



Application of the PIF Method in Seed Multiplication in *Xanthosoma sagittifolium* L. Schott: Effect of the Mass of the Corm Fragment and Realization of the Field Transfer Test

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Abstract: The unavailability of seeds is a real problem for farmers growing *Xanthosoma sagittifolium* L. Schott plants. To propose palliative solutions, this work aimed to produce seeds of *X. sagittifolium* by the PIF method. The growing medium during the production of the PIF plants was a mixture of wood shavings and sawdust (1: 1). 60 fragments of rhizomes for the white and red cultivars of *X. sagittifolium* were used, at a rate of 30 fragments/cultivar with 15 fragments of weight between [100-250] and 15 others between [250-500]. The PIF plants produced have been acclimatized. The experimental device was a complete randomized block in fields after the transfer with two repetitions. For each cultivar, three treatments were applied, control, arbuscular mycorrhizal fungi, and chicken manure. The agronomic growth parameters were evaluated every 30 days for three months. The results obtained showed that the number of weaned PIF plants was higher at D₆₀, for the rhizome fragments with a weight range of [250-500] with 03.13±00.83 and 04.13±00.12 weaned PIF plants/rhizome recorded in the red and white cultivars respectively. The PIF technique made it possible to produce 455 PIF from 60 rhizome fragments after 90 days for the two cultivars, with 222 PIF plants in the white cultivar and 233 PIF plants in the red. The highest agronomic growth parameters were noted in the PIF plants produced in propagators, also at the weight intervals of [250-500]. Mycorrhizal influenced the growth of the PIF plants in the field. In addition, the applied PIF technique made it possible to multiply by 7.58 times the 60 fragments of rhizomes used. It is therefore a way that could be explored in the multiplication of sanitized seed plants in *X. sagittifolium*.

Keywords: *Xanthosoma sagittifolium* (L.) Schott, PIF Method, PIF Plants, Corm Mass Interval, Field Transfer

1. Introduction

Xanthosoma sagittifolium (L.) Schott (cocoyam) is a tropical and subtropical root and tuber plant grown for its starchy bulbs [1]. Classified among the most important tuberous plants in the world [2, 3], Africa is the main

production center through Nigeria, Cameroon, and Ghana [2]. This plant is consumed in most African countries and its production has always been done by small farmers with limited resources mainly women. Formerly classified as a neglected plant [4, 5], authors are increasingly taking a vital interest in the cultivation of *X. sagittifolium*. It is recognized

that the tubers of this plant have a higher nutritional value than other root crops and basic tubers, especially in terms of starch quality [6, 7], protein source [8], and its digestibility [6]. We also noted its richness in minerals such as calcium, phosphorus, potassium, and magnesium [9, 8]. The leaves of *X. sagittifolium* are a source of protein, fiber, mineral elements, vitamins, antioxidants, and dietary fiber [10-12]. Researchers also attributed to these leaves a richness in apigenin. In addition, antihyperglycemic, antihypertensive, hypoglycemic, and prebiotic properties have also been recorded [13, 3].

Apart from its multiple interests, the production of *X. sagittifolium* contributes to food security in several producing countries. In Cameroon, and for some years now, the disastrous spectacle witnessed during all the cultivation campaigns is that of the unavailability of healthy or sanitized seeds for growers wishing to set up monoculture plantations of one hectare or even more. The seed most used so far is the fragment of corms or tubers of previous plants harvested in the field [15-18]. The major drawback of the use of corm fragments as a seed so far in almost the entire country is the possibility of transporting and transferring pathogens from one field to another [15, 16] because their health status is not known. Similarly, the use of tubers as seeds today would be the cause of the rise in the price of tubers on the markets. Among the many types of research carried out, most of it focuses on the production of healthy seeds. Progressive efforts are increasingly being made to improve the production of *X. sagittifolium*, including the use of *in vitro* culture in the production of vitroplants [16], the production of microtubers from vitroplants [19], the production of vitroplants using the TIS (Temporary immersion system) method [20] and the production of minitubers from acclimated vitroplants [21, 17]. These various interesting ways of industrial exploitation are very costly for the farmer. However, with regard to the boom in the production of seed plants in *Musa* sp., from the application of PIF techniques (a plant from stem fragment), the major interest would be to know what would be the response of fragments of corms of *X. sagittifolium* following application of this technique. [22], showed that the PIF technique could be done in two ways; therefore, regenerate a young plant from the sucker fragment or cause a proliferation of secondary buds on either side of the sucker following the removal of the main bud. Several advantages of this approach are appreciated. It is a less expensive technique compared to the *in vitro* culture technique and the culture technique in temporary immersion systems, it thus contributes to the rapid production of material of the *vivo*-plant type; can be carried out at any time of the year without difficulty [22-24], requires a reduced production space, contributes to the rapid production of desired varieties, allowing the exploitation of the majority of the buds of the plant and mass production of genetically identical suckers, in a clean environment and at a reduced time. The growing medium used in this technique is either wood chips or sawdust. Sometimes the mixture of wood chips plus sawdust at a 1:1 ratio was also recommended [24].

In addition, the application of this technique in *Colocasia esculenta* boosted seed production 4.99 times from the 120 corm fragments used [24]. However, could the weight of the corm fragments influence the production of these PIF plants? Moreover, what would be the response of these PIF plants to mycorrhization during acclimatization before transfer to the field? What types of fertilizers could be used to quickly improve their growth after transfer to the field? However, in seed production, having an associated fertilizer is an asset. It is in this perspective that the research work aimed to produce seeds of *Xanthosoma sagittifolium* L. Schott by the PIF method while highlighting the influence of the weight of the fragments of corm used and the evaluation of the action of certain fertilizers applied in fields after their transfer.

