

Alleviation of Drought Stress in German Chamomile (*Matricaria chamomilla* L.) in Response to Suppressive Oxidative Stress and Water Deficit-Induced Stomatal Closure by Exogenous Polyamines

Mohammad Javad Ahmadi-Lahijani^{1*}, Jafar Nabati¹, Saeed Moori², and Mohammad Kafi¹

ABSTRACT

Polyamines (PAs) are signaling molecules that exhibit promising roles in improving stress tolerance in plants. Limited information is available concerning the effects of the exogenous PAs on medicinal plants including chamomile. This experiment was carried out to study the effects of foliar application of PAs [Putrescine (Put), Spermidine (Spd), and Spermine (Spm)] on physiological and biochemical processes to understand the possible mechanisms concerning the water deficit stress [soil Field Capacity (FC) as control, 80% of FC (FC₈₀), and 60% of FC (FC₆₀)] alleviation in German Chamomile. We found that PAs partially inhibited water deficit-induced stomatal closure and induced antioxidant enzymes to eliminate the increased H₂O₂. Spd increased stomatal conductance (g_s) by 66, 65, and 35% at FC, FC₈₀, and FC₆₀, respectively, compared with the control. The increased g_s enhanced leaf net photosynthesis (A_N) by 52 and 86% at FC₈₀ and FC₆₀, respectively, compared with the control. The role of PAs in oxidative damage alleviation was approved by the negative correlation of leaf antioxidant activities and Malondialdehyde (MDA) and H₂O₂ content. According to the results, PAs function as stress-protecting compounds to instigate the antioxidative enzymes to scavenge stress-induced H₂O₂, improve membrane stability, and enhance water deficit tolerance. Generally, our results suggested that PAs could be potential growth regulators to alleviate mild to severe water deficit stress.

Keywords: Chlorophyll fluorescence, Enzymatic antioxidant, Hydrogen peroxide, Gas exchange variables, Non-photochemical quenching.

INTRODUCTION

Drought is considered the most crucial worldwide factor in plant production systems (Hafezi Ghehestani *et al.*, 2021). Water deficit stress affects the metabolism and growth of plants, agricultural ecosystems, and human societies (Tezara *et al.*, 1999). Various physiological and metabolic responses such as stomatal closure and decline in photosynthesis

and growth rate are induced in plants under water deficiency (Flexas and Medrano, 2002). Plant response to stressful conditions is initiated when the stress is recognized at the cellular level. In both unstressful and stressful environments, plants produce Reactive Oxygen Species (ROS) that react with proteins, lipids, and DNA and impair normal cellular functions (Apel and Hirt, 2004). Water deficit stress disturbs the balance between

¹ Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Islamic Republic of Iran.

² Plant Breeding Department, Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute (NSTRI), Tehran, Islamic Republic of Iran.

* Corresponding author; e-mail: mjahmadi@um.ac.ir



ROS production and scavenging in plants, leading to the accumulation of ROS and accelerating cell membrane damage and lipid peroxidation (Farooq *et al.*, 2009).

Polyamines (PAs) are classified as a group of phytohormone-like aliphatic amine natural compounds with aliphatic nitrogen structure and are considered secondary messengers in signaling pathways (Liu *et al.*, 2023). Generally, naturally occurring PAs in the higher plants including Putrescine (Put), Spermidine (Spd), and Spermine (Spm) are not only involved in numerous cellular and molecular processes in plants but also have been shown to improve plant tolerance to abiotic stresses (Baghalian *et al.*, 2011). PAs trigger several molecular, biochemical, and physiological responses of plants including increasing membrane stability and osmolyte accumulation, protection of photosynthetic apparatus, activation of antioxidant machinery, regulation of redox homeostasis, upregulation of stress-related genes, and promotion of plant stress tolerance (Alcázar *et al.*, 2020).

The interaction of PAs with membrane phospholipids induces membrane stability under stressful conditions. PAs play a vital role as signaling molecules that regulate several metabolic pathways. The abiotic stress adaptations are enhanced by the PAs' functions as stress signaling molecules (Pál *et al.*, 2015). Exogenously applied PAs increased antioxidant enzyme activities under various stressful conditions; which could reduce cell damage and enhance the stress tolerance of plants (Hassan *et al.*, 2018; Alcázar *et al.*, 2020). The accumulation of PAs under adverse conditions can directly act as an antioxidant in eliminating ROS or may activate the ROS-scavenging enzyme system (Liu *et al.*, 2023). Previous studies showed that exogenous PAs significantly increased the activity of antioxidants such as SOD, POD, and CAT and decreased ROS synthesis in *Vicia faba*, *Citrus reticulata*, *Arabidopsis thaliana*, and *Rosa damascene* (Hasan *et al.*, 2021; Liu *et al.*, 2023).

German chamomile (*Matricaria chamomilla*) is one of the most valuable

medicinal plants of the Asteraceae (Compositae) family with many applications in the pharmaceutical, nutritional, and cosmetic industries. Chamomile is relatively adaptable to a wide range of climates including arid and semi-arid regions (Das *et al.*, 1998). However, drought negatively affects chamomile performance and productivity. Although studies of PAs have been performed on various crops (Alcázar *et al.*, 2006; Farooq *et al.*, 2009; Liu *et al.*, 2023), the available information concerning the effects of PAs on medicinal plants is still limited. In this study, the effect of foliar application of polyamines on leaf gas exchanges, chlorophyll fluorescence, and physiological and biochemical processes was studied to understand the possible mechanisms concerning water stress alleviation in German chamomile.

MATERIALS AND METHODS

Experimental Site and Procedure

The experiment was carried out at the Greenhouse of the Research Center for Plant Sciences, Ferdowsi University of Mashhad, in 2021. Chamomile seeds (*cv.* Presov, obtained from Isfahan Natural Resources Research Center) were surface sterilized with 0.2% sodium hypochlorite solution for 5 min and rinsed three times with tap water. Ten chamomile seeds were sown in each 10 kg pot (20 and 25 cm in diameter and depth, respectively) filled with clay loam soil at a depth of 1 cm and thinned to 5 plants per pot, after establishment. Plants were grown under greenhouse conditions with day/night temperatures of 20/15±2°C, natural light (~800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ Photosynthetic Photon Flux Density (PPFD), photoperiod of 13/11 hours day/night, and relative humidity of 50±10%.

Soil Preparation and Treatments

Irrigation was applied until the full establishment (4-leaf), then, water deficit was

applied at three levels of control (FC_{100}), moderate stress (80% of FC; FC_{80}), and severe stress (60% of FC; FC_{60}) according to the method of Topp and Davis (1985). Plants were fertilized with the Hoagland nutrient solution once a week along with irrigation water. Polyamines were foliar applied as (a) Spermine [Spm], (b) Spermidine [Spd], (c) Putrescine [Put], and (d) Control, at a concentration of 10 μ M (Farooq *et al.*, 2009; Ali *et al.*, 2009). Ten mL of the solution was applied to each plant using a handheld sprinkler. The first spray was made at the 5-leaf stage and repeated at 15-day intervals until the flowering onset. The control plants were sprayed with distilled water. The experiments were carried out in three replicates. All physiological and biochemical data were taken from fully expanded leaves at the middle of the flowering stage.

Gas Exchange Parameters

Net photosynthetic rate (A_N), intercellular CO_2 concentration (C_i), Transpiration rate (T_r), and stomatal conductance (g_s) were measured between 9:00–11:00 hour using a portable photosynthetic system (ADC Bio Scientific Ltd, UK). Photosynthetically Active Radiations (PAR), air temperature, relative humidity, and CO_2 concentration inside the sensor head were set at 800 μ mol $m^{-2} s^{-1}$, $25 \pm 2^\circ C$, $50 \pm 5\%$, and 400 ± 20 ppm, respectively. Instantaneous (WUE_i) and intrinsic (A_N/g_s) water use efficiency were calculated by dividing A_N by T_r and g_s , respectively. Mesophyll conductance (g_m) was also calculated as A_N/C_i (Fischer *et al.*, 1998).

