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In situ PRODUCTION OF SHOOTS DERIVED FROM PLANTAIN STRAIN-SUCKERS BY STIMULATION WITH CYTOKININ SUBSTANCES

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ABSTRACT

Plantain (*Musa x paradisiaca* L., AAB genomic group) is a useful supplement to the nutritional balance of populations in developing countries. The objective of this study was to improve the multiplication rate of *in situ* plantain (*Musa x paradisiaca* L.) seeds through the supply of substances with cytokinin activity. Suckers of 10 cm height and related to the parent plant, called strain-suckers, were used to produce shoots. The pseudo-trunk of plantain tree was decorticated, then the apical meristem extracted. Four milliliters of each substance (6-Benzylaminopurine (BAP) ; Kinétine and Coconut water) were introduced in the cavity left by the extracted meristem. For each substances, the effect of the diameter of first-generation strain-suckers ($d < 9$; $9 < d < 12$ cm and $d > 12$ cm) was tested. This operation was repeated once so as to have the second and third generations. BAP induced the highest number of buds after three generations. The optimal concentration of BAP was 40 mg L^{-1}. Similarly, all concentrations of coconut water stimulated production of seedlings. Among different diameters of strain-suckers, the largest number of buds was induced with strain-suckers larger than 9 cm in diameter. The production of leafy shoots varied greatly, depending on the solutions tested and the size of strain-suckers used. After 3 to 4 months, seedlings obtained were ready to be transferred in the field.

Key Words: 6-Benzylaminopurine, coconut water, *Musa x paradisiaca*

RESUME

L'objectif principal de la présente étude est d'améliorer le taux de multiplication des semences *in situ* de bananier plantain (*Musa x paradisiaca* L.) par apport de substances à activité cytokinine. Les rejets baïonnettes de plus de 10 cm de haut et liés à la plante mère ont été répertoriés et appelés

souche-rejets. Le pseudo tronc de ces derniers a été décortiqué puis le méristème apical extrait. Dans la cavité laissée par l'extraction du méristème, 4 ml des solutions de Benzylaminopurine (BAP) ; de Kinétine (Kin) ou de l'eau de coco y ont été introduites. Cette opération a été répétée de sorte à disposer des rejets de deuxième et troisième génération. La substance induisant le plus grand nombre de bourgeons a ensuite été utilisée pour tester différents diamètres de rejets ($d < 9$ cm ; $9 < d < 12$ cm et $d > 12$ cm). La BAP a permis d'induire le plus grand nombre de bourgeons après trois générations. La concentration optimale de BAP a été de 40 mg L^{-1}. De même, le lait de coco a permis de stimuler la production de plantules. Parmi les différents diamètres de rejets, le nombre moyen le plus important de bourgeons a été induit avec les rejets de diamètre supérieur à 9 cm. La production de pousses feuillées a varié fortement selon les solutions testées et la taille des rejets utilisés. Au bout de 3 à 4 mois les plantules obtenues sont prêtes à être transférées au champ.

Mots Clés: Benzylaminopurine, eau de coco, *Musa x paradisiaca*

INTRODUCTION

Plantain (*Musa x paradisiaca* L., AAB genomic group) is a useful supplement to the nutritional balance of populations, especially in developing countries (Klotz and Gau, 2002). In Côte d'Ivoire, with an estimated production of nearly 1.6 million tonnes per year, plantain is the fourth most important food crop after yams, cassava and rice (FAOSTAT, 2017). With this production, Côte d'Ivoire is the third largest producer of plantain in West Africa, after Nigeria and Ghana (FAOSTAT, 2017) ; and the 8th in the world (Anonymous, 2015). It accounts for 20% of food production and 25% of all starchy foods consumed in the country (Perrin, 2015).

However, the supply of this commodity in the various local, sub-regional and even European markets remains far below the demand and in full demographic growth. In order to end the regular banana shortages in Côte d'Ivoire, it is necessary to increase plantain production through renewal and extension of cultivable areas. Thus, it becomes more than necessary to find planting materials for these surfaces. However, in the field, the number of suckers (3 or 6) produced naturally by plantain tree cannot meet the seed needs of farmers (Koné *et al.,* 2011). Farmers are regularly confronted with the problem of insufficient plants in Côte d'Ivoire (Koua *et al.,* 2019).

