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In vitro Environmental Factors Controlling Root Morphological Traits of Pineapple (*Ananas comosus* L. Merr)

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Abstract**—**Developing our knowledge of when pineapple roots grow can lead to improved water, fertilizer applications, and more precise culture management. This paper presents current understanding of morphological traits in pineapple roots, highlighting studies using incubation periods and various solid MS media treated with different sucrose concentrations and pH, which directly assess *in vitro* environmental factors. Rooting parameters had different optimal sucrose concentrations and incubation periods. All shoots failed to root in medium supplemented with sucrose at 5 g/L and no roots formed within the first 45 days in medium enriched with sucrose at 10 g/L. After 75 days, all shoots rooted in medium enriched with 10 and 20 g/L sucrose. Moreover, MS medium supplied with 20 g/L sucrose resulted in the longest and the highest number of roots with 27.3 mm and 4.7, respectively. Root function, such as capacity for P and N uptake, declined rapidly with root length. As a result, the longer the incubation period, the better the rooting responses would be.

Keywords**—**Environmental factors, *in vitro* rooting, pineapple, tissue culture.

I. Introduction

THE pineapple belongs to the bromeliad family, which contains 50 genera and about 2,500 known species. Main roots of pineapple only emerge within the first 12 months or less in *in vivo* growth culture. Generally, the different cultivars and medium strength play major role in rooting of pineapple and the cultivars might be the most important factor that determined the rooting process of initiation, development and plantlet growth [1]. The factors which control the root traits are important for designing an efficient environment for a faster and higher yield production of pineapple. Adventitious root formation is a complex process that is affected by multiple endogenous factors including phyto-hormones and environmental factors [2].

Not only incubation time, which is a considerable factor in root formation of pineapple, but also other supplements such as sucrose and pH are important. Although the highest proportion of the medium components is sucrose, the lowest concentration and shortest incubation period would reduce the cost of rooting stage and the overall cost of propagules production.

Rooting of pineapple have reported using sucrose and an incubation period combination of 10 g/L and 30 days [3], 20 g/L and 30 days [4], 30 g/L and 30 days [5], [6], 30 g/L and 45 days [7], 30 g/L and 60 days [8], 30 g/L and 75 days [9], 35 g/L and 30 days [10], 40 g/L and 60 days of incubation [11]. The effect of different sucrose concentrations and incubation periods were neither compared individually nor in combinations of the two factors. In addition, in many times the results were reported as general statement or only using one parameter such rooting percentage [3] and root number [12] for assessment of the rooting response.

During multiplication, pineapple decreased the medium pH to an equilibrium of 3.5 [13]. It is also expected to affect the root growing, but the influence of pH has not yet been tested at other value more than 5.7.

Hence, the main objectives of this study were: 1. To investigate the effect of different incubation periods and its interaction with sucrose concentrations and 2. To search for treatment that could simultaneously induce rooting at the best interaction of pH and sucrose.

II. Materials and Method

The present work was carried out at Institute of Biological Sciences, University of Malaya, Malaysia. The shoot explants of *Ananas comosus* were rinsed in distilled water for 20 minutes with addition of 1-2 drops of Tween-20. The explants were sterilized by rinsing in sodium hypochlorite (chlorox) solution of 70%, 50%, 30% and 10% for 5 min each. The explants were then soaked three times in sterile distilled water for 5 min. They were surface sterilized with 70% alcohol in the laminar flow. Finally the explants were rinsed again with sterile distilled water three times.

Stock culture that was maintained by sub-culturing every 60 days on MS medium supplemented with sucrose at 20 g/L and BAP at 2.0 mg/L was used as source of shoot explant of for rooting. Explants were cultured at density of three shoots per culture tube containing 6 mL of solid MS medium enriched with IBA at 2.0 mg/L and supplemented with sucrose at different concentrations of 5, 10, 15, 20, 25 and 30 g/L. The pH of the medium was adjusted to 5-6.5 using 1 N NaOH or 1N HCl. Autoclaving was carried out at 120°C and 20 psi for 20 min. After 30, 45, 60 and 75 days of incubation under constant temperature of 25ºC ± 1, 16 h photoperiod and 8 h dark period, three culture tubes from each sucrose treatment were collected for counting and measuring of the roots number, root percentage, root length and plantlets height. The compare means analyses, was performed for each experiment

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by using Duncan's multiple range tests ($p< 0.05$) through SAS 9.2 software.

