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Response of purslane (*Portulaca oleracea* L.) to boron toxicity

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ABSTRACT

This study was undertaken to determine the effects of increasing boron (B) in purslane (*Portulaca oleracea* L.). Two variety of purslane, Mercan (a variety cultivated widely) and Wild (a variety grown in nature spontaneously), were used for glasshouse experiment with the following treatments: 0, 5, 10, 25 mg B kg<sup>-1</sup>. Plant growth, total chlorophyll (Chl) and carotenoid (Car) reduced significantly in B-stressed plants. Total Chl and Car contents in Wild were greater than in Mercan. While shoot and root ratio (SRR) and membrane permeability (MP) were increased by applied B, relative water content (RWC) showed an indecisive trend in both varieties. Also, the content and uptake of B in shoot and root increased in parallel to increasing B levels, except for the highest B level in root. While uptakes of phosphorus (P) and potassium (K) decreased with increasing B levels, Calcium (Ca) uptake showed an indecisive trend in both varieties. Na uptakes increased in Mercan, whereas it decreased in Wild. The P and K uptakes were greater in Mercan, whereas the Ca and Na uptakes were greater in Wild. It was concluded that purslane showed different responses to increasing B levels and biomass production was greater in Mercan than in Wild.

Keywords:

Boron toxicity

Nutrient uptake

Plant growth

*Portulaca oleracea*

Purslane

Semizotunun (*Portulaca oleracea* L.) bor toksitesine tepkisi

ÖZET

Bu çalışma, semizotu bitkisinde (*Portulaca oleracea* L.) artan bor (B) düzeylerinin etkisini belirlemek için yapılmıştır. İki semizotu türü, Mercan (yaygın bir şekilde yetiştirilen bir tür) ve Yabani (doğada kendiliğinden yetişen bir tür) sera denemesi için kullanılmış ve 0, 5, 10, 25 mg kg<sup>-1</sup> B düzeyleri uygulanmıştır. Bor stresi altındaki bitkilerde bitki gelişmesi, toplam klorofil (Chl) ve karotenoid (Car) önemli derecede düşmüştür. Yabani çeşidin toplam Chl ve Car içerikleri Mercan çeşidinden fazla bulunmuştur. Artan B uygulamalarıyla her iki semizotu çeşidinde de, gövde kök oranı (SRR) ve membran geçirgenliği (MP) artarken, oransal su içeriği (RWC) dalgalı bir seyir izlemiştir. Ayrıca, kök ve gövdede bulunan B elementinin içerik ve alımları, kökteki en yüksek B düzeyi hariç, artan B düzeylerine paralel olarak artmıştır. Her iki çeşitte de fosfor (P) ve potasyum (K) alımları artan B düzeyleri ile düşerken, kalsiyum (Ca) alımı bir dalgalanma göstermiştir. Sodyum (Na) alımı Mercan çeşidinde artmış, yabani çeşitte ise düşmüştür. Fosfor ve potasyum alımları Mercan çeşidinde daha fazladır. Oysa kalsiyum ve sodyum alımları yabani çeşitte daha fazladır. Semizotu bitkisinin artan B düzeylerine farklı tepkiler gösterdiği ve Mercan çeşidindeki biokütle üretiminin yabani çeşitten daha fazla olduğu sonucuna varılmıştır.

Anahtar Sözcükler:

Bor toksisitesi

Besin alımları

Bitki gelişimi

*Portulaca oleracea*

Semizotu

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1. Introduction

Boron is an essential nutrient element for plants and taken by plants as boric acid (H<sub>3</sub>BO<sub>3</sub>) from the soil and transferred to upper organs via xylem (Raven, 1980) and majority of them accumulated in cell wall as H<sub>3</sub>BO<sub>3</sub> (Matoh, 1997). Many scientific data shows that B plays a critical role in cell wall structure and functions, especially in regulation of cell wall pore size, in both plants and animals (Brown et al., 2002; Goldbach and

Wimmer, 2007). Also, some evidences have been reported by Wang et al. (1999) who indicated the role of B in membrane integrity and membrane functions such as formation and maintenance of membrane potentials and membrane permeability. The transport of B from root to shoot increased linearly with increasing B concentration in rooting medium (Marschner, 1995). There is an exception for susceptible genotypes having higher B concentrations than less susceptible genotypes reported by Nable (1991) who investigated the B

toxicity resistance mechanism in barley genotypes. Either susceptible genotype accumulated higher B or tolerant genotype accumulated lower B may be associated with differences in membrane permeability or composition of cell wall and membrane (Nable and Paull, 1991).

