



Effect of Sucrose Concentrations and Incubation Periods on *in Vitro* Rooting of Moris Pineapple (*Ananas comosus*)

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Abstract: The effect of 6 sucrose concentrations (5, 10, 15, 20, 25, 30 g/l) over 4 incubation periods (30, 45, 60, 75 days) on *in vitro* rooting of Moris pineapple cultured in liquid half strength MS medium enriched with IBA at 2.0 mg/l was investigated. At all incubation periods, all shoots in medium enriched with sucrose at 5 g/l failed to root, and no roots formed within the first 30 days in medium enriched with sucrose at 10 g/l. After 30 days of incubation, the highest rooting percentage (66 %), tallest plantlets (23 mm tall), highest (3.4 roots) and longest (5.3 mm) root per shoot were obtained in medium enriched with sucrose at 25, 10, 15, 15 g/l respectively, while after 45 days, the highest of all rooting aspects (75 %, 32.3 mm tall, 3.7 roots, 7 mm long), were obtained in medium enriched with sucrose at 15 g/l. After 60 days, the highest rooting percentage (91.7 %) and tallest plantlets (36.7 mm tall) were obtained in medium enriched with sucrose at 20 g/l while highest roots per shoot (3.7 roots) and longest root (10.7 mm) were obtained in medium enriched with sucrose at 15 g/l. After 75 days, all shoots rooted (100 %) in medium enriched with sucrose at 10 and 20 g/l, while sucrose at 25 g/l resulted in tallest plantlets (46.3 mm tall) and at 20 g/l resulted in highest (4.7 roots) and longest roots (27.3 mm). At each incubation period, there was a different optimum sucrose enrichment for different rooting parameters.

Key words: Sucrose; Incubation Periods; *In Vitro* Rooting; Moris Pineapple; *Ananas comosus*.

INTRODUCTION

Different combinations of sucrose and incubation periods were used for the *in vitro* rooting of pineapple. *In vitro* rooting was reported using sucrose at 10 g/l and incubation for 30 days and 70 days (Soneji et al., 2002b), sucrose at 20 g/l for 30 days (Ko et al., 2006; Mengesha et al., 2013; Souza et al., 2016; Sunitibala Devi et al., 1997) and 70 days (Soneji et al., 2002b), sucrose at 30 g/l for 30 days (Almeida et al., 2002; Aydieh et al., 2000; Bhatia & Ashwath, 2000; Fitchet, 1990; Singh & Manual, 2000; Teng, 1997), 45 (Khan et al., 2004; Sripaoraya et al., 2003), 60 (Gangopadhyay et al., 2005; Hamad et al., 2013; Mathews & Rangan, 1979, 1981) and 75 days (Hamad & Taha, 2008) and sucrose at 35 g/l for 30 days (Kofi & Adachi,

1993) and sucrose at 40 g/l for 60 days of incubation (De Almeida et al., 1997). In all of these studies, each of the sucrose and incubation periods was fixed at one level and the focus was on hormone types and concentrations and medium strength. The effect of different sucrose concentrations and incubation periods were neither compared individually nor in combinations of the two factors. In addition, many times the results were reported as a general statement or the rooting response was assessed based only on one parameter, rooting percentage (Bhatia & Ashwath, 2000), root number (Danso et al., 2008). In some cases, two parameters, root number and length (Aydieh et al., 2000; Khatun et al., 1997) and in few cases, three parameters: rooting percent-

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age, root number, and length (Amin et al., 2005; Soneji et al., 2002a), were used for the assessment of rooting treatments. The plantlets' height, on the other hand, was rarely reported (Be & Debergh, 2006; Hamad et al., 2013; Souza et al., 2016). Sucrose and incubation period are not only important for its effect on rooting, but also for their effect on cost and management of the rooting stage and plantlets survival of acclimatization. Sucrose made the highest proportion of the medium components. And for the incubation period, lamps and air conditioning is required to maintain constant temperature and photoperiod. In large scale propagation, using higher sucrose and longer incubation would make the medium cost and electricity bill too expensive. Using the lowest possible concentration of sucrose and the shortest incubation period would reduce the cost of rooting stage and the overall cost of propagules production. The objective of this study is to compare the effect of different sucrose concentrations over different incubation periods on all rooting aspects of Moris pineapple cultivar. And to determine the best compromise of lowest possible concentration and shortest incubation period at which the best rooting responses, particularly the plantlets' height, could be obtained. The goal is to minimize the cost in terms of the sucrose amount and monthly electricity bill per single propagule.

