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Review

Can we really increase yields by making crop plants tolerant to boron toxicity?

Rob Reid [*]

School of Earth and Environmental Sciences, DX650 312, University of Adelaide, Adelaide 5005, Australia

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ABSTRACT

High boron concentrations in soil and in irrigation water reduce crop productivity in many areas of the world. Plant tolerance to boron toxicity has been identified in a range of genotypes and recent research has revealed a physiological mechanism behind this tolerance in cereals. Cultivars with high levels of expression of a gene encoding a boron-efflux transporter in roots and shoots have been reported to show tolerance to high boron in soils and in solution culture experiments conducted under controlled conditions in glasshouses and growth rooms. However, field trials of tolerant cultivars in rain-fed semi-arid environments have been disappointing with few showing even modest improvements in yield, and others showing either no effect or a decrease in yields.

Contents

1. Introduction

Boron is unusual among plant mineral nutrients in that it exists as an uncharged molecule, boric acid, at physiological pH. For other nutrients, the presence of a positive or negative charge limits membrane permeability and allows a degree of control over influx and efflux through membrane transporters. It is known that boric acid can easily pass directly across phospholipid bilayers [1] and it has recently been shown that fluxes of boric acid can be further accelerated by movement through aquaglyceroporins [2], channel-like proteins that permit the bi-directional movement of small neutral molecules such as arsenite, antimonite, glycerol and urea [3]. This high permeability and lack of control over boron entry into the plant creates problems when soils contain high levels of boron. Like most elements, boron becomes toxic to growth at high concentrations. Fortuitously, far more soils are deficient in boron than contain high boron levels. Deficiency can be easily corrected by foliar or soil fertilisation but high soil boron is problematic. Although boron is highly soluble and can be leached in high rainfall areas, it can persist in agricultural soils when rainfall is low, such as in southern Australia and in parts of the Middle East. In these areas, partial leaching pushes boron down the soil profile where roots then encounter toxicity in their search for water. In other regions where soil boron is only moderate, irrigation can increase boron toxicity if the irrigation water contains high concentrations of boron. Remediation of boron-toxic soils is impractical in most cases so solutions based on improved plant tolerance to boron have been investigated for several decades by plant breeders and more recently biotechnologists. Boron tolerance has been identified in many genotypes, and can be transferred by conventional breeding or molecular means to other cultivars, yet significant improvement in yields on boron-toxic soils have yet to be realised. The reasons behind this phenomenon are explored below.

2. Toxic effects of boron

The essentiality of boron for plant growth is predominantly (perhaps solely) due to its requirement in cell walls where it forms

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[*] Tel.: +61 8 83035290; fax: +61 8 83036222.
E-mail address: robert.reid@adelaide.edu.au.

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a structural component of the rhamnogalacturon II complex [4]. Strong evidence for a role of boron in other structural or metabolic processes is so far lacking. There is also no convincing evidence that toxicity is exerted through disruption of any process for which boron is required, notably its role in cell walls. More likely the toxicity of boron arises from its affinity for several key metabolites. In general terms, boron is relatively unreactive but it can form strong complexes with a handful of metabolites that have multiple hydroxyl groups in the *cis* conformation. Of these, ribose appears to be the most likely candidate for toxicity-related effects. *Cis* hydroxyls are exposed on the ribose moiety of the energy-carrying molecules ATP, NADH and NADPH, but photosynthesis and respiration are largely insensitive to concentrations of boron up to approximately 50 mM [5]. This makes it unlikely that binding to these molecules is strong enough to be the main cause of toxicity since cell division and expansion are inhibited in the range 1–5 mM boron [5]. A number of ribosomal proteins and transcription factors from Arabidopsis, which confer boron tolerance in yeast, have recently been identified [6]. It was suggested that boron interferes with transcription and/or translation by binding to *cis* hydroxyls on ribose molecules that are exposed during gene splicing and in t-RNA, and that these ribosomal proteins and transcription factors may prevent boron from binding, thereby protecting transcription and translation [6].

