Journal of Plant Process and Function, Vol. 12, No. 55, Year 2023 **page: 71-80**

Research Article

Biochemical responses of *Fragaria*× *ananassa* (Duch.) to mycorrhizal fungi and putrescine under salinity stress

Fatemeh Ishaqi [1], Azam Seyedi [2]*, Abolghasem Hamidi Moghaddam [2], Bahareh Parsa Mtolagh [3]

[1] Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Jiroft, Jiroft, Iran
[2] Department of Horticultural Science, Faculty of Agriculture, University of Jiroft, Jiroft, Iran
[3] Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Jiroft, Jiroft, Iran
(Received: 2022/04/02-Accepted: 2022/09/24)

Abstract

Salinity stress is one of the serious environmental stresses that can influence crop growth and physiological and biochemical parameters. In this study, the role of vesicular and arbuscular mycorrhizal fungi '*Glomus mossea*' and the putrescine polyamine in modulating the negative effects of salinity stress on physiological and biochemical parameters of strawberry plants were investigated. This study was done as a factorial experiment based on a completely randomized design with three replications in 2021 in the research greenhouse at the University of Jiroft. The studied factors included mycorrhizal fungi (0 and 30 g), putrescine (0 and 1.5 mM), and salinity levels (0 and 60 mM NaCl). Sodium (Na^+) and potassium (K^+) ions, and K^+/Na^+ in leaves and roots, photosynthetic pigments, relative water content (RWC), membrane stability index (MSI), reactive oxygen species (ROS), soluble sugar, anthocyanin, and proline in leaves were investigated. Simultaneous use of mycorrhizal fungi and putrescine by the more (170% compared to the control) accumulation of Na^+ in the roots decreased their transfer to the leaves and by increasing the ratio of K^+/Na^+ in the leaves, it reduced the accumulation of ROS and, as a result, increased the MSI and also maintained leaf photosynthetic pigments. Salinity increased total soluble sugars and the use of putrescine caused the accumulation of proline and anthocyanin in the leaves. Therefore, modulation of the negative effects of salinity stress by mycorrhizal fungi and putrescine indicates successful adaptation of strawberry plants to salinity stress in the presence of mycorrhizal fungi and putrescine. Finally, our results recommend the use of mycorrhizal fungi in saline areas and provide an important perspective for the use of putrescine in modifying salinity tolerance.

Keywords: Polyamine, Reactive oxygen species, Salinity tolerance, Strawberry

Introduction

Salinity stress is a major environmental factor that has been significantly affecting plant growth (Kumar *et al*., 2021). Although different strategies are followed to deal with salinity stress, most products do not grow well under salinity stress (Ferreira *et al*., 2019; Zahedi *et al*., 2020b; Roshdy *et al*., 2021). The key physiological responses of plants for dealing with salinity stress include osmotic adjustment with an accumulation of compatible solutes, known as osmolytes or osmoprotectants, increasing antioxidant enzyme activities and toxic ion elimination. In addition, plants under salinity stress produce a pool of reactive oxygen species (ROS) like superoxide anion radical ($O2^{-\bullet}$), hydrogen peroxide (H_2O_2), and hydrogen radical ($OH^\bullet$) (Mishra *et al.*, 2013). ROS affects the natural metabolic process through lipid peroxidation or oxidative damage to biological molecules, leading to imbalances in component levels and dysfunction of their general defense system (Islam *et al*., 2021; Bose *et al*., 2014). Retardation of root growth is the most critical change as it is the first organ exposed to salt stress to enhance the water extraction capacity in environments (Yuan *et al*., 2016). Finding ways to improve salinity is important for the production of crops. Salt stress is also a crucial factor that affects the growth of strawberry plants. The strawberry, *Fragaria* × *ananassa* Duch, is one of the most cultivated berry crops in the world, belonging to the Rosaceae family .

Polyamines (PAs) are organic polycations that involve two or more amino groups. The accumulation of polyamines in response to much abiotic stress is one of

*Corresponding Author, Email: a.seiedi@ujirotft.ac.ir

[DOR: 20.1001.1.23222727.1402.12.55.8.0]

the main remarkable metabolic signs in plants exposed to abiotic stress conditions responses. It has been reported PAs roles in the biosynthesis of DNA, RNA and protein, maintaining plant growth and development, delaying aging, and protecting the membrane from oxidative damage by removing free radicals in plants (Hussein *et al*., 2019; Islam *et al*., 2021). Polyamines are useful for protein homeostasis, detoxification of ROS and activation of antioxidant machinery under stress, thus providing tolerance to a wide range of stresses (Seo *et al*., 2019). The protective mechanism of the exogenous PA application in many crops against abiotic stresses is highlighted in the reviews by Todorova *et al.* (2015), Chen *et al*. (2019) and Islam *et al*. (2021). The external application of PAs in plants has been investigated by Alcazar *et al*. (2020) who found that small amines (putrescine, spermidine, spermine, and cadaverine) could regulate growth and various physiological processes, such as photosynthetic activity, osmolality accumulation, and antioxidant defense under abiotic stress.

Strawberry is a glycophyte (salt-sensitive) plant; therefore, its productivity is being severely affected in many regions of the world (Ferreira *et al*., 2019). The salinity of the water is maintained by soil particles, prevents it from being absorbed by plants, alters ionic balance, and causes nutritional deficiencies of K^+ and other ions, due to increased concentrations of Cl^- and Na^+ (Gupta and Huang, 2014).

Since saline land reclamation requires a lot of budget and time, the use of root symbiotic fungi can be of great help in mitigating the adverse effects of abiotic stresses. The symbiotic relationship between mycorrhiza fungi and plant roots is widely found in nature (Begum *et al*., 2019). The vesicular and arbuscular mycorrhizal fungi (VAMF) are biotrophic organisms that form vesicles, arbuscules, and the hyphae in roots, and also spores, and the hyphae in the rhizosphere. The formation of hyphae network by VAMF and their conjugation with the plant roots significantly increases the ability of roots in large areas of soil and improves plant growth (Bowles *et al*., 2016). This symbiosis induces the synthesis of secondary metabolites, being able to increase the plant's tolerance to biotic and abiotic stress (Begum *et al*., 2019). Also, they can induce the growth of plants via affect stomatal conductance, leaf water potential, relative water content (RWC), PSII efficiency, and CO_2 assimilation (He *et al*., 2017; Chandrasekaran *et al.*, 2019).

