



Research Paper

Agronomic and physio-biochemical responses to exogenous nitric oxide (NO) application in cauliflower under water stress conditions

Musa Seymen^{a,*}, Muhammet Erçetin^b, Duran Yavuz^c, Gülbanu Kıymacı^b, Necibe Kayak^d,
Abdurrahman Mutlu^b, Ertan Sait Kurtar^a

^a Selçuk University, Horticulture Department of Agriculture Faculty, Konya, Turkey

^b Selçuk University, Institute of Science, Department of Horticulture, Konya, Turkey

^c Selçuk University, Irrigation Department of Agriculture Faculty, Konya, Turkey

^d Sakarya University of Applied Sciences, Horticulture Department of Agriculture Faculty, Sakarya, Turkey

ARTICLE INFO

Keywords:

Antioxidant activity
Brassica oleracea L. var. botrytis
Biostimulant, Deficit irrigation
Photosynthetic activity

ABSTRACT

Water stress is the most significant abiotic stress factor that restricts agricultural cultivation, causes yield and quality losses, and negatively affects food supply security worldwide. Therefore, it is crucial to explore the effects of water stress on plants. In the present study, we determined the effects of varying concentrations of exogenous nitric oxide (NO) on the morphology, physiology, and biochemistry of cauliflower under different water stress conditions. Four irrigation levels (full irrigation at 100 % as a control – I100 % and water stresses at 20, 40, and 60 %, corresponding to I80 %, I60 %, and I40 %, respectively) and four concentrations of nitric oxide - NO (0, 50, 100, and 150 μM NO, namely, NO0 – control, NO50, NO100, and NO150) were evaluated. The applied water stress negatively affected different plant growth parameters, actual photosynthetic efficiency (PSII), and stomatal conductance (gs) and increased leaf temperature and hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and proline contents. NO application not only contributed positively to plant growth parameters under stress conditions but also improved PSII and gs. In addition, NO reduced the negative effects of water stress by inducing the antioxidant defense system. The 150 μM NO application contributed to plant growth under full irrigation and water stress conditions.

1. Introduction

The average temperature is constantly increasing due to global warming and climate change, making it difficult for food production to satisfy the increasing demands of the population (Shah and Smith, 2020). The negative effects of water stress caused by climate change result in productivity and quality losses in agricultural areas (Seymen, 2021; Kal et al., 2023; Kayak et al., 2023).

As a result of climate change, irregular rainfall, increased evaporation, and transpiration increase the water needs of plants (DeVincentis, 2020). In addition, the water requirements of plants increase by 4 to 4.5 % with a 1 °C increase in surface temperature (Shah et al., 2019). Although low levels of drought stress do not affect plant development, increased duration and severity of stress delay the rate of photosynthesis and significantly limit plant development by causing morphological and physiological damage (Rahman et al., 2021). In addition, severe water deficit in the root zone negatively affects plant growth by preventing the

uptake of nutrients from the soil (Hosseini et al., 2021).

In plants, drought causes certain biochemical and physiological changes in addition to limiting plant growth (Srivastava and Srivastava, 2014). Stress-induced disruption of the water balance leads to the formation of reactive oxygen species (ROS), which negatively affect membrane and protein functions (Yavuz et al., 2023). Apoplastic ROS are produced due to the involvement of homologous oxidase proteins that function during respiratory explosions in the plasma membrane. The resulting proteins create superoxide anions (O_2^-), either directly produced or by ascorbate peroxidase (APX) and superoxide dismutase (SOD), and cause the formation of hydrogen peroxide (H_2O_2), which is a nonradical reaction and a symptom of stress. Disruption of the ROS balance is known to negatively affect the photosynthetic electron transport rate and restrict the photochemical efficiency of PSII (Elkelish et al., 2021).

Plants have developed certain mechanisms to avoid the negative effects of stress, such as restricting vegetative parts, delaying growth,

* Corresponding author.

E-mail address: mseymen@selcuk.edu.tr (M. Seymen).

thickening leaves, altering root architecture, osmolyte synthesis, and reducing water potential (Yavuz et al., 2023). In addition, plants survive under stress conditions by synthesizing proline (Liu et al., 2011) and activating enzymatic (catalase [CAT], SOD, peroxidase [POD], ascorbate peroxidase) and nonenzymatic (glutathione, tocopherol, ascorbate) antioxidant defense systems (Seymen et al., 2023; Yavuz et al., 2023).

Nitrite oxide (NO) is a remarkable molecule that functions as an antioxidant and is produced as a physiological response under stress conditions; it is found in gaseous form in nature (Ekinçi et al., 2018). NO synthesized in plants reduces the reverse effects of ROS produced during stress by inducing transcriptional changes in the targets involved in the formation of ROS (Del Castello et al., 2019). In addition, NO prevents oxidative damage by promoting the secretion of antioxidant enzymes under stress conditions (Hu et al., 2007).

It maintains leaf water content under stress conditions, maintains photosynthetic activity by supporting chlorophyll content, improves lipoxygenase function, and minimizes membrane ion leakage (Sanchez-Romera et al., 2018; Lau et al., 2021). The application of NO to plants prevents water loss from the leaves by ensuring the closure of stomata and minimizing ion leakage (Hamurcu et al., 2020). Sodium nitroprusside (SNP, a NO donor) is an important defense molecule that maintains the balance between ROS and hormones and activates genes, controlling processes such as signal transduction and the emergence of defense mechanisms (Corpas et al., 2022).

The application of NO under water constraint conditions has been known to contribute positively to the growth and production of tomato (Jangid and Dwivedi, 2017), pepper (Yaşar and Üzal, 2021), watermelon (Hamurcu et al., 2020), broccoli (Munawar et al., 2019), and lettuce (Yavuz et al., 2023). However, the effects of NO application on cauliflower plants grown under water stress conditions have not been studied in detail.

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is a fiber- and carbohydrate-rich vegetable that significantly contributes to human nutrition. Approximately 25.5 million tons of cauliflower and broccoli were produced worldwide in 2021. With a production of 311,000 tons, Turkey is one of the leading producers of cauliflower worldwide (FAO, 2022). Cauliflower can be grown in open fields in all regions where high temperatures are absent or under cover when the necessary climatic conditions are met. As with other vegetable species, cauliflower requires a high water content and is negatively affected by water stress. Water stress negatively affects the head quality of cauliflower, thereby reducing its market value. Thus, certain practices are required to determine the appropriate irrigation program for cauliflower and reduce the negative effects of stress. It has been reported that 40 % water restriction negatively affects plant growth in broccoli, which is from the same family as cauliflower, and foliar as well as pre-sowing application

of NO could be helpful in upregulating the oxidative defense system (Munawar et al., 2019). In our study, we investigated the effects of increasing NO application under different water constraints on physiological, biochemical, and antioxidant enzyme activities in cauliflower.

2. Materials and methods

2.1. Study site and plant material

The experiment was conducted between March 7 and April 17, 2023, in a glass greenhouse of Selçuk University/Faculty of Agriculture, Konya (located at 38°01'49''N and 32°31'32''E), Turkey. The climate data inside the greenhouse during the experimental period are shown in Fig. 1. The pepper RZ F1 cultivar, which is a productive variety of cauliflower with high leaf quality, was used as the plant material. The variety is both table and industrial and is intensively cultivated.

2.2. Experimental design and growth conditions

The study was based on a randomized experimental design with three replications. A 4 × 4 factorial arrangement was used, consisting of four different irrigation levels (full irrigation at 100 % as a control – I100 % and water stresses at 20, 40, and 60 %, corresponding to I80 %, I60 %, and I40 %, respectively) and four concentrations of nitric oxide – NO (0, 50, 100, and 150 μM NO, namely, NO0 – control, NO50, NO100, and NO150). Two replicate pots were used.

Cauliflower was grown in pots (filled with 10 kg of soil) with a bottom diameter of 19 cm, an upper diameter of 29 cm, and a height of 24 cm. The soil used in the experiment contained 54.32 % clay, 22.35 % silt, and 23.18 % sand and had a clayey structure. The soil pH was 7.53, the electrical conductivity (EC) was 0.425 dS m⁻¹, the CaCO₃ content was 11.2 %, and the organic matter content was 3.36 %. The field capacity of the soil, which is sufficient in terms of macro- and microelements, was 36.60 %, the wilting point was 22.13 %, and the useful water capacity was 14.47 %.