3. Finding boron transporter genes that confer tolerance to boron toxicity

Over the past 20 years, there has been a vast amount of research devoted to trying to understand why some plants are tolerant to boron in the hope that the tolerance traits can be exploited to improve crop yields on boron-toxic soils. The history of this research and the important advances can be succinctly summarised as follows:

- 1988: Boron-tolerant cultivars of wheat and barley were identified and it was established that tolerance was correlated with reduced tissue accumulation of boron [7,8].
- 1999: Chromosomal regions related to boron tolerance in barley were mapped [9] with the main locus being associated with low tissue boron.
- 2004: The physiological basis for reduced boron accumulation in tolerant varieties was shown to be active efflux of boron from root cells [10]. Boron-efflux transporters that operated under deficiency conditions had been reported [11], but these transporters were degraded under high boron conditions [12].
- 2007: Based on sequence similarities with known boron-efflux transporters from rice, genes encoding boron-efflux transporters in wheat and barley, whose expression was closely correlated with boron tolerance, were reported [13]. It was proposed that the higher expression of the gene in a tolerant barley cultivar was due to amplification of the boron transporter gene [14]. Overexpression in Arabidopsis of a boron-efflux transporter in roots was shown to enhance tolerance [15].
- 2009: Originally, tolerance conferred by the boron-efflux transporter gene was attributed only to reduced root accumulation. An additional mechanism involving redistribution of boron from the symplast into the apoplast in leaves by the same efflux transporters, enhanced tolerance [16].

The end results of this research were the identification of tolerant cultivars, an understanding of a major mechanism of tolerance, and the ability to enhance tolerance by overexpression of genes encoding boron-efflux transporters. Most probably, these transporters are the same as those that normally function at low levels to provide boron for essential apoplastic processes such as cell wall development, but in tolerant cultivars their expression is greatly increased.

4. Laboratory science meets the real world

The superior performance of cultivars with high expression of boron-efflux transporter genes could easily be demonstrated under controlled conditions in a glasshouse and with homogeneous distribution of boron in soil, or in solution culture without other constraints such as salinity. However, field trials in rain-fed semi-arid environments that now extend over 10 years have failed to establish any substantial or consistent improvements in yields of these cultivars. The best-monitored example is the introgression of alleles carrying boron-tolerance genes from the highly tolerant Algerian landrace barley cultivar Sahara into high yielding varieties in southern Australia. Field trials have recorded a range of responses from higher yield, no change to reduced yield in the recipient cultivars [17–18]. One possibility for the lower-than-expected yields is that other genes with less desirable traits have been carried over from Sahara to the target cultivar, although no correlation was found between the amount of Sahara genome and yield in a number of backcross lines [17]. Theoretically, a transgenic approach in which only the boron-efflux transporter genes are inserted into high yielding cultivars should overcome this problem.

5. Dealing with multiple soil toxicities

There is some doubt that even the transgenic approach to increasing boron tolerance in crop species would generate significant improvements in productivity because of the co-occurrence of high salinity with most high boron soils, increasing with depth [19] (Fig. 1). Where salinity is the dominant toxicity, yield improvements are likely to be small unless cultivars are tolerant to both boron and salinity. A further confounding factor is the limitation imposed by water availability. High soil boron is usually associated with low rainfall, and vigorous early growth of boron-tolerant cultivars may simply deplete subsoil moisture and impose stresses at critical stages of later development [18]. Climatic variables are often intensified in regions with low rainfalls, oscillating between the extremes of drought and wet conditions, or rain falling only at the beginning or towards the end of a season. During years of higher rainfall, non-tolerant varieties

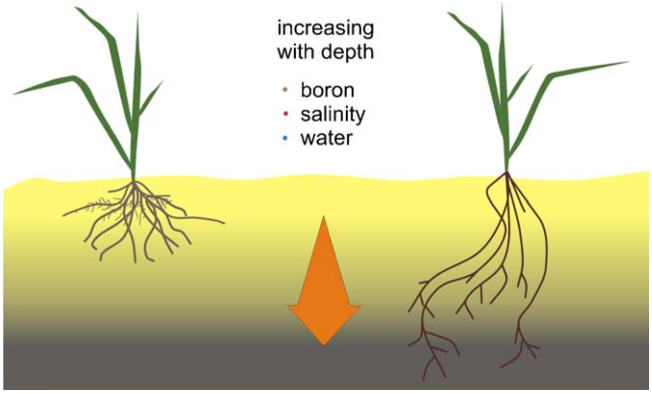

Fig. 1. Most soils containing high concentrations of B are also affected by salinity and low rainfall. B and Na concentrations usually increase down the soil profile which has an inhibitory effect on roots trying to access subsoil moisture. The effect of cellular B tolerance mechanisms on the pattern of development of roots early in the growing season may have positive or negative impacts on late season growth and yield.

