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العدد الثامــن عشر – 05/ مايو 2017

Biweekly changes in the *in vitro* shoot formation and growth pattern of pineapple (*Ananas comosus* L Merr) cv Smooth cayenne over 105 days of incubation on different hormone treatments

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Dear Dr. Abdelhamid M. Hamad.

Horticulture Department, Faculty of Agriculture, Omar Almukhtar University El-Baida, Libya

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Abstract

BAP (6-benzylaminopurine) at 2.25 mg l^{-1} applied singly, in combination with indole acetic acid (IAA) at 0.175; 0.75; 1.75 mg l^{-1} and in combination with IAA at 1.75 mg l^{-1} plus gibbrellic acid (GA_3) at 1.0 mg l^{-1} and BAP at 3.25 mg l^{-1} applied in combination with IAA at 1.75 and 3.75 mg l^{-1} were tested for their effect on the biweekly pattern of shoot formation (shoot per explant) and shoot growth (length and weight) of Smooth cayenne pineapple over 105 days of incubation. The highest shoot formation after 105 days of incubation were 31 shoots obtained in medium enriched with BAP at 2.25 mg l^{-1} plus IAA at 1.75 mg l^{-1} and the lowest were 10 shoots obtained in medium enriched with BAP at 3.25 mg l^{-1} plus IAA at 3.75 mg l^{-1}. The shoot formation over 105 days took place in cycles of high and low pulses ranged from none to 11 shoots per each two weeks. Different hormone treatments may result in equall shoot formation but induced significant different pattern of shoot formation. Both of single application of BAP at 2.25 mg. l^{-1} and combination of BAP at 2.25 mg l^{-1} plus IAA at 0.75 mg l^{-1} resulted in equal formation of 25 shoots, but the shoot formation at each two weeks of the 105 days incubation were 1; 6; 3; 4; 8; 2; 0 and 1; 5; 2; 5; 10; 1; 0 shoots respectively. Similar, combination of BAP at 3.25 mg l^{-1} plus IAA 1.75 mg/l and combination of BAP at 2.25 mg l^{-1}, IAA at 1.75 mg l^{-1} and GA_3 at 1.0 mg l^{-1} resulted in equal shoot formation (20 shoots) but different pattern. The total length and weight of shoots increased as the incubation increased and the pattern of the pulses of high and low gain per each two weeks in total length and total weight showed less fluctuation than that of the shoot formation pattern.

Key words: BAP; IAA; Shoot formation pattern; Incubation periods; *Ananas comosus*; Pineapple.

Introduction

Successfull *in vitro* shoot formation of pineapple had been reported by several researchers. Firoozabady and Gutterson (2003) reported a cyclic procedure of 15 days long multiplication while Fernando (1986) transferred the shoots to fresh medium each 21 days. Incubation for 30 (Gonzales *et al*., 2005; Firoozabady and Gutterson, 2003; Almeida *et al*., 2002; Soneji *et al*., 2002 a; Singh and Manual, 2000; Devi *et al*., 1997; Dewald *et al*., 1988; Bordoloi and Sarma, 1993; Kofi and Adachi, 1993; Fitchet, 1990; Zepeda and Sagawa, 1981), 35 (Khatun *et al*., 1997), 42 (Perez *et al*., 2009; Khan *et al*., 2004; Sripaoraya *et al*., 2003; Bhatia and Ashwath, 2002; Rahman *et al*., 2001; Teng, 1997; Aydieh *et al*., 2000; Escalona *et al*., 1999), 50 (Mathew and Rangan, 1981; Mathew *et al*., 1979), 60 (Hamad and Taha, 2008; Gangopadhaya *et al*., 2005; Be and Debergh, 2006; Hirimburgama and Wijesinghe, 1992; Mathew and Rangan, 1976), 64 (Omokoio *et al*., 2001), 70 (Soneji *et al*., 2002 b), 75 (Teixeira *et al*., 2006), 84 (Mhatre and Rao, 2002) and 112 days (Nelson, *et al*., 2015) were reported. Cytokinin particularly BAP was recommended singly (Nelson, *et al*., 2015: Akin-Idowu, *et al*., 2014: Zuraida, *et al*., 2011: Hamad and Taha, 2008; Be and Debergh, 2003; Bhatia and Ashwath, 2002; Almedia, *et.al*., 2002) and in combination with auxin (Dutta, *et al*., 2013; Perez, *et al*., 2012; Firoozabady and Gutterson, 2003; Rahman, *et.al*., 2001; Hirimburgawa and Wijesinghe, 1992) and natural additive (Singh and Manual, 2000; Bordoloi and Sarma, 1993, Zepeda and Sagawa, 1981). Kofi and Adachi (1993) reported that different hormone treatment could be recommended if different incubation period were used. BAP and KN induced the highest while 2iP induced the lowest shoot formation of Sugar loaf pineapple incubated for 30 days. However, if incubated for 60 days, BAP resulted in highest shoot formation, followed by 2iP while KN became the least effective hormone. Escalona *et al* (1999) reported a relation between explants density and incubation period and the obtained total of shoots and frequency of shoot sizes. The rate per week increased up to the 7th week and declined afterward. Moreover, Danso *et al* (2008) reported that the length of incubation period during multiplication stage effect the optimal types and concentration of the rooting hormone used during the *in vitro* rooting stage.