Cauliflower plants (in the planting stage) were obtained from a seedling company (Talya seedling-Antalya/Turkey). Equal amounts of irrigation were applied to the seedlings planted in pots. The volume of irrigation water applied to the subjects is provided, considering the experimental subject in which NO was applied (NO0) and the I₁₀₀ irrigation level was applied. In this context, the useful water capacity for NO0I100 % decreased to approximately 45 to 50 %, irrigation was applied, and the soil moisture in this trial reached the field capacity. Water deficit practices were started 22 days after planting the seedlings and applied for approximately 20 days. In the experiment, the soil moisture in the NO0I100 % experimental group was monitored using the

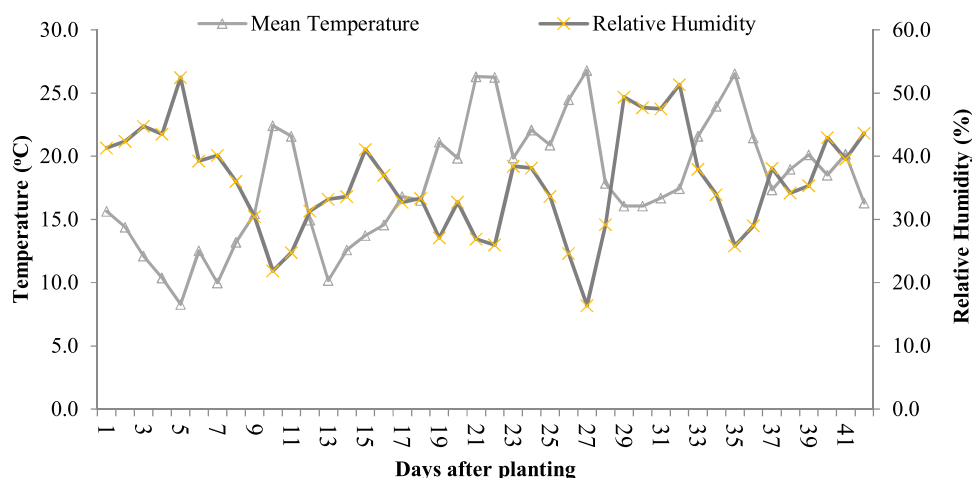


Fig. 1. Average temperature and humidity values of the experiment.

weighing method. During the trial, the pots were weighed with a digital scale with 1 g precision, and the lost water was calculated and applied to the pots. Afterward, 80, 60, and 40 % of the volume of water applied to the NO100 % trial subject was applied as irrigation water to the 180 %, I60 %, and I40 % experiment subjects, respectively, which received deficit irrigation.

The NO was applied twice in the evening on April 3 and April 10, after the water deficit appeared. All three application concentrations were calculated separately, dissolved in a sufficient volume of pure water, and applied to the plants by spraying on leaves (Hamurcu et al., 2020). The root collar rot was sprayed along with irrigation, whereas hoeing was performed once to combat weeds and aerate the roots.

2.3. Growth measurement

The cauliflower seedlings were harvested on April 17 by cutting them from the soil surface with scissors. The samples were taken to the laboratory, and certain plant agronomic data were measured immediately after the harvest. The fresh weights of root and shoot (g plant^{-1}) of five cauliflower plants harvested from each replicate were determined by weighing them separately using a precision scale. Next, the weighed samples were first kept in the shade, and dried in an oven at 65 °C for 72 h, and subsequently, their root dry weight (g plant^{-1}) and shoot dry weight (g plant^{-1}) were determined (Seymen, 2021). The root length (cm) was determined with a ruler in the cleaned roots, and the leaf area ($\text{cm}^2/\text{per leaf}$) was measured using the computer program (WinFOLIA computer program) using leaf samples collected just before harvest (Ipek et al., 2014).

2.4. Photosynthetic efficiency, stomatal conductance, leaf temperature, and chlorophyll fluorescence measurement

Actual photosynthetic efficiency (PSII), stomatal conductance – gs ($\text{mol m}^{-2} \text{s}^{-1}$), leaf temperature (°C), and chlorophyll fluorescence (Fv/Fm) were measured using the LI-COR brand LI-600 fluorometer device (LI-COR Inc., USA). The LI-600 are compact porometers with pulse-amplitude modulation fluorometers that simultaneously measure stomatal conductance and chlorophyll a fluorescence over the same leaf or needle area. The necessary measurements were made between 9.00 and 11.00 h in the morning, under cloudless (clear) weather conditions, before harvesting the old leaves. Chlorophyll fluorescence was measured on the same day after the plants were kept in the dark for half an hour (Liang et al., 2019).

2.5. Relative water content, membrane permeability, and pigment content assays

Next, we collected cauliflower leaves to determine the relative water content and membrane permeability using the method described by Lutts et al. (1996). To determine chlorophyll a and b, samples were ground using acetone according to the method described by Lichtenthaler and Buschmann (2001) and read on a spectrophotometer (Shimadzu-UV-6300PC) at 663 and 470 nm wavelengths. Jaspars formula ($\text{CT} = ((1000 \times \text{Abs}_{470}) - (2.27 \times \text{Chla}) - (81.4 \times \text{Chlb})) / 227$) was used to calculate the carotenoid content – CT (Witham et al., 1971).

2.6. Protein and proline assays

The ninhydrin method specified by Bates et al. (1973) was used to determine the proline content in cauliflower leaves. Protein analysis was performed using 0.5 g of cauliflower leaf samples and the Bradford method (1976).

2.7. Malondialdehyde and hydrogen peroxide assays

Leaves that had completed their growth before the cauliflower

harvest were collected. The MDA content was determined by measuring 2-thiobarbituric acid (TBAA) and lipid peroxidation using the method determined by Heath and Packer (1968). The readings were obtained using a spectrophotometer 532 and 600 nm. The hydrogen peroxide (H_2O_2) concentration was determined with the superficial residue used from the same mixture and calculated using a standard chart (Velikova et al., 2000).

2.8. Antioxidant enzyme activity assay

The samples were prepared from fresh leaves of cauliflower using the method described by Angelini and Federico (1989). The prepared samples were read at 560 nm according to the method described by Agarwal and Pveey (2004). The SOD activity was calculated by determining the content of the enzymes causing inhibition. The CAT activity was determined using the method described by Havir and McHale (1987). The resulting absorbance change was read at 240 nm. The POD activity was determined using the values obtained from measurements at 470 nm according to the method described by Chance (1955).

2.9. Water use efficiency

Water use efficiency (WUE, in g L^{-1}) values were calculated based on the shoot fresh weight of cauliflower (Silva et al., 2023) and ET.

$$WUE = \frac{SFW}{ET}$$

WUE= Water use efficiency (g L^{-1})

SFW= Cauliflower fresh weight (g plant^{-1})

ET= Evapotranspiration (L plant^{-1})

2.10. Data analysis

The data obtained were analyzed using the JMP-14 computer package program. Significant differences are grouped according to 5 % significance levels. The results obtained were interpreted according to importance groups, and the effects of water deficit and NO applications, as well as interactions, were determined statistically. Important parameters were determined by performing principal component analysis (PCA) on parameters obtained via the same statistical program. Important applications were interpreted using the loading plot and score plot graphics drawn from the resulting PC1 and PC2 components.

3. Results

3.1. Effects of exogenous nitric oxide (NO) on growth parameters under water stress

Water deficit and NO concentrations applied to cauliflower revealed statistically significant differences (Table 1). In addition to all water deficit reducing shoot fresh weight (SFW), I60 % and I40 % conditions caused significant losses in shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), and leaf area (LA). SFW obtained $41.09 \text{ g plant}^{-1}$ at I100 % and decreased by $14.25 \text{ g plant}^{-1}$ at I40 %. While $4.72 \text{ g plant}^{-1}$ SDW was obtained in the I100 % application, $2.38 \text{ g plant}^{-1}$ was obtained in the I40 % application. Similarly, compared to I40 % and I100 %, RFW, RDW, and LA decreased by 48.44, and 56 %, respectively. While the highest SFW of $30.95 \text{ g plant}^{-1}$ was obtained from the NO100 application, the highest SDW of 3.85, 3.84, and 378 g plant^{-1} was obtained from the NO50, NO100, and NO150 applications, respectively. The NO100 application increased the SDW, RFW, and RDW by 11, 18, and 20 %, respectively. The NO150 application contributed the most to RFW ($2.84 \text{ g plant}^{-1}$), and the most significant increase in RDW and LA was obtained from NO100 and NO150 applications. An evaluation of interactions showed that the NO100 application, which was generally applied under full irrigation conditions, significantly contributed to

Table 1
Effect of NO concentrations applied under deficit irrigation conditions on agronomic characteristics of cauliflower.

