Optimizing Potato (*Solanum tuberosum*), Plant Transplantation through Micropropogation

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**Authors’ contributions**

This work was carried out in collaboration among all authors. Author BS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MAFN managed the analyses of the study. Author TAD managed the literature searches. All authors read and approved the final manuscript.

**ABSTRACT**

**Aims**: A pot experiment was set to identify the most effective symbiotic couples during the acclimatization of *Solanum tuberosum* vitroplants inoculated with arbuscular mycorrhizal fungi (AMF).

**Methodology**: The answer to inoculation of 5 strains of MA fungi *Funnelliformis mosseae*, *Rhizophagus aggregatus*, *Rhizoglomus fasciculatum*, *Rhizoglomus intraradices*, *Rhizoglomus manihotis* is evaluated on potato varieties *Aïda*, *Atlas* and *Odessa*. For each variety, the experimental device was totally randomized with 5 replicates. The experiment lasted 3 months.

**Results**: The *F. mosseae* strain has the highest mycorrhizal dependencies with 81.51% and 70% respectively in the *Atlas* and *Aïda* varieties. *Adessa* is less than 40% dependent on all strains of fungi tested. In the *Atlas* variety, the *F. mosseae* strain significantly stimulates the aerial biomass of the plants, which reaches 723.32 mg compared to the other mycorrhizal and control strains. The production of minitubers was stimulated by mycorrhizal fungi. In the *Aïda* variety, the two strains *F. mosseae* and *R. aggregatum* averaged 2.80 and 2.72 minitubers per plant, respectively. With the *Atlas* variety, *F. mosseae* and *R. intraradices* result in the most significant production of minitubers.
Conclusion: The different species of fungi tested do not have the same level of efficiency towards these varieties. Nevertheless, they all promote plant development and potato production compared to non-inoculated controls. Mycorrhizal dependence is more observed with both Atlas and Aïda varieties.

Keywords: Arbuscular mycorrhizal fungi; potato; micropropagation; acclimatization; Glomus sp; Solanum tuberosum.

ABBREVIATIONS

AMF : Arbuscular mycorrhizal fungus
Fm : Funneliformis mosseae
Ra : Rhizophagus aggregatus
Rf : Rhizoglomus fasciculatum
Ri : Rhizoglomus intraradices
Ra : Rhizoglomus manihotis

1. INTRODUCTION

Micropropagation allows the propagation of identical genetic copy of a parent plant [1]. The vegetative multiplication of the potato in vitro is now almost worldwide used for seed production. We need plant nursery acclimatization in order to transfer the seedlings from in vitro to the field. In vitro conditions prior to acclimatization can modify the morphology and physiology of transplanted plantlets. The fragility of micropropagated plants during this phase shows the risks to have a sustained production in terms of quantity and quality. Therefore, it’s worthy to pay particular attention to the potential for the use of shrub mycorrhizal fungi to promote the acclimatization and survival of micropropagated plants [2]. Mycorrhization could be an efficient alternative to improve the rooting and survival of plantlets [3].

Most plant species have no problem to grow around mycorrhizal fungi. They play an important role in improving soil fertility and hydromineral nutrition, particularly the phosphorus intake of terrestrial plants. Improved phosphorus availability through mycorrhizal symbiosis brings about good yield. [4;5], by increasing tuber size or potato productivity [6]. In addition, mycorrhizal fungi might curb pathogens. With potatoes, in vitro, Rhizoglomus irregulare keeps Fusarium sambucium in check and even prevents it from harming the plant by blocking its toxin production. This Rhizoglomus as well control rhizoctonia.

Few researches have been done on a specific association between potato plants and mycorrhizal fungi during acclimatization. As a result, this study aims to identify the most effective symbiotic couples during the acclimatization of Solanum tuberosum vitroplants in plant nursery.

2. METHODOLOGY

2.1 Plant Stock

The different tubers such as Atlas, Aïda and Odessa are from GERMICOPA in France. These varieties adapt well to the agro-climatic conditions of Senegal. Atlas is a relatively late variety, Aïda is semi-late but early ripe, whereas Odessa is early to half-early [7]. Potato tubers were kept cold at 4°C for 4 months to dormant and treated with gibberellic acid at 10^{-2} M for 10 minutes to accelerate bud break [8]. Germination, germ disinfection process and production of potato vitroplants are done according to the protocol put forward by Sarr et al. [9]. Germs were placed in sterile culture glass tubes (22 × 150 mm) filled with 15 ml of Murashige and Skoog (MS) medium [10] while respecting the apicalbasal polarity original. This medium solidified with agar (8 g.l-1) was devoid of growth regulators and adjusted to pH 5.9 before sterilization (120°C for 30 min). The culture tubes were placed in a growth chamber (temperature 28°C, 16 h day photoperiod, light intensity 101.4 µmoles.m-2.s-1, relative humidity 55%).

