

*Full Length Research Paper*

# Effects of inoculation with some native arbuscular mycorrhizal fungi on tomato (*Solanum lycopersicum* L.) growth

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**In Burkina Faso, tomato (*Solanum lycopersicum* L.) sector plays a very important socio-economic role. However, its production is confronted by many constraints, among which is the soil poverty in mineral elements such as nitrogen and available phosphorus resulting in an increase in the area of land that is sown for this crop and an increase in the use of chemical inputs. In addition, chemical inputs have shown their limits with several environmental negative impacts. Therefore, this study was initiated to help improve sustainable agricultural production. In this study, tomato was grown in the greenhouse and inoculated with three natives' mycorrhizal inocula. The growth parameters were measured at 30 and 60 days after sowing. Above-ground, root and total biomass were assessed at 60 days after sowing. The results showed an improvement in height of 164.44%, in the collar diameter of 75.25%, in above ground biomass production of 540%, root biomass of 1061.97% and a total biomass of 638, 1% after inoculation. This study had shown promising results and merits further investigation *in situ*.**

**Key words:** Tomato, mycorrhizal inoculation, arbuscular mycorrhizal fungi, sustainable agriculture.

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.), is an herbaceous plant widely cultivated for its fruit and it represent one of the main food crops in the world. This crop is the second most important vegetable produced and consumed in Western countries (Willcox et al., 2003). With its products, tomatoes are one of the main food sources of carotenoids providing around 80% of the daily intake of lycopene, folate, ascorbic acid, flavonoids, a-tocopherol and potassium in the Western diet (Bramley, 2000; Willcox et al., 2003). In Burkina Faso, tomato is one of

the most important vegetable crops. However, its production encounters enormous problems such as the poverty of soils, particularly in phosphorus (P) and nitrogen (N) (Diem et al., 1981; Mikola, 1987), water deficit, wind and water erosion, as well as fungal, bacterial and viral diseases. Its production increased from 1,000 t in 2000 to 12,635 t in 2017 (FAO, 2020). The improvement in this production is linked to the area planted (from 1,000 ha in 2000 to 1,254 ha in 2017) since the yield has remained around 10 t/ha (FAO, 2020). In

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**Table 1.** Culture substrate physico-chemical characteristics.

Characteristics	Clay (%)	Total silt (%)	Total sand (%)	Total organic matter (%)	Total carbon (%)	Total nitrogen (%)	C/N	Total phosphorus (mg.kg <sup>-1</sup> )	Available phosphorus (mg.kg <sup>-1</sup> )	pH H <sub>2</sub> O (p/v: ½ :5)
Values	3.92	5.88	90.2	0.331	0.192	0.016	12	172.52	1.74	6.44

addition to the increase in the cultivated area, there is also the intensive use of chemical fertilizers to correct soil poverty and fight against tomato diseases. This results in huge losses for farmers. In addition, intensive agriculture has shown its limits: Soil and water pollution, the emergence of pesticide-resistant pathogens, high pesticide costs, risks to farmers' health, and weakening ecosystems (Pierre, 2012). Regulations are now in place to limit the use of these inputs. Therefore, maintaining crop productivity through agriculture that substitute chemical inputs use by the mobilization of biological processes is at the heart of the challenges of current agricultural research. Thus, tomato cultivation remains to this day, a major concern because its important contribution to food security and the increase in income of producers, especially in family farming. The current agriculture turns towards an ecological intensification which leans on the promotion of the ecological mechanisms by practices such as crop rotation, the cultural association or the biological control. The use of beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) is increasingly considered as one of the best sustainable organic farming practices (Haro et al., 2017). It is well established that the tomato forms a symbiosis with arbuscular mycorrhizal fungi (Dodzia et al., 2012; Khalloufi, 2017) while the beneficial effect of mycorrhizal symbiosis on plant growth and production has been the subject of several studies (Haro et al., 2016a, 2012, 2015, 2017, 2016b). Exploiting this symbiosis would be a possibility to improve tomato productivity. Thus, we became interested in the tomato mycorrhizal symbiosis with the aim of evaluating the effect of inoculation of this plant with arbuscular mycorrhizal fungi native to Burkina Faso on its growth.

