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ORIGINAL PAPER

# Changes in plant growth and mineral concentrations of soybean cultivars under waterlogging stress

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## Abstract

This study aimed to evaluate the response of four soybean cultivars (Arısoy, Cinsoy, Umut-2002, and Sarıgelin-1) in terms of their mineral and morpho-physiological properties to waterlogging stress during the early growing stage ($V_1$). 20-day-old plants were exposed to waterlogging for 14 days. The results showed that soybean cultivars reacted differently to waterlogging. The shoot and root fresh weight and chlorophyll rate were significantly lower in the waterlogged plants than in the control. No significant changes in shoot and root fresh weights of cv. Cinsoy was observed due to waterlogging while cv. Cinsoy had the highest chlorophyll rate. Waterlogging led to decreasing the phosphorus concentration, but Ca, Na, Zn, Mn, B, and Cu were enhanced. However, cv. Arısoy possessed higher K, P, Ca, Mg, S, and B levels than the control, and a significant reduction in Ca, S, Zn, Mn, B, Cu, and K/Na ratio was determined in the waterlogged plants. Phosphorus was decreased in all the cultivars except for cv. Arısoy. It was concluded that Arısoy and Cinsoy appeared more tolerant to waterlogging during the early growing stage compared to the other soybean cultivars, and the stress mainly affected the plant's mineral concentrations.

**Keywords**: flooding, *Glycine max* L., genotype, macronutrient, micronutrient, seedling development

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# INTRODUCTION

Soybean (*Glycine max* L.) is one of the most important annual legumes used for 18-24% oil and 36-45% protein content in seeds by oil, food, and feed industries throughout the world (Fehr 1980, Medic et al. 2014). Owing to the rich nutritional values of seeds, almost 1/3 of the edible oils and 2/3 of the protein source in the world are obtained from soybean (Sincik et al. 2008). In Türkiye, seed production of 182.000 tons in a sowing area of 44.000 ha in 2021 was not enough to meet the soybean demand, thus 1.9 billion dollars was paid for the import of approximately 2.5 million tons of seeds, 2.000 tons of oil, and 910.00 tons of meal (TUIK 2022). One of the most important reasons for the insufficient production of soybean is the limited cultivation area, mainly concentrated in the Mediterranean region, where climatic stresses such as waterlogging and extreme temperatures restrict the productivity of soybean.

Waterlogging, which defines excess water in the root zone due to firstly heavy rainfall and secondly irrigation water, limits plant growth by inhibiting oxygen, carbon dioxide, and light (Michael et al. 2009, Wu et al. 2017). The lack of adequate drainage facilities also enhances the severity of waterlogging stress (Kim et al. 2015, Sathi et al. 2022). Waterlogging is a significant abiotic stress factor for soybean cultivation, especially in the early growing stages, because 60-70% of the total precipitation occurs during the spring and summer seasons (Kim et al. 2015). However, the response of soybean to waterlogging resulting in yield loss is linked to the plant growth stage, duration of flooding, and cultivar tolerance. Soybean is considered to be a susceptible plant to waterlogging during early reproductive stages (Oosterhuis et al. 1990, Board 2008, Rhine et al. 2010, Hashimoto et al. 2020, Pasley et al. 2020), while Nguyen et al. (2012) reported that it leads to a reduction in seed yield by 17-40% at the vegetative stage. In addition, VanToai et al. (2010) determined that there were genotypic variations for flooding tolerance in soybean, and waterlogging resulted in seed yield loss of 40% and 80% for tolerant and sensitive genotypes, respectively. In this research, we focused on the changes in mineral contents and morpho-physiological responses of four native soybean cultivars under waterlogging stress.

# MATERIALS AND METHODS

A laboratory experiment was carried out in the Seed Science and Technology Laboratory of Eskişehir Osmangazi University, Türkiye, in 2022. Four soybean cultivars were used as materials, namely cv Cinsoy and Umut-2002 from Aegean Agricultural Research Institute, İzmir, cv.

