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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

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Running head: Polyamines alleviates drought stress in German Chamomile

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 (FC_{80}), and 60% of FC (FC_{60})] alleviation in German Chamomile. We found that PAs partially inhibited water deficit-induced stomatal closure and induced antioxidant enzymes to eliminate the increased H_2O_2. Spd increased stomatal conductance (g_s) by 66, 65, and 35% at FC, FC_{80}, and FC_{60}, respectively, compared with the control. The increased g_s enhanced leaf net photosynthesis (A_N) by 52 and 86% at FC_{80} and FC_{60}, 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 H_2O_2 content. According to the results, PAs function as stress-protecting compounds to instigate the antioxidative enzymes to scavenge stress-induced H_2O_2, 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 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. 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 µmol.m^{-1}.s^{-1} PPFD), photoperiod of 13/11 h day/night, and relative humidity of 50±10%.

Soil preparation and treatments

Irrigation was applied until the full establishment (4-leaf), and 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 µM (Farooq *et al.*, 2009; Ali *et al.*, 2009). 10 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 h 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 µmol.m^{-2}.s^{-1}, 25±2 ºC, 50±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 h. After 30 min 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 then 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 Eq. 1 (Lutts *et al.*, 2016):

$$EL\ (\%) = \frac{EC1}{EC2} \times 100 \qquad (1)$$

Here, EC_1 and EC_2 are the EC of the solution after 24 h and the autoclaved (120 °C for 20 min) samples, respectively.

Relative water content (RWC)

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

$$RWC\ (\%) = \left[\frac{Wf - Wd}{Wt - Wd}\right] \times 100 \qquad (2)$$

Here, W_f, W_t, and W_d are fresh weight, turgid weight, and oven-dried weight (at 70 ºC 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):

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

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 h. Leaf pigments content (Chlorophylls a, b, and carotenoids) were determined spectrophotometrically (U-2000, Hitachi Instruments, Tokyo, Japan) according to Lichthentaler and Wellburn (1983).

Leaf antioxidant enzymes

100 mg leaf fresh weight 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 H_2O_2 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 H_2O_2 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 12000×*g* for 15 min and the supernatant absorbance was read at 390 nm. The content of H_2O_2 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 $p \leq 0.05$ 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 FC_{60} diminished the untreated plant A_N by 40% compared with FC_{100} (Fig. 1A). PAs application increased A_N by ~60% compared with the untreated plants under FC_{100} (Fig. 2A). However, under FC_{80} and FC_{60}, Spd showed the greatest improving effect on A_N; Spd application enhanced leaf A_N by 52 and 86% at FC_{80} and FC_{60}, respectively, compared with the untreated plants (Fig. 1A). Chamomile leaf T_r reduced by 1.1 and 1.6 times, respectively, at FC_{80} and FC_{60} compared with FC_{100} (Fig. 1B). 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 (Fig. 1B).

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 (Fig. 1C). The lowest leaf g_m was observed when Spd and Spm were applied, respectively, at FC_{100} and FC_{80} (Fig. 1D). Although water deficit decreased C_i and C_i:C_a, PAs significantly increased Ci and C_i:C_a compared with the untreated plants (Fig. 3A 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} (Fig. 3C). Spm-treated plants showed 43% higher WUE_i at FC_{100} compared with the untreated plants; however, WUE_i reduced when PAs were applied under water deficit

conditions. A_N/g_s decreased by reducing 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 (Fig. 3D).

Leaf chlorophyll fluorescence (Chf)

Water deficit at the level of FC_{60} decreased F_v/F_m by 22% compared with FC_{100} (Fig. 4A). At FC_{80}, 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 (Fig. 4B). Spd-treated plants showed 85 and 65% lower leaf NPQ than the untreated plants, respectively, at FC_{80} and FC_{60}. Photochemical quenching (*qP*) reduced by 26 and 33% at FC_{80} and FC_{60}, respectively, compared with FC_{100} (Fig. 4C). The highest *qP* was observed in the Put-treated plants; 36, 69, and 34% higher than the untreated plants, respectively, at FC_{100}, FC_{80}, and FC_{60}. The linear electron transport rate, ETR, decreased by 24 and 48%, respectively, at FC_{80} and FC_{60} compared with FC_{100} (Fig. 4D). The greatest ETR was recorded in the Spm-followed by Put-treated plants at FC_{80} by on average ~25% higher than the untreated plants (Fig. 4D and 2A). However, at FC_{60}, Spd increased ETR by 39% compared with the untreated plants.

