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DIFFERENTIAL RESPONSE OF PINEAPPLE CULTIVARS TO PROPAGULES PRODUCTION USING STEM CUTTINGS

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ABSTRACT

Unavailability of pineapple planting materials has become one of the main bottlenecks in the exportation of the main pineapple cultivar produced in Benin. To increase the availability of homogenous planting materials, we determined the effects of stem cutting types and buds induction on the propagation of pineapple. Three stem cuttings types and two buds induction regimes were assessed on three cultivars following a split-split-plot design with four replicates. The growth data collected were subjected to analyses of variance followed by Student-Newman-Keuls test. We used the generalized linear models with Poisson distribution to analyse the count data and the generalized mixed effect models to analyse count data repeated in the times. Buds induction regime negatively affected pineapple stem propagation. Cutting types significantly affect propagules number and uniformity in all cultivars. Slice cuttings produced more uniform propagules. We also found a linear relationship between the propagules weight and height in Smooth Cayenne and Adjago cultivars. These results provide guidance for the production of uniform planting materials in pineapple particularly for Smooth Cayenne and Adjago's cultivars.

Keywords : *Ananas comosus*, vegetative propagation, uniformity, planting material, Smooth Cayenne.

RÉPONSE DIFFÉRENTIELLE DES CULTIVARS D'ANANAS À LA PRODUCTION DES REJETS A L'AIDE DE BOUTURES DE TIGE

RÉSUMÉ

L'indisponibilité des rejets d'ananas est devenue l'un des principaux goulots d'étranglement liés à l'exportation des principaux cultivars d'ananas produit au Bénin. Afin d'augmenter la disponibilité de matériel de plantation homogène, nous avons déterminé les effets des types de coupe de tiges, et de l'induction des bourgeons sur la propagation de l'ananas. Trois types de coupe de tiges et deux régimes d'induction au bourgeonnement ont été évalués sur trois cultivars suivant un dispositif expérimental en split-split-plot avec quatre répétitions. Les données de croissance recueillies ont été soumises à des analyses de variance et au test de Student-Newman-Keuls. Nous avons utilisé les modèles linéaires généralisés avec la distribution de Poisson pour analyser les données de comptage et les modèles linéaires généralisés à effets mixtes pour analyser les données de comptage répétées dans le temps. Le régime d'induction des bourgeons affecte négativement la propagation des tiges d'ananas. Les types de coupe affectent significativement le nombre de rejets et l'uniformité au niveau de tous les cultivars. Les boutures coupées en rondelles produisent un plus grand nombre de rejets uniformes. Nous avons également établi une relation linéaire entre le poids et la taille des rejets dans les cultivars Adjago et Cayenne Lisse. Ces résultats fournissent des indications pour la production de matériels de plantation uniformes dans l'ananas en particulier pour les cultivars Cayenne Lisse et Adjago.

Mots clés : *Ananas comosus*, propagation végétative, uniformité, matériel de plantation, Cayenne Lisse.

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INTRODUCTION

Ensuring that farmers have timely access to planting material of good quality is one of the most important elements for sustainable agricultural production (Kumar, 2008). However, the planting material available to smallholder farmers in many developing countries is often of insufficient quality, which undermines potential yields and overall harvest values and performance of crop production (Ogero, Gitonga, Mwangi, & Ombori, 2012). This situation is very critical for many tropical fruits including pineapple for which the demand has increased owing to recent policies for sustainable development and global economic crisis that created opportunities to increase fruits consumption for health benefits (Rana, Garforth, Sthapit, & Jarvis, 2007).

Pineapple (*Ananas comosus* L. Merr.) belongs to the Bromeliaceae family which includes about 2000 species (Givnish, Millam, Berry, & Sytsma, 2007). It is much appreciated all over the world for its excellent organoleptic qualities. Pineapple has diverse uses to millions of people. It is consumed fresh as dessert or enter into the preparation of molecules used for the treatment of diseases (Joy, 2010; Medina & Garcia, 2005). Several cultivars are produced in the world, but Smooth Cayenne is the only one that dominates the pineapple trade and industry (Botella & Smith, 2008). Although it is grown in more than 82 countries around the world, there is a remarkable lack of commercial cultivar of Smooth Cayenne (Soneji & Nageswara Rao, 2009).