However, little is known about the interactions of polyamines and mycorrhizal fungi on crops. Therefore, in this study, the effect of putrescine spraying on the strawberry plants that included and non-included VAMF in saline and non-saline conditions was investigated.

Materials and methods

Growth conditions and plant culture: The experiment was done in the research greenhouse of the University of Jiroft (35º 28ˈ N; 47º 57ˈ E, at an altitude of 625.6 m; subtropical) in 2021. The greenhouse temperature was 30/25±5 ºC (day/night) and the air's relative humidity was 85±5 Cº. This study was done as a factorial experiment based on a completely randomized design with three factors and three replications. Factors involved vesicular and arbuscular mycorrhizal fungi: *Glomus mosseae* (0 and 30 g/plant), putrescine (0 and 1.5 mM), and salinity (0 and 60 mM NaCl). Mycorrhizal fungi: *Glomus mosseae* were prepared by Green Biotechnology Company and used freshly.

'Parus' strawberry rooted plants (*Fragaria* × *ananassa* Duch.) were obtained from a nursery, Jiroft, Iran. Among them, the plants that were relatively uniform in size, crown diameter and number of leaves (they had 5 fully developed leaves) were selected for the experiment. One plant was used as an experimental unit and one replicated of each treatment and was planted, in 3 L volume plastic pots, filled with a mixture of soil and peat mass (95:5, V:V %). The plant's roots were treated with the mycorrhizal fungi (0 and 30 g of bed inoculated to the mycorrhizal fungi) at the planting time. The plants were irrigated immediately after planting. The volume of water required for each pot was determined by a pre-test based on the effluent to prevent salt accumulation near the roots, which was about 150 cc every two days. In order to ensure that the roots of the plants were colonized by mycorrhizal fungi, a pre-test was done and two weeks after planting, the roots of three plants that were inoculated by the mycorrhizal were investigated and the results showed that the roots were colonized about 10 to 15%. So two weeks after planting, the foliar spray of different putrescine concentrations was applied as control (water) and 1.5 mM once every week for two consecutive weeks, using a handgun sprayer (2 L in volume). Also, salinity treatment was started on the second week after planting and one day after the first stage of spraying the plants by putrescine. Salinity concentration (60 mM) was applied during the three stages (20, 40 and 60 mM) of irrigation. To preserve salinization at the correct level, before applying each subsequent saline irrigation, all the pots were leached with tape water to prevent salt accumulation over the experimental concentration. Salinity stress continued until salinity symptoms appeared as dryness at the edges of the lower leaves of the plants. Finally, the first and second fully expanded leaves of the terminal section of the plant were separated and immediately frozen in liquid nitrogen and maintained at -20ºC for further biochemical quantification. The third fully expanded leaf of the top of the plant was separated for evaluation by RWC and MSI. Then the plants slowly came out of the pots, and after washing the roots, the leaves separated from the roots and dried to evaluate the sodium and potassium in the root and leaf.

Determination of Na^+, K^+, and K^+/Na^+: The shoot and roots of harvested plants were quickly rinsed in distillation water to remove ions from the free space and

[DOR: 20.1001.1.23222727.1402.12.55.8.0]

gently blotted dry with a paper towel. To determine the ratio of K^+/Na^+ in shoots and roots, the organs were dried at 70 ºC for 48 h and the 50 mg dry weight was digested and turned to ashes at 600 ºC for 5 h in an electric oven. Na^+ and K^+ are resuspended in 10 ml of HCl (0.1 N) and the solution was filtered. Na^+ and K^+ were quantified by a flame photometer (Jons, 2001).

Determination of photosynthetic pigments: Chlorophyll and carotenoids of freeze-dried leaf samples were determined according to the method of Lichtenthaler (1987) by a spectrometer (UV/VIS, Perkin Elmer, USA) at 663, 645, 470 nm, and using the following formulas.

Chl a (mg/g) = (12.25 A663 – 2.79 A645) × V/W×1000
Chl b (mg/g) = (21.5 A645 – 5.1 A663) × V/W×1000
Total Chl (mg/g) = Chl a + Chl b
CARs (mg/g) = ((1000 A 470 – 1.8 Chla – 85.02 Chlb) / 198) × V/W×1000

Estimation of Leaf water status and membrane stability index: The relative water content (RWC) in the leaves was measured according to the method of Barrs and Weatherley (1962) using the following formula:

RWC (%) = (fresh weight - dry weight)/ (turgor weight-dry weight)×100.

The membrane stability index (MSI) was estimated using the formula:

MSI (%) =1- (EC1/EC2) × 100

Where EC1 is the electrical conductivity bridge after the leaves were heated at 40 ºC for 30 in a water bath, and EC2 is the electrical conductivity bridge after the leaves were boiled at 100 ºC for 10 in a water bath (Yue *et al*., 2019).

Estimation of osmotic adjustment molecules: The concentration of proline was determined by following the method by Carillo and Gibon (2011). The absorbance was taken at 520 nm, and calculations were done using an appropriate proline standard curve. The concentration of soluble sugar was determined by following the method Albalasmeh *et al.* (2013). The absorbance was taken at 315 nm, and calculations were done using an appropriate soluble sugars standard curve.

For anthocyanin determination was used Wagner method (1979). The absorption rate of the supernatant was read at 550 nm. Anthocyanin content was calculated using an extinction coefficient of 33000 mol^{-1} cm^{-1}.

Reactive oxygen species (ROS): The ROS content was monitored with the xylenol orange method has been described by Tirani and Haghjou (2019). To measure ROS, the first 0.05 g of fresh leaf tissue was completely grounded into porcelain mortar in an ice bath. Then one milliliter of 50 mM sodium phosphate buffer with pH 6.8 was added. The resulting homogeneous solution was poured into the Eppendorf and then centrifuged for 20 minutes at 10000 rpm at 4 ºC. Then, to 100 µl of the extract, 900 µl of fresh acid xylenol orange reagent was added. The optical absorption of the resulting solution was read at 560 nm. Blanc contained 100 µl of distilled water and 900 µl of fresh acid xylenol orange reagent. Different concentrations of H_2O_2 (30%) were used to draw the standard curve and the results were calculated and presented in micromoles per gram of fresh weight.