2. Material and Methods

2.1. Plant Material

The plant material used for this study consisted of fragments of corms of *X. sagittifolium*. The corm fragments are the part of *X. sagittifolium* plant, used as seed by farmers. These fragments of corms of white and red cultivars were collected from plants of ages varying between 6 and 12 months, in a peasant's field located in the locality of MBAMA, Arrondissement of ATOCK, Region of East Cameroon.

2.2. Cultivation of Corm Fragments of *X. sagittifolium* for the Production of *in Vivo* Plants Sanitized by the PIF Method

X. sagittifolium PIF sanitized *vivo* plants were produced in propagators following the method of [24]. White and red cultivars of *X. sagittifolium* were used. For each cultivar, 30 corm fragments were used. These corm fragments were cleaned and trimmed. They were soaked and disinfected in a 30‰ sodium hypochlorite solution for 40 minutes. Three rinses with water were carried out at 10, 15, and 30 minutes respectively. After disinfection, they were dried at room temperature for 24 hours. The masses of these different corm fragments were determined. These corms were subdivided into two batches of 15 fragments following a mass interval of [100-250] and [250-500]. A right-angle cross incision was also made on the main bud of each corm fragment to destroy its apical dominance. The seeding of its corm fragments was done in propagators built using wooden planks. Four propagators were made, one propagator per mass interval for each cultivar. The dimensions of the propagators were 2.5×1.5 m (length x width). The growing medium consisted of a mixture of wood shavings and sawdust at a 1:1 ratio. These propagators have been filled with growing medium. The sowing of the disinfected corm fragments in the culture substrate, according to their weight interval, was done randomly in these propagators. The seeded corm fragments were 50 cm apart at a right angle. The propagators were covered with transparent plastic throughout the manipulation. The frequency of watering the seeded corm fragments was

once every 72 hours. The growth of *X. sagittifolium* *in vivo* plants was monitored for 90 days. The growth parameters evaluated at D₃₀, D₆₀, and D₉₀ were the total number of buds having burst according to the mass intervals per cultivar, the variation in the number of buds produced over time, the average number of buds emitted/corm, the number of PIF plants weaned over time, the total number of PIF plants weaned/ mass interval, the total number of PIF plants weaned/cultivar, and the mean PIF plants weaned per corm.

2.3. Agronomic Growth Parameters Evaluated in PIF Plants Before Weaning

To assess the growth, agronomic parameters were evaluated at D₃₀, D₆₀, and D₉₀ in each propagator, according to the mass intervals [100-250] and [250-500]. The average height of the plants, the average number of leaves, the average leaf area, the average collar diameter, and the average leaf area were evaluated according to the method of [16, 17]. At the end of each month, weanings of the PIF plants of *X. sagittifolium* were carried out. Weaning consisted of detaching the vigorous PIF plant, presenting at least 2 to 3 well-opened leaves and having a size greater than or equal to 25cm from the corm fragment using a knife. The weaned plants were then cultured on a substrate consisting of a mixture of black soil and sterilized sand (2:1) in 1L polystyrene bags and were acclimatized for one month. Watering was carried out daily morning and evening, before transfer to the field.

2.4. Transfer of PIF Plants of *X. sagittifolium* in the Field

After 1 month of acclimatization, a field transfer test was carried out. The transferred PIF plants were subsequently fertilized. The experimental device was a randomized complete block. Two repetitions were carried out. For each cultivar of *X. sagittifolium* used, three treatments were applied: control, arbuscular mycorrhizal fungi (AMF), and chicken manure. The mycorrhizal PIF plants were inoculated during the one month of acclimatization, with a complex of strains of arbuscular mycorrhizal fungi (AMF), consisting of a mixture of strains of the genus *Glomus* (*Glomus etunicatum*, *Glomus microggregatum*, *Glomus intraradices*, *Glomus mosseae*, *Glomus claroideum*, and *Glomus geosporum*). However, chicken manure was applied in the field 14 days

before the next sowing [13, 18]. Every month and for three months, the agronomic growth parameters (average of the plant height, the number of leaves, the leaf area, and the collar diameter) were evaluated. The watering of the plants in the field was carried out every morning and every evening.

2.5. Statistical Analysis of Data

The results obtained were subjected to a descriptive analysis (Mean±standard deviation). The results were represented in the form of graphs and tables (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.

3. Results

3.1. Production of Sanitized Seed Plants of *X. sagittifolium* by the PIF Method

3.1.1. Evaluation of the Number of Buds Having Burst of *X. sagittifolium* over Time and According to the Weight of the Corms of the Cultivars

The evolution of the average number of buds having budded in *X. sagittifolium* varied at D₃₀, D₆₀, and D₉₀, according to the weight intervals of the white and red cultivars (Table 1). The maximum peak in the total number of buds having budded was recorded at first weaning (D₃₀), with 162 and 172 buds in the white cultivar, then 147 and 159 buds in the red cultivar for mass intervals between [100-250[and]250-500]. The maximum number of buds produced was recorded in the range of]250-500[g for both cultivars (Table 1). The average number of buds having budded per rhizome appears higher in the white cultivars compared to the red cultivar for the two mass intervals of rhizomes used (Figure 1). It is 10.80±01.90 and 11.47±02.19 buds and 9.80±01.74 and 10.60±01.89 buds in the white and red cultivars respectively for the mass intervals rhizome fragments between [100-250[and]250-500] at D₃₀ (Figure 1). This number of buds produced decreases over time through the weanings carried out.