Leaf RWC, ψ_O, and EL

Water deficit at FC_{80} and FC_{60} reduced leaf RWC by 13 and 22%, respectively, compared with FC_{100} (Fig. 5A). Put, Spm, and Spd enhanced leaf RWC by an average of ~25% compared with the untreated plants at FC_{100} (Fig. 5A and 2A). Water deficit at the level of FC_{60} reduced ψ_O by 45% compared with FC_{100} (Fig. 5B). In contrast, leaf ψ_O was enhanced in the PAs-treated plants. Put application increased leaf ψ_O 35% compared with the untreated plants at FC_{60} (Fig. 5B). Leaf EL was increased by 1.6 and 2.8 times at FC_{80} and FC_{60}, respectively, compared with FC_{100} (Fig. 5C). However, Spm and Spd decreased leaf EL by 17 and 28%, respectively, compared with untreated plants at FC_{60} (Fig. 5C).

Leaf photosynthetic pigment

A significant decrease was observed in leaf photosynthetic pigments content exposed to water deficit (Table 1). However, Spd increased leaf Chlt by 51, 60, and 79%, respectively, compared with the untreated plants at FC_{100}, FC_{80}, and FC_{60} (Table 1). The highest Chl a:b was observed in the put-treated plants at FC_{80}; 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 FC_{100} (Fig. 2B). 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 (Fig. 6A). Water deficit and PAs increased leaf antioxidant activity. Spd-treated plants showed the highest CAT at FC_{80} and FC_{60} than the untreated plants. Spd increased CAT by 82 and 100% compared with the untreated plants at FC_{80} and FC_{100}, respectively (Fig. 6A). Leaf POD activity showed an increasing trend by increasing the water deficit intensity and PAs application. At FC_{80} and FC_{60}, Spd- and Spm-treated plants showed the greatest POD, respectively, which were nearly double the untreated plants at the respective water deficit level. At FC_{80}, the highest APX activity was recorded in Spm- followed by Spd-treated plants by an average of ~35% over the untreated plants (Fig. 6C and 2B). Leaf SOD activity showed a similar trend as POD. The highest SOD activity was recorded in Spd- and Spm-treated plants at FC_{80} and FC_{60} by 42 and 36%, respectively, over the untreated plants at their respective water deficit levels (Fig. 6D).

Leaf MDA and H_2O_2 content

Water deficit increased leaf MDA content; water deficit at FC_{80} and FC_{60} increased leaf MDA content by 85% and 1.6 times, respectively, compared with FC_{100} (Fig. 6E). Although PAs-treated plant MDA also increased at FC_{80}, it remained almost unaltered at FC_{60} compared with FC_{80}. Leaf MDA content of Put, Spm, and Spd-treated plants were 38, 34, and 31%, respectively, lower than the untreated plants (Fig. 6E). Leaf H_2O_2 content was almost doubled at FC_{60} compared with FC_{100}. However, PAs treatments reduced leaf H_2O_2 content compared with the untreated plants (Fig. 6F). Spd application reduced leaf H_2O_2 by 18 and 10% compared with the untreated plants at FC_{80} and FC_{60}, respectively.

DISCUSSION

Abiotic stresses such as drought, cold, and K deficiency stresses simultaneously stimulate abscisic acid (ABA) and PAs biosynthesis (Li *et al.*, 2021; Réthoré *et al.*, 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 spayed 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 (Fig. 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 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 the increase in the antioxidant enzymes activities by PAs, when plants were fully irrigated, 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 (*SlWRKY1*, *SlHKT1*, *SlDREB2*, and *SlMYB102*), 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 H_2O_2 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 (Fig. 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 H_2O_2 elimination. PAs can potentially improve water deficit tolerance in chamomile. Furthermore, Spd showed the most effective impact in alleviating the water deficit effects.

ACKNOWLEDGMENT

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.

