Arbuscular Mycorrhizal Fungi Modulate Photosynthetic Gene Expression (*rbcl, rbcs, psbA, psbD*) to Enhance Salinity Tolerance in *Pistacia vera*

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Abstract

This study investigated the role of arbuscular mycorrhizal fungi (AMF) in mitigating salinity stress in *Pistacia vera* L. cv. Ohadi. A greenhouse pot experiment was conducted using a randomized complete block design with two factors: *Glomus mosseae* inoculation (inoculated or non-inoculated) and salinity (control or 12 dS m^{-1} NaCl). While salinity reduced AMF colonization from 74% to 39%, AMF-inoculated plants consistently exhibited superior performance compared to non-mycorrhizal plants. Salinity stress significantly decreased shoot and root biomass, total chlorophyll content, and net photosynthetic rate (Pn) in both groups. However, AMF symbiosis significantly ameliorated these negative effects, resulting in higher biomass, chlorophyll content, and a notably higher Pn (38.8% increase) under saline conditions. Furthermore, AMF inoculation altered the chlorophyll a/b ratio under salinity, suggesting an adaptive response in light-harvesting. Molecular analysis revealed that while salinity downregulated *psbA*, *psbD*, *rbcL*, and *rbcS* expression, AMF differentially upregulated *psbA* (under both conditions), *psbD* (specifically under salinity), and *rbcL* (under both conditions). Additionally, AMF improved shoot potassium (K) content and upregulated the expression of the *SKOR* gene, involved in K^+ transport, under both control and saline conditions. These findings demonstrate that AMF symbiosis enhances salinity tolerance in pistachio by improving K nutrition, modulating photosynthetic gene expression, and consequently, maintaining photosynthetic efficiency under stress.

Keywords: Pistachio, Mycorrhizae, photosynthesis, gene expression

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Introduction

Salinity stress represents a formidable and escalating threat to global agricultural productivity, impacting vast swathes of arable land and jeopardizing food security for a growing population. The accumulation of soluble salts, primarily sodium chloride (NaCl), in the soil solution imposes severe

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constraints on plant growth and development, disrupting essential physiological processes and ultimately leading to reduced yields and even plant mortality (Hao et al., 2021). Among the myriad detrimental effects of salinity, the inhibition of photosynthesis stands out as a primary mechanism underlying growth reduction. Elevated salt concentrations induce osmotic stress, limiting water uptake and causing stomatal closure, which in turn restricts CO_2 availability for carbon fixation (Zhao et al., 2021). Furthermore, salinity can lead to ionic toxicity, particularly from the accumulation of Na^+ and Cl^- ions within plant tissues, disrupting enzyme activity and damaging cellular structures, including the photosynthetic apparatus (Zhou et al., 2024). The intricate interplay of these factors results in a decline in photosynthetic efficiency, hindering the plant's ability to generate the energy required for growth and survival under saline conditions (Zahra et al., 2022).

Pistacia vera L., the pistachio tree, is a commercially significant crop cultivated in arid and semi-arid regions worldwide, valued for its nutritious nuts. However, these regions are frequently characterized by high soil salinity, rendering pistachio production particularly vulnerable to the adverse impacts of salt stress (Kashaninejad et al., 2006). While pistachio exhibits a degree of inherent salt tolerance compared to some other crops, elevated salinity levels demonstrably reduce its growth, yield, and nut quality (Igwegbe et al., 2023; Mandalari et al., 2021).Understanding the mechanisms underlying pistachio's response to salinity and developing strategies to enhance its tolerance are therefore crucial for ensuring sustainable production in salt-affected areas.

In the quest for sustainable solutions to mitigate salinity stress in agriculture, considerable attention has been directed towards harnessing the beneficial interactions between plants and soil microorganisms (Sadhana, 2014). Among these, arbuscular mycorrhizal fungi (AMF) stand out as ubiquitous and ecologically significant symbionts, forming mutualistic associations with the roots of the vast majority of terrestrial plant species, including *Pistacia vera*. These fungi establish an extensive network of hyphae that extend beyond the root zone, effectively increasing the plant's access to soil resources, particularly immobile nutrients like phosphorus (P) and micronutrients (Bhantana et al., 2021). Beyond nutrient acquisition, a growing body of evidence indicates that AMF symbiosis can confer enhanced tolerance to various abiotic stresses, including drought, heavy metal toxicity, and, crucially, salinity (Diagne et al., 2020; Wahab et al., 2023).

