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A short stature allele enhances tolerance to Zn deficiency and translocation of Zn in barley

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Background: Zinc (Zn) content is of great importance in healthy human diet, crop productivity and stress tolerance in soils with zinc deficiency. The genes used to increase unit area yield such as semi-dwarf 1 (*sdw1*) is commonly considered to reduce mineral content of grain.

Methods. In the present study, influence of *sdw1.d,* a widely used allele for short plant height in barley breeding, on zinc accumulation and tolerance to zinc deficiency were investigated. A near isogenic line of *sdw1.d* allele, its recurrent parent Tokak 157/37 and donor parent Triumph were grown in zinc-deficient and -sufficient hydroponic cultures. Two experiments were conducted until heading time and physiological maturity.

Results. In zinc-deficient conditions, *sdw1.d* allele increased shoot dry weight by 112.4 mg plant^{-1}, shoot Zn concentration by 0.9 ppm but decreased root Zn concentration by 6.6 ppm. It did not affect grain characteristics but increased grain Zn content. In zinc-sufficient conditions, *sdw1.d* allele increased shoot Zn content, and decreased root Zn content. *sdw1.d* did not affect grain weight but increased grain Zn concentration by about 30% under zinc-sufficient conditions. The results showed that *sdw1.d* allele has no negative effect on tolerance to zinc deficiency, and even promotes tolerance to zinc deficiency by more Zn translocation. It was revealed that *sdw1.d* allele improves Zn accumulation under both zinc-deficient and zinc-sufficient condition. The *sdw1.d* allele could contribute to solving the problems in plant growth and development caused by zinc-deficiency via improving tolerance to zinc-deficiency. It could also provide a better Zn biofortification .

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Abstract

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Keywords: Biofortification, Gibberellic acid, *Hordeum vulgare*, *HvGA20ox2*, Zinc, *sdw1.d,*

Introduction

Zinc has important roles in many metabolic and physiological events in plants, such as the function of a wide range of proteins, enzyme activation, and phytohormone synthesis (Kimura et al., 2023). Throughout the world, zinc deficiency in food is generally caused by grain-based diets that are low in Zn, resulting in Zn malnutrition. Various diseases caused by zinc deficiency were reported in 30% of the world's population (Xia et al., 2020). Half of the world's agricultural areas consist of soils deficient in zinc (Singh et al., 2023; Yadav et al., 2023). Production of cultivated plants in zinc-deficient areas results in both reduced unit area yields and food containing less zinc. Removing more zinc from the soil by crops and improving their ability to accumulate zinc in seeds is the best solution to these problems. Increased uptake and accumulation of zinc by crops can be improved by more effective use of genetic potential of crops.

Short stature genes have provided significant gains in grain yields of barley via lodging resistance and high harvest index. Semi-dwarf 1 (*sdw1*), which is widely used in barley to create shorter plants, was formed as a result of mutations in the *gibberellic acid (GA)-20 oxidase gene* (*HvGA20ox*2) (Xu et al., 2017). The *sdw1* alleles resulted in low amounts of bioactive gibberellic acid (Cheng et al., 2022). Gibberellic acid is an endogenous phytohormone that regulates plant height (Cheng et al., 2022) and induces Zn accumulation in grain (Mathpal et al., 2023). Therefore, relationship between *sdw1.d* allele and Zn accumulation and translocation need to be evaluated.

While grain zinc content is of great importance in terms of nutrition, it is also important for crop productivity, resulting in better seedling strength and stress tolerance in soils with Zn deficiency (Yadav et al., 2023). Loci affecting grain Zn accumulation (Khan et al., 2023; Sadeghzadeh, Rengel & Li, 2015) and leaf-to-grain Zn transport (Hussain et al., 2016) have been identified in barley. In these studies, since the parental lines carried the wild type allele or different mutant allele (*sdw1.d* and *sdw1.a*) of *sdw1* (Xu et al., 2017), it was reported that the region was not associated with zinc accumulation. A new mutant allele showing similar phenotypic properties as *sdw1* allele was mapped to the same locus as *sdw1*, and it was reported that the locus may be associated with a zinc-ion binding gene (Zhou et al., 2018). On the other hand, Wiegmann et al. (2019) and Herzig et al. (2019) determined that the *sdw1* allele of *Hordeum vulgare* ssp. *spontaneum* increased grain Zn content compared to *sdw1.d.* Wiegmann et al. (2019) stated that the lines having *sdw1* allele of *Hordeum vulgare* ssp. *spontaneum* contained higher zinc, but since these alleles cause low grain yields, this effect was due to the dilution effect. Therefore, the *sdw1* allele needs to be evaluated compared to the wild type allele found in cultivated varieties. However, the effect of *sdw1.d,* which is probably the most common *sdw1* allele in barley breeding (Xu et al., 2017), on Zn uptake is still unknown.

Zinc deficiency in plant negatively affects metabolic and physiologic process, thereby decreasing crop yields (Kimura et al., 2023). The most appropriate approach to eliminate the negative effects of zinc deficiency on crops is Zn fertilization. However, in developing countries where specialty fertilizers are not available, the way to produce crops in zinc-deficient soils is the use of cultivars with tolerance to zinc deficiency. Tokak 157/37 (hereafter Tokak) is a variety tolerant to zinc deficiency (Kinaci & Kinaci, 2005). The *sdw1.d* allele was transferred to Tokak and a near isogenic line (NIL) was developed (Kandemir et al., 2022). Since Tokak carries wild type alleles of *HvGA20ox2*, the effects of *sdw1.d* allele were clearly observed by Kandemir et al. (2022). All of the differences between a NIL and its recurrent parent for a trait is attributed to the introgressed gene region (Lu et al., 2020), and therefore, NIL is the best genetic material to evaluate the effects of an allele. Thus, the genetic background of Tokak provides an opportunity to study the effect of the *sdw1.d* allele on tolerance to zinc deficiency.

