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In vitro Rhizogenesis of Pineapple (*Ananas comosus L.*) `Smooth Cayenne`Cultivar

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ABSTRACT

This study aimed to develop a rhiyzogenesis protocol for the in vitromicropropagated pineapple plants and subsequently acclimatization the rooted shoots. Two auxins type with different concentrations were tested and several modifications were conducted to MS medium components. Significant differences were detected among auxins tested; 0.5 mg/l IBA was the optimal concentration for root number, followed by 0.1 mg/l NAA; MS free hormones achieved the best result of root length, followed by 0.1 mg/l NAA and 0.5 mg/l IBA respectively. Increase auxins concentration up to 2.0 mg/l inhibited root length. MS medium supplemented with 20.0 g/l sucrose was the optimal concentration for both root number and length with high significant differences among other sucrose levels tested, low sucrose concentrations inhibited root formation. Decreasing MS salts concentration gave the best results of root growth parameters; whereas MS medium free salts achieved the best result for root number, followed by quarter and half MS salts strength respectively; root formation was inhibited under high salts concentration. The rooted shoots were successfully acclimatized in different types of soil; the rooted shoots were transferred to plastic pots containing: clay, sand and a mixture of clay and sand with a ratio of (1:1). The highest percentage of survival shoots were obtained in sand (91%). The pineapple plants were established successfully under greenhouse condition.

Keywords: ananas comosus, micropropagation, MS medium, rooting, auxins, acclimatization,

Academic Discipline and Sub-Disciplines: Biotechnology

SUBJECT CLASSIFICATION: Plant tissue culture

TYPE (METHOD/APPROACH): **In vitro Experiment (**Full length research paper)

INTRODUCTION

Tissue culture is the only option to do to develop an efficient and economical micropropagation protocol for the large scale propagation of pineapples. In vitro propagation of pineapple for plantlet regeneration (Kiss etal., 1995; Firoozabady and Gutterson, 2003) and conservation (Souza et al., 2006) is well documented. It has comparative advantage over the traditional methods as it leads to the production of large numbers of disease-free uniform planting materials in a relatively shorter period independent of the season. The in vitro technique is found to be more efficient to overcome the problem of planting material shortage to attain the targeted extensive pineapple plantation.

Adventitious root formation is a complex process that is affected by multiple endogenous factors including phytohormones and environmental factors (Xuanet al., 2008). The nutrient medium serves as a source of assimilable carbon, nitrogen, and other minerals as well as regulatory compounds (Neumann et al. 2009). For in vitro rooting of Smooth Cayenne pineapple, some researchers have investigated different types of hormones and medium. MS phytagel solidified hormone free (Koet al., 2006), enriched with NAA at 1.0 mg/L (Hamad and Taha, 2008) and combination of 2.0 mg/L IBA and 2.0 mg/L IAA (Bhatia and Ashwath, 2002) led to root induction. Half strength solidified MS media without hormone (Zepeda and Sagawa, 1981) and enriched with combination of 0.5 mg/L IBA and 0.5 mg/L NAA (Firoozabady and Gutterson, 2003) also showed suitable result of rooting process. Full strength solid media without hormone (Almiedaet al., 1997), enriched with combination of 0.5 mg/L IBA and 0.5 mg/L NAA (Kansoet al., 2008) and half strength solid MS included 2.0 mg/L IBA (Akbar et al., 2003). Gangopadhyayet al., 2005 used full strength liquid MS media enriched with 2.0 mg/L IBA 2.0 and 0.4 mg/L KN for Elite cultivars.

The successful establishment of in vitro raised plants on the soil and later on field is the major success of in vitro propagation (Teixeira et al., 2001; Pospisilovaet al., 1999). Thus, in vitro pineapple plant-lets need to be acclimatized carefully.

This study was thus made with the objective of developing a defined rooting medium for the in vitromicropropagated pineapple shoots and to find a suitable soil for the rooted shoots acclimatization.

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MATERIALS and METHODS

The plant material and the nutrient medium

The explant used in this study was in vitro pineapple shoots variety"Smooth Cayenne"propagated by using the micropropagation protocol developed in the plant cell and tissue culture laboratory at the commission for biotechnology and genetic engineering, National Center for Research-Sudan.

Pineapple shoots were cultured in MS basal medium supplemented with 30.0 g/l sucrose and solidified with 6.0 g/l agar. The pH was adjusted to 5.8, then dispensed in measured amounts of 20 ml/test tube and then autoclaved at 121°C, 15 psi for 15 minutes.

