range of diseases including mildew, take-all and tan spot (for example, Simoglou and Dordas [2]). Zinc has also been found to reduce disease severity, but this may be due to a Zn effect on the pathogen rather than through changes to the plant metabolism [3]. Of the other micronutrients there is little clear evidence of an effect on disease response, although silicon may provide some protection to insect predation [3].

## 24.4 Areas of the World Most Susceptible to Nutrient Deficiencies or Toxicity

Several factors can lead to micronutrient deficiency in plants including low levels of the nutrients in the soil and low mobility or availability of the nutrients due to low solubility in the form required for uptake. Soil-microbe interactions can also influence the availability of the micronutrients. Where free  $\text{CaCO}_3$  is abundant in the soil chemistry, this can fix micronutrient cations, at a high soil pH the solubility of many micronutrients is reduced, and replenishment can be low if there is little organic matter in the soil. The impact of pH on nutrient availability is represented in Fig. 24.1.



**Fig. 24.1** Diagrammatic representation of the relationship between soil pH and micronutrient availability. (Modified with permission from *Plants in Action* [4] <http://plantsinaction.science.uq.edu.au>, published by the Australian Society of Plant Scientists)



**Fig. 24.2** World map showing the soil pH in wheat growing regions. (Dr. Kai Sonder, personal communication based on data from [5, 6])

The importance of soil pH in influencing both micronutrient deficiency and toxicity, is emphasized by the diversity of environments where wheat is grown. In Fig. 24.2 the soil pH in wheat growing regions is illustrated and shows that both high and low pH soil can be found. Acid soils are particularly prevalent in Europe, Eastern USA and southern Brazil while alkaline soils are found around the Mediterranean, the Middle East through to Western India, Northern China and Australia.

Estimating the full impact of micronutrient deficiencies is difficult. Although there has been extensive compositional analysis of soil in some regions, there are many areas where detailed information is lacking. A country level analysis found that “once the macronutrient deficiencies of soils are treated, Sillanpää [7] estimated that of the important agricultural soils of the world, 49% are deficient in zinc (Zn), 31% deficient in boron (B), 15% deficient in molybdenum (Mo), 14% deficient in copper (Cu), 10% deficient in manganese (Mn) and 3% deficient in iron (Fe).”

Globally, zinc deficiency is the most important for wheat production, and is particularly severe in Mediterranean-type and arid environments such as Turkey, Libya, and parts of India and Pakistan. Zn solubility in soils decreases with rising soil pH (Fig. 24.1) and high soil phosphorus can also induce Zn deficiency. Indeed, there is a link between Zn and P uptake, wheat plants under Zn deficiency will increase P uptake to a level that can be toxic [8]. This effect appears to be related to the observation that Zn deficiency up-regulates the expression of high affinity phosphate transporters [9].

Iron deficiency is seen in similar regions to Zn deficiency and occurs in calcareous soils, which cover extensive areas of crop production. As noted above, Fe availability is also strongly influenced by soil pH (Fig. 24.1).

Manganese deficiency can occur in coarse textured alkaline soils where it can be leached out of the soils. This can be a serious issue in regions where wheat is grown in rotation with rice and inundation of the soil can leach Mn into deep soil layers. Molybdenum deficiency is less widespread but can be serious in some regions, for example 44.67 million hectares in winter wheat production areas in China is regarded as Mo deficient [10].

In contrast, boron is a non-metal nutrient that is quite mobile in soils and can leach down the soil profile. Consequently, boron deficiency has been a problem in some humid climates, such as Bangladesh, Thailand and parts of China. Of all the micronutrients, boron has a particularly narrow range between deficiency and toxicity. Deficiency occurs where soluble B (boric acid) is below 0.5 mg/kg and toxicity occur at concentrations higher than 5.0 mg/kg.

## **24.5 Importance of Micronutrient Content of Grain for End Users**

A wide range of factors influence the nutritional quality of the wheat grain and processed products (see Chap. 12). Not surprisingly, the ability of wheat to effectively take up nutrients from the soil will impact on the overall nutrient composition of the plant and the harvested grain. Ensuring a healthy and nutritionally balanced plant, is fundamental to producing nutritious grain for human and other animal consumption. Most effort in elevating micronutrients composition of wheat grain has focused on zinc and iron and, in addition to Chap. 12, there are several good reviews covering this topic (for example, Yu and Tian [11]).