The mechanisms by which AMF alleviate salinity stress in host plants are multifaceted and encompass both nutritional and non-nutritional effects. Improved nutrient uptake, particularly of phosphorus, potassium (K), and essential micronutrients, can counteract the nutrient imbalances often induced by salinity (Sun et al., 2024). Specifically, maintaining adequate K^+ levels is critical under saline conditions, as Na^+ competes with K^+ for uptake and transport, disrupting K^+/Na^+ homeostasis and impairing vital cellular functions (Seutra Kaba et al., 2021). AMF have been shown to enhance K+ acquisition and improve K^+/Na^+ ratios in plants subjected to salinity, contributing to osmotic adjustment and mitigating ion toxicity (Seutra Kaba et al., 2021). Furthermore, AMF can influence plant water relations, potentially through improved hydraulic conductivity or altered aquaporin expression, leading to enhanced water uptake and maintenance of turgor pressure under osmotic stress (Liu et al., 2023). In addition, AMF colonization can stimulate the plant's antioxidant defense system, reducing oxidative damage caused by reactive oxygen species (ROS), which are generated under salinity stress (Wei et al., 2023b). Changes in phytohormone profiles, such as increased abscisic acid (ABA) and decreased ethylene levels, have also been implicated in AMF-mediated stress tolerance (Wei et al., 2023a).

Crucially, the impact of AMF on plant photosynthetic performance under salinity stress is gaining increasing recognition. Several studies have reported that AMF colonization can maintain or even enhance photosynthetic rates in plants exposed to salt, mitigating the inhibitory effects of salinity on this vital process (Fan et al., 2024). This improvement in photosynthetic capacity can be attributed to several factors, including enhanced nutrient status, improved water relations, and reduced oxidative stress, all of which contribute to the protection and efficient functioning of the photosynthetic machinery (RAZVI et al., 2023).

However, the specific molecular mechanisms underlying AMF-mediated modulation of photosynthesis under salinity stress, particularly at the level of gene expression, remain to be fully elucidated.

Photosynthesis relies on the coordinated expression of numerous genes encoding proteins involved in light harvesting, electron transport, and carbon fixation. Key components of the photosynthetic apparatus, such as photosystem II (PSII) and the Rubisco enzyme, are particularly vulnerable to salinity-induced damage (Vineeth et al., 2023). The psbA and psbD genes encode the D1 and D2 proteins, respectively, which form the reaction center core of PSII and are crucial for electron transport (Mukai et al., 2024). Salinity stress can lead to the downregulation of these genes, impairing PSII function and reducing photosynthetic efficiency. Similarly, the rbcL and rbcS genes encode the large and small subunits of Rubisco, the enzyme responsible for catalyzing the initial step of carbon fixation in the Calvin cycle. Salinity-induced reductions in rbcL and rbcS expression can limit Rubisco activity and, consequently, the rate of CO_2 assimilation (Xu et al., 2024). Furthermore, the SKOR gene, encoding a Shaker-like outward-rectifying K^+ channel, plays a crucial role in potassium transport and homeostasis in plants. Maintaining adequate K^+ levels is essential for various physiological processes, including stomatal regulation and enzyme activation, which are directly relevant to photosynthetic performance under salinity stress (Xu et al., 2024).

Therefore, this study aims to investigate the role of AMF (*Glomus mosseae*) in modulating the expression of key photosynthetic genes (rbcL, rbcS, psbA, psbD) and the potassium channel gene SKOR in *Pistacia vera* under salinity stress. By examining the interplay between AMF symbiosis, gene expression, and physiological parameters such as biomass, chlorophyll content, net photosynthetic rate, and shoot potassium concentration, we seek to gain a deeper understanding of the molecular mechanisms underlying AMF-mediated enhancement of salinity tolerance in this economically important crop. We hypothesize that AMF inoculation will mitigate the negative impacts of salinity on pistachio growth and photosynthesis by differentially regulating the expression of these crucial genes, thereby contributing to improved plant performance under saline conditions. This research will provide valuable insights into the intricate interactions between plants, AMF, and the environment, potentially paving the way for the development of novel strategies to enhance crop resilience in the face of increasing salinity challenges.

