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PHYSIOLOGICAL RESPONSE OF BASIL PLANTS (*OCIMUM BASILICUM* L.) TO DROUGHT

SUMMARY

Basil (*Ocimum basilicum* L.) is one of the most widely cultivated medicinal and aromatic species and the understanding of its physiological behavior under drought is of great importance for both basil producers and plant scientists. The aim of this study was to evaluate the physiological response of basil plants to different drought levels. This study found that drought significantly increased the activities of superoxide dismutase, guaiacol peroxidase, pyrogallol peroxidase and catalase, indicating that these enzymes play a prominent role in detoxification of reactive oxygen species and thus in plant drought tolerance. Exposure of basil plants to moderate and severe drought resulted in higher accumulation of proline and total phenolics and flavonoids, suggesting that basil plants under prolonged drought conditions tend to improve their drought tolerance by inducing the production of these compounds in leaves. Interestingly, mild drought did not negatively affect the photosynthetic pigment content as well as proline and total phenolic and flavonoid content in basil leaves as compared with control plants. In light of this fact, as well as the fact that the basil plants have the ability to produce protective macromolecules and antioxidants in high amounts in severe drought conditions, it can be concluded that basil plants tolerate drought conditions well.

Keywords: antioxidants, defense mechanisms, leaves, osmoregulation, water shortage

INTRODUCTION

Plants, as sessile organisms, are unable to escape unfavorable conditions in their environment; however, they have the ability to adapt to environmental change by regulating their own growth and development (Knudsen *et al.*, 2018).

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Over the course of the evolution plants have developed a very sophisticated mechanisms to sense and respond to environmental variations including drought caused either by water shortage or by the presence of excess salt in the soil (Lamers *et al.,* 2020). Regardless of the cause of drought, the fact is drought causes various biochemical and physiological changes in plants, which adversely affect crop yield and consequently agricultural production even in areas that are relatively well supplied with water resources. This problem has sharply increased over the past decades as a result of global warming (Gao *et al.,* 2018; Seleiman *et al.,* 2021).

Drought triggers a wide array of responses in plants ranging from molecular, cellular to whole-plant levels. The responses of plants to drought at the whole-plant level include, among others, a reduction in leaf area, stomatal closure, leaf folding and leaf abscission, all in order to reduce water loss (Wongla *et al.,* 2023). Under drought conditions, plants also transport more nutrients to the roots, improving their expansion and thus the ability to capture water from deeper soil layers. At the cellular level, plants tend to increase the production of osmolytes such as sugar alcohols and proline amino acid with a view to maintain homeostasis under drought conditions (Mahmood *et al.,* 2019). Plants also activate both enzymatic and non-enzymatic antioxidative defense system to scavenge reactive oxygen species (ROS) whose production in the plant cells is elevated under drought (Fetsiukh *et al.,* 2022). Enzymatic antioxidative defense system consists of numerous antioxidant enzymes such as superoxide dismutase, guaiacol peroxidase, pyrogallol peroxidase and catalase, which play a key role in ROS degradation (Rajput *et al.,* 2021). Non-enzymatic antioxidative defense system is comprised of different compounds with antioxidant properties such as ascorbic acid, carotenoids, glutathione, and phenolic compounds. Some of these compounds act directly in the ROS detoxification, while some act indirectly as cofactors for antioxidant enzymes (Chaudhary *et al.,* 2023).

The responses of plants to drought vary greatly among plant species which depend mainly on plant genotype, drought severity and duration as well as the interaction between plants and environment in which they grow (Farjam *et al.,* 2014). The plant response to drought is also highly influenced by the developmental stage of the plant (Bandurska, 2022). In this light, an understanding the physiological response of plants to water deficit is of a crucial importance for predicting plant behavior under drought. It is also a key determinant for improving crop productivity in a changing climate (Fahad *et al.,* 2017).

Basil (*Ocimum basilicum* L.) is one of the most widely cultivated medicinal and aromatic species in the world, with its origins traced back to India. It has been used as a spice throughout the world for centuries, and recently, there has been an increasing focus on its medicinal properties (Romano *et al.,* 2022).

