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Original Research Article

Growth, late blight and yield response of potato (*Solanum tuberosum* L.) to arbuscular mycorrhizal fungi inoculation in West region of Cameroon

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Abstract

The crop protection system in Cameroon is mainly based on the use of chemical pesticides which can lead to human and environmental health problems. Biological control is a low-cost and eco-friendly alternative control method that could be used to boost the production of quality potatoes. This study aims to test a biological control approach for potato growth, late blight, and yield using arbuscular mycorrhiza fungi (AMF) inoculants. To achieve this, a split-plot experimental design consisting of two factors: potato varieties (Pamela and Cipira) and AMF dose (0 g, 20 g, and 40 g per plant) was used. Results showed that the interaction between variety and AMF doses was significant for growth variables for the treatment Cipira × 20 g AMF/plant showing the highest plant height (48.0 cm) at the 4th week after sowing (WAS). In addition, the combination of variety and AMF doses significantly reduced late blight incidence and severity, with the best result exhibited by Pamela variety × 40 g AMF/plant (53% and 10%, respectively). The treatments also showed a significant effect on root colonization, with Pamela × 40 g AMF/plant exhibiting the highest arbuscular content in the root system (93%). In terms of yield, the interaction between variety and AMF doses had a significant effect on tuber yields, with a yield of 50 and 55 t/ha recorded for Pamela at 20 g of AMF/plant and 40 g of AMF/plant, respectively. These results show that farm management practices based on AMF inoculations could efficiently increase potato productivity in the Western Highlands of Cameroon.

Keywords: Arbuscular mycorrhizal fungi; root colonization; growth; late blight; potato; yield.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a tuberous herbaceous plant native to Latin America and one of the important crops in the world particularly in sub-Saharan Africa both in starter dishes (salads) and central menu (soups). It is the third most important crop grown for direct human consumption (Hardigan et al., 2017) and provides numerous essential nutrients including health-benefiting compounds like lycopene and phenolics (Galani et al., 2017). In Cameroon, it is estimated that about 50,000 to 65,000 hectares of land are used for its production (Martin et al., 1995). The most potato-producing regions are the North West and the West with more than 80% of the national production, estimated at 354 404 tons with an average yield of 16.40 t/ha in 2020 (FAO, 2022). This production remains low with respect to the national demand estimated to be one million tons of potatoes per year, representing thrice the present production. In response to this critical situation, several measures have been undertaken to boost production: importing 20 tons of improved seeds in 2013 and selecting and distributing two high-performance varieties (Cipira and Tubira) that boosted production (MINADER, 2022). Despite all these measures, there is still a production gap at the national level indicating that improved varieties alone may not be able to close up. Therefore, there is a need to combine the use of improved varieties together with appropriate cultivation practices to boost potato production.

Late blight caused by *Phytophthora infestans* is the major disease affecting potato production in the West region of Cameroon (Fontem and Gumedzoe, 1988). Plant protection against pests and diseases is a major issue in plant production. This issue remains essentially based on the use of synthetic products formulated by agrochemical companies (Haverkort et al., 2009). Intensive use of synthetic pesticides is admittedly effective and has led to a significant increase in yield, to the limitation of yield losses linked to pests, and to the protection of foodstuffs. However, they are increasingly being questioned for several reasons, particularly their toxicity on non-target organisms (birds, fish, pollinators, and humans), the development of resistance to pests and pathogens which causes them to lose their efficiency, and pollution of the environment (air, soil, water) by residues (Galani et al., 2021). Therefore, legislative and regulatory measures have been put in place regulating their use and considering the development of alternatives.

