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Full Length Research Paper

Screening *Aegilops-Triticum* species for Boron tolerance

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Boron toxic soils are a problem for wheat production in many regions of the world. Wild Triticeae relatives are known to provide gene pools for wheat germplasm improvement. To establish their potential for improving Boron tolerance, *Aegilops speltoides*, *Ae. longissima*, *Ae. sharonensis*, *Ae. bicornis*, *Ae. searsii*, *Ae. kotschyi*, *Ae. peregrina* ssp. *cylindrostachys*, *Ae. peregrina* ssp. *euvariabilis*, *Ae. geniculata* syn. *ovata*, *Ae. biuncialis*, *Ae. triuncialis*, and *Triticum turgidum* ssp. *dicoccoides* accessions collected from Israel, Turkey, Syria, Jordan, Egypt, Lebanon, and Iran were screened for Boron tolerance using a hydroponic system containing elevated levels of Boron. The results indicated that some *Ae. longissima*, *Ae. kotschyi*, *Ae. peregrina* ssp. *cylindrostachys*, *Ae. peregrina* ssp. *euvariabilis*, and *Triticum turgidum* ssp. *dicoccoides* accessions were tolerant to excess Boron. Tolerant Triticeae accessions can be useful sources of Boron tolerance for wheat improvement. The difficulties of screening wild *Triticum* and *Aegilops* species in hydroponics are discussed.

Key words: *Triticum*, *Aegilops*, boron tolerance, hydroponics.

INTRODUCTION

Boron is an essential plant micronutrient required for normal cell wall development, membrane function and other metabolic processes (Blevins and Lukazewski, 1998). Boron deficiencies and toxicities are well known to cause significant crop yield losses on a global scale (Sutton et al., 2007). Boron toxicity has been recognized as a yield limiting in the dry regions of West Asia and North Africa and is a problem associated with irrigation water in many parts of the world (Gupta et al., 1995 and Reid, 2010). Boron deficiencies are common in the sub-tropics and other regions with high rainfall (Blevins and Lukazewski, 1998), as in the light soils of Bangladesh (Jamjod et al., 2004). Boron deficiency has been shown to reduce seed set in wheat (*Triticum aestivum* L.) (Rerkasem and Jamjod, 1989; Abedin et al., 1994; Jahiruddin et al., 1995) caused by male sterility through impaired anthers and pollen development, and pollen germination (Rerkasem and Jamjod, 1989), and leading to redunction in yield in wheat and barley (*Hordeum vulgare* L.) (Paul et al., 1988; Yau, 2002).

Boron requirements vary among plant species, genotypes within a species (Rerkasem, 2002), and different environmental conditions (McDonald, 2010). Wheat varieties differ widely in their sensitivity to Boron uptake as well as to Boron deficiency (Rerkasem and Jamjod,

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1997; Kataki et al., 2001; Huang et al., 2000; Jefferies et al., 2000; Brown and Shelp, 1997; Subedi et al., 1999). The screening and selection of Boron efficient and/or tolerant wheat varieties, by wheat improvement programs, is likely to be the most cost effective approach to creating new cultivars that minimize the problem of yield loss due to Boron deficiency and/or toxicity.

Nutrient efficiency is defined as the ability of a genotype to develop normally and to yield well under a limiting supply of a specific nutrient (Stangoulis et al., 2000; Baligar et al., 2001). In a Boron efficiency study on *Brassica napus* L., Stangoulis et al. (2000) found that root length after 10 days in nutrient solution proved more reliable than other measures in determining genotype responses, which were corroborated by field-based Boron-efficiency studies. Seventy-nine accessions of wild *Aegilops* and *Triticum* species were evaluated in a hydroponic-based nutrient solution, for their ability to grow under varying levels of Boron. These wild Triticeae species constitute a series of secondary and tertiary gene pools of interesting and useful traits that can be exploited for wheat improvement programs. The objective of the present study was to screen the wild relatives of wheat and identify the accessions for further Boron tolerance laboratory and field studies.