Chlorophyll Fluorescence (Chf)

A portable fluorometer (PAM-2500, Walz, Effeltrich, Germany) was used to measure the dark- and light-adapted leaf chlorophyll fluorescence between 10:00–12:00 hour. After 30 minutes of dark adaptation, F_v/F_m was calculated as $(F_m - F_o)/F_m$, where F_m and F_o were the maximum

Fluorescence elicited by a saturating light pulse and steady-state chlorophyll fluorescence, respectively (Genty *et al.*, 1989). The maximum (F_m') and the steady-state (F_s) fluorescence signals were measured from the light-adapted leaves after 4 min of illumination with continuous red wavelength, non-saturating actinic light, and saturating pulses every 25 sec (Murchie and Lawson, 2013). To measure the minimal fluorescence after the PSI excitation (F_o'), the actinic light was turned off, and far-red pulses were applied. Photochemical quenching (qP) was calculated as $(F_m' - F_s)/(F_m' - F_o')$. Non-Photochemical Quenching (NPQ), which is a proportion of the rate of the thermal energy dissipation, was estimated as $(F_m - F_m')/F_m'$ (Van Kooten and Snel, 1990).

Electrolyte Leakage (EL)

Leaf EL was measured to determine leaf membrane damage using an Electrical Conductivity (EC) meter (Jenway Model 4510) according to Equation (1) (Lutts *et al.*, 2016):

$$EL (\%) = \frac{EC_1}{EC_2} \times 100 \quad (1)$$

Here, EC_1 and EC_2 are the EC of the solution after 24 hours and the autoclaved ($120^\circ C$ for 20 minutes) samples, respectively.

Relative Water Content (RWC)

Leaf RWC was estimated using Equation (2) (Smart and Bingham, 1974):

$$RWC (\%) = \left[\frac{W_f - W_d}{W_t - W_d} \right] \times 100 \quad (2)$$

Here, W_f , W_t , and W_d are fresh Weight, turgid Weight, and oven-dried Weight (at $70^\circ C$ until constant mass), respectively.

Leaf Osmotic Potential (ψ_o)

Leaf ψ_o was determined according to the freezing point depression method using an



osmometer (Wogel, model OM802.D). The leaf osmolytes content was calculated based on the van't Hoff equation, and the leaf water content was measured by Eq. (3):

$$\frac{mMol}{g} = \left[\left(-\frac{Op}{RT} \right) \times \left(\frac{WC}{1-WC} \right) \right] \quad (3)$$

Where, the osmolytes content is based on mM g⁻¹ dry weight, R is the universal gas constant (0.00831-liter MPa mol⁻¹ °K⁻¹), T is the absolute temperature (273 °K), Op is the leaf osmotic potential (MPa), and WC is the leaf Water Content. The solute potential was determined at room temperature (25°C).

Photosynthetic Pigments

Fresh leaves (100 mg) were homogenized in ethanol 70% and kept at 4°C for 24 hours. Leaf pigments content (Chlorophylls a, b, and carotenoids) were determined spectrophotometrically (U-2000, Hitachi Instruments, Tokyo, Japan) according to Lichtenthaler and Wellburn (1983).

Leaf Antioxidant Enzymes

Leaf fresh weight (100 mg) was ground in liquid nitrogen, and 1 mL potassium phosphate (0.1 M, pH= 7.8) containing 1 mM EDTA was added. The insoluble solids were removed by centrifuging the mixture in 12,000×g at 4°C (Sigma, model K18-3). The supernatant was kept at -80°C to assay the enzymatic antioxidant activities (Yamaguchi *et al.*, 1995). Leaf enzymatic antioxidants activity, including Ascorbate Peroxidase (APX, EC 1.11.1.11), Superoxide Dismutase (SOD, EC 1.15.1.1), Catalase (CAT, EC 1.11.1.6), and Peroxidase (POD, EC 1.11.1.7), were assayed by the methods described by Nakano and Asada (1981), Giannopolitis and Ries (1977), Cakmak and Horst (1991), and Ghanati *et al.* (2002), respectively.

Malondialdehyde (MDA) and H₂O₂ Content

One hundred mg of leaf fresh weight was used to measure leaf MDA by the methods

described by Jiang and Zhang (2001). Leaf MDA was measured by homogenizing leaf fresh weight in 5 mL of trichloroacetic acid (100 g⁻¹) containing 250 g L⁻¹ thiobarbituric acid. The supernatant absorbance was read at 532 nm spectrophotometrically (Jenway UV-Visible, Model 6305) and was corrected at A600. For H₂O₂ content measurement, leaf tissues (500 mg) were homogenized in an ice bath with 5 mL 0.1% (w:v) TCA. The homogenate was centrifuged at 12,000×g for 15 minutes and the supernatant absorbance was read at 390 nm. The content of H₂O₂ was given on a standard curve (Sergiev *et al.*, 1997).

Statistical Analysis

The experiment was carried out as a factorial (3 levels of water deficit×4 levels of PAs) arrangement in a randomized complete block design with three replications. The experiment was carried out twice and the pooled data were analyzed. Data were subjected to a two-way analysis of variance, and the LSD P≤ 0.05 was the test criterion for assessing differences between the means of the main and/or interaction effects using SAS v.9.4 software. Data was presented as ±SE.

RESULTS

Photosynthetic Parameters

Although the gas exchange parameters were reduced by the water deficit, they were significantly improved by the application of PAs. Water deficit at FC₆₀ diminished the untreated plant A_N by 40% compared with FC₁₀₀ (Figure 1-A). PAs application increased A_N by ~60% compared with the untreated plants under FC₁₀₀ (Figure 2-A). However, under FC₈₀ and FC₆₀, Spd showed the greatest improving effect on A_N. Spd application enhanced leaf A_N by 52 and 86% at FC₈₀ and FC₆₀, respectively, compared with the untreated plants (Figure 1-A).

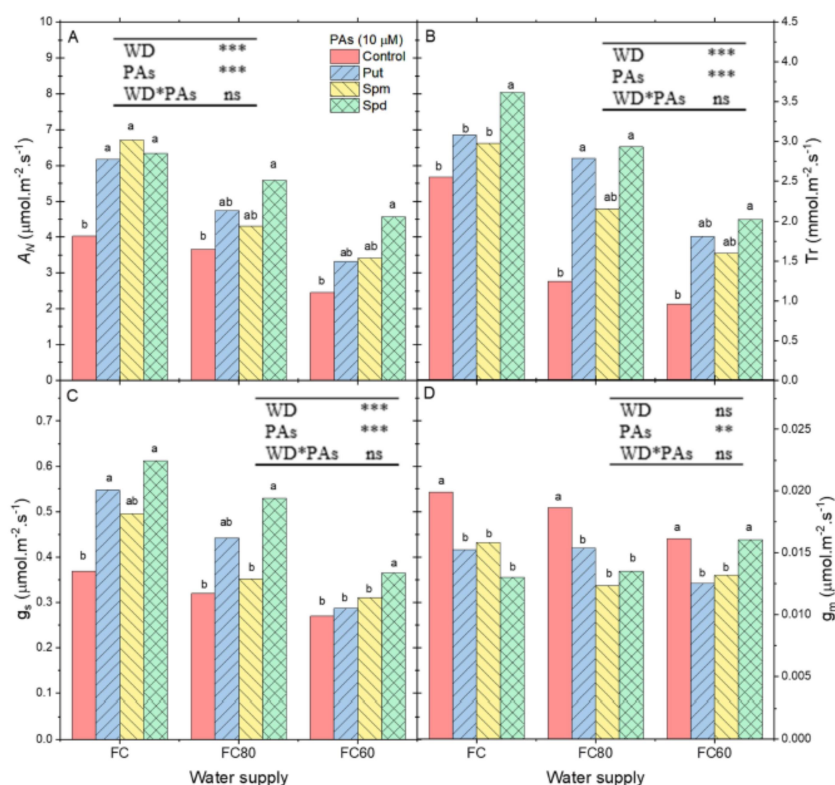


Figure 1. Changes in (A) Net photosynthetic rate, (B) Transpiration rate, (C) Stomatal conductance, and (D) Mesophyll conductance of German chamomile leaves exposed to water deficit and foliar application of polyamines. WD: Water Deficit, PAs: Polyamines. FC: Field Capacity, FC₈₀: 80% of FC, FC₆₀: 60% of FC. *, **, ***, and ns: Significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, and non-significant. Means with the same letters are not significantly different (LSD $P \leq 0.05$, $n = 9$).

