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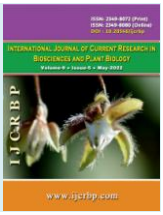

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Morphological and agronomical characterization of pineapple plants derived from *vitro* culture

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Abstract

In Côte d'Ivoire, pineapple occupies a prominent place after the coffee and cocoa binomial in exports. The country has long been the leading producer of smooth Cayenne in the European market. However, this dynamism is hampered by the lack of good quality rejects. To overcome this constraint, various methods of in *vitro* regeneration of plantlets have been initiated. Micro propagation and somatic embryogenesis allow massive production of plants of good sanitary quality. However, acclimatization of the *vitro* plants is a critical phase. This study was conducted to assess the growth parameters and success rate of *vitro* plants during acclimatization, as well as the growth potential of *vitro* plants in the field. To do so, the plants resulting from micropropagation and somatic embryogenesis were evaluated in greenhouse, shade and field. Plants from the mother suckers were used as controls in the field. After acclimatization, a 98% survival rate of the *vitro* plants was observed. In the field, the *vitro* plants behaved differently. The growth parameters of plants from somatic embryogenesis were significantly higher than those from micropropagation and plants from mother suckers. In addition, controlling acclimatization is an important asset for mass disposal of pineapple plants for the renewal of the Ivorian orchard. Thus, to develop pineapple cultivation in Ivory Coast, it would be interesting to use juvenile offspring from *vitro* cultures, in particular from somatic embryogenesis.

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Introduction

Pineapple [*Ananas comosus* var *comosus* (L. Merril)] (Kpéra et al., 2019), 8th world fruit production with nearly 13 million tonnes per year, is the subject of an export crop almost monovarietal with the smooth Cayenne variety (Brillouet, 2001). The smooth Pineapple *A. comosus* var. *cayenne* is cultivated in all tropical and subtropical countries for its fruit. It occupies a prominent place in Côte d'Ivoire after the coffee-cocoa binomial in exports.

In fact, the country exports on average 180,000 to 200,000 tonnes of fresh pineapple per year, i.e., 1.6% of

Ivorian exports (Ahoua, 2017). The importance of this production has earned Côte d'Ivoire the rank of leading exporter of fresh pineapple to the European market.

However, the smooth Cayenne fromCôte d'Ivoire, which in 1986 represented 97% of the European market, has fallen dramatically in recent years. In fact, from 213,620 tonnes in 1999, it fell to 60,000 tonnes in 2008, a decrease of 70% (O.C.D.E, 2008). This drop in the production of smooth Cayenne is partly due to the aging of the Ivorian orchard, but also to diseases and soil impoverishment.

Faced with these various problems, the renewal of the aging orchard seems essential. However, the natural spread of pineapple suckers in the field is insufficient to meet the needs of growers for quality planting material. To remedy these problems, different strategies have been developed to produce suckers free from diseases, such as *in vivo* and *in vitro* multiplication.

In vitro propagation provides an important advantage for the propagation and genetic improvement of pineapple. Micropropagation and especially somatic embryogenesis are efficient regeneration pathways in plants. These two types of propagation help to provide growers with healthy pineapple plants, improving yield and fruit quality. However, acclimatization of *vitro* plants is a critical phase of micropropagation (Khater, 2018).

In addition, the survival rate of seedlings is generally low (Dibi, 2011). But, Yapo (2013) reported a significant survival rate of pineapple seedlings from glass cultures of around 90%. However, this success does not match the agronomic performance of the acclimatized seedlings transferred to the field. Indeed, according to the work of Youmbi et al. (2005), the agronomic performances of *in vitro* plants transferred to the field differ according to the initial mode of multiplication for the same cultivar.

Also, did it seem useful to evaluate the behavior of *in vitro* pineapple plants resulting from micropropagation and somatic embryogenesis during acclimatization and in the field. This is with the aim of improving the quality and renewing the pineapple orchard which is aging in Côte d'Ivoire. Specifically, this involves evaluating the growth parameters and survival rate of seedlings during acclimatization, and also the morphological characteristics of *vitro* plants in the field.

Materials and Methods

Plant material

Two types of pineapple seedlings regenerated *in vitro* were used: seedlings from micropropagation and those from somatic embryogenesis. Traditional suckers served as controls in the field.

Acclimatization phase

Acclimatization consists of gradually adapting the *vitro* plants to the conditions prevailing in the greenhouse or outside. After removing the agar from the base of the plants, they are transferred to a substrate. The substrate used consists of an arable layer of fallow soil covered with vegetation. It was carefully mixed with sawdust. Acclimatization has two phases : a weaning phase and a rearing phase.