Selection of best hormone treatment are usually based on a broad spectrum approach in which high, intermediate and low concentration were compared. In all cases, the evaluation was based on counting of shoots produced after one fixed period of incubation. Since the optimal hormone treatment varied at different incubation, none of the recommended hormone treatments and incubation periods could be claimed as a universal treatment. Generally, the goal of an *in vitro* culture study is either developing a procedure that result in higher rate of multiplication at lower cost or investigation the physiobiochemical process of the *in vitro* shoots formation. Testing the effect of different hormones at one fixed incubation period or different incubations periods at one fixed hormone treatment on the *in vitro* multiplication without comparing of cost or monitoring the changes in shoot formation pattern over time neither support the selection of best hormone treatment and elucidation of physiological bases of its effect nor the reduction of the cost of multiplication. The objective of this study is to determine how long a hormone treatment could remain effective and when it does exert it's highest and lowest effect on the

in vitro shoot induction and growth over intervals of two weeks for a period of 105 days of incubation.

Materials and Methods

Explants and treatments

Nine liters of full strength MS (Murashige and Skoog, 1962) medium was prepared from stock solutions using 7 beakers. Each beaker contained 1260 ml of the medium, marked 1 to 7 and enriched with sucrose at 30 g/l and one of the following hormone treatments:

BAP alone at 2.25 mg. l^{-1}

BAP at 2.25 mg. l^{-1} in combination with IAA at 0.18, 0.75 and 1.75 at mg. l^{-1}

BAP at 2.25 mg. l^{-1} in combination with IAA at 1.75 mg. l^{-1} plus GA_3 at 1.0 mg. l^{-1}

BAP at 3.25 mg. l^{-1} in combination with IAA at 1.75 and at 3.75 mg. l^{-1}

The media were adjusted to pH 5.7 and the content of each beaker divided into 63 glass jars (20 x5 cm) each received 20 ml of medium and marked with the same number of beaker. Agar (0.14 g) was added to each jar and the jars were closed with autoclavable plastic lid and the media were autoclaved at 121^0 C and 1.5 kg /cm^2 for 25 minutes. A total of 441 *in vitro* obtained shoots from Smooth cayenne stock cultures were cultured, one shoot per jar and the jars were incubated under constant temperature of 25 ^{0} C and 16 hours of light (30 mmol m^{-2} s^{-1}) provided by cool white florescent lamps.

Data collection and statistical analysis

After each 15 days for a period of 105 days, 9 jars from each hormone treatment were taken out of the incubation room. The multiple shoots complex of each jar was picked and weighted (total weight per explant) and then separated into individual shoots for counting the shoots and measuring thier length. For each jar, the total shoots length was calculated by summation of length of all shoots and average shoot length computed by dividing the total shoot length over the shoot number. By the end of the experiment the data at all of the 15 day intervals were arranged in four tables, one table for total weight, one for total shoot number, one for total shoot length and one for average shoot length per jar (explant). Each table included 49 combinations (7 incubation periods and 7 hormone treatments) and the data obtained from each of the 9 jars. Data of each three jars were averaged to establish table with three replicates for each of the parameters. These tables were converted to tables of biweekly increase in shoot number, total shoot length and total weight for each of the different hormonal treatment by subtraction of the values obtained at replicate I, II and III of one incubation period from that of the replicate I, II and III of the previous incubation period. Before subjected to ANOVA analysis and Duncan Multiple Range Test, the data

were transformed by adding 0.1 to data of shoot number and 0.01 to data of total shoot length and weight.