MATERIALS AND METHODS

Plant material

Seventy-nine accessions from 12 wild species within the *Aegilops-Triticum* group were generously provided by Dr. Moshe Feldman, Department of Plant Sciences, The Weizmann Institute of Science, Rehovot 76100, Israel. These accessions were collected from several Mediterranean sources (Table 1). One rye (*Secale cereale* L. 'Blanco') obtained from the USDA-Sears Collection, University of Missouri was also included in the screening. Because of export problems and very limited seed, separate shipments of seed was obtained fom Dr. Feldman for the Missouri and Greece screening.
The wheat (*T. aestivum* L.) controls Cranbrook (susceptible), and G6 1450 and Halberd (tolerant) obtained from Dr. Tim Sutton, The Australian Centre for Plant Functional Genomics, Adelaide, Australia were only available for Boron evaluation in Greece.

At the Agricultural Agricultural University of Athens, the root tip growth for every accession was analyzed in a randomized complete block design involving three replications of four seeds each. At the University of Missouri, the root tip growth for every accession was analyzed in a randomized complete block design involving two replications of four seeds each.

Hydroponic screening

The hydroponic screening was done either in the laboratory (Agricultural University of Athens), or in a growth chamber with light set to 16 h day at 22ºC and 8 h night at 15ºC (University of Missouri, Columbia). At both locations, six to eight seeds of approximately the same size and appearance, were germinated prior to being placed in nutrient solution. Plump seeds from all wild species accessions with good endosperm development were used, which is critical for uniform germination and seedling growth. Seeds were surface-sterilized in a solution of 1.3% sodium hypochlorite (25% bleach solution) plus one drop of Tween 20 ® (surfactant) in distilled water for eight minutes and then thoroughly rinsed. Seeds were placed on filter paper in ~0.2% Pipracil (Piperacillin Sodium antibiotic) solution in distilled water in labeled petri dishes for germination. Petri plates were shifted to a refrigerator (4°C) as needed to slow seedling growth in order to maximize uniformity of germination among all accessions of all species. Healthy seedlings with roots 2 to 10 mm were removed from 4°C and placed into hydroponic treatments. The hydroponic nutrient solution consisted of 0.4 mM $CaCl_2$; 0.65 mM KNO_3; 0.25 mM $MgCl_2{\cdot}6H_20$; 0.1 mM $(NH_4)_2SO_4$; and 0.04 mM NH_4NO_3 in dionized water [21]. The nutrient solutions were supplemented with H_3BO_3 to a final concentration of 3 mM or 10 mM of Boron. All treatment solutions were aerated using commercially available fish aquarium air pumps and aeration stones. The nutrient solution was maintained at a neutral pH (~7.5) and constant concentration by changing the solutions daily. Four seedlings per accession were grown in 2 L of nutrient solution with 0, 3 or 10 mM Boron, respectively for ten days. The longest root of each plant was measured and its length recorded. In order to make the technique as simple as possible, all accessions were categorised as tolerant or susceptible based only on their seedling root growth in hydroponic solution containing Boron, as seedling root growth is the major parameter in establishing plant survival under Boron toxic conditions.

Statistical analysis of root length

Analysis of variance (ANOVA) was performed with the GLM procedure of SAS 9.1 program (SAS Institute, Cary, NY).

RESULTS AND DISCUSSION

Hydroponic screening

Substantial variation in seed size, kernel shape, germination rate, and seedling growth, even within the panicle of a single plant is characteristic of wild species including the cereal species (Table 2). Therefore, when conducting abiotic stress hydroponics experiments on wild cereal species one needs to very carefully select within the species, and within each individual accession for seeds that are uniform for size, shape, and especially germination. With careful selection for seed quality and germination uniformity, the results of hydroponic screening for abiotic stress can be useful (Tables 2 and 3), which will minimize the considerable variation observed in root length among and within accessions of the wild cereal species grown in hydroponics (Table 2).