There is a common belief that genes created by mutation and used in plant breeding to increase unit area yield decrease grain nutrient content (Wiegmann et al., 2019). Genes mutated to develop new alleles may cause loss of activity in traits other than the trait of interest (plant height for *sdw1.d*). In plant breeding, while increasing grain yields in various ways, nutritional quality characteristics such as the Zn content of grain are generally less taken into account. Therefore, in the present study, the effects of *sdw1.d* on Zn accumulation and tolerance to zinc deficiency were investigated.

Materials & Methods

Plant material and growth conditions

A near isogenic line of *sdw1.d* allele (*sdw1.d* NIL), the recurrent parent Tokak and the donor parent Triumph were used as the study material. NIL was developed by marker assisted backcrossing. Detailed information about the production of NIL can be found in Kandemir et al. (2022). Zn contents of seedling material were 30.4 ppm for Tokak, 41.0 ppm for *sdw1.d* NIL and 27.9 ppm for Triumph. To produce seedlings for hydroponic cultures, seeds were sterilized with 1% NaClO and rinsed three times with sterile distilled water. Seedlings were planted in sterile perlite and watered with distilled water until they reached about 10 cm length. Bunch of seven seedlings of about 10 cm height were dipped in 2.5 mM $CaSO_4$ to stimulate root growth (Cakmak et al., 1998) and transferred to hydroponic culture. Hydroponic cultures were carried out according to Cakmak et al. (1998).

The nutrient solution in hydroponic cultures contained 0.88 mM K_2SO_4, 2 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.25 mM KH_2PO_4, 0.1 mM KCl, 100 µM FeEDTA, 1 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.2 µM $CuSO_4$ and 0.02 µM $(NH_4)_6MoO_{24}$. No Zn was given to zinc-deficient hydroponic culture. 1 µM $ZnSO_4.7H_2O$ was used in zinc-sufficient hydroponic culture. Plants were grown in a temperature and light controlled (natural light supplemented by metal halide lamps) greenhouse at 22±2 °C for 16 h light (400 µmol m^{-2} s^{-1})/ 18 °C±2 for 8 h dark conditions. The nutrient solution was aerated with an air pump (50 l air min^{-1}) during the experiment. The nutrient solution was replaced every three days. Two experiments were conducted for vegetative parts such as roots and shoot (until ear emergence), and grain Zn content (until grain maturity). Each pot of hydroponic cultures containing the bunch of seven seedlings constituted one biological replicate. All experiments were conducted with three biological replications. Depending on the severity of zinc deficiency symptom and growth retardation in green parts, shoots and roots were harvested at the heading stage. In the experiment conducted to determine the grain Zn content, the grains were harvested when they matured.

Plant growth measurements

Zinc deficiency symptoms were expressed using a 1-5 scale (Cakmak et al., 1998). According to this scale, chlorotic and necrotic spots on leaves are as follows: 1=very severe, 2=severe, 3=mild, 4=slight and 5=very slight or absent. To determine the dry matter yields, harvested shoot and roots were washed with distile water and 0.01% HCl, and dried at 70 ˚C for 48 hours. After weighing the samples, root dry weight, shoot dry weight and grain weight per plant were determined. Plant height was determined by measuring the plants from the crown to the tip of the last grain in ear excluding the awn. The number of grains obtained from the bunch was divided by number of plants in the bunch, and the number of grains per plant was determined. To determine Zn concentrations of shoot, root and grain, the dry samples were ground in an agate mill and analyzed by wet digestion method in microwave (Mars 6, CEM, Matthews, NC) by H_2O_2-HNO_3 acid mixture. Zn concentrations (213.8 nm wavelength) were determined using ICP-OES (Varian, Vista Pro) (Cakmak et al., 1998).

Statistical analyses

Zinc-deficient and zinc-sufficient experiments were conducted in completely randomized design with three replications. Zinc-deficient and zinc-sufficient experiments were separately subjected to analysis of variance with JMP Pro 14 software (SAS Institute Inc$ NC, USA) according to the completely randomized blocks design. Comparisons among means were performed by Tukey test at 5% significance level.

Results

The *sdw1.d* effects in zinc deficiency

In zinc-deficient conditions, Tokak produced 882.1 mg plant^{-1}, *sdw1.d* NIL 994.4 mg plant^{-1} and Triumph 1067.1 mg plant^{-1} shoot dry weight. *sdw1.d* NIL produced 112.4 mg plant^{-1} higher shoot dry weight than cultivar (cv.) Tokak (Figure 1a). *sdw1.d* allele caused more shoot production. Triumph had a shoot Zn concentration of 8.8 ppm, Tokak 9.9 ppm and *sdw1.d* NIL 10.8 ppm (Figure 1b). Thus, *sdw1.d* allele resulted in 0.9 ppm higher shoot Zn concentration in zinc-deficient conditions in Tokak background. Tokak had a shoot Zn content of 8.7 µg plant^{-1} while Triumph had 9.4 µg plant^{-1} and *sdw1.d* NIL 10.7 µg plant^{-1} (Figure 1c). Triumph carrying *sdw1.d* allele did not differ from Tokak in shoot Zn content. *sdw1.d* allele increased shoot Zn content by 2 mg plant^{-1} compared to wild type allele. Root dry weights of *sdw1.d* NIL (119.5 mg) and Tokak (116.9 mg) were not different whereas Triumph produced 60% more root dry (192.7 mg) than Tokak and *sdw.1d* NIL (Figure 1d). The *sdw1.d* allele did not affect root dry weight. The *sdw1.d* NIL had a root Zn concentration of 11.7 ppm and Tokak 18.3 ppm. The *sdw1.d* allele reduced the root Zn concentration of Tokak by 6.6 ppm (Figure 1e). The root Zn concentration of Triumph (9.8 ppm) was not different from that of *sdw1.d* NIL. Therefore, *sdw1.d* allele caused lower root Zn concentration. Root Zn contents were 1.40 µg plant^{-1} in *sdw1.d* NIL, 1.87 µg plant^{-1} in Tokak and 2.14 µg plant^{-1} in Triumph (Figure 1f). The *sdw1.d* allele caused a prominent decrease in root Zn content.

