

SELECTION CRITERION TO ASSESS WHEAT BORON TOLERANCE AT SEEDLING STAGE: PRIMARY vs. TOTAL ROOT LENGTH

MILKA BRDAR-JOKANOVIĆ^{1*}, IVANA MAKSIMOVIĆ², EMILIJA NIKOLIĆ-DORIĆ², MARIJA KRALJEVIĆ-BALALIĆ² and BORISLAV KOBILJSKI¹

¹*Institute of Field and Vegetable Crops, Novi Sad, Serbia*

²*University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia*

**Corresponding author, E-mail: milkabrdar@yahoo.com, Current address: Institute for Vegetable Crops, Smederevska Palanka, Serbia*

Abstract

Excess boron may exhibit detrimental effect on wheat (*Triticum aestivum* L.). An effective technique for assessing the response of wheat genotypes to excess boron is required to create high yielding tolerant cultivars. Filter paper assay, based on seedling's primary root length reduction in the presence of excess boron, is commonly used for distinguishing tolerant and sensitive genotypes. The study was undertaken to investigate the effect of excess boron on both primary and lateral root length, number of roots, number of days from imbibition to germination and germination percentage on the sample of 25 wheat cultivars, differing in origin and boron tolerance. The experiment included control and three boron treatments. On average, excess boron reduced root length and number and had no effect on number of days from imbibition to germination and germination percentage; however, significant differences have been found among the genotypes. The imposed boron treatments demonstrated 5.2% stronger effect on lateral root length in comparison to primary root length. In 10 out of 25 cases, boron tolerance estimated from primary root length reduction was not consistent with the estimation from lateral root length reduction; therefore, total root length reduction may be more valuable selection criterion for boron tolerance in wheat.

Introduction

Boron is an essential micronutrient for vascular plants. Although a number of important functions in plant development have been proposed for boron, the exact role yet remains to be clarified. The most widely accepted are its roles in maintaining primary cell wall structure and integrity of plasma membranes, as well as supporting metabolic activities (Bolaños *et al.*, 2004; Roessner *et al.*, 2006).

Optimal soil boron concentration depends on various physical and chemical soil properties and plant species, as well as the genotype within the species. Both boron deficiency and toxicity affect crops and vegetables. While boron deficiency can be overcome by application of appropriate fertilizers, boron toxicity appears to be a more difficult problem. Boron laden soils occur naturally, however, certain anthropogenic factors, such as irrigation water containing excess boron, the use of fly ash as an ameliorant in agriculture, boron fertilizers used for correction of boron deficiency and surface mining may lead to excessive soil boron accumulation (Nable *et al.*, 1997; Yau & Ryan, 2008). Although the first report on boron toxicity symptoms was made in the 1930's (Christensen, 1934), the negative impact of excess boron on crops was not recognized as a serious problem until the early 1980's, when 17% barley (cultivar Clipper) yield reduction in South Australia has been attributed to high soil boron

concentration (Cartwright *et al.*, 1984). Detrimental effect of high boron concentrations on plants is often related to arid and semiarid environments. Besides for Australia, it has been reported for particular areas of North and South America, Asia, South Africa, Mediterranean and East Europe (Nable *et al.*, 1997; Yau and Ryan 2008). Furthermore, saline soils, which are serious problem in arid and semiarid areas throughout the world (Abro *et al.*, 2009; Sharif & Khan, 2009); aggravate the negative impact of boron on agricultural plants (Wimmer *et al.*, 2003). Boron laden soils may be ameliorated by leaching boron from soil or liming. Because those procedures are difficult, economically unfeasible and may be only a short term solution, number of authors proposed breeding tolerant cultivars as the best approach for solving the problem (Paull *et al.*, 1988; Nable *et al.*, 1997; Torun *et al.*, 2006; Yau & Ryan, 2008).

In wheat (*Triticum aestivum* L.) boron requirements are generally low; only a few ppm of soil boron may interrupt plant growth and development and, consequently, affect yield (Yau *et al.*, 1994). Nevertheless, the great variability that has been reported among wheat cultivars in terms of boron response (Paull *et al.*, 1988; Yau & Saxena, 1997; Torun *et al.*, 2006) makes possible developing tolerant cultivars. The first step in the breeding process is screening large number of wheat genotypes for tolerance to boron, for the purpose of selecting tolerant genotypes that can be exploited as starting material in the process of creating new boron tolerant high yielding cultivars.