MATERIALS AND METHODS

A half-strength MS medium (450 ml) enriched with IBA at 2.0 mg/l (Murashige and Skoog, 1962) was prepared and divided into 6 glass jars (75 ml each) marked 1 to 6. Sucrose at 5, 10, 15, 20, 25 and 30 g/l were added to each glass jar respectively. The medium was adjusted to pH 5.0 and autoclaved at 121⁰ C and 1.5 kg/ cm² for 20 minutes. The content of each glass jar was dispensed into 12 culture tubes (6 ml each) under a laminar cabinet using a sterilized syringe and the tubes marked with the same glass jar mark. Shoots from Moris pineapple stock cultures were cultured at a density of five shoots per culture. The cultures were incubated under constant temperature (25⁰ C) and 16 hours of

light provided by fluorescent lamps for 30, 45, 60 and 75 days. After 30 days of incubation, three culture tubes of each sucrose concentration were taken for data collection. The shoots were picked out to count the number of rooted shoots, number of roots per shoot, and to measure the roots length and plantlet height. A table for each rooting parameter of the different sucrose concentrations with three replicates was established and named the 30 days incubation tables. After 45, 60 and 75 days, three culture tubes of each sucrose concentration were taken out of the culture room, and a table for each rooting parameter of the different sucrose concentrations at each incubation period was established as were done after 30 days incubation. The tables of the same parameter obtained after different incubation periods (30, 45, 60 and 75 days) were combined in one table containing all combinations of sucrose and incubation periods and used for the analysis of variance and testing the significance of means by Duncan Multiple Range Test at $p \leq 0.05$ using SPSS statistical package No.11.

RESULTS

Two-way analysis of variance (Table 1) showed that both of the sucrose and incubation periods had a significant effect on all *in vitro* rooting aspects of pineapple. However, the sucrose seemed to have a higher influence on rooting percentage, root number, and length than the incubation period, while incubation periods affected plantlets' height more than the sucrose. The two factors exerted their influence independently from each other except in root length, where a significant interaction was detected at $p < 0.055$. Overall sucrose concentrations, incubation for 30 and 45 days, had statistically an equal effect in root formation (2 roots), elongation (4 mm) and plantlets height (24 mm) but the rooting percentage increased from 32 to 43.1 % as the incubation increased from 30 to 45 days (Table 2). Incubation for 60 days resulted in intermediate responses where 55.6 % of the shoots rooted, forming 2.5 roots/shoots, 6.7 mm long roots, and the plantlets reached 31.7 mm in

height. Incubation for 75 days, on the other hand, (76.4%), more roots per shoot (3.1 roots), longest roots (12.1mm long), and tallest plantlets (39.17 mm). Similarly, across all incubation periods, none of the shoots cultured on MS enriched with sucrose at 5 g/l produced roots, and the plantlets were the shortest (20.1 mm tall) of all sucrose concentrations. No significant difference between the plantlets' height in media enriched with sucrose at 10, 15, 20, 25, and 30 g/l (31.3 mm on average). In medium enriched with sucrose at 10 g/l, 35.4 % of the shoots rooted, forming 1.5 roots per shoot each 5.5 mm long, and the plantlets were 30.1 mm tall. No significant difference in rooting percentage in media enriched with sucrose at 15, 20, 25, and 30 g/l (68.75 % on average). Medium enriched with sucrose at 15 g/l resulted in highest root formation (3.8 roots) and medium enriched with sucrose at 10 g/l resulted in fewest roots (1.5 roots), while intermediate root formation (3 roots) was obtained in a media enriched with sucrose at 20 and 25 g/l. Medium enriched with sucrose at 20 g/l resulted in longest roots (11.5 mm) and those enriched with sucrose at 10 and 30 g/l resulted in shortest roots (5.4 mm), while intermediate root length (8.4 mm) obtained in media enriched with sucrose at 15 and 25 g/l.