Plant heights of *sdw1.d* NIL (52.0 cm), Tokak (61.7 cm) and Triumph (56.3 cm) were statistically different from each other (Figure 2a). The *sdw1.d* allele shortened the plant height of cv. Tokak by about 10 cm. Although *sdw1.d* NIL and Tokak did not show zinc deficiency symptom (symptom scale 5, Figure 2b), Triumph showed severe zinc deficiency symptom with a symptom scale point of 2.3. It was revealed that *sdw1.d* allele had no effect on tolerance to zinc deficiency while Triumph was highly sensitive to zinc deficiency. The number of grains per plant was similar in Tokak (23.7) and *sdw1.d* NIL (19.0) but was very low in Triumph (2.0) (Figure 2c). Grain weights of Tokak (36.0 mg) and *sdw1.d* NIL (36.9 mg) were not different (Figure 2d). The grain weight of cv. Triumph was 17.5 mg. The *sdw1.d* allele did not affect the grain weight of cv. Tokak under zinc-deficient conditions. Since Triumph produced very few grains in zinc-deficient conditions, grain Zn analysis could not be performed in Triumph. Grain Zn concentration of cv. Tokak (8.9 ppm) was less than that of *sdw1.d* NIL (11.7 ppm) (Figure 2e). The *sdw1.d* allele increased grain Zn concentration under zinc-deficient conditions. Grain Zn content of Tokak (0.33 µg plant^{-1}) was less than that of *sdw1.d* NIL (0.43 µg plant^{-1}). The *sdw1.d* allele increased grain Zn content about 25% under zinc-deficient conditions (Figure 2f).

The *sdw1.d* effects in zinc-sufficient conditions

In zinc-sufficient hydroponic culture, the differences in shoot dry weights were not significant (Figure 4a). The *sdw1.d* NIL had higher shoot Zn concentration (66. 9 ppm) than Tokak (59.3 ppm) or Triumph (53.2 ppm) (Figure 4b). The *sdw1.d* allele increased shoot Zn concentration approximately 7.6 ppm under zinc-sufficient conditions. Shoot Zn contents were 89.4 µg plant^{-1} in *sdw1.d* NIL, 79.3 µg plant^{-1} in Tokak and 74.2 µg plant^{-1} in Triumph (Figure 4c). In other words, the shoot Zn content of *sdw1.d* NIL was 10.1 µg plant^{-1} higher than Tokak. The *sdw1.d* allele increased the shoot Zn contents. Root dry weights were similar in Tokak (96.6 mg plant^{-1}) and *sdw1.d* NIL (106.7 mg plant^{-1}) (Figure 4d). The *sdw1.d* allele did not affect root dry weight. Triumph produced a higher root dry weight (130.9 mg plant^{-1}). Root Zn concentrations were similar in *sdw1.d* NIL (68.4 ppm) and Tokak (66.3 ppm), but lower in Triumph (45.9 ppm) (Figure 4e). The *sdw1.d* allele did not affect root Zn concentration. Root Zn content in *sdw1.d* NIL (6.51 µg plant^{-1}) was lower than Tokak (7.06 µg plant^{-1}) (Figure 4f). Thus, the *sdw1.d* allele decreased root Zn content.

Plant heights of *sdw1.d* NIL (59.7 cm), Triumph (67.7 cm) and Tokak (74.0 cm) were statistically different (Figure 5a). No zinc deficiency symptoms were observed in plants (Figure

5b). The *sdw1.d* allele decreased plant height about 15 cm. Numbers of grain per plant in Tokak (49.7) and *sdw1.d* NIL (56.1) were similar, while Triumph produced fewer grains (40.7) (Figure 5c). Grain weights were similar in *sdw1.d* NIL (39.0 mg) and Tokak (39.5 mg), but lower in Triumph (27.5 mg) (Figure 5d). The *sdw1.d* allele did not affect grain weight of cv. Tokak. Grain Zn concentration was lower in Tokak (72.5 ppm) than in *sdw1.d* NIL (92.5 ppm) (Figure 5e). The *sdw1.d* allele increased grain Zn concentration under zinc-sufficient conditions. The grain Zn content of *sdw1.d* NIL (3.66 µg $plant^{-1}$) was higher than Tokak (2.83 µg $plant^{-1}$) (Figure 5f). The *sdw1.d* allele increased the grain Zn concentration by about 30% under zinc-sufficient conditions.

Discussion

Zinc is one of the most important mineral elements required to complete of life cycle in plants and animals and human wealth (Joshi et al., 2023). In addition to efforts to improve unit area yields to feed the rising world population, the mineral content of grain must also be considered. Grain Zn biofortification is affected by many mechanisms (Kamaral et al., 2022). One of these mechanisms is endogenous phytohormones such as gibberellins that increase zinc uptake (Mathpal et al., 2023). The barley semi dwarf allele *sdw1.d* is associated with less gibberellic acid production and confers significant grain yield increases in barley (Xe et al., 2017). There is a well-characterized relationship between gibberellic acid and plant height (Cheng et al., 2022), Zn content (Mathpal et al., 2023). The gibberellic acid level of *sdw1.d* allele that reduces plant height may also affect Zn uptake and tolerance to zinc deficiency. Therefore, the effect of the *sdw1.d* allele was investigated in zinc-deficient and zinc-sufficient hydroponic cultures until ear emergence and harvest maturity.

In zinc deficiency, *sdw1.d* NIL did not show any necrotic symptoms and produced similar amounts of roots as in Tokak. An important indicator of response to zinc deficiency stress is more root production (Cakmak et al., 1999). Since *sdw1.d* NIL and its recurrent parent cv. Tokak, a zinc deficiency tolerant (Kinaci & Kinaci 2005), had similar symptom severity, the *sdw1.d* allele has no negative effect of on tolerance to zinc deficiency. The higher root production, intense necrotic leaf symptoms (Figure 3) and inability to produce seeds under zinc-deficient conditions indicate that Triumph is sensitive to zinc deficiency. Since there is no reliable relationship between grain or shoot Zn content and tolerance to zinc deficiency, zinc deficiency symptoms (necrotic symptoms and more root production) under zinc-deficient conditions are the best way to determine tolerance to this stress (Cakmak et al., 1998).