Sarıgelin-1 from Bahri Dağdaş International Agricultural Research Institute-Konya, and cv. Arısoy from Çukurova University, Faculty of Agriculture, Adana. The soybean cultivars were pre-germinated on filter paper moistened with distilled water in Petri dishes at 20°C for 48 h, and the seeds with radicle protrusion were transferred to plastic pots (0.5 L) filled with a mixture of sieved field soil:perlite:vermiculite (6:1:1 v:v:v), respectively. After transferring, they were fertilized with basal macronutrients N-P-K (8-8-8). Four plants from each cultivar were grown until the $V_1$ vegetative stage (at the first trifoliate stage described by Fehr et al. (1971)) in a growth chamber adjusted to temp. of 20°C/15°C day/night, respectively, with a range of 70 to 75% relative humidity. For waterlogging stress, 20-day-old plants were immersed in water up to 2 cm above the soil level and left for 14 days. Control plants were regularly irrigated every two days up to field capacity. Both waterlogged and control plants were allowed to grow at 10°C during the treatment so as to simulate low temperatures resulting from heavy rain conditions. Plant height, stem diameter (with digital caliper), leaf number, leaf temperature (with Trotec BP21 infrared thermometer), and chlorophyll rate (with Konica Minolta SPAD-502 portable chlorophyll meter) were measured 4 days after the waterlogging treatment. In addition, shoot and root fresh weights were determined after the roots and shoots of the plants were separated and cleaned. The samples of roots and shoot parts were dried in an oven set at 80°C for 24 h and their dry weights were measured. After dried shoot samples were ground, they were prepared for elemental analysis according to the wet-digestion method (Mertens 2005). Total element concentrations (K, P, S, Na, Ca, Mg, Fe, Zn, B, and Cu) of the samples were determined in an Inductively Coupled Plasma Optical Emission Spectrometer (Agilent's 5110 ICP-OES).

All data were analyzed according to a completely randomized design using the MSTAT-C (Michigan State University, v. 2.10) statistical program. The means were separated by Duncan's Multiple Range Test at $p<0.05$ level.

## RESULTS AND DISCUSSION

In the study, where waterlogging stress on soybean cultivars at the $V_1$ stage was tested, the plant's morphological parameters were significantly influenced by the cultivars and waterlogging treatment (Table 1). A two-way interaction of shoot and root fresh weight was significant. Among the cultivars, cv. Umut-2002 reached the highest plant height (18.4 cm) and cv. Cinsoy grew the shortest at 15.4 cm. A thinner stem diameter and the fresh shoot and root weights were obtained from the waterlogged plants of any variety and cv. Arısoy produced the minimum stem diameter, shoot, and root fresh weight. The stem diameter, root and shoot dry weights of waterlogged plants were significantly reduced compared to the control in all the cultivars (Tables 1, 2). Similarly, several researchers have reported that seedling development was significantly restricted under waterlogging stress in soybean

Table 1

Effects of waterlogging on soybean cultivars

| Factors | Plant height (cm) | Stem diameter (mm) | Shoot fresh weight (g plant$^{-1}$) | Root fresh weight (g plant$^{-1}$) |
|---|---|---|---|---|
| Stress (A) | | | | |
| Control | 16.8 | $4.15^{a}$ | $4.30^{a}$ | $5.31^{a}$† |
| Waterlogging | 17.2 | $3.36^{b}$ | $3.09^{b}$ | $4.16^{b}$ |
| Cultivars (B) | | | | |
| Arısoy | $17.4^{b}$ | $3.38^{b}$ | $3.39^{b}$ | $4.16^{c}$ |
| Cinsoy | $15.4^{c}$ | $4.02^{a}$ | $3.76^{a}$ | $4.63^{b}$ |
| Umut 2002 | $18.4^{a}$ | $3.87^{a}$ | $3.76^{a}$ | $5.41^{a}$ |
| Sarıgelin-1 | $17.0^{b}$ | $3.76^{a}$ | $3.87^{a}$ | $4.74^{b}$ |
| Analysis of Variance | | | | |
| A | ns | ** | ** | ** |
| B | ** | ** | ** | ** |
| A×B | ns | ns | ** | ** |

† Means followed by the same letter(s) are not significant at $p<0.05$. ** show significance level at $p<0.01$, ns – non-significant.