Materials and Methods

A greenhouse pot experiment was conducted to assess the effects of mycorrhizal inoculation and salinity on pistachio (*Pistacia vera* L., cv. Ohadi) growth. Greenhouse conditions included a day/night temperature of 34 °C/22 °C, 40±5% relative humidity, a 14/10 h light/dark cycle, and a maximum photosynthetic photon flux density of 1020 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The soil-sand mixture (pH 7.4, EC 1.7 $dS \cdot m^{-1}$) was sterilized at 121 °C for 20 minutes and contained 79.3% sand, 15.1% clay, 5.6% silt, 1.3% organic matter, and essential nutrients (e.g., 11.2 $mg \cdot kg^{-1}$ P, 151 $mg \cdot kg^{-1}$ K, 37 $mg \cdot kg^{-1}$ N).

The experiment followed a 2×2 factorial randomized complete block design with six replicates, testing mycorrhizal inoculation (inoculated vs. non-inoculated) and salinity levels (control EC 0.78 $dS \cdot m^{-1}$ vs. salt stress EC 12 $dS \cdot m^{-1}$). Pot positions were randomized every 5 days to reduce environmental variability. Seeds were surface-sterilized with 5% sodium hypochlorite, incubated at 30 °C for one week, and planted in pots containing a silty clay soil-sand mixture. After germination, seedlings were thinned to four per pot and irrigated every five days with municipal water (EC 0.78 $dS \cdot m^{-1}$) to maintain field capacity.

Arbuscular mycorrhizal (AM) fungal inoculum, *Glomus mosseae*, was obtained from the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM) and propagated in sterilized sand with corn plants as hosts. The inoculum consisted of soil, hyphae, spores (10–15 spores/g), and infected root fragments (71 % infection). During sowing, half of the pots received 100 g of *G. mosseae* inoculum, while the others received autoclaved inoculum (Afshar and Abbaspour, 2023). After three months of growth, salinity stress was

applied by irrigating with saline water (EC 12 $dS \cdot m^{-1}$) for one month. Control pots received non-saline water. Plants were harvested four months after sowing, washed, and analyzed for growth responses.

Shoot and root biomass were determined by carefully harvesting the plants, separating shoots and roots, washing the roots free of soil, and oven-drying both plant parts at 70 °C until constant weight was achieved. Chlorophyll content and the chlorophyll a/b ratio were quantified spectrophotometrically from fresh leaf tissue. A known weight of leaf material was extracted in 80% acetone, and the absorbance of the extract was measured at 663 nm, 645 nm, and 652 nm to calculate chlorophyll a and chlorophyll b concentrations using established equations. Net photosynthesis rate was measured on fully expanded, intact leaves using a portable photosynthesis system (LI-COR 6400XT) under controlled light and CO_2 conditions. Measurements were taken at a saturating light intensity (1000 µmol m^{-2} s^{-1}) and a constant CO_2 concentration (400 µmol mol^{-1}). For mycorrhizal assessment, fine root sections were segmented into 1 cm lengths, fixed and stained with trypan blue in lactophenol as per the protocols of Phillips and Hayman (Phillips and Hayman, 1970). The extent of mycorrhizal colonization was estimated using the gridline intersection technique described by Biermann and Linderman (Biermann and Linderman, 1981).

Total RNA was isolated from leaf tissues using the Easy Blue Absolute RNA Extraction Kit (iNtRON, South Korea) in accordance with the manufacturer's instructions. RNA integrity was verified by 1.0% agarose gel electrophoresis and quantified using a Nanodrop spectrophotometer (Eppendorf, Germany), with the A260/A280 ratio calculated to assess purity. Subsequently, first-strand cDNA synthesis was performed using the Power cDNA Synthesis Kit with gDNA Eraser PC5402 (iNtRON, South Korea). PCR primers specific to each target gene were designed based on *P. vera* sequences available in the NCBI GenBank database (Supplementary Table1). Quantitative real-time PCR (qRT-PCR) reactions were prepared by combining 0.4 µL of each primer, 10 µL of 2× Power SYBR Green PCR Master Mix, 2 µL of diluted cDNA template, and 7.2 µL of distilled water, and were conducted on an Applied Biosystems Step-One Plus system (ABI, CA, USA). The amplification protocol consisted of an initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 10 seconds and annealing/extension at 60 °C for 60 seconds. Each gene was analyzed in three biological replicates, and relative expression levels were quantified using the $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen (2001).