The higher grain zinc content in *sdw1.d* NIL grown under zinc deficiency condition compared to Tokat indicates that the *sdw1.d* allele enhances zinc translocation from roots to shoot and seed. The similar responses between the zinc deficiency tolerant Tokak and *sdw1.d* NIL shows that the *sdw1.d* allele has no negative effect on tolerance to zinc deficiency. On the other hand, *sdw1.d* NIL accumulated higher Zn in shoot and lower in the roots, produced more shoot and accumulated more Zn in the grain than cv. Tokak under zinc-deficient conditions. The results of the present study suggested that *sdw1.d* allele improves tolerance to zinc deficiency by increasing zinc translocation from roots to shoot and seed under zinc-deficient conditions, since more zinc accumulation in grains under zinc-deficient conditions is another indicator of zinc deficiency tolerance (Khan et al., 2023).

In sufficient zinc conditions, the differences in the shoot dry weights among genotypes were not significant since there was no stress in hydroponic culture. The *sdw1.d* allele was found to result in 15-20 cm shorter plant height in many studies (Cheng et al., 2023; Kandemir et al., 2022; Teplyakova et al., 2017). Shorter plant height is expected to result in less shoot. However, *sdw1.d* allele was found to increase the number of tillers in previous studies (Kandemir et al., 2022; Kuczyńska, Mikołajczak & Ćwiek, 2014). The lower plant height and higher number of tillers resulted in no change in shoot production. Although root Zn concentration of Tokak and *sdw1.d* NIL was similar, root Zn contents were lower in *sdw1.d* NIL. This is actually due to the partially lower root weights observed in *sdw1.d* NIL, although not statistically significant.

The *sdw1.d* allele increased shoot Zn concentration and content. The lower root Zn content and the higher shoot Zn content indicated that *sdw1.d* allele increases Zn translocation from roots to leaves. *sdw1.d* increased grain Zn concentration and contents with no difference in grain weights between *sdw1.d* NIL and Tokak. Low root Zn contents, high shoot and grain Zn contents indicated that the *sdw1.d* allele improves zinc translocation from roots to grain. Zhou et al. (2018) determined that the *sdw1* locus is related to Zn-ion binding. Wiegmann et al. (2019) reported that the *sdw1.d* allele decreased Zn uptake in populations produced from wild species *Hordeum vulgare* ssp. *spontaneum*, ssp. *agriocrithon* and cultivated cv. Barke carrying *sdw1.d* allele, and that this effect may be due to the dilution effect of the *sdw1.d* allele as a result of increased unit area yield. Consequently, due to the fact that the differences between the near isogenic line and its recurrent parent Tokak involve only the transferred gene region (Lu et al., 2020), the higher grain Zn contents measured in the *sdw1.d* NIL proved that the *sdw1.d* allele increases zinc accumulation. Xu et al. (2017) found that expression *HvGA20ox1* and *HvGA20ox3*, homologs of the *HvGA20ox2* gene, increased 2-fold and 3-fold, respectively, in *sdw1.d* mutants. The *HvGA20ox3* gene is located on chromosome 3HL (Dhanagond et al., 2019) and *HvGA20ox1* on chromosome 5HL (Göransson et al., 2019). QTLs related to zinc concentration, shoot and grain zinc content were found around the loci of these genes (Hussain et al., 2016; Sadeghzadeh, Rengel & Li, 2015). The increased expressions of *HvGA20ox1* and *HvGA20ox3* might have enabled the zinc, which is taken up more by the Tokak background in NIL, to be transported more into the grain.

Although Tokak uptakes more zinc under both zinc-deficient and zinc-sufficient conditions, translocation of zinc in the roots to the shoot and grain was low. The *sdw1.d* allele increased the translocation of zinc to the grain in the Tokak genetic background. Since the *sdw1.d* is a different form of a gene related to gibberellic acid, it can be concluded that altered levels of gibberellic acid increase zinc translocation. Endogenous hormones already have important effects on the uptake and accumulation of Zn. Sekimoto et al. (1997) found that Zn alters endogenous phytohormone levels, and stated that altered shoot amount under zinc-deficient conditions cannot be attributed to one phytohormone alone. Therefore, it is not known whether the short plant height and other effects of the *sdw1.d* allele are solely due to decreased levels of gibberellic acid or to the interaction of altering levels of gibberellic acid with other hormones. However, *sdw1.d* allele enhances zinc accumulation in shoot and grain under both zinc-deficient and zinc-sufficient conditions. The *sdw1.d* allele was known to have numerous pleiotropic effects such as delayed flowering and maturity, prostrate growth, higher level beta-glucan (Kuczyńska, Mikołajczak & Ćwiek, 2014), lower 1000-seed weight, higher number of ears per area (Kandemir et al., 2022). Increasing Zn translocation from roots to grain under both zinc-deficient and zinc-sufficient conditions is a new addition to the beneficial effects of this allele, providing a better Zn biofortification.

Conclusions

Barley produced in zinc-deficient areas results in not only reduced unit area yields but also in grains containing less Zn. The results of the present study showed that *sdw1.d* allele improved zinc uptake and tolerance to zinc deficiency in barley. The inference that the dilution effect of the yield-increasing effects of the alleles reduces the mineral content of the plants is not valid for the *sdw1.d* allele. However, this result is related to the *sdw1.d* allele. Since other *sdw1* alleles differ in the expression of the gene, they may react differently under conditions of Zn sufficiency and Zn deficiency. This can also alter the uptake of Zn when changing gibberellic acid levels interact with other hormones within the plant. While the *sdw1.d* allele could contribute to solving the problems in plant growth and development caused by zinc-deficiency via improving tolerance to Zn deficiency, it can also result in a better Zn grain biofortification whether zinc is sufficient or deficient in soil. These findings proved that the *sdw1.d* allele can be reliably used in plant breeding programs due to its zinc-related gains in addition to the benefits it provides in terms of short plant height.

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Figure 1

Shoot and root characteristics under zinc deficiency.