Both deficiency and toxicity of B in soils are a serious agricultural problem that limits crop yield and quality in different regions of the world. Plants exposed to excess B, especially under low rainfall conditions, show typical toxicity symptoms such as retarded development, chlorotic and necrotic patches in older leaves, decreased number, size and weight of fruit (Nable et al., 1997). Also, excess B caused an increase in MP and degradation in membrane integrity and structure (Karabal et al., 2003). It is possible to find lots of investigations related to the effect of B on plant growth and growing parameters, i.e. for tomato (Güneş et al., 1999), for carrot (Eraslan et al., 2007a), tomato and pepper (Eraslan et al., 2007b), for barley (El-Feky et al., 2012), for safflower (Ashagre et al., 2014).

The "Wild" purslane (*Portulacca oleraceae* L.) is represented 25 genera of succulent herbs and shrubs in *Portulacaceae* family. It is a widely distributed throughout all over the world where it grows all in cultivated fields, lawn and waste places up to 3850 m elevated (Mitich, 1997). Moreover, cultivated varieties of purslane are grown commercially in many countries and its fresh leaves and stems are cooked like spinach, and also consumed as green salad. Purslane with high nutritive value and antioxidant compounds, especially fatty acids and  $\beta$ -carotene (Liu et al., 2000; Simopoulos et al., 1992), deserves a special attention from nutritionist and agronomist and used for human food, animal feed and medicinal purpose.

The main aim of this study was to determine the response to increasing B treatments of two purslane cultivars. Some parameters, such as fresh weight (FW) and dry weight (DW) in shoot and root, membrane permeability (MP), relative water content (RWC), chlorophyll contents in leaf, B contents and mineral nutrient uptakes in shoot were evaluated.

## 2. Materials and Methods

### 2.1. Plant materials and treatments

The greenhouse experiment was carried out under natural light conditions in 2013 summer season at Kocaeli University, Arslanbey Campus (lat 40°42'33"N, long 30°01'37"E). Two different purslane cultivars were used for the experiment. Wild (*Portulacca oleracea* L.) seeds were collected from nature at the end of the previous summer season. The other purslane seeds (*P. oleracea* L. cv. Mercan), widely grown in Turkey, was supplied from the seed market. From both varieties 30 seeds have been sown into plastic pots containing 2000 g of air-dried soil. After good stands of the plants, young plants were thinned to 15 plants per pot.

Some properties of the experimental soil were as

follows: loam texture (sand/clay, 35.8/21.7 by dry weight); pH (1/2.5 soil/water) 7.34; EC, 508  $\mu\text{S cm}^{-1}$  (saturation extract); calcium carbonate ( $\text{CaCO}_3$ ), 17.29  $\text{g kg}^{-1}$ ; organic carbon, 6.25  $\text{g kg}^{-1}$  and total nitrogen (N), 0.86  $\text{g kg}^{-1}$ . Ammonium acetate ( $\text{NH}_4\text{OAc}$ )-extractable K, Ca, Mg and Na with were 100, 2151, 124 and 64  $\text{mg kg}^{-1}$ , respectively. Sodium bicarbonate ( $\text{NaHCO}_3$ )-available phosphorus (P) concentration was 12.43  $\text{mg kg}^{-1}$  and hot water extractable-B was 1.64  $\text{mg kg}^{-1}$ . Diethylene triamine penta acetic acid (DTPA)-extractable Fe, Mn, Zn and Cu were 24.28, 65.27, 2.09, and 1.17  $\text{mg kg}^{-1}$ , respectively. The soil properties were determined according to methods detailed in Page et al. (1982).

Four levels of B (0, 5, 10 and 25  $\text{mg kg}^{-1}$ ) as boric acid ( $\text{H}_3\text{BO}_3$ ) were applied to the soil. Three replications were performed for the experiments in completely randomized factorial design. For basal fertilization as ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), ammonium dihydrogen phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4$ ), and potassium sulfate ( $\text{K}_2\text{SO}_4$ ) were applied to the soil at 150, 75 and 150  $\text{mg kg}^{-1}$ , respectively. All these supplements were applied by spraying the solutions and mixing them into the soil. During the experimental period, soil was kept at approximately 70% of the field capacity with tap water.

