The Effect of an Arbuscular Mycorrhizal Inoculum on the Growth and Fruiting of \textit{Fragaria ananassa} (\textit{Weston}) \textit{Duchesne Ex Rozier} (\textit{Rosaceae}) in Morocco

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The effect of composite endomycorrhizal inoculums was studied on the strawberry plant growth under greenhouse conditions. After six months, a significative effect was observed. Leaves number, fruits number, fresh roots weight and fresh fruits weight under greenhouse conditions compared to controls were 28.9/11.2, 58/1, 12.59g/9.62g and 367g/230g. Mycorrhizal intensity, arbuscular content and spores number in mycorrhized and non mycorrhized strawberry seedlings were respectively 54.1, 28.3 and 220. The spores detected belong to eight species and four genera: \textit{Glomus}, \textit{Rhizophagus}, \textit{Gigaspora} and \textit{Scutellospora}.

Keywords: Mycorrhizal fungi; strawberry; arbuscular mycorrhizal fungi (AMF); spores; Morocco.
1. INTRODUCTION

The strawberry (*Fragaria ananassa. Duchesne*) is a perennial herbaceous plant belonging to the rosaceous family. It’s now cultivated in temperate and subtropical regions worldwide for its delicious fruit. It is among the most widely consumed fruits throughout the world [1].

In 2011, the worldwide commercial production of strawberries was 4.6 million metric tons (mmt), harvested from around 240,000 hectares [2]. Nearly 10 % of the area harvested was in the US; The US harvest was more than 5 times that of any other country. Other major producers include Turkey, Spain, Egypt, Korea and Mexico [1,2].

In Morocco, the strawberry represents a culture to high added value. It is practised in the perimeters of Loukkos and Gharb with dominance of the first one. Its surface does not stop increasing from one year to the next; it was 3,000 ha during the campaign sold with a 120,000 ton production [3]. This surface allows 420,000 US$ of output value and provides 5 million working days annually [3]. Seventy percent of the production is intended for the export [3]. The main customers are the European Union with development of other markets such England and Arab countries. These markets are known for their requirement regarding residues of pesticides in fruits and the product traceability [1,3].

Profitable strawberry production requires careful attention to many cultural practices. Variety, selection, weeds control, frost control, fertility and disease control. The management of this crop is intensive and there is the like likelihood of encountering a number of production difficulties in Moroccan fields’ case. Production costs are increasing because strawberry plants, chemical, fertilisers and almost all inputs are imported and their prices are much higher than in Europe [4]. Soils around the world and in many sites of plantations are increasingly being contaminated with hazardous products; such as agrochemicals, metals and other potentially toxic substances [5,6].

To have a production which meets export markets requirements and the consumer health [3], sustainable and healthy farming methods were identified and adopted [2] and they included the symbiosis with mycorrhizas.

Mycorrhizas are beneficial fungi growing in association with plant roots. They increase nutrient uptake not only by increasing the surface absorbing area of the roots, but also release powerful enzymes into soil that dissolve hard-to-capture nutrients, such as organic nitrogen (N), phosphorus (P), iron (Fe) and other "tightly brand" soil nutrients [7]. Arbuscular Mycorrhizal Fungi (AMF) are involved with a wide variety of other activities that benefit plant establishment, growth, enhancing plant health, vigour and minimising stress [2,8].

Despite the fact that in the recent years forest and agricultural species research have been developing quite intensively [9,10], there is only little data and researches on strawberry’s symbiosis with mycorrhizas.

Data suggest that the mycorrhization does not only have a positive effect on the growth parameters and the yields, but can also affect production quality [11].

Studies have showed an increase of strawberry plant productivity of *F. ananassa ‘Pajero’* using *in vitro* plant with the presence of AMF [12]. This natural alliance has the capacity of stimulating and enhancing growth plant [13].

Researches on the symbiosis between strawberry plants and mycorrhizas remain insufficient in spite of the important increase in the forest domain and genetic researches [14,15].

The purpose of this study is to determine the effect of the application of an arbuscular mycorrhizal inoculum on strawberry’s growth and fruiting in Morocco.

2. MATERIALS AND METHODS

The an arbuscular mycorrhizal 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 (*Camarosa*). 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 are rinsed with distilled water and cut in fragments from 1 to 2 mm of length. These roots were used as an inoculum.

Before the plantation, three grams (g) of barley roots fragments were incorporated on the top of
every jar, filled with mixture sterile sand and mycorrhized roots and intended to receive the strawberry plants. The control jars did not receive mycorrhized roots. After transplantation, all jars were transported to the greenhouse and watered regularly with distilled water. During six months, a regular counting of leaves, flowers and fruits was made. After six months, roots were collected, washed with clear water and dried on absorbing paper in laboratory conditions, and roots fresh weight and fruits were measured in (g).

