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Exogenous Nitric Oxide Donor Mitigates Drought Stress in Pot Marigold (*Calendula officinalis*) Via Enhanced Osmoregulation and Oxidative Stress Management

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Abstract

Elucidating the physiological and biochemical responses of ornamental plants to drought stress and nitric oxide-mediated amelioration strategies is essential for developing sustainable production systems under climate change scenarios. However, comprehensive assessments of sodium nitroprusside (SNP) effects on morpho-physiological traits, oxidative stress responses, and metabolic pathways in drought-stressed ornamentals remain limited. We evaluated the effects of foliar SNP applications at 0, 50, 100, and 150 µM on *Calendula officinalis* L. grown under three irrigation regimes: 100%, 70%, and 40% of field capacity (FC), followed by assessment of morphological parameters, water relations, photosynthetic pigments, oxidative stress markers, antioxidant enzyme activities, osmoprotectant accumulation, and GABA metabolism. Stress treatment and SNP concentration significantly influenced all measured parameters ($p \leq 0.01$). Particularly, 100 µM SNP consistently demonstrated superior ameliorative effects, maintaining higher plant height, shoot biomass, leaf area, and flower production under both well-watered and drought conditions. Under severe drought (40% FC), 150 µM SNP reduced RWC decline from 24.6% to 11.2% and effectively mitigated reductions in total chlorophyll (63.1%) and carotenoids (55.3%) compared to untreated controls. Moreover, SNP enhanced proline accumulation up to 3-fold and substantially reduced oxidative stress markers (H_2O_2, MDA, and EL) by up to 50%. Although drought stress upregulated antioxidant enzymes (CAT, APX, POD, and SOD) by 2.2–4.3 times, SNP application moderated these elevations, suggesting reduced oxidative burden. GABA metabolism also responded markedly, with SNP enhancing glutamate decarboxylase (GAD) activity and GABA content to 0.68 U mg^{-1} protein and 2.47 µmol g^{-1} FW, respectively, while downregulating GABA transaminase (GABA-T) activity to 0.13 U mg^{-1} protein. Principal component analysis revealed that 96% of total trait variance was explained by the first two components, clearly separating treatments by stress level and SNP concentration. We conclude that SNP significantly enhances drought tolerance in pot marigold through coordinated modulation of water relations, antioxidant systems, and GABA shunt pathway activation. The consistent superiority of specific SNP concentrations across multiple functional traits provides insights for optimizing foliar bio-stimulant applications, while the quantitative response patterns identified offer valuable parameters for developing stress-resilient ornamental production systems under changing climatic conditions.

Keywords Antioxidant · Carotenoid content · GABA · Morphological traits

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Introduction

Global climate change, particularly through rising temperatures and irregular precipitation patterns, has become one of the most significant environmental issues threatening agricultural production. Climate projections indicate that global temperatures will rise by 1–2 °C during the 21st century, and this increase will likely lead to more frequent droughts and extreme weather events, particularly in temperate regions where medicinal and ornamental plants are commonly cultivated (Pachauri et al. 2014). Drought stress can negatively affect key physiological processes such as photosynthesis, water balance, growth, and cellular metabolism in medicinal plants, leading to significant reductions in biomass and bioactive compound production (Farouk and Al-Huqail 2020; Shehzad et al. 2020). Furthermore, understanding the defense responses of plants under such stress conditions is crucial for the development of drought-tolerant cultivation strategies (Azizi et al. 2024). Drought stress induces physiological, biochemical, and molecular changes in plants, limiting growth and exacerbating oxidative damage through excessive accumulation of reactive oxygen species (ROS) (Rady et al. 2020; Farooq et al. 2020). In this context, *Calendula officinalis* L., commonly known as pot marigold, stands out as a key research species due to its medicinal importance and sensitivity to water stress. This herbaceous species belonging to the Asteraceae family is distinguished by a diverse range of phytochemicals, including calendulin, calendin, various flavonoids and flavonols, glycosides, and saponins, with demonstrated gastroprotective, antineoplastic, antibacterial, hepatoprotective, and hypoglycemic activities (Pourghasemian et al. 2023; Shahane et al. 2023; Azizi et al. 2024). Therefore, developing drought-resilient cultivation systems for pot marigold is crucial not only for maintaining the production of this valuable medicinal plant but also for ensuring the sustainability of bioactive compound availability.

Plants have evolved sophisticated defense mechanisms to mitigate oxidative stress by maintaining redox homeostasis through enzymatic and non-enzymatic antioxidant systems (Farouk and Omar 2020). This antioxidant network includes low molecular weight compounds such as ascorbic acid, phenolic compounds, anthocyanins, flavonoids, and enzymes including superoxide dismutase, catalase, and peroxidase (Farouk and Al-Huqail 2020; Farooq et al. 2020; Shehzad et al. 2020). Nonetheless, endogenous antioxidant capacity is often insufficient during extended drought, necessitating the use of exogenous agents to enhance stress tolerance. The role of external applications in regulating plant stress responses through modulation of antioxidative defense mechanisms, osmotic adjustment, and metabolic processes is critical. Therefore, investigating the effectiveness of different bioactive compounds in enhancing drought tolerance is valuable for identifying stress-mitigation strategies (Hasanuzzaman and Fujita 2013). Since medicinal plants are particularly vulnerable to drought-induced alterations in secondary metabolite biosynthesis, identifying compounds that can maintain both plant growth and bioactive compound production under water-limited conditions is essential for developing climate change-resistant cultivation systems. The use of nitric oxide donors represents a promising approach for enhancing drought tolerance in medicinal plant species. However, due to the complexity of plant responses to drought stress, comprehensive evaluation of multiple physiological and biochemical parameters is essential for understanding the mechanisms underlying stress mitigation strategies.

Sodium nitroprusside (SNP), a nitric oxide (NO) donor, is a critical signaling molecule involved in plant responses to various abiotic stresses. NO regulates numerous physiological processes, including seed germination, vascular differentiation, stomatal conductance, nutrient uptake, flowering, and fruit development (Niyoifasha et al. 2023). Additionally, NO activates antioxidative defense mechanisms, secondary metabolite biosynthesis, phytohormone signaling, and stress-responsive gene expression, often interacting synergistically with reactive oxygen species (ROS) pathways (Khan et al. 2023). Multiple studies have confirmed that exogenous SNP application enhances plant tolerance to drought and other environmental stresses (Hasanuzzaman and Fujita 2013; Farouk and Al-Huqail 2020; Al-Ashkar et al. 2024; Scialò et al. 2025). Despite these advances, limited information exists on the effects of SNP on the morphological, biochemical, and phytochemical traits of *C. officinalis* under drought conditions. Based on the crucial role of nitric oxide (NO) in modulating plant physiological and biochemical responses under abiotic stress, it is hypothesized that exogenous application of SNP, can enhance drought tolerance in *C. officinalis* by improving antioxidant defense, osmotic adjustment, and GABA metabolism. On the other hand, one of the most common NO releasers is sodium nitroprusside (SNP), which is relatively inexpensive and readily available and releases NO at intracellular pH which can be used on a wide scale to increase drought resistance in drought-sensitive medicinal plant *C. officinalis*. Specifically, it is expected that SNP treatment will mitigate oxidative damage, sustain photosynthetic efficiency, and promote the accumulation of compatible solutes under water deficit conditions. In this perspective, the objectives of the study are as follows: (I) to evaluate the effects of foliar application of sodium nitroprusside (SNP) at different concentrations on morphological, physiological, and biochemical responses of *C. officinalis* under drought stress; (II) to assess the impact of SNP treatment on oxidative stress markers,

antioxidant enzyme activities, and osmoprotectant accumulation during water deficit conditions; (III) to analyze the changes in GABA metabolism and related enzyme activities in response to SNP application under drought stress, with the goal of identifying potential mechanisms underlying stress tolerance; and (IV) to determine the effectiveness of different SNP concentrations in enhancing drought tolerance, providing valuable insights for optimizing cultivation strategies for pot marigold under water-limited conditions. Furthermore, limited water resources in Iran are a limiting factor in the cultivation of medicinal plants, which require irrigation. Hence, by achieving these objectives, the study seeks to contribute essential knowledge for developing sustainable production systems for this valuable medicinal plant, thus supporting the maintenance of bioactive compound availability in the face of climate-induced challenges.

Materials and Methods

Plant Materials and Treatments

Greenhouse experiments were conducted in 2025 at the research facility of the Department of Horticultural Sciences, Ilam University, Iran (coordinates: 33°39′24.3″N, 46°22′37.1″E; elevation: 1472 m above sea level). In order to ensure plant material quality, seeds of *C. officinalis* L., with a purity of 92%, germination rate of 90%, and thousand-seed weight of 12.8 g, were obtained from Pakan-Bazr Company. To control for potential pathogens, seed surface sterilization was performed using a 5% (v/v) sodium hypochlorite solution for 10 min, followed by three rinses with sterile distilled water. For seed germination, trays were prepared using a growing medium composed of coco peat and perlite mixed in equal proportions by volume (1:1). Once the seedlings reached the towe-leaf stage, they were transplanted into pots (20 cm in diameter and 25 cm in depth) filled with sandy loam soil, consisting of 55.80% sand, 34.85% silt, and 9.35% clay (Table S1) and grown under controlled greenhouse conditions, with temperature maintained at 23 ± 2 °C and relative humidity maintained between 60 and 65%. Weed control was performed manually throughout the plant growth period, and no fertilization was conducted during the experiment. The experiment was conducted as a factorial arrangement (4 levels of SNP concentrations × 3 levels of irrigation levels) whithin a Completely Randomized Design (CRD) with three replications. Each replication consisted of five pots for each treatment combination, and each pot (containing one plant) was considered as one experimental unit. In total, 180 pots were used for the experiment. These consisted of four SNP concentrations (0 µM as control, 50 µM, 100 µM, and 150 µM) and three irrigation levels corresponding to 100% (control), 70%, and 40% of field capacity (FC). Soil FC was determined following the method described by Pourmeidani et al. (2017). The procedure used is as described by the aforementioned authors. In brief, 5 kg of air-dried soil was oven-dried at 105 °C for 72 h in order to obtain dry weight. The soil was then saturated with water in containers and weighed every two hours over 36 h. Once weight stabilization was observed, a representative soil sample was collected for moisture content analysis. Soil moisture at FC was calculated using the formula:

$$\text{Soil water content (\%)} = [(\text{Fresh soil weight} - \text{Dry soil weight}) / \text{Dry soil weight}] \times 100 \quad (1)$$

On the basis of the 100% FC measurement, the corresponding water volumes for 70% and 40% FC were computed. Irrigation scheduling was based on pot weight and evapotranspiration estimates, with watering applied every two days. SNP spraying (0 µM SNP 'distilled water', 50 µM SNP, and 100 µM SNP) was carried out four times (at 25, 40, 55, and 70 days from transplanting), using hand-atomizer. The volume of spraying solutions (200 mL per pot) was applied to run-off and a few drops of surfactants. These SNP levels and spraying times were selected from a preliminary investigation for one month, where they were produced maximum plant biomass.

