Studies on Micropropagation of Pineapple (Ananas comosus L.)

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ABSTRACT

The present study was conducted at the Tissue Culture Laboratory, Horticulture, Research Institute, Agricultural Research Center (ARC), Egypt during the period from December 2013 to March 2016 to investigate the effect of different type Murashige and Skooge (MS), Gamborge (B5) and Woody plant media (WPM) at four salt concentrations (Full, ¼, ½ and ¼) of culture media on micropropagability of pineapple (Ananas comosus L. var. Smooth Cayenne) during establishment stage. Shootlet proliferations were investigated at different concentrations of 6-benzyl amino purine (BAP) and kinetine (Kin) at 0.25, 0.5, 1.0 and 2.0 mg/l for each, during two successive subcultures. Finally, rooting capacity were studied by various concentrations of indole butyric acid (IBA) and indole acetic acid (IAA) at 1.0, 2.0 and 3.0 mg/l on media containing activated charcoal. The rooted explants were transferred to greenhouse in peat moss and sand at various amounts 1:1, 1:2 and 2:1, respectively. The culture crowns were successfully disinfecting by Colorex 40% for 20 min with 77.77% survival and 100% free contamination. MS media at full strength was the best culture media showed shootlet number 1.75 shootlet/explant and shootlet length 2.25 cm with 9.25 leaf/shootlets. Among the different concentrations 2.0 mg/l BAP showed highest shoot proliferation of 56 and 42 shoots per explant at the first and second subculture, respectively. The longest shoot (5.5 and 5.5 cm) was produced in the two subcultures by the treatment combination of 0.25 mg/l BAP. The highest numbers of roots were produced by 1.0mg/l IAA were 10.67 roots/shootlet and the tallest length of roots were obtained for explants cultured on MS media containing IAA 3 mg/l. The individual rooted explants derived from plantlets were transferred to poly bags in the green house at mixture media after 7 days hardening in room temperature (28-30°C) and established plantlet was ready for planting.

Key words: Pineapple (Ananas comosus L. Smooth Cayenne), micropropagation, shoot proliferation, rooting stage, acclimatization

Introduction

Pineapple is one of the most economically important tropical fruits (Duval et al., 2001). This bromeliad is routinely propagated vegetatively by means of lateral shoots, basal suckers or crowns. Pineapple micropropagation can be considered to be easy, but the multiplication rate is low and it would take 8 years to obtain enough propagules from one mother plant (Almeida et al., 2002). In conventional breeding, clonal selection is tedious and requires several generations of backcrossing in order to develop pineapple varieties with desired traits. Being a vegetatively propagated plant, conventional hybridization techniques for the generation of better pineapple varieties are cumbersome and time consuming (Mhatre, 2007). Hence, the need to improve the multiplication rates of selected elite genotypes led to the development of tissue culture techniques for the Ananas comosus (L. Merr) pineapple (Almeida et al., 2002).

In vitro micropropagation of pineapple plantlets has many advantages over conventional methods of vegetative propagation. For instance, this technique allows an efficient and rapid increase of selected elite pineapple varieties. Many authors have reported successful production of pineapple via micropropagation system during the last few years (Firooozabady and Gutterson, 2003; Be and Debergh, 2006; Danso et al., 2008).

Tissue culture has been successfully applied to pineapple. It has the potential to produce millions of propagules per year. However, conflicting rat of multiplication and total plantlets production were reported and contradicting hormone treatments were recommended. A total production of plantlets, for instance, ranged from 40 (Dewald et al., 1988); 280 (Devi et al., 1997); 5000 (Zepeda and Sagawa, 1981); 40000 (Liu et al., 1989); 80000 (Kiss et al., 1995); 100000 (Sripaoraya et al., 2003) from single explant pear year. Others reported that starting by 1 (Bhatia and Ashwath, 2002; Dal-Vesco et al., 2001); 2 (Soneji et al., 2002); 10 (Drew, 1980); 22 (Firooozabady and Gutterson, 2003); 40 (Fitchet, 1990) and 80 explants (Almeida et al., 2002) atotal of 1 million, 10000, 1.25 million, 15757, 30000 and 161080 shoots could be obtained in 9, 6, 3, 7, 3 and 8 months respectively.