Results

After 15, 30 and 45 days of incubation, the best treatment for shoot formation was combination of BAP at 3.25 and IAA at 1.75 mg. l^{-1} and the least effective treatment was combination of BAP at 3.25, IAA at 3.75 mg. l^{-1}. They resulted in the highest formation of 4, 11 and 12 shoots and the lowest formation of 1, 3 and 7 shoots per explant respectively. After 60 and 90 days, single application of BAP at 2.25 mg. l^{-1} was the best treatment resulting in 15 and 25 shoots per explant respectively while after 75 and 105 the best treatments were combinations of BAP at 2.25, IAA at 0.75 mg. l^{-1} and BAP at 2.25, IAA at 1.75 mg. l^{-1} resulting in 24 and 31 shoots per explant respectively. The least effective treatment after 60, 75, 90 and 105 was combination of BAP at 3.25, IAA at 3.75 mg. l^{-1} resulting in 8, 9, 10 and 10 shoots per explant respectively. There was no increase in shoot formation after 75 days of incubation in medium enriched with BAP at 3.25, IAA at 3.75 mg. l^{-1} and after 90 days of incubation in medium enriched with BAP singly applied at 2.25 mg. l^{-1} and combination of BAP at 2.25 with IAA at 0.18 mg. l^{-1}. However, the shoot formation in medium enriched with BAP at 2.25, IAA at 1.75 mg. l^{-1}; BAP at 3.25, IAA at 1.75 mg. l^{-1} and medium enriched with BAP at 2.25, IAA at 1.75, GA_3 at 1.0 mg. l^{-1} increased as the incubation period increased up to 105 days. In fact, over 30 % of the shoot formation (11 of 31 shoots) in response to BAP at 2.25, IAA at 1.75 mg. l^{-1} occurred after 90 days of incubation.

The highest total weight after 15, 30, 45, 90 and 105 days of incubation were 0.44; 0.99; 1.45; 4.69 and 5.91 grams, all obtained respectively in medium enriched with combination of BAP at 3.25, IAA 1.75 mg. l^{-1} while after 60 and 75 days, the heaviest weight were 2.13 and 3.48 grams obtained in medium enriched with combination of BAP at 2.25, IAA at 1.75, GA_3 at 1.0 mg. l^{-1} and medium enriched with combination of BAP at 2.25, IAA 0.18 mg. l^{-1} respectively. The lowest total shoots weight after shorter incubation periods of 15, 30 and 45 days, were 0.20; 0.63 and 0.96 grams all obtained respectively in medium enriched with combination of BAP at 2.25, IAA 1.75, GA_3 at 1.0 mg. l^{-1} while at longer incubation period of 60, 75, 90 and 105 days, the lowest weight were 0.95; 1.18; 2.09 and 2.17 grams all obtained respectively in medium enriched with combination of BAP at 3.25, IAA at 3.75 mg. l^{-1}. On the other hand, the highest total length of shoots after 15 (28 mm), 30 (55 mm) and 45 (86 mm) days of incubation obtained in medium enriched with BAP 3.25, IAA 1.75 mg. l^{-1} while the highest total length after 75 (244 mm) and 90 (272 mm) days of incubation obtained in medium enriched with BAP at 2.25, IAA at 0.75 mg. l^{-1}. The highest total length of shoots after 60 days (135 mm) obtained in medium enriched with BAP alone at 2.25 mg. l^{-1} while after 105 days, medium enriched with combination of BAP at 2.25, IAA at 1.75 mg. l^{-1} resulted in the highest total shoot length (406 mm).

