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Behaviour of pineapple plants (*Ananas comosus* var comosus) resulting from *in vitro*budding and somatic embryogenesis during acclimatization phase and in the field

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ABSTRACT

The pineapple occupies a significant place in Côte d'Ivoire's economy. The quality of the plant material used in pineapple plantations is a guarantee of improved profitability and fruit quality. To raise this constraint, in vitro culture uses budding and somatic embryogenesis methods for a massive production of plants of good sanitary quality. However, during acclimatization, some major difficulties due to the rot of tissue culture plants and the nature of the substrate used disrupt production. To do this, different acclimatization substrates and the potentialities of Smooth Cayenne vitroplants were evaluated. During acclimatization under shade and in the field, survival rate of plantlets, growth and development parameters of vitroplants were compared. Suckers of field-grown of pineapple were used as controls. The results show a 98 % survival rate and the morphologically uniform plants were observed with potting soil mixed with sawdust in a 1:1 (v/v) ratio during weaning. And, the parameters observed showed no significant differences between vitroplants. However, the vegetative growth parameters in the field and those related to yield were significantly different between the three types of plants. The Productivity of plants regenerated by somatic embryogenesis are more efficient than those regenerated by in vitro budding. In addition, vitroplants have been more efficient than plants obtained traditionally. This difference in behaviour is due to the degree of rejuvenation of the plants by vitro methods. The potential for massive production of quality plantlets by in vitro cultivation is therefore an asset for the renewal of the Ivorian orchard in view of the revaluation of pineapple from Côte d'Ivoire. Acclimatization is a delicate and important step in in vitro culture. Its mastery will open up new possibilities for the farming world.

Keywords : Pineapple (*Ananas comosus* var comosus) - Côte d'Ivoire - *in vitro* regeneration - Substrates – Acclimatization

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INTRODUCTION

Pineapple [*Ananas comosus* var comosus (L.) Merril) Coppens and Leal] (Coppens and Leal, 2003), is native to South America. Pineapple is the eleventh most widely grown fruit (Kouadio, 2018). It ranks third among tropical fruits with a production of about 25.44 million tons (FAOSTAT, 2015). Pineapple culture occupies an important place in the economy of more than 80 countries. In Côte d'Ivoire, pineapple contributes for 0.6 % to national GDP and 1.6 % to agricultural GDP (MINAGRI, 2009). Indeed, the export of fresh pineapple generates nearly 34 billion FCFA of recipe per year (OECD, 2008). In Côte d'Ivoire, the smooth Cayenne variety is the most cultivated because of its adaptation to soil and climate conditions and especially for its yield potential and its appreciation on the international market (Leal and Coppens d'Eeckenbrugge, 1996). The quasi-hegemony of smooth Cayenne cultivation in Côte d'Ivoire has resulted, after several decades of intensive cultivation, without fallowing, a degeneration of the plant material with enormous phytosanitary risks. This has led to an ageing of the Ivorian orchard with its consequent decrease in production and quality. Thus, there was a drastic drop in production, of about 90.4 %, in 2014 compared to 1999 (Kouadio *et al*., 2018b). Faced with these problems, the renewal of the ageing orchard seems essential. To remove this constraint, the work of Yapo (2013) showed that *in vitro* regeneration is an effective alternative to provide producers with plants of good sanitary qualities in order to revive pineapple cultivation in Côte d'Ivoire. However, regenerated plants must undergo acclimatization, which is a critical phase of *in vitro* regeneration before being transferred to field. Indeed, the transition of vitroplants from *in vitro* conditions to very different natural conditions (substrate structure and texture, availability of nutrients, humidity and temperature, aggressiveness of micro and macro organisms absent in *vitro*, etc.) represents a physiological stress for plants(Bouare, 2008; Sissoko, 2009; Samber, 2010). Studies have also shown that the type of acclimatization substrate can influence the *ex-vitro* development of vitroplants (Dossoukpevi *et al*., 2015; Gnamien, 2016), thus affecting the success and profitability of micropropagation (Baiyeri, 2005). In addition, the survival rate of plantlets is generally low (Dibi, 2011). And, for the same cultivar, the agronomic performances of plants differ according to the initial propagation method (Youmbi *et al*., 2005). The general objective of this study is to evaluate the behaviour of vitroplants obtained by *in vitro* budding and somatic embryogenesis during acclimatization and in the field. The specific objectives are to evaluate the survival rate and parameters of vegetative growth and development of plantlets during acclimatization and in the field, and those related to vitroplant yield.

