UDC 575. 633.11
https://doi.org/10.2298/GENSR2202907K
Original scientific article

RESPONSE OF WHEAT GENOTYPES TO EXCESS BORON ESTIMATED BY *in vitro* CULTURE

Ankica KONDIĆ-ŠPIKA[1], Svetlana GLOGOVAC[1], Dragana TRKULJA[1], Ana MARJANOVIĆ-JEROMELA[1], Milica MARJANOVIĆ[2]

[1]Institute of Field and Vegetable Crops, Novi Sad, Serbia
[2]Gimnazija "Svetozar Marković", Novi Sad, Serbia

Kondić-Špika A., S. Glogovac, D.Trkulja, A.Marjanović-Jeromela, M. Marjanović (2022). *Response of wheat genotypes to excess boron estimated by in vitro culture*. - Genetika, Vol 54, No.2, 907- 919.

The objective of this study was to evaluate boron tolerance of wheat genotypes using mature embryo culture. The analysis involved 79 recombinant inbred lines of the International Triticeae Mapping Initiative (ITMI) population and three Serbian varieties with known boron (B) tolerance (Pobeda – sensitive S, Balerina -medium tolerant MT, and Nevesinjka – tolerant T). The evaluation was performed on a modified MS medium to which 15 mM of boric acid was added. The control medium contained no excess B. Callus fresh weight (CFW) and reduction of fresh callus weight (RFCW) were determined after one month of cultivation. ANOVA has shown highly significant effect of genotype, the media, and their interaction to callus tissue growth and also significant genotypic effect on RFCW. Majority of genotypes (39) had sensitive reaction to excess boron, twenty-three were medium tolerant, while four of them were tolerant. The obtained results can be potentially used for mapping QTLs associated with tolerance to excess B in wheat breeding program.

Key words: boron tolerance, mature embryo culture, RILs, *Triticum aestivum* L.

INTRODUCTION

Boron (B) is a metalloid essential for different plant functions. When present in suboptimal amounts it causes nutritional deficiency (ZIAEYAN and RAJAIE, 2009; WANG *et al*., 2015; LI *et al*., 2017), while in excess it may become toxic (NABLE *et al*., 1997; SAKAMOTO *et al*., 2011; FANG *et al*., 2016; CHOUDHARY *et al*., 2020). The dramatic differences in terms of boron requirements have been observed among plant species, as well as the genotypes within the

Corresponding author: Ankica Kondić-Špika, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia, E-mail:ankica.spika@ifvcns.ns.ac.rs

species, and the available soil boron which is deficient for one crop may exhibit toxic efects on another (BRDAR-JOKANOVIĆ, 2020). However, significant progress has been made in understanding B homeostasis in plants, which is of particular importance given the slight differences between B deficiency and toxicity (MATTHES *et al*., 2020). Boron toxicity is an environmental problem in areas throughout the world causing significant decrease in crop yield (MODY *et al*., 1993; SCHNURBUSCH *et al*., 2010; LIU *et al*., 2013).

Problem of boron excess naturally occurs in saline soils and soils obtained by sea withdrawal or evaporation of sea water (NABLE *et al*., 1997). Boron is probably the most toxic element in saline soils, where it frequently exceeds the tolerance level of not only sensitive but also semi tolerant and tolerant plants (MILJKOVIĆ, 1960). Although high B and salinity, as two individual abiotic stressors, have usually been studied separately (ZHANG *et al*., 2016; SONG *et al*., 2019; ARIF *et al*., 2019), some efforts in studying their joint impact on plant processes have been made (BEN-GAL and SHANI, 2002; YERMIYAHU *et al*., 2008; WIMMER and GOLDBACH, 2012; PANDEY *et al*., 2019). A common method of remediating high boron soils is through leaching. Since this is impractical in arid and semiarid regions, researchers have turned their attention to either enhancing plant tolerance to boron, or removal of boron from the soil by the plant's vegetative parts.

Boron uptake rate differs significantly among plant species, but also among genotypes belonging to the same plant species (STANGOULIS *et al*., 2000; ROESSNER *et al*., 2006). Tolerant genotypes typically accumulate less boron than sensitive ones (PAULL *et al*., 1988; JEFFERIES *et al*., 1999). By contrast, in several other studies it has been found that susceptibility to B toxicity in soil did not correlate with leaf or shoot concentration of B (YAU *et al*., 1995; TORUN *et al*., 2006; REID and FITZPATRICK, 2009). The observed differences might be due to the presence of different tolerance mechanisms in various plant species and genotypes. In the case of low B accumulation by tolerant genotypes, a mechanism of excess B exclusion is most probably at work. In other cases, internal tolerance mechanisms may be at work such as B adsorption (fixation) in cell walls or an antioxidant response (TORUN *et al*., 2006; GUNES *et al*., 2006; CERVILLA *et al*., 2007). Clear overview of recent advances in mechanisms and approaches for B toxicity alleviation in plants, as well as current limitations and suggestions for further research have been reviewed by HUA *et al.* (2021).