Data was statistically analyzed using the software SAS, version 9.4. Duncan's multiple range test was performed to determine the differences of 0.05 probability between treatments using the same program. Significant differences were indicated by different letters. To determination of simple correlation coefficients between the traits was used Minitab 16 software.

Results and discussion

Accumulation status of Na^+, K^+ and Na^+/ K^+ in leaf and root: The Na^+ content in the leaf showed a significant negative correlation with, K^+ content in the root, K^+/ Na^+ ratio in leaf and root, photosynthetic pigments, MSI and RWC, and a significant positive correlation with ROS and proline (Table 4). Na^+ content increased 1.5-1.8 folds in leaves of the plants under saline stress, compared with the control. In treated plants with mycorrhizal, putrescine and both, compared to control, Na^+ more accumulated in the roots and less transferred to the leaves. In untreated plants with mycorrhizal and putrescine, salinity increased Na^+ content by 85% compared with treated plants with both of them (Table 1).

The most content of Na^+ accumulated in the roots of the plants cotreated with mycorrhizal and putrescine under salinity stress (170% increased compared to the control). While the lowest amount of sodium transferred to the leaves under saline conditions was related to co-treated plants with mycorrhizal fungi and putrescine (Table 1). K^+ in leaf showed a significant positive correlation with carotenoid pigments and K^+/ Na^+ ratio in leaf (Table 4). Mycorrhizal fungi and their interaction with putrescine increased root K^+ content compared to control under normal and saline conditions (Table 1). Also, K^+/ Na^+ ratio in leaves increased in the plants treated with mycorrhizal fungi, putrescine, and both in normal and stress conditions. According to the Pearson correlation coefficient (Table 4) there was a significant and positive correlation between K^+/ Na^+ ratio in leaf with K^+/Na^+ ratio in the root (0.728^{**}), K^+/ Na^+ ratio in the leaf with Chl *a*, *b*, total and carotenoids (respectively: 0.743^{**}, 0.704^{**}, 0.745^{**} and 0.909^{**}) and also K^+/ Na^+ ratio in leaf with MSI (0.599^{**}) and RWC (0.717^{**}). There was a significant and negative correlation between K^+/ Na^+ ratio in leaves with ROS (-0.498^{*}) and soluble sugars (-0.432^{*}).

K^+ plays a major role in maintaining the turgor within the cell. Na^+ and K^+ have the same transport mechanism and under saline conditions, Na^+ concentration increases and competes with K^+ for the transporter, thereby decreasing the uptake of K^+ (Gupta and Huang, 2014). The strategy of high K^+/ Na^+ ratio is one of the pathways to high tolerance to salinity stress and survival in a plant (Almeid *et al*., 2017). These

[DOR: 20.1001.1.23222727.1402.12.55.8.0]

Table 1. Effect of mycorrhizal inoculated and exogenous putrescine on Na^+ and K^+ content, and Na^+/K^+ ratio in leaf and root of strawberry plants under salinity stress conditions.

Mycorrhizal (g/pot plant)	Putrecine (mM)	NaCl (mM)	Leaf Na^+ (mg/g DW)	Leaf K^+ (mg/g DW)	Leaf K^+/Na^+	Root Na^+ (mg/g DW)	Root K^+ (mg/g DW)	Root K^+/Na^+
control	control	control	7.23^{c}	10.18^{b}	1.34^{de}	7.56^{d}	2.78 ^{c}	0.37^{cd}
		60	13.35^{a}	11.53^{b}	0.86^{f}	8.33^{d}	2.61^{c}	0.31^{de}
	1.5	control	6.41^{d}	14.15^{a}	2.24^{b}	6.35^{d}	2.73^{c}	0.45 ^{bc}
		60	10.16^{b}	11.35^{b}	1.12^{ef}	13.34^{b}	3.17^{c}	0.24^{ef}
30	control	control	6.07^{d}	11.56^{b}	1.92^{c}	10.90^{c}	5.25^{a}	0.49^{ab}
		60	9.78^{b}	11.71^{b}	1.21^{de}	11.20^{bc}	4.14^{b}	0.37^{cd}
	1.5	control	4.56^{e}	11.31^{b}	2.50^{a}	8.59^{d}	4.64^{ab}	0.54^{a}
		60	8.04^{c}	11.45^{b}	1.43^{d}	20.41^{a}	4.29^{b}	0.21^{f}
Treatment			Significantly					
Mycorrhizal (M)			30.75**	0.52^{ns}	0.82**	90.36**	18.51**	0.02*
Putrescine (P)			21.67**	4.03 ^{ns}	1.43**	42.85**	0.001^{ns}	0.005 ^{ns}
Salinity (S)			104.70**	0.510^{ns}	4.31**	148.25**	0.54^{ns}	0.18**
M×P			0.43^{ns}	6.91*	0.045^{ns}	3.58^{ns}	0.34 ^{ns}	0.004^{ns}
M×S			2.08*	1.15^{ns}	0.013 ^{ns}	7.13^{ns}	1.10*	0.10*
P×S			1.92*	6.44*	0.38**	117.70**	0.69^{ns}	0.051**
M×P×S			1.20^{ns}	6.40*	0.30^{ns}	10.54*	0.008 ^{ns}	0.001^{ns}
Error			0.37	1.15	0.02	1.92	2.80	0.002
C.V.			7.38	9.20	9.82	12.80	17.80	13.60

Values represent the means ± standard errors of three independent replications (n=3). Different letters within the same column indicate significant differences at $P \leq 0.05$ among the treatments, according to Duncan's multiple range test.

results are consistent with the findings of Wagner *et al*. (2021) who instated Na^+ decreased and K^+/Na^+ increased in the leaf of inoculated plants with mycorrhizal fungi compared with non-inoculated plants.