Biological control measures such as arbuscular mycorrhizal fungi (AMF) have shown an increase in plant resistance against diseases (Anda et al., 2020; Dey and Ghosh, 2022). AMF are obligate root symbionts that form associations with about 80% of terrestrial plant species, including beans, maize, tomato, and potato. They have the ability to increase the root absorption surfaces of plants, facilitate their nutrition, and boost plant defence mechanisms and resistance of plants to diseases (Smith and Read, 2008). Although several studies have demonstrated the effects of AMF on root pathogens, only a few were related to leaf pathogens and the results were less conclusive (Whipps, 2004). In this study, the effect of an AMF on growth, late blight development, and tuber yield was evaluated on the two most cultivated potato varieties during the rainy season in the high plateau agroecological conditions of the western region of Cameroon.

MATERIALS AND METHODS

Collection of plants, mycorrhizas and chemical

The plant material used in this study was Pamela and Cipira potato varieties, obtained from the National Agricultural Research Institute for Development (IRAD) research station of Dschang. The AMF material was provided by the Biotechnology Center of the University of Yaoundé 1 based at Nkolbisson, Yaoundé, Cameroon. It consisted of a mixture of spores, root, and hyphae fragments from four pure strains of arbuscular mycorrhizal fungi (*Glomus hoi, Gigaspora margarita, Scutellosporadipur purascens,* and *Rhizophagus intraradices*). The inoculum concentration was 75 propagules/g. The synthetic fungicide, Bonsoin (30% of Chlorothalonil and 6% of Cymoxanil) was applied at the recommended dose of 1.50–1.75 kg of active ingredient/ha or 80 g of the fungicide for a 15 L knapsack sprayer.

Experimental design and crop management

The experimental site was cleared and marked out. Each pot (30 cm in diameter and 25 cm in height) was filled with 4.5 kg of topsoil previously sterilised in an autoclave at 121 °C for 2 hours. The pots were placed on woody support at 90 cm above the ground to avoid contamination by natural arbuscular mycorrhizal fungi from the soil (AMF). The design was completely randomised with a split-plot arrangement repeated three times. Each experimental unit consisted of ten pots. The main factor was potato varieties (Pamela and Cipira) while the secondary was the dose of AMF (0, 20, and 40 g of AMF/plant). The AMF dose 0 was considered a negative control whereas the fungicide Bonsoin was a positive control. Single and rooted pre-germinating seed tubers were sown at a depth of 8 cm in the pots containing the soil and the AMF inoculum was sprayed as a layer under the seed tuber. Poultry manure (5 t/ha) and mineral fertilisation (Yara Mila Complex, N-P-K: 13-13-21 at 600 kg/ha) were applied a week before sowing and three weeks after sowing (WAS), respectively. The insecticide Parastar (40 WP) (20 g/kg imidacloprid and 20 g/kg lambda-cyhalothrin) was used to control insects at the recommended dose of 50 g for a 15 L knapsack sprayer. Plants were allowed to be infected naturally by *P. infestans*.

Data collection

Growth variables

The following growth variables were collected at 4 weeks after sowing (WAS): the number of leaves, plant height, and leaf area whereas the number of buds and the root biomass were collected at 1 and 13 WAS, respectively. The height of the plants was taken using a 30 cm transparent plastic ruler. The leaf area was assessed using a grid made of wood, nails, and strings comprising 9 squares of 1 m side each (Tankou, 2006). The number of buds and leaves was determined by counting them on the plants sampled.

Disease incidence and severity

Late blight incidence and severity assessment started when the first symptom of the disease appeared in the field. Late blight incidence was determined using the formula, I = (NIP/TNP) × 100, where I = Incidence in %, NIP = Number of plants showing disease symptoms, TNP = Total number of plants considered (Djeugap et al. 2017). Late blight severity was determined by considering the proportion of the attacked surface on the plant to the total surface of the plant, expressed as a percentage using the Horsfall-Barrat modified rating scale (Zadoks and Schein, 1979). Disease variables were collected at 3 and 5 WAS.