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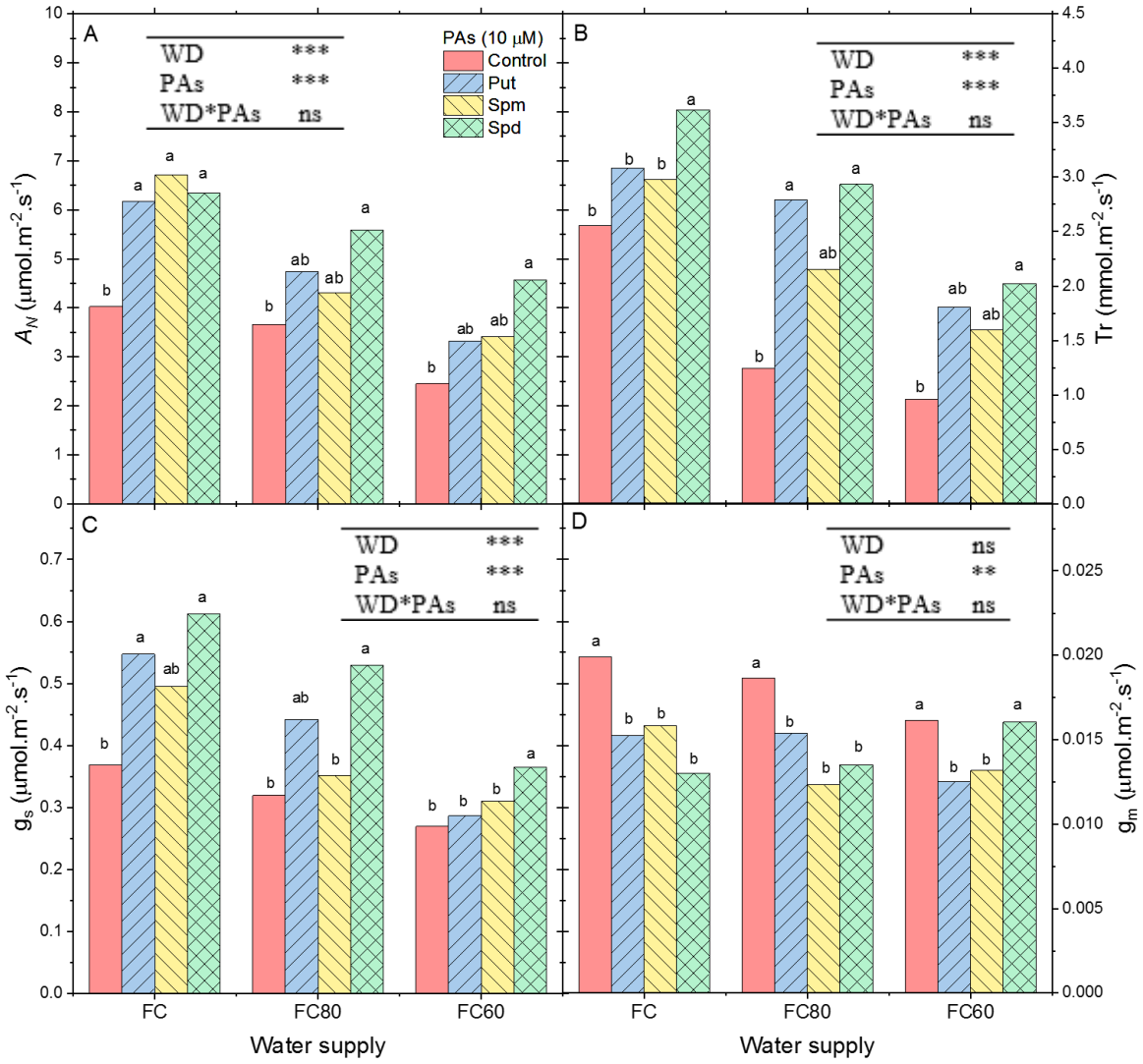

Fig. 1. Changes in (A) Net photosynthetic rate, (B) Transpiration rate, (C) Stomatal conductance, and (D) Mesophyll conductance of German chamomile leaves exposed to water deficit and foliar application of polyamines. WD: water deficit, PAs: polyamines. FC: field capacity, FC_{80}: 80% of FC, FC_{60}: 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.

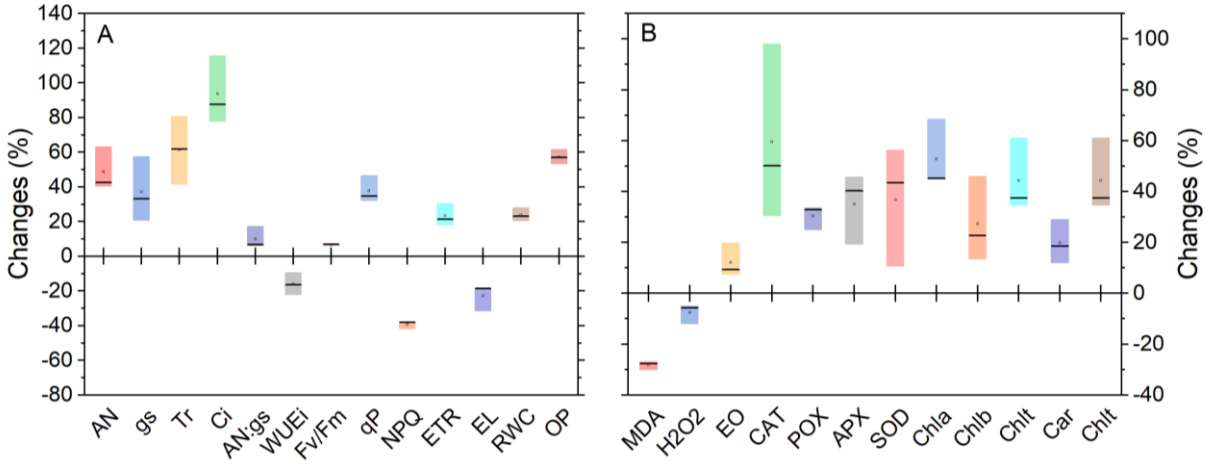

Fig. 2. Percent changes of (A) physiological and growth parameters, and (B) biochemical traits of German chamomile leaves affected by polyamines foliar application under water deficit levels relative to the untreated plants. A_N: Net photosynthetic rate, g_s: Stomatal conductance, T_l: Transpiration rate, C_i: Intercellular 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: Nonphotochemical 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.

