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The Potential Evaluation of Mycorrhizal Inoculation on the Growth of Young Cocoa Plants (*Theobroma cacao* L.) Hybrid P7 x IMC67 in Greenhouse

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ABSTRACT

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This research aims to evaluate the effectiveness of mycorrhizal inoculation alone or in combination with biofertilizers on the growth of cocoa seedlings. The study was carried out in the greenhouse at the Station d'Expérimentations Agronomiques of the University of Lomé from December 2020 to May 2021 following a completely randomized design. Cocoa beans (hybrid P7 x IMC67) were sown on substrates inoculated with *Rhizophagus aggregatum* and *Ben 10* strains at a single dose of 500 spores/pot or combined with two biofertilizers: FERTIPLUS (100 g/pot), AGROBIO (75 g/pot). The seedlings were monitored and their agronomic performance measured. The results show a good mycorrhization rate, 63.67% for *R. aggregatum*, 62% for *Ben 10*, 67% for *R. aggregatum* + AGROBIO and 60.34% for *Ben 10* + AGROBIO with mycorrhizal dependencies of 18.70%; 18.72%; 35.75% and 41.15% respectively. Furthermore, the results showed that the application of both mycorrhizal strains (*R. aggregatum* and *Ben 10*) alone or in combination with AGROBIO induced a significant increase ($p < 0.05$) in total fresh biomass, total dry biomass, height, collar circumference and number of leaves compared with the control. However, application of FERTIPLUS alone or in combination with the two mycorrhizal strains significantly ($p < 0.05$) depressed total fresh biomass, total dry biomass, height, crown circumference, taproot length and number of leaves compared with the control. This study shows that arbuscular mycorrhizal fungi provide important benefits to the plant, and can be used to improve the growth of young cocoa plants.

Introduction

The cocoa tree (*Theobroma cacao* Linn.) is a forest crop cultivated for its fruit, the cocoa bean, which is a raw material for the food, cosmetics and pharmaceutical

industries (Jagoret, 2011). The West African cocoa belt currently accounts for over 73% of global production (Wessel et Quist-Wessel, 2015) with the remainder coming from America and Asia. The area dedicated to cocoa cultivation has expanded significantly over the

past 60 years, rising from around 4.4 million hectares in the 1960 to almost 12 million hectares in 2021, for an annual production of over 5 million tons of merchantable cocoa (CIRAD, 2022).

In Togo, cocoa is one of the main agricultural cash crops for export, and a significant source of income for producers. More than 90% of cocoa production in Togo is carried out by small-scale farmers (Djiwa *et al.*, 2021) who cultivate an average area of 26,356.66 ha for a national production of around 12,674.43 tons of merchantable cocoa per year, with a low average yield of 506.83 kg/ha (DSID, 2018) compared with a potential of 1200 to 3500 kg.ha⁻¹ (CRAF, 2004).

The low productivity of cocoa orchards can be explained by several constraints such as ageing trees, crop losses due to pests, diseases and soil fertility degradation (Koudjega, 2013). In addition, the severity and length of the droughts recorded in the cocoa production zone have led to a drastic drop in yields and a high mortality rate among young seedlings when new plantations are set up. The main problem facing actors in Togo's cocoa sector is that existing cocoa orchards are past full production age and therefore no longer productive.

Several research programs have been developed to improve cocoa productivity in Togo. These programs include the introduction of drought-tolerant hybrids (IRCC, 1988), the development of agroforestry and the use of organic or mineral fertilizers (Koudjega, 2013; Adden, 2017; CRA-F, 2019). However, these efforts to improve cocoa production to meet future demand are hampered by the difficulty of supplying good-quality cocoa seedlings.

Indeed, fertilization management in the production of cocoa seedlings in nurseries often relies on mineral fertilizers and sometimes biofertilizers. However, to obtain good-quality seedlings, good treatment is essential, starting in the nursery, through the application of microorganisms such as arbuscular mycorrhizal fungi (AMF) or combined with fertilizers. AMF are telluric microorganisms that form symbiotic relationships with around 80% to 90% of terrestrial plants through root colonization (Brundrett, 2002; Berruti *et al.*, 2016). They can improve plant growth and increase yield, particularly on nutrient-poor acid soils, and substitute environmentally damaging chemical fertilizers (Nelly *et al.*, 2019). These fungi are natural biofertilizers that increase the uptake of nutrients (nitrogen, phosphorus,

zinc, etc.) and water from the soil to transfer them to the roots of the host plant (Jansa *et al.*, 2019; Zhou *et al.*, 2020) while providing protection against pathogens in exchange for the products of plant photosynthesis (Berruti *et al.*, 2016; Tchabi *et al.*, 2016; Wang *et al.*, 2017; Nenonene *et al.*, 2022). They improve drought tolerance and reduce sensitivity to toxic substances in the soil (Zhang *et al.*, 2006; Bucher, 2007). Consequently, the use of AMFs is essential for environmentally-friendly agriculture and could significantly reduce the use of chemical fertilizers and pesticides (Finlay *et al.*, 2008), while improving crop growth and yields (Grümberg *et al.*, 2015; Kim *et al.*, 2017; Tchabi *et al.*, 2022).