In vitro regeneration techniques have been widely used as alternative methods to traditional propagation, in developed countries and research centres (IITA, CARBAP, etc.). In recent years, *in vitro* technique have grown due to the ability to produce genetically uniform and healthy materials (Strosse *et al.,* 2008; Koné *et al.,* 2010; Koné, 2014; Shiv Shankar *et al.,* 2014). However, these techniques remain inaccessible to farmers because of high production costs and the technical requirements. Also, it is much more used to produce dessert banana plants that are commercially more profitable than plantain (Kwa, 2003).

In view of the complexity of the in *vitro* methods, *in vivo* propagation techniques were developed by the CARBAP (Kwa, 2003), in germoir as seedlings derived from fragments and mini-sets under tunnel by multiplication on decorticated strains. They have significantly increased the multiplication rate of plantain in Africa, especially Côte d'Ivoire (Koné *et al.,* 2016 ; Koné *et al.,* 2017 ; Koua *et al.,* 2019). However, the materials and financial means necessary for the establishment of such production systems are a hindrance for most small producers. The work done by Manzur-Macias (2001) and Koné (2014) showed that the possibility to adapt these techniques in the field.

Literature on the use of chemicals to stimulate *in situ* mass production of plantain

releases is virtually non-existent. Manzur-Macias (2001) used a solution of benzylaminopurine (BAP) to promote the mass production of leafy shoots in banana hybrid FIHA 20. Koné (2014) observed that urea solution used as a watering solution showed a proliferation of buds. However, this proliferation would be due more to rapid growth than to formation of new buds. Knowing that cytokinins are involved in bud formation, this study could provide information on cytokinin nature and / or doses adequate for the buds proliferation in plantain. The objective of the present study was to evaluate different substances with cytokinin activity on plantain seedling production in field conditions in Cote d'Ivoire.

MATERIALS AND METHODS

Study area. The study was conducted at Nangui Abrogoua University (UNA), located in Abidjan, in the southern of Côte d'Ivoire, at 5°17' and 5°31' North latitude and 3°45' and 4°22' West longitude. This site has a subequatorial climate. The climate is subdivided into four seasons; a long and a short rainy season, respectively, from March to July and from October to November; then a long and short dry season, respectively, from December to March and from August to September. The average annual temperature of the site is between 22 and 32 °C. Rainfall varies between 1100 and 1700 mm in the rainy season, and less than 150 mm of rain in the dry season.

Plant material. Plant materials used in this study consisted of seedlings of 7 to 8 months of plantain cultivar '' Corne 1 '' (genomic group AAB), according to the classification of CNRA (n.d.). It is commonly called in Cote d'Ivoire "Afoto ". The parcel was planted with plants of three months old. Suckers related to the mother plant, called here " strain-suckers ", were used. They are called strain-suckers because they are still in soil and related to the parent strain.

Stimulus substances used. The stimulating substances used were composed of solutions of 6-Benzylaminopurine (BAP), kinetin and fresh coconut water. Mature coconut fruits were bought on the market in Abobo (Abidjan-Côte d'Ivoire) for this purpose. Coconut water has been used for its rich composition of growth regulators (Ge *et al.,* 2006; Ma *et al.,* 2008; Yong *et al.,* 2009), and is easily available to poor small producers.

Plot preparation and planting. The experimental plot was previously left fallow for two years. The plot was weeded and plowed using a hand-hoe. Four kilogrammes of poultry droppings were placed in each hole of 50 cm x 50 cm. The droppings were left open for a week before the *in vivo* plants of three months and a size varying between 30 and 40 cm, were planted. Two months after planting, 4 kg of composted droppings were again placed around the plants. Ten grammes of carbofuran (Furadan-5G) was applied as a sanitary treatment (insecticide-nematicide) per plant six months after planting.

Manual watering with tap water was carried out every after three days in the dry season (15 L of water per banana tree). In the rainy season, watering was not necessary.

Obtaining strain-suckers. In order to increase the number of suckers to be stimulated, the mother strains underwent false decapitation at six months. A cleft was made at the base of the pseudostem to destroy the apical meristem of the parent strain six months after planting (Koné *et al.,* 2011).

