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Morphological Characterization of Arbuscular Mycorrhizal Fungi Associated with the Rhizosphere According to the Age of *Xanthosoma sagittifolium* L. Schott Plants in the Field

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Abstract

The objective of this work was to carry out a morphological characterization of arbuscular mycorrhizal fungi in the rhizosphere of *Xanthosoma sagittifolium* L. Schott plants. The plant material used was the white and red cultivars of *X. sagittifolium*, belonging to age intervals of 3 - 6, 6 - 9, and 9 - 12 months. Three harvest sites were chosen in the Central Region of Cameroon. In each site, soil from the rhizosphere and plant roots was collected in a randomized manner. In the field, the agronomic parameters were evaluated. The physicochemical characteristics of the soils, the mycorrhization index, and the morphological characterization of the mycorrhizal types of each site were carried out. The results obtained show that the agronomic growth parameters varied significantly using the Student Newman and Keuls Test depending on the harvest sites. The soils' pH in all sites was acidic and ranged between 4.6 and 5.8. The Nkometou site has a loamy texture while the Olembe and Soa sites have loam-clay-sandy and loam-clay textures respectively. The highest mycorrhization frequencies appeared at the Nkometou site, with 75 and 87.33% of the white and red cultivars plant roots at 6 - 9 and 3 - 6 months. The relative abundance of AMF arbuscular mycorrhizal fungal spores in the rhizosphere of *X. sagittifolium* plants varied with age and cultivar. There were 673

spores between 9 - 12 months in Nkometou in the red cultivar. Six AMF genera were identified in all the different soils collected: *Acaulospora* sp., *Funneliformis* sp., *Gigaspora* sp., *Glomus* sp., *Scutellospora* sp., and *Septoglomus* sp. The genus *Glomus* sp. was the most present at all age intervals in both cultivars.

Keywords

Xanthosoma sagittifolium L. Schott, Rhizosphere, Harvest Site, Arbuscular Mycorrhizal Fungi, Diversity

1. Introduction

Xanthosoma sagittifolium L. Schott is ranked among the most important tuberous plants in the world [1], Africa is the main source of production through Nigeria, Cameroon, and Ghana [2]. This plant is consumed in most African countries and its production is ensured by small farmers with limited resources and mainly women. Formerly classified among neglected plants authors are increasingly giving capital interest to the cultivation of *X. sagittifolium* [3] [4]. This speculation is a staple food for populations in tropical and subtropical regions, for human and animal nutrition. The leaves and tubers of the plant are the parts mainly consumed. The tubers are rich in minerals such as calcium, phosphorus, potassium, and magnesium [5] [6]. Leaves and flowers contain apigenin, which gives to plant anti-cancer and anti-diabetic properties [7] [8].

Mycorrhization tests show that an application of allochthonous arbuscular mycorrhizal fungi in *X. sagittifolium* plants contributes to improving not only their growth but also to boosting the production of seed minitubers [9] [10]. This application of mycorrhizal fungi in propagators also makes it possible to increase the production of PIF seed plants in *X. sagittifolium* and likewise their growth in the field [11] [12] [13]. However, by concluding that *X. sagittifolium* is a mycotrophic plant, [9] highlights the hypothesis of calling into question the knowledge of the biodiversity of indigenous arbuscular mycorrhizal fungi associated to the rhizosphere of *X. sagittifolium* plants. So many questions needed to be answered likewise; are there specific species of arbuscular mycorrhizal fungi associated with the rhizosphere of *X. sagittifolium*? Do communities of arbuscular mycorrhizal fungi in the rhizosphere vary depending on the soil type, plants age, harvest site, etc.? Do the white and red cultivars of *X. sagittifolium* establish symbiosis with the same types of arbuscular mycorrhizal fungi species? Exploring the diversity of indigenous arbuscular mycorrhizal fungi in the rhizosphere of *X. sagittifolium* can depend on the cultivars. That is why this research work focuses on the determination of the different potential genera of arbuscular mycorrhizal fungi associated with the rhizosphere of white and red cultivars in *X. sagittifolium*. More specifically, the investigations will make it possible to: evaluate the agronomic parameters plants of *X. sagittifolium* white and red cultivars

in the field following the age; the nature of the soils associated with the rhizosphere according to the different harvesting sites; and to determine a morphological characterization of different genus of arbuscular mycorrhizal fungi present in the rhizosphere.

2. Material and Methods

2.1. Study and Sampling Sites

The soil and root sample collection sites used are located in the Central region of Cameroon. The harvests were carried out in Nkometou, (Lékié Department, at geographical coordinates; 4˚5'29"N and 11˚36'16"E, with 600 m of altitude), Olembe (Mfoundi Department, at 3˚56'43"N and 11˚31'46"E, with 690 m of altitude), and Soa, (Department of Mefou and Akono at 3˚59'45"N and 11˚36'49"E, with 680 m of altitude) (Figure 1). These three sites belong to the forest agroecological zone with bimodal rainfall of Cameroon, characterized by a humid equatorial climate.

2.2. Evaluation of Agro Morphological Growth Parameters in *X. sagittifolium* Plants in the Field According to Harvest Sites

The plant material used consisted of plants of the white and red cultivars of *X. sagittifolium* with age intervals of 3 - 6, 6 - 9, and 9 - 12 months. These plants were chosen in the field in a randomized manner at each site. The field of each site had a minimum size of 1 ha. For each age group, agronomic growth parameters such as average plant height; the average diameter at the collar of the plants; the average number of leaves, the average leaf area; the average number of roots, and the average weight of the roots [9] [13] [14], were evaluated on 10 *X. sagittifolium* plants chosen at random in the field following the harvest sites.

