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Effect of Incubation Periods, Medium Volumes and Explants Density on *in Vitro* Shoot Formation and Growth and Cost of Multiplication of Moris Pineapple (*Ananas comosus* L.)

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Abstract: This study was conducted to investigate the effect of combinations of four incubation periods (30, 45, 60 and 75 days), three explants density (one, two and three) and four volumes of medium (3, 6, 9 and 12 ml) in the in vitro shoot formation per explant, total shoots and total cost per liter of medium and cost per shoot of Moris pineapple. The highest shoot per explant was obtained in combination of three explants in 12 ml of medium incubated for 60 days, three explants in 9 and in 12 ml of medium and one explant in 3 and in 9 ml of medium incubated for 75 days but at different total shoots (2750, 3667, 2750, 3667 and 1222 shoots), total cost (USA $25.85, 36.09, 28.39, 95.32 and 36.06) per liter of medium. Combinations in which three explants were used per culture resulted in lowest cost per shoot (1.0 cent) while combination in which one explant was used per culture, the cost per shoot was two to three times higher (2.6 and 2.9 cents). Combination of three explants, 3 ml of medium and 75 days of incubation, on the other hand, resulted in formation of only 6 shoots per explant (almost half of the highest obtained rate) but in highest total shoot per liter of medium (6000 shoots) at highest total cost (USA $95.32) and intermediate cost per shoot (1.6 cents). Combination of three explants, 6 ml of medium and 75 days resulted in formation of 10 shoots per explant, production of 5000 shoots per liter of medium at lowest cost per shoot (1.0 cent) and intermediate total cost per liter of medium (USA $ 50.68). Using of one explant in 12 ml for 30 days resulted in formation of 5 shoots per explant, highest cost per shoot (5.0 cents) and lowest total shoots (417 shoots) and lowest total cost (USA $ 20.73) per liter of medium.

Keywords: Total shoots; Total cost; Medium volume; Explants density; Incubation periods; Pineapple; *Ananas comosus.*

INTRODUCTION

A single multiplication cycle of pineapple ranged from a minimum of 15 days (Firoozabady & Gutterson, 2003) to a maximum of 112 days (Nelson et al., 2015) in which explants placed in as low as 10 (Hamad & Taha, 2003) and as high as 100 ml (Fernando, 1986) of MS medium. Yet 30 and 45 days and 20 and 25 ml of solid (Sripaoraya et al., 2003; Sunitibala Devi et al., 1997) and liquid medium (Be & Debergh, 2006; Pérez et al., 2009; Pérez et al., 2012; Teixeira et al., 2006) are the most commonly used cycle length and medium volume for *in vitro* shoot multiplication. Explants were used at density of 1(A. M. Hamad & R. Taha, 2008; A. M. Hamad & R. M. Taha, 2008a, 2008b), 2 (Be & Debergh, 2006; Soneji et al., 2002), 3 (Hamad & Taha, 2003, 2009), 4 (Khan et al., 2004), 5 (Daquinta et al., 1997; Pérez et al., 2009; Pérez et al., 2012), 8 (Dal Vesco et al., 2001), and 10 (Zuraida et al., 2011) explants per culture. Explants could be used intact (A. M. Hamad & R. Taha, 2008; Khan et al., 2004), halved single shoot

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(Hamad, 2017,(Almeida et al., 2002; Bhatia & Ashwath, 2002) and cluster of shoots (Escalona et al., 1999; Firoozabady & Gutterson, 2003; Hamad & Taha, 2009). Optimal hormone treatment varied at different incubation periods (A. M. Hamad & R. M. Taha, 2008b; Kofi & Adachi, 1993). The focus on these studies was mainly on testing hormone types, concentrations, and combinations and on comparing of solid and liquid media at fixed level of other factors and the assessment was based on the shoot formation rate and total shoot per one cycle (Escalona et al., 1999) and several cycles of multiplication (A. M. Hamad & R. Taha, 2008). Investigation of different combinations of explants density, volume of medium per culture, and incubation periods are not only important for their expected effect on shoot formation per explant but also on the possibly obtainable total shoots per liter of medium. The objective of this study was to investigate the effect of three explant densities (one, two and three explants per culture) and 4 volumes of medium (3, 6, 9 and 12 ml per culture) and four incubation periods (30, 45, 60 and 75 days) on the *in vitro* shoot formation per explants and on total shoots and cost per one liter of medium and cost per shoot of Moris pineapple.