Table 2

Effects of waterlogging on shoot and root dry weight and leaf number of soybean cultivars

| Factors | Shoot dry weight (mg plant$^{-1}$) | Root dry weight (mg plant$^{-1}$) | Leaf number (number plant$^{-1}$) |
|---|---|---|---|
| Stress (A) | | | |
| Control | $1062^{a}$ | $770^{a}$† | 3.38 |
| Waterlogging | $779^{b}$ | $554^{b}$ | 3.06 |
| Cultivars (B) | | | |
| Arısoy | 940 | 650 | $2.88^{b}$ |
| Cinsoy | 981 | 685 | $3.25^{ab}$ |
| Umut 2002 | 891 | 679 | $3.63^{a}$ |
| Sarıgelin-1 | 869 | 634 | $3.13^{ab}$ |
| Analysis of Variance | | | |
| A | ** | ** | ns |
| B | ns | ns | * |
| A×B | ** | ns | ns |

† Means followed by the same letter(s) are not significant at $p<0.05$. *, ** show significance level at $p<0.05$ and $p<0.01$, ns – non-significant.

(Hashiguchi et al. 2009), cotton (Ashraf et al. 2011), rapeseed (Liu et al. 2020), and cowpea (Jayawardhane et al. 2022).

A two-way interaction showed that lower root and shoot fresh weights were obtained from all soybean cultivars except for cv. Cinsoy, which obtained a similar weight in the control treatment (Figure 1*a*, *b*).

The shoot and root dry weights of the waterlogged plants were significantly reduced; however, no significant changes were observed in the leaf number (Table 2). Our results are confirmed by Júnior et al. (2022), who pointed out that aerial and root dry matter of soybean plants declined in waterlogged soil during the soybean reproductive stage. Also, Kim et al. (2015) found that the decrease in dry root and shoot dry weight was greater in waterlogging-sensitive cultivars compared to tolerant cultivars of soybean. The cultivar Umut-2002 had the highest mean leaf number, but the lowest number of leaves was counted in cv. Arısoy.

A significant reduction in the leaf chlorophyll rate was recorded under waterlogging stress (Table 3). The cultivar Umut-2002 had the lowest chlorophyll rate among the soybean cultivars. Generally, the chlorophyll rate of Arısoy, Umut-2002, and Sarıgelin-1 decreased under waterlogging stress (Figure 1*c*). Similar results were observed by Bacanamwo and Purcell (1999), who found that excessive water caused chlorosis and necrosis in the leaves of soybean. Sathi et al. (2022) and Ploschuk et al. (2022) emphasized that the SPAD values of soybean under flood stress decreased between 15% and 35%. The mean leaf temperature changed depending on waterlogging stress and cultivars, and it slightly decreased in the waterlogged plants while cv. Arısoy showed a higher mean leaf temperature than cv. Sarıgelin-1.

Table 3

Changes in chlorophyll rate and leaf temperature of soybean cultivars under waterlogging stress

| Factors | Chlorophyll rate (SPAD) | Leaf temperature (°C) |
|---|---|---|
| Stress (A) | | |
| Control | 27.8$^{a}$ | 24.9$^{a}$† |
| Waterlogging | 24.1$^{b}$ | 24.6$^{b}$ |
| Cultivars (B) | | |
| Arısoy | 26.4$^{a}$ | 25.0$^{a}$ |
| Cinsoy | 27.6$^{a}$ | 24.8$^{a}$ |
| Umut 2002 | 23.1$^{b}$ | 24.8$^{a}$ |
| Sarıgelin-1 | 26.9$^{a}$ | 24.4$^{b}$ |
| Analysis of Variance | | |
| A | ** | * |
| B | ** | ** |
| A×B | ** | ns |

† Means followed by the same letter(s) are not significant at $p<0.05$. *, ** show significance level at $p<0.05$ and $p<0.01$ respectively, ns – non-significant.