## MATERIALS AND METHODS

### Plant and fungal materials

Tomato variety AMIRAL F1-hybrid [variety of tropical, semi-tropical and Sahelian zones (produced/imported, packed and marketed by SAKATA SEED INDIA PVT., LTD)] was used. Tomato seeds were surface disinfected by soaking in 96% ethanol for 3 min, rinsed thoroughly with sterile distilled water and then disinfected in calcium hypochlorite solution (CaCl<sub>2</sub>O<sub>2</sub> at 3.3%, w/v) for 3 min and finally rinsed thoroughly with sterile distilled water before sowing. These seeds were sown at a rate of 4 seeds per pot.

Fungal material was composed of two efficient local AMF isolated

from the rhizosphere of cowpeas grown in Burkina Faso (Haro et al., 2012, 2017): Mycorrhizal complex [*Scutellospora* sp., *Gigaspora* sp., *Glomus* sp. (M1)] and *Glomus* sp. (M2). The inocula were obtained by multiplication of cowpea rhizosphere indigenous arbuscular mycorrhizal fungi (Haro et al., 2012). The inoculum constituted of spores, mycorrhizal root fragments and soil.

### Culture substrate

The growing substrate was a sterilized soil of Ouagadougou and its physical and chemical characteristics are presented in Table 1. Culture substrate was homogenized, sieved with a 2 mm sieve and sterilized at 121°C for 1 h.

### Test implementation

The test consisted of growing tomatoes in 2 L pots containing 2 kg of sterilized culture substrate (Table 1). The inoculation was carried out at the sowing time with 10 g of inocula [each inoculum constituted of spores, mycorrhizal root fragments and soil and was kept at room temperature (about 25°C)] (Haro et al., 2017) for each inoculated treatment. The inoculation consisted to place in the middle of each pot containing the culture substrate, 10 g of inoculum at 2 or 3 cm deep. Control pots were not inoculated. The experiment unit was composed of 3 treatments (two inoculated treatments (M1 and M2) and one control)] with 5 replicates per treatment. The tomato was sown at the rate of 4 seeds per pot and a wedge was carried out two weeks after the plants emergence to allow only one plant per pot. The experimental design used was a simple randomization complete block design. This experiment lasted 60 days (flowering stage).

### Grow parameters measurement

To estimate the effect of mycorrhizal inoculation on the tomato, the height, the diameter at the collar, the rate of relative growth in height and the rate of relative growth of the diameter at the collar were calculated at the 30th and at the 60th day after sowing. The relative growth rate in height (RGRH) was calculated according to the following formula:

$$RGRH = (H_f - H_i)/H_i$$

With H: height, i: initial and f: final.

The collar diameter was measured using a caliper at the separation zone between the root system and the aerial part at the 30th and at the 60th day after sowing. The relative growth rate of the collar diameter (TCRdc) was calculated by the following formula:

$$TCRdc = (Dcf - Dci)/Dci$$

With Dc = Diameter at the collar, I = initial and f = final.

**Table 2.** Tomato mycorrhizal frequency and intensity 60 days after sowing inoculated with 2 mycorrhizal inocula [mixed inocula (M1) and *Glomus* sp. (M2)].

Treatment	Mycorrhizal frequency (%)	Mycorrhizal intensity (%)
M1	86±4 <sup>a</sup>	40.5±4.07 <sup>a</sup>
M2	82±6.63 <sup>a</sup>	35.3±5.35 <sup>a</sup>
Control	0 <sup>b</sup>	0 <sup>b</sup>
Significance level	<0.000 1	<0.000 1

For the same parameter, data followed by the same letters are not significantly different according the Newman-Keuls test ( $p < 0.05$ ). Standard error of the mean ( $n = 5$ ).

**Table 3.** Plant height, the diameter at the collar, the rate of relative growth in height and the rate of relative growth of the diameter at the collar of tomato inoculated with 2 mycorrhizal inocula [mixed inocula (M1) and *Glomus* sp. (M2)].

