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Effect of Explants Length and Density on *in Vitro* Rooting of Pineapple *Ananas Comosus* L Merr

Abdelhamid M. Hamad

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

***Correspondence:** Abdelhamid M. Hamad, Horticulture Department, Faculty of Agriculture, Omar Al-Mukhtar University, El-Baida, Libya, Email: abdelhamidhamad@gmail.com

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Abstract

Pineapple explants of different length (5, 10, 15, and 20 mm long) were cultured at different densities (one, two, three, four, and five explants per culture) on full strength, agar solidified MS medium supplemented with sucrose at 30 g/l and IBA at 2.0 mg/l for 60 days. The rooting ability (rooting %, root number, and length) of the explants were not affected by the explants' length and explants' densities per culture. Irrespective of length and densities, 82 % of the explants rooted and produced 4.1 roots each 17.7 mm long. Moreover, overall explants lengths, plantlet height was not affected by explants density and were about 50.5 mm tall. On contrary, overall density, explant of different lengths developed into plantlets of significantly different height. Plantlets' height increased from 39.2 to 49.6; 55.9 and 57.4 mm as the explants length increased from 5 to 10; 15 and 20 mm respectively.

Keywords: Pineapple; *Ananas comosus;* Explants length; Explants density

Introduction

Previous studies showed that at each *in vitro* multiplication cycle of pineapple, shoots of various lengths ranged from 5 to 30 mm long were produced. The frequency of different shoot length varied depending on which length and density of explants were used for multiplication [1], hormone treatments [2], medium types [3], tissue culture system and incubation periods [4,5] and number of subcultures [6,7]. After four [7], five [8], six [9,10] and eight [11] cycles of multiplication hundreds of thousands is expected to be produced and 60 % of these shoots is expected to be shorter than 15 mm-long [1]. However, in all of the reported *in vitro* rooting results [9,12-16] selected particular shoots of equal or longer than 20 mm were used at fixed explants density per culture for testing of *in vitro* rooting stage. Commercialablity of the system cannot be achieved unless all shoots produced during multiplication stage irrespective of their length

could be *in vitro* rooted or elongated and survived the following acclimatization stage. The objective of this study is to test the effect of shoot length shorter than 20 mm (5,10,15 and 20 mm) at different explants density (one, two, three, four and five shoots per culture) on the shoots *in vitro* rooting and growth capability. Investigation of the shoot length and density effect on rooting response and plantlet height is an area that could combine the goal of elongation and rooting in one stage.

Materials and Methods

Stock cultures of Moris pineapples that were subcultured every 75 days on MS medium [17] enriched with sucrose at 30 g/l and BAP at 2.0 mg/l were used as a source of shoots. Multiple shoot clusters were removed out of culture tubes under airflow laminar, separated into individual shots, and arranged according to length into groups of 5, 10, 15, and 20 mm long shoots in a sterilized petri dish. Shoots of equal length were cultured at a density of one, two, three, four, and five shoots per culture tube containing 10 ml of agar solidified (7 g/l)-full strength MS medium enriched with IBA at 2.0 mg/l, sucrose at 30 g / l. and pH adjusted to 5.7. Each combination of shoot length and density was represented by 3 culture tubes. Cultures incubated under a photoperiod of 16 hours of light and constant temperature at 250 C. After 60 days, the shoots removed out of the cultures for counting root numbers, measuring roots length and plantlets height, and calculating the rooting percentage. The data converted to average per explant by dividing by the explants density. Analysis of variance and mean separation were made using SPSS statistical package No. 11.

