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Functional diversity of five species of arbuscular mycorhizal fungi in symbiosis with *Allium cepa* L.

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Abstract: *The ability of arbuscular mycorrhizal (AM) to improve plant growth is largely study but due to their large distribution across agro-ecolycals region in the world, more work still necessary to understand their functioning according to their origin, host plant and strain. Nursery experiments were conducted to study the response of Onion (Allium cepa L.) plant to the inoculation of five arbuscular mycorrhizal (AM) from Cameroon and England. Onion plants grown in pots in sterilized soil/sand substrate were inoculated with Glomus hoï, Glomus clarum, Glomus intraradices, Glomus sp. and Gigaspora margarita provided by the Department of Biological Sciences, University York, England and the Soil Microbiology Laboratory of the Biotechnology Centre, University of Yaoundé I Cameroon. A total of 90 pots made up with 5 pots in 3 replications each corresponding to each AM strains and the control were range in green house condition and watered every day with sterilized water until harvest. Samples of root and shoot were collected for root lengh, nitrogen, phosphorus, plant biomass measurement and acid phosphatase activity determination. Data were analyzed using SAS system for differences between means and correlation between parameters. Results indicate that G. clarum, G. intraradices and G. hoï increased the root length and stimulated the acid phosphatase activity of roots from 62 to 86% as compared to the control without inoculation. All the fungi species enhanced the root surface from 70 to 118% over the control. G. clarum improved nitrogen and phosphate uptake by 35 and 290 mg/plant respectively, and thus, enhanced the dry weight of onion bulbs by 83% compared to uninoculated treatment at 90 days after planting. Acid phosphatases and root length are indicated parameters for such study.*

Keywords: *Functional diversity, Arbuscular Mycorrhiza Fungi, Allium cepa, symbiosis*

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I. Introduction

Arbuscular mycorrhiza (AM) fungi form a mutual association called mycorrhiza with 80% of the terrestrial plant families and have a major influence on plant growth, diversity and health [2, 4, 7, 11, 26]. These fungi form essential components of sustainable soil-plant systems and improve crop growth and productivity [32, 3, 12, 27] .AMF deliver mineral nutrients to the host plant and obtain in return reduced carbon compounds derived from plant photosynthesis [8]. AMF are obligate symbionts of plants, and they can't yet be cultivated in absence of a host plant [28]. In the absence of a living host root, the growth and development of AMF is very limited [33]. The majority of cultivated food crops establish a symbiosis with mycorrhiza, which can be exploited to the benefit of agriculture [1, 15, 17].

AMF improve plants uptake of nutrients such as P, Zn, and Cu that are strongly sorbed on soil particles [28, 34, 35, 36, 37, 38]. AMF were also known to enhance N acquisition by the plant [39, 40, 41]. Among those nutrients, improvement of P nutrition of plants is the most recognized beneficial effect of AMF. These fungi can physically explore the soil with hyphal network finer than roots to access inorganic and organic P sources that are unavailable to non-mycorrhizal plants [42, 43, 44]. In particular, it is believed that plants with limited root hair development, such as cassava are generally dependent on AMF for P nutrition under all soil conditions [45]. Scientifics are still questioning whether AMF have any important effect on the solubilisation of nutrients bound in sparingly soluble mineral or organic forms [28, 46]. Although phosphatases, an enzyme involved in the catalysis of phosphate complex to the free phosphorus have been recently found to be exuded by axenically

growing mycelium of *Glomus intraradices* [47], it is not known whether this plays any important role in unsterile soil. The improved uptake of P from sparingly soluble forms might be rather due to the interactions of AMF with other soil microbiota (e.g P-solubilising bacteria [48, 49].