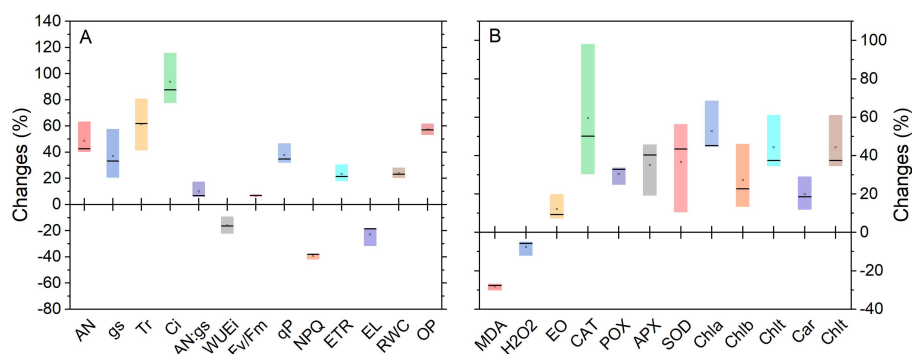


Figure 2. Percent changes of (A) Physiological and growth parameters, and (B) Biochemical traits of German chamomile leaves affected by polyamines foliar application under water deficit levels relative to the untreated plants. A_N : Net photosynthetic rate, g_s : Stomatal conductance, T_r : Transpiration rate, C_i : Interacellular CO_2 concentration, A_N/g_s : Intrinsic water use efficiency, WUE_i : Instantaneous Water Use Efficiency, F_v/F_m : Maximum photochemical quantum yield of PSII, qP : Photochemical quenching, NPQ : Non-Photochemical Quenching, ETR : Electron Transport Rate, EL : Electrolyte Leakage, RWC : Relative Water Contents, OP : Leaf Osmotic Potential, MDA: Malondialdehyde, H_2O_2 : Hydrogen peroxide, EO: Essential Oil, CAT: Catalase, POX: Peroxidase, APX: Ascorbate Peroxidase, SOD: Superoxide Dismutase, Chla: Chlorophyll a, Chlb: Chlorophyll b, Chlt: Total Chlorophyll, Car: Carotenoids.



Chamomile leaf T_r decreased by 1.1 and 1.6 times, respectively, at FC_{80} and FC_{60} compared with FC_{100} (Figure 1-B). The highest leaf T_r was observed in Spd-treated plants at FC_{100} ; 41, 17, and 21%, respectively, higher than the untreated, Put, and Spm-treated plants (Figure 1-B).

Foliar application of PAs reduced the diminishing effects of water deficit on g_s . Spd increased leaf g_s by 66, 65, and 35% at FC_{100} , FC_{80} , and FC_{60} , respectively, compared with the untreated plants (Figure 1-C). The lowest leaf g_m was observed when Spd and Spm were applied, respectively, at FC_{100} and FC_{80} (Figure 1-D). Although water deficit decreased C_i and $C_i:C_a$, PAs significantly increased C_i and $C_i:C_a$

compared with the untreated plants (Figures 3-A and -B). At FC_{100} and FC_{80} , C_i was the highest in the Spd-treated plants by 1.4 and 1.1 times higher than the untreated plants, respectively. The highest WUE_i was observed in the untreated plants at FC_{80} (Figure 3-C). Spm-treated plants showed 43% higher WUE_i at FC_{100} compared with the untreated plants; however, WUE_i decreased when PAs were applied under water deficit conditions. A_N/g_s decreased by the reduction in the soil moisture. The highest A_N/g_s was observed in Spm and Spd-treated plants at FC_{100} and FC_{60} , respectively, 24 and 38% higher than the untreated plants (Figure 3-D).

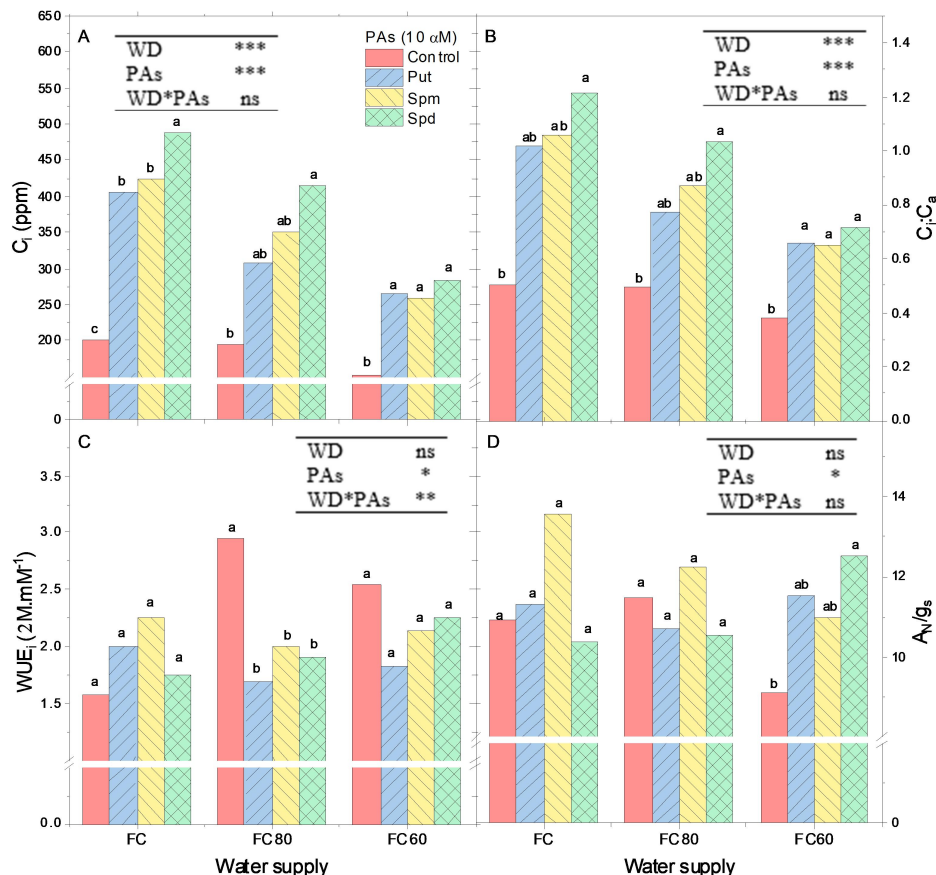


Figure 3. Changes in (A) Intercellular CO₂ concentration, (B) Intercellular to ambient CO₂ concentration, (C) Instantaneous water use efficiency, and (D) Intrinsic water use efficiency of German chamomile leaves exposed to water deficit and foliar application of polyamines. WD and other symbols as under Figure 1.

Leaf Chlorophyll Fluorescence (Chf)

Water deficit at the level of FC₆₀ decreased F_v/F_m by 22% compared with FC₁₀₀ (Figure 4-A). At FC₈₀, Put-treated plants showed the highest F_v/F_m , which was 13% higher than the untreated plants. Non-Photochemical Quenching (NPQ) increased by increasing the water deficit intensity (Figure 4-B). Spd-treated plants showed 85% and 65% lower leaf NPQ than the untreated plants, respectively, at FC₈₀ and FC₆₀. Photochemical quenching (qP) reduced by 26 and 33% at FC₈₀ and FC₆₀, respectively, compared with FC₁₀₀ (Figure 4-C). The highest qP was observed in the Put-treated plants; 36, 69, and 34% higher than the untreated plants, respectively, at FC₁₀₀, FC₈₀, and FC₆₀. The linear Electron Transport Rate

(ETR), decreased by 24% and 48%, respectively, at FC₈₀ and FC₆₀ compared with FC₁₀₀ (Figure 4-D). The greatest ETR was recorded in the Spm- followed by Put-treated plants at FC₈₀ by on average ~25% higher than the untreated plants (Figures 4-D and 2-A). However, at FC₆₀, Spd increased ETR by 39% compared with the untreated plants.