Tubers were removed from the solution, dried and placed in a sealed chamber, dark and airy at 25°C until germination. After sprouting, the germs of 1 to 2 cm in height were gently lifted tubers using a sterile scalpel and closed at their ends by dipping in a bath of liquid paraffin at 40°C. Disinfection of germs was carried out in a host of laminar airflow. First, they were immersed in distilled water with 20 drops of Tween 80 for 10 min water and then pre-soaked for 10 s in alcohol at 70°C before putting them in a solution of mercuric chloride (HgCl2) to 0.1% for 10 min. After disinfection, germs wiped and recovered, were sterilized on Whatman paper. Briefly,
2.1.1 Fungal material

The fungal material used is made up of 5 shrubby mycorrhizal fungi belonging to the collection of the Laboratory of Fungal Biotechnologies (LBC) of the Faculty of Sciences and Techniques (Dakar, Senegal). The characteristics that distinguish these stumps are as follows (Table 1):

### 2.1.2 Phases of culture and experimental device

The production of the inoculum of strains of AMF, is carried out in greenhouse. The corn (Zea mays L.) is used as a trap plant and the growing substrate is coarse beach sand with low phosphorus content. The substrate is previously washed with running water to desalinize and then sterilized at 120°C for 2 hours and put in pots of capacity 1.5 kg. Inoculation is done after taking out the corn plants by bringing 10 g of mycorrhizal inoculum in contact with the roots of the trap plant. The plants are then often watered to the capacity in the field and receive in addition every 15 days 100 ml of a nutrient solution of Long Ashton [11]. After 3 months, the plants are harvested by taking the substrate that will be dried and put in plastic bags and the roots that are carefully rinsed and placed in closed tubes. The degree of mycorrhization of the corn roots and the sporulation are checked under binocular magnification by non-destructive observations at magnification x 40. All collected samples are kept cold at 4°C until needed.

The inoculation is performed on the day the potato is transplanted into the greenhouse with the following conditions: day/night cycle of 12/12 h, 32/25°C and 40 to 50% air humidity. Plants were irrigated with tap water.

It is about putting 10 g of inoculum in a 2 to 3 cm hole in contact with the root system of each plant.

The answer to inoculation of 5 strains of AMF Funneliformis mosseae, Rhizophagus aggregatus, Rhizoglomus fasciculatum, Rhizoglomus intraradices, Rhizoglomus manihotis is evaluated on potato varieties Aïda, Atlas and Odessa. For each variety, the experimental device was totally randomized with 5 replicates. The experiment lasted 3 months and the following parameters were studied:

- The aerial biomass is dried for 48 hours in the oven at 80°C, in order to know the dry weight thanks to a precision balance by Sartorius trademark (Max range of 3100 g). The average number of minitubers produced is estimated by count.
- Relative mycorrhizal dependence (DMR) expresses the dry mass ratio (MS) of mycorrhizal and non-mycorrhizal plants in previously sterilized soil [12].

\[
\text{DMR} \% = \frac{\text{MS of plant mycorhize}}{\text{MS of non plant mycorhize}} \times 100
\]

### 2.2 Statistical Analysis

It is made using Xlstat Pro 2011 software (version 13.2, Addinsoft) and comparisons between averages are made with the Fischer LSD test at the 5% threshold.

<table>
<thead>
<tr>
<th>AMF Species</th>
<th>(T.H. Nicolson &amp; Gerd) C. Walker et A. Schubler</th>
<th>DAOM 227 131</th>
</tr>
</thead>
<tbody>
<tr>
<td>Funneliformis mosseae</td>
<td>(N.C. Schenke &amp; GS. Smith) C. Walker</td>
<td>DAOM 227 128</td>
</tr>
<tr>
<td>Rhizophagus aggregatus</td>
<td>(Thaxt.) C. Walker &amp; A. Schubler, 2010 (thaxt) Sievered, GA Silva &amp; Oehl</td>
<td>DAOM 227 130</td>
</tr>
<tr>
<td>Rhizoglomus fasciculatum</td>
<td>(Schenk &amp; Smith) Sievered GA Silva &amp; Oehl</td>
<td>DAOM 197 198</td>
</tr>
<tr>
<td>Rhizoglomus intraradices</td>
<td>Howeler, Sieverding &amp; Schenk</td>
<td>IR. 15</td>
</tr>
<tr>
<td>Rhizoglomus manihotis</td>
<td>Sievered GA Silva &amp; Oehl</td>
<td></td>
</tr>
</tbody>
</table>
3. RESULTS