Several studies have shown that cocoa trees develop mycorrhizal symbioses on which they depend for their growth and development (Iglesias *et al.*, 2011; Antonius *et al.*, 2015; Droh *et al.*, 2016; Djenatou *et al.*, 2020). However, to our knowledge, no study has been conducted on the efficacy of mycorrhizal inoculation in the production of cocoa seedlings in Togo. Thus, the objective of the present study is to evaluate the efficacy of two strains of AMF applied alone or in combination with biofertilizers on the growth of cocoa seedlings in greenhouse.

Materials and Methods

Plant Material, Fungi and Fertilizers

The plant material consists of cocoa beans (hybrid P7 x IMC67) selected and popularized in farming areas. The beans of this hybrid were obtained from ripe pods harvested in the seed fields of the Centre de Recherche Agronomique de la zone Forestière (CRA-F) at Zozokondzi in the Agou prefecture, Togo. The fungal material consisted of two strains of AMF: *Rhizophagus aggregatum* and *Ben 10*.

The *Rhizophagus aggregatum* strain was obtained at the Laboratoire Commun de Microbiologie (LCM) in Senegal, while the *Ben 10* strain is a local strain isolated from yam at the IITA laboratory and stored at the Laboratoire de Recherche sur les Agroressources et la Santé Environnementale (LARASE) at the University of Lomé (Tchabi *et al.*, 2008).

These strains were used because of their predominance in cocoa orchards (Cuenca et Meneses, 1996; Zako *et al.*, 2012; Amani *et al.*, 2023). These two AMF strains were applied alone or in combination with two biofertilizers:

an organic fertilizer based on poultry droppings (FERTIPLUS) and a growth biostimulant (AGROBIO) (Table 1).

Setting up the trial

The present study was conducted in a greenhouse at the Station d'Expérimentations Agronomiques de Lomé (SEAL) (6°10'25.52"N; 1°12'37.09"E) for six months (December 2020 to May 2021). The substrate consisted of a mixture of sieved topsoil (Table 2) and sea sand (1:2 v/v). This substrate was sieved and then sterilized at a temperature of 140°C for 2 hours before being potted into polyethylene bags measuring 27 cm x 11 cm at a rate of 3 kg/pot.

The trial was set up on a completely randomized design. Treatments consisted of the two CMA strains (*R. aggregatum* and *Ben 10*) combined or not with the two types of biofertilizers, for a total of nine (09) treatments replicated four times (Table 3).

FERTIPLUS and AGROBIO were applied one week before sowing the beans. At sowing time, 500 spores of each AMF strain were inoculated onto the substrates.

Assessment of AMF colonization of the root cortex

The level of AMF colonization of the root cortex was assessed six months after sowing. In the laboratory, the staining technique of Phillips and Hayman (1970) and Vierheilig *et al.*, (2005) was used. The roots of young plants were removed, rinsed with tap water and cut into small fragments of around 1 cm. These fragments were then placed in tubes containing 10% KOH and heated in a water bath at 90°C for 30 minutes. Next, they are rinsed with water to remove KOH, then placed successively in hydrogen peroxide (H₂O₂ at 10 vol) for 40 minutes and in hydrochloric acid (HCl at 1%) for 30 minutes. They are then rinsed again with water, stained with trypan blue (0.05%) and heated in a water bath for 30 minutes.

The prepared roots are then stored in tubes to which a few drops of glycerol have been added. Observations were made on 30 fragments per experimental unit using an optical microscope at 400X magnification. Mycorrhizal colonization rates (T%) were calculated using the following formula (Trouvelot *et al.*, 1986):
 $T (\%) = (\text{Number of mycorrhizal fragments}) / (\text{Number of observed fragments}) \times 100$

Assessment of growth parameters, fresh and dry biomass of seedlings

Six months after sowing, growth parameters (plant height, collar diameter (D) and number of leaves) were measured. The pots were then broken, the plants removed and the length of tap roots measured. Whole plants (leaves, stem and roots) were then harvested. They were immediately weighed to determine the total fresh biomass. They were then oven-dried at 70°C for 48 hours, and weighed again to obtain total dry biomass. Collar circumference was calculated by multiplying diameter by 3.14.

Assessment of relative mycorrhizal dependency

Relative mycorrhizal dependency (RMD) assesses the extent to which mycorrhizal symbiosis is likely to increase plant biomass under given environmental conditions (Plenchette *et al.*, 1983; Bâ *et al.*, 2001). It expresses the difference between the dry matter produced by the mycorrhized plant and that produced by the non-mycorrhized plant, as a function of the dry matter of the mycorrhized plant using the approach of Plenchette *et al.*, (1983), represented by the following equation:

$$RMD(\%) = \frac{DBIP - DBCP}{DBIP} \times 100$$

DBIP = Dry biomass of inoculated plants (g); DBCP = Dry biomass of control plants (g).

Bâ *et al.*, (2001) classified forest species according to their mycorrhizal dependence on AMFs into five classes: none (RMD = 0), marginal dependence (RMD < 25%), moderate dependence (RMD between 26% and 50%), relatively good dependence (RMD between 51% and 75%) and high dependence (RMD > 75%).

Statistical analysis

The data collected was entered into an Excel spreadsheet. Statistical analyses were carried out using R software version 4.0.2. The Shapiro Wilk and Levene tests were used to test the normality of the data and the homogeneity of the variances. When these conditions were met, an analysis of variance (ANOVA) was performed. The different means were discriminated and compared using the Student-Newmann-Keuls (SNK) test at the 5% threshold.

