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DETERMINATION OF THE RESPONSE OF *GYPSOPHILA ARROSTII* GUSS. TO BORON UNDER *IN VITRO* CONDITIONS

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Abstract

In this research, the response of the plant *Gypsophila arrostii* Guss. to boron (B) under *in vitro* conditions was examined. The seeds were cultured on MS medium including 0, 10, 20, 40, 80 mg B l^{-1}. Seedlings obtained from germinated seeds and grown in a culture medium for 8 weeks were analyzed. At the end of this period, stem length (cm), root length (cm), plant weight (g) and elemental content (mg kg^{-1}) of the plants were determined. According to the results, the seeds of *G. arrostii* Guss. could germinate on media with up to 80 mg B l^{-1}, and the seedlings demonstrated an ability to survive, *albeit* poorly, a dose of boron as high as 80 mg B l^{-1}. In the experiment, the highest stem length (7.5 cm) was obtained from the 20 mg B l^{-1} treatment and the highest stem fresh weight (0.9 g) and stem dry weight (0.19 g) were measured in the 10 mg B l^{-1} variant. No significant statistical difference was determined between the boron treatments in terms of root length, root fresh weight and root dry weight. Our evaluation of the elemental content of plants demonstrated that the amount of boron in the root and stem increased parallel to its increase in the growth media. In the 80 mg B l^{-1} treatment, 601.9 mg kg^{-1} boron in root and 1,035.4 mg kg^{-1} boron in stem were determined. Besides, it was discovered that the contents of K, Mg, Zn, Na in root decreased while the contents of P, B, Mn, Cu in root increased in response to the growing amount of boron in the environment. In response to the increasing boron concentrations, the content of K, P, Mn, Cu, Zn and S increased while the amount of Ca, Mg and Na in the plant stem decreased. Consequently, *G. arrostii* Guss. was found to be a boron hyperaccumulator, collecting boron in tissues (in the roots and stems), in which it resembled some other types of *Gypsophilla*.

Keywords: *Gypsophila arrostii* Guss., boron (B), *in vitro*, hyperaccumulator plant.

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INTRODUCTION

Numerous plants are used for economic purposes, for instance as medicinal plants, aromatic raw material, dyes, flowers, etc. An example is *Gysophila*, a genus of flowering plants, of which an outstanding number of 59 taxa grow in Turkey (Özcelik, Yildirim 2011). In fact, *Gypsophila*, known under the common name of baby's-breath, is the third largest group of plants in the carnation family *Caryophyllacea* that can be found in Turkey (Korkmaz et al. 2012). Some types of *Gypsophila* are considered to be ornamental plants.

Gypsophila are annual or perennial plants. The perennial species of *Gysophila* have a herbaceous structure with taproots that penetrate deep into the soil. Thus, the plants may prevent soil slide, loss of water and drainage problems.

The Turkish *Gypsophila* plants belong to one of the five different species: *G. bicolor, G. arrostii var. nebulosa, G. eriocalyx, G. perfoliata var. anatolica* and *G. venusta*. Saponosides obtained from the roots of these plants have been used for centuries as herbal raw product owing to a variety of and pharmacological effects they can produce, including antifungal and antibiotic action. *G. paniculata* L. and *G. arrostii* Guss. have long been used as an expectorant in Arabic countries. With the broad variety of properties, the genus *Gypsophila* is of interest to farmers, food engineers, chemists, pharmacists, florists, landscape architects, etc., as a drug, food product, cleaning product, or an ornamental plant in parks and gardens (İnan 2006).

Plants typically contain little boron, an element which has a narrow range between deficiency and toxicity, hence the difficulty plant growers encounter when trying to maintain an appropriate boron level in their crops. On the other hand, boron is an important micronutrient in agriculture and in the environment (Dogan 2012). In general, the demand of plants for boron is low and its high concentrations become toxic to plants (Nable et al. 1997). However, some *Gypsophila* types are resistant to cold, drought, salinity and even high boron content in soil, which means that these species can grow in very different habitats.

