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Vol. 14(20), pp. 1696-1701, 20 May, 2015
DOI: 10.5897/AJB2015.14571
Article Number: C4ECB4D52954
ISSN 1684-5315
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African Journal of Biotechnology

Full Length Research Paper

Responses of potatoes plants inoculated with arbuscular mycorrhizal fungi and litter in greenhouse

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Received 16 March, 2015; Accepted 6 May, 2015

A pot experiment was set to examine the impact of the foliar litter (*Hardwickia binata* and *Azadirachta indica)* and an *arbuscular mycorrhizal* (AM) fungus on the development of two varieties of potato plants (Aida, Atlas). Three litter doses (0, 25 and 50 g) were applied to the pots after bedding plantlets. The plants were inoculated with AM, *Glomus aggregatum*. Mycorrhizal colonization, shoot dry weight, size and number of minitubers were evaluated after 12 weeks on the potato growth. Results show that shoot dry weight of plants was improved by litter of the *H. binata* at 25 and 50 g. Thus, *A. indica* litter increased size of plants Aida at 50 g and the minitubers numbers Atlas at 25 g. On the other hand, root colonization decreased with increase in the dose of litter with both varieties of potato.

Key words: Arbuscular mycorrhizal fungi, potato, litter, micropropagation.

INTRODUCTION

Plant residues are an important source of nutrients (Musvoto et al., 2000). Leaf litter makes it possible to restore soils by a vertical transfer of minerals (Feller, 1995). The use of litter also stimulates the activity and development of soil microorganisms by a direct effect with the addition of carbon substrate in soil-vegetation systems (Vance and Chapin, 2001). The capacity of high fertilization of the litter is related to the type of organic matter use (Larkin and Tavantzis, 2013). Many tropical trees are used for agricultural purposes. The leaves of trees of *Hardwickia binata* Roxb, *Azadirachta indica* A. Juss, *Faidherbia albida* are used as green manure in Senegal. Some leaf litters used (*Andropogon gayanus*, Kunth and *Eragrostis tremula*, Steud) do not provide significant organic reserves whereas those of *F. albida* and *A. indica* show a high potential to improve the growth of the plants (Diallo et al., 2008).

For the plants like potato (*Solanum tuberosum*), which have a low root density and a strong potential of growth, the arbuscular mycorrhizal symbiosis may be of particular significance in coping with phosphorus and water deficiency stress in tropical soils. Arbuscular mycorrhizal

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Abbreviations: MS, Murashige and Skoog; **AM,** arbuscular mycorrhizal; **LBC,** laboratory of fungal Biotechnologies; **MC,** mycorrhizal colonization; **SDW,** shoot dry weight; **NM,** number of minitubers; **SM,** size of minitubers.

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Table 1. Characteristics of the soil used.

Component	Contents (%)
Clay	3.6
Silt	1.6
Fine silt	2.9
Fine sand	51
Coarse sand	40.9
Organic matter	1.06
Total carbon	2.5
Total nitrogen	0.3
Available phosphorus	3.1
pH (sol/water ratio 1:2)	6.7
pH (sol/KCl ratio 1:2)	4.5

(AM) fungi is known to increase yield and mineral nutrition. Their association makes it possible to supplement the nutrition of the plant in limiting elements (Diallo et al., 2010). Mycorhization of the potato vitroplants is compatible with P fertilization (Ndiaye et al., 2003). AM fungi can be ranked as *Glomus aggregatum*, *Glomus mosseae, Glomus versiforme* for improving yield as well as nitrogen, phosphorus, and potassium acquisition of *Solanum* cultivar (Diop et al., 2003). However, little information is available on the interactions between AM fungi with organic fertilization based on litter. Understanding this compatible is a trump to potatoes production.

The aim of this study was to investigate the effect of dual inoculation with fungi AM and litter on two varieties of micropropagated potatoes in greenhouse.

MATERIALS AND METHODS

Soil

Soil used in this study was collected at 5 to 20 cm depth from Sangalkam, (50 km from Dakar, Senegal). Soil was sterilized by autoclaving at 12°C for 1 h. Soil characteristics are given in Table 1.

