East African Scholars Journal of Agriculture and Life Sciences
Abbreviated Key Title: East African Scholars J Agri Life Sci
ISSN 2617-4472 (Print) | ISSN 2617-7277 (Online)
Published By East African Scholars Publisher, Kenya

Volume-6 | Issue-1 | Jan-2023 | DOI:10.36349/easjals.2023.v06i01.001

OPEN ACCESS

Original Research Article

Mycorrhization of *Xanthosoma sagittifolium* L. Schott Plants (White Cultivar): Evaluation of Their Effect on Acid Phosphatase Activity During Growth and Production of Minitubers

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Article History
Received: 05.11.2022
Accepted: 14.12.2022
Published: 10.01.2023

Journal homepage:
http://www.easpublisher.com

Quick Response Code

Abstract: This work's main objective was to evaluate the effect of certain strains of arbuscular mycorrhizal fungi on acid phosphatase activity during growth. The treatments applied were, control, *Glomus intraradices*, *Acaulospora tuberculata*, *Gigaspora margarita*, *Glomus* sp., and the mixture of these four AMF strains. Their effect on the growth of vitroplants was evaluated on days 60, 120, and 180 after inoculation. Mycorrhizal status following treatments and acid phosphatase activity were assessed. The minitubers obtained were subjected to a preservation and germination test. The results showed that the maximum growth peak of *X. sagittifolium* plants was obtained on day 120 for all treatments. The mixture of the four strains of AMF had more influence on the growth parameters with values of 18.40±0.54 Cm and 42.34±16.67 Cm^2, respectively for the average number of roots and leaf area. Mycorrhization did not significantly influence the mean number of leaves and mean plant height. Mycorrhizal structures were observed in all mycorrhizal treatments applied. The frequency of mycorrhization was very high in plants inoculated with Glomus sp. The evaluation of the phosphatase activity shows a significant activity at the level of the leaves with a peak of activity in the plants inoculated with *Glomus* sp. (47.72±0.07 µM/min/g of FW). Compared to the control, the number of minitubers produced was two to three times higher. The best tuberization percentage (80%) was obtained in plants inoculated with a mixture of AMF strains. The temperature of 7~10°C allowed the preservation of 50% of the germination capacity of the minitubers after two months. Based on the obtained results, it was concluded that fertilization with AMF is beneficial for the stimulation of minitubers production.

Keywords: *X. sagittifolium*, arbuscular mycorrhizal fungi, acid phosphatases, minituberization, and germination test.

INTRODUCTION

Xanthosoma sagittifolium L. Scott (cocoyam), appreciated for its nutritional value, is an important source of carbon for food security in Cameroon. Despite its place in the human diet, its intensive cultivation is hampered by various bacterial, fungal, and viral infections (Reyes Castro *et al.*, 2005). These constraints are the cause of the unavailability of healthy and pest-free seeds. Moreover, strategies for improving the cultivation of *X. sagittifolium* by conventional methods of genetic crossing have not been successful, in part because of the limited genetic diversity of existing varieties (Ngouo, 1988). The chemical control of these infectious agents, in addition to the very high costs of these products, would have an impact on environmental pollution and even the health of producers and consumers. In addition, the endemic nature of pathogens of *X. sagittifolium* such as *Fusarium oxysporum* (Saborío *et al.*, 2004, Djeuani *et al.*, 2018), *Rhizoctonia solani*, and especially *Pythium myriotylum* (Tambong and Höfte, 2001), microscopic fungi responsible root rot, remains the major constraint to its production in Cameroon, and even in Nigeria and Ghana. The use of mother or rhizome tubers (corms) or daughter tubers (cormels) as seeds remains a potential source of that disease spread. Given all these constraints, the urgency is to implement effective methods to improve the production of decontaminated

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and/or healthy seeds of *X. sagittifolium*. Suggestions for the production of these sanitized seeds, we noted chronologically, the application of biotechnological techniques for the production of vitroplants (Omokolo *et al*., 2003) and the production of microtubers by the technique of microtuberization (Tsafack *et al.*, 2009). However, the weight (0.2 to 0.9 g) and the relatively small size of these microtubers have been the major drawback in the conservation of their germination capacity (Tsafack *et al*., 2009; Coleman *et al.*, 2001). Minituberization, which is the classic intermediate step allowing the use of plant material from *in vitro* culture in open fields, has so far made it possible in *Solanum tuberosum* to produce tuber seeds of the considerable size called minitubers (Duffy and Cassells, 2000). Indeed, the size and physiology of minitubers bring them closer to traditional tubers. However, the development of a technical route for their optimal production in *X. sagittifolium* remains to be explored.

A combination of biotechnological techniques and the use of arbuscular mycorrhizal fungi (AMF) would appear as an alternative way to solve the problem of the unavailability of sanitized seeds. The ideal would be to see the impact of arbuscular mycorrhizal fungi on the development and production of *X. sagittifolium*. Knowing that *X. sagittifolium* is a plant that has a considerable demand for phosphorus assimilation from the soil, would the application of AMFs could influence the activity of the phosphatases involved in this mechanism? Increasingly, the use of AMFs in agriculture is presented as a sustainable and innovative solution for improving production. Indeed, these microorganisms, ubiquitous to almost all terrestrial plants, play a decisive role in improving water absorption by plants (Marulanda *et al*., 2003). They assist plants to increase antioxidant activities (Marulanda *et al*., 2007, Djeuani *et al.*, 2018), osmotic adjustment (Fernández-Lizarazo and Moreno-Fonseca, 2016), biological control of pathogens (Zhang *et al*., 2018), improve nitrogen nutrition, improve uptake and translocation of mineral nutrients from the soil to plants (Alguazil *et al*., 2006; Ceccarelli *et al*., 2010). The application of these arbuscular mycorrhizal fungi as inoculum in the soil has so far proved to be very promising in solving the problems of phosphorus and nitrogen deficiency in certain plants, whose growth is highly dependent on them. The AMFs present in the rhizosphere makes it possible to enlarge the surface of the soil exploitable by the roots of the plants while promoting the exudation of enzymes to mobilize inorganic phosphorus (Pi) (Collavino *et al*., 2010; Van der Heijden *et al*., 2015) like phosphatases. Phosphatases are ubiquitous enzymes involved in the absorption, assimilation, and metabolism of phosphorus in the root system of plants (Van Aarle *et al*., 2005). Their activity in the soil is inhibited or increased due to the addition of inorganic or organic phosphates respectively (Brandt *et al*., 2011; Margalef *et al*., 2021; Janes-Bassett *et al*., 2022). A distinction is made between acid and alkaline phosphatases. Involved in phosphorus metabolism in plants, acid phosphatases (EC 3.1.3.2) (APase) are present in intracellular spaces, cell walls, and inside the cell: in the amyloplast, mitochondria, nucleus, l Golgi apparatus, and the endoplasmic reticulum (Duff *et al.*, 1994; Sujkowska *et al*., 2006 and Tran *et al*., 2010). These are important indicators for plant responses to Pi deficiency in the soil. Lui *et al*., (2016), showed that the more Pi deficiency is noted in the soil, the more the secretion of acid phosphatases is carried out in the plant. Their secretion by the roots allows plants to mobilize organic phosphorus (P) in soils poor in P. Certain factors can influence the activity of APase in the rhizosphere, notably soil moisture, temperature, plant nutritional status, and fertilizer application (Kuzyakov and Blagodatskaya, 2015; Xu *et al.*, 2017). Furthermore, the presence of microorganisms in the rhizosphere is another important source of APase (Wei *et al*., 2018). Gao *et al*., (2020) demonstrated that the APase activity can be enhanced by the presence of AMFs in the soil. However, little information on the potential and diversity of arbuscular mycorrhizal fungi on growth, tuber production in *X. sagittifolium,* and their influence on APase activity are available. A better understanding of the symbiosis of arbuscular mycorrhizal fungi - *X. sagittifolium* would certainly facilitate the selection of specific AMFs that can be used as inoculum to improve plant development. It is therefore in this perspective that the objective of this work was to evaluate the effect of certain strains of arbuscular mycorrhizal fungi on acid phosphatase activity during growth. More specifically, it was to assess the action of four strains of arbuscular mycorrhizal fungi on the growth and induction of minituberization in vitro plants of *X. sagittifolium* produced *in vitro*, to evaluate their influence on the activity of acid phosphatases in the plants and to determine the best temperature conditions for better conservation of the germination capacity of these minitubers.

