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Effect of pH, Sucrose Concentrations and Medium States on *in vitro* Rooting of Pineapple (*Ananas comosus* (L) Merr) cv Queen

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Abstract: The effect of medium states (liquid, semi solid, solid), pH (5.0, 5.5, 6.0, 6.5) and sucrose concentrations (10, 20, 30, 40 g/l) on *in vitro* rooting of pineapple cultured in full strength MS enriched with IBA at 0.5 mg/l were investigated. According to average overall sucrose concentrations, overall pH adjustments, and at each combination of equal sucrose and pH, liquid medium was always super than solid and semisolid. The tallest plantlets (66 to 71 mm) obtained in liquid medium enriched with sucrose at 10 and 20 g/l both adjusted to pH 6.0; sucrose at 20 g/l and adjusted to pH 6.5 and sucrose at 30 g/l and adjusted to pH 5.0. All of the above combinations except sucrose at 20 and pH 6.0 resulted in 100% rooting. Sucrose at 30 g/l and pH 5.0 resulted in two times more (11 roots per shoot) and three times longer roots (39 mm) than the other treatments (5 roots each about 14 mm long). Each rooting parameter had different optimum combinations of medium state, sucrose and pH adjustment. For any parameter, proper pH adjustment could reduce the optimal sucrose enrichment from 30 to 20 and even to 10 g/l. Hence, pH adjustment is suggested as an important approach for reduction of *in vitro* rooting medium cost.

Keywords: Medium state; pH; Sucrose; *In vitro* rooting; Tissue culture; *Ananas comosus*

INTRODUCTION

In vitro rooting of pineapple were reported in medium solidified with phytagel at 2.5 g/l (Ko et al., 2006), agar at 6 (Rahman et al., 2001), agar at 7 (De Almeida et al., 1997) and 8 g/l (Akbar et al., 2003), in liquid medium using filter paper bridged (Soneji et al., 2002), sponge matrix (Gangopadhyay et al., 2005), Luffa matrix (Dutta et al., 2013), in static liquid medium; (Be & Debergh, 2006; Hamad et al., 2013 ; Hamad, 2019) and *ex vitro* in potting mix (Escalona et al., 1999; Liu et al., 1989). The effect of both of liquid and solid medium at full and half strength enriched with different auxin types and concentrations on the *in vitro* rooting of pineapple were compared (Hamad et al., 2013). Several other factors such as different combination of hormone types, concen-trations and medium strength (Bhatia & Ashwath, 2000; Firoozabady & Gutterson, 2003), shoot ages and cultivars (Hamad et al., 2013) , mix of commercial sugar with sucrose (Dutta et al., 2013) were found to induce significant effect on the *in vitro* rooting responses of pineapple. Medium pH adjustment in all of these cases was kept fixed at pH 5.7, and sucrose enrichments, on the other hand, most of the cases kept fixed at 30 g/l. In some cases, sucrose at 20 (Ko et al., 2006; Sunitibala Devi et al., 1997) and 40 g/l (De Almeida et al., 1997) in solid medium and at 20 (Soneji et al., 2002) and 35 g/l \ (Kofi & Adachi, 1993) in liquid medium was used for rooting.

In few cases the sucrose effect on rooting was compared at 2 \ (Mengesha et al., 2021), 3 (Zaied, 2007) and 4 (Almobasher, 2016; Hassan et al., 2018) different concentrations in solid medium and at 2 (Be & Debergh, 2006) and 6 (Hamad, 2019) different concentrations in liquid medium. (Hassan et al., 2018; Zaied, 2007) reported that rooting occurred in solid MS medium with no sucrose

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enrichment but the best rooting was in medium enriched with sucrose at 20 g/l. On the contrary, (Mengesha et al., 2021) reported that no rooting could be obtained and shoots died in solid MS devoid of sucrose and (Almobasher, 2016) not only reported no rooting in solid MS medium devoid of sucrose but also in medium enriched with sucrose at 10 g/ l and both found best root formation and length in medium enriched with sucrose at 20 g/l. (Dutta et al., 2013) recommended mix of commercial sugar at 2% with sucrose at 1 % in liquid with luffa support and Be and (Be & Debergh, 2006) recommended sucrose enrichment of 30 g/l for liquid MS medium. Hamad (2019) found that no rooting could be obtained in liquid MS medium enriched with sucrose at 5 g/ l and the optimum concentration ranged from 10 to 30 g/ l depending on the rooting parameters used for evaluation and varied according to the length of incubation period. In all of these studies of pineapple *in vitro* rooting, the medium pH was fixed at 5.7. The effect of different combinations of sucrose, medium states and pH adjustments still yet to be tested.