Boron-tolerant cultivars of wheat and barley have been identified (PAULL *et al*., 1988; KRALJEVIĆ-BALALIĆ *et al*., 2002; BRDAR *et al*., 2008; EMEBIRI *et al*., 2009; HAYES *et al*., 2015). However, in most cases the screening studies related to the B toxicity tolerance included only a few genotypes, mostly because the screening methods are labour intensive and prone to experimental error. STILES *et al*. (2010) recommended the use of the hydroponic system in a controlled greenhouse environment for boron tolerance evaluation, because it allows elimination of other limiting factors such as salinity, water availability, climatic conditions, or the difficulty in assessing the amount of available boron in soil. Tissue culture is also a promising approach for plant breeding toward resistance to different stress factors, and immature embryos are mostly used as explants in different plant species (AĞIL *et al*., 2022). In our previous study (KONDIĆ-ŠPIKA *et al*., 2010) we concluded that the method of *in vitro* embryo culture could be recommended for screening a large number of genotypes for breeding purposes. Therefore, in the present work, the same method was used for analysing the reaction of the International Triticeae

Mapping Initiative (ITMI) mapping population to excess boron in the medium. Three Serbian cultivars with known levels of boron tolerance from the previous study (cv. Nevesinjka-tolerant, cv. Balerina-medium tolerant and cv. Pobeda-sensitive genotype) were used as check genotypes for the method suitability and solidity, and as a criterion for line comparison and determination of their level of boron tolerance. The obtained results can be potentially useful for the detection of QTLs associated with tolerance to excess B in wheat breeding program.

MATERIALS AND METHODS

Plant material

In the study, 82 wheat (*Triticum aestivum* L.) genotypes were tested for boron tolerance. Most of the analysed material (79 recombinant inbred lines - RILs) belonged to ITMI population obtained from Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. The ITMI population is derived from a cross between the synthetic hexaploid wheat W-7984 and bread wheat cultivar Opata 85. For validation of the results obtained at the cellular level in the *in vitro* test, the analysis also included three domestic cultivars (Pobeda, Nevesinjka, and Balerina) with known boron tolerance or sensitivity determined in our previous study (KONDIĆ-ŠPIKA *et al*., 2010).

In vitro test

Sterilization and isolation of mature embryos were done as described by KONDIĆ-ŠPIKA *et al.* (2010). The isolated embryos were inoculated onto a modified MS (MURASHIGE and SKOOG, 1962) nutrient medium to which 15 mM boric acid was added. The medium contained the standard MS mineral solution, 0.6% agar, 3% sucrose, and it was supplemented with the following growth regulators: 2.4-dichlorophenoxyacetic acid (2.4-D) – 1.5 mgl^{-1}; naphthylacetic acid (NAA) – 0.5 mgl^{-1}, thiamin – 0.5 mgl^{-1} and glutamine – 150 mgl^{-1}. The medium for control group contained no excess boric acid. The experiment was conducted in two replications. Each replication consisted of five tubes (10x100mm) containing one embryo. The cultivation was performed in a growth chamber, at constant 25-27˚C, 1.500 lx and a 16h/8h light/dark photoperiod.

Callus fresh weight (CFW) was measured after one month of cultivation. The calluses were firstly transferred onto a filter paper, where the medium was removed. After that the calluses without medium remains were measured. Reduction of fresh callus weight (RFCW) on the medium with 15mMl^{-1} boric acid, in relation to the control was calculated by the formula:

$$RFCW = \frac{(FCW(control) - FCW(15\ mMl^{-1})) \times 100}{FCW(control)}$$

where:

RFCW - reduction in fresh callus weight

FCW(15 mMl^{-1}) - fresh callus weight at the medium with 15 mMl^{-1} of boric acid

FCW(control) - fresh callus weight at the control medium

Data processing

All the data were analysed using the program Statistica 11 (StatSoft, Inc. Corporation, Tulsa, OK, USA). Appropriate homogeneity tests, logarithmic transformation of data and variance analysis models were applied. The data of FCW and RFCW were subjected to cluster analysis using the UPGMA (un-weighted pair-group method with arithmetic average) algorithm in order to classify the genotypes into groups with different levels of boron tolerance.

RESULTS

The results of fresh callus weight (FCW) measurement of the 82 wheat genotypes from the *in vitro* test, as well as the reduction of fresh callus weight (RFCW) on the medium with 15mM of boric acid in relation to the control are shown in Table 1. As non-homogeneity of the variances was established by Cohran and Bartlett tests (Tab. 2), logarithmic transformation of data was performed. Homogeneity was achieved after the transformation (Tab. 2). The analysis of variance (Factorial ANOVA) has shown that the genotype, the media, and their interaction had highly significant effect on growth of callus tissue, expressed by FCW (Tab. 3). On the control medium the FCW varied from 4.1 mg (ITMI 100/99) to 84.8 mg (ITMI 43/00), with an average value of 25.2 mg. At the concentration of 15 mM of boric acid the FCW ranged from 1.3 mg in the line ITMI 54/00 to 42.1 mg in cv. Nevesinjka (Tab. 1)., with an average of 7.1 mg.

Table 1. Fresh callus weight (FCW) and the reductions of fresh callus weight (RFCW) of 82 wheat genotypes at different treatments: 1 – control medium without excess B, 2 - with 15 mM of boric acid.

