An Assessment of the Benefits and Period of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation and the Level of Fertilizer Influential Tomato Development (Solanum lycopersicum L.)

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Authors’ contributions
This work was carried out in collaboration among all authors. Authors OB, AK and KD designed the study, wrote the protocol and wrote the first draft of the manuscript. Author KNA managed the analyses of the study. Authors TS and SN managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT
Data on tomato fitness improvement by arbuscular mycorrhizal fungi (AMF) remain patchy. The present study was initiated to evaluate the effect of the period of AMF inoculation as well as the level of mineral manure on tomato growth. The experiment took place from June to October 2016, in the West African Science Service Center on Climate Change and Adapted Land Use greenhouse.

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AMF inocula were applied to seeds and/or transplants, each receiving three different levels of chemical fertilizer. The impact of the inoculation period and the level of fertilization, were assessed on plant growth parameters, including height, number of functional leaves, root-collar diameter, and root length. Observation of hyphae, arbuscules and vesicles was carried out by roots staining method and enabled the determination of mycorrhization parameters. Plants Mycorrhizal dependence was assessed with their fresh and dry mass. An analysis of variance and post ANOVA analysis was performed using the Newman-Keuls test (P= .05) for the comparison of means. The findings pointed that, when transplanting, the difference between mycorrhized plants and non-mycorrhized ones was very highly significant in terms of the height of the stem (P= .00), the length of the taproot, and the root collar diameter. The lower the level of manure was, the higher the frequency of infection has been (73.33% for MS1 and MSR1; 76.67% for MR1). Transplants growing without a supply of mineral manure expressed greater mycorrhizal dependence (66% for MSR1). Arbuscular mycorrhization of the tomato is profitable for its optimal development. The endomycorrhization of tomato can be done during sowing or transplanting with the same benefits but, with a low level of fertilizer. So, it’s necessary to control the intake of mineral manure because it influences the natural mycorrhization of plants.

Keywords: Tomato; arbuscular mycorrhizal fungi; chemical fertilizer; mycorrhizal dependence; endomycorrhization.

1. INTRODUCTION

Tomato plants can establish arbuscular mycorrhizal symbiosis [1], and the benefit of mycorrhization on their fitness been mainly described under stress [2]. The literature on the potential effects of arbuscular mycorrhizal fungi (AMF) on seedling growth in the nursery and the yield of vegetables grown above ground is very poor. However, some studies have shown that AMF could be of great importance for improving plant productivity, particularly in organic-biological production. Several authors have noted that AMF increased plant biomass [3,4,5], promoted flowering [6], and minimized deleterious effects of drought, frost, and other environmental stresses [7,8]. All these results reveal the importance of AMF in sustainable agriculture.

However, technical itineraries proposing endomycorrhizal inoculation to improve tomato production in Côte d'Ivoire are almost non-existent. Instead, chemical inputs are promoted, especially mineral fertilizers. This, without considering the likely impact of this approach, on the natural mycorrhization of plants. In addition, research for varietal improvement seems to overlook the biological trait represented by the plant's ability to establish a symbiotic endomycorrhizal relationship.

The challenges of agricultural sustainability and profitability lead us to know the best time of AMF inoculation to avoid the need for a chemical fertilizer application. The aim of this quest is to improve the living conditions of farmers by increasing their incomes. The objective of this present work is to assess on the tropimech variety of tomatoes (S. lycopersicum L):

- The benefit and the effect of the period of AMF inoculation;
- The effect of chemical fertilization on mycorrhization parameters and mycorrhizal dependence.

1.1 Study Site

The experiment took place from June to October 2016, in the WASCAL (West African Science Service Center on Climate Change and Adapted Land Use) greenhouse, located at the scientific and innovation center of the Félix Houphouët-Boigny University. The site is located in the city of Bingerville (District of Abidjan, South Côte d'Ivoire). Bingerville (~3.900000, 5.350000) has a tropical climate. The corresponding climate is of the subequatorial type [9]. The average annual precipitation is 1823 mm with an average temperature of 26.4°C, and the maximum rain is between May and June with 510 mm [10].