The "strain-suckers ", more than 10 cm high, have been recorded on plantain banana from seven to eight months. These suckers (Fig. 1a) were called first-generation buds (BG1). Using a thin blade knife disinfected with Ethanol 70%, the suckers were decorticated, cut transversely at least 2 cm from the collar, and their apical meristems extracted with a thin-blade knife (Fig. 1b, 1c and 1d). The explant thus prepared was used to receive the various stimulating solutions (Fig. 1 e). Each of the

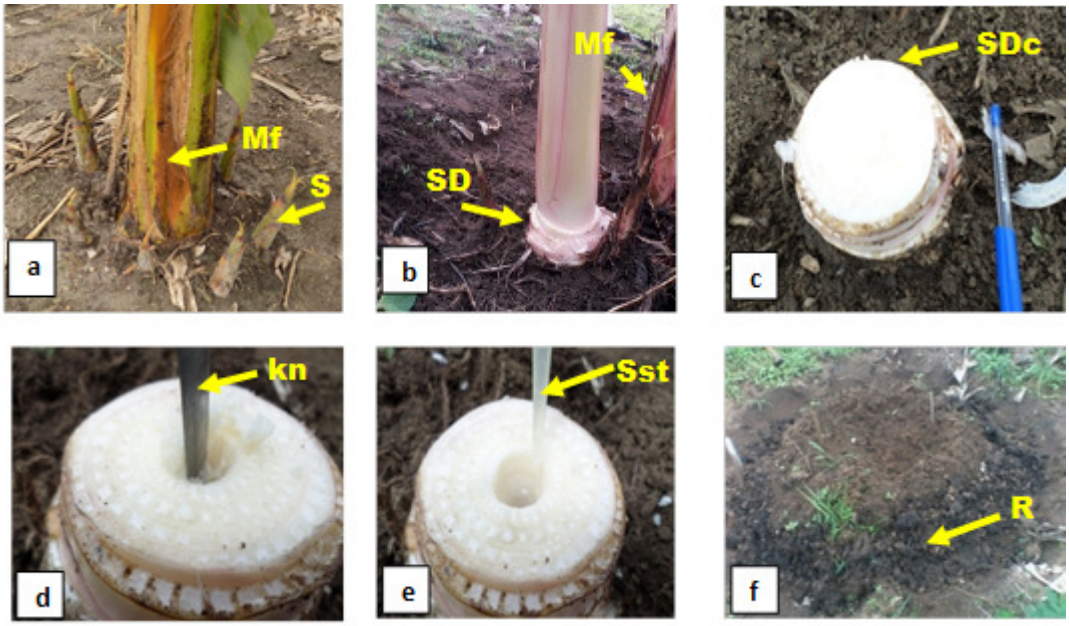

Figure 1. Stages of preparation of banana strains-suckers and application of hormonal substance for bud stimulation.

a = strain-sucker to treaty; b = shell strain-sucker; c = transversely cut strain-sucker; d = extraction of the meristem; e = injection of substance; f = treated strain-suckers covered with soil and manure; Mf = mother feet; S = strain-sucker; SD = strain-sucker decorticated; SDc = strain-sucker decorticated and pseudo cut trunk; kn = knife; Sst = syringe containing the stimulating solution; R = ridging around treated stumps eight months after planting.

strain-suckers was treated with only one type of stimulant.

Nature and concentration of stimulating substances. Using a syringe, 4 ml of the solution (BAP, Kinetin, coconut water or control) was introduced into the dug cavity in strain-sucker (Fig. 1 e). The different concentrations of BAP and Kin tested were 20, 40 and 60 mg L^{-1}. The coconut water was used pure, diluted 75 and 50%. The decorticated strain-sucker was then covered with a black bag for 24 hours. The bag was then removed and treated strain-suckers were recovered with the surface soil (Fig. 1 f). The control treatment was distilled water.

The buds induced on the strains-suckers are hereafter called buds of second generation (BG2). These buds developed to give leafy shoots, from which 20 cm high were decorticated again and treated with stimulating substances as described before. The buds obtained at the end of this treatment are hereafter called third generation buds (BG3). These (leafy shoots) were weaned and acclimatised.

Diameters of strain-rejects and shoots. The treatment that induced the greatest number of buds in the previous experiment (better interaction between Nature and concentration of stimulating substances) was chosen for the study of the different diameters of the strain-suckers. The strain-sucker diameters were measured with sliding calipers to determine their size. These measurements made it possible to calibrate the strain-suckers and to generate three size ranges, namely < 9 cm, 9 cm < diameter < 12 cm and diameter > 12 cm. These strain-suckers were then decorticated and

treated with the solution that induced the greatest number of buds.

