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Original article

Effects of seed priming on growth, nutrient uptake, and biochemical responses of *Astragalus fasciculifolius* Boiss under drought stress

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ABSTRACT

Astragalus fasciculifolius Boiss (*Astragalus*) is an important medicinal plant whose growth and development are negatively influenced by drought stress. To examine the potential of seed priming in inducing drought tolerance in *A. fasciculifolius*, a factorial experiment with four replications was conducted in a completely randomized design within a research greenhouse at Torbat Heydarieh University, Iran, in 2024. The experimental treatments were two drought stress levels (no stress: irrigation at 90 % of field capacity (FC), and drought stress: irrigation at 50 % of FC) and eight seed priming treatments including no priming (control), hydropriming (HP), priming with gibberellin (GA_3), salicylic acid (SA), potassium nitrate (KNO_3), zinc sulfate ($ZnSO_4$), humic acid (HA), and silicon dioxide nanoparticles (SiO_2-NPs). The results illustrated that drought stress significantly reduced the growth parameters and macro-elements absorption in *A. fasciculifolius*. However, seed priming with GA_3, $ZnSO_4$, and HA could moderate the adverse effects of water deficit, leading to significant improvements in plant height, aboveground biomass, and root nutrient uptake (N, P, K). This was associated with increased carotenoid and chlorophyll (Chl) levels. Seed priming with KNO_3 or SA did not significantly differ from the control treatment in terms of growth, physiological and biochemical parameters. The SiO_2-NPs treatment not only failed to induce drought stress tolerance in *A. fasciculifolius*, but also resulted in significantly lower growth parameters compared to the control. Essentially, the PCA results clearly demonstrated that seed priming with GA_3, $ZnSO_4$, and HA effectively mitigated the adverse effects of water deficit on *A. fasciculifolius* production, possibly through enhanced macro-nutrients uptake and increased photosynthetic pigment content.

1. Introduction

The Middle East and North Africa (MENA) region faces a dire situation regarding water resources (Akoumianaki-Ioannidou et al., 2021). Classified as the world's most water-stressed region, several MENA countries such as Iran rank among the most stressed globally. This vulnerability stems from naturally arid climates with limited freshwater supplies, coupled with high population growth and increasing water demand. Consequently, the regions experience a chronic imbalance between water availability and extraction, leading to severe drought stress and threatening regional stability (Salameh et al., 2021). Drought stress is a growing concern in agriculture, significantly impacting plant development, growth, and ultimately, yield (Ahluwalia et al., 2021). Since, water scarcity is a persistent challenge, particularly in semi-arid and arid regions, necessitating the development of innovative strategies to improve plant drought tolerance. Shifting the planting pattern to cultivation of drought-tolerant plants is an effective strategy for ensuring sustainable agriculture in drought-prone areas (IPCC, 2022).

A. fasciculifolius, a notable medicinal and industrial plant, is commonly grown in semi-arid and arid regions particularly in the MENA region such as Arabian Peninsula, Iran, Pakistan, Turkey, etc. (Oladi et al., 2017). This plant possesses numerous medicinal properties that can be beneficial in the treatment and prevention of various diseases. Some of its key medicinal properties include immune system boost, anti-inflammatory, stress reduction, cardiovascular health, blood sugar control and anti-cancer properties (Tayoub et al., 2021; Shahrani et al., 2021). *A. fasciculifolius* is known locally as a toothbrush (Nosrati et al., 2018). This designation reflects a historical practice among local communities where individuals employed stems and roots for oral hygiene

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purposes. Despite the relative drought tolerance of *A. fasciculifolius*, appropriate strategies should also be considered to ensure the proper and sustainable cultivation of this species in arid and semi-arid regions. In addition to plant breeding efforts, easily implementable practices such as seed priming can be employed to enhance plant resistance to drought stress.

Seed priming, a technique involving pre-treatment of seeds with various substances, emerges as a promising strategy to induce plant resistance to drought stress (Adhikari et al., 2024). This approach exposes seeds to mild stress conditions or specific compounds, triggering physiological and biochemical adaptations that improve tolerance to future drought events (Pourghasemian et al., 2020). Various seed priming approaches include osmopriming, hydropriming, nutrient priming, hormonal priming, biopriming, solid matrix priming, and magnetopriming (Waqas et al., 2019). These methods utilize inorganic and organic chemicals, plant extracts, and hormones to stimulate essential pre-germination metabolic processes and seedling growth. Priming with compounds improves water absorption, activates enzymes, and enhances antioxidant defenses, thereby enhancing drought and salinity tolerance and increasing yield in various plants (Marthandan et al., 2020). For example, studies highlight KNO_3 as an effective priming agent that enhanced germination, growth, vigor, and tolerance to salinity, drought, and extreme temperatures in crops such as maize (Saed-Moocheshi et al., 2014), rice (Ali et al., 2021) and cotton (Narejo et al., 2023).

Seed priming with phytohormones like GA_3 has also shown significant potential in mitigating drought stress effects on germination and earlier seedling growth (Aswathi et al., 2022; Rhaman et al., 2020). Previous studies suggest that ethylene may promote seed dormancy release, whereas GA_3 acts a crucial role in improving seed germination and vigor (Muniandi et al., 2018). Muniandi et al. (2018) observed severe growth retardation under drought stress, alleviated significantly by GA_3 priming. These treatments promoted root and shoot elongation, increased aboveground biomass, and enhanced seedling vigor parameter, emphasizing their role in enhancing seedling growth under adverse conditions. In addition to growth promotion, GA_3 priming induced antioxidant defense mechanisms in plant seedlings subjected to water deficiency. This included increased activities of key antioxidant enzymes, including catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase in both leaves and roots, which helped mitigate oxidative stress by regulating reactive oxygen species (ROS) and limiting lipid peroxidation (Lamlom et al., 2025).

Furthermore, osmotic adjustment played a crucial role in primings, as evidenced by increased concentrations of soluble proteins, proline, soluble sugars, and ion uptake in primed seedlings under drought stress (Chen and Jiang, 2010). These osmolytes help maintain cellular turgor and protect against dehydration, contributing to overall stress tolerance (Khan et al., 2019).

While some prior research has established the general benefits of seed priming in enhancing drought tolerance across various plant species, the novelty of this study lies in its specific focus on *A. fasciculifolius*, a significant medicinal plant endemic to arid and semi-arid regions, which has received limited attention in the context of drought stress mitigation through seed priming. Furthermore, this research uniquely investigates and compares the efficacy of a diverse range of priming agents in modulating growth, nutrient uptake, and key physiological and biochemical responses under water deficit. This study offers novel insights into targeted priming strategies for enhancing the resilience of this valuable plant to drought, contributing to its sustainable cultivation in water-scarce environments.

2. Materials and methods

2.1. Design and treatments

To investigate the potential of seed priming in inducing drought tolerance in *A. fasciculifolius*, a two-factor factorial experiment was conducted as a completely randomized design (CRD) with four replications in the physiology laboratory and research greenhouse of Torbat Heydarieh University, Iran in 2024. Prior to the treatments, plants were subjected to a preliminary drought stress experiment at 90 %, 70 %, 50 %, and 30 % field capacity (FC). As 30 % FC resulted in complete plant mortality, 50 % FC was selected as the stress level for subsequent treatments. The experimental treatments consisted of two drought stress conditions (full irrigation, 90 % FC and drought stress, 50 % FC) together with eight seed priming applications (no priming (control), priming with water (HP), gibberellin (GA_3): 400 mg L^{-1}, salicylic acid (SA): 400 mg L^{-1}, potassium nitrate (KNO_3): 3000 mg L^{-1}, zinc sulfate ($ZnSO_4$): 3000 mg L^{-1}, humic acid (HA): 400 mg L^{-1}, silicon dioxide nanoparticles (SiO_2-NPs): 400 mg L^{-1}). The concentrations employed for each priming treatment were derived from various references (Heidari and Sadeghi, 2014; Alam et al., 2022; Ziaei and Jafari, 2023; Ziaei et al., 2024).

