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EFFECTS OF INOCULATION WITH A COMPOSITE ARBUSCULAR MYCORRHIZAL INOCULUM ON PLANT TOLERANCE TO SALT INDUCED WATER STRESS IN STRAWBERRY

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ABSTRACT

Strawberry (*Fragaria × ananassa* Duch.) is an herbaceous biennial plant belonging to the *Rosaceae* family. The fruits are appreciated throughout the world because of their delicious taste and also for their high antioxidant content. Strawberry plant is considered particularly sensitive to drought and water stress. Water stress negatively influences the entire plant, causing possible drops in yield and can eventually cause plant mortality if left untreated. Salt induced water stress has generally a negative effect on plant growth and yield. Nowadays, different alternatives have been used to mitigate the effects due to salt stress, including inoculation of soil microorganisms such as arbuscular mycorrhizal fungi (AMF). These fungi have been shown to have a palliative effect on water stress damages. The AMF can constitute a positive recourse to plants with the presence of extra-matrix hyphae. The latter are finer than the plant roots, which facilitates the use of water-filled pores, otherwise inaccessible to the roots. The present study was carried out under greenhouse conditions with the aim of determining the effect of applying a composite mycorrhizal inoculum on strawberry plants stressed by drought. Three salinity levels were used (10 %, 25 % and 50 %) and the parameters measured were: mycorrhization status, root diameter, root length, salinity index, visual score and relative water content. The results showed that the salinity index, at the 50 % salinity level, was strongly correlated with root diameter and relative water content. Visual scores, for the three salt treatments, depended on mycorrhization status (yes/ no), root diameter, root length and relative water content. For the three salt treatments, the relative water content depended on the salinity index, root length and mycorrhization status (yes/ no). Based on the results obtained, the present study suggests a positive effect of inoculation with mycorrhizae on the salt stress tolerance of strawberries.

Key words: Strawberry, Arbuscular mycorrhizal fungi, Salinity, Roots, Drought, Water stress

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INTRODUCTION

Of the world's 1.5 billion hectares of cultivable land, 77 million hectares (5 %) do not furthering produce good crops owing to high salt content and 20% of the irrigated agricultural land is affected by salinity [1]. High soil salinity is a global environmental problem, especially for agricultural production, especially in arid and semi-arid areas covering more than 7% of the Earth's surface. Considered as a major factor, salt stress limits the growth and yield of agricultural crops [2]. Under saline conditions, plant growth is negatively affected, mainly due to osmotic stress. Low root water potential leads to water deficit within the plant, thus leading to a rapid decrease in cell water potential and volume [3]. and consequently, it results in a decrease in all vegetative and reproductive parameters, ultimately resulting in a decrease in yield [4]. Strawberry (*Fragaria × ananassa* -Duch.) is considered particularly sensitive to salt stress [5, 6]. Salt stress occurs when salts accumulate in the soil solution and their level reaches a threshold such that strawberry plants can no longer meet their water needs during the growing season. Salt-induced osmotic stress causes osmotic dehydration [3]. Visual signs of damage caused by salt accumulation in the soil include browning and drying of leaf edges, brittleness, delayed growth, and eventually the death of roots and seedlings. These observations will be widespread throughout the field when irrigation water salinity is high. Over-fertilization or application to wet foliage can also cause salt toxicity. A sodium concentration greater than 0.2% or chloride greater than 0.5% in plant tissue indicates salt toxicity [7].

Due to the scope of salinity affected soils and the deleterious effects of salinity on yields, methods to correct and/or improve crop production in salinity stressed soils are necessary. Management of soil salinity through land reclamation or improved techniques of irrigation provides only short-term solution and often remain expensive. Other methods like plant breeding for salt tolerance have been difficult and slow [1].