MATERIALS AND METHOD

Study site

The tests were conducted at the experimental station of Nangui Abrogoua University (formerly University of Nangui Abrogoua) (Abidjan, Côte d'Ivoire). The experimental site is located between latitude 6 ° 51 'N and longitude 5 ° 18' W (Figure 1). The climate is humid tropical type. Temperatures and average precipitation based on data recorded during the period March 2007 to February 2010 were 26.2 °C and 1504.61 mm of rainfall per year, respectively. The soil of the study site is ferruginous and loose type. The pH of this soil is more acidic on the surface. Its organic matter content varies from 2 to 3 % [17].

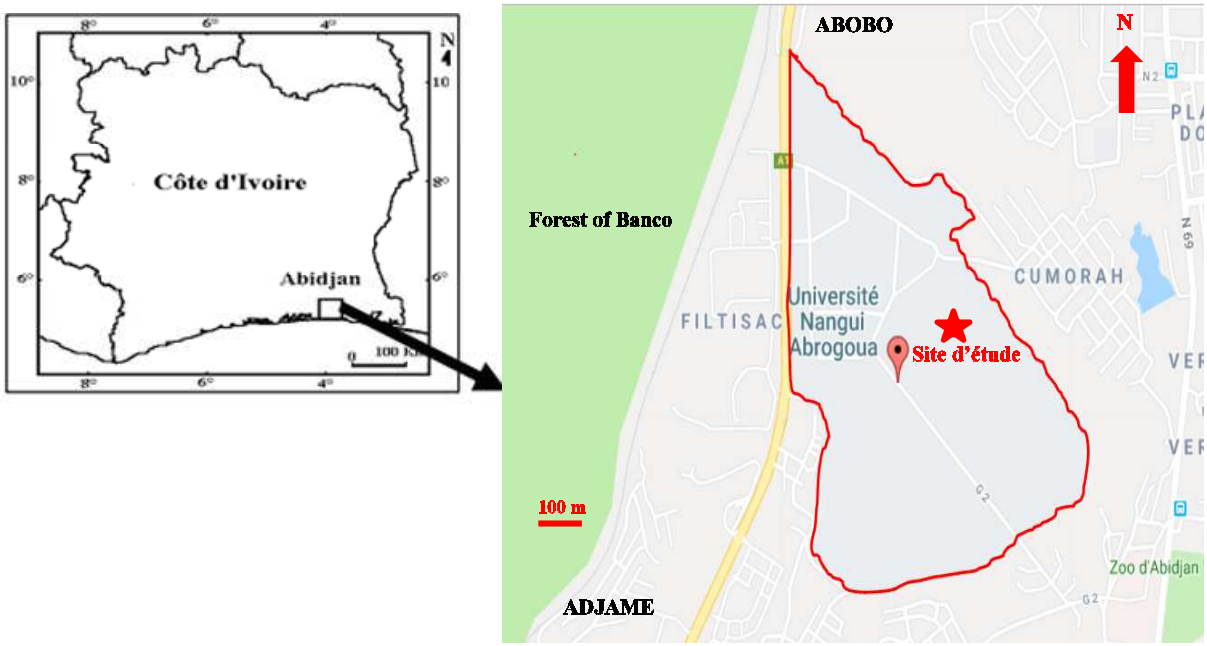

Figure 1. Location of the study site (Nangui Abrogoua University) in the Abidjan district

Source: N'cho, 2019

Plant material

The plant material consists of rooted smooth Cayenne vitroplants obtained by *in vitro* budding and somatic embryogenesis according to the routine protocol established in the laboratory (Yapo *et al*., 2011; Yapo, 2013). Traditional plants were used as controls in the field.

Methods

Study of the behaviour of different types of vitroplants on acclimatization substrates

Acclimatization of vitroplants was carried out in two stages: weaning and breeding.