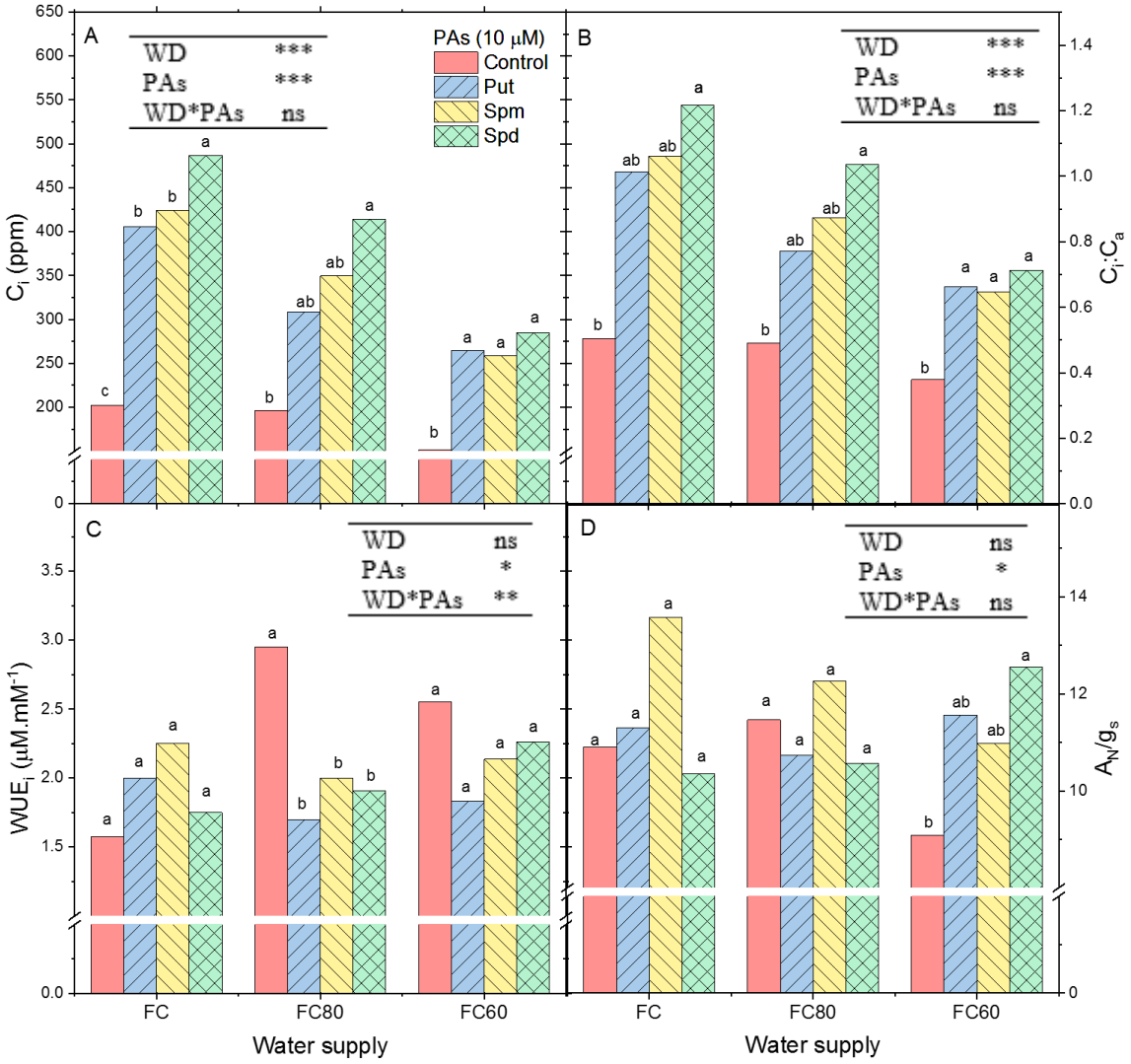

Fig. 3. Changes in (A) Intercellular CO_2 concentration, (B) Intercellular to ambient CO_2 concentration (C) Instantaneous water use efficiency, and (D) Intrinsic water use efficiency of German chamomile leaves exposed to water deficit and foliar application of polyamines. WD: water deficit, PAs: polyamines. FC: field capacity, FC_{80}: 80% of FC, FC_{60}: 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.