The different hormone treatments showed different shoot formation patterns over 105 days of incubation (Table, 2). For each treatment, there were alternative pulses of high

and low shoot formation and gain in total length and weight of shoots. However, the period at which the high and low pulses occurred and the magnitude of the pulses varied among the hormone treatments. The highest shoot formation of the different intervals of two weeks of the explants cultured in medium enriched with BAP alone at 2.25 mg. l^{-1}, combination of BAP at 2.25, IAA at 0.75 mg. l^{-1} and combination of BAP at 2.25, IAA at1.75 mg. l^{-1}, GA_3 at 1.0 mg. l^{-1} was 8; 10 and 7 shoots respectively all occurred in the same period between the 60- 75 days of the 105 days long incubation while the highest shoot formation in medium enriched with combination of BAP at 2.25, IAA at 1.75 mg. l^{-1} and combination of BAP at 3.25, IAA 1.75 mg. l^{-1} was 11 and 7 shoots occurred in the period between the 90- 105 and the 15- 30 days of the 105 days incubation respectively. On the other hand, the lowest shoot formation of the different two weeks intervals in medium enriched with BAP alone at 2.25 mg. l^{-1}, combination of BAP at 2.25, IAA at 0.175 mg. l^{-1} and combination of BAP at 2.25, IAA at 0.75 mg. l^{-1} was 2; 1 and 1 shoot occurred at the period between 75- 90; 60- 75 and 75 – 90 days of incubation respectively. In all of these hormone treatments no shoot formation occurred in the last 15 days of incubation (90- 105 days). For the medium enriched with combination of BAP at 2.25, IAA at 1.75 mg. l^{-1}, the lowest shoot formation per two weeks was 1 shoot occurred in the periods between 31- 45 and 76- 90 days. Furthermore, while in case of medium enriched with combination of BAP at 2.25, IAA at 1.75 mg. l^{-1} and medium enriched with combination of BAP at 3.25, IAA at 1.75 mg. l^{-1}, there were 3 pulses of high shoots formation (5, 7 and 11 shoots) and (7, 3 and 3 shoots) occurred at the period between 15- 30, 60- 75 and 90- 105 days of incubation respectively, shoots in the medium enriched with combination of BAP at 3.25, IAA at 3.75 mg. l^{-1} showed only one period of high shoot formation (4 shoots) occurred between 30 and 45 days of incubation after which the shoot formation was only one shoot per each two weeks. The other hormone treatments showed two pulse of high shoot formation occurred in the period between 15- 30 and 60- 75 days of incubation (Table, 2).

The increase in total shoot length and weight at each two week period showed also pattern of high and low pulses. The highest gain in total length per two weeks in medium enriched with combination of BAP at 2.25, IAA at 1.75 mg. l^{-1}, medium enriched with combination of BAP at 2.25, IAA at 1.75, GA_3 at 1.0 mg. l^{-1} and medium enriched with BAP alone at 2.25 mg. l^{-1} was 207; 96 and 76 mm respectively all occurred in the period between 90 and 105 days of incubation. For medium enriched with combination of BAP at 2.25, IAA at 0.75 mg. l^{-1} and combination of BAP at 3.25, IAA at 1.75 mg. l^{-1}, the highest gain in total shoots length was 118 and 59 mm occurred at the period between 60 to 75 and 75 to 90 days of incubation respectively. On the other hand, the highest gain in total weight per two weeks in medium enriched with combination of BAP at 2.25, IAA at 1.75 mg. l^{-1}, combination of BAP at 3.25, IAA at 1.75 mg. l^{-1} and combination of BAP at 2.25, IAA at 0.18 mg. l^{-1} was 3.11; 2.43 and 1.81 grams occurred respectively in the period between 90 -105; 75 - 90 and 60 - 75 days of incubation. Most important, in medium enriched with BAP alone at 2.25 mg. l^{-1} and medium enriched with combination of BAP at 3.25, IAA at 3.75 mg. l^{-1}, there were only one peak of total shoots weight gain (0.91 and 1.20) occurred between the 75 and 90 days and the last 15 days (90 to 105) of incubation respectively. Medium enriched with combination of BAP at 2.25, IAA at 0.18 mg. l^{-1} and combination of BAP at 2.25, IAA at

0.75 mg. l^{-1} resulted also in one peak each (1.81 and 1.52 grams) but in both media the peak occurred in the same period between the 60 and 75 days of incubation. On the contrary, the shoots cultured in medium enriched with combination of BAP at 2.25, IAA at 1.75 mg. l^{-1}, combination of BAP at 3.25, IAA at 1.75 mg. l^{-1} and with combination of BAP at 2.25, IAA at 1.75, GA_3 at 1.0 mg. l^{-1} showed two peaks of gain in the total shoots weight. In all of these treatments, one peak (3.11; 1.22 and 1.30 grams) occurred at the last 15 days of the 105 days of incubation. However, the other peak (1.17; 0.91 and 2.43 grams) occurred at different periods of incubation (45 to 60; 60 to 75 and75 to 90 days respectively).

