Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 12, No. 5, p. 1-15, 2018
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

Effect of arbuscular mycorrhizal fungi on the dynamics of hydrogen peroxide, the activities of catalase, ascorbate peroxidase and Guaïcol peroxidase in *Xanthosoma sagittifolium* L. Schott rhizome and root during growth

Djeuani Astride Carole[1,2*], Mbouobda Hermann Désiré[1,3], Omokolo Ndoumou Denis [1]

[1]*Department of Biological Sciences, University of Yaoundé I, Yaoundé, Cameroon*

[2]*Department of Plant Biology, University of Yaoundé I, Yaoundé, Cameroon*

[3]*Department of Biology, University of Bamenda, Bamenda, Cameroon*

Article published on May 13, 2018

Key words: Arbuscular mycorrhizal fungi, Growth, Catalase, Ascorbate peroxidase, Guaïcol peroxidase.

Abstract

The present study was conducted to determine the effects of four Arbuscular mycorrhizal fungi (AMF); *Glomus intraradices*, *Glomus* sp., *Gigaspora margarita* and *Acaulospora tuberculata* on the growth of *X. sagittifolium* (white cultivar) and stress enzyme expression. To evaluate the effect of that AMF on plant growth of the basal part of plant, the length (except rhizome), the fresh weight and the dry weight matter of roots and the rhizome were determine every 60 days for 180 days. The following experimental conditions were used *X. sagittifolium* + AMF and *X. sagittifolium* + AMF + Carbon source. The results obtained show that, mycorrhization significantly ($P<0.05$) affected the development of the basal part of the plant with increased length of the roots and mass of rhizome compared to the control. *Glomus* sp. and *G. intraradices* stimulated both the dry weight increase of roots and rhizomes respectively by 48 and 72% then 27 and 23.5% at day 180. *Glomus* sp. significantly stimulate the increased expression of hydrogen peroxide in rhizomes and roots in mycorrhizal *X. sagittifolium* plants. In presence of carbon source, significant values is 20.70 ± 1.38 $mM.min^{-1}.g^{-1}$ of FW obtained in roots of mycorrhizal plants with *A. tuberculata*. It was observed that addition of the carbon source significantly increased stress enzyme expression, with catalase been the most express enzyme compare to peroxidases. We conclude that the use of mycorrhizae in farming of *X. sagittifolium* may hold advantage of increasing the production and resistance against root rot disease.

***Corresponding Author:** Djeuani Astride Carole ✉ djeuani.uyi@hotmail.com

Introduction

Cocoyam (*Xanthosoma sagittifolium* L. Schott), is a tuberous herbaceous plant. Its nutrient richness covers almost all energy needs and up to 60% of the protein requirements of an adult (Sefa-dedeh *et al.,* 2004). It's well recognized as one of the starchy tubers ranked among the worlds largest.

In Cameroon *X. sagittifolium* is consumed in different forms depending on the locality and can be used as food for domestic animals such as pork because of its leaves and main tuber which are very fibrous for human consumption (Rodriguez *et al.*, 2009).

Anyway, (Englberger *et al.*, 2008) showed that the leaves of *X. sagittifolium* have great potential in the fight against hypertension, obesity, and diabetes because of their antioxidant properties. Njoku and Ohia (2007), also demonstrate that *X. sagittifolium* tuber consumption can contribute to the regulation of the acid-base balance of the human body because *X. sagittifolium* is the source of minerals: sodium (Na), calcium (Ca), magnesium (Mg) and potassium K). But despite these importance, increasingly, the decline in growth, production and the scarcity of healthy seeds have been attributed in *X. sagittifolium* to the presence of *Pythium myriotylum* (Tambong et Höfte, 2001), in the soil. Strategies are increasingly being addressed to combat this pathogen. Admittedly, Mbouobda (2011) and Djeuani (2009) have simultaneously shown that during the *X. sagittifolium* - *Pythium myriotylum* interaction, the accumulation of H_2O_2 significantly modifies the CAT, APX and G-POD activities through the application of Chitosan and Benzothiadiazole.

They reported that, if the antioxidant enzymes activities are high, more the defense mechanism is induced. Antioxidant enzymes play an important role in the growth and development of plants. In *X. sagittifolium*, Kamegne and Omokolo (2002) suggests that peroxidases are involved in organogenesis. Similarly, Tsafack 2010, noted the appearance of new isoforms of peroxidases which may play an important role during the process of microtuberization.

Several studies show the importance of oxidative enzymes in the development and defense process in plants. The involvement of antioxidant enzymes in detoxification reactions at the cellular level is influenced by the variation in the hydrogen peroxide (H_2O_2) content produced (Laloi *et al.,* 2004). H_2O_2 is an important signaling compound involved in plant interaction with pathogenic microorganisms, as well as symbiotic organisms such as rhizobium (Matamoros *et al.,* 2013), mycorrhizae (Zhang *et al.*, 2013; Armada *et al.,* (2016), root elongation and root hairs lengthening (Kwak *et al.,* 2003, Liszkay *et al.,* 2004).

According to El-Enany *et al.,* (2013), antioxidant enzymes can contribute in amelioration of water deficit and waterlogging stresses on *Vigna sinensis* plants. Kumar *etal.,* (2014), Armada *et al.,* (2016), reports that, mycorrhizae play a significant role in improving and increasing the activity of these antioxidant enzymes. For Zhang *et al.* (2013), the accumulation of H_2O_2 in clover roots, in response to AM fungal colonization gradually decreased with plant growth.

Its stability and high diffusion in the cell compartments allow it to be considered as a signal molecule in the stimulation of compounds such as catalase (CAT) (Kumar *et al.,* 2014), ascorbate peroxidase (APX) (Spanu and Bonfante-Fasolo, 1988; Liu *et al.,* 2016), guaicol peroxidase (G-POD) (Blilou *et al.,* 2000; Qiang *et al.,* (2006), glutathione reductase (Tsai *et al.,* 2005), etc… In mycorrhizal tobacco roots with *Glomus mosseae*, Blilou *et al.,* (2000) approved that the intake of mycorrhiza can stimulated an increase in CAT concentration in the colonized roots compared to the control.

