Polyamines (PAs) improve antioxidant enzyme activities of Chamomile (*Matricaria chamomila* L.) under water stress

Saeed Moori
Ph.D., Department of Agronomy and Plant Breeding, Lorestan University, Iran.

Mohammad Javad Ahmadi-Lahijani[1]
Assistant Professor, Department of Agrotechnology, Ferdowsi University of Mashhad, Iran.

Abstract

Water deficit is the most important abiotic stress that seriously affects plant production. Various strategies are proposed to reduce cell damage caused by the stresses to improve dehydration tolerance. Therefore, this study was carried out to investigate the effect of foliar application of polyamines (Putrescine, Spermine, and Spermidine; Put, Spm, and Spd, respectively) on the antioxidant enzyme contents in chamomile plants under water deficit (100, 80, and 60% of field capacity, I_{100}, I_{80}, and I_{60}, respectively). The results showed that water deficit increased the content of antioxidant enzymes. The highest leaf catalase and ascorbate peroxidase content were observed at I_{60Spd}. However, the highest leaf peroxidase and superoxide dismutase content were observed at I_{60Spm}. The application of polyamines had a positive effect on increasing antioxidant enzyme content in stressful conditions. According to the results, the foliar application of Spd had the most stimulating effect on increasing antioxidant enzyme content. It can be concluded that foliar application of polyamines led to an increase in the content of antioxidant enzymes and greater plant tolerance to water deficit.

Keywords: Antioxidant enzymes, drought stress, polyamines, chamomile.

[1] Corresponding author

Introduction

German chamomile (*Matricaria chamomila* L.) is one of the most valuable medicinal plants of the composite family with many pharmaceutical, nutritional, and cosmetics uses, and an important essential oil-bearing medicinal plant. Chamomile has adaptability to a wide range of climates and soil conditions, and its cultivation may be an alternative option in areas with drought and salinity problems. The production of essential oil depends on the metabolic state of the source tissues and may be integrated with the stress factors [1].

Among the hindering environmental factors of crop growth, horticultural and medicinal yield, drought is considered to be the most critical factor of production losses, especially in arid and semi-arid regions [2]. Limited water supply is a major environmental constraint in the crop productivity of medicinal plants. Moisture deficiency induces various physiological and metabolic responses, including stomatal closure, and decline in growth rate and photosynthesis [3]. Those changes lead to free radicles (ROS) production, which need to be scavenged. The antioxidant system is a defense system against free radicals [4]. Under non-stress conditions, plants maintain a balance between producing and scavenging ROS through a well-coordinated and rapidly responsive antioxidant system. Under these circumstances, the antioxidant enzymes; CAT, SOD, POD, and others, coordinate to promote low levels of free radicals to prevent cell damage [5].

Polyamines (PAs) are ubiquitous polycationic compounds that mediate essential aspects of cell growth, differentiation, and cell death in the organisms. Generally, naturally occurring PAs in higher plants; including Putrescine, spermidine, and spermine which are protonated at cytoplasmic pH. PAs are a group of phytohormone-like aliphatic amine natural compounds with aliphatic nitrogen structure and present in almost all living organisms, including plants [6]. Aliphatic polyamines are synthesized in both Prokaryotes and Eukaryotes. Among them, Putrescine (Put), spermidine (Spd), and spermine (Spm) are the most common. Moreover, Put and Spd are usually more abundant than Spm, which is often present at trace amounts. In plants, these polyamines are involved in numerous cellular and molecular processes [7]. Exogenous application of polyamines improved tolerance against abiotic stresses [8].

The present study was conducted to determine whether polyamines (Putrescine, Spermine, and Spermidine) foliar application alleviates the adverse effects of water stress on chamomile plants by instigating the leaf antioxidant enzymes.

Materials and methods

This research was conducted in the Faculty of Agriculture research greenhouse, the Ferdowsi University of Mashhad, in 2019 as a factorial experiment based on a randomized complete block design with three replications. The experimental factors were water stress at three levels (100, 80, and 60% of field capacity) and different polyamines (PAs), including Putrescine (Put), Spermine (Spm), and Spermidine (Spd) at a concentration of 0.1 mM [9]. The chamomile genotype was Shiraz cultivar, which was purchased from Pakan Bazar Isfahan Company.