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Generally, most pineapples are produced restrictively by using dormant axillary buds from crowns (Soneji, 2002; Sripaoraoy et al., 2003) and multiple shoot induction via in vitro produced leaf bases (Soneji et al., 2000). Almeida et al., (1997) reported that 3.0 mg/l 6-benzylaminopurine (BAP) combined with 2.0 mg/l indole acetic acid (IAA) produced the best results for the production of pineapple plantlets. The objective of the present work is to optimize a protocol for the micro propagation of pineapple cv. Smooth Cayenne, by the manipulation of different culture media at various concentrations in establishment stage and two growth regulators BAP or Kin, aiming to achieve a maximum rate of multiplication, ability to acclimatization in greenhouse and, therefore, improve the agronomical utilization of this cultivar.

Materials and Methods

This work was conducted in the Department of Tissue Culture and Germplasm Conservation Lab., Horticulture Research Center, Agriculture Researches Center, Giza, Egypt with co-operative of Horticulture Dep. Agriculture Faculty, Benha University, Egypt during the years of 2013-2016.

Plant materials

Pineapple’s crowns of Smooth Cayenne were obtained from Tropical Fruits Dep., Hort. Res.Institute (HRI) station in Giza, Egypt. The leaves were removed, and the crowns were washed by water to remove dust and dry matters and thoroughly washed in detergent. Afterwards the detergent was removed by using tap water for 1 hour and, subsequently, immersed in fungicide for one hour. Pineapple crown were sterilized with 10, 20, 30 and 40% Chlorox [sodium hypochlorite 5.2%] for 20 min or 0.1 and 0.2 % HgCl2 for 20 min with few drops of Tween 20 and rinsed three times with sterile distilled water. After oneweek incubation on free basal media of MS(Murashige and Skoog, 1962), B5 and WPMat 25°C ±2 under florescent lamps with light intensity of 3000 lux at 16 hrs photoperiods the optical vision contamination percentage was regarded, bud sprouting calculated, and browning percentage were observed as shown the next formula:

- Contamination % = [contamination jars/ total cultured jar] x 100
- Bud sprouting % = [number of explants sprouting/ total explants cultured in jar] x 100
- Browning % = [number of explants browning/ total explants cultured in jar] x 100

Establish growth media:

Sterilized crowns were cultured on MS medium (Murashige and Skoog, 1962), B5 (Gamborg, 1968) and WPM at four salt concentrations (full, ¼, ½ and ¾) each media free plant growth regulators and supplemented with 30 mg/l sucrose and 0.7% agar. The culture was incubated under 25°C ±2 under florescent lamps with light intensity of 3000 lux at 16 hrs photoperiods. The development of shoots was monitored every week. The numbers of shoots developed were counted after 8 weeks of culture and length of every shootlet formed with count of leaves obtained on every shootlet and percentages of explants formed roots as showed the next formula:

- Shoot number/explants = [shootlets formed per explants on jar] 
- Shootlets length cm = [shootlets length per explants cultured in jar] 
- Leaves number/shootlet = [count of leaves forming on shootlets per explants cultured in jar]

Shoot proliferation

Initiated shoots obtained from previous experiment and successive growth on suitable salt concentrations was sub cultured twice into this media containing different concentrations 0.25, 0.5, 1.0 and 2.0 mg/l of BAP or Kin, respectively. The number of regenerated plantlets were recorded after the first (4 weeks) and second (4 weeks) subcultures period. The average of plant height (cm), shoot number, leaves number and leaves area (cm²) formation were determined in this experiment.

- Leaves area cm² = [By Image J software program (Bakr, 2005. and O’Neal, et al., 2002).

Induction of rooting and acclimatization

For root induction, excised individual shoots were transferred in solidified MS basal medium without cutting leaves and supplemented with different concentrations of IAA or IBA (1.0, 2.0 and 3.0 mg/l) with 2 g/l activated charcoal. Three half-plants were placed in each jar (250 mm) containing 35 ml of the culture media. All the cultures were incubated at 25±2 °C under 16hours photoperiod at 30°C and white fluorescent lamps. Rooting percentage %, root number, and root length (cm) were recorded after 4 weeks incubation. Rooted explants were planted in pots containing a sterile soil containing peat and sand at 1:1, 1:2 and 2:1, respectively
and covered with transparence polypropylene package and kept in the greenhouse for 4 weeks acclimatization. At the first week one pore in the package was made and second weeks other one made and then removed the package at the end of third week, finally the package was removed and plants transfer in open field.

Rooting percentage% = [number of explants rooting / total explants cultured in jar] x 100

All experiments were arranged in completely randomized design. Each treatment was represented as three replicate jars with three explants per jar. Significant differences among the various treatments were compared using LSD test at 5% .