Photosynthetic pigments: The leaf chlorophyll content is an important physiological indicator for plant photosynthetic capacity and was significantly affected by all three factors (mycorrhizal, putrescine, and salinity). Salinity decreased chl *a*, chl *b*, total chlorophyll, and carotenoids in untreated and treated plants with mycorrhizal and putrescine. Exogenous putrescine improved photosynthetic pigments under the salinity stress conditions in non-inoculated plants. Also, mycorrhizal inoculated improved chl *a*, chl *b*, and total chlorophyll contents in the plants under normal and stress conditions compared to the untreated plants (Table 2). Under normal conditions the contents of chl *a*, chl *b* total chlorophyll and carotenoids increased significantly by 96, 83, 90, and 79%, respectively, in co-treated plants with mycorrhizal and putrescine compared to the control plants. Also, under saline conditions (60 mM NaCl), simultaneous application of mycorrhizal and putrescine increased the contents of chl *a*, chl *b*, total chlorophyll, and carotenoids by 35, 30, 34, and 21 % respectively, compared to the untreated plants (Table 2). Chlorophyll pigments had a significant and negative correlation with Na^+ and ROS in the leaves, and had a significant and positive correlation with MSI, RWC in the leaves, Na^+ in the roots, K^+ and K^+/Na^+ ratio in the leaves and roots. Also, there was a significant and positive correlation (0.895^{**}) between carotenoids and K^+ in the leaf (Table 4). Decrease in photosynthetic pigments under saline conditions mainly due to rapid disruption and inhibition of synthesis that exogenous putrescine can enhance salt tolerance by regulation of photosynthetic pathways (Bueno and Cordovilla, 2021; Islam *et al*., 2021). One reason for chlorophyll reduction during salinity stress induces the production of activated oxygen species which in turn destroys and decreases pigments. On the other hand, chlorophyll molecules decompose within chloroplasts and the thylakoid structure disappears (Cao *et al*., 2015; Nxele *et al*., 2017). Likely caused by repression of specific enzymes of the photosynthesis system and reduction of nutrient uptakes such as Mg and N for chlorophyll biosynthesis. Putrescine plays an essential role in protecting thylakoid membranes through a chlorophyll-protein complex site and positively impacts chlorophyll (Besford *et al.*, 1993). Previous studies showed that mycorrhizal (Zai *et al.*, 2012; Haque and Matsubara, 2018; Wang *et al*., 2019) and putrescine (Islam *et al.*, 2021) increased photosynthetic pigments in different plants under stress and normal conditions. The higher K^+/Na^+ ratio in the leaf of the plants treated by arbuscular mycorrhizal fungi and putrescine can help the maintenance of structural and functional chloroplasts, which is consistent with the results of Wang *et al.* (2021). Also, the improving effects of

[DOR: 20.1001.1.23222727.1402.12.55.8.0]

Table 2. Effect of soil application of Mycorrhizal and putrescine on contents of photosynthetic pigments in strawberry plants under salinity stress conditions.

Mycorrhizal (g/pot plant)	Putrecine (mM)	Salinity (mM NaCl)	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Total Chl (mg/g FW)	CARs (mg/g FW)
control	control	control	0.62 ^{d}	0.24 ^{cd}	0.87 ^{cd}	0.29 ^{bc}
		60	0.48 ^{e}	0.20 ^{d}	0.68 ^{e}	0.28 ^{c}
	1.5	control	0.66 ^{cd}	0.28 ^{bcd}	0.94 ^{cd}	0.33 ^{bc}
		60	0.62 ^{d}	0.25 ^{bcd}	0.87 ^{d}	0.31 ^{bc}
30	control	control	0.84 ^{b}	0.34 ^{b}	1.17 ^{b}	0.39 ^{b}
		60	0.75 ^{bc}	0.29 ^{bc}	1.04 ^{bc}	0.35 ^{bc}
	1.5	control	1.21 ^{a}	0.44 ^{a}	1.66 ^{a}	0.52 ^{a}
		60	0.65 ^{cd}	0.26 ^{bcd}	0.91 ^{cd}	0.34 ^{bc}
Treatment			Significantly			
Mycorrhizal (M)			0.42**	0.046**	0.759**	0.055**
Putrescine (P)			0.078 **	0.010 *	0.145**	0.012*
Salinity (S)			0.258**	0.032**	0.473**	0.026**
M×P			0.004 ^{ns}	0.0001^{ns}	0.003 ^{ns}	0.0005^{ns}
M×S			0.082**	0.009 *	0.148**	0.012*
P×S			0.049 **	0.005 ^{ns}	0.087**	0.009 ^{ns}
M×P×S			0.124**	0.009 *	0.203**	0.007 ^{ns}
Error			0.003	0.002	0.009	0.002
C.V.			7.96	16.32	9.33	14.60

Values represent the means ± standard deviation of three independent replications (n=3). Different letters within the same column indicate significant differences at P ≤ 0.05 among the treatments, according to Duncan's multiple range test. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (total Chl), and carotenoids (CARs). ns, *, and ** show non-significant, significant at 5 and 1% respectively. C.V., is coefficient variance.

mycorrhizal on photosynthetic pigments can be due to an increase in the plant's capacity to repair photosystem II (PSII) and the quantum efficiency of PSII under salinity stress by mycorrhizal via increasing the level of transcripts of chloroplast genes that encode antenna proteins in excitation energy transfer (Evelin *et al*., 2019).

Leaf water status, membrane stability index: RWC and MSI were significantly affected by salt stress (Table 3). Salinity decreased the content of RWC and MSI in all treatments. In the plants treated with mycorrhizal, putrescine, and both, the RWC and MSI content was higher than the untreated plants in the saline and normal conditions. In the salinity levels, the MSI content increased by 55 % in the plants treated with both mycorrhizal and putrescine compared with the untreated plants (Table 3). There was a positive and significant correlation (0.658**) between RWC and MSI. Also, there was a positive correlation between RWC and MSI with K^+ content in the root, K^+/Na^+ ratio in leaf and root and photosynthetic pigments, and a significant negative correlation with Na^+ leaf and ROS (Table 4). Membranes are the first place in the cell which are affected under stress conditions, and plant tolerance to stress conditions is determined by the plant's ability to protect the integrity of membranes under stress conditions. Under stress, soil water potential decreases, and plants prevent transpiration by using various mechanisms such as closing stomata, increasing stomatal resistance, and decreasing stomatal conductivity (Zahedi *et al.*, 2020a). Mycorrhizal fungi by restriction of Na^+ entrance of root to photosynthetic organs increases, water balance and mineral nutrition help to salt stress tolerance in colonized plants (Guerrero-Galan *et al*., 2019). Previous studies showed that mycorrhizal (Haque and Matsubara, 2018; Wang *et al*., 2019) and putrescine (Islam *et al*., 2021) improved RWC and MSI in different plants under stress and normal conditions.