Weaning

Weaning, which is the gradual adaptation of vitroplants to external conditions, was carried out in two phases:

For the first phase, three different acclimatization substrates were tested. These are sea sand, potting soil consisting of a soil layer of fallow land covered by vegetation and a mixture of potting soil and sawdust in a 1:1 (v/v) ratio. These substrates were autoclaved at 121 °C for 30 min at a pressure of 1 bar. After cooling, the substrates were placed in plastic bins previously perforated at the base and then sprayed with distilled water.The next day, plantlets about 12 cm long with well-developed roots were removed from the agar culture media. The roots were thoroughly rinsed with tap water to remove any traces of agar. Then, 20 plantlets from each *vitro* method were transplanted by tray containing the different substrates (Figure 2A). The bins were covered with a transparent plastic holder and placed in the culture room at 25 °C under a 12-hour photoperiod for two weeks. Water was supplied by sprinkling once every two days. A total of 120 vitroplants were used. After two weeks, the number of live plants was determined and the best substrate was retained for further

work.

The second phase consisted in evaluating the effect of the vitroplants origin during weaning in the bins containing only the best substrate previously retained. These bins were then transferred to a greenhouse at a temperature of 28 to 36 °C and a relative humidity of 77 to 85 % for eight weeks. Water was supplied by spraying at the rate of one watering per day. A total of 180 vitroplants were used at a rate of 30 vitroplants per type (origin of vitroplants) and per elementary plot. The test was conducted in a block device with three repetitions. The number of leaves, roots and height of each plant was determined.

Breeding

The weaned plantlets were transferred into 25 cm x 30 cm polyethylene bags (Figure 2B). These bags were previously filled with the best weaning substrate (potting soil + sawdust) and perforated to prevent excess water and root asphyxiation. The bags containing the plants were placed under shade. In this second stage of acclimatization, plant care is limited to spraying once every two 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.

The experimental setup put in place is a completely random block consisting of three trials with three repetitions each, where each type of plants corresponds to one test. For each trial, thirty plants were used. Each month, the number of leaves emitted by them and the number of plants that survived acclimatization were recorded. The survival rate (SR) of acclimatized pineapple plants was calculated according to the following formula:

Survival rate (%) = [number of acclimatized vitroplants / number of released vitroplants] × 100

Figure 2. Acclimatization of pineapple vitroplants

A: vitroplants transferred to substrate in bins; B: plantlets transferred to substrate in polyethylene bags placed under shade; C: acclimatized plant, ready to be transferred to the field

Study of the behaviour of the different types of plants in the field Establishment of the test

Three types of pineapple plants were used:

- pineapple plants regenerated by micropropagation (PM) and acclimatised;

- pineapple plants regenerated by somatic embryogenesis (PE) and acclimatised;

- plants from conventional propagation (PR), i. e. from pineapple mother plants, used as controls.

Thus, plants that averaged 30 cm in length were transferred to the field (Figure 2C). These plants were transplanted onto double line ridges in a three-repeat block system. These 5 m × 1 m ridges were previously amended with 1 kg of dolomite and 500 g of calcium triphosphate and then covered with black polyethylene film. The plants were staggered with a spacing of 25 cm on the line and 40 cm between the lines. An elementary plot consisted of 100 plants of each type of plant. The field is regularly maintained and a monthly fertilization of 2.5 g of potassium sulphate and 1 g of urea per plant has been carried out.

Evaluation of growth and development parameters

Every two weeks the height of the plants; the number of leaves emitted per plant; the length and width of leaf D were determined. After ten months of cultivation, TIF (floral induction treatment) was applied to induce uniform flowering of the plants. Approximately 150 days after TIF, the fruits were harvested and the total number of fruits harvested for each type of plant used was determined by counting. The mass of each of the harvested fruits was determined using a scale (Mettler-Toledo, B154 ®). Fruit with a mass greater than or equal to 800 g was considered commercially valuable and the rate was determined according to the following formula:

Commercial size fruit rate (FCIC) = 100 x (number of fruits harvested with a weight greater than 800 g/ number of fruits harvested).

The length of the fruit, the diameter of the fruit and the diameter of the central cylinder (or core) expressed in centimetres (cm) were measured using a caliper. Then, the yield (Rdt) was determined as follows:

Yield (kg/Ha) = mass of fruit of commercial size (Kg)/area (Ha)

Statistical analysis

For all experiments performed, STATISTICA 7.0 software was used for statistical analysis. The analysis of variance (ANOVA) revealed whether there was a difference between the factors studied. When a difference was observed, the Newman-keuls multiple rank test at the 5 % threshold was adopted to separate the averages. The percentage data were processed by the kruskal-wallis test.