Cost of rooting stage (Hamad, 2019) was expected to be three times cost of multiplication stage (Hamad, 2017a and b). In large scale production, the cost of medium during rooting could be decisive factor for feasibility of micropropagation. Sucrose and agar made the largest portion of the medium components and are very important items of the medium cost. Rooting could occur in solid and liquid medium. That is complete elimination of agar cost is possible. On contrary, sucrose is an obligatory requirement for *in vitro* rooting and indispensable component of the medium (Almobasher, 2016; Mengesha et al., 2021 ; Hamad, 2019). Reduction of sucrose cost could be done either by using of cheap sucrose alternatives (Dutta et al., 2013; Mengesha et al., 2021; Nelson et al., 2015) or by using the most possible minimum concentration. In fact, (Dutta et al., 2013) reported that compared to solid media enriched with sucrose at 30 g/l using of mix of commercial sugar with sucrose in liquid media with luffa matrix reduced the chemical cost of multiplication by 15 % and rooting cost by 62 %. Sucrose is just a one of several factors that could have independent and interaction effect on rooting (Hamad, et al 2013; Hamad, 2019). Minimum sucrose concentration that would maintain highest response for each rooting parameter is expected to be different at different combinations of other root effecting factors such as medium state and pH adjustments. The objective of this study is to compare the effect of different pH adjustments (5.0, 5.5, 6.0 and 6.5) and agar concentrations (0.0, 3.5, 7.0 g/l) on the rooting responses of Queen pineapple to different concentrations of sucrose (10, 20, 30, 40 g/l). The goal is to determine the pH of liquid medium that maintained the highest rooting responses at lowest sucrose concentration.

MATERIALS AND METHODS

One and half liter of MS medium (Murashige & Skoog, 1962) was enriched with IBA at 0.5 mg/l and divided into 4 beakers each received 312 ml and marked A, B, C, and D. Sucrose at 10, 20, 30 and 40 g/l were added to beakers A, B, C and D respectively. The content of each beakers divided equally (78 ml) into 4 glass jars marked with the same beaker symbol plus 1, 2, 3 and 4. The content of jars numbered 1, 2, 3 and 4 adjusted to pH 5.0, 5.5, 6.0 and 6.5 respectively. Then the content of each jar divided equally (26 ml) into another 3 glass jars marked with same symbol plus H, S and L. Agar at 3.5 and 7 g/l added to glasses marked with H and S respectively, and the glass marked L without agar. The glasses covered with autoclavable plastic lid and the medium was then sterilized at 121^{O} C and 1.5 kg/cm^2 for 25 minutes. The content of each glass dispensed under laminar into 3 culture tubes marked as glass**.** Three shoots from stock cultures were cultured per each culture tube. The cultures incubated un-

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der photo-period of 16 hours of light and constant temperature of 25^{O} C. After 60 days of incubation, the cultures removed from the incubation room, the shoots picked out of the cultures and placed over squared paper, for counting of the roots and measuring the root and shoot length. Each tube was considered as one replicate and the data except shoot length (plantlet height) were transformed to square roots (x+1) before analysis of variance. Analysis of variance and Duncan Multiple Range Test for significant of treatments at $p \leq 0.05$ were computed using SPSS statistical package No. 11.