Treatments	SFW (Shoot fresh weight) (g plant ⁻¹)	SDW (Shoot dry weight) (g plant ⁻¹)	RFW (Root fresh weight) (g plant ⁻¹)	RDW (Root dry weight) (g plant ⁻¹)	RL (Root length) (cm)	LA (Leaf area) (cm ²)
Irrigations (I)						
I100 %	41.09 ^A	4.72 ^A	2.71 ^A	0.41 ^A	21.94	64.90 ^A
I80 %	34.90 ^B	4.49 ^A	2.75 ^A	0.43 ^A	20.87	65.33 ^A
I60 %	22.80 ^C	3.35 ^B	1.77 ^B	0.31 ^B	20.41	46.44 ^B
I40 %	14.25 ^D	2.38 ^C	1.40 ^C	0.23 ^C	20.08	28.33 ^C
Nitric oxide (NO)						
NO0	26.12 ^c	3.47 ^b	1.83 ^d	0.30 ^c	18.72 ^b	40.49 ^c
NO50	28.38 ^b	3.85 ^a	2.05 ^c	0.33 ^b	21.98 ^a	52.25 ^b
NO100	30.95 ^a	3.84 ^a	2.26 ^b	0.36 ^{ab}	20.89 ^a	56.12 ^a
NO150	27.54 ^{bc}	3.78 ^a	2.84 ^a	0.37 ^a	21.72 ^a	56.14 ^a
I X NO (interactions)						
I100 %	NO0	39.61 ^{bc}	4.36 ^{bc}	1.91 ^{cde}	0.30 ^{def}	20.00 ^{d-g}
	NO50	42.00 ^b	5.06 ^a	2.68 ^b	0.39 ^{bc}	24.28 ^a
	NO100	48.00 ^a	5.23 ^a	2.79 ^b	0.47 ^a	23.00 ^{a-d}
	NO150	34.58 ^d	4.25 ^{bc}	3.47 ^a	0.47 ^a	20.50 ^{b-g}
I80 %	NO0	33.80 ^d	4.74 ^{ab}	2.63 ^b	0.44 ^{ab}	22.89 ^{a-d}
	NO50	34.13 ^d	4.48 ^{bc}	2.24 ^c	0.39 ^{bc}	19.00 ^{fg}
	NO100	37.18 ^{cd}	4.31 ^{bc}	2.85 ^b	0.38 ^c	19.39 ^{efg}
	NO150	34.49 ^d	4.44 ^{bc}	3.26 ^a	0.49 ^a	22.22 ^{a-e}
I60 %	NO0	17.91 ^g	2.67 ^e	1.59 ^{efg}	0.27 ^{efg}	14.17 ^h
	NO50	23.59 ^{ef}	3.39 ^d	1.59 ^{efg}	0.29 ^{def}	23.33 ^{ab}
	NO100	22.98 ^f	3.23 ^d	2.00 ^{cd}	0.34 ^{cd}	21.00 ^{b-f}
	NO150	26.76 ^e	4.12 ^c	1.90 ^{cde}	0.31 ^{de}	23.17 ^{abc}
I40 %	NO0	13.19 ^h	2.14 ^f	1.17 ^h	0.19 ^g	17.83 ^g
	NO50	13.83 ^h	2.47 ^{ef}	1.70 ^{def}	0.26 ^{gh}	21.33 ^{a-f}
	NO100	15.68 ^{gh}	2.60 ^{ef}	1.41 ^{gh}	0.23 ^{gh}	20.17 ^{c-g}
	NO150	14.33 ^h	2.32 ^{ef}	1.30 ^{gh}	0.21 ^h	21.00 ^{b-f}
LSD						
I	1.75**	0.26**	0.18**	0.03**	1.57 ^{ns}	2.33**
NO	1.75**	0.26*	0.18**	0.03**	1.57**	2.33**
I X NO	3.51**	0.52**	0.37**	0.06**	3.16**	4.66**

Statistically significant according to $P < 0.05^$ and 0.01^{**} . ns not significant. Uppercase letters indicate Irrigation, lowercase letters indicate nitric oxide, and italics indicate IXNO interaction for the Duncan groups.

plant growth. However, the NO150 application applied under 20 % water constraint (I80 %) conditions contributed to RFW, RDW, and root length (RL).

3.2. Effects of exogenous nitric oxide (NO) on photosynthetic efficiency, stomatal conductance, leaf temperature, and chlorophyll fluorescence under water stress

The highest chlorophyll fluorescence (Fv/Fm) in cauliflower was obtained from the I80 % (0.58) and I60 % (0.59) treatments. All water deficit conditions decreased the actual photosynthetic efficiency (PSII) and stomatal conductance (gs) and increased the leaf temperature (T leaf). Compared to the I100 % subject, the fully stressed subject (I40 %) exhibited decreases of 31 and 94 % in PSII and gs, respectively. The T leaf, on the other hand, reached 22.60 °C under full irrigation and increased to 30.12 °C under full stress. The highest PSII was obtained from the NO150 (0.64) and NO100 (0.66) treatments, and the highest gs was obtained from the NO50 (0.30 mol m⁻² s⁻¹) treatment. In addition to generally high PSII being obtained with NO applications under full irrigation conditions, NO50 and NO100 applications significantly contributed to PSII under the I60 water constraint. NO application contributed to the gs content under full irrigation and deficit irrigation conditions, except for I40 % (Table 2).

3.3. Effects of exogenous nitric oxide (NO) on RWC, MP, and pigment content under water stress

The highest MP was observed at 60 % water deficit (I40 %) (20.25 %) applied to cauliflower. The applications of NO did not contribute to the relative water content (RWC) of leaves. The NO150 application (19.71 %) increased the membrane damage (MP). An evaluation of the

interactions revealed that the NO50 application under full irrigation and severe water stress (I40 %) conditions increased the RWC (Table 2). Considering the chlorophyll a (Chla) content, the highest values were obtained for I60 % irrigation (23.96 mg g⁻¹), whereas chlorophyll b (Chlb) was higher than I60 % (9.28 mg g⁻¹) and I40 % (9.45 mg g⁻¹) irrigation subjects. The highest carotenoid content (CT) was obtained from the full irrigation application (4.36 mg g⁻¹), and water deficits reduced the CT. The NO applications reduced the content of Chla, Chlb, and CT. The NO applications did not contribute to chlorophyll contents, and the NO100 application at the I60 % irrigation level considerably elevated the content of Chla and Chlb. The highest CT was obtained from the I40 %NO0 application (Table 3).

3.4. Effects of exogenous nitric oxide (NO) on protein and proline contents under water stress

Water deficit applied to cauliflower increased its proline and protein contents. The maximum proline content was obtained from the severe water stress (I40 %) (340.26 µg g⁻¹) application, and the highest protein content was obtained from the I80 % application (58.71 µg g⁻¹). NO applications significantly reduced the proline content. While the highest proline content was obtained from NO0 application with 202.62 µg g⁻¹, the highest protein content was obtained from NO0 and NO100 applications, 49.88 and 50.89 µg g⁻¹, respectively. The highest proline content was obtained from NO0 and NO100 applications at the I40 % irrigation level, I80 %NO100 and I60 %NO0 applications resulted in the highest protein content (Table 3).

Table 2
Effect of NO concentrations applied under deficit irrigation conditions on physiological parameters in cauliflower.

Treatments	RWC (Leaf relative water content) (%)	MP (Membrane permeability) (%)	Fv/Fm (Chlorophyll fluorescence)	PSII (Actual photosynthetic efficiency)	gs (Stomatal conductance) (mol m ⁻² s ⁻¹)	T Leaf (Leaf temperature) (°C)
Irrigations (I)						
I100 %	68.46	17.74 ^B	0.48 ^C	0.73 ^A	0.53 ^A	22.60 ^D
I80 %	68.52	17.81 ^B	0.58 ^A	0.67 ^B	0.24 ^B	24.88 ^C
I60 %	67.80	18.52 ^B	0.59 ^A	0.66 ^B	0.17 ^C	27.25 ^B
I40 %	65.85	20.25 ^A	0.57 ^B	0.50 ^C	0.04 ^D	30.12 ^A
Nitric oxide (NO)						
NO0	69.23 ^a	18.48 ^b	0.57 ^a	0.60 ^b	0.20 ^c	26.13
NO50	73.32 ^a	17.78 ^b	0.54 ^b	0.47 ^c	0.30 ^a	26.37
NO100	64.48 ^b	18.35 ^b	0.55 ^b	0.66 ^a	0.26 ^b	25.90
NO150	63.60 ^b	19.71 ^a	0.56 ^a	0.64 ^{ab}	0.22 ^c	26.45
IXNO (interactions)						
I100 % NO0	65.05 ^{c-f}	17.35 ^{de}	0.54 ^f	0.72 ^{ab}	0.47 ^b	23.13
NO50	81.87 ^a	16.95 ^e	0.50 ^g	0.70 ^{bc}	0.75 ^a	22.60
NO100	60.06 ^f	19.13 ^{bcd}	0.42 ⁱ	0.75 ^{ab}	0.52 ^b	22.13
NO150	66.87 ^{c-f}	17.57 ^{de}	0.46 ^h	0.75 ^{ab}	0.38 ^c	22.57
I80 % NO0	76.76 ^{ab}	17.07 ^e	0.59 ^{cde}	0.76 ^a	0.15 ^{gh}	24.32
NO50	67.98 ^{b-f}	16.98 ^e	0.57 ^{def}	0.66 ^{cd}	0.22 ^{ef}	24.86
NO100	66.91 ^{c-f}	17.57 ^{de}	0.56 ^{ef}	0.65 ^{cde}	0.29 ^d	24.82
NO150	62.42 ^{ef}	19.62 ^{bc}	0.59 ^{cde}	0.60 ^{ef}	0.28 ^{de}	25.50
I60 % NO0	71.95 ^{bcd}	18.57 ^{cde}	0.54 ^f	0.50 ^g	0.13 ^h	27.80
NO50	70.09 ^{b-e}	17.49 ^{de}	0.60 ^{bcd}	0.63 ^{def}	0.17 ^{gh}	26.54
NO100	65.57 ^{c-f}	19.84 ^{bc}	0.58 ^{cde}	0.76 ^a	0.17 ^{gh}	27.26
NO150	63.60 ^{def}	18.17 ^{cde}	0.64 ^a	0.74 ^{ab}	0.19 ^{fg}	27.37
I40 % NO0	63.17 ^{def}	20.93 ^b	0.62 ^{ab}	0.42 ^h	0.03 ⁱ	29.27
NO50	73.35 ^{abc}	19.76 ^{bc}	0.49 ^g	0.59 ^f	0.04 ⁱ	31.46
NO100	65.36 ^{c-f}	16.87 ^e	0.61 ^{bc}	0.51 ^g	0.05 ⁱ	29.41
NO150	61.51 ^{ef}	23.46 ^a	0.55 ^f	0.47 ^{gh}	0.03 ⁱ	30.35
LSD						
I	4.74 ^{ns}	0.95 ^{**}	0.02 ^{**}	0.03 ^{**}	0.03 ^{**}	0.83 ^{**}
NO	4.74 ^{**}	0.95 ^{**}	0.02 ^{**}	0.03 ^{**}	0.03 ^{**}	0.83 ^{ns}
IXNO	9.48 [*]	1.90 ^{**}	0.03 ^{**}	0.05 ^{**}	0.07 ^{**}	1.68 ^{ns}

Statistically significant according to $P < 0.05^$ and 0.01^{**} . ns not significant. Uppercase letters indicate Irrigation, lowercase letters indicate nitric oxide, and italics indicate IXNO interaction for the Duncan groups.