### 2.2. Plant analyses

Six weeks later, plants were harvested properly and separated into shoots and roots for determining fresh and dry matter biomass. The shoots and roots were washed with running tap water and then three-times rinsed with de-ionized water to remove any soil particles attached to the plant surfaces. All materials were dried at 70°C in oven for at least three days, quickly measured for dry weights (DW) and separately grinded and kept for nutrient ions analyses. These ions were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Perkin Elmer Optima 2100 DV; Waltham, MA). Shoot and root ratio (SRR) was calculated by dry weights. Membrane permeability (MP) measurements with fresh matter were done before harvest. The MP (EC %) for the shoot disc samples was measured by the electrical conductivity (EC) method as described by Yan et al. (1996). Relative water content (RWC) was determined using a composite sample of leaved discs (1 cm), which were weighed to record fresh weight (FW), floated in distilled water for 4 h to determined turgid weight (TW) then oven-dried at 70 °C for 48 h to measure dry weight (DW). The RWC was calculated as follows:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW})$$

The ion uptake in the shoot was calculated as follows:

$$\text{Ion uptakes shoot or root (mg or } \mu\text{g plant}^{-1}) = \text{DW shoot or root} \times [\text{ion}] \text{ shoot or root}$$

### 2.3. Statistical analyses

The experimental design was a completely

randomized factorial design with three replicates and obtained data were analyzed by ANOVA. The differences were compared by Duncan's multiple-range test ( $\alpha$ : 0.05). The levels of significance are represented by \* at  $P < 0.05$ , \*\* at  $P < 0.01$ , and ns: non-significant. The statistical tests were performed by using MINITAB package program (Minitab Corp., State College, PA).

### 3. Results and Discussion

#### 3.1. Boron toxicity symptoms

Typical B toxicity symptoms were appeared on the oldest leaves as dark brown spots and necrotic lesions. The toxicity was not observed at 5 mg kg<sup>-1</sup> B levels in purslane cultivars. But, it intensified the leaf injury and spread to the younger leaves with increasing B level, especially 25 mg B kg<sup>-1</sup>. Similar leaf injury symptoms resulted in B toxicity reported by Eraslan et al. (2007a) in carrot, Gunes et al. (2009) in bean and Çikili et al. (2015) in peanut.

#### 3.2. Plant growth and biomass production

The shoot FW and DW of purslane cultivars decreased with increasing B levels (Table 1). At the highest B treatment, the reduction of shoot FW was 46.8% in Mercan and 68.0% in Wild, compared to control. Similarly, the shoot DW reduced in Mercan (64.5%) and in Wild (75.6%). Although these changes in shoot FW and DW, an interaction of B and cultivar was no significant. Irrespective of B treatments, shoot

FW and DW in Mercan was found greater than in Wild. Irrespective of cultivars, shoot FW and shoot DW was significantly reduced by B treatments. On the other hand, the root FW and DW diminished with increasing B levels. The reductions were over 80% in both purslane cultivars in comparison with control. But, the interaction of cultivar and B treatments was found significant in root FW. Irrespective of cultivars, the root FW and DW remarkably decreased with increasing of B levels. In our experiment, the shoot and root FW and DW of purslane cultivars were significantly reduced as a result of excess B. Similar results supporting these findings documented by Alpaslan and Gunes (2001) for tomato and cucumber, Eraslan et al. (2007a) for carrot, El-Feky et al. (2012) for barley, Çikili et al. (2015) for peanut.

The SRR of both cultivars enhanced with increasing B treatments (Table 1). These increments were 115.9% in Mercan and 57.1% in Wild at the highest B level, compared to control. But, the interaction of cultivars and B treatments was not significant in SRR. Irrespective of B treatments, significant difference was found in SRR of Mercan and Wild. Irrespective of cultivars, the SRR was linearly augmented with increasing B treatments and these increments in SRR were significant only at 25 mg kg<sup>-1</sup> B level. In parallel to our results, Cervilla et al. (2009) reported a significant increase in the SRR of two tomato cultivars. Hasnain et al. (2011) stated opposite results in mung bean cultivars. Ashagre et al. (2014) had no significant differences for SRR in safflower.

