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Impact of moderate soil drought on the dynamics and distribution of low molecular weight protectors in Triticum aestivum and Triticum spelta

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Abstract

Osmotic regulation, which involves low molecular weight protectors like amino acids and phenolic compounds, is one mechanism by which agricultural crops adapt to drought. We investigated the impact of moderate soil drought on the dynamics and distribution of free amino acids, phenols, and flavonoids in 18-day-old drought-resistant plants of *Triticum* aestivum L. cv. 'Podolyanka' and ecologically versatile *Triticum spelta* L. cv. 'Frankencorn'. Moderate soil drought resulted in a 20% increase in the total free amino acid content in spelt wheat shoots, while winter wheat exhibited minimal changes. Spelt wheat shoots showed significant increases in arginine, proline, phenylalanine, cysteine, and valine, whereas winter wheat exhibited substantial increases only in phenylalanine and tyrosine. Winter wheat shoots experienced notable decreases in arginine and glutamic acid, while methionine content in spelt wheat shoots decreased. Moderate soil drought induced an increase in the accumulation of total phenols and flavonoids. Spelt wheat roots showed a substantial increase in total phenols (53%), while winter wheat exhibited a significant increase (49%) in total phenols in shoots. Total flavonoid content increased in spelt wheat shoots and roots by 17%, and 38%, respectively, under moderate soil drought, whereas in winter wheat the increase was 70% in shootsand 10% in roots. Our findings on amino acid content suggest different sensitivities of cultivars to drought stress. The moderate increase in total phenolic compounds in spelt wheat shoots underscores the sensitivity of young *T. spelta* cv. 'Frankenkorn' plants to water deficit, contrasting with the more drought-resistant properties observed in *T. aestivum* cv. 'Podolyanka'.

1. Introduction

Soil drought is a prominent abiotic factor that significantly reduces agricultural crop production and poses a threat to global food security. One of the primary consequences of drought is the excessive generation of reactive oxygen species (ROS), which disrupt cellular structures and negatively impact respiration, photosynthesis, plant growth, and development. ROS induce oxidative damage to proteins, lipids, carbohydrates, and DNA, ultimately leading to plant mortality (Waraich et al. 2011; Reddy et al. 2014). Osmotic regulation is a key mechanism employed by plants to adapt to soil drought and detoxify ROS. It involves the accumulation and synthesis of various soluble substances within cells, thereby altering the osmotic potential (González-Chavira et al. 2018). Free amino acids (AAs) and phenolic compounds (PCs) are among the substances involved in osmotic regulation.

Accumulation of free AAs is a common response to abiotic stress. AAs play key roles in intracellular pH regulation, aiding in ROS detoxification, enzyme synthesis, and the formation of secondary metabolites. They also serve as energy donors in the tricarboxylic acid cycle, regulate various processes, and participate in the production of phytohormones and low-molecular-weight nitrogen compounds (Romanenko et al. 2023a). Stress-resistant plants tend to accumulate higher levels of free AAs than sensitive ones (Ali et al. 2019). Specific AAs have been found to have positive effects on plant acclimatization, suggesting their potential use as biostimulants in agriculture (Romanenko et al. 2023a).

PCs are a vast group of secondary metabolites that play essential roles in adaptation to environmental stress,primarily due to their antioxidant properties (Babenko et al. 2019). Among these compounds, flavonoids are the most abundant and biologically active, known for their unique chemical structure that gives them strong antioxidant and radical-neutralizing capabilities (Grace 2005). PCs act as non-enzymatic antioxidants, helping to protect cell membranes from oxidation by neutralizing ROS. This function is to maintain plant osmotic balance and provide comprehensive protection against oxidative stress. PCs also stimulate antioxidant reactions, involving both non-enzymatic and enzymatic components (Parvin et al. 2022). The accumulation of PCs varies among plant species depending on the intensity and type of stress, as well as their ability to scavenge ROS. This variability allows them to be used as stress markers to assess the severity of stressful conditions (Rahman et al. 2016; Parvin et al. 2022). Plants synthesize PCs actively to protect themselves from various abiotic stresses, which involves the activation of key genes and enzymes associated with PC biosynthesis (Chowdhary et al. 2021; Parvin et al. 2022). PCs are synthesized in plant tissues as an adaptive response to adverse environmental conditions (Naikoo et al. 2019).

Winter wheat (*Triticum aestivum* L.) is one of the world's primary crops, both in terms of cultivated area and consumption (FAO 2019). However, stressful conditions, such as drought, negatively impact wheat production. A 40% reduction in moisture can lead to yield losses of up to 20.6% (Daryanto et al. 2016). The growing trend of reviving, selecting, and reintroducing forgotten regional grain crops, particularly 'ancient' cereals, has significantly impacted modern wheat production. Among these cereals, *Triticum spelta* L. holds a prominent position. Spelt wheat exhibits favorable traits, such as successful crossbreeding with tetraploid wheat, contributing to the improvement of durum and soft wheat in breeding programs. It thrives in various cultivation conditions, making it suitable for cost-effective organic farming, and demonstrates resilience against natural and climatic environmental factors (Babenko et al. 2018). The demand for spelt has been growing rapidly in recent decades and is projected to continue increasing by about 5% annually (Wang et al. 2021), leading to the expansion of its production in semi-arid and arid regions (Technavio 2018).

The aim of our work was to analyze the impact of moderate soil drought on the dynamics and distribution of low molecular weight free AAs and PCs in the shoots and roots of young wheat cultivar 'Podolyanka' and spelt cultivar 'Frankencorn'. These varieties exhibit different levels of drought tolerance and are strategic cereals in terms of food security in Ukraine. The results of this study should enhance our understanding of the drought tolerance of these wheat species and their varieties in a changing climate. Additionally, this information will be valuable for future crop management, particularly in the arid regions of Ukraine.

