Effect of *Sorghum bicolor* mycorrhizae on development of tomato in nursery

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Abstract

Tomato is one of the most important vegetable crops in Burkina Faso. Nevertheless, tomato production is confronted with numerous biotic and abiotic constraints. So, it is essential to find ways of boosting its production, starting at the nursery. In nurseries, the use of mycorrhizal fungi can be an effective means of obtaining vigorous and healthy seedlings. This study was carried out with the aim of assessing the impact of endogenous mycorrhizal fungi on the growth and biomass of tomatoes in the nursery. To do so, sorghum (*Sorghum bicolor* L.), known as a hypermycotrophic plant, was used for the inoculum production of mycorrhizal fungi. In addition, the effect of direct association of sorghum and tomato was studied. The results showed that the application of mycorrhizal fungi significantly improved the emergence, growth, and leafing of tomatoes in the nursery. A height of 5.75 cm was observed in mycorrhizal plants as compared to 3.23 cm for non-mycorrhizal control plants. It was also shown that adding mycorrhizal inoculum to the seeds was much more effective than the direct association in the field with the sorghum plant and tomato. The results showed that endogenous mycorrhizal fungi significantly improved the emergence, plant height, leaf formation, and biomass production of tomato plants.

Keywords: Mycorrhizae, Nursery, *Sorghum bicolor*, Symbiosis, Tomato.

Introduction

Vegetable crops provide more than 33% of the agricultural production throughout the world (FAOSTAT, 2018). They also provide employment to 800 million people and thus help to fight against unemployment (Kanda *et al.*, 2014). Tomato (*Solanum lycopercicum* L.) is one of the most important vegetables. In 2018, tomato production was estimated at 182,256,458 metric tons obtained from 4,762,457 ha worldwide of which 11.40% was from Africa (FAOSTAT, 2018). In Burkina Faso, tomato is the second most cultivated vegetable after onion. Its production was 167,400 metric tons from a cultivated area of 10,284 ha (DGESS, 2019). Tomato is one of the most studied fleshy fruits, and it has assumed the status of functional food with minerals (iron, phosphorus), vitamins (B, C, E, K), essential amino acids, sugars, dietary fiber, as well as pigments (*β*-carotene; red lycopene) (Kowalczyk *et al.*, 2011). Its consumption is related to the reduced risk of cancer and cardiovascular diseases (Giovannetti *et al.*, 2012). However, tomato production is confronted with multiple constraints affecting yields in recent years. Pest and disease pressure have been identified as the major constraint due to crop losses (Kanda *et al.*, 2014; Mondédji *et al.*, 2015). In order to improve yield, farmers' first resort is the use of chemical pesticides (Mondédji *et al.*, 2015). However, the negative effects of chemical pesticides on humans, the environment and the insecticides resistance of bio-aggressors have been established (Agboyi *et al.*, 2016).

In nurseries, the use of mycorrhizal fungi can be an effective means of obtaining vigorous and healthy seedlings. Arbuscular mycorrhizal fungi have many benefits for plants, including improved water and mineral nutrition, and protection from biotic and abiotic stresses (Javaid *et al.*, 2000; Nadeem *et al.*, 2014). A control strategy to be adopted is to stimulate the plants defense system by inoculating arbuscular mycorrhizal fungi (AMF) (Berta *et al.*, 2014; Bona *et al.*, 2017). AM fungi form symbiotic associations with plants (Javaid and Khan, 2019). These fungi belong to the phylum Glomeromycota (Schüβler *et al.*, 2001). AMF colonize plant roots, but the symbiosis can affect the whole of plant physiology with detectable effects on shoots and fruits (Bona *et al.*, 2017). The aim of this study was to evaluate the impact of endogenous mycorrhizal fungi from *S. bicolor* on the development of tomato plants in the nursery.

Materials and Methods

Materials

The tomato variety Roma VF was used in the nursery. It is a strong and productive variety with a growth cycle of around 75 days. Its fruits are elongated, oval in shape, and have an average weight of 60–70 g. The main characteristic of this variety is its tolerance to fungal diseases such as mildew, and also to *Verticillium* and *Fusarium* infections.

The Kapelga variety of sorghum was used for the production of inoculum of mycorrhizal fungi. It was used as a trap plant for growing the mycorrhizal fungi that matched the inoculum. sorghum is known to be a mycorrhizotrophic plant (Plenchette and Morel, 1996), which can associate with various indigenous symbiotic fungi with a very high colonization rate. Kapelga is a traditional variety from Burkina Faso with an intermediate cycle of 100 to 105 days.