Under 30 days of incubation, none of the shoots rooted in medium enriched with sucrose at 5 and 10 g/l and the plantlets were the shortest (15.7

mm) compared to plantlets rooted in the other sucrose concentrations (22.3 mm). The highest rooting percentage (67%) was obtained in medium enriched with sucrose at 25 g/l while the highest roots per shoot (3.4 roots) and longest roots (5.3 mm) were obtained in medium enriched with sucrose at 15 g/l. For 30 days incubation, the best sucrose enrichment was 25 g/l according to rooting percentage and 15 g/l according to the other parameters (root per shoot, root length, and plantlet height). Under 45 days of incubation also none of the shoots rooted in medium enriched with 5 g/l. The highest rooting percentage (75%), roots per shoot (4 roots) and longest roots (7 mm) were obtained in medium-enriched with sucrose at 15 g/l and the tallest plantlets (32.3 mm) were obtained in medium enriched with sucrose at 20 and 15 g/l while medium enriched with sucrose at 5 g/l resulted in the shortest plantlets (19 mm) and a medium enriched with sucrose at 10 g/l resulted in the lowest rooting percentage (16.7 %), fewest roots per shoot (1 root) and shortest roots (1.7 mm). For 45 days incubation, the best sucrose enrichment was 15 g/l according to all parameters. Under 60 days of incubation, none of the shoots rooted in medium enriched with 5 g/l. The highest rooting percentage (91.7%), roots per shoot (4 roots), and tallest plantlets (36.7 mm) were obtained in medium enriched with 20 g/l and the longest roots (11.7 mm) were obtained in medium enriched with sucrose at 25 g/l.

Table (1). Significant of main and interaction effect of incubation periods and sucrose concentrations on *in vitro* rooting response of Moris pineapple cultures on a liquid half-strength medium enriched with IBA at 2.0 mg/l.

Factors	df	Rooting parameters			
		Plantlet height	Rooting %	Root No.	Root length
Incubation periods	3	6.4E-08**	9.8E-06**	0.0500*	0.0002**
Sucrose	5	0.0015**	2.3E-10**	7.3E-07**	7.5E-05**
Incubation*Sucrose	15	0.8063	0.0879	0.4769	0.0054**
Error	48				
Total	72				

Table (2). Effect of incubation periods and sucrose concentrations on *in vitro* rooting of Moris pineapple cultured on liquid half-strength MS medium enriched with IBA at 2.0 mg/l

