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A preliminary study on nickel tolerance of some barley genotypes

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ABSTRACT. In this study, nickel tolerance ($NiSO_4.7H_2O$) of some Turkish national barley (*Hordeum vulgare* L.) genotypes (Bülbül-89, Kalaycı-97, Karatay-94, Larende, Tarm-92, Tokak-157/37, Yesevi-93 and Zeynel Ağa) was investigated. Barley genotypes were exposed to different nickel concentrations [0 mM (control), 250, 500, 1000, 1500, and 2000 ppm]. Nickel toxicity significantly inhibited root and coleoptile growth in all barley genotypes in a concentration-dependent manner. However, root growth was much more inhibited by nickel applications in comparison with coleoptile growth, probably due to a higher level of sensitivity of root meristems against nickel toxicity or direct contact of roots with nickel ions in the growth medium. Root growth in the genotype Karatay-94 and coleoptile growth in the genotype Yesevi-93 was more remarkably reduced by nickel toxicity. Root and coleoptile growth in the genotypes Larende and Kalaycı-97 were less affected under nickel toxicity, respectively. In addition, nickel toxicity disturbed water relations in barley genotypes dependent on the organ type, as demonstrated by more severe inhibition in root fresh weight as compared to coleoptile fresh weight. These results could show that nickel toxicity reduced water uptake from growth medium in barley genotypes used in this study. Changes in dry weight of roots and coleoptiles indicated that nickel toxicity more severely decreased biomass accumulation in roots of barley genotypes. The calculated tolerance indices demonstrated that the genotype Kalaycı-97 is the most tolerant to nickel toxicity, while the genotype Karatay-94 is the most susceptible one.

Keywords: Barley; *Hordeum vulgare*; nickel tolerance; nickel toxicity; tolerance índices.

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Introduction

It has been reported that agricultural areas in the world are contaminated by heavy metals such as Cd, Zn, Cu, Ni, Pb, As, Cr, and Co (Huang et al., 2019). Some of these heavy metals, such as Ni, Cu, Co, and Zn are essential for plant growth and development (Doğru, 2020). Cd, Cr, Pb, and As, on the other hand, are not essential and do not fulfill any metabolic function in plants. Nickel (Ni) is the latest element considered essential for plant growth and development because of being a component of the enzyme urease (Deng et al., 2018). It forms about 0.008% of the earth's crust, 24th the most abundant element (Hedfi, Mahmoudi, Boufahja, Beyrem, & Aissa, 2007). Naturally, nickel is present in soils in the range of 3-100 ppm and water 0-0.005 ppm, respectively (Sachan & Lal, 2017). Although nickel is an essential microelement, a very low level of nickel is required by plants, being enough 0.1 ppm or lower-level concentration to support seed germination and plant growth (Deng et al., 2018). Therefore, nickel deficiency in agricultural soils rarely occurs. By contrast, the nickel concentration in soils may reach toxic levels due to industrial, natural, and anthropogenic activities. Nickel toxicity has been known to affect seed germination, seedling growth, biomass accumulation, photosynthetic pigment content, protein synthesis, photosynthesis, nitrogen metabolism, nutrient absorption, transpiration, and lead to oxidative stress in plants (Hasinur, Shamima, Shah, & Shigenao, 2005; Gajewska, Sklodowska, Slaba, & Mazur, 2006).

Seed germination is a crucial developmental stage determining a healthy crop establishment, vigour growth, and higher yield (Pavlova, Vila, Vila, Bani, & Xhaferri, 2018). It has been indicated that seed germination could be inhibited by higher nickel concentrations (Sachan & Lal, 2017). In addition, Shweti and Verma (2018) have declared that increasing levels of nickel decreased germination percentage, root and shoot growth, and biomass accumulation in wheat genotypes because of impairment of both digestion and

mobilization of seed reserves. It is rarely possible to remove heavy metal toxicity from natural environments. The strategies of plant breeding and genetic engineering are long-term and complex processes to develop heavy metal tolerance. Under these circumstances, an alternative way to increase yield potential under nickel toxicity could be to determine plant genotypes naturally tolerant to excess nickel. The most important cereal species providing the nutrition requirements for human health belong to the family Poaceae, such as rice, maize, wheat, barley, and sorghum etc. Among cereals, barley is the fourth largest crop after wheat, maize and, rice in the world and the second crop after wheat in Turkey (Baik & Ullrich, 2008). Thus, the objective of this study is to investigate the tolerance/sensitivity degrees to nickel toxicity of some national Turkish barley genotypes through some germination and growth parameters.

