

## Boron Tolerance in Wheat Accessions of Different Origin Estimated in Controlled and Field Conditions

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### ABSTRACT

This study was aimed to assess the effects of excess boron on 59 genetically divergent wheat accessions and to identify those with high and stable yields under a range of soil boron concentrations. The second aim was to test the applicability of a laboratory technique performed at juvenile stages of development in estimating field boron tolerance. The study comprised a control and three boron treatments, applied as 50, 100 and 150 mg boric acid L<sup>-1</sup> in laboratory, and 33.0, 67.0 and 133.0 kg boric acid ha<sup>-1</sup> in field trial. Yield performance and stability were evaluated using biplots from sites regression model, while interrelationships among analyzed parameters were assessed using path coefficient analysis. Parameters were mostly decreased by excess boron when compared to the control (seedling root length, seedling dry weight, grain number per spike, grain yield, flag leaf area, leaf area duration and grain weight). Significant increase was noted for seedling boron concentration and content, percentage of sterile spikelets per spike and number of spikes per m<sup>2</sup>. Spike length, number of spikelets per spike, and anthesis date remained unaffected. The majority of accessions with high and stable yields were of local origin, so, we conclude that adaptation to environmental factors other than elevated soil boron plays an important role in overall field boron tolerance. The effects of excessive external boron on boron accumulation noted at the seedling stage in laboratory studies corresponded to its effects on yield in field.

**Keywords:** Micro-element boron, Path coefficient, Sites regression model, *Triticum aestivum*, Yield stability.

### INTRODUCTION

Boron is an essential micronutrient for healthy growth and development of vascular plants. An inadequate boron supply may impair growth and, consequently, limit yield in agricultural plants including wheat. Soils that are low in boron can be ameliorated by application of appropriate fertilizers; but boron toxicity is a more difficult problem.

Boron toxicity symptoms were firstly described 80 years ago on barley (Christensen, 1934). However, the disorder in plant nutrition was not extensively investigated until the mid-

1980s, since the characteristic brown necrotic spots were previously often confused with leaf disease caused by *Pyrenophora teres* f. spp. *maculata*. When 17% of barley (cv. Clipper) yield loss in southern Australia was attributed to boron toxicity (Cartwright *et al.*, 1984), research on boron tolerance increased. Boron rich soils occur most commonly in arid and semi-arid regions of Australia, South and North America, South and East Europe, the Middle East, North Africa, India and the former USSR. Naturally-high soil boron most commonly originates from sea sediments and volcanoes and it is often found in association

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with saline soils. However, excess boron may be caused by anthropogenic activity, such as irrigation with water containing high concentrations of the element, application of fly ash used as an ameliorant in agriculture, boron fertilizers applied to correct boron deficiency, and surface mining. Although soils that are rich in boron can be ameliorated by liming or leaching, breeding tolerant cultivars has been proposed as the most economical approach for overcoming the problem (Yau and Ryan, 2008; Reid, 2010; Schnurbusch *et al.*, 2010; Masood *et al.*, 2012; Wimmer and Goldbach, 2012).

The first step in breeding tolerant cultivars is to establish if there is genetic variability in the tested material. Since screening large number of prospective candidates in field trials is time consuming and labor intensive, an effective screening technique for use under controlled environment conditions is desirable. Theoretically, boron-tolerant wheat genotypes should develop few or no symptoms of toxicity, contain relatively low amounts of boron in tissue, and grow or yield better in the presence of high concentrations of boron compared to sensitive lines. Therefore, in controlled environments, wheat accessions have most commonly been compared using symptom scores, boron concentration, dry weight and yield responses to excessive boron supply (Emebiri and Ogbonnaya, 2015; Ilyas *et al.*, 2015). Since the variation in wheat root elongation corresponds to the whole plant response to toxic external boron, the filter paper technique proposed by Chantachume *et al.* (1995) became one of the most frequently used screening procedures for assessing boron tolerance in wheat and barley. This rapid method is based on reduction in seedling root length in the presence of high external boron. It has been used for detecting phenotypic variability in boron tolerance, studying other traits related to boron tolerance (Rehman *et al.*, 2006; Brdar-Jokanović *et al.*, 2013), mapping chromosomal regions conferring boron tolerance in wheat and barley (Jefferies *et al.*, 2000), and to confirm the effects of genes involved in boron tolerance (Emebiri *et al.*, 2009). However, from the breeder's point of

view, the method is valuable only if the results can be related to plant reaction to excess boron in field conditions, primarily by means of yield. Therefore, the comparison of the wheat boron tolerance estimations based on this laboratory method with the boron tolerance in field would be of importance for further work on breeding high-yielding boron-tolerant wheat.

This study was undertaken to quantify the effects of excess boron on yield and yield-related traits in 59 divergent wheat accessions, and to identify lines characterized by high and stable yields under a range of soil boron concentrations. The second objective was to assess the effectiveness of estimating boron tolerance of lines of wheat in the field via a frequently used laboratory technique performed at the seedling stage.