### Result and Discussions

#### Disinfecting crowns

After removing the dust from crown and disinfecting by the various disinfectant materials like HgCl₂ and Colorex at different concentrations with stable time of emersion (20 min), the crown exposed to 40% Colorex was a healthy (77.77% survival) and free of contaminations (100%) as showed in Table (1), followed by Colorex 30% which resulted 77.7% survival and 4.4% contamination, as compared with the other additives of Colorex and/or HgCl₂.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Contamination %</th>
<th>Survival %</th>
<th>Browning %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorex 10</td>
<td>62.55</td>
<td>55.55</td>
<td>32.22</td>
</tr>
<tr>
<td>Colorex 20</td>
<td>63.81</td>
<td>12.22</td>
<td>14.81</td>
</tr>
<tr>
<td>Colorex 30</td>
<td>4.443</td>
<td>77.77</td>
<td>18.52</td>
</tr>
<tr>
<td>Colorex 40</td>
<td>0.00</td>
<td>77.77</td>
<td>22.18</td>
</tr>
<tr>
<td>HgCl₂ 0.1</td>
<td>66.66</td>
<td>18.52</td>
<td>33.33</td>
</tr>
<tr>
<td>HgCl₂ 0.2</td>
<td>55.55</td>
<td>8.907</td>
<td>18.52</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>32.83</td>
<td>31.21</td>
<td>22.12</td>
</tr>
</tbody>
</table>

*Fig. 1:* (a) Crown after disinfecting process, (b and c) crown sprouting after establish in MS medium

#### Establish successful growth on suitable salt concentrations

The successful explants of free pathogens cultured on three types of medium at four concentrations of salts were observed in Table (2). The data showed that the explants cultured on MS medium gave the best results of shoot number (1.75 shootlets/explant). On the other hand, both of B5 and WPM have low concentrations of salts at full strength that decreased the growth of shoot number to 1.25 and 1.5 shootlets/explant, respectively. Moreover, full strength of MS salts were increase the growth morphology shoot length (2.25 cm) compared with B5 and WPM which scored 1.917 and 1.57 cm, respectively. Also, leaves number were obtained 9.25, 8.47 and 7.75 leaf/shootlet for MS, B5 and WPM respectively.

On the other side, Full strength of salts was the best concentration used in the culture compared with the other concentrations which recorded the best number of shootlets 1.77, leaves number 10.0 and shootlet length 2.578 cm compared with the other concentrations.

On the interaction between type of media and their concentrations the data in table (2) reported that the best growth was observed with the explants cultured on MS media at full strength, it was scored 2.33 shootlet number/ explants with 3.067 cm length while ¾ strength gave the highest count of leaves 11.0 leaf/ shootlets compared with the other media salts.

These results are in coordination with findings of Andreu and Marin (2005) who demonstrated that culture medium composition influenced the multiplication rate of Prunus rootstock. Moreover, the obtained results agree with Hassan (2012) on two Ficus carica cultivars, Tange et al., (2008) on pyrus communis "Bartlett", Baiea(2002) on date palm. On the other hand, Fayek et al. (2007) stated that shoot tip and nodal segment
explants of three female jojoba clones were compared for its potentiality of in vitro propagation. The effect of media (2MS, MS, 3/4 MS, 1/2 MS, BS and N.N) was considered at proliferation. Data indicated that cultivating explants on 3/4 strength of MS nutrient medium is mainly recommended (Mustaffa et al. 2012). Finally, these results revealed that pineapple explants need high nutrient requirements to development and proliferation.

Table 2: Effect of medium type and salt concentrations on growth characters of Ananas comosus in vitro culture

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Mean Number of shoot</th>
<th>Mean Shoot length (cm)</th>
<th>Mean Number of leaves</th>
<th>Rooting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>BS</td>
<td>WP</td>
<td>Mean</td>
</tr>
<tr>
<td>Full</td>
<td>2.333</td>
<td>1.667</td>
<td>1.333</td>
<td>1.778</td>
</tr>
<tr>
<td>½ strength</td>
<td>1.667</td>
<td>1.333</td>
<td>2.000</td>
<td>1.667</td>
</tr>
<tr>
<td>⅓ strength</td>
<td>1.667</td>
<td>1.000</td>
<td>1.667</td>
<td>1.445</td>
</tr>
<tr>
<td>Mean</td>
<td>1.750</td>
<td>1.250</td>
<td>1.500</td>
<td>2.250</td>
</tr>
</tbody>
</table>