AMF root colonization variables

Six WAS, 5 over the 10 pots for each treatment were flooded with water so that the roots and all the tubers could easily be extracted. Root samples (about 5 g) were washed with running water, soaked in 10% KOH for 24 h at room temperature, then rinsed 3 times with tap water. Subsequently, they were soaked in 3.5% hydrogen peroxide for 30 min, then stained with blue ink acidified with 1% HCl (1:50) for 45 min at room temperature and stored in glycerol (50%) until observation. Thirty root fragments (1 cm long) of each root subsample (from 5 plants) per treatment were considered to estimate means. AMF root colonization indicators were: the frequency of mycorrhizal fragments (F %), arbuscule abundance (A%) in the entire root system, and the arbuscule abundance (a%) only in the root fragments that are mycorrhizal (Trouvelot et al., 1986).

Yield

Yield (t/ha) was recorded at harvest (13 WAS) on the remaining 5 plants per treatment by weighing the potato tubers harvested from each pot. In order to go from pot yields to yield per hectare, we used the extrapolation method. The following formula was used: Yield = number of pots per m^2 × number of plants per pot × number of stems per plant × number of runners per stem × number of tubers per plant × average tuber weight (Duchenne, 1990).

Data analysis

Data obtained were submitted to analysis of variance in the software R. The Shapiro-Wilk and Bartlett tests were carried out to verify the normality and the homogenetity respectively. When significant differences were observed, mean separation was performed using the least significant difference (LSD) test at $p < 0.05$.

RESULTS

Influence of AMF on potato growth

Globally, growth variables of the variety Cipira were significantly ($p < 0.05$) higher compared to the variety Pamela (Table 1). Number of buds or stems, number of leaves, leaf surface area, and plant height) were not significantly affected by AMF inoculation. However, variety had a significant effect on the number of leaves, leaf area, and plant height (Table 1). The interaction between variety and AMF doses was significant for growth variables (plant height), with treatment Cipira × 20 g AMF/plant showing the highest plant height in the 4th week after sowing (WAS) (48.20 cm). Pamela × 0 g of AMF/plant interaction (negative control) had the largest leaf area (270.40 cm^2) compared to other treatments. AMF had no significant effect on root biomass.

Influence of variety and AMF on potato late blight

Late blight incidence and severity were significantly higher in Cipira than in Pamela irrespective of the growth period. Interactions between varieties and AMF treatment had a significant ($p < 0.05$) effect on potato late blight incidence and severity at 3 WAS (Table 2). For instance, late blight severity was 10% and 25% at 5 WAS for Pamela and Cipira, respectively (Table 2). The combined effect of Pamela with 40 g of AMF/plant (severity = 10.33%) was comparable to Pamela × fungicide interaction (severity = 8.52%) on the late blight control, and it was higher than the effect of Cipira × Fungicide interaction at 5 WAS (Table 2). Late blight severity varied from 8.52% (Pamela × Fungicide interaction) to 25.12% (Cipira × 0 g/plant of AMF interaction).

Root colonization by AMF

Data analysis showed that AMF inoculations have a significant effect on mycorrhizal frequency, arbuscular content in the root system, and mycorrhizal

Table 1. Interaction effect between varieties and dose of arbuscular mycorrhizal fungi on potato growth variables, 4 weeks after sowing

Varieties	AMF treatments ($g.plant^{-1}$)	Number of buds/stems	Number of leaves	Leaf area (cm^2)	Plant height (cm)	Root biomass (g)
Pamela	0	4.00±1.10^{a*}	27±9^{a}	270.40±67.50^{a}	23.60±3.60^{b}	10.20±1.20^{a}
	20	2.20±0.40^{b}	19±3^{ab}	91.60±25.60^{b}	34.50±3.80^{b}	10.80±1.50^{a}
	40	2.50±0.41^{b}	12±5^{c}	88.80±30.50^{b}	21.60±8.80^{b}	9.40±1.10^{a}
	Fungicide	5.10±0.80^{a}	38±13^{a}	270.3 ± 80^{a}	46.20±5.30^{a}	10.00±1.0^{a}
	Mean of all the treatments	3.45±1.31	22±7	180.20±70.89	28.97±5.37	10.10±1.20
Cipira	0	4.20±1.10^{a}	29±8^{a}	268±66.70^{a}	40.30±5.40^{b}	10.60±2.40^{a}
	20	2.70±0.30^{b}	11^{c}	95.50±20.20^{b}	48.20±4.40^{a}	10.80±2.80^{a}
	40	2.00±0.90^{b}	10±2^{c}	91.80±25.60^{b}	43.20±3.90^{b}	10.30±2.20^{a}
	Fungicide	4.80±0.30^{ab}	36±14^{a}	360±105^{a}	44.80±0.50^{b}	10.00±1.00^{a}
	Mean of all the treatments	3.42±0.65	21±6	203.82±54.37	44.12±4.05	10.42±2.10
Varieties		S	S	S	S	S
AMF treatments		NS	NS	NS	NS	NS
Varieties × AMF treatments		NS	NS	NS	S	NS