Results and Discussion

Effect of the different treatments on the mycorrhization rate of the root cortex of young cocoa plants

Figure 1 shows the mycorrhization rate of the root cortex of young cocoa plants six months after sowing. A significant difference ($p < 0.05$) between the different treatments was observed for the rate of mycorrhization of the root cortex of the seedlings. The mycorrhization rate was higher for the application of *R. aggregatum* (63.67%), *Ben 10* (62%), *R. aggregatum* + AGROBIO (67%) and *Ben 10* + AGROBIO (60.34%). This demonstrates not only the high mycorrhization potential of the strains used, but also the ability of young cocoa plants to establish mycorrhizal symbioses. However, the root fragments of plants whose beans were sown on non-inoculated substrates were not mycorrhized. This can be explained by sterilisation, which would have destroyed all the AMF spores present in the soil used as a substrate. These results are in agreement with those obtained by Nelly *et al.*, (2019) and Djenatou *et al.*, (2020) who reported high mycorrhization rates (above 50%) on young cocoa plants whose substrates were inoculated with AMFs. However, our results do not agree with those of Iglesias *et al.*, (2011) and Droh *et al.*, (2016), who observed lower mycorrhization rates (below 50%) on young cocoa plants inoculated in greenhouses. This difference in results can be explained by the type of cocoa hybrid used, the type of fertiliser or the substrate used. In fact, the effectiveness of mycorrhizal symbiosis varies greatly depending on the variety of host plants, the strains of fungi (Lumini *et al.*, 2011), the physicochemical characteristics of the soil or substrate (Entry *et al.*, 2002; Sieverding, 2005; Khana *et al.*, 2006) and agricultural practices (ploughing, fertilisation) (Tchabi *et al.*, 2008).

Effect of the different treatments on the total fresh biomass, total dry biomass and mycorrhizal dependency of young cocoa plants

The different treatments significantly affected ($p < 0.05$) the total fresh biomass, total dry biomass and mycorrhizal dependency of young cocoa plants (Table 4). Inoculation of the substrates with the *R. aggregatum* + and *Ben 10* strains resulted in a gain in total fresh biomass of 26.47% and 27.94% respectively compared with the control. Similarly, the gain in total fresh biomass

due to applications of AGROBIO, *R. aggregatum* + AGROBIO and *Ben 10* + AGROBIO were 67.65%, 123.53% and 138.24% respectively compared with the control. However, the application of FERTIPLUS and *R. aggregatum* + FERTIPLUS resulted in a reduction in total fresh biomass of 22.06% and 1.47% respectively, whereas *Ben 10* + FERTIPLUS increased total fresh biomass by 4.41% compared with the control. With regard to total dry biomass, inoculation of the substrates with the *R. aggregatum* and *Ben 10* strains resulted in a gain in total dry biomass of 16.36% and 18.18% respectively compared with the control. Application of AGROBIO, *R. aggregatum* + AGROBIO and *Ben 10* + AGROBIO increased total dry biomass by 14.55%, 65.45% and 74.55% respectively compared to the control. On the other hand, the application of FERTIPLUS, *R. aggregatum* + FERTIPLUS and *Ben 10* + FERTIPLUS resulted in a decrease in total dry biomass of 20%, 30.91% and 16.36% respectively compared with the control. The application of *R. aggregatum* or *Ben 10* to the substrate without fertiliser resulted in a significant increase in total fresh biomass and total dry biomass compared with the control. These results are similar to those of several authors (Iglesias *et al.*, 2011; Antonius *et al.*, 2015; Droh *et al.*, 2016; Djenatou *et al.*, 2020) who reported that mycorrhizal inoculation stimulates biomass production in young cocoa plants. Furthermore, it was found that the production of fresh and dry biomass was greater with the application of *R. aggregatum* + AGROBIO and *Ben 10* + AGROBIO. This can be explained by the positive effect of the biofertiliser (AGROBIO) which, when applied alone or with the various mycorrhizal strains, probably provides nutrients that can be assimilated by the young seedlings. In fact, AGROBIO is a stimulator of biological activity in the soil which, once applied, gives the soil a good capacity to retain water, minerals and fertility over a long period.

Mycorrhizal dependency of young cocoa plants was higher for the application of *Ben 10* + AGROBIO (41.15%) and *R. aggregatum* + AGROBIO (35.75%). However, the combination of *R. aggregatum* + FERTIPLUS showed negative mycorrhizal dependence (-20.81%). These results are similar to those obtained by Iglesias *et al.*, (2011), who obtained marginal mycorrhizal dependence (37%) on young cocoa plants in greenhouses using fine roots collected from beneath the cocoa trees as fungal inoculum. These values are low compared to those obtained by Bâ *et al.*, (2001) on *Tamarindus indica* (between 51% and 75%) and on *Zizyphus mauritiana* (above 75%) whose substrates were

inoculated with *R. aggregatum*. Iglesias *et al.*, (2011) reported a mycorrhizal dependence of 73% on young seedlings of *Inga edulis* using fine roots of this plant as fungal inoculum. Assih *et al.*, (2022) obtained marginal mycorrhizal dependencies (less than 25%) on *Anacardium occidentale* seedlings with the strains *Glomus museae* (11.74%) and *R. aggregatum* (12.87%).