The collected data were analyzed using a one-way analysis of variance (ANOVA) conducted with SPSS software, version 22.0. Mean values, derived from six replicates per treatment, were reported alongside their standard errors (± S.E.) and presented in both tabular and graphical formats. To ascertain significant differences among the means, Tukey's Honestly Significant Difference (HSD) test was applied, with a significance threshold set at $p \leq 0.05$.

Results

Arbuscular mycorrhizal (AM) plants exhibited a mycorrhizal colonization rate of 74% under non-saline conditions, which significantly declined to 39% under a 12 dS m^{-1} NaCl treatment (Table 1). No mycorrhizal colonization was observed in non-mycorrhizal (NM) plants. Elevated salinity levels resulted in a reduction in shoot and root biomass in both NM and AM plants (Table 1). However, AM plants consistently demonstrated higher shoot and root biomass compared to NM plants across all salinity levels.

Salinity stress significantly reduced chlorophyll (Chl) content in both arbuscular mycorrhizal (AM) inoculated and un-inoculated pistachio seedlings. Nevertheless, under all salinity levels, AM-inoculated plants maintained a higher Chl content compared with un-inoculated plants, reflecting a protective effect of AMF against salt-induced chlorophyll degradation (Table 2). Additionally, salinity treatment significantly increased the Chl a/b ratio; under saline conditions, this increase was more pronounced in AM-inoculated plants than in uninoculated plants (Table 2). Salinity exerted a detrimental effect on the net photosynthetic rate (Pn) in both non-mycorrhizal (NM) and arbuscular mycorrhizal (AM) plants, with a more pronounced reduction observed in NM plants. Specifically, Pn declined by 22.6% in AM plants compared to a 50.4%

Table 1
The effects of *Glomus mosseae* arbuscular mycorrhizal fungi (AMF) inoculation on AM colonization percentage and biomass of pistachio plantlets were evaluated under two salinity conditions.

Treatment NaCl	AM status	AM colonization percentage (%)	Shoot biomass (g)	Root biomass (g)
Control	AM	74±5[a]	6.13±0.13[a]	4.21±0.14[a]
Control	NM	0.0	4.44±0.21[b]	3.52±0.17[b]
12 ds m^{-1}	AM	39±3[b]	3.46±0.12[c]	3.34±0.13[b]
12 ds m^{-1}	NM	0.0	2.55±0.18[d]	2.1±0.11[c]

Within each column, means with different superscript letters are significantly different ($p < 0.05$, LSD test).

Table 2
Effects of mycorrhizal inoculation and varying salinity levels on chlorophyll content, Chl a/b ratio, and net photosynthesis rate (Pn) in *Pistacia vera* L.

Treatment NaCl	AM status	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Chla/b	Pn (μ mol Co_2 m^{-2} s^{-1})
Control	AM	1.51 ± 0.02 a	0.59 ± 0.02 a	2.55±0.04c	8.78±0.35a
Control	NM	1.13 ± 0.04 b	0.47 ± 0.03 b	2.4±0.05c	8.31±0.21a
12 ds m^{-1}	AM	1.07 ± 0.02 b	0.33 ± 0.01 c	3.24±0.06a	6.73±0.3b
12 ds m^{-1}	NM	0.68 ± 0.04 c	0.22 ± 0.02 d	3.09±0.03b	4.12±0.4c

Within each column, means with different superscript letters are significantly different ($p < 0.05$, LSD test).