Screening wheat genotypes for tolerance to boron may be related to several impediments. The ultimate goal is to distinguish tolerant genotypes with superior performance on boron laden soils; however, screening large number of wheat genotypes in field experiments is time consuming and labor demanding process. Significant genotype x environment interactions and high possibility for experimental error due to uneven horizontal and vertical distribution of boron in soil, as well as narrow range between boron deficiency and toxicity (Kalayci *et al.*, 1998; Brennan & Adcock, 2004; Avci & Akar, 2005) make screening wheat genotypes in field conditions more difficult. Therefore, an effective screening technique in controlled environmental conditions would be more appropriate, however, neither universal laboratory technique nor universal selection criterion have been established yet. The phenotypic assessment of boron tolerance status in wheat included growing in pots containing boron contaminated soil (Paull *et al.*, 1988; Yau & Saxena, 1997; Torun *et al.*, 2006), in nutrient solution (Campbell *et al.*, 1998; Furlani *et al.*, 2003) and filter paper technique, proposed by Chantachume *et al.*, (1995). The technique is based on growing wheat seedlings on filter paper soaked with nutrient solutions containing high boron concentrations and measuring the longest root length reduction. It is a rapid, non destructive and economically feasible test that has been used for screening wheat genotypes for tolerance to boron (Chantachume *et al.*, 1995), research on traits related to tolerance to boron in barley (Rehman *et al.*, 2006), as well as for phenotypic variation analysis related to mapping chromosome regions conferring boron toxicity tolerance in wheat (Jefferies *et al.*, 2000).

However, the total root surface participates in water and nutrient uptake (Loes & Gahoonia, 2004). The estimate of wheat boron tolerance by original method of Chantachume *et al.*, (1995) excludes the possible effect of excess boron on lateral roots, concerned with their number and length. It raises the question does excess boron affects both primary and lateral root length to the similar extent? If not, estimating wheat boron tolerance on the basis of primary root length reduction would not be precise enough to distinguish tolerant and sensitive genotypes.

This study was undertaken in order to analyze and compare the effect of high boron concentrations on primary and lateral roots in 25 wheat genotypes differing in boron tolerance. Also the goal was to investigate the possible relationships among other wheat kernel characteristics (germination percentage, number of days from imbibition to germination and number of roots) and boron tolerance estimated from root length reduction in conditions of excess boron supply.

Materials and Methods

The experimental material consisted of 25 wheat (*Triticum aestivum* L.) cultivars. Thirteen of the tested cultivars originate from different countries (Stephens, USA; Trakija, Bulgaria; Bezostaja 1, Russia; Mironovska 808, Ukraine; Radika, Macedonia; Donjecka 48, Ukraine; Apache, France; Zitarka, Croatia; Fundulea 4, Romania; Pergamino Gaboto, Argentina; Magdalena, Hungary; Norin 10 / Brevor 14, USA; Renan, France) and the remaining 12 (Nevesinjka, Rapsodija, Milijana, Helena, Sonata, Kosuta, Partizanka, Simonida, Kantata, Sofija, Balerina, Pesma) are created at the Institute of Field and Vegetable Crops, Novi Sad, Serbia (local cultivars). The material originating from foreign countries presents either the cultivars included in the pedigree of high yielding local cultivars or the cultivars that are interesting from breeder's point of view due to high yield or other yield contributing character. The kernels were obtained from a field trial which did not include boron treatments.

The trial has been set by the filter paper method proposed by Chantachume *et al.*, (1995). The assay included control and three boron treatments. Three hundred kernels per treatment of each cultivar, subdivided in five replications, were surface sterilized with 70% EtOH (v/v) and pre germinated at 4°C for 48h and at 18°C for 24h. The kernels were imbibed on filter paper and soaked with boric acid solutions, concentrations of 0.93 (B 0, control), 50, 100 and 150 mg L⁻¹ (B 50, B 100 and B 150; treatments). All solutions included 0.5 mM Ca(NO₃)₂ × 4H₂O, 0.0025 mM ZnSO₄ × 7H₂O and 0.015 mM H₃BO₃. The kernels were germinated and the seedlings have grown in dark, under controlled conditions at 18°C, for eleven days.

The samples included 50 seedlings. The following traits have been analyzed: primary root length (cm), lateral root length (cm), number of seminal roots, number of days from imbibition to germination of at least 50% kernels and germination percentage. Lateral root length is expressed as sum of the lengths of all lateral roots. Root length reduction on three boron treatments, expressed as percentage of control, has been used as selection criterion for boron tolerance. Primary and lateral root reductions have been analyzed separately. The cultivars with average root reduction above upper quartiles have been estimated as boron tolerant (T), the cultivars with root reduction below lower quartiles as boron sensitive (S) and the remaining half of the cultivars have been classified as medium tolerant (MT) to excess boron.