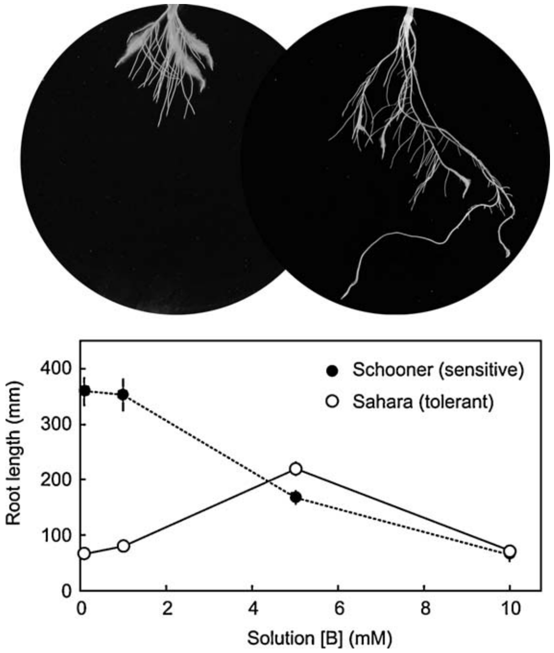

Fig. 2. Contrasting morphologies of root systems in barley cultivars and their responses to increasing concentrations of boron. At low boron concentrations the tolerant cultivar Sahara (top left image) has a short compact root system compared to the sensitive cultivar Schooner (top right image). Root growth of sensitive cultivars is reduced as boron concentrations increase but in the tolerant cultivar Sahara, moderate boron concentrations stimulate early root growth.

may be able to avoid toxic subsoils by directing root growth to the upper layers [18] so that yield differences do not become apparent.

The plasticity of root growth introduces a degree of unpredictability into responses to increasing soil boron. Different cultivars appear to employ a range of strategies when challenged with high boron, altering root morphology by increasing branching and development of finer roots, and varying the distribution of roots between topsoil and subsoil [20]. However, even cultivars classified as tolerant may avoid boron-toxic subsoil and proliferate roots in the topsoil, which seems to run counter to the expectation that tolerance would allow them to colonise a deeper profile [20]. Furthermore, large reductions in subsoil root growth do not necessarily result in significant reductions in subsoil water use [20].

A good example of the unpredictable nature of root growth in response to increasing boron is that displayed by the tolerant barley cultivar Sahara (Fig. 2). At low boron concentrations early growth of Sahara is characterised by a short and compact root system with many root hairs, and overall root length is quite low in comparison with other cultivars. The normal pattern of boron toxicity in other cultivars is inhibition of root growth as external boron increases, with sensitive cultivars more affected than tolerant cultivars. In Sahara, the opposite occurs; moderate boron concentrations strongly induce elongation of roots. The significance, if any, of this unusual response of root growth to boron tolerance is obscure. However, it does serve to reinforce the complexity of behaviour of plants growing in soil in which there are spatial variations in toxicities that may impact on how they manage limited water availability.

Rainfall itself can blur the differences between boron-tolerant and sensitive cultivars and complicate diagnosis of boron toxicity symptoms. A large fraction of total leaf boron can be leached by rain [16], so that the timing of rainfall events during the growing season could potentially have a major effect on the development of toxicity. This is undoubtedly one of the reasons for the poor correlation between leaf boron and leaf symptoms [21] and between leaf boron and yield. Another factor affecting symptom expression in leaves is the recently discovered redistribution of boron from symplast to apoplast which allows tolerant cultivars to accumulate up to 3-fold more boron in leaves before toxicity symptoms develop [16].

In summary, the acquisition of cellular mechanisms for protecting metabolic processes from the toxic effects of boron can result in physiological and morphological changes that influence the course of development in unpredictable ways that may expose the plant to a different range of stresses. It is clear that plants can be made tolerant to boron toxicity, and this may be useful in some situations such in crops irrigated with water containing high concentrations of boron but acceptable levels of salinity. However most boron-toxic soils occur in rain-fed semi-arid environments where low water availability and high salinity usually co-exist. Developing improved crops that can simultaneously accommodate three such stresses will not be an easy task.

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