Induction of CAT activities have also been observed in nodules of soybean roots colonized by *G. mosseae*

(Porcel *et al.,* 2003). Salzer *et al.* (1999) suggested that the increase of CAT activity in colonized roots of *Medicago truncatula* during symbiosis of AMF and inoculated plants could be associated with the concentration of H_2O_2 observed in arbuscule-containing cells. However, like catalase, Apel and Hirt, (2004); Porcel and Ruiz-Lozano, (2004); Armada *etal.*, (2016) approved that, synthesis of peroxidases can also be influenced in plant during mycorrhization. Spanu & Bonfante-Fasolo (1988) has evaluated peroxidase activity in cell walls in garlic plants colonized by *Glomus versiforme*. According to Garg and Chandel (2015) and Liu *et al.* (2016), the contribution of AMFs in the plant during growth significantly altered the APX activities compared to the control under biotic and abiotic constraints. In the same way, Qiang *et al.* (2006) found a decrease in G-POD activity in the roots of *Citrus tangerine*, mycorrhizae with *Glomus versiforme* during water stress. Moreover, (Lucca Zanardo *et al.,* 2009), showed that G-POD play an important role in root development.

In fact, it is increasingly shown that during plant growth, these stress enzymes play an important role in the prevalence of immune defense in plants. In addition, mycorrhizae have an influence on these enzymes via their direct effect on the production of hydrogen peroxides due to the presence of arbuscules Armada *et al.,* (2016), and their potential to enhance the rhizospheric soil characteristics considerably thereby affecting plant growth (Navarro *et al.,* 2013; Ahanger *et al.,* 2014).

The use of mycorrhizae in this work will confirm whether their considerable contribution to the field would be of crucial importance in the fight against endemic fungi of *Xanthosoma sagittifolium*. But, in *X. sagittifolium*, the used of arbuscular mycorrhizal fungus (AMF) during growth remain only an innovation. That is why the effect of *Glomus intraradices, Glomus sp., Gigaspora margarita* and *Acaulospora tuberculata* is evaluated on the growth of *X. sagittifolium* L. Schott, especially on the dynamics of hydrogen peroxide, the activities of catalase, ascorbate peroxidase and Guaïcol peroxidase.

Material and methods

Plant material, methods of inoculation, growth and parameters measured

The plant material used were constituted by rhizomes and roots collected on young plants *X. sagittifolium* vitroplants obtained through *in vitro* culture of the apex of white cultivar according to Omokolo *et al.* (1995) modified and Djeuani *et al.* (2014). After one month of acclimation, the vitroplants obtained were used to carry out mycorrhization. Four strains of mycorrhizae were used; *Glomus intraradices*, *Glomus* sp., *Gigaspora margarita* and *Acaulospora tuberculata*. Two culture conditions were applied.

In the first condition of experiments, plants of *X. sagittifolium* were cultivated with the fourth mycorrhizae individually, while in the second group, it was plant of *X. sagittifolium* + arbuscular mycorrhizal fungi + carbon source. In each bag of polyethylene (20 x 17 x 65 mm), containing a mixture of black earth + sand (1:1), a crucible of 5 cm was produced in which 30 g of each AMF strain were introduced. After a slight watering, vitroplants were seeded in each pot. For each treatment, 70 plants of *X. sagittifolium* were used. To appreciate the effect of mycorrhizal on the cocoyam growth, 3 plantlets of cocoyam seedlings were chosen at random every 60 days until 180 days. Once sacrificed, the morphological parameters evaluated in roots were the length, the weight fresh and the weight dry matter, and in rhizome; fresh rhizome matter and dry rhizome matter. A portion of the rhizomes and roots at each sampling will be stored at 4 ° C for biochemical analyzes. This during days 60, 120 and 180 respectively.

Biochemical analysis

Hydrogen peroxide content

The Hydrogen peroxide (H_2O_2) content was determined according to the method described by

Velikova and Loreto (2005). The plant material (1 g of rhizome or root) was ground in a porcelain mortar in the presence of 2 ml of 0.1% Trichloroacetic acid (TCA). After 30 min incubation, the mixture was centrifuged at 5000 g for 20 min.

The supernatant obtained constituted the hydrogen peroxide extract. For the determination of hydrogen peroxide, 0.5 ml of the extract obtained was added to a reaction mixture composed of 0.5 ml of phosphate buffer (10 mM, pH 7) and 1 ml of potassium iodide (KI, 1M). Absorbance reading was performed on the spectrophotometer (JENWAY 6305) at 390 nm against a blank in which the enzyme extract was replaced by the extraction buffer.

The activity was expressed in $mM.min^{-1}.g^{-1}FW$. The extinction coefficient $\epsilon = 10000\ M^{-1}.cm^{-1}$. For each extract, three readings were made.

Activities of catalase

From catalase activities, the method used is that described by Athar *et al.* (2008). In each tube, 2 ml of 18% H_2O_2 (prepared in 0.1 M phosphate buffer pH 7) was introduced, then 25µl of protein extract to the reaction medium added.

The absorbance was read at the spectrophotometer (JENWAY 6305) at 240 nm after 1 min. For each enzymatic extract, three readings were carried out and the activity of the CAT was expressed in $mM.mn^{-1}g^{-1}$ FW (molar extinction coefficient of $\varepsilon = 36\ M^{-1}\ cm^{-1}$).

Activities of ascorbate peroxidase

The *ascorbate peroxidase* (APX) activity was determined according to the method of Karyotou and Donaldson (2007). In each test tube 2.5 ml of reaction medium (H_2O_2 0.2%, 0.25 mM ascorbic acid and Phosphate buffer 0.06 M pH 7) was introduced. 10µl of protein extract was added. Mixed with the Vortex.

The absorbance was readed on the spectrophotometer (JENWAY 6305) at 290 nm against a white in which the enzyme extract was replaced by the extraction buffer. The activity of APX is expressed in $mM.mn^{-1}.g^{-1}$ FW. Molar extinction coefficient of $\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$.

Activities of Guaïcol peroxidases

The Guaïcol peroxidase assay was carried out according to the method of Thorpe *et al.* (1978). The reaction medium consisted of: 1 volume of 0.2% H_2O_2, 2 volumes of 1% Guaïcol, 5 volumes of phosphate buffer, 1/15 M, pH 6.8. In each test tube, 5 mL of the reaction medium was introduced and 10 µl of extract.

The absorbance was readed at 420 nm to the spectrophotometer against a white in which the extract was replaced by the extraction buffer.

Three repetitions were made per sample. The peroxidase content was expressed in $mM.mn^{-1}.g^{-1}$ of FW from a molar extinction coefficient of peroxidases $\epsilon = 26.6\ M^{-1}\ cm^{-1}$.

Statistical analysis

Results were expressed in for of Means ± SD, analysis were done by MS excel. Duncan Multiple Range Test with the least significant difference of 5 % ($P<0.05$) were used to compared results following treatments over time with SPSS 20.0.