The original data were submitted to regression analysis in order to describe the relationships between the traits and the increasing boron concentrations. The equations were adjusted to data obtained for control and boron treatments, using F coefficient magnitude as criteria for choosing the model (L, linear; Q, quadratic or C, cubic). Correlation coefficients among the analyzed traits have been calculated in order to explore their possible relationships. All calculations have been performed using Statistica 8.0 software package (StatSoft, University License, University of Novi Sad, 2009).

Results and Discussion

In the conditions of optimal boron supply (control) significant differences occurred among the cultivars for all the traits mentioned (Table 1). However, the interval of variation of primary (PRL) and lateral root length (LRL) was about two times narrower for local cultivars, compared to foreign (4.9 and 9.5 cm for PRL; 13.7 and 31.2 cm for LRL, respectively). Similarly, the number of roots (NR) ranged from 4 to 5 and from 3 to 6 axes for local and foreign cultivars, respectively. Since early rooting characteristics are likely to reflect wheat root architecture in later developmental stages (Mian *et al.*, 1994; Oyanagi, 1994), the fairly narrow intervals of variation that are noted for cultivars of local origin may be explained by selection during breeding for specific environmental conditions. On the other hand, the cultivars originating from foreign breeding institutions demonstrated major differences due to selection for adaptation to contrasting environments.

The applied boron treatments decreased linearly both PRL and LRL, with exception of cultivar Stephens (Table 2). Root length/boron treatments relation fit a quadratic model for the cultivar, suggesting susceptibility to low external boron concentrations (boron inefficiency), which may be a genetic characteristic of the cultivar. Except for Stephens, the extent of PRL reductions estimated for the studied cultivars coincides with the reductions reported for another set of wheat genotypes (Chantachume *et al.*, 1995). On average, the imposed boron treatments demonstrated 5.2% stronger effect on LRL in comparison to PRL. In addition, the growth of primary and lateral roots on boron treatments was not reduced to the same extent for all tested cultivars. Therefore, boron tolerance levels estimated from PRL reduction were not consistent with the levels estimated from LRL reduction in 10 out of 25 observed cases, highlighting the importance of total root length reduction on boron treatments as more valuable selection criterion for boron tolerance in wheat.

Overall NR decreased linearly with the increasing boron treatments (Table 2). However, the effect of boron treatments was quadratic, cubic or did not fit any applied regression equation for half of the cultivars tested, irrespective of their boron tolerance/sensitivity estimated from root growth reduction in boron enriched medium. Therefore, the decrease of wheat LRL in the presence of excess boron may be partly explained by the decrease in the NR; however, it was not always the case. For example, NR remained virtually the same with an increase in boron concentrations for sensitive cultivar Pesma, whereas significant reduction was noted in the case of also sensitive cultivar Renan.

None of the analyzed cultivars exhibited significant average NR increase on boron treatments. The results of the study of Manschadi *et al.*, (2008) performed on a set of 30 wheat genotypes imply the relation between root architectural traits (growth angle and number of seminal roots) and drought tolerance. Therefore, detrimental effect of high soil boron concentrations on wheat related to arid and semi-arid environments (Nable *et al.*, 1997) may be partly due to root number decrease; however, further studies are required to confirm the hypothesis.

Similar to other analyzed traits, number of days from imbibition to germination (ND) and germination percentage (GP) in conditions of optimal boron supply varied to the greater extent for foreign cultivars compared to local ones (2.2 and 5.2 days for ND, 27.7 and 40.7% for GP; for local and foreign cultivars, respectively), Table 1. Variation in wheat GP related to geographical origin has been reported by Ueno and Takahashi (1997). In addition, as the kernels of all cultivars included in our study have been obtained from a single field trial conducted in one season, the differences among cultivars in terms of GP on control may be partly due to different adaptation to particular environmental conditions.

Table 3. Correlation coefficients among primary root length (PRL), lateral root length (LRL), number of roots (NR), number of days from imbibition to germination (ND) and germination percentage (GP) in 25 wheat genotypes on control (above diagonal) and on boron treatments (below diagonal)

	PRL	LRL	NR	ND	GP
PRL		0.88**	ns	ns	ns
LRL	0.76**		0.59**	ns	0.49*
NR	ns	0.50*		ns	ns
ND	ns	ns	ns		-0.67**
GP	ns	0.49*	ns	-0.40*	

ns, *, ** insignificant, significant at 0.05 and 0.01 levels of probability, respectively

The effect of the applied boron treatments on ND was insignificant (Table 2). Nevertheless, for a half of the tested wheat cultivars, irrespective of their boron tolerance, the relation between the trait and the increasing boron concentration in the medium fit the linear regression model. Excess boron delayed germination in most of the cases. The exceptions were medium tolerant cultivars Milijana and Partizanka, which germinated approximately one day earlier on boron treatments with respect to control.