Evaluation of Growth Traits

At full bloom, occurring 90 days after sowing, various morphological traits were evaluated. Plant height, flower diameter, and root length were measured using a standard ruler, while leaf length and width were recorded. Following harvest, the fresh biomass of the aerial parts was determined using a precision digital balance. Shoot samples were then air-dried at room temperature (25–27 °C) for five days, after which their dry weight was recorded to assess biomass accumulation.

Relative Water Content Assessment

To determine the water status of leaf tissues, relative water content (RWC) was assessed following the procedure described by Ghoulam et al. (2002). Samples were collected from the upper canopy of plants within each treatment group. The fresh weight (Ww) of each leaf sample was immediately measured using a digital balance. To enable full hydration, samples were submerged in distilled water and incubated under light for 24 h to obtain the turgid weight (Wt). Excess surface moisture was then carefully removed before recording the turgid weight. Subsequently, samples

were oven-dried at 75 °C for 48 h in order to obtain the dry weight (Wd). The RWC was calculated using the following equation:

$$\text{RWC}\ (\%) = [(\text{Ww} - \text{Wd}) / (\text{Wt} - \text{Wd})] \times 100 \quad (2)$$

Total Chlorophyll and Carotenoid Concentration Measurement

To quantify chlorophyll and carotenoid contents in both shoot and petal tissues, the method outlined by Lichtenthaler (1987) was adopted with some modifications. Initially, 0.5 g of fresh plant material was collected and homogenized thoroughly in 15 mL of 80% (v/v) acetone. The homogenate was then centrifuged at 10,000 × g for 15 min at 4 °C using a Prism centrifuge (Labnet, USA) to obtain a clear supernatant. Absorbance readings of the extract were recorded using a UV-visible spectrophotometer (LABOMED UVS-2800) at wavelengths of 645 nm and 663 nm for chlorophyll, and 480 nm and 510 nm for carotenoids. Pigment concentrations were expressed as milligrams per gram of fresh weight (mg·g^{-1} FW) and calculated using the equations described by Lichtenthaler (1987):

$$\text{Total Chlorophyll} = [20.2 \times A_{645} + 8.02 \times A_{663}] \times V / (W \times 100) \quad (3)$$

$$\text{Total Carotenoids} = [7.6 \times A_{480} - 1.49 \times A_{510}] \times V / (W \times 100) \quad (4)$$

where A denotes absorbance at specific wavelengths, V represents the volume of the extract (mL), and W stands for the weight of the fresh tissue (g) used for extraction.

Oxidative Stress Assessment

Hydrogen Peroxide Quantification

To determine hydrogen peroxide (H_2O_2) concentrations in plant tissues, the method reported by Tariq et al. (2011) was employed. In brief, 1 g of fresh material was homogenized in 2 mL of acetone. The supernatant was then treated with a titanium-based reagent to reach a final concentration of 2% titanium (IV). Precipitation of the titanium–peroxide complex and excess titanium was induced by adding 0.2 mL of 17 M ammonium hydroxide. The resulting precipitate was washed five times with chilled acetone through repeated resuspension and decantation. After drying, the pellet was dissolved in 3 mL of 1 M sulfuric acid, and absorbance was measured at 410 nm using a spectrophotometer.

Evaluation of Membrane Integrity Via Electrolyte Leakage

Cell membrane stability was evaluated by assessing electrolyte leakage (EL) from shoot tissues following the procedure described by Tariq et al. (2011). Shoot samples of similar size were submerged in 25 mL of deionized water at room temperature for 4 h. The initial electrical conductivity (EC_1) of the bathing solution was then measured. Subsequently, samples were boiled for 30 min to ensure complete release of cellular ions, after which the final conductivity (EC_2) was recorded. Membrane permeability was calculated as the percentage of EL using the following formula:

$$\text{Electrolyte Leakage}\ (\text{EL}\%) = (EC_1/EC_2) \times 100 \quad (5)$$

Malondialdehyde Content

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) content as a thiobarbituric acid (TBA)-reactive substance. The method used is essentially the same as that proposed by Shao et al. (2006). Samples were homogenized in 5% (w/v) trichloroacetic acid (TCA), and the resulting supernatant was reacted with 5% TBA. The reaction mixture was incubated at 100 °C for 30 min and then cooled to room temperature. Absorbance was measured at 532 nm to quantify MDA, with readings at 600 nm used to correct for nonspecific turbidity. MDA levels were expressed as nanomoles per gram of fresh weight (nmol g^{-1} FW).

Assessment of Antioxidant Enzyme Activities

Antioxidant enzyme activities were measured spectrophotometrically. Fresh leaf tissue (500 mg) was homogenized in a potassium phosphate buffer (pH 7.0), then centrifuged. The resulting supernatant was used to assay catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase (SOD) activities, as well as total protein content.

CAT Activity

CAT activity was determined by measuring the decomposition of H_2O_2 at 240 nm. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2. A 50 µL aliquot of enzyme extract was added to bring the final volume to 1.5 mL. The decline in absorbance at 240 nm was monitored for 60 s, and CAT activity was calculated as the rate of absorbance decrease per minute per gram of fresh tissue (Aebi 1984).

POD Activity

POD activity was assayed using guaiacol as a substrate. The reaction mixture contained guaiacol and H_2O_2, and the increase in absorbance at 470 nm, due to the oxidation of guaiacol catalyzed by POD, was recorded spectrophotometrically. Enzyme activity was calculated based on the rate of change in absorbance per minute per gram of fresh tissue (Zhang and Davies 1989).

APX Activity

APX activity was determined spectrophotometrically by monitoring the decrease in absorbance at 290 nm, which reflects the oxidation of ascorbate. The reaction mixture contained phosphate buffer (pH 7.0), ascorbate, H_2O_2, and the enzyme extract. The rate of decline in absorbance per minute was used to calculate APX activity, expressed as units per gram of fresh tissue (Rao et al. 1997).

SOD Activity

SOD activity was evaluated by its capacity to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 12 µM methionine, 75 µM NBT, and 0.1 µM EDTA. To this mixture, 50 µL of enzyme extract and 10 µL of riboflavin were added. The reaction tubes were gently mixed and then exposed to fluorescent light for 15 min to initiate the photoreduction process. Absorbance was subsequently measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction under assay conditions. Enzyme activity was normalized to the protein concentration of the extract (Beauchamp and Fridovich 1971).

Determination of GABA Content and Associated Enzymatic Activities

The quantification of GABA was performed using a modified protocol based on Li et al. (2018). The method is in line with the procedure used by the aforementioned authors with some modifications. In brief, 1.0 g of frozen plant tissue was finely ground under liquid nitrogen and extracted with 10 mL of methanol at room temperature for 10 min. The homogenate was centrifuged at 12,000 ×g for 10 min at 25 °C. The supernatant was evaporated to dryness under a stream of nitrogen gas, and the residue was reconstituted in 1 mL of 70 mmol L^{-1} $LaCl_3$ solution. This suspension was centrifuged again (12,000 ×g, 15 min, 25 °C), and 0.8 mL of the clarified supernatant was combined with 0.16 mL of 1 mmol L^{-1} KOH, followed by a subsequent centrifugation step (12,000 ×g, 5 min, 25 °C). Then, 0.5 mL of this solution was mixed with 3 mL of potassium pyrophosphate buffer (0.1 mol L^{-1}, pH 8.6), containing α-ketoglutarate (1 mmol L^{-1}), $NADP^+$ (0.6 mmol L^{-1}), and 0.1 units of GABA transaminase (GABase). The enzymatic conversion of $NADP^+$ to NADPH was monitored spectrophotometrically at 340 nm, and GABA concentration was calculated using a standard calibration curve. For enzymatic activity assays related to GABA metabolism, 2.0 g of frozen tissue was ground in liquid nitrogen and extracted in 5 mL of 50 mmol L^{-1} Tris-HCl buffer (pH 9.1), supplemented with 10% glycerol, 5 mmol L^{-1} EDTA, 1 mmol L^{-1} dithiothreitol (DTT), 1 mmol L^{-1} phenylmethylsulfonyl fluoride (PMSF), and 0.5 mmol L^{-1} pyridoxal phosphate. The homogenate was centrifuged at 12,000 ×g for 20 min at 4 °C, and the resulting supernatant was used for subsequent enzymatic analyses.

Glutamate Decarboxylase Activity

For the assessment of glutamate decarboxylase (GAD) activity, the procedure reported by Wang et al. (2016) was adopted. Specifically, 0.1 mL of the enzyme-containing supernatant was mixed with 0.9 mL of 0.1 mol L^{-1} phosphate buffer (pH 5.8) containing 3 mmol L^{-1} L-glutamate as the substrate and 40 µmol L^{-1} pyridoxal phosphate as a coenzyme. The reaction mixture was incubated at 30 °C for 60 min in a thermostatically controlled water bath. The enzymatic reaction was halted by adding 0.1 mL of 0.5 mol L^{-1} trichloroacetic acid.

