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THE EFFECT OF BORIC ACID APPLICATION ON ECOPHYSIOLOGICAL CHARACTERISTICS OF SAFFLOWER VARIETIES (*CARTHAMUS TINCTORIUS* L.)

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ABSTRACT

In this study, different concentrations (0, 5, 10, 15 mM) of boric acid were applied on three varieties of safflower (Balcı, Dinçer, Remzibey), and germination rate, root-stem length, root-stem fresh and dry weight, root-stem biomass, seed vigor index, boric acid tolerance index and chlorophyll a, b and changes in total chlorophyll content were investigated. It was determined that a high boric acid concentration causes a decrease in the ecophysiological parameters examined in the safflower varieties. It was determined that the total chlorophyll content decreased in the Balcı and Remzibey varieties. According to the results, the most sensitive variety against boron is Remzibey, and the most tolerant variety is Balcı. The Dinçer variety is closer to the Balcı variety in terms of its boron tolerance.

KEYWORDS:
Boron, *Carthamus tinctorius* L., Germination, Seedling growth

INTRODUCTION

Safflower, known as one of the oldest oilseed plants cultivated by humans, is an important industrial plant [1, 2]. Safflower is a plant suitable for cultivation due to its high ability to grow in arid areas, high competition against weeds and low production cost [3, 4]. Nowadays, with global warming, the increase in soil salinity is giving the agriculture of stress-resistant plants prominence. Therefore, safflower cultivation becomes important in utilization of marginal soils [5].

Plants are under the influence of different biotic and abiotic stress factors in the areas where they grow naturally during their lifetime [6]. Abiotic stress is defined as environmental conditions that reduce growth and yield. Plant responses to abiotic stresses are dynamic and complex [7]. Factors such as drought, salinity, malnutrition, excess of nutrients, low and high temperature, radiation, soil and atmosphere pollution are among basic abiotic stresses that limit yield in crop production. One of the important abiotic factors that cause stress in plants is boron toxicity [8]. Boron is a micronutrient element that is absolutely essential for plants [9]. However, boron excess is an important agricultural problem which limits the plant's productivity since it creates a toxic effect [10]. The tolerance limits of boron in plants cannot be well established, and even a small amount of boron excess can cause severe damages to plants [11, 12].

In plants exposed to boron excess, viability, growth and development delay, deterioration of leaf morphology, irregularity in transpiration, permeability of membranes and severe oxidative damage are observed [13, 14]. The role of boron in plant growth and development is still not fully elucidated. Considering that the most important stage of the development of a healthy plant is germination and early development, it is very important to investigate the effects of boron in this period. In this context, in our study, three different varieties of safflower, which is an important industrial plant, were administered different concentrations of boric acid (0, 5, 10, 15 mM) and examined by comparing the changes in some ecophysiological parameters of the varieties.

MATERIALS AND METHODS

In the study, three different varieties of safflower (*Carthamus tinctorius* L.) were used as the experimental material. The seeds which were germinated were randomly selected and sterilized before planting. The seeds were kept in 10% NaOCl for 10 minutes and washed 3 times with distilled water [15]. In this study, a control group (distilled water) and 5, 10 and 15 mM of boric acid solutions were used. For germination tests, petri dishes with a diameter of 12 cm were used, and the inside of the petri dish was covered with two layers of blotting paper. Each petri dish was irrigated with 6 ml of the solution, and 100 seeds were used in each replicate. The experiments were

TABLE 1
Seed germination rates of safflower varieties applied boric acid

	Germination Rate (%)		
Application	Balcı	Dinçer	Remzibey
Control	88 ±3	98 ±3	92 ±3
5 mM	87 ±3	98 ±3	87 ±3
10 mM	85 ±5	98 ±3	75 ±5
15 mM	78 ±3	87 ±3	75 ±5

performed in a 16±8-hour photoperiod at 25±1 °C in a climate chamber for 21 days in three replicates. At the end of the 21 days, germination rate, root-stem length, root-stem fresh-dry weight and root-stem biomass were measured, and the seed vigor index and boric acid tolerance index were calculated [16-18]. The chlorophyll a, b and total chlorophyll content of the plant samples were determined according to the method described by Arnon (1949) [19]. The results were statistically analyzed by One-Way ANOVA (SPSS 21.0).