2.2. Plant materials and treatments applying

Before the experiment, *A. fasciculifolius* plants were collected from fields owned by the University of Saravan, located in Sistan and Baluchestan Province, Iran. The collected seeds were surface disinfected with distilled water in the laboratory, soaked in a 2 % solution of sodium hypochlorite, and after that washed with distilled water several times. The seeds were scratched slightly, soaked in prepared solutions, and shaken for 6 h (Saha et al., 2022). Next, the seeds were stored at room temperature for 48 h to reach the initial moisture content.

SiO_2 nanoparticles (SiO_2-NPs) with an average particle size of ~20 nm and a BET specific surface area of 20–50 m^2 g^{-1} were purchased from Sigma-Aldrich, USA (Product No. 637238; ≥99.5 % purity, trace metals basis). The nanoparticles were supplied as a dry powder and dispersed in distilled water to prepare a 400 mg L^{-1} stock suspension. The suspension was homogenized using an ultrasonic probe sonicator (Topsonics model UHP-1200, Iran) at 20 kHz for 30 min to minimize aggregation and ensure uniform dispersion. Working suspensions were freshly prepared before each priming treatment and maintained under continuous stirring to reduce sedimentation.

2.3. Sowing of seeds and growth conditions

Each experimental unit was a pot initially sown with eight seeds, which, after emergence and establishment, was thinned to four uniform plants. The seeds in each treatment were sown in pots containing 5 kg of soil under greenhouse conditions, which maintained a temperature of 15/25 °C, a 8/16 h dark/light cycle, with a relative humidity of 45–65 %. Illumination was provided by 400 W high-pressure sodium vapor lamps (Osram Sylvania, Lynn, MA, USA), resulting in a photosynthetic photon flux density of ~600 μmol m^{-2} s^{-1}. Table 1 represents the soil profile used in the pots. Drought stress treatments were imposed based on the gravimetric field capacity (FC) method (Rab et al., 2011). Ten pots (each with a recorded empty-pot mass, Wp) were filled with soil. Pots were saturated by adding water in excess until free drainage commenced; leachate was discarded. To minimize evaporative losses during equilibration, the pot surfaces were covered with aluminum foil. Pots were weighed periodically (e.g., every 2 h) and the stable weight after drainage ($\Delta Wt < 0.1$ %) was recorded as the weight at field capacity (Wfc). For determining the dry soil mass, soil from each pot was oven-dried at 105 ± 5 °C for 72 h to constant weight and weighed; the oven-dry soil mass was denoted Md. Field capacity was calculated as:

$$FC\ (\%) = \frac{(W_{fc} - W_p - M_d)}{M_d} \times 100 \quad (1)$$

where Wfc is the mass of pot + soil at field capacity (g), Wp is the empty-pot mass (g), and Md is the oven-dry soil mass (g). During the experi-

Table 1
Physico-chemical characteristics of the soil used in the pots.

Available potassium (mg kg^{-1})	Available phosphorus (mg kg^{-1})	Nitrogen (% DW^{-1})	Organic matter (% DW^{-1})	pH	EC* (dS m^{-1})	Bulk density (g cm^{-3})	Soil texture	Particle size percentage		
								Sand (%)	Silt (%)	Clay (%)
266	13.34	0.097	0.53	7.32	1.63	1.29	Clay	21.1	24.5	55.4

* :EC was determined in a saturated paste extract according to Richards (1954).

ment, to maintain soil moisture at each field capacity, pots were weighed daily, and the volume of water lost was replenished according to the respective irrigation treatments.

2.4. Measurements

Ten weeks after planting, the plants were harvested to monitor the growth parameters, including plant height, plant weight, leaf number per plant and some physiological and biochemical characteristics. Ten weeks after sowing, all plants from each pot (experimental unit) in each treatment were harvested. Growth traits, including plant height, plant weight, and leaf number per plant, were first measured, followed by the assessment of physiological and biochemical characteristics.

2.4.1. Photosynthesis pigments

Chlorophyll a, chlorophyll *b*, total chlorophyll, and carotenoids were determined according to Lichtenthaler (1987) and Lichtenthaler and Wellburn (1983). To this aim, fresh leaf tissue (0.2 g) was homogenized in 10 mL of 80 % (*v*/v) acetone and centrifuged at 10,000 ×*g* for 10 min. The absorbance of the supernatant was recorded at 663, 647, and 470 nm using a UV–Vis spectrophotometer (UNICO model 2100, USA) with a 1 cm pathlength cuvette.

$$\text{Chl a } (\text{mg L}^{-1}) = (12.25 \times A_{663}) - (2.79 \times A_{647}) \quad (2)$$

$$\text{Chl b } (\text{mg L}^{-1}) = (21.50 \times A_{647}) - (5.10 \times A_{663}) \quad (3)$$

$$\text{Carotenoids } (\text{mg L}^{-1}) = \frac{(1000 \times A_{470}) - (1.82\ \text{Chl a}) - (85.02\ \text{Chl b})}{198} \quad (4)$$

In these equations, A_{647}, A_{663}, and A_{470} represent optical densities at 647, 663, and 470 wavelengths, respectively.

To express concentrations per fresh tissue weight, the following conversion was applied:

$$\textit{Pigment } (\textit{mg g}^{-1}\ \textit{FW}) = \frac{(C \times V)}{(1000 \times W)} \quad (5)$$

where: C = pigment concentration in extract (mg L^{-1}), V = extraction volume (mL), W = fresh weight of tissue (g).

2.4.2. Nutrients absorption

The N content in *A. fasciculifolius* leaves was quantified using the Kjeldahl method, following the established protocol of Sáez-Plaza et al. (2013). This procedure involved the digestion of leaf samples with concentrated sulfuric acid, facilitated by a copper sulfate catalyst, to ensure the complete conversion of organic N to ammonium. The liberated ammonia was then subjected to distillation and subsequently titrated against a standardized acid solution. The N concentration within the original sample was determined stoichiometrically based on the volume of the standardized acid consumed during titration. N content was expressed as a percentage of dry weight (% DW).

P concentration in *A. fasciculifolius* leaves was determined using the molybdate-blue colorimetric method (Murphy and Riley, 1962). Approximately 0.4 g of oven-dried, ground plant material was weighed into porcelain crucibles and pre-heated on a hot plate until the samples turned black and tar-like. The crucibles were then placed in a muffle furnace, and the temperature was gradually increased at a rate of ~50 °C per hour until reaching 500 °C. Samples were maintained at this temperature for 6 h. After cooling, 3 mL of HCl was added to each crucible, and the mixture was heated on a hot plate at 85 °C for 30 min. The digests were subsequently diluted to 50 mL with 2 % HNO_3, filtered through Whatman No. 42 filter paper, and brought to a clear solution. An aliquot of the digest was reacted with ammonium molybdate and ascorbic acid reagents to form a blue-colored complex, and absorbance was recorded at 880 nm using a UV–Vis spectrophotometer (UNICO model 2100, USA). A calibration curve was prepared with KH_2PO_4 standards, and P content was expressed as a percentage of dry weight (% DW).