The plant material

The plant material consisted of potato tubers of two varieties (*S. tuberosum*), Atlas and Aida imported from GERMICOPA SA. (Quimper, France). These varieties are well adapted to agroclimatic conditions of Senegal. Dormancy was removed by chemical treatment (Bryan, 1989). Tubers were removed from the solution, dried and placed in a sealed chamber, dark and airy at 25°C until germination. After sprouting, the germs of 1 to 2 cm in height were gently lifted tubers using a sterile scalpel and closed at their ends by dipping in a bath of liquid paraffin at 40°C. Disinfection of germs was carried out in a host of laminar airflow. First, they were immersed in distilled water with 20 drops of Tween 80 for 10 min water and then pre-soaked for 10 s in alcohol at 70°C before putting them in a solution of mercuric chloride ($HgCl_2$) to 0.1% for 10 min. After disinfection, germs wiped and recovered, were sterilized on Whatman paper. Briefly, germs were placed in sterile culture glass tubes (22 × 150 mm) filled with 15 ml of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) while respecting the apical-basal polarity original. This medium solidified with agar (8 $g.l^{-1}$) was devoid of growth regulators and adjusted to pH 5.9 before sterilization (120°C for 30 min). The culture tubes were placed in a growth chamber (temperature 28°C, 16 h day photoperiod, light intensity 101.4 $\mu moles.m^{-2}.s^{-1}$, relative humidity 55%). After 4 weeks of culture, the explants formed, were cut into as many nodal cutting (3 to 7) and transplanted in jars with a capacity of 660 ml. Each jar contained 50 ml of MS medium. Older plantlets (10 days) and measuring approximately 7 cm were used as plant material (Figure 1).

Mycorrhizal inoculum

Mycorrhizal inoculum containing indigenous species of *G. aggregatum* was obtained from Laboratory of Fungal Biotechnologies (LBC) of the Plant Biology Department (University Cheikh Anta Diop / Senegal) was multiplied by using maize as host plant. Mycorrhizal inoculum consisted of rhizospheric soil mixture from pure culture containing spores, hyphae and mycorrhizal root fragments (an average of 40 spores per gram and 85% of roots infected) were used for the experiment.

Litter

Two trees litter were selected: *A. indica* A Juss (*Meliaceae*) and *H. binata* Roxb (*Fabaceae*). This tree species were chosen based on their availability and their leaves have good anti-microbial activity. Also, both trees are considered to be adaptable to diverse habitats and climatic conditions. Their leaves were taken when they fall between April and June. The leaves were immersed in 15% hydrogen peroxide solution for 90 s to reduce bacteria and fungi without phytotoxicity and dried in the host open air. Two weeks after, they were crushed to 0.75 mm fraction to accelerate decomposition upon contact with the ground and then stored in bags (Recous et al., 1995). Plant residues (carbon, nitrogen, hemicellulose, cellulose and lignin) were determined by the method of Van Soest (1963). The physical-chemical characteristics of litter used are given in the Table 2.

Experimental procedure

This study was conducted in a greenhouse of the department of Plant Biology (Cheikh Anta Diop University/Senegal) during 3 months. Soil was sterilized by autoclaving at 120°C for 1 h. All underwent incubation for seven months to accelerate the process of mineralization. Potato plant of two varieties, Aida and Atlas, were transplanted to the plastic pot with the same soil (5 kg). Each pot contained one plant. During this process, plants were inoculated with AM fungi *G. aggreegatum* by adding 20 g of inoculum directly in contact with the roots. Pot was laid in randomized block with ten replicates. Three factors were studied: (i) inoculation; (ii) litter and (iii) variety. Plants were grown in greenhouse with the following conditions: day/night cycle of 12/12 h, 32/25°C and 40 to 50% air humidity. Plants were irrigated with tap water.

Measured parameters

After sowing, roots and shoots were harvested separately. Fresh roots were taken to evaluate root colonization. Roots were first cleared with 10% KOH at 50°C for 60 min and then stained with 0.05% Trypan Blue at 50°C (Philips and Hayman, 1970). Mycorrhizal colonization (MC) root infection was evaluated under a

Figure 1. Older plantlets of potatoes Atlas on MS (Murashige and Skoog) medium 10 days in culture chamber (temperature of 28°C; 16 h day photoperiod, light intensity of 101.4 µmoles.m^{-2}.$s^{-1}$and relative humidity of 55%).

Table 2. Physical-chemical characteristics of leaves litter *Azadirachta indica* and *Hardwickia binata* used in the study.