Material and methods

Plant materials, growth conditions and experimental design

In this study, eight different barley (*Hordeum vulgare* L.) genotypes (Bülbül-89, Kalaycı-97, Karatay-94, Larende, Tarm-92, Tokak-157/37, Yesevi-93, and Zeynel Ağa) were used as plant material. The seeds were obtained from the Republic of Turkey Ministry of Agriculture and Forestry, Field Crops Central Research Institute, Ankara, Turkey. The treatments were factorial combinations of eight cultivars and six nickel ($NiSO_4.7H_2O$) levels (0, 250, 500, 1000, 1500, and 2000 ppm). The experimental design was a randomized complete block design with three replications per treatment. After sterilization in 5% sodium hypochlorite for 10 min., the seeds were washed with bi-distilled water and placed between filter papers in the Petri dishes. The filter papers were moistened with the appropriate solutions or bi-distilled water for 0 ppm nickel (control). In both cases, the total volumes of the appropriate solutions and bi-distilled water were 7 mL. Twenty-five seeds per dish were used for each treatment. The Petri dishes were put in the growth chamber in the dark at 25/20°C day/night temperature and 40-45 % relative humidity for 5 days.

Determination of growth and germination parameters

Measurement of root and coleoptile length was done with a millimetric ruler. The longest root was taken into consideration for measurement. Root and coleoptile length was expressed as cm $plant^{-1}$. After harvesting, barley seedlings were weighed for fresh weight (FW) determination. The dry weight (DW) of plants was measured after drying in a hot-air oven at 70ºC for 2 days. The tolerance indices (TI) were calculated on the basis of root length (RL) and shoot length (SL) (Bağcı, Ekiz, & Yılmaz, 2003; Ayhan, Ekmekçi, & Tanyolaç, 2007; Doğru & Kaçar, 2009). According to the calculated TI for each nickel treatment, eight different barley genotypes were scored between one and eight. One score was given to the genotype with minimum TI, while eight scores was given to the genotype with maximum TI. Then, the scores obtained for each nickel concentration for each genotype were added to obtain the total score. The amount of utilized (mobilized) seed reserve and conversion efficiency were calculated according to Soltani, Gholipoor, and Zeinali (2006).

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS 22.0 statistical software for Windows. To separate significant differences between means, LSD (least significant difference) test was used at $p = 0.05$.

Results

In the present study, nickel treatments reduced the root growth of all barley genotypes tested compared to the respective controls (Figure 1a). For example, 250 ppm nickel application led to the 17% reduction in the root length in the genotype Larende as compared to control, while the root growth of the genotype Bülbül-89 was more severely affected by 250 ppm nickel toxicity with a 49% lower than control (Figure 1a). In comparison with control, the root length of the genotypes Larende and Karatay-94 was decreased by 44% and 70%, and 75% and 86% as a result of 500 and 1000 ppm nickel toxicity, respectively (Figure 1a). In the genotype Kalaycı-97, root length was declined by 1500 and 2000 ppm nickel applications by 84% and 90%, respectively. As compared to the respective controls, 1500 ppm nickel toxicity resulted in a 91% decrease in the root length of the genotype Bülbül-89 and 2000 ppm nickel toxicity led to a 94% decline in the root length of the genotype Zeynel Ağa, respectively (Figure 1a).