Discussion

This study showed that the suitability of hormone treatment depended on how long the incubation period was and sound recommendation of hormone treatment could not be made unless different incubation periods were tested. Observing the explants growth responses over different incubation period showed that the different hormones treatments exerted its highest effect after different periods of exposure time and for each parameter, shoot formation, total shoot weight and length there were different optimum hormone treatment at different incubation periods (Table, 1). At shorter incubation periods of 15, 30 and 45 days, the best treatment for all parameters (shoot formation, total length and weight) was combination of BAP at 3.25, IAA at 1.75 mg. l^{-1} while at longer incubation of 60, 75 and 90 days, the best treatment was combination of BAP at 2.25, IAA at 0.75 mg. l^{-1}. After 105 days of incubation the best was BAP at 2.25, IAA at 1.75 mg. l^{-1}. Explants treated with BAP at 2.25 mg/l plus IAA at 0.75 mg. l^{-1} lost its shoot formation ability after 90 days of incubation while those treated with other hormone combinations remained capable of shoot formation. At incubation longer than 90 days, only three (BAP at 2.25, IAA at 1.75 mg. l^{-1} with and without GA_3 at 1.0 mg. l^{-1} and BAP at 3.25 mg. l^{-1} plus IAA at 1.75 mg. l^{-1}) of the seven treatments remained effective. That is in case of the other four hormone treatments, either the explants lost its capability of shoot formation or the hormones lost its effectiveness after 75 days of incubation. It is important to point out that while the explants cultured in medium enriched with BAP 3.25, IAA 3.75 mg. l^{-1} took 105 days to produce 10 shoots, the explants which cultured in medium enriched with BAP at 2.25 plus IAA at 1.75 and IAA at 0.75 mg.l^{-1} produced the same amount of shoots on only 45 days of incubation.

The results (Table, 2) showed that the shoot formation occurred at alternative pulses of high and low and different treatments differed on the time and magnitudes of the pulses. That is, the different hormone treatments not only resulted in different shoot formation per explant but also in the time when the hormone exerted its highest effect and when it regained or lost its effectiveness. Different treatments which resulted in equal total shoots after 105 days of incubation, had different shoot formation pattern. Explants treated with BAP alone at 2.25 mg. l^{-1} and with combination of BAP at 2.5 and IAA at 0.75 mg. l^{-1} both resulted in 25 shoots after 105 days of incubation. However, the biweekly shoot formations were 1; 6; 3; 4; 8; 2; 0 and 1; 5; 2; 5; 10; 1; 0 shoots respectively. Similar, explants treated with BAP at 3.25, IAA at 1.75 mg. l^{-1} and those treated with BAP at 2.25, IAA at 1.75 mg. l^{-1} plus GA_3 at 1.0 mg. l^{-1} produced 20 and 19 shoots after 105 days of incubation but had different shoot

formation pattern. In fact, in response to combination of BAP at 2.25 mg. l^{-1} plus IAA at 1.75 mg. l^{-1} 30% of the total shoots (11 of 31 shoots) were produced during the last15 days of the 105 days of incubation.

Determination of the pattern of shoot formation over incubation period could be used as guide line for more elaborate investigation of hormone mode of action. External addition of hormone is expected to change the internal hormonal balance of the explants in favor of shoot formation. However, unless the internal balance is identified, tissue culture would remain a mere try and error experiment and different treatments could be claimed as the best for shoot formation. Chemical and histological analysis revealed that exogenous application of BAP and NAA to pineapple leaf base (Hamasaki *et al*., 2005; Mercier *et al*., 2003) and etiolated node (Souza *et al*., 2003) caused a sharp increase in the endogenous content of IAA and Isopentenyladenine (Ip) within a week and that coincide with shoot formation. Shoots could be seen by naked eye within two weeks. The pulses of high and low shoot formation observed in this study are reflection of an endogenous changes in hormonal content, enzymes activity and mineral content of the explants and medium that need to be identified. Histological, chemical and radioactive techniques are usually used in investigation of metabolism and physiological events underline differentiation and developmental process. However, deriving of valuable information from such techniques depend mainly in selecting of proper time for monitoring the internal metabolic and histological changes and its association with the external parameters of growth. Monitoring the changes on the growth parameters over two week interval as it done in this study could help in selection of the most suitable hormone treatments and determination of the best times for conduction histological, enzymatic and radioactive analysis.