Boron effect on root growth

Since there were major differences between seed uniformity within and between the seed stocks and the experimental environments in Greece and Missouri, we will be only reporting on the Missouri data unless otherwise

Table 1. *Aegilops/Triticum* germplasm evaluated for their Boron tolerance response.

Specie	Country of origin	Number of accession
Aegilops speltoides	Israel	7
	Unknown	1
	Turkey	1
	Syria	1
Aegilops longissima	Israel	8
	Jordan	1
Aegilops sharonensis	Israel	9
Aegilops bicornis	Israel	2
	Egypt	3
Aegilops searsii	Israel	4
	Syria	1
Aegilops kotschyi	Israel	6
Aegilops peregrina ssp. *cylindrostachys*	Israel	4
Aegilops peregrina ssp. *euvariabilis*	Unknown	1
	Israel	9
Aegilops geniculata syn. *ovata*	Unknown	1
	Israel	2
	Lebanon	1
Aegilops biuncialis	Israel	5
Triticum turgidum ssp. *dicoccoides*	Israel	10
	Iran	1
	Turkey	1
Secale cereale 'Blanco'	Brazil	1
Triticum aestivum 'Cranbrook', G6 1450', Halberd'	Australia	3

Table 2. Mean, coefficient of variation and least significant difference for root length of of all genotypes analyzed in Missouri and Greece.

Genotypes (1-80)	Missouri			Greece
	0 mM B	3 mM B	10 mM B	3 mM B
Mean	10.2	11.5	4.0	3.0
CV	28.3	17.3	28.6	29.7
LSD	4.0	2.8	1.6	1.0

otherwise noted. The present study indentified three groups of root length variation, short, medium, and long among the 79 Triticeae accessions evaluated in Missouri (Table 3). Based on root length none of the *Aegilops biuncialis* accessions showed tolerance to Boron in the Missouri screening environment. *Aegilops geniculata* syn. *ovata* accessions showed variable response in screening environments and hence, no reliable conclusions could

Table 3. Average root length of 4 plants/accession of each *Aegilops* and *Triticum* accession under different Boron concentrations in hydroponic systems at the University of Missouri, Columbia.