RESULTS

Analysis of variance (Table 1) showed that sucrose had significant independent (direct) and dependent (indirect) effect via interaction with pH in all rooting parameters, and dependent (indirect) effect via interaction with agar (medium state) in two of the rooting parameters (root number and plantlet height). But the interaction in case of the other two parameters (rooting percentage and root length) was not significant. Medium state (agar) had significant independent effect (direct) in all rooting parameters except rooting percentage and dependent (indirect) effect via interaction with sucrose in two of rooting parameters (root number and plantlet height). Medium pH, on the other hand, did not have significant independent (direct) effect on any of the rooting parameters and did not interact significantly with agar concentrations (medium state). However, medium pH did have dependent (indirect) significant effect via interaction with sucrose in all rooting parameters, and via collective interaction with both of sucrose and agar (medium states) on all rooting aspects except the plantlets height. Overall sucrose concent-rations and pH adjustments (Table 2), liquid medium resulted in taller plantlets (51.06 mm), more (5.38 roots) and longer roots (18.25 mm) than solid medium (39 mm tall plantlets, 3.13 roots per shoots, 10.96 mm long each) but the rooting percentage of liquid (69.4 %) and solid (59 %) medium were not significantly different (Table 2). Similar, overall medium states and pH adjustments, the largest number (5.4 roots), longer roots (16.25 mm) and highest rooting percentage (76.3 %) obtained in medium enriched with sucrose at 20 and 30 g/l. However, sucrose at 20 g/l resulted in tallest plantlets (52.8 mm) and sucrose at 30 g/l resulted in an intermediate plantlet height (48.08 mm). Sucrose at 10 and 40 g/l resulted in the lowest rooting percentage (47.0 %), fewest (2.1 roots) and shortest (6.7 mm long) roots, but the plantlets on medium enriched with sucrose at 10 g/l was taller (42.33 mm) than that in medium with 40 g/l (31.9 mm). Overall medium states and sucrose, the pH adjustments of medium had no significant effect on any of the rooting parameters**.** On the other hand, overall pH adjustments, liquid medium enriched with sucrose at 30 g/l resulted in highest rooting percentage (88 %) and more (8.8 roots) and longest roots (29 mm) while semi solid enriched with sucrose at 10 g/l resulted in lowest rooting percentage (31.3 %), and fewest (1 root per shoot) and shortest roots (5.3 mm). However, the tallest plantlets (63 mm), on the other hand, obtained in liquid medium enriched with sucrose at 20 g/l while the shortest plantlets (28 and 30 mm) obtained in semi solid and liquid medium enriched with sucrose at 40 g/l. No significant different on plantlet height on solid medium enriched with sucrose at 10 and 40 g/l and semi solid enriched with sucrose at 10 g/l

Comparing of all combinations of sucrose, pH and medium states (Table 2) showed that the tallest plantlet (70 and 71 mm) was obtained in liquid medium enriched with sucrose at 10 and 20 g /l and adjusted to pH 6.0 and the most stunted plantlets (15.0 mm) obtained on semi solid enriched with sucrose at 40 g/l and adjusted to pH 5.0 and pH 6.0. On the other hand, all shoots (100 %) could be rooted in liquid media enriched with sucrose at 10 g/l and adjusted to pH 6.0; sucrose at 20

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g/l and pH 5.0 and pH *6*.5; sucrose at 30 g/l and pH 5.0 and on semi solid media enriched with sucrose at 20 g/l and adjusted to pH 6.5; sucrose at 30 g/l and pH 5.0 and sucrose at 40 g/l and pH 6.5. However, 100 % rooting obtained in liquid medium could be reversed to 22 % if enriched with sucrose at 10 g/l and adjusted to pH 5.5 and that of semi solid reversed to 11 % if enriched with sucrose at 40 g/l and adjusted to pH 5.0 and 6.0. Contrary, none of the combinations in which solid medium was used irrespective of sucrose and pH and none of the combination in which the media adjusted to pH 5.5 irrespective of sucrose and medium states resulted in 100 % rooting. The highest rooting percentage in solid media was 89 %, obtained in medium enriched with sucrose at 20 g/l and adjusted to pH 5.0 and sucrose at 30 g/l and pH 5.0 and pH 5.5. Highest root formation (10-11 roots) obtained in liquid medium enriched with sucrose at 20 and 30 g/l and adjusted to pH 5.0 and pH 5.5 and in solid medium enriched with sucrose a**t** 30 g/l and adjusted to pH 5.0. The lowest root formation (1 root) obtained in almost all (75 %) of the combinations in which the sucrose enrichment was 10 g/l and in 50 % of the combinations in which sucrose enrichment was 40 g/l. The longest root (39 mm) obtained on liquid medium enriched with sucrose at 30 g/l and adjusted to pH 5.0 and the shortest (3.0 mm) on semi solid medium enriched with sucrose at 10 g/l and adjusted to pH 6.5; sucrose at 40 g/l and adjusted to pH 5.0 and pH 6.0 and on solid medium enriched with sucrose at 10 g/l and adjusted to pH 5.5.

Table. (1). Significant of main and interaction effect of medium states, sucrose concentrations and pH of full strength MS medium on the *in vitro* rooting of Queen pineapple.