GABA Transaminase Activity

To determine GABA transaminase (GABA-T) activity, the method outlined by Pan et al. (2023) was employed. In this procedure, 0.2 mL of the enzyme extract was combined with 1.8 mL of 50 mmol L^{-1} Tris-HCl buffer (pH 8.2) containing 10% (v/v) glycerol, 16 mmol L^{-1} GABA, 4 mmol L^{-1} pyruvate, 1.5 mmol L^{-1} dithiothreitol, 0.75 mmol L^{-1} EDTA, and 0.1 mmol L^{-1} pyridoxal phosphate. The reaction mixture was incubated at 30 °C for 60 min to allow enzymatic activity. The reaction was stopped by adding 0.1 mL of 0.1 mol L^{-1} sulfosalicylic acid.

Data Analysis

The experiment was arranged as a factorial design within a completely randomized design (CRD) framework, with three replications per treatment. Data analysis was performed using SAS software version 9.4. Statistical significance was analyzed using two-way analysis of variance (ANOVA), and mean comparisons were performed using the least significant difference (LSD) test at a 5% significance

level. Assumptions of ANOVA were verified prior to analysis using the Shapiro–Wilk test for normality and Levene's test for homogeneity of variances. Additionally, Pearson's correlation network analysis was carried out using R software, and all graphical outputs were prepared using Origin software version 2021.

Results

SNP Promotes Plant Growth Traits Under Drought Stress

The interaction between water stress and SNP application was found to significantly influence pot marigold growth traits (Table S2). These tests revealed a general decline in all morphological parameters with increasing drought severity, with the notable exception of root length (Table 1). Interestingly, SNP treatments were shown to mitigate the adverse effects of water deficit effectively. Under well-watered conditions (100% FC), strong evidence was found that plants treated with 100 µM SNP exhibited the greatest plant height (26.29 ± 0.47 cm), significantly exceeding other treatments. Further analysis showed that plant height progressively decreased with reduced irrigation, reaching a minimum of 9.30 ± 0.54 cm under 40% FC without SNP treatment. The analysis revealed that water deficit from 100% to 40% FC caused a 56.3% reduction in plant height; however, pre-treatment with 100 µM SNP was found to curtail this decline to 19.7% relative to the control (100% FC without SNP). Similarly, the results showed that shoot fresh and dry weights were reduced by 52.8% and 61.1%, respectively, under severe drought and no SNP application. Despite these reductions, further analyses demonstrated that SNP treatments enhanced shoot biomass across all irrigation regimes. The most striking result to emerge from the data is that the highest fresh (45.55 ± 0.44 g plant^{-1}) and dry (17.53 ± 0.58 g plant^{-1}) weights were recorded in plants treated with 100 µM SNP at 100% FC, followed by 150 µM SNP at the same irrigation level.

The tests revealed that exposure to 40% FC significantly decreased leaf length and width by 33.2% and 38%, respectively, compared to untreated control plants. Remarkably, application of 100 µM SNP, a NO donor, was shown to effectively mitigate these reductions, limiting decreases to only 2.4% and 3.4% in leaf length and width, respectively, relative to untreated controls (Table 1). No significant differences were identified among other SNP concentrations under well-watered conditions, except for the 100 µM treatment. Conversely, the analysis showed that root length exhibited a positive response to water deficit, increasing by 35.7% and 71% under 70% and 40% FC, respectively, compared to 100% FC. Moreover, the results demonstrated that SNP application, particularly at 150 µM, further promoted root elongation beyond the effect of drought alone. The shortest root length (12.45 ± 0.70 cm) was observed in untreated plants at 40% FC, whereas the longest roots (28.07 ± 0.15 cm) were recorded in plants treated with 150 µM SNP under the same water deficit. Further examination revealed that flower diameter decreased with increasing drought severity, though treatments with 100 and 150 µM SNP produced comparable enhancements in flower size. The smallest flower diameter (2.98 ± 0.34 cm) was found in untreated plants subjected to 40% FC. The analysis showed that the number of flowers was significantly reduced under severe drought in untreated plants (4.25 ± 0.12) compared to

Table 1 Mean (±standard error) comparison of interaction effects of sodium Nitroprusside application and drought stress on growth traits of *Calendula officinalis*

Sodium nitroprusside (µM)	Drought stress (% FC)	Plant height (cm)	Shoot fresh Weight (g.plant^{-1})	Shoot dry Weight (g.plant^{-1})	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Flower diameter (cm)	Number of flower
0	100	21.33 ± 0.57^{de*}	36.69 ± 0.92^{d}	10.82 ± 0.19^{c}	10.55 ± 0.23^{c}	4.06 ± 0.28^{cd}	12.45 ± 0.70^{i}	4.56 ± 0.16^{cd}	7.33 ± 0.11^{d}
50	100	23.34 ± 0.58^{bc}	39.33 ± 0.63^{c}	12.01 ± 0.41^{c}	10.77 ± 0.30^{c}	4.06 ± 0.06^{cd}	13.95 ± 0.28^{h}	5.22 ± 0.32^{ab}	7.86 ± 0.39^{d}
100	100	26.29 ± 0.47^{a}	45.55 ± 0.44^{a}	17.53 ± 0.58^{a}	12.53 ± 0.19^{a}	5.11 ± 0.17^{a}	14.89 ± 0.33^{gh}	5.44 ± 0.19^{a}	11.96 ± 0.17^{a}
150	100	24.54 ± 0.42^{b}	42.32 ± 0.52^{b}	14.58 ± 0.50^{b}	11.13 ± 0.35^{bc}	4.32 ± 0.04^{bc}	15.53 ± 0.60^{fg}	5.50 ± 0.05^{a}	10.56 ± 0.32^{b}
0	70	16.32 ± 0.68^{g}	26.33 ± 0.64^{g}	7.11 ± 0.38^{fg}	8.41 ± 0.09^{d}	3.27 ± 0.11^{fg}	16.90 ± 0.28^{f}	3.25 ± 0.07^{gh}	5.60 ± 0.26^{f}
50	70	18.25 ± 0.43^{f}	28.60 ± 0.47^{f}	8.46 ± 0.52^{de}	9.07 ± 0.26^{cd}	3.80 ± 0.15^{de}	19.13 ± 0.47^{e}	3.80 ± 0.11^{ef}	6.52 ± 0.31^{e}
100	70	22.35 ± 0.55^{cd}	35.40 ± 0.56^{d}	13.93 ± 0.41^{b}	11.86 ± 0.60^{ab}	4.51 ± 0.19^{c}	21.32 ± 0.58^{d}	4.77 ± 0.15^{bc}	9.08 ± 0.15^{c}
150	70	20.61 ± 0.21^{e}	32.23 ± 0.63^{e}	11.44 ± 0.52^{c}	10.31 ± 0.31^{c}	3.94 ± 0.08^{cd}	23.39 ± 0.60^{c}	5.12 ± 0.15^{ab}	7.75 ± 0.08^{d}
0	40	9.30 ± 0.54^{j}	17.31 ± 0.60^{j}	4.20 ± 0.26^{h}	7.04 ± 0.35^{e}	2.94 ± 0.08^{g}	21.29 ± 0.58^{d}	2.98 ± 0.34^{h}	4.25 ± 0.12^{g}
50	40	12.81 ± 0.26^{i}	19.30 ± 0.57^{i}	6.21 ± 0.33^{g}	8.34 ± 0.29^{d}	3.43 ± 0.29^{ef}	24.29 ± 0.49^{c}	3.58 ± 0.15^{fg}	4.79 ± 0.08^{g}
100	40	17.11 ± 0.44^{fg}	26.99 ± 0.97^{fg}	9.11 ± 0.33^{d}	10.29 ± 0.27^{c}	3.92 ± 0.05^{cd}	26.46 ± 0.55^{b}	4.21 ± 0.10^{de}	5.70 ± 0.05^{f}
150	40	14.53 ± 0.42^{h}	22.33 ± 0.50^{h}	7.55 ± 0.29^{ef}	9.24 ± 0.16^{d}	3.92 ± 0.10^{cd}	28.07 ± 0.15^{a}	4.17 ± 0.03^{de}	5.66 ± 0.14^{f}
LSD		1.42	1.88	1.20	0.91	0.41	1.45	0.52	0.69
Mean		18.90	31.03	10.24	9.96	3.94	19.81	4.38	7.25

*Values in the same column followed by the same letter (s) are not significantly different at $P < 0.05$ according to the LSD test.

those grown at 100% FC (7.33 ± 0.11). Notably, these tests revealed that application of 100 µM SNP increased flower counts to 9.08 ± 0.15 and 5.70 ± 0.05 under 70% and 40% FC, respectively, compared to untreated plants at the same irrigation levels (Table 1).

SNP Improves Plant RWC and Pigments Under Drought Stress

A statistically significant interaction between SNP application and drought treatment was identified in influencing the RWC of *C. officinalis* leaves (Table S3). As shown in Fig. 1, the analysis revealed that leaf RWC decreased by approximately 7% and 24.6% in plants subjected to drought stress at 70% and 40% FC, respectively, compared to well-watered, untreated controls. Interestingly, pretreatment with SNP was found to ameliorate the drought-induced decline in RWC in a dose-dependent manner; specifically, treatment with 150 µM SNP was shown to reduce the RWC decrease to 5.5% at 70% FC and 11.2% at 40% FC relative to well-watered, untreated plants. Similarly, significant effects were observed on total chlorophyll (Fig. 2a) and carotenoid content (Fig. 2b), although the response was not entirely dose-dependent on SNP concentration. The tests revealed that severe drought stress caused a marked reduction in total chlorophyll and carotenoids by 63.1% and 55.3%, respectively, compared to well-watered, untreated plants. The analysis showed that application of 100 µM SNP resulted in a notable enhancement of both pigments under control (100% FC) and drought conditions. Consequently, the highest pigment concentrations were recorded in well-watered plants pretreated with 100 µM SNP, whereas the lowest levels were found in severely drought-stressed plants (Fig. 2a and b).

