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Native Arbuscular Mycorrhizal inoculation of Corn (*Zea mays* L.) cultivated in Burkina Faso

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ABSTRACT
Objective: This study was conducted with the aim to contribute to improving the productivity of corn by mycorrhizal inoculation.
Methodology and results: In this study, corn was grown in a greenhouse for 60 days and inoculated with five native inocula of arbuscular mycorrhizal fungi (AMF). The growth parameters were measured at the 30thand 60thdays after sowing. Shoot, root and total biomass were assessed at 60 days after sowing. The results show the M1 inoculum allowed an improvement corn height by 64.46% (30thday) and 93.2% (60thday), with the collar diameter by 22.76% (30thday) and 30.3% (60thday), relative growth rate in height by 169.49%, shoot biomass by 368%, root biomass by 366% and total biomass by 367% compared to the control.
Conclusion and application of findings: From this study, it emerged that the M1 inoculum is the most effective of all the inocula used. This study showed the potential of native AMF inocula to improve biomass production and it is necessary to deepen this study by *in situ* tests in order to be able to integrate it into the agricultural system.
Key words: Corn, inoculation, arbuscular mycorrhizal fungi, Burkina Faso

INTRODUCTION
In Burkina Faso, cereals are the staple food of the population and agriculture is based on these cereals (millet, sorghum, corn, rice and fonio). Agriculture contributes nearly 25% to the gross domestic product (GDP) and maize is the third cereal both in terms of cultivated area and in terms of production, after sorghum and millet (Elola, 2012; Ouedraogo, 2012). Production of this cereal increased from 423,494 t in 2000 to 1,700,127 t in 2018 (Figure 1) (FAO, 2020). For the same period, the area sown increased from 241,401 ha (2000) to 1,019,181 ha (2018) with a yield increasing from 1.75t/ha (2000) to 1.67 t/ha (2018) (Figure 1) (FAO, 2020). This shows that a continuous decline in the yield of corn. This decrease is due to the poverty or poor fertility of the soil in nutrients such as nitrogen and phosphorus (Bado, 2002) as well as to the rain conditions, which continues to deteriorate. In Burkina Faso for example, agriculture is still unproductive and dependent on the climate. It finds it difficult to meet the food needs of the population and guarantee the country's food security (Ouedraogo, 2012), hence the need to develop methods to improve agricultural production. This is how this study was initiated with the aim of helping to improve the corn

production since several studies have shown that it is a mycotrophic plant (Haro *et al.*, 2016; Haro *et al.*, 2017). In addition, the mycorrhizal symbiosis is capable of improving the growth and production of their host plant even when they grow on soils lacking in nutrients (Haro *et al.*, 2015; Haro *et al.*, 2016; Haro *et al.*, 2017; Haro & Sanon, 2020). Exploitation of this mycorrhizal symbiosis would be an opportunity to improve the growth and production of corn and this study will evaluate the effect of mycorrhizal inoculation on the growth of this plant.

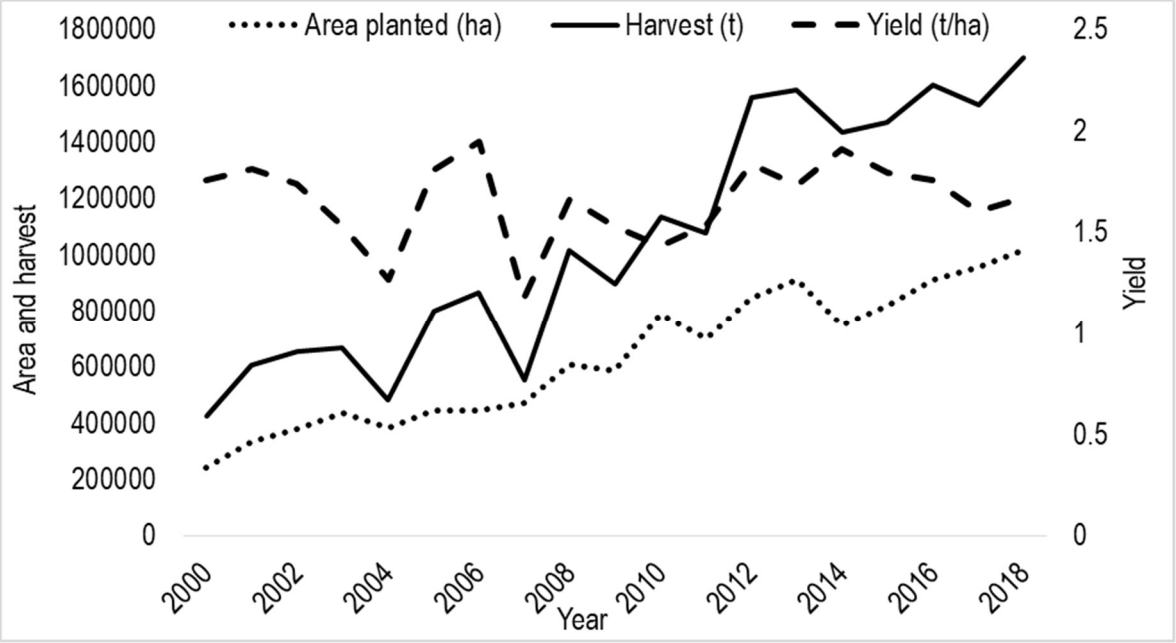

Figure 1: Corn production in Burkina Faso (area planted, harvested quantity and yield) from 2000 to 2018 (FAO, 2020)