Babaoglu et al. (2004) revealed that *G. sphaerocephala* Fenzl ex Tchihat plants could grow healthily even when their leaves contained 3,500 mg B kg^{-1}, whereas the species *Gypsophila perfoliata* L. and *Gypsophila sphaerocephala* Fenzl ex Tchihat demonstrated a potential ability to act as boron hyperaccumulator plants. This motivates researchers to explore *Gypsophila arrostii* as a possible boron hyperaccumulator. *Gypsophila arrostii* is the same type of plant as *G. perfoliata* L. and *Gypsophila sphaerocephala* Fenzl ex Tchihat, but it is easier to grow and therefore it could help ameliorate agricultural lands with B toxicity problem. Moreover, the problem of yield losses caused by boron toxicity in some regions where artificial fertilizers are overused could be resolved by growing *Gypsophila*. A study has shown that a Turkish ecotype of *Puccinellia distans* displayed exceptional boron tolerance, >1,250 mg B l^{-1} compared to <50 mg B l^{-1} for *Gypsophila arrostii* (Stiles et al. 2010, Padmanabhan et al. 2012).

The aim of the present study has been to determine the response of *Gypsophila arrostii* Guss., known to be a salt tolerant and boron accumulator plant, to soil doses of boron tested under *in vitro* conditions. Having explored the resistance mechanism in this plant, we could design a model for physiological research.

MATERIAL AND METHOD

Material

In this research, seeds of *Gypsophila arrostii* Guss., a flowering plant with commercial value, were sown in the Agricultural Biotechnology Lab at the Selcuk University, Turkey. The nutrient media were prepared using grade pure chemicals from Merck and Sigma.

Method

Preparation of the nutrient medium

The macronutrients NH_4NO_3 (1650 mg), KNO_3 (1900 mg), $MgSO_4 \cdot 7H_2O$ (370 mg), KH_2PO_4 (170 mg) and $CaCl_2 \cdot 2H_2O$ (440 mg) were added to each nutrient medium based on MS (Murashige, Skoog 1962). Adequate amounts of the micronutrients were mixed with stock solutions and added to each medium. The boron concentrations of 0, 10, 20, 40, 80 mg l^{-1} of the nutrient medium were adjusted by supplementing 0, 57.2, 114.4, 228.8, 457.6 mg l^{-1} boric acid (H_3BO_3).

The total composition was completed by adding 3% saccharose as a carbon source. The pH of the nutrient media was adjusted to 5.8 by using 1 N KOH or 1 N HCl. After adding 0.8% agar as a solidifying agent (technical agar DIFCO), each medium was sterilized at the pressure of 1.2 atm and kept for 20 minutes at the temperature of 121°C (Babaoglu et al. 2002).

Culture procedures

The seeds were sterilized and cultured in MS media, which contained 5 different (0, 10, 20, 40, 80 mg l^{-1}) concentrations of B. Culture containers (Magenta) were placed on shelves in a culture room, and kept under the following conditions: 16 hours photoperiod, 60% (±5) humidit, 24°C (±1) temperature and 50 μmol m^{-2} s^{-1} light density. The plants were grown for for 8 weeks. Afterwards, the root-stem length (cm), root-stem fresh and dry weight (g), and root-stem element content (mg kg^{-1}) in harvested plants were determined. The average values were submitted to variance analysis by the

MStat-C (Version 3 Michigan State University, USA) statistical software program and significant differences were checked by the LSD test (MSTAT-C 1980).

Determination of the content of elements in plants (mg kg^{-1})

After the plant parts (root and stem) were dried to constant weight in a drying cabinet with air circulation and the temperature of 70°C, they were weighed on precision scales (0.000), divided into batches of approximately 0.2 g, transferred to Teflon tubes and placed in a microwave oven (CEM--Mars x 5 model). There, they were digested for 25-30 minutes in a mixture of 5 ml nitric acid (HNO_3) and 2 ml H_2O_2 per sample. When the plant material was ashed, the tubes were replenished with pure water to the total volume of 25 ml, the whole content of each tube was filtered on filter paper, transferred to a labelled plastic container and kept in a refrigerator until analyses.

The samples were prepared by incineration in a microwave after being calibrated against standard solutions. They were analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry [ICP-AES (Varian-Vista Model, axiel)].

The samples were subjected to analysis of macro- and microelement contents with ICP-AES, which can determine 28 elements in a single reading. The data obtained from the determinations were submitted to calculations according to the following equation:

element content (mg kg^{-1}) = reading value x dilution factor (volume) / sample weight.

RESULTS AND DISCUSSION

Plant growth parameters

The variance analysis showed that the stem length and stem dry weight of plants grown in different boron treatments changed significantly at the significance level of 1%, while the results pertaining to the effect of boron application on the stem fresh weight were significant at the level of 5%, and the effects on the stem-root length and stem-root fresh and dry weight were insignificant (Table 1).