2. Materials and Method

2.1. Plant material and experimental design

The experiments were conducted between 2021 and 2022. We studied 18-day-old plants of winter wheat (*Triticum aestivum* L.) cv. 'Podolyanka' and spelt wheat (*Triticum spelta* L.) cv. 'Frankenkorn'. The 'Podolyanka' wheat cultivar is winter- and drought-resistant, high-yielding, and undemanding in terms of growing conditions. On the other hand, the 'Frankenkorn' spelt wheat cultivar is frost-resistant and ecologically versatile. We obtained wheat grains from the collection of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (Kyiv), while spelt grains were sourced from the collection of the National Center for Plant Genetic Resources of Ukraine (Kharkiv). The plant material was grown following the method described in the study by Romanenko et al. (2022).

2.2. Drought stress modeling and sampling

Soil drought was created by stopping watering 14-day-old plants for the next 4 days until the leaves wilted and the moisture content of the substrate was reduced by half (25–30%). On the 18th day, in the phase of 2–3 leaves, shoots and roots of 18 old-day plants without and after drought stress were investigated.

2.3. Free amino acids content determination

AAs were extracted from leaves that had been dried to its absolute dry weight. The extraction process involved incubating a 1-gram sample in a 3% sulfosalicylic acid solution followed by centrifugation at 4000 rpm for 30 minutes. The qualitative and quantitative composition of free AAs in the resulting supernatant was determined using ion-exchange liquid chromatography with an automatic amino acid analyzer T 339 (from the Czech Republic). AAs in the eluates were registered by detection with ninhydrin (Hare et al. 1985).

2.4. Total phenols and flavonoids determination

The total phenolic content was performed with Folin-Ciocalteu reagent, following the method described by Bobo-García et al. (2015). An 80% methanol solution served as the solvent. The quantitative phenolic content was then calculated based on the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry matter (mg GAE/g). The total flavonoid content was determined according to Smirnov et al. (2021). The quantitative content of flavonoids was assessed using the calibration curve for rutin, and the results were expressed in rutin equivalents (RE) per gram of dry matter (mg RE/g). Both phenolic and flavonoid measurements were conducted using a Jenway UV-6850 spectrophotometer (UK) at wavelengths of 750 nm and 397.6 nm.

2.5. Statistical analysis

The results of the statistical analysis are presented as mean (M) with the standard deviation (± SD). Significant differences were evaluated using one-way ANOVA and Tukey's test at $P < 0.05$. The statistical analysis was carried out using the Statistic v. 10.0 (Analytical Software, Tallahassee, Florida, USA).

3. Results

In the leaves of 18-day-old winter wheat and spelt plants, 17 AAs were identified. Under control conditions, aspartic and glutamic acids, glycine, alanine, and leucine prevailed in both *Triticum* species. However, the quantitative content of other AAs differed in their order of dominance (Fig. 1). Moderate soil drought influenced the total content of free AAs in spelt shoots, increasing it by 20%. In wheat, the increase was slightly higher than in the control (by 2%) (Fig. 2).

The quantitative content of dominant AAs significantly differed in both *Triticum* species under stress (Fig. 3). During drought, the amount of arginine (131%), proline (112%), phenylalanine (73%), cysteine (63%), histidine (33%), valine (47%), tyrosine (37%), isoleucine (35%), and glycine (28%) increased in spelt shoots (Fig. 3, a). In winter wheat, only phenylalanine (196%) and tyrosine (60%) showed significant increases, while proline (19%) and glycine (15%) increased slightly (Fig. 3, b). The least pronounced changes in *T. spelta* were observed for lysine, threonine, and glutamic acid. Aspartic acid, serine, alanine, and leucine content did not significantly ($P \leq 0.05$) differ from the control, but methionine decreased by 22% (Fig. 3, a). In the shoots of winter wheat, arginine (24%) and glutamic acid (21%) content decreased the most, while lysine, histidine, threonine, alanine, cysteine, valine, and leucine did not differ significantly ($P \geq 0.05$) from the control (Fig. 3, b).

The content of phenols and flavonoids in the shoots of control 18-day-old wheat plants was 10.31 mg GAE/g DW and 4.62 mg RE/g DW, respectively, while in spelt plants, it was 12.22 GAE/g DW and 5.92 RE/g DW. In the roots, these values were lower, amounting to 2.86 mg GAE/g DW and 1.29 mg RE/g DW for T. aestivum, and 3.7 GAE/g DW and 1.93 mg RE/g DW for *T. spelta*, respectively. Moderate soil drought activated the accumulation of phenol content in the organs of spelt and winter wheat, but the degree of increase in total phenols and flavonoids in these species of the Triticum genus differed significantly. In the shoots of *T. spelta*, total phenols increased only slightly compared to the control, by 7%, while in the roots, they increased by 53%, amounting to 13.04 and 5.68 GAE/g DW, respectively (Fig. 4, a, b). In the shoots of T. aestivum, total phenolics significantly increased by 49%, while in the roots, it remained close to the control (increased by 3%), equaling 15.38 and 2.95 GAE/g DW, respectively (Fig. 4, a, b).

Under the influence of moderate drought, the content of flavonoids in the shoots of spelt increased by 17% and amounting to 6.96 mg RE/g DW, but in the roots, it increased significantly by 38%, amounting to 2.67 mg RE/g DW (Fig. 5, a, b). In the shoots and roots of wheat under the influence of water deficit, there was also an increase in flavonoids, by 70% and 10%, respectively, amounting to 7.87 mg RE/g DW and 1.41 mg RE/g DW (Fig. 5, a, b).