Different in total weight between different hormone treatments and heaviest accumulation of weight at each two weeks interval of pineapple *in vitro* multiplication could be used as an indicator of possibly successfull acclimatization. Escolano, *et al*. (1999) and Dewald, *et al*. (1989) emphased the relation between size of shoots and successful *ex vitro* acclimatization of pineapple. Danso *et al*., (2008) reported also that the length of incubation period during the multiplication stage of pineapple effect the proper rooting hormone type and concentration during rooting stage. Using *Spathipuyllum floribundum*, Ramirez *et al*. (2001) reported that hormonal treatments that resulted in heavier shoots during multiplication stage resulted also in higher survival during acclimatization. Based on the shoot weigh gain (Table, 2), the shoots obtained from explants treated with BAP alone at 2.25 mg/l, combination of BAP at 2.25 and IAA at 0.75 mg/l and combination of BAP at 3.25 and IAA at 1.75 mg/l could possibly successfully acclimatized after any incubation period except 15 and 60 days. Shoots from explants treated with combination of BAP at 2.25 and IA at 1.75 mg/l could acclimatize after any incubation period except 45 and 90 days. On contrary, poor acclimatization is expected for those shoots obtained from explants treated with combination of BAP at 3.25 and I 3.75 mg/l after any incubation period except 90 days and for those treated with combination of BAP at 2.25, IAA at 1.75 and GA3 at 1.0 mg/l except 60 and 105 days. Moreover, the main purpose of pineapple tissue culture so far is propagules production. However, due to expected increase in the application of the pineapple bromelain for medical and industrial uses, the weight may soon become an important

parameter for selection of best treatment. Combination of BAP at 2.25 plus IAA at 1.75 mg. l^{-1} and BAP at 3.25 plus IAA at 1.75 mg. l^{-1} and longer incubation of 105 days seemed to be a best choice for biomass production (6 g/explant).

The main obstacle of micropropagation is the cost of production. However, recommendation of hormone treatment was usually based only on rate of multiplication.

Hormones differ not only in their effectiveness as *in vitro* shoot formation inducer but also in their price and longer incubation lead to more shoot formation but also in higher electricity cost (photoperiod and air condition). Several reports suggested an incubation period of 30 to 45 days. However, a tentative estimation of the cost after one mltiplication cycle (Table, 3) showed that none of the hormone treatments resulted in shoot cost less than one cent (0.01) unless the incubation was at least 75 days. After 75 and 90 days of incubation, the cost of production in case of singly applied BAP at 2.25 mg. l^{-1} and combination of BAP at 2.25 and IAA at 0.75 mg. l^{-1} was one cent (0.01). The cost per shoot in case of treatment BAP at 2.25, IAA at 1.75 mg. l^{-1} could be as low as one cent but only if the incubation was 105 days long. In these hormone treatments, the cost per shoot after the most commonly suggested incubation period of 30 and 45 days was three times higher (three cents per shoot). Knowing the changes on shoot formation and cost per shoot over incubation period, enable the propagator to select the proper incubation in term of cost and the physiologist could contemplate about the hormonal action mode or design a new experiment that would elucidate shoot formation pattern and physiology.