Weaning phase

The weaning phase consists of the gradual adaptation of the *vitro* plants to external conditions (light, atmosphere, substrate). Before placing the plants in a greenhouse, the following parameters were recorded on each seedling.

The number of roots

The number of sheets

Height (cm)

Then the seedlings were transplanted directly into tanks containing the substrate. The tanks were placed in a greenhouse for 8 weeks. Water was supplied by sprinkling at the rate of one watering per day. Every 5 days there was a supply of sawdust in the bins. A total of 100 *vitro* plants were used: 50 *vitro* plants from micro propagation and 50 from somatic embryogenesis. At the end of weaning the parameters mentioned above were evaluated.

Breeding phase

The rearing phase involves growing the *vitro* plants until they reach about 300 g. The weaned seedlings were transferred to polyethylene bags of dimension 25 x 30 cm. These bags were first filled with substrate (black earth plus sawdust) then placed under shade. The water

was supplied by sprinkling at the rate of watering every 2 days with tap water for 12 weeks. A monthly fertilization of 2.5 g of potassium sulphate and 1 g of urea per plant was carried out in the 2nd week of cultivation.

The parameters recorded on each plant were as follows :

The number of sheets issued

The survival rate of acclimatized pineapple plants.

The survival rate (TS) of the acclimatized pineapple plants was calculated according to the following formula :

NVA = Number of acclimatized *vitro* plants

NVS = Number of *vitro* plants that survived after acclimatization

Field experiment

The experimental plot is located at Nangui Abrogoua University (Abidjan, Côte d'Ivoire). Acclimatized *vitro* plants from micropropagation and somatic embryogenesis as well as traditional suckers were used.

Each type of seedling was placed on a block, with 3 repetitions. These blocks were completely randomized. Thus, an elementary plot consisted of 45 plants, or 15 plants of each type.

The cultivation of these suckers was done in ridge. The suckers were planted in staggered rows on the ridges. On each log, the distance between 2 suckers is 40 cm and 1 m between the rows. The field is regularly maintained.

A monthly fertilization of 2.5 g of potassium sulphate and 1 g of urea per plant was carried out. Every two weeks,the height, display, number of sheets issued, number of sheets and length and width of leaf Dwere recorded (Lacoeilhe and Py, 1974).

Statistical analysis

Statistical analyses were carried out using Statstica version 7.0 and the treatments were analyzed using a non-parametric analysis Kruskall-Wallis and Newman-Keuls, both at 5% significance level.

Results and Discussion

Growth characteristics of *vitro* plants during acclimatization

Weaning phase

After the weaning phase, the parameters measured (Table 1) are not significantly different. Seedlings produced by micropropagation behaved similarly to those produced by somatic embryogenesis during weaning.

Rearing phase

During the rearing phase (growth phase), the data obtained on the average number of leaves emitted (Table 2) shows that there is no significant difference.

Regarding the survival rate of the seedlings after acclimatization, Table 3 shows that all the seedlings have adapted identically to the external environment with a survival rate of approximately 98%.

Characteristics of growth and development of plants in the field

Number of sheets

Table 4 shows the average number of leaves of different pineapple plants as a function of time.

In the field, the results (Table 4) showed that the average number of leaves of the different types of plants is significantly different. Plants resulting from somatic embryogenesis have the highest average leaf number followed by those resulting from micropropagation. Finally, the traditional stalks had the lowest number of leaves.

Leaf emission rate (number of leaves emitted per month)

At the level of the leaf emission rate, the results indicate significant fluctuations *in vitro* plants characterized by a slowing phase from the 1st to the 3rd month and a more accelerated phase from the 4th to the 5th month. On the other hand, with traditional releases, the rate of emission was slow during the 4 months followed by a slight increase in the 5th month. However, with a significantly higher rate of leaf emission in plants

resulting from somatic embryogenesis than in plants resulting from micropropagation. We also note that during the 5 months of planting the plants resulting from micropropagation emit leaves exponentially while those resulting from micropropagation and traditional ones are more or less stationary.

Plant heights

In the field, the data obtained per month on the heights of the different plants (Table 6) show that the average heights of the plants produced by micropropagation and by somatic embryogenesis are significantly different from those of traditional suckers. But the plants produced by somatic embryogenesis had the highest heights.

Display of plants

Regarding display, the average values recorded (Table 7) indicate that those of the *vitro* plants are significantly different from those of the suckers. During the 1st and 2nd month the mean values of the different *vitro* plants are not significantly different, but from the 3rd month those of the plants produced by somatic embryogenesis are markedly higher than those of the plants produced by micropropagation.