## MATERIALS AND METHODS

Fifty-nine genetically divergent bread wheat (*Triticum aestivum* L.) accessions were assessed to provide a wide range of response to excess boron. These included: (i) high-yielding cultivars and lines of local origin (Institute of Field and Vegetable Crops, Novi Sad, Serbia); (ii) cultivars of worldwide origin selected for high yield or other yield-related characteristics, and (iii) lines from previous studies (Table 1).

The laboratory study used the method of Chantachume *et al.* (1995). Briefly, seed was surface sterilized with 70% EtOH (v/v) for 10 minutes, then with 5% H<sub>2</sub>O<sub>2</sub> (v/v) for 10 minutes, and rinsed with sterile water. Since all accessions included in the study were winter wheats, the seed was pre-germinated at 4°C for 48 hours and at 18°C for 24 hours, and then imbibed on filter paper soaked with boric acid solutions of the following concentrations: 0.93 (control), 50, 100, and 150 mg L<sup>-1</sup> (treatments). Each solution also contained 0.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>×4H<sub>2</sub>O, 0.0025 mM ZnSO<sub>4</sub>×7H<sub>2</sub>O and 0.015 mM H<sub>3</sub>BO<sub>3</sub>. Seed germination and growth of seedlings took place at 18°C

Table 1. The origin and boron tolerance of wheat accessions assessed in the study.

No.	Accession	Origin	Boron tolerance <sup>a</sup>		No	Accession	Origin	Literature source	Boron tolerance	
			Literature source	Laboratory trial					Laboratory trial	Field trial
1	Arija	NS <sup>b</sup> , local	-	MS (36.1)	31	Teodora	NS, local	-	T	T
2	Astra	NS, local	-	MT (33.3)	32	Vila	NS, local	MT, S <sup>c</sup>	MT	MS
3	Balerina	NS, local	MT, MS <sup>e</sup>	S (13.9)	33	NS 100/01	NS, local	-	MS	T
4	Cipovka	NS, local	-	MS	34	NS 73/02	NS, local	-	MT	T
5	Diva	NS, local	-	T (16.7)	35	NS 103/02	NS, local	-	MS	MS
6	Donna	NS, local	-	MS	36	NS 53/03	NS, local	-	MT	MT
7	Dragana	NS, local	-	T	37	Apache	France, foreign	-	MT (26.1)	S (34.8)
8	Etida	NS, local	-	MT	38	Bezostaja 1	Russia, foreign	S <sup>cd</sup>	MT	S
9	Evropa 90	NS, local	T, MT <sup>de</sup>	MT	39	Bolal 2973	Turkey, foreign	T <sup>e</sup>	S (34.8)	MS (47.8)
10	Helena	NS, local	-	MT	40	Condor	Australia, foreign	MS <sup>b</sup>	S	MT (8.7)
11	Janja	NS, local	-	MS	41	Dagdas	Turkey, foreign	T, MS <sup>c</sup>	S	MS
12	Kantata	NS, local	S <sup>e</sup>	MS	42	Fundulea 4	Romania, foreign	-	S	MS
13	Košuta	NS, local	-	MS	43	ITMI 22/00 <sup>c</sup>	ITMI, foreign	T <sup>e</sup>	MT	MS
14	Lana	NS, local	-	MT	44	ITMI 43/00	ITMI, foreign	T <sup>e</sup>	MS (21.7)	T (8.7)
15	Ljiljana	NS, local	MS <sup>e</sup>	T	45	Kalyan Sona	India, foreign	T <sup>de</sup>	MT	T
16	Milijana	NS, local	-	MS	46	Kenya Farmer	Kenya, foreign	S <sup>a</sup>	S	S
17	Mina	NS, local	-	T	47	Kirac 66	Turkey, foreign	T <sup>e</sup>	T (17.4)	MT
18	Nevesinjka	NS, local	T <sup>de</sup>	MT	48	Magdalena	Hungary, foreign	-	S	MS
19	NS 40 S	NS, local	-	S	49	Mironovska 808	Ukraine, foreign	-	MS	MS
20	Oda	NS, local	-	MT	50	Norin10/Brevor14	ITMI, foreign	MT <sup>e</sup>	MS	S
21	Partizanka	NS, local	S <sup>cd</sup>	MS	51	Peking 11	China, foreign	T <sup>de</sup>	T	S
22	Pesma	NS, local	T <sup>e</sup>	S	52	Pergamino Gaboto	Argentina, foreign	S <sup>e</sup>	S	MS
23	Pobeda	NS, local	MT, S <sup>de</sup>	MS	53	Radika	Macedonia, foreign	-	MS	MS
24	Rapsodija	NS, local	-	MT	54	Renan	France, foreign	-	S	MS
25	Renesansa	NS, local	MT <sup>de</sup>	S	55	Stephens	USA, foreign	-	T	S
26	Rusija	NS, local	-	S	56	Synthetic Norwich	ITMI, foreign	T <sup>e</sup>	MT	S
27	Simfonija	NS, local	-	T	57	Trakija	Bulgaria, foreign	S <sup>de</sup>	MT	MS
28	Simonida	NS, local	-	MS	58	Yanetzky Probat	ITMI, foreign	T <sup>e</sup>	T	MS
29	Sofija	NS, local	-	MS	59	Žitarka	Croatia, foreign	-	MS	S
30	Sonata	NS, local	-	MT	-	-	-	-	-	-