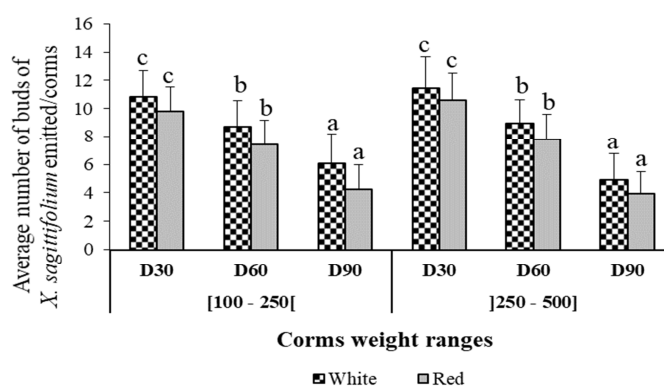


Figure 1. Variation in the average number of buds emitted per corm over time following weight intervals in the two cultivars (Data with the same letter in the graph for the mass intervals following days are not significantly different in the Duncan Test at 5%).

Table 1. Evaluation of the number of buds having burst and of PIF plants in *X. sagittifolium* weaned according to the cultivars and according to time (Number of corms used (NCU), Range of corm fragment weights interval (RCFW), variation in the number of buds emitted over time (VNBE), variation in the number of weaned PIF plants over time (VNWPP), Total number of buds emitted by corm weight interval (TNBE), Total number of weaned PIF plants by corm weight interval (TNWPP/WI), Total number of buds emitted per cultivar (TNBE/C), Total number of weaned PIF plants per cultivar (TNWPP/C), and Total number of weaned PIF plants (TNWPP).

Cultivars	NCU	RCFW	VNBE			VNWPP			TNBE	TNWPP/WI	TNBE/C	TNWPP/C	TNWPP
			D ₃₀	D ₆₀	D ₉₀	D ₃₀	D ₆₀	D ₉₀					
White	15	[100-250[162	130	91	23	39	31	162	93			
	15]250-500]	172	134	74	30	63	36	172	129			
	15	[100-250[147	111	64	38	39	32	147	109			455
Red	15]250-500]	159	117	59	46	58	20	159	124	306	233	

3.1.2. Average Number of PIF Plants Weaned from *X. sagittifolium* According to the Weight of the Corms of the Cultivars

The average number of weaned PIF plants per corm in *X. sagittifolium* varied according to the weanings carried out on D₃₀, D₆₀, and D₉₀. It was very high at D₆₀ for all corm weight intervals, for both cultivars (Figure 2). In the corm fragments used in the weight range between [100-250[, the average number of weaned PIF plants was higher in the red cultivar compared to the white cultivar with a significant value of 03.13 ± 00.83 (Figure 2). However, for the weight interval of between]250-500], the maximum of this average number of weaned PIF plants per corm was recorded in the white cultivar (04.13 ± 00.12) (Figure 2). Very few plants were weaned at the first weaning carried out on D₃₀ (Table 1). The significant peak in the average number of weaned PIF plants recorded was very high at D₆₀ corresponding to the second weaning for all the cultivars (Figure 2). The results showed that there were 39 PIF plants weaned in the two cultivars for the corm weight range of [100-250[g (Table 1). However, for the range of]250-500]g, 63 and 58 weaned *X. sagittifolium* PIF plants were recorded in the white and red cultivars, respectively. This number of PIF plants produced decreased at the third weaning carried out on D₉₀. From the 60 corm fragments used for the two cultivars (white and red), a total of 455 PIF plants of *X. sagittifolium* were produced; 222 for the white cultivar and 233 for the red cultivar

respectively.

The development of the PIF plants of *X. sagittifolium* produced varies according to the weight of the corm. The resumption of buds during the bud break of the corm fragments sown after one week of growth (Figure 3 A and B) depends on the cultivar. These buds evolved by first forming leaf sheaths after two weeks (Figure 3. C and D). The well-differentiated plants resulting from the growth of these different buds are of very varied sizes (Figure 3. E, F, and G). However, after acclimatization (Figure 3H), the growth of PIF plants would depend on their caliber since weaning (Figures 3I, J, and K).

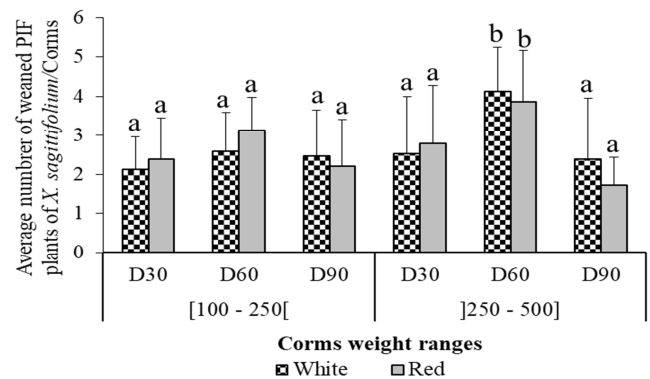


Figure 2. Number of average PIF plants weaned per corm over time following weight intervals in the two cultivars (Data with the same letter in the graph for the mass intervals following days are not significantly different in the Duncan Test at 5%).