## **24.6 Agronomic Approaches to Addressing Nutrient Deficiency**

There are several options for managing potential micronutrient deficiencies. These include applying micronutrients directly to the soil, as a foliar spray or through seed treatments. Soil fertilization can suffer from problems of nutrient availability and may require high doses of fertilizer. Foliar sprays are generally regarded as the most effective in improving yield and the nutritional status of the grain. An advantage of spraying is that farmers can wait to see if symptoms of nutrient deficiency become visible before spraying but this also means that spraying will occur at late crop developmental stages and this may be too late for some deficiencies to be corrected. Spraying can be high cost and not easily applied for resource poor farmers. Overall,

seed treatment is generally regarded as the best agronomic option for addressing micronutrient deficiency [12].

There are two basic approaches to treating seed to address micronutrient deficiency. A low technology and low-cost approach is known as seed priming, where wheat grains for sowing are soaked in a nutrient solution to partially rehydrate but avoiding allowing germination (reviewed in Farooq et al. [12]). Grain can then be redried to allow storage and transport. The simplicity of this method makes it suitable for on-farm application. Primed seed will usually germinate more rapidly and evenly than un-primed seed. This approach has been successfully used for zinc (use of 0.3% zinc sulphate), boron (0.008 M boric acid), manganese (0.1 M manganese sulphate), and copper (0.1 M copper sulphate) (reviewed in Farooq et al. [12]).

A more sophisticated approach to seed preparation is through seed coating (reviewed in Afzal et al. [13]). In recent years there has been significant improvement in seed coating technologies and, in addition to helping address micronutrient deficiencies, seed coating can also be used to apply fungicides, insecticides, nematicides and biostimulants. Wiatrak [14] evaluated polymer coating combined with a mixture of manganese, copper and zinc. The seed coating improved dry matter yield by 23%, N uptake by 25%, P uptake by 23% and grain yield was 2% higher than the control [14]. Seed coating does require some specialist equipment for the different methods of application: a dry powder applicator, rotary coater or drum coater. Seed dressing with a rotary coater is quite widely used on-farm and offers a simple method for applying micronutrients.

## 24.7 Genetic Approaches to Improving Nutrient Uptake

Nutrient use efficiency is defined as the ability of a cultivar to grow and yield well compared to a standard cultivar in soils deficient in the target nutrient. There does appear to be useful genetic variation in micronutrient efficiency for most micronutrients. Assessment and screening of germplasm has been primarily based on measuring yield of different cultivars in fields known to suffer from specific micronutrient deficiencies. In some cases, controlled environment, greenhouse or growth rooms, or hydroponic systems have been used to evaluate uptake efficiency. Since micronutrients are required in such small amounts, screening can be complicated by the nature of the growth medium being used since very low levels of micronutrients present in water or on equipment can influence the results. Further, the level of micronutrient in the seed used for sowing, will have a significant impact. Careful characterization is needed to ensure that differences observed in the plant performance are indeed related to the target micronutrient or to variation in the nutrient content of the seed used for the experiments. In addition, to considering the chemical and structural properties of the soil, when using soil-based screening methods, it is also important to consider the possible influence of soil microorganisms on micronutrient availability [15]. Advances in genomics technologies has provided an opportunity to explore the diversity of the microbial populations associated with plant roots. The plant-microbe interactions we see in agricultural systems have resulted from

co-evolution of plants and microbes in natural ecosystems and the combination of crop genomics with molecular microbiology offers options for modifying the interactions to improve the sustainability of crop production [16].