Weaning and acclimatisation of seedlings. The leafy shoots obtained after three generations were weaned and transplanted into nursery bags containing a mixture of sand and coffee parch. Weaning was done when the seedlings developed two to three leaves. With a sterilised knife, the shoots developed were gently weaned by cutting a small corm portion. Seedlings were transplanted into nursery bags of dimension 26.5 cm x 23.5 cm, and placed under mini-greenhouses for two weeks; before being transferred to a shade for four weeks (Koné *et al*., 2017). The acclimatisation substrate was composed of a mixture of sand and coffee parch in the proportions 1:1 (v / v). The leafy shoots were regularly watered (2 to 3 times a week) until they reached 30 cm, for transfer to the field.

Parameters evaluated. During the production of buds and leafy shoots, the parameters evaluated included: latency time (it is the time of bud induction or time taken for the induction of buds on an explant); number of leafy shoots or buds per strain-suckers (number of leafy shoots or buds produced per strain-suckers was counted); number of weaned leafy shoots (this is the number of weanable leafy shoots that have been assessed); rate of loss buds [(number of buds produced - number of shoots weaned) x 100 / number of buds produced]; and time to obtain leafy shoots (time required to produce a weanable leafy shoot). During acclimatisation, the survival rate (number of living plants / the total number of plants acclimatised) and the vigour index of the plants (Height / Diameter) were determined.

Field experimental design and data analysis. *Vivo* plants were planted in a completely randomised device, with a spacing of 2.5 m between plants. This experiment was repeated three times, with three replicates of ten mother strains per treatment.

For data analysis, the STATISTICA 7.1 software was used. Analysis of variance with one or two classification criteria (ANOVA 1 or 2) was used. Tukey's test ($P<0.05$) was used to compare significant difference among treatment means. For survival rate and loss rate, arcsin transformation (p=proportion) was performed before any analysis.

RESULTS

Development of leafy shoots. False decapitation doubled the number of suckers (Table 1); however, the decapitated plants did not allow for harvesting the banana bunches, and the control plants gave small bunch. After decorticated of strain-suckers, many buds appear three weeks later (Table 2).

The latency times and the mean time to obtain a leafy shoot were not statistically different ($P>0.05$). The number of leafy shoots per mother strain was more significant with

TABLE 1. Mean number of suckers produced in 11 months after desuckering and decapitated banana plant

Treatments	Mean number of suckers produced	Bunch weight (kg)
Desuckering	1.00 ± 0.00 c	13.77 ± 3.29 a
Control	10.11 ± 6.66 b	4.53 ± 1.23 b
Decapitated	26.76 ± 7.55 a	0.00 c

Means followed by the same letter are statistically identical to the 5% threshold (Tukey's test); Mean ± standard deviation; Control = plant in natural production; Decapitated = plant stimulated by false decapitation; Desuckering = the suckers are destroyed to obtain a quality diet

TABLE 2. Influence of stimulative solution on banana shoot production

Stimulative solution	Concentration	Latency time (days)	Time to obtain leafy shoots (days)	Total number of leafy shoots per mother foot
Water	0	11.70 ± 0.89 a	37.66 ± 13.76 a	57.55 ± 13.45 d
Coconut water	1	12.10 ± 1.20 a	35.7 ± 11.66 a	73.79 ± 14.46 c
	75 %	12.45 ± 2.11 a	37.42 ± 14.83 a	72.11 ± 9.36 c
	50 %	12.09 ± 2.02 a	36.90 ± 10.61 a	71.10 ± 13.88 c
Kinétine	60 mg L^{-1}	11.33 ± 3.12 a	34.93 ± 12.55 a	85.33 ± 2.58 b
	40 mg L^{-1}	12.56 ± 1.33 a	37.31 ± 16.02 a	83.4 ± 13.43 b
	20 mg L^{-1}	13.59 ± 4.18 a	35.12 ± 14.64 a	71.33 ± 5.70 c
BAP	60 mg L^{-1}	9.97 ± 4.92 a	34.17 ± 16.99 a	95.31 ± 11.16 a
	40 mg L^{-1}	12.62 ± 3.01 a	36.19 ±12.23 a	102.5 ± 17.99 a
	20 mg L^{-1}	13.66 ± 4.72 a	37.41 ± 15.53 a	73.87 ± 5.19 c
	P (0.05)	0.064832	0.051664	0.059338
P (0.05)		0.273300	0.707618	0.026933
P* (0.05)		0.053445	0.054321	0.003170

In the same column, the digits followed by the same letter are statistically identical to the 5% threshold (Tukey's test), Mean ± standard deviation; BAP = 6-Benzylaminopurine; P = probability of the main effects; P * = probability of interactions substance x concentrations

cytokinine solution than with the control (water) and Coconut water. It was clearly superior with concentrations above 40 mg L^{-1}.