Seeds were sown in plastic pots (35 cm dimensions) in a depth of 1 cm, and three pots were considered for each treatment containing 5 plants (n=15). The pots were placed in a greenhouse at 35/25 °C (day/night) and under natural light. Foliar application of PAs was made at three stages (four-leaf, eight-leaf, and flowering stages). Drought stress began when the plant reached the four-leaf stage. TDR was used to apply water stress treatment.

To measure the leaf antioxidant enzyme content, plant leaves were sampled seven days after the last treatment (flowering stage), and the samples were stored in a freezer at -80 °C. Leaf catalase (CAT), Peroxidase (POX), superoxide dismutase (SOD), and ascorbate peroxidase (APX) content were measured using the methods of [10], [11], [12], and [13], respectively.

Data were subjected to one-way analysis of variance and mean differences were compared by the least significant difference (LSD) testing using SAS *v*.9.1 software at $p \leq 0.05$. means are shown as ±SE.

Results and discussion

The results showed that the interaction effect of water deficit × foliar application of polyamines was significant on leaf antioxidant enzyme content (Table 1). The highest leaf CAT and APX were observed at I_{60Spd} (Figure 1). The use of polyamines, as it protects the plant from stress-induced water shortage, can help the plant's defense system cope with the stress. A study showed an increase in CAT activity due to the use of polyamines [14]. APX breaks down H_2O_2 into the water using ascorbate as a substrate. In practice, this enzyme transfers the excess electrons in H_2O_2 to dehydroascorbate and produces water. APX plays a crucial role in the glutathione-ascorbate cycle [15]. Increased activity of APX enzyme due to the use of polyamines has been reported in wheat and chamomile [16].

Table 1. ANOVA results of antioxidant enzymes of chamomile leaves under water deficit conditions and foliar application of polyamines.

S.O.V	df	CAT	POD	SOD	APX
Water stress	2	18.60^{**}	0.06^{ns}	1.86^{**}	36.35^{ns}
PAs	3	201^{**}	2.56^{**}	0.22^{**}	591^{**}
Stress× PAs	6	4.10^{**}	0.66^{**}	0.3^{**}	90.9^{**}
Error	24	0.44	10.7	0.0045	21.3
CV (%)	-	16.8	19.3	10.7	15.3

ns and **; represent non-significant and significant at 1%, respectively.

The highest leaf POX and SOD content were observed I_{60Spm} (Figure 1). Peroxidase is present in both the cytosol and chloroplasts, which can effectively remove oxygen free radicals. Increased POX activity has been reported in various studies under stress conditions [14]. Accumulation of peroxidase isozymes in the vacuole and cell wall causes these parts to become woody and thus leads to cell protection [17]. SOD is a key enzyme that scavenges superoxide radicals. SOD is the first line of defense that constitutes the elimination of ROSs in plants. The high expression of these enzymes can reduce the damage of ROSs to plants under stress conditions. Under drought stress, the activity of SOD in drought-resistant cultivars was higher than that in susceptible cultivars, which indicated that drought-tolerant varieties had a higher ability to scavenge superoxide free radicals [4]. Drought stress hinders enzyme activities by preventing the oxidative stress reduction mechanisms and cellular metabolism of plants. PAs binding to membrane proteins may stabilize the protein structure during stress and preserve photosynthetic activity [18].

Maintaining cellular membranes' integrity under drought stress is considered an integral part of the stress tolerance mechanism [19]. This is probably because PAs cause an increase in the activities of antioxidant enzymes, which, in turn, protect plants against the generation of ROS and membrane injury, or may result in the synthesis of other substances that have a protective effect on plant growth under stress. In addition to their properties as free radical scavengers, PAs were also reported to stabilize the biological membrane by directly binding to membrane phospholipids in stress conditions [20]. Polyamines are now being regarded as plant growth regulators and secondary messenger in signaling pathways and play an array of physiological roles in plant growth and development [19].