MATERIAL AND METHODS

Plant material

The plant material consisted of fragments of rhizomes of *X. sagittifolium* cultivar white harvested from a family farm in Mbama (located in Eastern Cameroon). The vitroplants were produced by *in vitro* culture of the apex according to the method of Omokolo *et al*., (1995) and Djeuani *et al*., (2014). After 45 days of growth, these vitroplants were cultured on black soil + sand substrate at a ratio of 2:1. This culture medium had a pH of 4.7, with an amount of assimilable phosphorus of 5.81mg/Kg, moisture content of 1.945%, and a C/N ratio of 1.47. Their acclimatization was carried out for a month.

Effect of different CMA strains on the growth and production of minitubers in X. sagittifolium

180 acclimatized vitroplants of *X. sagittifolium* were used. The treatments applied consisted of Control,

Acaulospora tuberculata, Glomus sp. Glomus intraradices, Gigaspora margarita, and the mixture of the four strains (1:1:1:1). 30 vitroplants were used per treatment. 30g of each strain of mycorrhizal fungus were inoculated by vitroplants. The mycorrhizal plants of each treatment were placed under a shade house. The experimental device was a complete block. The treatments were placed on a slab at a height of 0.5 m from the ground and 0.5 m apart. Every two days, the inoculated plants were watered. To assess the fertilizing potential of each treatment applied to growth, agronomic growth parameters such as plant height, number of leaves, leaf area, number of roots, total fresh biomass and total dry biomass, and mycorrhizal dependence calculated according to the method of Menge and Johnson (1978) and were evaluated at days 60, 120 and 180 respectively.

Histological analysis and assessment of the mycorrhizal status

The protocol of Phillips and Hayman (1970), modified by Vierheilig *et al.,* (1998) was used to assess the mycorrhizal status of each applied treatment. The root hairs of the roots of *X. sagittifolium* harvested following the applied treatments were cleaned, sectioned at a size of 0.5 to 1.0 cm, and were successively soaked in 10 $g.L^{-1}$ of KOH for 15 minutes at 90°C, then bleached with H_2O_2 (10%) for 3-6 hours and rinsed with HCl (10%). The dye used was Fuchsin acid $1g.L^{-1}$. Microscopic observations were made using a YVMEN brand optical microscope at 400X. 100 root fragments were observed per treatment and the mycorrhizal status was calculated according to the scale proposed by Trouvelot *et al.,* (1986).

Evaluation of the activity of acid phosphatases in the plant of X. sagittifolium during growth

Acid phosphatase activity was determined in the leaves, rhizomes, and roots of *X. sagittifolium* plants over time for all treatments applied according to the method of Kouadio *et al.,* (2006). The reaction medium consisted of 125 µl of 20 mM acetate buffer pH 5.6; 75 µl of 5 mM para-nitrophenyl phosphate (pNPP) prepared in the same buffer and 50 µl of enzymatic solution. The whole was incubated in a water bath for 10 min at 37°C while shaking. The reaction was stopped by adding 4 ml of 2% (w/v) sodium carbonate solution. The quantity of para-nitrophenol (pNP) released was measured with a spectrophotometer (brand JENWAY 6305) at 410 nm against a blank consisting of the reaction medium. Three readings were taken. The activity of the acid phosphatase was expressed in µM/min/g of the weight of fresh (FW) material. The molar extinction coefficient of acid phosphatase ϵ =18000 M/cm.

Harvesting and evaluation of growth parameters on the minitubers produced

The harvest of the minitubers following the treatments was carried out after 180 days when total yellowing of the leaves of the plants inoculated appeared. To evaluate the effect of different strains of arbuscular mycorrhizal fungi on the tuberization of plants, the following parameters were recorded; the number of minitubers produced, the number of inoculated plants having tuberized, the percentage of tuberization (Tsafack, 2010; Djeuani *et al*., 2014), the average number of minitubers per plant, average weight of minitubers per plant, and rate of increase in tuberization (Liu *et al*., 2018), average height and diameter of minitubers (Altindal and Karadogan, 2010; Tsafack, 2010, Djeuani *et al*., 2014).

Minituber storage conditions, parameters evaluated during the minituber storage and germination test

Independently of the treatment, the harvested *X. sagittifolium* minitubers were washed and drained. These minitubers were divided into two batches and stored for three months. This preservation test was carried out under two conditions; at room temperature and a temperature of 7~10°C (refrigerator). The parameter evaluated during the conservation of germination power was the average weight of the minitubers over time. Every month for three months, the minitubers were weighed using a KIKA brand scale to assess their water loss, and their weight was expressed in grams. Then, each month, the minitubers were subjected to a germination test to assess the degree of conservation of their germination capacity. For each storage condition, 30 minitubers were germinated in trays containing a mixture of black soil and sterile coarse sand (2:1). These tanks were placed under the shade at a photoperiod of 12/12 (day/night). The parameters measured were the number of minitubers having germinated and the percentage of germination.

Statistical analyzes

The results obtained were subjected to a descriptive analysis (Mean ± standard deviation). The results were represented in the form of tables (Microsoft Excel 2016 software). The evaluation of the weight loss of minitubers at different storage conditions over time was determined from a regression curve using Microsoft Excel 2016 software. The IBM SPSS Version 20.0 software was used to perform the statistical analyzes and to carry out the comparison of the means by an analysis of variance (ANOVA) according to the Student-Newman-Keuls test at the 5% threshold.