2. MATERIALS

2.1 Tomato Cultivar

The in-vivo evaluation of the inoculation effect of the arbuscular mycorrhizal fungi (AMF) was carried out on tomato seedlings of the Tropimech variety of Tomato (S. lycopersicum). Tropimech variety was chosen for its affordability and appreciation by growers. The seeds were
purchased from a seed company in Abidjan (South Côte d’Ivoire; 5.345317, -4.024429).

2.2 Mycorrhizal Inoculum

The material consists of arbuscular mycorrhizal fungi (AMF) propagules provided by “Inoculum plus” from Technopôle Agro-Environnement. The AMF species are presented in Table 1.

2.3 Mineral Manure

The mineral fertilizer used is NPK 15-9-20 + 3.8S + 1.8MgO + 0.02Zn + 0.02B + 0.02Mn. It is a commercial product labelled YaraMila.

3. METHODS

3.1 Seeds Treatment and Seedlings

The tomato seeds were washed with tap water and then immersed in 70% alcohol for 30 s. They were then transferred to a beaker containing 40% sodium hypochlorite and stirred for 10 min. The seeds were finally rinsed with distilled water and put to germinate in two large plastic tubs containing the substrate sand / potting soil in the proportions 6 / 1 for 30 days.

3.2 AMF Inoculation

3.2.1 During sowing

Before sowing, 150 g of the AMF inoculum was rigorously mixed with the substrate to obtain mycorrhized plants (M). In the second tray, there was no addition of mycorrhizal inoculum. The tomato plants from this tank are non-mycorrhized (NM) plants. The trays have been kept in a greenhouse, and the substrate was regularly watered to field capacity to keep it continuously moist.

3.2.2 During transplanting

The plants were transplanted into pots filled with a mix of sand / potting soil in the proportion 4/1. At this stage, AMF inoculations were carried out according to the following treatments:

- MS: inoculation during seedling stage only;
- MSR: inoculation during seedling and transplanting;
- MR: inoculation during transplanting only;
- NM: non-mycorrhized plants.

2 g of AMF inocula provided during transplanting (MSR and MR), were applied in each pot, in the planting hole made in the planting substrate.

For each inoculation treatment, three levels of fertilization named 1, 2, and 3 corresponding respectively to 0 g, 1 g, and 2 g applied separately onto six plants.

After germination, the plants were exposed in a greenhouse to ambient light only and were watered daily at pot capacity until the end of the experiment.

3.3 Observation of Hyphae, Arbuscules and Vesicles

Observation of endomycorrhizal infection was carried out by staining fine roots [11]. The observation was carried out on all mycorrhized plants at the end of the experiment.

3.4 Determination of Mycorrhization Parameters

At the time of transplanting and at the end of the experiment, the frequency of mycorrhization, the intensity of mycorrhization and the arbuscular abundance were determined.

Indeed, the roots of five plants chosen randomly from inoculation treatment (M) were cut and stained [11]. The same treatment is carried out with five not mycorrhizal (NM) plants. At the end of the experiment, the mycorrhizal colonization was estimated with 3 plants chosen randomly by

Table 1. Composition of the arbuscular mycorrhizal fungi consortium inoculated to tomato

<table>
<thead>
<tr>
<th>Number</th>
<th>Denominations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>- <em>Claroideoglomus etunicatum</em> (formerly G. etunicatum)</td>
</tr>
<tr>
<td>2</td>
<td>- <em>Glomus microaggregatum</em></td>
</tr>
<tr>
<td>3</td>
<td>- <em>Rhizophagus irregularis</em> (formerly G. intraradices)</td>
</tr>
<tr>
<td>4</td>
<td>- <em>Claroideoglomus claroideum</em> (formerly G. claroideum)</td>
</tr>
<tr>
<td>5</td>
<td>- <em>Funneliformis mossae</em> (formerly G. mossae)*</td>
</tr>
<tr>
<td>6</td>
<td>- <em>Funneliformis geosporum</em> (formerly G. geosporum)*</td>
</tr>
</tbody>
</table>

*Minimum number of fungal propagules: 1 million /kg (evaluated according to the Most Probable Number Test)*
treatment (MS1, MS2, MS3, MSR1, MSR2, MSR3, MR1, MR2, MR3).

