MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSE OF SPINACH PLANTS TO WATER STRESS*

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Abstract

The effects of different water stress (control, low, moderate and severe) on some morphological and physiological characteristics of spinach plants were evaluated. The results showed that water stress strongly affects spinach plant growth and metabolism. The increasing water stress decreased the leaf area, petiole length and biomass accumulation, resulting in dramatically lower spinach yields. Photosynthetic pigment contents increased in response to low and moderate level water stress; however, under severe stress conditions their content started to decrease. The increase in water stress also resulted in a higher proline accumulation as well as total phenolic and flavonoid contents. These results lead to the conclusion that spinach plants could produce protective macromolecules and antioxidants in large quantities even under severe drought conditions, suggesting that spinach can be considered a drought-tolerant plant species from a survival point of view.

Keywords: *antioxidants, drought, plant growth, stress adaption*

INTRODUCTION

Drought is one of the major constraints to crop production at global scale. Stress caused by drought induces various morphology, biochemistry and physiology changes in plant, leading to considerable reductions in plant growth and productivity. This problem has been specially pronounced in last decades because of global climate changes and intensifying competition for water resources by industrial, agricultural and other users (Luo *et al.*, 2022).

To overcome the hazard effects of drought, plants have evolved different strategies through evolution, including drought avoidance and tolerance (Adel and Carels, 2023). Drought avoidance is based on constitutive and acquired traits that reduce water loss

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from plants, such as stomatal closure, reduction in the plant leaf area and increase the root-to-shoot mass ratio. On the other hand, drought tolerance refers to the ability of the plant to maintain cell turgor and scavenge harmful substances through cell osmotic regulation and synthesis of protective substances and antioxidants. The traits vary among plant species depending mainly on their genotype, age, growth stage as well as drought severity and duration (Seleiman *et al.*, 2021; Bandurska, 2022).

Spinach (*Spinacia oleracea* L.) is one of the important leafy vegetable crops worldwide. It is a rich source of iron, calcium, magnesium, potassium, carotenoids, folic acid and vitamins K, C, A, E and B-6. As part of a healthy diet, it strengthens the immune system, improves bone health and can help to protect against cardiovascular disease and obesity (El-Sayed, 2020).

Interestingly, very few studies have thus far been carried out on the drought impacts on spinach plants, although drought is the main factor restricting its growth (Kabay, 2023). Since the spinach production and consumption has rapidly increased in recent times (FAOSTAT, 2022), it is important to pay attention to this issue. Without a good understanding of the plant physiological and biochemical response to drought, farmers and scientists are unable to improve water use efficiency in plant production, especially for plants that need a lot of water to grow such as spinach (Nasarullah *et al*., 2022).

The aim of this study was to evaluate the morphological and physiological response of spinach plants exposed to different levels of drought stress. The following morphological and physiological parameters were measured: fresh mass of aerial parts, leaf area, petiole length, yield, photosynthetic pigment contents including chlorophyll *a* content, chlorophyll *b* content and total carotenoid content, proline content, osmotic potential, total phenolic and flavonoid contents and total antioxidant capacity. We hypothesized that spinach plants exposed to drought would exhibit decreases in yield and photosynthetic pigment contents. We also hypothesized that the exposure of spinach plants to drought promotes the accumulation of the protective macromolecules and antioxidants in spinach leaves.

MATERIALS AND METHODS

Plant material

Spinach (*Spinacia oleracea* L. Rembrandt F1), a popular spinach variety, was used as the plant material in this study. It has green to dark green, smooth and oblong in shape leaves, and is especially intended for winter production. Its vegetation period ranges from 45 to 60 days from sowing.

Experimental site

The present study was carried out from December 2023 to March 2024 in a double-span polyethylene-covered greenhouse with natural ventilation at the agricultural experimental station of the Faculty of Agriculture and Food Sciences in Butmir near Sarajevo. The greenhouse was equipped with two side roll-up vents due to humidity regulation. The site is located at 43°49′34.42″ N and 18°19′18.48″ E, at an altitude of 505 m above sea level. The experiment was established on sandy loam soil comprising

32.9% clay, 33.5% silt and 33.6% sand. According to World Reference Base for Soil Resources (IUSS, 2015), the studied soil can be classified as Alluvium, indicating its formation from sediments deposited by flowing water. Soil chemical analysis was performed a few weeks before sowing. The following parameters of soil chemical properties were subject of analysis: soil reaction (soil pH in H_2O and 1 M KCl), organic matter content and content of available forms of phosphorus (P_2O_5) and potassium (K_2O). The examined soil chemical parameters are presented in Tab. 1.