4. Discussion

Plant stress response mechanisms are activated at the beginning of stress exposure to help overcome stressors. Plants accumulate a variety antioxidant compounds in their cytosol to reduce its osmotic potential and maintain cell turgor. In our study, under the effect of moderate soil drought on both

Triticum varieties, we observed a specific response in these which was expressed through the differential distribution of low-molecular-weight protectants, such as AAs, total phenols, and flavonoids.

In terms of the quantitative content of AAs, wheat was superior to spelt under both control conditions and stress. However, under the influence of short-term moderate drought in the shoots of spelt, the total content of free AAs significantly increased compared to the control (by 20%), while in the winter wheat there was no significant increase. This result indicates different sensitivities of *Triticum* varieties to drought stress, with 'Podolyanka' winter wheat being positioned as a more drought-resistant cultivar compared to 'Frankenkorn' spelt (Morgun et al. 2014).

Spelt is the oldest hexaploid species in the genus *Triticum*, from which all other cultivated species of wheat, including *T. aestivum*, were formed. It is considered a moisture and light-loving crop, but is less resistant to atmospheric and soil drought compared to wheat (Babenko et al. 2018). Previous research has shown that the pigment complex of 18-day-old ‘Podolyanka’ wheat seedlings exhibits greater drought resistance than that of ‘Frankenkorn’ spelt (Romanenko et al. 2023b). However, the root system of 18-day-old plants *T. spelta* cv. ‘Frankenkorn’ showed better recovery capacity after soil drought compared to that of ‘Podolyanka’ wheat (Kosakivska et al. 2023). Radzikowska et al. (2022) found that in the leaves of spelt plants 'Frankenkorn' cultivar, there was a significant loss of chlorophyll during soil drought; however, the decrease in the intensity of photosynthesis was the smallest compared to other spelt cultivars. Additionally, they noted that the 'Frankenkorn" was characterized by the weakest effect of drought on gas exchange parameters and a moderate decrease in the level of relative water content compared to other investigated spelt cultivars under the influence of soil drought.

The quantitative content of dominant AAs differed significantly in both *Triticum* species under stress. During drought, proline, arginine, cysteine, and phenylalanine mainly prevailed in the shoots of spelt, while tyrosine and phenylalanine were predominant in wheat. These amino acids are actively involved in antioxidant defense during drought (Kumar et al. 2021). However, our studies showed a specific response of the studied varieties in the quantitative composition of AAs under moderate soil drought. The significant increase in tyrosine and phenylalanine levels in 'Podolyanka' wheat suggests the activation of protein synthesis regulation to maintain cellular functions. Additionally, these AAs are involved in synthesizing various antioxidants that help scavenge ROS and reduce oxidative stress (Kumar et al. 2021). Tyrosine and phenylalanine also play a role in the synthesis of phenylpropanoids (Vogt 2010), which, in turn, generate numerous secondary metabolites such as stilbenes, monolignols, coumarins, phytoalexins, anthocyanins, flavonoids, and phenolic acids (Thakur et al. 2021). Furthermore, the significant increase in proline and arginine levels in spelt leaves during short-term drought stress indicates various adaptive responses and biochemical changes in the plant to cope with water scarcity. Proline acts as an osmolyte, maintaining cellular osmotic balance by regulating water uptake and retention, thus preventing cell dehydration (Hayat et al. 2012). Additionally, proline contributes to stabilizing proteins, enzymes, and cellular structures under stress, ensuring their integrity and functionality, while also scavenging ROS generated during drought stress (Szabados and Savoure, 2010). The study by Radzikowska et al. (2022) demonstrated that 'Frankencorn' spelt plants accumulate

significant levels of proline and anthocyanins under soil drought, and exhibit low levels of lipid peroxidation, indicating the protective role of these antioxidants in reducing damage and maintaining photosynthetic activity during drought. Generally, the accumulation of proline in plants under drought stress is associated with a decrease in the expression of *proline dehydrogenase* (*PDH*) genes, responsible for proline degradation, and an increase in the expression of *pyrroline-5-carboxylate synthase* (*P5CS*) genes, which encode key enzymes of proline biosynthesis (Forlani et al. 2019). Drought-induced proline accumulation is known to be regulated by an ABA-dependent signaling pathway (Hare et al. 1999). Osmotic stress triggers ABA accumulation, which then regulates *P5CS* gene expression (Forlani et al. 2019). Previously, it was found that endogenous ABA significantly increased in 18-day-old shoots of 'Frankencorn' spelt wheat during drought stress, unlike in 'Podolyanka' wheat (Kosakivska et al. 2023). It appears that the significant accumulation of proline in the leaves of the ‘Franckenkorn’ cultivar is a component of its strategy for coping with drought stress.

Lastly, the rise in arginine levels in spelt leaves may signal increased nitric oxide (NO) production, which plays a crucial role in regulating plant stress responses (Winter et al. 2015). In addition, arginine serves as a precursor for the synthesis of polyamines such as putrescine, spermidine, and spermine. These polyamines play crucial roles in various stress responses, including protection against oxidative damage and stabilization of membranes and proteins (Mattoo et al. 2015). Conversely, in wheat leaves, we observed a decrease in the levels of both arginine and glutamic acid. This reduction may be attributed to their conversion into other compatible soluble substances, particularly proline (Winter et al. 2015; Ali et al. 2019). Notably, proline exhibited a significant increase under short-term drought stress. Furthermore, spelt leaves showed a significant quantitative increase in cysteine and a decrease in methionine during drought stress. These changes indicate substantial metabolic adjustments occurring in response to water deficit. Cysteine, as the main metabolite, serves as a sulfur donor for the synthesis of methionine and glutathione (GSH) (Hell and Wirtz, 2011). GSH, in turn, plays a central role in ROS detoxification and actively protects cellular components from oxidative damage during drought stress (Noctor et al. 2012). The increased level of cysteine in spelt under drought stress may indicate its further involvement in GSH synthesis, as cysteine serves as its direct precursor (Noctor et al. 2012). Interestingly, a similar increase in cysteine levels has been reported in spring wheat plants during gradual drought conditions (Chen et al. 2004). On the other hand, the decrease in methionine in spelt leaves under short-term drought stress suggests its active participation in various metabolic changes in response to water scarcity. Unlike cysteine, the accumulation of free methionine in plant cells during stress is not directly associated with antioxidant defense mechanisms (Zagorchev et al. 2013). Methionine synthesis can be tightly regulated by the level and activity of the enzyme cystathionine γ-synthase (CGS), which is derived from cysteine (Amir and Hacham 2008). Additionally, methionine serves as a substrate for the synthesis of various polyamines (Alcázar et al. 2010) and plays a role in ethylene biosynthesis (Lin et al. 2010).