In recent years, there has been increasing tendency towards large-scale commercial production of pineapple in Benin owing to the increased demand in exportation. Two main cultivars are produced in the country: Smooth Cayenne and Sugarloaf (Perola). Smooth Cayenne is the most important cultivar often reported in Benin pineapple exportation (Achigan-Dako *et al*., 2014). In the last decade, Sugarloaf was also introduced in exportation toward Nigeria and Burkina, but the quantity is still low. Unfortunately, unavailability of adequate planting materials continues to be a major constraint that limits the large-scale exportation of Smooth Cayenne and the expansion of few existing farms in West Africa (Arinloye *et al*., 2012). It is very difficult to access uniform planting materials in large quantity due to the low rate of multiplication by conventional methods and lack of high quality propagules. The lack of homogenous planting materials is one of the main causes of heterogeneity of pineapple size leading to the low rate of exportation which is still around 2 % (Fassinou Hotegni *et al*., 2014).

A primary responsibility for research and development institutions is to propose to farmers adequate and cost-effective technologies to generate sufficient propagules for planting. Several techniques are available to produce uniform planting materials, including tissue culture. The use of tissue culture techniques has two advantages. It can be used to produce large number and uniform pineapple propagules in a relatively short period of time (Firoozabady *et al*., 2003), and can also be used to free planting material from pest and diseases (Swennen, 1990). However, as a sophisticated method, it requires skilled labor force (Vuylsteke, 1998). Tissue culture as a method of generating planting materials is very expensive and is not currently developed in Benin for smallholder farmers. There is therefore a pressing need for utilizing cost effective and simple techniques such as macro-propagation to produce planting materials in pineapple.

They are several techniques to speed up propagules production and vegetative growth in pineapple (Coppens d'Eeckenbrugge & Leal, 2003 ; Maerere, 1996). These techniques vary in their level of sophistication and proliferation rate of propagules. They tend to accelerate the initiation of axillary buds by removing inhibitory effects exerted by the apical meristem. Commonly used propagation techniques in pineapple include the mechanical destruction of terminal meristem, the fragmentation of propagules, and the stem cutting. The mechanical destruction of the terminal meristem with a gouge allows to produce about 6 to 8 propagules. The fragmentation of propagules is to subdivide propagules or crown into several part to produce about 4 or 6 others propagules (Maerere, 1996). The stem cutting provides more than ten propagules (Thiémélé *et al.*, 2013). Some of these techniques were based on the use of appropriate substratum to obtain a lot of planting materials but overlooked the uniformity of the propagules produced (Thiémélé *et al.*, 2013 ; Weerasinghe & Siriwardana, 2006). The use of the stem cutting method for increasing pineapple suckers at farm level was tested in Côte d'Ivoire (Thiémélé *et al.*, 2013). The stem cutting technique requires no specialized skills and can be used to produce large scale planting material in a relatively short period of time (Weerasinghe & Siriwardana, 2006). However, the issue of heterogeneity in planting material requires more attention to understand how the different cutting types affect the size and the weight of the propagules. Moreover, we do not know how different cultivars will respond to stem cutting propagation to yield uniform planting materials and how the height and weight of the propagules are affected by cutting types.

This study aims at evaluating the response of three pineapple cultivars on the production of uniform propagules using various stem cuttings. Here, we hypothesize that cultivars and propagules height and weight respond differently to the cutting types used.

MATERIALS AND METHODS

Plant material and stem pre-treatment

The stems of three cultivars collected from recently harvested plants were used. Cultivars included : 1) "Smooth Cayenne", a semi erect plant with smooth and numerous leaves producing large, sweet but acid fruits which weight on average (2 to 3 kg). It produces on average one or two propagules per plant per year ; 2) "Sugarloaf"(also known as "Pérola"), an erect plant with very spiny leaves that produces pyramidal fruits (0.7 to 2.2 kg) and many slips (10 to 16 per plant) (Coppens d'Eeckenbrugge & Leal, 2003 ; Maerere, 1996), and 3) “Adjago”, a semi erect and high plant with numerous smooth long and wide leaves that produces very large sweet and low acid fruits (2.5 to 4 kg). It produces very low rate of propagules, one or two suckers three months after harvest.

To gather plant materials for the experiment, stems were uprooted after fruit harvest and let in the field for three days to facilitate leaf removal. Leaves were removed from the stem and the remaining part was sunk into a mixture of Dimethoate (an insecticide) and Thiophanate methyl (a fungicide) respectively 50 ml and 80 g on 15 L of water during 30 min to prevent diseases and pest infestations. The treated samples were air-dried for one day.