The most widely used approach has been to grow out diverse bread and durum wheat accessions in environments known to be deficient in specific micronutrients and assess their performance using plots fertilized with the deficient nutrient as controls. The nutrient content of the plants and the grain is usually also measured to provide an indication of the nutrient uptake efficiency. For example, a screening of 24 genotypes in India under manganese deficient or sufficient (based on foliar sprays) conditions was used to identify lines able to maintain yield under Mn deficient conditions [17]. In this case, grain yield was related to grain Mn content and uptake with Mn efficiency and Mn uptake accounting for 86% and 66% of the yield differences under low Mn [17]. In another screen of 61 cultivars, 18 were identified as inefficient in Mn uptake, 21 as slightly and 11 as moderately efficient [18]. Similar results are seen for molybdenum efficiency with Mo efficient lines yielding 90% while Mo inefficient lines yielded only 50% under Mo deficient conditions compared to the same lines under Mo fertilization [10]. Genotypic variation in performance under boron deficiency based on seed set also ranges from 97% for efficient lines compared to only 11% in inefficient germplasm [19].

Field-based approaches to screening for nutrient efficiency can be complicated by other environmental and edaphic factors. For some micronutrients, pot trials in greenhouses can be used. For example, variations in Mn efficiency can be detected in pot trials by measuring plant biomass accumulation. Hydroponics or a supported hydroponic system can be used in some cases although there can be issues related to differences in root architecture and structure compared to soil grown plants. Shen et al. [20] screened 26 wheat cultivars for variation in responses to iron deficiency using plants grown initially in quartz sand and then transferred to a hydroponic system. This system allowed measurements of a number of physiological and biochemical factors associated with iron uptake and use including siderophore release and resulted in the identification of lines particularly tolerant to iron deficiency [20].

While good variation has been found in wheat germplasm collections for the efficient uptake and utilization of most micronutrients, the level of efficiency offered may not be sufficient to deal with deficiency in some regions. For example, several studies have identified genetic variation in the severity of a number of symptoms associated with copper deficiency [21]. In such cases, there may be an opportunity to explore wild or close relatives of wheat as a source for high efficiency. Cereal rye (*Secale cereale*) has been identified a possible source of high efficiency since it is able to grow well in environments known to be highly deficient in micronutrients. In the case of copper efficiency, a gene on rye chromosome 5RL provided good Cu efficiency when transferred into a wheat background [22].

The genetic control of micronutrient efficiency has been studied primarily from the perspective of enhancing the grain micronutrient content and this is impacted by both the uptake of the micronutrients by the plant and the translocation to the grain (see Chap. 12). Relocation of nutrients to the grain does not appear to be related to specific nutrients since accessions showing good translocation of Zn to the grain also show high levels of other nutrients (Chap. 12). In contrast, the genetic control



of micronutrient uptake appears to be specific for individual micronutrients since germplasm screening has not shown efficiency for multiple micronutrients although this may also be due to the lack of overlap between germplasm pools used in screening.

The broad spread in efficiency seen in germplasm screens, does suggest that efficiency is under complex genetic control, which could be due to multiple loci or high allelic diversity at a small number of loci. Results of genetic studies appear to be contradictory concerning the number of loci influencing micronutrient uptake efficiency. For example, a study of Zn accumulation using genome wide association study (GWAS) found seven loci associated with grain accumulation [23]. This complexity is reflected in the number of genes known to be associated with micronutrient uptake and transport with over 20 genes identified in wheat [24]. In contrast, single major genes have been identified as potential candidates for efficient uptake of copper, chloride and manganese where 42% of the total variation could be explained by a single locus in durum wheat [25].

Overall, our knowledge of the genetic control of micronutrient efficiency is largely based around work aimed at improving the micronutrient content of the grain (see Chap. 12) rather than uptake efficiency. Given the availability of alternative strategies for addressing micronutrient deficiency, largely through seed treatment or dressing, direct selection for micronutrient efficiency in breeding program is a generally a low priority.