Our results show that mycorrhizal dependence is higher when AGROBIO is combined with mycorrhizal strains, whereas it is lower when these strains are combined with FERTIPLUS. This situation can be explained by the low phosphorus content in the composition of AGROBIO (1.6 ppm) compared with FERTIPLUS (3 ppm). According to Smith *et al.*, (1992), the rate of colonisation is generally inversely correlated with the availability of phosphorus in the soil and, for some plants, colonisation is totally non-existent above a certain level of phosphorus. Douds *et al.*, (1995) reported that for a given soil and plant, there is a maximum amount of extractable phosphorus above which the plant's response to mycorrhization is zero, at least in terms of plant growth.

Effect of the different treatments on the height, Collar circumference, root length and number of leaves of young cocoa plants

Compared with the control, the different treatments had a significant influence ($p < 0.05$) on the height, collar circumference and number of leaves of cocoa plants six months after sowing of the beans (Table 5). Overall, these parameters were greater for treatments whose substrates had been inoculated with mycorrhizal strains (*R. aggregatum* and *Ben 10*) alone or in combination with AGROBIO. These results can be explained by the fact that AMFs contribute to better water nutrition for plants and also act as biofertilisers.

Indeed, when roots are extended by fungal hyphae, the volume of exploitable soil increases, thus creating the mycorrhizosphere, which significantly amplifies the zone in which the plant can extract water and nutrients and can explore deep layers inaccessible to the plant's roots (Lambers *et al.*, 2008; Hamza *et al.*, 2014).

Table.1 Chemical composition of FERTIPLUS and AGROBIO used

Parameters	FERTIPLUS	AGROBIO
Dry matter (%)	88	-
Organic matter (%)	65	59,51
Carbon (%)	37,70	34,6
Nitrogen (N)	4,2	5,04
Phosphorus (P ₂ O ₅)	3	1,6
Potash (K ₂ O)	2,8	1,68
Magnesium (MgO)	1	1,5
Calcium (CaO)	9	1,47
pH	6,4	6,48
C/N	9	6,9
Humidité (%)		7,2

The contribution of AMFs to the growth of young cocoa plants has been reported by several authors (Droh *et al.*, 2016; Nelly *et al.*, 2019; Djenatou *et al.*, 2020). This is probably due to the fact that there is a root increase during the growth of young plants, which, in symbiotic association, creates a network of filaments in the soil, thus enabling the absorption of water and elements that are not very mobile, such as phosphorus, zinc, copper and molybdenum, for the benefit of these plants (Sangay-Tucto, 2018; Wang-Bara *et al.*, 2021).

The symbiotic association between plant roots and AMFs

also contributes to improving nitrogen nutrition and the photosynthetic activity of the plant (Li *et al.*, 2020; Liang *et al.*, 2021). As a result, this symbiosis promotes the amplification of complex physiological effects that affect height, collar circumference, number of leaves and root length. Sangay-Tucto (2018) reported that controlled mycorrhization of *Caesalpinia Spinosa* with *Rhizophagus irregularis* significantly increased growth and nitrogen and phosphorus uptake compared to control plants. However, our results do not agree with those of Laminou Manzo *et al.*, (2009), who worked on other strains of mycorrhizae and other plant species and

showed that the addition of *Glomus intraradices* did not improve the height growth of *Acacia senegal* in the nursery.

These authors also stated that mycorrhizal additions did not have any particularly remarkable effects in stimulating the growth of seedlings in the nursery. According to these authors, this negative effect may be due either to a shortage of carbon substances diverted by the fungus (Plenchette, 1991), or to the introduction of

pathogens by mycorrhizal additions (Garbaye, 1991), or to a predominance of natural mycorrhization. The beneficial effect of AGROBIO applied alone or combined with mycorrhizal strains on the growth of young plants can be explained by the fact that the biofertiliser contains nutrients (NPK) that can be assimilated by young plants. These results corroborate those of Priou (2013) and Djenatou *et al.*, (2020), who showed that biofertilisers appear to be natural sources of NPK for plants.

Table.2 Physical and chemical characteristics of culture substrate

Parameters	values
pH (H ₂ O)	6,5
Electrical Conductivity (µs)	60
Carbon (%)	0,67
Organic mater (%)	1,16
Assimilable Phosphorus (ppm)	32,8
Nitrogen (%)	0,04
C/N	16,8
Potassium (ppm)	42,9
Calcium (ppm)	228,8
Magnesium (ppm)	223,46
Sodium (ppm)	20,47
Clay (%)	9,9
Silt (%)	1,6
Sand (%)	87,6

Table.3 The study treatments

N°	Treatements	Composition
1	<i>Control</i>	3 kg of substrate + 0 CMA + 0 Fertilizer
2	<i>FERTIPLUS</i>	3 kg of substrate + 100 grams of FERTIPLUS
3	<i>AGROBIO</i>	3 kg of substrate + 75 grams of AGROBIO
4	<i>Rhizophagus aggregatum</i>	3 kg of substrate + 500 spores of strain <i>R aggregatum</i>
5	<i>R. aggregatum + FERTIPLUS</i>	3 kg of substrate + 500 spores of strain <i>R aggregatum</i> + 100 grams of FERTIPLUS
6	<i>R. aggregatum + AGROBIO</i>	3 kg of substrate + 500 spores of strain <i>R aggregatum</i> + 75 grams of AGROBIO
7	<i>Ben 10</i>	3 kg of substrate + 500 spores of strain <i>Ben 10</i>
8	<i>Ben 10 + FERTIPLUS</i>	3 kg of substrate + 500 spores of strain 10 + 100 grams of FERTIPLUS
9	<i>Ben 10 + AGROBIO</i>	3 kg de substrate + 500 spores of strain <i>Ben 10</i> + 75 grams of AGROBIO