The K content in the plant samples was quantified using flame photometry, following the methodology described by Kalra (1998). Dried and ground leaf samples (~0.3 g DW) were placed in porcelain crucibles and ashed in a muffle furnace at 500 °C for 6 h. The resulting ash was dissolved in 10 mL 1 N HCl and heated on a hot plate at 85 °C for 30 min. The digest was filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask, rinsed with 2 % HNO_3, and made up to a final volume of 50 mL with 2 % HNO_3. If the measured concentration exceeded the calibration range, further dilutions with a known dilution factor were applied. K concentration was determined by flame photometry (Fater model 620C, Iran) at 766.5 nm. Calibration curves were prepared using KCl standards (0–10 mg L^{-1}). Results were expressed on a dry weight basis (% DW).

2.4.3. Proline contents

Proline concentration was determined following the methodology described by Bates et al. (1973). This involved homogenizing plant material in a 3 % aqueous sulfosalicylic acid solution, followed by centrifugation to remove tissue debris. The supernatant was then reacted with ninhydrin reagent and glacial acetic acid at 100 °C for a set incubation time. After stopping the reaction, a chromophore was extracted using toluene, and its absorbance was measured 520 nm wavelength using a spectrophotometer. Proline concentration was quantified from a standard calibration curve prepared with L-proline (0–100 μg mL^{-1}; $R^2 > 0.99$). Values were expressed as μg g^{-1} fresh weight (FW).

2.4.4. Total phenolic content

Total phenolic compounds were determined by the Folin–Ciocalteu method following Singleton et al. (1999) with minor modifications. Approximately 0.10 g fresh leaf tissue was homogenized in 5.0 mL of 80 % methanol and centrifuged (4000 *g*, 10 min). The supernatant was collected and used as the extract (V = 5.0 mL). In a test tube, 0.20 mL of extract was mixed with 1.0 mL of distilled water and 0.50 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water). After 5 min, 1.5 mL of 7.5 % (*w*/*v*) Na_2CO_3 was added. The mixture was incubated in the dark at room temperature for 120 min, and absorbance was read at 765 nm against a reagent blank (80 % methanol). A calibration curve was prepared using gallic acid (0–125 μg mL^{-1}). Total phenolic content was expressed as mg gallic acid equivalents (GAE) per g fresh weight (mg GAE g^{-1} FW).

2.4.5. Catalase (CAT) activity

CAT activity was measured according to the method of Aebi (1984). Fresh leaf tissue (0.5 g) was homogenized in 5 mL of 50 mM phosphate

buffer (pH 7.0) containing 1 mM EDTA and 1 % (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000 ×*g* for 10 min at 4 °C, and the resulting supernatant was used for enzyme assays. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 as substrate, and 100 µL of enzyme extract in a final volume of 3 mL. The decrease in absorbance was recorded at 240 nm for 1 min using a UV–vis spectrophotometer (UNICO model 2100, USA). The extinction coefficient of H_2O_2 (39.4 M^{-1} cm^{-1}) was used to calculate enzyme activity. One unit of CAT activity was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute under the assay conditions. The enzyme activity was expressed as units per mg of protein, with protein content determined using the Bradford method (Bradford, 1976).

2.5. Data analysis

Data were first tested for normality and homogeneity of variances. A two-way factorial ANOVA was performed to evaluate the main effects of drought stress, seed priming, and their interaction. When significant effects were detected, mean comparisons were conducted using Tukey's HSD post hoc test at α = 0.05. All statistical analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA).

To visualize the relationships between the different treatments and measured variables, a principal component analysis (PCA) was performed using R software (R statistical software (version 4.2.0; R Foundation for Statistical Computing, Vienna, Austria). The data were scaled and centered prior to PCA to ensure equal contribution of all variables. PCA was conducted based on treatment mean values across replications. The resulting biplot displays the scores of the different treatments along the principal components and the loadings of the variables, indicating their contribution to the principal components and their relationships with the treatments.

3. Results

3.1. Stress indicators

3.1.1. Proline

The proline content of *A. fasciculifolius* was significantly affected by the drought stress, seed priming and their interaction at the 1 % significance level (Supplementary Table S1).

No significant difference in proline content was observed among

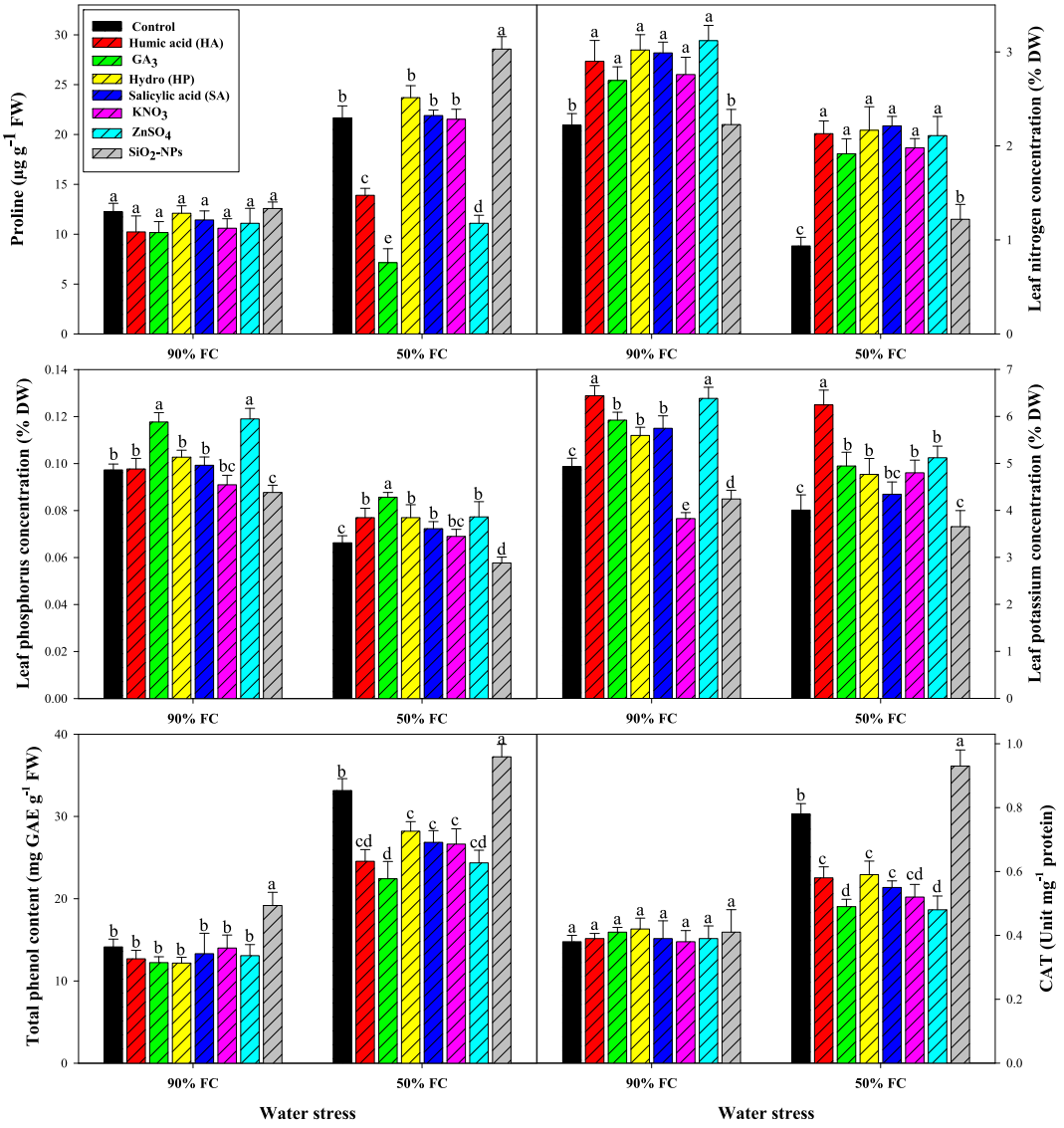


Fig. 1. Effect of drought stress and seed priming on nutrient (N, P and K) concentration, CAT activity and total phenolic content of *A. fasciculifolius*. Bars sharing a letter do not differ significantly at α = 0.05 (Tukey's HSD). Bars represent mean ± SD (n = 4 pots per treatment).