No.	Genotype	FCW (mg)		RFCW (%)	No.	Genotype	FCW (mg)		RFCW (%)
		1	2				1	2	
1	ITMI 1/00	21.2	5.1	75.9	44	ITMI 66/99	22.6	2.7	88.1
2	ITMI 2/00	33.7	3.3	90.2	45	ITMI 68/00	37.4	2.2	94.1
3	ITMI 3/00	12.7	5.0	60.6	46	ITMI 69/00	36.7	3.2	91.3
4	ITMI 4/00	11.5	2.5	78.3	47	ITMI 70/00	9.9	3.4	65.7
5	ITMI 6/00	6.3	2.9	54.0	48	ITMI 71/00	4.9	2.7	44.9
6	ITMI 9/00	9.4	4.9	47.9	49	ITMI 72/00	13.5	6.4	52.6
7	ITMI 10/00	15.3	9.5	37.9	50	ITMI 73/00	15.2	5.8	61.8
8	ITMI 12/00	10.1	2.8	72.3	51	ITMI 76/00	8.0	3.0	62.5
9	ITMI 13/00	19.2	4.8	75.0	52	ITMI 77/99	58.4	17.9	69.3
10	ITMI 14/00	15.7	6.7	57.3	53	ITMI 79/99	39.7	3.9	90.2
11	ITMI 15/00	16.8	3.6	78.6	54	ITMI 79/00	26.5	13.2	50.2
12	ITMI 16/00	23.4	5.3	77.4	55	ITMI 80/00	9.6	5.1	46.9
13	ITMI 17/00	27.7	6.9	75.1	56	ITMI 82/00	7.4	2.7	63.5
14	ITMI 18/00	40.4	15.3	62.1	57	ITMI 83/00	9.3	3.2	65.6
15	ITMI 19/00	25.1	9.6	61.8	58	ITMI 84/00	8.9	3.6	59.6
16	ITMI 20/00	28.6	5.1	82.2	59	ITMI 88/00	45.8	13.2	71.2
17	ITMI 22/00	26.5	10.4	60.8	60	ITMI 89/99	42.3	28.2	33.3
18	ITMI 23/00	27.8	13.8	50.4	61	ITMI 91/00	11.4	5.4	52.6
19	ITMI 24/00	16.1	5.2	67.7	62	ITMI 92/00	23.8	6.9	71.0
20	ITMI 26/00	14.3	4.9	65.7	63	ITMI 93/99	21.5	8.7	59.5
21	ITMI 28/00	17.7	5.9	66.7	64	ITMI 94/99	14.7	2.1	85.7

Table 1 continued.

22	ITMI 29/00	11.2	2.9	74.1	65	ITMI 95/00	77.5	12.9	83.4
23	ITMI 30/00	55.2	8.5	84.6	66	ITMI 96/99	68.8	20.1	70.8
24	ITMI 33/00	11.5	6.3	45.2	67	ITMI 99/00	5.3	2.6	50.9
25	ITMI 34/00	15.3	4.1	73.2	68	ITMI 100/99	4.1	2.4	41.5
26	ITMI 36/99	10.4	3.6	65.4	69	ITMI 103/00	8.8	3.1	64.8
27	ITMI 37/99	59.8	4.4	92.6	70	ITMI 105/00	35.8	7.7	78.5
28	ITMI 38/00	22.6	4.6	79.6	71	ITMI 106/00	14.7	6.2	57.8
29	ITMI 39/00	25.3	2.9	88.5	72	ITMI 107/00	26.3	8.2	68.8
30	ITMI 41/00	31.3	3.9	87.5	73	ITMI 108/00	9.6	3.1	67.7
31	ITMI 42/00	35.9	2.0	94.4	74	ITMI 109/00	23.2	6.3	72.8
32	ITMI 43/00	84.8	38.0	55.2	75	ITMI 110/00	5.2	2.8	46.2
33	ITMI 44/00	55.7	6.1	89.0	76	ITMI 112/00	26.2	5.1	80.5
34	ITMI 45/00	20.1	3.5	82.6	77	ITMI 113/00	16.3	3.9	76.1
35	ITMI 46/00	24.1	2.9	88.0	78	Opata/00	28.9	12.0	58.5
36	ITMI 47/00	12.3	2.6	78.9	79	Synth,/00	24.5	7.2	70.6
37	ITMI 49/00	13.0	2.0	84.6	80	Pobeda (S)	66.7	20.8	68.8
38	ITMI 50/00	54.6	2.5	95.4	81	Balerina (MT)	44.5	18.3	58.9
39	ITMI 51/00	18.1	3.3	81.8	82	Nevesinjka (T)	76.4	42.1	44.9
40	ITMI 54/00	4.8	1.3	72.9					
41	ITMI 59/00	25.9	9.7	62.5					
42	ITMI 60/00	17.7	4.9	72.3					
43	ITMI 62/00	7.4	2.3	68.9					

Table 2. Tests of homogeneity of variances for original and transformed FCW values

	Cochran C	Bartlett chi-sqr	Df[1]	P[2]
FCW (original)	0,154558	176,2782	160	0,179361
FCW (transformed)	0,100356	79,04393	81	0,540790

[1]Df - Degrees of freedom; [2]P – Probability

Table 3. Test of significance for effects of genotype, boron and their interaction (G x B) on fresh callus weight of 82 wheat genotypes.

Effect	SS	Df	MS	F	P
Intercept	339,8484	1	339,8484	251484,5	0,00
Genotype	26,1935	81	0,3234	239,3	0,00
Boron	25,9830	1	25,9830	19227,2	0,00
GxB	5,3859	81	0,0665	49,2	0,00
Error	0,2216	164	0,0014		

Significant reduction of callus growth was observed in all of the genotypes in the presence of excess B in the nutrient medium. The RFCW ranged from 33.3% in ITMI 89/99 to 95.4% in ITMI 50/00 (Tab. 1). Highly significant genotypic effect on RFCW was shown by the One-Way ANOVA (Tab. 4).