RESULTS

As seen in Table 1, when the germination rates of the seeds at different concentrations of boric acid were compared, it was determined that the numbers of germinated seeds decreased due to concentration increase in all varieties. The germination rate decreased from 88% to 78% in the Balcı variety, 98% to 87% in the Dinçer variety and 92% to 75% in the Remzibey variety depending on the concentration increase (Table 1).

When the root lengths were compared based on the varieties and concentrations, the highest (3.07 cm) root length was in the Balcı variety, whereas the lowest (0.84 cm) root length was found to belong to the Remzibey variety. In the Dinçer and Remzibey varieties, the root length decreased in all concentrations in comparison to the control group. In the Balcı and Remzibey varieties, the stem length decreased as the concentration of boric acid increased in comparison to the control group. In the Dinçer variety, the stem length at all concentrations decreased in comparison to the control group, while the 10 mM (1.42 cm) boric acid concentration with respect to 5 mM and 15 mM showed an increase (1.22; 1.11 cm respectively, Figure 1).

In the study, it was observed that there were differences among the varieties when the root lengths of the safflower varieties with the applied concentrations of boric acid were compared. It was determined that the root length significantly decreased at all boric acid concentrations in the Dinçer and Remzibey varieties and in the Balcı variety at 5 and 15 mM boric acid application concentrations in comparison to the control group (Figure 1, $p<0.05$).

When the root and body fresh-dry weights were compared in the safflower varieties, it was determined that changes occurred due to the increase in the concentration of boric acid. The highest root fresh and dry weights were determined in the control group (respectively 0.43 g; 0.07 g), while the lowest root fresh and dry weights were determined at 15 mM boric acid (respectively 0.04 g; 0.01 g). As the concentration of boric acid increased, the root fresh and dry weight of the Balcı and Remzibey varieties decreased in comparison to the control group. In the Dinçer variety, while the fresh weight showed a decrease at all concentrations according to the control group, the 10 mM boric acid application (1.45 mM) showed an increase with respect to 5 and 15 mM (respectively 1.13mM; 0.48 mM). It was determined that, as the concentration of boric acid increased, the Dinçer variety's root dry weigh was decreased in comparison to the control group (Figure 2). In boric acid application, the stem fresh and dry weights decreased as the concentration increased in all varieties of safflower. However, one notable result was that the stem fresh weight of the Dinçer variety increased at the 10 mM boric acid application in comparison to 5 and 15 mM, although the result was below the control. This result was very important (Figure 2). In our study, the root-stem fresh and dry weights were adversely affected by boric acid application in all varieties of safflower ($p<0.05$).

When the biomass of the treated plants were calculated, it was determined that the highest root biomass was in the Dinçer variety in the control group (532 g / ha), and the lowest root biomass was in the Dinçer variety at a concentration of 15 mM of boric acid (93 g/ha). The highest stem biomass was found to be at a concentration of 10 mM boric acid in the Balcı variety (2937 g/ha), while the lowest body biomass was found at a concentration of 15 mM of boric acid (1288 g/ha). The root and stem biomass in all varieties at the applied boric acid concentrations was determined to decrease in comparison to the control group (Figure 3).