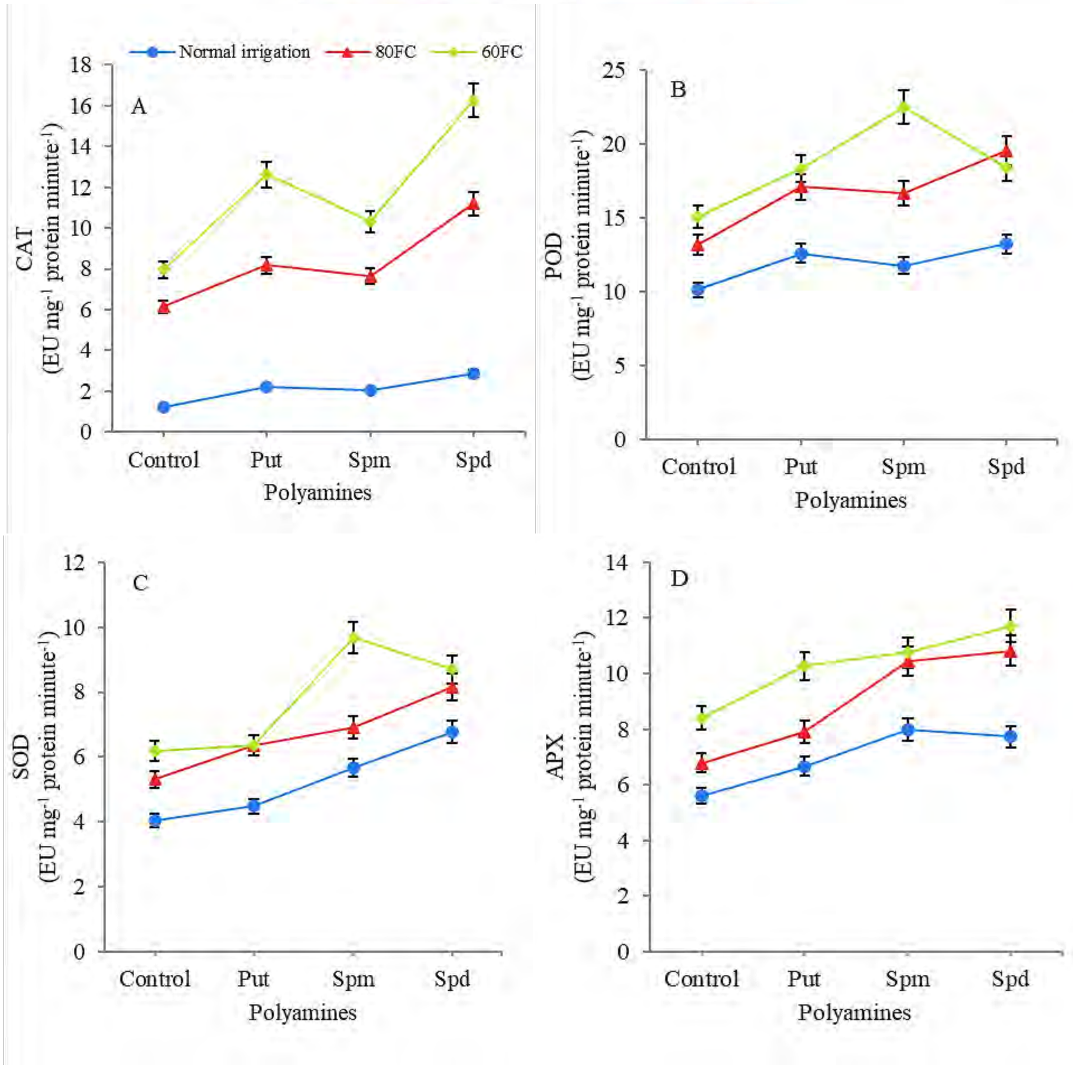

Figure 1. Effects of water deficit and foliar application of polyamines (Putrescine (Put), spermine (Spm), and (Spd) spermidine at a concentration of 0.1 mM) on leaf catalase (A), peroxidase (B), superoxide dismutase (C), and ascorbate peroxidase (D) content of chamomile. Data are means of three replicates and ±SE. LSD ($P < 0.05$).