Sheet width D

Concerning the average values of the width of the leaf D (Table 8) indicate that the *vitro* plants had the best average values on the traditional stalks. But from the 3rd month, the mean values of the width of the leaf D of the plants produced by somatic embryogenesis were higher than those of the plants produced by the micropropagation.

Length of sheet D

The average lengths of the D leaves recorded (Table 9) show that there is a significant difference between *vitro* plants and traditional stalks. However, the average lengths of plants produced by somatic embryogenesis are greater than those of plants produced by micropropagation. During acclimatization, the parameters recorded on seedlings resulting from micropropagation evolved in a similar manner to those recorded on seedlings resulting from somatic embryogenesis. This can be explained by the fact that the two types of plants are anatomically and physiologically similar. This result is in agreement with the work of Ferreira et al., (2017) who showed that during acclimatization, the *vitro* plants resulting from pistachio leaves behave in the same way as those resulting from buds or cotyledons. During acclimatization the roots of the pineapple plants developed well (Yabor et al., 2020). The *vitro* plants adapted well to outdoor conditions as the survival rate of the acclimatized pineapple seedlings was 98%. These results are similar to those of Sripaoraya et al., (2003) also reported a 96% seedling follow-up rate during pineapple regeneration via embryogenesis and organogenesis. So, we can say that the cultivation conditions adopted during this work would certainly be beneficial to the development and growth of pineapple plants. The high survival rate would be due to a good acclimatization of the tissue culture plants. Indeed, the substrate used would be ideal for good growth and roots. This would therefore have allowed a good development of the seedlings in the field. The study of the agronomic characteristics of pineapple plants in the field showed a significant difference between seedlings from *vitro* cultures and those from traditional stalks. In fact, most of the plantlets produced by micropropagation and by somatic embryogenesis survived after their transfer to the field and exhibited growth and development parameters clearlysuperior to those obtained from traditional suckers (controls) (Baiyeri (2005)). These results seem to show the important role of *vitro* methods in the growth and development of pineapple plants. Indeed, the *vitro* methods would cause a rejuvenation or a juvenilization of pineapple seedlings, as mentioned by Yapo et al., (2011). This appears to give increased vigor to the seedlings. Indeed, according to Yapo (2013), the harder the explants on a culture medium, the greater the juvenilization. Alvarez-Gutiérrez et al., (2021) also reported that *in vitro* banana plants from axillary buds and those from apical buds all performed better than traditional stalks. However, the results show a difference in the development of plants produced by micropropagation from those produced by somatic embryogenesis. Indeed, in the field, the *vitro* plants produced by somatic embryogenesis have growth characteristics, that is to say the height of the plants, the width and length of the leaf D, more important than those produced by micropropagation. Plants produced by somatic embryogenesis also have developmental characteristics, i.e. the number of living leaves, the number of leaves emitted higher than those produced by micropropagation.

Table 1. Seedling growth characteristics after weaning.

Parameters	Seedlings from micropropagation		Seedlings from somatic embryogenesis	
	Before weaning	After weaning	Before weaning	After weaning
Sheets Number	11.34±3.83a	12.72±3.52a	13.00±3.64b	14.04±3.01a
Root number	6.53±3.74a	12 .52±4.47a	7.94±3.22b	15.22±13.85a
Height	2.21±0.75a	2.87±0.86a	1.88±0.47a	2.89±0.50a

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

Table 2. Characteristics of the average number of leaves emitted by the seedlings during rearing as a function of time.

Time (months)	Seedlings from micropropagation	Seedlings from somatic embryogenesis
1	1.68±0.63a	1.83±0.66a
2	1.86±0.66a	1.95±0.35a
3	2.06±0.57b	1.93±0.52b

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

Table 3. Survival rate of pineapple plants after acclimatization.

Origin of *vitro* plants	Number of acclimated seedlings	Seedling survival rate (%)
Micropropagation	50	98a
Somatic embryogenesis	50	98.3a

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

Table 4. Average number of leaves of different pineapple plants as a function of time.