3.5. Effects of exogenous nitric oxide (NO) on malonaldehyde and hydrogen peroxide contents under water stress

Water stress was negatively affected by the stress due to elevated MDA and hydrogen peroxide (H₂O₂) contents. While the highest MDA content was obtained from the I40 % application with 0.99 μmol g⁻¹, similarly, the highest H₂O₂ content was obtained from the I40 % application with 2.33 nmol ml⁻¹. The application of NO50 reduced the MDA content. Although the applications of NO do not contribute significantly to reducing MDA and H₂O₂ contents under water deficit conditions, NO50 and NO100 applications reduced the MDA content under 20 % water deficit (I80 %) (Table 4).

3.6. Effects of exogenous nitric oxide (NO) on antioxidant enzyme activity under water stress

The application of NO serves as a signaling molecule in increasing the content of antioxidants under water-stress conditions. We observed a significant increase in the activity of catalase (CAT) and peroxidase (POD) under water deficit conditions. According to full irrigation conditions, at the highest water stress (I40 %), was observed an increase of approximately 36, 4, and 436 %, respectively. Thus, the NO150 application increased the levels of antioxidants. In the NO150 application, CAT, SOD, and POD activities were obtained as 1071, 2575, and 5204 EU g⁻¹, respectively. The highest activity of SOD and POD was obtained from I40 %NO150, which resulted in severe water stress, and the highest NO concentration was applied (Table 4).

3.7. Water use efficiency

Water use efficiency (WUE, in g L⁻¹) values were calculated based on the shoot fresh weight of cauliflower and the volume of water applied. WUE ranged from 4.63 g L⁻¹ (I40 %NO0) to 12.56 g L⁻¹ (I100 %NO100). The maximum WUE value was found in I100 %NO100, where the highest leaf fresh weight was obtained, followed by I100 %NO50 (11.30 g L⁻¹). As the concentration of NO increased in I100 %NO150 (9.04 g L⁻¹) applications, the WUE values of cauliflower decreased. The lowest WUE values were obtained at the I40 % irrigation level, where severe water stress was applied, and no significant difference was found between NO applications. The application of I60 %NO150 (8.24 g L⁻¹) is an important irrigation strategy to obtain the highest benefit from unit water in regions with limited water resources (Fig. 2).

3.8. Principal component analysis (PCA) results

Parameters obtained following NO application to cauliflower under deficit irrigation conditions were subjected to PCA (Table 5). The five components of the study explained 84.07 % of the variance. In addition to the plant development parameters in the first component, PSII and gs exhibited a positive correlation, whereas T leaf, MDA, proline (PL), and POD displayed a negative correlation. In the second component, RWC and color pigments were strongly positively correlated, whereas membrane permeability (MP), SOD, and POD were strongly negatively correlated. The loading plot graph drawn to determine the correlation between parameters is depicted in Fig. 3. A powerful positive correlation was observed between positive plant development parameters and PSII and gs. These parameters

Table 3
Effect of NO concentrations applied under deficit irrigation conditions on pigment, proline, and protein contents in cauliflower.

Treatments	Chla (Chlorophyll a) (mg g ⁻¹)	Chlb (Chlorophyll b) (mg g ⁻¹)	CT (Carotenoid) (mg g ⁻¹)	PL (Proline) (µg g ⁻¹)	PT (Protein) (µg g ⁻¹)	
Irrigations (I)						
I100 %	24.43 ^B	8.02 ^C	4.36 ^A	89.33 ^D	29.29 ^C	
I80 %	24.31 ^B	8.51 ^B	4.23 ^B	111.59 ^C	58.71 ^A	
I60 %	25.21 ^A	9.28 ^A	4.18 ^B	141.05 ^B	52.76 ^B	
I40 %	23.96 ^B	9.45 ^A	4.24 ^B	340.26 ^A	53.59 ^B	
Nitric oxide (NO)						
NO0	27.57 ^a	9.75 ^a	4.91 ^a	202.62 ^a	49.88 ^{ab}	
NO50	24.88 ^b	9.29 ^b	4.16 ^c	152.36 ^c	49.95 ^c	
NO100	24.63 ^b	9.31 ^b	4.33 ^b	183.72 ^b	50.89 ^a	
NO150	20.82 ^c	6.92 ^c	3.61 ^d	143.52 ^c	48.63 ^b	
<i>I X NO (interactions)</i>						
I100 %						
	NO0	27.52 ^{ab}	9.59 ^{cd}	4.76 ^{bc}	102.00 ^{fg}	25.28 ⁱ
	NO50	25.08 ^{cd}	8.12 ^{ef}	4.50 ^{de}	91.11 ^g	25.59 ⁱ
	NO100	22.77 ^{ef}	7.65 ^f	3.78 ^g	128.70 ^e	28.38 ^t
	NO150	22.39 ^f	6.72 ^g	4.41 ^{de}	35.50 ^h	37.92 ^h
I80 %						
	NO0	27.39 ^{ab}	10.27 ^{bc}	4.80 ^{bc}	176.22 ^d	58.42 ^{bc}
	NO50	26.10 ^{bc}	9.68 ^{cd}	4.43 ^{de}	138.48 ^e	51.78 ^f
	NO100	23.69 ^{def}	7.55 ^f	4.32 ^{ef}	43.26 ^h	65.55 ^a
	NO150	20.06 ^g	6.56 ^g	3.37 ^h	88.40 ^g	59.12 ^b
I60 %						
	NO0	27.73 ^a	9.69 ^{cd}	4.84 ^b	170.72 ^d	63.74 ^a
	NO50	22.33 ^f	8.52 ^e	3.58 ^{gh}	97.08 ^{fg}	47.41 ^g
	NO100	27.97 ^a	10.77 ^{ab}	4.58 ^{cd}	176.86 ^d	54.26 ^{def}
	NO150	22.81 ^{ef}	8.15 ^f	3.72 ^g	119.57 ^{ef}	45.61 ^g
I40 %						
	NO0	27.67 ^a	9.45 ^d	5.23 ^a	361.56 ^a	52.09 ^{ef}
	NO50	26.03 ^{bc}	10.85 ^{ab}	4.13 ^f	282.75 ^c	55.04 ^{de}
	NO100	24.12 ^{de}	11.26 ^a	4.62 ^{bcd}	386.09 ^a	55.36 ^{cd}
	NO150	18.02 ^h	6.23 ^g	2.96 ⁱ	330.62 ^b	51.88 ^{ef}
LSD						
I		0.77 ^{ns}	0.35 ^{**}	0.12 [*]	12.77 ^{**}	1.62 ^{**}
NO		0.77 ^{**}	0.35 ^{**}	0.12 ^{**}	12.77 ^{**}	1.62 ^{**}
<i>I X NO</i>		1.54 ^{**}	0.70 ^{**}	0.25 ^{**}	13.54 ^{**}	3.24 ^{**}

Statistically significant according to $P < 0.05^$ and 0.01^{**} . ns not significant. Uppercase letters indicate Irrigation, lowercase letters indicate nitric oxide, and italics indicate IXNO interaction for the Duncan groups.

displayed a powerful negative correlation with H₂O₂, MDA, PL, protein (PT), and T leaves formed under stress conditions. The first component was the most important component, displaying the effect of stress. The second component exerted the effects of NO application; the levels of antioxidants increased, and the levels of color pigments decreased with increasing NO application in the negative zone. There was a strong negative correlation between antioxidants and color pigments in the presence of NO. When the loading plot graph drawn from PC1 and PC2 was examined, the full irrigation issues best explained the plant growth in cauliflower. In addition, NO application under full irrigation conditions contributed positively to plant growth. However, NO150 application under deficit irrigation conditions contributed to the antioxidant defense system (Fig. 4).

4. Discussion

Drought is one of the most important abiotic stress factors that severely limits plant productivity and quality. The most important symptoms in plants following water deficit are reduced turgor pressure, leaf growth, and photosynthesis rate. In addition, the increased severity of drought causes mechanical, metabolic, and oxidative damage in plants (Aksoy, 2008).