LSD 5% 0.3970 0.1484 1.192 44.41
0.4584 0.1713 1.376 51.28
0.7940 0.2967 2.383 88.82

Fig. 2: Effect of salts concentration and types of growth media of Ananas comosus in vitro culture

Shoot proliferation and multiplication

All concentrations of BAP and kinetin showed formation of shoots ranging from 12.33 to 56.00 as shown in (Table 3). On the first subculture, MS medium supplemented with 2 mg/l BAP produced the highest number of shoots development on the base of explants and formation cluster (56 shootlet/explant) followed by kinetin 2 mg/l (47.0). The additive BAP at 0.25 mg/l also induced shootlet elongation to the highest length 5.5 cm followed by kin at 2 mg/l (4.7 cm) compared with MS free (control) (0.7 cm). Other growth character leaves number was increased to the maximum number 21 leaves/shootlets for the cluster cultured on MS media containing 2 mg/l Kin. Moreover, all concentrations of Kin (0.25 to 2 mg/l) supplemented into medium required extended time (4 weeks) to increasing leaves area from 4.2 to 5.8 cm².

At the second subculture the growth has more development of shootlet numbers, shootlet length, leaves number and leaves area. The application 2 mg/l BAP increased number of shootlets to 42.0 and leaves area 7.4 cm², while length of shootlets was increased to 5.5 cm with 0.25 mg/l BAP and leaves number to 9.66 for kin at 2 mg/l. At the end of incubation period some vitrification symptoms were observed (Fig. 4) that returns to increment number of leaves that decrease or competitive some growth factor (etc. Light, salts). The highest concentration of BAP in the medium was more stimulatory to shoot development than the lowest concentration of this growth regulator. These results corroborate with those obtained by Be and Debergh (2006). They reported that more axillary shoots were produced per inoculums when BAP concentration was increased. Generally, cytokinins are known to stimulate cell division and axillary bud proliferation (Kyte and Kley, 1996), thereby resulting to significant shoot formation at the expense of root development. According to Firoozabad and Gutterson (2003), addition of BAP in MS medium was essential for the regeneration plantlets from shoot apices of pineapple. Zuraida et al. (2011) working on Maspine pineapple by treated with BAP 0, 0.5, 1.0, 2.0 and 5.0 mg/l, they found that the highest number of shoots was observed on the medium containing 5 mg/l BAP (7 plantlets). Therefore, Smooth Cayenne pineapple could easily produce in vitro shoots from crown at 5 mg/l BAP. Cytokinin alone in the culture medium induces shoot formation in many plants. MS medium supplemented with 3.0 mg/l BA was suitable for micropropagation of Ficus benjamina vars. Natasja and Starligh (Rzepka-Plevnes and Kurek, 2001). Almeida et al., (2002) introduced the pineapple explants in MS medium supplemented with 2 mg/l BAP and then sub cultured for multiplication in solid and liquid MS medium with different BAP concentration (0-1.5 or 3 mg/l). To maximize the number of plantlets obtained by micropropagation, Danso et al. (2008) studied various combinations of N6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA) in solid or liquid cultures. They reported that liquid cultures required 5.0 mg/l BAP to increase the multiplication rate. Khan et al. (2004) found that BAP 0.5 mg/l was better for the pineapple number and length of shoots/explants and IBA at 1.5 mg/l level was best for roots initiation. Enrichment of
media with 0.54 – 2.69 μm NAA and 0.44 μm BA was obtained for enhanced regeneration by using nodules regenerated shoots for the pineapple (Teng and Yu, 1996). Firoozabody and Gutterson (2003) used a combination of 1.5 mg/l BA and 0.5 mg/l NAA to produce the highest rate of shoot multiplication for pineapple explants, about three to four folds monthly.

**Table 3:** Effect of BAP and kinetin concentrations on some growth characters of *Ananas comosus* in vitro culture

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Subculture I</th>
<th>Subculture II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot length</td>
<td>Shoot No.</td>
</tr>
<tr>
<td>Control</td>
<td>0.700</td>
<td>12.33</td>
</tr>
<tr>
<td>BAP 0.25</td>
<td>5.500</td>
<td>16.00</td>
</tr>
<tr>
<td>BAP 0.5</td>
<td>4.600</td>
<td>17.00</td>
</tr>
<tr>
<td>BAP 1.0</td>
<td>4.033</td>
<td>25.00</td>
</tr>
<tr>
<td>BAP 2.0</td>
<td>3.400</td>
<td>56.00</td>
</tr>
<tr>
<td>Kin 0.25</td>
<td>3.233</td>
<td>21.00</td>
</tr>
<tr>
<td>Kin 0.5</td>
<td>2.267</td>
<td>25.00</td>
</tr>
<tr>
<td>Kin 1.0</td>
<td>4.267</td>
<td>32.00</td>
</tr>
<tr>
<td>Kin 2.0</td>
<td>4.700</td>
<td>47.00</td>
</tr>
</tbody>
</table>