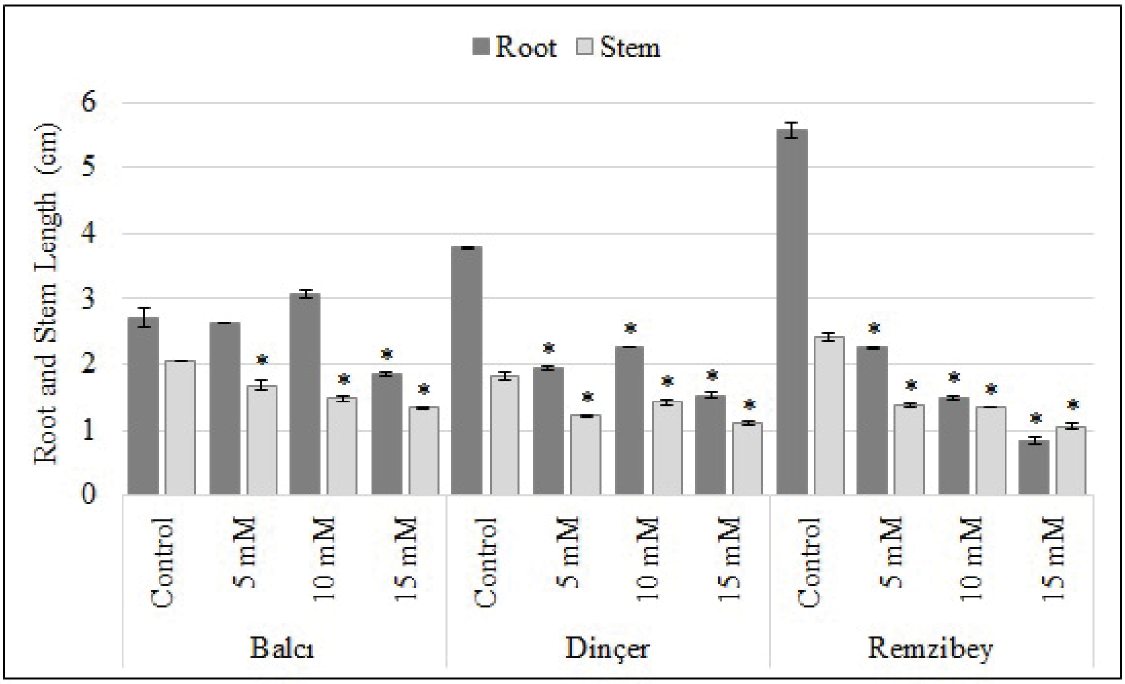

FIGURE 1
Root and stem lengths of safflower varieties applied boric acid (*p<0.05)

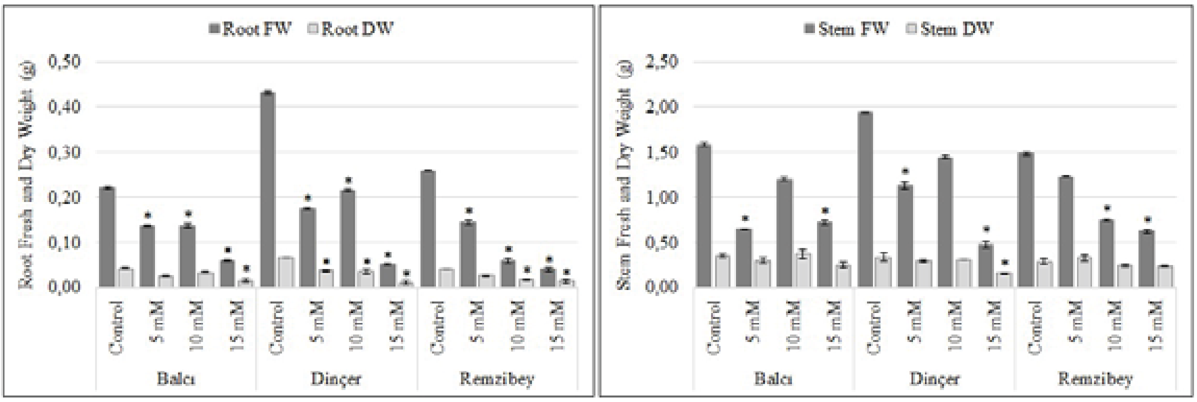

FIGURE 2
Root and stem fresh-dry weights of safflower varieties applied boric acid (FW: Weight, DW: Dry Weight; *p<0.05)