III. Results

A. Effect of Sucrose Concentrations and Incubation Periods

Individual combinations of sucrose levels and incubation periods indicated different optimal level of sucrose for each rooting parameter (Table I). After 30 days of incubation the plantlet height was 15.7 mm at MS media supplemented with 5g/L sucrose, which was increased to 19.0, 21.3 and 24.3 mm as the cultures were kept to 45, 60 and 75 days respectively. Incubation at 30 days and increasing the sucrose up to 30 g/L did not convey any substantial result on the plantlets height. However, the suppressive effect of the high sucrose concentration could be avoided by extending the incubation from 15 day to 30 days. The highest root number (4.7 roots/shoot) and longest roots (27.3 mm) of all treatments obtained with medium enriched with sucrose at 20 g/L and incubated for 75 days. The root number and root length declined to 3 roots and 11.3 mm by increment of sucrose to 30 g/L.

The highest rooting percentage after 30, 45 and 60 days of incubation were observed in a media enriched with sucrose 25, 15 and 20 g/L with 66.7%, 75.4% and 91.7 %, respectively, which were declined to 33.3%, 48.2% and 66.7 % at media enriched with 30 g/L sucrose.

In medium enriched with sucrose at 10 g/L, the tallest plantlets obtained after 60 days incubation. Moreover, the tallest plantlet in the other sucrose concentrations obtained after 75 days of incubation. Extending the incubation from 60 days to 75 days increased the plantlet height in the medium enriched with 15 and 20 g/L.

The tallest plantlets (46.3 mm) obtained with medium enriched with sucrose at 25 g/L, while the highest rooting percentage (100 %) in the media enriched with sucrose at 10 and 20 g/L.

B. Effect of Different pH, Sucrose Concentrations

The highest rooting percentage in solid media was 89.2 %, which was obtained at the medium enriched with 40 g/L sucrose and pH 6.0 (Table II). Rooting percentage as low as 34.1 % observed in the medium enriched with sucrose at 10 g/L and pH 5.0.

The highest root formation (11.2 roots/shoot) occurred in the medium enriched with sucrose at 30 g/L and pH 5.0. The longest root (26.3 mm) obtained at the medium enriched with sucrose at 30 g/L and pH 5.0. According to plantlets height, the medium supplemented with 30 g/L sucrose and pH 5.5 showed the best treatments with 56.3 mm.

IV. Discussion

Sucrose or other carbon source, optimal degree of temperature and light intensity are obligatory requirement during in vitro micropropagation. Although sucrose at 20 and even 10 g/L in the MS medium and 70 days of incubation resulted in an excellent *in vitro* rooting respons of Queen Pineapple [1], enrichment of medium with sucrose at 30 g/l and incubation for 30 days is still the most common practice for *in vitro* rooting of pineapples.

TABLE I
Mean Comparison of Sucrose Concentrations and Incubation Periods for *in vitro* Rooting of Pineapple

Sucrose (g/l)	Incubation period (Days)				
	30	45	60	75	Average
Plantlets height (mm)					
5	15.7 b	19.0 c	21.3 c	24.3 c	20.1 B
10	23.0 a	28.0 ab	34.0 a	35.3 b	30.1 A
15	21.7 a	32.3 a	36.0 a	43.0 a	33.3 A
20	22.3 a	31.3 a	36.7 a	43.7 a	33.5 A
25	20.3 a	19.7 c	29.7 b	46.3 a	29.0 A
30	22.0 a	25.7 b	32.7 ab	42.3 ab	30.7 A
Average	20.8 C	26.0 BC	31.7 B	39.17 A	
Rooting (%)					
5	11.4 d	8.5 d	9.5 e	15.2 c	10.1 D
10	12.3 d	16.7 d	25.1 d	100 a	35.4 B
15	50.3 b	75.4 a	75.2 b	91.7 a	72.9 A
20	41.7 c	50.1 c	91.7 a	100 a	70.8 A
25	66.7 a	66.7 b	75.4 b	91.7 a	75.0 A
30	33.3 cd	48.2 c	66.7 c	75.2 b	56.3 B
Average	37.1 C	39.9 C	56.6 B	77.4 A	
Root No.					
5	1.2 c	1.2 c	1.3 c	1.1 c	1.1 C
10	1.4 c	1.1 c	1.1 c	4.2 a	1.5 C
15	3.7 a	4.2 a	3.3 ab	4.3 a	3.8 A
20	2.2 b	1.7 bc	4.1 a	4.7 a	3.1 AB
25	2.7 b	3.3 ab	2.7 b	3.1 b	2.9 B
30	2.3 b	2.3 b	2.7 b	2.7 b	2.5 B
Average	1.9 B	2.2 B	2.4 AB	3.2 A	
Root length (mm)					
5	1.3 c	1.1 d	1.3 c	2.1 d	1.5 C
10	1.2 c	4.3 c	1.7 c	16.2 b	5.6 B
15	5.3 ab	7.1 a	10.7 a	10.3 c	8.3 AB
20	5.7 a	3.7 cd	9.3 ab	27.3 a	11.5 A
25	6.2 a	5.3 bc	11.7 a	10.7 c	8.4 AB
30	4.7 b	5.7 b	8.1 b	3.3 d	5.3 B
Average	4.1 C	4.5 C	7.1 B	11.7 A	