Table 1. Chemical properties of the studied soil

Parameter	Unit	Value	Characteristics
pH KCl	pH unit	7.5	neutral
pH H_2O	pH unit	6.7	reaction
organic matter	%	2.5	medium level
P_2O_5	mg/100 g	35.05	high level
K_2O	mg/100 g	30.04	high level

In accordance with these results the following fertilizer recommendations were given and carried out identical to all plots: the mineral NPK (10:20:30) fertilizer was added in amount of 5 kg/100 m^2 (amounts of fertilizers were recalculated based on examined soil plot area).

Experimental design and treatments

Spinach was seeded on 27 December 2023 in 1 m wide and 20 m long seedbeds at 5 cm between the plants and 20 cm between the rows. The distance between the seedbeds was 1.5 m. Four drought (water stress) levels were tested: (T1) optimum watering - 90% field capacity; (T2) low water deficit stress - 75% field capacity; (T3) moderate water deficit stress - 50% field capacity; and (T4) severe water deficit stress - 25% field capacity. Tensiometers (Soil Moisture Equipment Corp., Santa Barbara, USA) were used to monitor field capacity.

Plants were exposed to different irrigation regimes for 40 days, starting at 10 days after sowing and then re-watered until harvest. The drip irrigation system was used, supported with the water pump. In the winter period, from the end of January until the middle of February 2024, due to very low temperatures, the water was given by manual watering.

Plant sampling and analysis

At the time of technological maturity (sixty days from sowing), the spinach plants were harvested manually (above-ground spinach parts). Morphological parameters and yield were analyzed immediately after harvest. The fresh mass of spinach plants was determined using a digital electronic balance (RADWAG WPX 4500 with 0.01 g accuracy) and the total yield was calculated by summing the single plant mass from each experimental unit. The measurement of the petiole length was performed using an appropriate ruler, while the leaf area was measured using the Millimeter Graph Paper

Method (Pandey and Singh, 2011). A total of 30 spinach plants from each experimental unit were sampled to study the physiological traits: proline content, osmotic potential, photosynthetic pigment contents, total phenolic and flavonoids contents and total antioxidant capacity.

Proline estimation

Proline content was measured according to the method of Bates *et al*. (1973). Fresh leaf samples (0.5 g) were homogenized in 10 mL of aqueous sulfosalicylic acid 3% (w/v), and then filtered through a glass-fiber filter to a plastic test tube. Afterwards, 2 mL of filtrate was mixed with 2 mL of ninhydrin reagent (2.5 g of ninhydrin in 40 mL orthophosphoric acid 6 M and 60 mL of glacial acetic acid) and 2 mL of glacial acetic acid in a test tube and incubated for 1 h at 100 °C. Following incubation, 4 mL of toluene was added to the solution and vigorously mixed by vortex for 30 sec. The reddish layer of mixture was transferred to cuvette and absorbance was read at 520 nm in an Ultrospec 2100 Pro UV–Vis spectrophotometer (Amersham Pharmacia Biotech Biochrom Ltd., Holliston, MA) using toluene as blank. A proline standard curve ranging from 0 to 5 µg/mL proline was used to determine the proline levels of each sample, and then the obtained values were recalculated on fresh mass (µg/g FM).

Leaf osmotic potential estimation

Leaf osmotic potential of spinach leaves was measured according to the method of Ball and Oosterhuis (2005). Ten fully expanded spinach leaves from each experimental unit were collected by hand. Within 10 min of detachment, twenty leaf discs (6 mm diameter) were punched out of spinach leaves and then frozen at -100 °C in a refrigerator freezer. After 24 h, the filter paper disc was inserted between thawed leaf discs and pressed in a vice until a filter paper disk was saturated with the expressed sap. The osmotic potential of this liquid was then measured with a vapor pressure osmometer (Wescor, Logan, UT, USA).