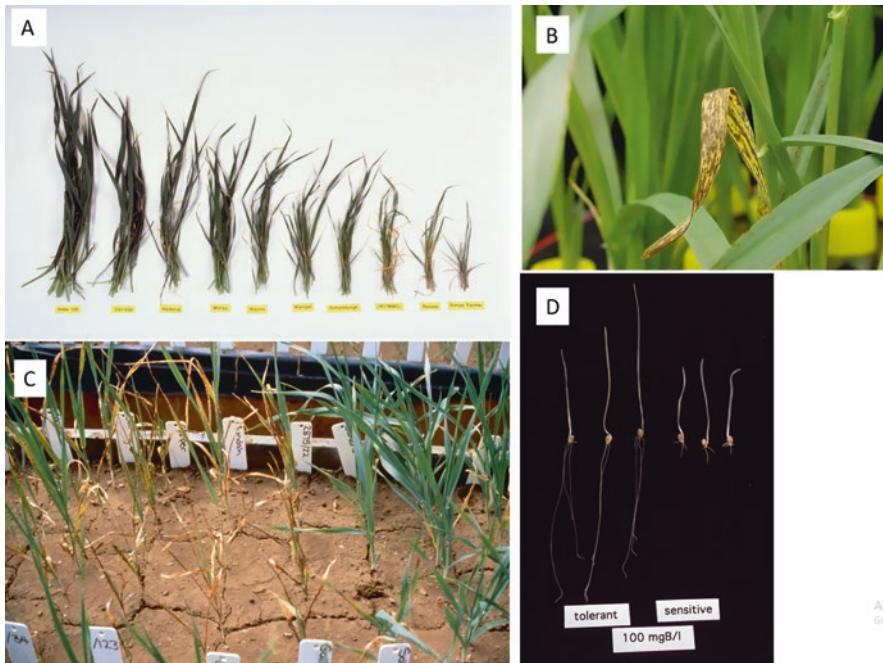
## 24.8 Micronutrient Toxicity

Micronutrient toxicity occurs when the level of soluble nutrients in the soil exceeds a tolerance threshold. The most important micronutrient toxicities are aluminium, boron and manganese, with Al and B the most significant for wheat production areas. Salinity is also a major and increasing problem in many regions but is not regarded as a micronutrient toxicity. In contrast to nutrient deficiencies, there are few management or agronomic options for ameliorating toxicities. In the case of Al toxicity due to soil acidity, liming is an option but is largely used only in wealthy countries. Genetic solutions to micronutrient toxicity problems represent the primary option for control. This is reflected in the extensive work that has been undertaken into the elucidation of the genetic control of toxicity tolerance. For both B and Al tolerance, the genes controlling tolerance have been isolated and their mode of action extensively studied.

Mn toxicity does affect some wheat producing areas where soils are acid and waterlogged or poorly drained. The symptoms of Mn toxicity include reduced growth, interveinal chlorosis, leaf tip necrosis and brown spots on mature leaves [26]. There is genetic variation for Mn toxicity tolerance based on hydroponic screens and screening for tolerant germplasm in a breeding program is feasible [27]. However, Mn toxicity tends to be transient and is not considered a major breeding objective. In contrast, Al and B toxicity tolerance are significant breeding objectives in many wheat growing regions.

### 24.8.1 Boron Toxicity

Boron can accumulate to toxic levels in dry environments on alkaline soils of marine or volcanic origin and, in some cases, as a result of long-term irrigation [28]. The main form of boron in soil solution is as  $B(OH)_3$  or boric acid. Globally, more areas are affected by boron deficiency than toxicity. However, toxicity occurs in many areas where wheat is grown, including, southern Australia, the Middle East from Turkey to Israel, areas in Peru and Chile, parts of Russia and central Asia, and on the ferralsols of India [28]. Boron toxicity symptoms are characterized by leaf necrosis moving from the leaf tips inwards due to the deposition of boron in tissues at the end of the plant transpiration stream (Fig. 24.3b). High soil boron also causes severe root stunting in susceptible lines (Fig. 24.3d). There are very few viable options for ameliorating boron toxicity apart from extensive leaching with low B water [28]. Fortunately, there is good genetic variation for boron tolerance in bread and durum wheat (Fig. 24.3a). In a study in Australia involving an extensive wheat germplasm