In this research, *G. arrostii* plants developed in media dosed with different amounts of boron. After eight weeks of the growing period, the following values were determined in the plants (Table 2).

As seen in Table 2, the longest stem length (7.5 cm) was grown by the plants from the 20 mg l^{-1} boron treatment. At the lower and higher doses, the stem length was shorter, and the shortest one was obtained in the 80 mg B l^{-1} treatment. As seen in this table, *G. arrostii* Guss. reacted similarly in the

Table 1

The variance analysis results of *Gypsophila arrostii* plant progress in different B treatments

Parameters	DF	MS	F	CV
Stem length	4	16.893	10.6354**	0.24
Root length	4	2.020	2.3142	0.20
Stem fresh weight	4	0.322	4.8347*	0.57
Root fresh weight	4	0.052	2.9741	0.68
Stem dry weight	4	0.011	7.8559**	0.41
Root dry weight	4	0.001	3.3190	0.29

** significance level of 1%, * significance level of 5%

Table 2

Plant development of *G. arrostii* in different doses of B application

Parameters	B (mg l^{-1})					LSD value
	0	10	20	40	80	
Stem lenght (cm)	4.8 *AB*	6.3 *A*	7.5 *A*	6.3 *A*	1.4 *B*	3.45
Root lenght (cm)	5.3	5.2	5.1	4.3	3.3	-
Stem fresh weight (g)	0.5 *ab*	0.9 *a*	0.5 *ab*	0.2 *b*	0.1 *b*	0.49
Root fresh weight (g)	0.22	0.34	0.30	0.09	0.03	-
Stem dry weight (g)	0.08 *B*	0.19 *A*	0.09 *B*	0.07 *B*	0.01 *B*	0.09
Root dry weight (g)	0.06	0.09	0.07	0.07	0.01	-

LSD groups to doses of 10, 20, and 40 mg l^{-1} of boron in terms of the stem length. In brief, the stem length was longer in response to boron concentrations up to a certain level. With respect to the root length, it was noted that the root development was retarded as the doses of boron increased, but the differences were found to be statistically insignificant. According to some former studies, the root length of several types of plants decreased as B doses applied increased. Yorgancilar and Babaoglu (2005) concluded that the root length of plants grown without B in the substrate did not show more intensive growth, but the value of this trait declined as the B dose in the substrate increased. Moreover, according to their study, higher B doses accelerated the development of capillary roots up to a certain concentration, but excess B caused deformations of roots. Consequently, shorter root length is recorded at higher B doses. Nonetheless, the root development in the analyzed plant progressed even at a dose of 80 mg B l^{-1}.

At the end of the experiment, it was determined that the length of *G. arrostii* Guss. seedlings grown under boron toxicity conditions had decreased. Ari Baykal and Oncel (2006) observed a shorter length of seedlings of 2 genotypes that belonged to two wheat types (*Triticum aestivum* L. cv. Kıraç 66 and *Triticum durum* Desf. cv. Kunduru 1149) caused by an application of toxic doses of boron.

The weight of stems grown by *G. arrostii* Guss. plants varied depending on the B doses. In brief, it was lower in response to an application of 20 mg l^{-1} dose and over, while the highest fresh weight of stems (0.9 g) was obtained from plants grown with 10 mg B l^{-1} in the medium. Thus, the stem fresh and dry weight decreased parallel to an increase in boron doses. Nevertheless, *G. arrostii* Guss. plants resist boron pollution quite well. YORGANCILAR and BABAOGLU (2005) reported that an average stem weight decreased as the B application doses increased.

Despite the lack of statistically significant differences between the boron treatments in terms of the root fresh and dry weight of *G. arrostii* Guss., the root development of plants slowed down due to an application of 20 mg B l^{-1} or higher doses. GÜNES et al. (2000) stated that B application into substrate in which 8 corn genotypes were grown in a greenhouse increased the plants' boron intake and concentration but decreased their dry weight. In the experiment by YORGANCILAR and BABAOGLU (2005), depending on B doses, dry weight of wheat decreased in some types but there was no evident change in some other types of wheat.