Microorganisms living in the rhizosphere play a crucial role in promoting plant growth and soil fertility. Arbuscular mycorrhizal fungi (AMF) have been shown to form symbiotic and mutualistic associations with the roots of approximately 80% of plant species. They are obligate biotrophs [8]. Symbioses of halotolerant bacteria with plant roots also promote plant resistance to salt stress. Due to growth-promoting properties in stressed environments, halotolerant bacteria can have a positive effect on plant growth and promote nutrient uptake. The use of microorganisms is an economical and ecological approach to release stress from cultivated plants in salt-impacted agricultural fields [1].



These kinds of interactions tend to increase salinity tolerance of strawberry and confirm the beneficial potential of this biotechnology in horticulture under arid conditions [9].

Studies have demonstrated the potential of AMF and inoculants in inducing strawberry vegetative growth response [10]; the response being dependent on cultivar–AM species combinations and environmental conditions [11, 12].

While, the impact of AMF on diverse vegetable crops was described in the past, the deduction of their general significance for these crops in horticulture is missing so far [13].

This study aimed at determining the effect of a composite mycorrhizal inoculum on strawberry plants tolerance to salt induced water deficiency.

MATERIALS AND METHODS

Materials and Procedures

This study was conducted under greenhouse conditions of the Botanic, Biotechnology and plants protection Laboratory, Sciences Faculty, University IbnTofail, Kenitra, Morocco. Ibn Tofail University located in Kenitra, a coastal city in north-western Morocco, in the Gharb-Chrarda-Beni Hssen region. The university campus is in the southeast area of Kenitra, near the Maâmora Forest. The geographic coordinates of the greenhouse are approximately (x=390546; y=405958). The study was conducted using a completely randomized block design with multiple repetitions of each treatment. Each repetition corresponded to an individual experimental unit consisting of a pot containing a plant.

Mycorrhizal Inoculum

A composite endomycorrhizal inoculum collected from the soil and the roots samples of the olive trees rhizosphere in different Moroccan olive groves was used to inoculate strawberry plants. The barley was used as a plant host for the inoculum multiplication. Barley grains were disinfected in 5 % hypochlorite sodium solution during 2 minutes and left to germinate in plastic jars filled with mixture sterile sand and mycorrhized roots. After four weeks, barley roots were rinsed with distilled water and cut in fragments. These roots were used as an inoculum.

Salt treatments

Forty-four (44) days after transplanting, salt was gradually brought (for one week) into the irrigation solution to reduce osmotic shock to final concentrations of 0 %, 10 %, 25 %, 50 %. The watering was carried out every two days, to compensate losses due to evapotranspiration but also to allow a leaching of salt.



Saline Stress Sensitivity Index (SSSI)

The response of mycorrhized strawberry plants to salinity was assessed through biomass and the salt sensitivity index (SSSI), which reflects the relationship of treatment sensitivity to inoculum (expressed as relative salt biomass deficit or RBD) to average sensitivity of all treatments with inoculum or salinity intensity index (IIS): $ISSS = RBD / SII$; with $RBD = (bT_t - bT_s) / bT_t$ where BT is the average total biomass of a treatment with the given inoculum in the absence of salt (t) or under saline stress (s) and $SII = (M_t - M_s) / M_s$. M being the average total biomass of all treatments with inoculum in the absence of salt (t) or under saline stress(s) [14].

Relative water content (RWC)

The relative water content (RWC) of the plants is closely related to the foliar water potential (Ψ) and thus a parameter that varies depending on the stress state. This relative water content expressed as % fresh material (MF) is determined according to the following formula:

$$RWC = 100 (MMF - MMS) / (MFT - MS)$$

MMF: mass of the fresh material of the sample;

MMS: mass of the dry matter of the sample;

MFT: mass of the fresh material of the sample at full turgidity.

Foliar salt damage as visual score

At the end of the experiment, foliar salt damage (leaf edge burn, necrosis, and discoloration) was rated using a visual score of each plant from 0 to 5, where 0 = dead; 1 = over 90% foliar damage; 2 = moderate (50% to 90%) foliar damage; 3 = slight (less than 50%) foliar damage; 4 = good quality with minimal foliar damage; 5 = excellent without any foliar damage.