The correlation (of Spearman of rank R) between the different treatments applied for the studied parameters were carried out using the Software XLSTAT 2007.lnk Version 10.0. Principal component analysis (PCA) made it possible to present, in a two-dimensional plan, the arrangement of the parameters evaluated according to the treatments and this using the XLSTAT Software.lnk Version 10.0. In order to group plants treatment x data analysis.

Results

Effect of AMF used on X. sagittifolium roots during growth and mycorrhization

As shown in Table 1, there is considerable variation of the length, the dry and fresh biomass. Mycorrhization

significantly influenced root length in all mycorrhizal *X. sagittifolium* plants in the presence or absence of the carbon source.

This influence is highly significant at 5% between D_{120} and D_{180}. In D_{120}, a maximum average root size was recorded in plants of *X. sagittifolium* + *A. tuberculata* (18.06 ± 2.78 cm) and plants of *X. sagittifolium* + *A. tuberculata* + Source of carbon (19 15 ± 1,59cm). As for the average dry and fresh biomass of the roots, it is very important in presence of *Glomus* sp. However, concerning the average dry biomass in root, the results show that there is no significant difference between all the applied arbuscular mycorrhizal fungi treatments. In D120, there is a significant variation in fresh root biomass.

Table 1. Parameters collected on harvested roots during growth and mycorrhization time.

Parameters collected on harvested roots during time									
Treatments apply	Day 60			Day 120			Day 180		
	Length of roots (Cm)	Fresh root matter (g)	Dry root matter (g)	Length of roots (Cm)	Fresh root matter (g)	Dry root matter (g)	Length of roots (Cm)	Fresh root matter (g)	Dry root matter (g)
Control	14,18±1,01 a	1,61±0,07 ab	0,19±0,08 a	13,92±2,19 a	1,73±0,33 a	0,20±0,10 a	11,66±0,72 a	1,13±0,25 a	0,11±0,04 a
G. intraradices	16,65±3,14 ab	1,45±0,54 a	0,19±0,08 a	16,43±1,82 b	1,85±0,48 ab	0,22±0,14 a	12,91±0,29 b	1,85±0,87 ab	0,18±0,08 ab
Glomus sp.	15,89±3,63 ab	1,68±0,37 ab	0,20±0,07 a	17,27±1,75 b	3,07±0,92 cd	0,37±0,17 a	15,09±0,49 c	2,43±0,28 c	0,21±0,23 ab
Gi. margarita	16,78±2,93 b	2,11±0,51 bc	0,25±0,05 a	17,69±0,89 b	3,02±0,60 cd	0,36±0,27 a	17,66±1,02 d	2,13±0,40 b	0,16±0,16 ab
A. tuberculata	16,56±2,36 ab	1,80±0,23 abc	0,21±0,15 a	18,06±2,78 bc	1,99±0,45 ab	0,24±0,16 a	15,21±0,47 c	1,59±0,85 ab	0,18±0,14 ab
Mean	16,01	1,73	0,20	16,67	2,33	0,27	14,50	1,82	0,16
F value	3, 45	2,00	0,30	9,95	6,09	1,01	9,86	3,42	0,32
P	0,01	0,13	0,87	0,000	0,002	0,42	0,000	0,02	0,85
	(*)	(ns)	(ns)	(***)	(**)	(ns)	(***)	(*)	(ns)
Control + S	15,07±2,34 a	1,40±0,40 a	0,16±0,10 a	13,75±2,87 a	1,67±0,29 a	0,20±0,08 a	11,00±0,53 a	1,22±0,62 a	0,11±0,05 a
G. intraradices + S	16,01±2,29 a	1,54±0,31 ab	0,18±0,06 a	16,87±1,81 b	2,01±0,49 ab	0,24±0,26 a	13,63±0,52 b	2,58±0,86 b	0,25±0,13 ab
Glomus sp. + S	16,28±4,81 a	2,69±0,42 d	0,32±0,23 a	17,88±1,89 bc	3,33±0,39 cd	0,41±0,07 a	15,28±3,54 bc	2,70±0,52 c	0,39±0,18 b
Gi. margarita + S	17,12±3,54 a	2,14±0,53 bc	0,25±0,20 a	17,99±0,75 bc	3,24±0,56 cd	0,39±0,11 a	18,26±0,46 d	2,50±0,42 b	0,30±0,21 ab
A. tuberculata + S	16,02±3,02 a	1,98±0,31 abc	0,24±0,13 a	19,15±1,59 c	2,56±0,44 bc	0,30±0,13 a	16,42±2,96 cd	2,42±0,62 b	0,29±0,26 ab
Mean	16,10	1,95	0,23	17,12	2,56	0,30	14,91	2,28	0,26
F value	0,81	7,99	0,84	0,37	13,45	1,75	8,56	0,11	1,47
P	0,52	0,01	0,51	0,84	0,000	0,17	0,000	0,97	0,24
	(ns)	(*)	(ns)	(ns)	(***)	(ns)	(***)	(ns)	(ns)

ns: non-significant at the threshold of 5%; *: significant at the threshold of 5%; **: highly significant at the threshold of 5%; ***: very highly significant at the threshold of 5%. Data sharing the same letter in the same column were not significantly different at 5% level (Duncan's multiple range tests) *S: Carbon source.

Effect of AMF used on X. sagittifolium fresh and dry rhizome during growth and mycorrhization

In rhizomes, fresh and dry biomasses increased gradually over time. There is no significant difference (Duncan's multiple range tests at 5%) in the variation of the average fresh and dry biomass in rhizome of *X. sagittifolium* plants mycorrhized with *Glomus intraradices, Glomus sp., Gigaspora margarita* and *Acaulospora tuberculata* (Table 2). In general, *Glomus intraradices* shows the higher record of fresh and dry biomass. The increase in this fresh biomass leads to an increase in dry biomass. However, considerable and significant variation of fresh and dry biomass were recorded in *Glomus* sp. (9.47±1.24and 3.24±0.79 g) to the D_{60} and *G. intraradices* (19.25±2.72 and 7.82±1.93 g) to the D_{180}. Nevertheless, in the presence of the carbon source, there is a highly significant difference in the fresh biomass of *X. sagittifolium* rhizomes at D_{60} and D_{180}. Similarly, this fresh biomass is significant in *G. intraradices* compared to other treatments, with 9.92±0.63; 15.18±1.20 and 19.64±0.97 (g) at D_{60}, D_{120} and D_{180} respectively. (Table 2). But, at D_{120}, there was no significant difference for all applied treatments and in the two culture conditions used (Table 2).