Incubation periods (Days)	Sucrose concentrations (g/l)						Average
	5	10	15	20	25	30	
Plantlet height (mm)							
30	15.7 h	23.0 defgh	21.7 defgh	22.3 defgh	20.3 efgh	22.0 defgh	20.8 C
45	19.0 gh	28.0 cdefgh	32.3 abcdefg	31.3 abcdefg	19.7 fgh	25.7 defgh	26.0 C
60	21.3 defgh	34.0 abcdefg	36.0 abcde	36.7 abcd	29.7 bcdefgh	32.7 abcdefg	31.7 B
75	24.3 defgh	35.3 abcdef	43.0 abc	43.7 ab	46.3 a	42.3 abc	39.17 A
Average	20.1 B	30.1 A	33.3 A	33.5 A	29.0 A	30.7 A	
Rooting %							
30	0.0 f	0.0 f	50 bcde	41.7 cdef	66.7 abcd	33.3 cdef	32.0 D
45	0.0 f	16.7 ef	75 abc	50 bcde	66.7 abcd	50 bcde	43.1 C
60	0.0 f	25 def	75 abc	91.7 ab	75 abc	66.7 abcd	55.6 B
75	0.0 f	100 a	91.7 ab	100 a	91.7 ab	75 abc	76.4 A
Average	0.0 C	35.4 B	72.9 A	70.8 A	75.0 A	56.3 A	
Root No.							
30	0.0 d	0.0 d	3.4 abc	1.7 bcd	2.7 abcd	2.3 abcd	1.7 B
45	0.0 d	1 cd	3.7 abc	2.0 abcd	2.7 abcd	2.3 abcd	2.0 B
60	0.0 d	1 cd	4.0 ab	4 ab	3.3 abc	2.7 abcd	2.5 AB
75	0.0 d	4 ab	4.3 ab	4.7 a	3 abc	2.7 abcd	3.1 A
Average	0.0 D	1.5 C	3.8 A	3.1 AB	2.9 AB	2.5 BC	
Root length (mm)							
30	0.0 d	0.0 d	5.3 cd	3.7 cd	6 cd	3.0 cd	3.0 B
45	0.0 d	1.7 cd	7 bcd	5.7 cd	5.3 cd	5.7 cd	4.2 B
60	0.0 d	4.3 cd	10.7 bc	9.3 bcd	11.7 bc	4.7 cd	6.8 B
75	0.0 d	16 b	10.3 bcd	27.3 a	10.7 bc	8.0 bcd	12.1 A
Average	0.0 C	5.5 B	8.3 AB	11.5 A	8.4 AB	5.3 B	

Data were means of 15 shoots cultured at a density of 5 shoots per culture tube containing 6 ml of liquid, half-strength MS medium enriched with IBA at 2.0 mg/l.

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

In summary, no rooting was obtained in medium enriched with 5 g/l no matter how long the shoots were incubated. None of the shoots in medium enriched with 10 g/l produced roots during the first 30 days of incubation, and only a few shoots rooted after 45 and 60 days. However, all shoots rooted if the incubation extended to 75 days. The lowest root formation (1 root per shoot) and root elongation (1.7 mm long) obtained in medium enriched with sucrose at 10 g/l and incubated for 45 and 60 days and the shortest plantlets (15.7 mm) obtained in medium enriched with sucrose at 5 and 10 g/l for 30 days. The highest rooting percentage (100 %), the highest root formation (4.7 roots/shoot), root elongation (27.3 mm) and tallest plantlets (46.3

mm) were all obtained after 75 days incubation but at different sucrose enrichment, 10, 20, 20 and 25 g/l respectively.

DISCUSSION

Enrichment of medium with sucrose at 30 g/l and incubation for 30 days which is the most common practice for *in vitro* rooting was not the proper treatment for Moris pineapple. It resulted in the rooting of only 33 % of the shoots, and the plantlets were shorter than 25 mm. At any fixed incubation period sucrose at 15, 20 and 25 g/l were a better choice for rooting than sucrose at 30 g/l, and at any fixed sucrose content any incubation periods resulted in a better rooting response than the 30-day long incubation (Table

2). Out of 24 combinations of sucrose concentrations and incubation periods, sucrose at 30 g/l and incubation for 30 days resulted in lower a rooting percentage than 15 of the combinations, shorter plantlets than 13, fewer roots than 8, and shorter roots than 9 of the tested combinations. Incubation for 75 days was the best incubation period for all rooting parameters. However, each parameter had different optimal sucrose concentration. The tallest plantlets (46.3 mm) were obtained in medium enriched with sucrose at 25 g/l, the highest (4.7 roots) and the longest (27.3 mm) roots were obtained in medium enriched with sucrose at 20 g/l, while the highest rooting percentage (100 %) was obtained in medium enriched with sucrose at 10 and 20 g/l. Not only at each incubation period different rooting parameters had different optimum sucrose, but also the same rooting parameter had different optimum sucrose at different incubation periods. The best sucrose concentration for the highest rooting percentage of shoots incubated for 30, 45, 60, and 75 days was 25, 15, 20, and 10 g/l respectively. On the contrary, the best sucrose concentration for plantlet height of the shoot incubated for 30, 45, 60 and 75 days was 10, 15, 20, and 25g/l respectively. The optimum sucrose concentration for root formation (root number) and root elongation (root length) if shoots were incubated for 30, 45, and 60 days was 15 g/l, and if shoots were incubated for 75 days was 20 g/l.