We demonstrated that cauliflower growth significantly decreased as water stress increased. When the severe water stress issue was examined compared to the full irrigation issue, the SFW, SDW, RFW, RDW, and LA decreased by approximately 65 %, 50 %, 48 %, 44 %, and 56 %, respectively. The application of NO reduced the effect of water stress on plant growth parameters. The highest SFW of 30.95 g plant⁻¹ was obtained from the NO100 treatment, while the highest RFW of 2.84 g plant⁻¹ was obtained from the NO150 treatment. SDW and RL were positively affected by all NO applications. A study reported that NO

application under drought stress conditions in two different watermelon genotypes contributed to plant fresh and dry weight (Avşaroğlu, 2016). Similarly, NO application under drought stress conditions contributes to plant growth in tomato (Jangid and Dwivedi, 2017), pepper (Kaya et al., 2019), and chard (Ekinci et al., 2020) plants. NO protects plants against damage due to oxidative stress via different biological means (Kumar et al., 2010). It can easily penetrate the cell membrane due to its lipophilic nature and functions as both an intracellular and extracellular messenger, regulating multiple physiological and chemical processes such as dormancy, growth and development, aging, respiration, photosynthesis, programmed cell death, and the antioxidative defense system (Yavuz et al., 2023).

Researchers have reported that NO application prevents or reduces water loss by increasing stomatal conductance under drought-stress conditions (Munemasa et al., 2015). Although this decrease prevents water loss, it increases the leaf temperature due to a decreased transpiration rate. Similarly, in our study, leaf temperature increased by 33 % under severe water stress conditions. In addition, compared to the full I100 % application, the I40 % application resulted in PSII and GS reductions of 31 and 94 %, respectively. Our study revealed that although water deficit in cauliflower significantly restricted stomatal conductance, the applied NO increased stomatal conductance. In addition, NO50 and NO100 significantly contributed to PSII. One study reported that the application of 150 µM NO significantly reduced water loss under drought stress conditions (Mata and Lamattina, 2001). Melatonin and NO applied under drought-stress conditions in soybeans reduced the accumulation of ROS, increased photosynthetic efficiency, and protected the plants from cellular damage (Imran et al., 2021). An experiment conducted on tomatoes revealed an accumulation of NO in the stomata because of microscopic examinations of leaves under drought-stress conditions (Ahmed et al., 2021). NO is a considerable

Table 4

Effect of NO concentrations applied under deficit irrigation conditions on malondialdehyde and hydrogen peroxide content, and antioxidant enzyme activities in cauliflower.

Treatments	MDA (Malondialdehyde) ($\mu\text{mol g}^{-1}$)	H ₂ O ₂ (Hydrogen peroxidase) (nmol mL^{-1})	CAT (Catalase) (EU g^{-1})	SOD (Superoxide dismutase) (EU g^{-1})	POD (Peroxidase) (EU g^{-1})
Irrigations (I)					
I100 %	0.77 ^D	1.65 ^D	888 ^B	2412 ^{AB}	1162 ^D
I80 %	0.83 ^C	1.79 ^C	527 ^C	2434 ^A	3190 ^C
I60 %	0.93 ^B	1.88 ^B	1251 ^A	2276 ^B	4677 ^B
I40 %	0.99 ^A	2.33 ^A	1208 ^A	2514 ^A	6503 ^A
Nitric oxide (NO)					
NO0	0.88 ^a	1.73 ^c	852 ^b	2347 ^b	2557 ^d
NO50	0.85 ^b	2.06 ^a	902 ^b	2308 ^b	3671 ^c
NO100	0.91 ^a	1.88 ^b	1049 ^a	2405 ^b	4101 ^b
NO150	0.90 ^a	2.00 ^a	1071 ^a	2575 ^a	5204 ^a
I X NO (interactions)					
I100 %					
NO0	0.71 ^h	1.79 ^f	644 ^c	2348 ^b	877 ^l
NO50	0.77 ^{gh}	1.54 ^g	580 ^{cd}	2455 ^b	1084 ^{kl}
NO100	0.89 ^{de}	1.27 ^{hi}	1173 ^{ab}	2424 ^b	1444 ^j
NO150	0.71 ^h	2.01 ^e	1156 ^{ab}	2421 ^b	1245 ^{jk}
I80 %					
NO0	0.88 ^e	1.28 ^{hi}	355 ^d	2465 ^b	1781 ^l
NO50	0.79 ^g	2.57 ^{ab}	571 ^{cd}	2405 ^b	2339 ^h
NO100	0.81 ^{fg}	2.07 ^{de}	650 ^c	2405 ^b	3021 ^g
NO150	0.86 ^{ef}	1.25 ⁱ	533 ^{cd}	2462 ^b	5621 ^c
I60 %					
NO0	0.96 ^{cd}	1.55 ^g	1149 ^{ab}	2376 ^b	3408 ^f
NO50	0.72 ^h	2.06 ^{de}	1367 ^a	1991 ^c	4608 ^d
NO100	1.03 ^b	1.43 ^{gh}	1121 ^{ab}	2444 ^b	4200 ^e
NO150	1.03 ^b	2.51 ^b	1371 ^a	2295 ^b	6493 ^b
I40 %					
NO0	0.98 ^{bc}	2.31 ^c	1261 ^{ab}	2202 ^{bc}	4163 ^e
NO50	1.12 ^a	2.07 ^{de}	1093 ^b	2384 ^b	6653 ^b
NO100	0.90 ^{de}	2.73 ^a	1255 ^{ab}	2350 ^b	7741 ^a
NO150	0.98 ^{bc}	2.24 ^{cd}	1225 ^{ab}	3122 ^a	7456 ^a
LSD					
I	0.03 ^{**}	0.09 ^{**}	128 ^{**}	141 [*]	161 ^{**}
NO	0.03 ^{**}	0.09 ^{**}	128 ^{**}	141 ^{**}	161 ^{**}
I X NO	0.06 ^{**}	0.18 ^{**}	257 ^{**}	282 ^{**}	322 ^{**}

Statistically significant according to $P < 0.05^$ and 0.01^{**} . ns not significant. Uppercase letters indicate Irrigation, lowercase letters indicate nitric oxide, and italics indicate IXNO interaction for the Duncan groups.

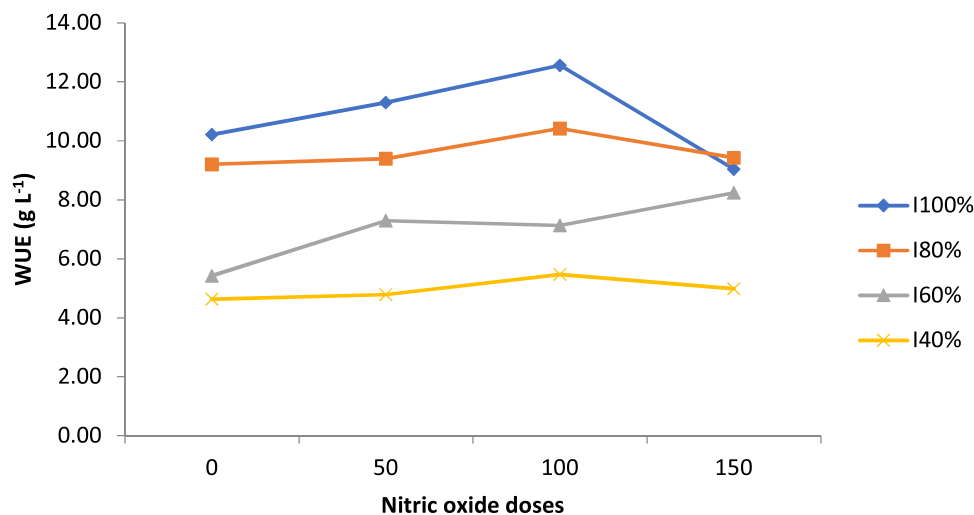


Fig. 2. Effect of NO concentrations applied under deficit irrigation conditions on WUE in cauliflower.

second messenger that has been implicated in stomatal functions in various plant species (Shi et al., 2015). In addition, ABA functions as an endogenous modulator preventing water loss from plants by regulating stomatal closure because of ABA synthesis (Daszkowska-Golec and Szarejko, 2013). NO produced by NR1 regulates ABA-dependent stomatal closure by functioning as a signaling molecule (Neill et al., 2008). Thus, exogenously applied NO provides tolerance to drought conditions depending on the plant species (Sharma et al., 2020).

In our study, the highest Chla concentration of 25.21 mg g⁻¹ was

obtained from the I60 % application, while the highest Chlb concentrations of 9.28 and 9.45 mg g⁻¹ were obtained from the I60 % and I40 % applications, respectively. All applied water stresses decreased the CT content. It has been reported that changes in chlorophyll a and b contents occur due to the inhibition of chlorophyll biosynthesis and enzymes, the degradation of pigments caused by the disruption of chloroplast membrane integrity, and the interruption of other metabolic activities (Foyer and Shigeoka, 2011). Carotenoids are molecules that are located together with chlorophyll in leaves, work indirectly in

Table 5

PCA results obtained from agronomic, physiological, and biochemical parameters of NO concentrations applied to cauliflower under deficit irrigation conditions.