RESULTS AND DISCUSSION

Evaluation of the vitroplants behaviour on acclimatization substrates after weaning

Evaluation of substrate effect on the survival rate of vitroplants

The results of the various tests carried out made it possible to highlight the behaviour of the vitroplants on the substrates tested (Table 1). The mixture of potting soil and sawdust was the most favourable to the recovery of vitroplants in an *ex vitro* environment with a survival rate of 87.5 %. Thus, this substrate (potting soil + sawdust) was used for further work.

Table 1. Survival rate of acclimatized vitroplants on different substrates

Acclimatization substrates	Number of acclimatized plantlets	Plant survival rate (%)
Sea sand	40	17,5±1,82a
Potting soil	40	47,5±3,83b
Potting soil + sawdust	40	87,5±2,17c

±S: standard error; in the same column the averages followed by the same letter are statically identical to the 5% threshold (Newman-keuls test).

Evaluation of the vitroplants origin on growth and development parameters

During acclimatization, the parameters measured (Table 2) did not change significantly from beginning to end of weaning. Plantlets produced by bud culture behaved in the same way as those produced by somatic embryogenesis.

Table 2. Evolution of growth parameters of different types of vitroplants during weaning

Parameters	**Origin of vitroplants**			
	***In vitro* budding**		**Somatic embryogenesis**	
	Before weaning	***After weaning***	***Before weaning***	***After weaning***
Number of sheets	11,34±3,83a	12,72±3,52a	13,00±3,64a	14,04±3,01a
Number of roots	6,53±3,74a	12 ,52±4,47b	7,94±3,22a	15,22±13,85b
Height (cm)	12,08±0,75a	12,87±0,86a	12,08±0,47a	12,89±0,50a

±S: standard error; on the same line the averages followed by the same letter are statically identical to the 5 % threshold (Newman-keuls test).

Evaluation of the vitroplants behaviour on acclimatization substrates during breeding Survival rates of vitroplants after acclimatization

According to the results reported in Table 3, whatever their origin, all the plantlets used adapted identically to the external environment (potting soil + sawdust) with a survival rate of about 98 %.

Table 3. Survival rate of different types of pineapple vitroplants after acclimatization

Origin of vitroplants	**Number of acclimatized plants**	**Plant survival rate (%)**
Growing buds	60	98,0±1,63a
Somatic embryogenesis	60	98,3±0,96a

The values followed by the same letter are not significantly different at p = 0.05; the values represent the average of 3 repetitions; ±S: standard error; in the same column the means followed by the same letter are statically identical to the 5% threshold (Newman-keuls test).

Average number of leaves emitted per month

The analysis of Table 4 shows that during the breeding phase (growth phase), the average number of leaves emitted was not significantly influenced by the origin of the vitroplants.

Table 4. Average number of leaves emitted by each vitroplant during the breeding phase

Time	Number of leaves emitted	
	by plants obtained by *in vitro* budding	by plants obtained by somatic embryogenesis
1st month	1,68±0,63a	1,83±0,66a
2nd month	1,86±0,66a	1,95±0,35a
3rd month	2,06±0,57a	2,09±0,52a

±S: standard error; on the same line the averages followed by the same letter are statically identical to the 5% threshold (Newman-keuls test).

Evaluation of the behaviour of the different types of plants in the field

Evaluation of the effect of plant origin on growth and development parameters in the field

Number of leaves

The results presented in Figure 3 show that in the field the average number of leaves was very significantly influenced by the origin of the plant. Thus, plants from *vitro* methods induced the highest number of leaves during the test compared to those from traditional plants (suckers). However, the average number of leaves of plants produced by somatic embryogenesis is higher than that of plants produced by *in vitro* budding.