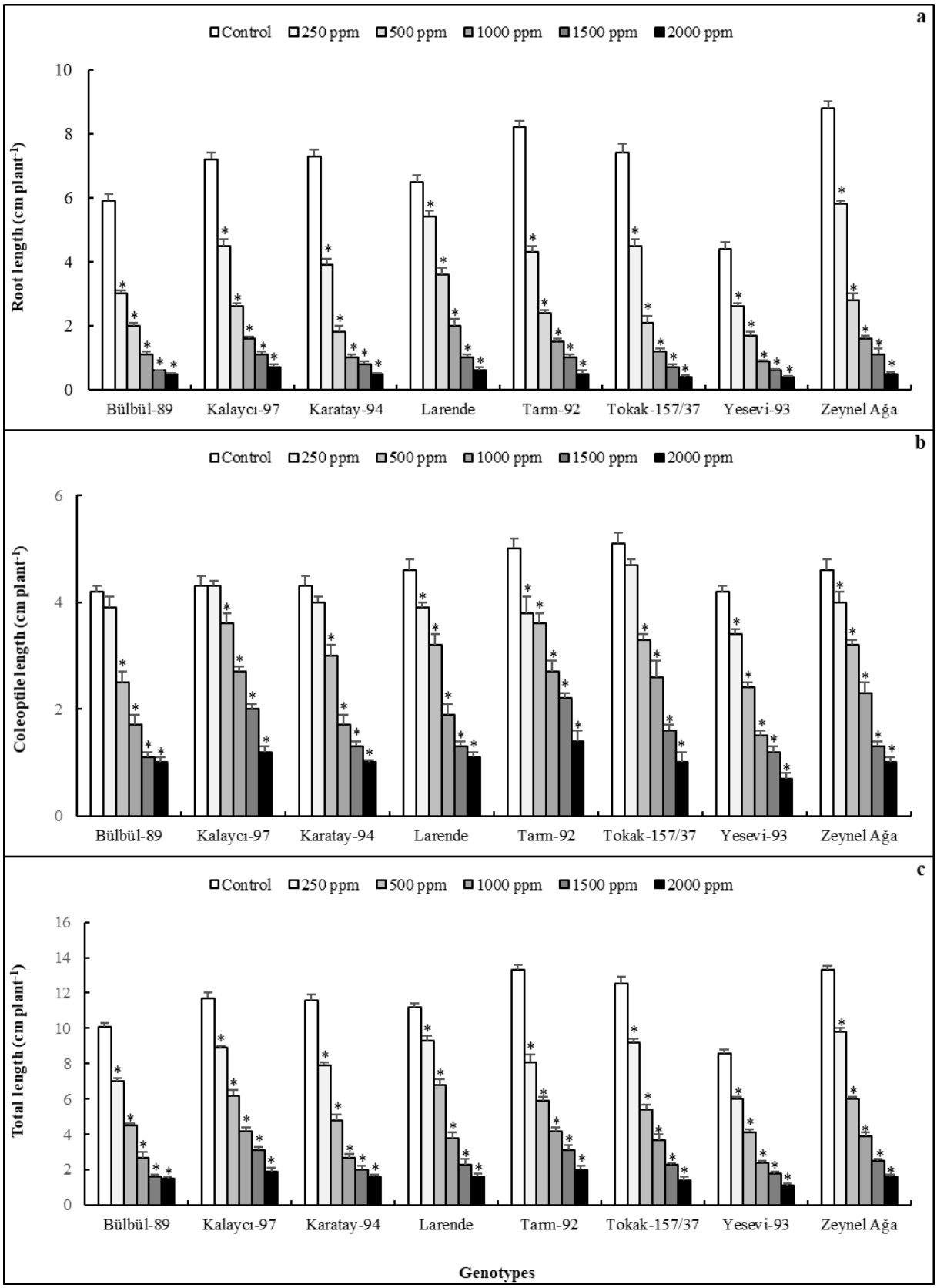

Figure 1. The effect of nickel toxicity on (a) root length, (b) coleoptile length, and (c) total length in barley genotypes [Significant differences from controls ($p < 0.05$) are marked with an asterisk. Abbreviation and statistical evaluation are the same for the following figures].

Similar to root growth, nickel concentrations applied to barley genotypes remarkably reduced coleoptile length in a dose-dependent manner under 250 ppm nickel toxicity, except Bülbül-89, Kalaycı-97, Karatay-94 and Tokak-157/37 (Figure 1b). In the genotype Kalaycı-97, coleoptile growth was considerably declined by 250, 500, 1000, and 1500 ppm nickel treatments by 3%, 21%, 40% and 56%, respectively (Figure 1b). The

coleoptile length of the genotype Tarm-92 was decreased by 250 ppm nickel application by 25% compared to control. Both 500 and 1000 ppm nickel applications led to 42% and 65% reduced coleoptile length in the genotype Yesevi-93 in comparison with controls (Figure 1b). In the genotype Bülbül-89, the coleoptile length was 75% lower than the control due to 1500 ppm nickel toxicity. In the genotypes Tarm-92 and Yesevi-93, the coleoptile length was 72% and 84% lower than control plants as a result of 2000 ppm nickel treatment, respectively.