Colonization of 1 cm long of 10 root fragments was checked for mycorrhization and evaluated under a microscope [12]. In this method, the frequencies of infection are classified from 0 to 5, and the arbuscular abundance from A0 to A3 (Fig. 2).

The frequency of the infection $F$ (%) or percentage of the number of endomycorrhizal root fragments was calculated as follows:

$$F(\%) = \frac{n_0}{N} \times 100$$

Where $N$ = number of fragments observed; $n_0$ = number of mycorrhizal fragments.

The root fragments intensity of mycorrhization $M$ (%) corresponding to the proportion of colonized cortex was calculated as follows:

$$M(\%) = \frac{95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1}{N}$$

Where $n_5$ = number of fragments noted 5; $n_4$ = number of fragments noted 4; $n_3$ = number of fragments noted 3, $n_2$ = number of fragments noted 2, $n_1$ = number of fragments noted 1 and $N$ = number of fragments observed.

The root system intensity of mycorrhization $m$ (%) or absolute intensity of mycorrhization was determined as follows:

$$m(\%) = \frac{M \times N}{n_0}$$

The root fragments arbuscular abundance $a$ (%) or relative arbuscular content of infection corresponding to the proportion of the root fragment cortex containing arbuscules;

$$a(\%) = \frac{(100m_3 + 50m_2 + 10m_1)}{100}$$

Where $m_3$, $m_2$, $m_1$ are the intensities of absolute mycorrhization, classified A3, A2, A1 respectively, with

$$m_i = \frac{95n_5Ai + 70n_4Ai + 30n_3Ai + 5n_2Ai + n_1Ai}{n_0}$$

The root system arbuscular abundance $A$ (%) or absolute arbuscular content of infection corresponding to the proportion of the entire root system cortex containing arbuscules:

$$A(\%) = a \times \frac{M}{100}$$

Fig. 1. Notation of mycorrhizal infection and richness in arbuscular, (a): Mycorrhizal infection (classified from 0-5); (b): Wealth in arbuscular (classified from A0-A3)
3.5 Evaluation of the Effect of AMF Inoculation on Tomato Plants Growth

The impact of mycorrhizal inoculation was assessed through plant growth parameters such as the stem height, the taproot length, the collar diameter, and the number of functional leaves. The measures were performed at transplanting, then at the end of the experiment. At the end of the experiment (60 days after transplanting), plants were removed from the growing medium and weighed to determine the fresh biomass.

The stem height and the length of the taproot were determined respectively for both the two first with a measuring tape and the root collar diameter by a caliper. The functional leaves were simply counted.

3.6 Mycorrhizal Dependence

The plants were removed from their growing medium, and the fresh mass of the whole plants (roots, stems, and leaves) was determined by simple weighing using a precision electronic balance (10⁻⁶). The plants were then placed in an oven at 70°C for 3 days to determine the dry mass.

The values obtained were used to calculate the relative dependence on mycorrhization (DM) defined as follows [13]:

\[
DM = \frac{\text{dry mass of mycorrhizal plants} - \text{dry mass of non–mycorrhizal plants}}{\text{dry mass of non–mycorrhizal plant}} \times 100
\]

3.7 Statistical Analyzes

The data collected were subjected to an analysis of variance with one classification criterion (ANOVA I) using Statistica version 7.1 software. A post ANOVA analysis was performed using the Newman-Keuls test (\(P = .05\)) for the comparison of means and the Wilks lambda multivariate analysis of variance (\(P = .05\)).

4. RESULTS

4.1 Measurement of Growth Parameters at Transplanting

When transplanting, the measurements carried out revealed that mycorrhized plants were more developed than the non-mycorrhizal plants (Table 2). The differences noted in the mean values were highly to very highly significant in terms of the height of the stem (\(P = .00\)), the length of the taproot (\(P = .00\)), and the root collar diameter (\(P = .00\)). In terms of the number of functional leaves (\(P = .11\)), the differences observed were not significant.