MATERIALS AND METHODS

Full strength MS(Murashige & Skoog, 1962) medium enriched with sucrose at 20 g/l and BAP at 2.0 mg/l was adjusted to pH 5.0, sterilized at 121 ^{0}C and 1.5 kg /cm^2 for 25 minutes and dispensed under laminar air flow cabinet into 144 culture tubes. Each 36 of the 144 cultures tubes received 3, 6, 9 and 12 ml of the medium. One, two and three 15 to 20 mm long shoots were taken from 6-months old Moris stock cultures and placed in each 9 of the 36 culture tubes that received an equal volume of medium. The cultures incubated under constant temperature 25 ^{0}C and 16 hours of light provided by cool white fluorescent lamps. After 30 days of incubation, 9 cultures from each combination of explants density and volumes of medium were randomly picked and used for data collection. The multiple shoots complex of each explant was picked out, placed over a squared paper and separated into individual shoots for counting and measuring the length of shoots.

Data were used to establish two tables, one for total shoots and one for total shoot length per culture after 30 days incubation period. The table of total shoots per culture was converted to table of shoot formation per explant by dividing by the number of explants per culture tube and to table of total shoot per liter of medium by multiplying by 1000 and dividing by the medium volume per culture. Table of total shoot length was converted to table of shoot length per explant by dividing by the total shoots per culture. Each of these tables is two factors table (explants and volumes). After 45, 60 and 75 days of incubation, the same procedures were repeated to establish tables for total shoots and total shoots length per culture and shoots formation and shoot length per explant after 45, 60 and 75 days of incubation. The two factors tables (explants, volumes) of the same parameters (shoot formation, shoot length per explant and total shoots per liter of medium) at each incubation period were combined to obtain three factors tables (explants, volumes, and incubation) and the data were subjected to analysis of variance and separation of means by Duncan Multiple Range Test (Duncan, 1955) at $p \leq 0.05$ using SPSS statistical package No 11. Table of total shoots per liter of medium was used for estimation of total cost and cost per shoot.

RESULTS

Table 1 showed that five different combinations of explants density and medium volumes per culture and incubation periods resulted in highest shoot formation per explant (11 shoots). One of these combinations made of three explants per culture incubated in 12 ml of medium for 60 days. In the other four

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combinations, the incubation period was 75 days but of a different combination of explants density and medium volume per culture. One explant in 3 and 9 ml and three explants in 9 and 12 ml of medium. In all of these five combinations, the incubation period was longer than 45 days and none included density of two explants per culture. On the other hand, six different combinations resulted in the lowest shoot formation per explant (3 shoots).

In these combinations, the incubation was shorter than 60 days, the medium less than 9 ml, and the explants density was more than one explant per culture. At a fixed density of one explant, if the medium dispensed at 3 ml per culture, each 15 days increase in the incubation (30, 45, 60 and 75 days) increased the shoot formation per explant (4, 8, 9 and 11 shoots). However, if the medium dispensed at 6 ml, incubation for 45 days resulted in more shoots per explant (6 shoots) than incubation for 30 days (4 shoots) but equal to that obtained after 60 days (6 shoots). Extending the incubation to 75 days increased the shoot formation to 8 shoots. When the medium was dispensed at 9 ml, incubation for 30, 45 and 60 days resulted in equal shoots formation per explant (8 shoots) and extending the incubation to 75 days increased the shoots formation to 11 shoots per explant. In cultures containing 12 ml of medium, increasing the incubation from 30 to 45 and 60 days increased the shoots formation from 5 to 6 and 9 shoots per explants.