Statistics analysis

The collected data were statistically processed using IBM SPSS Statistics software at $p < 0.05$.

RESULTS AND DISCUSSION

Saline Stress Sensitivity Index (SSSI)

The SSSI determines the sensitivity of strawberry plants inoculated with mycorrhizal fungi to salinity induced stress. The results showed that the salinity index, at the level of 50% of salinity, was highly correlated to the roots diameter and the RWC (table 1).

In multivariate analysis, mycorrhization status (yes/no), roots diameter, roots length and RWC were the independent variables associated with salinity index for salinity treatments of 50% and 25 % and to the mycorrhization status (yes/no), roots diameter and roots length for salinity level 10% (Table 2).



It is concluded that AMF symbiosis significantly increases salinity tolerance in strawberry plants. It confirms the potential of mycorrhizal biotechnology for horticulture in arid regions as mentioned in Li Fan and al., study [15].

In another study, mixing two AMF species increased root and shoot mass to a greater extent compared to using each species alone at low salinity (0–50 mM), but reduced fruit quality. They found that at higher levels (100–200 mM), *R. irregularis* species has a palliative effect against salt stress and improved fruit quality significantly compared to the other AMF species [16].

The same finding coincides for other crops, as mentioned in Jalaludin study [17]. For mycorrhized corn and for mycorrhized lettuce plants inoculated by *Glomus sp.* Roots biomass in plants treated with arbuscular mycorrhizae was significantly higher compared to control plants and this could be explained by the possible stimulation of root development to enable the plant to withstand high salinity levels by improving its hydro-mineral nutrition [18, 19].

Visual score

Damages caused by the salt treatment and observed on plants are presented on figure 1. The visual score for the three salt treatments was correlated to the roots diameter, the roots length and the RWC (Table 1). Visual score was dependent to the mycorrhization status (yes/no), the roots diameter, the roots length and the RWC for the three salt treatments (Table 2).

During drought, mycorrhizal symbiosis can alter osmotic and elastic properties in leaves [20, 21, 22] and roots cells [23], and accumulation of key solutes in leaves and roots cells [24, 25].

Regarding the promotion of gas exchange and improved drought resistance, the plant/AMF symbiosis may allow leaves to maintain a normal water balance (closer to the responses of unstressed controls) and to fix more carbon during periods of water stress [24, 26].

The alternative that the roots of AMF treated plants could access water inaccessible to the roots of non-inoculated plants is highly supported. Better root growth of AMF-treated plants in dried soils could be linked to better exploitation of non-free water, with mycorrhizae allowing access to soil water below the permanent wilting of roots of non-mycorrhizal plants [27, 28, 29].

According to the results obtained this study and existing similar work, we can say that the salt tolerance of the plants was largely related to the presence of AMF that improved the nutrition of plants under saline stress.





Figure1: Salt injury symptoms in strawberry leaves: brown & brittle leaf margins

Relative water content (RWC)

For the 10% and 25% salt treatment, the RWC was correlated to the intensity of mycorrhization, the spores' number and the salinity index. However, for 50% salt treatment, the RWC was correlated to the intensity of mycorrhization, the spores' number, the roots diameter, roots length and the visual score (table1).

In multivariate analysis, and for the three salt treatments, the RWC was dependent to the index of salinity, the roots length and the mycorrhizal status (yes/no) (table2).

The moisture characteristic of a soil depends on the size and distribution of its pores, or capillary space [30]. Because AMF affect soil structure, it seems logical to suspect that AMF colonization of a soil might affect its moisture retention properties and, in turn, the plants behaviour, especially when the soil is relatively dry.

Furthermore, Cinta *et al.* [31] found that the RWC of whole strawberry plants was 11% higher in AMF-colonized seedlings compared to those not colonized by AMF. They have concluded that this effect is related to higher water in mycorrhized roots treated with AMF and suggested that mycorrhized roots must have a higher concentration of water-soluble compounds or a different distribution in the cell compartments than non-AMF roots.