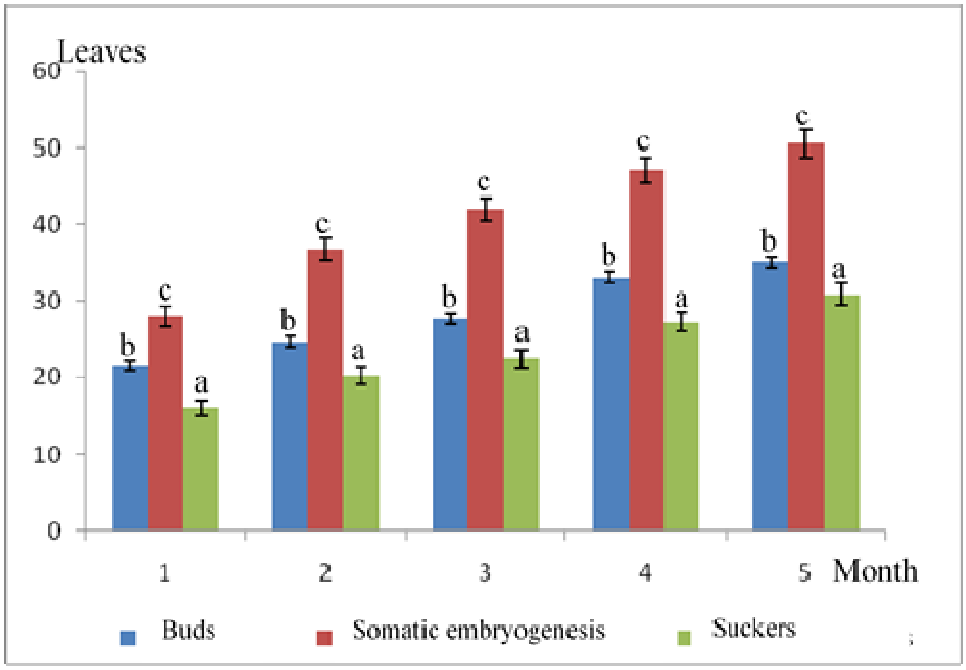

Figure 3. Average number of leaves of the different pineapple plants

Histograms with different letters are significantly different at the 5% threshold (Newman keuls test)

Rhythm of monthly foliar emission

The foliar emission rhythm was significantly influenced by the origin of the plants used (Figure 4). Thus, the foliar emission rhythm of vitroplants was significantly higher than that of traditional plants (suckers). However, the kinetics of emission from somatic embryogenesis was greater than that of plants produced by bud culture. Significant fluctuations were observed during foliar emission in vitroplants. These fluctuations are characterized by a slowdown phase from the first to the third month and a more accelerated phase from the third month. The rhythm of foliar emission from traditional plants has been slow for five months.

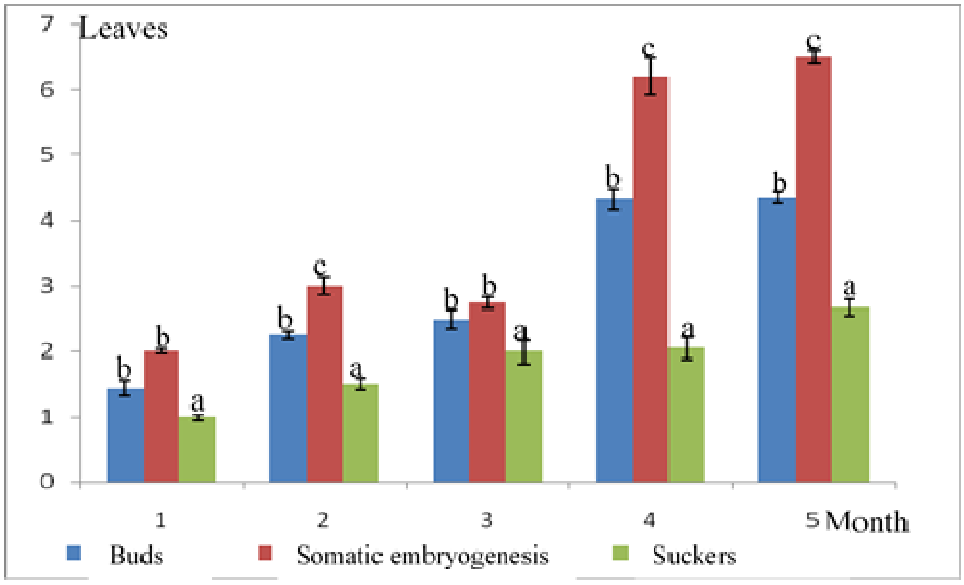

Figure 4. Rhythm of foliar emission of different pineapple plants

Histograms with different letters are significantly different at the 5% threshold (Newman keuls test)

Height oh plants

The data collected per month in the field show that the average heights of plants produced by bud culture and somatic embryogenesis are significantly different and higher than those of traditional discharges. However, plants obtained from somatic embryogenesis had the highest mean heights (Figure 5).