Specie	Genotype	Average root length# (cm)			Specie	Genotype	Average root length (cm)		
		0 mM	3 mM	10 mM			0 mM	3 mM	10 mM
Ae. speltoides	TS-01	17.3	12.0 (3.1)*	5.2	*Ae. kotschyi*	TKK-06	9.0	12.3 (3.8)	5.5
	TS-02	12.6	8.8 (1.1)	6.0		TKK-11	8.1	10.8 (3.6)	6.5
	TS-41	13.6	12.2 (3.2)	4.0		TKK-17	11.4	14.0 (4.2)	6.3
	TS-43	11.2	9.7 (1.4)	2.3		TKK-21	10.1	15.9 (3.1)	5.7
	TS-47	5.9	8.7 (2.5)	3.5					
	TS-76	19.0	8.9 (2.8)	5.6	*Ae. peregrina ssp. cylindrostachys*	TKC-01	9.4	12.1 (2.4)	4.6
	TS-100	13.5	12.7 (2.6)	4.5		TKC-04	7.9	10.2 (2.3)	3.0
	TS-117	10.5	13.0 (1.9)	4.8		TKC-06	9.2	16.4 (3.7)	2.7
	TS-118	14.0	9.8 (1.1)	4.3		TKC-08	8.1	14.0 (2.4)	2.2
	TS-132	14.7	6.6 (2.1)	2.2					
					Ae. peregrina ssp. euvaribilis	TKE-02	6.8	10.9 (3.4)	3.4
Ae. longissima	TL-01	15.7	11.0 (2.7)	3.5		TKE-03	12.7	17.5 (4.6)	3.9
	TL-02	9.0	9.0 (2.4)	0.0		TKE-12	10.9	12.4 (4.1)	2.9
	TL-04	9.3	9.7 (4.3)	6.7		TKE-19	10.3	15.5 (2.7)	2.9
	TL-05	11.1	8.8 (1.0)	3.7		TKE-22	11.5	0.0 (2.1)	7.0
	TL-07	10.3	8.6 (2.6)	5.6		TKE-24	13.6	14.5 (2.0)	3.6
	TL-09	15.4	14.0 (1.8)	10.3		TKE-42	8.8	14.0 (4.7)	6.3
	TL-17	17.4	11.6 (3.4)	10.9		TKE-46	9.0	13.1 (4.4)	4.7
	TL-21	14.6	10.3 (4.8)	5.0		TKE-62	10.3	0.0 (4.8)	0.0
	TL-24	6.8	13.2 (2.2)	3.5		TKE-66	9.4	11.3 (2.0)	2.8
Ae. sharonensis	TH-01	9.6	9.3 (1.8)	3.5	*Ae. geniculata syn. ovata*	TO-01	8.9	15.0 (2.8)	3.9
	TH-02	12.2	9.8 (2.7)	2.0		TO-07	16.3	14.0 (3.6)	4.6
	TH-03	19.8	12.8 (1.6)	3.6		TO-13	2.0	13.8 (3.8)	1.3
	TH-04	10.4	12.9 (2.1)	6.6		TO-44	3.3	10.3 (5.1)	1.9
	TH-07	14.9	13.0 (3.0)	5.6					
	TH-10	14.0	16.7 (3.6)	5.4	*Ae. biuncialis*	TN-03	3.4	9.3 (2.9)	1.9
	TH-11	15.9	13.9 (2.4)	4.6		TN-13	3.2	7.4 (3.0)	2.0
	TH-15	22.8	14.3 (2.0)	6.4		TW-03	1.9	10.3 (3.0)	0.6
	TH-17	19.2	16.8 (2.9)	7.5		TW-09	1.6	9.0 (2.3)	1.0
						TW-11	1.2	8.3 (2.1)	0.7
Ae. bicornis	TB-04	13.8	14.1 (3.1)	4.3					
	TB-05	9.9	12.0 (2.3)	6.3	*T. dicoccoides*	TTD-04	4.5	12.7 (2.5)	4.5
	TB-07	10.0	12.6 (3.8)	6.2		TTD-09	5.1	0.0 (3.3)	2.6

Table 3. Contd'

Ae. bicornis	TB-10	15.5	13.4 (3.3)	5.9		TTD-12	4.1	12.5 (3.8)	1.6
	TB-12	8.9	14.0 (3.2)	5.1		TTD-15	3.8	12.3 (3.7)	2.3
						TTD-24	2.3	5.0 (3.5)	0.6
	TE-03	9.5	10.0 (3.1)	6.6		TTD-25	2.2	12.1 (2.8)	2.1
Ae searsii	TE-09	13.5	12.6 (4.3)	4.3	*T. dicoccoides*	TTD-30	2.2	12.9 (2.6)	1.4
	TE-21	21.8	15.0 (2.9)	4.4		TTD-47	5.4	15.3 (4.3)	1.4
	TE-27	15.5	11.1 (3.3)	5.5		TTD-48	4.4	8.3 (3.8)	0.7
	TE-36	18.1	12.4 (0.0)	3.3		TTD-54	2.0	9.6 (4.0)	1.6
						TTD-64	1.8	15.0 (31.)	1.4
						TTD-68	1.9	9.6 (3.5)	1.8
Ae. kotschyi	TKK-01	16.5	16.3 (5.2)	7.8					
	TKK-03	19.3	13.6 (5.3)	6.6	*S. cereale (rye)*	Blanco	3.8	8.8 (3.7)	1.2
						Cranbrook	-	(4.2)	-
					T. aestivum	G6 1450	-	(7.8)	-
						Halberd	-	(7.8)	-

* denotes data collected on 4 plants/accession at the Athens Agricultural University, Athens, Greece.