The AMF colonisation roots were quantified using Philips and Hayman technique [16]. The roots were carefully washed with tap water, cut into segments of 1 centimeter (cm) in length, and submerged in a solution of 10% potassium hydroxide (KOH) for 45 minutes at 90ºC. They were then washed again in tap water. To bleach those with excess pigment roots; were submerged in hydrogen peroxide (H2O2 10%). After this, root segments were placed in a beaker containing 100 milliliter (ml) of distilled water and 0.05 (g) of Cresyl blue, transferred to a 90°C water bath and incubated for 15 minutes.

Thirty fragments, chosen at random, served for the microscopic observation and the determination of mycorrhizal parameters. The mycorrhization frequency (% F), the mycorrhiza intensity (% M), the arbuscular content (% TA) and the content in vesicles (%TV) were calculated according to Trouvelot and al. (1986) index of mycorrhization [17].

Spores were extracted following the wet sieving method described by Gerdemann and Nicolson [18]. In a 1 liter (L) beaker, 100 (g) of each soil was submerged in 1 (L) of tap water and stirred for 1 minute with a spatula. After 10 to 30 seconds of settling, the supernatant passes through a sieve of four levels by decreasing the size of the stitches 500, 200, 80 and 50 microns. This operation is repeated twice.

The same soil sample was again submerged, stirred, and the wet sieving is repeated 3 times. Deposition in the used sieve contained the maximum of spores; it was recovered with 20 (ml) of the sucrose solution (40%) and transferred to centrifuge tubes again for 15 to 30 seconds. The supernatant is collected on a sieve of 50 microns using a water jet [19].

The characteristic structures (color, shape, size and number of separation membranes...) of the spores were outlined by mounting between slide and slide 0.1 ml.

A preliminary identification of the spore’s type was made based on the criteria proposed and available in various databases [20].

2.1 Statistical Analysis

The statistical analyses were made by analysis of the variance in a single criterion of classification (anova1) at the level of 5 % with the software STATISTICA.

3. RESULTS

3.1 Mycorrhization Effect on the Agronomic Parameters of Strawberry Plants

After 6 months of inoculation, a significant effect is observed on inoculated plant’s growth compared to the control ones. Indeed, the mean values of leaves, fruits, and the weight of roots and fruits of the inoculated plants are superior to control plants. They reached respectively 28.9 against 11.2, 58 against 1, 12.59 against 9.62, and 367 against 230 (Table 1).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mycorrhized Plants</th>
<th>Non Mycorrhized Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of leaves</td>
<td>28.9 a</td>
<td>11.2 b</td>
</tr>
<tr>
<td>Number of fruits</td>
<td>5 a</td>
<td>1 b</td>
</tr>
<tr>
<td>Fresh roots weight (g)</td>
<td>12.59 a</td>
<td>9.62 b</td>
</tr>
<tr>
<td>Fresh fruits weight (g)</td>
<td>367 a</td>
<td>230 b</td>
</tr>
</tbody>
</table>

2 results with the same letter don’t differ significantly at the threshold of 5%

3.2 Evaluation of the Inoculation Effect on the Mycorrhization Parameters of Strawberry Plant

The microscopic observation of roots fragments after 10 months of inoculation, allowed to, highlight the presence of various mycorrhizal structures; arbuscular structures, internal and external hyphae, spores, and the endophytes.
Mycorrhizal frequency, mycorrhizal intensity, arbuscular content, and spores number in mycorrhized strawberry seedlings were respectively 100, 54.1, 28.3 and 200 against 78, 37, 8.5 and 98 in non mycorrhized plants (Table 2).

### Table 2. Inoculation effect on mycorrhization parameters of Strawberry plants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mycorrhized Plants</th>
<th>Non Mycorrhized Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycorrhizal frequency (%)</td>
<td>100 a</td>
<td>78 b</td>
</tr>
<tr>
<td>Mycorrhizal Intensity (%)</td>
<td>54.1 a</td>
<td>37 b</td>
</tr>
<tr>
<td>Arbuscular content (%)</td>
<td>28.3 a</td>
<td>8.5 b</td>
</tr>
<tr>
<td>Spores number</td>
<td>220 a</td>
<td>98 b</td>
</tr>
</tbody>
</table>

*Results with the same letter don’t differ significantly at the threshold of 5%*

The observed spores belong to eight species being part of four different genera: *Glomus, Rhizophagus, Gigaspora* and *Scutellospora* with an abundance of the genera *Glomus* (Fig. 1).

Mycorrhized plants showed a good growth and a good fruiting in comparison with non mycorrhized plants. This observation is justified through the parameters presented on Fig. 2.

The visual inspection of strawberry plants allowed us highlighting a difference in roots vigor, volume, and the date of blooming between mycorrhized and non mycorrhized plants (Fig. 2).