In general, the concentration of boron in *G. arrostii* plans grown under boron toxicity conditions increases. ARI BAYKAL and ONCEL (2006) determine that the percentage of dry matter decreased and the boron concentration increased at the termination of an experiment in which boron had been applied to substrate in which of 2 genotypes of two wheat types (*Triticum aestivum* L. cv. Kıraç 66 and *Triticum durum* Desf. cv. Kunduru 1149) were grown.

In conclusion, *Gypsophila arrostii* Guss. can resist, at least to a certain extent, a boron dose of 40 mg l^{-1} when all the plant development parameters are assessed as a whole.

Plant element content

Root element content

After the root B content of *Gypsophila arrostii* seedlings was determined by ICP-AES, the B amounts were calculated as mg kg^{-1} and the average values obtained by statistical analysis are given in Table 3. When the effects of boron application on *G. arrostii* plants were examined, it was demonstrated that the lowest B intake in the root was 10.5 mg kg^{-1} at the 0 B dose, and the highest one was 601.9 mg kg^{-1} at the 80 mg l^{-1} dose. It was observed that B accumulation in the plant roots increased linearly to the increase in B concentrations.

Our analysis of the elements in the plant roots showed that in different B application variants, the root content of Na, B, K, Cu, Mn, S was significant at 1% level, while P, Mg and Zn were significant at 5% level. The amount of Ca was statistically insignificant (Table 4).

As seen in Table 3, while the content of K, Mg, Zn and Na in the plant root decreased proportionally to the increasing B doses, the content of P, B, Mn and Cu increased. Certain fluctuations in the content of Ca and S were

Table 3

Elements in roots of *Gypsophilla arrostii*

B (mg l^{-1})	Macroelements (mg kg^{-1})				Microelements (mg kg^{-1})					
	Ca	K	P	Mg	B	Mn	Cu	Zn	Na	S
0	3302.4	26082.1*AB*	6419.2*bc*	1689.2*a*	10.5E	144.9*BC*	4.3*BC*	200.4*ab*	2336.7*A*	3464.4*b*
10	3697.1	28592.2*A*	5712.1*bc*	1873.8*a*	57.7D	131.7*C*	4.3*BC*	212.8*a*	1721.4*B*	3689.8*b*
20	2992.4	27672.5*AB*	5506.3*c*	1419.2*ab*	98.4C	180.6*AB*	4.0*C*	175.0*bc*	1848.2*B*	3433.1*b*
40	3146.8	22873.0*BC*	7424.1*ab*	1021.5*b*	212.0B	163.3*ABC*	7.5*A*	188.7*abc*	1806.8*B*	4367.6*a*
80	2613.2	18104.6*C*	8535.3*a*	967.1*b*	601.9A	208.7*A*	6.5*AB*	161.0*c*	1305.6*C*	3801.5*b*
Mean	3150.4	24664.9	6719.4	1394.2	196.1	165.84	5.32	187.58	1803.74	3751.28

S $LSD_{0.05}$: 543.7; Na $LSD_{0.01}$: 381.2; Zn $LSD_{0.05}$: 29.61; Mg $LSD_{0.05}$: 5532.0.; Mn $LSD_{0.01}$: 46.79; P $LSD_{0.05}$:1729.; K $LSD_{0.01}$:5327; Cu $LSD_{0.01}$: 2.447; B $LSD_{0.01}$: 36.96

Table 4

Variance analysis table

Parameters	Root				Stem			
	DF	MS	F	CV	DF	MS	F	CV
Ca	4	476743.227	1.6558	0.17	4	444650.228	9.7386**	0.05
K	4	54544218.823	14.4250**	0.08	4	10147063.515	7.7226**	0.04
P	4	4777991.221	5.6637*	0.14	4	5474730.373	17.4650**	0.12
Mg	4	479179.265	5.5510*	0.21	4	206214.895	9.2461**	0.08
B	4	171016.005	939.4769**	0.07	4	574581.118	308.2873**	0.07
Mn	4	2751.128	9.4330**	0.10	4	7690.888	11.2323**	0.11
Cu	4	7.311	9.1567**	0.17	4	2.459	6.9144*	0.23
Zn	4	1252.972	5.0657*	0.08	4	5726.829	39.3651**	0.09
Na	4	405739.349	20.9603**	0.08	4	48672.088	3.1412	0.10
S	4	427280.912	5.1235**	0.08	4	329411.078	9.5016**	0.07

** significance level of 1%, * significance level of 5%

obserevd. Gezgin and Hamurcu (2006) stated that boron acted antogonistically with N, Ca, Mg, Fe and Mn but demonstrated synergic interactions with P, K, S, Zn and Cu. These results are somewhat different from our findings. The differences could have arisen from measuring the root element content in plants grown in closed jars in tissue culture.