Considering the fact that AMF cannot be cultivated without a host plant, it is preferably important to study them in association with a host plant with strong dependence, in order to understand the mechanisms by which this symbiosis is established. Therefore, onion (*Allium cepa* L.) that occupies the second position among the vegetable in terms of production after tomato [50], was selected as host plant for the characterisation of some AMF isolates. In Cameroon, 80% of onion production is located in the Far North province where averagely 70000 tons are being yielded yearly [31], but is yet enough to satisfy the population needs. This study was conducted to study the influence of five AM strain on the growth of onion (*Allium cepa* L.) at different periods of time. We tested the infectivity of the five AM, their influence on plant biomass production, bulbs yield, nitrogen and phosphorus uptake by the host plant. We finally study the influence of those AM on root length and acid phosphatase activity.

II. Materials and Methods

II.1. Substrate preparation and characteristics

Soil was collected from the top layer (0–15 cm) in the campus nursery of the Faculty of Science, University of Yaoundé 1 in Cameroon, sieved with the 5 mm diameter sieve and mixed with sand following a ratio of 3:1(V/V) respectively. The mixture was steam-sterilised twice at 100°C for 45 min and used to fill plastic at the rate of 1.5 Kg per pot. Basic physical and chemical properties of the mixture were as follows: pH (H_20), 6.1; organic mater, 17.2 $g.kg^{-1}$; organic carbon, 10 $g.kg^{-1}$; nitrogen, 0.8 $g.kg^{-1}$; available P, 10 $mg.kg^{-1}$; CEC, 5.11 $cmol.kg^{-1}$; Ca, 5.01, Mg, 0.32, K, 0.20, Na, 0.07 $cmol.kg^{-1}$.

II.2. Plant and AM material

Onion bulbs used in this survey (Sturon variety) was originated from England, with diameter range of 15-22 mm

Glomus hoi inoculum was provided by the Department of Biological Sciences, University York, England and the *Glomus clarum, Glomus intraradices, Glomus aggregatum* and *Gigaspora margarita* isolates was provides by the Soils Microbiology Laboratory of the Biotechnology Centre of the University of Yaoundé 1.

II.3. Treatments and growth conditions

The experiment was carried out in a greenhouse of the Biotechnology Center of University of Yaoundé I. The experiment was a complete factorial design which was comprised of six treatments (five AMF and the control) with three replicates for each treatment. Each treatment was a pool of 5 pots with 3 replications containing one plant per pot. Inoculation with AMF consisted of layering each sowing hole (10 cm deep) at the middle of the pot with 10g of soil containing 100 spores of each of the AMF species per pot. After inoculation one onion bulb was put in the each pot, close to the AMF inoculums. The sowing holes of the control treatment received 10g of sterilized soil. In all, ninety pots were arranged in a randomized complete block design were obtained. All pots were well watered and Hoagland's nutrient solution[10] was applied every three weeks throughout the growth period of plants.

II.4. Evaluation of studied parameters

Root colonisation was estimated at 15, 30, 60 and 90 days after planting (DAP) using the method described by Kormanik and Mc Graw, 1982 [13]. At 30, 60 and 90 DAP, plants were harvested and oven dried at 70°C during 48 hours for the evaluation of plant biomass. 1g of each roots harvested at the same dates within the same periods were scanned with a UMAX 2000 apparatus and the pictures treated with a DTSCAN software. Data on the root length system were thus obtained by calculation for root biomass.

The measurement of the total activity of acid phosphatases in fresh roots at 30, 60 and 90 DAP was obtained according to Tabatabaï and Juma, 1988 [29].

The total phosphorus and nitrogen of the whole plant harvested at 30, 60 and 90 DAP were measured respectively by the method of Okalebo and *al*., 1993 [21] and Devani and al., 1989 [5].

II.5. Statistical analysis

Data were statistically analysed using SAS computer software. Analysis performed were comparison of means using the least significant difference (LSD), after the analysis of variance (ANOVA). The correlations between parameters wee also performed using the same program.