be drawn about *Ae. geniculta* syn. *ovata* Boron tolerance. The remaining species showed medium levels of Boron tolerance with some accessions within a species showing very high levels of Boron tolerance. Thirty-four genotypes exhibited good root growth in 3 mM Boron. *Aegilops sharonensis* accessions (TH-07, TH-10, TH-11, TH-15 and TH-17), *Aegilops longissima* accessions (TL-17, and TL-24), *Aegilops kotschyi* accessions (TKK-01, TKK-03, TKK-06, TKK-17, and TKK-21), *Aegilops bicornis* accessions (TB-04, TB-07, TB-10, and TB-12), *Aegilops triuncialis* accession (TW-03), *Aegilops searsii* accessions (TE-09 and TE-21), *Aegilops peregrina* ssp. *cylindrostachys* accessions (TKC-06), *Aegilops peregrina* ssp. *euvariabilis* accessions (TKE-03, TKE-12,TKE-42, and TKE-46), *Ae. geniculata* syn. *ovata* accessions (TO-07, and TO-13), and *Triticum dicoccoides* accessions (TTD-12, TTD-15, TTD-47, and TTD-64) all showed better root growth in both the Missouri and Greece screening environments (Table 3). The most tolerant accessions in the study showing tolerance to 10 mM boron in Missouri were *Ae. longissima* TL-09, and TL-17.

Observations from the limited number of accessions analyzed in Missouri indicated that wheat improvement programs would probably not find the species *Aegilops speltoides*, *Ae. biuncialis*, or *Ae. triuncialis*, useful as parents for improving tolerance to Boron in wheat.

However, analysis of a much larger number of accessions from all species, including *Ae. speltoides*, *Ae. biuncialis* and *Ae. triuncialis* may yield additional sources of Boron tolerant germplasm. The highest levels of Boron tolerance to 10 mM were observed in *Ae. longissima* accessions (TL-09 and TL-17). In general, accessions of *Aegilops bicornis*, *Ae. kotschyi, Ae. peregrina* ssp. *cylindrostachys, Ae. peregrina* ssp. *euvaribilis,* and *Ae. geniculata* species showed high levels Boron tolerance. It is clear that, when evaluating non-domesticated members of the *Triticeae* tribe, large numbers of accessions and quantities of seeds, containing well-developed endosperm, for each species are required. In additon, to establish the optimum level of Boron to maximize growth and yield, a multitude of Boron concentrations between 0 mM and 10 mM need to be tested. The present results confirmed those of Miwa et al. (2007), who showed that 10 mM boric acid severely limits root growth. We see no reason to screen beyond the 10 mM level for Boron tolerance.

In the present study several *Triticeae* accessions averaged well over 7.0 mm of root length, indicating reason to screen beyond the 10 mM level for Boron tolerance.

In the present study several *Triticeae* accessions averaged well over 7.0 mm of root length, indicating that accessions of wild *Triticeae* species showed considerable Boron tolerance under hydroponic screening conditions.

Evaluating Triticeae germplasm pools for Boron tolerance at the seedling root growth stage, using hydroponics is a useful screening technique, but careful selection of the seed within and between accessions is needed to prevent the vagaries of seed quality from influencing root growth analyses in hydroponics. The present study established that several species including *Ae. sharonensis, Ae. kotschyi*, *Ae. bicornis*, *Ae. peregrina* ssp. *cylindrostachys, Ae. peregrina* ssp. *euvariabilis*, *Ae. geniculta*, *Ae. longissima*, and *T. dicoccoides* contained accessions that expressed Boron tolerance, and that there were major differences in Boron tolerance between and within accessions of the various *Aegilops/Triticum* species. However, problems were noted in evaluating wild Triticeae species for Boron tolerance that were not noted by Sutton et al. (2007) in their studies on wheat. The high degree of variation between and within wild cereal species involved differences in endosperm development and quality, seed viability, seedling growth rate, germination rate, etc., and made it difficult to obtain uniform root growth under hydroponic growing conditions whether or not Boron was added to the hydroponic solution. This was noted in Table 3 where many accessions showed on average a shorter root growth in 0 Boron than in 3 mM Boron, which was due to the vagries of seed development and germination. Nevertheless, several wild *Triticeae* species expressed sufficient Boron tolerance to be considered as useful candidates for wheat improvement programs striving to produce improved Boron tolerant wheat cultivars. For wheat, grain yield under field conditions is the critical measure, and any results using root length measurements as a parameter for quickly screening germplasm for boron tolerance will ultimately have to be field-tested.