Estimation of photosynthetic pigments

Photosynthetic pigments content including chlorophyll *a*, chlorophyll *b* and total carotenoids content was determined according to the method described by Lichtenthaler and Welburn (1983). 0.2 g of fresh leaves was extracted with 10 mL of pure acetone using a mortar. The pigment extracts were filtered through a coarse filter paper into a 25 mL volumetric flask and diluted to the mark with extract solution (pure acetone). The resulting extracts were assayed spectrophotometrically at 662 nm, 645 nm, and 470 nm. Concentrations of chlorophyll *a*, chlorophyll *b* and total carotenoids (mg/mL) were determined using the following equations:

$$c\ (\text{chlorophyll } a) = 9.784 \times A_{662} - 0.990 \times A_{644}$$

$$c\ (\text{chlorophyll } b) = 21.426 \times A_{644} - 4.650 \times A_{662}$$

$$c\ (\text{total carotenoids}) = 4.695 \times A_{440} - 0.268 \times (c\ \text{chlorophyll } a + c\ \text{chlorophyll } b)$$

The obtained values were then recalculated to fresh mass of leaves (mg/g FM).

Extraction of phenolic compounds

At maturity stage (60 days after sowing), the leaves were picked and dried in air and then the leaves were grinded and stored in paper bags until extraction. The extraction was performed in Erlenmeyer flasks (100 mL) using a 30% aqueous solution of ethanol (1 g of air-dried leaves in a 40 mL of extract solution). The flasks were capped and incubated in a water bath at 40 °C for 2 h. Thereafter, the extracts were filtered through a coarse filter paper into a 25 mL volumetric flask and then diluted to the mark with extract solution. The extracts thus obtained were used for the estimation of the total phenolic and flavonoid content, and for the total antioxidant capacity.

Total phenolic content estimation

The total phenolic content was determined by the Folin-Ciocalteu method (Ough and Amerine, 1998). 0.25 mL of extract, 15 mL of distilled water, and 1.25 mL of Folin-Ciocalteu's reagent (previously diluted 1:2, reagent: distilled water) were placed and mixed thoroughly into a 25 mL flask for 5 min. The flask was incubated at room temperature for 15 min in the dark and then 3.75 mL of saturated sodium carbonate solution (Na_2CO_3) was added. Afterwards, the flask was filled to the mark with 30% ethanol and heated in a water bath at 50 ºC for 30 min. After cooling to room temperature, the resulting mixtures were assayed spectrophotometrically at 765 nm. The gallic acid standard curve ranging from 0 to 500 mg/L was used to determine the total phenolic content of each sample, and then the obtained values were recalculated on fresh mass (mg eq. GA/100 g FM).

Total flavonoid content estimation

The total flavonoid content was determined by the Aluminium chloride colorimetric assay (Zhishen *et al*., 1999). 1 mL of extract, 4 mL of distilled water and 0.3 mL 5% $NaNO_2$ were placed and mixed thoroughly in a 10 mL flask. After 5 min. 0.3 mL 10% $AlCl_3$ was added, and the mixture was incubated at room temperature for 5 min. Then 2 mL of 1 M NaOH was added, and the flask was filled to the mark with distilled water. After 15 min, the resulting mixture was assayed spectrophotometrically at 510 nm. The catechin standard curve ranging from 0 to 100 mg/L was used to determine the total flavonoid content of each sample, and then the obtained values were recalculated on fresh mass (mg eq. C/100 g FM).

Total antioxidant capacity estimation

The ferric reducing antioxidant power (FRAP) assay was used to estimate the total antioxidant capacity (Benzie and Strain, 1996). 240 µL of distilled water, 80 µL of extract, and 2080 µL of FRAP reagent (0.3 mol/L acetate buffer (pH = 3.6), 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mmol/L $FeCl_3$ x 6 H_2O in a ratio 10:1:1) were added into a 10 mL Erlenmeyer flask and then heated in a water bath at 37 ºC for 5 min. After cooling to room temperature, the resulting mixtures were assayed spectrophotometrically at 595 nm. The $FeSO_4$ x $7H_2O$ standard curve ranging from 0 to 2000 µmol/L was used to determine the total antioxidant capacity of each sample and then the obtained values were recalculated on fresh mass (µmol Fe^{2+}/100 g FM).

Statistical analysis

All assays were performed in triplicates and the results were expressed as means ± standard deviation. The significance of differences was determined via one-way analysis of variance and least-significant-difference test (LSD test) using SPSS 22.0 software package program (IBM, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Growth and morphological parameters

As expected, the fresh mass of aerial parts of spinach plants were adversely affected by the drought. The highest fresh mass was obtained in the control (no-stress) treatment, whereas the lowest was recorded in spinach plants exposed to severe drought stress conditions. Reduction in fresh mass in spinach plants due to drought has also been reported earlier in numerous studies (Ors and Suarez, 2017; Yavuz *et al*., 2022).