Changes in the content of various AAs under drought conditions have been reported in many cultivated cereal plants (Wingler et al. 1999; Yang et al. 2000; Kusaka et al. 2005; Bowne et al. 2012; Raorane et al. 2015; Casartelli et al. 2018; Michaletti et al. 2018). Increased proline content under drought conditions is a common feature of most cereal crops (Marcińska et al. 2013). Wheat plants, in particular, accumulate

proline in the leaves more than other osmoregulators, including glycine betaine, soluble carbohydrates, and proteins (Farshadfar et al. 2008). Significant accumulation of AAs and proline, in particular, has been found in wheat genotypes with different levels of drought tolerance (Mattioni et al. 1999; Kumar et al. 2017; Babenko et al. 2020). Proline accumulates in both leaves and roots of drought-tolerant and drought-sensitive wheat genotypes during water deficit, with higher content in the drought-tolerant genotype, along with total amino acid content (Kang et al. 2019). Drought-tolerant and drought-sensitive wheat genotypes have been reported to accumulate amino acids differently in leaves during drought: the heat-tolerant wheat genotype significantly accumulated alanine, isoleucine, leucine, lysine, and tyrosine, while the intolerant genotype accumulated isoleucine and phenylalanine (Kang et al. 2019). The quantitative content of amino acids, primarily proline, tryptophan, and branched-chain amino acids (leucine, isoleucine, valine), increased during soil drought in all drought-tolerant and sensitive wheat varieties (Bowne et al. 2012; Michaletti et al. 2018). Aromatic amino acids, especially tryptophan, as well as phenylalanine and tyrosine, also accumulated under water deficit in the aboveground tissues of wheat (Bowne et al. 2012; Rahman et al. 2017; Kang et al. 2019).

The synthesis of PCs is one of the strategies used by plants under unfavorable environmental conditions to avoid drought-induced oxidative damage (Kumar et al. 2020). PCs levels have been reported to be used as an indicator of drought tolerance (Varela et al. 2016; Liu et al. 2018; Upadhyay et al. 2020). We have shown that moderate soil drought induced the accumulation of PCs in the organs of both *Triticum* species, but the nature of changes in the content of total phenols and flavonoids differed. In the shoots of *T. spelta*, total phenolics and flavonoids increased by 7% and 17%, respectively, while in *T. aestivum*, they increased by 49% and 70%. In spelt roots, total phenolics and flavonoids increased by 53% and 38%, respectively, and in winter wheat, by 3% and 10%. Thus, the reaction of *Triticum* species differed in shoots and roots under the influence of water deficit, and the data obtained indicate the formation of a specific reaction of these *Triticum* species to the effect of moderate soil drought. In 'Podolyanka' winter wheat plants, this process was more active in shoots, while in 'Frankencorn' spelt, it was more active in the roots. The results of our studies are in agreement with the data obtained by Gregorová et al. (2015) in winter wheat plants of Slovak selection SK-196, for which a significant increase in the content of PCs in shoots and a moderate increase in the roots were found under the influence of severe soil drought. The authors position the studied wheat variety as drought-tolerant and note that the enhanced synthesis of PCs in shoots helps to protect photosynthetic tissues from oxidative stress and dehydration. The increased content of total phenols and flavonoids was found in drought-sensitive and drought-tolerant winter wheat genotypes (Chakraborty and Pradhan, 2012; Hameed et al. 2013; Ma et al. 2014), but it is the drought-tolerant genotypes that are characterized by PC hypersynthesis (Guo et al. 2020; Upadhyay et al. 2020). It is worth noting that in the shoots of *T. aestivum* cv. 'Podolyanka', there is an increased level of tyrosine (by 60% compared to the control) and phenylalanine (by 196% compared to the control), and these aromatic amino acids are directly involved in the synthesis of phenylpropanoids, which in turn are the substrate for the synthesis of flavonoids (Vogt 2010; Thakur et al. 2021). In the case of *T. spelta* cv. 'Frankenkorn', the shoots also contain an increased level of phenylalanine by 73% compared to the control, but the total content of this amino acid in spelt remains lower compared to winter wheat. The

predominance of these two aromatic amino acids, as well as phenols and flavonoids in wheat leaves, indicates a coordinated response of wheat plants to stress conditions caused by short-term drought.