Table 4. One-Way ANOVA for effect of genotype on reduction of fresh callus weight (RFCW) of 82 wheat genotypes

Effect	SS	Df	MS	F	P
Intercept	522305,9	1	522305,9	157522,0	0,00**
Genotype	15987,7	81	197,4	59,5	0,00**
Error	271,9	82	3,3		

Formation of fresh callus weight was affected by tolerance of genotypes to excess boron and genotype callusing ability, expressed at the control medium. Sixteen genotypes expressed low callusing ability at the control medium (FCW ≤ 10 mg) and they were excluded from further analyses. The rest of the genotypes (66) were classified by UPGMA dendrogram. Genotypes with RFCW ≤ 50.0% were considered as tolerant (T), from 50.1 to 70.0% as medium tolerant (MT) and ≥ 70.1% as sensitive (S). The most of the classified genotypes had S (39) or MT (23) reactions to excess boron, while only 4 genotypes (3 ITMI lines and cultivar Nevesinjka) exhibited tolerant reactions to excess B. The level of tolerance of Serbian cultivars Nevesinjka and Balerina was confirmed in this study, while cultivar Pobeda exhibited MT instead of S reaction. However, this cultivar had RFCW value 68.8%, very close to the proposed classification value for sensitive cultivars (70.1%).

Using the UPGMA clustering method genotypes were classified into five groups based on FCW and RFCW values (Figure 1). In the first group, S genotypes were found in the largest number (27), while one genotype was MT. The mean RFCW of this group was 78.5% and average FCW on the control medium was 20.8 mg. The second group included 17 MT genotypes and 2 T genotypes, separated in sub-group (ITMI10/00 and ITMI33/00), with average RFCW of 57.6% and FCW on the control medium of 18.5 mg. In the third group, there were nine S genotypes with the highest average RFWC (91.3%) and relatively high average FCW on the control medium (45.4 mg). Seven genotypes, of which four were MT and three were S, with an average RFCW value of 69.2% and FCW on the control medium 57.4 mg, singled out in the fourth group. In the fifth group, there were one MT and one T genotype. At the very bottom of the dendrogram, one genotype stood out (ITMI 89/99) because it had the lowest RFCW value (33.3%) among all tested genotypes.

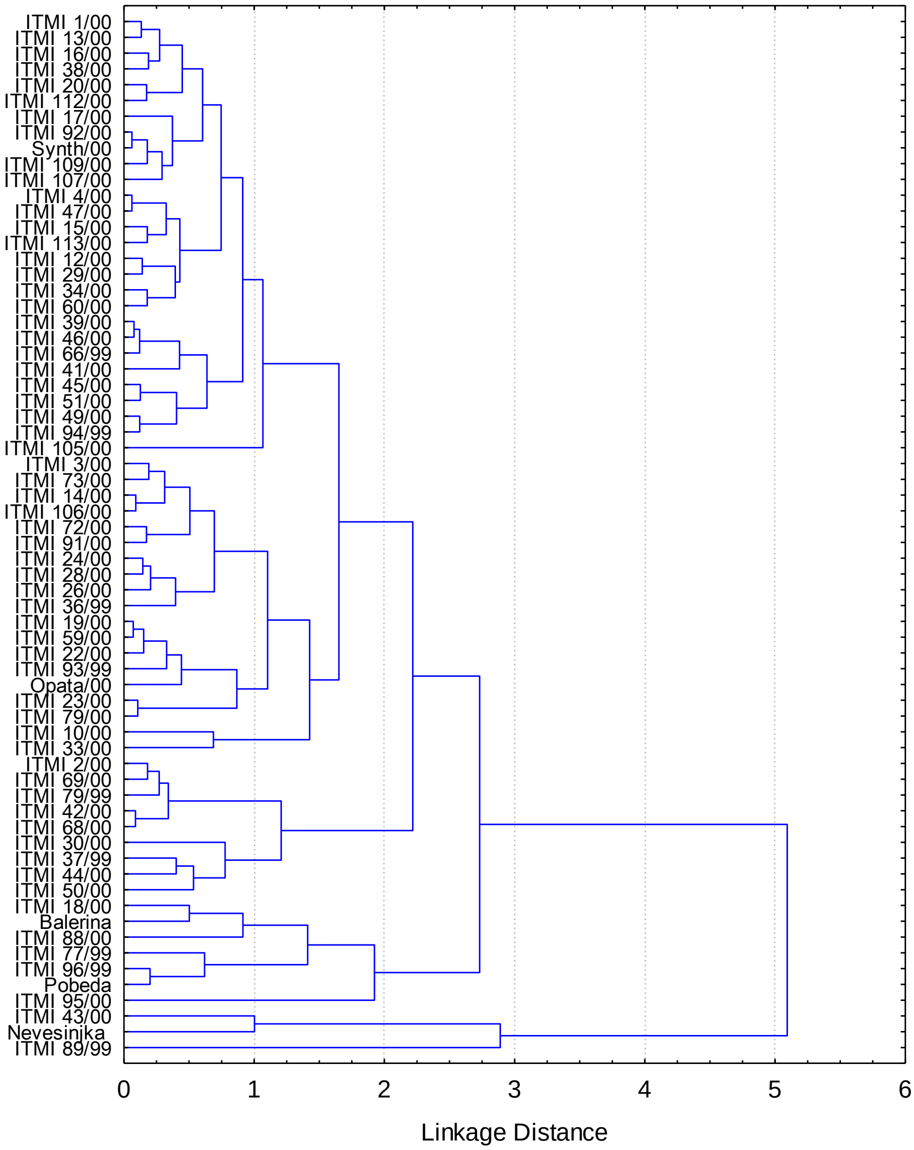

Figure 1. UPGMA cluster for boron tolerance of 66 wheat genotypes.