Similarly, the applied boron treatments had no effect on mean GP of the tested cultivars. However; linear, quadratic or cubic regression of GP on boron treatments was significant for all individual cases, with the great majority of the cases being negative. Numerous quadratic and cubic fits suggest the inconsistent reaction of GP to imposed treatments. Excess boron significantly delayed coleoptile emergence, but had no effect on GP in pot experiments conducted on seven bread (Paull *et al.*, 1988) and nine durum wheat genotypes (Yau & Saxena, 1997). Partial disagreement between the results concerning GP that are obtained in this study and the results published by other authors may be attributed to genetic characteristics of the material tested. In addition, the differences may be the consequence of the experimental procedures. Boron concentrations applied in pot experiments are usually lower than concentrations applied in filter paper assays. Increasing boron treatments caused lower GP for six corn, carrot and tomato genotypes tested by filter paper technique (Bañuelos *et al.*, 1999).

The cultivars with higher GP took less time to germinate in both control and boron treatments. In addition, GP was positively correlated to LRL (Table 3), implying seed viability as a possible factor for wheat boron tolerance, at least at seedling stage. It is in accordance with the results of filter paper assay performed on 23 barley genotypes, where boron tolerant genotypes exhibited higher GP comparing to sensitive ones (Rehman *et al.*, 2006). LRL was positively correlated to NR and PRL, as expected.

Significant differences concerning all analyzed traits have been found among the tested wheat cultivars, in other words, almost each cultivar responded differently to increasing external boron concentrations, which makes the selection of boron tolerant genotypes quite difficult. The phenomenon has been reported for other traits related to boron tolerance or boron use efficiency in cereals that have been tested in greenhouse and field experiments (Kalayci *et al.*, 1998; Avci & Akar, 2005; Soylu *et al.*, 2005; Torun *et al.*, 2006; Korzeniowska, 2008; Mehmood *et al.*, 2009).

Conclusions

Considering (i) the importance of assessment of the level of boron tolerance at early stages of wheat development, (ii) stronger effect of imposed boron treatments on lateral root length in comparison to primary root length and (iii) the fact that in two fifths of the analyzed cases boron tolerance levels estimated from primary root length reduction were not consistent with the levels estimated from lateral root length reduction; one may conclude that total root length reduction is more reliable selection criterion for wheat tolerance to excess boron than the primary root length reduction.

References

- Abro, S.A., A.R. Mahar and A.A. Mirbahar. 2009. Improving yield performance of landrace wheat under salinity stress using on-farm seed priming. *Pak. J. Bot.*, 41(5): 2209-2216.
- Avci, M. and T. Akar. 2005. Severity and spatial distribution of boron toxicity in barley cultivated areas of Central Anatolia and Transitional zones. *Turk. J. Agric. For.*, 29:377-382.
- Bañuelos, G.S., H.A. Ajwa, L. Caceres and D. Dyer. 1999. Germination responses and boron accumulation in germplasm from Chile and the United States grown with boron-enriched water. *Ecotoxicol. Environ. Saf.*, 43:62-67.
- Bolaños, L., K. Lukaszewski, I. Bonilla and D. Blevins. 2004. Why boron? *Plant Physiol. Biochem.*, 42:907-912.
- Brennan, R.F. and K.G. Adcock. 2004. Incidence of boron toxicity in spring barley in Southwestern Australia. *J. Plant Nutr.*, 27:411-425.
- Campbell, T.A., A.J. Rathjen, J.G. Paull and A.K.M.R. Islam. 1998. Method for screening bread wheat for tolerance to boron. *Euphytica*, 100:131-135.
- Cartwright, B., B.A. Zarcinas and A.H. Mayfield. 1984. Toxic concentrations of boron in a red-brown earth at Gladstone, South Australia. *Aust. J. Soil Res.*, 22:261-272.
- Chantachume, Y., D. Smith, G.J. Hollamby, J.G. Paull and A.J. Rathjen. 1995. Screening for boron tolerance in wheat (*T. aestivum*) by solution culture in filter paper. *Plant Soil*, 177:249-254.
- Christensen, J.J. 1934. Non-parasitic leaf spots of barley. *Phytopath.*, 24:726-742.
- Furlani, Á.M.C., C.P. Carvalho, J.G. De Freitas and M.F. Verdial. 2003. Wheat cultivar tolerance to boron deficiency and toxicity in nutrient solution. *Sci. Agric.*, 60:359-370.
- Jefferies, S.P., M.A. Pallotta, J.G. Paull, A. Karakousis, J.M. Kretschmer, S. Manning, A.K.M.R. Islam, P. Langridge and K.J. Chalmers. 2000. Mapping and validation of chromosome regions conferring boron toxicity tolerance in wheat (*Triticum aestivum*). *Theor. Appl. Genet.*, 101:767-777.
- Kalayci, M., A. Alkan, I. Çakmak, O. Bayramoğlu, A. Yilmaz, M. Aydin, V. Ozbek and H. Ekiz. 1998. Studies on differential response of wheat cultivars to boron toxicity. *Euphytica*, 100:123-129.
- Korzeniowska, J. 2008. Response of ten winter wheat cultivars to boron foliar application in a temperate climate (South-West Poland). *Agron. Res.*, 6:471-476
- Løes, A.K. and T.S. Gahoonia. 2004. Genetic variation in specific root length in Scandinavian wheat and barley accessions. *Euphytica*, 137:243-249.
- Manschadi, A.M., G.L. Hammer, J.T. Christopher and P. DeVoi. 2008. Genotypic variation in seedling root architectural traits and implication for drought adaptation in wheat (*Triticum aestivum* L.). *Plant Soil*, 303:115-129.
- Mehmood, E.-U.-H., R. Kausar, M. Akram and S.M. Shahzad. 2009. Is boron required to improve rice growth and yield in saline environment? *Pak. J. Bot.*, 41(3): 1339-1350.
- Mian, M.A.R., E.D. Nafziger, F.L. Kolb and R.H. Teyker. 1994. Root size and distribution of field-grown wheat genotypes. *Crop Sci.*, 34:810-812.
- Nable, R.O., G.S. Bañuelos and J.G. Paull. 1997. Boron toxicity. *Plant Soil*, 193:181-198.