5. Conclusions

The results of our studies showed that under moderate soil drought, differential changes occur in the accumulation and distribution of low molecular weight protectants (AAs, phenols, and flavonoids) in the organs of 18-day-old winter wheat 'Podolyanka' plants and spelt wheat 'Frankencorn'. Wheat and spelt reacted differently to drought, indicating their specific response and potential for adaptation to water deficit. The quantitative content of dominant amino acids differed significantly in both Triticum species under both control and stress conditions. Under drought, proline, arginine, cysteine, and phenylalanine dominated in the shoots of spelt wheat, while tyrosine and phenylalanine dominated in winter wheat. It was found that under control conditions, the phenolic compound content was higher in the organs of the ecologically plastic 'Frankenkorn' spelt. Under drought, the content of stress-induced phenolic compounds in the shoots of drought-tolerant winter wheat 'Podolyanka' increased significantly, while in 'Frankenkorn' spelt, it increased in the roots. Quantitative and qualitative changes in the content of AAs and PCs indicate the involvement of these compounds in the formation of the response to moderate soil drought and correspond to the individual traits of drought tolerance of winter wheat 'Podolyanka' and spelt 'Frankenkorn' due to their genetic characteristics and level of drought tolerance. The significant increase in proline, coupled with a moderate increase in total PC compounds in the shoots of 'Frankenkorn' spelt, suggests that this variety employs an individual strategy in responding to water deficit compared to T. aestivum cv. 'Podolyanka', which exhibited higher drought resistance.

Declarations

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

Iryna V. Kosakivska and Kateryna O. Romanenko conceived and designed the experiments and wrote the main manuscript text. Kateryna O. Romanenko, Lidia M. Babenko and Oleksandr E. Smirnov conducted

all experiments. Kateryna O. Romanenko carried out the analytical work prepared all figures in paper, analyzed and interpreted the data, provided critical review of the entire manuscript. All authors read and approved the final manuscript.

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Figures

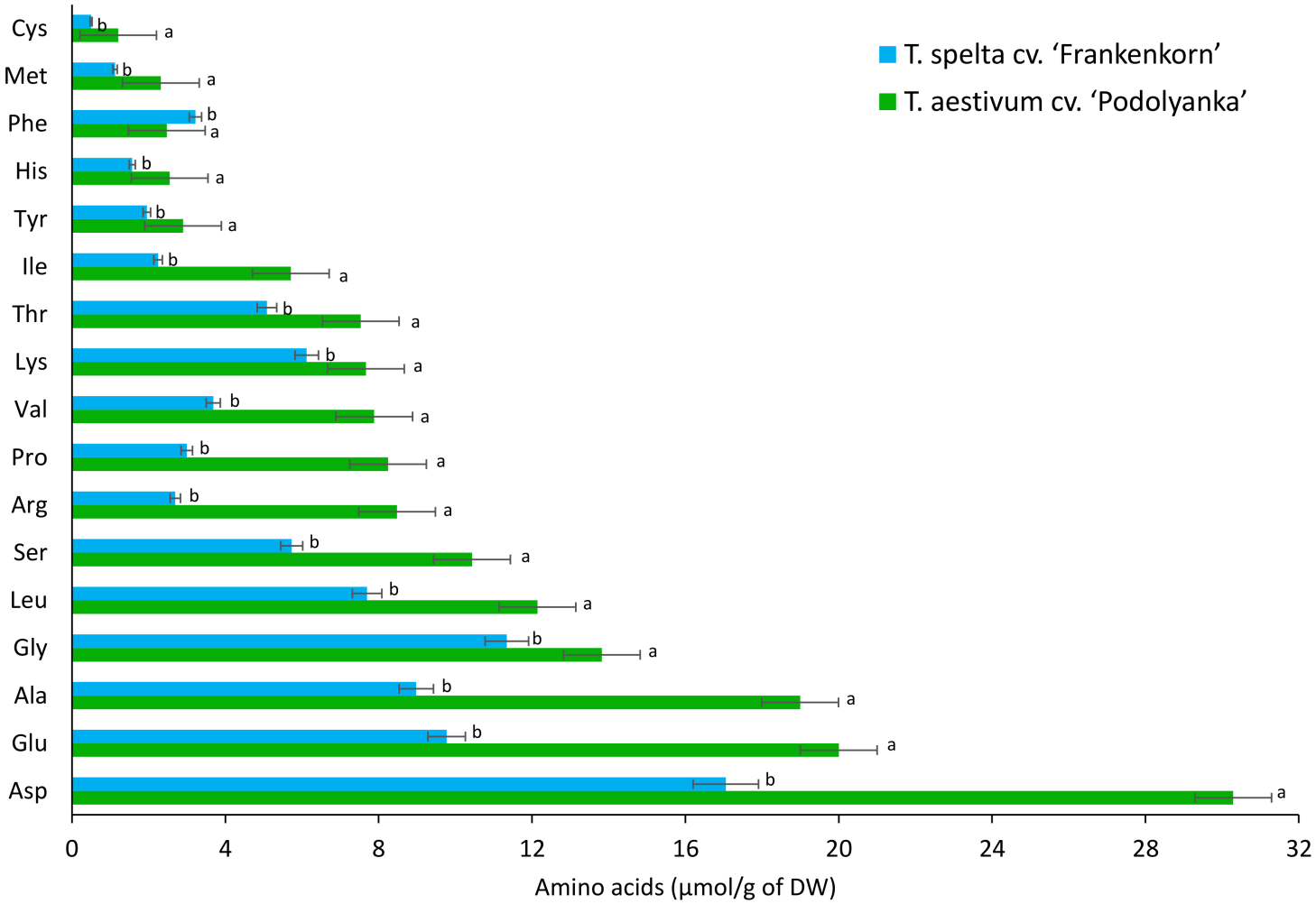


Figure 1

Certain AA content under control conditions in the shoots of 18-day-old plants of *T. aestivum* cv. 'Podolyanka' and *T. spelta* cv. 'Frankencorn'

Abbreviations for AAs: Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine. The data are mean values (n = 3), the bars represent standard deviation (SE); columns for *T. spelta* 'Frankencorn' and *T. aestivum* cv. 'Podolyanka' indicated with different letters (*a* and *b*) are significantly different at $P < 0.05$ (Tukey's test) from each other.