Factors	df	Rooting parameters (p values)			
		Plantlet height	Rooting %	Root No.	Root length
Medium states	2	0.0001**	0.1310	9.09E-07**	5.14E-05**
Sucrose conc.	3	5.19E-08**	0.0001**	1.53E-12**	7.59E-09**
pH	3	0.8404	0.5973	0.4395	0.1909
State*Sucrose	6	0.0103*	0.4861	0.0055**	0.1382
State*pH	6	0.5682	0.2698	0.2666	0.4854
Sucrose*pH	9	1.07E-05**	0.0238 **	1.23E-06**	0.0004**
State*Sucrose*pH	18	0.2315	0.0085**	2.39E-05**	0.0038**
Error	96				
Total	144				

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Table (2). Effect of medium states, sucrose concentrations and pH of full strength MS medium on the *in vitro* rooting of Queen pineapple

Medium states	pH	Sucrose (g/l)				
(Agar/l)		10	20	30	40	Average
Plantlet height (mm)						
Liquid	5	55 bc	56 bc	66 ab	22 ef	49.75 AB
(0.0 g/l)	5.5	61 abc	57 bc	60 abc	22 ef	50 AB
	6	70 a	71 a	56 bc	31 cdef	57 A
	6.5	38 cde	68 ab	38 cde	46 cd	47.5 AB
	Average	56 AB	63 A	55 AB	30.25 D	51.06
Semi solid	5	52 bcd	53 bc	56 bc	16 f	44.25 AB
(3.5 g/l)	5.5	32 cdef	42 cd	55 bc	24 ef	38.25 B
	6	38 cde	65 ab	48 bcd	15 f	41.5 AB
	6.5	27 def	52 bcd	27 def	58 abc	41 AB
	Average	37.25 CD	53 AB	46.5 BC	28.25 D	41.25
Solid	5	33 cdef	36 cde	51 bcd	32 cdef	40.5 AB
(7 g/l)	5.5	28 def	42 cd	56 bc	27 def	38.25 B
	6	30 cdef	43 cd	27 ef	42 cd	35.5 B
	6.5	44 cd	39 cde	37 cde	48 bcd	42 AB
	Average	33.75 CD	42.5 BCD	42.75 BCD	37.25 CD	39.06
	Grand aver	42.3	52.8	48.08	31.91	43.7
Rooting %						
Liquid	5	44.3 abcde	100 a	100 a	33.3 abcde	69.4 NS
(0.0 g/l)	5.5	22.3 de	89 abc	96.3 ab	37 abcde	61.2 NS
	6	100 a	44.3 abcde	77.7 abcd	78 abcd	75 NS
	6.5	33.3 abcde	100 a	78 abcd	78 abcd	72.3 NS
	Average	49.98 BCD	83.08 AB	88.0 A	56.58 ABCD	69.41
Semi solid	5	66.7 abcde	77.7 abcd	100 a	11 e	63.8 NS
(3.5 g/l)	5.5	22 bcde	55.7 abcde	55.7 abcde	44.3 abcde	44.4 NS
	6	22 bcde	89 abc	77.7 abcd	11 e	49.9 NS
	6.5	14.7 de	100 a	55.7 abcde	100 a	67.6 NS
	Average	31.3 D	80.6 AB	72.3 ABC	41.6 CD	56.42
Solid	5	33.3 abcde	89 abc	89 abc	44.3 abcde	63.9 NS
(7.0 g/l)	5.5	22 bcde	77.7 abcd	89 abc	55.3 abcde	61 NS
	6	44.3 abcde	77.7 abcd	44.3 abcde	89 abc	63.8 NS
	6.5	78 abcd	33.3 cde	33.3 cde	44.3 abcde	47.3 NS
	Average	44.4 CD	69.4 ABC	63.9 ABCD	58.3 ABCD	59
	Grand Aver	41.89	77.69	74.78	52.16	61.63
Root number						
Liquid	5	1 hi	10 ab	11 a	1 hi	5.75 AB
(0.0 g/l)	5.5	1 hi	10 ab	11 a	3 defgh	6.25 A
	6	5 abcdef	3 defgh	7 abcd	4 cdefg	4.75 B
	6.5	1 hi	6 abcde	6 abcde	6 abcde	4.75 B
	Average	2 CDE	7.3 AB	8.8 A	3.5 CD	5.38
Semi solid	5	1 hi	3 defgh	8 abc	0 i	3 CD
(3.5 g/l)	5.5	1 hi	2 efghi	3 defgh	2 efgh	2 D
	6	0 i	7 abcd	6 abcde	1 hi	3.5 BC
	6.5	0 i	5 abcdef	2 efgh	4 cdefg	2.75 CD
	Average	0.5 E	4.25 CD	4.75 BC	1.75 DE	2.81
Solid	5	1 hi	5 bcdef	11 a	1 hi	4.5 B
(7 g/l)	5.5	1 hi	2 efgh	7 abcd	4 cdefg	3.5 BC
	6	2 efgh	2 efgh	1 hi	6 abcde	2.75 CD
	6.5	4 cdefg	1 hi	1 hi	1 hi	1.75 D
	Average	2 CDE	2.5 CDE	5 BC	3 CD	3.12
	Grand aver	1.5	4.68	6.18	2.75	3.77

