# Effects of arbuscular mycorrhizal fungal strains on the growth of cowpea [*Vigna unguiculata* (L.) Walp.] in the presence of Tilemsi natural phosphate (TNP).

## Abstract

The growth of cowpea [*Vigna unguiculata* (L.) Walp.] was evaluated in response to the inoculation of seeds at sowing with an arbuscular mycorrhizal fungus strain (*Glomus aggregatum*) in the presence of Tilemsi natural phosphate (TNP). The soil collected from the large cultivation plot of IPR/IFRA in Katibougou served as the substrate after sterilization. Sowing was done with 3 cowpea seeds per pot, and after one week, two plantlets were kept in each pot. The pots were placed on metallic tables in the greenhouse of the Soil Microbiology Unit (LMS) of LaboREM-Biotech for the two months of the experiment.

The results showed that, despite a low rate of root colonization by the mycorrhizal fungi (F: 80.75%; I: 7.31), the average growth of inoculated plants was more significant compared to non-inoculated plants. The results also indicate that the fungus + natural phosphate treatment significantly increased the average number of inflorescences, aerial, and root biomasses. The treatment with the fungus alone was more significant in terms of the frequency and intensity of mycorrhization. However, as biomass predicts yield, this study suggests that the mycorrhizal biofertilizer provides important benefits to the plant by solubilizing the TNP. Mycorrhizal fungi can thus be used to improve the growth and yield of cowpea in several agricultural perimeters after TNP application.

**Keywords** : Mycorrhization, Fertilization, Cowpea.

## I. INTRODUCTION

The constantly increasing African population forces farmers to make lands more and more productive. Sub-Saharan Africa alone accounts for nearly 200 million people (FAO, 2010). This results in undernourishment combined with limited and degraded land resources (Le Cacheux, 2011; Fitter, 2012). In reality, agricultural production on soils poor in nutrients and deficient in water yields very low returns.

Currently, the high productivity of intensive agriculture is greatly dependent on chemical inputs, particularly mineral phosphate fertilizers, which enrich the soils with the necessary nutrients for plant growth (Childers et *al*, 2011; Cordell et *al*, 2009; Gilbert, 2009; Roy-

Bolduc and Hijri, 2010). Agriculture's dependence on these chemical inputs leads to negative environmental impacts. To avoid a food crisis and achieve sustainable growth, more appropriate and environmentally friendly high-yield technologies have been recommended (Bationo et *al*., 1998; Scherr and McNeely, 2008; Brussaard et *al*., 2010). These fall within the framework of efforts undertaken by researchers and extension workers to improve global food security.

Cowpea (*Vigna unguiculata* (L) Walp) is one of the main legumes cultivated worldwide. Its cultivation area is located between latitude 35°N et 30°S of the equator (Singh et *al*, 1997). It is an annual plant with an upright or creeping habit. The leaves, as well as the green pods and seeds of cowpea, are a good source of protein, vitamins, and minerals for human and animal consumption (Cissé and Hall, 2003). Furthermore, during the dry season, in some regions of West and Central Africa, the monetary value of stored cowpea haulms becomes very high (Cissé and Hall, 2003). Its cultivation, like that of other legumes, can help restore soil fertility thanks to its high potential for biological nitrogen fixation (Bado, 2002; Graham and Vance, 2003).

Despite the numerous studies conducted on this species, the average yield per hectare of cowpea cultivation remains relatively unstable. It varies between 50 to 550 kg/ha in Africa and depends on climatic conditions, varieties, cropping systems, and the level of use of inputs and pesticides (Cissé and Hall, 2003). The low yields observed are due, among other things, to low rainfall, soil deficiency in nitrogen and phosphorus, which are essential for the plant's nutrition and production, as well as low soil fertility and extensive agriculture without soil fertility restoration (FAO, 2003 ; Bationo et *al*., 1998).