In this study, drought also caused a reduction in leaf area and this reduction was more pronounced with increasing drought duration (Tab. II). Yang *et al*. (2021) have reported that the decrease in leaf area is mainly due to reduced cell division and expansion under drought conditions, which results in decreased spinach yield. These findings strongly support the hypothesis that spinach plants require regular watering to keep spinach growing fast. Contrastingly, if drought conditions persist, the lack of water in leaves will adversely affect cell growth, resulting in dramatically lower spinach yields (Sun *et al*., 2023). This study's results agree with such observations.

Table 2. Effects of water stress on plant morphological characteristics

Treatment*	Fresh mass of aerial parts (g per plant)	Leaf area (cm^2)	Petiole length (cm)	Yield (kg/m^2)
T1 (90% FC)	$93.3 \pm 10.2^{a**}$	80.9 ± 11.5^{a}	13.5 ± 0.3^{a}	8.9 ± 1.3^{a}
T2 (75% FC)	50.7 ± 7.1^{b}	49.4 ± 10.1^{b}	8.6 ± 0.1^{b}	4.6 ± 0.7^{b}
T3 (50% FC)	34.2 ± 8.0^{c}	32.2 ± 8.4^{c}	6.4 ± 0.2^{c}	3.0 ± 0.8^{c}
T4 (25% FC)	18.5 ± 9.9^{d}	11.2 ± 7.3^{d}	2.7 ± 0.2^{d}	1.5 ± 0.9^{d}
$LSD_{0.05}$	12.71	9.28	1.42	1.44

*Treatment: (T1) optimum watering - 90% field capacity; (T2) low stress - 75% field capacity; (T3) moderate stress - 50% field capacity; and (T4) severe stress - 25 % field capacity

**The mean followed by different letters in the same column indicate a significant difference at $P < 0.05$.

Drought also decreased the petiole length as compared to non-water stress treatment. Predictably, petiole length significantly decreased with increasing drought duration. Reduction in petiole length due to drought has also been reported in other studies for a number of plant species (Durigon *et al*., 2019; Enkhbat *et al*., 2022). In essence, reduced total biomass, leaf area, and petiole length are all plant mechanisms for improving water use efficiency and reducing damage under drought stress conditions (Farooq *et al*., 2009; Lovelli *et al*., 2017).

Physiological parameters

Proline, an amino acid, plays a highly important role in plants (Chahine *et al*., 2021). It stabilizes the osmotic differences between the cell's surroundings and cytosol, thus enhancing the cell's potential to maintain water without hampering the normal metabolism (Hayat *et al*., 2012). Besides acting as an osmoprotectant, proline acts as an effective quencher of reactive oxygen species. Due to its unique properties, proline greatly improve plants' ability to overcome drought stress conditions, thus the rapid increase in proline accumulation is one of the most significant responses in plants to water stress (Elmasry and Al-Maracy, 2023). In this study, the lowest values of proline content were observed in the control untreated spinach plants (FC 90%), and highest in the spinach plants under severe stress (FC 25%) (Tab. 3).

Table 3. Effects of water stress on plant physiological characteristics

Treatment*	Proline (µg/g)	Water potential (Mpa)	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Carotenoids (mg/g)
T1 (90% FC)	1.36 ± 1.2^{c**}	-0.56 ± 0.1^{a}	0.97 ± 0.3^{c}	0.43 ± 0.2^{c}	0.36 ± 0.1^{b}
T2 (75% FC)	19.01 ± 7.4^{b}	-0.72 ± 0.1^{b}	1.24 ± 0.1^{bc}	0.53 ± 0.2^{c}	0.45 ± 0.1^{b}
T3 (50% FC)	24.24 ± 9.0^{b}	-0.78 ± 0.1^{b}	1.96 ± 0.1^{a}	1.05 ± 0.2^{a}	0.69 ± 0.2^{a}
T4 (25% FC)	230.54 ± 9.9^{a}	-1.19 ± 0.2^{c}	1.29 ± 0.2^{b}	0.81 ± 0.1^{b}	0.61 ± 0.1^{a}
LSD $_{0.05}$	12.01	0.11	0.28	0.22	0.14

*Treatment: (T1) optimum watering - 90% field capacity; (T2) low stress - 75% field capacity; (T3) moderate stress - 50% field capacity; and (T4) severe stress - 25 % field capacity