Litter	Lignin (%)	Cellulose (%)	Hemicellulose (%)	C (mg/g)	N (mg/g)	C/N
Azadirachta indica	22	20.1	11	440.6	14.5	30.3
Hardwickia binata	18.3	17.6	11.3	424.1	21.0	20.2

binocular microscope by grid-line intersect method according to Giovannetti and Mosse (1980). The shoot dry weight (SDW) was measured after oven-drying at 75°C for 72 h. The minitubers were counted and means of number of minitubers per plant (NM) were calculated. The average size of minitubers (SM) per plant was measured using a caliper. Analysis of variance was carried out with the software XLSTAT (version 13.2). The comparisons between the averages were done using the software by Fischer LSD test at 5%.

RESULTS

Litter effect on plant development

A statistical analysis performed shows that the litter has a significant effect on biomass plants potato mycorrhizal (R^2 = 0.96, p <0.001). For both varieties, the contribution of litter (*H. binata* or *A. indica*) increased significant shoot dry weight. The application of litter *H. binata* at a dose of 50 g, provides the greatest biomass (1654.00 mg) in the mycorrhiza Atlas range (Table 2).

Litter effect on yield

The litter input causes a significant increase in the number of minitubers products by mycorrhized potato seedlings. Litter *H. binata* gave the largest number of minitubers (3.83 to 25 g) (Table 3). In plants both

Table 3. Shoot dry weight (SDW), Number of minitubers of plant (NM) and Size minitubers (SM) of plant potato (Atlas, Aida) during three months under different treatment of litter (*Azadirachta indica, Hardwickia binata*) inoculed or not with *Glomus aggregatum*.

Inoculation Treatment	Litter treatment (g)	Atlas			Aida		
		SDW (mg)	NM	SM (cm)	SDW (mg)	NM	SM (cm)
M+	Control	661^{e}	2.10^{d}	2.14^{c}	220^{g}	2.06^{d}	1.53^{def}
	Hb25	698^{d}	2.80^{a}	3.39^{a}	610^{b}	3.83^{a}	1.54^{de}
	Hb50	1654^{a}	2.50^{b}	3.41^{a}	960^{a}	2.87^{b}	1.82^{cd}
	Ai25	758^{c}	2.75^{a}	2.45^{b}	412^{d}	2.50^{c}	2.80^{b}
	Ai50	1584^{b}	2.30^{c}	2.40^{b}	541^{c}	2.87^{b}	3.17^{a}
M-	Control	418^{h}	1.30^{f}	1.20^{g}	194^{h}	1.13^{e}	1.04^{g}
	Hb25	470^{g}	1.41^{f}	1.33^{e}	204^{h}	1.41^{e}	1.18^{fg}
	Hb50	475^{g}	1.45^{f}	1.35^{de}	195^{h}	1.41^{e}	1.45^{fef}
	Ai25	513^{f}	1.73^{e}	1.27^{f}	256^{f}	1.36^{ef}	1.67^{de}
	Ai50	515^{f}	1.65^{e}	1.37^{d}	277^{e}	1.53^{e}	2.02^{c}

In column, values followed by the same letters are not significantly different (Fischer's protected LSD P < 0.05). M+ = Inoculated with *Glomus aggregatu;* M- = non inoculated with *Glomus aggregatum.* Hb25=*Hardwickia binata* 25 g litter; Hb50=*Hardwickia binata* 50 g; Ai25= *Azadirachta indica* 25 g litter; Ai50=*Azadirachta indica* 50 g l.

Table 4. Root colonization mycorrhizal rate of potato plants (Aida, Atlas) during three months at different litter addition levels of *Azadirachta indica* and *Hardwickia binata*.

Litter treatment	Inoculation treatment	Mycorrhizal colonization (%)	
		Aida	Atlas
Control	M-	0	0
Hb0	M+	21.66^{a}	23.33^{b}
Hb25	M+	13.33^{c}	14.66^{c}
Hb50	M+	08.00^{d}	08.00^{f}
Ai0	M+	16.27^{b}	24.16^{a}
Ai25	M+	09.33^{d}	13.50^{d}
Ai50	M+	07.33^{d}	08.83^{e}

In column, values followed by the same letters are not significantly different (Fischer's protected LSD P < 0.05). M+ = Inoculated with *Glomus aggregatum;* M- = non inoculated with *Glomus aggregatum.* Hb25=*Hardwickia binata* 25 g litter; Hb50=*Hardwickia binata* 50 g; Ai25= *Azadirachta indica* 25 g litter; Ai50=*Azadirachta indica* 50 g litter.

varieties, the average size of minitubers increased when the amount of litter *H. binata* increased. With the addition of litter A. *indica*, only the size of the Atlas minitubers variety improved with the increase of litter (Table 3).