SNP Reduced Oxidative Damage by Modulation of Biomarkers Under Drought Stress

To evaluate the potential of SNP in mitigating oxidative stress induced by drought, levels of proline, H_2O_2, MDA, and EL were quantified. The analysis revealed that all these parameters showed significant increases under severe drought stress (40% FC), rising approximately 3-, 3.1-, 4.1-, and 2-fold, respectively, compared to plants grown under well-watered conditions (100% FC) without SNP application (Figs. 3a–d). The results showed that foliar application of 150 µM SNP further enhanced proline accumulation, with the highest proline content (6.1 µmol g^{-1} FW) observed in plants subjected to severe drought stress and SNP treatment (Fig. 3a). Conversely, further analysis demonstrated

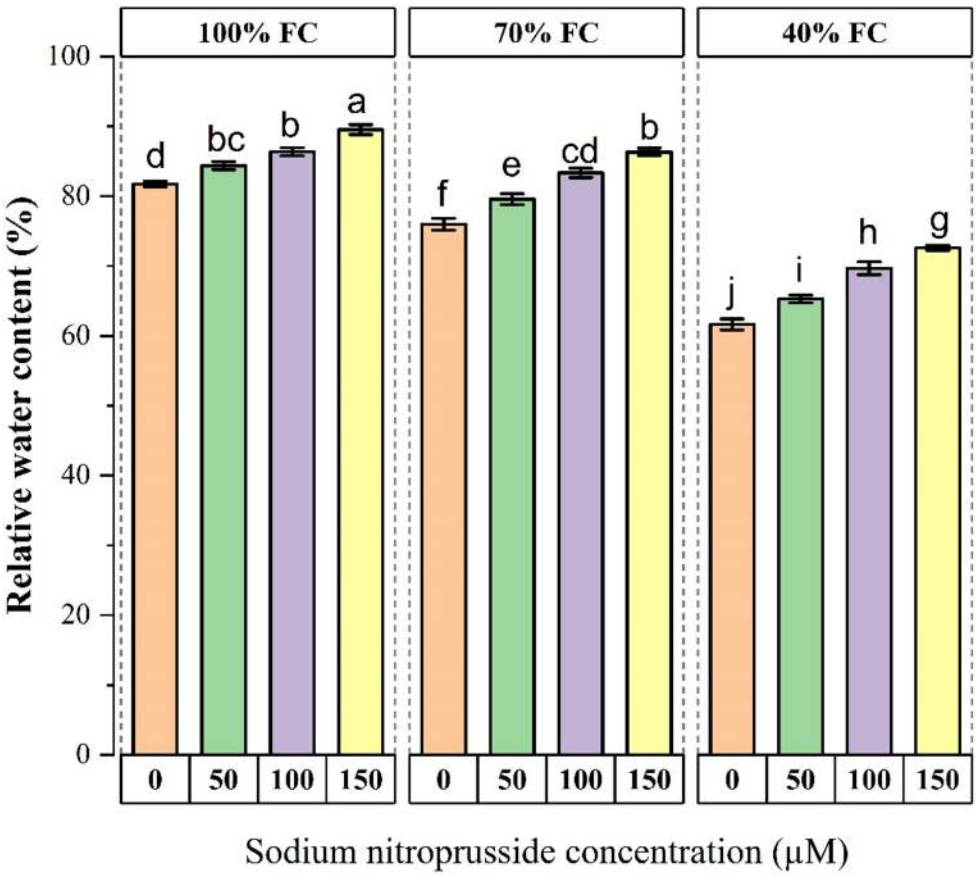


Fig. 1 The effect of sodium nitroprusside treatment on relative water content in pot marigold under different drought stress conditions (Means ± SE with at least one common letter at the 5% probability level of LSD test show no significant difference)

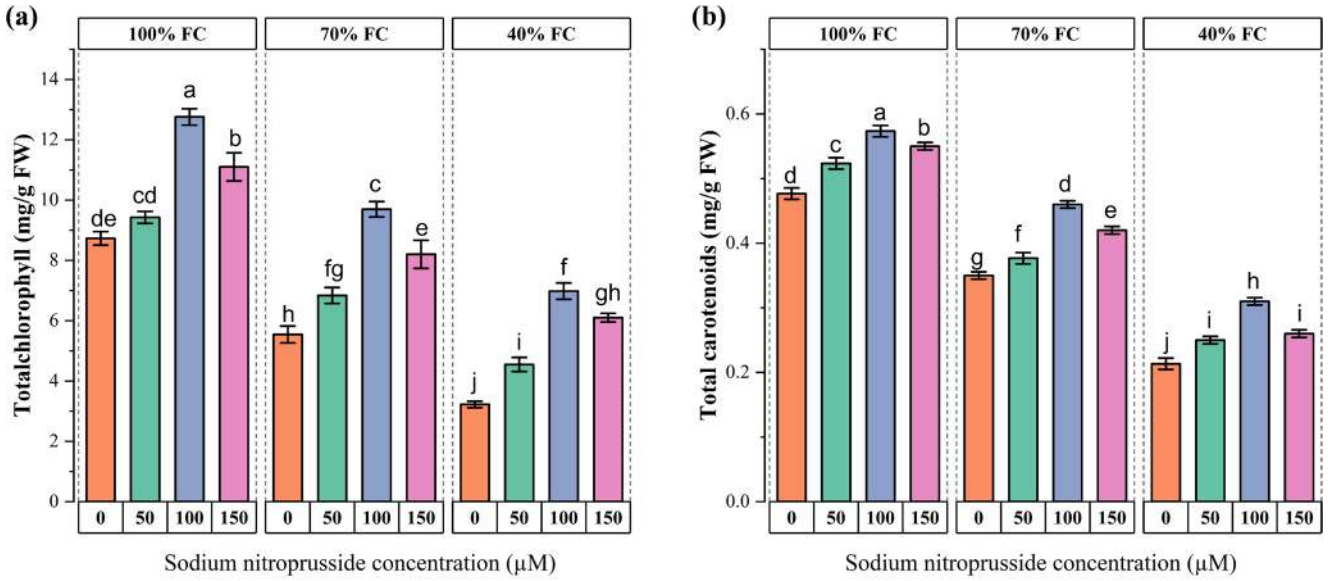


Fig. 2 The effect of sodium nitroprusside treatment on chlorophyll content (**a**) and carotenoids content (**b**) in pot marigold under different drought stress conditions (Means ± SE with at least one common letter at the 5% probability level of LSD test show no significant difference)

that H_2O_2, MDA, and EL levels were markedly reduced following SNP treatment, particularly at the 150 µM concentration. The highest levels of these oxidative stress markers were recorded in drought-stressed plants (40% FC) without SNP, while plants pretreated with 100 and 150 µM SNP under well-watered conditions (100% FC) exhibited the lowest values for these parameters (Figs. 3b–d). The analysis revealed a minor difference between the effects of 100 and 150 µM SNP under well-watered conditions, suggesting that SNP's protective effects are more pronounced under drought stress than in optimal irrigation.

SNP Decreased Plant Antioxidative Enzymes Under Drought Stress

The tests revealed that drought stress induced a significant upregulation of antioxidant enzyme activities in pot marigold, with CAT, APX, POD, and SOD increasing by approximately 2.2-, 4.1-, 4.3-, and 3.3-fold, respectively. However, the analysis showed that foliar application of SNP at different concentrations reduced the elevated activities of these enzymes in a concentration-dependent manner under drought conditions, whereas no significant differences were observed among SNP treatments in well-watered plants (100% FC) (Figs. 4a–d). Consequently, the lowest antioxidant enzyme activities were generally recorded in SNP-treated plants under non-stress conditions, while the highest activities were found in untreated plants subjected to severe drought stress (40% FC), as shown in Figs. 4a–d.

SNP Increased Plant GAD and GABA while Decreasing GABA-T Under Drought Stress

According to Fig. 5, the analysis revealed that SNP treatment had a significant effect on GAD activity, GABA-T activity, and GABA content in pot marigold under varying drought stress conditions (Table S3). The tests showed that as drought severity increased, both GAD activity and GABA content rose (Fig. 5a and c), whereas GABA-T activity declined (Fig. 5b). Furthermore, the results demonstrated that foliar application of SNP, particularly at 150 µM, significantly enhanced GAD activity and GABA content. The most striking result to emerge from the data is that plants grown under 40% FC and treated with 150 µM SNP exhibited the highest GAD activity (0.68 U mg^{-1} protein) and GABA content (2.47 µmol g^{-1} FW), as shown in Fig. 5a and c. Conversely, the analysis revealed that GABA-T activity decreased in a dose-dependent manner in response to SNP treatment, with the highest activity (0.53 U mg^{-1} protein) recorded in control plants without SNP and the lowest activity (0.13 U mg^{-1} protein) observed in severe drought-stressed plants treated with 150 µM SNP (Fig. 5b and 6).