Like a number of other plant species, basil is often exposed to adverse environmental conditions, including primarily the soil water deficiency. Therefore, the aim of this study was to evaluate the physiological response of

basil plants to different levels of drought stress. We hypothesized that drought-affected basil plants would exhibit increases in physiological performance measures such as proline content, total phenolic and flavonoid content, total antioxidant capacity and antioxidant enzymatic activities in leaves. We also hypothesized that drought-affected basil plants would exhibit declines in photosynthetic pigment content in leaves as compared to non-stressed basil plants.

MATERIAL AND METHODS

Experimental design

The experiment was conducted from the end-August 2023 to mid-September 2023 in a greenhouse at the experimental station of the Faculty of Agriculture and Food Science in Sarajevo (43°49′34.41″ N and 18°19′18.47″ E). Over the course of the experiment, air temperature in greenhouse was maintained at 25 ± 4 °C during the day and 18 ± 4 °C during the night. White shade cloth was used to reduce light and heat intensity during hot days.

Basil plants used in the experiment were produced in the nursery near the greenhouse and there was no observed significant difference between basil plants in terms of vigor, size and appearance. Before setting up an experiment, the basil plants were in pre-flowering stage (Figure 1).

Figure 1. Basil plants used in the experiment

For the drought treatment experiment, basil plants were grown in pots (8 cm diameter × 12 cm height, one plant per plot) previously filled with substrate Florabella (Klasmann-Deilmann, Germany).

Four drought stress levels were applied in the experiment and each level included twelve individuals: (1) non-stress (70-80% of the maximum substrate water-holding capacity was designated as the control (non-stress) treatment), (2) mild drought stress (three days without water), (3) moderate drought stress (six days without water), and (4) severe drought stress (nine days without water). After drought treatments, basil plants were regularly watered until the end of experiment (18th day of experiment).

The leaves of basil plants were collected twice over the course of the experiment: on the 9th day of experiment and on the 18th day of experiment (after a 9-day water recovery period). During the 9th day of the experiment, fresh leaves of basil plants were sampled to study the following physiological traits: proline content, photosynthetic pigment content including chlorophyll *a*, chlorophyll *b* and total carotenoids, and the activities of following antioxidant enzymes; superoxide dismutase, guaiacol peroxidase, pyrogallol peroxidase and catalase. For this purpose, three fully developed and healthy leaves from each basil plant were collected and immediately frozen in liquid nitrogen and kept at -80 °C until analysis.

After sampling, basil plants in all drought treatments were re-watered to 70-80% substrate water-holding capacity for the next nine days to assess the impact of drought treatments on the phenolic and flavonoid biosynthesis in plants. At the end of the drought/re-watering experiments (18th day of experiment), five fully developed leaves from each basil plant leaves were cut, dried in oven at 40 °C for 48 h, ground into powder with an electric blender, and then placed in paper bags until analysis.

Chemicals

Potassium dihydrogen phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), bovine serum albumin, Bradford reagent, dithiothreitol (DTT), polyvinylpyrrolidone-40 (PVP-40), guaiacol, pyrogallol, hydrogen peroxide, cytochrome C oxidase, ethylenediaminetetraacetic acid (EDTA), xanthine and xanthine oxidase and were obtained from Sigma-Aldrich (St. Louis, MO, USA). The other chemicals used throughout the analysis, i.e. ninhydrin reagent, proline amino acid, glacial acetic acid, acetone, ethanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminum chloride, sodium hydroxide, catechin, iron (III) chloride, 2,4,6-tripyridyl-s-triazine and iron (II) sulfate heptahydrate were obtained from Merck (Darmstadt, Germany).

Protein extraction and determination

0.5 g fresh leaves of basil plants were frozen in liquid nitrogen and then ground into a fine powder using a pestle and mortar. In order to separate the soluble proteins from the homogenized powder, 1.5 mL of a 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM DTT and 0.05% PVP-40 was added. The homogenate was centrifuged at 10,000 × g for 10 min at 4 °C to obtain a clear supernatant. The resulting supernatant fraction was then transferred to a new tube for protein concentration and antioxidant enzyme activity measurements. Protein concentration was measured using the Bradford method with bovine serum albumin serving as the reference standard (Bradford, 1976).