Extending the incubation to 75 days did not increase the shoots formation resulted in equal shoot formation as that of 60 days incubation (9 shoots). At density of one, the highest shoot formation (11) obtained when medium dispensed at 3 and 9 ml and culture incubated for 75 days. At fixed density of two explants, dispensing the medium at 3 ml per culture and incubation for 30 and 45 days resulted in equal shoots formation per explant (3 shoots) and increasing the incubation to 60 and 75 days increased the shoots formation to 4 and 6 shoots per explant respectively. If the medium dispensed at 6 ml, incubation for 30, 45 and 60 days resulted in equal shoots formation per explant (4 shoots) and increasing the incubation to 75 days increased the shoots formation to 5 shoots per explant while if medium dispensed at 9 ml, increasing the incubation from 30, 45 to 60 days increased shoot formation from 3 to 5 and 6 shoots but extending the incubation to 75 days did not increase the shoots formation more than that obtained after 60 days (6 shoots).

Dispensing the medium at 12 ml and incubation for 45 and 60 day resulted in equal shoot formation (5 shoots) and both were more than the shoot formation (3 shoots) after 30 days of incubation. Extending the incubation to 75 days doubled the shoot formation (9 shoots) compared to that obtained after 45 and 60 days of incubation. At a density of two explants, the highest shoot formation (9 shoots) obtained when medium dispensed at 12 ml and incubated for 75 days. Similar, the response of explants when three explants were used per culture were also varied depending on the volume of medium and incubation periods. When the medium dispensed at 6 and 9 ml per culture, each 15 days increase in the incubation (30, 45, 60 and 75 days) increased the shoot formation per explant (3, 4, 6 and 10 shoots) and (5, 6, 7 and 11 shoots) respectively. However, when the medium dispensed at 3 ml, incubation for 30 and 45 days resulted in equal shoots formation per explant (3 shoots) and increasing the incubation to 60 and 75 days increased the shoots formation to 4 and 6 shoots per explant respectively. If the medium dispensed at 12 ml per culture, increasing the incubation from 30 to 45 and 60 days increased the shoots formation from 5 to 7 and 11 shoots per explant, but extending the incubation to 75 days did not increase the shoots formation more than that obtained after 60 days (11 shoots).

At a density of three, the highest shoot for-

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mation (11) obtained when medium dispensed at 9 ml and incubated for 75 days and when medium dispensed at 12 ml and incubated for 60 days. Converting the shoot formation per explant to total shoot production per liter of medium (Table, 2) showed that the five combinations (3 explants in 12 ml and incubation for 60 days and 3 explants in 9 and 12 ml and one explants in 3 and 9 ml and incubation for 75 days) which resulted in the highest shoot formation per explant (11 shoots) resulted in different total shoots per liter of medium (2750, 3667, 2750, 3667 and 1222 shoots). Three of these combinations (three explants in 9 and 12 ml of medium for 75 days and three explants in 12 ml of medium for 60 days) resulted in lowest cost per shoot 0.1 cent and lowest total cost (USA $ 25.85; 36.09; 28.39) while the cost per shoot of the other two combinations was three-time higher (2.6 and 2.9 cents) and the total cost was USA $ 95.32 and 36.09 (Table, 2).

On the contrary, combination in which the shoot formation was 5 shoots less than the highest obtainable shoot formation (three explants in 3 ml of medium for 75 days) resulted in the highest total shoots per liter (6000 shoots) but at highest total cost (USA $ 95.32) and the cost per shoot was two times (1.6 cent) the possible lowest cost per shoot (0.9 cent).