Table 2. Parameters collected on harvested rhizomes during growth and mycorrhization time.

	Parameters collected on harvested rhizomes					
	Day 60		Day 120		Day 180	
Treatments apply	Fresh rhizome matter (g)	Dry rhizome matter (g)	Fresh rhizome matter (g)	Dry rhizome matter (g)	Fresh rhizome matter (g)	Dry rhizome matter (g)
Control	8,55±0,42 a	2,93±0,89 a	12,74±2,07 a	4,06±0,97 a	14,72±2,87 a	5,71±1,62 a
G. intraradices	9,37±0,52 ab	3,20±1,09 ab	15,00±0,80 abc	5,17±1,02 abc	19,25±2,72 cde	7,82±1,93 abc
Glomus sp.	9,47±1,24 ab	3,24±0,79 ab	15,07±1,52 bc	5,12±1,38 abc	15,79±2,55 ab	7,08±1,68 ab
Gi. margarita	9,05±0,69 ab	3,09±0,32 ab	14,14±1,07 abc	5,27±1,08 abc	16,63±2,41 abcd	6,32±1,69 a
A. tuberculata	8,58±0,67 a	2,93±0,89 a	13,93±1,01 abc	4,46±1,40 ab	14,87±3,21 a	6,01±1,51 a
Mean	9,00	3,07	14,18	4,82	16,25	6,58
F value	1,54	0,151	2,38	0,98	2,21	1,28
P	0,228 (ns)	0,960 (ns)	0,08 (ns)	0,43 (ns)	0,10 (ns)	0,31 (ns)
Control + S	8,59±0,69 b	2,98±0,80 ab	13,16±2,02 ab	4,24±1,12 a	16,05±1,84 abc	6,22±2,64 a
G. intraradices + S	9,92±0,63 c	3,39±0,85 b	15,18±1,20 bc	5,36±1,01 abc	19,64±0,97 de	8,13±2,07 abc
Glomus sp. + S	9,55±0,73 bc	3,26±0,86 ab	15,10±1,21 bc	5,39±0,78 abc	18,62±1,54 bcde	8,09±0,82 abc
Gi. margarita + S	9,42±0,85 bc	3,24±0,96 ab	14,51±1,92 abc	5,77±0,86 abc	17,41±1,35 abcde	6,94±1,30 ab
A. tuberculata + S	6,18±0,53 a	2,11±0,64 a	14,67±1,73 abc	5,33±1,35 abc	17,36±3,01 abcde	6,82±1,36 ab
Mean	8,73	2,99	14,52	5,21	17,81	7,24
F value	23,22	1,91	1,20	1,49	93,03	1,13
P	0,000 (***)	0,14 (ns)	0,33 (ns)	0,24 (ns)	0,000 (***)	0,36 (ns)

ns: non-significant at the threshold of 5%; *: significant at the threshold of 5%; **: highly significant at the threshold of 5%; ***: very highly significant at the threshold of 5%. Data sharing the same letter in the same column were not significantly different at 5% level (Duncan's multiple range tests) *S: Carbon source.

Hydrogen peroxide (H_2O_2) content and the activities of CAT, APX and G-POD in the rhizome and the roots of X. sagittifolium plant

AMF applied during the growth of *X. sagittifolium* plants has influenced the activities of the antioxidant enzymes. It is found that the hydrogen peroxide content was very low in the controls (Table 3). But in the rhizome, it increases progressively over time, while in the roots, this content evolves in gauss curve, between the D_{60} and the D_{180}. In the rhizome, at 5% (Duncan's multiple range tests at 5%), the contribution of mycorrhiza only greatly stimulated the production of these H_2O_2, in *Glomus* sp., during the first 120 days of growth.

Table 3. Hydrogen peroxide (H_2O_2) content on *X. sagittifolium* harvested rhizomes and roots during growth and mycorrhization time.

	Hydrogen peroxide (H_2O_2) content in $mM.min^{-1}.g^{-1}$ of FW					
Treatments apply	Rhizomes			Roots		
	Day 60	Day 120	Day 180	Day 60	Day 120	Day 180
Control	4,50±0,31 a	09,34±0,48 ab	09,86±0,78 ab	8,20±0,22 a	09,30±0,82 a	08,94±0,14 b
G. intraradices	8,24±0,00 b	09,10±0,48 ab	10,76±1,12 b	8,76±0,05 ab	13,14±0,36 b	06,90±0,06 a
Glomus sp.	9,14±0,19 c	10,10±0,19 b	10,76±0,36 b	9,92±0,00 b	14,42±0,19 c	10,08±0,32 c
Gi. margarita	8,54±0,02 b	09,26±0,70 ab	08,72±0,04 a	9,92±0,16 b	16,84±0,16 d	08,86±0,18 b
A. tuberculata	8,32±0,05 b	08,50±0,02 a	10,78±0,74 b	9,16±0,11 b	12,92±0,08 b	09,92±0,40 b
Control + S	4,88±0,08 a	11,08±0,45 b	11,38±1,00 ab	8,28±0,00 a	09,76±0,67 a	08,94±0,02 a
G. intraradices + S	8,84±0,11 b	13,06±0,31 b	15,18±0,70 c	8,94±0,02 a	13,38±0,02 b	09,82±0,50 a
Glomus sp. + S	8,70±0,14 b	10,40±1,06 ab	15,42±1,08 c	9,32±0,05 ab	12,86±0,14 b	10,88±0,04 b
Gi. margarita + S	9,22±0,08 c	08,48±0,33 a	09,86±0,34 a	9,02±0,14 ab	13,12±0,33 b	09,62±0,30 a
A. tuberculata + S	9,58±0,14 b	08,92±0,16 a	13,22±0,22 bc	9,10±0,02 ab	20,70±1,38 c	09,22±0,22 a

Data sharing the same letter in the same column were not significantly different at 5% level (Duncan's multiple range tests) *S: Carbon source.

The presence of the carbon source has influence that content of H_2O_2 in rhizomeat day 60, in plant mycorrhize with *A. tuberculata*, (9.58 ± 0.14 mM.min^{-1}.g^{-1} of FW), at D_{120} in plant mycorrhize with *G. intraradices* (13.06 ± 0.31 mM.min^{-1}.g^{-1} of FW) and at day 180 in plant mycorrhize with *Glomus* sp., (15.42 ± 1.08 mM.min^{-1}.g^{-1} of FW). However, in the roots, this H_2O_2 content, rather, present all the significant values of all treatments applied to D_{120}, the most important is 20.70 ± 1.38 mM.min^{-1}.g^{-1} of FW in plant mycorrhize with *A. tuberculata* + carbon source.