**Fig. 24.3** Boron toxicity symptoms and screening. Genetic diversity in boron tolerance is illustrated through the images of leaves from plants grown in high boron soil (a). The lines shown, from left to right, are India 126, G61450 (landraces from India and Greece respectively), Australian cultivars Halberd, Moray, Wyona, Warigul, Schomburgk, WI\*MMC, Reeves and an African landrace, Kenya Farmer. The leaf symptoms of boron toxicity (b) are characterized by necrosis proceeding inward from the leaf tip. Screening for tolerance can be undertaken by growing seedlings in high boron soil boxes (c) or using a hydroponic screen. In boron sensitive lines, high boron severely inhibits root growth (d)

collection grown at 233 sites over 12 years, varieties tolerant to boron were found to yield around 16% more than intolerant genotypes in regions where boron toxicity was known to be a problem [29]. Since symptoms of susceptibility to high soil boron are visible in seedlings, with tolerant lines showing no or reduced symptoms, hydroponic screens (Fig. 24.3d and Exercise 24.9.1) or sowing seeds in seedling trays containing high boron soil (Fig. 24.3c) can be used as simple and rapid screens.

In bread wheat, tolerance is predominantly conferred by the *Bo1* gene which is thought to have originated in wheat varieties in Australia in the early twentieth century. This gene is located on chromosome 7BL in the bread wheat variety Halberd [30] and is also found on 7BL in durum wheat cultivar Lingzhi [31]. A further locus for tolerance was identified in a bread wheat landrace G61450 [32]. The underlying genes have been isolated and characterized [33]. The gene encodes a root-specific boron transporter that appears to function by pumping boron out of the root thereby preventing excess boron from entering the transpiration stream. Interesting, the tolerance locus found in cultivated wheat appears to have arisen via several genomic changes involving tetraploid introgression, dispersed gene duplication, and changes in gene structure resulting in variation in gene expression. The extensive allelic variation seen in the 7BL gene, has resulted in the range in tolerance responses represented in Fig. 24.3a.

A survey of allelic diversity in advanced breeding lines in Australian breeding germplasm, identified the deployment of four different alleles at the *Bo1* locus on 7BL. The allele *Bo1-B5b* was the most widely used in southern Australia where boron toxicity is an issue but was almost completely absent in advanced lines in the Northern regions where the *Bo1-B5g* allele dominated [33]. These results suggest that there is active selection against the boron tolerance allele in regions where soil boron is present at non-toxic levels and this likely reflects the narrow range between deficiency and toxicity for this element.

Through the isolation of the *Bo1* gene and characterization of allelic diversity at this locus, breeders can make use of diagnostic markers to ensure the appropriate level of tolerance or efficiency is present in their breeding lines [33].