*Means in a column followed by the same letter are not significantly different according to the LSD test at a 5% probability threshold. AMF = arbuscular mycorrhizal fungi. WAS = weeks after sowing. S = Significant; NS = Not significant. Values are means ± standard deviation.

Table 2. Interaction effect between varieties and dose of arbuscular mycorrhizal fungi on the incidence and severity of potato late blight 3 and 5 weeks after sowing

Varieties	AMF treatments ($g.plant^{-1}$)	Disease Incidence (%)		Disease Severity (%)	
		3 WAS	5 WAS	3 WAS	5 WAS
Pamela	0	68.60±10.50^{a*}	100.00^{a}	7.87 ± 3.21^{b}	20.23 ± 3.50^{a}
	20	46.60±9.50^{bc}	80 ± 11.00^{b}	0.00^{c}	14.15 ± 2.10^{b}
	40	33.30±7.20^{c}	53.30 ± 7.50^{d}	0.00^{c}	10.33 ± 2.50^{c}
	Fungicide	35.60±11.50^{c}	47.20 ± 6.40^{d}	3.13 ±2.30^{b}	8.52 ± 2.10^{c}
	Means of all the treatments	46.02±9.67	70.12±6.22	2.75±1.37	13.30±2.55
Cipira	0	56.60±7.80^{a}	82.60 ± 10.50^{b}	14.22 ±4.40^{a}	25.12 ±4.20^{a}
	20	60.10±8.30^{a}	86.30 ± 9.50^{b}	0.00^{c}	18.73 ± 2.30^{ab}
	40	47.60±8.50^{bc}	68.60 ± 80.00^{c}	0.00^{c}	15.64 ± 3.30^{b}
	Fungicide	46.30±7.30^{c}	55.30 ± 7.70^{d}	5.56 ± 2.60^{b}	16.12 ± 3.10^{b}
	Means of all the treatments	52.65±7.97	73.20±26.92	4.94±1.65	18.90±3.22
Varieties		S	S	S	S
AMF treatments		NS	NS	S	NS
Varieties × AMF treatments		S	NS	S	NS

*Means in a column followed by the same letter are not significantly different according to the LSD test at a 5% probability threshold. AMF= arbuscular mycorrhizal fungi. WAS = weeks after sowing. S = Significant; NS = Not significant. Values are means ± standard deviation.

part of root fragments. Roots of the variety Pamela were more adapted to AMF colonization than those of the Cipira. Mycorrhizal frequency of potato root varied from 20.12% for Cipira × 0 g/plant of AMF to 86.66% for Pamela × 40 g/plant of AMF interaction (Table 3). Roots of control and fungicide treatments were shaken and no AMF colonization was observed. Treatment based on Pamela × 40 g/plant of AMF interaction exhibited the significantly ($p < 0.05$) highest arbuscular content in the root system (A = 93.64) and arbuscular content of mycorrhizal part of root fragments (a = 84.13). The lowest A and values were observed on Cipira × 0 g of AMF/plant interaction (A = 1.71 and a = 1.11) followed by Cipira × 20 g of AMF/plant (A = 1.75 and a = 1.23) (Table 3).