**The mean followed by different letters in the same column indicate a significant difference at $P < 0.05$.

The results of this study also showed that the proline content in spinach leaves increased significantly with increasing drought stress duration. Accordingly, the proline content in spinach leaves in treatments T2, T3, and T4 was 13.9, 17.8 and 169.5, times respectively higher than the control group (T1). These results indicate that cellular water uptake in prolonged drought stress conditions became more complex, and therefore, plants accumulate a large amount of osmotic active substance such as proline, which helps in maintaining cell water potential under water-deficit conditions (Chun *et al*., 2018). Numerous studies have also found evidence for a positive relationship between proline accumulation and drought treatment duration (Fu *et al*., 2018; Hosseinifard *et al*., 2022).On the other hand, an inverse relationship was observed between the level of water stress and the water potential of the leaves, indicating that drought restricts water supply and thus decreases leaf water potential (Ding *et al*., 2021). The control treatment i.e. no water stress treatment (T1) registered the highest leaf water potential value, whereas the severe stress treatment (T4) recorded the lowest water potential value (Tab.

III). These results are in line with those reported by Reyes *et al*. (2018). Hence, the decline of leaf water potential can be used as an important indicator of plant drought stress.

In this study, the photosynthetic pigment contents (chlorophyll *a*, chlorophyll *b* and total carotenoids) in plants showed considerable variations in response to the drought. The content of chlorophyll *a* and chlorophyll *b* in spinach leaves first rise in response to low-to-moderate level water stress (T2, T3) and then start to decrease with increasing drought stress duration (T4). A similar pattern was also found in total carotenoid contents; however, the changes in total carotenoid contents between spinach plants exposed to moderate and severe water stress were not statistically significant (Table 3). These results were inconsistent with the previously reported that drought reduces leaf photosynthetic pigment contents regardless of the stress level (Baccari *et al*., 2020; Juzoń *et al*., 2020). Anjum *et al*. (2011) reported that the water deficit due to drought induces the degradation of the thylakoid membrane within chloroplasts, resulting in chlorophyll breakdown.

Contrastingly, several studies have shown results where some plants including spinach exhibit increased chlorophyll content under low-level drought stress conditions (Xu and Leskovar, 2015; Rustioni and Bianchi, 2021). Interestingly, Zhang *et al*. (2014) did not observed any changes in chlorophyll content in spinach as affected by drought. In summary, these findings indicate that photosynthetic pigment contents in plants can vary significantly under water stress conditions, which primarily depends on water stress duration and intensity as well as plant tolerance to drought. Plants that maintain a relatively higher chlorophyll content under drought are generally have higher photosynthetic efficiency, and thus stronger tolerance to drought (Li *et al*., 2006). From this point of view, spinach could be considered as a drought-tolerant plant and this finding is in line with the previous studies of Yousif *et al*. (2010) and Kovár and Olšovská (2020). It is, nevertheless, important to note that the intense or prolonged drought period will undoubtedly negatively affect the chlorophyll synthesis in plants, resulting in decreased photosynthetic efficiency.

Interestingly in this study, the carotenoid content begins to decline at the same time as chlorophyll *a* and chlorophyll *b* content, but at a much slower rate, indicating that the chlorophyll content decreases faster than carotenoid content (Lichtenthaler and Babani, 2022). The ability to maintain carotenoid content can enhance plant tolerance to drought considering the fact that carotenoids protect chlorophyll pigments from photo-oxidation (Crupi *et al*., 2023). Numerous studies have also demonstrated that carotenoids play an important role in the mechanisms protecting the photosynthetic apparatus against reactive oxygen species initiated by various harmful environmental factors (Latowski *et al*., 2011; Dumanović *et al*., 2021). Moreover, this is of special significance, because the increase of reactive oxygen species in the plant cells causes oxidative damage and ultimately cell death (Huang *et al.,* 2019).

In the present study, drought stress remarkably increased the amounts of total phenolic and flavonoid contents in spinach leaves (Tab. 4). Increased synthesis of phenolic compounds under drought has also been reported in a number of plant species,

indicating that accumulation of phenolic compounds is one of the strategies adopted by plants to counteract drought stress (Cramer *et al*., 2011; Nicolas-Espinosa *et al*., 2023).