Means followed by different letters are significantly different at an alpha level of 0.05 according to Tukey’s test. The error bars indicate the standard error.

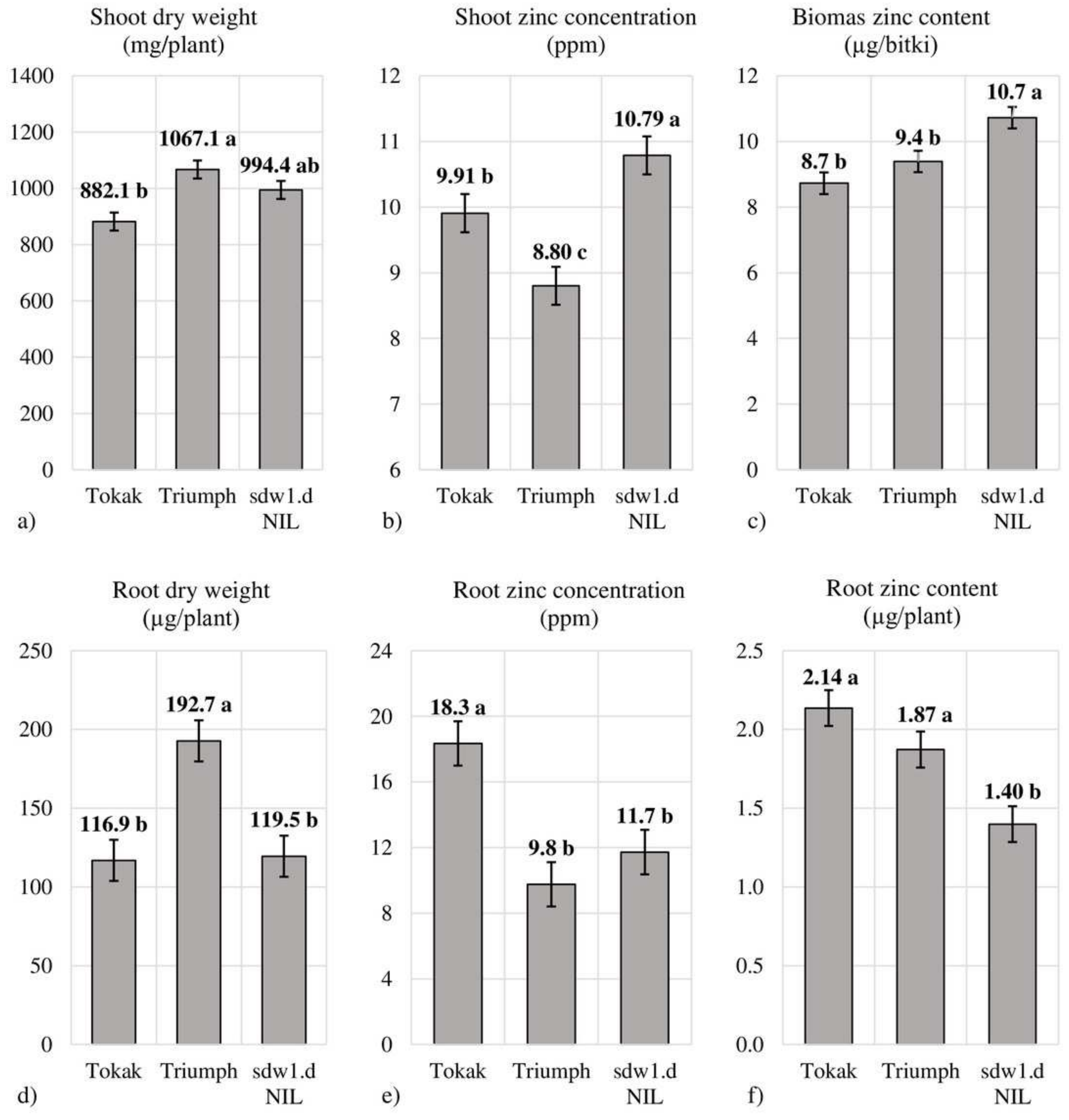

Shoot dry weight
(mg/plant)
1400
1200
1000
800
600
400
200
0
882.1 b
1067.1 a
994.4 ab
Tokak
Triumph
sdw1.d NIL
a)
Shoot zinc concentration
(ppm)
12
11
10
9
8
7
6
9.91 b
8.80 c
10.79 a
Tokak
Triumph
sdw1.d NIL
b)
Biomas zinc content
(µg/bitki)
12
10
8
6
4
2
0
8.7 b
9.4 b
10.7 a
Tokak
Triumph
sdw1.d NIL
c)
Root dry weight
(µg/plant)
250
200
150
100
50
0
116.9 b
192.7 a
119.5 b
Tokak
Triumph
sdw1.d NIL
d)
Root zinc concentration
(ppm)
24
20
16
12
8
4
0
18.3 a
9.8 b
11.7 b
Tokak
Triumph
sdw1.d NIL
e)
Root zinc content
(µg/plant)
2.5
2.0
1.5
1.0
0.5
0.0
2.14 a
1.87 a
1.40 b
Tokak
Triumph
sdw1.d NIL
f)

Figure 2

Plant height, zinc deficiency symptom and grain characteristics under zinc deficiency.

Means followed by different letters are significantly different at an alpha level of 0.05 according to Tukey’s test. The error bars indicate the standard error. *Zinc deficiency symptom scale: (necrotic patches on leaves) 1 = very severe, 2 = severe, 3 = mild, 4 = slight and 5 = very slight or absent). Since Triumph produced very few grains in zinc deficiency conditions, grain Zn analysis could not be performed.