RESULTS AND DISCUSSION

Results

Evaluation of growth and agronomic parameters of X. sagittifolium according to the different treatments applied

The analysis of the results of growth and agronomic parameters in *X. sagittifolium* plants showed that the application of the strains of fungi; (*Glomus intraradices, Acaulospora tuberculata, Gigaspora margarita, Glomus* sp., as well as their mixture) had a

significant effect (P<0.05) on the growth parameters, in particular the average leaf area and the average height of the plants evaluated (Table 1). These growth parameters showed their maximum growth peak at D_{120}. The average number of roots obtained was high in the presence of the mixture of the four CMA strains (18.40 ± 0.54 cm) at 120 days of growth. No significant difference in the influence of these different treatments on the average number of leaves was observed. The effect of the different treatments on the average leaf area shows a significant increase compared to the control of 75.09% in *G. intraradices*, 58.90% in *Acaulospora tuberculata*, and 57.49% in *Gigaspora margarita*, 80.38% in *Glomus* sp. and 86.63% for the mixture between D_{60} and D_{120}. Analyzes of fresh and dry biomass showed an increase over time (Table 2). The applied mycorrhizal treatments significantly improved the fresh weight of the plants compared to the control. The maximum weight of 42.50 ± 5.87 g on D_{180}, in the presence of *G. intraradices*, was noted. Similarly, the dry biomass value was significant at D_{180}, with 19.10 ± 2.68 g for the mixture of the four AMF strains used.

Evaluation of different structures of arbuscular mycorrhizal fungi observed on roots of X. sagittifolium plants and assessment of the mycorrhizal status

Histological observations showed that the control plant showed no mycorrhizal structure (Figure 1A). However, for different mycorrhizal treatments applied, mycorrhizal structures were observed in the roots. The presence of hyphae (Figure 1. B, C, D, E, and F), arbuscules (Figure 1. G, H, I, J, and K), and spores were noted in plants inoculated with *Glomus* sp. (Figure 1. L), and with the mixture of the four AMF strains (Figure 1. M). Similarly, cells in aggregates of 4 subglobose to ovoid to clavate were observed in plants inoculated with *Gigaspora margarita*. (Figure 1. N and W) and about 18 subglobose to ovoid to clavate, borne by hyphae in plants inoculated with the mixture of the four strains (Figure 1. P). These cells in aggregates characteristic of Gigasporaceae were the most observed in plants inoculated with the strained mixture.

Analysis of mycorrhizal status showed that mycorrhizal dependence in plants following the mycorrhizal treatments applied increased over time, with peaks at D_{180} (Table 2). The highest dependence was recorded in plants inoculated with the treatment mixture of the four treatments (39.22%). The frequency of mycorrhization (F%), and the intensity of mycorrhization of the root system (M%), were very high on D_{120}, with values of 70.33%, and 18.18% in plants inoculated with the *Glomus* sp. Furthermore, the maximum peak of the intensity of mycorrhization in the root fragments (m%) was recorded at D_{120}, with 39.03% in the presence of the mixture of the four AMF strains. A significant peak was noted in the plants inoculated with the treatment mixture of four strains of arbuscular mycorrhizal fungi at D_{180} with a value of 19%. However, it was noted that the content of arbuscules in the root fragments was very low with values below 3% (Table 2).

Acid phosphatase activities in plants inoculated with X. sagittifolium during growth

Acid phosphatase activities varied significantly during growth following applied arbuscular mycorrhizal fungi treatments (Figure 3). These acid phosphatases showed very high activity in leaves compared to rhizomes and roots (Figure 3 A, B, and C). In the leaves, these phosphatase activities increased over time for all the treatments with significant values at D_{180}. Maximum peaks were recorded in plants inoculated with *Glomus* sp. (47.72±0.07 µM/min/g of FW) and the mixture of the four strains of CMA (47.50±0.39 µM/min/g of FW) (Figure 3A). However, in the rhizomes, the activity of these acid phosphatases presented its maximum at D_{160} in almost all the mycorrhizal treatments applied with peaks of 27.88±0.31 and 27.88±0.47µM/min/g of PMF, respectively in plants inoculated with *Acaulospora tuberculata* and *Glomus* sp. (Figure 3B). In the roots, these activities were high compared to the rhizome. It is noted that it increases over time, thus presenting significant peaks at 5%, the maxima of which were 30.55±0.33; 30.22±0.31, and 30.11±0.22 µM/mn/g of WF respectively in *X. sagittifolium* plants inoculated with *Acaulospora tuberculata*, *Glomus* sp., and the mixture of the four AMF strains at D_{180} (Figure 3C).

Tuberization assessment

After 180 days of culture, the results showed that the strains *Acaulospora tuberculata*, *Glomus* sp., *Glomus intraradices*, *Gigaspora margarita*, and their mixture contributed to the production of minitubers in plants inoculated with *X. sagittifolium* compared to control plants (Table III). These minitubers showed variable sizes depending on the treatments applied. The number of minitubers produced compared to the control (17 minitubers), almost doubled with values of 35, 39, and 37 minitubers respectively in the plants of *X. sagittifolium* inoculated with *Acaulospora tuberculata*, *Glomus* sp., and *Glomus intraradices*. In the plants inoculated with the mixture of four strains of AMF, it is found that the number of minitubers produced is almost three times that produced by the control (45 minitubers). The average number of minitubers per plant increased significantly in all treatments compared to the control (0.56±0.18 minitubers) (Table III). The tuberization percentages greater than 50% were obtained in the presence of *Glomus intraradices* (60.00%), *Acaulospora tuberculata* (60.00%), and the mixture of the four strains (73.33%). The average mass of the minitubers, the rate of increase in tuberization, and the average size of the minitubers per plant were more significant at 5% in the plants inoculated with the mixture of the four strains, with respectively 4.14±1.46 g, 61.59% and 3.12±1.82cm (Table III). Similarly, the

average diameter of the minitubers was significantly higher (5%) in all the treatments compared to the control with significant values of 1.97 ± 0.92 and 1.97 ± 0.80 cm respectively in the presence of *Acaulospora tuberculata* and a mixture of the four strains.

Analysis of the conservation of minitubers and evaluation of the germination test

During the storage of the minitubers at room temperature, and at 7~10°C, the results showed that the average weight of the minitubers gradually decreased over time (Figure 3 A and B). At room temperature, the regression curve of the mean weight of the minitubers shows a gradual decrease which increases over time (Figure 3A). This average weight loss was 37.48; 47.24 and 68.55% respectively at months 1, 2, and 3 of storage. In the third month, almost all of the minitubers stored at room temperature showed a weight of less than 1g. At 7~10°C storage, the results showed that the average weight of the minitubers decreased slightly over time. The regression curve of the variation in weight (Figure 3B) showed a slight decrease in the weight of minitubers stored between 7-10°C compared to those stored at room temperature. A correlation rate of 85.93% was recorded in the 1st month of storage, i.e. 14.07% water loss. During the 2nd and 3rd months of storage, the results showed 29.25 and 36.89% weight loss (Figure 3B). It was noted that after the 3rd month, the average weight of the minitubers was around 1.7 g.