Table 3. Hydrogen peroxide (H_2O_2) content on *X. sagittifolium* harvested rhizomes and roots during growth and mycorrhization time.

Hydrogen peroxide (H_2O_2) content in mM.min^{-1}.g^{-1} of FW						
Treatments apply	Rhizomes			Roots		
	Day 60	Day 120	Day 180	Day 60	Day 120	Day 180
Control	4,50±0,31 a	09,34±0,48 ab	09,86±0,78 ab	8,20±0,22 a	09,30±0,82 a	08,94±0,14 b
G. intraradices	8,24±0,00 b	09,10±0,48 ab	10,76±1,12 b	8,76±0,05 ab	13,14±0,36 b	06,90±0,06 a
Glomus sp.	9,14±0,19 c	10,10±0,19 b	10,76±0,36 b	9,92±0,00 b	14,42±0,19 c	10,08±0,32 c
Gi. margarita	8,54±0,02 b	09,26±0,70 ab	08,72±0,04 a	9,92±0,16 b	16,84±0,16 d	08,86±0,18 b
A. tuberculata	8,32±0,05 b	08,50±0,02 a	10,78±0,74 b	9,16±0,11 b	12,92±0,08 b	09,92±0,40 b
Control + S	4,88±0,08 a	11,08±0,45 b	11,38±1,00 ab	8,28±0,00 a	09,76±0,67 a	08,94±0,02 a
G. intraradices + S	8,84±0,11 b	13,06±0,31 b	15,18±0,70 c	8,94±0,02 a	13,38±0,02 b	09,82±0,50 a
Glomus sp. + S	8,70±0,14 b	10,40±1,06 ab	15,42±1,08 c	9,32±0,05 ab	12,86±0,14 b	10,88±0,04 b
Gi. margarita + S	9,22±0,08 c	08,48±0,33 a	09,86±0,34 a	9,02±0,14 ab	13,12±0,33 b	09,62±0,30 a
A. tuberculata + S	9,58±0,14 b	08,92±0,16 a	13,22±0,22 bc	9,10±0,02 ab	20,70±1,38 c	09,22±0,22 a

Data sharing the same letter in the same column were not significantly different at 5% level (Duncan's multiple range tests) *S: Carbon source.

Similarly, it is noted that, whether in the rhizomes or the roots, the CAT activities are the most expressed compared with that of the peroxidases (Ascorbate and Guaïcol peroxidases) (Table 4 and 5). In rhizomes, these activities increase progressively over time and also vary significantly according to AMF treatments.

Table 4. Activities of CAT, APX and G-POD (mM.min-1.g-1 of FW) on harvested rhizomes during growth and mycorrhization.

Activities of CAT, APX and G-POD (mM.min^{-1}.g^{-1} of FW) on harvested rhizomes									
Treatments apply	CAT			APX			G-POD		
	Times in days			Times in days			Times in days		
	60	120	180	60	120	180	D 60	120	180
Control	4461,11±0,03 c	4494,44±0,05 c	4911,11±0,72 a	51,35±0,95 c	49,64±0,35 bc	38,64±0,92 b	07,57±0,09 c	04,79±0,19 a	05,37±0,60 a
G. intraradices	5938,88±0,22 d	6333,33±0,01 d	7333,33±1,02 ab	71,92±0,90 d	60,50±0,75 c	48,07±0,78 bc	13,31±0,01 d	11,31±0,07 c	13,25±0,05 c
Glomus sp.	3638,88±0,02 b	5294,44±0,03 c	7872,22±0,96 ab	14,71±0,80 a	30,78±0,39 ab	15,00±1,00 a	07,30±0,10 c	07,51±0,02 ab	08,96±0,39 b
Gi. margarita	2594,44±0,14 a	3600,00±0,23 b	9283,33±0,36 b	19,57±0,80 ab	32,00±0,00 ab	56,71±0,14 c	01,20±0,10 a	05,39±1,05 ab	13,65±0,57 c
A. tuberculata	2872,22±0,24 a	2822,22±0,21 a	6683,33±0,67 ab	74,78±0,99 d	45,64±0,80 abc	57,21±0,50 c	05,64±1,03 b	10,31±0,99 c	14,72±0,11 c
Control + S	4688,88±0,84 b	7216,66±0,16 c	7272,22±0,89 a	49,71±1,09 a	44,35±0,41 b	43,42±0,57 b	07,51±0,08 c	03,30±0,07 a	06,15±0,26 a
G. intradices + S	6588,88±0,03 cd	6838,88±0,05 c	8772,22±0,31 bc	89,35±0,12 b	73,57±0,80 c	57,14±0,55 c	07,03±0,53 c	07,32±0,23 b	09,61±0,08 b
Glomus sp. + S	4433,33±0,03 b	6100,00±0,10 b	7750,00±0,12 ab	20,14±0,60 a	16,57±0,23 a	17,35±0,64 a	09,71±0,27 d	10,21±0,12 c	10,20±0,80 b
Gi. margarita + S	6138,88±0,25 c	2705,55±0,21 a	9022,22±0,02 bc	37,92±0,31 a	22,92±0,29 a	54,00±0,71 c	03,15±0,12 b	03,96±0,32 a	07,48±0,33 a
A. tuberculata + S	1205,55±0,14 a	2666,66±0,28 a	9011,11±0,03 bc	32,50±0,70 a	29,14±0,40 ab	21,42±0,14 a	00,89±0,05 a	09,38±0,87 c	14,28±0,75 c

Data sharing the same letter in the same column were not significantly different at 5% level (Duncan's multiple range tests) *S: Carbon source.

They are significant in plant mycorrhize with *G. intraradices* and *Gi. margarita*. On D_{180}, the best activities of catalase was in plant mycorrhize with *Gi. margarita* (9283.33 ± 0.36 mM.min^{-1}.g^{-1} of FW) and plant mycorrhize with *Gi. margarita* + carbon source (9022.22 ± 0.02 mM.min^{-1}.g^{-1} of FW). While in the

roots, this catalase activity decreases rather over time, thus exhibiting considerable decreases in activity in D_{120}. Very important on D_{60}, this decreased catalase activity, showed little reduction, in plants treated with *Glomus* sp. + carbon source (Table 5).