DISCUSSION

The results have shown differences between genotypes in their callus growth both on the control medium and in the presence of the excess boron. By analysing the effect of different culture media on callusing ability in barley, HAN *et al*. (2011) found significant variation between genotypes in callus induction from mature embryos in the optimum medium. Significant differences in callusing ability were also determined by KACEM *et al*. (2017) between durum wheat genotypes, under the water stress condition. Also, it is very important that differences among the genotypes in their reactions to excess boron were detectable at the cellular level in the *in vitro* culture. These results are in agreement with our previous findings (KONDIĆ-ŠPIKA *et al*., 2010), as well as with the results of similar studies on different plant species (SOTIROPOULOS *et al*., 1998 ; MARTÍNEZ-CUENCA *et al*., 2015; KOCAOĞLU KAVAS *et al*., 2016).

The most of the genotypes had very sensitive and medium tolerant reaction to boron excess, which was in agreement with reactions of the parent genotypes of the ITMI population, Synthetic (S) and Opatha (MT). However, three ITMI lines had tolerant reaction to excess boron, probably due to mutations or somaclonal variations, which occurred during the cultivation. The analysis of genotype classification revealed that although the largest number of S genotypes was in the same, first group, some of S genotypes were also present in the third group. Similarly, the highest number of MT genotypes was grouped together, but a certain number was found in the group with S as well as with T genotypes. Significant differences in callusing ability on the control medium were identified among genotypes belonging to the same boron tolerance group (S, MT and T) and these differences were the reason for such inconsistences in the grouping of the genotypes. More precisely, among the genotypes with the lowest FCW on the control medium, there were genotypes from all three B tolerance categories, and the same was observed for the genotypes with medium and the highest FCW values on the control medium. These results indicated that greater callusing ability did not mean greater tolerance for excess B and vice versa. Based on that, it can be concluded that the response of genotypes to excess B in this research, tested by *in vitro* culture, was primarily conditioned by genetic factors associated with B tolerance. Obtained results further indicate usefulness of *in vitro* culture in testing B effects. However, the differences between the sensitive and the medium tolerant genotypes might have been more conspicuous with boron concentrations other than the concentration used in our experiment. A study of wheat root culture had shown that the concentrations of 15 and 25 mM could be considered as adequate limit values when assessing sensitive, medium sensitive and tolerant genotypes for root elongation, lateral branching and callus growth in root explants (HUANG and GRAHAM, 1990). However, it had not been clarified whether these concentrations are adequate when other plant tissues and organs are used as explants in *in vitro* culture. The concentrations used in our study were selected on the basis of the results of MARJANOVIC (2005), which indicated that the concentrations of 15 and 30 mM of boric acid could be effective for differentiating genotypes with different levels of tolerance. However, as the concentration of 30 mM turned out to be lethal, concentrations of 10, 15, and possibly 20 mM of boric acid could be recommended for further work.

Considering adverse effects of excess B on plant processes and their productivity, breeding for tolerance is of particular importance. Examining the tolerance of as many wheat genotypes as possible, and its close relatives, would enable breeders to develop tolerant varieties.

Our research, as well as research by other authors (YAU and ERSKINE, 2000; BOGACKI *et al*., 2013; DE ABREU-NETO *et al*., 2017), have proven the existence of genotype variability in tolerance to excess B. Valuable source of diversity and useful alleles in plant breeding to biotic and abiotic stressors are wild relatives. *Aegilops*, being the closest wheat relative, was used in the study of KHAN *et al.* (2021) with the aim to identify most tolerant accessions to B toxicity. High level of variation among accessions in response to B toxicity was determined and two tolerant accessions were proposed as potential genotypes for improvement of B toxicity tolerance in wheat breeding programs.

For the improvement of breeding efficiency it is also very important to identify genes and QTLs potentially associated with the boron tolerance in wheat. EMEBIRI and OGBONNAYA (2015) performed GWAS on 333 synthetic hexaploid wheat lines using DarT markers and discovered two novel regions on chromosome 1AL conferring B tolerance in wheat. However, they also emphasized the limitations of their study and further challenges including characterization of materials and validation of results. In the research of OCHIAI *et al*. (2008) RILs showed to be good mapping population in QTL analysis and identification of gene conferring tolerance toward B toxicity. RILs are widely used in the studies focused on assessment of tolerance to different types of abiotic factors (SHANMUGAVADIVEL *et al*., 2017; ASIF *et al*., 2020; KONG *et al*., 2021). Our results determining the boron tolerance level of different RILs from the ITMI mapping population, could also contribute to the detection of genes and QTLs involved in the wheat reaction to the excess boron.