Supplementary Table 1. Primer sequence used for qRT-PCR analysis

Gene	Gene ID (GenBank)	sequences (5'-3')	Amplicon (bp)
rbcL	33127488	F: TCCGAGTCACTCCTCAACCT R: ATCGGTCCACACAGTTGTCC	96
rbcS	116124297	F: TGAGTGCAAGAAGGCGTACC R: GACAAAGGCCATGCACTGAG	85
psbA	33127471	F: TAGCACTGAATAGGGAGCCG R: ATTCCAGGCTGAGCACAACA	86
psbD	33127591	F: GGCCTGAAGCACAAGGAGAT R: AGTCCGAAAGCACCGTGTAG	85
SKOR	116117994	F: CTCACCCTGTTGCGTTTGTG R: ACGATTGGCAATGAAGTGAAGC	91
EF1α	116114399	F: TCACTGCGGTTGTAAGCTGT R: TTTGGGGTTGTGGCATCCAT	110

decrease in NM plants under saline conditions (Table 2). Under control (non-saline) conditions, no significant difference in Pn was detected between NM and AM plants. However, under salinity stress, AM plants exhibited a 38.8% higher Pn compared to NM plants (Table 2).

The expression of *psbA* and *psbD* was downregulated in both non-mycorrhizal (NM) and arbuscular mycorrhizal (AM) plants under salinity treatments (Fig. I). AM fungi upregulated the expression of *psbA* under both non-saline and saline conditions, while the expression of *psbD* was specifically enhanced by mycorrhizal colonization under NaCl treatment. Salinity also downregulated the expression of *rbcL* and *rbcS* in NM plants, but only reduced the expression of *rbcL* in AM plants (Fig.I). The expression level of *rbcL* was significantly higher in AM plants compared to NM plants under both control and NaCl treatments, whereas the expression of *rbcS* remained similar between NM and AM plants across all salinity levels.

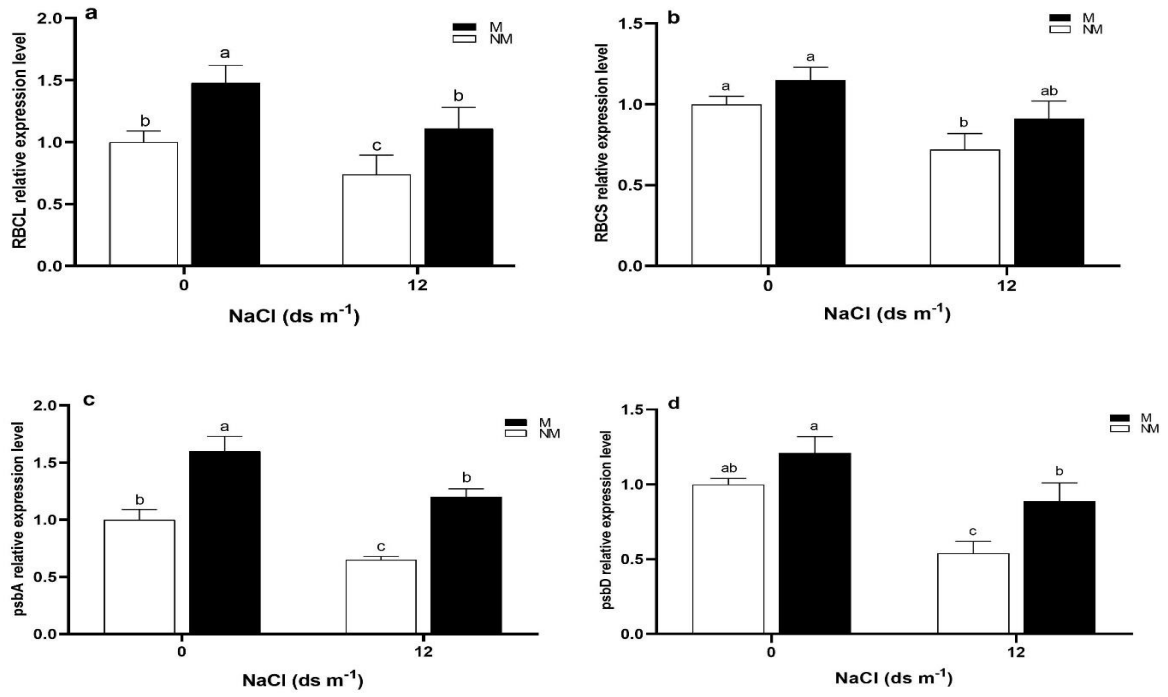

Fig. I. Effects of Salinity and *Glomus mosseae* Inoculation on the Expression of *rbcL, rbcS, psbA and psbD* Genes in Pistachio Shoots. Different letters within each gene denote statistically significant differences ($P < 0.05$).