### 24.8.2 Aluminium Toxicity

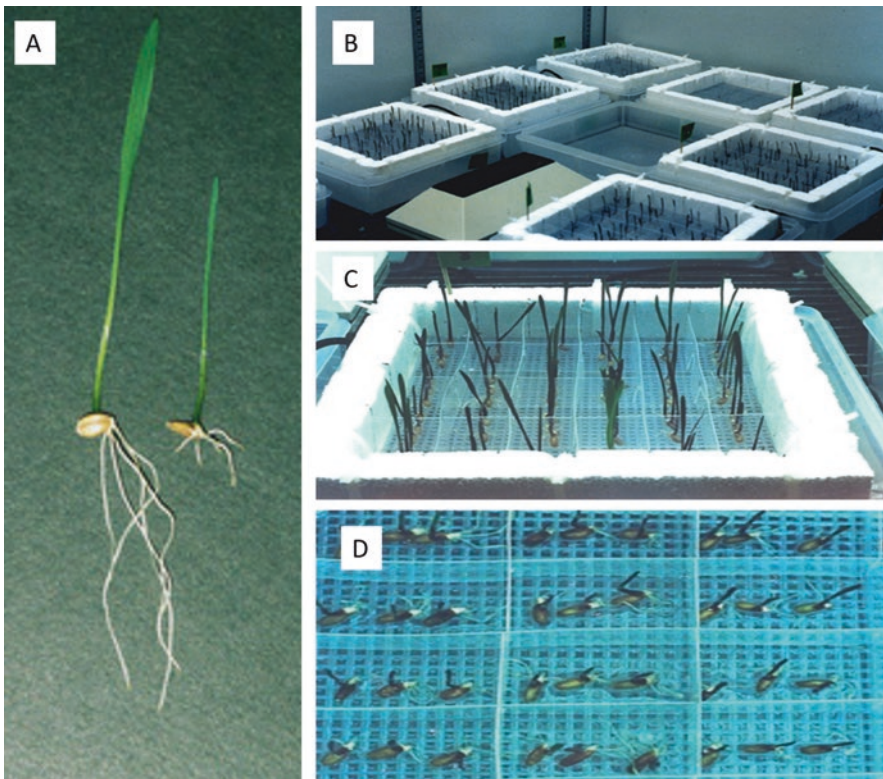
Aluminium is highly abundant in soils and under normal conditions it remains in an insoluble form as Al-oxyhydroxides or as clay minerals. However, at low pH (below 4.5) Al can become soluble as the highly toxic  $Al^{3+}$  cation. In this toxic form, Al can block root growth and severely hinder plant growth and development. Al toxicity is one of the most widespread limitations to crop production and ranks with salinity and water stress in the extent of its effect. Acid soils have been estimated to affect around 30% of the world's cropping area and in many regions, the area affected is increasing as a result of farming practices [34]. In Europe and North America, lime ( $CaCO_3$ ) is widely used to reduce soil acidity. If the pH can be raised to 6 or 7,  $Al^{3+}$  will be insoluble and no longer a problem. However, in poorer regions, particularly

in South America and Sub-Saharan Africa, liming is not an option and soil acidity is a major limitation to production.

Al toxicity primarily affects root growth with strong inhibition of root hair development and root branching (Fig. 24.4a). Seeds will often germinate and appear normal, but as the inhibition of root growth becomes more severe, plants will start to wilt. The strong impact of Al on root growth means that a simple hydroponic screen can be used to identify tolerant germplasm (Fig. 24.4b, c, d). The regions of the root affected by Al are areas where cells are dividing and expanding, around the root tip, and the elongation and root hair zones.

It is important to note that Al can also have a negative impact on the uptake and transport of a range of nutrients in wheat. There is also some evidence that the severity of Al toxicity can be influenced by the uptake efficiency of several nutrients, particularly iron [35].

There is considerable variation in tolerance to Al in both bread and durum wheat although the genetic control differs. The ability of some wheat cultivars to tolerate Al is related to the exclusion of Al from the root tip. A major locus for tolerance is



**Fig. 24.4** Symptoms and screening for Al toxicity tolerance. The severe inhibitory effect of Al on root growth is shown (a). The reduced seedling growth is also apparent. Screening for Al tolerance can be readily undertaken using a hydroponic system shown in b, c and d

found on 4DL of bread wheat but most studies indicate that there are a number of other genes that can also influence the level of tolerance [36]. The gene at the 4DL locus, *TaALMT1*, encodes a transporter protein that serves as a ligand-activated anion channel [37]. This gene is constitutively expressed in root apices at a higher level in tolerant compared in intolerant genotypes. The mode of action is through the release of malate anions from the root apices which appears to chelate the  $Al^{3+}$  in the apoplast to render it harmless [38]. Several studies have explored the location and impact of other genes with possible loci on 5AS, 6AL, 7AS, 2DL and 3DL (reviewed in Ryan [26]). These additional loci may have potential in lifting the level of tolerance in wheat but currently, selection has focused on the *TaALMT1* locus on 4DL.

Ryan (2018) suggested a number of options for increasing the current level of tolerance found in wheat germplasm including the search for novel alleles, given the known diversity at this locus and evidence that rye (*Secale cereale*) has a far higher level of tolerance than its close relative wheat. Pyramiding Al-tolerance loci, and the possibility of using genetic engineering or gene editing to enhance expression of *TaALMT1*, are additional options.