Superoxide dismutase activity assay

Superoxide dismutase (SOD) activity was determined spectrophotometrically using the method of McCord and Fridovich (1969). This method was based on the SOD ability to inhibit reduction of cytochrome C by superoxide anions generated from xanthine-xanthine oxidase system. The assay reaction mixture (850 - 1.000 µl) consisted of 50 mM phosphate buffer (pH 7.8),

0.1 mM EDTA, 50 µM xanthine, 10 µM cytochrome C, and xanthine oxidase in amounts sufficient to cause an increase in absorbance of 0.025 min^{-1} at 550 nm. The protein extract (10 - 50 µl) were added to the reaction mixture and the rate of reduction of cytochrome c was followed spectrophotometrically at 550 nm. The SOD activity was expressed in units of SOD activity (U) per mg of protein. One unit of SOD activity was defined as the amount of SOD needed to inhibit 50% of the reduction rate of cytochrome C.

Catalase activity assay

Catalase (CAT) activity was determined spectrophotometrically using the method of Aebi (1984). The reaction medium was prepared by mixing 950 µl of the reaction mixture (10 mM H_2O_2 in 50 mM potassium phosphate buffer pH 7.0) and 50 µl of protein extract. CAT activity was assayed by observing the rate of decrease in absorbance at 240 nm at an interval of 10 sec up to 120 sec, as a consequence of H_2O_2 decomposition. The results were expressed as micromoles decomposed H_2O_2 per min per mg of protein.

Guaiacol peroxidase activity assay

Guaiacol peroxidase (GPOD) activity was determined spectrophotometrically using the method of Chance and Maehly (1954). 900 µl of the reaction mixture (50 mM potassium phosphate buffer pH 7.0 supplemented with 18 mM guaiacol and 5 mM H_2O_2) and 100 µl of protein extract were mixed to obtain reaction medium. GPOD activity was assayed by observing the rate of increase in absorbance at 470 nm at an interval of 15 sec up to 180 sec, as a result of guaiacol oxidation. The results were expressed as micromoles tetraguaiacol (product of guaiacol oxidation) per min per mg of protein.

Pyrogallol peroxidase activity assay

Pyrogallol peroxidase (PPOD) activity was determined spectrophotometrically using the method of Maehly and Chance (1954). The reaction medium was prepared by mixing 950 µl of the reaction mixture (50 mM potassium phosphate buffer pH 7.0 amended with 20 mM pyrogallol and 1 mM H_2O_2) and 50 µl of protein extract. PPOD activity was assayed by observing the rate of increase in absorbance at 430 nm, at an interval of 15 sec up to 180 sec, as a result of pyrogallol oxidation. The results were expressed as micromoles purpurogallin (product of pyrogallol oxidation) per min per mg of protein.

Proline estimation

Proline content was determined spectrophotometrically using the method of Bates et al. (1973). 0.5 g fresh leaves of basil plants leaves were homogenized in 10 mL of 3% aqueous sulfosalicylic acid, and then filtered through a glass microfiber filter to a plastic test tube. 2 mL of filtrate was mixed with 2 mL of freshly prepared ninhydrin reagent (2.5 g of ninhydrin in 40 mL 6 M orthophosphoric acid and 60 mL of glacial acetic acid) and 2 mL of glacial acetic acid in a test tube and then the mixture was incubated for 1 h at 100 °C. After incubation, 4 mL of toluene was added, the mixture was gently mixed with a vortex for 30 sec and then left at room temperature for 15 min. The upper reddish layer of mixture was transferred to cuvette and absorbance was read at 520 nm

using toluene as blank. A proline standard curve ranging from 0 to 5 μg mL^{-1} was used to determine the proline levels of each sample, and the obtained values were than recalculated to fresh mass of leaves (μg g^{-1} FM).