Reactive oxygen species: In the untreated plants with mycorrhizal and putrescine, ROS content increased significantly in saline conditions. In the treated plants with mycorrhizal, putrescine and both, there was no significant difference between ROS content in saline and normal conditions and the co-treated plants with mycorrhizal and putrescine significantly prevented enhancing ROS in the saline conditions compared to the all treated (Table 3). ROS showed a significant negative correlation with photosynthetic pigments and K^+/Na^+ ratio in the root and a significant positive correlation with Na^+ content in the leaf (Table 4). Putrescine protects the plasma membrane by reducing ROS and Na^+ accumulation and increases antioxidant activities and photosynthetic capacity in the plant under salinity conditions (Alcazar *et al*., 2020; Islam *et al*., 2021). Abiotic stresses trigger the overproduction of ROS in

[DOR: 20.1001.1.23222727.1402.12.55.8.0]

Table 3. Effect of mycorrhizal inoculated and putrescine spraying on relative water content (RWC), memberane stability index (MSI), proline, soluble sugars, and anthocyanin content in strawberry plants under salinity stress conditions.

Mycorrhizal (g/pot plant)	Putrescine (mM)	NaCl (mM)	RWC (%)	MSI (%)	ROS (µmol/g FW)	Proline (µmol/g)	Soluble sugars (mg/g FW)	Anthocyanin (µmol/g)
control	control	control	74.6 ^{bc}	86.7^{ab}	0.52^{bc}	44.2 ^{e}	0.17 ^{bcd}	0.54 ^{ab}
		60	72.1 ^{c}	57.7 ^{c}	0.59^{a}	70.6 ^{b}	0.24 ^{a}	0.48 ^{b}
	1.5	control	80.7 ^{a}	89.2 ^{ab}	0.52 ^{bc}	58.0 ^{d}	0.16 ^{d}	0.62 ^{a}
		60	76.9 ^{abc}	85.5 ^{b}	0.55 ^{ab}	66.0 ^{c}	0.23 ^{ab}	0.58 ^{ab}
30	control	control	79.0 ^{ab}	87.9 ^{ab}	0.53 ^{abc}	57.0 ^{d}	0.18 ^{bcd}	0.50 ^{b}
		60	79.3 ^{ab}	85.8 ^{b}	0.55 ^{ab}	79.6 ^{a}	0.24 ^{a}	0.50 ^{b}
	1.5	control	81.7 ^{a}	91.1 ^{a}	0.47 ^{c}	70.5 ^{b}	0.18 ^{bcd}	0.52 ^{b}
		60	76.7 ^{abc}	89.4 ^{ab}	0.48 ^{c}	73.5 ^{b}	0.15 ^{d}	0.54 ^{ab}
Treatments					Significantly			
Mycorrhizal (M)			57.25**	462.26**	0.007*	658.24**	0.001 ^{ns}	0.104 ^{ns}
Putrescine (P)			44.79**	516.24**	0.009*	102.30**	0.004 ^{ns}	0.024*
Salinity (S)			44.52**	499.13**	0.005*	1353.75**	0.010**	0.001 ^{ns}
M×P			43.76**	203.99**	0.003^{ns}	1.22 ^{ns}	0.001 ^{ns}	0.008 ^{ns}
M×S			0.98^{ns}	314.14**	0.001^{ns}	29.68*	0.003 ^{ns}	0.006 ^{ns}
P×S			16.55*	246.33**	0.0005^{ns}	542.16**	0.002 ^{ns}	0.0006 ^{ns}
M×P×S			6.45 ^{ns}	231.57**	0.0003^{ns}	0.48 ^{ns}	0.002 ^{ns}	0.003 ^{ns}
Error			3.27	6.58	0.001	3.63	0.001	0.003
C.V.			2.33	3.04	6.35	2.93	17.28	10.8

Values represent the means ± standard errors of three independent replications (n=3). Different letters within the same column indicate significant differences at P ≤ 0.05 among the treatments, according to Duncan's multiple range test. ns, *, and ** show non-significant, significant at 5 and 1% respectively. C.V., is coefficient variance.

plant cells, which becomes a major challenge for optimal plant growth. Generally, ROS damages molecular and cellular components via oxidation of biological molecules such as carbohydrates, lipids, proteins, enzymes, and DNA and causes plant death (Sachdev *et al*., 2021). Proline is the main osmotic adjustment molecule with a low molecular weight that plays a crucial role in regulating redox potential (Hong-Bo *et al*., 2006), scavenging hydroxyl radical (Kishore *et al.*, 2005), thus mitigating oxidative damage and stabilizing the cell membrane (Zhu *et al*., 2023) under stress conditions. Proline accumulation is a vital indicator for plant response to salt stress to protect them from such damage (Forlani *et al*., 2019). In this study, osmolyte proline accumulated in the leaf under salinity conditions, and exogenous putrescine and spermidine treatment increased the accumulation of more proline, which indicates an adaptation to salinity (Sathiyaraj *et al*., 2014; Islam *et al*., 2021). Accumulation of proline may represent a major physiological adaptation in plants for osmotic adjustment and homeostasis of redox, such as proline increases in N levels and endogenous proline content (Kaur and Asthir, 2015). Besides, amino acids play a pivotal role in protein biosynthesis (Yang *et al*., 2020).

Osmotic adjustment molecules: Salinity induced proline accumulation in strawberry leaves (Table 3), wherein the level of this amino acid grew with salinity, and there was a significant positive correlation between proline and Na content in the root (Table 4). Application of mycorrhizal fungi and putrescine increased proline content significantly compared with nontreated plants in normal and saline conditions. Accumulation of proline is a common physiological response in plants exposed to various abiotic stress and has different roles under stress conditions, such as stabilization of proteins, membranes, subcellular structures, protecting cellular functions by scavenging reactive oxygen species (ROS) (Kaur and Asthir, 2015). An increase in proline could be due to de novo synthesis, decreased degradation, lower utilization, or hydrolysis of proteins (Kaur and Asthir, 2015). The accumulation of proline and also sugar under salinity stress are adaptive mechanisms that can improve salt tolerance in plants (Karimian and Samiei, 2021). Previous studies showed that mycorrhizal fungi (Haque and Matsubara, 2018; Wang *et al.*, 2019) and putrescine (Bueno and Cordovilla, 2021; Islam *et al*., 2021) increased proline in the different plants under stress and normal conditions. Salinity treatment increased significantly soluble sugars in the untreated plants and were treated with mycorrhizal and putrescine alone. But in the co-treated plants with both mycorrhizal and putrescine salinity not only couldn't increase soluble sugars but also decreased non-significantly soluble sugars. There was a positive significant correlation between soluble sugars with Na^+ and ROS in the leaf, and a significant negative correlation between soluble sugars with K^+/Na^+ ratio and MSI in the leaf