Conclusion

According to the results, Spd and Spm were more effective than Put for reducing the effects of water deficit stress. The use of polyamines due to increased antioxidant activity plays a vital role in reducing oxygen free radicals (hydroxyl, hydrogen peroxide, and superoxide) produced under drought stress conditions. Therefore, it can be suggested that the application of polyamines as foliar spraying under stress conditions can be a practical step to save water consumption in the water shortage crisis in agriculture by reducing the damage caused by water stress.

References

[1] Sangwan, N., A. Farooqi, F. Shabih, and R. Sangwan, Regulation of essential oil production in plants. Plant growth regulation, 2001. 34(1): p. 3-21.
[2] Reddy, A.R., K.V. Chaitanya, and M. Vivekanandan, Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of plant physiology, 2004. 161(11): p. 1189-1202.
[3] Flexas, J. and H. Medrano, Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. Annals of botany, 2002. 89(2): p. 183-189.
[4] Moori, S. and M.J. Ahmadi-Lahijani, Hormopriming instigates defense mechanisms in Thyme (Thymus vulgaris L.) seeds under cadmium stress. Journal of Applied Research on Medicinal and Aromatic Plants, 2020. 19: p. 100268.
[5] Garg, N. and G. Manchanda, ROS generation in plants: boon or bane? Plant Biosystems, 2009. 143(1): p. 81-96.
[6] Gill, S.S. and N. Tuteja, Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant physiology and biochemistry, 2010. 48(12): p. 909-930.
[7] Bouchereau, A., A. Aziz, F. Larher, and J. Martin-Tanguy, Polyamines and environmental challenges: recent development. Plant science, 1999. 140(2): p. 103-125.
[8] Amri, E. and M.J. Mohammadi, Effects of timing of drought stress on pomegranate seedlings (Punica granatum L. cv Atabaki) to exogenous spermidine and putrescine polyamines. African Journal of Microbiology Research, 2012. 6(25): p. 5294-5300.
[9] Khoshbakht, D., M. Asghari, and M. Haghighi, Influence of foliar application of polyamines on growth, gas-exchange characteristics, and chlorophyll fluorescence in Bakraii citrus under saline conditions. Photosynthetica, 2018. 56(2): p. 731-742.
[10] Bailly, C., Active oxygen species and antioxidants in seed biology. Seed Science Research, 2004. 14(2): p. 93-107.
[11] Kalpana, R. and K. Madhava Rao, On the ageing mechanism in pigeonpea (Cajanus cajan (L.) Millsp.) seeds. Seed Science and Technology (Switzerland), 1995.
[12] Rao, K.M. and T. Sresty, Antioxidative parameters in the seedlings of pigeonpea (Cajanus cajan (L.) Millspaugh) in response to Zn and Ni stresses. Plant science, 2000. 157(1): p. 113-128.
[13] Nakano, Y. and K. Asada, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and cell physiology, 1981. 22(5): p. 867-880.
[14] Ruiz, J.M., M. Baghour, and L. Romero, Efficiency of the different genotypes of tomato in relation to foliar content of Fe and the response of some bioindicators. Journal of Plant Nutrition, 2000. 23(11-12): p. 1777-1786.
[15] Noctor, G. and C.H. Foyer, Ascorbate and glutathione: keeping active oxygen under control. Annual review of plant biology, 1998. 49(1): p. 249-279.
[16] Asada, K., Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant physiology, 2006. 141(2): p. 391-396.
[17] Gaspar, T., C. Penel, F.J. Castillo, and H. Greppin, A two-step control of basic and acidic peroxidases and its significance for growth and development. Physiologia plantarum, 1985. 64(3): p. 418-423.
[18] Hamdani, S., H. Yaakoubi, and R. Carpentier, Polyamines interaction with thylakoid proteins during stress. Journal of Photochemistry and Photobiology B: Biology, 2011. 104(1-2): p. 314-319.
[19] Farooq, M., A. Wahid, and D.-J. Lee, Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiologiae Plantarum, 2009. 31(5): p. 937-945.