Experimental site and design and data collection

The study was carried out at the Faculty of Agronomic Sciences, University of Abomey-Calavi (06°25.029’N and E 002°20.430’E) in Benin, which has a subequatorial climate with a succession of four seasons (two rainy seasons and two dry seasons), a rainfall varying between 850 mm and 1160 mm.

The trials were set up from July 11 to November 10, 2014 and repeated from February 20 to June 30, 2015. We used a split-split plot experimental design with four replicate blocks. The main factor was bud induction vs. no bud induction. Bud induction consisted in sinking the cuttings in water and bagging them for five days before planting. Water is required for optimal hydrolysis of food storage macromolecules and for increased enzymatic activities. It helps buds to break their dormancy and increase sprouting capacity (Lang, Early, Martin, & Darnell, 1987). Bagging the stem after sinking in water might help cuttings to maintain favorable conditions for better sprouting.

The stem cutting type was the second factor, with three modalities: whole stems, longitudinally cut stems, and slice cuttings (Figure 1).

Figure 1. Pineapple stem cuttings : A- Cleaved cuttings ; B- slice cuttings C- whole cuttings.

The third factor involved cultivars ("Sugarloaf", "Smooth Cayenne", and "Adjago"). For each block, a total of 18 experimental plots were prepared. The trial was installed in a ferralitic soil. A set of 10 stems was planted in two rows on each experimental plot. Whole stems and longitudinal sections were about 15 cm long, and slice cuttings had 3 cm diameter.

Data collection started two weeks after stem planting. Data collected included the time of first budding, the number of propagules, the height and length of the propagules, the number and width of the leaves. These data were collected on at least five propagules per plot each week during twelve consecutive weeks. After three months, all propagules were removed, counted and weighed per plot.

Data analysis

Descriptive statistics such as frequency, means, standard deviation, and percentages were used to analyze the data. To test the effect of cutting types, cultivars and bud induction method on sprouting time, we used the generalized linear model with binomial distribution and for number of propagules, and number of leaves, we used the generalized linear model (glm) with Poisson distribution (Anders & Huber, 2010 ; Myers & Montgomery, 1997). The effect of the same factors on the propagule leaf length and width were tested using linear mixed effects models. Allometric relationships were tested between plant height and propagule weight to understand how these relationships evolve for cutting types, cultivars and buds induction regimes.

RESULTS

Effects of cutting types, bud induction on sprouting ability of pineapple cuttings

Sprouting time

The sprouting time varied from 17 to 40 days. On average it was 22 days when cuttings were planted with no bud induction and 31 days when cuttings were sunk in water and bagged. Bud induction delayed sprouting time. The shortest sprouting time (17 days) was observed in "Sugarloaf" for whole cuttings. Propagules obtained from whole stem cuttings grew rapidly in all cultivars. Cutting types have significant effects on sprouting time ($p < 0.001$). Whole and cleaved cuttings sprouted earlier whereas slice cuttings sprouted later (Table 1). The analysis of deviance using the generalized linear models with Poisson distribution showed a highly significant three-way interaction for cutting types, buds induction and cultivars ($p < 0.001$) on sprouting time.

Table 1. Sprouting time (number of days between planting and first buds)

Bud induction regime	Type of materials	Cultivars	Sprouting time (Days)
No induction	Whole	Sugarloaf	17
		Smooth Cayenne	20
		Adjago'	18
	Cleaved	Sugarloaf	18
		Smooth Cayenne	24
		Adjago'	23
	Slices	Sugarloaf	20
		Smooth Cayenne	24
		Adjago'	23
Induction	Whole	Sugarloaf	NA
		Smooth Cayenne	27
		Adjago'	23
	Cleaved	Sugarloaf	NA
		Smooth Cayenne	38
		Adjago'	27
	Slices	Sugarloaf	NA
		Smooth Cayenne	39
		Adjago'	33

Number of propagules

The number of propagules produced varied according to the bud induction regime, cutting types and cultivars (Figure 2). The number of propagules varies from 2 to 16 per stem for whole cuttings after three months. The mean number of propagules is presented for each treatment combination in Table 2.

Bud-induced stems produced lower propagules number in all cutting types. Bud-induced stems of Sugarloaf did not produce any propagules because stem decayed. Slice cuttings produced a higher number of propagules (more than 10 per stem) than the other cuttings. Moreover, those propagules were uniform. The analysis of deviance performed with generalized linear mixed models on the number of propagules showed a highly significant three-way interaction for cutting types, buds induction and cultivars ($p < 0.001$).