Estimation of photosynthetic pigment content

The content of chlorophyll *a*, chlorophyll *b* and total carotenoids was determined spectrophotometrically using the method of Lichtenthaler and Weliburn (1983). 0.2 g of fresh basil leaves was extracted with 10 mL of pure acetone using a pestle and mortar. After that, the extract was filtered through coarse filter paper into a 25 mL volumetric flask and diluted to the mark with extract solution (pure acetone). The filtrates were assayed spectrophotometrically at 662, 645 and 470 nm and the concentrations of chlorophyll *a*, chlorophyll *b* and total carotenoids (mg mL^{-1}) were determined using the following equations:

c (chlorophyll *a*) = 9.784 x A662 - 0.990 x A644

c (chlorophyll b) = 21.426 x A644 - 4.650 x A662

c (total carotenoids) = 4.695 x A440 - 0.268 x (c chlorophyll *a* + c chlorophyll *b*)

The obtained values were than recalculated to fresh mass of leaves (mg g^{-1} FM).

Extraction of phenolic compounds

1 g of ground and dried basil leaves was placed in an Erlenmeyer flask (100 mL) and macerated with 40 mL of 30% ethanol solution for 6 h with frequent shaking and then left to stand at room temperature for 18 h. Thereafter, the extract was filtered through a coarse filter paper into a 25 mL volumetric flask and then diluted to the mark with extract solution. Each extract was tested for total phenolic and flavonoid content and total antioxidant activity.

Total phenolic content estimation

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu method (Ough and Amerine, 1988). 0.1 mL of extract, 6 mL of distilled water, and 0.5 mL of Folin-Ciocalteu's reagent (previously diluted 1:2 with distilled water) were addedto a 10 mL flask and then mixed thoroughly for 5 min. After that, 1.5 mL of saturated solution of Na_2CO_3 was added and then the flask was filled to the mark with 30% ethanol solution and left at room temperature for 1 h. Thereafter, the resulting mixtures were assayed spectrophotometrically at 765 nm. The gallic acid standard curve ranging from 0 to 500 mg L^{-1} was used to determine the total phenolic content of each sample, and then the obtained values were recalculated to fresh mass of leaves (mg eq. GA 100 g^{-1} FM).

Total flavonoid content estimation

The total flavonoid content was determined spectrophotometrically using the Aluminium chloride colorimetric assay (Zhishen *et al.*, 1999). 1 mL of extract, 4 mL of distilled water, 0.3 mL 5% $NaNO_2$ and 0.3 mL 10% $AlCl_3$ were placed into a 10 mL flask and mixed thoroughly for 5 min. Then 2 mL of 1 mol L^{-1} NaOH was added and the flask was filled to the mark with distilled water. After 10 min, the resulting mixtures were assayed spectrophotometrically at 510 nm. The catechin standard curve ranging from 0 to 100 mg L^{-1} was used to

determine the total flavonoid content of each sample, and then the obtained values were recalculated to fresh mass of leaves (mg eq. C 100 g^{-1} FM).

Total antioxidant capacity estimation

The total antioxidant capacity was determined spectrophotometrically using the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). A 10 mL volumetric flask was filled with 200 µL of distilled water, 100 µL of extract, and 2000 µL of FRAP reagent (0.3 mol L^{-1} acetate buffer (pH = 3.6), 10 mmol L^{-1} TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mmol L^{-1} $FeCl_3$ x 6 H_2O in a ratio 10:1:1) and then left at room temperature for 15 min. The resulting mixtures were assayed spectrophotometrically at 595 nm. The $FeSO_4$ x $7H_2O$ standard curve ranging from 0 to 2000 µmol L^{-1} was used to determine the total antioxidant capacity of each sample and then the obtained values were recalculated to fresh mass of leaves (µmol Fe^{2+} 100 g^{-1} FM).

Statistical analysis

All assays were performed in triplicates and the results were expressed as means ± standard deviation. The significant differences between treatments were determined using the one-way analysis of variance (ANOVA) and least significant difference (LSD) test at 5% level of probability.

RESULTS

The study found that proline content significantly increased in basil leaves under both moderate drought and severe drought (Table 1). In this regard, the proline content in basil leaves under moderate (T3) and severe drought (T4) was 0.62 and 3.25 times higher, respectively, than the control group (T1).