Pearson Correlation and PCA

The Pearson correlation analysis revealed significant and biologically meaningful relationships among morphological and biochemical traits of *C. officinalis* under varying drought and SNP treatments (Fig. 6). The correlation matrix was constructed using mean values of all measured variables, including photosynthetic traits, oxidative stress

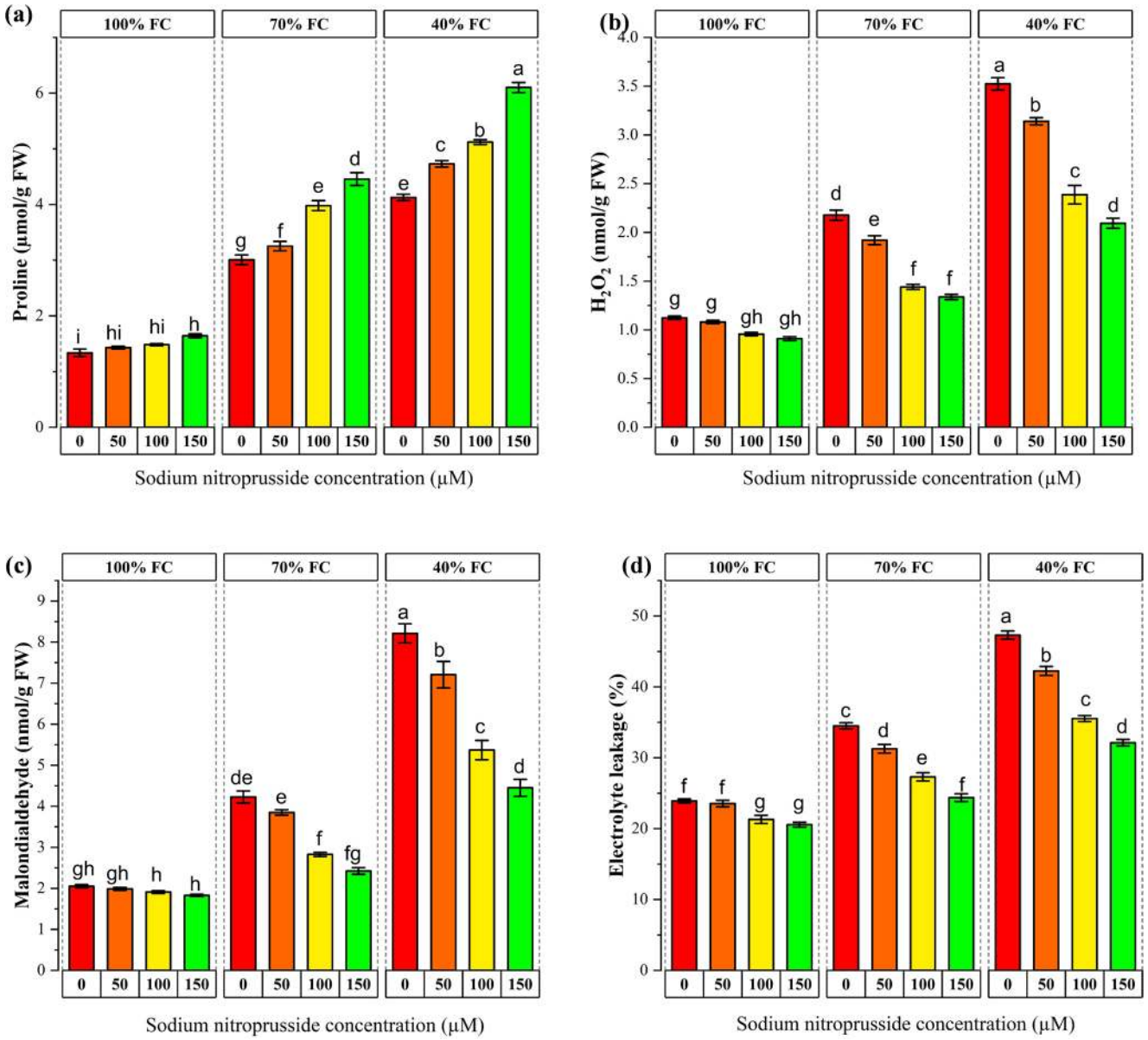


Fig. 3 The effect of sodium nitroprusside treatment on proline (**a**), H_2O_2 (**b**), malondialdehyde (**c**), and electrolyte leakage (**d**) in pot marigold under different drought stress conditions (Means ± SE with at least one common letter at the 5% probability level of LSD test show no significant difference)

markers (e.g., H_2O_2, MDA), antioxidant enzyme activities (SOD, CAT, POD), osmoprotectants, and GABA metabolism–related enzymes. Significant correlations were selected based on a threshold of $|r| \geq 0.70$ and $p < 0.05$, and visualized as a network using software R. The analysis showed that morphological traits such as shoot dry weight, number of flowers, shoot fresh weight, and plant height exhibited strong positive correlations ($r > 0.90$, $p < 0.001$) with photosynthetic pigments (chlorophyll and carotenoids) and relative water content (RWC). This finding confirms that better water status and pigment retention enhance growth and yield components. Further analysis demonstrated that GABA pathway-related traits (GABA, GAD, GABA-T), proline content, and root length were highly correlated, reflecting coordinated osmotic adjustment under stress. The tests revealed that antioxidant enzymes (CAT, APX, POD, and SOD) correlated positively with oxidative stress markers (MDA, H_2O_2, and EL), suggesting a tightly regulated defense network. Interestingly, these oxidative markers showed strong negative correlations with growth and physiological parameters, especially shoot dry weight, RWC, and pigment content, highlighting the adverse effects of oxidative damage on plant performance. Overall, the data underscore the integrative role of antioxidant systems and

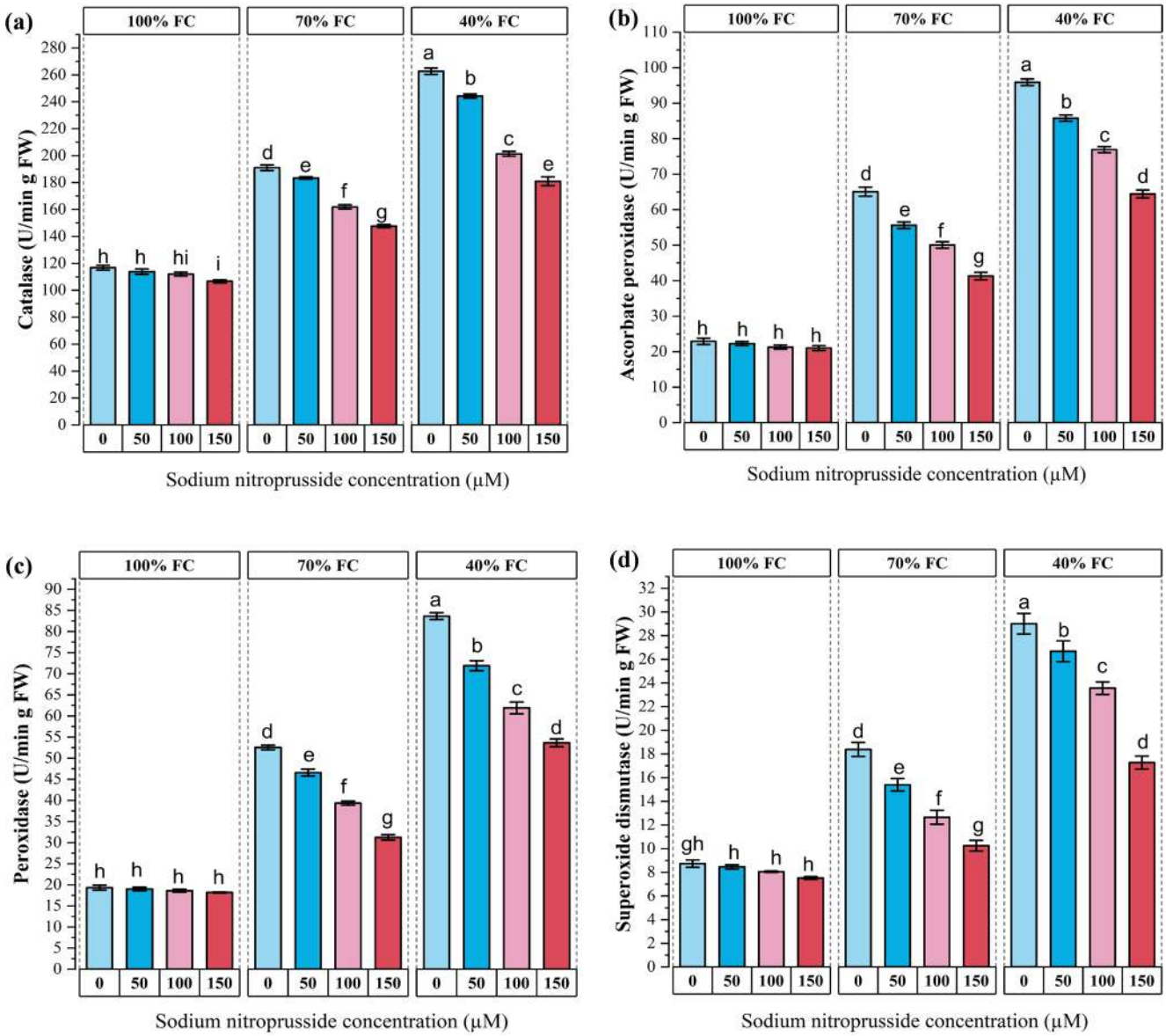


Fig. 4 The effect of sodium nitroprusside treatment on catalase (**a**), ascorbate peroxidase (**b**), peroxidase (**c**), and superoxide dismutase (**d**) activity in pot marigold under different drought stress conditions (Means ± SE with at least one common letter at the 5% probability level of LSD test show no significant difference)

osmolyte accumulation in promoting growth and drought resilience in *C. officinalis* under SNP treatment. PCA further elucidated relationships among traits across SNP concentrations and drought levels. The analysis revealed that the first two components explained 96% of total variance (PC1 = 79.3%, PC2 = 16.7%). The PCA bi-plot distinctly separated treatments by response patterns: the results showed that moderate to high SNP concentrations (100 and 150 µM) under mild to moderate drought correlated with elevated GABA and proline levels, indicating enhanced osmotic and stress tolerance. The analysis demonstrated that severe drought, with low or no SNP, clustered near oxidative stress indicators (MDA, H_2O_2, EL, POD, CAT, APX), reflecting increased cellular damage. Remarkably, well-watered plants treated with higher SNP doses were positively associated with photosynthetic pigments and RWC, indicating improved physiological status. These findings confirm that exogenous SNP, especially at 100–150 µM, enhances drought tolerance in *C. officinalis* by modulating antioxidant defenses and osmoprotectant accumulation (Fig. 7).