various priming treatments in non-stressed plants (Fig. 1). Drought stress induced a remarkable increase in proline content in all the seed priming treatments compared to non-stress conditions. Under drought stress conditions, the highest proline content (28.56 μg g^{-1} FW) was recorded in the SiO_2-NPs treatment, suggesting that this priming agent failed to mitigate drought-induced stress and may have intensified the adverse effects of water stress. Seed priming with SA, HP, and KNO_3 was also ineffective in limiting the drought-induced accumulation of proline. In contrast, priming with HA, GA_3, and $ZnSO_4$ significantly reduced leaf proline content under drought stress compared to the unprimed control. Under water deficit conditions, GA_3 treatment resulted in the lowest proline content (7.15 μg g^{-1} FW) among all treatments.

3.1.2. Total phenolic content

Concentration of total phenol in *A. fasciculifolius* leaf was significantly affected by drought stress ($P < 0.01$), seed priming ($P < 0.01$) and their interaction ($P < 0.01$) (Supplementary Table S1).

Irrigation at 50 % FC significantly increased the total phenolic content across all seed priming treatments (Fig. 1). Under full irrigation conditions, only seed priming with SiO_2-NPs showed a significant increase in total phenolic content compared to the other priming treatments. Under water deficit conditions, the highest total phenolic content was observed in the SiO_2-NPs treatment (37.25 mg GAE g^{-1} FW), followed by the no priming treatment (33.15 mg GAE g^{-1} FW). The other treatments reduced the content of this parameter compared to the control, with GA_3 priming being the most effective among them.

3.1.3. CAT activity

ANOVA results showed that drought stress ($P < 0.01$), priming ($P < 0.01$) and interaction effect of drought stress × priming ($P < 0.01$) had significant effects on CAT activity (Supplementary Table S1).

Drought stress dramatically stimulated CAT enzyme activity in all the priming treatments (Fig. 1). For instance, CAT activity in the control treatment increased by approximately 79 %. In fully irrigated plants, CAT enzyme activity did not differ significantly across the various seed priming treatments. Under 50 % FC irrigation, all seed priming treatments significantly decreased CAT enzyme activity compared to the control, except for the SiO_2-NPs treatment, which resulted in a significant increase in CAT activity. Priming *A. fasciculifolius* seeds with $ZnSO_4$ and GA_3 exhibited the lowest enzyme activity (0.48 Unit mg^{-1} protein) in this condition.

3.2. Physiological parameters

3.2.1. Nutrient absorption

Based on the ANOVA results, absorption of N, P and K were significantly affected by drought stress, seed priming and interaction effect of drought stress × seed priming (Supplementary Table S2).

Exposure the plants to water deficit significantly reduced leaf N, P, and K uptake across all seed priming treatments (Fig. 1). In 90 % FC irrigation, N uptake by *A. fasciculifolius* leaves was significantly stimulated by all seed priming treatments except SiO_2-NPs. The highest N concentration (3.12 %) was recorded for seed priming with $ZnSO_4$, which increased N uptake by 41 % compared to the control. There was no significant difference in N concentration between seed priming with SiO_2-NPs and the non-primed seeds. Fluctuations in leaf N concentration under drought stress followed a similar trend to those observed under non-stress conditions. However, under stress conditions, seed priming with SiO_2-NPs also caused an increase in N uptake, although the magnitude of this increase was considerably lower than that observed for other priming treatments. In this irrigation condition, seed priming with SA comprised the maximum N concentration of leaf (2.21 %).

In 90 % FC irrigation, priming with $ZnSO_4$ and subsequently GA_3 had considerably higher P concentration than other treatments (Fig. 1). Other priming methods had no significant difference with the control in this regard. Compared to full irrigation conditions, drought stress significantly reduced P uptake in all seed priming treatments. However, all priming treatments except SiO_2-NPs were able to enhance P uptake under water deficit conditions. Among them, GA_3 was the most effective treatment, showing a significant improvement compared to the others (Fig. 1).

When the plants were well watered, seed priming with SiO_2-NPs and KNO_3 restricted K absorption compared to the control treatment. However, the other priming treatments improved leaf K concentration. HA (6.44 %) and SA (6.38 %) exhibited the highest K concentrations under 90 % FC irrigation. When the plants were subjected to drought stress, all seed priming treatments except SiO_2-NPs significantly enhanced K uptake in leaves compared to the control. Seed priming with HA were substantially included the highest K concentration (6.24 %) among the treatments. Seed priming with KNO_3, which had the lowest concentration of leaf K under non-stress conditions, significantly enhanced K uptake in leaves under drought stress.

3.2.2. Photosynthesis pigments

Chlorophyll (a and b), carotenoid contents and the Chl a/b ratio were significantly influenced by the drought stress, seed priming and combined effect of drought stress × seed priming at the 1 % significance level (Supplementary Table S3).

On average, chlorophyll (a and b) and carotenoid contents were significantly elevated by drought stress. In full irrigation regime, priming seeds with SiO_2–NPs declined the photosynthesis pigments content than non-primed plants, with the lowest concentrations of chlorophyll *a* (0.709 mg g^{-1} FW), chlorophyll *b* (0.114 mg g^{-1} FW), and carotenoids (0.060 mg g^{-1} FW) observed in this treatment. No significant difference was observed between non-priming treatment and other priming treatments in terms of photosynthetic pigments in no stress conditions at 90 % FC irrigation. During drought stress condition, the highest of all the three photosynthesis pigments content was found in GA_3, followed by HA and $ZnSO_4$ treatments. The minimum contents of them were recorded in SiO_2-NPs treated plants, which exhibited even lower levels than the non-primed control.

In all seed priming treatments, Chl b content was significantly higher under drought stress conditions compared to non-stress conditions. Under both 90 % FC and 50 % FC conditions, seeds primed with SiO_2-NPs exhibited the lowest Chl b content (Fig. 2). The maximum values of Chl b contents in stress condition were obtained in HA, $ZnSO_4$ and GA_3 treatments (0.272, 0.264, and 0.246 mg g^{-1} FW, respectively). The other treatments contained less than 0.222 mg g^{-1} FW of Chl b (Fig. 2).

Carotenoid content showed similar trends to Chl a, reaching its highest levels under severe stress conditions in GA_3 (0.111 mg g^{-1} FW) and HA (0.104 mg g^{-1} FW) treatments, followed by $ZnSO_4$ treatments (0.100 mg g^{-1} FW). Seed priming with SA, water, and KNO_3 showed no significant difference compared to non-primed seeds under 90 % FC, whereas under 50 % FC, they significantly increased carotenoid content compared to the control.

Although drought stress increased Chl a, Chl b, and carotenoid contents across different seed priming treatments, the Chl a/b ratio decreased under 50 % FC irrigation compared to 90 % FC (Fig. 2). Seed primed with SA included the highest Chl a/b ratios (6.97) in no stress conditions. Under these conditions, seed priming with KNO_3, $ZnSO_4$, and SiO_2-NPs did not show a significant difference compared to that treatment. Under 50 % FC conditions, the lowest Chl a/b ratio was observed in non-primed seeds (4.48), which did not differ significantly from seeds primed with HA, water, KNO_3 and $ZnSO_4$. Under these conditions, the highest chlorophyll *a*/b ratio was observed in seeds primed with GA_3, SA, and SiO_2-NPs, which differed significantly from the other seed priming treatments. Except for the control treatment, the chlorophyll *a*/b ratios were significantly lower in the primed seeds under 50 % FC compared to 90 % FC, under the same priming conditions (Fig. 2).