Peroxidase activities were less expressed during mycorrhization (Table 4 and 5). However, APX activities were more important than G-POD activities. APX activity decreased considerably over time, with significant values in the presence of carbon source in rhizome and root. Moreover, in the rhizomes and the roots, the G-POD activities were weakest synthesize. They vary very little. This G-POD activity is low in the presence of the carbon source compare to catalase and APX activities.

Table 5. Activities of CAT, APX and G-POD (mM.min-1.g-1 of FW) on harvested roots during growth and mycorrhization.

Activities of CAT, APX and G-POD (mM.min^{-1}.g^{-1} of FW) on harvested roots									
Treatments apply	CAT			APX			G-POD		
	Times in days			Times in days			Times in days		
	60	120	180	60	120	180	D 60	120	180
Control	0616,00±0,03 a	1938,88±0,60 b	2111,11±0,10 a	10,64±0,11 a	10,28±0,21 a	07,92±0,64 a	4,58±0,99 ab	4,47±0,35 a	1,07±0,24 a
G. intraradices	4444,44±0,01 d	3000,00±0,06 c	2877,00±0,23 a	53,78±0,52 e	45,07±0,72 b	35,85±0,28 c	7,83±2,12 c	4,75±0,10 a	2,60±0,24 b
Glomus sp.	4455,55±0,09 d	3111,11±0,06 c	3122,22±0,81 a	17,98±0,50 b	37,64±1,42 b	18,14±0,85 b	4,06±0,56 ab	6,32±0,09 ab	4,72±0,28 d
Gi. margarita	2988,88±0,17 b	1427,77±0,00 ab	3177,78±0,14 a	22,50±0,90 c	36,71±0,00 b	65,14±0,42 c	2,42±0,38 a	6,48±0,35 ab	2,06±0,32 b
A. tuberculata	3050,00±0,18 b	1094,44±0,02 a	2861,11±0,42 a	26,28±0,81 d	15,64±0,29 a	20,42±0,57 b	5,84±0,14 bc	8,07±0,91 b	3,56±0,15 c
Control + S	1011,11±0,04 a	2244,44±0,20 b	2716,00±1,08 a	06,07±0,50 a	06,71±0,84 a	06,21±0,35 a	05,19±0,30 ab	01,29±0,06 a	0,27±0,03 a
G. intraradices + S	5161,11±0,03 d	3400,00±0,10 c	3305,55±0,35 ab	69,92±0,91 c	57,50±0,98 b	44,64±1,07 c	03,96±1,01 a	03,28±0,11 b	3,29±0,37 c
Glomus sp. + S	5144,44±0,15 d	3616,66±0,07 c	5094,44±0,59 c	23,28±0,21 b	19,07±0,14 a	20,07±0,21 b	05,26±0,51 b	03,62±0,12 b	2,72±0,64bc
Gi. margarita + S	2750,00±0,25 b	1494,44±0,03 a	3538,88±0,63 ab	16,92±0,71 b	10,28±0,62 a	24,00±0,00 b	12,17±0,22 d	05,53±0,82 c	1,27±0,12 a
A. tuberculata + S	3294,44±0,02 c	1311,11±0,25 a	2816,66±0,32 a	89,28±1,48 d	80,14±0,50 c	46,00±0,42 c	07,18±0,29 c	10,27±0,70 d	1,57±0,06 ab

Data sharing the same letter in the same column were not significantly different at 5% level (Duncan's multiple range tests) *S: Carbon source.

Moreover, the analyze in a factorial plane (1X2) shows important variability of all the biochemical parameters in plant of *Xanthosoma sagittifolium* inoculated with AMF and AMF + carbon source in rhizomes and roots (Fig. 1). A contribution of the total variability of 83.35% for *X. sagittifolium* + CMA plants only and 78.95% in the presence of the carbon source in the rhizomes, (Fig 1 A and C). While in the roots, this contribution is 76.36 and 75.12% respectively (Fig.1 B and D). Analysis of the contributions to the axes showed that the main biochemical parameters associated with axis 1, in the rhizome, are; CAT and G-POD in mycorrhizal plants only, in the presence of the carbon source CAT and H_2O_2. In axis 2, In the rhizomes for both conditions and in the absence or presence of the carbon source, two groups are distinguished (Fig 1A and C). Group 1, comprising on the one hand the *X. sagittifolium* plants inoculated with *G. intraradices* (Fig. 1A) and on the other hand the *X. sagittifolium* plants inoculated with *G intraradices* + the carbon source (Fig. 1C), respectively influencing the APX activities on day 60. Group 2, including, *X. sagittifolium* plants inoculated with *A. tuberculata*, *G. intraradices*, *Gi. margarita* and *Glomus* sp. influencing on the D_{180} respectively the content of $H_2O_2$and the CAT and G-POD activities, in the absence or presence of the carbon source (Figs 1A and C). Nevertheless, in the roots, APX, G-POD and H_2O_2 show 39.82% variability in *X. sagittifolium* + CMA plants and 46.08% variability in *X. sagittifolium* + CMA + carbon source plants in the axis 1. Is recorded, 36.54% and 29.04% of variability in the axis 2, respectively in the absence or presence of the carbon source (Fig.1 B and D). In these roots, two groups are also distinguished (Fig. 1B and D). Group 1, encompasses the plants of *X. sagittifolium* inoculated with *G. intraradices* and *Glomus* sp. and the plants of *X. sagittifolium* + *A.*

tuberculata on day 160, linked and influencing the CAT activities in the absence of the carbon source (Fig. 1B). In the presence of the carbon source, this CAT activity is influenced by mycorrhizae in plants of *X sagittifolium, A. tuberculata, G. intraradices, Gi. margarita* and *Glomus* sp, in D180. On the 60th, *A. tuberculata* (Fig. 1D). Thus, for group 2, the H_2O_2 content and the G-POD activities are influenced on day 120, by strains of *A. tuberculata*, *G. intraradices, Gi. Margarita* and *Glomus* sp applied (Fig. 1B). In the presence of the carbon source, this H_2O_2 content and the G-POD activities are influenced by *G. intraradices*, and *Glomus* sp by D120, and then by *Glomus* sp at D60 (Fig. 1D).

It is also apparent from these principal component analyzes (PCAs) that there is a positive and highly significant correlation (P <0.05) between CAT and G-POD in rhizomes of *X. sagittifolium* plants + CMA, positive and very significant between CAT and H_2O_2 in rhizomes of *X. sagittifolium* plants + CMA + carbon source (Table 6 A and C).