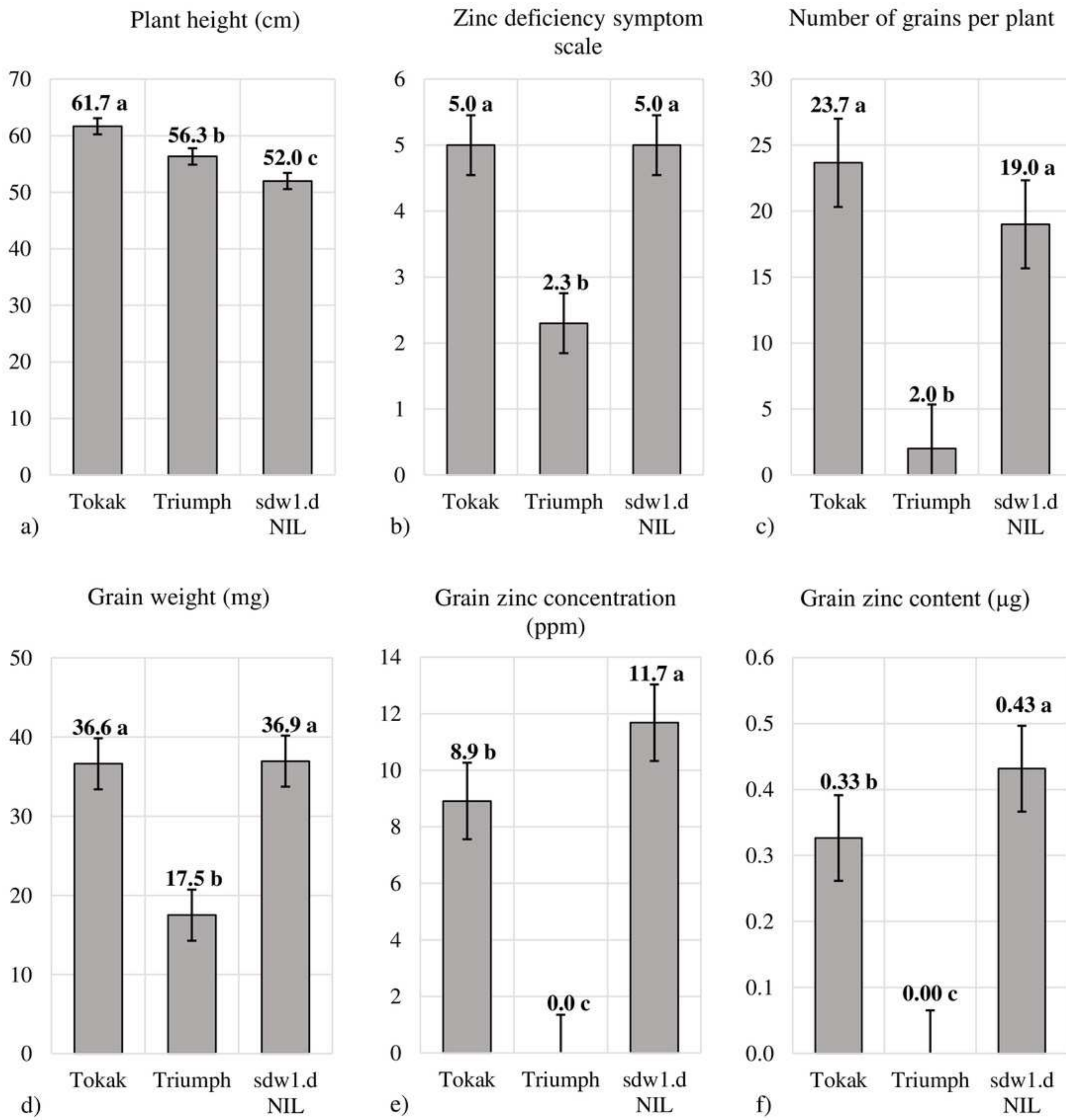

Plant height (cm)
70
60
50
40
30
20
10
0
61.7 a
56.3 b
52.0 c
Tokak
Triumph
sdw1.d NIL
a)
Zinc deficiency symptom scale
6
5
4
3
2
1
0
5.0 a
2.3 b
5.0 a
Tokak
Triumph
sdw1.d NIL
b)
Number of grains per plant
30
25
20
15
10
5
0
23.7 a
2.0 b
19.0 a
Tokak
Triumph
sdw1.d NIL
c)
Grain weight (mg)
50
40
30
20
10
0
36.6 a
17.5 b
36.9 a
Tokak
Triumph
sdw1.d NIL
d)
Grain zinc concentration (ppm)
14
12
10
8
6
4
2
0
8.9 b
0.0 c
11.7 a
Tokak
Triumph
sdw1.d NIL
e)
Grain zinc content (µg)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.33 b
0.00 c
0.43 a
Tokak
Triumph
sdw1.d NIL
f)

Figure 3

Genotypes a) Tokak 157/37, b) *sdw1.d* NIL, and c) Triumph under zinc-deficient (Zn-) and zinc-sufficient (Zn+) conditions.

The black arrow (in figure c) indicates the zinc deficiency symptom in leaves of Triumph.