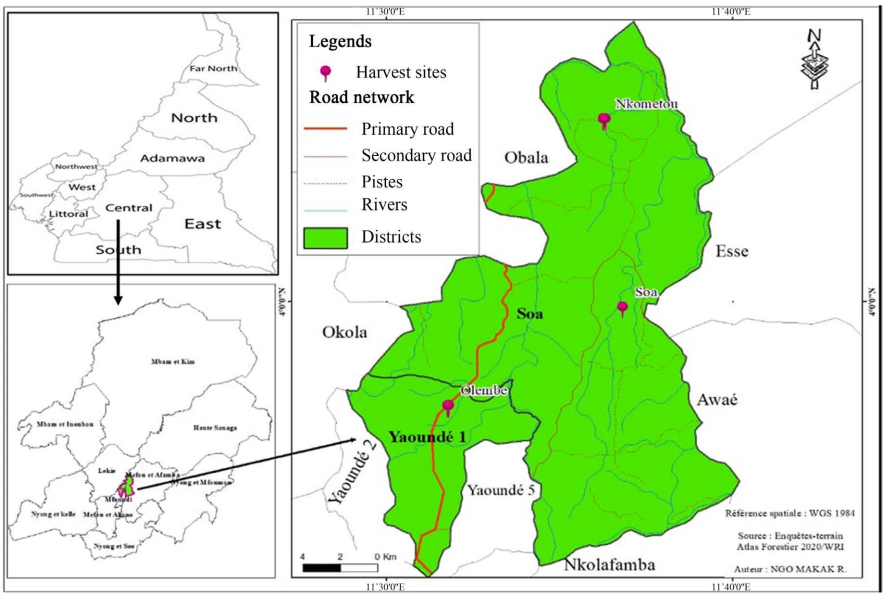

Figure 1. Geographical location of rhizosphere soil and root sampling sites of *Xanthosoma sagittifolium* plants.

From these same previously selected plants, the soil of the rhizosphere, as well as root fragments were taken for laboratory analyses.

2.3. Sampling and Analysis of Physicochemical Soil Parameters

Each sample of these soils was taken at a depth of 0 - 20 cm using an auger at the level of the rhizosphere of the *X. sagittifolium* plants. For each age group (3 - 6, 6 - 9, and 9 - 12 months) of the targeted plants in each harvest site, three repetitions were carried out. These soils by age group will then be mixed. 500 g, will be bagged then labeled, and brought back to the laboratory for analysis. Analyzes of the physicochemical characteristics of these soils were carried out at the University of Dschang, by the Soil Analysis and Environmental Chemistry Research Unit of the Faculty of Agronomy and Agricultural Sciences. The physical parameters of the soil (sand, clay and silt contents) and chemical parameters (pH-H_2O and pH-KCl), organic carbon (CO) content, total nitrogen content (N), exchangeable base content (Ca, Mg, K and Na), cation exchange capacity (CEC) and assimilable phosphorus content (P Bray II), exchangeable base sum (EBS) and the rate of saturation (V), were determined and analyzed according to the standards of [15] [16] [17] protocols.

2.4. Determination of the Mycorrhizal Status of Plant Root Fragments in *X. sagittifolium* Histological Analysis and Assessment of Mycorrhizal Status

The root hairs of *X. sagittifolium* of the white and red cultivars collected in the field at each site were cleaned, drained, and sectioned to a size of 0.5 to 1.0 cm. The protocol of [18] was used. These root hairs were soaked in 10 $g \cdot L^{-1}$ KOH for 15 minutes at 90˚C, then rinsed with HCl (10%). The dye used was trypan blue 1 $g \cdot L^{-1}$. Microscopic observations were carried out using the HYMEN brand optical microscope at 400X. 100 of these fragments were observed for each age group and per site. Mycorrhizal status was calculated following the scale proposed by [18]. The parameters F%, M%, m%, a%, A%, v% and V% were calculated.

2.5. Extraction, Enumeration, and Characterization of Spores

The method of [19] was used for the extraction and enumeration of spores in the soil. This method is based on sieving the soil through a set of superimposed sieves having different mesh diameters; 0.5; 0.25; 0.125 and 0.0625 µm. 10 mL of pellet from each soil sample sieved according to the age groups (3 - 6, 6 - 9, and 9 - 12 months), will be observed under a binocular magnifying glass with an X40 objective. An exhaustive count of spores for each age group was carried out in the watch glasses. This operation was carried out in 10 fields and an average was calculated based on the number of spores found. Thus, the total number of spores/soil collected was evaluated from the sum of the number obtained in sieves 2, 3, and 4 (0.25; 0.125; 0.0625 µm). Spore density was determined for each soil sample. The different spores resulting from the extraction and enumeration of soils were described morphologically according to the identification keys of

[20], from the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi [21], and the European Bank of Glomales. The classification of species was made using the method of [22]. Species diversity was measured using the species richness of the diversity indices of [23]. The Shannon index (H) varying from 0 (a single species, or one species very largely dominant over all the others) to log S (when all the species have the same abundance) was calculated according to the following formula:

$$H = -\sum_{i=1}^{S} ni/N \, Log\left(ni/N\right)$$

Where: S = total number of species; Ni = number of individuals of gender (i) in a given category; N = total number of individuals of all genders in a given category; log2 = logarithm to base 2; H = Shannon diversity index.

2.6. Statistical Analyzes

The results obtained were the subject of a descriptive analysis (Mean ± standard deviation). The results are represented in the form of graphs and tables (Microsoft Excel 2013 software). The IBM SPSS Version 20.0 software was used to carry out the statistical analyses and to compare the means using an analysis of variance (ANOVA) using the Student-Newman-Keuls test at the 5% threshold.

3. Results

3.1. Agronomic Parameters Recorded in the White and Red Cultivars of *X. sagittifolium* in the Field in the Different Harvest Sites Following the Age Month Intervals

The average plant height varied significantly at $P < 0.05$ by the Student Newman and Keuls Test in plants of the two cultivars of *X. sagittifolium*. We see that this height increases depending on the age interval and the site (Table 1). Low average height values were recorded for both cultivars between 3 - 6 months of growth. *X. sagittifolium* plants presented greater average heights at the Nkometou site compared to those of Soa and Olembe. The plants of the white cultivar appear larger than those of the red cultivar. We note significant average values of 133.13 ± 040.60 and 158.73 ± 007.54 cm, between 6 - 9 and 9 - 12 months respectively in the Nkometou site. The average diameter at the collar showed maximum values greater than 7cm at the age interval of 9 - 12 months, in *X. sagittifolium* plants from the Nkometou site (07.53 ± 01.02 cm) in the cultivar white. In plants of the cultivar, it is 07.74 ± 02.66 and 07.65 ± 01.35 cm, respectively at the age interval of 9 - 12 months, in Olembe and Soa. The average number of leaves did not vary significantly in the Student Newman and Keul Test in all cultivars (Table 1).

Generally speaking, the average leaf area of the leaves increases significantly ($P < 0.05$) following the age interval for the white and red cultivars (Table 1). At the age interval of 6 - 9 months, this average leaf area of leaves appears very large in both cultivars. A maximum of 1840.52 ± 371.41 cm^2 is recorded, at a growth

Table 1. Variation in agronomic parameters of white and red cultivars of *X. sagittifolium* plants in the field following age intervals.