[DOR: 20.1001.1.23222727.1402.12.55.8.0]

Table 4. Pearson correlation coefficient between some of evaluated parametrs

	Chl *a* (1)	Chl *b* (2)	Chl total (3)	CARs (4)	Na leaf (5)	Na root (6)	K leaf (7)	K root (8)	K/Na leaf (9)	K/Na root (10)	ROS (11)	MSI (12)	RWC (13)	Proline (14)	Soluble sugars (15)
1	0.918**														
2	0.994**	0.956**													
3	0.912**	0.933**	0.933**												
4	-0.723**	-0.682*	-0.724**	-0.600**											
5	-0.108ns	-0.156ns	-0.123ns	-0.080ns	0.095 ns										
6	-0.025ns	0.067ns	0.000 ns	0.895**	-0.122ns	-0.147ns									
7	0.642 **	0.552 **	0.628 **	0.904 **	-0.517**	0.401 *	-0.039ns								
8	0.743**	0.704**	0.745 **	0.909 **	-0.898**	-0.254ns	0.422*	0.434*							
9	0.672**	0.674**	0.683 **	0.683 **	-0.633**	-0.668**	0.194ns	0.362ns	0.728 **						
10	-0.552**	-0.576*	-0.567**	-0.597**	0.623**	-0.174ns	0.111 ns	-0.337ns	-0.498 *	-0.259ns					
11	0.508*	0.458 *	0.503 *	0.368 ns	-0.803**	0.204 ns	0.101 ns	0.461 *	0.599 **	0.274 ns	-0.567**				
12	0.612**	0.546**	0.604**	0.483*	-0.674**	-0.084ns	0.297 ns	0.432 *	0.717 **	0.518 **	-0.390ns	0.658**			
13	0.142 ns	0.086 ns	0.129 ns	0.189 ns	0.338 ns	0.442 *	0.062 ns	0.281 ns	-0.194ns	-0.234ns	0.027 ns	-0.165ns	0.144ns		
14	-0.199ns	-0.165ns	-0.193ns	-0.192ns	0.604 **	-0.108ns	0.208 ns	-0.132ns	-0.432 *	-0.140ns	0.427*	-0.434*	-0.305ns	0.339ns	
15	-0.103ns	0.031ns	-0.069ns	-0.035ns	0.189 ns	-0.013ns	0.133 ns	-0.328ns	0.142 ns	-0.154ns	-0.194ns	0.307ns	0.157ns	-0.194ns	-0.329ns

****, *, and ns show significant in P≤ 0.01%, P≤ 0.05% and no significant respectively**
Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (total Chl), and carotenoids (CARs).

(Table 4). Rahimi and Biglarifard (2011) reported that salinity treatment increased significantly soluble sugars in strawberry plants (cv. Camarrosa).

Controlled foliar spraying of putrescine can trigger physiological processes and induce osmotic adjustment molecules like proline, total soluble sugars, and total soluble protein (Chen *et al*., 2019) along with antioxidant enzymes to maintain the optimum level of ROS (Islam *et al*., 2021). Putrescine reduces cell membrane damage by lowering lipid peroxidation, which was confirmed through reduced MDA (Islam *et al*., 2021). We found that the leaf concentration of proline and soluble sugars was significantly higher in the co-treated plants with both mycorrhizal and putrescine compared to the control plants (Table 2). Accumulation of soluble sugars and amino acids, spatially proline, is a well-known response to osmotic stress in plants (Cirillo *et al*., 2021). Proline as a buffer of cellular redox potential and ROS scavenger, stabilizing membranes and proteins, and can also induce the expression of salt stress-responsive genes, in particular genes with proline responsive elements in their promoters (Carillo, 2018). In addition, proline can rapidly metabolize when no longer required to supply energy, carbon, and nitrogen to recover and repair stress-induced damage (Carillo and Gibon, 2011). Also, the plants co-treated with both mycorrhizal and putrescine showed higher CO_2 assimilation rates and lower fresh weight reduction compared to untreated plants under saline conditions. These results point to a strong modulation of plant metabolism induced by anthocyanins, which act as a checkpoint for many responses that lead to higher resilience to salinity. Anthocyanin increased proportionally by putrescine application under normal and saline conditions compared to control. Also, under saline conditions, the application of putrescine, mycorrhizal fungi, and both of them increased proportionally anthocyanin compared to the control (Table 3).

Conclusion

Simultaneous use of mycorrhizal fungi and putrescine by the accumulation of Na^+ in the roots decreased the transfer of Na^+ to the leaves. Also, the increasing K^+/Na^+ ratio in leaves decreased ROS, and increased MSI and RWC, and maintained photosynthetic pigments. Key osmolytes such as total soluble sugars and proline. The application of putrescine improved the accumulation of proline, total soluble sugars, and anthocyanin in plant leaves. Therefore, modulation of physio-biochemical processes reduced the level of ROS, which indicates a successful adaptation of strawberry plants to salinity stress. Finally, our results support the use of bio-inoculants in saline horticultural areas and provide an important prospect for the use of putrescine in modulating salinity tolerance

References

Albalasmeh, A. A., Berhe, A. A., & Ghezzehei, T. A. (2013). A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate Polymers, 97*(2), 253-261. https://doi.org/10.1016/j.carbpol.2013.04.072.

Alcazar, R., Bueno, M., & Tiburcio, A. F. (2020). Polyamines: Small amines with large effects on plant abiotic stress tolerance. *Cells, 9*, 2373. https://doi.org/10.3390/cells9112373.

Almeid, D. M., Oliveira, M. M., & Saibo, N. J. M. (2017). Regulation of Na^+ and K^+ homeostasis in plants: Towards improved salt stress tolerance in crop plants. *Genetics and Molecular Biology, 40* (1), 326-345. https://

[DOR: 20.1001.1.23222727.1402.12.55.8.0]

10.1590/1678-4685-GMB-2016-0106.