Root fresh and dry weight of barley genotypes used in this study showed significant decreases in response to nickel applications compared to the respective controls (Figures 2a and b). At 250 ppm nickel treatment, for instance, led to 22% in the genotype Larende and 64% in the genotype Tokak-157/37 lower fresh weight than controls. The fresh weight of the genotype Karatay-94 and Tokak-157/37 under 500 ppm nickel toxicity was 56% and 79% lower than the respective controls, respectively (Figure 2a). 1000 ppm nickel treatment resulted in a decline in the fresh weight of the genotype Karatay-94 (82%) and the genotype Tarm-92 (%89). The fresh weight of the genotypes Kalaycı-97 and Tarm-92 was remarkably changed by 1500 ppm nickel, with a reduction of 87% and 92% compared to controls, respectively (Figure 2a). When compared to the respective controls, the root fresh weight of the genotype Kalaycı-97 was 91% lower while it was reduced in the genotype Zeynel Ağa by 96% by 2000 ppm nickel application (Figure 2a). The genotype Zeynel Ağa represented a 12% lower root dry weight as compared to control, while the genotype Kalaycı-97 showed 45% lower root dry weight under 250 ppm nickel toxicity (Figure 2b). Compared to the respective controls, the root dry weight was remarkably decreased by 500 ppm nickel application by 29% and 61% in the genotypes Zeynel Ağa and Tarm-92, respectively. At 1000 ppm nickel toxicity, it was found that the root dry weight of the genotypes Karatay-94 and Zeynel Ağa was decreased by 72% and 54%, respectively (Figure 2b). The root dry weight of the genotype Kalaycı-97 was declined by 70% due to 1500 ppm nickel treatment, while it was decreased by 88% in the genotype Karatay-94 (Figure 2a). 2000 ppm nickel application led to 80% lower root dry weight in the genotype Kalaycı-97 and 93% lower root dry weight in the genotype Zeynel Ağa, respectively (Figure 2b).

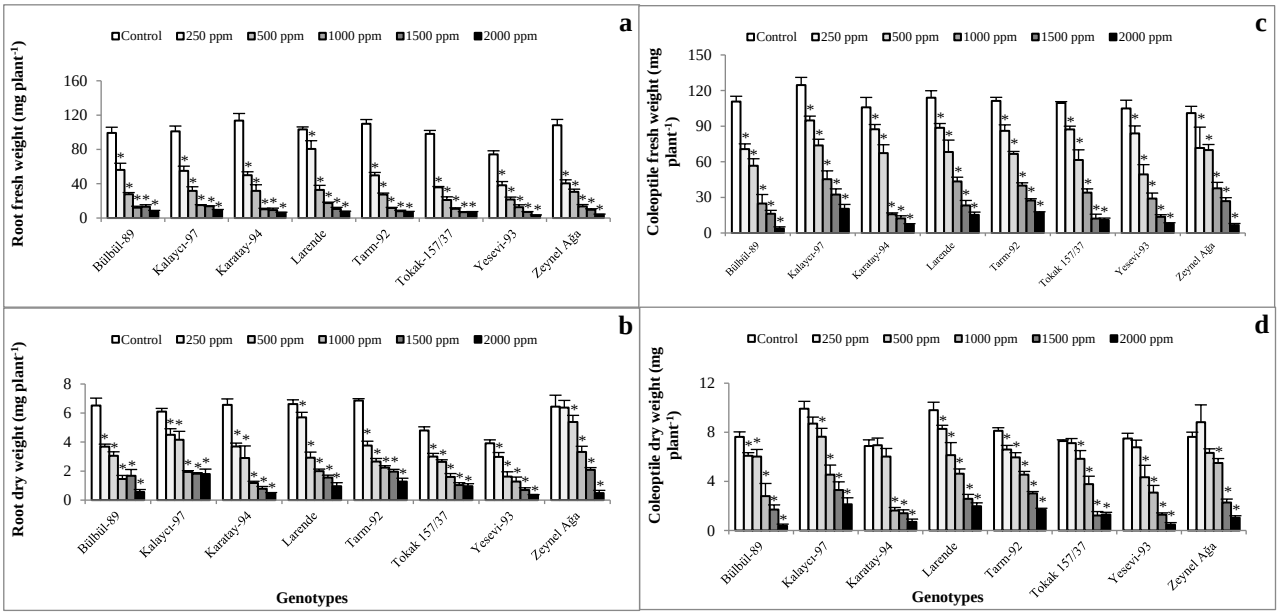

Figure 2. The effect of nickel toxicity on (a) root fresh weight, (b) root dry weight, (c) coleoptile fresh weight, and (d) coleoptile dry weight in barley genotypes.