Table 1. Effects of drought on proline and leaf photosynthetic pigment contents in basil plants

Drought treatment	Proline (µg g^{-1} FM[2])	Chlorophyll *a* (mg g^{-1} FM)	Chlorophyll *b* (mg g^{-1} FM)	Carotenoids (mg g^{-1} FM)
T1 (control)	30.03 ± 2.01^{c}	1.02 ± 0.25^{a}	0.72 ± 0.06^{ab}	0.50 ± 0.05^{b}
T2 (mild)	30.61 ± 1.12^{c}	1.00 ± 0.15^{a}	0.85 ± 0.34^{a}	0.65 ± 0.11^{a}
T3 (moderate)	48.11 ± 10.1^{b}	0.73 ± 0.09^{b}	0.67 ± 0.06^{b}	0.52 ± 0.05^{b}
T4 (severe)	97.67 ± 14.91^{a}	0.56 ± 0.14^{c}	0.35 ± 0.13^{c}	0.35 ± 0.06^{c}
$LSD_{0.05}$[1]	6.91	0.13	0.14	0.05

[1]Averages denoted by the same letter in the same column indicate no significant difference ($P < 0.05$)

[2]fresh mass

This study also showed that moderate and severe drought negatively affects the chlorophyll *a* content in basil leaves. As compared with chlorophyll *a*, the content of chlorophyll *b* in basil leaves was first raised in response to mild drought stress and then began to decrease with increasing drought duration. Total carotenoid contents in basil leaves have followed a similar pattern as chlorophyll *b* in response to drought (Table 1).

Antioxidant enzyme changes in response to drought stress in basil leaves were determined by observing the activities of following antioxidative enzymes; SOD, PPOD, GPOD and CAT. SOD activity in leaves of basil plants exposed to drought was significantly higher than those under non-stress conditions (control group). This study also found that SOD activity under mild and moderate drought stress was significantly higher than those under non-stress and severe drought conditions (Table 2).

Table 2. Antioxidant enzyme changes in response to drought stress in basil leaves

Drought treatment	SOD[2] activity (U min^{-1} mg^{-1} protein)	PPOD activity (µmol min^{-1} mg^{-1} protein)	GPOD activity (µmol min^{-1} mg^{-1} protein)	CAT activity (µmol min^{-1} mg^{-1} protein)
T1 (control)	4.08 ± 1.76^{c}	3.03 ± 1.98^{c}	0.15 ± 0.04^{c}	0.100 ± 0.025^{b}
T2 (mild)	11.42 ± 5.22^{a}	16.28 ± 3.81^{a}	0.26 ± 0.17^{b}	0.188 ± 0.030^{a}
T3 (moderate)	10.11 ± 4.32^{a}	15.61 ± 5.56^{a}	0.35 ± 0.14^{b}	0.178 ± 0.019^{a}
T4 (severe)	7.73 ± 4.22^{b}	11.52 ± 9.66^{b}	0.50 ± 0.11^{a}	0.176 ± 0.022^{a}
$LSD_{0.05}$[1]	2.53	3.55	0.10	0.018

[1]Averages denoted by the same letter in the same column indicate no significant difference ($P < 0.05$)

[2]SOD (superoxide dismutase), PPOD (pyrogallol peroxidase), GPOD (guaiacol peroxidase), CAT (catalase)

PPOD activity showed similar patterns of change to those observed for SOD activity. PPOD activity in leaves of basil plants first increased during mild drought, but then decreased, especially under severe drought conditions.

Drought treatment also caused a significant increase in GPOD activity compared with well-watered basil plants. In this study, GPOD activity in leaves of stressed basil plants gradually increased, reaching the highest value under severe drought stress.

Drought also significantly increased CAT activity in leaves of basil plants as compared to well-watered plants, regardless of drought duration. However, the differences in CAT activity among the drought treatments were not significant.