- Oyanagi, A. 1994. Gravitropic response growth angle and vertical distribution of roots of wheat (*Triticum aestivum* L.). *Plant Soil*, 165:323-326.
- Paull, J.G., B. Cartwright and A.J. Rathjen. 1988. Responses of wheat and barley genotypes to toxic concentrations of soil boron. *Euphytica*, 39:137-144.
- Rehman, S., T.I. Park, Y.J. Kim, Z.W. Seo and S.J. Yun. 2006. Inverse relationship between boron toxicity tolerance and boron contents of barley seed and root. *J. Plant Nutr.*, 29:1779-1789.
- Roessner, U., J.H. Patterson, M.G. Forbes, G.B. Fincher, P. Langridge and A. Bacic. 2006. An investigation of boron toxicity in barley using metabolomics. *Plant Physiol.*, 142:1087-1101.
- Sharif, F. and A.U. Khan. 2009. Alleviation of salinity tolerance by fertilization in four thorn forest species for the reclamation of salt-affected sites. *Pak. J. Bot.*, 41(6): 2901-2915.
- Soylu, S., B. Sade, A. Topal, N. Akgün, S. Gezgin, E.E. Hakki and M. Babaoğlu. 2005. Responses of irrigated durum and bread wheat cultivars to boron application in a low boron calcareous soil. *Turk. J. Agric. For.*, 29:275-286.
- Torun, A., A. Yazici, H. Erdem and I. Çakmak. 2006. Genotypic variation in tolerance to boron toxicity in 70 durum wheat genotypes. *Turk. J. Agric. For.*, 30:49-58.
- Ueno, K. and H. Takahashi. 1997. Varietal variation and physiological basis for inhibition of wheat seed germination after excessive water treatment. *Euphytica*, 94:169-173.
- Wimmer, M.A., K.H. Mühling, A. Läuchli, P.H. Brown and H.E. Goldbach. 2003. The interaction between salinity and boron toxicity affects the subcellular distribution of ions and proteins in wheat leaves. *Plant Cell Environ.*, 26:1267-1274.
- Yau, S.K. and J. Ryan. 2008. Boron toxicity tolerance in crops: a viable alternative to soil amelioration. *Crop Sci.*, 48:854-865.
- Yau, S.K. and M.C. Saxena. 1997. Variation in growth, development, and yield of durum wheat in response to high soil boron. I. Average effects. *Aust. J. Agric. Res.*, 48:945-949.
- Yau, S.K., J. Hamblin and J. Ryan. 1994. Phenotypic variation in boron toxicity tolerance in barley, durum and bread wheat. *Rachis*, 13:20-25.

(Received for publication 10 February 2010)