The coleoptile fresh weight of the genotypes Karatay-94 and Bülbül-89 was significantly decreased by 17% and 36% in response to 250 ppm nickel application, respectively (Figure 2c). 500 ppm nickel treatment led to a 31% decrease in the genotype Zeynel Ağa and a 53% decrease in the genotype Yesevi-93 compared to the respective controls (Figure 2c). At 1000 ppm nickel concentration, the genotype Larende showed 62%, and the genotype Karatay-94 showed 75% decline in coleoptile fresh weight, respectively. 1500 ppm nickel toxicity led to a 73% decrease in the coleoptile fresh weight in the genotype Zeynel Ağa as compared to control. Similarly, the coleoptile fresh weight of Karatay-94 was 88% lower than the respective control (Figure 2c). In the genotypes Kalaycı-97 and Zeynel Ağa, the fresh weight of coleoptile was declined, with a reduction of 84% and 93%, respectively. Compared to respective controls, 250 and 500 ppm nickel treatments led to 8% and 14% decreased coleoptile dry weight in the genotype Tokak-157/37 (Figure 2d). The dry weight of coleoptile

in the genotype Zeynel Ağa decreased by 28% in response to 1000 ppm nickel application (Figure 2d). Similarly, the same nickel concentration led to 72% lower coleoptile dry weight in the genotype Karatay-94 as compared to control. At 1500 ppm nickel concentration, the genotype Tarm-92 showed a 63% decrease and the genotype Yesevi-93 showed a 83% decrease in the dry weight of coleoptile in comparison with control (Figure 2d). The dry weight of coleoptile in the genotype Tarm-92 was declined by 2000 ppm nickel concentration by 72%, and it was decreased in the genotype Yesevi-93 by 84% as compared to the respective controls (Figure 2d).

250 ppm nickel treatment did not significantly affect the amount of utilized seed reserve in the genotypes Bülbül-89, Karatay-94, Tokak-157/37, Yesevi-93, and Zeynel Ağa (Figure 3a). In the genotypes Kalaycı-97, Larende and Tarm-92, however, it was remarkably decreased by 250 ppm nickel application compared to controls (Figure 3a). At 500 ppm nickel concentration, the genotype Yesevi-93 showed a 20% decrease, and the genotype Larende showed 46% decrease in the utilized seed reserve content compared to the respective controls. Compared to the respective controls, the utilized seed reserve content in the genotype Larende was declined by 1000, 1500, and 2000 ppm nickel concentrations by 59, 63 and 70%, respectively. The seed reserve content in the genotype Tarm-92 decreased by 27% in response to 1000 ppm nickel concentration (Figure 3a). At 1500 ppm nickel treatment, a 35% decreased content of the utilized seed reserve was observed in the genotype of Bülbül-89 as compared to control. The genotype Zeynel Ağa represented a 37% decline in the content of the utilized seed reserve as a result of 2000 ppm nickel application compared to control (Figure 3a). The conversion efficiency of the genotypes Bülbül-89, Karatay-94, Tarm-92, and Yesevi-93 was not affected by 250, 500, and 1000 ppm nickel concentrations compared to the respective controls (Figure 3b). Similarly, the conversion efficiency in the genotypes Larende, Tokak-157/37, and Zeynel Ağa were not changed by 250, 500, 1000 and 1500 ppm nickel toxicity (Figure 3b). All nickel concentrations used in this study were found to be ineffective on the conversion efficiency in the genotype Kalaycı-97 as compared to control.