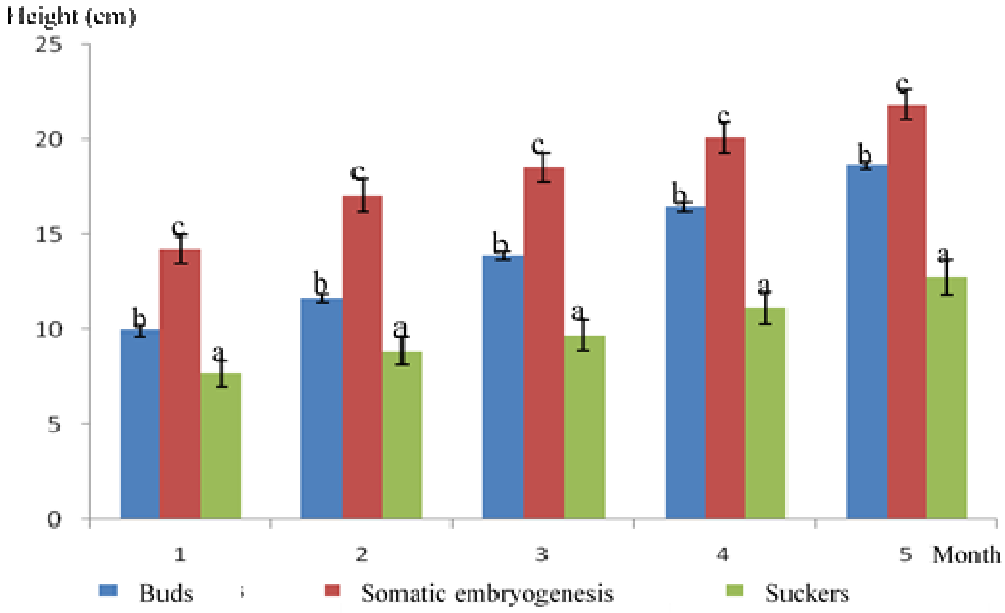

Figure 5. Height of the different pineapple plants

Histograms with different letters are significantly different at the 5% threshold (Newman keuls test)

Width of leaf D

The results reported in Table 5 show that the width growth of leaf D was significantly different depending on the origin of the plants. Thus, the vitroplants had the best average values of the width of the leaf D compared to those of the plants resulting from traditional plants. Concerning the average leaf D width of plants obtained by *vitro* methods (*in vitro* budding and somatic embryogenesis), the analysis of the variances revealed no significant difference during the first two months of culture. But from the third month, the average leaf width (D) of the somatic embryogenesis plants was higher than that of the bud culture plants.

Table 5. Leaf width (D) of the different types of pineapple plants in the field

Leaf width (D) (cm)			
Time	**plants obtained by *in vitro* budding**	**plants obtained by somatic embryogenesis**	**plants obtained by traditional budding**
1st month	2,91±0,16b	3,01±0,98b	2,02±0,97a
2nd month	3,99±0,98b	4,17±1,03b	3,26±1,69a
3rd month	5,02±0,27b	6,48±0,78c	3,70±1,93a
4th month	5,98±1,29b	7,13±1,41c	5,12±1,35a
5th month	7,26±1,33b	8,01±1,36c	6,83±1,78a

±S: standard error; on the same line the averages followed by the same letter are statically identical to the 5% threshold (Newman-keuls test).

Length of leaf D

The average length of leaf D was significantly influenced by the origin of the plants (Table 6). The

average lengths of the leaves (D) of the plants obtained by the vitroplants are higher than those of the leaves D of the plants resulting from the traditional plants. The results also show that the average lengths of the plants produced by somatic embryogenesis are greater than those of plants produced by bud culture.

Table 6. Leaf length (D) of the different pineapple plants in the field

Leaf length (D) (cm)			
Time	**plants obtained by *in vitro* budding**	**plants obtained by somatic embryogenesis**	**plants obtained by traditional budding**
1st month	30,48±06,64a	33,10±10,78b	29,50±08,89a
2nd month	48,29±03,23b	57,98±08,17c	41,79±11,77a
3rd month	69,68±06,24b	88,76±11,99c	59,10±12,21a
4th month	88,49±08,16b	100,30±09,55c	73,07±12,50a
5th month	97,84±05,07b	117,71±13,40c	82,42±10,32a

±S: standard error; on the same line the averages followed by the same letter are statically identical to the 5% threshold (Newman-keuls test).