Mineral phosphate fertilizers are very expensive and inaccessible to farmers because of their low purchasing power. However, several African countries have significant deposits of natural phosphates (NP), a potential, cheaper source of phosphorus for farmers, which could reduce dependence on chemical fertilizers, reduce pollution risks, and thus enhance local fertilizing resources such as Tilemsi natural phosphates in Mali, Matam in Senegal, and Kodjari in Burkina Faso (Koné et *al*. 2021, 2019, 2018 ; Babana and Antoun, 2006, 2005 ; Dianou and Bâ, 1999 ; Asimi and Kambou, 2000).

Nevertheless, the utilization of natural phosphates (NP) by plants is not always easy because of their low solubility (Bagayoko and Coulibaly, 1995). To improve NP solubility, several strategies are used, including inoculation with arbuscular mycorrhizal fungi (AMF) (Strullu, 1991 ; Babana and Antoun, 2003). These microorganisms, naturally present in soils, form symbioses with the majority of terrestrial plants, including cultivated ones (Smith and Read, 2008). They play an important role in water and mineral nutrition (Smith and Read, 2008), protecting host plants against pathogens (Sikes et *al*., 2009) and against drought (Augé et *al*., 2001). In addition, they improve ecosystem sustainability by enhancing soil structure (Wilson et *al*., 2009) and reducing nutrient losses that can be caused by runoff (Van der Heijden, 2010).

This study is part of the effort to improve cowpea growth. For this reason, the use of a selected arbuscular mycorrhizal fungus strain, *Glomus aggregatum*, as a biological fertilizer, coupled with mineral fertilization based on Tilemsi natural phosphate, could ensure sustainable management of this soil's fertility and increase the growth of cowpea, an important plant in human diet.

## II. MATERIALS AND METHODS

### 1. Study Site and Soil Sampling

The study was conducted at the Soil Microbiology Unit (LMS) of LaboREM-Biotech at the Faculty of Sciences and Techniques (FST) of the University of Sciences, Techniques and Technologies of Bamako (USTT-B). The soil was sampled from the 0-30 cm horizon in Katibougou (Koulikoro/Mali), 60 km from Bamako. After sterilization in an autoclave for 20 minutes at 120º C (2 times), it was distributed into 5 kg plastic pots with holes in the bottom, at a rate of 3.75 kg per pot. The characteristics of this soil are mentioned in Table 1.

**Table 1: Some physicochemical characteristics of the soil used.**

| | Clay (%) | Sand (%) | C (%) | N (%) | C/N | pH | Ca (mg) | Mg (mg) | P (ppm) | K (mg) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Value** | 10.4 | 57.6 | 0.2 | 0.03 | 3.9 | 5.8 | 10 | 1.8 | 2.48 | 9.3 |

**Source**: SACKO/AUF 2014 project data

### 2. Plant Material

The cowpea variety used belongs to the collection of the Rural Economy Institute (IER) of Mali. It is KOROBALEN, a short-cycle variety (65 to 70 days) cultivated in the 300 to 800mm isohyet.

### 3. Fungal and Fertilization Materials

The inoculum used is the *Glomus aggregatum* (AMF) strain. This inoculum comes from the collection of the Soil Microbiology Unit of the Laboratory of Research in Microbiology and Microbial Biotechnology/LaboREM-Biotech. This mycorrhizal fungus strain was isolated from an *Acacia mangium* plantation and has proven effective in plants and crops (Sacko et *al*., 2012, 2014). It was multiplied on millet in a culture chamber according to the method described by Bâ et *al*. (1996).

The inoculum consists of a mixture of spores, hyphae, millet root fragments colonized by *Glomus aggregatum* (Ga), and sand. At the time of sowing, 20g of this mixture was added per pot, as well as 20g of TNP 30% ($P_2O_5$). The TNP used comes from Bourem in the Tilemsi valley in Northern Mali. It is used in its powder form at 30% ($P_2O_5$), 10% of elements smaller than 40µmand 60% of elements smaller than 60µm. It exhibits a solubility of 0.007% in water.