[20] Hussain, S.S., M. Ali, M. Ahmad, and K.H. Siddique, Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. Biotechnology advances, 2011. 29(3): p. 300-311.

[1] Sangwan, N., A. Farooqi, F. Shabih, and R. Sangwan, Regulation of essential oil production in plants. Plant growth regulation, 2001. 34(1): p. 3-21.
[2] Reddy, A.R., K.V. Chaitanya, and M. Vivekanandan, Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of plant physiology, 2004. 161(11): p. 1189-1202.
[3] Flexas, J. and H. Medrano, Drought‐ inhibition of photosynthesis in C3 plants: stomatal and non‐ stomatal limitations revisited. Annals of botany, 2002. 89(2): p. 183-189.
[4] Moori, S. and M.J. Ahmadi-Lahijani, Hormopriming instigates defense mechanisms in Thyme (Thymus vulgaris L.) seeds under cadmium stress. Journal of Applied Research on Medicinal and Aromatic Plants, 2020. 19: p. 100268.
[5] Garg, N. and G. Manchanda, ROS generation in plants: boon or bane? Plant Biosystems, 2009. 143(1): p. 81-96.
[6] Gill, S.S. and N. Tuteja, Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant physiology and biochemistry, 2010. 48(12): p. 909-930.
[7] Amri, E. and M.J. Mohammadi, Effects of timing of drought stress on pomegranate seedlings (Punica granatum L. cv Atabaki) to exogenous spermidine and putrescine polyamines. African Journal of Microbiology Research, 2012. 6(25): p. 5294-5300.
[8] Bouchereau, A., A. Aziz, F. Larher, and J. Martin-Tanguy, Polyamines and environmental challenges: recent development. Plant science, 1999. 140(2): p. 103-125.
[9] Khoshbakht, D., M. Asghari, and M. Haghighi, Influence of foliar application of polyamines on growth, gas-exchange characteristics, and chlorophyll fluorescence in Bakraii citrus under saline conditions. Photosynthetica, 2018. 56(2): p. 731-742.
[10] Bailly, C., Active oxygen species and antioxidants in seed biology. Seed Science Research, 2004. 14(2): p. 93-107.
[11] Kalpana, R. and K. Madhava Rao, On the ageing mechanism in pigeonpea (Cajanus cajan (L.) Millsp.) seeds. Seed Science and Technology (Switzerland), 1995.
[12] Rao, K.M. and T. Sresty, Antioxidative parameters in the seedlings of pigeonpea (Cajanus cajan (L.) Millspaugh) in response to Zn and Ni stresses. Plant science, 2000. 157(1): p. 113-128.
[13] Nakano, Y. and K. Asada, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and cell physiology, 1981. 22(5): p. 867-880.
[14] Ruiz, J.M., M. Baghour, and L. Romero, Efficiency of the different genotypes of tomato in relation to foliar content of Fe and the response of some bioindicators. Journal of Plant Nutrition, 2000. 23(11-12): p. 1777-1786.
[15] Noctor, G. and C.H. Foyer, Ascorbate and glutathione: keeping active oxygen under control. Annual review of plant biology, 1998. 49(1): p. 249-279.
[16] Asada, K., Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant physiology, 2006. 141(2): p. 391-396.
[17] Gaspar, T., C. Penel, F.J. Castillo, and H. Greppin, A two‐ step control of basic and acidic peroxidases and its significance for growth and development. Physiologia plantarum, 1985. 64(3): p. 418-423.
[18] Hamdani, S., H. Yaakoubi, and R. Carpentier, Polyamines interaction with thylakoid proteins during stress. Journal of Photochemistry and Photobiology B: Biology, 2011. 104(1-2): p. 314-319.
[19] Farooq, M., A. Wahid, and D.-J. Lee, Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiologiae Plantarum, 2009. 31(5): p. 937-945.
[20] Hussain, S.S., M. Ali, M. Ahmad, and K.H. Siddique, Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. Biotechnology advances, 2011. 29(3): p. 300-311.