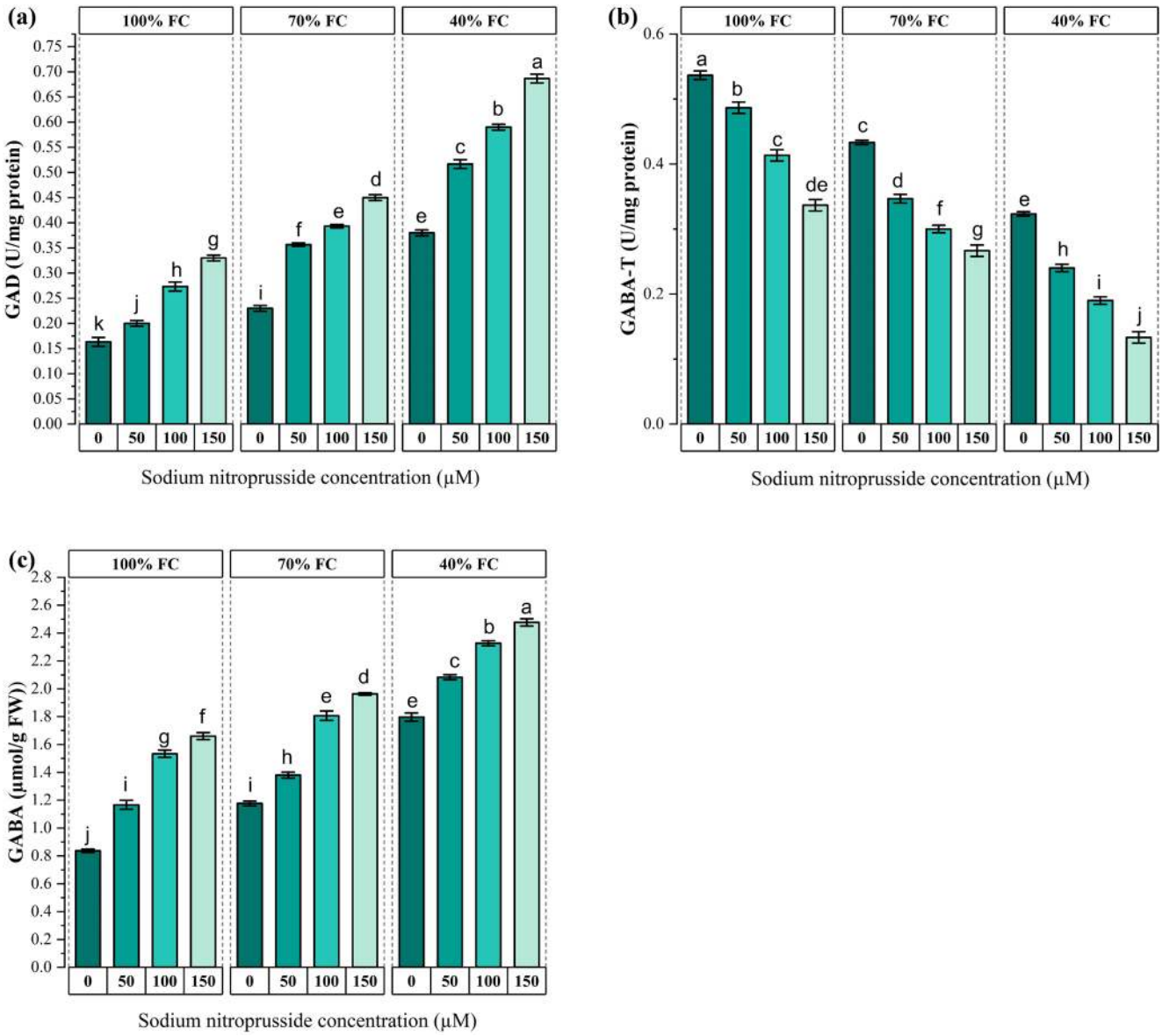


Fig. 5 The effect of sodium nitroprusside treatment on GAD activity (**a**), GABA-T activity (**b**), and GABA content (**c**) in pot marigold under different drought stress conditions (Means ± SE with at least one common letter at the 5% probability level of LSD test show no significant difference)

Discussion

SNP and Morphological Traits Under Drought Stress

Our findings demonstrate that exposure to water deficit substantially impairs the morphological characteristics of *C. officinalis*; however, exogenous application of SNP appears to alleviate some of these detrimental effects by enhancing various growth parameters. These results are consistent with previous studies conducted on *Silybum marianum* and *Helianthus annuus*, where SNP treatment was found to improve vegetative growth indices (Zangani et al. 2023; Shehzad et al. 2023). The beneficial effects of SNP can be attributed to its capacity to reduce transpirational water loss and membrane damage, thereby stabilizing plant water status and improving performance under drought conditions.

It is proposed that NO, released from SNP, acts as a crucial signaling molecule involved in delaying senescence by modulating ethylene biosynthesis (Leshem and Wills 1998). Furthermore, there is evidence to suggest that NO influences cell division and enlargement, processes which depend heavily on cellular hydration and turgor pressure (Long et al. 2020). These physiological roles may well account for the stem elongation observed in SNP-treated *C. officinalis*. As NO increases the activity of exo- and endo-beta-glucanase in the wall cells, and the glycosidic bond between glucose units in the wall is broken by this enzyme,

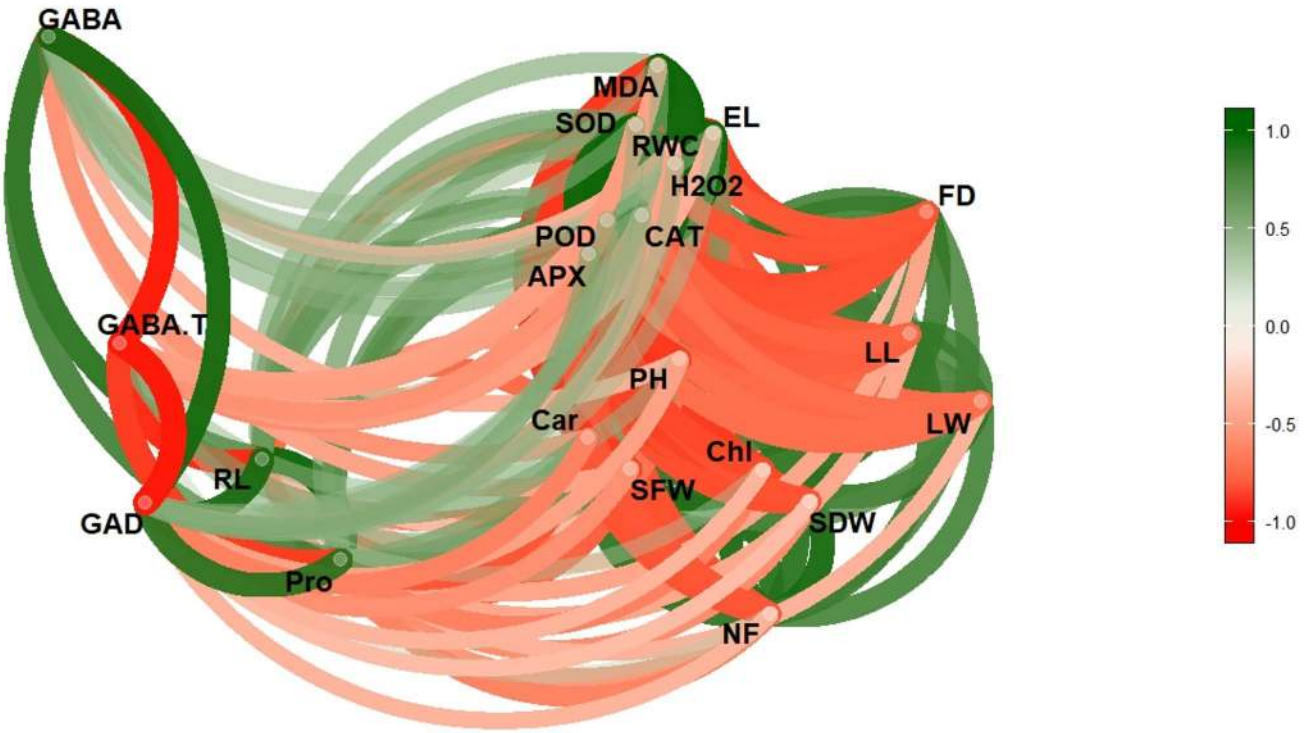


Fig. 6 Network correlation representation of morphological and biochemical traits of pot marigold under drought stress conditions and sodium nitroprusside application. PH: Plant height; SFW: Shoot fresh Weight; SDW: Shoot dry Weight; RL: Root length; FD: Flower diameter; NF, Number of flower; LL: Leal length; LW: Leaf width; RWC: Relative water content; Pro: Proline; EL: Electrolyte leakage; Chl: Chlorophyll; Car: Carotenoid; MDA; Malondialdehyde; CAT: Catalase; SOD: Superoxide dismutase; POD: Peroxidase; APX: Ascorbate peroxidase; GABA: γ-aminobutyric acid; GABA-T: GABA transaminase; GAD: Glutamate acid decarboxylase

which leads to weakening of the wall and cell expansion (Hayat et al. 2014). In addition, NO released from SNP, by accumulating in the endoderm of stem cells, can regulate the auxin pathway or interact with auxin, and thus can affect plant growth (Domingos et al. 2015). Notably, treatment with 100 µM SNP resulted in greater shoot biomass accumulation compared to other concentrations, suggesting that this dose may be optimal for promoting growth under limited moisture conditions. The reduction in fresh and dry shoot biomass under drought conditions has been well documented in marigold plants (Eghlima et al. 2024), which corroborates our findings regarding the general decline in growth traits under water stress.