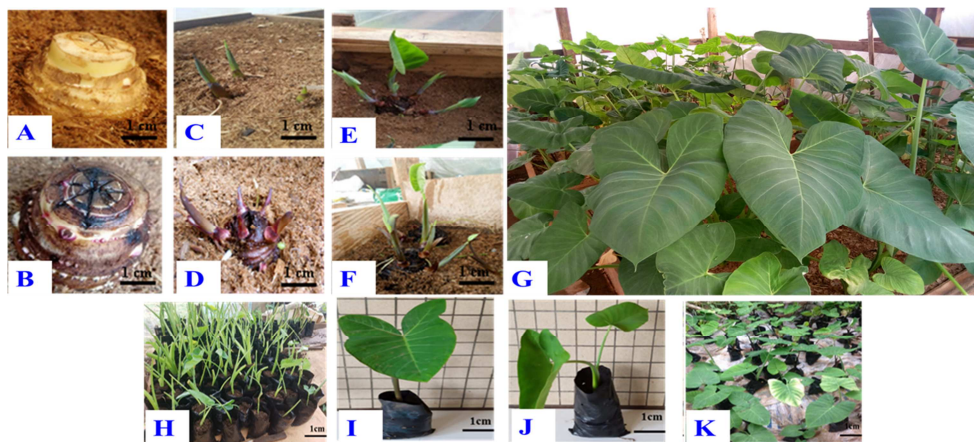


Figure 3. Growth stage in *X. sagittifolium* by the PIF method. Fragment of the corm cultured in the propagators, cultivar white (A) and cultivar red (B) showing the beginning of budding after one week. Two-week-old buds in white (C) and red (D) cultivars, bayonet rejection of white (E) and red (F) cultivar corms, the appearance of PIF plants as propagators (G), PIF plants weaned and placed potted on sterile substrates for acclimatization (H), PIF plants after one month of acclimatization: white (I) and red (J) and plants ready for transfer to the field (K).

3.1.3. Agronomic Growth Parameters Evaluated in PIF Plants of *X. sagittifolium* Cultivars White and Red in Propagators in Shade According to Weaning Time

The analysis of the results of the agronomic parameters evaluated in the PIF plants of the white and red cultivars produced on D₃₀, D₆₀, and D₉₀ of weaning in the propagators varied significantly in Duncan's multiple-range tests at 5% depending on the corm weight ranges used (Table 2). The average height of plants, the average diameter of the collar, the average number of leaves, and the average leaf area, were higher at D₆₀ corresponding to the second weaning. For the corm weight intervals of [100-250], peak values of 33.58±03.01 and 35.40±02.76cm were recorded for the average height of weaned PIF plants, then 245.96±026.62 and 279.65±041.94cm² for mean leaf area of PIF plants in propagators in white and red cultivars respectively. However, the maxima of the average diameter of the collar of the PIF plants and of their average number of leaves were 1.53±0.25cm and 1.56±0.10 at D₃₀ in the white cultivar and 1.86±0.11cm, and 1.83±0.03 at D₆₀ in the

red cultivar (Table 2). It should be noted that the highest agronomic growth parameters were registered in PIF plants produced from corm fragments with weight intervals of [250-500]. On D₆₀, the maxima of the average size (50.31±03.81cm), the average collar diameter (2.29±0.15cm), and the average number of leaves (2.25±0.05) were recorded in the red cultivar. The maximum average surface area was high during the three weaning periods in the white cultivar at D₃₀ (308.55 ± 001.90 cm²), D₆₀ (493.12 ± 042.28 cm²) and D₉₀ (440.05 ± 053.14 cm²) compared to the red cultivar (Table 2). The agronomic parameters, average height, and average leaf area evaluated in the plants of *X. sagittifolium* presented higher values in the PIF plants of the two cultivars produced from the fragments of corms of weight intervals of [250-500] compared to those of intervals of [100-50]. Moreover, there is no significant difference between the growth parameters diameter and the average number of leaves determined at 5% in Duncan's multiple-range tests (Table 2).

Table 2. Agronomic growth parameters evaluated in PIF plants of *X. sagittifolium* cultivars white and red in shade house propagators during weaning.

Cultivars	Range of corm fragment weights	Weaning time	Average heights of PIF plants (cm)	Average collar diameter (cm)	Average number of leaves	Average leaf area (cm ²)
White	[100-250[D ₃₀	27.43±02.26a	1.53±0.25a	1.56±0.10a	228.54±063.38ab
		D ₆₀	33.58±03.01a	1.36±0.26a	1.25±0.13a	245.96±026.62bc
		D ₉₀	30.40±01.68a	1.30±0.02a	1.35±0.03a	205.11±033.38a
]250-500]	D ₃₀	40.54±05.71b	1.85±0.13ab	1.75±0.05ab	308.55±001.90e
		D ₆₀	40.51±06.69b	2.08±0.36ab	2.00±0.01ab	493.12±042.28g
		D ₉₀	39.68±02.20ab	1.89±0.70ab	1.85±0.15ab	440.05±053.14f
Red	[100-250[D ₃₀	32.16±01.37a	1.79±0.42a	1.82±0.12ab	205.82±020.52a
		D ₆₀	35.40±02.76a	1.86±0.11ab	1.83±0.03ab	279.65±041.94d
		D ₉₀	31.59±01.17a	1.85±0.01ab	1.90±0.08ab	233.06±077.69b
]250-500]	D ₃₀	43.21±01.10c	2.01±0.22ab	2.11±0.13ab	283.06±047.88cd
		D ₆₀	50.31±03.81d	2.29±0.15ab	2.25±0.05ab	473.88±029.20g
		D ₉₀	48.88±03.39d	2.21±0.07ab	2.21±0.19ab	316.83±076.67e
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						
Day (D)						

3.2. Agronomic Growth Parameters Evaluated in PIF Plants of *X. sagittifolium*, White and Red Cultivars After Transfer to the Field

The growth of *X. sagittifolium* PIF plants transferred to fields was significantly influenced by the fertilizers applied. The results showed that, for all the treatments used, the parameters; the average height of the plants, the average diameter of the collar, the average number of leaves, and the average leaf area, increase over time (Table 3). In *X. sagittifolium* white cultivar PIF plants, applied arbuscular mycorrhizal fungi had the greatest influence on mean plant height compared to control treatments and chicken manure. The maximum was 37.60±04.15cm on D₉₀. However, the maxima of the average diameter of the collar, the average number of leaves, and the average leaf area were

1.91±0.55cm, 1.93±0.72, and 238.58±42.07cm² respectively at D₉₀, in PIF plants fertilized with chicken manure was noticed. In the red cultivar, the growth values evaluated were very high on D₉₀ in the PIF plants fertilized with hen droppings in the fields. No significant difference in the effect of the treatments on the parameters, the average diameter of the collar, and the average number of leaves were noticed. The low average of the leaf area was obtained in the Control treatment on D₃₀ with, respectively, 080.35±03.27 and 082.34±08.05cm² in the white and red cultivars (Table 3). Fertilizer treatments of arbuscular mycorrhizal fungi and chicken manure applied had the greatest influence on the growth of plant height and leaf area of plants in the field. The leaf surfaces are large in the PIF plants at D₉₀, in the red cultivar.