Leaf RWC, ψ_o , and EL

Water deficit at FC₈₀ and FC₆₀ reduced leaf RWC by 13 and 22%, respectively, compared with FC₁₀₀ (Figure 5-A). Put, Spm, and Spd enhanced leaf RWC by an average of ~25% compared with the untreated plants at FC₁₀₀ (Figures 5-A and 2-

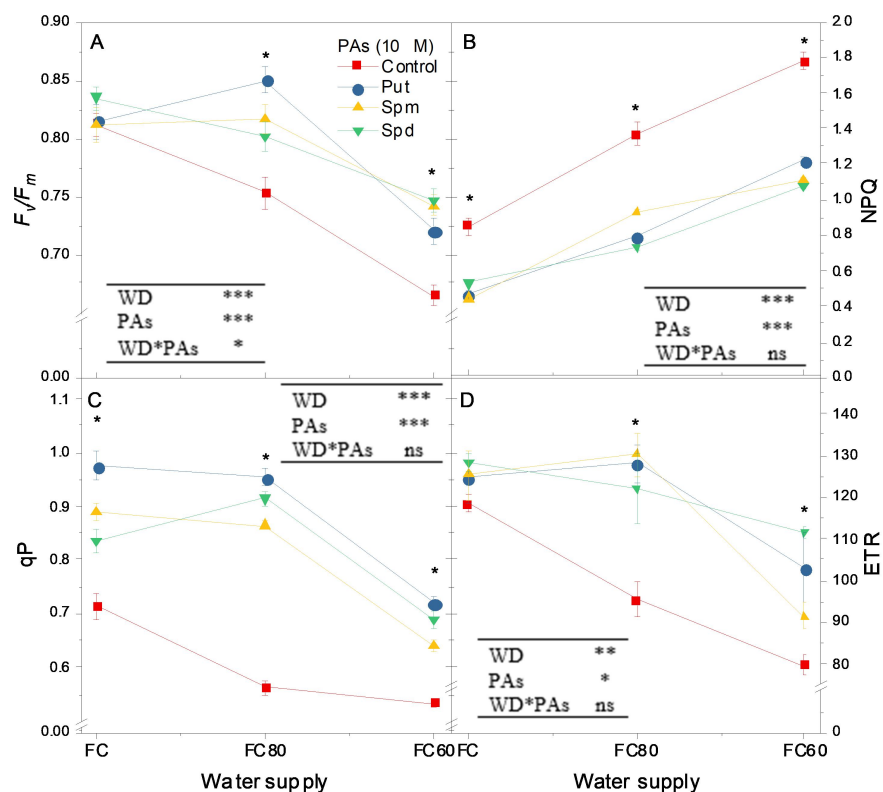


Figure 4. Changes in (A) Maximum photochemical quantum yield of PSII, (B) Nonphotochemical quenching, (C) Photochemical quenching, and (D) Linear electron transport rate of German chamomile leaves exposed to water deficit and foliar application of polyamines. FC: Field Capacity, FC₈₀: 80% of FC, FC₆₀: 60% of FC. WD and other symbols as under Figure 1.

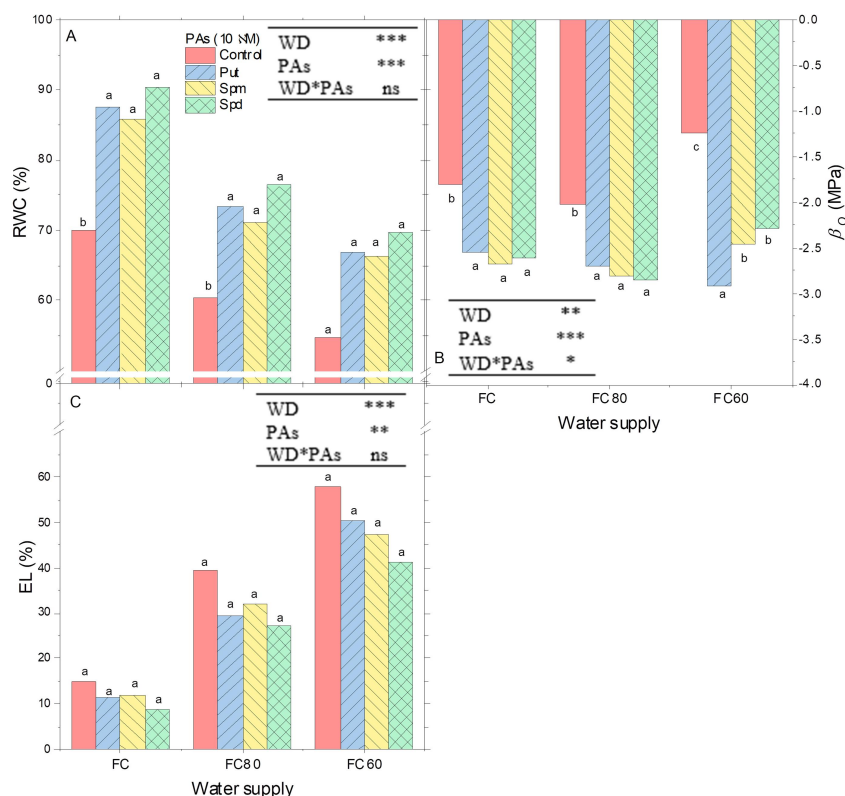


Figure 5. Changes in (A) Relative water content, (B) Osmotic potential, and (C) Electrolyte leakage of German chamomile leaves exposed to water deficit and foliar application of polyamines. WD and other symbols as under Figure 1.

A). Water deficit at the level of FC₆₀ reduced ψ_o by 45% compared with FC₁₀₀ (Figure 5-B). In contrast, leaf ψ_o was enhanced in the PAs-treated plants. Put application increased leaf ψ_o by 35% compared with the untreated plants at FC₆₀ (Figure 5-B). Leaf EL was increased by 1.6 and 2.8 times at FC₈₀ and FC₆₀, respectively, compared with FC₁₀₀ (Figure 5-C). However, Spm and Spd decreased leaf EL by 17 and 28%, respectively, compared with untreated plants at FC₆₀ (Figure 5-C).

Leaf Photosynthetic Pigment

A significant decrease was observed in leaf photosynthetic pigments content exposed to water deficit (Table 1). However, Spd increased leaf Chl_t by 51, 60, and 79%, respectively, compared with the untreated plants at FC₁₀₀, FC₈₀, and FC₆₀ (Table 1).

The highest Chl a:b was observed in the put-treated plants at FC₈₀; 32, 13, and 18% higher than the untreated, Spm-, and Spd-treated plants, respectively. Water deficit increased leaf carotenoid content on average by ~18% compared with FC₁₀₀ (Figure 2-B). The highest leaf carotenoid content was observed in Spd-treated plants at all water-deficit levels (Table 1).

Enzymatic Antioxidant

Leaf enzymatic antioxidant activity was significantly influenced by the water deficit, foliar application of PAs, and their interaction (Figure 6-A). Water deficit and PAs increased leaf antioxidant activity. Spd-treated plants showed the highest CAT at FC₈₀ and FC₆₀ than the untreated plants. Spd increased CAT by 82 and 100% compared with the untreated plants at FC₈₀ and FC₁₀₀,

Table 1. Effect of water deficit and foliar application of polyamines on photosynthetic pigments of German chamomile leaves.^a

Water deficit	PAs (10 µM)	mg g ⁻¹ FW				Chlorophyll a:b
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	
FC	Control	0.71	0.31	1.03	0.64	2.41
	Put	0.93	0.36	1.29	0.75	2.58
	Spm	0.98	0.39	1.38	0.78	2.50
	Spd	1.09	0.46	1.55	0.82	2.39
LSD		0.46	0.23	0.46	0.23	1.32
FC ₈₀	Control	0.53	0.26	0.79	0.75	2.02
	Put	0.83	0.27	1.11	0.86	3.38
	Spm	0.72	0.29	1.01	0.89	2.58
	Spd	0.90	0.37	1.27	0.96	2.42
LSD		0.41	0.21	0.54	0.46	2.21
FC ₆₀	Control	0.33	0.20	0.54	0.76	1.64
	Put	0.54	0.25	0.78	0.80	2.07
	Spm	0.58	0.27	0.84	0.88	2.14
	Spd	0.66	0.30	0.96	0.99	2.25
LSD		0.35	0.15	0.43	0.51	1.29
Water deficit		***	***	***	ns	ns
Polyamines		***	**	***	*	ns
WD×PAs		ns	ns	ns	ns	ns

^a FC: Field Capacity, FC₈₀: 80% of FC, FC₆₀: 60% of FC, PAs: Polyamines, Put: Putrescine, Spm: Spermine, Spd: Spermidine, and WD: Water Deficit. *, **, ***, and ns: Significant at P≤0.05, P≤ 0.01, P≤ 0.001, and non-significant. Means with the same letters are not significantly different (LSD P≤ 0.05).

respectively (Figure 6-A). Leaf POD activity showed an increasing trend by increasing the water deficit intensity and PAs application. At FC₈₀ and FC₆₀, Spd- and Spm-treated plants showed the greatest POD, respectively, which were nearly double the untreated plants at the respective water deficit level. At FC₈₀, the highest APX activity was recorded in Spm- followed by Spd-treated plants by an average of ~35% over the untreated plants (Figures 6-C and 2-B). Leaf SOD activity showed a similar trend as POD. The highest SOD activity was recorded in Spd- and Spm-treated plants at FC₈₀ and FC₆₀ by 42 and 36%, respectively, over the untreated plants at their respective water deficit levels (Figure 6-D).