The effects of waterlogging stress on macronutrients in shoot parts of soybean cultivars are collated in Table 4. A significant increase was determined for Na, Ca, and S although the P content decreased when the plants were exposed to waterlogging. Two-way interaction was significant in the concentrations of K, K/Na, P, Ca, Mg and S. Sodium (Na) was only higher in waterlogged plants than in control. The response of soybean cultivars to waterlogging for K content was varied. In waterlogged plants, the K content increased in cv. Arısoy, but decreased in Cinsoy and Sarıgelin-1 (Figure 1*d*). On the other hand, the K/Na ratio was not changed in cv. Arısoy; it deminished in the cultivars Cinsoy and Sarıgelin-1 (Figure 1*e*). The P content of all soybean cultivars except for cv. Arısoy was reduced by waterlogging (Figure 1*f*). An increased S content was measured in waterlogged plants of all soybean cultivars (Figure 2*c*).

Table 4

Main effects of waterlogging on macronutrient concentrations of soybean cultivars

| Factors | K (g kg$^{-1}$) | Na (g kg$^{-1}$) | K/Na | P (g kg$^{-1}$) | Ca (g kg$^{-1}$) | Mg (g kg$^{-1}$) | S (g kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Stress (A) | | | | | | | |
| Control | 19.9 | $0.20^{b}$ | $97.6^{a}$ | $4.16^{a}$ | $11.7^{b}$ | 3.91 | $1.87^{b}$† |
| Waterlogging | 19.0 | $0.22^{a}$ | $88.3^{b}$ | $3.45^{b}$ | $13.3^{a}$ | 4.11 | $2.31^{a}$ |
| Cultivars (B) | | | | | | | |
| Arısoy | 19.0 | 0.21 | $89.8^{b}$ | $3.61^{c}$ | $11.1^{b}$ | $3.57^{c}$ | $1.94^{b}$ |
| Cinsoy | 18.9 | 0.21 | $89.8^{b}$ | $3.95^{b}$ | $12.9^{a}$ | $4.01^{b}$ | $2.20^{a}$ |
| Umut 2002 | 18.9 | 0.21 | $90.3^{b}$ | $4.27^{a}$ | $13.4^{a}$ | $4.43^{a}$ | $2.35^{a}$ |
| Sarıgelin-1 | 20.8 | 0.21 | $102.0^{a}$ | $3.39^{c}$ | $12.6^{a}$ | $4.02^{b}$ | $1.88^{b}$ |
| Analysis of Variance | | | | | | | |
| A | ns | * | ** | ** | ** | ns | ** |
| B | ns | ns | * | ** | ** | ** | ** |
| A×B | ** | ns | ** | ** | ** | ** | ** |

† Means followed by the same letter(s) are not significant, *, ** show significance level at $p<0.05$ and $p<0.01$ respectively, ns – non-significant.

Low oxygen amounts due to excess water in the soil prevent root respiration, which limits the energy required for nutrient uptake and transport (Boru et al. 2003). Our results agree with Board (2008), who found a decrease in K and P concentrations of soybean in the $V_4$ stage (4th node beginning with the unifoliolate node on the main stem, as described by Fehr et al. (1971)) under flooding stress. Similarly, Rhine et al. (2010) emphasized that soybean leaf P content was lower in 8-day flooding stress than in control. On the other hand, we observed a significant increase in Mg, S, and Ca concentrations in aerial parts of seedlings (Figure 2*a*, *b*, *c*), Board (2008) reported considerable reductions in these minerals. Our results are in agree-