Table 3. Agronomic parameters of growth in PIF plants of *X. sagittifolium* (White and Red cultivars) after transfer to the field over time.

Cultivars	Treatments	Weaning time	Average heights of PIF plants (cm)	Average collar diameter (cm)	Average number of leaves	Average leaf area (cm ²)
White	Control	D ₃₀	25.25±07.42a	1.33±0.16a	1.63±0.12a	080.35±03.27a
		D ₆₀	26.67±02.05a	1.35±0.14a	1.74±0.18a	232.74±07.64b
		D ₉₀	31.87±09.09ab	1.54±0.19a	1.85±0.19a	173.46±06.95ab
	Chicken manure	D ₃₀	34.27±01.35ab	1.54±0.17a	1.75±0.15a	238.82±10.40c
		D ₆₀	28.84±08.72a	1.31±0.10a	1.31±0.11a	215.91±26.74b
		D ₉₀	35.48±06.28b	1.91±0.15ab	1.93±0.12ab	238.58±12.07c
	AMF	D ₃₀	32.68±03.23ab	1.49±0.17a	1.80±0.16ab	146.56±11.76a
		D ₆₀	35.53±09.29b	1.37±0.16a	1.65±0.11a	189.68±15.55ab
		D ₉₀	37.60±04.15c	1.67±0.16a	1.83±0.13ab	142.05±17.83a
	Control	D ₃₀	25.70±08.14a	1.46±0.19a	2.25±0.15b	082.34±08.05a
		D ₆₀	24.28±07.86a	1.33±0.29a	1.86±0.11ab	150.63±14.54a
		D ₉₀	27.36±07.63a	1.67±0.18a	1.90±0.11ab	179.96±10.95ab
Red	Control	D ₃₀	29.45±08.75a	1.64±0.12a	1.78±0.13a	121.05±12.81a
		D ₆₀	26.60±07.90a	1.66±0.17a	1.90±0.15ab	201.32±12.38b
		D ₉₀	34.37±09.32ab	2.09±0.13b	1.98±0.13ab	225.28±08.16b
	Chicken manure	D ₃₀	28.30±08.79a	1.49±0.02a	1.90±0.12ab	122.68±11.15a
		D ₆₀	27.03±08.86a	1.49±0.17a	1.80±0.16ab	146.56±21.76a
		D ₉₀	33.10±07.32ab	2.35±0.19b	2.35±0.10b	152.06±11.40a
	AMF	D ₃₀				
		D ₆₀				
		D ₉₀				
	Control	D ₃₀				
		D ₆₀				
		D ₉₀				

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

Day (D)

AMF: arbuscular mycorrhiza fungi

4. Discussion

The results obtained show that the bud burst of the corm fragments sown according to the defined mass intervals was 100% in the two cultivars. This means that these fragments of *X. sagittifolium* used had a good sanitary state. The resumption of dormant buds on either side of the fragment during the first early stages of growth would be the result of favorable culture conditions in the propagators, thus facilitating the adsorption of water inside these corm fragments by the osmosis phenomenon. Moreover, the number of buds emitted is higher in corm fragments weighing between [250-500]. This suggests that, when the fragment of corms is large, the more it has a maximum of buds. It should be noted that the budburst of these buds was carried out on a culture substrate used consisted of a mixture of shavings and sawdust. This substrate is rich in lignocellulosic material with a C/N ratio between 100-500 and poor in mineral elements due to its slow mineralization. According to [25], this high C/N ratio would indicate a slow mineralization of carbon, a slow decomposition of organic matter, and therefore an immobilization of the nitrogen necessary for growth. Therefore, during the resumption of germination and the proliferation of the emitted PIF plants, several physiological phenomena are brought into play, not only in the corm fragment but also influenced by the conditions of the production environment of the PIF plants which is the propagator. In addition, [26] reported that in *Musa* sp., the proliferation of shoots would directly depend on the physiological state of the suckers or bulbs used, their sanitary state, the environmental conditions, and also the genotypes used. However, the recovery of these PIF plants requires the availability of the nutrients necessary for their growth. To this end, the bud break and the growth of the

emitted buds would be partly stimulated by the phenomenon of osmosis in the corm fragments, which would help to hydrolyze the starch chains of the starchy parenchyma of the corm, facilitating the release of glucose. This glucose, used in the process of respiration, will be degraded to provide the CO₂ necessary for these first stages of growth. Therefore, the recovery would be dependent on the physiological state of the corm fragments and the cultivar used. This explains why the maximum number of buds emitted was greater for fragments of corms of weight varying between [100-250] and [250-500] in the white cultivar, compared to the red cultivar. Moreover, in potatoes for example, [27], affirmed that the physiological behavior of tuber seeds is closely linked to the variety. [28], attributed the emission of the high number of seedlings emitted also in the explants of *Musa* sp., to the quantity of the important nutrient reserves contained in the bulbs of weight ranging from 1 to 1.5 kg.