Table 3. Antioxidant properties of basil plants under drought conditions

Drought treatment	Total phenolics (mg 100 g^{-1} FM^{2})	Total flavonoids (mg 100 g^{-1} FM)	Total antioxidant capacity (µmol Fe^{2+} 100 g^{-1} FM)
T1 (control)	$32.88 \pm 1.19^{c*}$	2.43 ± 0.69^{b}	50.47 ± 1.94^{c}
T2 (mild)	33.07 ± 0.77^{bc}	2.51 ± 0.12^{b}	52.41 ± 3.22^{bc}
T3 (moderate)	37.91 ± 0.98^{a}	2.98 ± 0.06^{a}	58.04 ± 3.16^{a}
T4 (severe)	35.00 ± 0.21^{b}	2.78 ± 0.10^{a}	54.79 ± 3.16^{b}
$LSD_{0.05}$[1]	2.21	0.26	2.84

[1]Averages denoted by the same letter in the same column indicate no significant difference ($P < 0.05$)

[2]fresh mass

In the current study, total phenolic content significantly increased in basil leaves under moderate and severe drought as compared with non-stressed basil plants. On the other hand, mild drought stress did not affect the total phenolic content of basil leaves compared to control conditions (Table 3).

The total flavonoid content in basil leaves showed a similar pattern of change as that observed for total phenolic content. The study results also showed that the total antioxidant capacity (FRAP value) was higher in drought-stressed plants as compared to control plants. Under moderate stress, the increase was more noticeable, and then under severe and mild drought stress.

DISCUSSION

Effects of drought on proline and leaf photosynthetic pigment contents in basil plants

Drought strongly affects all aspects of plant physiology which leads, among other things, to osmotic imbalance in plant cells. Most scientists agree that plants under drought stress conditions tend to maintain the osmotic balance and turgor pressure in plant cells by increasing the accumulation of proline as one of the major organic osmolytes (Ashraf and Foolad, 2007; Chahine *et al.*, 2021; Spormann *et al.*, 2023). Apart from acting as an excellent osmolyte, proline enhances cell membrane stability and reduces the production of reactive oxygen species. It also acts as a signaling/regulatory molecule able to trigger specific gene expression essential for plant responses to stress conditions (Kavi Kishor *et al.*, 2022).

In this study, the proline content in basil leaves significantly increased under both moderate drought and severe drought (Table 1). Numerous studies have also reported a similar increase in proline content in different plants subjected to drought (Fu *et al.*, 2018; Hosseinifard *et al.*, 2022). Interestingly, in this study, mild drought stress did not significantly affect proline accumulation in basil leaves. Considering the fact that proline is a reliable biomarker of stress (Hayat *et al.*, 2012), these results indicate that basil plants tolerate short-term drought conditions well. These results are in line with those of Kalamartzis et al. (2020) and Driesen et al. (2023).

In the current study, moderate and severe drought drastically decreased the content of all the studied photosynthetic pigments including chlorophyll *a*, chlorophyll *b* and total carotenoids in basil plants as compared to control treatment. These results are basically consistent with the previous studies (Dias *et al.*, 2018; Hu *et al.*, 2023), suggesting that exposure of plants to long-term drought conditions causes a considerable damage to photosynthetic pigments and thus to plant photosynthetic capacity. Most scientists believe that drought induces loss of thylakoid cell membrane structural integrity within chloroplast, resulting in degradation of photosynthetic pigments (Chaves *et al.*, 2009; Li *et al.*, 2020). In addition, drought negatively affects the plant's ability to take up nitrogen, iron and other nutrients necessary for the synthesis of photosynthetic pigments as well as for maintaining the structure and function of chloroplasts (Umair Hassan *et al.*,

2020), and this is probably an additional reason for the decrease of the photosynthetic pigments content under long-term drought conditions.

In this study, mild drought stress did not negatively affect chlorophyll *a*, chlorophyll *b* and total carotenoid contents in basil leaves (Table 1). Moreover, total carotenoid contents in basil leaves under mild drought stress were significantly higher ($p < 0.05$) than those in the corresponding controls (non-stressed plants). Numerous studies have reported that carotenoids play a key role in protecting photosynthetic apparatus from photodamage caused by drought-induced oxidative stress (Latowski *et al.*, 2011; Ashikhmin *et al.*, 2023), and therefore increasing or maintaining the content of total carotenoids in the leaves under drought conditions is of great importance for photosynthetic efficiency. In addition, given that the carotenoids act both as light-harvesting pigments and as stress signals in plants, the maintenance of total carotenoid contents in leaves of plants exposed to drought is considered to be an important indicator for assessing plant drought tolerance (Mibei *et al.*, 2016). From this point of view, basil can be regarded as a drought-tolerant plant. However, it is important to note that prolonged drought conditions have a serious negative impact on the photosynthetic pigment synthesis, which ultimately leads to a decrease in their content in the leaves (Wang *et al.*, 2018). This hypothesis is supported by the results of the current study.