Experimental design

The experiments were carried out during the rainy season in 2022 under semi-control conditions using trays. Trays measuring 100 cm long, 50 cm wide, and 20 cm deep were used for growing sorghum. Each tray contained a 10 cm layer of sand followed by a 5 cm layer of soil taken at 10–25 cm from an uncultivated area covered by a 5 cm layer of sand. In order to produce reproductive spores of AMF, sorghum was sown according to a simple design using three trays. Three rows of the test plants were planted per tray with 10 cm spacing between the two adjacent rows. There were 12 bunches in each row, 8 cm apart.

To assess the impact of mycorrhization on tomato plants, a three-replicated experimental design was used. It included three treatments *viz.* uninoculated tomato plants (control treatment), sorghum and tomato plants association, and tomato plants inoculated with AMF. These treatments were carried out in three trays with layers of soil and sand as described above. Both the control and mycorrhizal treatments, each accommodated four rows of 20 tomato plants per replicate giving 240 experimental plants per tray or per treatment. In the sorghum-tomato association treatment, the four rows per replicate were replaced by four double rows (with 2 cm between rows); the first one containing sorghum and the other, tomato plants.

Production of inoculum of native mycorrhizal fungi from sorghum

After an *in vitro* germination test, sorghum seeds were sown at a depth of 3 cm from the substrate, and the whole experiment was regularly watered in order to induce germination and the emergence of seedlings. After emergence, the plants were regularly watered with water as needed over up to two-month. After two months of monitoring, the time required for root development, and the establishment of mycorrhizal symbiosis, the sorghum plants were subjected to water stress for 10 days to induce extensive production of reproductive spores. After the water stress period, the sorghum plants were watered to facilitate the extraction of mycorrhizal inoculum from the roots.

Samples analyses of sorghum roots and mycorrhizal inoculum preparation

A fraction of harvested roots was carefully washed and cut into 1 cm segments for fungal structure (vesicles and mycelium) observation according to Philips and Hayman (1970) method. The roots were washed in dilute vinegar for 5 min, then placed in a 0.05% trypan blue solution for 30 min at 90 °C in order to give blue color to the chitin of the cell walls. Finally, the root fragments (1 cm) were placed between the slide and coverslip in glycerin and observed under an optical microscope at a magnification of 400 mm to identify characteristic structures of AMF.

The second fraction of sorghum roots were stored in a dry place before being used for the mycorrhizal inoculation process. To do this, an extract of AMF was prepared. This extract was obtained by grinding 4 g of sorghum roots in 1 L of tap water.

Tomato nursery, mycorrhizal inoculation, and treatments

After confirming the colonization of sorghum roots by mycorrhizal fungi, all the roots of sorghum plants were dug up, cut into thin fragments, and stored in a dry place to serve as inoculum in the tomato nursery. To evaluate the impact of sorghum mycorrhization on tomato growth, we set up an experimental plan comprising a sorghum-tomato association, tomato in monoculture on soil amended with mycorrhizal inoculum from sorghum and a control. Seeds of tomato were sown in trays according to the experiment design described above. The trial consisted of three replicates. In both the control and mycorrhization trays, there were 10 lines with 20 bunches per line in each replication. In the association tray, in contrast to the other two, a double line of 2 cm was planted in place of each row, one of which was planted with sorghum and the other with tomato (sorghum-tomato association). For the treatment tray with mycorrhizal inoculum, 4 g of sorghum roots were mixed with one liter of distilled water. The resulting suspension was used to irrigate the tray each day for three days before sowing. After sowing, three watering sessions with the inoculum suspension were carried out before using water for further irrigation. The nursery was monitored for 18 days and parameters were measured. After emergence, plant maintenance consisted of watering the control treatment and the sorghum-tomato association treatment with tap water, while an extract of AMF from sorghum roots has been associated with plant watering in mycorrhizal treatment.

Statistical analysis

Statistical analyses were performed using the statistical software R version 4.1.1 (R Core Team, 2023). Analyses of nonlinear regression between treatments were performed, testing different link functions, to fit germination with the gnls function, using the nmle package. Using plant counts, the average number of plants emerged and per treatment was determined. Average tomato plant height was obtained by measurement. Biomass was determined for each treatment by weighing whole tomato plants. Data were analyzed using analysis of variance (ANOVA) procedures and differences between treatments and means were compared using Tukey's HSD test at a 5% level of significance.

Results

Detection of mycorrhizae

Fungal structures in the roots of sorghum were found to be dark blue in color when observed under magnification 400X. These fungal structures were differentiated into a branched structure named "arbuscules" or a ball called "vesicle" formed essentially of hyphae (Fig. 1). These structures were found from all samples of sorghum roots.