Figure.1 Mycorrhization rate of the root cortex of young cocoa plants six months after sowing

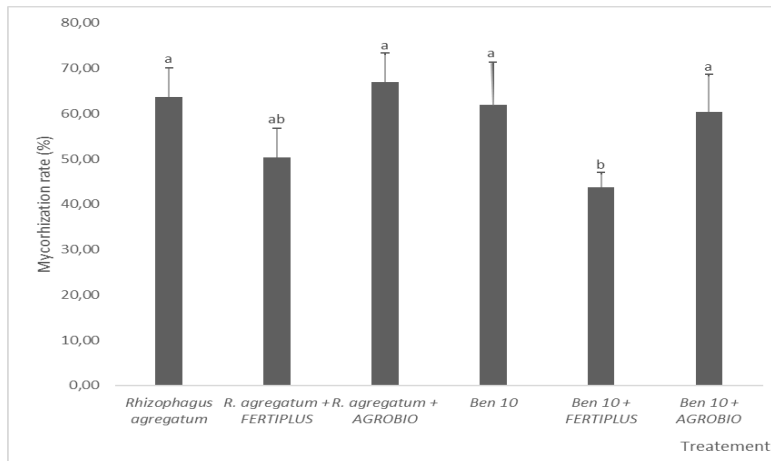


Table.4 Effect of different strains of AMF on total fresh biomass, total dry biomass and relative mycorrhizal dependency six months after inoculation

Treatments	Total fresh biomass (g)	Total dry biomass (g)	Mycorrhizal dependency (%)
<i>Control</i>	17,00 ± 3,92 b	13,75 ± 1,26 bc	-
<i>FERTIPLUS</i>	13,25 ± 4,65 b	11,00 ± 1,63 bc	-
<i>AGROBIO</i>	28,50 ± 3,79 ab	15,75 ± 2,75 b	-
<i>R. aggregatum</i>	21,50 ± 4,73 b	16,00 ± 2,16 b	18,70 ± 12,96 b
<i>R. aggregatum + FERTIPLUS</i>	16,75 ± 3,40 b	9,50 ± 0,58 c	-20,81 ± 10,99 d
<i>R. aggregatum + AGROBIO</i>	38,00 ± 10,10 a	22,75 ± 4,99 a	35,75 ± 14,33 a
<i>Ben 10</i>	21,75 ± 5,12 b	16,25 ± 2,99 b	18,72 ± 18,85 b
<i>Ben 10 + FERTIPLUS</i>	17,75 ± 2,87 b	11,50 ± 1,00 bc	8,00 ± 10,00 c
<i>Ben 10 + AGROBIO</i>	40,50 ± 19,19 a	24,00 ± 3,27 a	41,15 ± 9,89 a
P-value	p < 0,001	p < 0,001	p < 0,001

*Means with the same letter in the same column are not significantly different at the 5% threshold using the Newman-Keuls test.

Table.5 Effects of mycorrhizal inoculation on plant height, crown circumference, root length and number of leaves six months after inoculation.

Treatments	Plant length (cm)	Collar circumference (cm)	Root length (cm)	Number of leaves
<i>Control</i>	35,89 ± 1,77 c	2,25 ± 0,31 a	22,00 ± 2,83 a	16 ± 1 a
<i>FERTIPLUS</i>	28,94 ± 2,50 d	1,74 ± 0,2 b	20,00 ± 4,40 a	13 ± 0 bc
<i>AGROBIO</i>	35,80 ± 1,48 c	2,32 ± 0,19 a	21,00 ± 3,37 a	14 ± 1 b
<i>R. aggregatum</i>	39,22 ± 1,71 bc	2,11 ± 0,15 a	28,50 ± 5,26 a	16 ± 1 a
<i>R. aggregatum + FERTIPLUS</i>	29,85 ± 3,88 d	1,98 ± 0,26 ab	27,50 ± 4,93 a	13 ± 0 bc
<i>R. aggregatum + AGROBIO</i>	44,38 ± 3,37 a	2,28 ± 0,18 a	27,00 ± 2,16 a	14 ± 1 b
<i>Ben 10</i>	45,14 ± 3,95 a	2,38 ± 0,14 a	29,50 ± 10,08 a	16 ± 1 a
<i>Ben 10 + FERTIPLUS</i>	28,60 ± 0,71 d	1,70 ± 0,20 b	26,00 ± 4,69 a	12 ± 0 c
<i>Ben 10 + AGROBIO</i>	42,05 ± 1,66 ab	2,31 ± 0,13 a	25,25 ± 4,99 a	13 ± 0 bc
P-value	p < 0,001	p < 0,001	0,138	p < 0,001

*Means with the same letter in the same column are not significantly different at the 5% threshold using the Newman-Keuls test.

However, the application of FERTIPLIS alone or combined with the different mycorrhizal strains significantly reduced the height and collar circumference of the plants. The results also showed that the different treatments applied had no significant effect ($p > 0.05$) on tap root length. This depression in growth parameters due to the application of organic fertiliser (FERTIPLUS) compared with the control shows that the nutrients it contains cannot be assimilated by young cocoa seedlings.