Treatment	Height 1 (cm)	Height 2 (cm)	RGRH (%)	Diameter 1 (mm)	Diameter 2 (mm)	TCRdc (%)
M1	14.5±1.55 <sup>a</sup>	35.7±2.67 <sup>a</sup>	1.54±0.24 <sup>a</sup>	2.53±0.32 <sup>a</sup>	3.54±0.3 <sup>a</sup>	0.45±0.16 <sup>a</sup>
M2	12.6±1.95 <sup>a</sup>	20±4.71 <sup>ab</sup>	1.46±0.25 <sup>a</sup>	2.2±0.28 <sup>a</sup>	2.87±0.35 <sup>b</sup>	0.21±0.14 <sup>a</sup>
Control	10.2±1.06 <sup>a</sup>	13.5±4.15 <sup>b</sup>	0.74±0.36 <sup>a</sup>	1.61±0.14 <sup>a</sup>	2.02±0.28 <sup>b</sup>	0.4±0.14 <sup>a</sup>
Significance level	NS	0.004	NS	NS	0.01	NS

For the same parameter, data followed by the same letters are not significantly different according the Newman-Keuls test ( $p < 0.05$ ). Standard error of the mean ( $n = 5$ ). Height 1 and 2: Height measured respectively at 30 and 60 days after sowing. Diameter 1 and 2: diameter at the collar measured respectively at 30 and 60 days after sowing. NS: Not significant.

### Shoot, root and total biomass measurement

At 60 days after sowing, each plant was carefully removed in order to recover the aerial part and all the roots of the plants. All these parts were dried in an oven at 70°C for 72 h for the measurement of shoot, root and total biomass. After the biomass measurement, the roots were used for the mycorrhizal infection study.

### Staining for mycorrhizal colonization

About 10 g of roots from each treatment were thoroughly washed and placed in falcon tubes and then cleared using 10% KOH. They were heated in 90°C water bath for one hour. The roots were washed with tap water. Staining was then done by adding 0.05% trypan blue in lactic acid and heating in 90°C water bath for 30 min (Phillips and Hayman, 1970) and the observation was done under microscope (OLYMPUS BH-2) (magnification = 10x). The mycorrhizal frequency and intensity were estimated by Trouvelot et al. (1986) method.

### Statistical analysis

Data were statistically analyzed using a one-way analysis of variance (ANOVA) with XLSTAT 2018 statistical software, and the means were compared using the Newman-Keuls test ( $p < 5\%$ ).

## RESULTS

### Mycorrhization frequency and intensity evaluation

Table 2 shows the frequency and intensity of mycorrhization of the tomato. The frequency of

mycorrhization was quite high (86% for M1 inoculum and 82% for M2 inoculum) while the intensity of mycorrhization remains low (40.5% for M1 inoculum and 35.3% for M2 inoculum) (Table 2). Statistical analyzes show significant differences between the different treatments. However, the roots of the control treatments are not mycorrhized (Table 2).

### Effects of the tested AMF on the tomato growth

Table 3 presents the results of the measurements of the height, the relative growth rate in height, the collar diameter and the relative growth rate of the collar diameter of the tomato inoculated with native arbuscular mycorrhizal fungi. The results showed that the plant height varied according to the inoculum used and over time. At 30 days after sowing, the statistical analysis does not show any significant difference ( $P > 0.05$ ) between the different treatments, neither for the height nor for the collar diameter (Table 3 and Figure 1). At 60 days after sowing, the differences appear between the different treatments for both the height and the collar diameter and the highest values were obtained for inoculation with M1 inoculum (35.7 cm for the height and 3.5 mm for the collar diameter). This inoculum improved the tomato height growth by 164.44% and that collar diameter by 75.25% compared to controls (Table 3). However, M2 inoculum did not improved tomato growth compared to the control (Table 3). Moreover, the statistical analysis did not show any significant difference ( $P > 0.05$ ) between



**Figure 1.** Tomatoes plants 60 days after sowing and inoculated with 2 mycorrhizal inocula [mixed inocula (M1) and *Glomus* sp. (M2)].

the different treatments either for the relative growth rate in height, or for the relative growth rate in the collar diameter. In general, the M1 inoculum improved the tomato growth (Table 3 and Figure 1).

#### Assessment of shoot, root and total biomass

The results on shoot, root and total biomass are presented in Table 4. These results varied according to the treatments. Statistical analysis showed significant differences between the different treatments. The highest values were obtained with M1 inoculum (Table 4). This inoculum improved the shoot biomass by 540%, the root biomass by 1061.97% and the total biomass by 638.1% compared to the control (Table 4). However, M2 inoculum did not improved tomato biomass production compared to the control (Table 4).

#### DISCUSSION

The objective of this study was to assess the effects of mycorrhizal inoculation with native strains from Burkina Faso on tomato growth. The mycorrhizal results showed that the roots of the tomato were highly colonized by the mycorrhizal strains used. The absence of mycorrhizal

infection on the controls roots showed that this treatment was free from any mycorrhizal contamination. The growth and biomass stimulation between the different treatments and the control could be attributed to the effect of arbuscular mycorrhizal fungi inoculation.