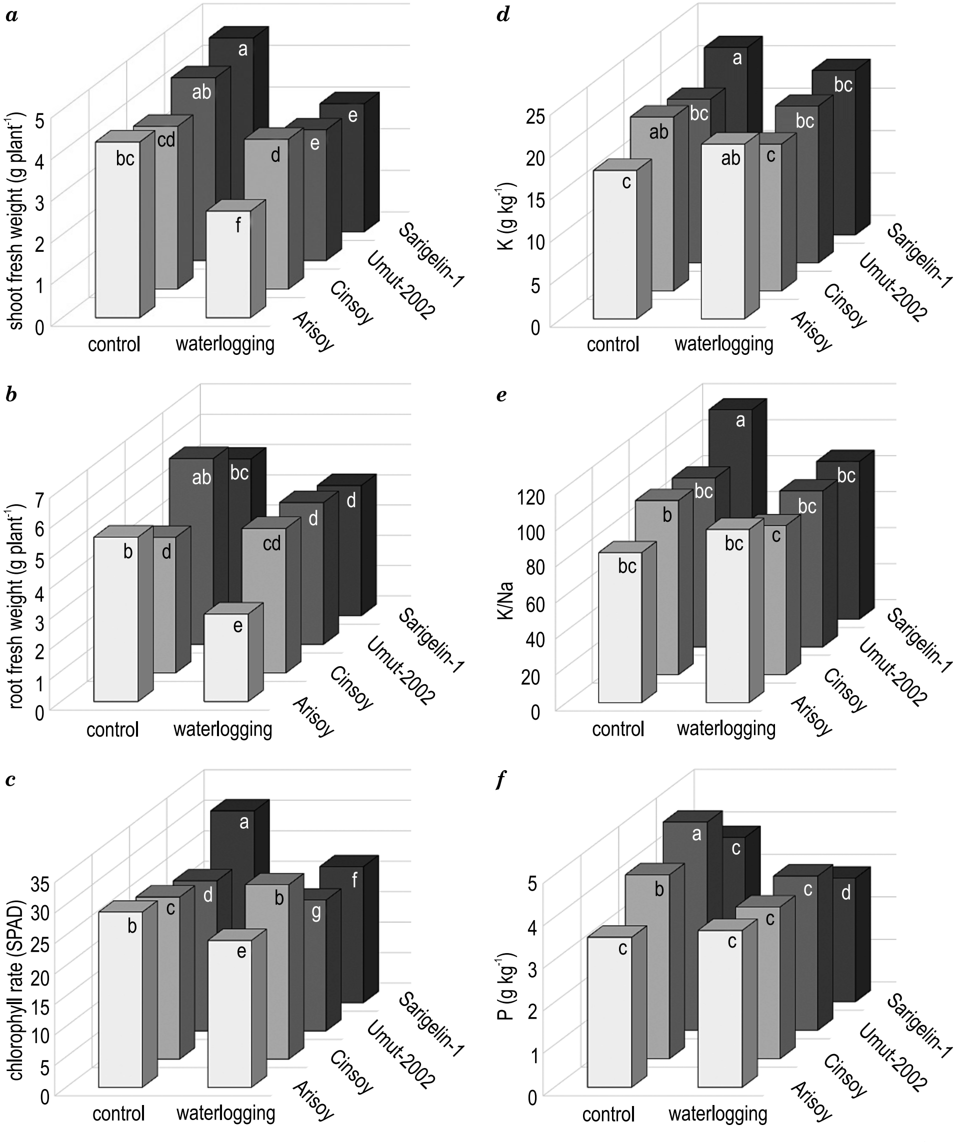

Fig. 1. The interaction effects of cultivar × waterlogging on shoot fresh weight: *a* – g plant$^{-1}$, *b* – root fresh weight (g plant$^{-1}$), *c* – chlorophyll rate (SPAD value), *d* – K (g kg$^{-1}$), *e* – K/Na ratio, *f* – P (g kg$^{-1}$) contents in aerial parts of seedlings. The letter(s) in each column show significance levels at $p<0.05$

ment with the findings of Milroy et al. (2009), who reported an increased Na content of cotton leaves in waterlogging stress.