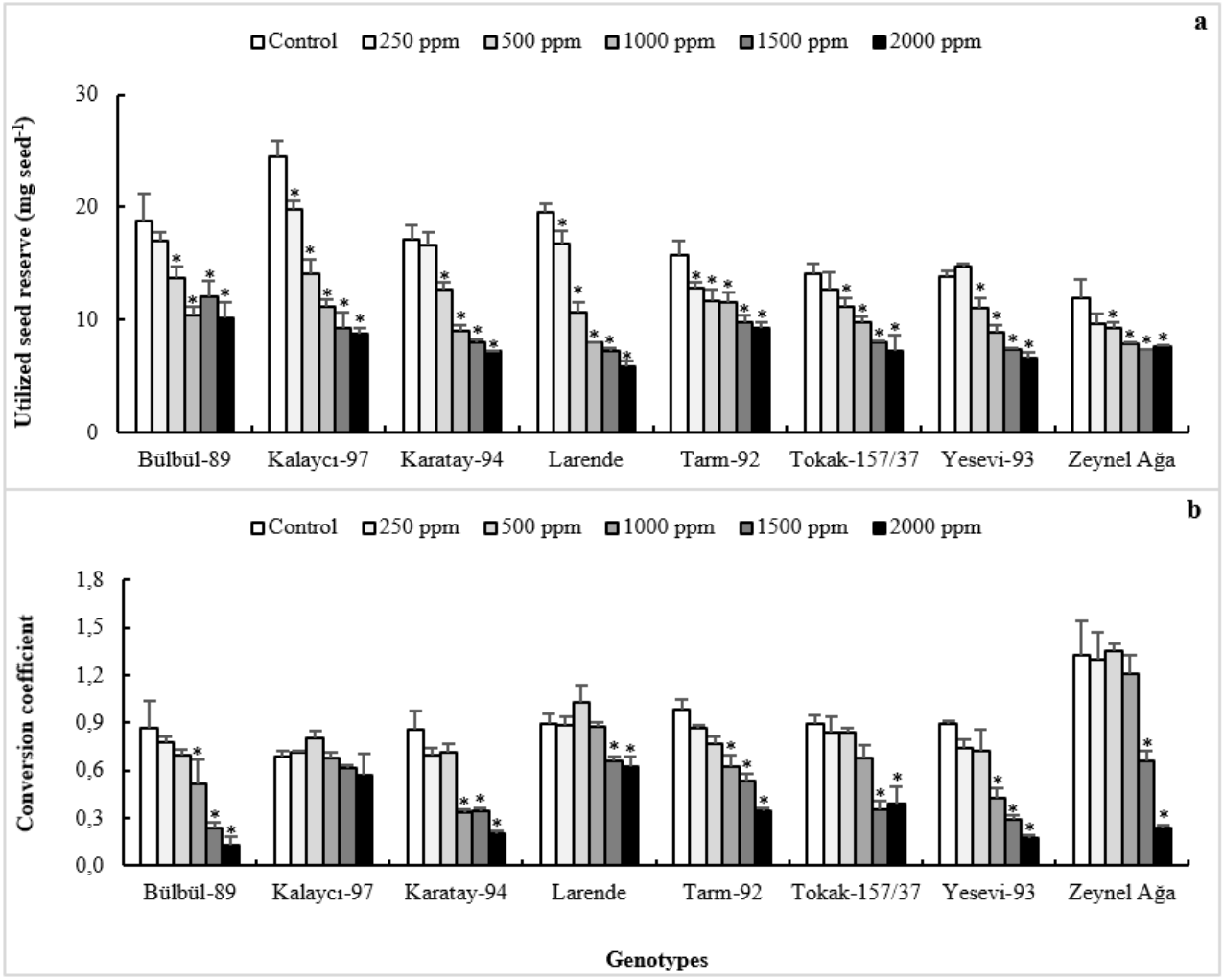

Figure 3. The effect of nickel toxicity on (a) utilized seed reserve, and (b) conversion coefficient in barley genotypes.

According to the tolerance indices (TI) calculated on the basis of root length, the genotype Karatay-94 had the lowest score (11) among all barley genotypes used in our study (Table 1). As for TI calculated on the basis of coleoptile length, the lowest score (7) was observed in the genotype Yesevi-93. On the contrary, the genotype Larende represented the highest score of the root TI (38), whereas the highest score of the coleoptile TI (39) was observed in the genotype Kalaycı-97. According to the total TI, however, the genotypes Kalaycı-97 and Karatay-94 had the scores 74 and 33, respectively.

Table 1. Tolerance indices and total scores of the barley genotypes.