DISCUSSION

This study demonstrated that unless the effect of explants density, volume of medium and incubation period were tested at combinations of different levels, physiological studies of shoot formation and commercial protocol for *in vitro* multiplication could neither be developed nor verified. The results showed that changing the level of any one of these factors lead to a different optimum combination of the other two factor for highest shoot formation per explants (Table, 1), total shoots production and total cost per liter of medium and cost per shoot (Table, 2). Hence, to achieve the different goals for physiologist and propagators, dispensing of medium per culture should be made in accordance with the intended density of explants and incubation period and vice versa. According to shoot formation per explant, five different combinations of explants density, medium volume per culture and incubation periods (Table, 1) resulted in the highest shoot formation per explant (11 shoots). According to rates, either one of these five combinations could be recommended as the best treatment. However, Table 2 showed that higher shoot formation per explant may result in lower total shoots and lower shoot formation may result in higher total shoots per liter of medium. Combination of three explants, 3 ml and 75 days of incubation resulted in formation of only 6 shoots per explants (half of the highest obtained rate) but produced the highest total shoots (6000 shoots) while combination of one explant, 9 ml and 75 days of incubation resulted in highest shoot formation (11 shoots) but low total shoots (1222 shoots) per liter of medium. Which one is the more important than the other? The rate per explant or the total shoots per liter of medium depend on the researcher goal. Physiology judged by rate while for management and profitability of production a compromise between highest total shoots and lowest total cost per liter and cost per shoot are very crucial.

Two of the five combinations which resulted in the highest shoot formation (11 shoots) beside it resulted in different total shoots (3667 and 1222 shoots) and total cost (USA $ 95.32 and 36.09), the cost per shoot was 3 times higher (2.6 and 2.9 cents) than the lowest obtainable cost per shoot. The other three combinations resulted also in different total shoots (3667, 2750 and 2750 shoots) and total cost (USA $ 36.09; 28.39 and 25.85) per liter of medium (Table, 2) but in lowest cost per shoot (0.1 cent). In other words, if the combinations assessment was based on shoot formation per explant, there is a risk of recommending combination which produced shoots at three times higher cost per shoot

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(2.6 cents) than the possibly obtained lowest cost (0. 9 cent), and losing of possibly produced 2445 shoots (3667- 1222) and extra spending of USA $ 69.47 (95.32-25.85**)** per liter of medium.

On the other hand, if assessment was based on total shoots per liter of medium (Table, 2), combination of three explants, 3 ml and 75 days of incubation resulted in highest total shoots (6000 shoots), but in lower shoot formation per explant (6 shoots) and higher cost per shoot (1.6 cents) and highest total cost (USA $ 95.32) per liter of medium. That is, there is a risk of recommending combination which suppressed the shoot formation per explant, produced shoots at two times higher cost per shoot than the possibly obtained lowest cost (0. 9 cents) and at highest total cost (USA $ 95.32). The cost per shoot could be reduced to 0.1 cent, the total cost of USA $ 50.68 and the shoot formation increased to 10 shoots per explant if the medium dispensed at 6 instead of 3 ml, but the total shoots declined from 6000 to 5000 shoots per liter.

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Table (1). Effect of medium volumes, incubation periods and explants density per culture on the *in vitro* shoots formation per explant and shoot length of Moris pineapple.

MS	**Incubation periods (days) and explants density**											
(ml)	30			45			60			75		
	1	2	3	1	2	3	1	2	3	1	2	3
Shoots per explants												
3	4 e	3 e	3 e	8 bcde	3 e	3 e	9 bcd	4 e	4 e	11 b	6 cde	6 cde
6	4 e	4 e	3 e	6 cde	4 e	4 e	6 cde	4 e	6 cde	8 bcde	5 de	10 bc
9	8 bcde	3 e	5 de	8 bcde	5 de	6 cde	8 bcde	6 cde	7 bcde	11b	6 cde	11 b
12	5 de	3 e	5 de	6 cde	5 de	7 bcde	7 bcde	5 def	11 b	9 bcd	9 bcd	11 b
Shoot length (mm)												
3	8.7bcd	11.7abcd	9.3bcd	9.7bcd	11.7abcd	12.3abcd	10 bcd	13.7abcd	11.7abcd	10.7abcd	14.7 abc	15 ab
6	8.3 cd	8.3 cd	9.3bcd	10 bcd	9 bcd	8 d	11.3abcd	9.7 bcd	12.7abcd	14.7 abc	10.3 bcd	15 ab
9	7.3 d	7 d	7.3 d	7.7 d	7.7 d	8 d	9.3 bcd	12.3abcd	12.3abcd	12.7abcd	13.3abcd	13.7abc
12	8 d	7.7 d	7 d	9 bcd	9 bcd	8.7 bcd	9 bcd	11.7abcd	12 abcd	10 bcde	13.3abcd	16.7 a