During recovery, almost all of the buds present on the corm fragment bud out. It can be seen that this total number of buds having budded decreases over time. The decrease in the number of buds emitted over time could be explained by the fact that when some of these buds grow to give PIF plants, others remain in the bud state. Under natural conditions, the growth of the apical bud in *X. sagittifolium* plants inhibits the growth of axillary buds present in the axils of the leaf sheaths. Thus, despite the sectioning of the apical bud before germination, there would exist during the resumption, an apical dominance between the buds emitted, and this is what would explain the varying and progressive growth observed in the buds broken over time. Similar results were obtained in *Colocasia esculenta* [24]. The average number of weaned PIF plants per corm fragment was 3 to 4 on D₆₀. The maximum weaned PIF plant was also obtained on the same day at all the weight intervals used. However, corm fragments ranging in weight between [250-500] in both

cultivars produced more plants compared to those in the weight range of [100-250]. The weight of the corm fragment is a very crucial factor in achieving seed production of *X. sagittifolium* by the PIF method. The larger the fragment of the corm, the more the availability of carbohydrates, making it possible to meet, in terms of cost, the needs in CO₂ appear important for the growth of PIF plants in the propagators. It should be noted that in the field, this weight of the corm fragment would depend on several factors such as the age of the plant, the environmental conditions such as the physicochemical parameters of the soil, the hydromineral nutrition, and the microbial activity of the rhizosphere in *X. sagittifolium*.

Despite the destruction of the apical dominance of the main bud at the beginning of the culture, the PIF plants from the emitted buds showed growth at different speeds. This is what reflects the significant difference in Duncan's multiple-range tests, for the growth parameters evaluated. The results, therefore, show that there would be an apical dominance between the different buds emitted, so the consequence would be the significant difference observed between the number of PIF plants weaned in the first month, in the second, and in the third month of cultivation. The number of PIF plants produced decreased over time for all cultivars. From 60 fragments of corms of weight varying between [100–250] and [250-500] in the two cultivars, 455 PIF plants were produced and weaned with 222 PIF plants for the white cultivar and 233 PIF plants for the red cultivar. The application of the technique makes it possible to multiply by 7.58 times the number of fragments of corms used at the start. Similar results were obtained in *C. esculenta* [24], where the results showed that from the PIF method, 120 corm fragments were multiplied by 4.99. The number of weaned PIF plants is higher on D₆₀. We noticed that it drops sharply at D₉₀. As in *C. esculenta*, the weaning of PIF plants in *X. sagittifolium* consisted of removing only mature and vigorous PIF plants, presenting an ability to be able to quickly resume during acclimatization. Consequently, the number of weaned PIF plants raised on D₆₀ is justified by the fact that at the beginning of bud burst, the majority of plants having budded on the corm fragment after 30 days of growth will not all be weaned. Similarly, these PIF plants that have not been weaned would continue to exert inhibition on the existing buds that have not quickly taken over. The results of the analysis of the agronomic parameters of growth during the production of the PIF plants in the propagators showed that the parameters, average height of the plant, and leaf area varied significantly ($P < 0.05$) over time. The corm fragment intervals [250-500] showed better growth parameters. At this interval, the plants were also more vigorous. This would perhaps reflect that the physiological and biochemical phenomena associated with the development of the plant would therefore also be dependent on the weight of the fragment. Whether in the shade house or in the field, the result shows that there is no significant difference between the parameters of average collar diameter and the average number of leaves in those two conditions. However, the

average leaf area varied in the two cultivars. The leaf areas were larger in the shade house than in the field. In the shade, on a fragment of corms of *X. sagittifolium*, the appearance of PIF plants varying between 3 to 12 was observed. However, the quantity of carbohydrates present in these corms would be partly insufficient. Therefore, these large leaf surfaces observed in the shade in PIF plants would contribute to maximizing photosynthesis, in order to be able to cover carbohydrate needs and circumvent a potential competition with regard to CO₂ needs, necessary for growth. However, the quantity of carbohydrates present in these corms would be partly insufficient. Therefore, these large leaf surfaces observed in the shade in PIF plants would contribute to maximizing photosynthesis, in order to be able to cover carbohydrate needs and circumvent a potential competition with regard to CO₂ needs, necessary for growth, development, and the flourishing of all PIF plants from the same corm fragment. In the field, the plants are solitary and therefore are not exposed to competition with respect to the CO₂ available for growth. In the field, the treatments applied influenced the growth of PIF plants. The chicken manure treatment had the greatest influence on the parameters evaluated compared to the control and the AMF treatment in the field. Chicken manure would make mineral matter directly available to plants. These results are in agreement with those of [29], who have shown that an exogenous supply of chicken manure improves the availability of nutrients and creates favorable conditions for good plant development. [30, 31] demonstrated that the combined use of poultry manure and mineral manure would increase the yield of the maize plant compared to the application of organic or mineral manure alone. Similarly, in *X. sagittifolium*, [13], also recorded in the field, a significant increase in all growth parameters in the presence of hen manure treatments, compared to NPK and control treatments. In the PIF plants inoculated with the AMF of the white and red cultivars, a weak influence of these agronomic growth parameters was observed during the 90 days of growth. This could be explained by the fact that there may be an antagonistic action between the allochthonous AMF complex brought as fertilizer and the autochthonous AMFs of the soil substrate of the field on the one hand, and on the other hand, it is known that the richness in mineral elements of the soil such as phosphorus [32-34], its physicochemical composition [35, 36], soil microbiota [37] would influence the action of AMF. This invisible action of AMFs on the growth of PIF plants in the field may also suppose that the strains used, perhaps influence other internal physiological phenomena in plants.

5. Conclusion

At the end of the investigations carried out in this research, it should be noted that the production of seed plants of *X. sagittifolium* by the PIF method requires the use of fragments of corms of good sanitary condition. The use of corm fragments weighing more than or equal to 250g or 500g makes it possible to have a maximum of buds. From the 60

fragments of corms of weight varying between [100-250[and]250-500] at a rate of 30 fragments of corms per cultivar of *X. sagittifolium*, 455 PIF plants were produced and weaned. From these results, the technique made it possible to multiply the number of starting corm fragments by 7.58 times. The technique is an efficient approach to propose for the production of seeds in *X. sagittifolium*. Mastering the sanitary and physiological state of the corm fragments, and knowing their origin, are very crucial in the realization of this technique. After transfer to the field, it can be seen that the types of fertilizers applied influenced the growth of PIF plants in both varieties.