It seems likely that biomass reduction under drought is linked to the vulnerability of young leaves to water scarcity (Cechin et al. 2006), and SNP's beneficial effects could be explained by improved water uptake and distribution. However, our findings indicate that SNP's effects are dose-dependent: while 100 µM produced superior growth performance, higher concentrations (e.g., 150 µM) failed to further improve performance and might potentially induce nitrosative stress, as excessive NO can have cytotoxic effects (Wang et al. 2016). This dual role of NO as a signaling molecule at low concentrations and a stressor at high concentrations has also been reported by Wimalasekera and Scherer (2022). As anticipated, root architecture proved to be key for drought adaptation, enhancing water acquisition and anchorage through deeper roots, altered configurations, and enlarged vessel elements (Kalra et al. 2024). In line with this expectation, the greatest root elongation (28.07 ± 0.15 cm) was observed in plants treated with 150 µM SNP under severe drought (40% FC), suggesting that SNP may stimulate root growth as a compensatory response to water limitation. With regard to reproductive traits, the highest flower yield and diameter were recorded in well-watered plants treated with 100 µM SNP, followed by 150 µM treatment. These floral parameters declined with increasing drought severity, which can be attributed to reduced leaf area and smaller leaf dimensions under drought conditions, thereby limiting photosynthesis and assimilate translocation to reproductive organs, as previously reported by Shubhra et al. (2004). There is strong probability that NO delivered via SNP influences auxin signaling, potentially accumulating in the stem endodermis and interacting with auxin metabolism, which may enhance vegetative and reproductive growth. SNP application has also been associated with improved nitrogen-carbon balance, resulting in higher photosynthetic rates, chlorophyll content, Rubisco

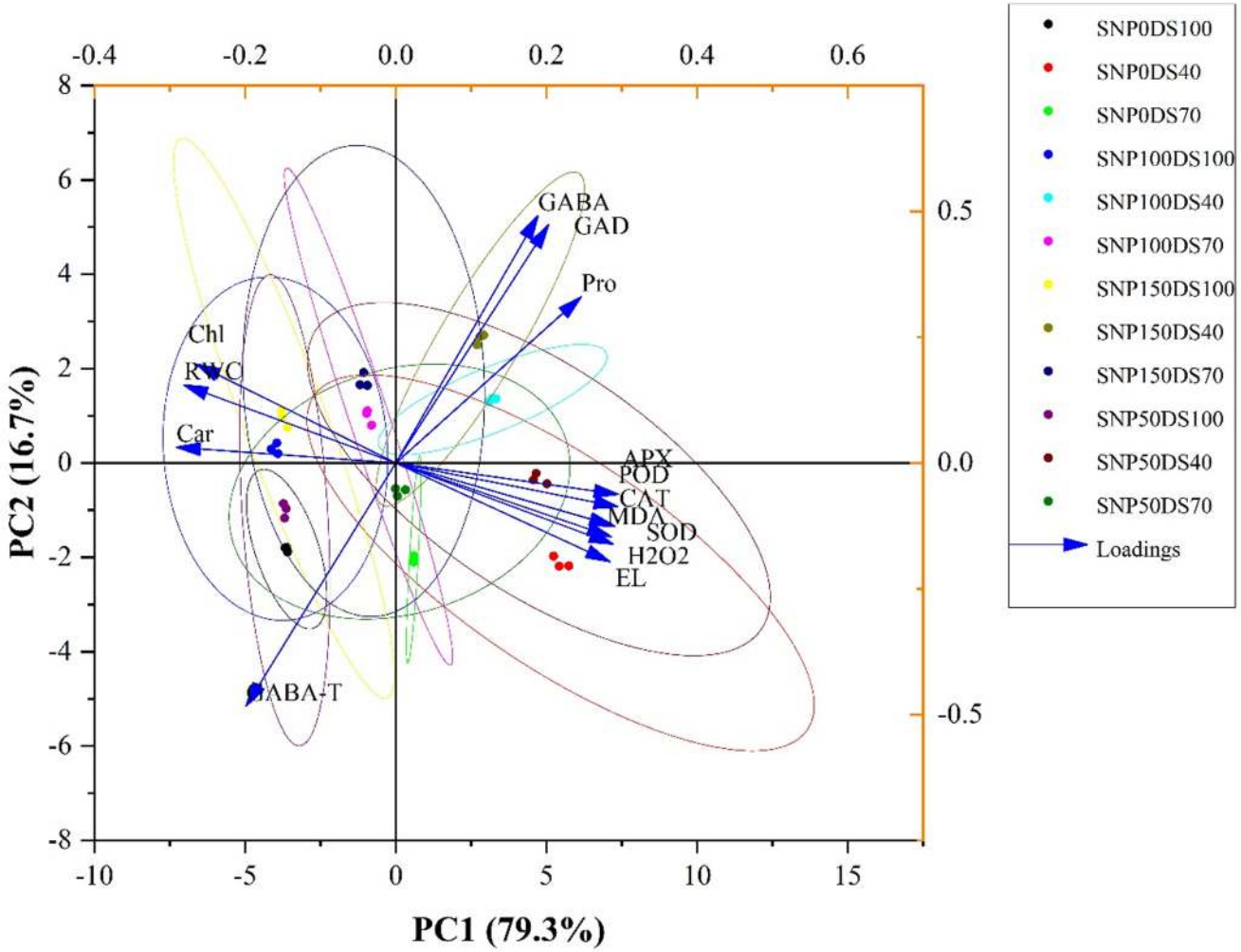


Fig. 7 PCA biplot of the analyzed parameters in pot marigold after exposure to sodium nitroprusside under different drought stress conditions. SNP: Sodium nitroprusside; SNP_0: without sodium nitroprusside; SNP_{50}: 50 µM; SNP_{100}: 100 µM; SNP_{150}: 150 µM; D: Drought stress; DS_{100}: 100% field capacity; DS_{70}: 70% field capacity; DS_{40}: 40% field capacity;

activity, biomass accumulation, and overall plant development (Sami et al. 2021).

SNP and Physiological Traits Under Drought Stress

The comparative analysis revealed a significant decline in leaf RWC under drought stress, which was expected given the water-limiting conditions. However, foliar application of 150 µM SNP consistently resulted in the highest RWC values across both well-watered and water-limited conditions (Fig. 1). These findings are in good agreement with Shehzad et al. (2023), who reported an 8–10% improvement in water status in *Helianthus annuus* treated with 100 µM SNP. Our study corroborates these results, recording a 13.3% increase in RWC under 40% FC in marigold plants treated with 150 µM SNP compared to untreated controls. Comparable enhancements in plant water retention following SNP application have been documented in sunflower (Cechin et al. 2015) and *Malus* rootstocks (Zhang et al. 2016). RWC serves as a key physiological indicator for drought stress, as water deficit typically induces stomatal closure to reduce transpiration and conserve leaf water. It would appear that SNP facilitates this process by modulating intracellular calcium levels via cyclic guanosine monophosphate (cGMP) and cyclic ADP-ribose (cADPR) pathways. Evidence using calcium chelators such as EGTA suggests that SNP acts upstream of Ca^{2+}mobilization. This is consistent with Neill et al. (2003a), who demonstrated that NO functions as a secondary messenger in abscisic acid (ABA)-mediated signaling in *Arabidopsis thaliana*. Furthermore, inhibition of NO by scavengers like carboxy-PTIO increases stomatal aperture (Neill et al. 2003b), which lends support to NO's role in promoting stomatal closure.

Despite increasing drought severity, plants treated with 100 µM SNP maintained higher total chlorophyll (Fig. 2a) and carotenoid (Fig. 2b) contents under both control and

drought conditions. In contrast to this beneficial effect, 150 µM SNP did not exert the same positive influence, which underscores the importance of dose optimization. This dose-dependent response concurs with findings reported by Đurić et al. (2023), where 250 µM SNP reduced carotenoid levels in *Impatiens walleriana* under PEG-induced drought. As expected, drought-induced decline in photosynthetic efficiency is primarily due to reduced stomatal conductance and impaired photosynthetic biochemistry. Stomatal closure, triggered by low leaf water potential and ABA signaling, limits CO_2 uptake and carbon assimilation. These physiological responses vary with plant water regulation strategies, such as isohydric versus anisohydric behavior, with isohydric plants being more susceptible to carbon limitation and anisohydric plants to hydraulic failure (Zhang et al. 2020). There is satisfactory agreement between our findings and previous research showing that drought disrupts photosynthesis by degrading chlorophyll, damaging the photosynthetic apparatus, inhibiting photochemical reactions, and reducing Calvin cycle enzyme activity (Cechin et al. 2015). These effects were particularly evident in our study under severe drought conditions. Nevertheless, SNP application has been shown to preserve photosynthetic pigments during drought, as observed in Oriental lily (Wang et al. 2015) and cucumber (Fan et al. 2012). This preservation can probably be attributed to the antioxidative properties of NO, which scavenges ROS and protects pigments from oxidative damage (Kim and Lee 2005). Furthermore, it is reasonable to assume that SNP releases iron upon dissociation, which may enhance chlorophyll stability under stress by increasing iron availability (Sheokand et al. 2010; Santisree et al. 2019).