Table 1. Effects of increasing B treatments on shoot and root growth, and shoot: root ratio of purslane cultivars

Cultivars	Boron (mg kg <sup>-1</sup> )	Shoot		Root		SRR
		FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	
Mercan	0	46.09	3.72	4.39 a	0.41	8.97
	5	44.95	3.21	3.63 ab	0.34	9.53
	10	41.90	2.74	2.11 c	0.24	11.23
	25	24.51	1.32	0.75 d	0.07	19.37
Wild	0	27.92	2.70	3.65 ab	0.40	6.83
	5	24.02	2.22	2.50 c	0.33	6.83
	10	20.15	1.90	2.61 bc	0.33	5.73
	25	8.94	0.66	0.61 d	0.06	10.73
<i>F</i> - test significance		ns	ns	*	ns	ns
Mercan		39.36	2.75 •	2.72	0.27	12.28 •
Wild		20.26	1.87	2.34	0.28	7.53
<i>F</i> - test significance		**	**	ns	ns	**
	0	37.01 a	3.21 a	4.02 a	0.41 a	7.90 b
	5	34.49 a	2.72 ab	3.07 b	0.34 b	8.18 b
	10	31.03 a	2.32 b	2.36 b	0.29 b	8.48 b
	25	16.73 b	0.99 c	0.68 c	0.07 c	15.05 a
<i>F</i> - test significance		**	**	**	**	**

The values are mean of three replicates. Different letters in column indicates significant differences according to Duncan's multiple-range test ( $\alpha$ : 0.05). The differences between cultivars (•) were assessed by t-test ( $\alpha$ : 0.05).

### 3.3. Membrane permeability, relative water content and chlorophyll content

There was a significant interaction between B treatments and cultivars on the MP and its content increased up to 10 mg kg<sup>-1</sup> B level in both cultivars (Table 2). The increment of MP could be explained by membrane damage due to excess B. These results agree with previous finding of Çikili et al. (2015) for peanut plants, Eraslan et al. (2007b) for tomato and pepper, Gunes et al. (2009) for bean and Kaya et al. (2009) for tomato. Also, Karabal et al. (2003) informed a regular increase in electrolyte leakage from root and leaf of barley with toxic B level. But, in both cultivars, there was a non-significant difference between control and the highest B level. It might be that plant sterols reduced the MP of leaves at the highest B level. Cholesterol, a strong plant sterol, enhances membrane mechanical coherence and inhibits the passive transmembrane permeability in eukaryotic plasma membranes (Mouritsen et al., 1995). Also, the increase of MP value in Mercan was greater than in Wild with 5 and 10 mg kg<sup>-1</sup> B levels. It could be explain by genotypic differences in cultivars.

The RWC is a good indicator for plant water status and interaction of B treatments and cultivars was significant in RWC (Table 2). However, the RWC showed an indecisive trend in both cultivars. For example, minimum RWC rate in Wild was found by 5

mg kg<sup>-1</sup> B level. On the other hand, the RWC in both cultivars was significantly augmented by 25 mg kg<sup>-1</sup> B level.

At the highest B level, the increment in RWC could be explained by inability to achieve turgor conditions as a result of disintegration of cell membrane of plant in B-toxic conditions. In parallel to our results, Hamurcu et al. (2015) found a high rate of RWC at the highest B levels in hydroponically grown watermelon plant. Also, Kaya et al. (2009) reported similar results in tomato.

Total Chl and Car contents decreased markedly up to 10 mg kg<sup>-1</sup> in Mercan cultivar. Total Chl and Car contents were decreased significantly with 10 and 25 mg kg<sup>-1</sup> B levels in wild culture (Table 2). When compared to average of cultivars, the contents of total Chl and Car in Wild was remarkable greater than in Mercan. Regardless of cultivars, increasing B levels decreased total Chl and Car content, but the decreases in both parameters were found significantly in 10 mg kg<sup>-1</sup> B level. These reductions could be explained by the negative effects of excess B on decreases in leaf chlorophyll and in photosynthetic rate, volume of mesophyll cells, and cell damage (Sotiropoulos et al., 2002). Also, the decreases in total Chl with increasing B treatments reported by Supanjani (2006) for hot pepper, Nagesh et al. (2012) for French bean and Çikili et al. (2015) for peanut. Additionally, Hajiboland et al. (2011) reported that presence of B increased the Car rate in B deficient tea plants.