<sup>a</sup>T, Tolerant; MT, Moderately Tolerant; MS, Moderately Sensitive; S, Sensitive. <sup>b</sup>NS, Institute of Field and Vegetable Crops, Novi Sad, Serbia. <sup>c</sup>ITMI, International Triticeae Mapping Initiative population. Percentages of local and foreign accessions exhibiting respective levels of boron tolerance are given in brackets. <sup>d</sup>Pot trial (Paull *et al.*, 1988), <sup>e</sup>Pot and filter paper (Chantachume *et al.*, 1995), <sup>f</sup>Field, pot and nutrient solution (Kalayci *et al.*, 1998), <sup>g</sup>Field (Kraljević-Balalić *et al.*, 2004), <sup>h</sup>*in vitro* (Kondić-Špika *et al.*, 2010).



for 11 days in the dark. The experimental layout was a completely random design, with five replicates and 60 seeds per experimental unit. The growth and boron content of seedling roots were assessed by measuring total root length (cm), root dry weight (mg), boron concentration ( $\text{mg kg}^{-1}$  dry matter) and content ( $\mu\text{g seedling}^{-1}$ ). Boron concentration was determined using an ICP spectrophotometer, after digestion of the plant material in nitric acid. Boron content was determined by multiplying the boron concentration by dry weight. Boron tolerance was estimated from mean root length reduction in boron treatments relative to the control, as follows: < 10% Tolerant (T), 10-20% Moderately Tolerant (MT), 20-30% Moderately Sensitive (MS), and > 30% Sensitive (S).

The two-year field trial (2005/2006 and 2006/2007) was conducted at Rimski Šančevi Experimental Station, Institute of Field and Vegetable Crops, Novi Sad, Serbia (45° 20' N, 19° 51' E, 84 m altitude). The soil type was a fertile chernozem, containing 0.76  $\text{mg kg}^{-1}$  hot water extractable boron at 0-30 cm depth and 0.53  $\text{mg kg}^{-1}$  at 30-60 cm (common for this soil type). Weather data for the two wheat growing seasons, collected from a meteorological station about 500 m from the field, are presented in Table 2.

The trial was set in a randomized complete block design with three replications. The trial consisted of three concentrations of boron [33  $\text{kg H}_3\text{BO}_3 \text{ ha}^{-1}$  (B1), 67  $\text{kg}$  (B2), 133  $\text{kg}$  (B3)] and a control (B0), applied immediately after sowing by watering the plots with boric acid dissolved in distilled water. Each plot was 1.2  $\text{m}^2$  (1 m width, 1.2 m length), consisting of six rows with 20 cm

between rows. Plant spacing within rows was 2 cm.

Grain yield ( $\text{g m}^{-2}$ ), primary yield components (grain weight, grain number per spike, number of spikes per  $\text{m}^2$ ) and yield-related traits (anthesis date - days from January 1 to anthesis, flag leaf area, leaf area duration (days from flowering to complete loss of leaf green coloration), spike length, number of spikelets per spike, percentage of sterile spikelets per spike) were recorded. Spike analyses were performed on 10 randomly collected spikes per plot. Mean yield reduction relative to the control was used as the selection criterion for estimating boron tolerance, using the following rating scale: < 3% Tolerant (T), 3-6% Moderately Tolerant (MT), 6-9% Moderately Sensitive (MS), and > 9% Sensitive (S).

The plots were seeded on October 24, 2005, and October 25, 2006. Standard agronomic practices for the region were applied (fertilization, weed, insect and disease management). The plots were harvested at maturity on July 12-19, 2006, and June 22-23, 2007.

Besides calculating basic statistical parameters, the data were assessed using analysis of variance to confirm that there were significant Genotype-Environment (GE) interactions for each variable (not shown), as a precognition for employing biplot analysis. The LSD test was used for comparison of means. A Site Regression model (SREG) (Crossa and Cornelius, 1997) and the corresponding two-dimensional biplots were used to evaluate mean performance and stability of genotypes across treatments and years. The biplot analysis treats genotype and genotype x

**Table 2.** Weather data for 2005/2006 and 2006/2007 wheat growing seasons and 30-year average (1981-2010). Source: Republic Hydrometeorological Service of Serbia, Rimski Šančevi experimental station.