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References

- [1] Si H, Zhang N, Tang X, Yang J, Wen Y, Wang L and Zhou X. (2018). Transgenic Research in Tuber and Root Crops. Genetic Engineering of Horticultural Crops, First Edition PP: 225-248. <https://doi.org/10.1016/B978-0-12-810439-2.00011-8>
- [2] FAOSATAT. (2021). Food and Agriculture Organization of the United Nations Statistical Database; Statistical Division; FAO: Rome, Italy; Available online: <http://www.fao.org/statistics/en/>
- [3] Otekunrin OA, Sawicka B, Adeyolu AG and L Rachon. (2021). Cocoyam [*Colocasia esculenta* (L.) Schott]: Exploring the Production, Health and Trade Potentials in Sub-Saharan Africa. *Sustainability* 13 (8): 4483. <https://doi.org/10.3390/su13084483>
- [4] Nyadanu D and Lowor ST. (2015). Promoting the competitiveness of neglected and underutilized crop species: a comparative analysis of the nutritional composition of indigenous and exotic leafy and fruit vegetables in Ghana. *Genetic Resources and Crop Evolution* 62: 131-140. <https://doi.org/10.1007/s10722-014-0162-x>
- [5] Thais CLDS, Tayse FFDS, Maria IR, Ana LTGR, Daniela AN, Marta CTD, Elenice CECS, Gunter K, Alessandra BR and Helena TG. (2021). A study of the bioactive potential of seven neglected and underutilized leaves consumed in Brazil. *Food chemistry* 364: 130350. <https://doi.org/10.1016/j.foodchem.2021.130350>
- [6] Ariwaodo CA, Ezeama CF and Nwabueze TU. (2017). Morphology, Rheology and Functional Properties of Starch from Cassava, Sweet Potato and Cocoyam. *Asian Journal of Biology* 3 (3): 1-13.
- [7] Jacob OA and Adeleke OA. (2019). Isolation and Characterization of Starch obtained from Cocoyam cultivated at Akungba Akoko, Ondo State, Nigeria. *Nutrition & Food Science International Journal* 8 (2): 555732.
- [8] Opara LU. (2003). Edible aroids: Post-harvest operations. Rome, Italy: Food and Agriculture Organization of the United Nations.
- [9] Sefa-Dedeh S and Agyir-Sackey EK. (2002). Starch structure and some properties of cocoyam (*Xanthosoma sagittifolium* and *Colocasia esculenta*) starch and raphides. *Food Chemistry* 79: 435-444. [https://doi.org/10.1016/S0308-8146\(02\)00194-2](https://doi.org/10.1016/S0308-8146(02)00194-2)
- [10] Ekwe KC, Nwosu KI, Ekwe CC and Nwachukwu I. 2009. Examining the underexploited values of cocoyam (*Colocasia* and *Xanthosoma* spp.) for enhanced household food security, nutrition and economy in Nigeria. In: Jaenicka, H., Ganry, J., Zeledon-Hoeschle, I. and Kahara, R. (Eds.). Proceedings of the international symposium on underutilized plants for food security, income and sustainable development. *Acta Horticulture* 86: 71-78.
- [11] Adepoju OT and Oludo MD. (2016). Comparative study and improving dietary diversity of Nigerians through consumption of three non-conventional green leafy vegetables. *American Journal of Food and Nutrition* 4: 5-15.
- [12] Temesgen M, Retta N and Tesfaye E. (2016). Effect of pre-curing on nutritional and antinutritional composition of taro leaf. *International Journal of Food Science and Nutrition* 1 (1): 5-11.
- [13] Gwan ME, Djeuani AC, Tene TPM, Boudjeko T and Omokolo ND. (2019). Field performance of *Xanthosoma sagittifolium* L. Schott minitubers grown under the influence of poultry manure and NPK fertilizers: changes in the content of some secondary metabolites. *Journal of Biology Agriculture and Healthcare* 9 (20): 30-42. <http://dx.doi.org/10.7176/JBAH/9-20-05>
- [14] Jehannara C, Nicola G, Benavent-Gil Y and Rosell CM. (2021). Aroids as underexplored tubers with potential health benefits. *Advances in Food and Nutrition Research* 97: 319-359.
- [15] Onokpise OU, Wutoh JG, Ndzana X, Tambong JT, Meboka MM, Sama AE and Bruns M. (1999). Evaluation of Macao cocoyam germplasm in Cameroon. In J. Janick (Ed.), Perspectives on new crops and new uses. Alexandria, VA: ASHS Press, (pp. 394-396).
- [16] Omokolo, N. D., T. Boudjeko and T. Tsafack. (2003). In vitro tuberization of *Xanthosoma sagittifolium* (L.) Schott: effects of phytohormones, sucrose, nitrogen and photoperiod. *Scientia Horticulturae* 98: 337-345. [https://doi.org/10.1016/S0304-4238\(03\)00066-9](https://doi.org/10.1016/S0304-4238(03)00066-9)
- [17] Djeuani AC. (2018). Les champignons mycorhiziens à arbuscules et la minitubérisation chez *Xanthosoma sagittifolium* L. Schott: évolution de quelques paramètres biochimiques au cours de ce processus. Thèse de doctorat/PhD, Département de biologie végétale, Faculté des Sciences, Université de Yaoundé 1 (Cameroun), 167p.
- [18] Amama AB. (2021). Production des plants assainis chez *Xanthosoma sagittifolium* (L.) Schott à partir de la méthode de PIF. Master, Université de Yaoundé I. 67p.
- [19] Tsafack TJJ. (2010). Microtuberisation chez *Xanthosoma sagittifolium* L. Schott et analyse de quelques aspects physiologiques et biochimiques. Thèse de Doctorat/PhD. Université de Yaoundé I. 150p.