III. Results

III.1. Root colonisation by AM fungi

The analyses of figure 1 show that 20% of roots were colonised by *Glomus intraradices* and *Glomus hoï* at 15 days after planting (DAP). This root colonization increased to 35% at 30 DAP and remained always above that of the other isolates. There was an important activity in *Glomus clarum* and *Glomus aggregatum* at 30 and 60 DAP with 65% of roots colonized at 60 DAP. The root colonization in plants inoculated with *Glomus clarum*, *Glomus intraradices* and *Glomus aggregatum* was 70% and 50% in inoculated roots plants with *Gigaspora margarita* and *Glomus hoï* at 90 DAP. At this stage two groups of isolates from their rate of root colonisation were distinguished: the fast root colonizing (*Glomus clarum*, *Glomus aggregatum* and *Glomus intraradices*), and the slow root colonizing isolates (*Glomus hoï* and *Gigaspora margarita*).

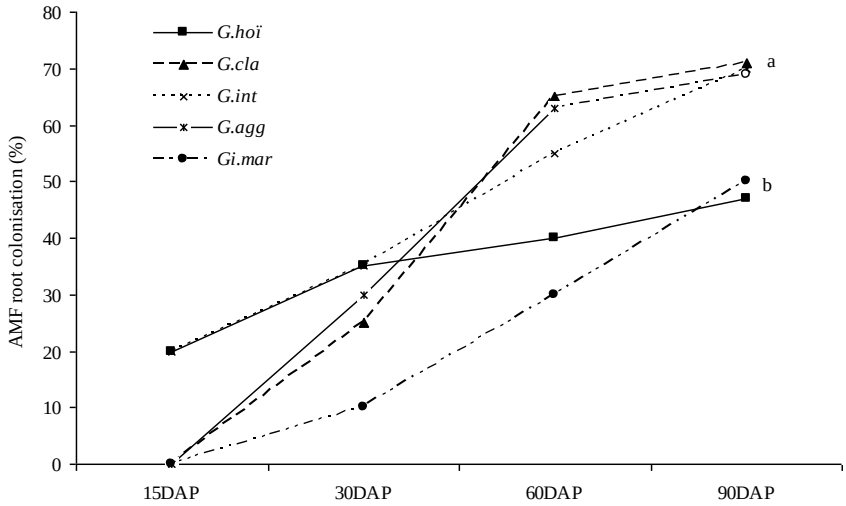

Figure 1. Root colonization of *Allium cepa* by different AMF species at 15, 30, 60 and 90 day after planting

III.2. Evolution of root length

The results obtained from figure 2 reveal a progressive effect of mycorhization on the roots growth. At 15 DAP, uninoculated plants showed a more developed root system (19.9 dm) than that of inoculated plants with *Glomus hoï, Glomus aggregatum* and *Gigaspora margarita*. *Glomus hoï* isolates were more active with 79 and 67 % increased root length compared to the control, respectively at 30 and 60 DAP. At 90 DAP, *Glomus hoï* remained more active with 190 % root length growth improvement compared to 80 % for other *Glomus* species.