Cultivars	Harvest sites	Age month interval	Growth parameters evaluated					
			Average plant height (cm)	Average collar diameter (cm)	Average number of leaves	Average leaf area (cm^2)	Average number of roots	Average root weight (g)
White	Nkometou	3 - 6	062.30 ± 18.16bc	02.36 ± 00.51a	01.66 ± 00.57a	483.59 ± 095.12ab	17.00 ± 05.29ab	16.23 ± 01.64b
		6 - 9	133.13 ± 40.60e	05.00 ± 00.45b	03.00 ± 01.00a	1534.72 ± 237.04cd	31.66 ± 08.14bc	90.69 ± 05.64c
		9 - 12	158.73 ± 07.54f	07.53 ± 01.02c	03.00 ± 01.00a	1840.52 ± 371.47d	62.33 ± 09.01d	186.38 ± 26.94c
	Olembe	3 - 6	057.24 ± 01.82bc	02.06 ± 00.98a	02.00 ± 01.00a	406.83 ± 093.02ab	16.80 ± 07.00ab	01.78 ± 00.54a
		6 - 9	038.60 ± 09.38ab	01.28 ± 00.55a	01.60 ± 00.57a	197.53 ± 029.77ab	15.40 ± 03.60ab	04.24 ± 00.34a
		9 - 12	103.15 ± 1.35d	05.25 ± 03.18b	03.50 ± 00.50a	1402.57 ± 324.17cd	50.00 ± 49.49bcd	23.96 ± 14.04b
	Soa	3 - 6	024.50 ± 10.40a	00.96 ± 00.17a	01.80 ± 00.00a	137.87 ± 019.21ab	26.20 ± 01.73bc	09.79 ± 00.92a
		6 - 9	074.66 ± 05.68c	04.48 ± 00.25b	03.83 ± 00.94a	758.78 ± 143.27abc	44.66 ± 17.21bcd	316.65 ± 27.13e
		9 - 12	121.6 ± 08.88de	05.44 ± 00.46b	03.20 ± 00.81a	1091.20 ± 25.08bc	42.60 ± 07.09bcd	200.69 ± 08.73d
Red	Nkometou	3 - 6	062.83 ± 07.25bc	01.43 ± 00.60a	01.66 ± 00.57a	228.01 ± 041.73ab	12.66 ± 07.3ab	07.70 ± 01.34a
		6 - 9	102.76 ± 12.91d	04.30 ± 01.57b	02.33 ± 00.57a	681.86 ± 148.32abc	67.33 ± 40.06bcd	108.91 ± 28.75c
		9 - 12	118.33 ± 04.61de	06.93 ± 00.05bc	04.00 ± 01.00a	1294.21 ± 289.74bc	106.00 ± 12.76f	223.59 ± 16.14 d
	Olembe	3 - 6	054.52 ± 12.56b	01.80 ± 00.45a	01.80 ± 00.57a	293.89 ± 074.20ab	09.80 ± 01.52 a	04.26 ± 00.66a
		6 - 9	078.35 ± 21.78c	04.05 ± 00.66b	01.75 ± 00.57a	500.99 ± 167.78ab	29.75 ± 13.22abc	52.85 ± 04.13b
		9 - 12	125.24 ± 38.58e	07.74 ± 02.66c	03.20 ± 00.88a	1542.34 ± 115.29c	71.20 ± 27.50cd	164.26 ± 15.32c
	Soa	3 - 6	026.25 ± 02.80a	01.14 ± 00.15a	01.85 ± 00.47a	152.91 ± 050.93a	19.57 ± 07.37ab	13.75 ± 00.20a
		6 - 9	070.00 ± 05.39bc	04.10 ± 00.60b	03.00 ± 00.00a	862.56 ± 287.74bc	37.16 ± 09.50abc	133.25 ± 01.82c
		9 - 12	133.11 ± 05.29e	07.65 ± 01.35c	03.50 ± 00.81a	2564.55 ± 417.76d	98.00 ± 39.87e	625.42 ± 18.86f

Student Newman Keuls tests.
Data sharing the same letter in the same column and for each treatment were not significantly different at the 5% level.

age interval of 9 - 12 months, in plants of the white cultivar of *X. sagittifolium*, from the Nkometou site. In plants of the red cultivar, it is 3119.33 ± 517.76 cm^2 at the age interval of 9 - 12 months at the Soa site. However, the results show an increase in the values of the number and average weight of roots over time (Table 1). The average number of roots varies significantly ($P < 0.05$) with a maximum value of 106.00 ± 012.72, recorded in plants of the red cultivar from the Nkometou site. However, for the average weight of fresh roots harvested, although it is low for the age interval of 3 - 6 months, it should be noted that the significant values are 316.65 ± 27.13 and 625.42 ± 18.86 g, respectively for plants of white and red cultivars from the Soa site at the age interval of 6 - 9 and 9 - 12 months (Table 1).

3.2. Physicochemical Characteristics of the Soil at Different Sites

The physical properties of the soils studied varied according to the three sites (Table 2). From the analysis triangle of the mineral texture of the soils, we note

Table 2. Physicochemical analyses of the soils of *X. sagittifolium* plant rhizosphere in each harvest site. Sand (S%), clay (L%), and Loam (L%) contents. pH (aqueous (pH-H_2O) and acid (pH-KCl)), carbon (C), total nitrogen (N), phosphorus (P), organic matter (OM). Exchangeable bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+); Cation exchange capacity (CEC); Assimilable phosphorus content (P Bray II); Exchangeable Base Sum (EBS), Saturation Rate (V).