At the end of this study, it emerged that the various local strains of AMF applied alone or in combination with AGROBIO significantly improved the agronomic performance of young cocoa plants. This provides a basis for future studies on evaluating the resistance of mycorrhizal cocoa seedlings of this variety (hybrid P7 x IMC67) to biotic and abiotic stresses in a real environment.

Authors Contributions

This work was carried out in collaboration among all authors. Authors Pana KADANGA and Atti TCHABI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author Komlan Adigninou ABLEDE, Etienne Blaise M'boumba, Komivi Exonam AMETEFÉ and Moubarak KONDOW managed the analyses of the study. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Conflict of Interest: The authors declare no competing interests.

References

Adden A. K., 2017. Amélioration de la productivité des vergers de cacaoyers (*Theobroma cacao* Linn.)

pour une gestion forestière durable au Togo. *Sciences du Vivant [q-bio]*. Ecole Supérieure d'Agronomie de l'Université de Lomé, 2017.

Amani C. Y. F., Alban M'bo K. A., Cherif M., Koné D. et Kouamé C. 2023. Diversité des Champignons Mycorrhiziens à Arbuscule Associés aux Cacaoyers (*Theobroma cacao* L.) en Côte d'Ivoire. *European Scientific Journal*, ESJ, 19 (27), 179. <https://doi.org/10.19044/esj.2023.v19n27p179>.

Antonius S., Saraswati P., Sudirman Yahya S. and Novita A. T., 2015. Inoculation of Arbuscular Mycorrhizal Fungi Increase the Growth of Cocoa and Coffee Seedling Applied with Ayamuru Phosphate Rock. *Journal of Agricultural Science* ; 7 (5) : 1-12.p. <https://doi.org/10.5539/jas.v7n5p199>

Assih A., Nenonene A. Y. Bokobana A., 2022. Mycorrhizal Inoculation Effect on Water Deficit Tolerance of Cashew Seedlings (*Anacardium occidentale* L.) and Soil Nutrients Availability. *Asian Journal of Agricultural and Horticultural Research*. <https://doi.org/10.9734/AJAHR/2022/v9i4199>. ISSN: 2581-4478. pp 112-122.

Bâ A., Guissou T., Duponnois R., Plenchette C., Sacko O., Sidibé D., Sylla K. and Windou B., 2001. Mycorrhization contrôlée et fertilisation phosphatée : applications à la domestication du jujubier. *Fruits*, 56, 261–269.

Berruti, A., Lumini, E., Balestrini, R., Bianciotto, V., 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Front. Microbiol.*, 6, 1559. <https://doi.org/10.3389/fmicb.2015.01559>

Brundrett M. C., 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytol.*, 154(2) : 275-304. <https://doi.org/10.1046/j.1469-8137.2002.00397.x>

Bucher M., 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytol.*, 173(1) : 11-26. <https://doi.org/10.1111/j.1469-8137.2006.01935.x>

CIRAD (Centre de coopération International pour la Recherche Agronomique pour le Développement), 2013. Améliorer les systèmes agroforestiers en zone tropicale humide : les cas de cacaoyers et des caféiers. CIRAD. 2p.

CRA-F 2019. Centre de Recherche Agronomique de la zone Forestière. Rapport annuel 2019