4. DISCUSSION

Our findings suggest that strawberry's growth and fruiting are positively affected by the application of a composite endomycorrhizal inoculums. Mycorrhization status was the strongest factor influencing both growth and fruiting of strawberry.

In our study, one hundred percent of strawberry’s roots were mycorrhized. This finding indicates the existence of an active indigenous mycorrhizal fungus in a natural symbiosis with the strawberry.

Indeed, only limited information is available on indigenous mycorrhizal fungi association with strawberry, and data focused about the field of strawberry symbiosis with mycorrhizas remain weak [15].

As our study, Derkowska reported that mycorrhizal frequency, mycorrhizal intensity, and arbuscular content of strawberry seedlings were more important in mycorrhized seedlings than non mycorrhized ones (87.78 %; 3 %, 1.17 % and wide groups of vesicles) [14].

The increase of plant growth and fruit production was observed after plants inoculation with arbuscular mycorrhizal fungi (AMF). It was also reported by other authors [21-22]. Mycorrhized strawberry seedlings showed a higher volume of roots than non mycorrhized seedlings. Some AMF endophytes increase the absorptive area of the plant, and act as extensions to the root system [23]. Roots colonised by mycorrhizal fungi were better formed and had more lateral roots [22]. These results indicate that mycorrhization has a beneficial effect on growth and development of the root system, plant mineral status, and plant growth and yielding. The same results were identified by other studies [14,24]. Mycorrhized strawberry seedlings have high number of leaves, flowers, and fruits in the greenhouse and all have good and earlier fruiting. Oppositely, non inoculated seedlings have maintained a slower growth. Chantal Hamel had also reported that AMF improve and anticipate strawberry productivity [25].

Statistical analysis showed a positive correlation between the inoculation by a composite mycorrhizal fungus and strawberry’s growth and fruiting. This result joins what had been demonstrated by other studies [14]. AMF improves plant growth through increasing uptake of phosphorus, reducing soil borne diseases fungi [26], and enhancing absorption of water and other nutrient from the soil of entire strawberry plants [15,27].

In a study elaborated in a nursery on strawberry plants; mycorrhized plants showed a densification of roots, a better absorption of nutrient, and a quality improvement of plants resumption after plantation, a better tolerance in hydric stress, and an increase of productivity and fruit size [7].

Same effect had been noted on other crops like tomato [28] and banana [29]. The beneficial effect of the mycorrhizal symbiosis on the water status of tomato plants stimulated plant growth [28]. In banana, mycorrhization resulted in significantly better plant growth even in the presence of endoparasitic nematodes [29].
Mycorrhizal fungi, which are active in the rhizosphere, take part in the cycles and transfer of mineral elements in the soil and into the roots [30]. AMF can stimulate plant growth, especially in soils with lower fertility [31]. It was also documented that positive effect of mycorrhizas on plants is mainly due to improved Phosphorus uptake [18,31]. In our study, fertilisers input were absent to optimise conditions for AMF development and to avoid other vegetative production or earlier flowering.

Spores number was more important in mycorrhized soils than non mycorrhized ones. It showed that the rate of colonisation by the inoculums was higher than by the indigenous mycorrhizas. Differences on AMF density could be explained by structure complexity of the underground component of the soil. The same result was observed by Zhao, Z.W et al. [32]. In fact, species have different growth responses to AMF [33]. In a natural ecosystem, diversity of the AMF community could potentially affect the way plant species coexist, and therefore become a determinant of plant community structure [33]. It appears very clearly that additional studies are needed in this field.

Some limitations should be pointed in our study. These results remain dependent on the conditions of the experiment (choice of a single variety, use of a composite endomycorrhizal inoculum, and seedlings breeding at the greenhouse far from the natural interactions which can affect the growth and the productivity of strawberry plants). Despite those limits, our study delivers a precious glimpse about the positive effect of arbuscular mycorrhizal fungi on strawberry’s growth and fruiting. Further studies are warranted to verify and confirm these results and should include other components of this subject, particularly, molecular factors, different species of mycorrhizas with large culture spectre and large samples.

![Fig. 1. Occurrence frequency of the mycorrhizal species in strawberry plants](image1)

![Fig. 2. Mycorrhized (A) and non mycorrhized (B) strawberry seedlings on the same date](image2)
5. CONCLUSION

Strawberry’s growth and fruiting are positively affected by the presence of arbuscular mycorrhizal fungi in the soil. Mycorrhization status seems to be the strongest factor influencing both good growth and fruiting quality of strawberry. Strawberry symbiosis with mycorrhizas represents a safe way not only to preserve soil from chemical products and protect costumers’ health from residues of toxic elements in contaminated food, but also to promote and optimise growth and fruiting of strawberry. It seems to be that arbuscular mycorrhizal fungi should be used in current systems of strawberry farming, and be extended to other speculations of horticulture.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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