Stem element content

After *G. arrostii* plant seedling stem B contents were determined by means of an ICP-AES device, B contents are calculated as mg kg^{-1} and the average vaues obtained by result of statistic analysis were given in Table 5. The significant elements at the level of % 1 in the different B applications in the result of element analysis of plant stems were Ca, B, K, Mn, S, P, Mg and

Table 5

Elements in stems of *Gypsophilla arrostii*

B (mg l^{-1})	Macroelements (mg kg^{-1})				Microelements (mg kg^{-1})					
	Ca	K	P	Mg	B	Mn	Cu	Zn	Na	S
0	4645.3*A*	29857.4*AB*	4963.0*BC*	2107.8*A*	9.5*D*	238.1*B*	2.8*ab*	125.7*BC*	1249.8	2560.3*AB*
10	4386.2*A*	27524.7*B*	3203.6*D*	2019.5*A*	286.4*C*	224.7*B*	2.2*bc*	97.8*C*	1316.5	2250.1*B*
20	4179.9*AB*	27467.4*B*	3666.9*CD*	1841.9*A*	500.8*B*	185.8*B*	1.3*c*	94.4*C*	1283.1	2168.6*B*
40	4193.5*A*	31740.8*A*	5538.3*AB*	1925.0*A*	958.4*A*	251.7*B*	3.0*ab*	134.0*B*	1333.0	2618.7*AB*
80	3600.4*B*	30174.7*AB*	6502.6*A*	1431.4*B*	1035.4*A*	324.0*A*	3.7*a*	202.8*A*	1019.9	3001.2*A*
Mean	4201.1	29353.0	4136.3	1865.1	558.1	244.86	2.6	130.94	1240.46	2519.78

Ca $LSD_{0.01}$: 585.4; B $LSD_{0.01}$: 118.3; Mn $LSD_{0.01}$: 71.69; Mg $LSD_{0.01}$: 409.1; Zn $LSD_{0.01}$: 33.04; S $LSD_{0.01}$: 510.1; P $LSD_{0.01}$: 1534.0; K $LSD_{0.01}$: 3140; Cu $LSD_{0.05}$: 1.123

Zn, in the level of % 5, Cu was found as important and Na element was found to be statistically insignificant (Table 4).

When the effect of boron application on the content of elements in *Gypsophila arrostii* Guss. stems was considered, the lowest B intake in stem B content was 9.5 mg kg^{-1} at 0 B and the highest B intake rose to 1,035.4 mg kg^{-1} with 80 mg l^{-1} dose (Table 5). NABLE et al. (1990) stated that B intake increased linearly as the B concentration increases in the range of 0.618-395 mg of boric acid l^{-1}. These results support our findings. BABAOGLU et al. (2004) reported that *Gypsophila perfoliata* L. and *Gypsophila sphaerocephala* Fenzl ex Tchihat plants have the potential to be a hyperaccumulator plant and the plants develop healthily even when approximately 3,500 mg B kg^{-1} is found in leaves of *G. sphaerocephala* Fenzl ex Tchihat. *G. arrostii* Guss. Belongs to the same type as *Gypsophila sphaerocephala* Fenzl ex Tchihat and *Gypsophila perfoliata* L., but it is easier to cultivate. As it was assessed to contain 1,035.4 mg kg^{-1} B in its stem at 80 mg B l^{-1} dose, it could be a B hyperaccumulator plant.

Regarding the content of the other elements in the plant, the amounts of K, P, Mn, Cu, Zn and S in the plant stem increased while the amount of Ca, Mg and Na in the plant stem decreased following an application of the increasing boron doses to the media. GEZGIN and HAMURCU (2006) stated that boron acted antogonistically with N, Ca, Mg, Fe and Mn but had synergic interactions with P, K, S, Zn and Cu. These results are similar to the present findings.

As a result, this study done under tissue culture conditions and in closed jars gives general knowledge about the elemental nutrition of *G. arrostii*. However, the contents of some elements can show variability in a greenhouse and a field. Therefore, *in vitro* studies can provide us with prior knowledge to results of greenhouse and field cultivation. This is because environmental factors of plant cultivation in land conditions vary depending on climate conditions.