CONCLUSIONS

Based on this study, it was found that examined wheat genotypes had different callusing ability and different responses to increased B content. Tolerance of the genotypes determined in the *in vitro* test should be checked in field conditions. Variability in RILs response toward excess B followed by molecular evaluation using DNA markers could allow mapping of QTL_s associated with B toxicity tolerance in wheat breeding programs.

ACKNOWLEDGMENTS:

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant numbers: 451-03-68/2020-14/20003 and 451-03-9/2021-14/200032) and by the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. We are grateful to Dr. Andreas Borner and Dr. Marion Roder from IPK, Gatersleben, for providing us with ITMI population seeds, tissue culture chemicals and for their helpful suggestions during the experiment.

Received, November 14th, 2021
Accepted April 10th, 2022

REFERENCES

AĞIL F., M., ÖRGEÇ, F.P., KARAKAŞ, S.K., VERMA, N., ZENCIRCI (2022): In vitro mature embryo culture protocol of einkorn (*Triticum monococcum* ssp. *monococcum*) and bread (*Triticum aestivum* L.) wheatunder boron stress. Plant Cell, Tissue and Organ Cult., *148*:293–304.

ARIF, M.R., M.T., ISLAM, A.H.K., ROBIN (2019): Salinity stress alters root morphology and root hair traits in Brassica napus. Plants, *8*:192.

ASIF M.A., M., GARCIA, J., TILBROOK, C., BRIEN, K., DOWLING, B., BERGER, R.K., SCHILLING, L., SHORT, C., TRITTERMANN, M., GILLIHAM, D., FLEURY, S.J., ROY, A.S., PEARSON (2020): Identification of salt tolerance QTL in a wheat RIL mapping population using destructive and non-destructive phenotyping. Funct. Plant Biol., *48***:** 131-140.

BEN-GAL, A., U., SHANI (2002): Yield, transpiration and growth of tomatoes under combined excess boron and salinity stress. Plant Soil, *247*: 211–221.

BOGACKI, P., D.M., PECK, R.M., NAIR, J.H., KLAUS, H., OLDACH (2013): Genetic analysis of tolerance to Boron toxicity in the legume *Medicago truncatula*. BMC Plant Biol., *13*: 54.

BRDAR, M., I., MAKSIMOVIĆ, M., KRALJEVIC-BALALIC, B., KOBILJSKI (2008): Boron tolerance in twelve NS wheat cultivars. Acta Agric. Serbica, *13* (25): 17-23.

BRDAR-JOKANOVIĆ, M. (2020): Boron toxicity and deficiency in agricultural plants. Int. J. Mol. Sci., *21*(4): 1424.

CERVILLA, L.M., B., BLASCO, J.J., R'IOS, L., ROMERO, J.M., RUIZ (2007): Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. Ann. Bot., *100*: 747-756.

CHOUDHARY, S., A., ZEHRA, M., NAEEM, M., MASROOR, A., KHAN, T., AFTAB (2020): Effects of boron toxicity on growth, oxidative damage, antioxidant enzymes and essential oil fingerprinting in *Mentha arvensis* and *Cymbopogon flexuosus*. Chem. Biol. Technol. Agric., *7*:8.

DE ABREU NETO J.B., M.C., HURTADO-PEREZ, M.A, WIMMER, M., FREI (2017): Genetic factors underlying boron toxicity tolerance in rice: genome-wide association study and transcriptomic analysis. J. Exp. Bot., *68* (3):687–700.

EMEBIRI, L., P., MICHAEL, D., MOODY (2009): Enhanced tolerance to boron toxicity in two-rowed barley by marker assisted introgression of favorable alleles derived from Sahara 3771. Plant Soil, *314*:77–85.

EMEBIRI, L.C., F.C., OGBONNAYA (2015): Exploring the synthetic hexaploid wheat for novel sources of tolerance to excess boron. Mol. Breed., *35*: 68.

FANG, K., W., ZHANG, Y., XING, Q., ZHANG, L., YANG, Q., CAO, L., QIN (2016): Boron Toxicity Causes Multiple Effects on *Malus domestica* Pollen Tube Growth. Front. Plant Sci., *7*: 208.

GUNES, A., G., SOYLEMEZOGLU, A., INAL, E.G., BAGCI, S., COBAN, O., SAHIM (2006): Antioxidant and stomatal responses of grapevine (*Vitis vinifera* L.) to boron toxicity. Sci. Hortic., *110*: 279-284.

HAN, Y., X.L., JIN, F.B, WU, G.P., ZHANG (2011): Genotypic differences in callus induction and plant regeneration from mature embryos of barley (*Hordeum vulgare* L.). J Zhejiang Univ Sci B (Biomed & Biotechnol), *12*(5):399-407.

HAYES, J.E., M., PALLOTTA , M., GARCIA , M.T., ÖZ, J., RONGALA, T., SUTTON (2015): Diversity in boron toxicity tolerance of Australian barley (*Hordeum vulgare* L.) genotypes. BMC Plant Biol., *15*:231.

HUA T., R., ZHANG, H., SUN, C., LIU (2021): Alleviation of boron toxicity in plants: Mechanisms and approaches. Crit. Rev. Environ. Sci. Technol., *51*(24): 2975-3015.

HUANG, C., R., GRAHAM (1990): Resistance of wheat genotypes to boron toxicity is expressed at the cellular level. Plant Soil, *126*:195-300.

JEFFERIES, S.P., A.R., BARR, A., KARAKOUSIS, J.M., KRETSCHMER, S., MANNING, K.J., CHALMERS, J.C., NELSON, A.K.M.R., ISLAM, P., LANGRIDGE (1999): Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.). TAG, *98*:1293-1303.