Each combination of medium volume, explants density and incubation period consisted of nine culture tubes.
Explants (shoots) were cultured in culture tubes containing static liquid full strength MS medium enriched with sucrose at 20 g/ l and BAP at 2.0 mg/l and pH adjusted to 5.0 and incubated under constant temperature (25 ^{0} C) and 16 hours of light.
Means followed by same letters were not significantly different as tested by Duncan Multiple Range Test at $p \leq 0.05$.

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Table (2). Effect of medium volumes, incubation periods and explants density per culture on the total shoots and total cost per liter of medium and cost per shoot of Moris pineapple

MS	**Incubation periods (days) and explants density**											
(ml)	30			45			60			75		
	1	2	3	1	2	3	1	2	3	1	2	3
Total shoots per liter												
3	1333hij	2000efghi	3000cde	2667defg	2000efghi	3000cde	3000cde	2667defg	4000bc	3667cd	4000 bc	6000a
6	667 j	1333 hij	1500ghij	1000 ij	1333 hij	2000efghi	1000 ij	1333 hij	3000 cde	1333hij	1667fghi	5000b
9	889 ij	667 j	1667fghij	889 ij	1111 hij	2000efghi	889 ij	1333 hij	2333efgh	1222hij	1333 hi	3666cd
12	417 j	500 j	1250 hij	500 j	833 ij	1750fghij	583 j	833 ij	2750 def	750 j	1500ghi	2750def
Total cost per liter (USA $**)**												
3	64.58	64.58	64.58	74.83	74.83	74.83	85.07	85.07	85.07	95.32	95.32	95.3
6	35.27	35.27	35.27	40.41	40.41	40.41	45.54	45.54	45.54	50.68	50.68	50.68
9	25.85	25.85	25.85	29.26	29.26	29.26	32.68	32.68	32.68	36.09	36.09	36.09
12	20.73	20.73	20.73	23.29	23.29	23.29	25.84	25.84	25.84	28.39	28.39	28.39
Cost per shoot (USA $**)**												
3	0.048	0.032	0.022	0.028	0.037	0.025	0.028	0.032	0.021	0.026	0.024	0.016
6	0.053	0.026	0.023	0.040	0.030	0.020	0.045	0.034	0.015	0.038	0.030	0.010
9	0.029	0.039	0.015	0.033	0.026	0.015	0.037	0.024	0.014	0.029	0.027	0.010
12	0.050	0.041	0.016	0.046	0.028	0.013	0.044	0.031	0.009	0.038	0.019	0.010

Each combination of medium volume, explants density and incubation period consisted of nine culture tubes.
Explants (shoots) were cultured in culture tubes containing static liquid full strength MS medium enriched with sucrose at 20 gm/ l and BAP at 2.0 mg/l pH adjusted to 5.0 and incubated under constant temperature (25 ^{0} C) and 16 hours of light.
Means followed by same letters were not significantly different as tested by Duncan Multiple Range Test at $p \leq 0.05$.
Total shoots per liter computed by multiplying the average total shoots per culture tube by 1000 and dividing by the volume of medium per culture tube
Cost estimate included only the variable cost items (Medium, culture tubes, labor and electricity)

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That is losing of 1000 possibly produced shoots but saving of USA $ 44,64 per liter of medium. Similar, if one or two explants were used per culture, the highest total shoots per liter of the medium was (3667and 4000 shoots respectively) and the lowest cost per shoot in both cases (2.6 cents) obtained when medium dispensed at 3 ml per culture and incubated for 75 days. In a case when two explants were used, the cost per shoot could be reduced to 1.9 cents if the medium dispensed at 12 instead of 3 ml, but the expected total shoot per liter of medium declined from 4000 to 1500 shoots (Table, 2).