During storage, minitubers stored at 7~10°C were found to have whitish and pinkish scales or buds (Figure 4B). The best germination rates were recorded in the 1st month at room temperature (70.00%) and 7~10°C (93.33%). In the 2nd month, at 7~10°C, the germination rate was 50%. However, after 3 months of storage, the germination percentage was almost zero for all conditions (Table IV). Furthermore, the results showed that the minitubers stored at 7~10°C germinated faster. Plants were grown after two months of storage and germinated 13 days after sowing. The results also showed that minitubers stored at 7~10°C grow faster (Figure 4 G and H) and have larger sizes than those from minitubers stored for one month at room temperature. Moreover, after one month of growth, the roots were more developed in plants from storage at 7~10°C compared to those at room temperature.

Table I: Agronomic growth parameters evaluated in *X. sagittifolium* plants inoculated with the different strains of AMF over time

Growth parameters					
Treatments apply	**Times (Days)**	**The average number of roots (NR)**	**Average Number of leaves (NL)**	**Average Leaves surface (SL) (cm^2)**	**Average Height of plants (HP) (cm)**
Control	**60**	14.40±1.51 a	2.50±0.61 a	21.01±11.27 a	17.70±1.77 a
	120	12.40±1.67 a	2.52±0.73 a	34.72±06.16 b	25.00±1.77 ab
	180	10.40±3.64 a	1.98±0.82 a	39.72±08.88 c	22.91±1.58 ab
Acaulospora tuberculata	**60**	15.80±3.36 a	2.62±0.80 a	23.23±11.37 a	18.28±1.23 a
	120	16.60±0.89 a	2.62±0.66 a	39.44±14.46 b	25.84±1.09 ab
	180	11.40±2.07 a	2.02±0.55 a	38.49±11.75 b	23.56±1.98 ab
Glomus sp	**60**	15.20±4.11 a	2.62±0.80 a	33.31±10.47 a	19.43±1.60 a
	120	15.40±1.85 a	3.00±0.57 a	41.44±12.23 b	28.54±1.03 b
	180	11.80±2.28 a	2.40±0.94 a	38.43±10.49 ab	24.57±2.74 ab
Glomus intraradices	**60**	15.60±2.60 a	2.50±0.83 a	29.67±10.72 a	17.04±2.39 a
	120	13.80±1.92 a	2.54±0.61 a	39.51±09.29 b	27.74±2.12 b
	180	10.00±2.54 a	2.30±0.76 a	38.57±10.42 b	24.37±2.14 ab
Gigaspora margarita	**60**	13.80±2.58 a	2.52±0.86 a	23.48±10.71 a	19.40±1.24 a
	120	16.40±0.54 a	2.56±0.81 a	40.84±12.69 b	26.92±1.36 ab
	180	10.60±3.84 a	2.06±0.96 a	37.98±11.25 b	25.05±1.09 ab
The mixture of four strains	**60**	15.60±3.36 a	3.36±0.82 a	36.55±11.73 a	20.26±2.24 a
	120	18.40±0.54 a	3.04±0.75 a	42.34±16.67 b	27.76±2.24 ab
	180	13.00±1.87 a	2.74±1.06 a	40.16±15.51 b	25.70±3.48 ab
Duncan's multiple range tests Data sharing the same letter in the same column and for each treatment were not significantly different at the 5% level					

Table II: Fresh and dry weight matter of *X. sagittifolium* in response to mycorrhization during growth and assessment of mycorrhizal status following treatments

Treatments Apply	Times (Days)	Average Of Total Fresh (g)	Average Of Total Dry (g)	Ratio F/D	DM (%)	F (%)	M (%)	a (%)	m (%)	A (%)
Control	**60**	17.26±3.13 a	03.81±1.14 a	4.53	0.00	0.00	0.00	0.00	0.00	0.00
	120	24.49±3.33 b	04.53±0.90 a	5.40	0.00	0.00	0.00	0.00	0.00	0.00
	180	32.04±4.35 c	11.61±1.31 b	2.75	0.00	0.00	0.00	0.00	0.00	0.00
Acaulospora tuberculata	**60**	22.47±1.33 a	03.09±0.46 a	7.27	12.32	53.66	17.19	08.40	32.03	1.50
	120	27.33±0.28 b	05.29±0.57 a	5.16	14.38	64.66	17.88	14.50	25.42	0.24
	180	28.26±0.94 b	14.35±2.64 b	1.95	19.10	26.33	01.66	13.50	04.38	2.35
Glomus sp	**60**	22.74±1.48 a	04.67±0.19 a	4.81	16,56	74.66	07.62	10.80	34.65	1.85
	120	30.22±0.49 b	05.92±2.46 a	5.10	23,49	70.33	17.17	06.70	39.03	0.30
	180	28.39±0.95 b	16.17±2.64 b	1.75	28,22	44.00	04.60	15.20	14.54	1.15
Glomus intraradices	**60**	18.78±0.90 a	04.30±0,97 a	4.36	08.63	36	08.98	14.70	24.94	2.36
	120	25.31±1.14 b	05.24±1.13 a	4.83	13,53	47	16.10	11.30	34.26	0.45
	180	42.50±5.87 c	15.13±5.36 b	2.80	23.26	38	04.04	13.70	09.18	1.23
Gigaspora margarita	**60**	21.08±0.85 a	04.18±0.42 a	4.94	11.49	22.00	01.92	12.10	02.57	1.44
	120	29.05±1.59 b	05.71±1.71 a	3.86	20.65	44.00	11.97	08.30	18.51	0.04
	180	38.11±2.57 c	14.62±2.58 b	2.39	20.96	31.66	00.51	18.10	01.95	0.34
The mixture of four strain	**60**	19.26±1.20 a	04.58±0.88 a	4.20	10.58	35.00	03.79	13.70	10.82	2.94
	120	30.86±0.80 b	06.20±1.87 a	4.97	26.85	61.66	18.18	16.50	29.49	0.90
	180	35.44±0.26 c	19.10±2.68 b	1.85	39.22	46.33	05.46	19.00	11.79	0.72

Duncan's multiple range tests
Data sharing the same letter in the same column and for each treatment were not significantly different at the 5% level