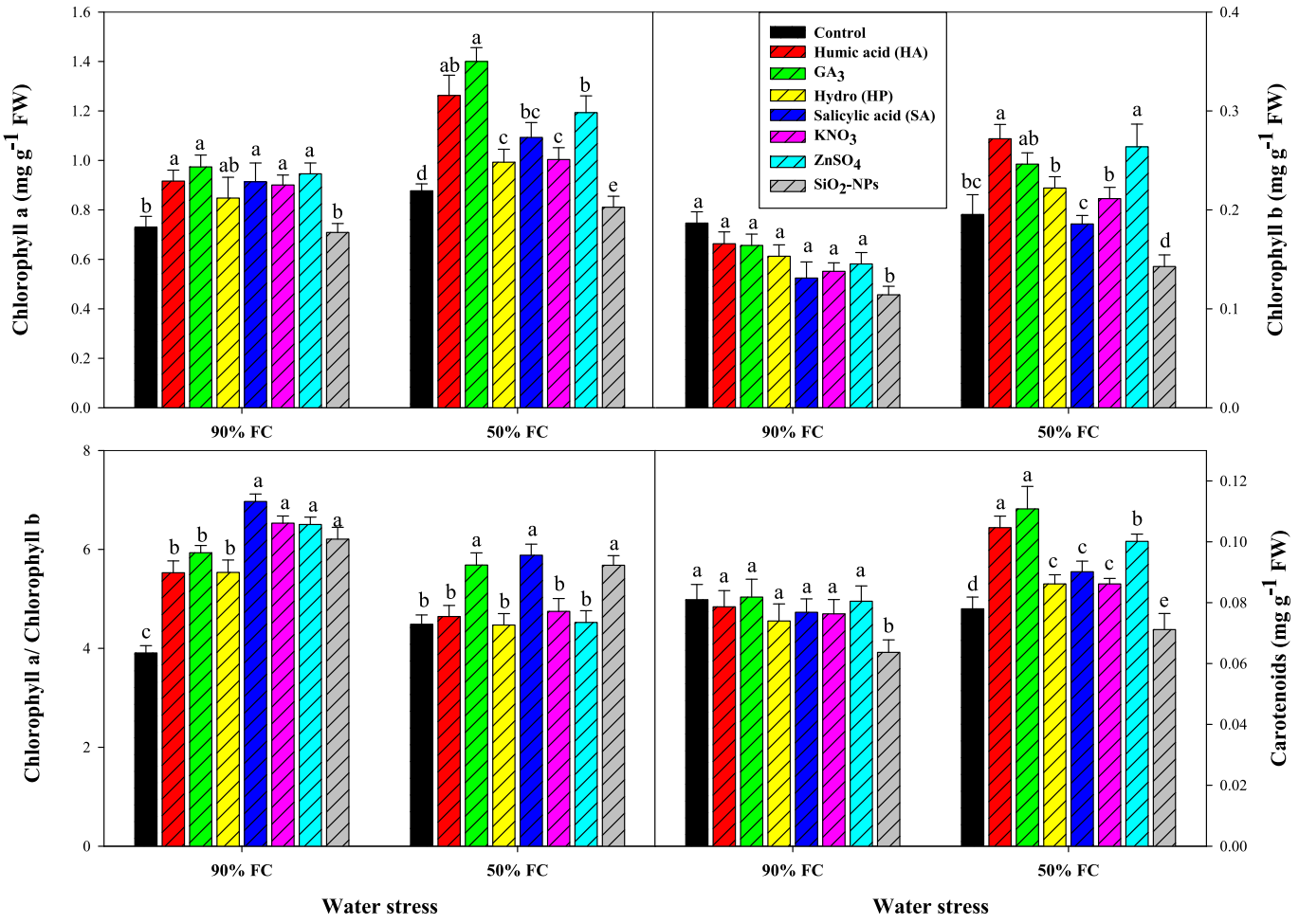


Fig. 2. Effect of drought stress and seed priming on photosynthesis pigments of *A. fasciculifolius*. Bars sharing a letter do not differ significantly at α = 0.05 (Tukey's HSD). Bars represent mean ± SD (n = 4 pots per treatment).

3.3. Vegetative characteristics

3.3.1. Plant height

Drought stress ($P < 0.01$), priming ($P < 0.01$) and their interaction ($P < 0.01$) had significant effects on plant height of *A. fasciculifolius* (Supplementary Table S4).

According to the results of mean comparisons, the plant height was significantly declined by 12.2 % as drought stress intensified from 90 % to 50 % FC (Fig. 3). In the full irrigation regime, with the exception of the SiO_2-NPs treatment, which led to a significant decrease in plant height compared to the control treatment, no significant difference was observed between the other treatments. Under 50 % FC condition, all the seed priming treatments led to an improvement in plant height compared to the non-primed plants, but seed priming with SiO_2-NPs did not contain a positive effect on plant height. This trait was the highest in GA_3, KNO_3, and $ZnSO_4$ treatments (12.53, 11.73, and 11.56 cm, respectively), followed by SA, HA, and HP treatments. The control and SiO_2-NPs treatments represented the lowest plant height of <10 cm (Fig. 3).

3.3.2. Leaf number per plant

The analysis of variance revealed that leaf number per plant of *A. fasciculifolius* was significantly affected by drought stress ($P < 0.01$) and seed priming ($P < 0.01$) and drought stress × seed priming ($P < 0.01$) (Supplementary Table S4).

Drought stress meaningfully declined the leaf number per plant in all the priming treatments (Fig. 3). On average, a 13.1 % reduction in the trait was observed as drought stress intensified from 90 % to 50 % of FC.

Among seed priming treatments, the highest values of leaf number per plant were recorded in GA_3, HA, and $ZnSO_4$ treatments (7.58, 6.58, and 5.58 leaves per plant, respectively). The other treatments were assigned to the same statistical group in terms of this trait (Fig. 3). The number of leaves per plant was negatively affected by seed priming with SiO_2-NPs and KNO_3, showing a significant reduction compared to the non-primed control.

3.3.3. Plant dry weight

The simple and interaction effects of drought stress and seed priming treatments were significant on the plant dry weight of *A. fasciculifolius* at the 1 % significance level (Supplementary Table S4).

Drought stress caused a notable reduction in plant dry weight across all seed priming treatments compared to plants irrigated at 90 % FC. In non-stress condition, only seed priming with GA_3 significantly caused an increase (23.4 %) in plant dry weight compared to the non-priming treatment. On the other hand, seed prime with SiO_2-NPs not only did not have a positive effect on this parameter, but also reduced that by about 16 % compared to the control treatment.

When the plants were exposed to drought stress, priming the seeds with HP, GA_3, SA, SiO_2-NPs, KNO_3 and $ZnSO_4$ could mitigate the negative impacts of drought stress and lead to a significant improvement (13 %, 33 %, 12 %, 19 %, 22 % and 24 %, respectively) in the plant dry weight compared to the no primed treatment. The lowest plant dry weight (0.730 g plant^{-1}) was recorded for the SiO_2-NPs treatment (Fig. 3).