Barrs, H. D., & Weatherley, P. E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences, 15* (3), 413-428.

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. *Frontiers in Plant Science*, *10*, 1068. https://doi.org/10.3389/fpls.2019.01068.

Besford, R. T., Richardson, C. M., Campos, J. L., & Tiburcio, A. F. (1993). Effect of polyamines on stabilization of molecular complexes in thylakoid memberanes of osmotically stressed oat leaves. *Planta, 198*, 201-2016.

Bose, J., Rodrigo-Moreno, A., & Shabala, S. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. *Journal of Experimental Botany*, 65, 1241–1257. https:// doi: 10.1093/jxb/ert430.

Bowles, T. M., Barrios-Masias, F. H., Carlisle, E. A., Cavagnaro, T. R., & Jackson, L. E. (2016). Effects of arbuscular mycorrhizale on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. *Science of the Total Environment, 566*, 1223–1234. https://doi: 10.1016/j.scitotenv.2016.05.178.

Bueno, M., & Cordovilla, M. P. (2021). Spermidine pretreatments mitigate the effects of saline stress by improving growth and saline excretion in *Frankenia pulverulenta*. *Agronomy, 11* (8), 1515. https://doi.org/10.3390/ agronomy 11081515.

Cao, B. L., Ma, Q., Zhao, Q., Wang, L., & Xu, K. (2015). Effect of silicon on absorbed light allocation, antioxidant enzymes and ultrastructure of chloroplast in tomato leaves under simulated drought stress. *Scientia Horticulturae, 194*, 53-62. https://doi.org/10.1016/j.scienta.2015.07.037.

Carillo, P. (2018). GABA shunt in durum wheat. *Frontiers in Plant Science, 9*, 100. https://doi.org/10.3389/fpls.2018.00100.

Carillo, P., & Gibon, Y. (2011). Protocol: Extraction and Determination of Proline. PrometheusWiki.

Cirillo, V., D'Amelia, V., Esposito, M., Amitrano, C., Carillo, P., Carputo, D., & Maggio, A. (2021). Anthocyanins are key regulators of drought stress tolerance in tobacco. *Biology, 10*(2), 139. https:// doi: 10.3390/biology10020139.

Chandrasekaran, M., Chanratana, M., Kim, K., Seshadri, S., & Sa, T. (2019). Impact of arbuscular mycorrhizal fungi on photosynthesis, water status, and gas exchange of plants under salt stress—a meta-analysis. *Frontiers in Plant Science*, *10*, 457. https://doi.org/10.3389/fpls.2019.00457.

Chen, D., Shao, Q., Yin, L., Younis, A., & Zheng, B. (2019). Polyamine function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in Plant Science, 9*, 1945. https://doi.org/10.3389/fpls.2018.01945.

Evelin, H., Devi, T. S., Gupta, S., & Kapoor, R. (2019). Mitigation of salinity stress in plants by arbuscular mycorrhizal symbiosis: Current understanding and new challenges. *Frontiers in Plant Science, 10*, 470. https://doi.org/10.3389/fpls.2019.00470.

Ferreira, J. F. S., Liu, X., & Suarez, D. L. (2019). Fruit yield and survival of five commercial strawberry cultivars under field cultivation and salinity stress. *Scientia Horticulturae, 243*, 401-410. *https://doi.org/10.1016/j.scienta.2018.07.016*.

Forlani, G., Trovato, M., Funck, D., & Signorelli, S. (2019). Regulation of proline accumulation and its molecular and physiological functions in stress defence. *Osmoprotectant-Mediated Abiotic Stress Tolerance in Plants*, 73-97. https://doi.org/10.1007/978-3-030-27423-8-3.

Hong-Bo, S., Xiao-Yan, C., Li-Ye, C., Xi-Ning, Z., Gang, W., Yuan, Y., Chang-Xing, Z., & Zan-Min, H. (2006). Investigation on the relationship of proline with wheat anti-drought under soil water deficits. Colloids Surf. B: *Biointerfaces, 53*, 113-119. https://doi.org/10.1016/j.colsurfb.2006.08.008.

Guerrero-Galan, C., Calvo-Polanco, M., & Dagmar Zimmermann, S. (2019). Ectomycorrhizal symbiosis helps plants to challenge salt stress conditions. *Mycorrhizal, 29*, 291–301.

Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *International Journal of Genomics*, ID701596. https://doi.org/10.1155/2014/701596.

Haque, S. I., & Matsubara, Y. (2018). Salinity tolerance and sodium localization in mycorrhizal strawberry plants. *Communications in Soil Science and Plant Analysis, 49* (22), 2782-2792. https://doi.org/10.1080/00103624.2018.1538376.

He, F., Sheng, M., & Tang, M. (2017). Effects of Rhizophagus irregularis on photosynthesis and antioxidative enzymatic system in *Robinia pseudoacacia* L. under drought stress. *Frontiers in Plant Science, 8*, 183. https://doi.org/10.3389/fpls.2017.00183.

Hussein, H. A. A., Mekki, B. B., El-Sadek, M. E. A., & El Lateef, E. E. (2019). Effect of L-Ornithine application on improving drought tolerance in sugear beet plants. *Heliyon, 5* (10), e02631. https://doi.org/10.1016/j.heliyon.2019.e02631.

Islam, M. J., Ryu, B. R., Azad, M. O. K., Rahman, M. H., Rana, M. S., Lim, J. D., & Lim, Y. S. (2021). Exogenous putrescine enhances salt tolerance and ginsenosides content in Korean ginseng (*Panax ginseng* Meyer) sprouts. *Plants, 10*, 1313. https://doi.org/10.3390/plants10071313.

Jons, J. (2001). Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press, Boca Raton. USA. https://doi.org/10.1201/9781420025293.

Karimian, Z., & Samiei, L. (2021). Mitigation of salt stress by mycorrhizal inoculation on *Nitraria schoberi* as a native landscape plant in the arid regions. *Desert*, *26*(1), 16-27. https://doi.org/10.22059/JDESERT.2020.284473.1006735.

Kaur, G., & Asthir, B. (2015). Proline: A key player in plant abiotic stress tolerance. *Biologia plantarum*, *59*, 609-619. https://doi.org/10.1007/s10535-015-30549.