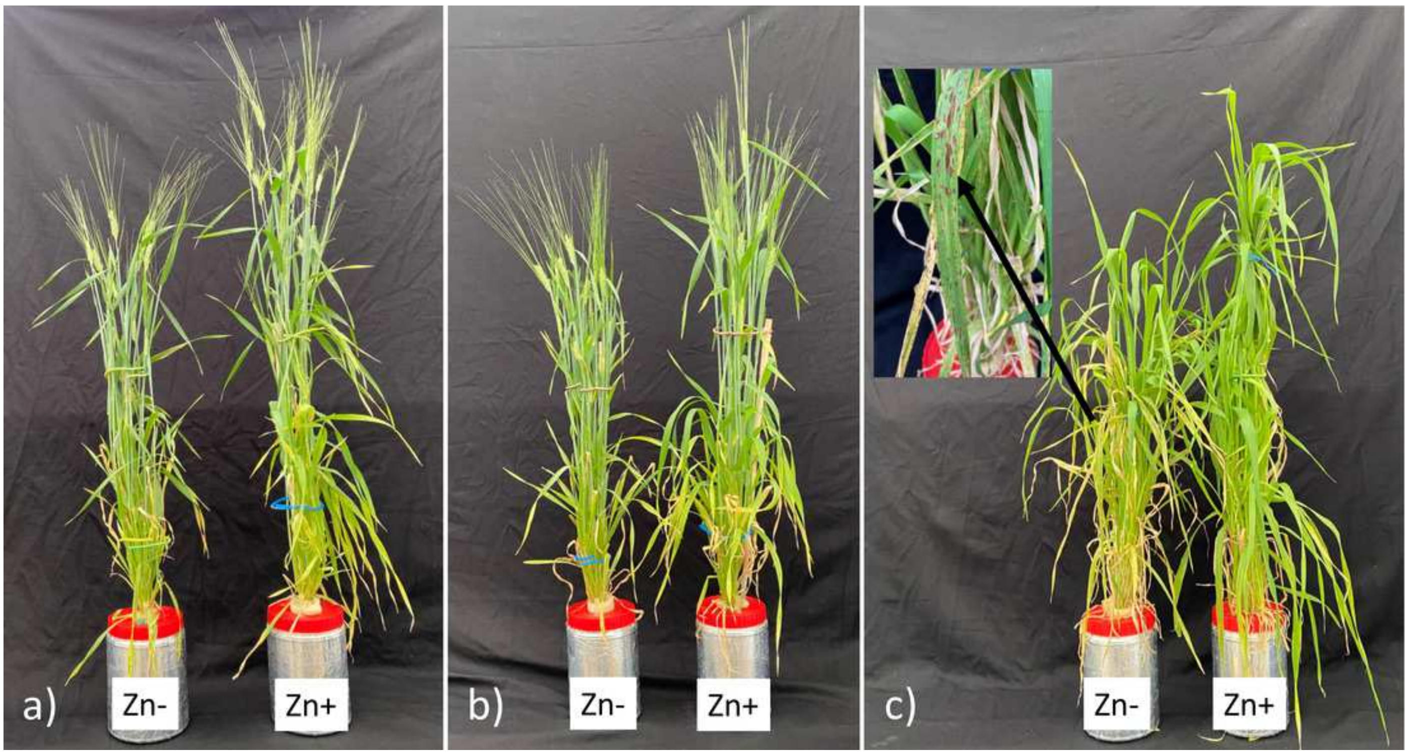
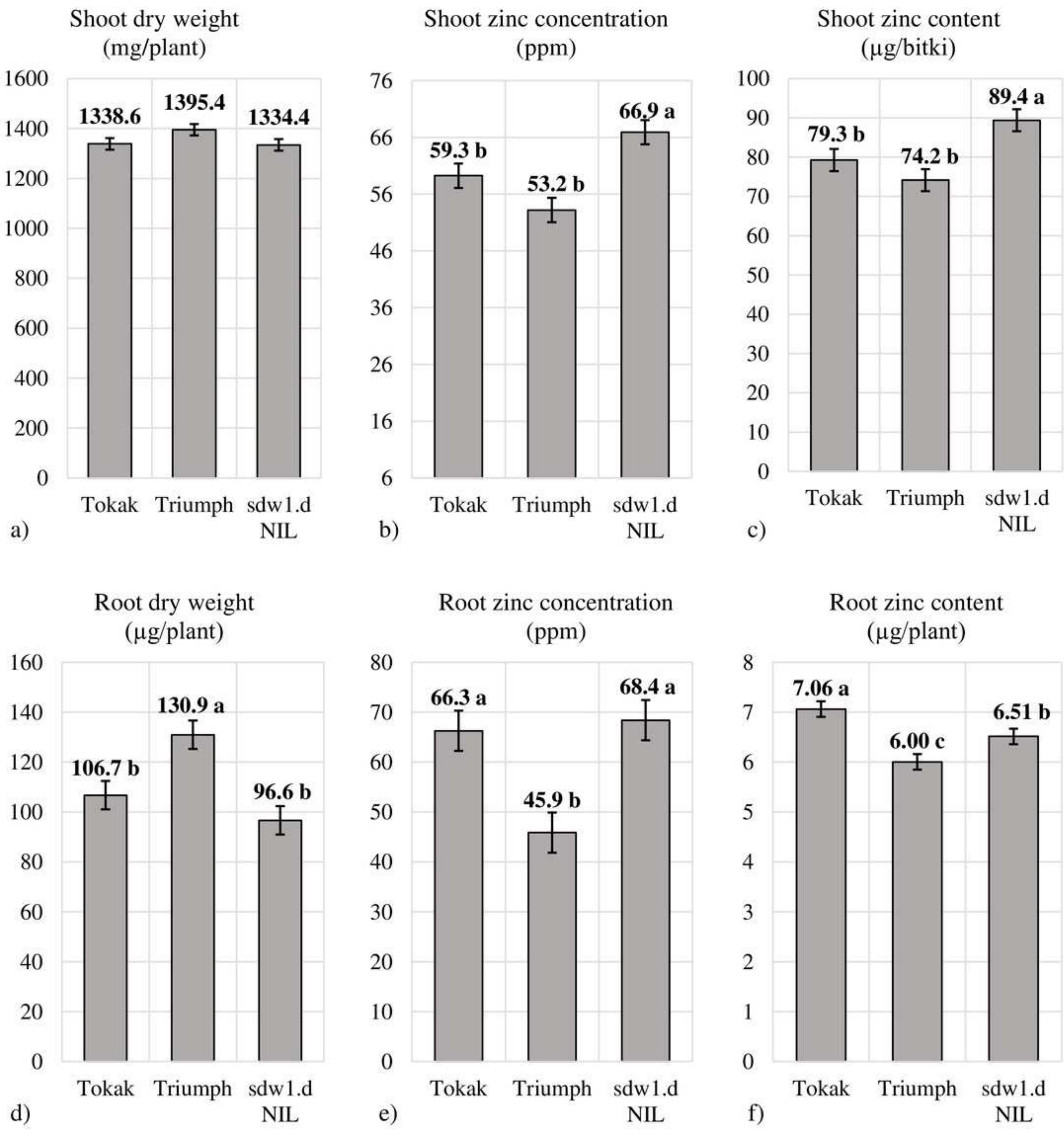


Figure 4

Shoot and root characteristics under zinc-sufficient conditions.