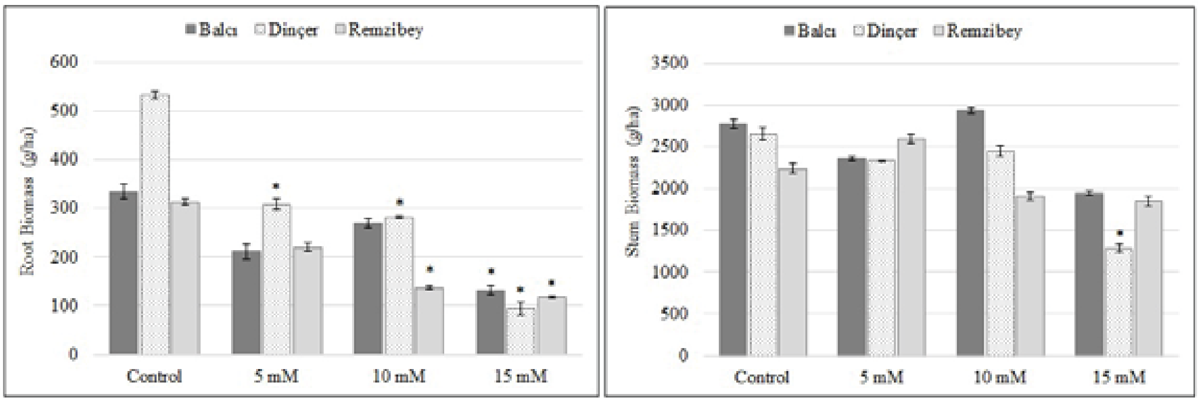

FIGURE 3
Root and stem biomass of safflower varieties applied boric acid (*p<0.05)

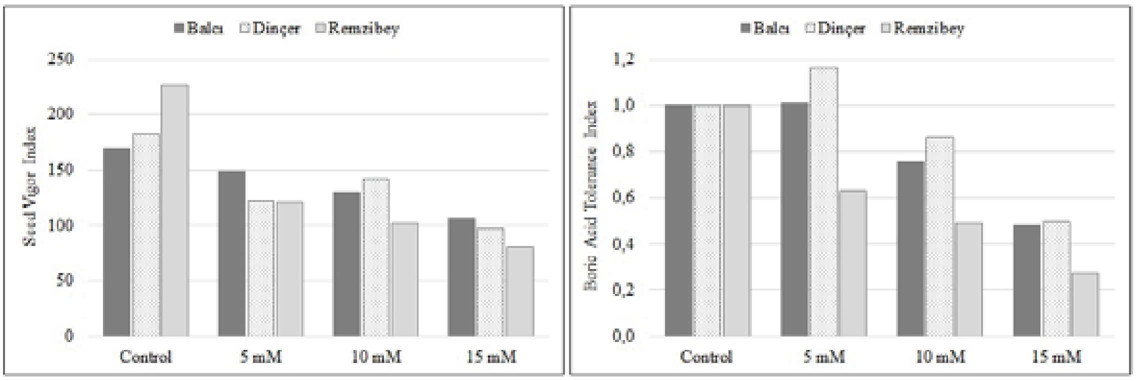

FIGURE 4
Seed vigor index and boric acid tolerance index of safflower varieties applied boric acid

In our study, it was determined that the seed vigor index decreased in all varieties in comparison to the control group due to the increasing boric acid concentrations (Figure 4). Among the varieties, the most affected variety by boron stress was Remzibey, while the least affected variety was Balcı. However, it was determined that the seed vigor index was increased in the application of 10 mM boric acid (142) in the Dinçer variety with respect to 5 and 15 mM (respectively 122 and 98). The highest and lowest seed vigor index values were determined in the control group and 15 mM boric acid in the Remzibey variety (respectively 227 and 80) (Figure 4). When the boric acid tolerance indices of the safflower varieties were calculated, it was determined that the highest boric acid tolerance (1.17) was at 10 mM boric acid in the Balcı variety, and the lowest boric acid tolerance (0.27) was at 15 mM boric acid application in Remzibey. The tolerance index of the 10 mM boric acid concentration in the Balcı variety (1.17) was found to be higher than that of the control group (1). When the concentrations were compared, it was determined that the tolerance index was the highest at 10 mM of boric acid application for all varieties. (Figure 4).