Kumar, S., Li, G., Yang, J., Huang, X., Ji, Q., Liu, Z., Ke, W., & Hou, H. (2021). Effect of salt stress on growth, physiological parameters and ionic concentration of water dropwort (*Oenanthe javanica*) cultivars. *Frontiers in Plant Science*, *12*, 660409. https://doi.org/10.3389/fpls.2021.660409.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, *148*, 350-382. *https://doi.org/10.1002/0471142913.faf0403s01*.

Mishra, P., Bhoomika, K., & Dubey, R. S. (2013). Differential responses of antioxidative defense system to prolonged salinity stress in salt-tolerant and salt-sensitive Indica rice (*Oryza sativa* L.) seedlings. *Protoplasma*, *250*, 3–19. https://doi.org/10.1007/s00709-011-0365-3.

Nxele, X., Klein, A., & Ndimba, B. K. (2017). Drought and salinity stress alters ROS accumulation, water reaction, and osmolyte content in sorghum plants. *South African Journal of Botany*, *108*, 261-266. *https://doi.org/10.1016/j.sajb.2016.11.003*.

Rahimi, A., & Biglarifard, A. (2011). Impacts of NaCl stress on proline, soluble sugars, photosynthetic pigments and chlorophyll florescence of strawberry. *Advances in Environmental Biology*, *5*(4), 617-623.

Roshdy, A. E. D., Alebidi, A., Almutairi, K., Al-Obeed, R., & Elsabagh, A. (2021). The effect of salicylic acid on the performances of salt stressed strawberry plants, enzymes activity, and salt tolerance index. *Agronomy*, *11*, 775. *https://doi.org/10.3390/agronomy11040775*.

Sathiyaraj, G., Srinivasan, S., Kim, Y. J., Lee, O. R., Parvin, S. Balusamy, S. R. D., Khorolragchaa, A., & Yang, D. C. (2014). Accumulation of hydrogen peroxide enhances salt tolerance by activating defence-related proteins in *Panax ginseng* CA Mayer. *Molecular Biology Reports, 41*, 3761-3771. https://doi.org/10.1007/s11033-014-3241-3.

Seo, S. Y., Kim, Y. J., & Park, K. Y. (2019). Increasing polyamine cnontents inhanced the stress tolerance via reinforcement of antioxidative properties. *Frontiers in Plant Science*, *10*, 1331. https://doi.org/10.3389/fpls.2019.01331.

Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., & Hasanuzzan, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidunts*, *10*(2), 277. https://doi.org/10.3390/antiox10020277.

Tirani, M., & Haghjou, M., (2019). Reactive oxygen species (ROS), total antioxidant capacity (AOC) and malondialdehyde (MDA) make a triangle in evaluation of zinc stress extension. *Journal of Animal and Plant Sciences*, *29*, 1100-1111.

Todorova, D., Katerova, Z., Alexieva, V., & Sergiev, I. (2015). Polyamines- possibilities for application to increase plant tolerance and adaptation capacity to stress. *Genetic and Plant Physiology*, *5*, 123–144.

Wagner, G. J. (1979). Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanin in protoplasts. *Plant Physiology*, *64*(1), 88-93. *https://doi.org/10.1104/pp.64.1.88*.

Wang, H., An, T., Huang, D., Liu, R., Xu, B., Zhang, S., Deng, Siddique, X. K. H. M., & Chen, Y. (2021). Arbuscular mycorrhizal symbioses alleviating salt stress in maize is associated with a decline in root-to-leaf gradient of Na^+/K^+ ratio. *BioMed Central Plant Biology, 21*,457. https://doi.org/10.1186/s12870-021-03237-6.

Wang, J., Fu, Z., Ren, Q., Zhu, L., Lin, J., Zhang, J., Cheng, X., Ma, J., & Yue, J. (2019). Effects of Arbuscular mycorrhizal fungi on growth, photosynthesis, and nutrient uptake of *Zelkova serrate* (Thunb) Makino seedling under salt stress. *Forests, 10*, 186. https://doi.org/10.3390/f10020186.

Yang, Q., Zhao, D., & Liu, Q. (2020). Connections between amino acid metabolisms in plants: Lysine as an example. *Frontiers in Plant Science, 11*, 928. https://doi.org/10.3389/fpls.2020.00928.

Yuan, Y., Zhong, M., Shu, S., Du, N., Sun, J., & Guo, S. (2016). Proteomic and physiological analyses reveal putrescine responses in roots of cucumber stressed by NaCl. *Front. Plant Science*, 7, 1035. doi: 10.3389/fpls.2016.01035.

Yue, J. M., You, Y. Y., Zhang, L., Fu, Z. Y., Ang, J. P., Zhang, J. C., & Report, D. G. (2019). Exogenous 24-epibrassinolide alleviates tolerance in *Robinia psedacacia* L. seedling. *Journal of Plant Growth Regulation*, *38*(2), 1-14. https://doi.org/ 10.1007/s00344-018-9881-0.

Zahedi, S. M., Moharrami, F., Sarikhani, S., & Padervand, M. (2020a). Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. *Scientific Reports, 10* (1), 17672. https://doi.org/ 10.1038/s41598-020-74273-9.

Zahedi, S. M., Hosseini, M. S., Abadia, J., & Marjani, M. (2020b). Melatonin foliar sprays elicit salinity stress tolerance and enhance fruit yield and quality in strawberry (*Fragaria* × *ananassa* Duch.). *Plant Physiology and Biochemistry*, *149*, 313-323. *https://doi.org/10.1016/j.plaphy.2020.02.021*.

Zai, X. M., Zhu, S. N., Qin, P., Wang, X. Y., Che, L., & Luo, F. X. (2012). Effect of *Glomus mossea* eon chlorophyll content, chlorophyll fluorescence parameters, and chloroplast ultrastructure of beach plum (*Prunus maritime*) under NaCl stress. *Photosynthetica, 50*(3), 323-328. https://doi.org/ 10.1007/s11099-012-0035-5.

Zhu, J., Cai, Y., Wakisaka, M., Yang, Zh., Yin, Y., Fang, W., Xu, Y., Omura, T., Yu, R., & Zheng, A. L. T. (2023). Mitigation of oxidative stress damage caused by abiotic stress to improve biomass yield of microalgae: A review. *Science of the Total Environment, 896*. https://doi.org/10.1016/j.scitotenv.2023.165200.

[DOR: 20.1001.1.23222727.1402.12.55.8.0]