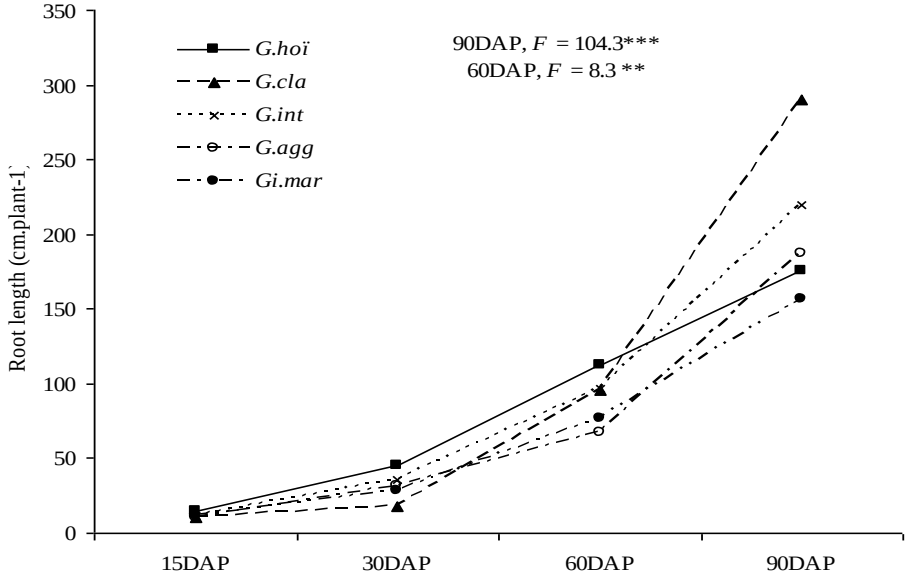

Figure 2. Root length of *Allium cepa* as influenced by different AMF species at 15, 30, 60 and 90 days after planting (DAP).

III.3. Acid phosphatases activity

The statistical analysis of data indicated that the root acid phosphatase activity of uninoculated plants at 15 DAP was significantly higher than that of inoculated plants (Table I). However, at 30 DAP the root acid phosphatase activity of uninoculated plants was instead significantly lower than that of AMF inoculated plants. This activity increased from 17 to 330 % compared to that of the control. At 60 DAP, the root acid phosphatase activity decreased in both inoculated and uninoculated plants, but remained significantly greater in the former. *Glomus hoï* and *Glomus clarum* were the most active isolates inducing acid phosphatase in plant root with respectively 86 and 62 % increases.

Table I. Root acid phosphatases activity induced by different AMF species in *Allium cepa* at 15, 30, 60 and 90 days after planting (DAP).

Treatments	acid phosphatase activity (µmole/mn/plant)		
	15DAP	30DAP	60DAP
Control	0.34a	0.28d	3.36d
Glomus hoï	0.31ab	1.20a	6.26a
Glomus clarum	0.25cd	0.62c	5.47b
Glomus intraradices	0.21d	0.59c	4.25c
Glomus aggregatum	0.27bc	0.33d	4.00c
Gigaspora margarita	0.11[e]	0.72b	3.95c
F value	*18.21*	*134,86*	*118.13*
Significance	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*
LSD (5%)	*0.05*	*0.08*	*0.30*

Means followed by the same letter within columns are not significantly different at 5%, (n = 3).

III.4. Phosphorus and nitrogen uptake

AMF had a positive and significant influence on plant phosphorus uptake at 30, 60 and 90 DAP (Table II). *Glomus clarum* was the most active isolate in this respect. It enabled inoculated plants to uptake phosphorus two, three and five times more than uninoculated plants respectively at 30, 60 and 90 DAP. There was no significant difference in the phosphorus uptake between the 30 and 60 DAP. However, from the 60 DAP, *Glomus clarum* was the most efficient isolate allowing absorption of 290 mg phosphorus/plant, against only 65 mg phosphorus uptaked/uninoculated plant. Inoculated plants with *Glomus intraradices*, *Glomus aggregatum* and *Gigaspora margarita* took up more than 100 mg phosphorus/plant, while *Glomus hoï* allowed the host plant to accumulate less than 100 mg phosphorus/plant.

Similarly, AMF isolates allowed inoculated plants to quickly uptake nitrogen more than uninoculated plants from 30 to 60 DAP (Table II). *Glomus clarum* inoculated plant uptook nitrogen 14 fold more than the control. At 90 DAP, the nitrogen accumulation in *Glomus clarum* and *Glomus aggregatum* inoculated plants increased by 74 % compared to that of the control. This increment varied from 10 to 27 % with other AMF isolates. From 30 to 90 DAP, plants inoculated with *Glomus clarum* and *Glomus hoï* had the same nitrogen absorption rate. *Glomus aggregatum* was very active in stimulating nitrogen accumulation in plants from 60 to 90 DAP.

Table II. Shoot phosphorus uptake induced by different AMF species in *Allium cepa* at 15, 30, 60 and 90 days after planting (DAP).