KACEM, N.S., F., DELPORTE, Y., MUHOVSKI, A.,DJEKOUN,B., WATILLON (2017): *In vitro* screening of durum wheat against water-stress mediated through polyethylene glycol. J. Genet. Eng. Biotechnol., 15(1): 239–247.

KHAN, M.K., A., PANDEY, M., HAMURCU, Z.Z., AVSAROGLU, M., OZBEK, A.H., OMAY, F., ELBASAN, M.R., OMAY, F., GOKMEN, A., TOPAL, S., GEZGIN (2021): Variability in Physiological Traits Reveals Boron Toxicity Tolerance in Aegilops Species. Front. Plant Sci., *12*:736614.

KOCAOĞLU KAVAS, M., M., YORGANCILAR (2016): Determination of the response of Gypsophila arrostii Guss. to boron under in vitro conditions. J. Elem., *21*(1): 67-76.

KONDIĆ-ŠPIKA, A., B., KOBILJSKI, M., MANOJLOVIĆ, N., HRISTOV (2010): In vitro evaluation of boron tolerance in wheat (*Triticum aestivum* L.) genotypes. Field and Vegetable Crop Research, *47* (1): 85-91.

KONG, W., C., ZHANG, S., ZHANG, Y., QIANG, Y., ZHANG, H., ZHONG, Y., LI (2021): Uncovering the Novel QTLs and Candidate Genes of Salt Tolerance in Rice with Linkage Mapping, RTM-GWAS, and RNA-seq. Rice, *14*:93.

KRALJEVIC-BALALIC, M., O., LJUBOTIN, R., KASTORI, N., MLADENOV (2002): Genetic diversity of *Triticum* sp. regarding boron concentration. In: Proc. of Eucarpia, Cereal Section Meeting, Salsomaggiore, Italy, pp.198-202.

LI, M., Z., ZHAO, Z., ZHANG, W., ZHANG, J., ZHOU, F., XU, X., LIU (2017): Effect of boron deficiency on anatomical structure and chemical composition of petioles and photosynthesis of leaves in cotton (*Gossypium hirsutum* L.). Sci. Rep., *7*: 4420.

LIU, G.D., R.D., WANG, L.C., LIU, L.S., WU, C.C., JIANG (2013): Cellular boron allocation and pectin composition in two citrus rootstock seedlings differing in boron–deficiency response. Plant Soil, *370*: 555–565.

MARJANOVIC, M. (2005): Wheat tolerance testing to boron by an *in vitro* technique and molecular markers-microsatellites. M. Sc. Thesis, University of Novi Sad, Faculty of Agriculture, Serbia.

MARTÍNEZ-CUENCA, M-R., B., MARTÍNEZ-ALCÁNTARA, A., QUIÑONES, M., RUIZ, D.J., IGLESIAS, E., PRIMO-MILLO *et al*. (2015): Physiological and Molecular Responses to Excess Boron in *Citrus macrophylla* W. PLoS ONE, *10*(7): e0134372.

MATTHES, M.S., J.M., ROBIL, M.S., PAULA (2020): From element to development: the power of the essential micronutrient boron to shape morphological processes in plant. J. Exp. Bot., *71*(5):1681–1693.

MILJKOVIĆ, N. (1960): Characteristics of Vojvodina saline soils and the problem of boron in them. Ph.D. Thesis,University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia, (In Serbian).

MOODY, D.B., A.J., RATHJEN, B., CARTWRIGHT (1993): Yield evaluation of a gene for boron tolerance. In: Randall,P., E.Delhaize, R.A. Richards and R.Munns (eds.) Genetic aspects of plant mineral nutrition, Kluwer Academic Publ, Dordrecht, The Netherlands

MURASHIGE, T., F., SKOOG (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., *15*: 473-497.

NABLE, R.O., G.S., BANUELOS, J.G., PAULL (1997): Boron toxicity. Plant Soil, *193:*181-198.

OCHIAI, K., S., UEMURA, A., SHIMIZU, Y., OKUMOTO, T., MATOH (2008): Boron toxicity in rice (*Oryza sativa* L.) I. Quantitative trait locus (QTL) analysis of tolerance to boron toxicity. TAG, *117* (1): 125-33.

PANDEY, A., M.K., KHAN, E.E., HAKKI, S., GEZGIN, M., HAMURCU (2019): Combined Boron Toxicity and Salinity Stress—An Insight into Its Interaction in Plants. Plants (Basel): *8*(10): 364.

PAULL, J.G., B., CARTWRIGHT, A.J., RATHJEN (1988): Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica, *39*: 137-144.

REID, R., K.L., FITZPATRICK (2009): Redistribution of boron in leaves reduces boron toxicity. Plant Signal. Behav., *4*:1091–1093.

ROESSNER, U., J.H., PATTERSON, M.G., FORBES, G.B., FINCHER, P., LANGRIDGE, A., BACIC (2006): An investigation of boron toxicity in barley using metabolomics. Plant Physiol., *142*: 1087-1101.

SAKAMOTO, T., Y.T., INUI, S., URAGUCHI, T., YOSHIZUMI, S., MATSUNAGA, M., MASTUI, M., UMEDA, K., FUKUI, T., FUJIWARA (2011): Condensin II alleviates DNA damage and is essential for tolerance of boron overload stress in Arabidopsis. Plant Cell, *23*: 3533–3546.