Parameter	2005/2006	2006/2007	1981-2010
Mean daily temperature (°C)	9.2	10.6	8.6
Minimum temperature (°C)	-14.0	-6.0	-3.1
Maximum temperature (°C)	34.0	36.0	28.1
Mean daily temperature during grain filling (°C)	19.2	21.4	19.6
Sum of precipitation (mm)	498.4	390.9	465.3
Sum of precipitation during grain filling (mm)	174.4	170.5	154.4

environment interaction as two sources of variation relevant to genotype evaluation (Yan and Tinker, 2006). Each boron treatment in each year was treated as a separate entity prior to performing the regression and biplot analysis. Path coefficient analysis was performed to investigate the interrelationships among the analyzed wheat traits. Statistical analysis was carried out using R software (R Development Core Team, 2011).

## RESULTS

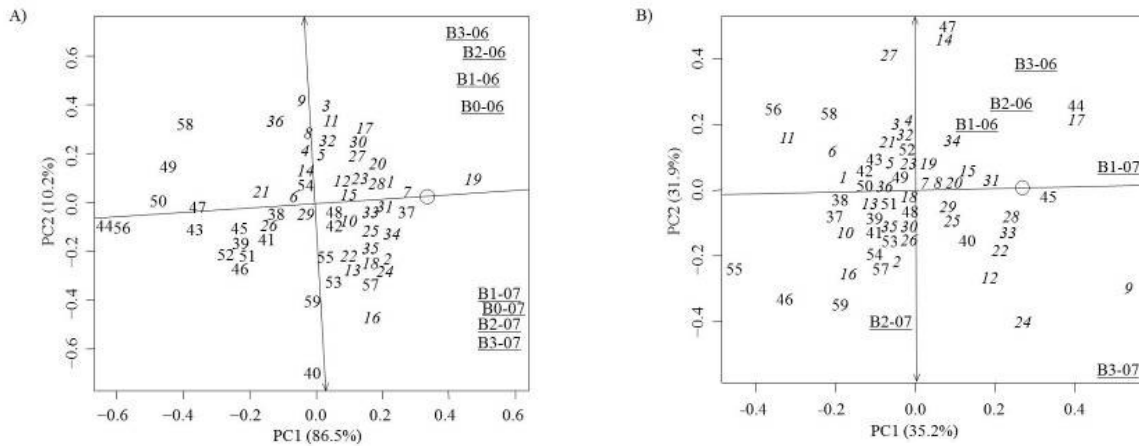
The average effects of boron treatments on wheat are presented in Table 3. Excess boron significantly reduced the majority of the analyzed parameters in both laboratory and field trials. Treatments with boron increased only boron concentration and content, number of sterile spikelets per spike, and number of spikes per m<sup>2</sup>. Spike length, number of spikelets per spike and anthesis date were not affected.

As the most important agronomic trait, wheat yield obtained from the field trial was further analyzed using SREG model and the corresponding two-dimensional biplots. Biplots are used for visualizing genotype response to the particular environment, as well as for evaluating mean performance and stability (Yan and Tinker, 2006). Each genotype had a significant genotype x environment interaction in ANOVA (not shown), which is an important prerequisite for using this analysis. Yield performance is depicted in Figure 1-A. Each treatment x year combination (including the control) was evaluated as a separate entity; so, eight entities were included in the analysis of each genotype. The correlation between any of the environments is represented by the cosine of the angle between their vectors. Acute angles imply positive, obtuse negative, and right angles indicate no correlation. In this study, the control and the three boron treatments imposed in the first growing season were strongly positively correlated forming a group. However, the