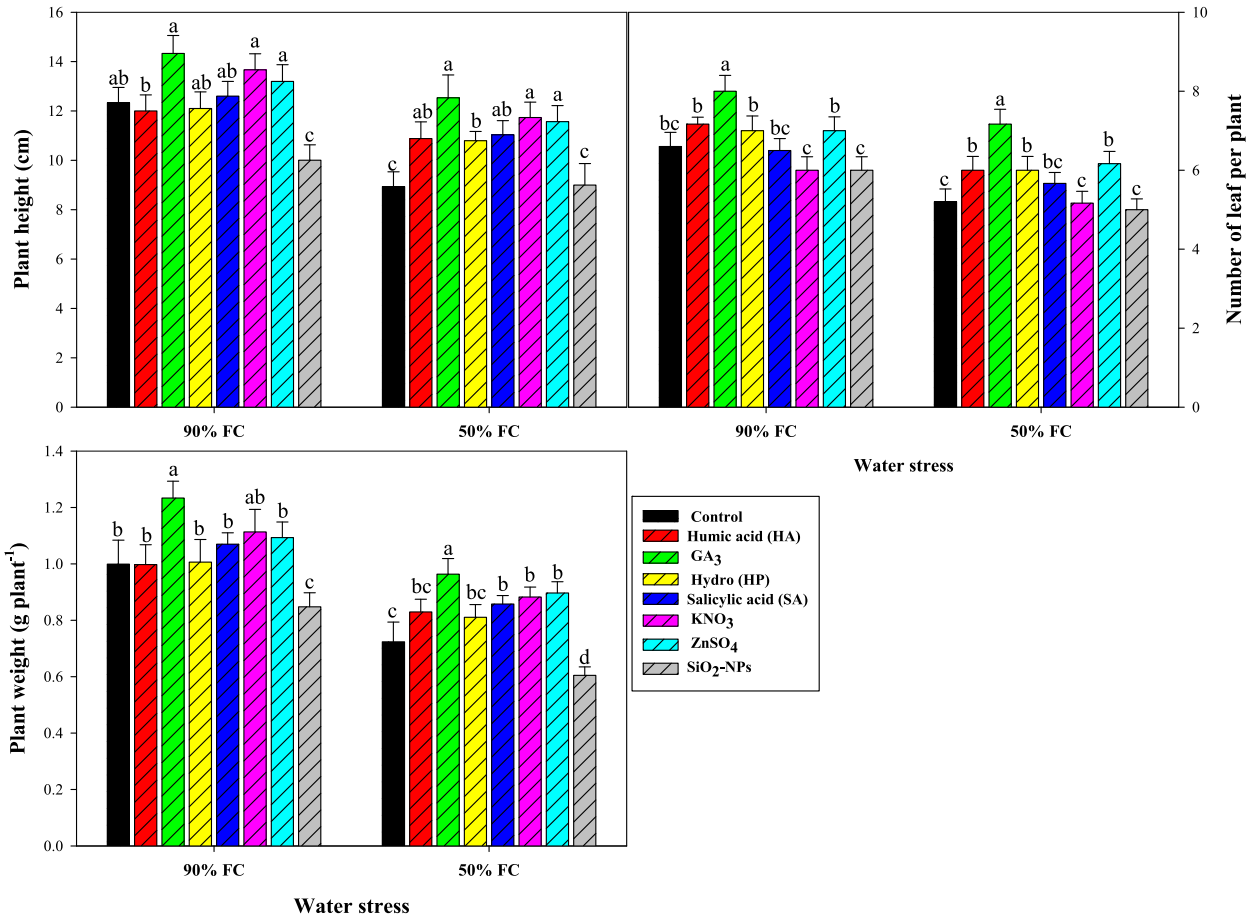


Fig. 3. Effect of drought stress and seed priming on plant height and weight, and number of leaf per plant of *A. fasciculifolius*. Bars sharing a letter do not differ significantly at α = 0.05 (Tukey's HSD). Bars represent mean ± SD (n = 4 pots per treatment).

3.4. PCA-based discrimination of treatments and variables

The first two principal components together explained 83.1 % of the total variance, with PC1 accounting for 57.4 % and PC2 for 25.7 % (Fig. 4). The PCA loadings revealed that growth-related parameters (plant height, leaf number, shoot dry weight, and nutrient uptake of N and P) were strongly and negatively associated with PC1, indicating that treatments located on the negative side of PC1 were characterized by superior growth performance (Table 2). The results of the PCA indicated that under non-stress conditions, only the GA_3 priming treatment exhibited a positive impact on growth-related traits such as plant height, leaf number, and shoot dry weight (Fig. 4). In contrast, treatments including SA, Hydropriming and KNO_3 showed no noticeable difference compared to the non-primed plants. The SiO_2-NPs treatment was positioned in the negative region of PC1, indicating a negative impact *A. fasciculifolius* sp. The SiO_2-NPs treatment was located on the negative side of PC1, suggesting a detrimental influence on various investigated traits of *A. fasciculifolius*. Under drought stress, seed priming with GA_3 and HA proved to be the most effective treatments, contributing to the enhancement of all photosynthetic pigments. This was consistent with the high positive loadings of chlorophyll *a*, chlorophyll *b*, and carotenoids on PC2, where GA_3 and HA treatments clustered in the upper positive quadrant. Seed priming with $ZnSO_4$ also showed a substantial positive effect on a wide range of physiological and biochemical traits and was positioned in the positive quadrant of both PC1 and PC2. Likewise, KNO_3 and SA treatments were positively associated with total phenolic content and CAT activity, appearing in the positive regions of both principal components. This pattern corresponded to the strong positive loadings of phenols (0.358) and CAT (0.332) on PC1, suggesting that these treatments stimulated stress-response pathways rather than growth promotion (Table 2). In contrast, the SiO_2-NPs treatment under drought stress appeared distant from the vectors of positively correlated traits, indicating no beneficial effects on any of the measured parameters and even exerting a negative impact compared to the non-primed seed.

4. Discussion

The results illustrated that drought stress had a significant detrimental effect on the growth characteristics of *A. fasciculifolius* plants, as manifested by a substantial reduction in leaf number, plant height, and dry weight across all seed priming treatments compared to 90 % FC irrigation conditions (Fig. 3). Water limitation exerts a profound influence on all morphological, cellular, biochemical, and physiological aspects of plants (Kapoor et al., 2020). In this investigation, water deficit negatively impacted plant growth through disrupting the uptake and translocation of essential nutrients, such as nitrogen, phosphorus, and potassium, and concomitantly altering leaf chlorophyll content. The decline in nutrient uptake during water deficit is a multifaceted phenomenon resulting from the interplay of various physiological and biochemical mechanisms, including photosynthetic stomatal closure (Zhou et al., 2013), generation of ROS (Impa et al., 2012), encompassing reduced soil water potential (Ding et al., 2021), altered hormonal balance (Ramachandran et al., 2020), diminished activity of nutrient uptake-related enzymes (Cao et al., 2018) and nutrient immobilization in soil (Kumar et al., 2024). Furthermore, growth retardation when the plant exposure to drought stress is further exacerbated by disruptions in cell elongation and division (Anjum et al., 2017).