The mean of parameters with same small letters were not significantly different as per Duncan's multi-range test at $P<0.05$

The total mean of the concentration with same capital letters were not significantly different.

Although, [13] demonstrated that rootless shoots could be successfully hardened and *ex vitro* acclimatized with the 80 mm plant height or longer, none of the rootless and even the rooted shoots (plantlets) were grown longer than 60 mm.

Be and Debergh [14] demonstrated that the electricity cost of incubation during multiplication and rooting stage of pineapple, could be entirely eliminated by outdoor incubation under lath house in tropical regions. Kodym et al. [15] reported that diverting of natural light into an enclosed room by using of tubular could substitute for artificial light in banana cultures. To lower the micropropagation cost of sugar cane [16] and chrysanthemum [17], table sugar was suggested as a cheaper sucrose alternative.

Enrichment of the solid medium with sucrose at 30 g/L and adjusting of the pH to 5.7 are often used in *in vitro* culture [18], and there is no particular supporting data for the using of pH value and sucrose concentration in each species. Soneji et al. [1] demonstrated that high root induction could be obtained in liquid filter paper enriched with sucrose at 20 g/L. However, this study compared their results with sucrose at 30 g/L and different pH values, which confirms the effective role of pH in pineapple growing. Rooting of pineapple was also reported in liquid medium, but the shoots were supported by a sponge matrix [8] and filter paper bridge [1], [18]. However, using of liquid medium with adjusting to specific pH value might be an even better method for reducing the concentration of sucrose and other alternatives. Also, [19] reported that the shoot size also affected all rooting parameters and taller shoots exhibited higher rooting percentage, root number and root length compared to shorter shoots in Paulownia. Konan et al. [20] reported the improvement of oil palm rooting by changing the coupling factors of shoot sizes.

TABLE II
MEAN COMPARISON OF SUCROSE AND PH CONCENTRATIONS FOR IN VITRO ROOTING OF PINEAPPLE

	pH	Sucrose (g/L)				
		10	20	30	40	Average
Plantlet height (mm)						
	5	33.1 b	36.2 b	51.1 a	32.1 b	40.5 A
	5.5	28.4 b	42.3 a	56.3 a	27.4 bc	38.25 AB
	6	30.2 b	43.1 a	27.2 c	42.1 a	35.5 B
	6.5	44.1 a	39.1 ab	37.1 b	48.2 a	42 A
	Average	33.75 B	42.5 A	42.75 A	37.25 AB	
Rooting (%)						
	5	34.2 c	89.1 a	89.1 a	44.3 bc	63.9 A
	5.5	22.4 d	77.7 b	88.5 a	53.7 b	61.5 A
	6	44.3 b	72.1 b	44.3 b	89.2 a	63.8 A
	6.5	78.1 a	35.3 c	34.2 c	41.8 c	47.3 B
	Average	44.7 C	69.4 A	63.9 AB	58.3 B	
Root No.						
	5	1.1 c	5.2 a	11.2 a	1.2 c	2.1 C
	5.5	1.3 bc	2.1 bc	7.2 b	4.3 b	2.5 BC
	6	2.1 b	2.3 b	1.5 c	6.1 a	5.2 A
	6.5	4.0 a	1.3 c	1.1 c	1.4 c	3.1 B
	Average	2.3 C	2.8 BC	5.4 A	3.7 B	
Root length (mm)						
	5	10.1 a	15.2 a	26.3 a	6.1 b	14.5 A
	5.5	3.2 c	14.1 a	24.2 a	7.2 b	12.3 AB
	6	7.3 b	11.3 b	6.2 b	14.3 a	9.5 B
	6.5	12.1 a	7.2 c	7.3 b	6.2 b	8.3 B
	Average	8.4 C	11.9 B	15.8 A	8.5 C	

The mean of parameter with same small letters were not significantly different as per Duncan's multi-range test at P<0.05
The total mean of the concentration with same capital letters were not significantly different.

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