Leaf MDA and H₂O₂ Content

Water deficit increased leaf MDA content: water deficit at FC₈₀ and FC₆₀ increased leaf

MDA content by 85% and 1.6 times, respectively, compared with FC₁₀₀ (Figure 6-E). Although MDA of PAs-treated plant also increased at FC₈₀, it remained almost unaltered at FC₆₀ compared with FC₈₀. Leaf MDA content of Put, Spm, and Spd-treated plants were 38, 34, and 31%, respectively, lower than the untreated plants (Figure 6-E). Leaf H₂O₂ content was almost doubled at FC₆₀ compared with FC₁₀₀. However, PAs treatments reduced leaf H₂O₂ content compared with the untreated plants (Figure 6-F). Spd application reduced leaf H₂O₂ by 18 and 10% compared with the untreated plants at FC₈₀ and FC₆₀, respectively.

DISCUSSION

Abiotic stresses such as drought, cold, and K deficiency stresses simultaneously stimulate Absciscic Acid (ABA) and PAs biosynthesis (Li *et al.*, 2021; Réthoré *et al.*,

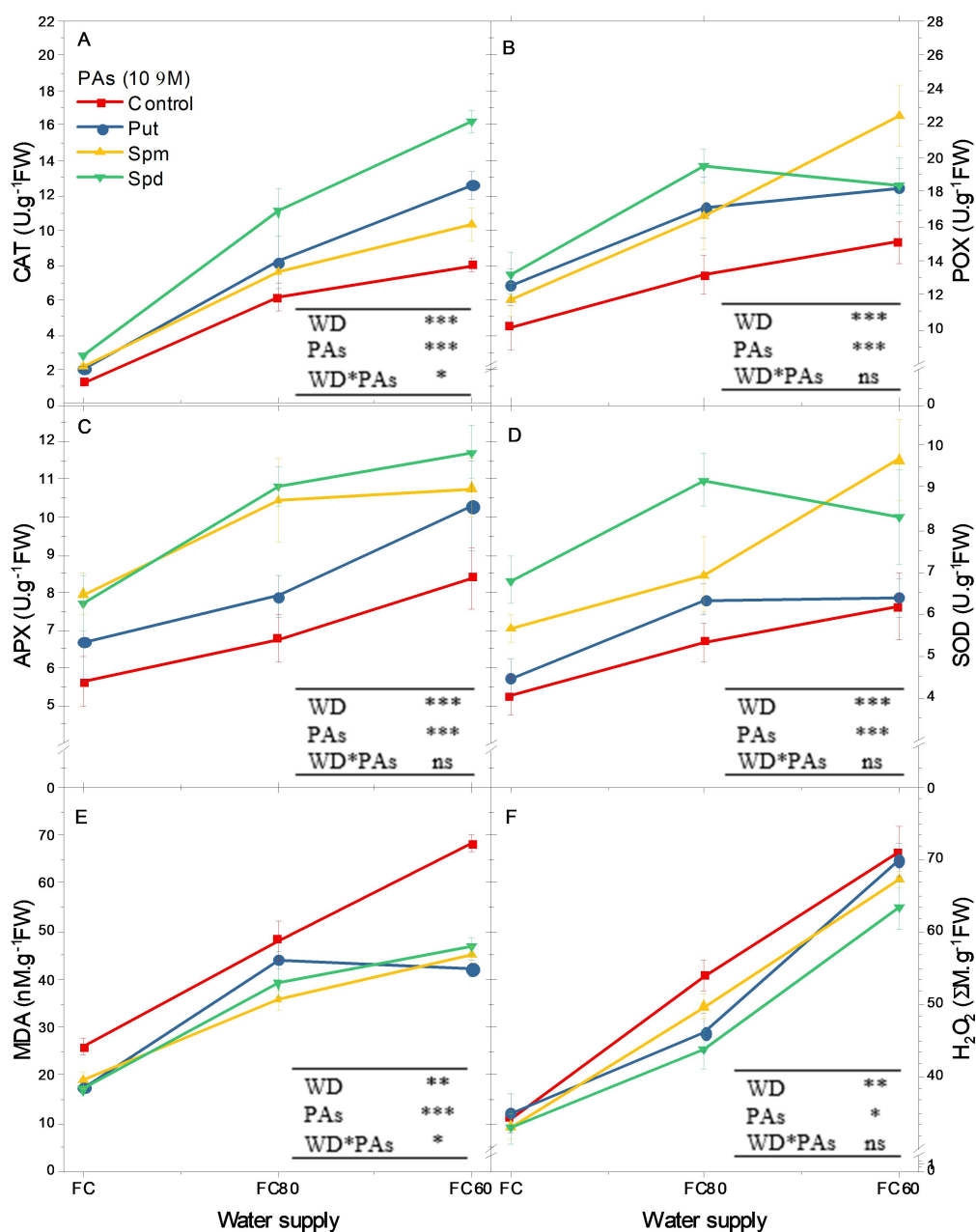


Figure 6. Changes in the activity of (A) Catalase, (B) Peroxidase, (C) Ascorbate peroxidase, (D) Superoxide dismutase, the content of (E) Malondialdehyde, and (F) Hydrogen peroxide German chamomile leaves exposed to water deficit and foliar application of polyamines. FC: Field Capacity, FC₈₀: 80% of FC, FC₆₀: 60% of FC. WD and other symbols as under Figure 1.

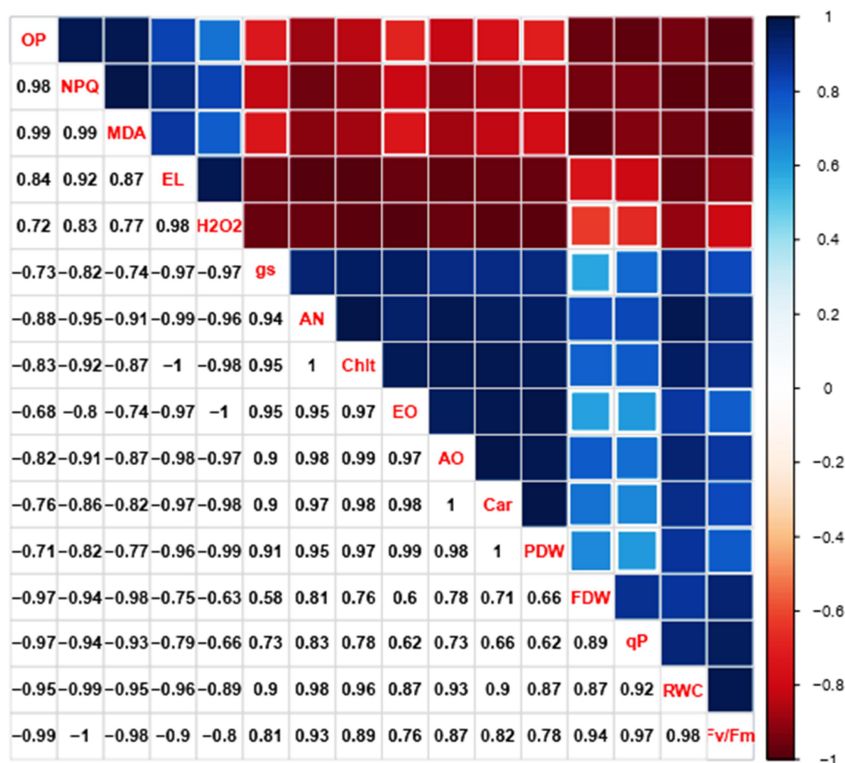


Figure 7. Corplot analysis of OP: Leaf Osmotic Potential, NPQ: Non-Photochemical Quenching, MDA: Malondialdehyde, EL: Electrolyte Leakage, H2O2: Hydrogen peroxide, gs: Stomatal conductance, AN: Net photosynthetic rate, Chlt: Total Chlorophyll, EO: Essential Oil, AO: Antioxidant enzymes, Car: Carotenoids, qP: Photochemical quenching, RWC: Relative Water Contents, F_v/F_m : Photochemical quantum yield of PSII german chamomile plants under water deficit and polyamines foliar application.