Number of generation. Figure 2 shows the evolution of the number of buds produced during the 1st, 2nd and 3rd generations, according to the stimulative solutions. This production of buds from the first to the third generation was carried out for about 60 days. These numbers, initially low in generation 1 (approximately 26 buds) for all treatments, increased in generations 2 and 3. However, this increase was neither arithmetic, nor geometric, because it followed no mathematical rule. Despite this difference from generation, the variation between numbers of buds for treatments was very important ($P<0.001$). This was noticed based on the standard deviations which were large. However, buds production was also observed without hormonal treatment (controls: single water). Thus, number of shoots varied between 45 to 60.

Strain-suckers diameter and leafy shoot proliferation. The number of buds produced and leaf shoots weaned by strain-suckers on mother strains are shown in Table 3. The number of induced buds varied from 10 to 70, depending on strain-suckers diameter. The highest number was obtained with diameters greater than 9 cm. Irrespective of the treatment applied, the average number of buds and weaned shoots produced, increased with increasing diameter of the shoots. Among the treatments tested, BAP gave the highest responses in terms of buds produced and weaned shoots. In our study, the number of

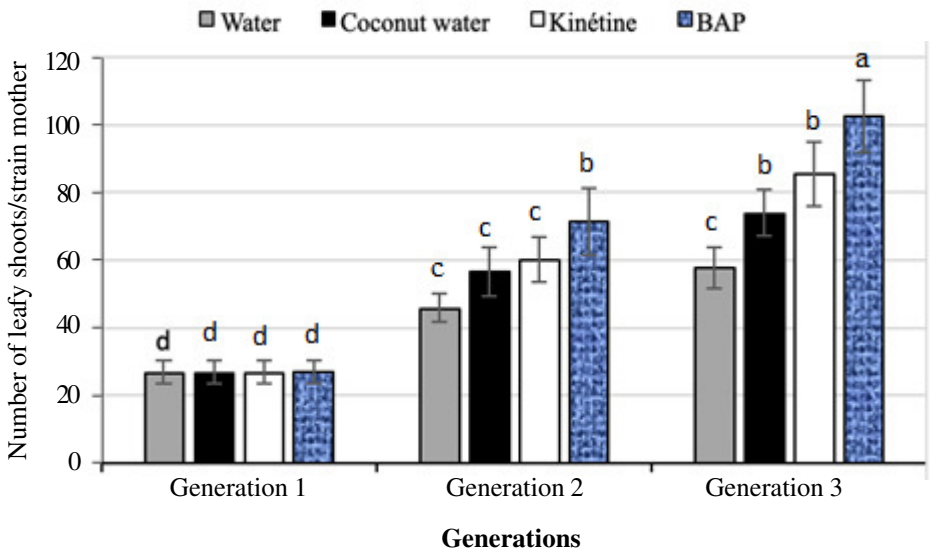

Figure 2. Evolution of the number of banana leaf shoots on stimulatives solutions over three generations.

Generation 1 values were obtained after false decapitation of the mother plant. Histograms with the same letter are statistically identical to the 5% threshold (Tukey's test), Mean ± standard deviation, P < 0.001, BAP = 6-Benzylaminopurine.

shoots weaned was less than that of the buds produced.

Of all cytokinin-active solutions, BAP favoured the production of buds and shoots the most. However, the number of wilted leaf shoots was only influenced by the diameter of the strain-sucker because treatment probability threshold was greater than 0.05 (P = 0.220518).

Rate of bud loss depended on the diameter of the strain-suckers (Table 3). Stumps of small diameters (< 9 cm) showed a very significant (P< 0.0001) rate of loss; while strains of great dimension expressed a low rate of loss.