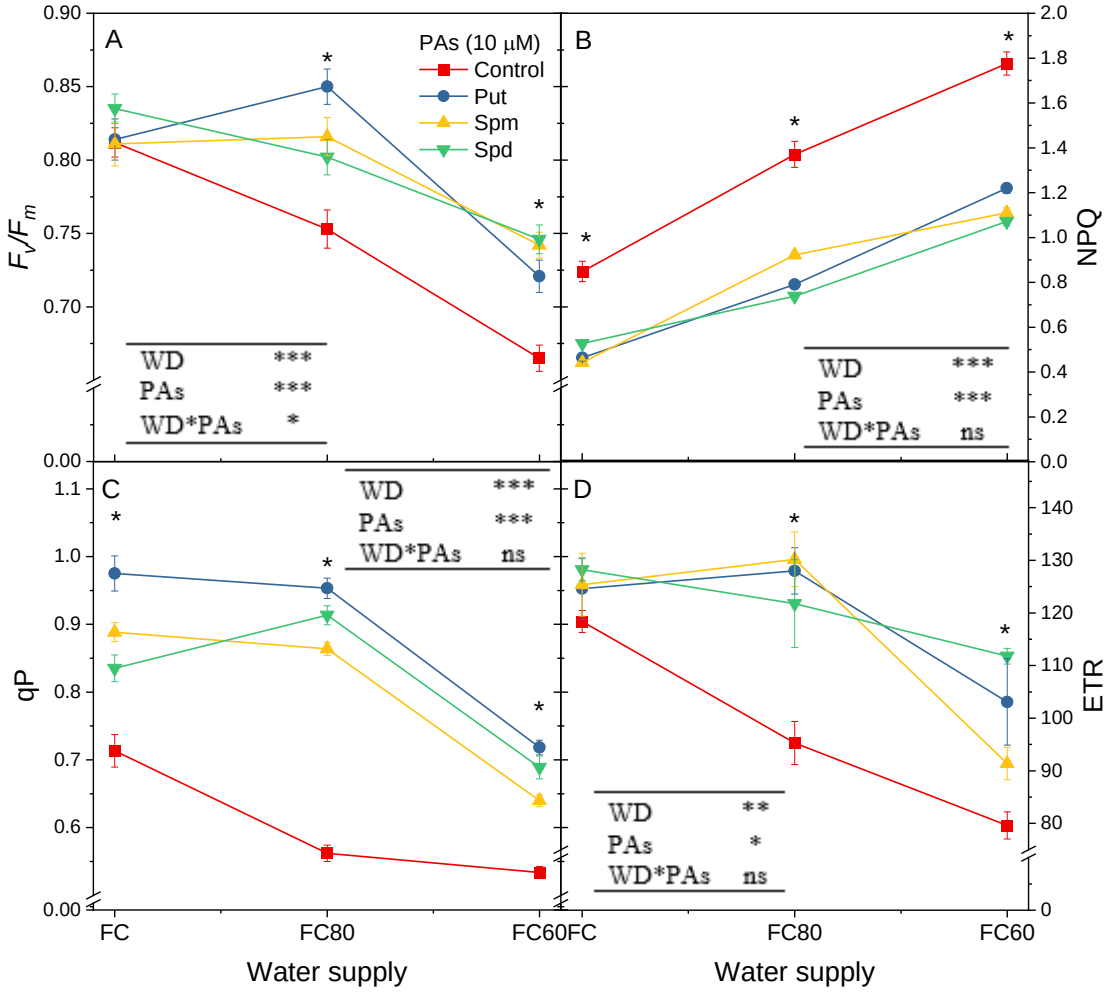

Fig. 4. Changes in (A) Maximum photochemical quantum yield of PSII, (B) Nonphotochemical quenching, (C) Photochemical quenching, and (D) Linear electron transport rate of German chamomile leaves exposed to water deficit and foliar application of polyamines. FC: field capacity, FC_{80}: 80% of FC, FC_{60}: 60% of FC. WD: water deficit, PAs: polyamines. *, **, ***, and ns: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and non-significant. Error bars indicate the differences between the water deficit levels, ±SE. n=9.