2021; Zhu *et al.*, 2020). It has been supposed that PAs and ABA either alone or synergically induce stomatal closure to increase plant tolerance during stressful conditions (Gong *et al.*, 2021). However, most recent findings revealed that the ABA-induced stomatal closure is directly inhibited by PAs in *Vicia faba*, and exogenous applications of PAs could reopen stomata even if they were partially closed by ABA treatment (Liu *et al.*, 2023). We observed that exogenously sprayed PAs stimulated leaf g_s of the water deficit-stressed chamomile plants, resulting in the improved leaf A_N , T_r , F_v/F_m , ETR, and qP , and decreased NPQ, which may indicate the alleviating role of PAs on drought-induced stomatal closure. Spd or Spm increased g_s and photosynthesis of Chinese dwarf cherry but did not affect the F_v/F_m under drought stress (Yin *et al.*, 2014). Those observations indicate that PAs

can enhance photosynthesis by inhibiting stomatal closure without affecting the stability of the photosynthetic system.

PAs are involved in plant protection against different environmental stresses (Baghalian *et al.*, 2011; Farooq *et al.*, 2009). PAs with acid-neutralizing, antioxidative, and membrane-stabilizing properties positively influence photosynthetic efficiency under stressful conditions (Mapelli *et al.*, 2008). Exogenously application of Put increased the net photosynthetic rate of basil (*Ocimum basilicum* L.) plants under drought stress, while electrolyte leakage was reduced (Darabi *et al.*, 2020). PAs with high net positive charges can stabilize PSII proteins such as D_1 and D_2 and, by binding to membrane proteins, can stabilize the structure of the proteins during stress (Hamdani *et al.*, 2011).



The reductions in F_v/F_m and qP were correlated with an increase in NPQ (Figure 7). A decline in F_v/F_m indicates photoinhibition damage resulting from the incident PPFD when plants are exposed to environmental stresses (Wang *et al.*, 2018). We found that water-deficit-induced NPQ was ameliorated by the PAs, meanwhile, leaf qP of PAs-treated plants was improved under water deficit conditions. Sang *et al.* (2016) found that the NPQ of water-stressed cucumber (*Cucumis sativus*) leaves treated with Spd was enhanced, indicating that Spd can accelerate the dissipation of absorbed light under drought conditions. Water deficit leads to a decrease in the ETR and the generation of excess excitation energy (Tezara *et al.*, 2005). The ETR of PAs-treated plants was higher than the untreated plants under water deficit, indicating that the PSII reaction center of PAs-treated leaves maintained high activity. The negative charges of LHCII (Light harvesting complexes of photosystem II) can be neutralized with the PA's positive charges, resulting in the LHCII complexes quenching by minimizing the exertion between the complexes (Hamdani *et al.*, 2011).

Exogenously applied PAs significantly increased leaf enzymatic antioxidant activities. Enzymatic antioxidant activity and higher ROS scavenging ability were enhanced by exogenously applied Spd in cucumber roots under stressful conditions (Wu *et al.*, 2018). In a study on tomato plants under heat stress, exogenous application of Spd regulated various signal transduction factors mainly associated with genes related to stress signaling pathways such as hormonal and sugar metabolisms (Cheng *et al.*, 2012). To decrease the ROS content during stressful conditions, the concentration of several nonenzymatic antioxidants, such as ASA and lycopene, as well as enzymatic antioxidants including SOD, POD, APX, and CAT activity can be enhanced by PAs (Hasan *et al.*, 2021). ABA or Spd treatment alone decreased the activities of both POD and CAT, which might be due to their contribution to the

ROS accumulation induced by ABA or Spd. However, Spd could activate the antioxidant enzymes to scavenge H_2O_2 induced by ABA in the presence of ABA (Liu *et al.*, 2023). Our results also showed that, when plants were fully irrigated, the increase in the antioxidant enzymes activities by Pas was not as high as the water-stressed plants, which might be due to their lower ABA content. However, under stressful conditions, higher ABA content probably activates the antioxidant enzymes to scavenge higher H_2O_2 . Alcázar *et al.* (2006) also found that Put accumulation was mainly an ABA-dependent metabolic response during drought.

PAs are regulators of redox homeostasis that play a dual role in plant oxidative stress (Saha *et al.*, 2015). Although PAs might be responsible for cellular breakdown due to generating the strong oxidizers H_2O_2 under stressful conditions, H_2O_2 can act as a signaling molecule involved in stress signal transduction (Groppa and Benavides, 2008). The H_2O_2 -mediated signaling pathway, which is involved in salt stress-responsive genes (SIWRKY1, SIHKT1, SIDREB2, and SIMYB102), was induced by exogenous Spd in tomato plants and significantly reduced the adverse effects of salt stress (Raziq *et al.*, 2022). Working on *Salvia officinalis* L. under drought stress revealed that the enzymatic activities of APX and CAT were strongly coordinated (Mohammadi-Cheraghabadi *et al.*, 2021).

PAs foliar application alleviated the ROS-induced membrane damage and reduced leaf MDA, EL, and H_2O_2 . MDA and H_2O_2 accumulation can be indicators of cell damage. A lower leaf MDA and H_2O_2 content led to lower membrane damage and leaf EL. PAs can accelerate the antioxidant enzyme activities to protect plants against the oxidative damages and membrane injury or may enhance the biosynthesis of protective substances under stressful conditions. Tomato (*Solanum lycopersicum*) leaf MDA content and EL were decreased by exogenous Spd under drought stress (Sang *et al.*, 2016). Besides their properties

as free radical scavengers, PAs also stabilize biological membranes by binding to membrane phospholipids under stressful conditions (Pál *et al.*, 2015). Moradi Peynevandi *et al.* (2018) also observed that exogenously applied PAs significantly decreased leaf H₂O₂ and MDA contents and enhanced the membrane stability of cold-stressed stevia (*Stevia rebaudiana* Bertoni) plants.

Leaf Chla was decreased greater than Chlb content under water deficit conditions. This might be due to a greater susceptibility of Chla than Chlb to stress or the generation of Chlb through the degradation of Chla products (Sen *et al.*, 2014; Shahba *et al.*, 2010). This eventually led to a decrease in Chla:b. It has been reported that PAs could protect the functional and structural integrity of chloroplasts and slow down the rate of photosynthetic pigment degradation (Li *et al.*, 2014; Nahar *et al.*, 2015). In our experiment, Spd foliar application increased the carotenoid content of leaves. Carotenoids are also among the essential compounds playing a role in protecting photosynthesis and stress-signaling pathways. Leaf carotenoid content was positively correlated with leaf A_N, chlorophyll fluorescence, and antioxidative enzyme activities (Figure 7). Due to an increase in leaf carotenoid content under PAs application, it seems that carotenoids are likely to prevent chlorophyll degradation due to their protective role (Dhar *et al.*, 2020).

CONCLUSIONS

Our results revealed that exogenously applied PAs improved the drought tolerance of chamomile plants in multiple ways. PAs improved leaf water status and alleviated oxidative damage on the biological membranes by instigating leaf antioxidant content and reducing membrane damage. The results here indicated that exogenously applied PAs act as regulators to prevent chlorophyll degradation, protect the

photosynthetic antenna and PSII structure, and improve the photosynthetic efficiency of chamomile plants. Leaf antioxidant activities were found to be determining and effective mechanisms to alleviate water deficit effects on chamomile plants, induced by PAs. PAs inhibit water deficit-induced stomatal closure through antioxidant enzyme-dependent H₂O₂ elimination. PAs can potentially improve water deficit tolerance in chamomile. Furthermore, Spd showed the most effective impact in alleviating the water deficit effects.

ACKNOWLEDGEMENTS

The research leading to these results received funding from the Vice President for Research and Technology of Ferdowsi University of Mashhad under Grant Agreement No N.1/53684.

REFERENCES

1. Alcázar, R., Bueno, M. and Tiburcio, A. F. 2020. Polyamines: Small Amines with Large Effects on Plant Abiotic Stress Tolerance. *Cells*, **9**(11): 1-20.
2. Alcázar, R., Cuevas, J. C., Patron, M., Altabella, T. and Tiburcio, A. F. 2006. Absciscic Acid Modulates Polyamine Metabolism under Water Stress in *Arabidopsis thaliana*. *Physiol. Plant*, **128**(3): 448-455.
3. Ali, R., Abbas, H. and Kamal, R. 2009. The Effects of Treatment with Polyamines on Dry Matter and Some Metabolites in Salinity-Stressed Chamomile and Sweet Majoram Seedlings. *Plant Soil Environ.*, **55**(11): 477-483.
4. Apel, K. and Hirt, H. 2004. Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. *Ann. Rev. Plant. Biol.*, **55**: 373-399.
5. Baghalian, K., Abdoshah, S., Khalighi-Sigaroodi, F. and Paknejad, F. 2011. Physiological and Phytochemical Response to Drought Stress of German Chamomile (*Matricaria recutita* L.). *Plant Physiol. Biochem.*, **49**(2): 201-207.