Despite the plants employ diverse mechanisms to survive in water

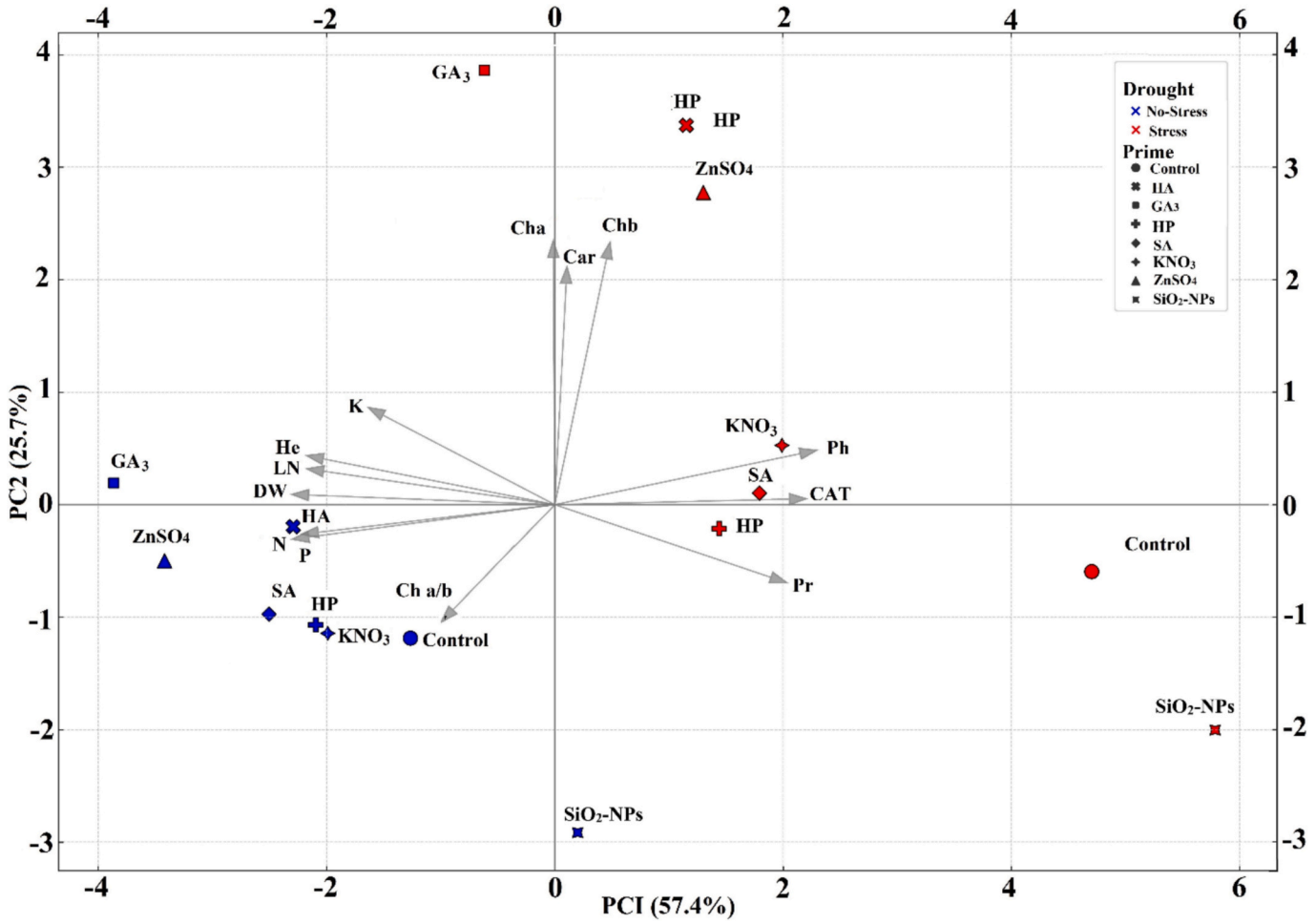


Fig. 4. Biplot of the PCA results in discrimination of treatments and variables.
(Red point: drought stress condition, blue point: no stress condition; pH: Total phenolic content, CAT: Catalase, Pr: Proline, Ch: Chlorophyll, Car: Carotenoid, N: Nitrogen, P: Phosphorus, K: Potassium, DW: Dry weight, LN: Leaf number, He: Plant height)

Table 2
Principal component loadings of growth, physiological, and biochemical traits of *Astragalus fasciculifolius* under drought stress and seed priming treatments.

Variable	PC1	PC2
Plant height	−0.326	0.097
Leaf number	−0.320	0.110
Dry weight	−0.349	0.019
Chlorophyll a	0.015	0.521
Chlorophyll b	0.075	0.532
Chl a/b	−0.201	−0.258
Carotenoids	0.021	0.532
Proline	0.311	−0.136
N	−0.330	−0.040
P	−0.347	−0.058
K	−0.242	0.211
Phenols	0.358	0.075
Catalase	0.332	−0.014

PCA was performed using standardized treatment mean data. PC1 and PC2 explained 57.4 % and 25.7 % of the total variance, respectively.

scarcity conditions, research has demonstrated that seed priming with various biostimulants can enhance the tolerance and adaptation rate of those to drought stress (Mansour et al., 2023). This study also corroborated the positive function of certain priming agents for *A. fasciculifolius* seeds in enhancing adaptation to water deficit stress, which will be discussed further.

The findings of this study confirmed a significant increase in proline contents under drought stress compared to full irrigation conditions in the non-primed treatment (Fig. 1). Proline, a compatible osmolyte, accumulates in plants under drought stress to maintain cell turgor pressure (Kayak et al., 2023), scavenge ROS, act as a signaling molecule for stress-responsive gene expression (Ghosh et al., 2022), regulate plant metabolism (Furlan et al., 2020) and stabilize cell membranes. In contrast, under conditions of water scarcity, plants whose seeds were primed with HA, GA_3 and $ZnSO_4$ showed significantly lower proline contents compared to those in the non-primed treatment. Moreover, these pretreated plants did not exhibit a significant increase in proline content when subjected to drought stress. This observation could be attributed to the possibility that plants primed with these substances (HA, GA_3 and $ZnSO_4$) employed alternative mechanisms that rendered them less susceptible to stress conditions, thereby obviating the need for a substantial increase in stress-related metabolites such as proline. The reduction in proline production during seed priming with HA, GA_3 and $ZnSO_4$ may indicate that the application of these materials has mitigated the negative effects of drought stress, thereby decreasing the need for proline synthesis. In other words, with the improvement in plant condition, there is no longer a necessity for proline production.

Under water deficit 50 % FC irrigation conditions, all the seed priming techniques considerably improved macronutrients uptake (N, P, K) compared to the non primed plants. So, priming seed with SiO_2-NPs caused to a differential effect, leading to a decrease in elements uptake. This contrasting response may be due to the distinct physiological mechanisms triggered by the different priming agents (Nile et al., 2022).

Among the treatments tested, primed seed with HA exhibited the highest efficacy in promoting nutrient uptake, followed by GA_3 and $ZnSO_4$. The increased nutrient uptake observed with the mentioned treatments under drought stress may be linked to their positive impact on plant growth (Abbasi et al., 2019), root system development (Khodadadi et al., 2020) and stress tolerance (Zia et al., 2021). Priming the plant seeds with HA, GA_3, and $ZnSO_4$ revealed as effective techniques to alleviate the depressing impacts of drought stress on nutrient uptake and potentially improve *A. fasciculifolius* productivity under unfavorable environmental conditions.

Furthermore, compared to the control, priming seeds with GA_3, followed by HA, and then $ZnSO_4$ significantly enhanced the content of photosynthetic pigments in water deficit condition (Fig. 2). In most cases, drought stress typically leads to a decrease in content of photosynthetic pigments (Chavoushi et al., 2020). Conversely, some drought-tolerant plant species may exhibit elevated chlorophyll content under drought stress conditions (Ahmed et al., 2020). This phenomenon could be attributed to an increased content of resources towards maintaining photosynthetic activity (Keller et al., 2021). The application of HA, GA_3, and $ZnSO_4$ appears to stimulate the allocation of resources towards the production of photosynthetic pigments.