Table 2. Effects of increasing B treatments on some growth parameters measured in leaves of purslane cultivars

Cultivar	Boron (mg kg <sup>-1</sup> )	MP (%)	RWC (%)	Total Chl (mg g <sup>-1</sup> FW)	Car (mg g <sup>-1</sup> FW)
Mercan	0	30.3 c	79.3 cd	0.152 b	0.077 b
	5	57.6 a	84.1 b	0.130 b	0.065 c
	10	56.0 a	81.1 bc	0.095 c	0.051 d
	25	30.2 c	89.5 a	0.153 b	0.076 b
Wild	0	26.8 c	81.2 bc	0.175 a	0.087 a
	5	39.9 b	76.2 d	0.181 a	0.090 a
	10	44.3 b	83.0 bc	0.136 b	0.069 c
	25	30.8 c	84.3 b	0.150 b	0.076 b
<i>F</i> - test significance		*	**	**	**
Mercan		43.5	83.5	0.133 **	0.067 **
Wild		35.5	81.2	0.161	0.080
<i>F</i> - test significance		**	*	**	**
	0	28.6 b	80.3 b	0.164 a	0.082 a
	5	48.8 a	80.2 b	0.156 a	0.077 a
	10	50.1 a	82.1 b	0.115 b	0.060 b
	25	30.5 b	86.9 a	0.152 a	0.076 a
<i>F</i> - test significance		**	**	**	**

The values are mean of three replicates. Different letters in column indicates significant differences according to Duncan's multiple-range test ( $\alpha$ : 0.0). The differences between cultivars were assessed by t-test ( $\alpha$ : 0.01)

### 3.4. Boron content and uptake in purslane root and shoot

The shoot B content enhanced linearly in both cultivars with increasing B treatments; however,

interaction of B treatments and cultivars on the shoot B content were not found significantly (Figure 1a). With increasing B treatments, shoot of Mercan absorbed more B than Wild. Irrespective of cultivars, increasing B

treatments augmented significantly the shoot B content. A high B content in shoot of both cultivars could be explained by increasing B in root media (Salinas et al., 1986) and having high B accumulation capacity (Stiles et al., 2010). Absorbed B was relocated to upper organs and accumulated in there at the end of transpiration stream (Reid et al., 2004).

Boron uptake of plants is related primarily to soluble B concentration in rooting medium. In comparison with control, a sharp increase in shoot B uptake was found up to 10 mg kg<sup>-1</sup> B level in both cultivars (Figure 1b). This case might be explained by decreases of ion absorption as a result of growth reduction caused by B toxicity and/or an increment of ion efflux due to damages of plasma membrane properties of the plants. Irrespective of B treatments, the increase in Mercan was markedly higher (almost 2-fold) than Wild. Irrespective of cultivars, shoot B uptake increased with increasing B treatments. A possible explanation of these differences between cultivars could be explained by differences in cell membrane permeability and composition of

membrane and cell wall (Nable and Paul, 1991). Also, a linear relationship between B treatments and B uptakes was reported by Carr et al. (2011) in pea plants and Tariq and Mott (2006) in radish.

Root B content augmented with increased B levels, except for the highest B level (Figure 1c). Irrespective of cultivars, while 5 and 10 mg kg<sup>-1</sup> B levels increased the root B content compared to control, 25 mg kg<sup>-1</sup> B level decreased it. The interaction of cultivars and B treatments on root B uptake was not considerable; however, 25 mg kg<sup>-1</sup> B level decreased significantly the root B uptake by 87.0%, compared to control (Figure 1d). This result could be explained by root B absorption capacity and B-complex formations inside or outside the roots (Hu and Brown, 1997). Reports showed that soluble B in growing media absorbed easily by roots (El-Feky et al., 2012) and increased B concentrations in shoot for sunflower and tomato seedlings (Keleş et al., 2011).