6. Cakmak, I. and Horst, W. J. 1991. Effect of Aluminium on Lipid Peroxidation, Superoxide Dismutase, Catalase, and Peroxidase Activities in Root Tips of Soybean (*Glycine max*). *Physiol. Plant.*, **83**(3): 463-468.
7. Cheng, L., Sun, R. -R., Wang, F. -Y., Peng, Z., Kong, F. -L., Wu, J., Cao, J. -S. and Lu, G. 2012. Spermidine Affects the Transcriptome Responses to High Temperature Stress in Ripening Tomato Fruit. *J. Zhejiang Univ. Sci. B.*, **13**(4): 283-297.
8. Darabi, F., Abbasi, N. and Zarea, M. J. 2020. Evaluation of the Effect of Putrescine and Brassinosteroid on Induction of Drought Tolerance and Physiological Changes in Basil Plant (*Ocimum basilicum* L.). *Environ. Str. Crop Sci.*, **13**(4): 1183-1202.
9. Das, M., Mallavarapu, G. and Kumar, S. 1998. Chamomile (*Chamomilla recutita*): Economic Botany, Biology, Chemistry, Domestication and Cultivation. *J. Med. Arom. Plant. Sci.*, **20**: 1074-1109.
10. Dhar, M. K., Mishra, S., Bhat, A., Chib, S. and Kaul, S. 2020. Plant Carotenoid Cleavage Oxygenases: Structure-Function Relationships and Role in Development and Metabolism. *Brief. Func. Gen.*, **19**(1): 1-9.
11. Farooq, M., Wahid, A. and Lee, D. -J. 2009. Exogenously Applied Polyamines Increase Drought Tolerance of Rice by Improving Leaf Water Status, Photosynthesis and Membrane Properties. *Acta Physiol. Plant.*, **31**(5): 937-945.
12. Fischer, R., Rees, D., Sayre, K., Lu, Z. M., Condon, A. and Saavedra, A. L. 1998. Wheat Yield Progress Associated with Higher Stomatal Conductance and Photosynthetic Rate, and Cooler Canopies. *Crop Sci.*, **38**(6): 1467-1475.
13. Flexas, J. and Medrano, H. 2002. Drought-Inhibition of Photosynthesis in C₃ Plants: Stomatal and Non-Stomatal Limitations Revisited. *Ann. Bot.*, **89**(2): 183-189.
14. Genty, B., Briantais, J. -M. and Baker, N. R. 1989. The Relationship between the Quantum Yield of Photosynthetic Electron Transport and Quenching of Chlorophyll Fluorescence. *Biochim. Biophysic. Acta (BBA)-Gen. Sub.*, **990**(1): 87-92.
15. Ghanati, F., Morita, A. and Yokota, H. 2002. Induction of Suberin and Increase of Lignin Content by Excess Boron in Tobacco Cells. *Soil Sci. Plant Nut.*, **48**(3): 357-364.
16. Giannopolitis, C. N. and Ries, S. K. 1977. Superoxide Dismutases: I. Occurrence in Higher Plants. *Plant Physiol.*, **59**(2): 309-314.
17. Gong, L., Liu, X. -D., Zeng, Y. -Y., Tian, X. -Q., Li, Y. -L., Turner, N. C. and Fang, X. -W. 2021. Stomatal Morphology and Physiology Explain Varied Sensitivity to Absciscic Acid across Vascular Plant Lineages. *Plant Physiol.*, **186**(1): 782-797.
18. Groppa, M. and Benavides, M. 2008. Polyamines and Abiotic Stress: Recent Advances. *Amino Acid*, **34**(1): 35-45.
19. Hafezi Ghehestani, M. M., Azari, A., Rahimi, A., Maddah-Hosseini, S. and Ahmadi-Lahijani, M. J. 2021. Bacterial Siderophore Improves Nutrient Uptake, Leaf Physiochemical Characteristics, and Grain Yield of Cumin (*Cuminum cyminum* L.) Ecotypes. *J. Plant Nutr.*, **44**(12): 1794-1806.
20. Hamdani, S., Yaakoubi, H. and Carpentier, R. 2011. Polyamines Interaction with Thylakoid Proteins during Stress. *J. Photochem. Photobiol. B: Biol.*, **104**(1-2): 314-319.
21. Hasan, M. M., Alharbi, B. M., Alhaithloul, H. A. S., Abdulmajeed, A. M., Alghanem, S. M., Al-Mushhin, A. A., Jahan, M. S., Corpas, F. J., Fang, X. -W. and Soliman, M. H. 2021. Spermine-Mediated Tolerance to Selenium Toxicity in Wheat (*Triticum aestivum* L.) Depends on Endogenous Nitric Oxide Synthesis. *Antioxidants*, **10**(11): 1-22.
22. Hassan, F., Ali, E. and Alamer, K. 2018. Exogenous Application of Polyamines Alleviates Water Stress-Induced Oxidative Stress of *Rosa damascena* Miller var. trigintipetala Dieck. *South Afr. J. Bot.*, **116**: 96-102.
23. Jiang, M. and Zhang, J. 2001. Effect of Absciscic Acid on Active Oxygen Species, Antioxidative Defence System and Oxidative Damage in Leaves of Maize Seedlings. *Plant Cell Physiol.*, **42**(11): 1265-1273.
24. Li, M., Wang, C., Shi, J., Zhang, Y., Liu, T. and Qi, H. 2021. Absciscic Acid and Putrescine Synergistically Regulate the Cold Tolerance of Melon Seedlings. *Plant Physiol. Biochem.*, **166**: 1054-1064.