Litter effect on mycorrhizal colonization

A mycorrhizal root colonization was influenced by the amount of litter made. It gradually decreased with increasing amount of litter. The litter input inhibits root colonization of potato by AM fungi *G. aggregatum* (Table 4). Control plants have obviously not been colonized by *G. aggregatum.* Analysis of variance showed a significant interaction between inoculation litter and variety (Table 5).

DISCUSSION

The litter has a stimulatory effect on the biomass, the number and size of minitubers. This stimulation of growth can be explained by a greater availability of minerals. Indeed, litter stimulates the activity and diversity of soil microorganisms (Shiralipour et al., 1992; Carpenter-Boggs et al., 2000). In turn, this microbial community degrades organic matter and release mineral elements to promote the development of plants (Samba, 2001; Diallo et al., 2005). Although, both bacteria and fungi contribute to litter decomposition, fungi are thought to use available C substrates more efficiently than bacteria. In our experimentation, this is the *G. aggregatum* that ensure this role, AM fungi may be involved both in decomposition processes and in the capture of the less mobile amino-

Table 5. Effects of different factors interactions on variables based on analysis of variance; root mycorrhizal colonization (MC), shoot dry weight (SDW), size minitubers (SM) and number of minitubers of plant (NM).

Factor	MC	SDW	SM	NM
R^2	0.967	0.999	0.935	0.941
F	152.465	6111.385	75.008	83.381
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Variety*G. *aggregatum**litter	152.465 < 0.0001	6111.385 < 0.0001	75.008 < 0.0001	83.381 < 0.0001

acids or ammonium ions. AM fungi hyphal were shown to facilitate enhancement of N capture from the litter, the N gain in the plants being linearly related to hyphal density in the organic matter (Read and Moreno, 2003). Both litters used also have a positive impact on the performance of potato mycorrhiza earth. However, that stimulatory effect of litter *H. binata* is more significant than *A. indica*. The same trend was obtained by Diallo et al. (2008) on the growth of maize and millet. This can be explained by the higher C/N ratio in *A. indica* than *H. binata.* In fact, when the nitrogen in the soil is low, microorganisms assimilation of soil mineral nitrogen reduces and crop yields decline. So, the nature of the litter determines its efficiency, due to the variable biochemical characteristics of plant residues (Williams, 1974) which interesting correlates to the anti-microbial activity of both the trees with the arbuscular mycorrhizal fungi.

Micropropagated potato plants can benefit from inoculation with AM fungi (Vosátka et al., 2000). These finding are also supported by our recent observations. Our results have showed that, the litter inhibits mycorrhizal roots of potato at 25 g. This inhibition increases with the amount of litter made. Impact is more significant at Atlas with litter *A. indica*. Several authors demonstrated that the mycorrhizal colonization can decrease in fertile medium (Duke et al., 1994). The release of soluble sugars in the decomposition of lignin litter is another nutrient for the fungus. This can happen for carbon plant and directly meet the needs of sugars from the litter. Thus, the fungi increasing decomposition of litter and the total P uptake by the plant is as important as when the mycorrhizal contribution was supplied with P in organic form (Read and Moreno, 2003).

We found that the decline of mycorrhiza is a concomitant expression of good performance metrics of the potato. This once again confirms that the benefits of mycorrhizae are not always related to a more intense roots colonization inside (Plenchette et al., 1982). These benefits may be explained by a better decomposition of organic matter or a good viability inoculation of *G. aggregatum* (Schädler et al., 2010). Inoculation of micropropagation potato plants with AM fungi during the transfer from *in vitro* conditions may improve the viability of potato and their physiological state (McArthur and Knowles, 1993; Ndiaye et al., 2005). This viability may be increased by activity of antimicrobial of the litter of both trees. The addition of litter is compatible with potato mycorrhizae. However, to increase the potential of the inoculation in practical production of potato, it is necessary to consider the growth response of different potatoes varieties, as well as appropriate combination of litter and AM fungi. Also, biochemical tests will be determined to see nutritional contents of *S. tuberosum*.

Conclusion

Our experiment shows that the contribution of organic fertilizers based on litter *H. binata* or *A. indica* is compatible with a good expression of the mycorrhizal roots of potato obtained by micropropagation. This beneficial effect is a function of the dose and the nature of the litter made. *H. binata* litter has a significant positive impact on the biomass and size of minitubercules. The results of this study contribute to the understanding of the biological processes involved in litter decomposition with AM fungi.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEGEMENT

This work is a part of the author's thesis. The study was finance by FNRAA Senegal (contrat: 09/AP03M10202 J).

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