The results on tomato growth show that inoculation improved the growth of this plant. Statistical analysis of plants height measured at 30 days after sowing did not show any significant difference between inoculated plants and control. This can be explained by the fact that the substrate used contained the necessary nutrients which were directly accessible to the plant's roots. These results are in agreement with those of Haro et al. (2012) who showed that the plant will not find a need to form the mycorrhizal symbiosis if the nutrients are available in the environment. This also explains the absence of significant differences in the rate of relative growth in height and the rate of relative growth in the collar diameter of the tomato. However, the statistical analysis on the height and the collar diameter of the plants measured at 60 days after sowing show significant differences between the different treatments. This can be explained by the fact that the growth of the plants over time has led to the depletion of the mineral elements directly accessible by their roots. Thus, the mycorrhizal symbiosis was established and developed gradually with the exhaustion of the mineral elements in the soil; hence

**Table 4.** Plant shoot, root and total biomass 60 days after the sowing of tomato inoculated with 2 mycorrhizal inocula [mixed inocula (M1) and *Glomus* sp. (M2)].

Treatment	Shoot biomass (g)	Root biomass (g)	Total biomass (g)
M1	0.79±0.17 <sup>a</sup>	0.33±0.07 <sup>a</sup>	1.12±0.18 <sup>a</sup>
M2	0.29±0.11 <sup>b</sup>	0.22±0.1 <sup>ab</sup>	0.41±0.11 <sup>a</sup>
Control	0.12±0.07 <sup>b</sup>	0.03±0.02 <sup>b</sup>	0.15±0.07 <sup>b</sup>
Significance level	0.009	0.016	<0.001

For the same parameter, data followed by the same letters are not significantly different according to the Newman-Keuls test ( $p < 0.05$ ). Standard error of the mean ( $n = 5$ ).

the effectiveness of the mycorrhizal strains in improving the growth in height and the collar diameter of the tomato plants at 60th day after sowing. Similar results have been found by Haro et al. (2012) on cowpeas. The highest values obtained with M1 inoculum, can be explained by the high mineral absorption power of the M1 inoculum.

For shoot, root and total biomass, M1 inoculum showed the best efficiency. As with the height and collar diameter measured at the 60th day after sowing, the effectiveness of the M1 inoculum on stimulating the production of biomass could be explained by the improvement of the tomato mineral nutrition by this inoculum. These results are in agreement with those of Ndoye et al. (2016) who showed that inoculation with *G. verruculosum*, *G. manihotis* and *R. irregularis* significantly improved the biomass of fonio plants. Similar results were found by Laminou Manzo et al., (2009) who showed the effectiveness of *Glomus intraradices* on the production of total biomass of *Acacia raddiana*, *Acacia nilotica*, *Acacia senegal* and *Prosopis chilensis*.

It appears that M1 was the most effective of all the inocula used in this study. This could be explained by the fact that M1 contains three AMF genera (*Scutellospora* sp., *Gigaspora* sp., *Glomus* sp.) whose effects can be added compared to M2 which has only one genus (*Glomus* sp.). Similar results were found by Haro et al. (2012) who showed that native strains containing at least 2 genera have effects that can add up compared to monospecific inocula.

The M2 inoculum did not improve either the tomato growth or the biomass production. This can be explained by the ineffectiveness of *Glomus* sp. in the tomato mineral nutrition improvement, which is justified by a host preference for endomycorrhizal fungi. Similar results were found by Haro et al. (2012) on cowpea.

## Conclusion

From these results, it generally appears that mycorrhizal inoculation improves the growth and production of tomato biomass. This study showed that Burkina Faso native mycorrhizal strains can improve both the production of biomass and the growth in height and in the collar diameter. Although, it has shown promising results, this

study deserves to be supplemented by *in situ* tests which would make it possible to assess the effect of this inoculation on the tomatoes production.

## CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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## REFERENCES

- Bramley PM (2000). Is lycopene beneficial to human health? *Phytochemistry* 54(3):233-236. DOI: 10.1016/S0031-9422(00)00103-5
- Diem HG, Guèye I, Gianinazzi-Pearson V, Fortin JA, Dommergues YR (1981). Ecology of VA mycorrhizae in the tropics: The semi-arid zone of Senegal. *Acta Oecologica / Oecologia Plantarum* 16:53-62.
- Dodzia BK, Attia T, Kodjoa TA, Affoha A, Mawuenaa G (2012). Impact de la fumure organique appliquée seule et en combinaison avec une souche indigène de champignon mycorrhizien arbusculaire *Glomus mosseae* sur *Meloidogyne* spp, principal nématode parasitaire de la tomate au Togo. *Journal of Applied Biosciences* 55:3973-3986.
- FAO (2020). Agricultural production, crop primary database. Food and Agricultural Organization of the United Nations, Rome. Agricultural production, crop primary database. Food and Agricultural Organization of the United Nations, Rome. <http://www.fao.org/faostat/fr/#data/QC>: consulted on 01/2020.
- Haro H, Sanon KB, Blagna F, Fofana B (2016a). Effect of native arbuscular mycorrhiza fungi inocula on the growth of Cowpea [*Vigna unguiculata* (L.) Walp.] in three different agro ecological zones in Burkina Faso. *Journal of Applied Biosciences* 108:10553-10560. DOI: <http://dx.doi.org/10.4314/jab.v108i1.8>
- Haro H, Sanon KB, Diop I, Kane A, Dianda M, Hounngandan P, Neyra M, Traoré A (2012). Réponse à l'inoculation mycorrhizienne de quatre variétés de niébé [*Vigna unguiculata* (L.) Walp.] cultivées au Burkina Faso et au Sénégal. *International Journal of Biological and Chemical Science* 6(5):2097-2112. DOI: <http://dx.doi.org/10.4314/ijbcs.v6i5.18>
- Haro H, Sanon KB, Krasova-Wade T, Kane A, N'Doye I, Traoré AS (2015). Réponse à la double inoculation mycorrhizienne et rhizobienne du niébé (variété, KVX396-4-5-2D) cultivé au Burkina Faso. *International Journal of Biological and Chemical Science* 9(3):1485-1493. DOI: <http://dx.doi.org/10.4314/ijbcs.v9i3.31>
- Haro H, Sanon KB, Le Roux C, Duponnois R, Traoré AS (2017). Improvement of cowpea productivity by rhizobial and mycorrhizal

- inoculation in Burkina Faso. *Symbiosis*: 1-14. DOI: 10.1007/s13199-017-0478-3
- Haro H, Sanon KB, Traoré AS (2016b). Amélioration de la productivité du niébé par l'inoculation rhizobienne et mycorrhizienne à Yakouta, Nord du Burkina Faso.
- Khalloufi M (2017). L'étude des effets combinés des phytohormones et du sel sur des plantes de tomate (*Solanum lycopersicum* L.) inoculées ou non par des mycorhizes à arbuscules (AMF). Proyecto de investigación, Universidad de Murcia. p. 210.
- Laminou Manzo O, Ibrahim D, Campanella B, Paul R (2009). Effets de l'inoculation mycorrhizienne 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.. *GeoEcoTrop Journal* 33:115-124.
- Mikola P (1987). Mycorrhizae under tropical stresses. *Angewandte Botanik* 61(1-2):15-23.
- Ndoye F, Diedhiou AG, Gueye M, Fall D, Barnaud A, Sy MO, Noba K, Diouf D, Kane A (2016). Réponse du fonio blanc (*Digitaria exilis* Stapf) à l'inoculation avec des champignons mycorrhiziens à arbuscules en conditions semi-contrôlées. *Journal of Applied Biosciences* 103(1):9784-9799. DOI: <http://dx.doi.org/10.4314/jab.v103i1.1>
- Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55(1):158-161.
- Pierre S (2012). Evaluation in vitro du potentiel bioprotecteur des champignons mycorrhiziens à arbuscules contre le flétrissement bactérien de la tomate. Faculté d'Ingénierie biologique, agronomique et environnementale, Université catholique de Louvain. p. 69.
- Trouvelot A, Kough JL, Gianinazzi-Pearson V, Gianinazzi S (1986). Mesure du taux de mycorrhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. *Mycorrhizae: Physiology and Genetics*, pp. 217-221.
- Willcox JK, Catignani GL, Lazarus S (2003). Tomatoes and cardiovascular health. *Critical Reviews in Food Science and Nutrition* 43(1):1-18. DOI: 10.1080/10408690390826437