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Medium states	pH	Sucrose (g/l)				
(Agar/l)		10	20	30	40	Average
Root length (mm)						
Liquid	5	14 bcd	24 abc	39 a	8 cde	21.3 A
(0.0 g/l)	5.5	9 cde	27 abc	28 abc	6 cde	17.5 AB
	6	16 abcd	12 bcd	37 ab	12 bcd	19.3 A
	6.5	4 de	20 abcd	12 bcd	25 abc	15.3 AB
	Average	10.8 CD	20.5 AB	29 A	12.8 BCD	18.25
Semi solid	5	9 cde	14 bcd	24 abc	3 e	12.5 AB
(3.5 g/l)	5.5	4 de	11 bcd	8 cde	7 cde	7.5 B
	6	5 de	25 abc	14 bcd	3 e	11.8 AB
	6.5	3 e	25 abc	8 cde	15 bcd	12.8 AB
	Average	5.3 D	18.75 AB	13.5 BC	7 CD	11.15
Solid	5	10 bcd	15 bcd	26 abc	6 cde	14.3 AB
(7.0 g/l)	5.5	3 e	14 bcd	24 abc	7 cde	12 AB
	6	7 cde	11 bcd	6 cde	14 bcd	9.5 AB
	6.5	12 bcd	7 cde	7 cde	6 cde	8 B
	Average	8 CD	11.8 BCD	15.75 BC	8.3 CD	10.96
	Grand aver	8.03	17.01	19.41	9.36	13.45

Means of each rooting parameter followed by small letters and overall average followed by capital letters were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test.
NS. (Not significant).

DISCUSSION

Previous reported studies of *in vitro* rooting assessed the rooting treatment based on one parameter, rooting percentage (Bhatia & Ashwath, 2000), root number (Danso et al., 2008). In some cases two parameters, root number and length (Almobasher, 2016; Aydieh et al., 2000; Khatun et al., 1997) and in few cases three parameters: rooting percentage, root number and length (Amin et al., 2005; Soneji et al., 2002) were used for assessment of rooting treatments. Results (Table, 2) showed that assessment of rooting treatment based on one or two parameters could not be claimed as best rooting treatment. Different combinations of sucrose, pH and medium state could be recommended based in which parameter was used for assessment of treatments. Out of 48 combinations, seven resulted in 100 % rooting, five in tallest plantlets (65 to 71 mm), five in highest root number (10 – 11 roots) and two in longest roots (37, 39 mm). Not only at any fixed pH and medium state, different rooting parameters have different optimal sucrose enrichment and each single rooting parameter could have several optimum combinations of pH, medium state and sucrose enrichment but also combination which optimum for one parameter could suppress or promote another one, two or three rooting parameter. Two combinations (liquid enriched with sucrose at 10 g/l, pH 6.0; sucrose at 20 g/l, pH 6.5) was optimal for rooting percentage (100 %) and plantlet height (68 and 70 mm) but suppressed root number from 11 to 5 and 6 roots and root length from 39 to 16 and 20 mm respectively. Other two combinations (liquid enriched with sucrose at 20 g/l pH 5.0 and sucrose at 30 g/l, pH 5.5) was optimum for rooting percentage (100 and 96 %) and root number (10 and 11 roots) but suppressed the plantlet height from 70 to 56 and 60 mm and root length from 39 to 24 and 28 mm respectively. Semi sold medium enriched with sucrose at 20 g/l and adjusted to pH 6.5, sucrose at 30 g/l and pH 5.0 and sucrose at 40 g/l and pH 6.5 was optimum only for rooting percentage (100 %). Liquid medium enriched with sucrose at 20 g/l and adjusted to pH 6.0 was optimum only for plantlet height (71 mm). Liquid medium enriched with sucrose at 20 and 30 g/l and adjusted to pH 5.5 and solid medium enriched with sucrose at 30 g/l and adjusted to pH 5.0 was optimum only for root number (10 to11 roots). Liquid medium

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enriched with sucrose at 30 g/l and adjusted to pH 6.0 was optimum only for root length (37 mm).