**Table 3.** Effect of boron treatments on seedling growth and yield components of wheat <sup>a</sup>.

Origin of tested material	Seedling root length (cm)	Seedling weight (mg)	Seedling dry weight (mg)	Seedling B concentration (mg kg <sup>-1</sup> )	Seedling B content (µg seedling <sup>-1</sup> )	Seedling B content (µg seedling <sup>-1</sup> )	Grain yield [g (m <sup>2</sup> ) <sup>-1</sup> ]	Grain weight (mg)	Number of grains per spike
Serbian (NS)	35.6 ± 0.5 <sup>Aa</sup>	11.0 ± 0.2 <sup>Aa</sup>	15.53 ± 0.61 <sup>Aa</sup>	15.53 ± 0.61 <sup>Aa</sup>	0.17 ± 0.01 <sup>Aa</sup>	0.17 ± 0.01 <sup>Aa</sup>	1094.7 ± 14.1 <sup>Aa</sup>	44.3 ± 0.4 <sup>Aa</sup>	46.9 ± 0.5 <sup>Aa</sup>
Foreign (For)	32.1 ± 1.1 <sup>Ab</sup>	9.6 ± 0.3 <sup>Ab</sup>	16.22 ± 0.78 <sup>Aa</sup>	16.22 ± 0.78 <sup>Aa</sup>	0.16 ± 0.01 <sup>Aa</sup>	0.16 ± 0.01 <sup>Aa</sup>	801.2 ± 35.3 <sup>Ab</sup>	39.0 ± 0.7 <sup>Ab</sup>	42.4 ± 1.2 <sup>Ab</sup>
Serbian (NS)	28.8 ± 0.5 <sup>Ba</sup>	10.5 ± 0.2 <sup>Ba</sup>	158.10 ± 2.03 <sup>Ba</sup>	158.10 ± 2.03 <sup>Ba</sup>	1.63 ± 0.04 <sup>Ba</sup>	1.63 ± 0.04 <sup>Ba</sup>	1069.1 ± 9.8 <sup>Ba</sup>	43.7 ± 0.3 <sup>Ba</sup>	44.2 ± 0.4 <sup>Ba</sup>
Foreign (For)	24.6 ± 1.0 <sup>Bb</sup>	8.9 ± 0.3 <sup>Ba</sup>	177.76 ± 4.80 <sup>Bb</sup>	177.76 ± 4.80 <sup>Bb</sup>	1.52 ± 0.06 <sup>Ba</sup>	1.52 ± 0.06 <sup>Ba</sup>	765.8 ± 23.4 <sup>Bb</sup>	38.2 ± 0.5 <sup>Ba</sup>	41.2 ± 0.8 <sup>Bb</sup>
	Number of spikes per m <sup>2</sup>	Spike length (cm)	Number of spikelets per spike	Number of spikelets per spike	Sterile spikelets (%)	Sterile spikelets (%)	Anthesis date (days from Jan. 1)	Leaf area duration (days from anth.)	Flag leaf area (cm <sup>2</sup> )
Serbian (NS)	549.8 ± 8.8 <sup>Aa</sup>	9.9 ± 0.1 <sup>Aa</sup>	20.7 ± 0.1 <sup>Aa</sup>	20.7 ± 0.1 <sup>Aa</sup>	8.4 ± 0.3 <sup>Aa</sup>	8.4 ± 0.3 <sup>Aa</sup>	132.5 ± 0.1 <sup>Aa</sup>	36.5 ± 0.2 <sup>Aa</sup>	26.8 ± 0.4 <sup>Aa</sup>
Foreign (For)	495.5 ± 14.2 <sup>Ab</sup>	9.8 ± 0.2 <sup>Ab</sup>	20.6 ± 0.3 <sup>Ab</sup>	20.6 ± 0.3 <sup>Ab</sup>	9.9 ± 0.5 <sup>Ab</sup>	9.9 ± 0.5 <sup>Ab</sup>	135.0 ± 0.4 <sup>Ab</sup>	32.2 ± 0.6 <sup>Ab</sup>	23.9 ± 0.6 <sup>Ab</sup>
Serbian (NS)	573.5 ± 6.1 <sup>Ba</sup>	9.7 ± 0.1 <sup>Aa</sup>	20.6 ± 0.1 <sup>Aa</sup>	20.6 ± 0.1 <sup>Aa</sup>	11.2 ± 0.2 <sup>Ba</sup>	11.2 ± 0.2 <sup>Ba</sup>	132.4 ± 0.1 <sup>Aa</sup>	35.9 ± 0.1 <sup>Ba</sup>	26.4 ± 0.2 <sup>Ba</sup>
Foreign (For)	500.2 ± 9.5 <sup>Bb</sup>	9.6 ± 0.1 <sup>Aa</sup>	20.4 ± 0.2 <sup>Aa</sup>	20.4 ± 0.2 <sup>Aa</sup>	11.2 ± 0.4 <sup>Bb</sup>	11.2 ± 0.4 <sup>Bb</sup>	135.1 ± 0.3 <sup>Aa</sup>	31.7 ± 0.4 <sup>Ba</sup>	23.4 ± 0.4 <sup>Ba</sup>

<sup>a</sup> Means±SE are shown. For each trait, means corresponding to control and boron treatments that are followed by different uppercase letters are significantly different at  $p < 0.05$ . Within the



**Figure 1.** Mean performance and stability of wheat accessions for (A) Yield and (B) Relative yield in boron treatments (% of the control). Arabic numbers correspond to the number of the accessions listed in Table 1; accessions of local origin are in italic. B0-06, B1-06, B2-06, B3-06, B0-07, B1-07, B2-07, B3-07: entities corresponding to the control and boron treatments for 2005/2006 and 2006/2007 growing seasons, respectively.

group was not correlated to the second group consisting of the entities corresponding to the second season of the experiment.