Genotypes	Root					Coleoptile					Total score
	Tolerance indices (%)					Tolerance indices (%)					
	250 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm	250 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm	
Bülbül-89	51.4 (1)*	34.2 (5)	18.1 (4)	9.3 (1)	8.5 (6)	93.1 (7)	59.1 (2)	39.4 (3)	25.4 (1)	24.5 (6)	36**
Kalaycı-97	63.1 (6)	36.4 (6)	21.3 (7)	15.7 (8)	9.6 (8)	96.7 (8)	79.2 (8)	59.6 (8)	44.2 (8)	26.0 (7)	74
Karatay-94	54.1 (3)	25 (1)	14.3 (1)	10.6 (3)	7.3 (3)	91.4 (5)	69.7 (5)	38.4 (2)	29.4 (5)	23.6 (5)	33
Larende	82.8 (8)	55.7 (8)	30.0 (8)	15.5 (7)	8.6 (7)	84.0 (3)	68.8 (4)	40.5 (4)	28.1 (3)	22.7 (3)	55
Tarm-92	51.8 (2)	28.9 (3)	18.2 (5)	11.5 (4)	7.4 (4)	75.2 (1)	70.4 (7)	53.4 (7)	42.7 (7)	28.2 (8)	48
Tokak 157/37	60.7 (5)	28.8 (2)	15.7 (2)	9.6 (2)	6.0 (2)	92.4 (6)	64.7 (3)	50.0 (5)	30.4 (6)	18.6 (2)	35
Yesevi-92	58.1 (4)	37.3 (7)	21.3 (6)	14.0 (6)	8.1 (5)	81.5 (2)	57.6 (1)	35.3 (1)	27.3 (2)	16.4 (1)	35
Zeynel Ağa	65.8 (7)	31.4 (4)	17.9 (3)	12.7 (5)	5.8 (1)	87.3 (4)	70.2 (6)	50.4 (6)	29.2 (4)	22.8 (4)	44

*Values in the parenthesis indicates the scores given according to tolerance índices. **The highest score indicates the lowest damage and the lowest score indicates the highest damage.

Discussion

It has been well documented that excess nickel concentrations inhibit early seedling growth in plants (Ashraf, Sadiq, Hussain, Ashraf, & Ahmad, 2011; Sethy & Ghosh, 2013; Shweti & Verma, 2018; Doğru, Altundağ, & Dündar, 2021a; Doğru, Altundağ, & Dündar, 2021b). In the present study, root and coleoptile growth and fresh and dry weights of roots and coleoptiles of barley genotypes were partially reduced by nickel concentrations. In addition, these reductions were entirely concentration-dependent. And it has been observed that there is a great variation between barley genotypes under nickel toxicity in terms of root and coleoptile length. Khan et al. (2020) have shown that nickel uptake is accelerated with increasing nickel concentration applied in rice plants. Previous studies have also indicated that heavy metal accumulation capacity may differ between plant species and genotypes (Krstic, Stankovic, Igic, & Nikolic, 2007; Khan et al., 2020). Therefore, the genotypes Kalaycı-97 and Larende, which generally represent a higher growth rate in roots under nickel toxicity, may be accepted to accumulate less amount of nickel in their roots. It is also possible that the genotypes Kalaycı-97 and Larende may excrete organic acid to the growth medium, thus preventing nickel ions from entering plants (Sachan & Lal, 2017). Our results showed that root growth of barley genotypes was much more inhibited than coleoptile growth, probably due to direct contact of roots with nickel ions in the growth medium and inhibition of root respiration (Khan et al., 2020). This result is in accordance with the previous studies (Seregin, Kozhevnikova, Kazyumina, & Ivanov, 2003; Shweti & Verma, 2018). In the genotypes Kalaycı-97 and Tarm-92, with the higher coleoptile growth rate under nickel toxicity, translocation of nickel ions to the leaves may mostly be limited as a protective mechanism. In addition, the poorly developed root systems in barley plants under nickel toxicity may restrict the capacity of the seedlings to absorb nutrients and hamper further seedling growth. Ahmad, Hussain, Ashraf, Ahmad, and Ashraf (2009) have reported that nickel is an active competitor of a number of essential macro- and micronutrients concerning their uptake and distribution by plant roots. It has been reported that Ni toxicity led to the damage in the cell nucleus and nucleolus in the root tips of tomato. Similarly, Shi and Cai (2009) have explored that excessive heavy metal treatments change the plasticity of the cell wall, which consequently impair cell division and/or elongation. Thus, nickel toxicity may be responsible for the decreased root and coleoptile growth rate by interfering with mitotic activity in our study (Gautam, Rathoure, Chhabra, & Pandey, 2017). It has been reported that nickel toxicity alters all energy-required processes in plant cells during germination and consequently leads to decreased radicle and plumule growth (Singh, Mall, & Singh, 2006; Talukdar, 2011). One of the explanatory reasons for these results may be the accumulation of photoassimilates in the seeds as a result of the reduced starch hydrolysis and sucrose transport as reported earlier (Roitto, Rautio, Julkunen-Tiito, Kukkola, & Huttunen, 2005). In addition, the reduced root length has been found to be responsible for the reduced total plant length in the present study.