MATERIAL AND METHODS

Plant and fungal materials: The local variety of corn was used. Corn 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 ($CaCl_2O_2$ 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 five native arbuscular mycorrhizal fungi (AMF) isolated from the rhizosphere of cowpeas cultivated in Burkina Faso (Haro *et al.*, 2012; Haro *et al.*, 2017): mycorrhizal complex [*Scutellospora* sp., *Gigaspora* sp., *Glomus* sp. (M1); *Glomus* sp., (M2); *Gigaspora* sp., *Glomus* sp., *Entrophospora* sp. (M3); *Gigasporas* p., *Glomus* sp., *Racocetra* sp. (M4) and *Glomus* sp., *Acaulospora* sp, *Racocetra* sp (M5). The inocula were obtained by multiplication of 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 physico-chemical characteristics were as follows: clay (%) 3.92, total silt (%) 5.88, total sand (%) 90.2, total organic matter (%) 0.331, total carbon (%) 0.192, total nitrogen (%) 0.016, C/N 12, total phosphorus (mg.kg-1) 172.52, available phosphorus (mg.kg−1) 1.74 and pH H_2O: 6.44. Culture substrate was homogenized, sieved with a 2 mm sieve and sterilized at 121 °C for 1 h.

Greenhouse experiment: The experiment was conducted for 60 days in 4 litre pots containing 4 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 to 3 cm deep. Control pots were not inoculated. There were ten replicates per treatment (n =

(5 mycorrhizal inocula + 1 control) x 10 replicates =60 plants). The corn 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. To estimate the effect of mycorrhizal inoculation on the corn, 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 = (Hf - Hi)/Hi

with H: height, i: initial, f: final.

The collar diameter was measured using a calliper 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: f: final.

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 hours 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 minutes (Phillips & 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 analysed 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 parameters: The results of corn mycorrhization are presented in Table 1. In general, corn is highly mycorrhized with a frequency of mycorrhization reaching 100% and an intensity of mycorrhization reaching 74.2% with M1 inoculum. Statistical analyses show significant differences between the different treatments and the control roots are not mycorrhized.

Table 1: Corn mycorrhizal frequency and intensity 60 days after sowing inoculated with 5 mycorrhizal inocula (M1, M2, M3, M4 and M5).

Treatments	Mycorrhizal frequency (%)	Mycorrhizal intensity (%)
Control	0^{d}	0^{d}
M1	100^{a}	74.2 ± 2.43^{a}
M2	95.33 ± 1.64^{b}	37.21 ± 2.36^{b}
M3	92.33 ± 1.14^{b}	38.45 ± 1.66^{b}
M4	85.33 ± 2.02^{c}	20.61 ± 2.29^{c}
M5	91.43 ± 2.1^{b}	20.71 ± 0.98^{c}
Significance level	<0.0001	<0.0001

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 = 10).

Corn growth and biomass production parameters measurement: Tables 2 and 3 presents the results of corn growth and biomass production. For growth in height and the collar diameter, the statistical analyses show significant differences between the different treatments. At the 30thday after sowing, the highest values were obtained with M4 inoculum for the height (32.02 cm) and for the collar diameter (4.8 mm). M4 inoculum improved corn height by 64.46%and collar diameter by 22.76% compared to the control. At the 60thday after sowing, the highest values were obtained with M1 inoculum for the height (66.75 cm) and for the collar diameter (7.44 mm). This inoculum allows an improvement corn height by 93.2% and collar diameter by 30.3% compared to the control. Statistical analyses also show significant differences for the relative growth

rate in height while no significant difference was observed for the relative growth rate of the collar diameter. Inoculum M1 obtained the highest value of the relative growth rate in height of corn (3.18%) and improved it by 169.49% compared to the control. Concerning the biomass production of corn, statistical analyses also show significant differences between the different treatments and the highest values were obtained with M1 inoculum for the shoot biomass (8.09 g), root biomass (4.1 g) and total biomass (12.19 g). This inoculum improved the shoot biomass by 368%, root biomass by 366% and total biomass by 367% compared to the control.

Table 2: Plant height, the collar diameter, the rate of relative growth in height and the rate of relative growth of the diameter at the collar of corn inoculated with 5 mycorrhizal inocula (M1, M2, M3, M4 and M5).