Influence of variety and AMF on potato yield

In the absence of AMF inoculation, the Pamela variety showed a significantly higher yield (42 t/ha) than the Cipira (27 t/ha). The interaction effect between variety and AMF revealed that the highest potato tuber yield was obtained with Pamela both at 20 g (50 t/ha) and 40 g of AMF/plant (55 t/ha) (Figure 1).

Table 3. Effect of variety and dose of arbuscular mycorrhizal fungi on root colonization of potato

Varieties	AMF treatments (g/plant)	Mycorrhization frequency (%)	Arbuscular content in the root system (A%)	Arbuscular content of the mycorrhizal part of root fragments (a%)
Pamela	0	43.33±17.86[c*]	29.07±25.11[c]	17.25±8.730[b]
	20	66.66±13.11[b]	72.96±23.41[b]	75.61±25.90[a]
	40	86.66±11.54[a]	93.64±5.92[a]	84.13±32.60[a]
	Fungicide	56.63±12.23[bc]	29.07±9.11[c]	17.34±6.10[b]
	Mean of all the treatments	63.32±13.68	68.68±26.88	48.58±18.33
Cipira	0	20.12±3.45[d]	1.71±0.11[d]	1.11±0.12[d]
	20	26.66±8.57[d]	1.75±0.22[d]	1.23±0.10[d]
	40	53.35±13.83[bc]	15.39±7.62[cd]	9.22±4.80[bc]
	Fungicide	40.24±10.16[c]	15.96±4.60[cd]	9.33±5.00[bc]
	Mean of all the treatments	35.09±9	8.70±3.13	5.22±2.50
Varieties		S	S	S
AMF treatments		S	S	S
Varieties × AMF treatments interactions		NS	S	S

*Means in a column followed by the same letter are not significantly different according to the LSD test at a 5% probability threshold. AMF= arbuscular mycorrhizal fungi. WAS = weeks after sowing. S = Significant; NS = Not significant. Values are means ± standard deviation.

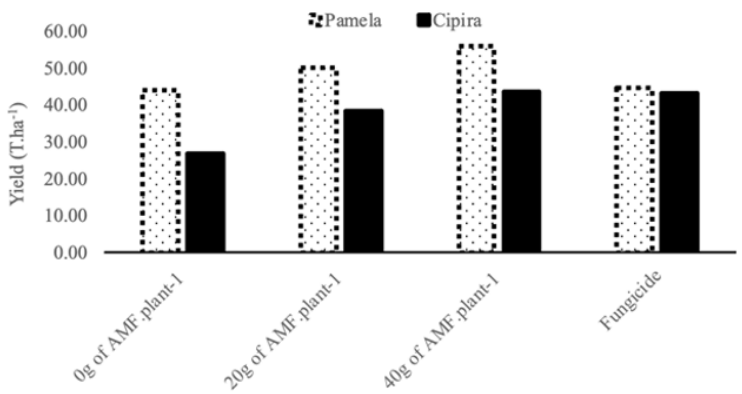

Figure 1. Interaction effect of variety and AMF on potato yield

DISCUSSION

Effect of variety and AMF inoculations on potato growth variables

It is known that mycorrhizal fungi affect the growth of most plant species in various ways. The results of the present study demonstrated the beneficial effects of a composite of AMF (*G. hoi, G. margarita, S. dipurpurascens,* and *R. intraradices*) on some growth variables, late blight and yield of two potato varieties in the West region of Cameroon. Results showed a significant increase over non-inoculated plants with respect to the number of buds/stem at emergence (varietal effect), leaf area, and plant height. Previous studies have demonstrated the role of mycorrhizal symbiosis in improving the uptake of phosphorus, nitrogen, and trace elements in tomatoes (Balliu et al., 2015). In this case, only the interactive effect of variety and AMF has an influence on plant height at 4 WAS. This may also be due to the host preference of AMF species as reported by previous studies (Earanna, 2001; Gracy and Bagyaraj, 2005).