Treatments	Phosphorus uptake (mg/plant).		
	30DAP	60DAP	90DAP
Control	0.23d	0.85c	3.19e
Glomus hoï	0.54c	1.20c	5.56d
Glomus clarum	1.85a	3.17a	14.47a
Glomus intraradices	0.23d	2.80a	8.58b
Glomus aggregatum	1.02b	2.32b	7.14c
Gigaspora margarita	0.63c	2.95a	4.52[e]
F value	86.43	49.13	532.0
Significance	*P* < 0.0001	*P* < 0.0001	*P* < 0.0001
LSD (5%)	0.20	0.42	0.53

Means followed by the same letter within columns are not significantly different at 5%, (n = 3).

Table III. Shoot nitrogen uptake induced by different AMF species in *Allium cepa* at 15, 30, 60 and 90 days after planting (DAP) (mg/plant)

Treatments	Nitrogen uptake (mg/plant)		
	30DAP	60DAP	90DAP
Control	1.1c	10.6c	20.0c
Glomus hoï	4.3b	26.3a	29.6b
Glomus clarum	14.0a	16.0b	25.0bc
Glomus intraradices	2.2c	10.2c	22.7c
Glomus aggregatum	2.0c	12.7bc	39.5a

Gigaspora margarita	5.2b	14.0bc	25.0bc
F value	82.43	21.05	17.03
Significance	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
LSD (5%)	1.6	3.9	5.15

Means followed by the same letter within columns are not significantly different at 5%, (n = 3).

III.5. Biomass production

The effect of AMF isolates on *Allium cepa* biomass production was significant (Table IV). At 30 DAP, the plant biomass accounted by *Glomus clarum* was increased by 83 %, followed by *Glomus intraradices* and *Gigaspora margarita* (66 %). In contrast, the biomass of plants inoculated with *Glomus hoï* and *Glomus aggregatum* was slightly lower than that of the control. Between 30 and 60 DAP, no significant difference was observed between the biomass of inoculated and uninoculated plants. Differences between treatments re-appeared at 90 DAP with *Glomus clarum* enhancing the plant biomass by 79 % (9.4 mg/plant) compared to the control. Here, again the difference between plants inoculated with *Glomus margarita*, and uninoculated plants was not significant ($p > 0.05$).

Table IV. Shoot dry weight induced by different AMF species in *Allium cepa* at 15, 30, 60 and 90 days after planting (DAP)

Treatments	Shoot dry weight (mg/plant)		
	30DAP	60DAP	90DAP
Control	0.6bc	2.1ab	5.3c
Glomus hoï	0.4c	1.5b	7.1bc
Glomus clarum	1.1a	2.2ab	12.4a
Glomus intraradices	1.0ab	2.5a	10.8a
Glomus aggregatum	0.5c	1.8ab	8.3b
Gigaspora margarita	0.7abc	2.0ab	7.5bc
F value	4.32	1.19	12.55
Significance	$P < 0.0176$	$P < 0.036$	$P < 0.0002$
LSD (5%)	0.41	0.98	2.25

Means followed by the same letter within columns are not significantly different at 5%, (n = 3).

IV. Discussions

Considering their high infective potential, *Allium cepa* L roots are quickly and highly colonized by *Glomus clarum*, *Glomus intraradices* and *Glomus intraradices*, *Glomus hoï* and *Gigaspora margarita* slowly and weakly colonize roots. The symbiosis was soon, establish between *Glomus hoï* ,*Glomus intraradices* and *Allium cepa* L. (less than 15 days after planting) while other isolates get symbiosis with the host plant 15 DAP. Talking about the competition in AMF species, [1] have suggested that the first species to colonize the roots of the host plant presents a substantial advantage on other competitors. It is thus possible that the two isolates *Glomus hoï* and *Glomus intraradices*, which start earlier the symbiosis with *Allium cepa* L. are more competitive than others. As soon as the symbiosis is established, the root length of inoculated plants increased for all the treatments. This difference in root length increment between inoculated and uninoculated plants could be explained by the ability of isolates to form extra-root hyphae or to synthesize growth hormones. In other words, *Glomus hoï* might have stimulated more mechanisms involve in plant development than other. Tawaraya and *al.*, 2001 [30] have also observed an enhanced root length in inoculated plants of *Allium fistulosum* with *Glomus fasciculatum* than in uninoculated control. One of the most direct consequences of this improvement of root length system in inoculated plants is the enhancement of the nutrients absorption area as reported by [24, 23]. This increment of the nutrient absorption area is related to the fact that mycorrhizae form external hyphae in some host species that develop beyond the exploration zone of roots. There was a highly significant correlation between the mycorrhizal root colonization and the root length system ($r = 0.81$; $p < 0.0001$) on one hand and the root surface area ($r = 0.69$; $p < 0.0001$) on the other. This observation support the high relationship between those parameters and their potential simultaneous action in plants root especially *Allium* species for growth.