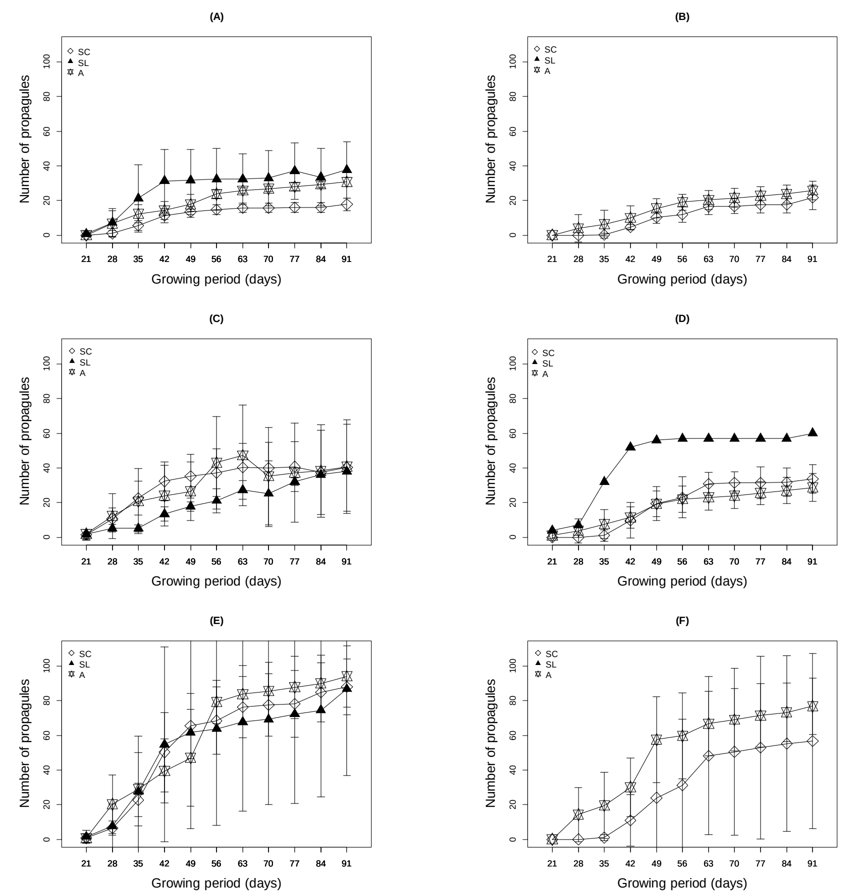

Figure 2. Variation of the number of propagules per cultivar, cutting types and buds induction according to growing period. A : Whole stem with no buds induction ; B : Whole stem with buds induction ; C : Cleaved stem with no buds induction ; D : Cleaved stem with buds induction ; E : Stem cutting in slices with no buds induction; F : Stem cutting in slices with buds induction. A : Adjago, SC : Smooth Cayenne, SL : Sugarloaf

Table 2. Effect of treatment on mean number of propagules

Stem Treatment	Mean number of propagules per stem with standard errors								
	Smooth Cayenne			Sugarloaf			Adjago		
	Whole	Cleaved	Slices	Whole	Cleaved	Slices	Whole	Cleaved	Slices
No induction	3.7 ± 0.22	5.60 ± 1.57	10.8 ± 1.01	3.77 ± 1.67	5.10 ± 1,67	8.67 ± 3.12	3.7 ± 0.33	4.07 ± 1.70	12.50 ± 3.39
Buds induction	2.18 ± 0.45	3.37 ± 5.12	4.1 ± 3.17	NA	NA	NA	2.57 ± 0.33	3.87 ± 0.49	7.67 ± 1.03

Effects of cutting types, bud induction on plant growth parameters

Plant height

As main factors, bud induction regimes, cutting types and cultivars had no significant effect on plant 3.2. Effect of bud induction and cutting types on early plant growth in pineapple cultivars height (p = 0.082). Bud induction regime did not improve propagule height in any cultivar whatever the cuttings types. Plant heights were 58.5 ± 0.12 cm, 42.6 ± 0.32 cm and 33.3 ± 0.38 cm respectively for whole stems, cleaved stems and slices after three months. Bud induction was detrimental for Sugarloaf especially with the whole and slices cuttings (Figure 3).