Table 4. Effects of water stress on antioxidant properties of spinach leaves

Treatment[1]	Total phenolics (mg/100 g)	Total flavonoids (mg 100/g)	Antioxidant capacity (μmol Fe^{2+}/100 g)
T1 (100% FC)	46.5 ± 4.6[d**]	12.7 ± 0.9[c]	337.1 ± 11.1[d]
T2 (75% FC)	67.9 ± 5.8[c]	14.4 ± 1.8[c]	456.6 ± 49.2[c]
T3 (50% FC)	78.4 ± 4.5[b]	17.4 ± 3.5[b]	481.9 ± 56.1[b]
T4 (25% FC)	132.4 ± 15.1[a]	26.3 ± 3.3[a]	769.7 ± 75.9[a]
$LSD_{0.05}$	6.81	1.73	22.64

[*]Treatment: (T1) optimum watering - 90% field capacity; (T2) low stress - 75% field capacity; (T3) moderate stress - 50% field capacity; and (T4) severe stress - 25 % field capacity
[**]The mean followed by different letters in the same column indicate a significant difference at $P < 0.05$.

Study results also showed that the longer drought stress duration caused a higher increase in total phenolic and flavonoid contents. These results are expected since it is generally known that phenolic compounds play an important role in plant protection against reactive oxygen species (Andabaka *et al*., 2022), and plants therefore tend to increase their accumulation under stress conditions (Sarker and Oba, 2018; Misra *et al*., 2023). On the other hand, an opposite observation was found in several studies where exposure of plants to drought decreased the total phenolic and flavonoid contents in leaves, especially under intense drought periods (Król *et al*., 2014; Seleiman *et al*., 2021). Namely, the exposure of plant to long-term drought conditions rapidly triggers the state of stress in plants, leading to physiological destabilization, oxidative damage and disrupted metabolism. Therefore, the ability of the plants to synthesize phenolic compounds and other antioxidant substances in such conditions is significantly reduced. However, the results of this study revealed that the spinach plants could produce phenolic compounds in high amounts even in long-term drought conditions.
In this study, drought caused an increase in total antioxidant capacity (FRAP values) in comparison with control (non-stress) treatment. The study findings also showed that the FRAP values in spinach leaves increased with increasing drought stress levels. A similar pattern was observed in the relationship between drought stress levels and total phenolic and flavonoid contents, indicating that phenolic compounds greatly contribute to the antioxidant activity of plants. These results are consistent with the findings of previous studies (Lyu *et al*., 2023; Zeng *et al*., 2023).

CONCLUSIONS

The water stress resulted in reduction in spinach growth parameters and biomass accumulation and this reduction was more pronounced with increasing drought duration. The content of chlorophyll *a*, chlorophyll *b* and total carotenoid content

increased in response to low and moderate level water stress; however, under severe drought stress conditions their content started to decrease. The increase in water stress also resulted in a higher proline accumulation as well as phenolic and flavonoid contents, regardless of the stress levels. These points lead to the conclusion that spinach plants have the ability to produce protective macromolecules and antioxidants in high amounts even in severe drought conditions, suggesting that spinach could be considered as a drought-tolerant plant species from a survival point of view.

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MORFOLOŠKI I FIZIOLOŠKI ODGOVOR BILJAKA ŠPINATA NA VODNI STRES

Sažetak

U ovom istraživanju ispitivan je uticaj različitih nivoa vodnog stresa (nizak, umjeren i visok) na određene morfološke i fiziološke karakteristike biljaka špinata. Rezultati su pokazali da izlaganje biljaka špinata vodnom stresu snažno utiče na njihov rast i metabolizam. S povećanjem intenziteta vodnog stresa smanjivala se površina lista, dužina peteljke i biomasa biljke, što je rezultiralo znatno nižim prinosima špinata. Sadržaj fotosintetskih pigmenata u listovima špinata se povećao u uslovima niskog i umjerenog vodnog stresa. Međutim, u uslovima visokog nivoa vodnog stresa njihov sadržaj je počeo padati. Povećanje vodnog stresa takođe je rezultiralo većom akumulacijom prolina, kao i ukupnim sadržajem fenola i flavonoida. Ovi rezultati dovode do zaključka da biljke špinata imaju sposobnost sintetisati veliku količinu zaštitnih makromolekula i antioksidanasa čak i u uslovima kada su duže vremena izložene suši, iz čega proizilazi da se špinat s gledišta egzistencije može smatrati biljnom vrstom s visokim stepenom tolerancije na vodni stres.

Ključne riječi: *antioksidansi, suša, biljni rast, adaptacija na stres*