Acclimation of leafy shoots. Data for survival rate, leaf height and leaf vigour index obtained after treatment of different strains-suckers with cytokinin-activity solutions, are presented in Table 4. The survival rate and height of the leafy shoots at the end of acclimation were only influenced by the diameter of strain-suckers. Thus, these parameters increased with the diameter of strain-suckers. However, survival rate varied between 80 and 98%, regardless the diameter of the strain-sucker. The same was true for the height of the shoots, which varied between 10 and 15.5 cm. The statistical difference revealed in vigour indices was not significant (P>0.05).

DISCUSSION

The time to induce buds was the same for all the treatments used. The nature of the stimulations could not influence the sensitivity of strain-suckers tissue in the first moments. The treatments, therefore, had no effect on the reaction time of the buds. This study found that cytokinin activity substances stimulated the proliferation of the buds. This could be explained by the fact that the hormones, water and mineral salts continued to be transported from the roots to the apex; though, there were no more young cells to

TABLE 3. Influence of banana strain-suckers diameter on shoot production in the presence stimulatives solutions

Stimulatives solutions	Diameter of treated strain-suckers (cm)	Mean number of buds produced	Mean number of weaned leafy shoots	Rate of loss buds(%)
Water	d < 9	12.95 ± 2.10 h	7.22 ± 3.76 g	42.24 a
	9 < d < 12	26.61 ± 1.73 e	24.91± 4.9 d	9.4 ef
	d > 12	26.5 ± 3.53 e	26.47 ± 5.21 d	0.99 g
Coconut water	d < 9	14.5 ± 0.58 g	11.44 ± 3.92 f	21.1 c
	9 < d < 12	26.2 ± 2.49 e	24.81 ± 6.77 d	5.31 f
	d > 12	37.6 ± 2.83 d	33.99 ± 8.20 c	9.6 e
Kinétine	d < 9	23.00 ± 0.93 f	16.52 ± 4.76 e	28.17 b
	9 < d < 12	26.61 ± 2.00 e	25.20 ± 8.14 d	5.3 f
	d > 12	48.13 ± 3.06 b	42.69 ± 12.82 b	11.3 de
BAP	d < 9	23.34 ± 2.70 ef	16.57 ± 6.77 e	30 b
	9 < d < 12	39.22 ± 3.84 c	33.61 ± 9.52 c	12.3 d
	d > 12	69.57 ± 4.06 a	59.90 ± 11.61 a	11.9 de
	P (0.05)	0.008164	0.001862	< 0.0001
P (0.05)		0.006442	0.220518	< 0.0001
P* (0.05)		0.003060	0.043501	< 0.0001

In the same column, the digits followed by the same letter are statistically identical to the 5% threshold (Tukey's test), Mean ± standard deviation; BAP = 6-Benzylaminopurine; d = calibration of the diameters of the strain-reject; P = probability of the main effects; P * = probability of interactions substance x strain diameter-rejects

metabolise them. Cytokinin activity substances, water and minerals accumulated in what remained of the stem that had been decapitated. These substances stimulated cells in the youngest buds (those closest to the destroyed apex) that were kept dormant by the action on the apical bud. The stimulated cells then began to divide, produce auxin and reform the connection with the xylem. This resulted in growth recovery of new buds formed (Heller *et al.*, 1990; Meyer *et al.*, 2008). Decapitation, followed by the addition of substances with cytokinic activity, could have modified the ratio cytokinins/auxins that would have more controlled cell proliferation to produce more buds, rather than their growth.

The highest bud numbers were obtained with BAP (Table 2), reflecting the ability of this hormone to stimulate the induction of buds. Manzur-Macias (2001) demonstrated the possibility of producing a large number of buds on FHIA-20 plantain, using BAP at a concentration of 40 mg L^{-1}. BAP produced more buds than kinetin. The favourable effect of BAP, in comparison with other cytokinins such as kinetinin and 2iP (N6- [2-Isopentenyl] adenine), has also been observed by many authors (Resmi and Nair, 2007; Farahani *et al.*, 2008; Buah *et al.*, 2010). BAP is not easily broken down; thus persists in the environments where it is introduced. Klem *et al.* (2000) reported that BAP was a chemically stable cytokinin in tissue culture, whereas most

TABLE 4. Influence of the combined effect of stimulative solution and strain-suckers diameter on survival and vigor of leafy shoots in acclimation