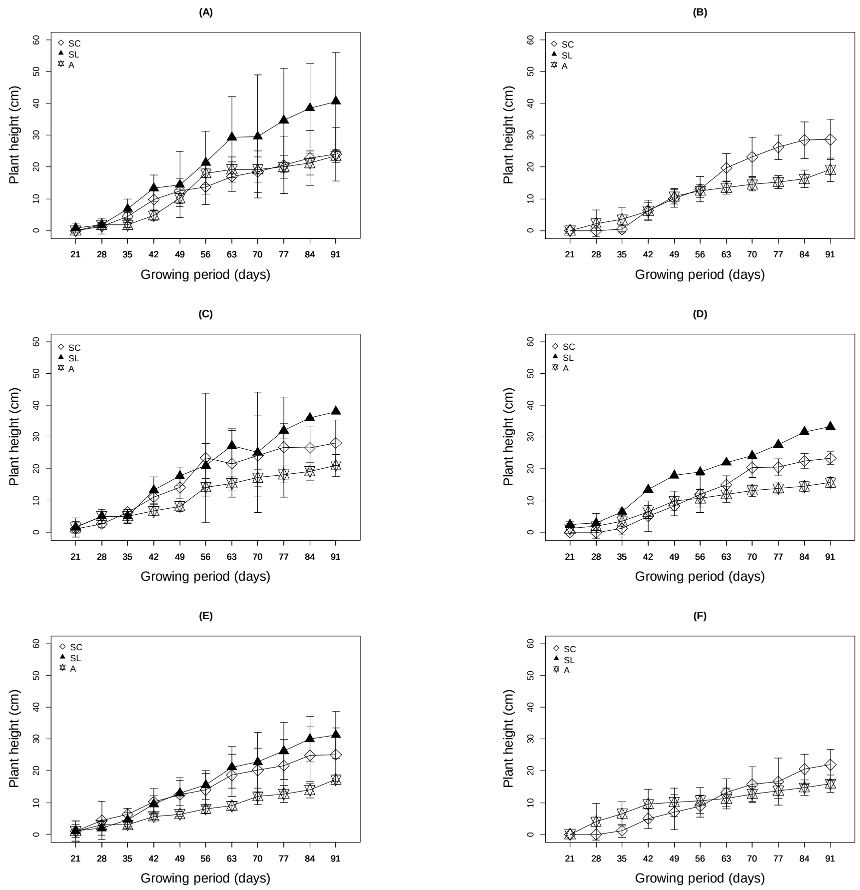

Figure 3. Variation of plant height per cultivar, cutting types and buds induction according to growing period. A : Whole stem planting with no buds induction ; B : Whole stem with buds induction ; C : Cleaved stem and planting with no buds induction ; D : Cleaved stem with buds induction; E: Stem cutting in slices planting with no buds induction ; F : Stem cutting in slices with buds induction. A : Adjago, SC : Smooth Cayenne, SL : Sugarloaf.

Leaf length and width

One average, leaf length was 25.65 ± 0.46 cm and 36.52 ± 0.24 cm respectively with buds induction and without buds induction. Bud induction regimes have negative effect on propagules growth in all three cultivars. Cuttings types did not have significant effect on growth ($P = 0.069$). The analysis of deviance indicated highly significant three-way interaction ($P < 0.001$) between bud induction regimes, cutting types and cultivars for leaf length and width (Figure 4).

Number of leaves

The analysis of deviance indicated significant three-way interaction of buds induction regime, cutting types and cultivars on the variation of leaf number ($P = 0.000$). As main factors buds induction regime and cutting types did not have any effect on leaf number ($p = 0.66$). However, leaf number significantly changed with cultivars ($p = 0.02$). On average, 15 to 36 leaves were produced in 'Smooth Cayenne' and 'Adjago while 12 to 25 were produced in 'Sugarloaf' (Figure 5).

Effect of the buds induction regime and cutting types on the propagules weight

Three weight categories of propagules were produced after three months: the first weight category ranged between 300 and 200 g, the second between 200 and 100 g and the third below 100 g (Table 3). The propagules weight significantly varied among cutting types ($p < 0.001$). Whole stems produced high-weight propagules (3.7 ± 10.26) but in limited number. Cleaved cuttings produced intermediate propagules (5.1 ± 16.75) while slice cuttings produced many low-weight propagules (8.6 ± 31.29). Bud induction regime had no significant effect on propagules weight ($p = 0.087$).

Allometric relationships between plant height and propagules weight for the three cultivars showed that the regression lines have equal slope ($p = 0.096$) but differed significantly in elevation ($p = 0.004$) and no significant shift among lines ($p = 0.052$) whatever the treatment (Figure 6).