- CRA-F. 2004. Centre de Recherche Agronomique de la zone Forestière. Point sur la recherche cacaoyère au Togo. Kpalimé, 22 p.
- Cuenca, G., Meneses, E. (1996). Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant Soil* 183, 315–322 (1996). <https://doi.org/10.1007/BF00011447>
- Djenatou P., Ngho Ndooh J. P., Kosma P. Eddy Léonard Ngonkeu Mangaptche E. L. N., 2020. Evaluation of the Inoculation Effect of Arbuscular Mycorrhizal Fungi on the Growth of Cocoa Seedlings (*Theobroma cacao* L.) in the Nursery. *International Journal of Sciences*. <https://doi.org/10.18483/ijSci.2352>.
- Djiwa O., Pereki H. et Guelly K. A. 2021. Perceptions ethnoculturelles des services écosystémiques rendus par les agroforêts à base de cacaoyer au Togo. *Biotechnol. Agron. Soc. Environ.* 2021 25(3), 208-222 (15p). <https://doi.org/10.25518/1780-4507.19153>
- Douds Jr., D. D., Galvez, L., Janke, R. R., Wagoner, P. 1995. Effect of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. *Agriculture, Ecosystems and Environment* 52, 111–118. [https://doi.org/10.1016/0167-8809\(94\)00550-X](https://doi.org/10.1016/0167-8809(94)00550-X)
- Droh G., Kouassi A., Kouadjo Z G C., Zézé A., Nguetta, A S. and Sanders I. R., 2016. Effects of two types of AMF on growth of cocoa seedlings (*Theobroma cacao* L.) in greenhouses. *Global Journal of advanced Research*, 3, (3) : 157-164.
- DSID, 2018. (Direction de la Statistique, de l'Informatique et de la Documentation) Évaluation des superficies et des rendements de café et de cacao, campagne agricole 2017-2018. DSID/MAEH/PNIASA/Banque Mondiale, Lomé, Togo, 76 p. CRAF, 2004
- Entry, J., Rygiewicz, P., Watrud, L. and Donnelly, P. (2002). Influence of adverse soil conditions on the formation and function of Arbuscular mycorrhizas. *Advances in Environmental Research*, 7, 123-138. [https://doi.org/10.1016/S1093-0191\(01\)00109-5](https://doi.org/10.1016/S1093-0191(01)00109-5)
- Finlay R. D., 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *J. Exp. Bot.*, 59(5) : 1115–1126. <https://doi.org/10.1093/jxb/ern059>
- Garbaye J., 1991. Utilisation des mycorrhizées en sylviculture. In : Strullu DG., Garbaye J., Perrin P., Plenchette C. Les mycorrhizes des arbres et plantes cultivées. Paris : *Lavoisier*, 197-248.
- Grümbert B. C., Urcelay C., Shroeder M. A., Vargas-Gil S. et Luna C. M., 2015. The role of inoculum identity in drought stress mitigation by arbuscular mycorrhizal fungi in soybean. <https://doi.org/10.1007/s00374-014-0942-7>
- Hamza N. Application des mycorrhizes arbusculaires en culture maraîchère cas de la pastèque (*Citrullus lanatus*). Mémoire de Magister, Université Ferhat Abbas Sétif, Algérie ; 2014.
- Iglesias L., Salas E., Leblanc HA., and Pekka Nygren P., 2011. Response of *Theobroma cacao* and *Inga edulis* seedlings to cross-inoculated populations of arbuscular mycorrhizal fungi. *Agroforest Syst*, 83,63–73. <https://doi.org/10.1007/s10457-011-9400-9>.
- IRCC-TOGO. 1988. Institut de Recherche sur les filières Café et Cacao Togo. Rapport annuel. Kpalimé pp. 20-21.
- Jagoret P., 2011. Analyse et évaluation des systèmes agroforestiers complexes sur long terme : Application aux systèmes de culture à base de cacaoyers au centre Cameroun. Thèse, Université Montpellier SUPAGRO, 288p.
- Jansa, J., Forczek, S. T., Rozmoš, M., Püschel, D., Bukovská, P., and Hřšelová, H., 2019. Arbuscular mycorrhiza and soil organic nitrogen : network of players and interactions. *Chemical and Biological Technologies in Agriculture*, 6(1), 1-10. <https://doi.org/10.1186/s40538-019-0147-2>.
- Khana, M., and Delowara, S. 2006. Effect of edaphic factor son root colonization and spore population of arbuscular mycorrhizal fungi. *Bulletin Institute Tropical Agriculture*, 29, 97-104.
- Kim S. J., Eo J. K., Lee E. H., Park H. et Eom A. H., 2017. Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. *Mycobiology* 45 (1) : 20–24. <https://doi.org/10.5941/MYCO.2017.45.1.20>
- Koudjega T., 2013. Détermination de formules d'engrais adaptées aux trois sous zones cacaoyères du Togo. Mémoire du Diplôme d'Etudes Approfondies en Biologie du Développement à l'Université de Lomé – Togo. 97p.
- Lambers, H., Raven, J.A., Shaver, G.R. and Smith, S.E. 2008. Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution*, 23: 95-103. <https://doi.org/10.1016/j.tree.2007.10.008>
- Laminou Manzo, O., Ibrahim, D., Campanella B. et Paul