4.2 Evolution of Agronomic Parameters

4.2.1 Evolution of the stem height

At the end of the experiment, there was a significant difference in the stem height between the treatments (\(P = .00\)). The NM1 plants remained the smallest and followed by the MS1 and MR1 plants, respectively (Fig. 2).

The Newman and Keuls test identified the homogeneous groups of treatments. Plants having received a double mycorrhization but without fertilizer (MSR1) had a higher average height than plants MS3 and NM3 even if the differences were not significant (Table 3).

4.2.2 Evaluation of the number of leaves

A significant leaf fall was noted in the NM3 plants between 45 and 60 days. On the contrary, in MR3 plants this number increased. The lowest numbers of functional leaves were obtained from plants that did not receive mineral manure. The number of functional leaves of NM1 plants (non-mycorrhized and non-fertilized) was much lower than that of other plants (Fig. 3).

4.2.3 Evaluation of the collar diameter

The stem collar diameter increased during the experiment, regardless of the treatment (Fig. 4). The collar diameter of the NM1 plants was the smallest, followed consecutively by the plants MS1, MSR2, MR1, and MSR3 (Fig. 5). The differences observed were very highly significant with \(P = .00\).

4.2.4 Root length evaluation

Means root length of mycorrhized plants was 6.89 cm versus 4.25 cm for non-mycorrhized one at transplanting, and respectively 17.83 versus 14.80 cm at the end of the experiment (Table 4). During transplanting and at the end of the experiment, the roots of mycorrhized plants were longer than those of non-mycorrhized plants (Fig. 6). Results presented in Table 4 show very highly
significant differences at transplanting ($P = .00$) and at the end of the experiment ($P = .00$).

### 4.3 Effect of the Chemical Fertilizer Level

At the end of the experiment (60 days after transplanting), the results obtained without chemical fertilizer were different from those obtained with mineral fertilizer (Fig. 8). The higher the level of chemical fertilizer was, the greater the fresh and dry mass of the plants. Wilk Lambda MANOVA gave the fertilizer level $P = .00$. The level of chemical fertilizer had a highly significant effect on the measured agronomic parameters (Fig. 7). The post hoc test of Newman-Keuls on the fresh mass data specifies the differences between the supply of chemical fertilizer (fertilizer levels 2 and 3) and no supply of chemical fertilizer (fertilizer level 1) as highly significant. Besides, the differences between the effects of the chemical fertilizer levels 1 and 2 were significant (Fig. 8).

### 4.4 Effect of Time of Inoculation

The graph of all effects (Fig. 9) showed differences between plants depending on the time of inoculation. The vertical bar indicates 0.95 confidence interval and $P = .00$ and the Wilks lambda test = .00. Similarly, the univariate results showed that the period of inoculation had a very significant effect on the agronomic parameters as well as plant masses with $P = .00$ for all the measured parameters.

### 4.5 Study of Mycorrhization

#### 4.5.1 Mycorrhization parameters at transplanting

The plants inoculated during transplanting with the AMF showed mycorrhizal structures in their roots. However, the frequency of infection and the arbuscular abundance were very low, less than 1% (Table 5).

#### 4.5.2 Mycorrhization parameters at the end of the experiment

The lower the level of manure is, the higher the frequency of infection (Table 6) has been. Thus, the lowest frequencies of infection were obtained with the level of manure 3 and the highest with level 1. There was a positive correlation between the frequency of mycorrhization and the root system arbuscular abundance (Table 6).

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**Fig. 2. Evolution of the average stem height of mycorrhized plants (M) and not-mycorrhized (NM) during the experiment**

NM: plant not mycorrhized; MS: plants mycorrhized at seedlings; MR: plants mycorrhized at transplanting; MSR: plants mycorrhized at seedlings and transplanting; 1, 2 and 3: Fertilizer levels 1, 2 and 3.
Table 2. Means value of growth parameters at transplanting

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Height (cm)</th>
<th>Taproot length (cm)</th>
<th>Number of leaves</th>
<th>Stem collar diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycorrhized</td>
<td>8,09 ± 0,20</td>
<td>6,89 ± 0,35</td>
<td>3,94 ± 0,15</td>
<td>1,06 ± 0,04</td>
</tr>
<tr>
<td>Non mycorrhized</td>
<td>5,22 ± 0,14</td>
<td>4,26 ± 0,22</td>
<td>3,64 ± 0,11</td>
<td>0,68 ± 0,02</td>
</tr>
</tbody>
</table>