SNP and Cell Oxidative Biomarkers Under Drought Stress

As water availability declined, *C. officinalis* plants exhibited marked increases in oxidative stress indicators, including proline accumulation (Fig. 3a), H_2O_2 levels (Fig. 3b), MDA content (Fig. 3c), and EL (Fig. 3d). Notably, foliar application of SNP further elevated proline levels, especially under severe drought and at 150 µM concentration, which suggests a signaling role for NO in activating proline biosynthesis. This observation is in line with prior reports by Cechin et al. (2015). It is well established that proline serves as a crucial osmolyte, aiding osmotic adjustment, stabilizing proteins and membranes, and scavenging ROS to protect cells from oxidative damage (Krasensky and Jonak 2012). The SNP-induced increase in proline may be explained by NO-mediated upregulation of key biosynthetic enzymes such as P5CS, which is central to proline production under abiotic stress (Lei et al. 2007). In contrast to the proline response, other oxidative stress markers, H_2O_2, MDA, and EL, were significantly reduced in plants subjected to severe drought (40% FC) when treated with 150 µM SNP. The lowest levels of these markers occurred in well-watered plants pretreated with the same SNP dose (Figs. 3b–d), indicating that NO application mitigated oxidative damage. This suppression reflects improved cellular redox balance and enhanced membrane stability. These findings are consistent with protective effects of SNP reported in soybean (Qu et al. 2023) and tomato (Sundararajan et al. 2022) under drought stress. The reduction in H_2O_2 and MDA levels in SNP-treated plants supports the hypothesis that exogenous NO reduces ROS accumulation, thereby limiting lipid peroxidation and preserving membrane integrity. Consequently, this leads to decreased ion leakage, as reflected by lower EL values. There is evidence to suggest that NO interacts with membrane components, enhancing membrane fluidity and resilience by modulating phospholipid bilayers and improving elasticity (Leshem and Haramaty 1996). These interactions contribute to better physiological performance and growth under stress conditions. Our findings substantiate the role of SNP in reinforcing plant defense mechanisms through antioxidant regulation. By reducing oxidative markers and maintaining membrane function, SNP enhances ion transport and minimizes cellular damage during drought. This supports Liu et al. (2014), who suggested that NO not only stabilizes cellular structures but also activates protective responses that buffer plants against oxidative stress.

SNP and Antioxidative Enzymes Under Drought Stress

Sodium nitroprusside is one of the important NO producers, capable of producing about 2 µmol/L of NO radical for every 0.5 mmol/L of sodium nitroprusside (Ruan et al. 2002). As anticipated, the enzymatic antioxidant defense system, including CAT, APX, POD, and SOD, was significantly activated in *C. officinalis* under drought stress. However, following foliar application of SNP, the activities of these enzymes decreased in a concentration-dependent manner, as illustrated in Figs. 4a–d. One of the reasons for the positive effect of NO on the activity of antioxidant enzymes is the role of this substance in stimulating the activity of iron-containing enzymes (Farooq et at., 2009). NO can also maintain cellular structures by improving cell wall flexibility, affecting phospholipids, and improving membrane fluidity. In addition, it has been reported that NO can react with alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals of lipids, leading to direct inhibition of lipid peroxidation (Shehab et al., 2010). NO can also react directly with free radicals such as $O_2^{\bullet-}$ to produce peroxynitrite ($ONOO^-$), which can act as a messenger molecule in response to stress or in the regulation of protein activity (Fan and Liu 2012). It is well known that SOD

plays a vital role by converting superoxide radicals (O_2^-) into H_2O_2, which is then detoxified by CAT, POD, and APX into less reactive molecules. The observed decline in antioxidant enzyme activities with increasing SNP concentrations suggests a complex regulation mechanism, which reflects the variability reported in the literature. For instance, while Sundararajan et al. (2022) documented enhanced enzyme activity after SNP application, Đurić et al. (2023) found minimal effects. revious studies have highlighted nuanced responses of antioxidant enzymes to NO levels: Neill et al. (2008) reported that elevated NO influences POD activity and stomatal behavior, while Shang et al. (2022) showed that CAT activity increases over time depending on NO concentration. Our results are in good agreement with findings by Lubyanova and Allagulova (2024), who observed SNP-mediated modulation of redox-related enzymes in wheat.

The positive correlation between oxidative stress markers (such as MDA and H_2O_2) and antioxidant enzyme activity in our study indicates a coordinated response where increased ROS triggers detoxification pathways. It would seem that the beneficial effects of SNP pre-treatment arise from its modulation of hormonal signaling, enhancement of water use efficiency, and upregulation of protective proteins such as dehydrins, as suggested by Maslennikova et al. (2017). Despite these benefits, we are aware that SNP has certain limitations; its decomposition releases iron and cyanide, which can be phytotoxic at higher concentrations due to chemical instability (Keisham et al. 2019). Nevertheless, when applied optimally, SNP proves to be a cost-effective NO donor with documented advantages across various plant species (Lubyanova and Allagulova 2024). Its effectiveness may be attributed to both directly scavenging ROS and indirectly enhancing non-enzymatic antioxidants like proline, which was elevated in our study. There is strong probability that by reducing ROS, such as H_2O_2, SNP helps alleviate oxidative stress under drought conditions (Cechin et al. 2015). Interestingly, in contrast with our findings, Liu et al. (2014) demonstrated that NO application under drought not only increased endogenous NO levels but also activated antioxidant defenses, acting both as a signaling molecule and radical scavenger. Additionally, it can be suggested that SNP-derived NO reacts with superoxide radicals to form peroxynitrite, a less reactive compound, thereby reducing cytotoxicity from ROS accumulation. This NO-mediated interaction mitigates oxidative damage and delays programmed cell death, as demonstrated by Beligni et al. (2002) in barley.

SNP Effects on GAD, GABA, and GABA-T Under Drought Stress

Increased drought severity led to a simultaneous rise in glutamate GAD activity and GABA accumulation, both of which showed a positive correlation with higher concentrations of exogenously applied SNP, as illustrated in Fig. 5a and c. Conversely, the activity of GABA transaminase (GABA-T), the enzyme responsible for GABA catabolism, consistently declined under intensified drought stress and with increasing SNP levels. The lowest GABA-T activity was observed in plants grown at 40% FC and treated with 150 µM SNP (Fig. 5b). This inverse relationship between GABA-T activity and GAD activity/GABA content suggests a shift favoring GABA accumulation under drought and SNP treatment, which is consistent with findings by Paul and Ekambaram (2005). It is well established that GABA, a non-protein amino acid, plays multifunctional roles in plant physiology, including regulation of growth, development, and adaptive responses to environmental stresses (Guo et al. 2023). Drought and other environmental stressors can cause cytosolic acidification (to pH ~ 5.8) and elevated intracellular Ca^{2+} levels, both of which are known activators of GAD that promote GABA biosynthesis (Guo et al. 2023). Additionally, GABA accumulation may be linked to a decline in polyamines caused by oxidative degradation (Balfagón et al. 2022) or generated via a non-enzymatic pathway involving proline.

Our findings support this mechanism, as proline (Fig. 3a) and GABA (Fig. 5c) contents increased in parallel under drought and SNP treatments. According to Signorelli et al. (2015), proline can interact with hydroxyl radicals, leading to its decarboxylation into pyrrolidin-1-yl, which then serves as a substrate for proline dehydrogenase (PDH), facilitating GABA production via an alternative route. The positive association between SNP application and GABA accumulation under drought stress can be explained by nitric oxide's regulatory effect on GAD activity. Acting as a secondary messenger, NO can induce GAD expression and activity, often in synergy with signaling molecules such as H_2O_2 during abiotic stress, thus enhancing stress tolerance (Kabała and Janicka 2024). There is evidence to suggest that GABA is involved in intricate crosstalk with various plant hormones, including indole-3-acetic acid (IAA), abscisic acid (ABA), ethylene, jasmonic acid (JA), salicylic acid (SA), gibberellins (GAs), cytokinins (CKs), brassinosteroids (BRs), and strigolactones. It is proposed that NO may regulate GABA either upstream by stimulating its biosynthesis or downstream through post-translational modifications, such as reversible S-nitrosylation of biosynthetic enzymes or activation of protein kinases that govern the GABA pathway (Kabała and Janicka 2024).

Conclusions

Our study demonstrated that exogenous SNP application effectively enhances drought tolerance in pot marigold (*C. officinalis*) through multiple coordinated physiological and biochemical mechanisms. The foliar application of SNP, particularly at optimal concentrations, significantly mitigated drought-induced growth inhibition and maintained plant productivity under water deficit conditions. Under severe drought stress, SNP treatments substantially reduced plant height decline while improving shoot biomass, leaf dimensions, and reproductive parameters including flower number and diameter. The protective effects of SNP were mediated through enhanced water relations and photosynthetic capacity maintenance. SNP pretreatment effectively preserved relative water content and photosynthetic pigments (chlorophyll and carotenoids) under drought stress, with higher concentrations providing superior protection against water deficit-induced physiological deterioration. This improved physiological status translated to better growth performance and stress resilience. While drought stress elevated oxidative damage markers (H_2O_2, MDA, electrolyte leakage), SNP treatment significantly reduced these indicators while simultaneously decreasing the hyperactivation of antioxidant enzymes (CAT, APX, POD, SOD), suggesting more efficient cellular protection with lower metabolic cost. Concurrently, SNP enhanced the GABA metabolic pathway by increasing GAD activity and GABA content while decreasing GABA-T activity, indicating improved osmoregulation and stress signaling. These findings have significant implications for ornamental plant cultivation under increasing drought stress scenarios associated with climate change. The cost-effective application of SNP as a foliar treatment represents a practical management strategy that could be readily adopted by growers to maintain pot marigold quality and yield under water-limited conditions. The identified optimal SNP concentration range provides flexibility for implementation across different cultivation systems. Future research should investigate the temporal dynamics of SNP effects throughout the growing season and explore the underlying gene expression patterns regulating GABA metabolism and antioxidant responses. Additionally, field validation studies across diverse environmental conditions would strengthen the practical applicability of these greenhouse findings and potentially identify cultivar-specific responses to SNP treatment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-025-12029-3.

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Author contributions AHM: methodology, greenhouse, and lab work, data curation, data analysis. GE: conceptualization, supervision, data curation, analysis, reviewing, and editing; HSH: methodology, reviewing, and editing. FA and OK: data analysis, reviewing, and editing. MM: conceptualization, methodology, data curation, reviewing, and editing.

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Data Availability Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Declarations

Competing Interest The authors declare that they have no conflicts of interest related to this work.

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