Under drought stress, the plant's antioxidant (CAT enzyme activity) and non-enzymatic (total phenolic content) defense systems were significantly activated. The most significant enhancement of these systems was observed in the unprimed and SiO_2-NPs seed priming treatments. In other seed priming treatments, there was no substantial increase in total phenolic content and CAT activity compared to non-stress conditions. It is hypothesized that application of these substances, particularly GA_3 and $ZnSO_4$, reduced the plant's need to activate its antioxidant defense system by alleviating the detrimental effects of drought stress.

Overall, the findings of this study clearly demonstrate that among the used priming among the tested priming treatments, seed priming with HA, GA_3, and $ZnSO_4$ effectively alleviates alleviated the detrimental effects of drought stress on *A. fasciculifolius* growth and yield. The observed improvements in this plant growth parameters and yield attributes can be attributed to the multifaceted mechanisms involved in the priming treatments. These mechanisms will be discussed below.

Seed priming with HA has appeared emerged as a promising and eco-friendly approach to enhance plant growth and drought stress tolerance. This approach offers several advantages, including improved nutrient availability through chelation and enhanced root uptake of nutrients (Izhar Shafi et al., 2020). Additionally, HA promotes the growth of beneficial soil microbes (Galambos et al., 2020), which contribute to nutrient cycling, improve plant root health, and potentially enhance stress tolerance mechanisms. Furthermore, HA priming stimulates the production of stress hormones and antioxidants, enabling plants to cope with drought stress by mitigating oxidative damage (Abu-Ria et al., 2024). Priming seed with HA plays a proven role in motivating seed germination and root development by promoting cell division and elongation (Jindo et al., 2012). Stronger roots allow crops to access nutrients and water more effectively, especially under drought stress conditions (Shen et al., 2020). The efficacy of seed priming with HA in mitigating the deleterious effects of drought stress on the growth, productivity, and overall health of medicinal and industrial plants has been extensively substantiated in various investigations.

Seed priming with GA_3 emerges as an encouraging strategy to promote plant growth and adapted them to drought stress. GA_3 acts in multiple ways: it jumpstarts germination by breaking seed dormancy and activating enzymes for seedling development (Thakur et al. 2022). This leads to faster emergence of stronger seedlings that can better compete for resource absorption. Additionally, GA_3 improves radical and root growth, allowing plants to access crucial water and nutrients more efficiently, especially during water deficit (Ma et al., 2018). GA_3 may also influence plant water management by regulating stomatal conductance, potentially enhancing drought tolerance (Salvi et al., 2021). Furthermore, GA_3 can trigger the genes expression associated with stress defense, helping plants scavenge harmful ROS and maintain cellular function under water deficit conditions (Emamverdian et al., 2020; Niharika et al., 2021). Overall, GA_3 priming provides a multifaceted approach to recover plant growth and resilience by promoting faster development, efficient resource acquisition, and improved stress tolerance (Mearaji et al., 2021).

The results illustrated that priming the seed with $ZnSO_4$ can be offered as an approach to enhance plant growth and drought tolerance. The potential mechanisms may involve enhanced Zn uptake during imbibition, attributed to its direct presence in the priming solution (Rai-Kalal and Jajoo, 2021). Zn, a crucial micronutrient, plays a vital role in enzyme activation, protein synthesis, and auxin metabolism, ultimately promoting plant growth (Feizi et al., 2025). Additionally, Zn stimulates root development, leading to a more extensive root system that improves water and nutrient acquisition, particularly under drought stress (Umair Hassan et al., 2020). Furthermore, $ZnSO_4$ priming enhances the activity of antioxidant enzymes (CAT, SOD) due to the role of Zn as a cofactor (Singhal et al., 2021). This bolstered antioxidant defense system helps plants scavenge free radicals and mitigate oxidative damage caused by drought stress. Zn can also stimulate the production of osmoprotectants like proline and glycine betaine, which aid in maintaining cell turgor pressure and osmoregulation during drought (Jan et al., 2022). Overall, $ZnSO_4$ priming enhances Zn availability, thereby promoting nutrient metabolism, strengthening root development for improved water and nutrient uptake, and mitigating the detrimental effects of drought stress by boosting antioxidant defenses and osmoprotectant synthesis (Zhang et al., 2021).

Seed priming with SiO_2-NPs exhibited no alleviating effect on drought-induced damage and, in contrast, significantly suppressed plant growth under both drought and well-watered conditions relative to the unprimed control. These negative effects were accompanied by decreased chlorophyll content, increased levels of MDA, enhanced CAT activity, elevated total phenolic compounds, and decreased uptake of essential nutrients, all of which could be indicative of oxidative stress. Although SiO_2-NPs have been widely reported to enhance plant growth and stress tolerance, mechanistically, it can also intensify ROS generation within chloroplast and mitochondrial membranes by promoting electron leakage (Gao et al., 2023). The resulting ROS accumulation causes lipid peroxidation—reflected by higher MDA levels—leading to membrane damage, ionic leakage, and reduced efficiency of nutrient transport systems. In this study, the observed rise in CAT activity and phenolic content by SiO_2-NPs application can suggests an antioxidant defense response, which, although protective, appears insufficient to fully mitigate oxidative injury, thus contributing to decreased photosynthetic performance and growth. Furthermore, surface interactions between SiO_2-NPs and nutrient ions may disrupt membrane electrochemical gradients and nutrient homeostasis (Ali, 2024), consistent with the observed reductions in leaf N, P, and K concentrations. Therefore, even under stable dispersion and in the absence of agglomeration, the persistent presence of SiO_2-NPs during seed priming can impose metabolic costs through ROS-mediated oxidative imbalance and nutrient uptake disruption.

5. Conclusion

This study revealed that drought stress significantly affected the physiological and growth characteristics of *A. fasciculifolius*. However, seed priming with GA_3, $ZnSO_4$, and HA effectively alleviated these adverse effects by reducing proline accumulation and maintaining higher levels of chlorophyll and carotenoids. Priming agents enhanced plant biomass and growth under stress conditions, which may be associated with a reduced requirement for CAT activity compared to the non-primed treatment. In addition, GA_3, $ZnSO_4$, and HA priming agents improved key growth parameters and nutrient uptake (N, P, K) under drought conditions.

Considering the diverse medicinal and health properties of the *A. fasciculifolius*, and its potential as an economically viable crop, it is recommended that farmers, particularly in arid and semi-arid regions facing water scarcity, incorporate its cultivation into their cropping patterns. To ensure drought resistance, it is advised to prime the seeds of this plant with substances such as GA_3, $ZnSO_4$ and HA prior to sowing. As a cost-effective and scalable technology, seed priming provides an accessible solution for diverse farming scales, bolstering resilience against increasing climate change-induced drought events.

CRediT authorship contribution statement

Seyed Masoud Ziaei: Writing – original draft, Methodology, Conceptualization. **Rooholla Moradi:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Hossein Sahabi:** Writing – review & editing. **Mohsen Zaferanieh:** Software, Resources, Data curation. **Majid Jafari:** Writing – review & editing, Writing – original draft, Conceptualization.

Limitations and future perspectives

This study has several limitations that should be acknowledged. The experiment was conducted on a single plant species and within one growing season under greenhouse conditions, which may not fully reflect field-scale variability. In addition, the nutrient analysis was restricted to the major macronutrients (N, P, and K), and no microscopic or imaging techniques were employed to verify the distribution or internalization of SiO_2 nanoparticles in plant tissues. Future research should therefore include multi-species and multi-season field experiments, evaluate a broader nutrient profile, and incorporate imaging or spectroscopic analyses to trace nanoparticle behavior. Moreover, assessing a dose–response relationship for SiO_2-NPs under both greenhouse and field conditions will help to identify optimal concentrations that enhance stress tolerance without imposing phytotoxic effects.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare they have not used any of the Generative AI tools in creating this article.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aoas.2025.100397.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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