The content of micronutrients such as Zn, Mn, B, and Cu of the waterlogged plants was higher than that of the unstressed plants, with an insignificant Fe content (Table 5). Fe, B, and Cu concentrations were affected

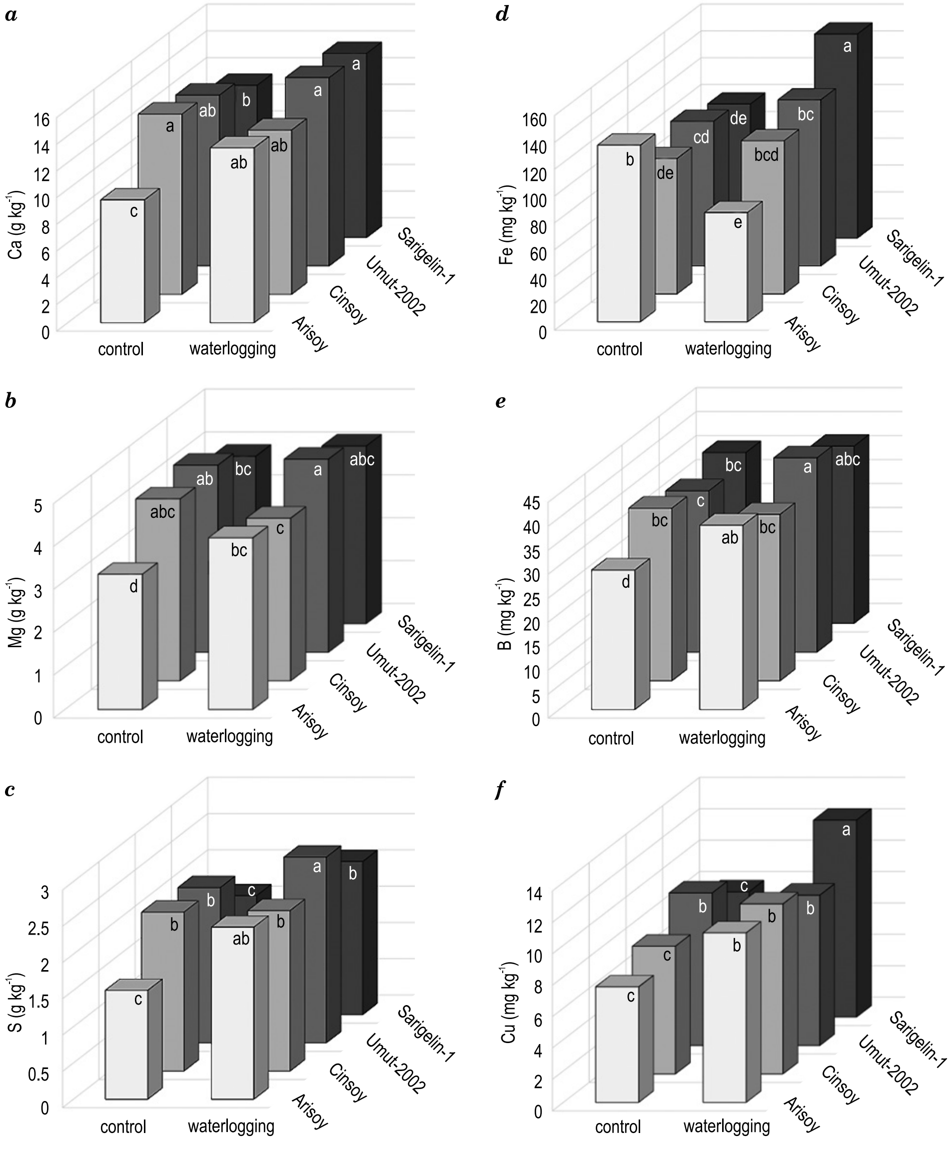

Fig. 2. The interaction effects of cultivar × waterlogging on: *a* – Ca (g kg$^{-1}$), *b* – Mg (g kg$^{-1}$), *c* – S (g kg$^{-1}$), *d* – Fe (mg kg$^{-1}$), *e* – B (mg kg$^{-1}$), *f* – Cu (mg kg$^{-1}$) contents in aerial parts of seedlings. The letter(s) in each column show significance levels at $p<0.05$

by the cultivar × waterlogging interaction. Waterlogging stress caused an improvement in the Fe content of cv. Sarıgelin-1, but cv. Arısoy had a higher Fe content in unstressed plants (Figure 2*d*). Additionally, the highest B and Cu contents were obtained from the plants subjected to waterlogging (Figure 2*e*, *f*). The findings on micronutrient changes due to waterlogging are varied, after generally word a reduction in Cu, Zn, and B was

Table 5

Main effects of waterlogging on micronutrient concentrations of soybean cultivars

| Factors | Zn (mg kg$^{-1}$) | Fe (mg kg$^{-1}$) | Mn (mg kg$^{-1}$) | B (mg kg$^{-1}$) | Cu (mg kg$^{-1}$) |
|---|---|---|---|---|---|
| Stress (A) | | | | | |
| Control | 43.0$^{b}$ | 110 | 47.8$^{b}$ | 33.6$^{b}$ | 8.30$^{b}$† |
| Waterlogging | 50.0$^{a}$ | 118 | 55.1$^{a}$ | 37.7$^{a}$ | 10.93$^{a}$ |
| Cultivars (B) | | | | | |
| Arısoy | 46.3 | 107$^{b}$ | 47.5$^{b}$ | 33.8 | 9.08 |
| Cinsoy | 46.3 | 108$^{b}$ | 48.9$^{b}$ | 35.4 | 9.48 |
| Umut 2002 | 48.0 | 116$^{ab}$ | 58.3$^{a}$ | 37.1 | 9.64 |
| Sarıgelin-1 | 45.0 | 126$^{a}$ | 51.0$^{b}$ | 36.3 | 10.30 |
| Analysis of Variance | | | | | |