As seen in Figure 5, while the amount of chlorophyll a, b and total chlorophyll decreased at 5 mM compared to the control group in the Dinçer variety, a value more than the control was determined at the 10 and 15 mM boric acid applications. The amount of chlorophyll a, b and total chlorophyll in the Remzibey cultivar decreased compared to the control at 5 and 15 mM boric acid applications, and it was found to be higher than the control at 10 mM. Likewise, the amount of chlorophyll a, b and total chlorophyll was lower than the control at all three boric acid concentrations in the Balcı variety, while the chlorophyll a, b and total chlorophyll content at the 10 mM boric acid concentration was lower than the control but higher than the other concentrations. One of the most striking results in this study was that the amount of chlorophyll in the application of 10 mM of boric acid in Dinçer and Remzibey was higher than the control, whereas it was lower than the control in the Balcı variety but higher than those at the other two concentrations. Thus, there was a relationship between the application of 10 mM boric acid and the amount of chlorophyll (Figure 5).

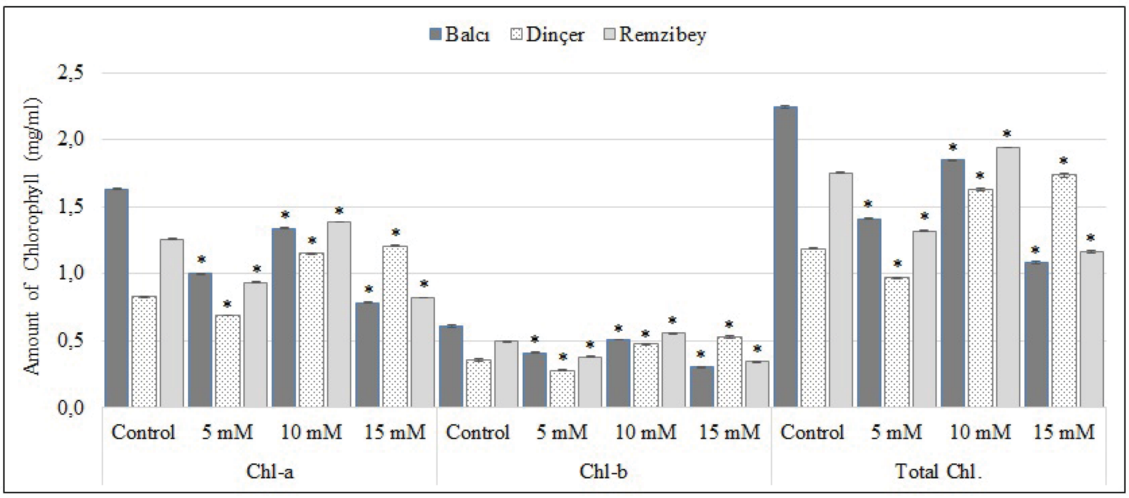

FIGURE 5
Amount of chlorophyll a, b and total chlorophyll of safflower varieties applied boric acid (*p<0.05)

DISCUSSION

Seed germination, which is one of the most critical events in the life cycle of a plant, is very sensitive to stress factors [20, 21]. The effect of boron stress at the germination phase shows variation between varieties of the safflower plant. In the Balcı and Remzibey varieties, as the boron concentration increased, the germination rate decreased in comparison to the control group, whereas, in the Dinçer variety, this decrease was observed only at the highest boron concentration (15 mM, Table 1). This situation may be explained by the fact that the resistance of plants against boron stress shows a change according to plant species and varieties [22]. The results obtained from the study were consistent with the literature on safflower germination with boric acid application [23-25]. It was also stated that low concentrations of boron increased germination [26- 28]. In the case of our study, it is possible to explain the low germination percentage of the seeds in the Balcı and Remzibey varieties by the influence on the seed water intake of boron and by its negative effect on the seed's metabolism required to remove the sleeping state.