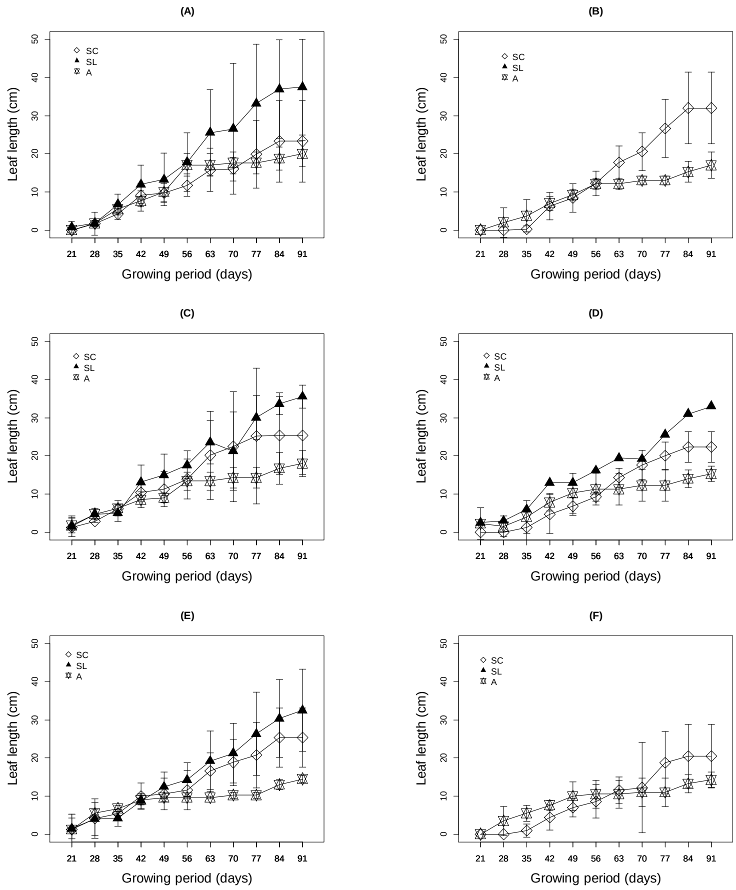

Figure 4. Leaves length variation per cultivar, cutting types and buds induction according to growing period. A: Whole stem planting with no buds induction ; B : Whole stem with buds induction; C: Cleaved stem and planting with no buds induction ; D : Cleaved stem with buds induction ; E : Stem cutting in slices planting with no buds induction ; F : Stem cutting in slices with buds induction. A : Adjago, SC : Smooth Cayenne, SL : Sugarloaf.

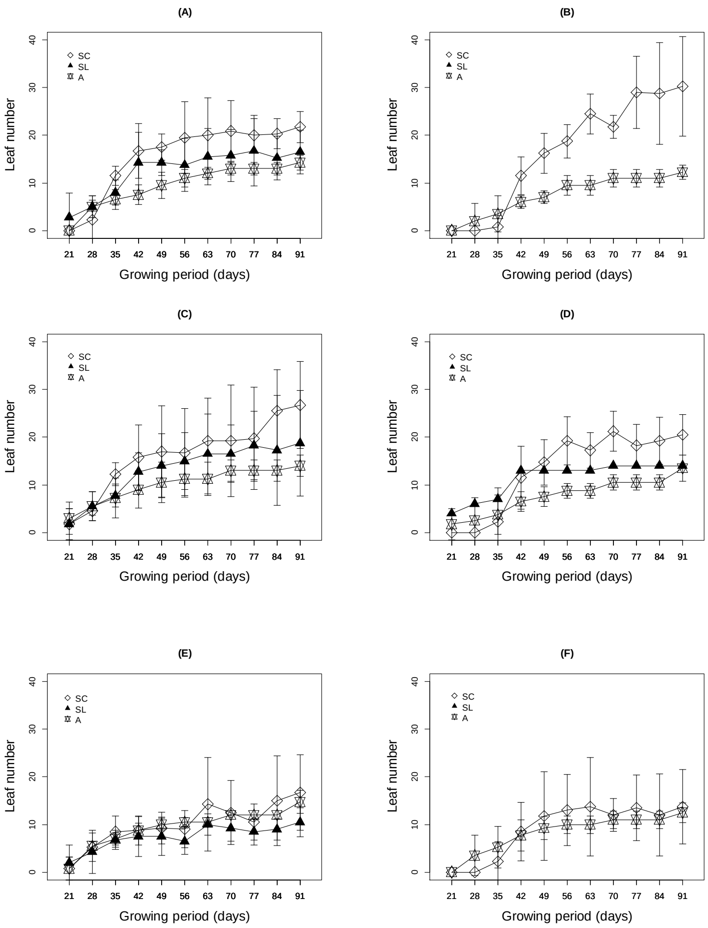

Figure 5. Leaves number variation per cultivar, cutting types and buds induction according to growing period. A: Whole stem planting with no buds induction; B: Whole stem with buds induction; C: Cleaved stem and planting with no buds induction; D: Cleaved stem with buds induction; E: Stem cutting in slices planting with no buds induction; F: Stem cutting in slices with buds induction. A : Adjago, SC : Smooth Cayenne, SL : Sugarloaf