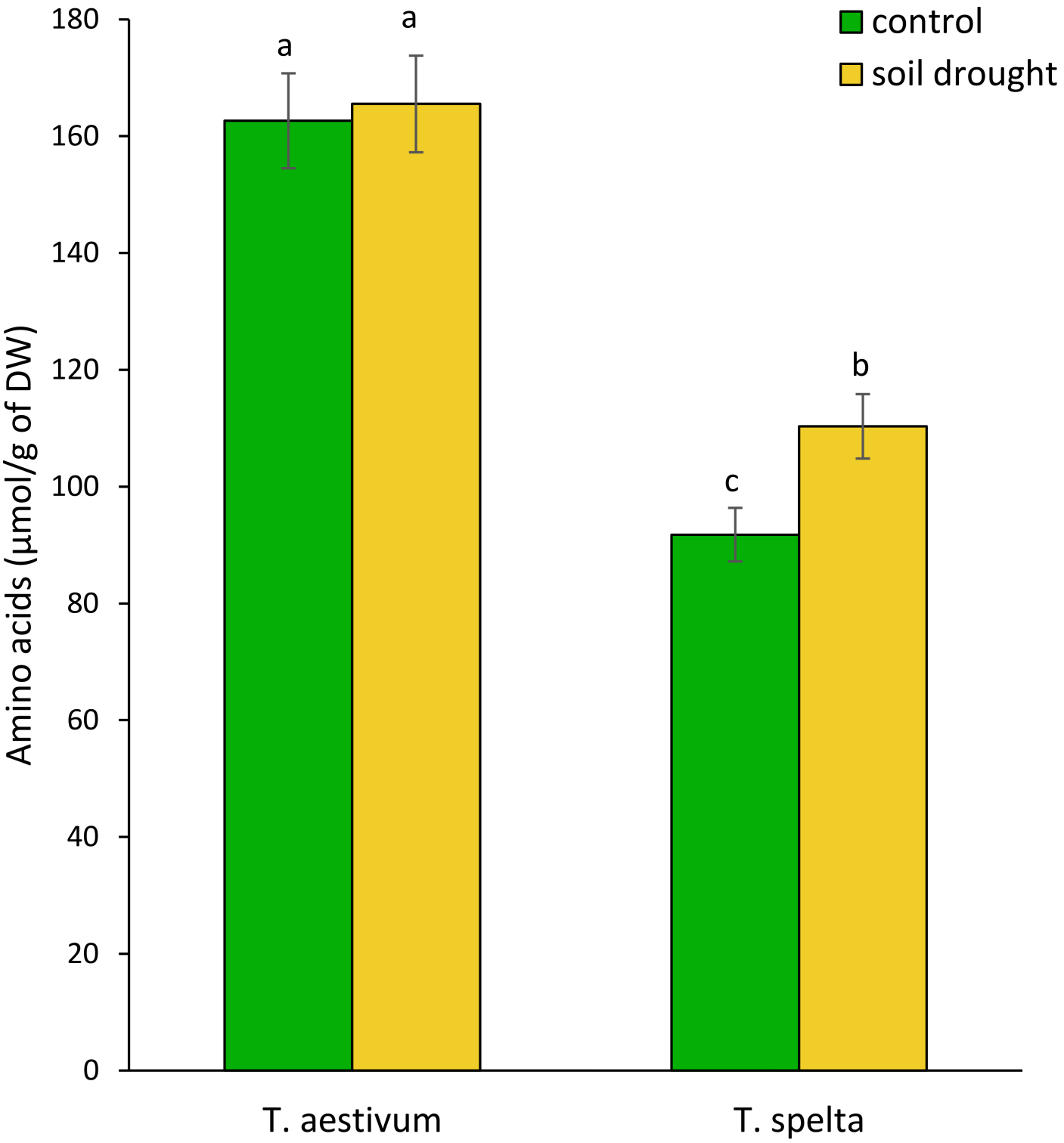


Figure 2

Effect of moderate soil drought on AA content in the shoots of 18-day-old plants of *T. aestivum* cv. 'Podolyanka' and *T. spelta* cv. 'Frankencorn'. The data are mean values (n = 3), the bars represent standard deviation (SE); different letters on the top of columns indicate statistically significant differences at $P \leq 0.05$ (Tukey's test)