Evaluation of plants origin effect on the physical characteristics of fruits

All pineapple plants used in this trial (15 months of cultivation), from planting to harvesting, were able to produce fruit (Figure 6). However, the physical characteristics of the fruits differ according to the origin of the cultivated plants (Table 7). Thus,vitroplants have a better yield (6743 kg/ha and 5979 kg/ha for fruits FE and fruits FM respectively) than plants resulting from traditional budding (4992 kg/ha). The results also show that the comparison of the average weights of the three types of fruits revealed that fruits FE have significantly higher weights (1124 g) than fruits FM (996 g) and fruits FR (832 g). Similarly, the length and diameter of the three types of fruit evolved in parallel with the weight of the fruit. In addition, 65 % of fruits FE had a significantly higher commercial interest rating than fruits FM (53.70 %) and fruits FR (43 However, the results revealed no significant difference between the central cylinder (heart) of the three types of fruit. The results obtained show that the vitroplants have developed fruits of physical characteristics significantly higher than those of the fruits of the traditional plants.

Figure 6. Fruiting of field-acclimatized pineapple plants

A: fruiting of pineapple plants acclimated to the field; B: mature pineapple fruit

Table 7. Physical characteristics of pineapple fruits from the three types of plants

Parameters	Different types of pineapple fruit		
	Fruits EN	**Fruits FM**	**Fruit FE**
Average weight (g)	832±4,71a	996±2,17b	1124±1,52c
Yield (kg /ha)	4992±9,37a	5979±4,33b	6743±7,61c
Length (cm)	11,89±1,11a	12,55±2,28b	14,00±1,52c
Fruit diameter (cm)	09,00±1,08a	10,00±1,17b	11,50±0,71c
Heart diameter (cm)	03,00±0,68a	03,00±0,19a	02,80±0,27a
TFIC (%)	43,00±1,43a	53,7±2,80b	65,00±1,33c

Fruits FR (fruits from conventional plants); Fruits FM (fruits from micropropagated plants); Fruits FE (fruits from plants propagated by somatic embryogenesis). TFIC = Fruits of commercial interest size ±S: standard error; on the same line the averages followed by the same letter are statically identical to the 5% threshold (Newman-keuls test).

DISCUSSION

The objective of this study was to evaluate the behaviour of vitroplants obtained by *in vitro* budding and somatic embryogenesis during acclimatization and in the field. Tests carried out during acclimatization reveal that the nature of the substrate used for our tests greatly influenced the weaning of vitroplants. Thus, the mix of potting soil and sawdust was the most favourable for the recovery of vitroplants (87.5 %). This suggests that the combination of substrates would be beneficial for plantlet recovery during acclimatization. Similar results have been reported by several authors(Souayah *et al.,* 2004; Sidibé *et al*., 2013; Ayoliè *et al*., 2016)in various plant species (banana, tomato...). Indeed, mixing sawdust with potting soil would improve the physical properties (structure and texture) of the substrate. The latter becomes much airier and less compact (asphyxiating). This allows better water retention and therefore promotes a high survival rate of vitroplants compared to sand and potting soil alone (El Hamdouni *et al*., 2000). In addition, the decomposition of the wood would release mineral elements that enrich the acclimatization substrate. The improvement of these physical and trophic parameters of the substrate by adding sawdust, coupled with strict control of environmental conditions (temperature and humidity), have favoured the survival of vitroplants on this substrate.

In addition, sand was the least favourable to the recovery of pineapple vitroplants during weaning (17.5 %). This can be explained on the one hand by the fact that a material with a granular structure such as sand offers a higher resistance to root penetration than a material with a fibrous structure such as sawdust (Folliot and Marchal, 1990)or lumpy material such as potting soil. On the other hand, the salt contained in the sea sand would have induced salt stress at the origin of certain metabolic disturbances (reduction of root development, inhibition of hydromineral nutrition, disturbance of photosynthesis, reduction of cell division ...) leading to the death of several plantlets on this substrate (Ould Mohamdi *et al*., 2011 ; Achour *et al*., 2015 ; Kouadio *et al.*, 2018a). These results are contrary to those reported by Gnamien (2016)in pistachio and Kouadio (2018)in smooth cayenne. These authors mentioned that sand alone favoured a better adaptation of vitroplants. However, they reported that the combination of sand with any other substrate causes some

compaction that reduces root aeration. This has a negative influence on the recovery of vitroplants.