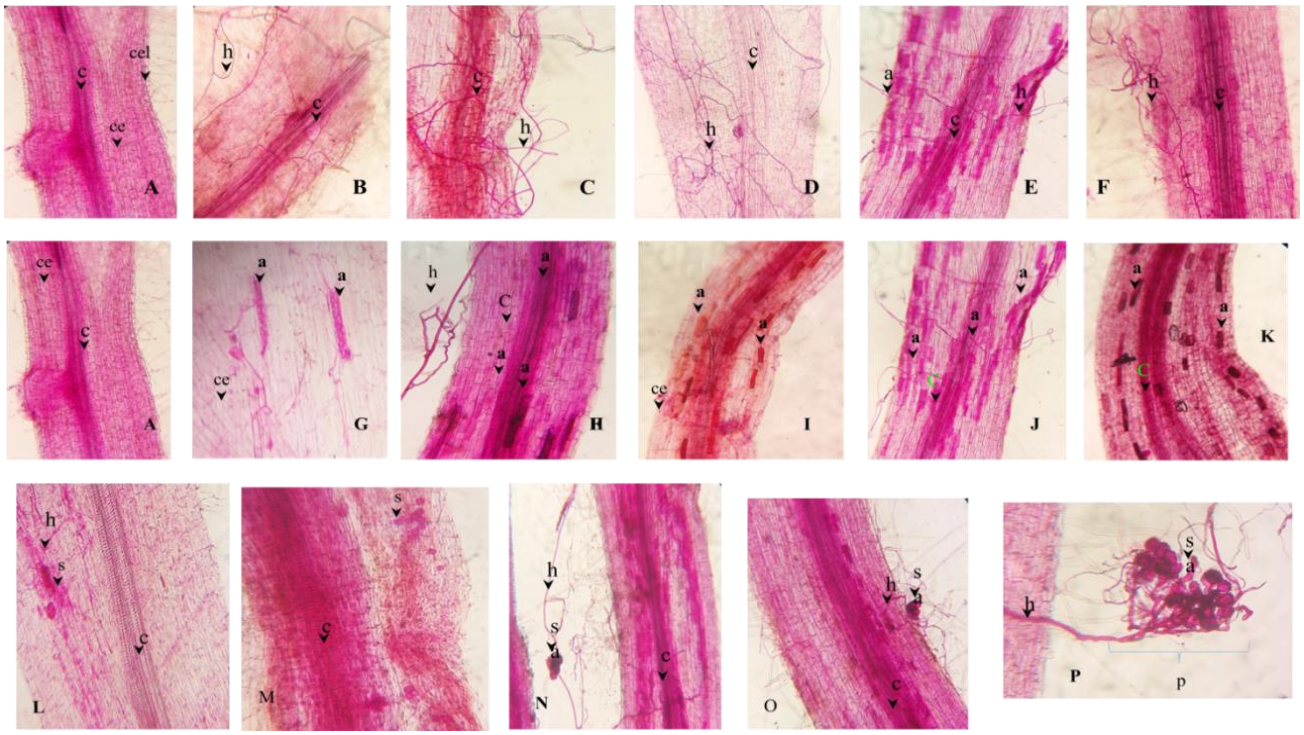

Figure 1: Microscopic observations at 400X of *Xanthosoma sagittifolium* root structures stained with Fuchsin acid. Control (A). Hyphae structures in plants inoculated with *Glomus intraradices* (B), *Acaulospora tuberculata* (C), *Gigaspora margarita* (D), *Glomus* sp. (E), and the mixture of four strains on that AMF (F). Arbuscular observed: in plants inoculated with *Glomus intraradices* (G), *Acaulospora tuberculata* (H), *Gigaspora margarita* (I), *Glomus* sp. (J), and the mixture of four strains on that AMF (K). Spore was observed: in plants inoculated with *Glomus* sp. (E), and with the mixture of the four strains of AMF (G). Appressorium (a), plant cells of the hypodermis (Ce), plant cells of the epidermis (cel), cortex (C), and hyphae (h). Arbuscular (a), branched pedicel (p), spore sac or spore mother (s), and spore (s)

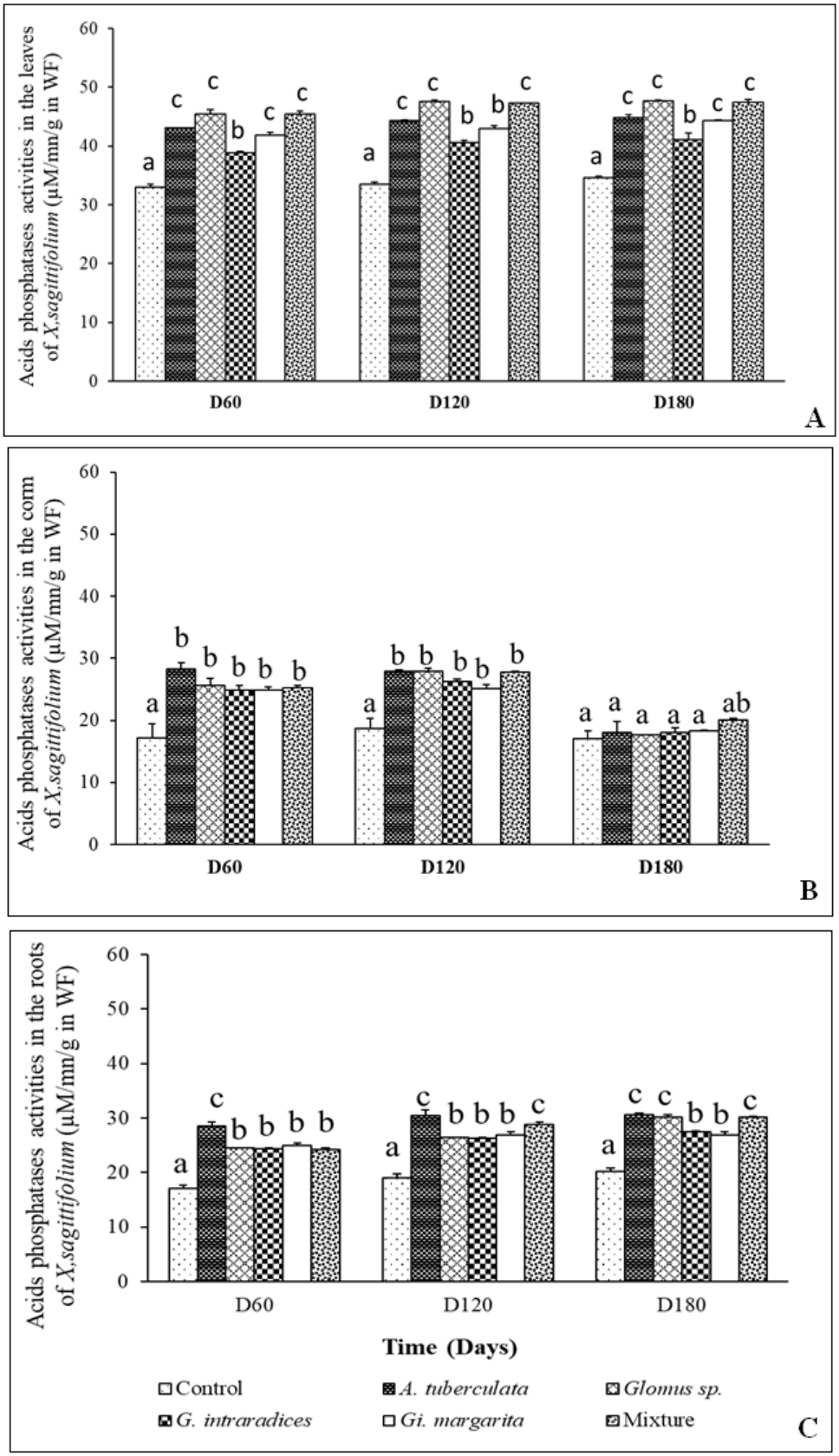

Figure 2: Acid phosphatase activities were evaluated in plants of *X. sagittifolium* inoculated during growth. Leaves (A), rhizomes (B), and roots (C)