## 24.9 Exercises

### 24.9.1 *Support the Diagnosis of Micronutrient Deficiencies in Wheat*

The internet provides a good resource for identifying the symptoms of micronutrient deficiency. Conduct an image search using the follow terms “wheat” plus “deficiency” plus “symptoms” plus “zinc” or “iron” or “boron”, or “copper” or “nickel” or “chlorine” or “manganese” or “molybdenum”. Assemble the images showing symptoms and prepare a description of the key phenotypes. Focus on the leaf symptoms and try and provide a description that allows differentiation of the symptom’s characteristic for each deficiency.

### 24.9.2 *Establish a Filter-Based System for Screening Wheat Accessions for Tolerance to Boron Toxicity*

A simple procedure is described below for screening wheat accessions for boron tolerance. In selecting germplasm to screen, you will usually find that landraces from the eastern Mediterranean and North Africa and elite germplasm from Southern Australia have some level of tolerance, while European and North American cultivars are quite sensitive. This filter-paper method of screening can also be used to assess aluminium toxicity tolerance.



Use three treatment levels for the solution culture-root length assay at 100 mg B l<sup>-1</sup> (B100), 50 mg B l<sup>-1</sup> and 0 mg B l<sup>-1</sup> (B0). Seedling root lengths of wheat varieties will respond consistently at the concentrations: 50, 100. A control treatment (B0) was included to account for genetic variation of root length in the absence of boron toxicity. Seeds of each line should be surface sterilized with 5.0% sodium hypochlorite and pre-germinated for 8 days at 4 °C in Petrie dishes on filter paper soaked in water. After the 8 days, take three evenly germinated seeds, for each accession, and place these embryo-downwards at a spacing of 2 cm across the middle of filter paper (Ekwip 32 x 46 cm grade R6) soaked in either the B0 or B100 solutions. The base solution used in both the control (B0) and high-concentration treatment (B100) must include 0.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.0025 mM ZnSO<sub>4</sub> and 0.015 mM H<sub>3</sub>BO<sub>3</sub>, following the method of Chantachume et al. [39]. For the B50 and B100 treatments, add the appropriate additional H<sub>3</sub>BO (50 or 100 mg per litre). The filter papers were rolled and covered with aluminium foil, then stored upright at 15 °C for 12 days. After the 12 day period, unroll the filter paper and measure the length of longest root of each seedling. Use the ration of the root length in the controls (B0) to the B50 and B100 treatments as the measure of boron toxicity tolerance.

## 24.10 Key Concepts

- Micronutrients are critical for plant growth and are not always easy to identify. Multiple strategies can be employed to address deficiency or toxicity problems.
- Deficiency is usually managed through seed priming or coating, or foliar sprays when symptoms first show.
- Many studies have identified extensive genetic variation in micronutrient uptake efficiency but use of this germplasm is not a high priority for most breeding program.
- The prime focus of micronutrient uptake and transport has been on enhancing the nutritional value of wheat grains for humans.
- Nutrient toxicity is most appropriately managed through genetic improvement of wheat since agronomic approaches are generally inefficient, short-term and expensive.
- The major genes controlling boron and aluminium toxicity tolerance have been cloned and their mode of action well characterized.

## 24.11 Conclusions

Micronutrients are essential for plant growth and development. There is also good evidence that several micronutrients play an important role in disease responses. Therefore, ensuring wheat plants have access to sufficient levels of all eight micronutrients is critical for production. Extensive genetic variation is known for both

nutrient use efficiency, but agronomic approaches are often effective in dealing with deficiencies. Consequently, breeding for micronutrient efficiency generally takes a low priority relative to the many other traits assessed in a breeding program.

In contrast, breeding represents the main strategy for managing the impact of boron, or aluminium toxicity. Toxicity due to high levels of manganese can also be an issue in some regions but is not regarded as a major international problem for wheat production. Given the importance of boron and aluminium toxicity, there has been considerable effort in identifying sources of tolerance and defining the genetic and biochemical mechanisms of tolerance. The major genes controlling toxicity tolerance have been isolated and allelic diversity explored in large germplasm collections. Diagnostic markers are now available for the major tolerance loci and these are extensively deployed in breeding program that target regions susceptible to boron or aluminium toxicity.

## References

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