Table 3. Frequency of weight categories in propagules produced

			Weight category (g)			
Cultivar	Treatment	Cutting type	300-200	200-100	< 100	Total
		Whole	66	34	16	116
		Cleaved	35	111	20	163
	No induction	Slice	18	149	209	376
		Whole	8	79	16	103
		Cleaved	7	88	20	115
Adjago	Buds induction	Slice	0	109	198	307
		Whole	55	86	20	151
		Cleaved	0	156	48	204
	No induction	Slice	0	112	235	347
		Whole	0	0	0	0
		Cleaved	0	0	60	60
Sugarloaf	Buds induction	Slice	0	0	0	0
		Whole	45	26	0	71
		Cleaved	15	125	21	161
	No induction	Slice	0	122	230	352
		Whole	13	63	12	88
		Cleaved	0	52	83	135
Smooth Cayenne	Buds induction	Slice	0	0	127	127

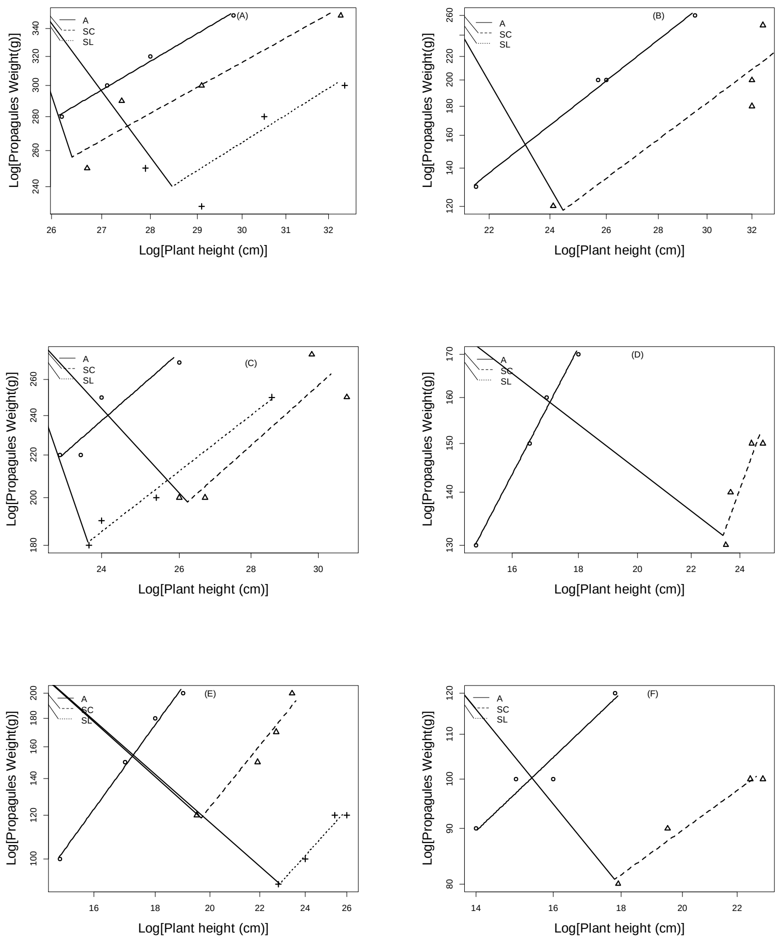

Figure 6. Allometric relationship between propagules weight and plant height for buds induction regimes, and cutting types in the three cultivars. A: Whole stem planting with no buds induction; B: Whole stem with buds induction; C: Cleaved stem and planting with no buds induction; D: Cleaved stem with buds induction; E: Stem cutting in slices planting with no buds induction; F: Stem cutting in slices with buds induction. A : Adjago, SC : Smooth Cayenne, SL : Sugarloaf

DISCUSSION

The need for adequate planting materials is the main limit for large-scale production of pineapple.