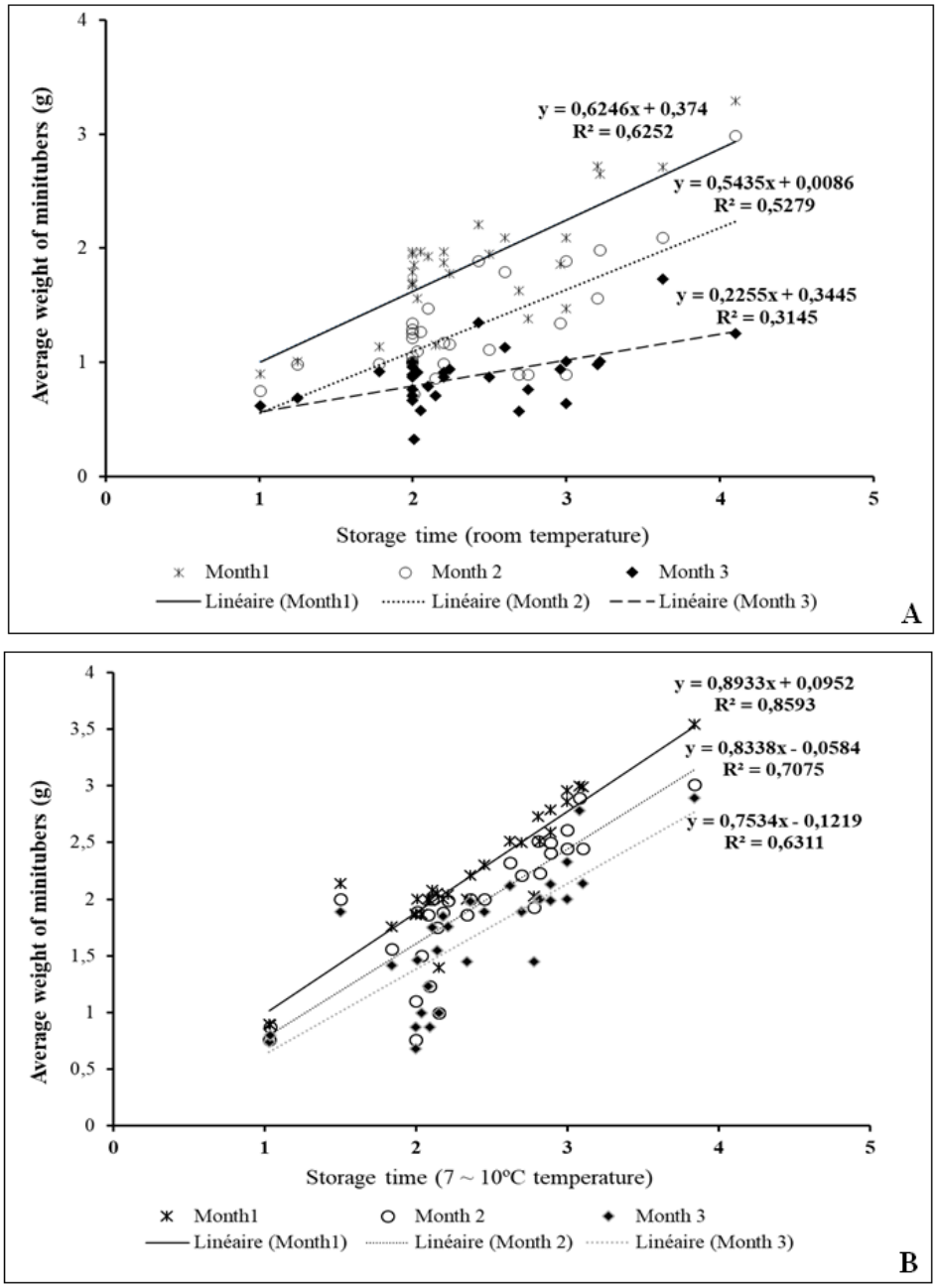

Figure 3: Regression curves of the mean weight of X. sagittifolium minitubers as a function of storage time. Room temperature (A) and at 7~10 °C (B)

Table III: Parameters evaluated on harvested minitubers

Treatments apply	NP	Parameters evaluated on *X. sagittifolium* minitubers harvested							
		NM	NTP	PT (%)	ANM	AMW (g)	I (%)	AHM (cm)	ADM (cm)
Control	**30**	17	15	50,00%	0,56±0,18 a	1,59±1,14 a	/	1,06±0,36 a	0,97±0,39 a
A. tuberculata	**30**	35	18	60,00%	1,16±0,64 a	2,44±1,40 b	34,84	2,58±0,99 c	1,97±0,92 c
Glomus sp.	**30**	39	16	53,33%	1,30±0,99 b	3,00±1,81 c	47,00	2,18±1,16 b	1,61±1,00 c
G. intraradices	**30**	37	18	60,00%	1,23±0,76 ab	2,84±1,44 b	44,01	2,04±0,71 b	1,50±0,74 b
Gi. margarita	**30**	33	17	53,33%	1,06±0,67 a	2,60±1,38 b	38,84	2,10±1,24 b	1,28±0,51 b
The mixture	**30**	45	22	73,33%	1,50±0,63 b	4,14±1,46 c	61,59	3,12±1,82 d	1,97±0,80 c

Duncan's multiple range tests, Data sharing the same letter in the same column were not significantly different at the 5% level
NP (Number of cultivated plants), NM (Number of minitubers), NTP (Number of tuberous plants), PT (Percentage of tuberization), ANM (Average number of minitubers plants), AMW (Average minitubers weight/plants (g)), I (Increased rate), AHM (Average height of Minitubers) and ADM (Average diameter of minitubers).