Time (months)	Leaves number of plants from		
	Micropropagation	Somatic embryogenesis	Traditional stalks
1	21.56±4.91b	27.97±8.13c	16.02±6.17a
2	24.72±4.94b	36.77±8.47c	20.20±6.67a
3	27.65±5.32b	41.91±8.74c	22.50±7.21a
4	33.01±5.41b	47.14±10.02c	27.26±7.72a
5	35.07±6.01b	50.62±10.92c	30.90±8.02a

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

Table 5. Foliar emission rate of the different pineapple plants as a function of time

Time (months)	Number of leaves emitted by plants from		
	Micropropagation	Somatic embryogenesis	Traditional stalks
1	1.43±0.98b	2.00±0.24b	0.00±0.00a
2	2.25±0.83b	3.00±0.87c	1.50±0.56a
3	2.49±0.63b	2.74±0.56b	2.00±0.55a
4	4.32±1.30b	6.20±1.67c	2.05±0.42a
5	1.98±0.70b	2.82±0.61b	2.67±0.70a

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

Table 6. Heights of the different pineapple plants as a function of time,

Time (months)	Height (cm) of plants from		
	Micropropagation	**Somatic embryogenesis**	**Traditional stalks**
1	9.95±2.09b	14.22±4.47c	7.68±4.11a
2	11.66± 1.76b	17.05±4.83c	8.87±4.46a
3	13.92 ±1.79b	18.51±4.74c	9.68±4.61a
4	16.47±1.81b	20.09±4.62c	11.15±5.06a
5	18.64±1.61b	21.81±4.61c	12.75±5.26a

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

Table 7. Display of leaves of different pineapple plants in the field.

Time (months)	Spreading of leaves (cm)		
	Micropropagation	**Somatic embyogenesis**	**Traditional stalks**
1	48.19±13.16b	49.01±20.98b	40.40±15.97a
2	50.56±13.98b	50.87±23.03b	36.70±17.69a
3	49.30±12.27b	62.48±23.78c	37.70±23.93a
4	43.08±11.29b	75.43±17.41c	38 .12±22.35a
5	53.46±11.33b	74.04±15.36c	35.63±20.78a

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

Table 8. Leaf widths D of pineapple plants.

Time (months)	Leaf width D (cm)		
	Micropropagation	**Somatic embyogenesis**	**Traditional stalks**
1	1.86±0.41b	2.31±0.51c	1.58±0.66a
2	2.48±3.28a	2.69±0.49c	1.76±0.78a
3	2.25±0.30b	2.70±0.45c	1.86±0.65a
4	2.23±0.38a	3.40±0.53c	2.33±0.73a
5	2.60±0.48b	3.40±0.43c	2.32±0.78a

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

Table 9. Length of leaves D of pineapple plants as a function of time.

Time (months)	Length of leaves D (cm)		
	Micropropagation	**Somatic embryogenesis**	**Traditional stalks**
1	28.48±6.64a	38.10±12.78b	24.50±8.89a
2	29.29±6.23b	38.98±12.17c	23.79±11.77a
3	31.68±6.24b	40.76±13.99c	25.10±12.21a
4	33.49±6.16b	48.30±9.55c	26.07±12.5a
5	39.84±9.07b	51.71±13.40c	27.42±14.32a

± S: standard error; on line the means followed by the same letter are statically identical at 5% (Newman-Keuls test). Experiment was triplicate.

This difference in the development of *vitro* plants in the field would be due to the degree of rejuvenation of the plants. Indeed, after obtaining the plants *in vitro* by micropropagation (8 weeks), the leaves were used to induce somatic embryogenesis (24 weeks). The longer time spent by the explants on the culture medium during somatic embryogenesis would have made it possible to obtain pineapple plants with a greater degree of rejuvenation than those resulting from micropropagation (bud culture).

This would explain the good behavior of plants produced by somatic embryogenesis in the field of those produced by micropropagation. Plants from traditional stalks had the low rates of growth and development. This is certainly because of the accumulation of pesticide residues and the use of rejects collected from the pineapple plants in the hut, that is to say the low rate of juvenization.

Perspectives

At the end of this work, it was noted that the *vitro* plants produced by micropropagation and those produced by somatic embryogenesis showed similar growth and development during acclimatization. Their 98% survival rate and the absence of variants observed during the weaning and rearing phases make buds and calluses potential explants for the *vitro* methods of *Ananas comosus* (L.) Merril var. cayenne smooth. Observations made on agronomic characteristics in the field showed significant differencesthroughout our study. The *vitro* plants were more efficient than the traditional suckers (controls).

The overall results of this study proved that acclimatization is a delicate and important step in the cultivation of *vitro* plants, because the best pineapple plants that come out of the lab can suffer enormous damage during acclimatization. Therefore, cultivation techniques should be mastered during this phase in order to be able to provide producers with quality rejects.

For a good development of pineapple cultivation in Côte d'Ivoire, it would therefore be interesting to replace traditional suckers with suckers with juvenile characteristics, i.e., from *vitro* cultures, in particular from somatic embryogenesis. To complete this work, it would be interesting to seek to reduce the duration of acclimatization by testing different substrates in view of a good control of the cultivation of pineapple plants.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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