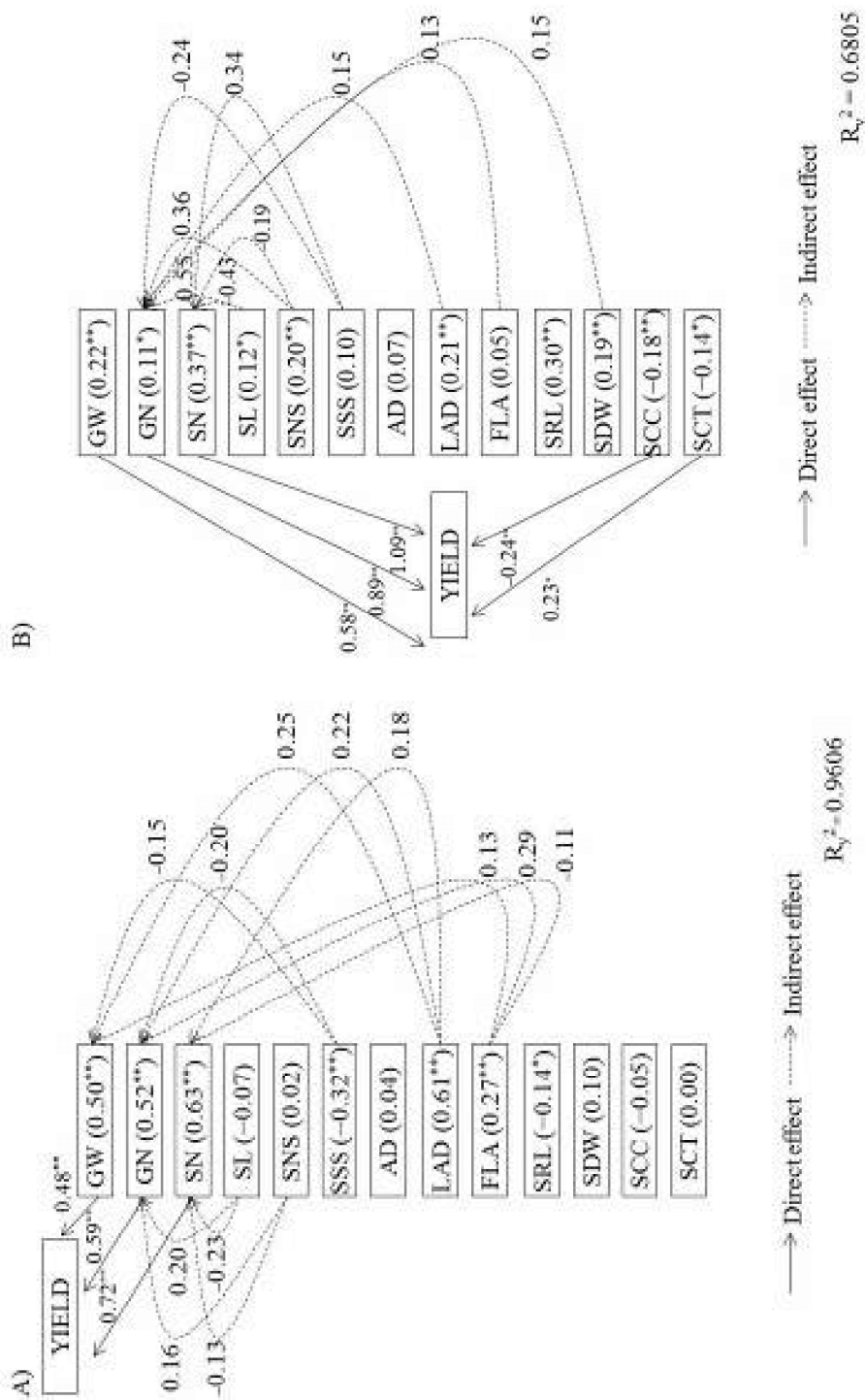
The positive direction of the abscissa line indicates better mean performance, while positive direction of the ordinate line indicates stability. Accessions closer to the abscissa were characterized by higher stability and vice versa. With the exception of few cases (37 – Apache), the accessions of local origin (7 – Dragana, 1 – Arija, 28 – Simonida, 31 – Teodora) out-yielded foreign cultivars and had higher yield stability (Figure 1-A).

To further investigate the effect of boron on wheat, the relative yield in selected treatments (% relative to the control) was examined (Figure 1-B). This value represents yield response to excess boron, regardless of the absolute value of yield for that line. Three sectors were apparent; the first one is comprised of the three treatments from 2005/06 and the lowest concentration treatment from 2006/2007. The second and the third sectors consist of one treatment each from 2006/2007. This indicates that elevated soil boron had a small effect on yield in generally more favorable season of 2005/2006. Higher temperatures, especially

during grain filling, and lower precipitation were recorded for 2006/2007, relative to 2005/2006 (Table 2). Accessions with the most stable yield across the soil boron levels were Kalyan Sona, Simonida and Teodora (45, 28, and 31, respectively).

According to the path coefficient analysis employed to assess the effectiveness of estimating boron tolerance in the field via laboratory technique performed at the seedling stage (Figures 2-A and -B), all three primary yield components (grain weight, grains per spike, number of spikes per m<sup>2</sup>) had significant direct positive effects on yield at both optimal and elevated boron supply. Those effects were stronger and the differences in their contribution to yield formation were more pronounced in treatments where boron was applied.