Harvest sites	pH		EBS ($cmol{\cdot}kg^{-1}$)	CEC ($cmol{\cdot}kg^{-1}$)	K^+ (%)	Ca^{2+} (%)	Mg^{2+} (%)	Na^+ (%)	V (%)	OM (%)	P (%)	N (%)	C (%)	C/N	Physical analysis			Texture
	H_2O	KCl													Clay (%)	Loan (%)	Sand (%)	
Nkometou	5.8	4.7	5.54	1.32	15.5	3.21	0.98	0.03	35.74	3.18	5.81	1.26	1.85	1.47	23.25	46.75	30	Loamy
Olembe	5.8	4.6	5.6	2.05	16.45	2.45	1.08	0.02	34.04	3.54	40.54	1.75	2.05	1.17	31.25	9.5	59.25	Loamy-clayey-Sandy
Soa	5.6	4.8	5.22	1.85	17.85	2.15	1.21	0.01	29.24	3.89	168.99	0.455	2.26	4.96	35.75	40	24.25	Loamy-Clayey

that the Nkometou site has a loamy texture, with 23.25% clay, 46.75% silt, and 30% sand. The Olembe and Soa sites have respectively loam-clay-sand textures (31.25% clay, 9.5% silt, 59.25% sand) and loamy-clay (35.75%). % clay, 40% silt, and 24.25% sand). The soils of the different study sites showed an acidic character. The pH of water and hydrochloric acid is between 4.6 and 5.8 (Table 2). The results of these soils from the three *X. sagittifolium* cultivation sites have an average organic matter content. We record 3.18; 3.54 and 3.89% respectively in Nkometou, Olembe, and Soa. The Nkometou site appears richest in total nitrogen. The C/N ratio is very high at the Soa site with a peak of 4.96 but appears very low in the Nkometou (1.74) and Olembe (1.17) sites. The results also show that assimilable phosphorus was very high in the soil of the Soa site with a value of 168.99%. However, it should be noted that the low value of phosphorus content is recorded at the Nkometou site (5.81%). As for the soil of the Olembe site, it is moderately poor in phosphorus (40.54%). Furthermore, according to the standard proposed by Calvet and Villemin (1986), the three sites have high CEC contents. Furthermore, the sum of exchangeable bases varied very little with normal contents. It is 5.54 (0.03% Na^+; 3.21% Ca^{2+}; 0.98% Mg^{2+}; 1.32% K^+) for the Nkometou site, 5.6 (0.02% Na^+; 2 .45% Ca^{2+}; 1.08% Mg^{2+}; 2.05% K^+) for Olembe and 5.22 (0.01 Na^+; 2.15% Ca^{2+}; 1.21 Mg^{2+}; 1.85 K^+) for Soa.

3.3. Mycorrhizal Status of Plant Roots Following Harvest Sites as a Function of Harvest Age Month Interval

The results of the mycorrhizal status assessed varied according to the three harvest sites for the white and red cultivars (Table 3). The value of the percentage of vesicular intensity of AMF appears very low. The highest mycorrhization frequencies (F%) appear at the Nkometou site. The maxima were 75 and 87.33% respectively in the root hairs of plants of white and red cultivars at 6 - 9 and 9 - 12 months. At Nkometou, we note a higher intensity of mycorrhization compared to the Olembe and Soa sites (Table 3). The maxima are 40.73% for the mycorrhizal intensity (M%) for Nkometou red cultivar plants of 3 - 6 months, 61.02% for the mycorrhizal intensity in the root system (m%) at age interval of

Table 3. Mycorrhizal status of the roots of white and red cultivars *X. sagittifolium* plants, according to the age interval and according to the harvest site. F%: Frequency of mycorrhizal colonization, M%: Intensity of mycorrhizal colonization, m%: Intensity of mycorrhizal colonization in the root system, a%: Arbuscular content, A%: Arbuscular intensity in the root system, v%: Vesicular intensity of the mycorrhizal part and V%: Vesicular intensity in the root system. Numbers followed by the same letter in the same column are not significantly different in the Newman and Keuls Student Test (5%).

Cultivars	Harvest sites	Age month interval	Mycorrhizal status of the roots						
			F (%)	M (%)	m (%)	a (%)	A (%)	v (%)	V (%)
White	Nkometou	3 - 6	68.00	29.68	43.70	14.30	04.27	00.06	00.01
		6 - 9	75.00	26.34	35.07	15.35	04.11	00.05	00.01
		9 - 12	49.33	11.80	24.22	20.86	02.58	00.50	00.05
	Olembe	3 - 6	22.50	01.02	04.55	14.75	00.30	00.00	00.00
		6 - 9	30.00	03.84	10.43	25.66	01.31	00.00	00.00
		9 - 12	28.00	08.50	29.07	04.95	00.31	00.25	00.01
	Soa	3 - 6	23.33	00.43	01.97	14.96	00.08	00.00	00.00
		6 - 9	37.66	02.86	06.11	25.23	00.61	00.53	00.02
		9 - 12	56.00	17.14	30.92	17.30	02.40	01.06	00.16
Red	Nkometou	3 - 6	87.33	40.73	46.58	24.13	10.01	04.66	02.09
		6 - 9	82.66	35.33	42.68	40.00	13.55	02.86	00.92
		9 - 12	76.33	26.35	33.89	40.23	10.45	08.03	01.91
	Olembe	3 - 6	37.00	21.78	61.02	11.43	02.74	03.20	00.47
		6 - 9	31.66	13.60	61.02	25.73	02.09	03.00	00.42
		9 - 12	54.33	15.00	25.64	23.96	03.08	00.10	00.02
	Soa	3 - 6	49.00	05.57	10.29	12.86	00.06	00.00	00.00
		6 - 9	17.66	02.43	11.65	34.03	00.97	00.03	00.00
		9 - 12	44.33	08.11	17.33	09.36	00.92	00.10	00.01

3 - 6, and 6 - 9 months in plants from the Olembe site and 13.55% for arbuscular intensity in the root system (A%) at the age interval of 6 - 9 months for plants of the red Nkometou cultivar.

3.4. Relative Abundance and Diversity Index of Rhizosphere Arbuscular Mycorrhizal Fungi in *X. sagittifolium* Plants Following Harvest Sites as a Function of Harvest Age Month Interval

Graphs of the relative abundance of AMF spores in the rhizosphere of plants of *X. sagittifolium* cultivars white and red following the harvest site, several spores vary from one harvest site to another depending on age and cultivar (Figure 2(A) and Figure 2(B); Figure 3). In the white cultivar, the maxima were observed in plants aged between 9 - 12 months. It is 470,280, and 673 spores respectively at the site of Nkometou, Olembe, and Soa. Likewise, the results also show that the minimums were recorded in plants aged 3 - 6 months in Nkometou with a value of 112 spores in 50 g of soil (Figure 2(A)). In the red cultivar, the maxima are