Mycorrhization effect on seeds emergence

The assessment of the emergence of tomato seeds 18 days after sowing (DAS) is shown in Fig. 2. Results showed positive effect of mycorrhization treatment on tomato seed emergence (Fig. 2). At six DAS, the tomato seeds treated with mycorrhizae started to emerge. This growth trend was observed up to 10 DAS (Fig. 2). With the sorghum-tomato association treatment, first emergence was recorded at nine DAS, compared to 10 DAS for the control, followed by an evolutionary phase to reach a plateau at 14 DAS. However, the number of emerged plants was still smaller than that of tomato plants in association with sorghum at 18 DAS. The best emergence was recorded in the mycorrhization followed by the association and the lowest emergence was obtained by the control. Comparative analyses 18 DAS showed significant differences ($P \leq 0.001$) between the number of emerged plants in the mycorrhization treatment and the number of emerged plants in control treatments. Wherever, no significant difference was found between the numbers of plants in the control and the association treatments.

Mycorrhization effect on the heights and number of leaves of tomato plants

Table 1 shows the effects of the treatments on the height and number of leaves of tomato plants. The use of the mycorrhizal extract had a strong impact on their growth, with a very significant increase in height, with an average of 5.75 cm compared with 3.23 cm for the control. The association between tomato and sorghum treatment resulted in an average of 4.15 cm height of the tomato plants. The results showed the height of tomato plants was higher in the mycorrhizal treatment and sorghum-tomato association treatment compared to the control ($P \leq 0.004$). Also, the number of plant leaves was higher in the mycorrhizal and sorghum-tomato association treatments compared to the control. The number of leaves per plant on average was 7.27 cm in the mycorrhizal treatment, 4.73 cm in the association, and 3.3 cm in the control. There was no significant difference between the mycorrhization and association treatments for these two parameters.

Mycorrhization effect on fresh plants biomass in nursery

The highest average biomass (0.18 g) was recorded from tomato plants treated with mycorrhizae while tomato produced average biomass of 0.04 g and 0.08 g per plant from the sorghum-tomato association and control treatments, respectively. Based on the comparative analyses, a significant difference ($P \leq 0.024$) was found between the biomass of tomato plants from mycorrhizal treatment and those from sorghum-tomato association treatment. No significant difference was observed between the control treatment and the two other treatments (Table 2).

Discussion

Sorghum is a plant with high mycorrhizal activity. That was confirmed through the results in this study with the detection of mycorrhizal structures. Indeed, sorghum roots favor a significant multiplication of mycorrhizal spores (Watts-Williams *et al.*, 2021). The positive impact of mycorrhization on tomato emergence has been shown in this work as reported by Leventis *et al.* (2021) and Wang *et al*. (2022). This might be explained by the fact that mycorrhizal fungi would have the ability to act on the germinative power of seeds by facilitating dormancy lifting. A positive impact of mycorrhizae on the growth of tomato plants has been highlighted. This stimulation of plant growth by mycorrhizal strains is a consequence of improved nutrition through transfers of water and mineral elements. In particular, phosphorus and nitrogen, and improved uptake of trance elements that are not very mobile in soils, such as copper and cobalt, from the mycorrhization to the host plant (Nwoko, 2014).

The results also showed that inoculation with mycorrhizae fungi significantly improved leafing and biomass production of tomato plants. Indeed, in some plants, mycorrhizal colonization leads to an increased branching of the root system, a shortening of adventitious roots, an increase in fine roots or a transformation of the root architecture related to the respective proportions of the root categories (Chen, 2021). This facilitates access to water and nutrients, and thus the development of seedlings. These benefits were also highlighted by Al-Karaki (2006) and Nzanza *et al.* (2011), who reported that mycorrhization has a positive impact on the development of tomato seedlings as well as an improvement in plant photosynthesis. Improved photosynthetic activities would result in better fruiting and subsequently a good yield at harvest.

Moreover, the results showed that mycorrhization recorded the highest number of emerged plants, better height growth, better leaf formation, and better biomass production of the tomato plants compared to the sorghum-tomato combination. This could be explained by the fact that mycorrhization stimulated better nutrition and increased branching of the seedling root system (Chen, 2021). However, in the case of the sorghum-tomato association, the limited time available did not allow the sorghum to produce enough mycorrhizae fungi to stimulate the various growth parameters of the tomato. The cultivation of mycorrhizae fungi lasts two to three months, the time needed to develop the roots and establish a mycorrhizal symbiosis (Konvalinková *et al*., 2015). Moreover, competition for nutrients and mycorrhizae fungi between sorghum and tomato would occur due to their proximity to each other. In addition, the study as a whole highlighted the contribution of arbuscular mycorrhization to the development of tomatoes. Good plant development is very often associated with better plant health, as some studies have suggested. Indeed, mycorrhization appeared to reduce the impact and/or severity of damage caused by some root phytopathogenic fungi and nematodes. Symbiosis compensates for damage caused by root phytopathogens. The protection conferred would not only be local but also systemic (Schouteden, 2015).