Results

Analysis of variance (Table, 1) indicated that both explant densities and explant length had no significant direct (independent) or indirect (dependent) effect via interaction with each other in rooting percentage, root number, and length. Average overall combinations of explants length and density showed that about 82 % of the combinations developed 4 roots each about 17.7 mm long (Table, 2). However, while overall explants length, the plantlets height were not significantly affected by explant densities with an average of 50.5 mm tall, overall explants density, there was a significant increase in plantlets height from 39.2 to 49.6; 55.9 and 57.4 mm as the explants length increased from 5 to 10; 15 and 20 mm long (Table, 2). The tallest plantlets (53.33 mm) developed from the 5 mm explants obtained at a density of four while the tallest plantlets (76 mm) from the 15 mm long explants obtained at a density of three explants per culture. The tallest plantlet in the case of 10 and 20 mm long explants were 57.7 and 61.7 mm obtained at a density of one and two explants per culture respectively. According to plantlet height, the 20 different combinations could be separated into three significantly different groups. Combinations that resulted in the tallest plantlets (76 mm) were15 mm long explant and density of three. Combinations that resulted in shortest plantlets about 35 mm tall (27 to 41 mm) were 5 mm long explant cultured at a density of one, two, and five explants per culture; 10 mm long explants cultured at a density of two and 15 mm long explants cultured at a density of four explants per culture. The rest of the other combinations resulted in an intermediate plantlet height of about 53.0 mm tall (45 to 61 mm). The difference in plantlet height was related in particular to the 5 and 15 mm long explant more than the density. The height of the plantlets which developed from 5 and 15 mm long explants when cultured at a density of one was 34.7 and 48.3 mm and respectively increased to 45.3 and 76 mm when cultured at a density of three explants per culture. However, while the height of the plantlet developed from the 5 mm long explant declined to 35 mm when density increased to five explants, the height of the plantlets developed from the 15 mm long explants declined to 41.7 mm when density increased to four but increased to 60 mm when density increased to five explants per culture. At a density of one and five explants per culture, the height of the plantlets developed from the 10, 15, and 20 mm long explants was not significantly different (about 53.7 mm tall), but was 1.5 times

taller than the plantlets developed from the 5 mm long explants (35 mm tall). At a density of two explants per culture, the 5 and 10 mm long explants resulted in shorter plantlets (about 32.5 mm tall) than that from the 15 and 20 mm long explants (57.7 mm tall). At the density of three and four, there was no significant difference between the height of the plantlets that developed from the 5, 10, and 20 mm long explants (about 53.4 mm tall). The height of the plantlets which developed from the 20 mm long explants was not significantly affected by explants density per culture with an average of about 57.4 mm tall (45.3 to 61.7 mm).

Table 1: Significant of main and interaction effect of explants length and density on *in vitro* rooting of Moris pineapple.

Factors	**df**	**Rooting parameters (**$p \leq 0.05$)			
		Rooting %	Root No	Root length	Plantlet height
Explants densities	4	0.9863	0.9611	0.1328	0.4137
Explants length	3	0.8539	0.6402	0.9826	**0.**0246*****
Density*Length	12	0.4876	0.8419	0.7856	0.5195
Error	40				
Total	60				

Table 2: Effect of explant length and density on the in vitro rooting of pineapple.

Explants	**Explants length (mm)**				
Density	5	10	15	20	Average
Plantlet height (mm)					
1	34.67 b	57.67 ab	48.33 ab	58.67 ab	49.83 NS
2	27.67 b	37.33 b	53.67 ab	61.67 ab	45.08 NS
3	45.33 ab	51.67 ab	76.00 a	61.00 ab	58.5 NS
4	53.33 ab	49.00 ab	41.67 b	60.33 ab	51.08 NS
5	35.00 b	52.33 ab	60.00 ab	45.33 ab	48.17 NS
Average	39.20 B	49.60 AB	55.90 A	57.40 A	50.50
Rooting %					
1	100	66.7	66.7	100	83.33 NS
2	66.7	100	83.3	100	87.50 NS
3	77.7	100	77.7	66.7	80.50 NS
4	91.7	91.7	75.0	50	77.08 NS
5	86.7	86.7	93.3	60	81.66 NS
Average	84.53 NS	89.0 NS	79.2 NS	75.33 NS	82.00
Roots No					
1	3.3	2.7	4.0	5.3	3.83 NS
2	3.0	3.3	4.0	5.0	3.83 NS
3	4.3	4.3	6.3	3.3	4.58 NS
4	3.0	4.7	4.3	4.7	4.17 NS
5	3.3	5.0	6.0	2.3	4.17 NS
Average	3.4 NS	4.0 NS	4.93 NS	4.13 NS	4.1
Root length (mm)					
1	9.3	12.7	9.7	14.0	11.4 B
2	8.3	12.3	19.7	19.0	14.8 AB
3	20	15.0	23.7	17.3	19.0 AB
4	29	19.3	18.3	31.3	24.5 A
5	16	28.0	22.3	9.3	18.9 AB
Average	16.53 NS	17.47 NS	18.73NS	18.2 NS	17.7

The number represents the mean of nine explants per treatment. Means of the same parameters followed by same small letters and overall averages followed by same capital letters were not significantly different at $p \leq 0.05$ as tested by Duncan Multiple Range Test., NS = not significant.