REFERENCES

Abedin MJ, Jahiruddin M, Hoque MS, Islam MR, Ahmed MU (1994). Application of boron for improving grain yield of wheat. Progress Agric., 5:75-79.

Baligar VC, Fageria NK, He ZL (2001). Nutrient use efficiency in plants. Soil Sci. Plant Anal., 32: 921-950.

Blevins DG, Lukazewski KM (1998). Boron in plant structure and function. Ann. Rev. Plant Physiol. Plant Mol. Biol., 49:481-500. (doi:10.1146/annurev.arplant.49.1.481)

Brown PH, Shelp BJ (1997). Boron mobility in plants. Plant Soil, 193: 85-101.

Gupta UC, Jame YM, Campbell CA, Leyshon AJ, Nicholaichuk W (1995). Boron toxicity and deficiency: a review. Can. J. Soil Sci., 65: 381–409.

Huang L, Pant J, Dell B, Bell RW (2000). Effects of Boron deficiency on anther development and floret fertility in wheat (*Triticum aestivum* L. `Wilgoyne'). Ann. Bot., 85: 493-500.

Jahiruddin M, Ali MS, Hossain MA, Ahmed MU, Hoque MM (1995). Effect of boron on grain set, yield and some other parameters of wheat cultivars. Bangladesh J. Agric. Sci., 22: 179-184.

Jamjod S, Niruntrayagul S, Rerkasem B (2004). Genetic control of boron efficiency in wheat (*Triticum aestivum* L). Euphytica, 135: 21-27.

Jefferies SP, Pallotta MA, Paull JG, Karakousis A, Kretschmer JM (2000). Mapping and validation of chromosome regions conferring boron toxicity tolerance in wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 101: 767-777.

Kataki PK, Upreti HK, Bhatta MR (2001). Soil boron deficiency induced wheat sterility in Nepal: response to boron and nitrogen application. J New Seeds, 3: 23–39.

McDonald GK, Eglinton JK, Barr AR (2010). Assessment of the agronomic value of QTL on chromosomes 2H and 4H linked to tolerance to boron toxicity in barley (*Hordeum vulgare* L.) Plant Soil, 326: 275-290.

Miwa K, Takano J, Omori H, Seki M, Shinozaki K, Fujiwara T (2007). Plants tolerant of high boron levels. Science, 318: 1417.

Paul JG, Cartwright B, Rathjen AJ (1988). Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica 39:137-144.

Reid R (2010). Can we really increase yields by making crop plants tolerant to boron toxicity? Plant Sci., 178:9-11.

Rerkasem B, Jamjod S (1989). Correcting boron deficiency induced ear sterility in wheat and barley. Thai J. Soils Fert., 11: 200-209.

Rerkasem B, Jamjod S (1997). Genotypic variation in plant response to low boron and implications for plant breeding. Plant Soil, 193: 169-180.

Rerkasem B (2002). Boron nutrition of crops and genotypic variation in boron efficiency. In: Goldbatch *et. al.* (ed.), Boron in Plant and Animal Nutrition. Kluwer Aca. Pub., New York, pp. 269-280.

Stangoulis JCR, Webb MJ, Graham RD (2000). Boron efficiency in oilseed rape: II. Development of a rapid lab-based screening technique. Plant Soil, 225:253–261. (DOI 10.1023/A:1026593528256)

Subedi KD, Gooding MJ, Gregory PJ (1999). Boron accumulation and partitioning in wheat cultivars with contrasting tolerance to boron deficiency. Plant Soil, 214: 141-152.

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: 1446.

Yau SK (2002). Interaction of boron-toxicity, drought, and genotypes on barley root growth, yield, and other agronomic characters. Aust. J. Agric. Res., 53: 347-354.