Tables 1 and 2 showed that using of explants at a density of one and two per culture did not serve the propagator goal of highest total shoots and lowest total cost per unit of medium. A density of three explants, on the other hand, could serve the propagator goal only if the medium dispensed at volume larger than 3 ml per culture and the incubation is longer than 45 days. Lowest cost per shoot is very crucial for both small and big companies while the production of highest total shoots is depending on the company budget, client demands, market size, and time of delivery. Depending on his budget and obligations, propagator could select the most suitable combination and decide the proper management of *in vitro* multiplication system.

The shoot formation at each fixed explants density depended on medium volumes, and at each fixed volume of medium depended on the explants density and on both cases on how long the cultures were incubated. Certain volumes of medium per culture and certain incubation periods are optimum for certain explants density and not for the others. Shoot formation per explant reflects how the different factors directly or via interaction with each other affect the process of shoot formation. At a fixed density of one explant and incubation of 30 days, the shoot formation increased as the volume of medium increased up to 9 ml per culture and declined at 12 ml (Table, 1). This decline in shoot formation if a volume of medium was higher than 9 ml indicated that the number of some ions of the medium nutrient or hormone content of the 12 ml reached inhibitory level. However, if one explant incubated for 45, 60 and 75 days, the decline of shoot formation started if the volume of medium was higher than 3 ml per culture. Using a larger volume of medium enhance the shoot formation in case of shorter incubation but retard the shoot formation in case of longer incubation. It seemed that the promotion effect of a larger volume of medium when explants incubated for a shorter period could be reversed by longer incubation.

Explants during the first 30 days of incubation might have inefficient nutrient uptake and require a larger volume of medium while after 30 days of incubation explant developed efficient nutrient uptake and absorbed enough nutrient even if the volume per culture was too low. In addition, after 30 days of incubation, the explants may release certain extracts that interact with certain components of the medium to produce an inhibitor of shoot formation. On the contrary, at a fixed density of three explants, the highest shoot formation at any of the incubation period obtained when medium dispensed at the largest volume of medium (12 ml) per culture. The shoot formation increased as the volume of medium per culture increased. This may indicate that presence of three explants would share the content and reduce the amount of certain medium components below its inhibitory level. At a density of one explant per culture, two different volumes of medium (3 and 9 ml) at same incubation period (75 days) could be used to induce one explant to express its highest shoot formation ability (11 shoots). At a density of three explants, also two different but larger volumes of medium (9 and 12 ml) at same incubation (75 days) and same medium volume (12 ml) but different incubation period (60 and 75 days) resulted in highest shoot formation. One of these volumes of

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medium (9 ml) and one of these incubation periods (75 days) is shared by the two different explants density. All of these different combinations of medium volume and incubation periods resulted in highest and equal shoot formation (11 shoots) whether the explant was one or three.

In case of two explants per culture, none of the combinations resulted in 11 shoots. But also, two different volumes of medium and two different incubation periods could be used to induce highest shoot formation per explant (9 shoots). Medium dispensed at 9 ml and incubation for 60 days and dispensed at 12 ml and incubation for 75 days. However, when the response of one and three explants was compared, it showed that depending on the volume of medium and incubation period, one explant per culture could result in higher, equal or less shoot formation rate than that of three explants per culture and vice versa (Table, 1). The effect of medium volume and explants density on shoot formation could be related to the shortage (competition) or oversupply (inhibition) of medium components. The effect of incubation period is a result of a complicated relationship of growth, changes of medium components and vessel atmosphere over time. If the cultures containing less than 12 ml and the incubation period less than 75 days, using of one explant resulted in higher rate of shoot formation than using three explants per culture. The low rate of shoot formation of the three explants may due to competition for nutrient or hormone content of the medium. On the contrary, if the culture contained 12 ml of medium irrespective of the incubation period (30, 45, 60 and 75 days), using of three explants resulted in higher rate of shoot formation per explant than using one explant per culture. The hormone or ions content of the larger volume of the medium reached an inhibitory level for the one explant and suppressed its shoot formation. However, irrespective of using smaller or larger volume of medium per culture if the culture incubated for 75 days, three explants resulted in higher rate of shoot formation than one explant. In this case, competition and over supply could not explain the result. Over longer incubation, the three explants might release an extract that overcomes shortage as well as the inhibitory effect of medium components and enhances the shoot formation of the three explants more than one explant. (Konan et al., 2007) reported that when more than one explants were used per culture, explants size-related cofactor control the *in vitro* rooting ability of oil palm. The higher shoot formation of the three explants than the one at certain medium volume and incubation and lower at other indicated presence of explants density- related cofactors. Promotion or inhibition nature of the cofactor depended on the volume of medium dispensed per culture and incubation period. Elucidation the role of medium volume, explants density and incubation period in the shoot formation process require future investigation using specifically selected combinations and retesting their effect using medium of different strength and pH adjustments in connection with a chemical analysis of medium and explants and histological investigation over incubation.