Generally, *in vitro* rooting is done for propagation purposes or physiological studies and best treatment should be judged based on parameters that serve the goals of propa-gators and physiologist. For propagation purposes selection of rooting treatment will be in favor of low cost and particular parameter quality which is very essential for higher survival of acclimatization. Assess-ment based on one parameter will be in favor of liquid over solid medium; lower over higher sucrose; shorter and fewer over longer and more roots for easy handling of acclimatization stage. If assessment based on rooting percentage (100 %) the cheapest combination was sucrose at 10 g/l and pH 6.0 and if for tallest plantlets (70 mm) the cheapest was also liquid medium enriched with sucrose at 10 and pH 6.0. For highest roots number (10 roots), the cheapest one was liquid medium enriched with 20 g/l and adjusted to pH 5.0 and 5.5**.** Two treatments resulted in longest roots, 39 and 37 mm. (liquid enriched with sucrose at 30 g/l adjusted to pH 5.0 and pH 6.0) and both were of equal cost (equal sucrose enrichment). If, more than one parameters included for assessment of rooting treatments, the choice will be compromise between possible best response of both parameters and low cost of the treatment. For both of rooting percentage (100 %) and plantlet height (70 mm), the best conciliation for both parameters, and low cost would be liquid medium enriched with sucrose at 10 g/l and adjusted to pH 6.0. For rooting percentage (100 %) and root number (10 roots), the best compromise between best results and low cost would be liquid medium enriched with sucrose at 20 g/l and adjusted to pH 5.0. None of the combinations was best for three and only one combination (liquid medium enriched with sucrose at 30 g/l and adjusted to pH 5.0) was the best compromise for all of four rooting parameters (100 % rooting, 66 mm tall plantlets, 11 roots, 39 mm long). It is clear that in liquid medium, simple manipulation of medium pH could reduce the optimum sucrose from 30 g/l to 20 g/l by adjusting the medium to pH 6.5 and even to 10 g/l by adjusting to pH 6.0. Obtaining 100 % of rooting and 70 mm tall plantlets with 5 roots each 16 mm long in liquid medium enriched with sucrose at 10 g/l by adjusting the medium to pH 6.0 is very important approach for reduction of *in vitro* rooting cost. It is simpler and easier approach than using of cheap sucrose alternative at fixed pH of 5.7 as mean of cost reduction in micropro-pagation of pineapple (Dutta et al., 2013; Mengesha et al., 2021; Nelson et al., 2015), banana (Kodym & Zapata-Arias, 2001) and several plant species (Gangopadhyay et al., 2002).

Future investigation of combinations of low concentration range of 5 to 20 g/l of cheap sucrose alternative and wider pH range of 3.5 to 8.0 may lead to optimum sucrose enrichment lower than 10 g/l, and substantial reduction in cost of rooting medium and worth being tested. Since cost of rooting (Hamad, 2019) is expected to be about three times cost of multiplication (Hamad, 2017a and b) any effort for minimizing the cost of rooting will be very important for reduction of total cost of micropropagation. In large scale production any small reduction of sucrose enrichment per liter of medium could be turning point for commercially feasible micropropagation system.

In most of *in vitro* rooting studies the relation between the rooting parameter and survival during acclimatization was not tested. A little attention was paid to determine which rooting parameter is more important than the others for survival, and what are the lower limit of the parameter required for survival. Nevertheless, (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

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Vesco et al., 2001; DeWald et al., 1988; Ko et al., 2006; Soneji et al., 2002) respectively reported that over 90 % of 35, 50, 60, 70 and 80 mm tall plantlets survived acclimatization stage. For pineapple, plantlet height is probably more crucial for acclimatization survival. However, the effect of different rooting treatment on the plantlet height was rarely reported (Hamad, 2019; Be & Debergh, 2006; Hamad et al., 2013; Hassan et al., 2018), and was not tested as factor for survival of acclimatization stage. For better selection of treatments and proper micropropagation system, rooting and acclimatization treatments should be evaluated in connection with each other. After rooting stage the plantlets should be separated into groups according to root number, root length and plantlet height before being subject to acclimatization treatments. Assessment of rooting. Treatments should not only base on comparison of parameters, but in which rooting treatment and which rooting parameter resulted in highest acclimatization survival. Selection of best rooting treatment should be based on particular parameter with specific quality, which result in highest survival than the other parameters.