The decrease in root lengths may be due to the fact that an excessive amount of boron has a toxic effect on plant roots [10, 29]. This situation prevents root development. This is because boron toxicity creates a decrease in the mitotic activity of root end meristem cells, and this decrease is known to reduce the rate of root elongation by affecting cell division [30]. Choi et al. (2007) [31] stated that boron toxicity decreases sugar intake at the root, which in turn causes the osmotic pressure to change, thereby preventing cell growth and root prolongation. In our study, according to the stem length data, the variety least affected by the application of boric acid was Balcı, while the most affected variety was Remzibey. According to the results obtained from the study and information of the literature, the cause of the differences in the root and stem lengths among the varieties may have been different amounts of boron accumulation in the cells and tissues of different safflower varieties [11]. Due to the decrease in root elongation, it is considered that the underdeveloped roots may have negative effects on body development and cause formation of plants with shorter stem [10, 23]. In our study, when the stem lengths of the safflower varieties were compared, it was determined that, as the concentration of boric acid in all varieties increased, there was a statistically significant decrease in the stem length compared to the control group (Figure 4.1, $p<0.05$).

Yaman et al. (2012) [23], in their study with four different safflower varieties (Yenice, Shifa, Dinçer and Remzibey) and ten different boron doses (0, 7.5, 15, 22.5, 30, 37.5, 45, 60, 75 and 90 ppm), stated that increasing boron doses increase the root and stem length of the safflower up to a certain level, but the root and body length decreases after a certain dose. In the same study, it was reported there were differences in the root and stem lengths among the varieties. Campbell et al. (1988) [32] determined that boron application, which was increased, decreased root and body development. A high amount of boron has a physiological effect on plants, and boron reduces root and body growth by preventing the intake of some nutrients [33].

In a study with wheat, it was determined that the root fresh and dry weight decreased significantly when the boron concentration increased above 0.5 mg/L [34]. In boron applications above 0.25 mg/L, as the boron concentration increased, the root fresh and dry weights decreased significantly. In also studies on other plants, it was reported that, as the boron concentration increases, root and stem fresh and dry weights decrease [26, 35, 36].

The decrease in the root-stem fresh and dry weight due to increasing boric acid concentrations in the safflower varieties may be explained by the decrease in the intake of necessary nutrients due to limiting of root development by excess boric acid, and consequently, slowing of development [23]. The boron requirement of each plant is different, and the tolerance limits of the element boron are very variable. The difference between the varieties in terms of the root-stem wet and dry weights of the safflower varieties used in our study depends on this variability. Since the element boron is associated with cell elongation and meristematic growth in a plant, it may be thought that high concentrations of boron may cause negative effects on cells and hinder cell proliferation and growth [37]. In this case, the root and shoot cannot grow in the plant, and as the boron concentration increases, the root-body age and dry weight decrease.

In the study, it was noteworthy that the Balcı root and stem biomass and Dinçer stem biomass were higher in the application of 10 mM of boric acid than the 5 mM and 15 mM boric acid concentrations. Biomass is the mass of biological material that occurs when green plants transform and convert solar energy into chemical energy with photosynthesis [38]. Boron application inhibits stem cell division and cell elongation in plants, thus preventing root growth [39]. The root development of the safflower plant is adversely affected by boron, and boron prevents the transport of sufficient nutrients and water to the stem. In this case, as the photosynthesis in the stem also slows down, there is not enough nutrients to be stored, and the stem fresh and dry weights are reduced [30]. Since stem biomass has a direct relationship to root weight, it may be affected by boron stress, and the stem biomass decreases as the concentration increases. As this decline in root development directly affects root biomass, root biomass may also have decreased.