The variety influences plant height irrespective of the growth stage throughout the growth cycle of the potato. In addition, plant growth is a function of several factors, including varietal characteristics, the environment, and agricultural practice (Beninal et al., 2011). The Cipira variety exhibited the highest plant height during the growth cycle. This result is similar to that of Beninal et al. (2011) who established that the height of potato plants varies depending on the growth stage, variety, fertilisation, diseases, and pests. The number of leaves was also influenced by the variety × AMF interactions. Indeed, the number of leaves decreased by 4WAS with the appearance of the disease and the attack of the pests (white flies) which caused necrosis and death of the infected leaves.

Similar results were reported in other species, such as *Ocimum basilicum, Lactuca sativa, Lonicera confusa,* and *Vicia faba* (Ferahani et al., 2008; Hazzoumi et al., 2015). AMF increases plant biomass under biotic stress as it could compensate for the damage caused by pathogenic fungi (Jung et al., 2012; Dowarah et al., 2021). These results were also obtained on potatoes by Ferjaoui (2010). Cipira × 40 g AMF/plant interaction recorded the highest number of leaves. In general, AMF increases the root surface area for the absorption of nutrients and therefore, facilitates plant nutrition. Tomato inoculated with AMF has shown increased leaf area, nitrogen, potassium, calcium and phosphorus content (Balliu et al., 2015) and confers vigour to plant host (Prasad et al., 2017). AMF inoculation also increases access of roots to a large soil surface area, causing improvement in plant growth, availability and translocation of various nutrients (Bowles et al., 2016).

Effect of potato variety and AMF on potato late blight

Generally, the incidence and severity of late blight were lower in potato plants inoculated with AMF throughout the growing season. AMFs are known to stimulate crop resistance to diseases. Ferjaoui et al. (2010) obtained similar results and established the fact that late blight appears often in the earlier stage of the growing cycle of potatoes irrespective of cultivars. At this stage, the plants are young, rich in cellulose, and very sensitive. However, there are potato varieties that are less susceptible than others. The Pamela variety × 40 g AMF/plant interaction was the most resistant to late blight. AMF reduces late blight on inoculated potato plants compared to non-inoculated plants. It is known that AMF induces disease control in indirect and direct ways. Indirectly by enhancing plant growth and vigour through improved nutrition and competition with other harmful microorganisms. They are also able to produce anti-fungal and anti-bacterial compounds (Bencherif et al., 2019; Kaur and Suseela, 2020) and toxins that act against pathogenic organisms (Weng et al., 2022), thus AMF which are bio-fertilisers are bio-inoculants acting as bio-pesticide in sustainable crop productivity (Barrow, 2012). However, it is also important to note that the use of insecticides to control white flies could significantly reduce the effect of AMF.

Effect of potato variety and AMF on potato yield

The mycorrhiza-based inoculation of potatoes significantly increased potato yield compared to non-inoculated. Variety × AMF interaction gave the highest yield (55.5 t/ha). Pamela variety × 40 g of AMF/plant showed the highest tubers yield. This could be explained both by the genetic potential of each variety (Benniou and Benamara, 2001) and phosphorus intake due to AMF (Balliu et al., 2015). Moreover, mycorrhization leads to better exploitation and absorption of the nutrients with a positive impact on the chlorophyll content, and at the same time, they provide potato plants with improved water exploitation, thus increasing yield (Hazzoumi et al., 2015).

CONCLUSION

This study showed that the commercialized composite of AMF (*Glomus hoi, Gigaspora margarita, Rhizophagus intraradices, Scutellosporadi purpurescens*) can be used as biofertilisers to improve potato growth and yield and as a biological control agent against potato late blight under screen house conditions. In field conditions, farm management practices based on AMF treatments may represent a sustainable mode to improve soil fertility, disease control, and crop yield, while preserving the agricultural environment.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to research, authorship, and publication of this article.

ETHICAL COMPLIANCE

The authors have followed ethical standards in conducting the research and preparing the manuscript.

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