However, in another study with *Jatropha*, the established symbiosis with AMF fungi has been shown to improve water absorption [32]. In results of this study, we have shown that incorporation of mycorrhiza in roots affect significantly the RWC. Our result matches with other studies where AMF symbiosis was shown to influence the plant water relationship by altering the osmotic balance of cells [33].

Salinity stress conditions undoubtedly limit plant productivity [10] and, therefore, the role of AMF as a biological resource for improving plant salt stress tolerance, is gaining economic importance worldwide.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

In this study and for all strawberry plants, the RWC and the visual score were reduced by the increased salinity of the irrigation water. Plant tolerance to salt stress was observed in plants inoculated with a composite mycorrhizal inoculum.

However, this knowledge requires a better understanding of how plants and AMF intimately interact with each other under salt stress and how this interaction leads to physiological changes in plants. This technique is of significant importance for developing sustainable cropping systems that effectively exploit AMF to improve overall plant health under water stress.

A significant number of studies and reports summarize the comparative water relations between plants receiving AMF and those not receiving it. However, the influence of AMF symbiosis on water relations and soil water retention properties for horticultural crops remains understudied.

This study deserves to be extended to compare the results obtained under controlled conditions with those obtained in the field. These results could be used in the fight against soil salinity by exploiting the benefits of AMF on the water supply of plants. These are promising results that need to be studied in depth in order to maximize the exploitation of this natural symbiosis.

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Conflicts of Interest

The authors declare no conflict of interest.



Table 1: Univariate analysis defining factors associated with strawberry salinity index and visual score

		Salinity index		Visual score	
		R	P	r	P
Salinity level 10%	Roots diameter (cm)	-0.682	0.002	0.543	0.02
	Roots length (cm)	-0.683	0.002	0.506	0.03
	RWC (%)	-0.511	0.03	-	-
	Mycorrhization intensity %	0.683	0.002	0.546	0.01
	Spores number	0.548	0.01	0.504	0.03
Salinity level 25%	Roots diameter (cm)	-0.632	0.005	0.482	0.04
	Roots length (cm)	-0.567	0.01	-	-
	RWC (%)	-0.677	0.002	0.646	0.004
	Mycorrhization intensity %	0.653	0.003	-	-
	Spores number	0.607	0.008	-	-
Salinity level 50%	Roots diameter (cm)	-0.762	<0.0001	-	-
	Roots length (cm)	-0.697	0.001	-	-
	RWC (%)	-	-	0.489	0.03
	Mycorrhization intensity %	0.588	0.01	-	-
	Spores number	0.625	0.006	-	-

RWC: Relative water content
r: Correlation coefficient
p: The significance threshold

Table 2: Linear regression analysis with salinity index and visual score as dependent variables and mycorrhization status, RWC, roots diameter and roots length as independent variables

		Salinity index		Visual score	
		B	p	β	p
Salinity level 10%	Roots diameter (cm)	-0.666	0.003	0.523	0.002
	Roots length (cm)	-0.846	<0.0001	0.566	0.01
	RWC (%)	-0.857	<0.0001	-	-
	Mycorrhization status (yes/no)	-0.991	<0.0001	0.610	0.007
Salinity level 25%	Roots diameter (cm)	-0.581	0.01	0.476	0.04
	Roots length (cm)	-0.695	0.001	0.537	0.02
	RWC (%)	-0.677	0.002	0.679	0.002
	Mycorrhization status (yes/no)	-0.889	<0.0001	0.713	0.001
Salinity level 50%	Roots diameter (cm)	-0.796	<0.0001	0.500	0.03
	Roots length (cm)	-0.721	0.001	0.497	0.03
	RWC (%)	-0.812	<0.0001	0.652	0.003
	Mycorrhization status (yes/no)	-0.937	<0.0001	0.589	0.01

RWC: Relative water content

β : Regression coefficient

p: The significance threshold

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