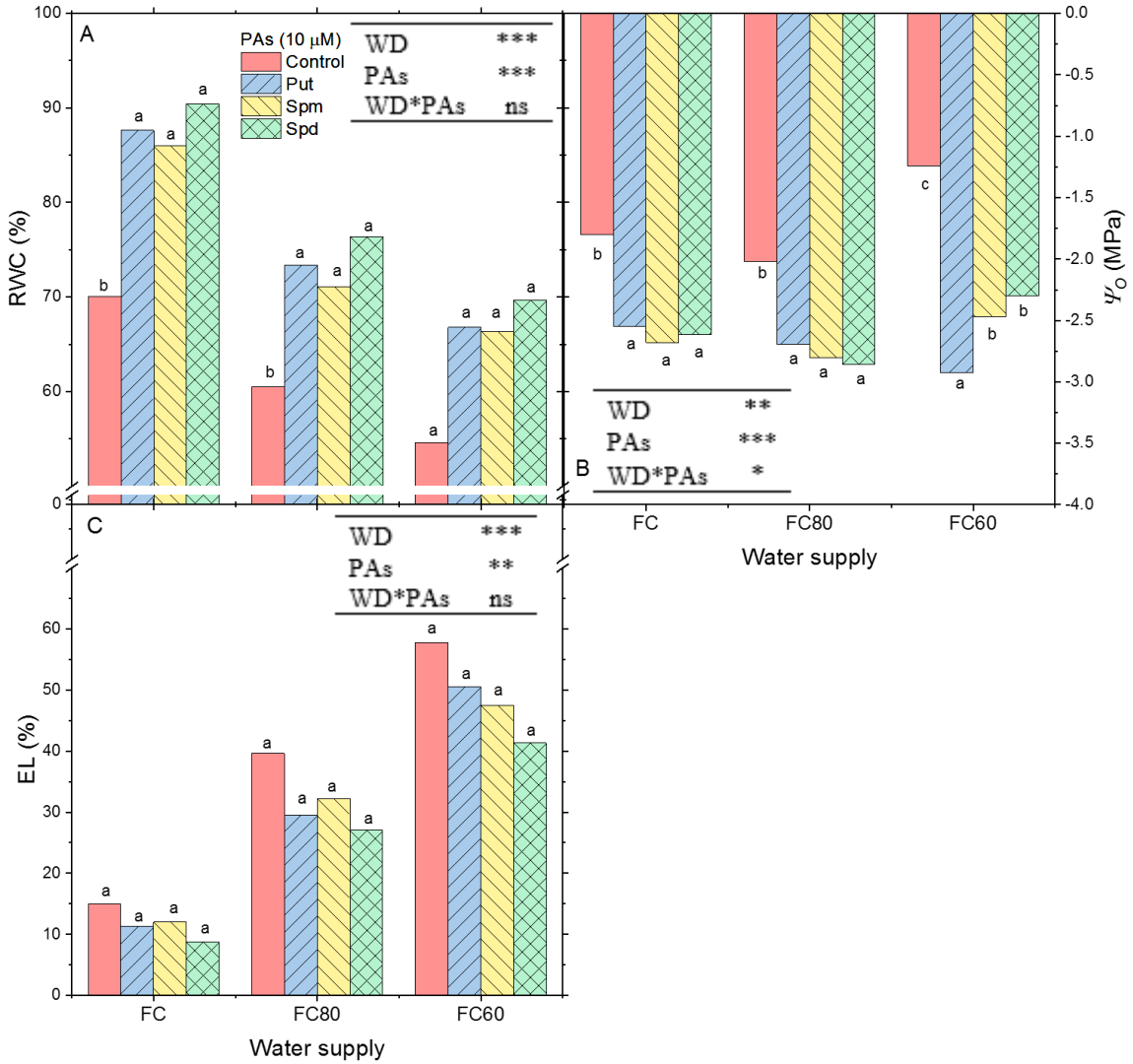

Fig. 5. Changes in (A) Relative water content, (B) Osmotic potential, and (C) Electrolyte leakage of German chamomile leaves exposed to water deficit and foliar application of polyamines. WD: water deficit, PAs: polyamines. FC: field capacity, FC_{80}: 80% of FC, FC_{60}: 60% of FC. *, **, ***, and ns: 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.