Since the seed vigor index is related to the germination and root-stem length of a plant, the decrease in these two properties negatively affects the seed vigor index. Boron affects cell proliferation, cell division and meristematic growth in plants [40, 41]. Yaman et al. (2012) [23] found that, in their study with aspir, when the boron level was above a certain degree, the seed vigor index of all varieties decreased, and there were differences between the varieties. Similarly, Mirshekari (2012) [42] showed in their study on wheat that, as the boron concentration increases, the seed vigor index decreases. The seed vigor index is an important factor for early development of plants and rapid resistance formation [43].

The boric acid tolerance index is a parameter that shows the resistance of a plant to boric acid. The tolerance of plants against any ecological factor varies from species to species or even in varieties of the same species. Ashagre et al. (2014a) [19] reported that wheat tolerance index increased at low boron levels, and the boron tolerance index decreased as the boron concentration increased. Similarly, Shaikh et al. (2013) [44] found that the tolerance index decreases as the boron concentration increases in their studies on wheat. The boric acid tolerance index was consistent with the other ecological results of the study. While the variety that tolerated boric acid most was Balcı, it was found that Remzibey had the lowest tolerance.

It is known that boron has an effect on chlorophyll-related metabolic events in plants and changes in the chlorophyll content and photosynthesis in leaves exposed to boron stress [33]. The exact amount of boron required for a plant may be toxic or inadequate for other plants [45]. Some plant species and varieties of these species have developed a tolerance mechanism that is still unknown against boron. Oluk et al. (2006) [46], in their study on sunflower, reported that the amount of chlorophyll decreased in boron deficiency but increased in boron excess. It is known that the membrane structure of chloroplasts is deteriorated, and the proteins and enzymes responsible for chlorophyll synthesis are inhibited under boron stress. It has been reported in the literature that this situation causes imbalance in the PS II complex protein, and it consequently decreases both chlorophyll a, b and total chlorophyll synthesis in photosynthetic structures [47]. In another study, another reason for reduction of chlorophyll content under boron stress was explained by the increase in the activity of the chlorophyllase enzyme under stress conditions [48, 49]. Many studies in the literature supported these results [50, 51]. However, in this study, it is difficult to explain the reason for the increase in the amount of chlorophyll at increasing boric acid concentrations in the Balcı variety. The effects of different levels of boric acid on varieties may be explained by the differences in the anatomical structures of the varieties, differences in secretory cells, differences in the tolerances of varieties to boric acid and differences in genomes [52].

CONCLUSIONS

In this study, changes in ecophysiological parameters were investigated by applying three different boric acid concentrations on three safflower varieties. It was determined that high boric acid concentrations caused a decrease in germination, root-stem length, root fresh-dry weight, stem fresh-dry weight, root-stem biomass and seed vigor index parameters in the safflower varieties, and it was determined that boric acid excess affected the early growth phase negatively. According to the boric acid tolerance index, it was determined that the most sensitive variety against boron was Remzibey, and the most tolerant variety was Balcı. The Dinçer variety was closer to the Balcı variety in terms of its tolerance to boron. The fact that the results of the boric acid tolerance index were in parallel with the results of the growth parameters showed that these properties may play a role in determining varieties resistant to boron. It was determined that the chlorophyll a, b and total chlorophyll results were connected with the 10 mM boron application, and it was observed that the amount of chlorophyll a, b and total chlorophyll decreased at different boric acid concentrations in the Dinçer and Remzibey varieties in comparison to the control group. This study, which reveals changes in safflower germination and in early developmental stage due to the varying amounts of boron, will contribute to abiotic stress studies which reveal the safflower-boron relationship.

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