Since different rooting parameters have different optimum combinations of sucrose and incubation periods, different combinations could be recommended depending on whether one, two, or all of the parameters were used for assessment. For obtaining over 90 % rooting and roots longer than 10 mm, the choice would be between sucrose at 10 g/l and incubation for 75 days and sucrose at 15 g/l and incubation for 60 days. For the formation of more than three roots per shoot, the choice is sucrose at 10 g/l and incubation for 75 days and sucrose at 15 and incubation for 30 days. Plantlets obtained after 45 days of incubation in medium enriched with sucrose at 15 and 20 g/l and after 60 and 75 days of incubation irrespective of sucrose enrichment

were taller than 35 mm. Sucrose at 15 g/l and incubation for 45 days and sucrose at 10 g/l and incubation for 60 days was the best compromise for lowest sucrose and shortest incubation in which plantlets were taller than 35 mm. The combination of sucrose and incubation for lowest cost of rooting (Table, 3) appears to be one of the following: the low sucrose level of 10 g/l but longer incubation of 75 days, intermediate sucrose of 15 g/l and shortest incubation of 45 days and high sucrose of 20 g/l and intermediate incubation of 60 days. Low sucrose reduces the sucrose cost, but long incubation increases the monthly electricity bill. Selection among these alternatives would depend on the cost of each added or saved 5 g/l of sucrose and the electricity bill of each increase or decrease of 15 days of incubation according to the price prevailed in the district at which the propagation takes place. The calculation of cost (Table, 3) showed that the cost of rooting was mainly due to incubation periods more than the concentration of sucrose. At a density of 5 shoots per culture, the minimum rooting cost per shoot was 3.95 cents, obtained when the shortest incubation (30 days) and lowest sucrose (5 g/l) was applied while the highest cost was 5.88 cents when the longest incubation (75 days) and highest sucrose (30 g/l) was applied. At any incubation period, the difference in rooting cost per shoot at different sucrose concentrations was negligible (less than 0.02 cents), while incubation of 30 days decreased the cost by 2 cents compared to 75 days of incubation. In a previous report, we estimated the multiplication cost per shoot after 4 cycles of multiplication to be 0.9 (Hamad, 2017a) and if one liter of medium was used, to be 1.6 (Hamad, 2017b) cents. This study showed that the rooting cost per shoot (rooting stage) ranges from 3.95 to 5.88 cents, which is 2 to 6 times the multiplication cost per shoot (multiplication stage). Hence, to efficiently minimize the cost of micropropagation, the effort should be concentrated on reducing the cost of rooting rather than the cost of multiplication. Otherwise, the complaints about the high cost of micropropagated materials would always be there even if the

multiplication was fully automated and at the highest rate.

Table (3). Effect of incubation periods and sucrose concentrations on the rooting cost per shoot of Moris pineapple

Incubation periods (Days)	Sucrose concentration (g/l)						Average
	5	10	15	20	25	30	
	Rooting cost per shoot (cents)						
30	3.95	3.97	3.98	4.00	4.02	4.03	3.99
45	4.57	4.58	4.60	4.62	4.63	4.65	4.61
60	5.18	5.20	5.21	5.23	5.25	5.26	5.22
75	5.80	5.81	5.83	5.85	5.86	5.88	5.84
Average	4.87	4.89	4.91	4.92	4.94	4.96	4.92

Mediums were prepared in glass jars, autoclaved at 121°C and 1.5 kg/cm² for 20 minutes and dispensed into culture tubes under a laminar cabinet. Shoots were rooted at a density of 5 shoots per culture tubes containing 6 ml of liquid half-strength MS medium enriched with IBA at 2.0 mg/l. and incubated under constant temperature (25°C and 16 hours of light provided by cool white fluorescent lamps.