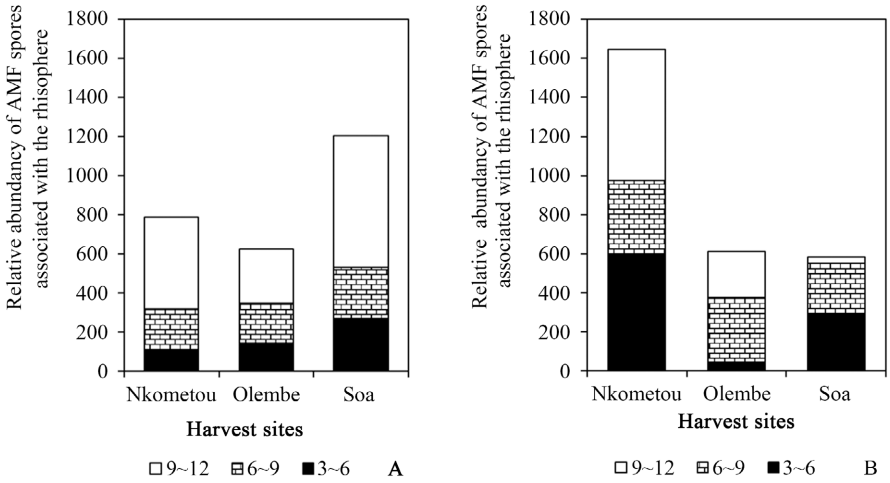

Figure 2. Relative abundance of AMF spores following the age intervals of the white and red cultivars of *Xanthosoma sagittifolium* in the three harvest sites.

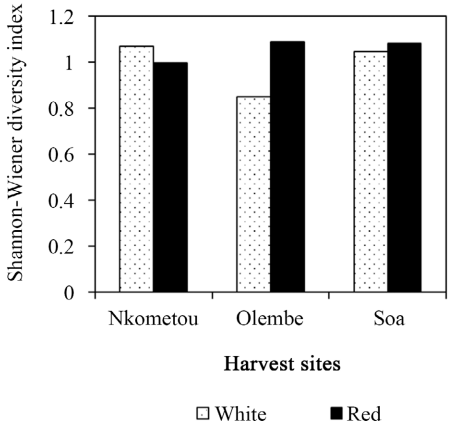

Figure 3. Shannon and Weiner diversity index in the three harvest sites.

notably at the age interval of 9 - 12 months (673 spores), 6 - 9 months (331 spores), and 3 - 6 (294 spores) respectively in the plants of *X. sagittifolium* in Nkometou, Olembe and Soa (**Figure 2(B)**). Low relative abundance was observed in plants aged 9 - 12 in the Soa site (33 spores). The Shannon and Weiner diversity index in the three collection sites presents a specific diversity of AMF of the rhizosphere in the plants of the white and red cultivars of *X. sagittifolium* in the Soa site compared to the Nkometou and Olembe (**Figure 3**).

3.5. Morphological Appearance of the Spores of AMF Associated with the Rhizosphere of Plants of the White and Red Cultivar of *X. sagittifolium* Following the Age Month Intervals

Observations of AMF spores under a microscope at 200 µm showed significant variation between the spores associated with the rhizosphere of plants of white and red cultivars of *X. sagittifolium*, particularly in terms of shapes, colors, walls, and slits. spores. 6 genera of AMF have been recorded; *Glomus* sp. *Acaulospora* sp., *Scutellospora* sp., *Septoglomus* sp. *Gigaspora* sp., and *Funneliformis* sp

(Figure 4 and Figure 5). In white cultivars, of these six genera, the 5 AMF genera recorded are *Acaulospora* sp., *Glomus* sp., *Gigaspora* sp., *Scutellospora* sp., and *Septoglomus* sp. (Figure 4). While in the red cultivar, the results present *Acaulospora* sp., *Gigaspora* sp. *Glomus* sp. *Funneliformis* sp., and *Scutellospora* sp. (Figure 5). *Glomus* sp. spores were the most abundant genera in the rhizosphere of both cultivars at all ages. It also appears most represented at the rhizosphere of *X. sagittifolium* at Olembe and Soa, compared to Nkometou at the age interval of 6 - 9 and 9 - 12 (Figure 4 and Figure 5).

3.6. Correlation Study between the Evaluated Parameters

The factor plot of the principal component analysis of the data confirms a positive correlation between agronomic parameters and mycorrhizal status (Figure 6). This linear correlation, thanks to Pearson's rank coefficient R, indicates a highly significant correlation at 5% at the age interval 9 - 12 months in the three sites, between the parameters; average size of plants, average number of leaves, average leaf surface, average diameter at the collar, average number of roots and average weight of roots, in the white cultivar plants from the Nkometou site and the same in the red cultivar plants from the sites by Olembe and Soa. Furthermore, it appears that all the parameters of the mycorrhization index are significantly correlated with the relative abundance of spores, in white cultivar plants from the Nkometou site, at the age interval, 3 - 6, 6 - 9, and 9 - 12 months. However, the analysis of the Pearson correlation between the physicochemical