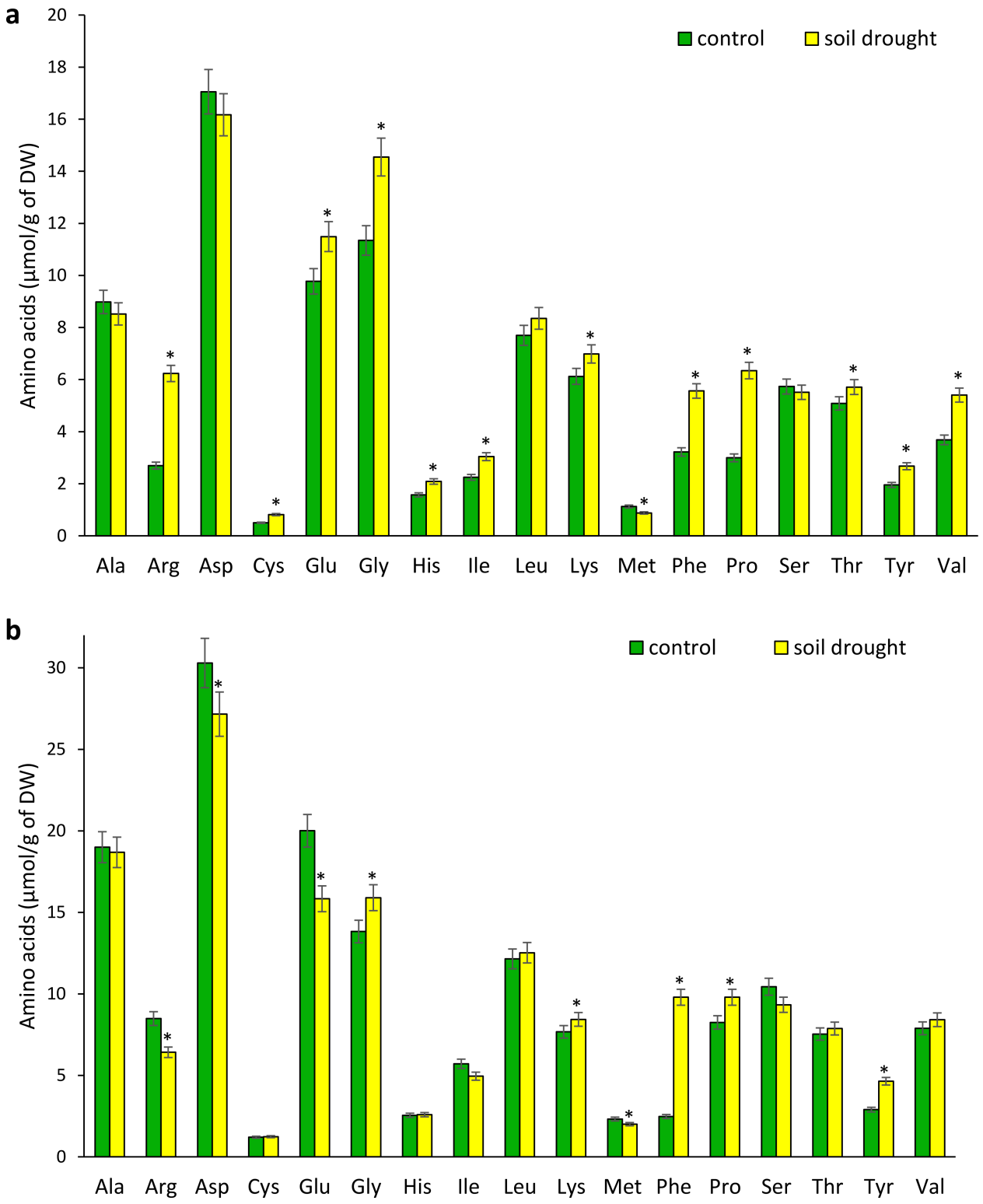


Figure 3

The effect of moderate soil drought on certain AA content in the shoots of 18-day-old plants of *T. spelta* cv. 'Frankenkorn' (a) and *T. aestivum*cv. 'Podolyanka' (b). Thedata are mean values (n = 3), the bars represent standard deviation (SE); asterisk * show significant differences at P < 0.05 vs. control. The absence of an asterisk means there is no statistically significant difference between the "soil drought" group and the control (P > 0.05)