- [20] Niemenak N, Noah AM and Omokolo DN. (2013). Micropropagation of cocoyam (*Xanthosoma sagittifolium* L. Schott) in temporary immersion bioreactor. *Plant Biotechnology Reports* 7 (3): 383. <https://doi.org/10.1007/s11816-013-0272-5>
- [21] Djeuani AC, Mbouobda HD, Niemenak N, Fotso, Elian Hubert, Ngaha EC, Abilogo M and Omokolo ND. (2014). Effect of carbon source on minituberization of cocoyam (*Xanthosoma sagittifolium*): Analysis of soluble sugars and amino acids contents. *Current Research in Microbiology and Biotechnology* 2: 519-526.
- [22] Kwa M. (2002). Techniques horticoles de production de masse de plants de banane: la technique des plants issus de fragments de tige (PIF). Fiche technique CARBAP 4 p.
- [23] Bonte E, Verdonck R and Gregoire L. (1995). La multiplication rapide du bananier et du plantain au Cameroun. *Tropicultura* 13 (3): 109-116.
- [24] Djeuani AC, Mbouobda HD, Nzie O, Ngoi JK and Niemenak N. (2021). Use of Oyster shell ash of *Crassostrea* spp. in mass production of sanitized seedlings of *Colocasia esculenta* L. Schott by the PIF method and influence of some fertilizers during their growth in acclimation. *Open Access Library Journal* 8 (11): 1-15. <https://doi.org/10.4236/oalib.1108008>
- [25] Bakayoko S, Abobi AHD, Konate Z and Toure NU. (2019). Effets compares de la bouse de bovins séchée et de la sciure de bois sur la croissance et le rendement du maïs (*Zea mays* L.). *Agronomie Africaine* 8: 1-10.
- [26] Bawoumodom PITB, Komi O, Rassimwai P and Atalaesso B. (2020). Macro-propagation of Dessert Bananas (Dankodu and Tsikodu) and Plantain (Savé) (*Musa* Spp.) by PIF Technique in Togo, West Africa. *Agricultural and Biological Sciences Journal* 6 (4): 195-201.
- [27] Delaplace P, Fauconnier ML and Patrick du Jardin. (2008). Méthodes de mesure de l'âge physiologique des tubercules semences de pomme de terre (*Solanum tuberosum* L.). *Biotechnology, Agronomy and Society and Environment* 12 (2): 171-184.
- [28] Bangata BNM, Ngbenelo N and Mobambo KN. (2019). Evaluation du potentiel de prolifération d'explants de différentes dimensions de bananier plantain (*Musa* sp. cv. AAB) par la macropropagation en conditions semi contrôlées. *Revue Africaine d'environnement et d'Agriculture* 2 (2): 25-31.
- [29] Žydelis R, Lazauskas S, Volungevičius J and Povilaitis V. (2019). Effect of organic and mineral fertilisers on maize nitrogen nutrition indicators and grain yield. *Zemdirbyste-Agriculture* 106 (1): 15-20.
- [30] Jan MF, Asad AK, Waqas L, Ahmadzai MD, Ahmad H and Haroon J. (2018). Impact of integrated potassium management on plant growth, dry matter partitioning and yield of different maize (*Zea mays* L.) hybrids. *Pure and Applied Biology* 7 (4): 1277-1285.
- [31] Gomgnimbou APK, Bandaogo AA, Coulibaly K, Sanon A, Ouattara S et Nacro HB. (2019). Effets à court terme de l'application des fientes de volaille sur le rendement du maïs (*Zea mays* L.) et les caractéristiques chimiques d'un sol ferrallitique dans la zone sud-soudanienne du Burkina Faso. *International Journal of Biological and Chemical Science* 13 (4): 2041-2052.
- [32] Bainard LD, Bainard JD, Hamel C and Gan Y. (2014). Spatial and temporal structuring of arbuscular mycorrhizal communities is differentially influenced by abiotic factors and host crop in a semi-arid prairie agroecosystem. *FEMS Microbiology Ecology* 88: 333-344.
- [33] Zhao H, Li X, Zhang Z, Zhao Y, Yang J and Zhu Y. (2017). Species diversity and drivers of arbuscular mycorrhizal fungal communities in a semi-arid mountain in China. *PeerJ* 5: e4155.
- [34] Peña Venegas RA, Lee SJ, Thuita M, Mlay DP, Masso C, Vanlauwe B, Rodriguez A and Sanders IR. (2021). The Phosphate Inhibition Paradigm: Host and Fungal Genotypes Determine Arbuscular Mycorrhizal Fungal Colonization and Responsiveness to Inoculation in Cassava with Increasing Phosphorus Supply. *Frontiers in Plant Science* 12: 693037.
- [35] Neina D. (2019). "The Role of Soil pH in Plant Nutrition and Soil Remediation", *Applied and Environmental Soil Science*, 9 pages.
- [36] Velázquez MS, Fabisik JC, Abarca CL, Allegrucci N and Cabello M. (2020). Colonization dynamics of arbuscular mycorrhizal fungi (AMF) in *Ilex paraguariensis* crops: Seasonality and influence of management practices. *Journal of King Saud University - Science* 32 (1): 183-188.
- [37] Svenningsen NB, Watts-Williams SJ, Joner E, Battini, Efthymiou F, Aikaterini CP, Nybroe C, Ole and Jakobsen I. (2018). Suppression of the activity of arbuscular mycorrhizal fungi by the soil microbiota. *ISME J* 12: 1296-1307.