SCHNURBUSCH, T., J., HAYES, T., SUTTON (2010): Boron toxicity tolerance in wheat and barley: Australian perspectives. Breed. Sci., *60*: 297–304.

SHANMUGAVADIVEL, P.S., S.V., AMITHA MITHRA, P., CHANDRA, M.K., RAMKUMAR, T., RATAN, M., TRILOCHAN, K.S., NAGENDRA (2017): High Resolution Mapping of QTLs for Heat Tolerance in Rice Using a 5K SNP Array. Rice, *10*:28.

SONG, B., X., HAO, X., WANG, S., YANG, Y., DONG, Y., DING, Q., WANG, X., WANG, J., ZHOU (2019): Boron stress inhibits beet (*Beta vulgaris* L.) growth through influencing endogenous hormones and oxidative stress response. Soil Sci. Plant Nutr., *65*: 346–352.

SOTIROPOULOS, T.H.E., I.N., THERIOS, K.N., DIMASSI (1998): The effect of toxic boron concentrations on shoot proliferation of in vitro kiwifruit shoot tip cultures. Advances in Horticultural Science, *12*(4): 196–200.

STANGOULIS, J.C.R., H.S., GREWAL, R.W., BELL, R.D., GRAHAM (2000): Boron efficiency in oilseed rape. I. Genotypic variation demonstrated in field and pot grown *Brassica napus* L. and *Brassica juncea* L. Plant Soil, *225*: 243-251.

STILES, A.R., D., BAUTISTA, E., ATALAY, M., BABAOGLU, N., TERRY (2010): Mechanisms of boron tolerance and accumulation in plants: A physiological comparison of the extremely boron-tolerant plant species, *Puccinellia distans*, with the moderately boron-tolerant *Gypsophila arrostil*. Environ. Sci. Technol., *44* (18): 7089- 7095.

TORUN, A.A., A., YAZICI, H., ERDEM, I., ÇAKMAK (2006): Genotypic variation in tolerance to boron toxicity in 70 durum wheat genotypes. Turk. J. Agric. For., *30*: 49-58.

WANG, N., C., YANG, Z., PAN, Y., LIU, S., PENG (2015): Boron deficiency in woody plants: various responses and tolerance mechanisms. Front. Plant Sci., *6*:916.

WIMMER, M.A., H.E., GOLDBACH (2012): Boron-and-Salt interactions in wheat are affected by boron supply. J. Soil Sci. Plant Nutr., *75*: 171–179.

YAU, S.K., M.M., NACHIT, J., RYAN, J., HAMBLIN (1995): Phenotypic variation in boron toxicity tolerance at seedling stage in durum wheat (*Triticum durum*). Euphytica, *83*: 185-191.

YAU, S.K., W., ERSKINE (2000): Diversity of boron-toxicity tolerance in lentil. Gen. Res. Crop Evol., *47*: 55–61.

YERMIYAHU, U., A., BEN-GAL, R., KEREN, R.J., REID (2008): Combined effect of salinity and excess boron on plant growth and yield. Plant Soil, *304*:73–87.

ZHANG, P., M., SENGE, Y., DAI (2016): Effects of Salinity Stress on Growth, Yield, Fruit Quality and Water Use Efficiency of Tomato under Hydroponics System. Reviews in Agricultural Science, *4*: 46- 55.

ZIAEYAN, A.H., M., RAJAIE (2009): Combined effect of Zinc and Boron on yield and nutrients accumulation in corn. Int. J. Plant Prod., *3*(3): 35-44.

REAKCIJA GENOTIPOVA PŠENICE NA SUVIŠAK BORA U KULTURI *in vitro*

Ankica KONDIĆ-ŠPIKA[1], Svetlana GLOGOVAC[1], Dragana TRKULJA[1], Ana MARJANOVIĆ-JEROMELA[1], Milica MARJANOVIĆ[2]

[1]Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija
[2]Gimnazija "Svetozar Marković", Novi Sad, Srbija

Izvod

Cilj ovog istraživanja bio je da se proceni tolerantnost genotipova pšenice na suvišak bora primenom kulture zrelog embriona. Analizom je obuhvaćeno 79 rekombinantnih inbred linija ITMI mapirajuće populacije i 3 sorte poznate tolerantnosti na bor (Pobeda – osetljiva, Balerina – srednje tolerantna i Nevesinjka – tolerantna) poreklom iz Srbije. Procena tolerantnosti je rađena na modifikovanoj MS hranljivoj podlozi u koju je dodato 15 mM borne kiseline. Kontrolna podloga nije sadržala suvišak bora. Sveža masa kalusa i redukcija sveže mase kalusa, određene su nakon mesec dana gajenja. ANOVA je pokazala veoma značajan efekat genotipa, hranljive podloge i njihove interakcije na rast kalusa, kao i značajan genotipski efekat na redukciju sveže mase kalusa. Većina genotipova (39) je imala osetljivu reakciju na suvišak bora, 23 su bila srednje tolerantna dok su 4 genotipa bila tolerantna. Dobijeni rezultati se potencijalno mogu koristiti za mapiranje gena i QTL-ova povezanih sa tolerancijom na suvišak B u oplemenjivanju pšenice.

Primljeno 14.XI.2021.
Odobreno 10. IV. 2022.