Means followed by different letters are significantly different at an alpha level of 0.05 according to Tukey's test. The error bars indicate the standard error.

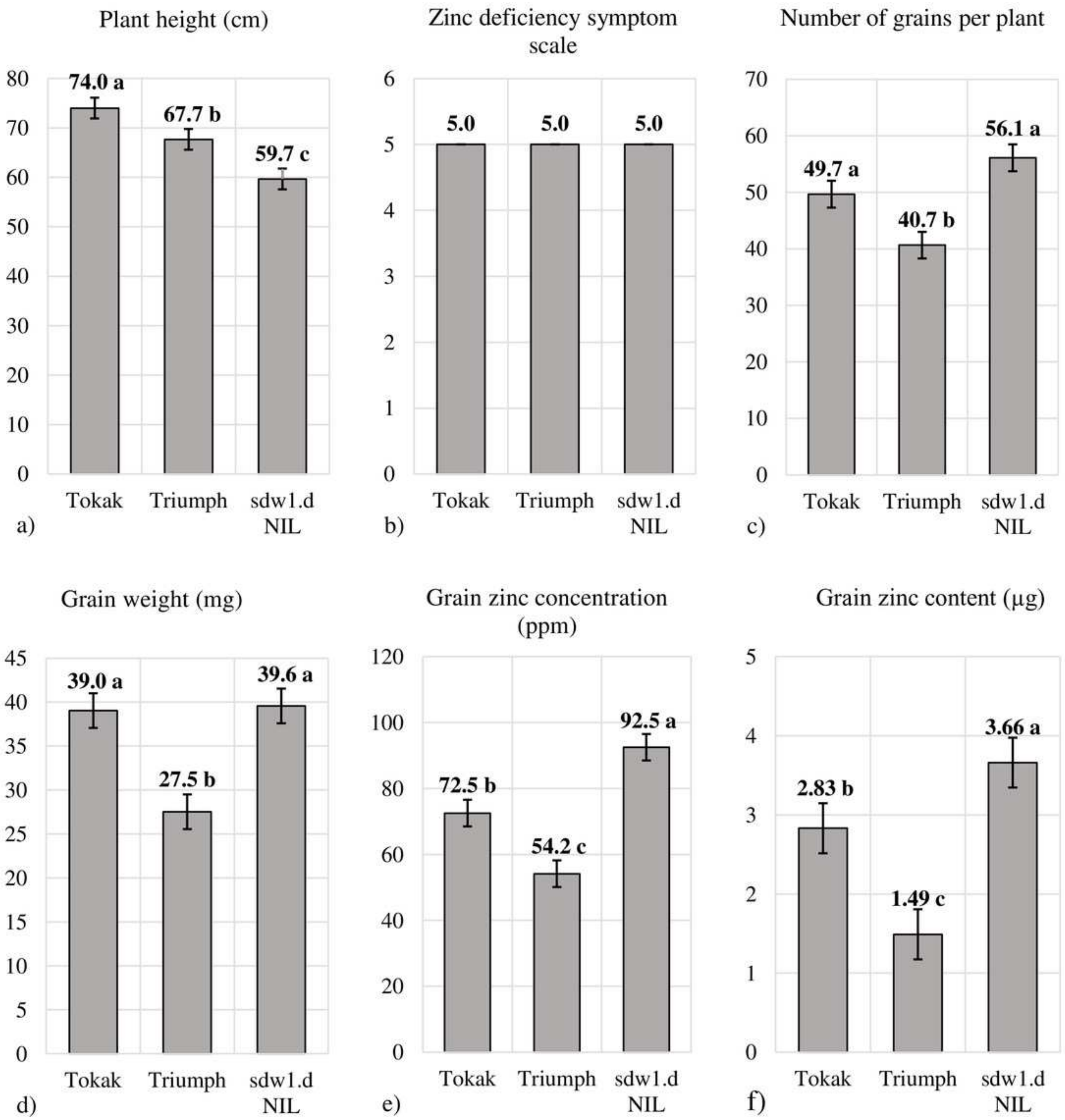


Shoot dry weight (mg/plant)
1600
1400
1200
1000
800
600
400
200
0
1338.6
1395.4
1334.4
Tokak
Triumph
sdw1.d NIL
a)
Shoot zinc concentration (ppm)
76
66
56
46
36
26
16
6
59.3 b
53.2 b
66.9 a
Tokak
Triumph
sdw1.d NIL
b)
Shoot zinc content (µg/bitki)
100
90
80
70
60
50
40
30
20
10
0
79.3 b
74.2 b
89.4 a
Tokak
Triumph
sdw1.d NIL
c)
Root dry weight (µg/plant)
160
140
120
100
80
60
40
20
0
106.7 b
130.9 a
96.6 b
Tokak
Triumph
sdw1.d NIL
d)
Root zinc concentration (ppm)
80
70
60
50
40
30
20
10
0
66.3 a
45.9 b
68.4 a
Tokak
Triumph
sdw1.d NIL
e)
Root zinc content (µg/plant)
8
7
6
5
4
3
2
1
0
7.06 a
6.00 c
6.51 b
Tokak
Triumph
sdw1.d NIL
f)

Figure 5

Plant height, zinc deficiency symptom and grain characteristics under zinc sufficient conditions.

Means followed by different letters are significantly different at an alpha level of 0.05 according to Tukey's test. The error bars indicate the standard error. *Zinc deficiency symptom scale: (necrotic patches on leaves) 1 = very severe, 2 = severe, 3 = mild, 4 = slight and 5 = very slight or absent.).


Plant height (cm)
74.0 a
67.7 b
59.7 c
Tokak
Triumph
sdw1.d NIL
a)
Zinc deficiency symptom scale
5.0
5.0
5.0
b)
Number of grains per plant
49.7 a
40.7 b
56.1 a
c)
Grain weight (mg)
39.0 a
27.5 b
39.6 a
d)
Grain zinc concentration (ppm)
72.5 b
54.2 c
92.5 a
e)
Grain zinc content (μg)
2.83 b
1.49 c
3.66 a
f)