LSD 5%: 0.2948 3.111 0.8826 1.805 0.09321 13.18 1.682 1.670

**Fig. 3:** Effect of BAP and Kin concentrations on growth proliferation of *Ananas comosus* in vitro culture

**Fig. 4:** Effect of subculture on growth proliferation of *Ananas comosus* and vitrification of shoots during the second subculture

**Rooting induction and proliferation**

According to the data in Table (4) all the explants treated with IAA and IBA have insignificant effect of rooting percentage values but the length of roots and number were varied between treatments. The effect of IAA on shootlets elongation was observed in Table (4) and Fig. (5), which showed that applied IAA at 2.0 mg/l gave the maximum length of explants 2.9 cm compared with control (0.8667 cm). Also, IAA at 1.0 and 2.0 mg/l increased the leaves number to 12 leaf/shootlets for each compared with control (10.67 leaf/shootlets).

On the other hand, IBA gave a negative effect of shoot length and leaves number by decreased the values to (1.43, 1.06 and 1.26 cm) and (6.33, 5.0 and 5.33 leaf/shootlet).

On the other hand, the number of root formation on the base of shootlet gave a significant effect after treated with IAA and IBA, all IBA treatments have no significant effect on root numbers (3.0, 4.33 and 3.33 root/shootlet) while the tallest root was obtained with IBA treatment at 3 mg/l (13.6 cm) as shown in Fig. (5). In this application there are many investigators discussed this work, Pieriket al. (1984) and Dansoet al. (2008) reported that in vitro rooting of pineapples can be enhanced by an addition of auxins such as NAA, IBA or combination of NAA and IBA in the medium. The marked improvement in the mean number of roots produced...
when NAA and IBA were applied in combination may have resulted from the fact that these hormones can act either in concert or synergistically for the induction of *in vitro* roots (Danso et al., 2008).

### Table 4: Effect of IAA and IBA concentrations on rooting characterizations of *Ananas comosus* in *vitro* culture

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot length</th>
<th>Leaves No.</th>
<th>Rooting %</th>
<th>Root No.</th>
<th>Root length cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.8667</td>
<td>10.67</td>
<td>100.00</td>
<td>1.667</td>
<td>6.500</td>
</tr>
<tr>
<td>IAA 1.0 mg/l</td>
<td>2.500</td>
<td>12.00</td>
<td>100.00</td>
<td>8.900</td>
<td>7.400</td>
</tr>
<tr>
<td>IAA 2.0 mg/l</td>
<td>2.900</td>
<td>12.00</td>
<td>100.00</td>
<td>11.00</td>
<td>8.420</td>
</tr>
<tr>
<td>IAA 3.0 mg/l</td>
<td>2.300</td>
<td>11.67</td>
<td>100.00</td>
<td>8.667</td>
<td>12.24</td>
</tr>
<tr>
<td>IBA 1.0 mg/l</td>
<td>1.433</td>
<td>6.333</td>
<td>100.00</td>
<td>3.000</td>
<td>9.983</td>
</tr>
<tr>
<td>IBA 2.0 mg/l</td>
<td>1.067</td>
<td>5.000</td>
<td>100.00</td>
<td>4.333</td>
<td>12.17</td>
</tr>
<tr>
<td>IBA 3.0 mg/l</td>
<td>1.267</td>
<td>5.333</td>
<td>100.00</td>
<td>3.333</td>
<td>13.60</td>
</tr>
<tr>
<td>LSD 5 %</td>
<td>0.2516</td>
<td>3.747</td>
<td>NS</td>
<td>2.902</td>
<td>2.480</td>
</tr>
</tbody>
</table>

![Image](image_url)

**Fig. 5:** Effect of IAA and IBA concentrations on growth of *Ananas comosus* roots of *in vitro* culture.

### Acclimatization

Transfer of pineapple plantlets with sterile roots to the greenhouse conditions showed almost 100% of survival success for all treatments except with those adapted on peat: sand 1:2 and 1:1 scored 88.88% for the explant rooted on media containing IAA at 3.0 mg/l and peat: sand 1:2 scored 88.88% for the shootlet rooted on media containing IAA at 1.0 mg/l, respectively (Table 5 and Fig. 6). The explants adapted on polypropylene package containing 2 peats:1 sand obtained from IAA 1.0 mg/l gave the highest number of shoots 1.3 shoot/shootlet. While those produced from IAA at 2.0 mg/l cultured on mixture containing 1:1 and 1:2 of peat and sand or rooted plantlets produced from IAA at 3.0 mg/l and cultured on same mixture gave the tallest adapted plantlets 2.22, 2.12, 2.09 and 2.0 cm, respectively.