## 4. Culture Conditions

In this study, two factors were examined :

- **Inoculation with 2 modalities :**
  - Control (without inoculant).
  - Inoculation with *Glomus aggregatum* (Ga) at a rate of 20g of inoculum per pot.
- **Fertilization with TNP at 2 variations levels:**
  - Control (without fertilizer).
  - Fertilization with PNT at 20g per pot.

The experimental design was a Fischer block with four treatments of 10 repetitions. Three cowpea seeds were sown directly per pot. Thinning occurred on the 10th day after sowing, leaving 2 plants per pot. Watering was done according to demand, and the experiment lasted 2 months.

## 5. Measured Parameters

To evaluate biomasses and mycorrhization parameters, a sampling of five homogeneous plants per treatment was conducted on the 60th day after sowing. The plants were uprooted, and the aerial and root parts of each plant were separated. They were then placed in labeled envelopes, dried in an oven at 70°C for 72 hours, and then weighed. The roots of the plants from each treatment were stained according to the method described by Phillips and Hayman (1970), and the slides were then read using the scale described by Trouvelot et *al*. (1986).

### 6. Statistical Analyses

The results were analyzed using ANOVA in R2.101 softwares, and the NEWMAN-KEULS test, at a 5% threshold, was used to compare the means.

## III. Results

### 1. Influence of Endomycorrhizal Inoculation on Growth Parameters

The effect of inoculation on aerial and root biomasses was determined on the 60th day after sowing (DAS). The results obtained show that all inoculated plants allowed an increase in biomasses on the 60th DAS compared to the controls. The results of the variance analysis show significant effects of the *Glomus aggregatum* x Tilemsi Natural Phosphate interaction on the production of cowpea plant biomasses at 2 months (Table 2).

**Table 2: Analysis of variance of dry aerial and root biomasses of cowpea plants after 2 months of culture.**

| Treatments | Dry Aerial Biomass | Dry Root Biomass |
|---|---|---|
| **Ga + TNP** | 6.04 a | 2.10 a |
| **Ga** | 2.82 c | 1.64 b |
| **TNP** | 4.00 b | 1.90 ab |
| **Control** | 2.68 c | 0.70 c |

**Legend** : Values followed by the same letter are not statistically different from each other at the 5% threshold according to the Tukey Contrasts Test. Ga = *Glomus aggregatum*; TNP = Tilemsi Natural Phosphate.

### 2. Mycorrhization Parameters of Cowpea Plants

At 60 days of culture, the intensities and frequencies of mycorrhization were determined. For all treatments, the results showed low mycorrhization intensities (less than 10%) with very high frequencies (>70%). The variance analysis between the inoculation and phosphate fertilization factors showed significant effects of the *Glomus aggregatum* x Tilemsi Natural Phosphate interaction on the mycorrhization parameters of cowpea plants (Table 3).

**Table 3: Mean values of Mycorrhization Frequencies and Intensities of cowpea plants per pot after 2 months of culture.**

| Treatments | Mycorrhization Frequency (%) | Mycorrhization Intensity (%) |
|---|---|---|
| **Ga + TNP** | 76.0 a | 6.68 a |
| **Ga** | 85.5 b | 7.94 b |

## IV. DISCUSSION

This work evaluated the response of cowpea to endomycorrhizal inoculation with *G. aggregatum* in the presence of TNP in the greenhouse. The results show that inoculation with *G. aggregatum* and TNP application significantly improves cowpea biomasses in the greenhouse (+81%). Thus, significant effects of the *Glomus aggregatum* x Tilemsi Natural Phosphate interaction on the biomass production of cowpea plants at 2 months were observed. Similar results have been observed on cowpea under semi-controlled conditions by (Haro et *al*., 2012) and also on other plant species (Bâ et *al*., 2001 ; Avio et *al*., 2006 ; Koné et *al*. 2021, 2019, 2018).