Root induction experiments

Two types of auxins (NAA and IBA) were used in different concentrations (0.0, 0.1, 0.5, 1.0, 1.5, 2.0 mg/l) for both of them. Different levels (0.0, 10.0, 20.0, 30.0 and 40.0 g/l) of sucrose were tested and several modifications of MS salts strengths were tested (0.0, 0.25X, 0.5X, 1.0X and 2.0X). Cultures were then maintained in the incubation room at 25±2° C under 16/8 photoperiod.

Acclimatization

Rooted shoots were carefully washed under tap water, and then transferred to small plastic pots containing three types of soil: 1) sand, 2) clay, 3) mixture of clay and sand with a ratio of (1:1), and covered with plastic bags to save humidity.

Data analysis

Data were collected for the in vitro treatments after six weeks and analyzed using analysis of variance (ANOVA) on excel program. Means were separated using Duncan's Multiple Rang Test at 5% probability level. Survival rate percentage was recorded for the acclimatized shoots.

RESULTS and DISCUSSION

In vitro micropropagation protocol was developed for pineapple plant variety"Smooth Cayenne"(Halaet al., 2009). For root induction; two auxins types (NAA and IBA), sucrose and MS medium salts with different concentrations from all of them were used. Results showed significant differences between auxins tested; MS medium supplemented with 0.5 mg/l IBA achieved the highest mean value of root number (4.0 a); followed by 0.1 and 1.5 mg/l NAA with mean value of (3.7 ab) and (3.5 ab) respectively (Table 1), followed by 0.5 mg/l, 1.0 mg/l NAA and 0.1 mg/l IBA with (3.2 b) mean value for each of them (Table 1), the least treatment induced roots was 1.0 mg/l IBA with (1.7 d) mean value (Table 1, Plate1). MS free hormones gave the high mean value of root length (1.7 a), followed by 0.1 mg/l NAA (1.1 b) and 0.5 mg/l IBA (0.6 c) mean values respectively. Auxins concentrations increased up to 2.0 mg/l inhibited root length (Table 1, Plate 1).

Our findings were in agreement with the finding of Kiss et al., 1995 who reported that regenerated plantlets were rooted on a growth regulator free MS medium and residual shoots of the initial explants could be recycled by rooting on a growth regulator free MS medium; but in contrast with the finding of (Saifulla et al., 2004) who employed several concentrations of IBA in the rooting experiments; the greatest average number of roots was obtained in a media containing 1.0 mg/L IBA; this is in contrast to the finding by (Devi et al., 1997) of good rooting on MS medium supplemented with 9.84 µM IBA. Also (Abdel hamidet al., 2013) reported that the best treatment for Smooth Cayenne was 1.0 mg/l NAA and IBA at 0.5 and indicated that the cultivars had significant direct effect on root number, root length and plantlets height, although interaction of hormone types and concentrations had no significant relation with root length however, it had significant indirect effect on root number and plantlets height.

In sucrose test; the best result in both root number (2.83 a) and root length (2.67 a) was obtained in MS medium supplemented with 20.0 g/l sucrose with high significant differences among other sucrose levels tested (Table 2, Plate 1); followed by adding 30 g/l sucrose and 40 g/l sucrose with mean values of (2.0 b), (2.17 b) and (1.50 c) for both root number and root length respectively (Table 2). Decreasing sucrose concentration to the minimum or culturing in medium free of sucrose inhibited root formation (Table 2, Plate 1).

In agreement with our results; Bridgen, 1994 reported that sucrose is efficiently up-taken across the plasma membrane, it has been used as the only energy source in most of the tissue culture studies with the concentration of 2-5%. The carbon sources serve as energy and osmotic agents to support the growth of plant tissues (Lipavska and Konradova, 2004). Roots have an essential role and function in plant life and development through water and nutrients supply from the environment to the whole plant; addition of energy source types and levels into the in vitro rooting media influenced pineapple root growth, well pineapple root growth was measured on MS media supplemented with analytic grade sucrose and table sugar (Schiefebeidet al., 1997). In addition Mengeshaet al., 2013 reported that the energy sources with varied concentration strongly influenced the in vitro growth and subsequent acclimatization of pineapple plantlets. Analytic grade sucrose and table sugar at 3% performed well for in vitro survival rate; shoot amplification; rooting ability and acclimatization.