Medium pH adjustment determined the optimum sucrose enrichment for each rooting parameter on different medium states (Table, 2) and could switch the nature of sucrose and medium state interaction from enhancing to retarding rooting responses. In all medium states (liquid, semi solid and solid) in which the pH was adjusted to 5.0 and 5.5, the optimum sucrose for rooting %, root number, root length and plantlet height was 30 g/l. However, if the medium pH was adjusted to 6.0, the optimum sucrose for rooting responses in solid and semi solid was 20 g/l, while in liquid medium the optimum sucrose was 10 g/l. If the medium pH adjusted to 6.5, the optimum sucrose for rooting response in liquid and semi solid was 20 g/l, while in solid medium was 10 g/l. Adjustment of semi solid medium pH to (5.0 and 6.0) and enrichment with low (10 g/l) and high sucrose (40 g/l) promoted rooting percentage but retarded root number while in liquid medium improved both process of rooting % and root number. Solid medium, on the contrary, suppressed root number if enriched with sucrose at 10 and 40 g/l and suppressed rooting percentage but improved root number if enriched with sucrose at 20 and 30 g/l. Semi solid, on the other hand, improved both process of rooting percentage and root number. All shoots in liquid medium enriched with sucrose at 10 g/l and adjusted to pH 6.0 and sucrose at 30 g/l and adjusted to pH 5.0 rooted (100 %) and developed into 66 and 70 mm tall plantlets. However, the second treatment resulted in two times (11 roots) more and longer (39 mm) roots than the first treatment (5 roots, 16 mm). Shoots cultured in liquid medium enriched with sucrose at 20 g/l and adjusted to pH 6.0, and in medium enriched with sucrose at 10 g/l and adjusted to pH 5.5 developed in taller plantlets (71 and 61 mm) but these treatments failed to induce more than 45 % rooting. Being seven combinations resulted in 100 % rooting but in different number and length root, implied that root initials might have been formed in all of these combinations, but its growth arrested under some combinations of sucrose and pH and promoted under others. Low and high sucrose did not support root development**.** Histological study of apple *in vitro* rooting showed that initiation of root initials depended on IBA while development and growth of roots depended on sucrose enrichment (Harbage et al., 1993).

At fixed sucrose of 10, 20 and 30 g/l, increasing pH up to 6.5 decreased rooting percentage. while at fixed pH of 5.0, 5.5, 6.0 and 6.5 increasing sucrose enrichment up to 30 g/l increased rooting percentage. However, on the contrary at fixed sucrose of 40 g/l increasing medium pH up to 6.5 cause an increase of rooting percentage. This indicated that higher sucrose enrichment suppressed rooting and that suppression could be allevi-

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ated by increasing medium pH. Increasing of rooting in medium enriched with sucrose at 40 g/l by increasing the medium pH indicated that the pH interference kept sucrose accessibility and /or absorbed amount of sucrose below the limit that retard root initiation and development. Statistical analysis (Table 1) showed that sucrose had independent significant effect on rooting percentage and interacted significantly with pH, while pH did not had significant independent effect on rooting percentage. Medium state, on other hand, neither had independent effect nor interacted with sucrose or with pH. Root number and root length, on the other hand, was under independent effect of both sucrose and medium state and interaction of sucrose with pH and sucrose with medium state.

On the same time, all of the three parameters was not effected by pH and interaction of pH with medium state but all of the three rooting parameters (rooting %, root number and root length) was under collective significant interaction of the three factors (sucrose medium state and pH). This indicated that the collective interaction occurred in two steps: First, the sucrose interacted with pH to produce an intermediate product or condition that trigger initiation process of root premordia (significant effect in rooting percentage). Second, medium state interacted with the product or condition resulted from sucrose pH interaction and facilitate root growth and development. Neither the effect of medium state alone nor the interaction of medium state with sucrose or pH on the rooting percentage was significant (Table, 1) Medium states. did not effects the process of root premordia initiation, but facilitated root growth and penetration of internal tissues of the shoots. Once emerged, the root elongation in liquid was faster than in solid media. The importance of medium pH for *in vitro* rooting is not understood yet. The effect varied among different plants and media. Lowering of pH from 5.7 to 4.7 reduced the rooting percentage of Geraldton wax from 63 to 20 % (Page & Visser, 1989), while lowering the pH from 5.7 to 4.0 increased the rooting percentage of Australian woody plants from 28 to 100 % (Williams et al., 1985). In a solid WPM medium enriched with sucrose at 30 g/l, lowering the pH from 5.7 to 3.5 decreased the rooting of *Choisya ternata* by up to 60 % and Delphinium by 15 % (Leifert et al., 1992). At fixed concentration of sucrose (30 g/l), using of solid and liquid MS medium and adjusting the pH to range of 4.2 up to 6.2 did not affect the rooting percentage of *Maranta leuconeura* cv Kerchoviana (Bennett et al., 2003; Ebrahim & Ibrahim, 2000) reported that the lower rooting percentage (62 %) and few roots (4 roots) of *Eucalyptus glabulus* were mainly due to presence of NHNO. In medium devoid of NHNO, rooting increased to 94% and roots to 7 roots per shoot over pH range of 4.0 to 6.0. (Harbage et al., 1993) noticed that the optimal pH for root formation of Gala apple varied at different concentration of IBA. Increasing the sucrose concentration shifted the *in vitro* rooting dose-response curve of Jork 9 apple to auxin to the right (Calamar & De Klerk, 2002). Our results indicated that for *in vitro* rooting of pineapple, the rooting dose response curve to sucrose could be shifted by medium pH and the sucrose concentration could be minimized by adjusting the medium to proper pH.