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تأثير فترة التحضين، حجم الوسط وكثافة العزلة على تكوين ونمو الفريعات وتكلفة انتاج مرحلة التضاعف في مزرعة أنسجة انااناس صنف موريس

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المستخلص : أجريت هذه الدراسة لمعرفة درجة تأثير توليفات مكونة من أربعة فترات تحضين (30 ، 45 ، 60 و75 يوم) ثلاث درجات كثافة من العزلة (واحد ، اثنين و ثلاثة) وأربع كميات من الوسط (3 ، 6 ، 9 و 12 مل) علي عدد الفريعات المتكونة من العزلة الواحدة من أناناس صنف موريس وكذلك تأثيرها علي العدد الاجمالي للفريعات. التكلفة الاجمالية وتكلفة إنتاج الفريع الواحد فيما لو استخدم لتر واحد من الوسط. توليفة من ثلاث عزلات في 12 مل من الوسط احتضنت لمدة 60 يوم، ثلاث عزلات في 9 وفي 12 مل من الوسط وعزلة واحدة في 3 وفي 9 مل من الوسط احتضن كل منها لمدة 75 يوم ادت للحصول علي أعلي عدد من الفريعات المتكونة من العزلة الواحدة (11 فريع) ولكن اختلفت في العدد الاجمالي من الفريعات (2750 ، 3667، 2750، 3667 و 1222 فريع) وفي اجمالي التكلفة (25.85، 95.32، 28.39، 36.09 و 36.09 دولار امريكي علي التوالي). التوليفات التي استخدمت فيها ثلاث عزلات في المزرعة الواحدة أدت إلى اقل تكلفة انتاج للفريع الواحد (1.0 سنت) بينما التوليفات التي استخدم فيها عزلة واحدة تضاعفت تكلفة انتاج الفريع الواحد مرتين إلى ثلاث مرات (2.6 و 2.9 سنت). من ناحية أخري توليفة من ثلاث عزلات ، 3 مل من الوسط و 75 يوم تحضين أدت إلى الحصول علي 6 فريعات فقط (نصف أعلي متوسط فريعات امكن الحصول عليه) ولكن على أعلى اجمالي عدد من الفريعات (6000 فريع) وبأعلى اجمالي تكلفة (95.32 دولار) فيما لو استخدم لتر من الوسط وبتكلفة 1.6 سنت للفريع الواحد. تحضين ثلاث عزلات في 6 مل من الوسط لمدة 75 يوم أدي للحصول علي 10 فريعات من العزلة الواحدة وانتاج 5000 فريع وإجمالي تكلفة 50.68 دولار فيما لو استخدم لتر من الوسط وبتكلفة 1.0 سنت للفريع الواحد. استخدام عزلة واحدة و 12 مل من الوسط والتحضين لمدة 30 يوم أدى إلى الحصول علي 5 فريعات من العزلة الواحدة وبأعلى تكلفة إنتاج للفريع الواحد (5.0 سنت) ولكن أدني اجمالي عدد فريعات (417 فريع) وأقل اجمالي تكلفة (20.73 دولار) فيما لو استخدم لتر واحد من الوسط .

الكلمات المفتاحية : إجمالي الفريعات، التكلفة الإجمالية، حجم الوسط، كثافة العزلة، فترات التحضين، أناناس.

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