Table IV: Germination test in *Xanthosoma sagittifolium* minitubers harvested after three months of storage at room temperature and 7 ~ 10 ° C

Germination test				
Time (month)	**Conservation condition**	**Number of minitubers in germination**	**Number of minitubers germinated**	**Percentage of germination (%)**
1	Room temperature	**30**	21	70,00
2	Room temperature	**30**	10	33,33
3	Room temperature	**30**	0	00,00
1	7~10°C in freezer	**30**	28	93,33
2	7~10°C in freezer	**30**	15	50,00
3	7~10°C in freezer	**30**	1	03,33

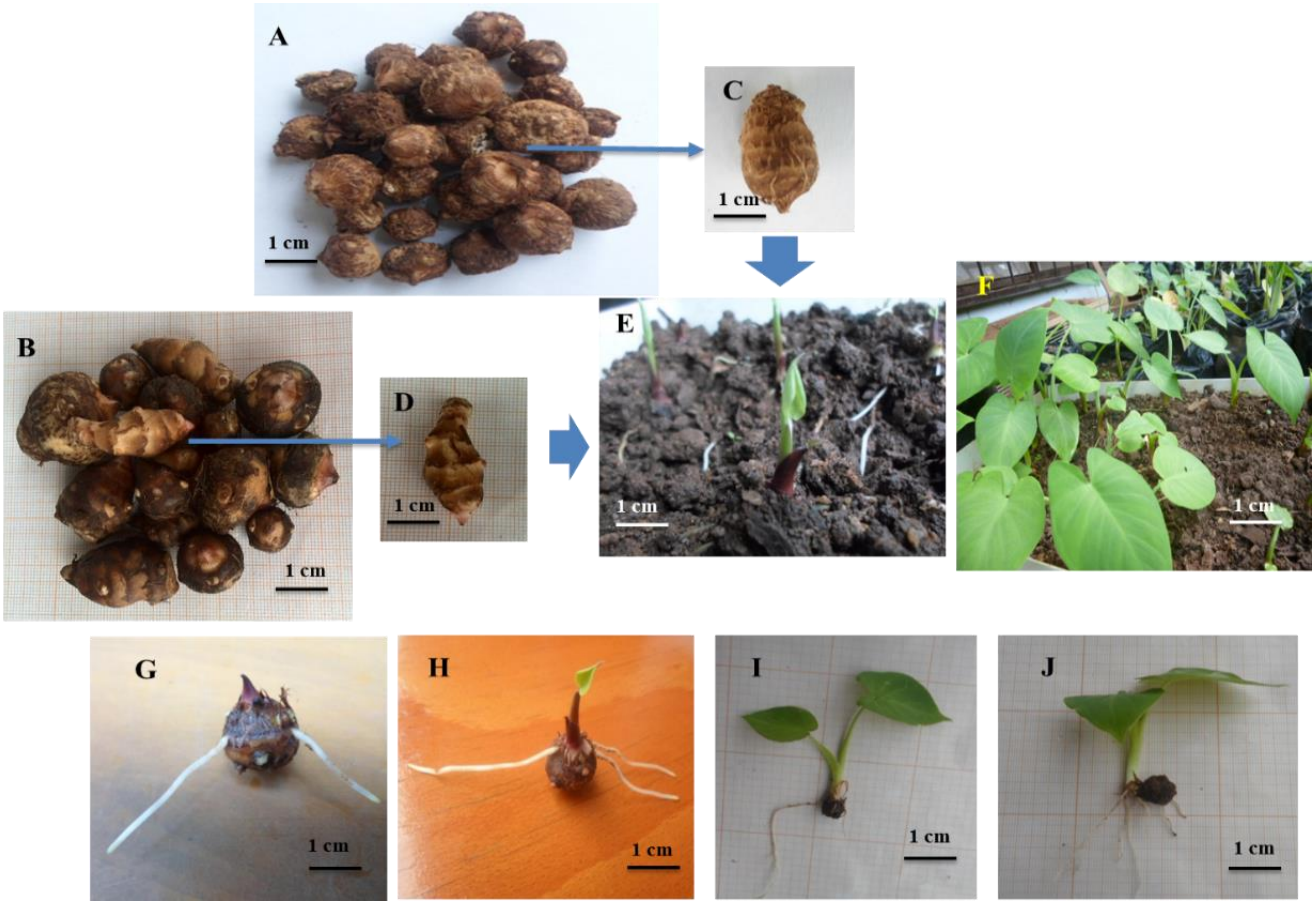

Figure 4: Germination test in *X. sagittifolium* minitubers after a month of growth. Minitubers are stored at room temperature (A and C); minitubers are stored at 7-10 ° C (B and D). The aspect of the growth after one week (E, G, and H) and after one month (F, I, and J)

DISCUSSION

The general objective of this research was to evaluate the effect of some strains of mycorrhizal biofertilizers on the growth and production of minitubers in *X. sagittifolium*, which can be used as sanitized seeds. The contribution of these mycorrhizal strains *Acaulospora tuberculata*, *Glomus* sp., *Glomus intraradices*, *Gigaspora margarita*, and their mixture significantly influenced growth and minituberization.

The agro-morphological parameters showed that the application of these arbuscular mycorrhizal fungi did not significantly influence ($P<0.05$) the average number of leaves and the average size of *X. sagittifolium* plants. The frequency of leaf appearance observed would be attributed to the leaf renewal of the plant during its growth. It was noted that the mean plant leaf area was higher in mycorrhizal plants compared to control plants. Chagnon, (2015); Begum *et al*., (2019); Higo *et al.*, (2020), and Jansa *et al*., (2020) noted that AMFs play a beneficial role in enhancing growth in plants. The increase in leaf area thus observed would be attributed to the stimulation of the production of growth hormones, influenced by the water and mineral nutrition of plants linked to the presence of AMFs, to maximize the leaf area available to carry out photosynthesis. in response to the demand for carbohydrates by these

AMFs. It should be noted that apart from the fact that the plant must feed the AMFs with carbohydrates, it must also store its reserves in the tubers. In *X. sagittifolium*, leaf area values were higher in plants inoculated with the mixture of the four AMF strains. This made it possible to highlight the existence of a synergistic action of these arbuscular mycorrhizal species used vis-à-vis the plant. The renewal of the leaves would have led to a reduction in the size of the plants. This result is contrary to that of Rafiq Lone *et al.,* (2015), who showed that the addition of *G. intraradices* or *G. mosseae* in the growing medium gradually improves the size of *Solanum tuberosum* plants. Moreover, the stimulation of the growth of *X. sagittifolium* plants during symbiosis would be linked to the increase in the root absorption surface in response to the increase in the number and size of the roots. However, the growth of the roots would depend directly on the humidity, the oxygen, the temperature, and the sugars sent by the leaves. Brunner (2001) showed that the presence of mycorrhizae in the rhizosphere had the effect of stimulating root growth.

The fresh and dry biomasses varied according to the treatments applied. The contribution of AMF positively and significantly influenced the production of biomass. For all these treatments, results obtained showed that the biomass increased significantly over time. According to Domokos *et al.,* (2018), this increase in biomass as being the product of the improvement in the absorption of nutrients and water directly involved in the process of photosynthesis. The highest fresh and dry biomasses were recorded at D_{120} and D_{180}, in inoculated plants with the mixture of the four AMF strains. This result would suggest the absence of an antagonistic effect between the strains (*Acaulospora tuberculata, Glomus* sp., *Glomus intraradices, Gigaspora margarita*) constituting the mixture. We would rather speak of a synergistic action, which made it possible to understand that; the more AMF strains there are in the substrate, the more the supply of the plant with nutrients and water would be improved.