Stimulatives solutions	Diameter of treated strain-suckers (cm)	Survival rate of leafy shoots (%)	Mean hight of leafy shoots (cm)	Mean vigour index of leafy shoots
Water	d < 9	82.34 ± 8.16 cd	10.79±2.42 b	8.79 ± 3.74 a
	9 < d < 12	84.17± 7.03 bc	12.75±2.86 ab	8.53 ± 1.98 ab
	d > 12	96.77 ± 9.55 a	15.53±4.42 a	8.87 ± 2.11 ab
Coconut water	d < 9	84.66 ± 8.29 bc	12.95±3.55 ab	8.77 ± 2.17 ab
	9 < d < 12	82.87 ± 7.67 cd	13.33±4.30 ab	7.98 ± 3.15 ab
	d > 12	93.99 ± 6.72 a	15.31±3.52 a	8.06 ± 2.82 ab
Kinétine	d < 9	83.26 ± 8.63 cd	14.18±3.40 a	8.06 ± 2.16 ab
	9 < d < 12	85.09 ± 8.36 bc	14.75±2.84 a	8.81 ± 2.29 a
	d > 12	96.98 ± 6.72 a	15.18±3.42 a	9.08 ± 1.46 a
BAP	d < 9	86.75 ± 7.90 bc	12.41±4.00 ab	8.90 ± 3.18 a
	9 < d < 12	80.99 ± 9.52 d	13.75±3.55 ab	7.98 ± 3.33 ab
	d > 12	97.90 ± 6.87 a	15.90±4.22 a	8.96 ± 3.65 a
	P(0.05)	0.0002700	< 0.001	0.067077
P(0.05)		0.2086442	0.060167	0.051089
P*(0.05)		0.0151060	0.590560	0.020597

In the same column, the digits followed by the same letter are statistically identical to the 5% threshold (Tukey's test), Mean ± standard deviation; BAP = 6-Benzylaminopurine; d = calibration of the diameters of the strain-reject; P = probability of the main effects; P * = probability of substance x strain diameter-rejects interactions

other purine cytokinins (ketins or isopentenyl adenosine) were considered chemically unstable. Cronauer-Mitra and Krikorian (1984) suggested that BAP and other exogenous cytokinins appear to be the main factors affecting bud multiplication in banana.

The influence of diameter of strain-suckers was noted on the mean number of buds and the time of leafy growth (Table 3). In fact, the greatest number of buds was obtained with strain-suckers of diameters between 9 and 12 cm, and those greater than 12 cm. Diameters less than 9 cm produced the smallest number of buds. This could be explained by the fact that these rejects, being less developed, had very few differentiated growth points. These vegetative points would have taken longer to develop into buds (Kwa, 1998 ; Koné, 2014). Discards of large diameters produced rapid buds. This could be explained by the fact that these latter would present, mature latent buds. These buds would divert, water and mineral salts to ensure their growth, at the expense of undifferentiated growth points.

BAP at 40 mg L^{-1} gave the highest numbers of buds (Table 2). This concentration, therefore, made it possible to induce more buds in our experimental conditions in plantain. The mean number of buds increased with application of 40 mg L^{-1} of BAP to generation 3. This is due to the fact that buds come from leafy shoots of generation 2. The shoots of this generation were treated when their diameters reached at least one centimeter. This

uniformity of the diameter of the leafy shoots would significantly limit the trophic competition between the rejects used to induce the buds.

A strong interaction was noted between the stimulating substances and the diameter of the strain-suckers (Table 3). These observations would assume sensitivity of the substances to large strain-suckers. The organogenic responses of large suckers would be due to the fact that they have more mature buds sensitive to stimulating substances, rich in phytohormone. The latter would more easily stimulate the two morphogenetic fields involved in bud formation (Kwa, 1993). According to the latter, the morphogenic buds formation sites are activated or dormant depending on whether or not the plant is in favorable conditions which could trigger bud formation.

CONCLUSION

The results of the present study show that the potential for *in situ* bud induction depends on the nature and concentration of the hormonal substances. The most important responses are expressed with BAP at the concentration of 40 mg L^{-1}. The induction of releases from generation 1 to generation 2 varies greatly depending on the size of the discharges used. Discard diameters between 9 to 12 cm or greater than 12 cm favour maximum bud induction. The bud production protocol established during this study lasts a mean of 2.5 months from generation 1 to generation 3; with an mean production potential of 300 leafy shoots.

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