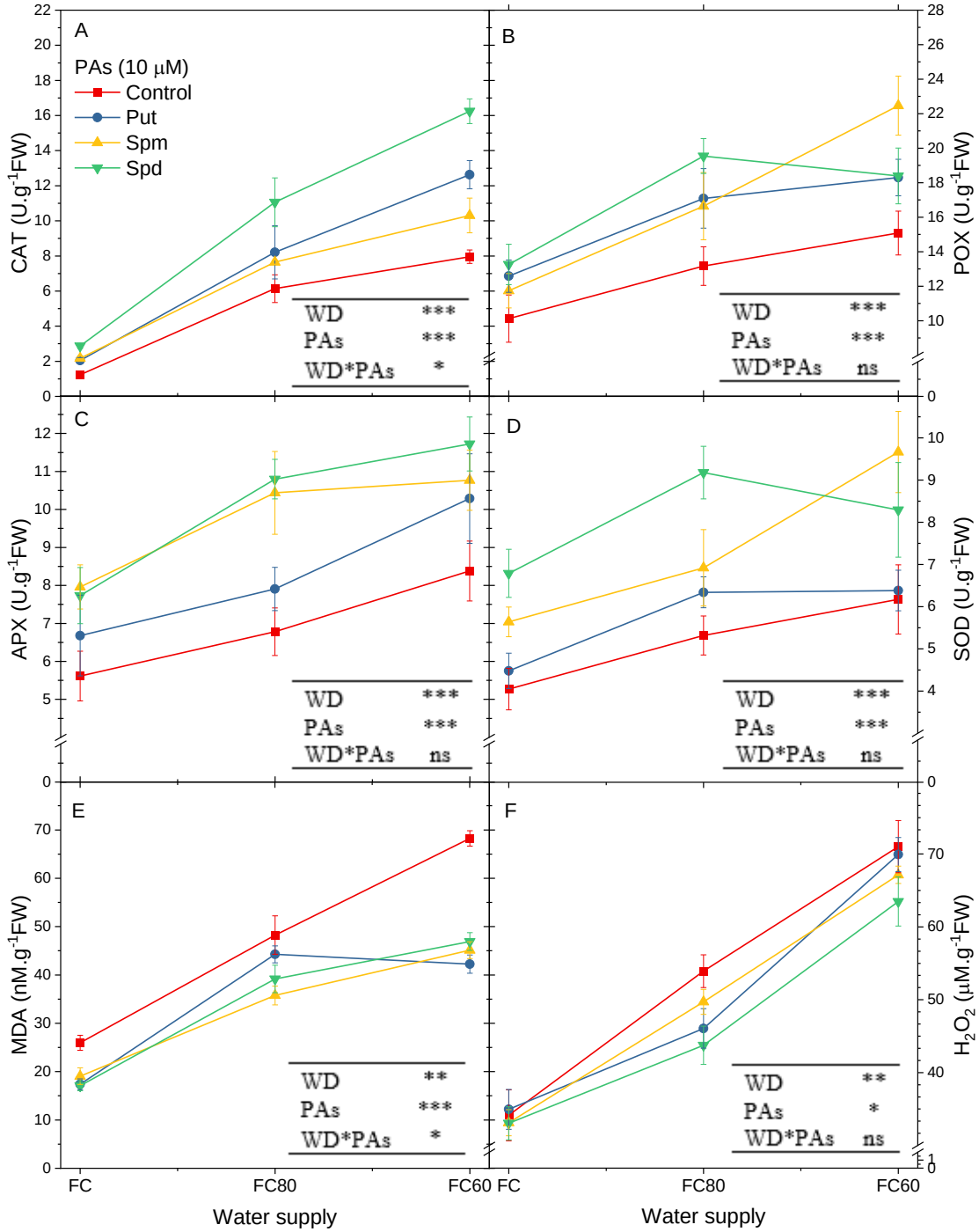

Fig. 6. Changes in the activity of (A) Catalase, (B) Peroxidase, (C) Ascorbate peroxidase, (D) Superoxide dismutase, the content of (E) Malondialdehyde, and (F) Hydrogen peroxide German chamomile leaves exposed to water deficit and foliar application of polyamines. FC: Field capacity, FC_{80}: 80% of FC, FC_{60}: 60% of FC. WD: water deficit, PAs: polyamines. *, **, ***, and ns: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and non-significant. Error bars indicate the differences between the water deficit levels, ±SE. n=9.