Different concentrations of MS salts were tested for pineapple root induction. Results showed that MS medium free salts gave the optimum result (4.50 a) of root number with high significant differences compared to other salt strengths tested (Table 3), followed by quarter and half MS salts strength with (3.33 b) mean value of root number for both of them, the normal concentration of MS medium (1X) achieved (2.17 c) mean value of root number, the increasing of MS salts up to 2X inhibited root formation and there were no significant differences obtained between salt strengths for root length (Table 3),

Our results were in agreement with Abdel hamidet al., 2013 who reported that the effect of shoot age in quarter, half and full strength of two MS medium types (solid, liquid) supplemented with different concentrations of auxins and

cytokinins on two pineapple cultivars (Smooth cultivars were examined to identify the optimal combination for root formation. All the shoots of both cultivars rooted in solid medium enriched with 2.0 mg/L IBA at quarter strength for Smooth Cayenne and half strength for Morris.

Rooted shoots were removed from the rooting medium after six weeks of incubation and transferred to plastic pots containing three types of autoclaved soil (1- sand, 2- clay, 3- mixture of sand and clay with a ratio of 1:1) and covered with plastic bags to maintain humidity and were kept under culture room conditions for one week; shoots were successfully acclimatized in the tested soils, 80 % of the plants survived and the highest survival percentage was achieved in sand soil 91%. The rooted shoots were established well in the greenhouse condition and all plants were morphologically normal (Figure 2. d).

Table 1. Effect of auxins on the in vitro rooting of pineapple

Auxin type	Concentration (mg/l)	Root number (Mean±SE)	Root length (cm) (Mean±SE)
0.0	0.0	2 .3±0.7 c	1.7±0.2 a
NAA	0.1	3.7±0.6 ab	1.1±0.3 b
	0.5	3.2±0.5 b	0.3±0.1 de
	1.0	3.2±0.6 b	0.3±0.1 de
	1.5	3.5±0.4 ab	0.5±0.1 cd
	2.0	2.2±0.8 cd	0.3±0.0 de
IBA	0.1	3.2±0.4 b	0.4±0.1 cd
	0.5	4.0±0.4 a	0.6±0.1 c
	1.0	1.7±0.2 d	0.3±0.0 de
	1.5	2.0±0.4 cd	0.4±0.1 cd
	2.0	2.2±0.2 cd	0.3±0.1 de

Means with the same letter(s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Table 2. In vitro rooting of pineapple shoots in different concentrations of sucrose after 8 weeks.

Sucrose concentrations (g/l)	Root number (Mean±SE)	Root length (cm) (Mean±SE)
0.0	0.01±0.0 d	0.01±0.0 d
10	0.01±0.0 d	0.01±0.0 d
20	2.83 ±0.5 a	2.67±0.6 a
30	2.00 ±0.3 b	2.17±0.3 b
40	1.50±0.5 c	1.50±0.6 c

Means with the same letter(s) in the same column are not significantly different at 5% using Duncan Multiple Rang test.

Table 3. . In vitro rooting of pineapple shoots in MS salts strengths after 8 weeks.

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MS salts Strengths	Root number (Mean±SE)	Root length (cm) (Mean±SE)
0.0	4.50±0.2 a	3.67±0.2 a
0.25X	3.33±0.6 b	3.83±0.7 a
0.5X	3.33±0.4 b	3.67±0.3 a
1X	2.17±0.2 c	1.17±0.4 b
2X	0.01±0.0d	0.01±0.0 c

Means with the same letter(s) in the same column are not significantly different at 5% using Duncan Multiple Range Test.

Figure 1. In vitrorhizogenesis of pineapple shoots

(a) Rooting in MS medium supplemented with 0.5 mg/l IBA. **(b)** Rooting in MS medium supplemented with 0.1 mg/l NAA. **(c)** Rooting in MS medium free hormone. **(d)** Rooting in MS medium supplemented with 20 g/l sucrose. **(e)** Rooting in MS medium free salts. **(f)** Rooting in 0.25X MS medium. **(g)** Rooting in 0.5X MS medium.

Figure 2. Rooted shoots acclimatization.

(a) In vitro pineapple plants

(b) (c) Acclimatization in:1- clay, 2- sand, and 3- mix. of clay and sand (1:1) (from left to right).

(d) Pineapple plants successfully established in the greenhouse condition.

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