Items	PC1	PC2	PC3	PC4	PC5
Eigenvalue	10.14	3.82	1.64	1.58	1.29
Percentage of variance	46.13	17.37	7.46	7.19	5.90
Cumulative variance	46.13	63.50	70.97	78.16	84.07
Eigenvectors					
SFW	0.302	-0.017	-0.016	-0.034	-0.033
SDW	0.300	-0.011	-0.036	0.094	0.073
RFW	0.269	-0.093	0.156	0.185	-0.081
RDW	0.276	-0.075	0.156	0.209	0.006
RL	0.136	-0.191	-0.231	0.079	0.615
LA	0.290	-0.107	0.033	0.170	-0.028
RWC	0.071	0.271	0.138	0.046	0.323
MP	-0.171	-0.303	0.305	-0.212	0.005
Fv/Fm	-0.151	0.083	-0.217	0.495	-0.020
PSII	0.247	0.044	-0.075	0.066	0.325
gs	0.272	0.003	-0.065	-0.279	0.038
T Leaf	-0.302	-0.046	0.050	0.075	0.138
Chla	-0.014	0.477	0.159	-0.092	0.071
Chlb	-0.117	0.405	0.025	0.033	0.292
CT	-0.004	0.465	0.109	-0.117	-0.154
MDA	-0.210	-0.051	0.286	0.012	0.375
H ₂ O ₂	-0.168	0.003	-0.459	0.051	-0.189
PL	-0.266	0.019	0.084	-0.154	0.130
PT	-0.168	0.023	0.285	0.548	-0.170
CAT	-0.181	-0.121	-0.335	-0.266	0.036
SOD	-0.033	-0.300	0.423	-0.139	-0.000
POD	-0.248	-0.205	-0.103	0.231	0.190

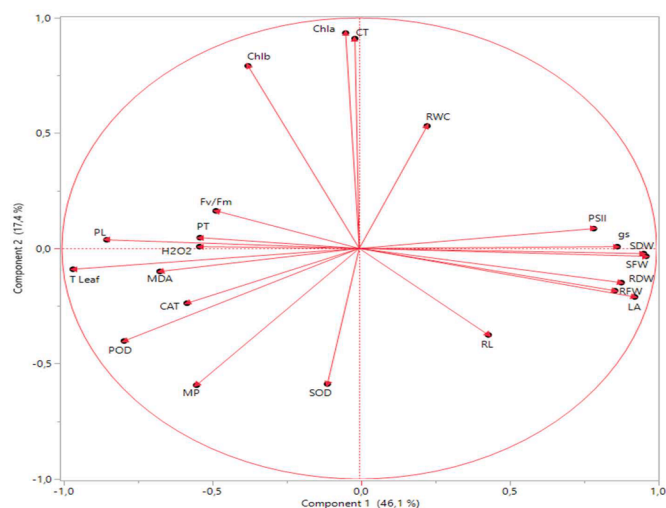


Fig. 3. Loading plot graph obtained from agronomic, physiological, and biochemical parameters of NO concentrations applied to cauliflower under deficit irrigation conditions.

photosynthesis, and have antioxidant properties (Keyvan, 2010). In a study conducted on cabbage, it was reported that drought stress caused an increase in Chla and CT but a decrease in Chlb (Seymen et al., 2023). In a study conducted on broccoli, it was reported that drought stress significantly reduced the Chla and Chlb contents, while NO application had a positive effect under control conditions but had no positive effects under stress conditions (Munawar et al., 2019). In our study, it was observed that the applied NO did not contribute to the pigment content; this situation was related to the genetic structure, and while it contributed positively to the pigment content in some species, it did not contribute to the pigment content in others.

The application of NO to cauliflower plants activated several defense mechanisms, including enzymatic and nonenzymatic antioxidant mechanisms, to suppress the harmful effects of ROS. We detected a

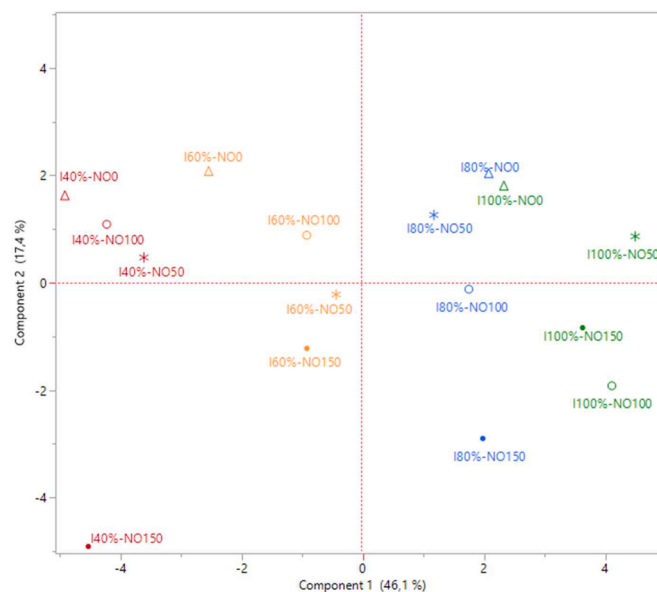


Fig. 4. Score plot graph obtained from agronomic, physiological, and biochemical parameters of NO concentrations applied to cauliflower under deficit irrigation conditions.

significant increase in the proline content with increasing water stress. When the amount of proline was $89.33 \mu\text{g g}^{-1}$ under full irrigation conditions, it increased to $340.26 \mu\text{g g}^{-1}$ under severe stress conditions. The protein content was highest at the 180 % irrigation level of $58.71 \mu\text{g g}^{-1}$. Proline is an abundant amino acid in tissues under stress conditions and has been reported to participate in the detoxification of free O₂ radicals in plants (Knörzer et al., 1999). Considering that tolerance to drought increases with increasing proline content and that the amount of proline decreases with the disappearance of drought, this finding implies that proline plays an important role in the adaptation of plants to drought-related oxidative stress. A study conducted on soybean plants reported that the amount of proline increased as the duration of drought stress increased (Doğan and Avu, 2013). Similarly, a study conducted on soybeans reported a significant increase in the amount of proline under drought stress conditions (Rezayian et al., 2020). A study on cauliflower revealed that the proline and protein contents significantly improved as water stress increased. The application of NO significantly reduced the proline content and the negative effects of water stress.

Several studies have reported a significant increase in the content of MDA and H₂O₂ under stress conditions, which are important indicators of the negative effects of stress (Kayak et al., 2023; Seymen et al., 2023; Yavuz et al., 2023). Significant increases in the MDA and H₂O₂ contents were observed as the severity of water deficit increased in cauliflower. Compared to full irrigation, severe water stress increased the MDA and H₂O₂ contents by approximately 28 % and 41 %, respectively. The disruption of the water balance due to stress causes the formation of ROS, which restricts the organization of membrane and protein functions (Yavuz et al., 2023). A disrupted ROS balance impairs the rate of photosynthetic electron transport and limits plant growth by reducing the photochemical efficiency of PSII (Elkelish et al., 2021).

Numerous studies have shown that enzymatic antioxidants protect against ROS in plants exposed to drought stress. Under full irrigation conditions, at the highest water stress (140 %), an increase of approximately 36, 4, and 436 %, respectively, was observed. In the NO150 treatment, the CAT, SOD, and POD activities were 1071, 2575, and 5204 EU g⁻¹, respectively. A study conducted on Chard revealed that the activities of the enzymes SOD, CAT, and POD increased under drought stress. These authors reported that NO application increased the activities of CAT, POD, and SOD under all water deficit conditions (Ekinci et al., 2020). A study conducted on soybeans revealed that in addition to

increasing MDA and H₂O₂ contents, a significant increase in proline content was noted under drought conditions. The application of NO has been reported to reduce the destructive effects of ROS by promoting antioxidant defense mechanisms (Rezayian et al., 2020). NO functions as a signaling molecule to reduce the ROS-related contradiction of stress by inducing antioxidant enzymes such as SOD and POD (Del Castello et al., 2019). Furthermore, the applied water deficit significantly reduced the increase in CAT and POD activities. Overall, NO150 application prevented the negative effects of ROS by ensuring the highest CAT, SOD, and POD activities.

In this study, the application of 100 µM NO increased the WUE at all irrigation levels except for I60 %. On the other hand, increasing the NO dose by 150 µM increased the WUE only in the treatments where the I60 % irrigation level was applied and caused a decrease in the WUE at other irrigation levels. These results show that 100 µM NO is the threshold for achieving high WUE under full and limited irrigation in cauliflower. In agreement with our findings, 100 µM NO applied to soybeans has been reported to increase WUE (Sousa et al., 2020). Stomatal conductance is known to be an effective factor affecting WUE in water-stressed plants. It has been reported that exogenous NO induces stomatal closure in lettuce grown under deficit irrigation, thereby resulting in significant increases in WUE (Yavuz et al., 2023). In some studies, it has been reported that NO positively affects stomatal-related physiological properties and thus provides water efficiency by protecting plants against water stress (Joudoi et al., 2013; Sun et al., 2017). Our results showed that exogenous NO significantly contributed to water efficiency under limited irrigation conditions as well as under full irrigation conditions.

PCA is an analysis method that provides important information in many stress studies (Seymen et al., 2019; Kal et al., 2023). In the present study, the cumulative variance loadings of the first two components were greater than 63.50 %, indicating that PCA produces effective results in evaluating the data. The first component, which explained 46.13 % of the total variance, was accepted as the component explaining water stress. Research has shown that the first component reveals the effects of stress (Yavuz et al., 2020; Seymen, 2021; Seymen et al., 2023; Yavuz et al., 2023). While full irrigation issues were included in the positive part of the first component, stress issues were included in the negative part.