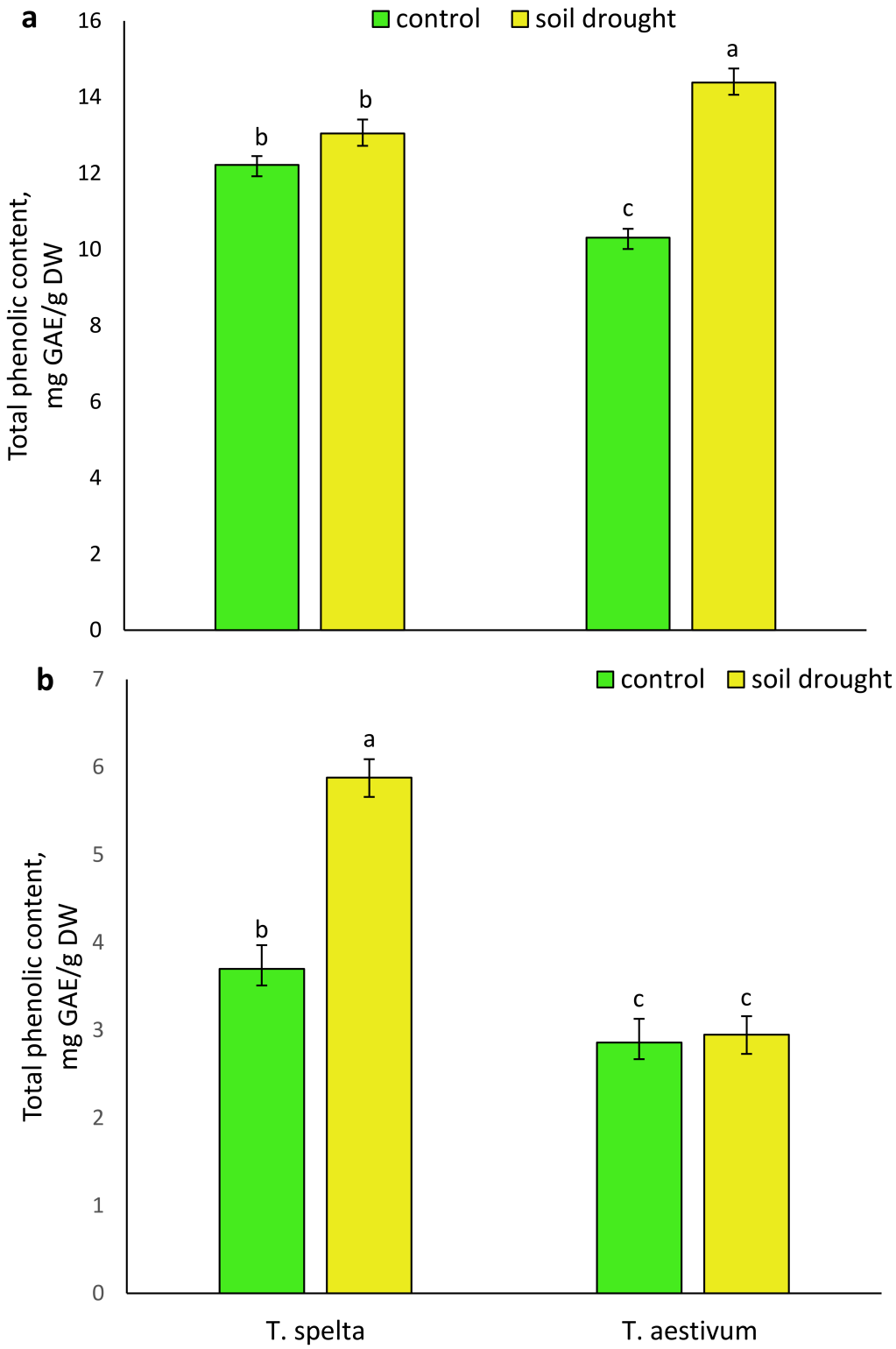


Figure 4

Effect of moderate soil drought on the total phenol content in shoots (a) and roots (b) of 18-day-old plants of *T. spelta* cv. 'Frankenkorn' and *T. aestivum* cv. 'Podolyanka'. The data are mean values (n = 3), the bars represent standard deviation (SE); different letters on the top of columns indicate statistically significant differences at $P \leq 0.05$ (Tukey's test)

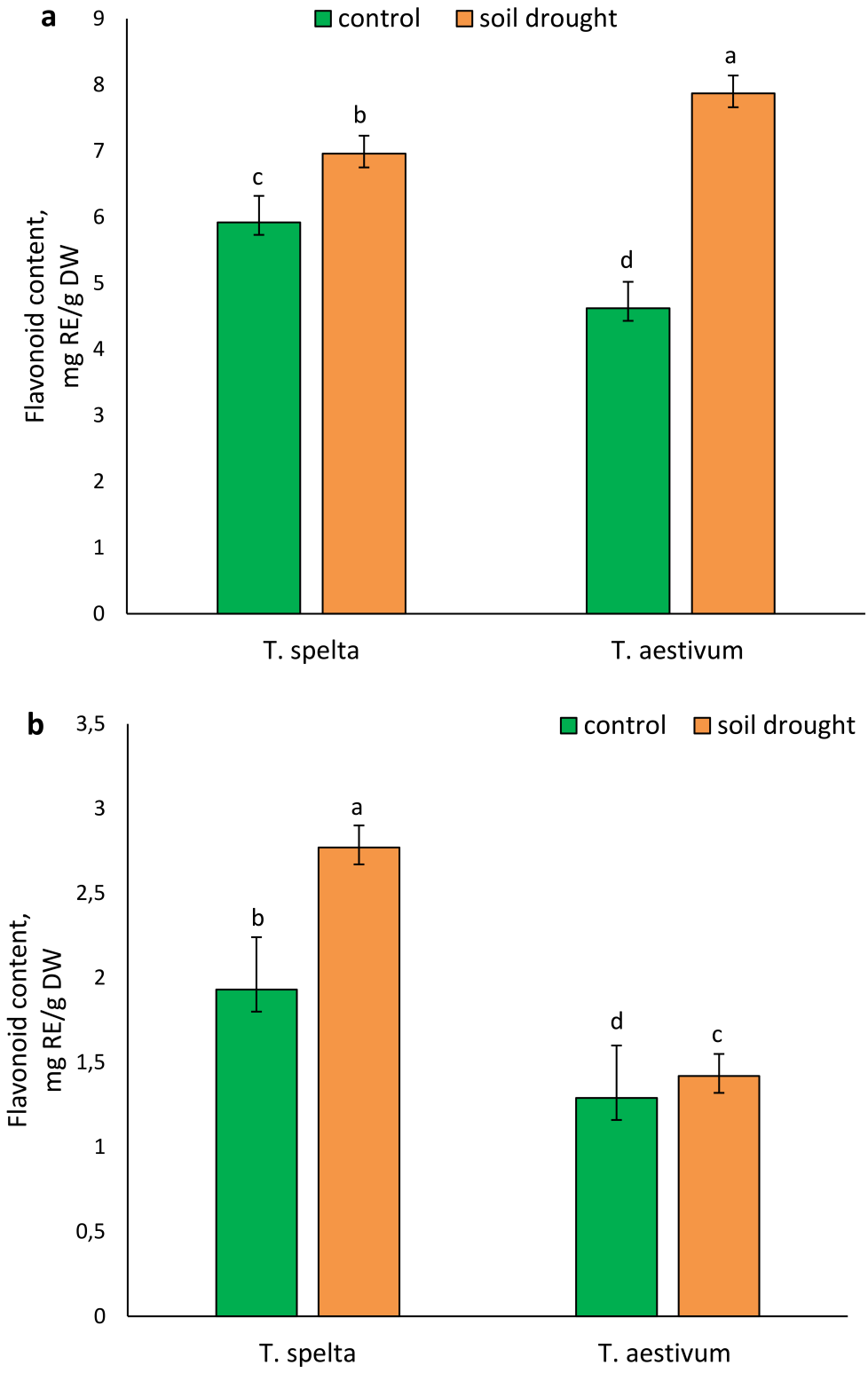


Figure 5

Effect of moderate soil drought on flavonoid content in shoots (a) and roots (b) of 18-day-old plants of *T. spelta* cv. 'Frankenkorn' and *T. aestivum* cv. 'Podolyanka'. The data are mean values (n = 3), the bars represent standard deviation (SE); different letters on the top of columns indicate statistically significant differences at $P \leq 0.05$ (Tukey's test)