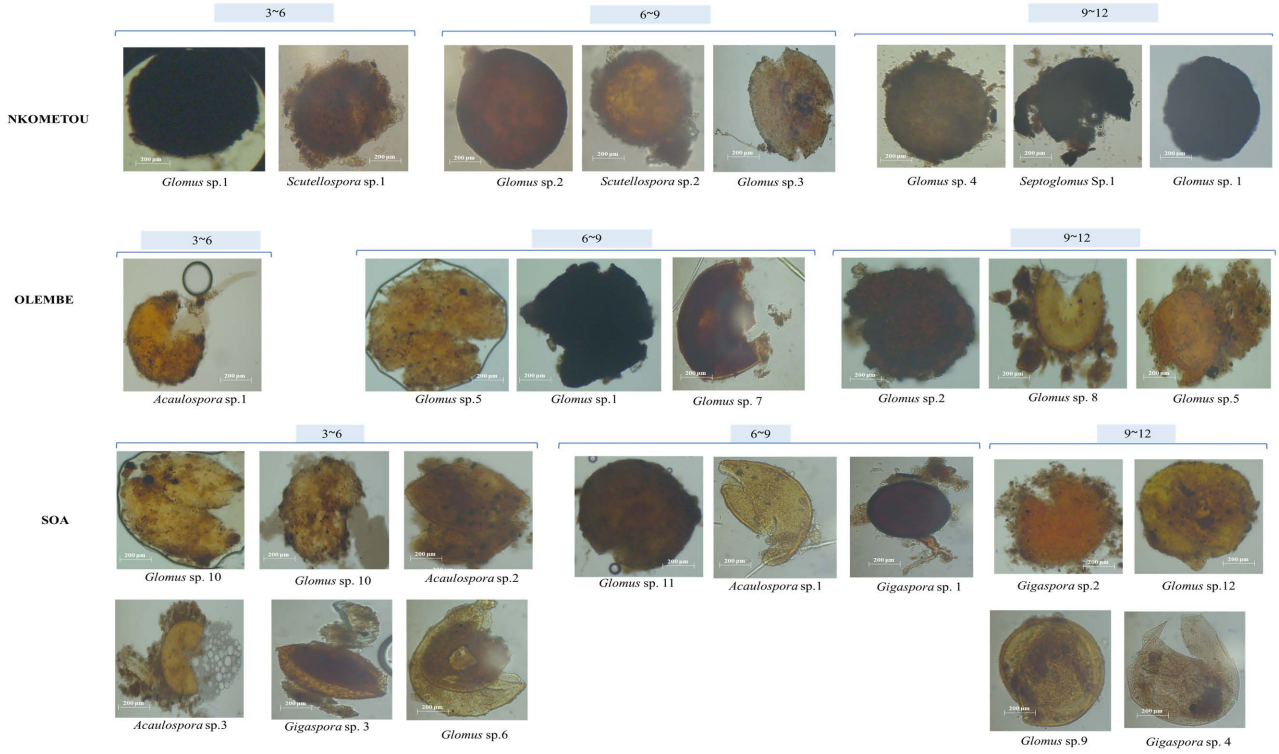

Figure 4. Morphological appearances of the arbuscular mycorrhizal fungi spores associated with the rhizosphere of the white cultivar of *X. sagittifolium* plants following the age intervals observed with a Leica brand light microscope at 200 µm.

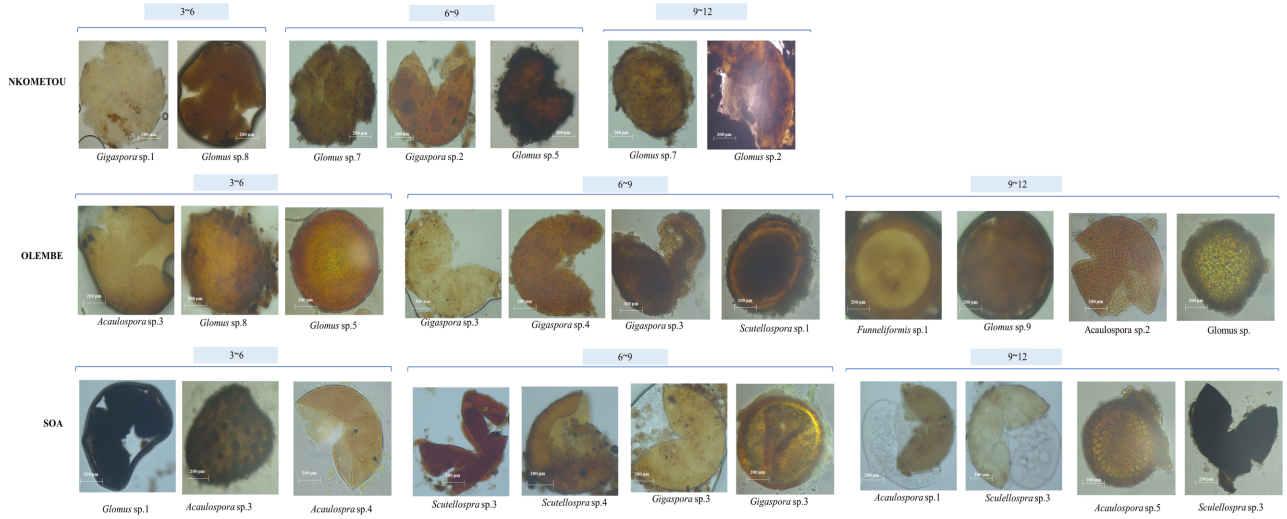

Figure 5. Morphological appearances of the arbuscular mycorrhizal fungi spores associated with the rhizosphere of the red cultivar of *X. sagittifolium* plants following the age intervals observed with a Leica brand light microscope at 200µm.

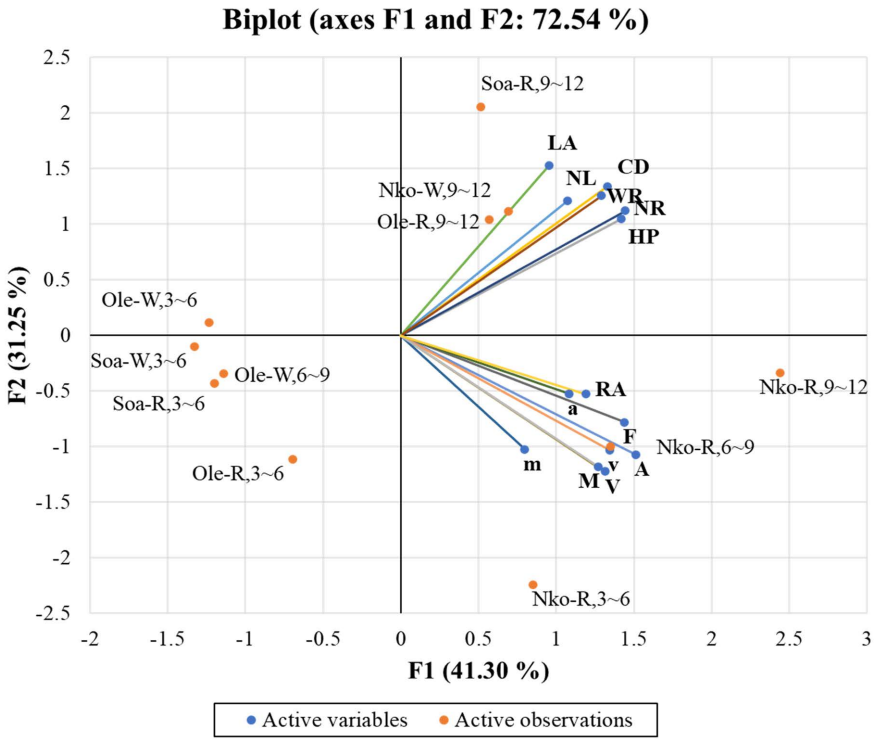

Figure 6. Pearson correlation between agronomic parameters, mycorrhizal status, and relative abundance. R: red, Bl: white; LA: leaf area; NL: Number of leaves; DC: Diameter of the collar; WR: Weight of Root: NR: Number of roots; RA: relative abundance, F: Frequency of mycorrhizal colonization, M: Intensity of mycorrhizal colonization, m: Intensity of mycorrhizal colonization in the root system, a: Arbuscule content, A: Arbuscular intensity in the root system, v: Vesicular intensity of the part mycorrhizal and V%: Vesicular intensity in the root system.