It is also reported to be negative and non-significant in the rhizome under both conditions between H_2O_2 and APX. However, in the roots, all correlations between the biochemical parameters evaluated are not significant (Table 6 B and D).

Table 6. Correlation matrix (Pearson) between the various biochemical parameters measured in rhizomes of mycorrhizal and mycorrhizal *X. sagittifolium* plants + carbon source, during growth, minituberization and mycorhization. H_2O_2: Hydrogen peroxide, CAT: catalase, APX: ascorbate peroxidase, G-POD: Guaïcol peroxidase, P <0.05 (*) significant, P <0.01 (**): significant highly correlated and P <0.001 (***): significant highly correlated.

Cocoyam + AMF									
A					C				
Variables	H_2O_2	CAT	APX	G-POD	Variables	H_2O_2	CAT	APX	G-POD
H_2O_2	1				H_2O_2	1			
CAT	0,364	1			CAT	0,608**	1		
APX	-0,272	0,160	1		APX	0,043	0,282	1	
G-POD	0,209	0,725***	0,474	1	G-POD	0,373	0,485	0,202	1
Cocoyam + AMF + Carbon source									
B					D				
Variables	H_2O_2	CAT	APX	G-POD	Variables	H_2O_2	CAT	APX	G-POD
H_2O_2	1				H_2O_2	1			
CAT	-0.232	1			CAT	-0.394	1		
APX	0.136	0.459	1		APX	0.376	0.159	1	
G-POD	0.513	-0.069	0.128	1	G-POD	0.390	-0.259	0.312	1

Discussion

The recorded parameters on the growth and development of rhizomes and roots showed a considerable variation in size and dry and fresh biomass, depending on the four strains of mycorrhizal fungus used. In mycorrhizal *X. sagittifolium* plants, this growth was significantly influenced by *Glomus* sp and *Gi. margarita*, whether in the presence or not of the carbon source. These results are in agreement with those of Rafiq Lone *et al.* (2015), which showed that in *Solanum tuberosum*, fresh and dry biomass increases during mycorrhization and over time. Mathur and Vyas (1999), justified this increase in fresh, dry biomass as the product of improved nutrient and water uptake that are directly involved in the mechanism of photosynthesis in plants thanks to the CMA. The best fresh and dry biomass values were recorded in *Glomus* sp. and *Gigaspora margarita*, and exhibiting appreciable peaks in the presence of the carbon source. In the rhizome, fresh and dry biomasses increase gradually over time. This increase in fresh biomass also results in an increase in

dry biomass. A continuous and significant increase in the average root size in *Gi. margarita* + carbon source was notice. Similar results were obtained in *Solanum melongena* mycorrhizae with *cc* It should be noted that there was a significant difference in mean root size between AMF treatments applied on D_{60}, D_{120} and D_{180}. Significant value was observed on D_{120} with *A. tuberculata*. Wimalarathne *et al.* (2014), also showed that in the presence of *Funneliformis mosseae*, the roots of *Piper nigrum* grew more rapidly than controls.

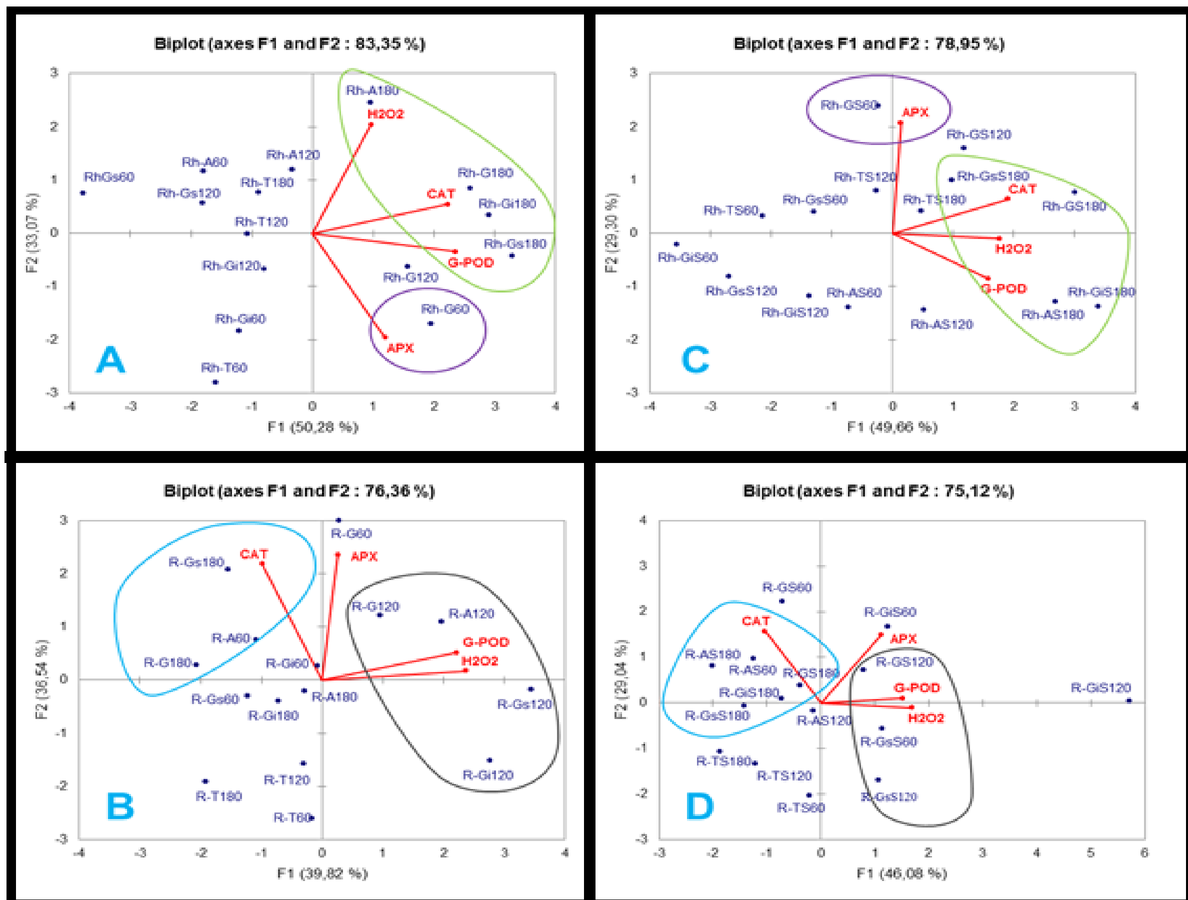

Fig. 1. Factor diagram of Principal Component Analysis (PCA) data on the effect of AMFs (A and B) and AMF + carbon source (C and D) on growth and mycorrhization in rhizomes (Rh) and *X. sagittifolium* roots (R). Control (T), *Glomus intraradices* (G), *Gigaspora margarita* (Gi), *Glomus* sp. (Gs) and *Acaulospora tuberculata* (A). Source of carbon (S).