3.1 Mycorrhizal Dependence

After three months of farming, potato varieties proved to be mycotrophic. Root colonization as observed with binocular loupes, consisted mainly of coiled intraroot hyphae and blisters characteristic of the infection. The control treatments did not show mycorrhizal propagules (Fig. 1).

The *F. mosseae* strain has the highest mycorrhizal dependencies with 81.51% and 70% respectively in the *Atlas* and *Aïda* varieties. *Adessa* is less than 40% dependent on all strains of fungi tested (Fig. 2).

3.2 Aerial Biomass

Potato plants show satisfactory vegetative development throughout their cultivation. No signs of necrosis are observed. The dry biomass of the inoculated plants is significantly higher than that of the control plants in all varieties. However, it varies with the inoculated strain. In the *Atlas* variety, the *F. mosseae* strain significantly stimulates the aerial biomass of the plants, which reaches 723.32 mg compared to the other mycorrhizal and control strains.

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**Fig. 1. Appearance of mycorrhizal propagules of atlas roots**

**Fig. 2. Mycorrhizal dependence of three varieties of greenhouse potato**

*Fm = Funneliformis mosseae, Ra= Rhizophagus aggregatus, Rf= Rhizoglomus fasciculatum, Ri= Rhizoglomus intraradices, Ra= Rhizoglomus manihotis. Bars followed by the same letters are not significantly different (Fischer’s protected LSD P < 0.05)*
At the level of the variety Aïda, *F. mosseae* and *R. aggregatum* cause a significant increase in aerial biomass with respectively 715.06 and 657.23 mg.

In Adessa, the most significant biomass is obtained with strain *R. intraradice* 531.86 mg followed by *F. mosseae* 505.1 mg. *R. fasciculatum*, *R. manihotis* and *R. aggregatum* do not significantly influence plant biomass (Table 2).

### 3.3 Number of Minitubers

Inoculated treatments have great impact on the number of minitubers produced by all varieties compared to controls. This positive impact also has variability depending on the symbiotic strain.

In the Aïda variety, the two strains *F. mosseae* and *R. aggregatum* averaged 2.80 and 2.72 minitubers per plant, respectively. The effect of the other strains does not differ significantly from their controls. With the Atlas variety, *F. mosseae* and *R. intraradice* result in the most significant production of minitubers. However, in the Adessa variety, the *R. intraradice* strain produces more minitubers (Table 3).

### 4. DISCUSSION

#### 4.1 Plant Development and Production

The inoculation of the potato with the five fungal strains in the nursery has positively stimulated the aerial biomass of the vitroplants. The Atlas and Aïda varieties benefited more from the introduction of inocula endomycorhiziens than the Adessa variety. These greenhouse results show that different potato varieties can exhibit variable growth response following endomycorrhizian inoculation [13;14].

Among the strains used *F. mosseae* resulted in higher dry biomass production followed by *R. aggregatum*. Similar results were obtained from the work of [15] and [16]. Elsewhere, under artificial conditions Rhizoglomus intraradices has been shown to be more effective in increasing the growth and nutrition of potato plants than *F. mosseae* [17].

### Table 2. Shoot dry weight (mg) plant potato (Atlas, Aïda and Adessa) during three months under different treatment of *Glomus* sp.