- R., 2009. Effets de l'inoculation mycorhizienne du substrat sur la croissance et la résistance au stress hydrique de cinq espèces fixatrices de dunes : *Acacia raddiana* Savi ; *Acacia nilotica* (L.) Willd. Ex Del. var. *adansonii* ; *Acacia senegal* (L.) Willd ; *Prosopis chilensis* Stunz. et *Bauhinia rufescens* Lam. *Geo-Eco-Trop.*, 33, 115–124.
- Li S., Yang W., Guo J., Li X., Lin J., Zhu X., 2020. Changes in photosynthesis and respiratory metabolism of maize seedlings growing under low temperature stress may be regulated by arbuscular mycorrhizal fungi. *Plant Physiol Biochem.*, 154, 1-10. <https://doi.org/10.1016/j.plaphy.2020.05.025>.
- Liang B. B., Wang W. J., Fan X. X., Kurakov A. V., Liu Y. F., Song F. Q., 2021. Arbuscular mycorrhizal fungi can ameliorate salt stress in *Elaeagnus angustifolia* by improving leaf photosynthetic function and ultrastructure. *Plant Biol.*, 23(1) : 232-241. <https://doi.org/10.1111/plb.13164>.
- Lumini E., Vallino M., Alguacil M. M., Romani M. et Bianciotto V. 2011. Different farming and water regimes in Italian rice fields affect arbuscular mycorrhizal fungal soil communities. *Ecological Applications*, 21: 1696-1707. <https://doi.org/10.1890/10-1542.1>
- Nelly S. A., Angelbert D. C., Consorcia E. R., 2019. Growth response of cacao (*Theobroma cacao* L.) plant as affected by bamboo biochar and arbuscular mycorrhizal fungi in sterilized and unsterilized soil. *Biocatalysis and Agricultural Biotechnology*, 22, 101347. <https://doi.org/10.1016/j.bcab.2019.101347> <http://www.elsevier.com/locate/bab>.
- Nenonene Y. A., M'boumba E. B., Tahaba N. D. and Tchabi A., 2022. Evaluation of the Effectiveness of Arbuscular Mycorrhizian Fungi and Organic Fertilizers on Nematodes (*Meloidogyne spp*) Phytoparasites and Agronomic Performance of Chili Pepper (*Capsicum Annuum* L.) in South-Togo. *International Journal of Innovative Science and Research Technology*, 7(12) : 10p. <https://doi.org/10.5281/zenodo.7490655>
- Phillips J. M. and Hayman D. S., 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55,158-161. [https://doi.org/10.1016/s0007-1536\(70\)80110-3](https://doi.org/10.1016/s0007-1536(70)80110-3)
- Plenchette C. 1991. Utilisation des mycorhizées en agriculture et horticulture. In : Strullu DG., Garbaye J., Perrin P., Plenchette C. Les mycorhizes des arbres et plantes cultivées. Paris : Lavoisier, 131-196.
- Plenchette C., Fortin J. A., Furlan V., 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhiza dependency under field conditions. *Plant Soil*, 70, 199–209. <https://doi.org/10.1007/BF02374780>
- Priou L., 2013. Multiplication des mycorhizes arbusculaires en milieu liquide et solide afin d'améliorer la formulation de biofertilisants. *Sciences agricoles*. 2013. ffdumas-00975007.
- Sangay-Tucto S., 2018. Étude de l'impact des symbioses mycorhizienne et rhizobienne dans la domestication du Tara, *Caesalpinia spinosa* L. Interactions entre organismes. Université Montpellier ; Universidad Peruana Cayetano Heredia, 2018. Français. NNT : 2018MONTG080
- Sieverding, E. (2005). *Glomus badium* a new sporocarpic mycorrhizal fungal species from European grassland with higher soil pH. *Journal of Applied Botany and Food Quality* 79(1).
- Smith, S. E., Robson, A. D., Abbott, L. K. (1992). The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. *Plant and Soil* 146, 169-179. <https://doi.org/10.1007/BF00012010>
- Tchabi A., Hountondji FCC, Ogunsola B, Lawouin L, Coyne D, Wiemken A, Oehl F, 2016. The Influence of Arbuscular Mycorrhizal Fungi Inoculation on Micro-Propagated Hybrid Yam (*Dioscorea spp.*) Growth and Root Knot Nematode (*Meloidogyne spp.*) Suppression. *Int. Journal of Current Microbiology Applied Science* 5(10): 267-281. <https://doi.org/10.20546/ijemas.2016.510.030>
- Tchabi A., Coyne D., Hountondji F., Lawouin L., Wiemken A., Oehl F., 2008. Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. *Mycorrhiza*, 18(4):181-95. <https://doi.org/10.1007/s00572-008-0171-8>
- Tchabi A., M'boumba E. B., Olowotche N. and Kadanga P., 2022. Bioferlizing and Nematodes Control Potentials of Four Native Isolates of Arbuscular Mycorrhizal Fungi on Sweet Pepper (*Capsicum annuum*) in Togo. *International Journal of Plant*

- and Soil Science*. 13p.
<https://doi.org/10.9734/ijpss/2022/v34i242610>
- Trouvelot, A., Kough, J. L. et Gianinazzi-Pearson, V., 1986. Mesure du taux de mycorhization VA d'un système racinaire. Recherche de méthode d'estimation ayant une signification fonctionnelle. *Proceedings of the 1st european symposium on mycorrhizae, Dijon*, 1-5 July 1985, 217–221.
- Vierheilig, H., Schweiger, P., and Brundrett, M., 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum*, 125(4), 393–404.
<https://doi.org/10.1111/j.1399-3054.2005.00564.x>
- Wang, W., Shi, J., Xie, Q., Jiang, Y., Yu, N., Wang, E., 2017. Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. *Mol. Plant* 10 (9), 1147 – 1158.
<https://doi.org/10.1016/j.molp.2017.07.012>
- Wang-Bara B., Amedep D., Housseini D. J., Mana G. G. 2021. Evaluation des effets des doses de mycorhizes sur les paramètres de croissance et de la production de trois variétés de Voandzou dans la localité de Dschang, Ouest Cameroun. *European Scientific Journal*, 17(17):
<https://doi.org/10.19044/esj.2021.v17n17p213>.
- Wessel M., Quist-Wessel F. P. M. 2015. Cocoa production in West Africa, a review and analysis of recent developments. *NJAS - Wageningen Journal of Life Sciences* 74 and 75, 1-7.
<https://doi.org/10.1016/j.njas.2015.09.001>
- Zako B. I. M. S., Tié B. T., Zirih G. N., Kouadjo Z. C. G., Fossou K. R and Adolphe Z. 2012. Arbuscular mycorrhizal fungi associated with *Theobroma cacao* L. in the region of Yamoussoukro (Côte d'Ivoire). *African Journal of Agricultural Research* Vol. 7(6).
<http://www.academicjournals.org/AJAR>.
<https://doi.org/10.5897/AJAR11.2057>. February, 2012 pp. 993-1001.
- Zhang X. H., Lin A. J., Chen B. D., Wang Y. S., Smith S. E., Smith F. A., 2006. Effects of *Glomus mosseae* on the toxicity of heavy metals to *Vicia faba*. *J. Environ. Sci.*, 18(4) : 721-726.
- Zhou J., Zang H., Loeppmann, S., Gube M., Kuzyakov Y., and Pausch, J. (2020). Arbuscular mycorrhiza enhances rhizodeposition and reduces the rhizosphere priming effect on the decomposition of soil organic matter. *Soil Biology and Biochemistry*, 140, 107641.
<https://doi.org/10.1016/j.soilbio.2019.107641>.

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