parameters of the soils, the relative abundance of spores, and the Shannon index, presents a contribution from axis 1 (Fact. 1) of 75.23% and axis 2 (Fact. 2) by 24.77%. That is a total of 100% variability (Figure 7). It, therefore, appears at the

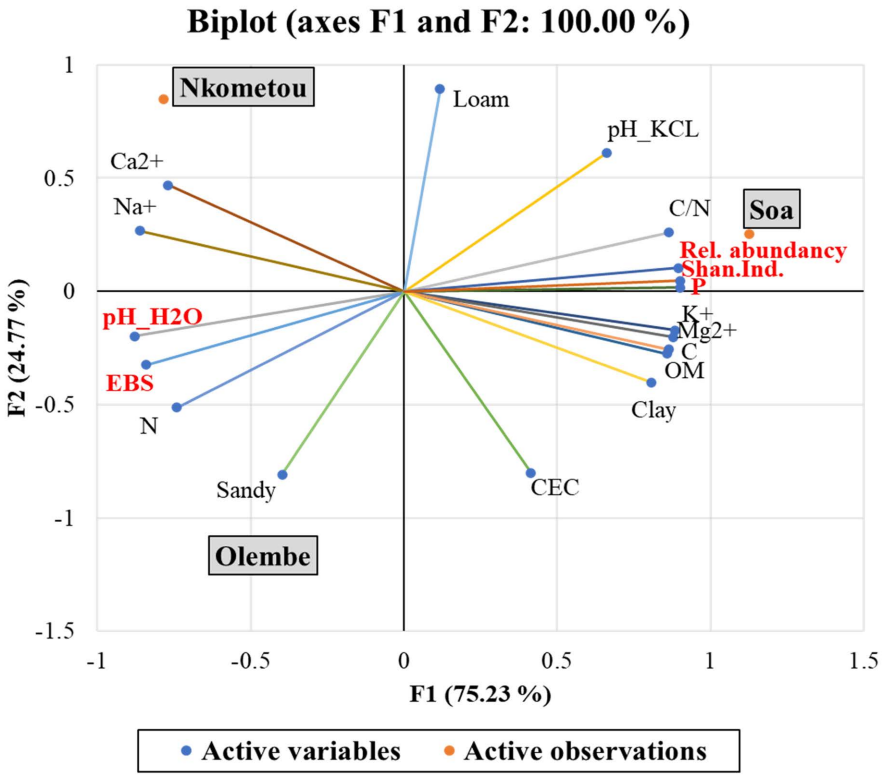

Figure 7. Pearson correlation between soil analysis parameters, relative spore abundance, and Shannon index. pH (aqueous (pH-H_2O) and acid (pH-KCl)), carbon (C), total nitrogen (N), phosphorus (P), organic matter (OM). Exchangeable bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+); Cation exchange capacity (CEC); Assimilable phosphorus content (P Bray II); Exchangeable Base Sum (EBS), Saturation Rate (V). Relative abundancy (Rel. abundancy) and Shannon index (Shan. ind.).

Soa site, a positive and significant correlation between the parameter's assimilable phosphorus content, the abundance of spores, and the Shannon index (Figure 7). However, in Olembe, there is a significantly negative correlation for the pH-H_2O and EBS parameters.

4. Discussion

The morphological growth parameters varied significantly by the Student's and Keuls' Test at $P < 0.05$. These parameters increase over time in all sites and are important at the Nkometou site compared to the Olembe and Soa sites. In Nkometou, the average plant sizes, collar diameter, leaf area, and number of plant roots presented higher values in the white cultivar compared to the red cultivar. This increase in growth parameters observed would be the result of the physiological response of the *X. sagittifolium* plants, to the availability of the nutrients necessary for their growth on one hand in the soils of these sites and on the other hand, the presence of microorganisms in these soils which are in symbiosis with *X. sagittifolium* plants, would contribute to the improvement of their hydromineral nutrition. These beneficial soil microorganisms provide favorable conditions for plant development [24]. The very high average number of roots

between 9 - 12 months, in both cultivars and all sites, would explain that at this age, *X. sagittifolium* plants would need more nutrients to ensure not only its hydromineral nutrition but also storage in tubers. Furthermore, this variation in agronomic growth parameters recorded between month intervals in white and red cultivars, at all sites, could also be the result of variation in physicochemical characteristics such as; the pH, the sum of exchangeable bases, the C/N ratio, etc. in the soils of these different sites. [25], have also shown that soil pH and hydromineral nutrition significantly influence growth in poplar (*Populus tremuloides*), jack pine (*Pinus banksiana*), and white spruce (*Picea glauca*) seeds grown on sand. According to [26], the nature and properties of the soil play a critical role in plant growth.

The results of soil analysis present physicochemical characteristics that differ relatively from one site to another. The soils of the Nkometou site have a loamy texture, compared to those of Olembe and Soa, which are loamy-clay-sandy and loamy-clayey respectively. The textures of these three sites appear suitable for the cultivation of *X. sagittifolium*. According to [27] and [28], these soil texture types are easily well-drained and well-aerated. [29] and [30], showed that growth and optimal tuberization in *X. sagittifolium* require light soils, rich in organic matter, well-drained, deep, well loosened and having a pH between 5.5 and 6.5. Chemical analyses show that the different soils of the three sites studied have acidic pHs, with values between 5.6 and 5.8 for pH (H_2O) and 4.6 and 4.8 for pH (HCl). The majority of Cameroonian soils are acidic [31]. According to [28], *X. sagittifolium* plants exhibit optimum growth and production at a pH varying between 5.5 and 6.5 (pH-H_2O). However, it has been shown that pH (5.8 to 6.3) is satisfactory for good biological activity and nutritional exchanges in the soil [26] [32]. The soils of these three harvest sites had a C/N ratio < 6, which would reflect the rapid decomposition of organic matter. These soils were very rich in total nitrogen with values all > 0.25. The Olembe site, with a loamy-clayey-sandy texture, had a greater sand richness compared to the other sites and likewise a higher total nitrogen content. [33] emphasize that, in sandy soils, which are soils with great aeration, the mineralization of nitrogen is very rapid. According to the fertility scale for assimilable phosphorus of [34], the assimilable phosphorus contents varied significantly. The values recorded were 5.81 ppm (Nkometou), 40.54 ppm (Olembe), and 168.99 ppm (Soa). The soils of the Nkometou site appeared poor in phosphorus. [35], mentioned that the soils poverty in phosphorus and also the impact of acidity on the CEC of the soil are characteristic of tropical soils. In the soils of the different sites studied, the CEC is higher in Olembe compared to Nkometou and Soa. Of the four exchangeable bases determined, the percentage of Potassium (K^+) is higher compared to Calcium (Ca^{2+}), Magnesium (Mg^{2+}), and Sodium (Na^+). These minerals are involved in important physiological processes for plants: photosynthesis, fruitification, cell permeability, and ionic balances [36]. This high K^+ content is very beneficial for *X. sagittifolium* plants. [36] and [37] showed that potassium not only contributes to strengthening cell walls but also to increasing leaf surface area and consequently,