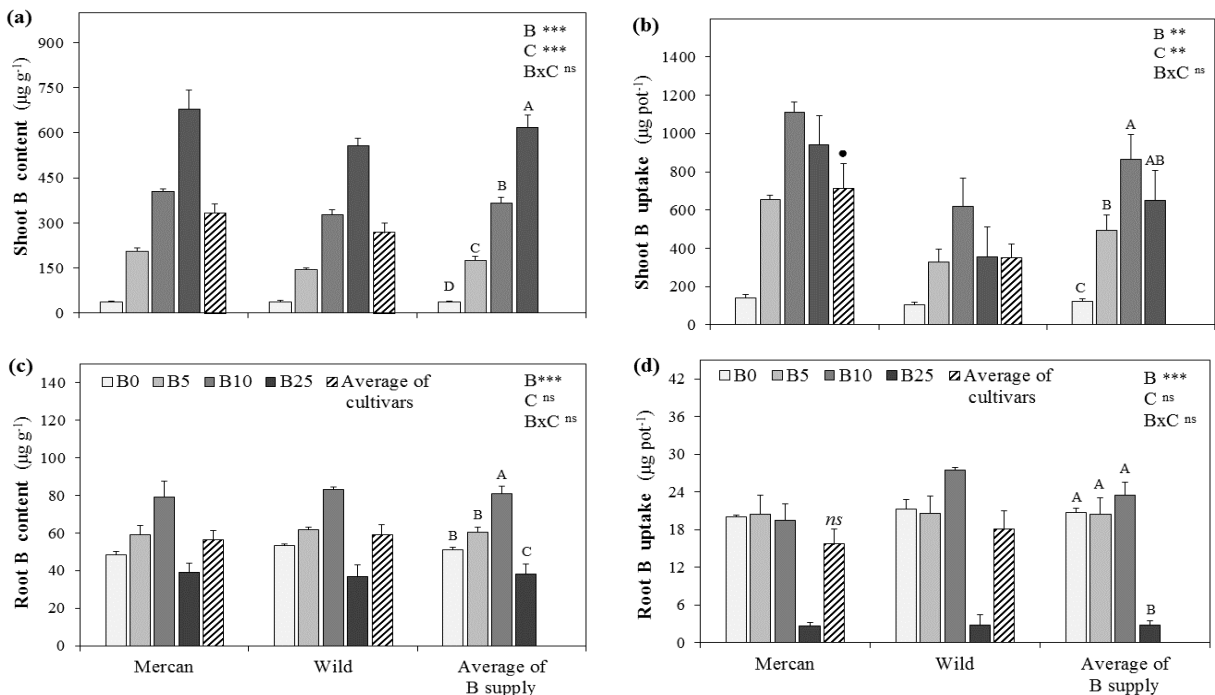


Figure 1. Effects of increasing B treatments on boron content and uptake of shoot and root in purslane cultivars. The values are means of three replicates and the bars represent their standard errors (Mean  $\pm$ SE, n = 3). Different letters on the column indicates significant differences according to Duncan's multiple-range test ( $\alpha$ : 0.05)

### 3.5. Mineral nutrient uptakes of purslane shoots

Effects of increasing B treatments on the uptake of P, Ca, K and Na were given in the Figure 2. As compared to control, the greatest decrease in P uptake was seen at 25 mg kg<sup>-1</sup> B level. But, no considerable interaction between B treatments and cultivars on P uptake of purslane was found (Figure 2a). Irrespective of B treatments, P uptake of Mercan was markedly

higher than of Wild. Irrespective of cultivar, the effect of the highest B level on P uptake was significant. On the other hand, 5 mg kg<sup>-1</sup> B level increased the shoot Ca uptake, but the other B levels decreased in both cultivars (Figure 1b). Irrespective of B treatments, Ca uptake of Wild was greater than Mercan cultivar. Also, K uptake decreased with increasing B levels in both cultivars, but no interaction was found (Figure 1c). Irrespective of B treatments, K uptake in Mercan remarkably was greater

than in Wild. To the detriment of cultivars, the highest B level decreased K uptake by 78%, compared to control. Additionally, high B significantly decreased concentration of Ca, P and K in tomato plants (Kaya et al., 2009).

Increasing B treatments augmented the Na uptake in Mercan cultivar, however; it decreased markedly in

Wild (Figure 2d). The Na uptake could be inhibited by excess B, due to genotypic differences. Benlloch et al. (1991) revealed cultural differences in tolerance of olive to Na and excess B in rooting medium. Similar findings, increase of Na content with increasing B, reported by Ismail (2003) for maize and sorghum; Samet et al. (2013) for beans were agree with these results.