This positive effect of inoculation in the presence of TNP on the growth of cowpea plants is largely due to a better improvement in phosphate nutrition. Indeed, arbuscular mycorrhizal fungi are likely to increase the growth and production of cultivated plants by improving their water and mineral nutrition and protecting them against pathogens (Dommergues et *al*., 1999; Smith and Read, 2008; Sikes et *al*., 2009). These results on cowpea are consistent with those of (Smith et *al*., 2000) and (Avio et *al*., 2006) on *Medicago sativa*. They also align with those of (Sampath Kumar et *al*, 2002; Prakash et *al*, 2004; Boureima et *al*, 2007) who observed an improvement in sesame biomass in the greenhouse following mycorrhizal inoculation. They also corroborate those of (Sampath Kumar et *al*., 2002; Koné et *al*. 2021, 2019, 2018), which showed that the association with different genera of arbuscular mycorrhizal fungi (*Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora*) allowed for greater biomass at both the root and aerial parts of local varieties of sesame, *maize*, and *sorghum*. Our results are identical to those of (Prakash et *al*., 2004; Koné et *al*. 2021, 2019, 2018) who obtained good assimilation of natural phosphate and indirectly an intense absorption of nitrogen nutrients with an improvement in the biomass of mycorrhized sesame, sorghum, and maize plants coupled with natural phosphate application in the greenhouse. Indeed, the results allowed for a total biomass in all mycorrhized plants superior to the controls.

Despite the lower mycorrhization parameters in inoculated plants, a significant positive effect on cowpea plant growth was observed. Smith et *al*. (2004) concluded that mycorrhization intensities are not always correlated with plant growth. Similarly, significant effects of the *Glomus aggregatum* x Tilemsi Natural Phosphate interaction on the mycorrhization parameters of cowpea plants were observed. Indeed, the plant's response to mycorrhization depends on the mycorrhizal fungus species (Plenchette et *al*., 1982; Krishna and Dart, 1984) but also on the host plant species (Plenchette et *al*., 1983; Krishna et *al*., 1985).

Our results are consistent with those of (Subba Rao et *al.*, 1985; Plenchette et *al*., 2000; Koné et *al*. 2018, 2019, 2021), who observed very significant growth stimulations on millet, sorghum, and maize. Also (Sampath Kumar et *al*., 2002; Koné et *al.* 2021, 2019, 2018) obtained an infectivity of (97%) with the *Glomus* genus on local varieties of sesame, maize, and sorghum.

Inasmuch as the symbiosis improves water and mineral nutrition, the plant will not find it necessary to form this symbiosis if nutrients are available in the environment. Thus, according to (Gianinazzi-Pearson and Gianinazzi, 1986; Plenchette, 1982; Strullu, 1991), this symbiosis will gradually establish and develop with the depletion of nutrients directly accessible to the plant roots in the soil. Corroborating our experimentation, (Dianou and Bâ, 1999; Asimi and Kambou, 2000) with Burkina phosphate, as well as (Babana and Antoun, 2006, 2005; Koné et *al*. 2021, 2019, 2018) with Tilemsi natural phosphate, observed a positive influence of these natural phosphates associated with mycorrhizal fungi on the growth of cowpea, wheat, maize, and sorghum.

## V. CONCLUSION

This work allowed for the study of the greenhouse effect of inoculating a Malian variety of *Vigna unguiculata* with a fungal strain *G. aggregatum* in the presence of Tilemsi natural phosphate. Inoculation with the endomycorrhizal fungus, *Glomus aggregatum*, improves the growth of cowpea cultivated on Katibougou soil thanks to better phosphate nutrition. This suggests a great ability of the introduced symbiont. Hence, this biofertilizer could be recommended to farmers to improve the phosphate nutrition of phosphorus-deficient soils.

Thus, in tropical countries where soil acidity is a limiting factor for plant growth, the inoculation of plants with mycorrhizal fungus strains appears as an alternative to the use of chemical fertilizers for sustainable agriculture and long-term preservation of soil fertility.

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