Salinity stress significantly reduced the potassium (K) content in the shoots of both mycorrhizal and non-mycorrhizal pistachio plants. However, at both control and saline conditions, mycorrhizal plants exhibited higher K content compared to non-mycorrhizal plants. Specifically, under control conditions, mycorrhizal plants showed a 9.6% increase in K content, while under salinity stress, the increase was 21.8 %. This demonstrates the critical role of mycorrhizal symbiosis in enhancing K uptake and mitigating the adverse effects of sodium chloride-induced salinity (Fig. II). In shoots, salinity stress downregulated the expression of *SKOR* in both non-mycorrhizal and arbuscular mycorrhizal plants (Fig. II). However, AM symbiosis significantly upregulated the expression of *SKOR* across all salinity levels compared to NM plants, highlighting the protective role of mycorrhizal association in modulating gene expression under saline conditions.

Discussion

Our study demonstrates that arbuscular mycorrhizal (AM) plants exhibit a significant decline in mycorrhizal colonization rate from 67% to 43% under elevated salinity conditions, while consistently displaying higher shoot and root biomass compared to non-mycorrhizal (NM) plants across all salinity levels. These findings underscore the importance of AM fungi in mitigating the adverse effects of salinity stress on plant growth, as previously reported by Thangavel et al. and Karima et al.

(Karima et al., 2023; Thangavel et al., 2022). Notably, our results align with those of Wang et al. (2019), who observed enhanced biomass production and salt tolerance in sweet sorghum inoculated with AM fungi (Wang et al., 2019). However, the magnitude of mycorrhizal colonization decline in our study is more pronounced than that reported by Kumar et al. (2015), suggesting potential variations in AM fungal strains or plant species-specific responses to salinity stress (Kumar et al., 2015). The observed decline in mycorrhizal colonization under salinity stress may be attributed to the disruption of nutrient uptake and exchange between the plant and AM fungi, leading to reduced fungal growth and colonization (Thangavel et al., 2022). Despite this decline, AM plants continued to exhibit improved growth and biomass

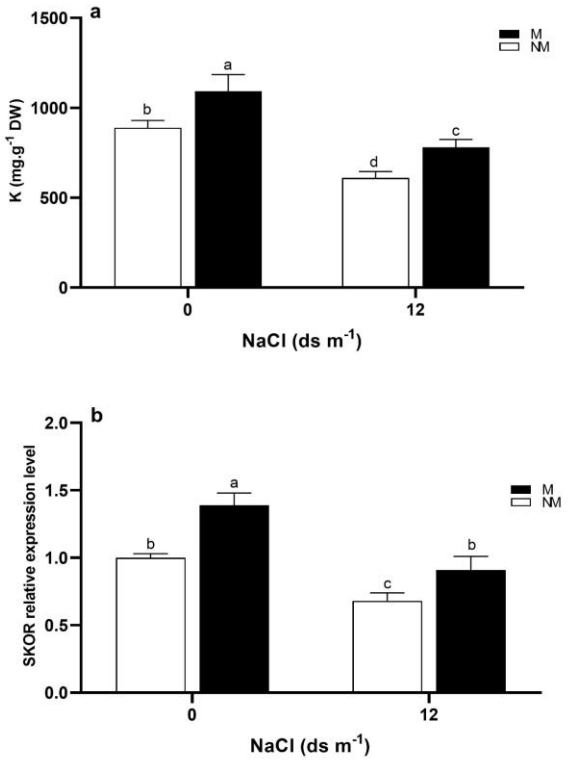

Fig. II. . Effects of Salinity and *Glomus mosseae* Inoculation on K content and the Expression of *SKOR* Genes and in Pistachio Shoots. Different letters within each gene denote statistically significant differences ($P < 0.05$).

production, likely due to the enhanced nutrient acquisition and antioxidant defense mechanisms conferred by the AM symbiosis (Karima et al., 2023).