The selection of the best combination of incubation and sucrose should depend on whether the purpose of the experiment would be the reduction of propagation cost and obtaining plantlets that could survive acclimatization or physiological study of rooting. Minimum sucrose and shorter incubation which result in plantlet quality above that required for highest acclimatization survival are very important for low-cost micropropagation, while the determination of the time at which rooting takes place, and monitoring of increase in root number and length, and plantlet height over time, are important for the physiological study of roots and proper timing of histological and physiobiochemical analysis. Published studies did not elaborate on the relationship between rooting percentage, root per shoot and root length, and plantlet survival. It is not known which one or two of these parameters are very crucial for the survival of acclimatization. (Escalona et al., 1999) reported that the survival percentage of *ex vitro* acclimatized rootless shoots increased from 20 to 100 % as the size of the shoots increased from 20 to 80 mm long. (Be & Debergh, 2006; Dal Vesco et al., 2001; DeWald et al., 1988; Ko et al., 2006; Soneji et al., 2002a) respectively reported that over 90 % of 35, 50, 60, 70 and 80 mm long-rooted pineapple shoots survived the acclimatization stage. For pineapple, plantlet height is probably more crucial for acclimatization survival. Therefore, the selection should be for a combination of sucrose and an incubation period that re-

sults in plantlets taller than the minimum required height (35 mm) for survival at the lowest cost. If plantlets taller than 35 mm are suitable for successful acclimatization, there will be two combinations of sucrose and incubation period (sucrose at 15 g/l and 60 days and sucrose at 15 g/l and 75 days) to choose from.

But if the plantlet has to be taller than 40 mm, sucrose at 15 g/l and incubation for 75 days is the cheapest combination. In this study, all of the plantlets obtained after each incubation, irrespective of sucrose enrichments and its rooting response, were used for acclimatization. Following Hamad, (2016) recommendation, plantlets were placed in pots full of sand under a polyethylene enclosure for 21 days and a shade house for 60 days. The survival of plantlets that were rooted for 30, 45, 60 and 75 days was 70.0; 80.5; 84.5, and 94.3 % respectively. Some of the plantlets of the 30 days incubation were rootless, and some were with fewer and shorter roots and the plantlets were less than 35 mm in height. The low survival of these plantlets (70 %) may be due to one or more of these rooting parameters. If the survival of acclimatization is not of major interest, as in the case of physiological study of rooting, different sucrose enrichment has to be used at different incubation periods depending on which parameter was of major interest. After 45 days incubation, sucrose at 15 g/l, and after 60 days of incubation, sucrose at 20 g/l was the best enrichment for obtaining taller plantlets, highest rooting percentage and

more and longest roots while after 30 and 75 days of incubation, three sucrose enrichments could be used depending on the parameter of major interest. In the case of 30 days incubation, sucrose at 10 g/l for tallest plantlet, sucrose at 25 g/l for highest rooting percentage, sucrose at 15 g/l for more and longer roots, while in the case of 75 days incubation, sucrose at 25 g/l for tallest plantlets, sucrose at 10 g/l for highest rooting percentage and sucrose at 20 g/l for more and longer roots.

The optimum sucrose for rooting percentage under 30, 45, 60, and 75 days of incubation was 25, 15, 20 and 10 g/l (Table, 2). On the contrary, the optimum sucrose for plantlet height under 30, 45, 60, and 75 days of incubation was 10, 15, 20, and 25 g/l. This means that high sucrose enrichments improved shoot rooting (rooting percentage) under a short incubation regime but retarded the shoot growth (plantlet height). While under a long incubation regime they retarded shoot rooting but improved shoot growth. This indicated that the two processes of root initiation and plantlet elongation did not occur simultaneously, but one of them followed the other. Under low sucrose enrichment, the shoot directed the sucrose use for shoot growth and then for root initiation, and under high sucrose enrichment, the shoot directed the sucrose use first for root initiation and then for shoot growth. Both of the two parameters increased as the incubation periods increase up to 75 days, but the time at which most shoots rooted and the highest increase in plantlet height occurred varied over each 15 day period among the different combinations of incubation period and sucrose concentration. Being that no rooting occurred in medium enriched with 5 g/l even if the incubation period increased to 75 days while the plantlet height reached 24 mm tall indicated that initiation of the rooting process in the presence of 5 g/l requires shoots longer than 24 mm. Failure of shoots to form roots for the first 30 days in medium enriched with sucrose at 10 g/l while 75 % of shoots form roots in the period between 60 and 75 days indicated that the shoot length limit for rooting in the presence of sucrose at 10 g/l is 35 mm long shoots. Noticing the peri-