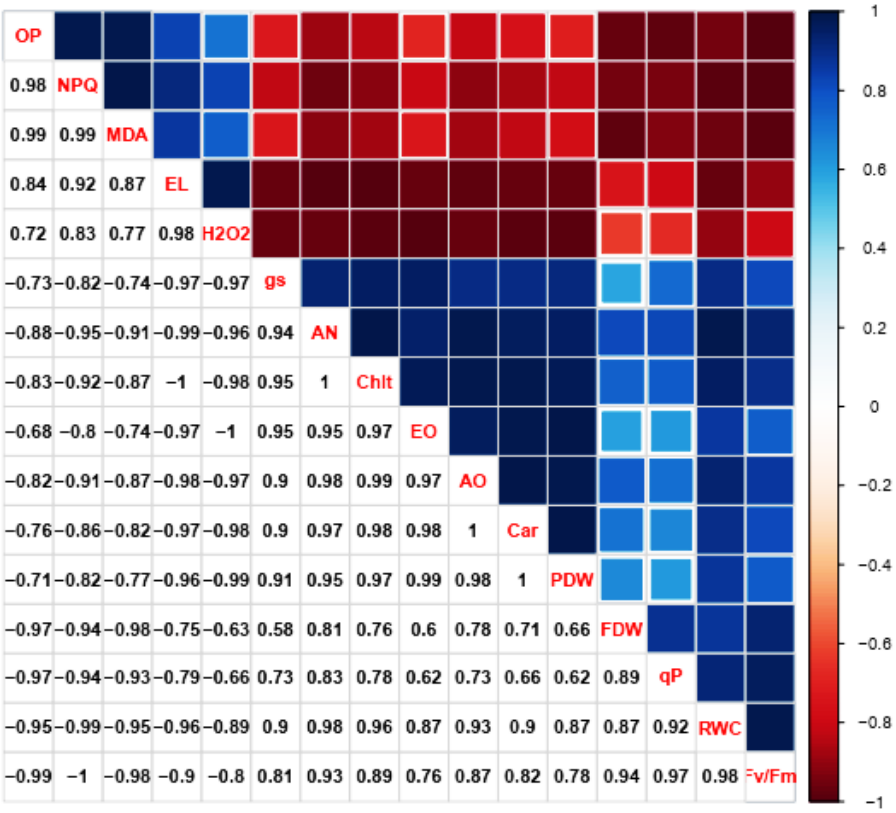

Fig. 7. Corplot analysis of OP: Leaf osmotic potential, NPQ: Nonphotochemical quenching, MDA: Malondialdehyde, EL: electrolyte leakage, H2O2: Hydrogen peroxide, gs: Stomatal conductance, A_N: Net photosynthetic rate, Chlt: Total chlorophyll, EO: Essential oil, AO: Antioxidant enzymes, Car: Carotenoids, 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.

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

Water deficit	PAs‡ (10 µM)	mg.gFW^{-1}				
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	Chlorophyll a:b
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

† FC: field capacity, FC_{80}: 80% of FC, FC_{60}: 60% of FC, ‡ PAs: polyamines. □ Put: putrescine, Spm: spermine, Spd: spermidine. WD: water deficit, PAs: polyamines. *, **, ***, 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 $p \leq 0.05$.

کاهش تنش خشکی در بابونه آلمانی (*Matricaria chamomilla* L.) در پاسخ به تنش اکسیداتیو و بسته‌شدن روزنه ناشی از کمبود آب توسط کاربرد خارجی پلی‌آمین‌ها

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چکیده

پلی‌آمین‌ها مولکول‌های پیامرسانی هستند که نقش‌های امیدوارکننده‌ای در بهبود تحمل به تنش در گیاهان نشان داده‌اند. اطلاعات محدودی در مورد اثرات کاربرد خارجی پلی‌آمین‌ها بر روی گیاهان دارویی از جمله بابونه در دسترس است. این آزمایش به‌منظور بررسی اثرات محلول‌پاشی پلی‌آمین‌ها [پوترسین (Put)، اسپرمیدین (Spd) و اسپرمین (Spm)] بر فرآیندهای فیزیولوژیکی و بیوشیمیایی برای درک مکانیسم‌های احتمالی مربوط به کاهش اثرات تنش کمبود آب [ظرفیت مزرعه (FC) به عنوان شاهد، 80 درصد ظرفیت مزرعه (FC80) و 60 درصد ظرفیت مزرعه (FC60)] در بابونه آلمانی انجام شد. نتایج نشان داد که پلی‌آمین‌ها تا حدی بسته‌شدن روزنه ناشی از کمبود آب را مهار می‌کند و آنزیم‌های آنتی‌اکسیدانی را برای از بین بردن افزایش پراکسیدهیدروژن القا می‌کند. اسپرمیدین هدایت روزنه‌ای را در FC، FC80 و FC60 به ترتیب 66، 65 و 35 درصد در مقایسه با شاهد افزایش داد. افزایش هدایت روزنه‌ای فتوسنتز خالص برگ را در FC80 و FC60 به ترتیب 52 و 86 درصد در مقایسه با شاهد افزایش داد. نقش پلی‌آمین‌ها

در کاهش تنش اکسیداتیو با همبستگی منفی فعالیت‌های آنتی‌اکسیدانی برگ و محتوای مالون‌دی‌آلدئید و پراکسیدهیدروژن تایید شد. با توجه به نتایج، پلی‌آمین‌ها به عنوان ترکیبات محافظ تنش عمل می‌کنند تا آنزیم‌های آنتی‌اکسیدانی را برای حذف پراکسیدهیدروژن ناشی از تنش، بهبود پایداری غشاء و افزایش تحمل کمبود آب تحریک کنند. به‌طورکلی، نتایج نشان داد که پلی‌آمین‌ها می‌توانند تنظیم‌کننده‌های بالقوه رشد برای کاهش تنش کم‌آبی خفیف تا شدید باشند.

کلیدواژگان: فلورسانس کلروفیل؛ آنتی اکسیدان آنزیمی؛ پراکسیدهیدروژن؛ متغیرهای تبادل گاز؛ خاموشی غیر فوتوشیمیایی