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Table (1) Effect of hormone treatments and incubation periods on the *in vitro* shoot formation rate and total shoot length and weight of Smooth cayenne pineapple

Hormones treat. (mg. l^{-1})	**Incubation periods (Days)**						
	15	30	45	60	75	90	105
Shoots per explants							
BAP(2.25)	2 rq	8 lmno	11 ijkl	15 fghi	23 bcd	25 b	25 b
BAP(2.25) IAA(0.18)	2 rq	6 lmnopr	10 jklm	13 ghik	14 ghij	17 efg	17 efg
BAP(2.25) IAA(0.75)	2 rq	7 lmnop	9 klmn	14 ghij	24 bc	25 b	25 b
BAP(2.25) IAA(1.75)	2 rq	7 lmnop	8 lmno	12 hijkl	19 def	20 cde	31 a
BAP(3.25) IAA(1.75)	4 oprq	11 ijkl	12 hijkl	13 ghik	16 efgh	17 efg	20 cde
BAP(3.25) IAA(3.75)	2 rq	5 noprq	8 lmno	8 lmno	9 klmn	10 jklm	11 ijkl
BAP(2.25) IAA(1.75) GA_3 (1.0)	1 q	3 prq	7 lmnop	9 klmn	16 efgh	17 efg	19 def
Total shoot length (mm)							
BAP (2.25)	18 uv	41 stuv	79 oprqstu	135 ijklmno	208 efgh	225 cdef	301 b
BAP (2.25) IAA (0.18)	18 uv	37 stuv	70 prqstuv	117 klmnopr	98 mnoprqs	168 fghijk	137 ijklmno
BAP (2.25) IAA (0.75)	18 uv	42 stuv	74 prqstuv	126 jklmnop	244 cde	272 bc	300 b
BAP (2.25) IAA (1.75)	18 uv	42 stuv	48 qstuv	108 lmnoprq	188 efghi	180 fghij	406 a
BAP (3.25) IAA (1.75)	28 tuv	55 qstuv	86 noprqst	104 lmnoprq	145 ijklmn	204 efgh	214 defg
BAP (3.25) IAA (3.75)	18 uv	16 v	40 stuv	56 qstuv	61 rqstuv	72 prqstuv	75 prqstuv
BAP(2.25) IAA(1.75) GA_3 (1.0)	15 v	18 uv	62 rqstuv	95 mnoprqs	160 ghijkl	153 hijklm	266 bcd
Total shoot weight (g)							
BAP (2.25)	0.28 kl	0.8 ijkl	1.36 fghijkl	1.65 efghijkl	2.25 defghij	3.03 bcdefg	4.23 bc
BAP (2.25) IAA (0.18)	0.27 kl	0.97 ijkl	1.1 hijkl	1.67 efghijkl	3.48 bcde	33.52 bcde	3.85 bcd
BAP (2.25) IAA (0.75)	0.28 kl	0.73 ijkl	1.45 fghijkl	1.66 efghijkl	3.18 bcdef	3.71 bcd	4.5 abc
BAP (2.25) IAA (1.75)	0.27 kl	0.93 ijkl	0.96 ijkl	1.75 efghijkl	2.66 cdefghi	2.99 bcdefgh	6.1 a

BAP (3.25) IAA (1.75)	0.44 jkl	0.99 ijkl	1.45 fghijkl	1.80 efghijkl	2.26 defghij	4.69 ab	5.91 a
BAP (3.25) IAA (3.75)	0.27 kl	0.51 jkl	0.89 ijkl	0.95 ijkl	1.18 ghijkl	2.09 defghijkl	2.17 defghijk
BAP(2.25) IAA(1.75) GA_3 (1.0)	0.20 k	0.63 jkl	0.96 ijkl	2.13 defghijkl	2.18 defghijk	2.18 defghijk	3.48 bcde

Data represent means of 9 shoots individually cultured on 20 ml of agar solidified (7 g/l) full strength MS medium enriched with sucrose at 30 g/l).

Means of the same parameter followed by the same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test.

Table (2). Effect of the hormone treatments on the biweekly increase in shoots formation and total shoot length and weight of Smooth cayenne pineapple over 105 days of incubation.