This study also showed that, in general, the origin of vitroplants (plants from bud culture and plants from somatic embryogenesis) did not significantly influence their behaviour (identical survival rate: 98 %, same number of leaves and roots and same height growth) during the growth phase (breeding). This suggests that both types of vitroplants are anatomically and physiologically similar. Plants from bud culture would therefore have the same agronomic characteristics as those from somatic embryogenesis. Similar results have been reported by Chatibi *et al (*1995)in pistachio trees. Indeed, the work of these authors has shown that during acclimatization, vitroplants derived from pistachio leaves behave in the same way as those derived from buds or cotyledons. Admittedly, in *in vitro* culture, the survival rate of plantlet after acclimatization is generally low (Dibi, 2011). However, our study showed a 98 % pineapple plantlet survival rate after acclimatization. This result is rare in *in vitro* culture, but several authors have reported similar plantlet survival rates in other plants (Jain *et al*., 2002; Le Van *et al*., 2004). Similarly, Sripaoraya *et al* (2003)reported plantlet survival rates of 96 % during pineapple regeneration via embryogenesis and organogenesis. The growing conditions applied during this work would certainly be beneficial to the development and growth of pineapple plants.

Concerning the evaluation of the behaviour of vitroplantsin the natural environment (*in planta*), the results of this study reveal that all pineapple plants, whatever of their origin, survived after their transfer to the field. Although somaclonal variations were observed by Smith *et al* (2002), no phenotypic variation in pineapple plants was observed in this study. All the plants were able to produce fruit after 15 months of culture. However, the physical characteristics of the fruit differ according to the origin of the grown pineapple plants. Thus, compared to the FR fruits produced by pineapple plants resulting from conventional propagation (PR), i. e. from suckers taken from pineapple mother-plants, the FM and FE fruits produced by pineapple plants from *vitro* cultures (micropropagation and somatic embryogenesis) had higher fruit weights and yields. These results seem to reveal the primordial role of *vitro* methods in the material savings observed in fruits from vitroplants. This improvement in yield would come from the physiological potential of the vitroplants because all the plants were cultivated under the same conditions. Indeed, after obtaining the vitroplants by micropropagation (8 weeks), the leaves were used to induce somatic embryogenesis (24 weeks). Since *vitro* cultures allow plants to be rejuvenated (Dibi, 2011), the longer time spent by explants on culture media during somatic embryogenesis would have made it possible to obtain pineapple plants with a higher degree of rejuvenation than those from micropropagation. Therefore, the rejuvenation of vitroplants seems to confer more intense physiological potentialities as reported by (Dibi, 2011) in rubber tree. In fact, the juvenility induced by *in vitro* culture has increased the cell division capacity of floral tissues (ovaries, sepals and bracts), stimulated intense synthesis of certain molecules such as sugar and increased fruit size more rapidly. The results reveal an intense differentiation of floral organs in vitroplants compared to traditional plants (suckers). This would explain the best yields obtained by vitroplants. These results are in agreement with the work of Youmbi *et al.* (2005)who showed that banana plantlets had a good yield compared to traditional plants.

CONCLUSION:

Acclimatization is a delicate and important step in *in vitro* culture, the mastery of which will open up new possibilities for the farming world. This study showed that the mixture of potting soil and sawdust allows the best development of pineapple plants for both types of vitroplants considered during weaning. During breeding (growth), the origin of vitroplants (plants resulting from the

micropropagation by culture of terminal buds and plants resulting from somatic embryogenesis) does not influence their behaviour (survival rate, growth and development). In the field, the study showed that the degree of rejuvenation of plants by *vitro* methods seems to influence vegetative development and the differentiation of floral organs. However, the impact of *in vitro* culture on the biochemical parameters of fruits should be established in order to fully appreciate the influence of the rejuvenation of plants induced by *vitro* culture in pineapple.

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