| A | ** | ns | ** | ** | ** |
| B | ns | * | ** | ns | ns |
| A×B | ns | ** | ns | ** | ** |

† Means followed by the same letter(s) are not significant, *, ** show significance level at $p<0.05$ and $p<0.01$ respectively, ns – non-significant.

reported by Kozlowski and Pallardy (1985), while Fe and Mn concentrations were found to have increased in soybean under such conditions (Board 2008). In contrast, Milroy et al. (2009) determined that B, Fe, and Mn in cotton decreased due to waterlogging. This might be explained by inhibiting the uptake of nutrients from the soil by plants or the absence of oxygen in the root zone, which limited the mobilization of the plant nutrition to the growing embryonic axis. Also, different plant species, growing stages, and exposure durations to waterlogging might be the reasons for differences in mineral compositions.

## CONCLUSION

Flooding, which is one of the most common abiotic stresses associated with global climate change, adversely influences the productivity of crop plants. In the study, seedling growth parameters and mineral compositions of four soybean cultivars grown in Türkiye were investigated for waterlogging stress during the early growing stage. The results revealed that plant growth was depressed by waterlogging and mineral compositions were different from treated and untreated (control) plants. Also, soybean cultivars responded differently to waterlogging. It was concluded that the cultivars Arısoy and Cinsoy should be preferred because they appeared more tolerant to waterlogging than the cultivars Sarıgelin-1 and Umut-2002.

## Conflict of interest

The authors declare no conflict of interest.

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