Our findings revealed that buds induction did not have positive effect on pineapple planting materials production contrary to findings reported by de Faÿ *et al.* (2000) with Picea abies . In pineapple, the bud induction technique used rather delayed sprouting. Maerere (1996) reported that temperature and light were essential factors that cause the differences observed in the resumption of pineapple buds. Those factors could have consequences on sprouting capacity. Apart from temperature and light, soil composition (medium) was also essential for sucker development. However, in our study only one soil type was used.

Smooth Cayenne and Adjago sprouted after bud induction unlike Sugarloaf. The three cultivars did not have the same sprouting ability. Smooth Cayenne and Adjago had similar behavior certainly because of the type of the propagules they produced naturally. Adjago and Smooth Cayenne produce suckers whereas Sugarloaf produces slip. Smooth Cayenne required more time for sprouting. It seems that Smooth Cayenne suffers from apical dormancy which delays and reduces sprouting. Probably, this can explain the very low rate of propagation observed on-farm. Our study also showed that buds sprouted within three weeks in all three cultivars. The same results were found by Thiémélé *et al.* (2013) and Weerasinghe and Siriwardana (2006) with Smooth Cayenne and Queen "Victoria".

For all three cultivars, whole stem cuttings produced few propagules; cleaved stem produced more, but slice cuttings produced the highest number of propagules. The same result was obtained by Abdullah, Hossain, and Bhuiyan (2006) and Weerasinghe and Siriwardana (2006) who concluded that stem size affects significantly the number of produced propagules. Moreover, the number of propagules changed according to the cultivar. Smooth Cayenne and Adjago produced more propagules than Sugarloaf. The low number of propagules produced by Sugarloaf can be explained by the fact that the cultivar produces a lot of slips, which may reduce its capacity of producing suckers. But this has to be demonstrated. Sugarloaf stem did not have the same aptitude as other cultivars. Naturally, the cultivar produced a lot of slips that can be calibrated for large-scale production. However, for mass propagation with stem cuttings it would be interesting to use growth regulator such as indole-3-butyric acid (IAA) and indole-3-acetic acid (IBA) to accelerate the production of uniform planting material. Kumar (2008) and Abdullah *et al.* (2006) observed that stem cuttings with pre-formed buds produced more propagules and roots than those without pre-formed ones only in the case they was treated with IBA. It is the most common exogenously applied plant growth regulator especially preferable in "in vivo" conditions (De Klerk, Van der Krieken, & De Jong, 1999).

Whole stem cuttings produced propagules with high weight while cleaved stem cuttings and slice cutting produced average and low weight propagules respectively. Cleaved stem cuttings produced more propagules than the others in vivo techniques (Weerasinghe & Siriwardana, 2006). However, propagules of those cuttings exhibited average weight. This could be the consequence of the amount of foods reserves available in the stems. The role of nutriment availability in pineapple early growth was also highlighted by Allen *et al.*(2006). There was no effect of genotypes on propagules' weight. (Weerasinghe & Siriwardana, 2006) indicated that after four months, slice stems produced three to five propagules of 100 g. In our study slices cuttings produced in average eight propagules in three months. They could be used to increase the production of uniform propagules weight. This achievement is an important gain and would contribute to improve fruit size and harvest time(Robert *et al.*, 1999). The leaf number and growth in propagules were cultivar-dependent. This is since each cultivar had different characteristic.

Despite the numerous studies carried on pineapple, there are still many bottlenecks regarding pineapple propagation. Studies were carried out to assess which part of pineapple stem was more productive (Achigan Dako *et al.*, 2014), or the effect of substratum on planting material for rapid propagation (Firoozabady *et al.*, 2003). Our study focused on stem cutting types that can be used to produce uniform pineapple planting materials. It underlined the importance of the fragmentation on the propagation rate and the propagules weight.

Smooth Cayenne is more requested for exportation but produces few propagules in relatively long time. Based on our findings, pineapple planting materials production system could be established to fulfill producers' needs. A recent prospection and collect made by Agbangla (2013) showed that Benin have about six different cultivars concentrated in the low-cultivated region. Our technique could also be used to conserve the pineapple germplasm available in Benin.

CONCLUSION

Our findings indicated that stem cutting types affect the number and weight of propagules obtained. Bud induction affects propagules production negatively. Overall, Smooth Cayenne and Adjago were more prone to cutting propagation than Sugarloaf. Slice cuttings gave numerous and uniform propagules. Further utilization of our results will certainly ease the production of propagules for increased pineapple production.

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