Table 3. Homogeneous groups at the level of the stem height at the end of the experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root length</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM1</td>
<td>10,75 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS1</td>
<td>28,13 e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR1</td>
<td>35,17 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM3</td>
<td>39,78 ab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS3</td>
<td>40,12 ab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR1</td>
<td>41,37 ab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSR1</td>
<td>41,67 ab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSR2</td>
<td>41,67 ab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS2</td>
<td>45,82 ac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM2</td>
<td>46,20 ac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSR2</td>
<td>47,23 ac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR3</td>
<td>47,25 ac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR2</td>
<td>51,50 c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MS: mycorrhizal at seedlings only; MSR: mycorrhizal plants at seedlings and transplanting; MR: mycorrhizal plants at transplanting only; NM: not mycorrhizal plants; 1, 2 and 3: Chemical manure levels 1, 2 and 3

Fig. 3. Evolution of the number of functional leaves during the experiment

NM: plant not mycorrhized; MS: plants mycorrhizal at seedlings; MR: plants mycorrhizal at transplanting; MSR: plants mycorrhizal at seedlings and transplanting; 1, 2 and 3: Fertilizer levels 1, 2 and 3
Table 4. Length of the root at transplanting and at the end of the experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transplanting</th>
<th>End of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycorrhized</td>
<td>6.89 ± 0.35 a</td>
<td>17.83 ± 0.64 a</td>
</tr>
<tr>
<td>Non mycorrhized</td>
<td>4.25 ± 0.22 b</td>
<td>14.80 ± 0.78 b</td>
</tr>
</tbody>
</table>

Fig. 4. Evolution of the stem collar diameter

NM: not mycorrhizal; MS: mycorrhizal at seedlings; MR: mycorrhizal at transplanting; MSR: mycorrhizal at seedlings and transplanting; 1, 2 and 3: Chemical manure levels 1, 2 and 3

Fig. 5. Variations in the collar diameter of the collar according to the period of AMF inoculation

NM: non-mycorrhizal plant; MS: seed-inoculated plant; MR: Plant mycorrhized at transplanting; MSR: seed-inoculated plant and transplanting; 1, 2 and 3: fertilizer levels 1, 2 and 3
**Fig. 6.** Root length at transplanting and at the end of the experiment in mycorrhized plants and non-mycorrhized plants.

**Fig. 7.** Effect of the level of mineral manure

| Mf: Fresh mass; Ms: Dry mass; 1, 2, and 3: Fertilizer levels 1, 2, and 3 |

**Table 5. Parameters of subculture mycorrhization**

<table>
<thead>
<tr>
<th></th>
<th>F (%)</th>
<th>M (%)</th>
<th>m (%)</th>
<th>a (%)</th>
<th>A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>M</td>
<td>34.00</td>
<td>58.00</td>
<td>70.54</td>
<td>0.55</td>
<td>0.32</td>
</tr>
</tbody>
</table>

NM: non mycorrhized plant; M: mycorrhized plant; F: frequency of infection; M (%): Intensity of mycorrhization; m (%) : root system intensity of mycorrhization; a (%): root fragments arbuscular abundance; A (%): root system arbuscular abundance
Fig. 8. Fresh masses of plants obtained depending on the level of fertilization
(1): 0 g of manure added to the growing medium; (2): 1 g of manure added to the growing medium; (3): 2 g of manure added to the growing medium

Fig. 9. Graph of agronomic parameters according to the treatments
NM: non-mycorrhized plant; MS: seed-inoculated plant; MR: Plant mycorrhized at transplanting; MSR: Seed-inoculated plant and transplanting; DC: Collar diameter; Mf: Fresh mass; Ms: Dry mass; 1, 2 and 3: fertilizer levels 1, 2 and 3
4.5.3 Mycorrhizal dependence (DM)

The determination of the fresh and dry masses preceded the calculation of the DRM for each situation of mycorrhization. The vertical bar indicates 0.95 confidence interval and $P = .00$ and the Wilks lambda test = .02. The period of inoculation had a significant impact on the development of the plants and the fresh masses (Fig. 10).