Cytological analysis of *X. sagittifolium* root fragments after staining revealed mycorrhizal structures such as intracellular hyphae as well as arbuscules and spores. Similar results were observed by AL-Hadidi *et al.* (2021) in *Ipomoea batatas*. Cells in aggregates of 4 to 18 subglobose to ovoid to clavate characteristics of Gigasporaceae were also observed. Their abundance in the mixture of strains would be the result of the synergistic action of all the AMF strains applied. The more AMFs are complex, the more they have the possibility of producing all their characteristic structures. Analyzes of the frequency of mycorrhization showed that it was significant in *X. sagittifolium* inoculated with *Glomus* sp., with a peak frequency of approximately 75% on D_{60}. The values of this frequency and also that of the intensity of mycorrhization decreased over time. The variation of the frequency and intensity maxima showed that there is no specific effect between the white cultivar of *X. sagittifolium* and the AMF strains applied. Chagnon (2015), has also shown that there is rarely a specific effect between an AMF and a plant species, because AMFs are ubiquitous, having the ability to colonize most terrestrial plants. This root colonization decreases over time, and can only be explained by the fact that during growth, the plant would have carried out root renewal. The arbuscule contents recorded were very high in the plants inoculated with the mixture of the four AM strains. Thus, the abundance of arbuscules was also observed in plants inoculated with the mixture treatment. Their abundant presence would be due to the number of AMF strains constituting the mixture. Mycorrhizal dependence (MD) results in inoculated *X. sagittifolium* plants were less than 50% for all mycorrhizal treatments. The maximum values are obtained in plants inoculated with the mixture of the four AMF strains (39.22%). These values made it possible to classify *X. sagittifolium* as being a plant that is moderately dependent on the mycorrhizal association. Similar results are obtained in *Coffea arabica* (Jaramillo and Osorio 2009) with mycorrhizal dependence values below 50%. The results obtained could be justified by the fact that the mycorrhizal associations are not specific to a given type of plant. Thioye *et al.*, (2018), showed that the DM values in mycorrhizal plants depend on the type of AMF used and their ability to sufficiently uptake mineral elements for plant nutrition. However, the high MD observed on D_{120} in all the mycorrhizal treatments would reflect the cost in terms of nutrient requirements during the symbiosis of *X. sagittifolium* strain of AMF, to be able to carry out both tuberization and feed the mycorrhizal fungus with carbon at the same time. arbuscules.

The results showed that mycorrhization stimulated the production of minitubers. The percentages of tuberization were greater than 50% (those obtained in the controls). The double or even triple mycorrhization of *X. sagittifolium* concerning the number of minitubers was obtained in the controls (17 minitubers). The highest number of minitubers was recorded in plants inoculated with the mixture of the four AMF strains. This increase in production would be the direct result of improved plant development and stimulation of photosynthesis due to the synergistic action of AMFs. The minitubers obtained presented a significant weight and size in the plants inoculated with the mixture of the four strains of AMF. These results are similar to those of Liu *et al.,* (2018), who pointed out that inoculation of potato plants with *Rhizophagus irregularis* and *Glomus proliferum* would have increased biomass, tuber size, tuber ratio, and plant growth under low content conditions. in phosphorus. Moreover, it should be noted that this association is increasingly beneficial in the production of potato seeds (Nurbaity *et al.,* 2019).

Acid phosphatase activity is very high in the leaves, rhizomes, and roots of plants inoculated with *X. sagittifolium* compared to control plants. This increase in phosphatase activity in plants during symbiosis would be directly proportional to the levels of mycorrhizal colonization. These results are in agreement with those of Smith *et al.,* (2011) and Gosling *et al.,* (2013), which showed that the increase in acid phosphatase activities is a function of the assimilation of Phosphate (P) by the plant during symbiosis. These very high acid phosphatase activities in the leaves could be the product of degradation or recycling of pre-existing forms of organic P stored in the leaf vacuoles of *X. sagittifolium* in response to perceived Pi deficiency at the soil level. In the rhizomes, the activities of acid phosphatases decrease after 120 days of cultivation. However, in the roots, it is found that this activity of acid phosphatases is very high compared to the rhizomes. The presence of these acid phosphatases in the rhizomes of *X. sagittifolium* could be explained by their translocation from the leaves of the plant to the roots. In leaves and roots, these acid phosphatase activities increase over time. It is observed that this activity of acid phosphatases in the rhizomes, unlike the leaves and roots, decreases after 120 days of growth. Tran *et al.,* (2010) have also shown that once secreted by the roots, acid phosphatases play a significant role in the degradation of organic forms of phosphorus in the rhizosphere. The presence of arbuscular mycorrhizal fungi in the rhizosphere of inoculated *X. sagittifolium* plants would have facilitated the secretion of these acid phosphatases at the root level compared to control plants.

However, the analyzes of the minituber preservation tests showed that the average weight of the minitubers gradually decreases over time. This decrease in the average weight varied less at 7~10°C, compared to the ambient temperature. The germination capacity of the minitubers was almost nil in the two treatments after three months of storage. This decrease in the average weight of the minitubers and the loss of germination power were attributed to the loss of water which would therefore have led to a loss of their physiological potential. Segnou *et al.,* (2012) think that the deterioration of the physiological state observed cannot be stopped, but can be more or less delayed by suitable storage conditions.

CONCLUSION

The individual application of the strains of *Acaulospora tuberculata, Glomus* sp., *Glomus intraradices, Gigaspora margarita,* and their mixture made it possible to improve the growth of the foliar surface of the leaves and also the production of the minitubers in *X. sagittifolium*. These considerable caliber minitubers can be used as sanitized seeds. The mixture of the four AMF species had a synergistic effect on plant growth and productivity. The conservation of the minitubers at a temperature of 7~10°C made it possible to preserve for two months about 50% of the germination power. Very high acid phosphatase activities in leaves and roots are thought to be related to Pi deficiency in the growing medium.

Thanks

The authors would like to thank the Laboratory of Plant Physiology and Biochemistry, for the supply of the material used in this study, the *X. sagittifolium* work team for their dynamism, and the Laboratory of Biological Control and Applied Microbiology, IRAD Cameroon, for updating the arrangement of strains of all AMFs used.

Conflict of Interest: The authors declare that there is no conflict of interest.

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Cite This Article: Djeuani, A. C, Mbouobda, H. D, Ngonkeu, M. E. L, Niemenak, N (2023). Mycorrhization of *Xanthosoma sagittifolium* L. Schott Plants (White Cultivar): Evaluation of Their Effect on Acid Phosphatase Activity During Growth and Production of Minitubers. *East African Scholars J Agri Life Sci*, *6*(1), 1-14.