the chlorophyll content of leaves in plants.

The results show that the mycorrhizal status values varied depending on the cultivars and the age range of the plant. The work of [9] showed that *X. sagittifolium* is a mycotrophic plant. This variation in mycorrhizal status values would be influenced not only by the root renewal that plants make during their growth but also by the properties of the soil. The high (in Nkometou) or low (in Olembe and Soa) mycorrhization frequencies in the two cultivars at all age intervals could be explained by the fact that the mycorrhizal infection is the result of the AMF response. to the effects of hormones emitted by plants. Generally, the high frequency of mycorrhization in plants from 6 - 9 months can be explained by the fact that at this age, the plants already carry out the physiological phenomenon of tuberization (storage). However, this storage in plants requires good photosynthesis. However, in symbiosis, *X. sagittifolium* plants should also respond in terms of carbohydrate cost to AMF. In plants of the red cultivar, from the Nkometou site, the highest frequency of mycorrhization was observed in plants aged 3 - 6 months. This could be justified by the fact that these plants were located on an anthill. [38], show that the presence of ants in the soil would influence the symbiosis through the process of bioturbation, which, due to the high temperatures in the anthills, would not only lead to the accumulation of mycorrhiza spores. In the soil, ants and termites act like earthworms; they contribute to the establishment of the symbiosis between plants and microorganisms through the release of phytohormones [39].

Results for relative spore abundance varied from site to site. This relative abundance of spores appears very high in plants of *X. sagittifolium*, with an age interval of 9 - 12 months in the three sites in plants of the white cultivar. However, we note a value of 673 spores/50g of rhizosphere soil in plants from the NKOMETOU site in plants of the red cultivar. This high abundance observed could be explained by the fact that the lower the phosphorus levels in the soil, the higher the quantity of spores appears. Similar results were obtained by [40], with Bambara groundnut plants. The analysis of this abundance made it possible to understand the richness and diversity of the AMF communities present in each site. This variation could also be attributed to environmental conditions. Moreover, [41] report that this abundance of spores depends on the physicochemical properties of the soil. Likewise, the availability of nutrients in the soil would influence the presence of spores. According to [42], loamy and sandy soils with nutrient deficiency would have a high number of AMF propagules and species. The variation in spore density could also be attributed to the presence of plant cover, which would contribute to the creation of a vast network of hyphae and interconnections with the roots of host plants, improving not only biomass but also the microbial activity of the soil [43]. The soils of Nkometou and Soa showed a higher quantity of spores compared to the soil of Olembe. Additionally, the soil at the Soa site showed a higher amount of assimilable phosphorus and an acidic pH at a value of 5.6. However, at this pH value, the phosphorus in the soil is in dihydrogen phosphate form, which can be assimi-

lated by the plant. The results of this work reveal a spore density estimated at 5457 spores for the total of 150 g of soil for the three collection sites, this density is significantly higher than that obtained by [44] (around 4045 spores per 100 g of soil) under cowpea cultivation in the different agroecological zones of Benin.

The six AMF genera were identified in the two cultivars of *X. sagittifolium* in the three study sites following the age intervals. These were the genera *Acaulospora* sp., *Funneliformis* sp., *Gigaspora* sp., *Glomus* sp., *Scutellospora* sp., and *Septoglomus* sp., belonging to three families; *Acaulosporaceae*, *Gigasporaceae,* and *Glomeraceae*. These different types of AMF identified were not present in all the harvested sites. It is noted that, in the rhizosphere of plants with white cultivars, the AMF genera recorded were *Acaulospora* sp., *Gigaspora* sp., *Glomus* sp., *Scutellospora* sp., and *Septoglomus* sp. Whereas, in plants of the red cultivar, these are the genera *Acaulospora* sp., *Gigaspora* sp., *Glomus* sp., *Funneliformis* sp., and *Scutellospora* sp. The genus *Septoglomus* sp., was only present in the rhizosphere of *X. sagittifolium* plants of the white cultivar while *Scutellospora* sp. only appeared in the rhizosphere of the red cultivar plants. However, the genus *Glomus* sp. appeared most in the rhizosphere of *X. sagittifolium* at the sites of Olembe and Soa, compared to Nkometou at the age intervals of 6 - 9 and 9 - 12. The work of [45] showed that the soils of Cameroon are richer in AMF of the genus *Glomus* sp. Similarly, [46] highlighted the richness of the AMF of *Glomus* sp. in the rhizosphere of *Gossypium hirsutum* L. plants in North Cameroon. This abundance of the *Glomus* sp. genus could be explained by the fact that this species of AMF can multiply or reproduce and adapt to extreme conditions and acidic soils. Furthermore, the Shannon index greater than 1 obtained at the Soa site explains the diversity of AMF observed associated with the rhizosphere of *Xanthosoma sagittifolium* plants.

5. Conclusion

This pioneering study made it possible to highlight a few genera of AMF associated with the rhizosphere of white and red cultivars of *Xanthosoma sagittifolium* L. Schott plants in the field. Of the three study sites with different physicochemical properties, it should be noted that the genus of AMF, which was more represent in all age intervals is the genus *Glomus* sp. The genus *Septoglomus* sp., was only present in the rhizosphere of *X. sagittifolium* plants of the white cultivar while *Scutellospora* sp. only appeared in the rhizosphere of plants of the red cultivar.

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Conflict and interest

The authors declare no conflicts of interest regarding the publication of this paper.

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