Enzymatic antioxidant responses to drought in basil plants

Under drought stress conditions, many physiological processes in plants, including photosynthesis, respiration as well as nutrient uptake and translocation, are negatively affected (Phansak *et al.*, 2021). These physiological disorders unavoidably led to overproduction of ROS that can cause damage to all classes of biomolecules (Oguz *et al.*, 2022).

However, over the course of evolution, plants have evolved complex defense systems against the accumulation and production of ROS, and among them, the enzymatic ROS-scavenging system is certainly one of the most important (Nadarajah, 2020). This defense system consists of numerous enzymes that have the ability to protect plant cells from the harmful effects of ROS. Some of them, such as SOD, CAT and peroxidases (PODs) are of great importance for maintaining the level of ROS in plant cells.

SOD acts as the first line of intracellular defense against superoxide radical anions which are produced in the large amounts in the early stage of stress as a result of the one-electron reduction of molecular oxygen. This enzyme catalyzes the dismutation of superoxide radical (O_2^-) to molecular oxygen (O_2) and less toxic hydrogen peroxide (H_2O_2) which is subsequently decomposed to H_2O by CAT or PODs. However, their mechanisms for converting H_2O_2 into H_2O are not the same; PODs convert H_2O_2 into H_2O using a wide variety of substrates as an electron donor, while CAT does not require an electron donor for the conversion of H_2O_2 into H_2O (Gusti *et al.*, 2021; Ren *et al.*, 2016).

In this study, SOD activity in leaves of basil plants under drought conditions was significantly higher than those under non-stress conditions (Table

2). The study results also showed that SOD activity under mild and moderate drought stress was significantly higher than that under non-stress and severe drought conditions, indicating that SOD activity first increased under mild and moderate drought, and then began to decrease under severe drought conditions. A similar pattern of changes in SOD activity was observed in leaves of *Medicago sativa* L. (Tina *et al.*, 2017) and *Ilex verticillata* (L.) A.Gray. subjected to drought (Xie *et al.*, 2023). These results strongly support the hypothesis that SOD play a crucial role in detoxification of ROS in plant cells in the early stages of drought. This finding is also consistent with a number of other studies that have reported high SOD activity at the initial stage of plant exposure to drought (Lu *et al.*, 2010; Ighodaro and Akinloye, 2018; Duong *et al.*, 2023).

In this study, PPOD activity has followed a similar pattern as SOD in response to drought. PPOD activity first increased during mild drought, but then decreased, especially under severe drought conditions (Table 2). These findings provide further support for the hypothesis that prolonged drought conditions cause an imbalance in the plant's defense system which, among other things, leads to a decrease in SOD and PPOD activity (Kapoor *et al.*, 2020). These findings align with those reported by Liu *et al.* (2011) and Ulusu et al. (2022).

In the current study, drought treatment caused a significant increase in GPOD activity compared to control (non-stressed plants). Interestingly, GPOD activity in leaves of stressed basil plants gradually increased over the course of the experiment, reaching the highest value under severe drought stress, which was inconsistent with the pattern observed for SOD and PPOD activity. Namely, in comparison with GPOD activity, SOD and PPOD activity was the highest under mild drought stress.

In this study, drought also caused a significant increase in CAT activity compared to well-watered plants, regardless of drought duration. In the scientific literature, there are many reports on CAT activity in plants under drought conditions and they are extremely heterogeneous. CAT activity has been shown to increase, remain unchanged, or decrease under drought depending mainly on the experimental conditions and plant species (Jiang and Zhang, 2002; Luna *et al.*, 2004; Song *et al.*, 2022; Mishra *et al.*, 2023). In this regard it is important to note that CAT has a very low affinity for H_2O_2, which means that it is effective only at high concentrations of H_2O_2. This is perhaps one of the reasons for the low CAT activity in the initial phase of stress, observed in earlier studies. On the other hand, CAT has a very fast turnover rate. One molecule of this enzyme can convert more than 2 million molecules of H_2O_2 to H_2O per second, indicating that CAT is one of the most important parts of the enzymatic antioxidant defense system in plants (Racchi, 2013). The results of this study support this hypothesis.