As for biomass accumulation, fresh and dry weights of roots and coleoptiles of barley seedlings were reduced by nickel applications. Also, these reductions were proportional to nickel concentrations. Fresh and dry weights of roots and coleoptiles of barley seedlings were decreased as nickel concentrations in the growth medium increased. Similar to root and coleoptile length, root fresh and dry weights were more severely affected by nickel toxicity in our study. This result indicates that nickel toxicity inhibited root metabolism more severely than coleoptiles in barley genotypes used in this study. Siddiqui, Al-Whaibi, and Basalah (2011) and Ashraf et al. (2011) have declared that higher nickel concentrations reduced fresh and dry weight in wheat and sunflower plants, respectively. Doğru and Kaçar (2019) have reported that a lower level of root fresh weight indicates severe impairment of water uptake from growth medium. Therefore, changes in root and coleoptile fresh weights clearly indicated that nickel toxicity affected water relations between barley plants and growth medium, depending on the nickel concentration, organ type, and genotype. It has been well known that heavy metal toxicity affects various plant processes, including osmotic homeostasis in plant cells (Sethy & Ghosh, 2013). Ahmad et al. (2009) have stated that Ni toxicity-induced growth inhibition in plants could be ascribed to the down-regulation of protein synthesis and activities of some key enzymes responsible for mobilization of food reserves in seeds. In germinating seeds, various glycolytic and proteolytic enzymes are produced to control the rate of seed germination and seedling growth under different environmental conditions. α-amylase, for example, is the most important hydrolytic enzyme and is involved in starch hydrolysis to produce soluble sugars, which in turn are readily oxidized in cellular respiration to yield energy for radicle and shoot growth (Ashraf, Naveed, Ashraf, & Akram, 2009). The yielding soluble sugars also play an important role in cellular osmotic adjustment in germinating seeds and at the early seedling growth stage. Proteases, on the other hand, are responsible for the hydrolysis of stored seed proteins to free amino acids. These amino acids are then used for the synthesis of proteins and enzymes required for growth and development (Cantón, Suárez, & Cánovas, 2005). In the present study, the seed reserves' utilization in barley genotypes was decreased as the nickel concentration increased. Conversion coefficient in barley genotypes, however, was reduced only at higher nickel concentrations, except the genotype Kalaycı-97. These results indicated that hydrolytic and proteolytic reactions slowed down in barley seeds due to nickel toxicity. It is probable that curtailed food mobilization and utilization restricted the growth of barley seedlings at the early stage in this study. Ashraf et al. (2011) have reported that α-amylase and protease activity were decreased by nickel toxicity in sunflower plants. So, the decreased level of utilization of seed reserves and conversion coefficient could be due to the decreased activities of hydrolytic and proteolytic enzymes in our study.

Schützendübel et al. (2001) have stated that the most sensitive and rapid physiological response in plants to cadmium toxicity is the changes in root length. However, different plant species and even genotypes of the same species generally represent variation concerning the uptake rate and translocation of metals. For this reason, both root and coleoptile scores of tolerance indices (TI) were considered to determine the tolerance/sensitivity degree of barley genotypes tested in this study. The genotypes Karatay-94 and Yesevi-93 had the lowest scores of TI (11 and 7) calculated on the basis of root and coleoptile length, respectively. This result indicates that root and shoot meristems are highly sensitive to nickel toxicity, and they represented the highest nickel toxicity-induced damage. Growth of root and coleoptile of the genotypes Larende and Kalaycı-97, on the other hand, was less affected by nickel toxicity, with scores of 38 and 39, respectively.

Conclusion

To sum up, our results showed that root and coleoptile lengths and fresh and dry weights of barley genotypes were partially reduced and decreased in a concentration-dependent manner. In addition, utilization of seed reserves and conversion coefficient were also decreased, probably due to inhibition of the hydrolytic enzymes in barley seeds. On the basis of tolerance indices, it has been concluded that the genotype Kalaycı-97 is the most tolerant to nickel toxicity, whereas Karatay-94 is the most sensitive one.

Acknowledgements

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