5. Conclusion

Cauliflower was negatively affected by the applied water stress, and increased water stress resulted in significant decreases in agronomic characteristics. In addition, water stress restricted important physiological parameters, such as stomatal conductance and actual photosynthetic efficiency. Thus, cauliflower is sensitive to water stress. In addition to the contribution of applied nitric oxide to agronomic characteristics, nitric oxide also regulates stomatal conductance and actual photosynthetic efficiency. Drought considerably elevated the content of proline, protein, catalase, and peroxidase activity. In addition, nitric oxide applied to reduce the negative effects of reactive oxygen species occurring under water stress conditions triggered the antioxidant defense system. Principal component analysis revealed that nitric oxide application contributed to plant development under full irrigation conditions. Cauliflower plants are significantly affected by water shortage conditions, and 150 µM nitric oxide contributed to the antioxidant defense system and reduced the negative effects of stress.

CRedit authorship contribution statement

Musa Seymen: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Data curation. **Muhammet Erçetin:** Formal analysis, Data curation. **Duran Yavuz:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Gülbanu Kıymacı:** Validation, Formal analysis, Data curation. **Necibe Kayak:** Formal analysis, Data curation. **Abdurrahman Mutlu:**

Formal analysis, Data curation. **Ertañ Sait Kurtar:** Writing – review & editing, Resources, Funding acquisition.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

Acknowledgements

This research was funded by the SÜ. BAP (Project No. 23401015). This manuscript contains certain data from Muhammet Erçetin's Master's thesis.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2024.113116](https://doi.org/10.1016/j.scienta.2024.113116).

References

- Agarwal, S., Pandey, V., 2004. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. *Biol. Plant.* 48 (4), 555–560. <https://doi.org/10.1023/B:BIOP.0000047152.07878.e7>.
- Ahamed, G.J., Li, X., Mao, Q., Wan, H., Zhou, G., Cheng, Y., 2021. The SIWRKY81 transcription factor inhibits stomatal closure by attenuating nitric oxide accumulation in the guard cells of tomato under drought. *Physiol. Plant* 172 (2), 885–895. <https://doi.org/10.1111/pp.13243>.
- Aksoy, E., 2008. Master's Thesis. Middle East Technical University Institute of Science, Department of Biology.
- Angelini, R., Federico, R., 1989. Histochemical evidence of polyamine oxidation and generation of hydrogen peroxide in the cell wall. *J. Plant Physiol.* 135 (2), 212–217. [https://doi.org/10.1016/S0176-1617\(89\)80179-8](https://doi.org/10.1016/S0176-1617(89)80179-8).
- Avşaroglu, Z.Z., 2016. Doctoral Dissertation. Harran University, Institute of Science, Department of Biology.
- Bates, L.S., Waldren, R.P., Teare, I., 1973. Rapid determination of free proline for water-stress studies. *Plant Soil.* 39 (1), 205–207. <https://doi.org/10.1007/BF00018060>.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72 (1-2), 248–254.
- Chance, B., 1955. Assay of catalase and peroxidase. *Methods Enzymol.* 2, 765–775. [https://doi.org/10.1016/S0076-6879\(55\)02300-8](https://doi.org/10.1016/S0076-6879(55)02300-8).
- Corpas, F.J., Rodríguez-Ruiz, M., Muñoz-Vargas, M.A., González-Gordo, S., Reiter, R.J., Palma, J.M., 2022. Interactions of melatonin, reactive oxygen species, and nitric oxide during fruit ripening: an update and prospective view. *J. Exp. Bot.* 73 (17), 5947–5960. <https://doi.org/10.1093/jxb/erac128>.
- Daszkowska-Golec, A., Szarejko, I., 2013. Open or close the gate - stomata action under the control of phytohormones in drought stress conditions. *Front. Plant Sci.* 4, 138. <https://doi.org/10.3389/fpls.2013.00138>.
- Del Castello, F., Nejankin, A., Cassia, R., Correa-Aragunde, N., Fernández, B., Foresi, N., Lamattina, L., 2019. The era of nitric oxide in plant biology: twenty years tying up loose ends. *Nitric. Oxide* 85, 17–27. <https://doi.org/10.1016/j.niox.2019.01.013>.
- DeVincenzis, A.J., 2020. Doctoral Dissertation. University of California, Davis.
- Dogan, M., Avu, A., 2013. Against drought stress effect of antioxidant enzymes of boron. *Artvin Çoruh Univ. Faculty Forest. J.* 14 (1), 94–103.
- Ekinçi, M., Ors, S., Yildirim, E., Turanc, M., Sahin, U., Dursun, A., Kula, R., 2020. Determination of physiological indices and some antioxidant enzymes of chard exposed to nitric oxide under drought stress. *Russian J. Plant Physiol.* 67 (4), 740–749. <https://doi.org/10.1134/S1021443720040056>.