Non-enzymatic antioxidant responses to drought in basil plants

Under drought conditions, plants also activate a non-enzymatic antioxidant defense system to achieve redox homeostasis in plant cells. The non-enzymatic antioxidant defense system of the plant comprises a variety of non-enzymatic antioxidant molecules including ascorbic acid, carotenoids, glutathione and

phenolic compounds. They not only protect the plant cells against ROS, but also play a prominent role in plant growth and development by modifying numerous signaling pathways related to cell division, cell elongation, abscission and cell death (Kasote *et al.,* 2015). Among them, phenolic compounds appear to be the most important since they exhibit direct and indirect antioxidant properties. In this regard, phenolic compounds can directly neutralize ROS through electron donation; however, they also exhibit indirect antioxidant activity by inducing the production of endogenous antioxidant enzymes (Kumar and Goel, 2019).

In the current study, exposure of basil plants to moderate and severe drought conditions caused a significant increase in total phenolic content as compared with control plants (Table 3). Increased synthesis of the phenolic compounds in plants exposed to prolonged drought has been observed in numerous studies (Park *et al.,* 2023; Šamec *et al.,* 2021), indicating that the accumulation of phenolic compounds in plant cells is one of the important defense strategies adopted by plants to cope with deleterious effects of drought (Ahlawat *et al.,* 2023).In this study, mild drought stress did not affect the total phenolic content of basil leaves compared to control conditions. Given the fact that phenolic accumulation in plants serves as a reliable biomarker of stress (Jańczak-Pieniążek *et al.,* 2022), the absence of significant changes in total phenolic content between mild drought and control treatment indicates that basil plants tolerate short-term drought conditions well. This hypothesis is also supported by the results of the proline and photosynthetic pigment content analysis.

The results of this study demonstrated that severe drought caused a significant decrease in the total phenolic content of basil leaves as compared with moderate drought; however, the total phenolic content in basil plants under severe drought conditions was still significantly higher than in well-watered plants. Król et al. (2014) reported that exposure of plants to long-term drought stress can cause disturbances in phenolic biosynthetic pathways, which consequently leads to a decrease in their content in leaves. Several other studies also provide evidence that long-term progressive drought negatively affects the synthesis of phenolic compounds in plants (Gutierrez-Gonzalez *et al.,* 2010; Chennupati *et al.,* 2012).In this study, the total flavonoid content showed a similar pattern of change as that observed for total phenolic content. The obtained results were expected since flavonoids are one of the most abundant and widespread phenolic compounds in plants (Mutha *et al.,* 2021). The study results also showed that the total antioxidant capacity (FRAP value) in the leaves of basil plants increased significantly in all drought stress treatments as compared with control plants. The increase was more noticeable under moderate stress, followed by severe and mild drought stress. The same pattern was observed in the relationship between drought stress levels and total phenolic and flavonoid contents, indicating that phenolic compounds are one of the main contributors to the antioxidant capacity of plants. These findings align with those of earlier research (Lyu *et al.,* 2023; Zeng *et al.,* 2023).

CONCLUSIONS

The study revealed that exposure of basil plants to drought significantly increased the activities of SOD, GPOD, PPOD and CAT in plant leaves, indicating that these enzymes play an important role in ROS detoxification and thus in plant drought tolerance. Exposure of basil plants to moderate and severe drought also resulted in higher accumulation of proline and total phenolics and flavonoids, indicating that basil plants under prolonged drought stress conditions tend to improve their drought tolerance by inducing the production of these compounds in leaves. The study results also revealed that moderate and severe drought had a negative effect on the content of photosynthetic pigments, i.e. chlorophyll *a*, chlorophyll *b* and total carotenoids. In the current study, mild drought did not negatively affect the content of photosynthetic pigments as well as proline and total phenolic and flavonoid content in basil leaves. These findings lead to the conclusion that basil plants tolerate drought conditions well. This conclusion is further supported by the fact that the basil plants have the ability to produce protective macromolecules and antioxidants in high amounts even in severe drought conditions.

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