<table>
<thead>
<tr>
<th></th>
<th>Atlas</th>
<th>Aïda</th>
<th>Adessa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>402.23 cd</td>
<td>388.05 cd</td>
<td>388.04 de</td>
</tr>
<tr>
<td>Fm</td>
<td>723.32 a</td>
<td>715.06 a</td>
<td>505.11 d</td>
</tr>
<tr>
<td>Ra</td>
<td>677.08 b</td>
<td>657.23 b</td>
<td>455.02 de</td>
</tr>
<tr>
<td>Rf</td>
<td>635.13 bc</td>
<td>628.02 bc</td>
<td>450.20 de</td>
</tr>
<tr>
<td>Ri</td>
<td>625.05 bc</td>
<td>621.04 bc</td>
<td>531.86 c</td>
</tr>
<tr>
<td>Ra</td>
<td>634.03 bc</td>
<td>630.05 bc</td>
<td>456.50 de</td>
</tr>
</tbody>
</table>

*Fm = Funneliformis mosseae, Ra= Rhizophagus aggregatus, Rf= Rhizoglomus fasciculatum, Ri= Rhizoglomus intraradices, Ra= Rhizoglomus manihotis. In column numbers followed by the same letters are not significantly different (Fischer's protected LSD P < 0.05)*

### Table 3. Number of minitubers plant potato (Atlas, Aïda and Adessa) during three months under different treatment of *Glomus* sp.

<table>
<thead>
<tr>
<th></th>
<th>Atlas</th>
<th>Aïda</th>
<th>Adessa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.22 d</td>
<td>1.55 d</td>
<td>1.05 c</td>
</tr>
<tr>
<td>Fm</td>
<td>1.91 a</td>
<td>2.80 a</td>
<td>1.62 ab</td>
</tr>
<tr>
<td>Ra</td>
<td>1.72 c</td>
<td>2.72 b</td>
<td>1.56 b</td>
</tr>
<tr>
<td>Rf</td>
<td>1.79 b</td>
<td>2.66 bc</td>
<td>1.50 b</td>
</tr>
<tr>
<td>Ri</td>
<td>1.81 b</td>
<td>2.02 c</td>
<td>1.66 a</td>
</tr>
<tr>
<td>Ra</td>
<td>1.69 c</td>
<td>2.62 bc</td>
<td>1.52 b</td>
</tr>
</tbody>
</table>

*Fm = Funneliformis mosseae, Ra= Rhizophagus aggregatus, Rf= Rhizoglomus fasciculatum, Ri= Rhizoglomus intraradices, Ra= Rhizoglomus manihotis. In column, values followed by the same letters are not significantly different (Fischer's protected LSD P < 0.05)*
The production of minitubers was stimulated by mycorrhizal fungi. This increase can be explained by an accumulation of tuber reserves [18;19]. This effect varies depending on the fungal symbiote and potato variety. Significant production is noted in the comparative Atlas and Aida varieties of the Adessa variety. This positive impact is more pronounced with strains F. mosseae and R. aggregatum.

4.2 Mycorrhization

The potato vitroplants studied were mycotrophic after 3 months of cultivation. This compatibility of mycorrhizal fungi with potato vitroplants was previously observed in a controlled environment [15].

We observed an absence of mycorrhizal colonization of the roots of the vitroplants in the control treatments. This shows that the approach adopted has effectively maintained the culture system without fungal contamination.

Our results also indicate significant differences in levels of endomycorrhizian colonization among the different potato varieties used. Atlas was more compatible with mycorrhization followed by Aida. Adessa has the lowest mycorrhizal dependence. This intraspecies variability has already been demonstrated in several cultivated species [20], including potatoes [21].

In other parts, the five strains colonized the roots of the potato plants differently. Strains F. mosseae and R. aggregatum are more successful at root colonization of potato Atlas and Aida varieties. Mycorrhizal dependence observed in all varieties did not reach 100%. Previous work by [22] on the mycorrhization of potatoes reveals that this species, although poorly colonized by MA fungi, can show notable morphological and physiological responses in the presence of fungal symbiotes. This dependence noted for better growth can be, among other things, explained by the low root density of the plant compared to its strong growth potential. In addition, the difference in dependence observed within the same variety has been described in several studies [23;24].

5. CONCLUSION

The three varieties Aïda, Atlas and Adessa adapt well to the local conditions of Senegal. The results of our work have shown that the fungi introduced as inoculants during the transplant of potato plants in the greenhouse, optimize their development and production of minitubers.

The different species of fungi tested do not have the same level of efficiency towards these varieties. Nevertheless, they all promote plant development and potato production compared to non-inoculated controls. Mycorrhizal dependence is more observed with both Atlas and Aida varieties. The R. mosseae strain is more compatible with these two varieties than the other strains tested.

These inocula endomycorrhiziens, applied to the potato crop, could improve yields or, at least, reduce chemical inputs, including fertilizers. This mycorrhizal optimization could also help improve tuber quality by providing protection against telluric diseases and limiting the development of infections.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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