Hormone treatments (mg. l^{-1})	**Growth per two weeks over 105 days of incubations**						
	(1-15)	(16-30)	(31-45)	(46–60)	(61–75)	(76-90)	(91-105)
Shoots per explant.							
BAP(2.25)	1 f	6 bcde	3 def	4 cdef	8 abc	2 ef	0 f
BAP(2.25) IAA(0.18)	1 f	4 cdef	4 cdef	3 def	1 f	3 def	0 f
BAP(2.25) IAA(0.75)	1 f	5 cdef	2 ef	5 cdef	10 ab	1 f	0 f
BAP(2.25) IAA(1.75)	1 f	5 cdef	1 f	4 cdef	7 abcd	1 f	11a
BAP(3.25) IAA(1.75)	4 def	7 abcd	1 f	1 f	3 def	1 f	3 def
BAP(3.25) IAA(3.75)	1 f	3 def	3 def	0 f	1 f	1 f	1 f
BAP(2.25) IAA(1.75) GA_3(1.0)	1 f	2 ef	4 cdef	2 ef	7 abcd	1 f	2 ef
Total shoots length (mm)							
BAP(2.25)	3 f	23 def	38 cdef	56 bcdef	73 bcde	17 def	76 bcd
BAP(2.25) IAA(0.18)	3 f	19 def	33 cdef	21 def	35 cdef	8 def	37 cdef
BAP(2.25) IAA(0.75)	3 f	24 def	32 cdef	52 cdef	118 b	28 def	28 def
BAP(2.25) IAA(1.75)	3 f	24 def	6 ef	60 bcdef	63 bcdef	28 def	207 a
BAP(3.25) IAA(1.75)	13 def	27 def	31 cdef	18 def	41 cdef	59 bcdef	10 def
BAP(3.25) IAA(3.75)	3 f	4 f	19 def	15 def	5 ef	11 def	4 f
BAP(2.25) IAA(1.75) GA_3(1.0)	0 f	3 f	44 cdef	33 cdef	65 bcdef	10 def	96 bc
Total shoots weight (g)							
BAP (2.25)	0.08 b	0.52 ab	0.56 ab	0.29 b	0.60 ab	0.78 ab	1.20 ab

BAP (2.25) IAA (0.18)	0.07 b	0.7 ab	0.13 b	0.57 ab	1.81 ab	0.04 b	0.33 b
BAP (2.25) IAA (0.75)	0.08 b	0.48 ab	0.69 ab	0.21 b	1.52 ab	0.53 ab	0.79 ab
BAP (2.25) IAA (1.75)	0.07 b	0.66 ab	0.03 b	0.79 ab	0.91 ab	0.33 b	3.11 a
BAP (3.25) IAA (1.75)	0.24 b	0.55 ab	0.46 ab	0.36 b	0.46 ab	2.43 ab	1.22 ab
BAP (3.25) IAA (3.75)	0.07 b	0.24 b	0.38 b	0.06 b	0.23 b	0.91 ab	0.07 b
BAP (2.25) IAA (1.75) GA_3(1.0)	0.00 b	0.43 b	0.33 b	1.17 ab	0.06 b	0.00 b	1.30 ab

Data represent means of 9 shoots individually cultured on 20 ml of agar solidified (7 g/l) full strength MS medium enriched with sucrose at 30 g/l.

Increase on shoots formation per explant, total length and weight of shoots at each increment of two weeks were calculated by substraction of the values obtained at one incubation from that of the previous one. (Incubation for 30 – Incubation for15 days)

and (Incubation for 45 – incubation for 30 days) and so on.

Means of the same parameter followed by the same letter were not significantly different at $p \leq 0.05$ according to DMRTest.

Table (3). Estimated variable cost per shoot of Smooth cayenne pineapple after different incubation periods in different hormone treatments

Hormone treatments (mg/l)	**Incubation periods** (Days)					
	30	45	60	75	90	105
	Cost/ shoot (USA $)					
BAP (2.25)	0.03	**0.02**	**0.02**	**0.01**	**0.01**	**0.02**
BAP (2.25) IAA (0.18)	0.04	**0.03**	**0.02**	**0.02**	**0.02**	**0.02**
BAP (2.25) IAA (0.75)	0.03	0.03	**0.02**	**0.01**	**0.01**	**0.02**
BAP (2.25) IAA (1.75)	0.03	0.03	**0.03**	**0.02**	**0.02**	**0.01**
BAP (3.25) IAA (1.75)	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
BAP (3.25) IAA (3.75)	0.04	0.03	0.04	0.04	0.04	0.04
BAP (2.25) IAA (1.75) GA_3 (1.0)	0.08	0.04	0.03	**0.02**	**0.02**	**0.02**

The calculated cost included only the variable items of cost (Jars, MS, agar, sucrose, hormones, wages of labor and electricity cost for operating of autoclave, laminar and incubation room).