Discussion

In some of the reported *in vitro* rooting of pineapple, treatments were some time assessed by one parameter, rooting percentage [9], root number [18]. In some cases two parameters, root number and length [19-21] and in other cases three parameters: rooting percentage, root number and length [15,22], were used for assessment of rooting treatments. This study showed that none of these parameters indicated any significant different among the 20 combinations of different explant length and density (Table, 1). After 60 days of incubation, about 82 % of the explants, irrespective of explants length and density, formed 4 roots each 17.7 mm long. Assessment of *in vitro* rooting treatments by their effect on plantlets height was rarely reported [12,16]. However, plantlet height was the only parameter that separated the 20 combinations of explant length and density into three significantly different groups. The average plantlets height of the first group was 76 mm while height of the second and third group was 53 and 35 mm respectively. Significant interaction between explant length and density was not detected, yet presence of more than one explants in one culture resulted in significant different plantlet height. Such different was related in particular to the 5 and 15 mm long explant more than the density. At density of three shoots per culture, the rooting of oil palm was under influence of coupling factors of shoot sizes [23]. That is rooting of one shoot of oil palm effected the rooting of the others and the best rooting obtained when the shoots in a single culture were of different sizes. If all of the three shoots were of small size, the shoots failed to root. However, if the culture contained one small plus two of long or intermediate shoots, all shoots rooted and developed into longer plantlets. In this study no mixing of explants of different length was tested. However, while different density induced no significant effect in any of the rooting parameters, different explant length induced significant different in plantlets height. The coupling effect in case of pineapple may not be crucial for root induction but for plantlet growth. The coupling effect between density and length of explants of pineapple on plantlet height may not merely competition for nutrient but through release of component that could alleviate or compensate the competition for the nutrient.

Rooting stage is carried mainly to improve plantlet survival of the subsequent acclimatization stage. However, the survival are usually assessed in relation to the effect of types and mixing ratio of rooting substrates used during acclimatization stage [24,25] rather than relation between survival and rooting parameters. Nevertheless, *in vitro* propagation studies of pineapple indicated that about 95 % of the plantlets which were taller than 30 mm [2], 50 [3], 70 mm [26] and 80 mm [12] survived acclimatization. Moreover, it was reported that none of the shoots shorter than 30 mm survived and only 40, 60 and 70 % of the 50, 60 and 70 mm long shoots could survive direct transfer to *ex vitro* acclimatization respectively while all shoots (100 %) longer than 80 mm survived [4]. Elongation stage with different procedures [4,10,27,28] was suggested to facilitate separation of shoot, enhance growth, increase shoot length and to improve *ex vitro* survival and acclimatization. These imply that plantlet height could be better indicator than root number and length for expectation of possible survival of acclimatization. The results of this study (Table, 2) showed that explants length within a range of 5 to 20 when cultured at density of five explants would result after 60 days of incubation in simple rooting medium in plantlet taller than 35 mm and up to 60 mm. A plantlet height within the range reported with high survival of acclimatization. Cost of rooting stage [16] was expected to be three times cost of multiplication stage [29,30]. Using of five explants per culture, in addition to maintain plantlets height taller than 35 mm with 4 roots, it minimize cost of rooting per plantlet in term of amount of medium, number of vessel and shelving space. To minimize cost of rooting stage further studies is needed to determine the maximum possible explants density not only in relation to explant

length but also in relation to lowest medium strength, smallest medium volume per culture, shortest photoperiod and lowest light intensity that would result in plantlets taller than the minimum height required for acclimatization survival in shortest incubation period. Any little reduction of cost of rooting stage in particular that accompanied with increase in plantlet height is very essential for reduction of total cost of micro-propagation and improving of acclimatization survival and worth being tried.

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