od at which 50 % of the shoots rooted indicated that the required shoot length for rooting in medium enriched with 15, 20, and 20 g/l was 22 mm. Similarly, it can be noticed that the required shoot length for rooting of over 75 % of shoots in medium enriched with 10, 15, 20, 25, and 30 g/l was 35, 32, 37, 30, and 42 mm respectively. It seemed that the rooting started after the shoots attain a certain size, and the sucrose is not involved in rooting directly, but by enhancing the shoot to reach the size required for the shoot to gain root forming ability.

Sucrose was involved in controlling the time at which the root initiation occurred and also the pattern of root formation and elongation over time. In medium enriched with sucrose at 5 g/l, the shoots failed to root even if the incubation extended to 75 days. In medium enriched with sucrose at 10 g/l, no rooting occurred in the first 30 days and only 25 % rooted in the first 60 days of incubation, but all of the shoots rooted when the incubation extended to 75 days. That is, 75 % of rooting occurred in the last 15 days of incubation. Incubation shorter than 75 days will result in poor rooting. On the contrary, in medium enriched with sucrose at 15 g/l, 75 % of rooting occurred within the first 45 days of incubation, no rooting occurred in the period between 45 and 60 days and only 17 % rooted in the period between 60 and 75 days of incubation. Under this sucrose enrichment, incubation should be terminated after 45 days. In medium enriched with sucrose at 20 and 25 g/l, 92 % of shoots rooted within the first 60 days and only 8 % rooted in the period between 60 and 75 days of incubation. In medium enriched with sucrose at 30 g/l, 67 % of shoots rooted in 60 days, 8 % in the period between 60 and 75 days, and 25 % of the shoots failed to root even if the incubation extended to 75 days. That is if the medium is enriched with sucrose at 20, 25, and 30 g/l the incubation should be terminated after 60 days of incubation.

Similarly, depending on sucrose enrichment, the process of root formation (root number), and elongation (root length) ranged from did not start, stopped after 30, 45 or 60, days to be a continu-

ous process for 75 days. In medium enriched with sucrose at 10 g/l, no root formation occurred in the first month and most of the roots occurred in the last 15 days of the 75 days incubation (60 to 75 days). On the contrary, in medium enriched with sucrose at 30 g/l, shoot rooting occurred in the first month and no root formed after that even if the incubation extended to 75 days. In medium enriched with sucrose at 15 and 25 g/l, root formation occurred in alternative cycles. Root formation occurred in the first month of incubation and the period between 45 – 60 days, and no formation occurred in the period between 30- 45 and 60- 75 days of incubation. In medium enriched with sucrose at 20 g/l, root formation was a non-stop continuous process of alternative slow and fast root growth over 75 days of incubation. Similarly, root elongation was also a continuous non-stop process for 75 days in medium enriched with 10 and 20 g/l. However, in medium enriched with sucrose at 15, 25, and 30 g/l no root elongation occurred after 60 days of incubation. Daily and weekly records of the changes in rooting parameters would determine the time of fast, slow and no growth, and the best time for histological and biochemical analysis.