Transplants growing without a supply of mineral manure expressed greater mycorrhizal dependence (Fig. 11). The MD of the other plants transplanted on supports improved by the addition of mineral manure was almost null.

Table 6. Mycorrhization parameters at the end of the experiment

<table>
<thead>
<tr>
<th></th>
<th>NM1</th>
<th>NM2</th>
<th>NM3</th>
<th>MS1</th>
<th>MS2</th>
<th>MS3</th>
<th>MSR1</th>
<th>MSR2</th>
<th>MSR3</th>
<th>MR1</th>
<th>MR2</th>
<th>MR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>73.33</td>
<td>56.67</td>
<td>33.33</td>
<td>73.33</td>
<td>53.33</td>
<td>46.67</td>
<td>76.67</td>
<td>63.33</td>
<td>20.00</td>
</tr>
<tr>
<td>M (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>22.03</td>
<td>8.60</td>
<td>2.40</td>
<td>18.93</td>
<td>6.13</td>
<td>1.05</td>
<td>18.50</td>
<td>6.87</td>
<td>1.30</td>
</tr>
<tr>
<td>m (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>30.04</td>
<td>15.18</td>
<td>7.20</td>
<td>25.81</td>
<td>11.49</td>
<td>2.25</td>
<td>24.13</td>
<td>10.84</td>
<td>6.50</td>
</tr>
<tr>
<td>a (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>78.93</td>
<td>76.83</td>
<td>43.33</td>
<td>88.72</td>
<td>63.86</td>
<td>47.30</td>
<td>74.59</td>
<td>77.00</td>
<td>40.77</td>
</tr>
<tr>
<td>A (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>17.39</td>
<td>6.61</td>
<td>1.04</td>
<td>16.79</td>
<td>3.91</td>
<td>1.55</td>
<td>13.80</td>
<td>5.29</td>
<td>0.53</td>
</tr>
</tbody>
</table>

NM: non mycorrhized plant; M: mycorrhized plant; F: frequency of infection; M (%): Intensity of mycorrhization; m (%): root system intensity of mycorrhization; a (%): root fragments arbuscular abundance; A (%): root system arbuscular abundance. NM: non-mycorrhized; MS: seed-inoculated plant; MR: Plant mycorrhized at transplanting; MSR: seed-inoculated plant and transplanting; 1, 2 and 3: Fertilizer levels 1, 2 and 3

Fig. 10. Effect of the period of inoculation on fresh masses

NM: non-mycorrhized plant; MS: seed-inoculated plant; MR: Plant mycorrhized at transplanting; MSR: seed-inoculated plant and transplanting; 1, 2 and 3: fertilizer levels 1, 2 and 3

Fig. 11. Histograms of mycorrhizal dependence as a function of inoculation period and level of fertilization

NM: non-mycorrhized plant; MS: seed-inoculated plant; MR: Plant mycorrhized at transplanting; MSR: seed-inoculated plant and transplanting; 1, 2 and 3: fertilizer levels 1, 2 and 3
5. DISCUSSION

The AMF inoculum contains infectious strains. The formation of mycelium and arbuscules shows that arbuscular mycorrhizae are formed between the inoculated AMF and the Tropimech variety of Tomato. This result is consistent with previous findings pointed out Tomatoes as mycotrophic plants [1].

Inoculation resulted in measurable changes in growth parameters. The inoculated plants responded positively to mycorrhization in the early stage leading to better development compared to non-mycorrhized plants. These data are consistent with past results demonstrating the interest of an early mycorrhization [14,15,16,17,18]. Plant bioaugmentation by arbuscular mycorrhizae has also been reported to improve the qualities of seedlings in nurseries [19,17,18].

The number of leaves, which plays an essential role in seedling growth and development, was not significantly affected by the two treatments (M and NM). However, the inoculated tomato plants showed higher values of the number of leaves.