Although several traits of agronomic importance were correlated to yield, none of them affected it directly. The correlations may be explained by various indirect effects on yield. For example, spike length and number of spikelets per spike affected yield indirectly; positively via number of grains per spike and negatively via number of spikes per m<sup>2</sup>. This was true for both the control and boron treatments. However, in



**Figure 2.** Direct and indirect effects of the analyzed traits on wheat yield, in A) control and B) boron treatments. GW, Grain Weight; GN, Number of Grains per Spike; SN, Number of Spikes per m<sup>2</sup>; SL, Spike Length; SNS, Number of Spikelets per Spike; SSS, Percentage of Sterile Spikelets per Spike; AD, Anthesis Date; LAD, Leaf Area Duration; FLA, Flag Leaf Area; SRL, Seedling Root Length; SDW, Seedling Dry Weight; SCC, Seedling Boron Concentration; SCT, Seedling Boron Content. Correlation coefficients with yield are indicated in parentheses (r) with \*, \*\*, \*\*\*: Significant at P<0.05 and 0.01, respectively.



the control, the effects were mutually of similar strength resulting in the absence of significant correlations with yield. In the boron treatments, however, more pronounced differences in the strength of these effects resulted in significant correlations with yield.

In the laboratory assessment of seedlings, none of the traits had a direct effect on yield in the control. In the boron treatments, a direct negative effect of boron concentration and a direct positive effect of boron content on yield were observed.

## DISCUSSION

The effects of the imposed boron treatments on the wheat traits are generally in accordance to those reported previously for barley, triticale, and common and durum wheat (Chantachume *et al.*, 1995; Jefferies *et al.*, 2000; Corrêa *et al.*, 2005; Rehman *et al.*, 2006; Yau and Ryan, 2008; Emebiri *et al.*, 2009; Coscun *et al.*, 2014). Contrary to our observations, the abovementioned authors reported reductions in number of spikes per m<sup>2</sup>, delay in heading date, and no change in flag leaf area in durum wheat lines exposed to high soil boron. The discrepancies may be due to different experimental designs, the applied boron doses, genetic differences among the tested materials, and number of accessions included in the analyses. The current study was performed on 59 divergent accessions, and the Serbian varieties (Röder *et al.*, 2002) and foreign accessions in the study were selected to be highly heterogeneous, so, for the majority of the parameters, it included a wider range of variation than previous reports.

Since high and stable yield represents the most important goal of wheat breeders and producers, yield data were further analyzed. The biplot method of yield data analysis and interpretation is commonly used in trials dealing with multiple treatments, seasons and/or locations (Kendal, 2015; Rasoli *et al.*, 2015). In this study, eight

genotype × treatment combinations formed two uncorrelated groups. The groups consisted of four entities each, corresponding to the two years of the experiment. Therefore, yield differences were more pronounced between the two years of the study than among the boron treatments. Such a distribution demonstrated the importance of seasonal variation in temperature and precipitation in yield formation, and was in accordance, although not explicitly stated, to the results of previous field trials (Kalayci *et al.*, 1998; McDonald *et al.* 2010).

The biplot analysis of the relative yield in selected treatments (% of control, regardless of absolute value) showed comparatively stronger effect of boron treatments on wheat in the season characterized by higher temperatures and lower precipitation, which supports the conclusion of previous studies that drought accompanied with high air temperatures aggravates the negative effect of soil boron on yield (Yau and Ryan, 2008; Reid, 2010).

Yield stability across growing seasons and soil boron levels was also considered, because boron levels exhibiting detrimental effect on yield may vary depending upon soil type and characteristics such as moisture and pH. Boron concentrations that reduced root growth of barley by 10% varied approximately ten-fold among the 19 analyzed soils (Mertens *et al.* 2011). In addition, the range between boron toxicity and deficiency is narrow. Both disorders may occur within the same locality, even within the same season (Avci and Akar, 2005). Barley plants with extreme toxicity symptoms may occur in the field at the distance of less than 10 m from plants exhibiting no symptoms (Brennan and Adcock, 2004). Consequently, cultivars adapted to a wide range of soil boron concentration are preferred. The generally better mean performance and stability of local accessions was somewhat expected and can be explained by the fact that the accessions of local origin represent the highest-yielding cultivars developed mainly



through experiments conducted at the same location as this study and, therefore, highly adapted to the agro-ecological conditions. On the other hand, foreign accessions were high-yielding cultivars of worldwide origin, or boron tolerance checks chosen on the basis of literature data. The importance of adaptation to various agro-ecological factors in yield formation under the elevated soil boron was illustrated by the difference in boron tolerance of local and foreign accessions under controlled conditions and in the field. In the laboratory, 50.0% of local and 43.5% foreign accessions were assessed as boron tolerant or moderately tolerant. However, 55.6% local and only 17.4% of foreign accessions fell into those categories when assessed in the field. In other words; whereas approximately the same number of local accessions exhibited good boron tolerance in both laboratory and field, more than a half of the foreign accessions that were tolerant in laboratory were not tolerant in the field.