Although the weight and length of the shoots used during rooting were not taken into consideration, (Ramirez-Malagon et al., 2001) found that heavy shoots of *Spathiphyllum floribundum* obtained during multiplication had a higher chance of survival during acclimatization. Hamad (2017c) found that the period of the highest increase in shoot weight and length of Smooth cayenne pineapple over each 15 days of 105 days of incubation varied depending on the hormone treatments and occurred after the shoots incubated for more than 45 days. Weight, length, and density of shoots at the beginning of the rooting stage may have great influence in its rooting response to sucrose and incubation period and proposed for future testing. (Hamad et al., 2013) found that the different rooting parameters of Moris pineapple had different optimum hormone type and concentration and medium strength and the rooting percentage ranged from 30 to 100 %, root number from 1 to 10 roots, root length from

5 to 65 mm and plantlet height from 19 to 65 mm. For taller plantlets they recommended the use of IBA at 0.5 mg/l in quarter strength solid MS and IAA at 2.5 mg/l in full strength liquid MS. Quarter strength MS and IAA at 2.5 mg/l is suggested for future testing in combination with shoots of different length and density under different photoperiods at 5, 10 and 15 grams of sucrose per liter of medium over 30 and 45 days of incubation. Such testing may come up with a treatment that overcomes the no rooting in medium enriched with sucrose at 5 g/l and incubated for 30 days, or shorten the incubation in medium enriched with sucrose at 10 g/l from 75 to 30 days and would reduce the cost and result in plantlets with the quality required for survival of acclimatization.

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تأثير تركيزات السكرز وطول فترة التحضين على تجذير الأناناس صنف Moris (أناناس كوموسوس) مخبرياً

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المستخلص: اختبر تأثير ستة تركيزات من السكرز (5، 10، 15، 20، 25 و 30 جرام/لتر) وأربع فترات تحضين (30، 45، 60 و 75 يوماً) في تجذير فروع أناناس صنف Moris مخبرياً والمزروع في نصف قوة وسط موريسبيوز و سكوق MS المزود بهرمون IBA بتركيز 2.0 مل/لتر. في كل فترات التحضين الأربعة فإن الفريعات المزروعة في الوسط المزود بسكرز بتركيز 5 جرام/لتر فشلت في تكوين جذور. كذلك لم تتكون جذور في الوسط المزود بسكرز بتركيز 10 جرام/لتر خلال 30 يوماً من التحضين. بعد 30 يوماً من التحضين أعلى نسبة تجذير (66%) وأطول نبيتته (23 ملم) وأكثر عدد (3.4 جذر) وأطول (5.3 ملم) جذور تم الحصول عليها في وسط مزود بسكرز بتركيز 25، 10، 15، و 15 جرام/لتر على التوالي، بينما بعد 45 يوماً من التحضين فإن أعلى قيمة لأي وجه من أوجه التجذير الأربعة (75%، 32.3 ملم، 3.7 جذر، بطول 7 ملم) تم الحصول عليها في وسط مزود بسكرز بتركيز 15 جرام / لتر. بعد 60 يوماً أعلى نسبة تجذير (91.7%) وأطول نبيتته (36.7 ملم) تم الحصول عليهما في وسط مزود بسكرز بتركيز 20 جرام/لتر بينما أكثر عدد (3.7 جذر) وأطول (10.7 ملم) جذور تم الحصول عليها في وسط مزود بسكرز بتركيز 15 جرام/لتر. بعد 75 يوماً كل الفريعات أعطت جذوراً (100%) عندما كان الوسط مزوداً بسكرز بتركيز 10 و تركيز 20 جرام/لتر بينما تزويد الوسط بسكرز بتركيز 25 جرام/لتر أدى للحصول على أطول نبيتته (46.3 ملم) وسكرز بتركيز 20 جرام/لتر أدى إلى أكبر عدد (4.7 جذور) وأطول (27.3 ملم) جذور. عند كل طول فترة تحضين هناك تركيز مثالي مختلف من السكرز لكل وجه من أوجه التجذير المختلفة.

الكلمات المفتاحية: سكرز، فترة التحضين، التجذير مخبرياً، الأناناس، أناناس كوموسوس.