- Ekinci, M., Selda, Ö.R.S., Turan, M., Yıldırım, E., 2018. Effects of nitric oxide applications on increasing tolerance to abiotic stress conditions in plants. *Yüzüncü Yıl Univ. J. Agric. Sci.* 28 (2), 254–265. <https://doi.org/10.29133/yyutbd.427960>.
- Elkelish, A., Ibrahim, M.F., Ashour, H., Bondok, A., Mukherjee, S., Aftab, T., El-Gawad, H.G.A., 2021. Exogenous application of nitric oxide mitigates water stress and reduces natural viral disease incidence of tomato plants subjected to deficit irrigation. *Agronomy* 11 (1), 87. <https://doi.org/10.3390/agronomy11010087>.
- FAO, 2022. Food and Agriculture organization of the United Nations: home, production statistics. Date of access: September 7, 2022. <https://www.fao.org/faostat/en/#data>.
- Foyer, C.H., Shigeoka, S., 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol.* 155, 93–100. <https://doi.org/10.1104/pp.110.166181>.
- Hamurcu, M., Khan, M.K., Pandey, A., Ozdemir, C., Avsaroglu, Z.Z., Elbasan, F., Gezgin, S., 2020. Nitric oxide regulates watermelon (*Citrus lanatus*) responses to drought stress. *Biotech.* 10 (11), 1–14. <https://doi.org/10.1007/s13205-020-02479-9>.
- Havir, E.A., McHale, N.A., 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol.* 84 (2), 450–455. <https://doi.org/10.1104/pp.84.2.450>.
- Heath, R.L., Packer, L., 1968. Photo peroxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125 (1), 189–198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1).
- Hosseini, S.M., Paydar, M.M., Triki, C., 2021. Implementing sustainable ecotourism in Lafour region, Iran: applying a clustering method based on SWOT analysis. *J. Clean. Prod.* 329, 129716. <https://doi.org/10.1016/j.jclepro.2021.129716>.
- Hu, K.D., Hu, L.Y., Li, Y.H., Zhang, F.Q., Zhang, H., 2007. Protective roles of nitric oxide on germination and antioxidant metabolism in wheat seeds under copper stress. *Plant Growth Regul.* 53 (3), 173–183. <https://doi.org/10.1007/s10072-007-9216-9>.
- Imran, M., Latif Khan, A., Shahzad, R., Aaqil Khan, M., Bilal, S., Khan, A., Lee, I.J., 2021. Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants. *AoB Plants* 13 (4), plab026. <https://doi.org/10.1093/aobpla/plab026>.
- Ipek, M., Pirlak, L., Esitken, A., Figen Dönmez, M., Turan, M., 2014. Plant growth-promoting rhizobacteria (PGPR) increase yield, growth and nutrition of strawberry under high-calcareous soil conditions. *J. Plant Nutr.* 37 (7), 990–1001. <https://doi.org/10.1080/01904167.2014.881857>.
- Jangid, K.K., Dwivedi, P., 2017. Physiological and biochemical changes by nitric oxide and brassinosteroid in tomato (*Lycopersicon esculentum* Mill.) under drought stress. *Acta Physiol. Plant.* 39, 1–10. <https://doi.org/10.1007/s11738-017-2373-1>.
- Joudoi, T., Shichiri, Y., Kamizono, N., Akaïke, T., Sawa, T., Yoshitake, J., Iwai, S., 2013. Nitrate cyclic GMP modulates guard cell signaling in Arabidopsis. *Plant Cell* 25 (2), 558–571. <https://doi.org/10.1105/tpc.112.105049>.
- Kal, Ü., Dal, Y., Kayak, N., Yavuz, D., Türkmen, Ö., Seymen, M., 2023. Application of nitrogen for mitigating the adverse effects of flooding stress in lettuce. *J. Plant Nutr.* 46 (20), 4664–4678. <https://doi.org/10.1080/01904167.2023.2240831>.
- Kaya, C., Ashraf, M., Wijaya, L., Ahmad, P., 2019. The putative role of endogenous nitric oxide in brassinosteroid-induced antioxidant defence system in pepper (*Capsicum annuum* L.) plants under water stress. *Plant Physiol. Biochem.* 143, 119–128. <https://doi.org/10.1016/j.plaphy.2019.08.024>.
- Kayak, N., Kal, Ü., Dal, Y., Yavuz, D., Seymen, M., 2023. Do proline and glycine betaine mitigate the adverse effects of water stress in spinach? *Gesunde Pflanzen* 75, 97–113. <https://doi.org/10.1007/s10343-022-00675-6>.
- Keyvan, S., 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *J. Animal Plant Sci.* 8 (3), 1051–1060.
- Knörzer, O.C., Lederer, B., Durner, J., Böger, P., 1999. Antioxidative defense activation in soybean cells. *Physiol. Plant* 107, 294–302. <https://doi.org/10.1034/j.1399-3054.1999.100306.x>.
- Kumar, P., Gupta, R.C., Kumari, S., Singhal, V.K., 2010. Impact of chromatin transfer and spindle abnormalities on pollen fertility and pollen size in *Plantago lanceolata* L. *Cytologia* 75 (4), 421–426.
- Lau, S.E., Hamdan, M.F., Pua, T.L., Saidi, N.B., Tan, B.C., 2021. Plant nitric oxide signaling under drought stress. *Plants* 10 (2), 360. <https://doi.org/10.3390/plants10020360>.
- Liang, D., Ni, Z., Xia, H., Xie, Y., Lv, X., Wang, J., Lin, L., Deng, Q., Luo, X., 2019. Exogenous melatonin promotes biomass accumulation and photosynthesis of kiwifruit seedlings under drought stress. *Sci. Hortic.* 246, 34–43. <https://doi.org/10.1016/j.scienta.2018.10.058>.
- Lichtenthaler, H., Buschmann, C., 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry*. <https://doi.org/10.1002/0471142913.faf0403s01>. F4-3.
- Liu, C., Liu, Y., Guo, K., Fan, D., Li, G., Zheng, Y., Yu, L., Yang, R., 2011. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six Woody plant species in karst habitats of Southwestern China. *Environ. Exp. Bot.* 71 (2), 174–183. <https://doi.org/10.1016/j.envexpbot.2010.11.012>.
- Lutts, S., Kinet, J., Bouharmont, J., 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann. Bot.* 78 (3), 389–398.
- Mata, C.G., Lamattina, L., 2001. Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiol.* 126 (3), 1196. <https://doi.org/10.1104/pp.126.3.1196>.
- Munawar, A., Akram, N.A., Ahmad, A., Ashraf, M., 2019. Nitric oxide regulates oxidative defense system, key metabolites and growth of broccoli (*Brassica oleracea* L.) plant under water limited conditions. *Sci. Hortic.* 254, 7–13. <https://doi.org/10.1016/j.scienta.2019.04.072>.
- Munemasa, S., Hauser, F., Park, J., Waadt, R., Brandt, B., Schroeder, J.I., 2015. Mechanisms of abscisic acid-mediated control of stomatal aperture. *Curr. Opin. Plant Biol.* 28, 154–162. <https://doi.org/10.1016/j.pbi.2015.10.010>.
- Neill, S., Barros, R., Bright, J., Desikan, R., Hancock, J., Harrison, J., 2008. Nitric oxide, stomatal closure, and abiotic stress. *J. Exp. Bot.* 59, 165–176. <https://doi.org/10.1093/jxb/erm293>.
- Rahman, A., Rath, A., Nambiar, R., Mishra, P.K., Anoop, A., Bhushan, R., 2021. Signatures of natural to anthropogenic transition in lake sediments from the Central Himalaya using stable isotopes. *Appl. Geochem.* 134, 105095. <https://doi.org/10.1016/j.apgeochem.2021.105095>.
- Rezayian, M., Ebrahimzadeh, H., Niknam, V., 2020. Nitric oxide stimulates antioxidant system and osmotic adjustment in soybean under drought stress. *J. Soil. Sci. Plant Nutr.* 20, 1122–1132. <https://doi.org/10.1007/s42729-020-00198-x>.
- Sanchez-Romera, B., Porcel, R., Ruiz-Lozano, J.M., Aroca, R., 2018. Arbuscular mycorrhizal symbiosis modifies the effects of a nitric oxide donor (sodium nitroprusside; SNP) and a nitric oxide synthesis inhibitor (N^ω-nitro-L-arginine methyl ester; L-NAME) on lettuce plants under well watered and drought conditions. *Symbiosis* 74 (1), 11–20. <https://doi.org/10.1007/s13199-017-0486-3>.
- Seymen, M., 2021. Comparative analysis of the relationship between morphological, physiological, and biochemical properties in spinach (*Spinacea oleracea* L.) under deficit irrigation conditions. *Turk. J. Agric. Forest.* 45 (1), 55–67. <https://doi.org/10.3906/tar-2004-79>.
- Seymen, M., Yavuz, D., Dursun, A., Kurtar, E.S., Türkmen, Ö., 2019. Identification of drought-tolerant pumpkin (*Cucurbita pepo* L.) genotypes associated with certain fruit characteristics, seed yield, and quality. *Agric. Water. Manage.* 221, 150–159. <https://doi.org/10.1016/j.agwat.2019.05.009>.
- Seymen, M., Yavuz, D., Eroglu, S., Ari, B.Ç., Tanrıverdi, Ö.B., Atakul, Z., Issi, N., 2023. Effects of different levels of water salinity on plant growth, biochemical content, and photosynthetic activity in cabbage seedling under water-deficit conditions. *Gesunde Pflanzen* 75 (4), 871–884. <https://doi.org/10.1007/s10343-022-00788-y>.
- Shah, A., Smith, D.L., 2020. Flavonoids in agriculture: chemistry and roles in, biotic and abiotic stress responses, and microbial associations. *Agronomy* 10 (8), 1209. <https://doi.org/10.3390/agronomy10081209>.
- Shah, K., Chaturvedi, V., Gupta, S., 2019. Climate change and abiotic stress-induced oxidative burst in rice. *Advances in Rice Research for Abiotic Stress Tolerance*. Woodhead Publishing, pp. 505–535. <https://doi.org/10.1016/B978-0-12-814332-2.00025-3>.
- Sharma, A., Kapoor, D., Wang, J., Landi, M., Zheng, B., Yan, D., 2020. Nitric oxide mediated mechanisms adopted by plants to cope with salinity. *Plant Biol.* 64, 512–518. <https://doi.org/10.32615/bp.2020.070>.
- Shi, K., Li, X., Zhang, H., Zhang, G., Liu, Y., Zhou, Y., 2015. Guard cell hydrogen peroxide and nitric oxide mediate elevated CO₂-induced stomatal movement in tomato. *New Phytologist* 208, 342–353. <https://doi.org/10.1111/nph.13621>.
- Silva, M.G.D., Costa, L.F.D., Soares, T.M., Gheyri, H.R., 2023. Growth and yield of cauliflower with brackish waters under hydroponic conditions. *Rev. Bras. Eng. Agric. Ambient.* 27, 663–672. <https://doi.org/10.1590/1807-1929/agriambi.v27n9p663-672>.
- Sousa, L.F., Menezes Silva, P.E., Lourenço, L.L., Galmés, J., Guimarães, A.C., Silva, A.F., Farnese, F.D.S., 2020. Improving water use efficiency by changing hydraulic and stomatal characteristics in soybean exposed to drought: the involvement of nitric oxide. *Physiol. Plant* 168 (3), 576–589. <https://doi.org/10.1111/ppl.12983>.
- Sun, L., Li, Y., Miao, W., Piao, T., Hao, Y., Hao, F.S., 2017. NADK2 positively modulates abscisic acid-induced stomatal closure by affecting accumulation of H₂O₂, Ca²⁺ and nitric oxide in Arabidopsis guard cells. *Plant Sci.* 262, 81–90. <https://doi.org/10.1016/j.plantsci.2017.06.003>.
- Srivastava, S., Srivastava, M., 2014. Morphological changes and antioxidant activity of Stevi are baudiana under water stress. *Am. J. Plant Sci.* 5 (22), 3417. <https://doi.org/10.4236/ajps.2014.522357>.
- Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sci.* 151 (1), 59–66. [https://doi.org/10.1016/S0168-9452\(99\)00197-1](https://doi.org/10.1016/S0168-9452(99)00197-1).
- Witham, F.H., Blaydes, D.F., Devlin, R.M., 1971. *Experiments in Plant Physiology*. Van Nostrand Reinhold Compan, New York, USA, pp. 55–56.
- Yaşar, F., Üzal, Ö., 2021. Effects of nitric oxide application on antioxidant enzyme activities of pepper plants under drought stress. *ISPEC J. Agric. Sci.* 5 (4), 846–853. <https://doi.org/10.46291/ISPECJASvol5iss4pp846-853>.
- Yavuz, D., Seymen, M., Süheri, S., Yavuz, N., Türkmen, Ö., Kurtar, E.S., 2020. How do rootstocks of citron watermelon (*Citrus lanatus* var. *citroides*) affect the yield and quality of watermelon under deficit irrigation? *Agric. Water. Manage.* 241, 106351. <https://doi.org/10.1016/j.agwat.2020.106351>.
- Yavuz, D., Seymen, M., Kal, Ü., Atakul, Z., Tanrıverdi, Ö.B., Türkmen, Ö., Yavuz, N., 2023. Agronomic and physio-biochemical responses of lettuce to exogenous sodium nitroprusside (SNP) applied under different irrigation regimes. *Agric. Water. Manage.* 277, 108127. <https://doi.org/10.1016/j.agwat.2022.108127>.