However, in the case of reactive oxygen species, the content of hydrogen peroxide is very low in the controls. But in the rhizome, this content increases progressively over time, whereas in the roots, it evolves in gauss curve, between the D_{60} and the D_{180}. This accumulation of H_2O_2 content is very high in the presence of the carbon source and varies considerably. Li *et al.* (2008), have shown that various environmental stresses cause an accumulation of H_2O_2 in the different tissues of the plant, and that the regulation of this accumulated H_2O_2 level is extremely important and can modified the metabolism of the plant. In excess in the plant, they are converted into a toxic molecule such as the oxide (-OH) ion, if it is not quickly removed from the plant cells (Sharma and Dubley, 2004; Bárzana *et al.*, 2015). Its strong presence in the basal part of the plant on the D_{120} of mycorrhization. It's much more recorded at the root level, in *X. sagittifolium* + AMF plants and in *X. sagittifolium* + AMF + carbon

source. It is also noted that the most important values of these H_2O_2, were significant in *Gi. margarita* and *A. tuberculata* + carbon source. However, in the rhizomes, *Glomus* sp. has the best stimulation of the synthesis of these hydrogen peroxides. This presence of H_2O_2 in rhizomes is attributed not only to the presence of mycorrhization, but also may be the result of a number of phenomena related to growth, such as tuberization.

Moreover, the high H_2O_2 content recorded on the D_{120} of growth and mycorrhization would be attributed to the establishment of several colonization sites for AMF used at the root level of *X. sagittifolium* plants during colonization, which also triggered the synthesis of ROS and consequently the increase in H_2O_2, or the presence of the arbuscule and the existence of several senescent arbuscules in the root cells. Fester and Hause (2005) have note the accumulation of H_2O_2in the intracellular hyphae of *G. intraradices* present in the cytoplasm of the roots of *Zea mays*. They concluded that once produced, these H_2O_2 diffuse easily through the thin wall of the hyphae of the arbuscular branches. Moreover, this significant variation in H_2O_2 would undoubtedly have led to the significant variation of antioxidant enzymes such as catalase, ascorbate peroxidase and Guaïcol peroxidases evaluated. Similarly, in rhizomes or roots, these levels of H_2O_2 are more influenced by CAT activities compared to peroxidases (Ascorbate and Guaïcol peroxidases).

The results also showed that the catalase activities vary significantly according to the applied mycorrhizal arbuscular treatments. In rhizomes, they increased gradually over time, while in the roots they decreased. CATs are also recognized for their specific role in protecting cells against toxic effects. They contributed particularly to the detoxification of the cells by the neutralization of the oxidized radicals accumulated in the cytoplasm. Chen *et al.* (1993) reported that the CAT activity depends on the presence of H_2O_2 in the medium. In the rhizome, the observed increase is correlated with the increase in H_2O_2. But the decrease in this root-level CAT activity may be due to the decrease in the peroxide content observed in our results or to the production of reactive oxygen species arising during hypersensitive responses during the penetration of mycorrhizal hyphae into the *X. sagittifolium* roots. Moreover, Shim *et al.* (2003) suggested that this decline in catalase activity is a phenomenon related to the accumulation of salicylic acid in oxidatively stressed plants. Similar results were observed by Atsushi *et al.* (2007), at the roots of Radish, in the presence of *Gi. margarita*. However, Patai *et al.,* (2016), obtained contrary results, showing that CAT activities in the roots also increased in *Lactuca sativa* plants inoculated with *Funneliformis mosseae*. However, with respect to peroxidase activities, they are very low compared to CAT activities in roots and rhizomes (Table 4 and 5). Apel and Hirt (2004) demonstrated that when APX and G-POD activities are favored in plants, CAT activities are reduced and vice versa. APX activities are more important than G-POD activities. Whether one is in the roots or in the rhizomes, one decreases this activity considerably over time. They were very high at the beginning of my growth and mycorrhization, showing significant values in *A. tuberculata* and *G. intraradices* + carbon source. Patai *et al.*, (2016), obtained decreased APX activities in the roots also decreased after mycorrhizal lactate plants (*Lactuca sativa*) with *Funneliformis mosseae*. Reduction of this APX activity in the roots suggests a decrease in oxidative stress in root cells caused by a low presence of arbuscular structures. However, for G-POD activities, despite their very low presence, they remain significant in rhizomes, compared to roots. Their considerable increase between D_{120} and D_{180} could be characterized by the initiation of tuberization. Tsafack (2010), showed that during microtuberization of *X. sagittifolium*, the initiation of the tubers was accompanied by an activation of Guaicol-peroxidase (G-POD) activities, presenting new iso-peroxidases. Similar results were obtained during bulbification in *Allium sativum* by Coleniowski (2001). It has been demonstrated by Kamegne and Omokolo (2002) that peroxidases are

involved in organogenesis in *X. sagittifolium*. However, contrary to the increase in G-POD activity observed in rhizomes, there is a considerable decrease in this activity in the roots. This decrease is correlated negatively and significantly with the increase in CAT activities in *X. sagittifolium* plants + AMF. Qiang *et al.* (2006) also found a decrease in G-POD activity in the roots of *Citrus tangerine*, mycorrhizae with *Glomus versiforme* during water stress.

Conclusion

Mycorrhization influenced growth in *X. sagittifolium*, especially AMFs of the genus *Glomus*. From this study, the *Glomus intraradices* treatment influenced the morphological parameters evaluated, such as the fresh and dry mass in the rhizomes, while in the roots the effect of *Glomus* sp. The elimination of hydrogen peroxide produced during growth and mycorrhization in the roots and rhizomes of *X. sagittifolium* was more influenced by catalase activities. Similarly, the comparison with the control shows that the presence of mycorrhizae further induced the synthesis of these H_2O_2, and therefore that of CAT, APX and G-POD.

Acknowledgements

Authors are indebted to *X. sagittifolium* research Team of the Laboratory of Plant Physiology and Biochemistry, Department of Biological Sciences, Ecole Normale Supérieure (ENS), University of Yaoundé 1 for their participation in this research.

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