Treatments	Height 1 (cm)	Height 2 (cm)	RGRH (%)	Diameter 1 (mm)	Diameter 2 (mm)	TCRdc (%)
Control	19,47±1,43^{c}	34,55±1,65^{b}	1,18±0,26^{ab}	3,91±0,19^{b}	5,71±0,17^{c}	0,6±0,12^{a}
M1	27,22±2,85^{b}	66,75±6,02^{a}	3,18±1,51^{a}	4,68±0,3^{a}	7,44±0,42^{a}	0,68±0,12^{a}
M2	25,22±1,18^{bc}	49±3,1^{ab}	1,1±0,24^{ab}	4,61±0,12^{a}	6,21±0,18^{bc}	0,38±0,05^{a}
M3	22,32±0,92b^{c}	60,52±10,8^{a}	1,82±0,43^{ab}	4,26±0,1^{ab}	6,79±0,14^{b}	0,63±0,06^{a}
M4	32,02±1,57^{a}	51,02±2,54^{ab}	0,69±0,11^{b}	4,8±0,19^{a}	6,63±0,15^{b}	0,43±0,06^{a}
M5	21,92±1,38^{bc}	59,67±4,29^{a}	2,06±0,42^{ab}	4,32±0,13^{ab}	6,55±0,18^{b}	0,55±0,07^{a}
Significance level	<0.0001	0.004	0.05	0.004	<0.0001	NS

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 = 10).

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

Table 3: Corn shoot, root and total biomass 60 days after the sowing of corn inoculated with 5 mycorrhizal inocula (M1, M2, M3, M4 and M5).

Treatments	Shoot biomass (g)	Root biomass (g)	Total biomass (g)
Control	1,73±0,16^{c}	0,88±0,09^{b}	2,61±0,24^{b}
M1	8,09±1,15^{a}	4,1±0,58^{a}	12,19±1,73^{a}
M2	3,08±0,35^{bc}	1,43±0,18^{b}	4,51±0,52^{b}
M3	4,62±0,79^{b}	2,25±0,44^{b}	6,86±1,23^{b}
M4	3,85±0,47^{bc}	1,86±0,27^{b}	5,72±0,74^{b}
M5	4,33±0,42^{bc}	2,13±0,26^{b}	6,46±0,68^{b}
Significance level	<0,0001	<0,0001	<0,0001

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 = 10).

DISCUSSION

The mycorrhizal results showed that corn is strongly mycorrhized and no mycorrhization was observed in the control roots. This can be explained by the absence of an endomycorrhizal type contaminant on all treatments because mostly plants react with fungi. Therefore, the differences observed in growth and biomass production parameters could be attributed to the effect of mycorrhizal inocula. These results corroborate with those of Haro & Sanon (2020). In addition, Wang & Qiu (2006) have shown that around 90% of terrestrial plants are affected by endomycorrhizal symbiosis. At 30th day after sowing, inoculation with M4 provided the best stimulation of corn growth in height and collar diameter. This can be explained by the effectiveness of this inoculum in improving the corn mineral nutrition which results in improved plant growth. These results are in agreement with those of Haro & Sanon (2020) which showed that mycorrhizal inoculation improved sesame

growth and its biomass production. Haro *et al.* (2016) are also showing improvement in cowpea growth and biomass production by mycorrhizal inoculation. At 60th day after sowing, it was inoculation with M1 that allowed the best improvement corn height, relative growth rate in height, collar diameter and biomass production. These results can be explained by a better efficacy of this inoculum. These results are in agreement with those of Koda *et al.* (2018) who showed the beneficial role of mycorrhizal inoculation on the growth and seed yield of corn. The performance of M1 is due to its better ability to improve the mineral absorption of corn. These results are in agreement with those of Haro *et al.* (2017). The variability of the response of maize to inoculation with different fungal inocula is justified by the host preference of endomycorrhizal fungi (Haro *et al.*, 2012). These results corroborate those of Assogba *et al.* (2017) who showed the potential of arbuscular mycorrhizal fungi for improving corn productivity. Ndoye et al. (2016) who showed that fonio responds differently to mycorrhizal inoculation depending on AMF species used have found similar results. These results suggest that M1 is the most effective inoculum of all the inocula used. These results corroborate those of Haro *et al.* (2012) who showed that the Ya inoculum allowed a better improvement of the cowpea biomass compared to all the inocula used.

CONCLUSION

This study, conducted with the objective of testing the response of corn to endomycorizal inoculation, showed that the use of native mixed AMF inocula corn growth in height, collar diameter and biomass production compared to the control. This is a contribution to improving the corn productivity. It emerges from this study that the potential of native AMF in improving corn biomass productivity is immense, especially for Burkina Faso, hence the need to accelerate its integration into the agricultural system. It would be interesting to complete this study with field tests that would make it possible to evaluate the effect of inoculation with native AMF on the corn grain productivity.

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