Finally, the large count of leaves formed in adapted plantlets was 6.55 leaf/shootlet for those produced on mixture containing 2:1 peat: sand and rooted from treated with IAA 3.0 mg/l.

These results were in conformity with Folliot and Marchal (1990), they found that the peatmoss was the best in pineapple acclimatization compared with eight substrates used in the experiment. Similarly, acclimatization of MD2 pineapple rooted plantlets in jiffy peatmoss pots resulted in an optimal growth and establishment in greenhouse (Danso et al., 2008). Increasing number and length of leaves and optimal growth of plantlets *in vivo* was highly correlated with artificial and commercial soils especially peat moss. This was probably attributed to the unique features of peat moss, of being of homogeneous composition, high structural stability, high capacity for retaining water and air, low and easily adjustable pH and nutrient status and lack of pathogens, insects, pests and weed seeds (Anon, 2008). The development of aerial part and optimal survival percentage of *in vitro* plantlets was observed in a mixture of soil, peatmoss and sand in jackfruit plant (Hamed et al., 2007), peat moss and perlite in a proportion of (1:1) in amelanchier plantlets (Staruvey and Lineberger, 1985) and grapevine plantlets (Kalatejari et al., 2006), sand: silt in 1:1 ratio in pineapple plant (Idris, 2002) and rice husk + sand in pineapple plant (Khoa et al., 2004). On the contrary, Amin et al. (2005) and Tavares et al. (2008) established pineapple and bromeliads respectively in sand with good growth of plantlets. This was probably attributed to genotypic differences or the type and structure of the soil mixes.

The established plants were exposed to an open field environment where they showed rapid growth after the acclimation period in greenhouse (Figure 6). This system is presently being introduced for the large-scale production of this specific pineapple cultivar. Most of the obstacles currently found in conventional pineapples
micropropagation have been overcome. Thus, an efficient and viable protocol was established for the mass propagation of Smooth Cayenne pineapple.

Table 5: Effect of IAA concentrations and media composition on rooting characterizations of *Ananas comosus* in *vitro* culture

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Survival %</th>
<th>Shoot No.</th>
<th>Shoot length cm</th>
<th>Leaves No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA 1.0 Peat 1: Sand 1</td>
<td>100.0</td>
<td>1.2</td>
<td>1.66</td>
<td>6.22</td>
</tr>
<tr>
<td>IAA 1.0 Peat 2: Sand 2</td>
<td>88.88</td>
<td>0.9</td>
<td>1.71</td>
<td>5.36</td>
</tr>
<tr>
<td>IAA 1.0 Peat 2: Sand 1</td>
<td>100.0</td>
<td>1.3</td>
<td>1.21</td>
<td>4.66</td>
</tr>
<tr>
<td>IAA 2.0 Peat 1: Sand 1</td>
<td>100.0</td>
<td>0.78</td>
<td>2.22</td>
<td>4.44</td>
</tr>
<tr>
<td>IAA 2.0 Peat 1: Sand 2</td>
<td>100.0</td>
<td>0.66</td>
<td>2.12</td>
<td>5.11</td>
</tr>
<tr>
<td>IAA 2.0 Peat 2: Sand 1</td>
<td>88.88</td>
<td>0.77</td>
<td>1.98</td>
<td>4.33</td>
</tr>
<tr>
<td>IAA 3.0 Peat 1: Sand 1</td>
<td>88.88</td>
<td>1.1</td>
<td>2.09</td>
<td>4.09</td>
</tr>
<tr>
<td>IAA 3.0 Peat 1: Sand 2</td>
<td>88.88</td>
<td>1.2</td>
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<td>3.98</td>
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<tr>
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<td>100.0</td>
<td>1.0</td>
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<td>6.55</td>
</tr>
</tbody>
</table>

LSD 5%  NS  0.067  0.0233  0.0566

Fig. 6: Effect of IAA concentrations and media composition on rooting characterizations of *(Ananascomosus)* in *vitro* culture

References