This study investigated the influence of arbuscular mycorrhizal fungi (AMF) on photosynthetic parameters of pistachio seedlings subjected to salinity stress. The principal findings revealed that while salinity diminished chlorophyll (Chl) content in both AM-inoculated and un-inoculated seedlings, AM-inoculated plants consistently exhibited higher Chl levels across all salinity treatments, indicating AMF's protective role against salt-induced chlorophyll breakdown. Furthermore, salinity augmented the Chl a/b ratio, an effect amplified in AM-inoculated plants. Net photosynthetic rate (Pn) also decreased under salinity in both groups, but the reduction was notably less severe in AM plants (22.6%) compared to NM plants (50.4%), resulting in a 38.8% higher Pn in AM plants under salt stress. This aligns with a significant body of existing research highlighting AMF's role in mitigating salt stress through enhanced photosynthetic function. For instance, Sheng et al. (2008) demonstrated similar positive effects of AMF on photosynthesis and water status in maize under salt stress (Sheng et al., 2008). The observed increase in the Chl a/b ratio in AM plants under saline conditions may suggest a shift towards more efficient light-harvesting complexes. Likewise, Peng et al. (2024)recently reported positive effects of AMF on photosynthetic characteristics of cotton seedlings exposed to saline-alkali stress, further validating the broad applicability of AMF in alleviating abiotic stresses (Peng et al., 2024). This present study supports these findings, specifically in pistachio, offering similar evidence across diverse plant species. While Chen et al. (2017)explored the role of AMF in black locusts, emphasizing improvements in K^+/Na^+ homeostasis alongside photosynthesis, the current study focused primarily on photosynthetic pigments and Pn, suggesting a broader suite of physiological benefits conferred by AMF. In comparison to Zong et al. (2023)who reported AMF improvement through osmotic tolerance, antioxidant activity, and photosynthesis, the current study focuses on the effects of the photosynthetic mechanisms in detail, therefore contributing to the body of knowledge by exploring specific pathways. A plausible mechanism for the observed improvement in photosynthetic performance in AM plants under salinity involves enhanced nutrient uptake, particularly phosphorus, crucial for chlorophyll synthesis and photosynthetic enzyme activity, and improved water relations, mitigating the negative impacts of osmotic stress imposed by salinity. Moreover, AMF can enhance antioxidant activity, protecting photosynthetic machinery from oxidative damage, as seen by Wang et al. (2020), which could contribute to maintaining higher Chl content. The findings of this study possess practical implications for pistachio cultivation in saline-prone areas.

This investigation demonstrates that salinity stress negatively impacts the expression of crucial photosynthetic genes, psbA, psbD, rbcL, and rbcS, in plants, while arbuscular mycorrhizal (AM) symbiosis offers a degree of mitigation against these detrimental effects. Specifically, salinity induced

downregulation of psbA and psbD, encoding components of photosystem II, in both mycorrhizal and non-mycorrhizal plants; however, AM fungi notably enhanced psbA expression under both control and saline conditions and specifically augmented psbD expression under salinity stress. Similarly, the genes rbcL and rbcS, involved in carbon fixation, experienced reduced expression under salinity in non-mycorrhizal plants, although AM colonization buffered the decline of rbcS and significantly elevated rbcL expression compared to non-mycorrhizal counterparts in both control and stressed environments. These findings align with Ren et al. (2019), who observed complex transcriptional responses in *Sesbania cannabina* to salinity and AM symbiosis, including alterations in photosynthetic gene expression, though specific gene responses may vary between species. Our results contrast with observations by Wu et al. (2022) in *Populus euphratica*, where sex-specific responses were noted, highlighting the potential for nuanced interactions dependent on plant characteristics. Furthermore, the enhanced rbcL expression with AM colonization under stress is consistent with Chen et al. (2017), who linked improved photosynthesis in mycorrhizal black locusts under salt stress to enhanced water status and ion homeostasis. The observed upregulation of psbA and psbD under salinity by AM fungi suggests a mechanism involving protection or enhanced repair of the photosystem II complex, potentially through improved antioxidant capacity or nutrient acquisition, as implicated by Rashad et al. (2021)in their study of stress-responsive gene expression in mycorrhizal banana plantlets. The differential regulation of rbcL versus rbcS by AM fungi further suggests a targeted modulation of the Calvin cycle, potentially favoring the large subunit encoded by rbcL. These results advance our understanding of AM-mediated salinity tolerance by revealing specific molecular targets within the photosynthetic apparatus. This enhanced photosynthetic capacity under stress, facilitated by AM fungi, has significant implications for improving plant productivity in saline environments, particularly in agricultural systems challenged by increasing soil salinization. While Winicov and Button (1991)documented the accumulation of photosynthetic gene transcripts in salt-tolerant alfalfa cells in response to NaCl, the current study illuminates the differential regulatory role of AM symbiosis, offering a more comprehensive view of plant adaptation strategies to salinity.