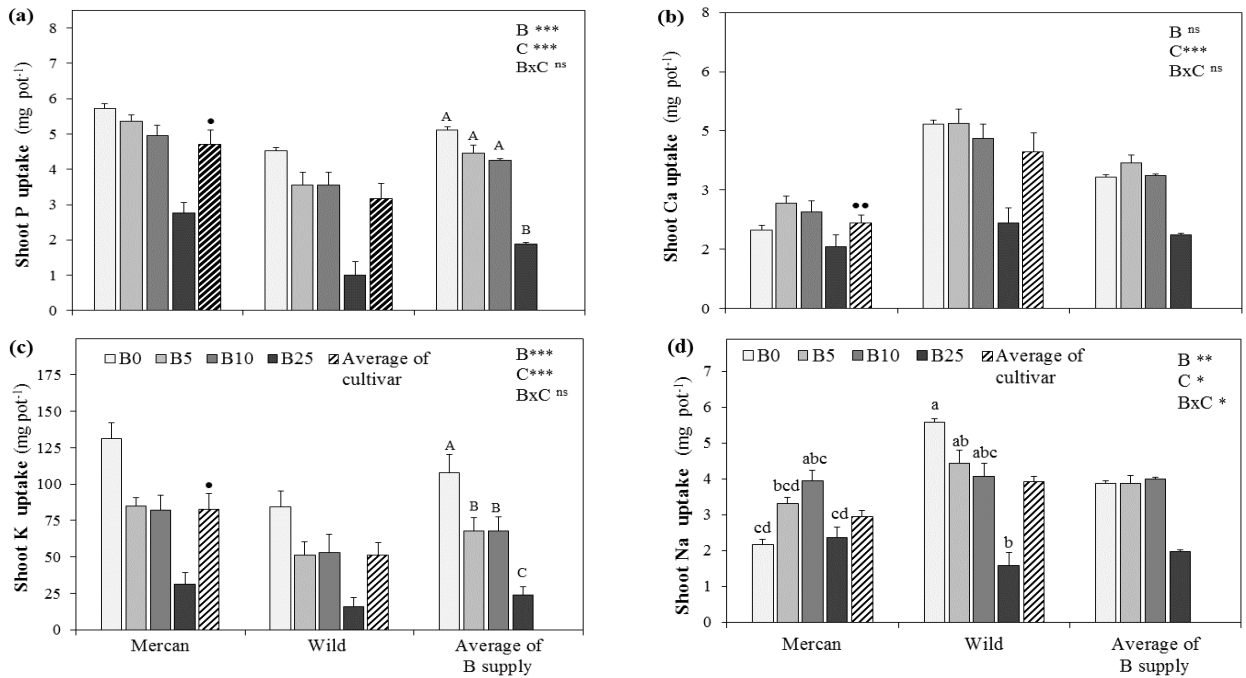


Figure 2. Effects of increasing B treatments on the uptake of P, Ca, K and Na in purslane cultivars. The values are means of three replicates and the bars represent their standard errors (Mean  $\pm$ SE, n = 3). It indicates that small letters for interactions between B treatments and cultivars, capital letters for average of B supply.

## 5. Conclusion

Biomass production and leaf parameters could be accepted good predictor for toxic effects of B. Plant growth, photosynthetic pigments, MP and mineral balance of purslane cultivars significantly affected by the detrimental effect of excess B and plants exhibited visual symptom of B toxicity. While shoot and root biomass, total Chl and Car significantly decreased in B-stressed plants, the SRR and the MP were increased. Total Chl and Car contents in Wild were greater than in Mercan.

While the P and K uptakes of B-stressed plants decreased in both cultivars, the Ca uptake showed an indecisive trend. The Na uptakes increased in Mercan, whereas it decreased in Wild. The P and K uptakes were greater in Mercan, whereas the Ca and Na uptakes were greater in Wild. Purslane is an important cultivable plant and showed different responses to increasing B treatments. In general, wild varieties is known more strong to stress conditions. When considered the shoot and root biomass production, our results showed that Mercan cultivar, commonly grown in Turkey, was less

affected from toxic effect of B than Wild. For more information about purslane that has short vegetable cycle and high fresh mass yield ought to be carried out more greenhouse and field studies.

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