Plants that were not mycorrhized (NM1) and did not receive mineral manure showed the lowest growth compared to mycorrhized plants that did not receive mineral manure (MS1, MSR1, MR1). Indeed the mycorrhizosphere, which results from an extension and probably of a ramification of the root system of the mycorrhized plant, has a positive correlation with the volume of soil whose reserves are accessible to the plant, called volume of colonized soil [20]. Thus, the area of nutrient sampling from mycorrhized plants, for the benefit of the plant, is more extensive than that of non-mycorrhized plants. So, the ability of seedlings for better development depends on the ability of the roots to withstand structural and functional changes on the one hand and to access water and nutrient reserves on the other ground [21]. The roots have been significantly longer in the inoculated plants compared to the uninoculated controls, and this could have improved the absorption of nutrients and ensured better growth. It has been reported that inoculation at the initial stage of plant development could promote arbuscular mycorrhizal symbiosis, resulting in increased plant growth in the nursery and improved performance after planting in the field [22].

In terms of root length, seed inoculation showed the best results with fertilization levels 2 and 3 than double inoculation and transplanting inoculation. Seed-inoculated plants showed the best results due to the early establishment of the symbiotic relationship. When the symbiotic relationship is established in the early stages of the plant's development, it results in better growth. Several authors pay particular attention to the depth of rooting [23], making it possible to search for water in depth, especially when its presence is limited in the surface layers of the soil. In the literature, the ability to maintain a high number of primary roots under water stress is considered to allow better access to water by the plant. However, several authors pay particular attention to the depth of rooting [23], making it possible to seek water in depth when its presence is limited in the surface layers of the soil [24]. This would be true even if this depth is only reached by a single taproot [25].

The number of leaves was relatively low when the plant was not mycorrhizal, and the rate of fertilization was high. The leaves are the primary support for photosynthesis. A better growing mycorrhizal plant offers more leaves. The number of leaves, which plays a vital role in seedling growth and development, was not significantly different between treatments, even though the inoculated tomato plants exhibited higher leaf number values. Such results have already been obtained in other works [26].

The average weight of the fresh biomass of the inoculated Tomato not supplied with minerals (19.85 g plant⁻¹) was higher and very significantly different from the non-mycorrhized control and not fertilized (.65 g plant⁻¹). The total dry mass of the tomato seedlings increased significantly with the inoculation of AMF compared to the control. The total dry biomass of AMF-inoculated seedlings increased compared to the control, which indicates better potential performance in the field. Thus, the highest mycorrhizal dependence was obtained in the absence of mineral manure (F1). In a soil rich in mineral elements, there is almost no difference in growth between a mycorrhized plant and non-mycorrhized one. This is why, in the event of the contribution of mineral manure (F2, F3), the MD is almost zero. This shows that the plant is less dependent on mycorrhiza for its growth.

Similarly, the mycorrhization rates obtained in our study are low and lower than those obtained with the Tomato cultivated in the soil, 35.99%
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[27]. This rate decreases with the level of manuring. Then, the interest of the endomycorrhizal symbiotic relation would be more marked when the soil is relatively poor in mineral elements in particular phosphorus [28,29]. The double mycorrhization increases by the second contribution of AMF to transplanting the chances of establishing the symbiotic relationship. Thus, the highest value is obtained in a double mycorrhization (MSR1) situation and the lowest in the seedlings-mycorrhization (MS1) situation.

6. CONCLUSION

The practice of endomycorrhization of tomatoes, which is a reality in many countries, should be in Côte d'Ivoire. This prospective study revealed the need for better management of the AMF-fertilizer pair in order to ensure better development of the tomato.

The interest of arbuscular mycorrhization in the growth and development of tomato plants has again been demonstrated. In addition, the influence of mineral manure on the plant's ability to establish a symbiotic endomycorrhizal relationship has been proven: the higher the manuring rate, the lower the mycorrhization rate and vice versa. And, this has the effect of reducing the plant's dependence on mycorrhization for its development. This ultimately poses the problem of the loss of the ability to establish this symbiotic relationship by the plant due to the systematic supply of mineral manure by the farmers. As for the appropriate moment for the contribution of AMF, it can be done from the semi but also, when transplanting the plants in association with a moderate supply of manure to be quite beneficial for the plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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