This study reveals that salinity stress significantly diminishes potassium (K) accumulation in the shoots of pistachio plants, irrespective of mycorrhizal status; however, arbuscular mycorrhizal (AM) symbiosis substantially enhances K content in shoots under both control and saline conditions, demonstrating a crucial role in mitigating salt-induced K deficiency. Specifically, mycorrhizal plants exhibited a nearly 10% increase in shoot K concentration in non-saline environments and a notable 22% increase under salinity stress compared to their non-mycorrhizal counterparts. Concurrently, the expression of SKOR, a gene encoding an outward-rectifying K^+ channel involved in K^+ release into the xylem, was downregulated by salinity in both plant groups. Crucially, AM symbiosis significantly upregulated SKOR expression across all salinity levels, suggesting a mycorrhizal-mediated enhancement of K^+ translocation to the shoot. These findings corroborate those of Ghorbani et al. (2019), who demonstrated that Piriformospora indica improved K^+/Na^+ homeostasis in tomato under salinity, although they did not specifically examine SKOR expression. Our results are also consistent with Santander et al. (2021), who observed differential regulation of cation transporters by AM fungi in lettuce under salinity, further supporting the notion that mycorrhizae influence ion transport mechanisms. While Selvakumar et al. (2018) highlighted the role of spore-associated bacteria in regulating maize root K^+/Na^+ homeostasis during AM symbiosis, our findings point directly to the fungal influence on SKOR expression in the shoot. The observed upregulation of SKOR by AM fungi, even under salinity-induced downregulation, suggests a mechanism whereby the symbiosis either directly stimulates SKOR transcription or counteracts the suppressive effects of salinity on SKOR expression, potentially through improved nutrient status or hormonal signaling, as suggested by Karima et al. (2023) in their work on legume species. This enhanced K+ delivery to the shoot, facilitated by elevated SKOR expression in mycorrhizal plants, is likely a pivotal factor contributing to the observed higher shoot K content and improved salinity tolerance. The implications of

these results are significant for enhancing pistachio cultivation in salt-affected regions, as promoting AM symbiosis could be a viable strategy for improving K nutrition and, consequently, plant performance under salinity stress.

Conclusion

In conclusion, this study demonstrates the multifaceted benefits of arbuscular mycorrhizal (AM) symbiosis in enhancing the salinity tolerance of *Pistacia vera*. While salinity stress negatively impacted mycorrhizal colonization, biomass, chlorophyll content, and net photosynthetic rate (Pn), AM plants consistently outperformed non-mycorrhizal (NM) plants across all parameters. The AM-mediated improvement in salinity tolerance is attributed to a combination of physiological and molecular mechanisms. Firstly, AM symbiosis significantly enhanced potassium (K) uptake and maintained higher shoot K concentrations, crucial for osmotic adjustment and enzyme function under salinity. This was further supported by the upregulation of the *SKOR* gene, facilitating K^+ translocation to the shoot. Secondly, AM fungi modulated the expression of photosynthetic genes, mitigating the detrimental effects of salinity on the photosynthetic apparatus. Specifically, the upregulation of *psbA*, *psbD*, and *rbcL* in AM plants likely contributed to the observed higher chlorophyll content and enhanced Pn under salt stress. The increased Chl a/b ratio in AM plants under salinity suggests an adaptation strategy to optimize light harvesting under stress conditions. These findings underscore the potential of utilizing AM fungi as a biotechnological tool to improve pistachio cultivation in saline environments by bolstering photosynthetic efficiency and nutrient acquisition.

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