Path coefficient analysis was performed to investigate if the effects of excess boron observed at juvenile stages of wheat development can be used to estimate boron tolerance in the field. It was also used to assess the effects of traits of agronomic importance on yield. The advantage of this analysis is that it simultaneously considers relationships among all traits. In contrast, Pearson's correlation coefficients examined the relationship between only two traits. Although all three primary yield components had direct positive effects on yield at both optimal and elevated boron supply, those effects were stronger and the differences in their contribution to yield formation were more pronounced in the boron treatments. This supports the hypothesis of approximately equal contribution of yield components to yield under favorable environmental conditions and compensatory effect among them in stressful conditions, e.g. high temperatures and water shortage (Talebi *et al.*, 2010). The compensatory effects among traits of agronomic importance observed in this study have been

also reported by Ali *et al.* (2008) and Yagdi (2009).

Since none of the seedling traits investigated in the laboratory had direct effect on yield in the control, we conclude that boron accumulation in seedlings at optimal boron supply is not a useful indicator of boron tolerance in the field. This result does not support previous speculation about lower boron accumulation in tolerant lines of wheat and barley (Rehman *et al.*, 2006). In the boron treatments, a direct effect of boron concentration (negative) and content (positive) on yield was observed. The negative effect was expected, because high internal boron has been associated with boron susceptibility in cereals, which favored the hypothesis of reduced accumulation as the mechanism of tolerance (Padmanabhan *et al.*, 2012). On the other hand, the positive effect of boron content on yield may be related to the opposing hypothesis of an internal tolerance mechanism (Roessner *et al.*, 2006; Pang *et al.*, 2010). Although these results imply the effectiveness of the employed laboratory technique for estimating wheat boron tolerance in the field, further research on boron uptake and accumulation is required to clarify the relations among boron concentration and content assessed at seedling stage and yield obtained in field conditions. Nevertheless, the effects of excess boron on wheat observed at juvenile stages of development in controlled conditions can be related to yield response to elevated soil boron in the field. However, besides widely used root length reduction as selection criterion for assessing tolerance, attention should be paid to genotypic differences concerning boron accumulation, with boron concentration and content as promising selection criteria.

## CONCLUSIONS

Excess boron decreased root length and dry weight of wheat seedlings under controlled conditions, as well as grain yield,



weight, and number per spike, leaf area duration and flag leaf area of adult plants under field conditions. Excess boron also increased boron concentration and content in seedlings, and the number of spikes per m<sup>2</sup> and percentage of sterile spikelets per spike in adult plants. In most cases, the wheat accessions showing high and stable yields across growing seasons and soil boron levels were of local origin. This indicates that adaptation to specific agro-ecological conditions has an important effect on boron tolerance in the field. Also, boron accumulation at the seedling stage may be a useful indicator of yield response to elevated soil boron in the field.

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## برآورد مقاومت به عنصر بور در نمونه های گندم از مبادی مختلف در محیط کنترل شده و مزرعه

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### چکیده

هدف این پژوهش ارزیابی اثر مقادیر زیاد بور روی ۵۹ نمونه مختلف گندم و شناسایی گندم هایی بود که در دامنه ای از غلظت های بور در خاک عملکرد ثابت یا بالایی دارند. هدف دیگر پژوهش، آزمودن کاربردی بودن یک تکنیک آزمایشگاهی در مراحل اولیه رشد برای برآورد مقاومت به بور در شرایط مزرعه بود. تیمار های آزمایش شامل تیمار شاهد و سه سطح بور بود (۵۰، ۱۰۰، و ۱۵۰ میلی گرم در لیتر در آزمایشگاه، و در آزمون های مزرعه ای ۳۳، ۶۷، و ۱۳۳ کیلو گرم اسید بوریک در هکتار). ارزیابی عملکرد و ثبات آن با استفاده از بای پلات از مدل رگرسیون جایگاه ها (sites regression model) انجام شد در حالیکه روابط متقابل پارامترهای تجزیه شده با کاربرد تجزیه ضرایب مسیر (path coefficient analysis) ارزیابی شد. زیادی بور غالباً پارامترهای طول ریشه گیاهچه ها، وزن خشک گیاهچه ها، تعداد دانه در سنبله، عملکرد دانه، مساحت برگ، پرچم، مساحت برگ، و وزن دانه ها را در مقایسه با تیمار شاهد کاهش داد. همچنین، افزایش معناداری در غلظت و مقدار موجود بور در گیاهچه، درصد سنبلچه های عقیم در سنبله، و تعداد سنبله ها در متر مربع مشاهده شد. اما اثری روی طول سنبله، تعداد سنبلچه ها در سنبله، و تاریخ گلدهی به چشم نخورد. از آنجا که مبداء بیشتر نمونه های گندم که عملکرد ثابت یا بالایی داشتند محلی بود، نتیجه گرفتیم که در شرایط مزرعه، سازگاری با عوامل محیطی (به غیر از زیاد بودن مقدار بور در خاک) نقش مهمی در تحمل کلی گندم به بور بازی میکند. اثر مقادیر زیاد بور در محیط رشد روی انباشت بور در گیاهچه ها که در آزمون های آزمایشگاهی مشاهده شد با اثر آن روی عملکرد در شرایط مزرعه همخوانی داشت.