Phosphatases are enzymes that catalyses the liberation of the inorganic phosphate ions from the soil. The root acid phosphatase activity of *Allium cepa* L. was significantly increased in AMF-inoculated than in the control. The greatest acid phosphatase activity was recorded in *Glomus hoï*. The acid phosphatase activity induced by mycorrhizae was reported to be considered as a marquer for the evaluation of the efficacy of the symbiosis [52, 53]. A positive and significant correlation was observed between the root acid phosphatase activity and the mycorrhizal root colonization ($r = 0.67$; $p < 0.0001$), the root length system ($r = 0.75$; $p < 0.0001$), and the root surface area ($r = 0.82$; $p < 0.0001$).

inoculated with *Glomus clarum* absorbed more phosphorus than those inoculated with other AMF isolates at 90 DAP, indicating a mycorrhizal contribution to the assimilation of this nutrient. These results are in conformity with those of [9, 16], who obtained 50 and 230 % increment of phosphorus in *Allium cepa* L. Similarly, Ngonkeu, 2003 [18] have reported 27 fold increased phosphorus in *Allium* inoculated with *Glomus*

intraradices compared to uninoculated plants grown on poor substratum. Other studies show increase in P uptake following AMF inoculation [14, 30]. The fact that our plants were cultured in pots may explain the low phosphorus absorption by *Glomus hoï* inoculated plants despite their high root acid phosphatase activity and root length system.

Glomus clarum and *Glomus intraradices* were more efficient in improving nitrogen absorption in inoculated *Allium cepa* L. The abilities of *Glomus clarum*, *Glomus intraradices* and *Gigaspora margarita* to colonize early the root, enabled them to induce an effect of 4 – 79% on plant biomass at 90 DAP. Almost 500% of enhanced growth was obtained in *Allium* inoculated with *Glomus caledonium* and *Glomus epigaeus* [23], against 235 % to 176 % on the same plant inoculated with *Glomus clarum* and *Glomus intraradices* [18], compared to 600 % obtained by [22] in inoculated *Allium cepa* L. Van Aarle et *al.,* 2002 [53] think that the higher the root acid phosphatase activity, the higher the phosphorus content in plant tissues, and the higher the plant biomass of AMF-inoculated plants. A high and positive correlation between the mycorrhizal root colonization, the root length system, the root acid phosphatase activity and the root surface area is a clear indication of the potential of these AMF isolates on *Allium cepa* L production.

V. Conclusion

The results obtained from this study suggest that *Glomus intraradices* produce more spores than others, and with *Glomus clarum* having the most elevated infective propagules. *Glomus clarum, Glomus* sp. and *Gigaspora margarita* highly colonized *Allium cepa* L roots. All the isolates considerably stimulated the root acid phosphatase activity, enhanced the root length system with the high performance attributed to *Glomus hoi* and *Glomus clarum* respectively. The plant biomass was also improved by all the AMF isolates with the predominance of *Glomus clarum* isolate. In general, *Glomus clarum-Allium cepa*-symbiosis presented the best functional characteristics and may be recommended for improved *Allium cepa* growth and production. Repeated experiments in field conditions under different soil types are requested to give more support to this useful mutual inter-relationship.

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