CONCLUSIONS

The root-stem length and the fresh-dry weight of the plant *Gypsophila arrostii* Guss. increased up to a certain level and then decreased with the increasing B concentrations. Nonetheless, the plant grew even with the application of 80 mg B l^{-1}. *G. arrostii* Guss. revealed the characteristics of a hyperaccumulator plant by accumulating B in the tissues (root and stem) of the plant. It was observed that the *G. arrostii* plant incorporated boron with a linear increase both in the stem (9.5-1,035.5 mg kg^{-1}) and the root (10.5--601.9 mg kg^{-1}), considering the results of its response to 0 and 80 mg l^{-1} B, respectively. According to these results, *G. arrostii* Guss. acts as a hyperaccumulator plant for boron. Therefore, its potential of being used for phytoremediation of agricultural areas that suffer from boron toxicity has been verified by this study.

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REFERENCES

Ari Baykal S. and Oncel I. 2006. *Changes of soluble phenolic and soluble protein amounts on the tolerance of boron toxicity in wheat seedlings*. Çukurova University, Faculty of Sciences-Letters, Journal of Natural Sciences, 7(1): 13-25.

Babaoglu M,, Yorgancilar M., Akbudak M.A. 2002. *Plant biotechnology*. I. *Applications of tissue culture*. Chapter 1. *Tissue culture: basic laboratory techniques*. Selçuk University Foundation Publications. ISBN: 975-6652-04-7, 1-35

Babaoglu M., Gezgin S., Topal A., Sade B., Dural H. 2004. *Gypsophila sphaerocephala Fenzl ex Tchihat.: A boron hyperaccumulator plant species that may phytoremediate soils with toxic B levels*. Turk J. Agric For., 28: 273-278.

Dogan M. 2012. *The effect of different boron practices on the germination of capparis spp. and Carthamuss* spp. *seeds*. Süleyman Demirel University, J. Natur. Appl. Sci., 16(2): 154-161.

Gezgin S., Hamurcu M. 2006. *The importance of the nutrient elements ınteraction and the ınteractions between boron with the other nutrient elements in plant nutrition*. Selçuk University, J. Agric. Faculty, 20(39): 24-31.

Günes A., Alpaslan M., Ozcan H., Çikili Y. 2000. *Tolerance to boron toxicity of maize (Zea mays* L.*) cultivars widely cultivated in Turkey*. Turk. Agric. Foresty, 24: 277-282.

İnan M. 2006. *Determination of saponin content and root yield different origin soapwort (Gypsophila* sp.*) species at the Çukurova conditions*. Çukurova University, Institute of Natural and Applied Sciences (PhD Thesis).

Korkmaz M., Ozcelik H., Ilhan V. 2012. *Habitat properties of some Gypsophila L. (Caryophyllaceae) taxa of Turkey*. Res. J. Biol. Sci., 5(2): 111-125.

MSTAT-C. 1980. *MStat User's Quides Statistics (Version 5 ed.)*. Michigan State Universty. Michigan, USA.

Murashige T. and Skoog F. A. 1962. *Revised medium for rapid growth and bioassays with tobacco tissue cultures*. Physiol Plant, 5(3): 473-497.

NABLE O. R., LANCE R. C. H., CARTWRIGHT B. 1990. *Uptake of boron and silicon by barley genotypes with differing suspectibilities to boron toxicity*. Ann. Bot., 66(1): 83-90.

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

OZCELIK H., YILDIRIM B. 2011. *Opinions on conservation, economical importance and using possibility of Turkish Soapworts (Gypsophila* L. *and Ankyropetalum* Fenzl spp.*)*. Süleyman Demirel University, Faculty of Forestry Journal, 12: 57-61.

PADMANABHAN P., BABAOGLU M., TERRY N. 2012. *A comparative transcriptomic analysis of the extremely boron tolerant plant Puccinellia distans with the moderately boron tolerant Gypsophila arrostil.* Plant Cell Rep., 31: 1407-1413.

STILES A.R., BAUTISTA D., ATALAY E., BABAOGLU M., TERRY N. 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: 7089-7095.

YORGANCILAR M., BABAOGLU M. 2005. *The responses of some wheat varieties to boron as determined by tissue culture*. Institute of Natural and Applied Sciences (PhD Thesis).