25. Li, X., Gong, B. and Xu, K. 2014. Interaction of Nitric Oxide and Polyamines Involves Antioxidants and Physiological Strategies against Chilling-Induced Oxidative Damage in *Zingiber officinale* Roscoe. *Sci. Hortic.*, **170**: 237-248.
26. Lichtenthaler, H. K. and Wellburn, A. R. 1983. Determinations of Total Carotenoids and Chlorophylls a and b of Leaf Extracts in Different Solvents. *Biochem. Soc. Trans.*, **11**: 591-592.
27. Liu, X. D., Zeng, Y. Y., Zhang, X. Y., Tian, X. Q., Hasan, M. M., Yao, G. Q. and Fang, X. W. 2023. Polyamines Inhibit Absciscic Acid-Induced Stomatal Closure by Scavenging Hydrogen Peroxide. *Physiol. Plant.*, **175**(2): e13903.
28. Lutts, S., Benincasa, P., Wojtyla, L., Kubala, S., Pace, R., Lechowska, K., Quinet, M. and Garnczarska, M. 2016. Seed Priming: New Comprehensive Approaches for an Old Empirical Technique. In: "*New Challenges in Seed Biology - Basic and Translational Research Driving Seed Technology*", (Eds.): Araujo, S. and Balestrazzi, A. Published by InTech, PP. 1-46
29. Mapelli, S., Brambilla, I., Radyukina, N., Ivanov, Y. V., Kartashov, A., Reggiani, R. and Kuznetsov, V. V. 2008. Free and Bound Polyamines Changes in Different Plants as a Consequence of UV-B Light Irradiation. *Gen. App. Plant Physiol.*, **34**(1-2): 55-66.
30. Mohammadi-Cheraghabadi, M., Modarres-Sanavy, S. A. M., Sefidkon, F., Rashidi-Monfared, S. and Mokhtassi-Bidgoli, A. 2021. Improving Water Deficit Tolerance of *Salvia officinalis* L. Using Putrescine. *Sci. Rep.*, **11**(1): 1-15.
31. Moradi Peynevandi, K., Razavi, S. M. and Zahri, S. 2018. The Ameliorating Effects of Polyamine Supplement on Physiological and Biochemical Parameters of *Stevia rebaudiana* Bertoni under Cold Stress. *Plant Prod. Sci.*, **21**(2): 123-131.
32. Murchie, E. H. and Lawson, T. 2013. Chlorophyll Fluorescence Analysis: A Guide to Good Practice and Understanding Some New Applications. *J. Exp. Bot.*, **64**(13): 3983-3998.
33. Nahar, K., Hasanuzzaman, M., Alam, M. and Fujita, M. 2015. Exogenous Spermidine Alleviates Low Temperature Injury in Mung Bean (*Vigna radiata* L.) Seedlings by Modulating Ascorbate-Glutathione and Glyoxalase Pathway. *Int. J. Mol. Sci.*, **16**(12): 30117-30132.
34. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.*, **22**(5): 867-880.
35. Pál, M., Szalai, G. and Janda, T. 2015. Speculation: Polyamines Are Important in Abiotic Stress Signaling. *Plant Sci.*, **237**: 16-23.
36. Raziq, A., Din, A. M. U., Anwar, S., Wang, Y., Jahan, M. S., He, M., Ling, C. G., Sun, J., Shu, S. and Guo, S. 2022. Exogenous Spermidine Modulates Polyamine Metabolism and Improves Stress Responsive Mechanisms to Protect Tomato Seedlings against Salt Stress. *Plant Physiol. Biochem.*, **187**: 1-10.
37. Réthoré, E., Jing, L., Ali, N., Yvin, J.-C., Pluchon, S. and Hosseini, S. A. 2021. K Deprivation Modulates the Primary Metabolites and Increases Putrescine Concentration in *Brassica napus*. *Front. Plant Sci.*, **12**: 681895.
38. Saha, J., Brauer, E. K., Sengupta, A., Popescu, S. C., Gupta, K. and Gupta, B. 2015. Polyamines as Redox Homeostasis Regulators during Salt Stress in Plants. *Front. Environ. Sci.*, **3**: 1-13.
39. Sang, Q., Shu, S., Shan, X., Guo, S. and Sun, J. 2016. Effects of Exogenous Spermidine on Antioxidant System of Tomato Seedlings Exposed to High Temperature Stress. *Russ J Plant Physiol.*, **63**(5): 645-655.
40. Sang, T., Shan, X., Li, B., Shu, S., Sun, J. and Guo, S. 2016. Comparative Proteomic Analysis Reveals the Positive Effect of Exogenous Spermidine on Photosynthesis and Salinity tolerance in Cucumber Seedlings. *Plant Cell Rep.*, **35**(8): 1769-1782.
41. Sen, G., Eryilmaz, I. E. and Ozakca, D. 2014. The Effect of Aluminium-Stress and Exogenous Spermidine on Chlorophyll Degradation, Glutathione Reductase Activity and the Photosystem II D₁ Protein Gene (psbA) Transcript Level in Lichen *Xanthoria parietina*. *Phytochem.*, **98**: 54-59.
42. Sergiev, I., Alexieva, V. and Karanov, E. 1997. Effect of Spermine, Atrazine and Combination between Them on Some



- Endogenous Protective Systems and Stress Markers in Plants. *Compt. Rend. Acad. Bulg. Sci.*, **51(3)**: 121-124.
43. Shahba, Z., Baghizadeh, A. and Yosefi, M. 2010. The Salicylic Acid Effect on the Tomato (*Lycopersicum esculentum* Mill.) Germination, Growth and Photosynthetic Pigment under Salinity Stress (NaCl). *J. Stress Physiol. Biochem.*, **6(3)**: 4-16.
44. Smart, R. E. and Bingham, G. E. 1974. Rapid Estimates of Relative Water Content. *Plant Physiol.*, **53(2)**: 258-260.
45. Tezara, W., Marín, O., Rengifo, E., Martínez, D. and Herrera, A. 2005. Photosynthesis and Photoinhibition in Two Xerophytic Shrubs during Drought. *Photosynthetica*, **43(1)**: 37-45.
46. Tezara, W., Mitchell, V., Driscoll, S. and Lawlor, D. 1999. Water Stress Inhibits Plant Photosynthesis by Decreasing Coupling Factor and ATP. *Nat.*, **401(6756)**: 914-917.
47. Topp, G. and Davis, J. 1985. Time-Domain Reflectometry (TDR) and Its Application to Irrigation Scheduling. *Adv. Irrig.*, **3**: 107-127.
48. Van Kooten, O. and Snel, J. F. 1990. The Use of Chlorophyll Fluorescence Nomenclature in Plant Stress Physiology. *Photosynth. Res.*, **25(3)**: 147-150.
49. Wang, Z., Li, G., Sun, H., Ma, L., Guo, Y., Zhao, Z., Gao, H. and Mei, L. 2018. Effects of Drought Stress on Photosynthesis and Photosynthetic Electron Transport Chain in Young Apple Tree Leaves. *Biol. Open*, **7(11)**: 1-9.
50. Wu, J., Shu, S., Li, C., Sun, J. and Guo, S. 2018. Spermidine-Mediated Hydrogen Peroxide Signaling Enhances the Antioxidant Capacity of Salt-Stressed Cucumber Roots. *Plant Physiol. Biochem.*, **128**: 152-162.
51. Yamaguchi, K., Mori, H. and Nishimura, M. 1995. A Novel Isoenzyme of Ascorbate Peroxidase Localized on Glyoxysomal and Leaf Peroxisomal Membranes in Pumpkin. *Plant Cell Physiol.*, **36(6)**: 1157-1162.
52. Yin, Z. P., Li, S., Ren, J. and Song, X. S. 2014. Role of Spermidine and Spermine in Alleviation of Drought-Induced Oxidative Stress and Photosynthetic Inhibition in Chinese Dwarf Cherry (*Cerasus humilis*) Seedlings. *Plant Growth Regul.*, **74**: 209-218.
53. Zhu, M. -D., Zhang, M., Gao, D. -J., Zhou, K., Tang, S.-J., Zhou, B. and Lv, Y. -M. 2020. Rice OsHSFA3 Gene Improves Drought Tolerance by Modulating Polyamine Biosynthesis Depending on Absciscic Acid and ROS Levels. *Int. J. Mol. Sci.*, **21(5)**: 1-16.

کاهش اثرات تنش خشکی در بابونه آلمانی (*Matricaria chamomilla* L.) در پاسخ به تنش اکسیداتیو و بسته شدن روزنه ناشی از کمبود آب توسط کاربرد پلی آمین ها

محمد جواد احمدی لاهیجانی، جعفر نباتی، سعید موری، و محمد کافی

چکیده

پلی آمین ها مولکول های پیامرسانی هستند که نقش های امیدوارکننده ای در بهبود تحمل به تنش در گیاهان نشان داده اند. اطلاعات محدودی در مورد اثرات کاربرد خارجی پلی آمین ها بر روی گیاهان دارویی از جمله بابونه در دسترس است. این آزمایش به منظور بررسی اثرات محلول پاشی پلی آمین ها [پوترسین (Put)، اسپرمیدین (Spd) و اسپرمین (Spm)] بر فرآیندهای فیزیولوژیکی و بیوشیمیایی برای درک مکانیسم های احتمالی مربوط به کاهش اثرات تنش کمبود آب [ظرفیت مزرعه (FC) به عنوان شاهد، ۸۰ درصد ظرفیت

مزرعه (FC80) و ۶۰ درصد ظرفیت مزرعه (FC60) [در بابونه آلمانی انجام شد. نتایج نشان داد که پلی آمین ها تا حدی بسته شدن روزه ناشی از کمبود آب را مهار می کند و آنزیم های آنتی اکسیدانی را برای از بین بردن افزایش پراکسید هیدروژن القا می کند. اسپرمیدین هدایت روزه های را در FC، FC80 و FC60 به ترتیب ۶۶، ۳۵ و ۶۵ درصد در مقایسه با شاهد افزایش داد. افزایش هدایت روزه های فتوسنتز خالص برگ را در FC80 و FC60 به ترتیب ۵۲ و ۸۶ درصد در مقایسه با شاهد افزایش داد. نقش پلی آمین ها در کاهش تنش اکسیداتیو با همبستگی منفی فعالیت های آنتی اکسیدانی برگ و محتوای مالون دی آلدئید و پراکسید هیدروژن تایید شد. با توجه به نتایج، پلی آمین ها به عنوان ترکیبات محافظ تنش عمل می کنند تا آنزیم های آنتی اکسیدانی را برای حذف پراکسید هیدروژن ناشی از تنش، بهبود پایداری غشاء و افزایش تحمل کمبود آب تحریک کنند. به-طور کلی، نتایج نشان داد که پلی آمین ها می توانند تنظیم کننده های بالقوه رشد برای کاهش تنش کم آبی خفیف تا شدید باشند.