Conclusion

Results of this study showed that endogenous mycorrhizal fungi significantly improved the emergence, plant height, leaf formation, and biomass production of tomato plants in the nursery. This confirmed the ability of arbuscular mycorrhizal fungi to stimulate plant development. Nevertheless, it would be useful to determine the frequency of mycorrhization in tomato roots and to evaluate the impact on yield at harvest.

Author's contributions

KK conceived research idea. KHS and SN conducted experiments and wrote the manuscript. AO analyzed the data. TAN and KK supervised all the studies. All the authors read and approved the manuscript.

Conflict of interests

The authors declare that there is no conflict of interest.

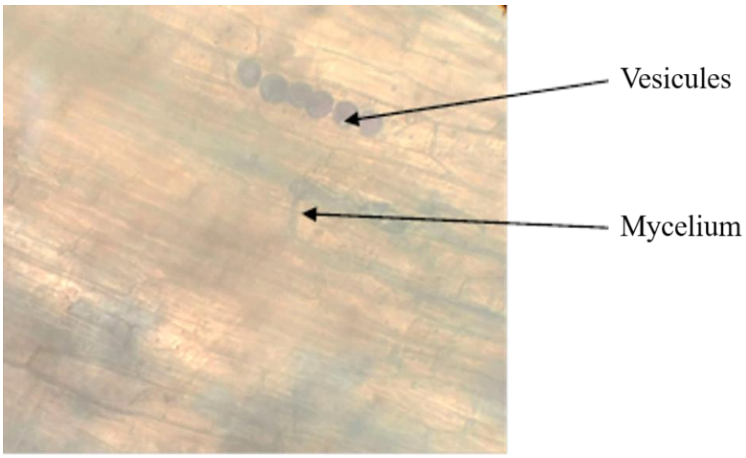

Fig. 1: Vesicles of mycorrhiza in the *Sorghum bicolor* root.

Table 1: Mycorrhization effect on tomato plants height.

Treatments	Height (cm)	Leaves number
Association	4.15 ± 1.18 [c]	4.73 ± 1.49 [c]
Mycorrhiza	5.75 ± 1.57 [a]	7.27 ± 2.09 [a]
Control	3.23 ± 0.49 [b]	3.33 ± 0.9 [b]

In a column, values with different letters are significantly different at $P \leq 0.05$ as determined by Tukey test.

Table 2: Mycorrhization effect on tomato plants biomass in nursery at 18 days after sowing in semi-control condition.

Treatments	Biomass (g)
Sorghum-tomato association	0.04 ± 0.01 [b]
Mycorrhizae	0.18 ± 0.02 [a]
Control	0.08 ± 0.01 [ab]

In a column, values with different letters are significantly different at $P \leq 0.05$ as determined by Tukey test.

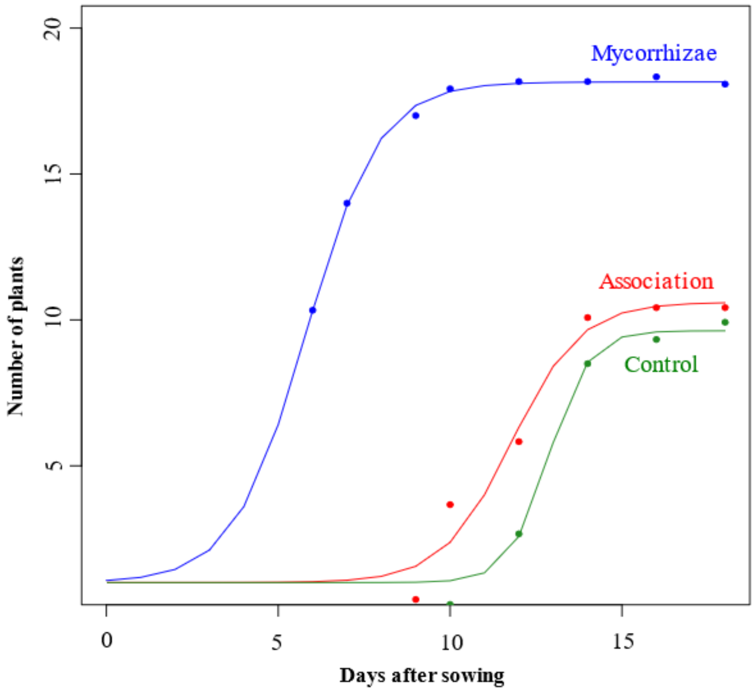

Fig. 2: Tomato seeds emergence evolution according to the number of days after sowing and the uninoculated tomato plants (Control), sorghum and tomato plants association (Association) and tomato plants inoculated with arbuscular mycorrhizal fungi (mycorrhizae) treatments.

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