Most of the reported rooting studies used either solid or liquid medium at full or half strength at fixed sucrose of 30 g/l and pH 5.7 and recommended different concentration and combination of rooting hormones. For assessment of rooting treatments some used only one parameter and others used two or three rooting parameters and come up with different recommendations for *in vitro* rooting depending in which parameter was used for assessment and which factors was included in testing of rooting. In this study, liquid medium enriched with sucrose at 30 g/l and adjusted to pH 5.0 was optimum for all rooting parameters, (100 % rooting, 66 mm tall plantlet with11 roots per shoot each 39 mm long.

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However, none of the treatments recommended in previous studies or in this study could be adopted as a universal treatment for rooting unless the cost of rooting kept at its lowest. The crucial rooting parameter for survival of acclimatization identified, and the mode of the factor effect on each of the three physiological steps of rooting elucidated. Future studies of *in vitro* rooting should focus in determining the relation between rooting parameters and percentage of survival during acclimatization. The two stages should be studied in connection with each other. Determining of optimum combination for each single rooting parameter as done in this study will help in selection of the most proper treatment and best timing for histological, physiological and biochemical study of root formation steps.

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تأثير درجة الحموضة وتركيز السكروز وحالة الوسط على التجذير المخبري للأناناس صنف كوين

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المستخلص: تم بحث تأثير حالة الوسط (سائل، صلب وشبه الصلب)، ودرجة الحموضة (5.0، 5.5، 6.0 و6.5)، وتركيز السكروز (10، 20 ، 30 و40 جرام / لتر) على تجذير عزلات أناناس مزروعة في وسط موريشيوس، وسكوج (MS) يحتوي على هرمون حمض اندول بيوتيرك (IBA) بتركيز 0.5 مللي جرام / لتر. طبقا للمتوسط العام لكل تركيزات السكروز، ودرجات حموضة الوسط، والمتوسط عند كل توليفة متساوية المحتوى من السكروز، ودرجة الحموضة فإن الوسط السائل كان أفضل من الوسط الصلب، والوسط شبه الصلب. أقصي طول للنبيتات الناتجة (66 – 71 ملم) تم الحصول عليها باستعمال الوسط السائل المحتوي على سكروز 10 جرام / لتر، ودرجة حموضة 6.0، والوسط السائل المحتوي على سكروز 20 جرام / لتر ودرجة حموضة 6.5، والمحتوي على سكروز 30 جرام / لتر، ودرجة حموضة 5.0. كل من هذه التوليفات السابقة الذكر ما عدا توليفة سكروز 20 جرام / لتر، ودرجة حموضة 6.0 أدت إلى تجذير كل العزلات (100 %). توليفة سكروز 30 جرام، ودرجة حموضة 5.0 ضاعفت عدد الجذور مرتين (أحد عشر جذرا بالعزلة)، وطول الجذور ثلاث مرات (29 ملم) مقارنة بالتوليفات الأخرى، والتي أدت إلى تكوين خمسة جذور بمتوسط طول 11 ملم. كل مقياس من مقاييس التجذير له متطلبات مثالية من توليفة خاصة من السكروز، ودرجة الحموضة، وحالة الوسط، وتختلف من مقياس تجذير، ومقياس تجذير آخر. لكل مقياس من مقاييس التجذير توجد درجة حموضة أكثر ملاءمة من غيرها، والتي تؤدي لخفض تركيز السكروز المطلوب من 30 إلى 20 وحتى إلى 10 جرام / لتر. لهذا فإن اختيار درجة حموضة وسط التجذير تعتبر مدخلا مهما لخفض تكلفة التجذير.

الكلمات المفتاحية: حالة الوسط، درجة الحموضة، السكروز، التجذير، الأناناس.

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