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The Role of Cytokinin and IAA in the Propagation of Three Varieties of Finger Lime (*Citrus Australasica*) by Tissue Culture

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Abstract

The Experiment was carried out in the plant tissue culture laboratory of the Department of Horticulture and Garden Engineering / College of Agriculture / University of Diyala, to propagate three varieties of Finger Lime (*Citrus australasic*) (Green, Red and Black*) in vitro*, during Initiation stage, the effect of different concentrations of Kin was studied. If the concentration of 4 mg L^{-1} gave the highest response rate, the largest number and length of shoots, the largest number of leaves, and the lowest percentage of yellowing and leaves drop , the red variety had the highest response rate while The black variety gave the highest number and length of shoots and number of leaves and the lowest percentage of yellowing and leaves drop , During the multiplication stage, the effect of concentrations of IAA or BA was studied in combination with the best concentration of Kin in multiplying single node explants explant and planting them in MS medium. The results showed that after 6 weeks, the highest rate of number and length of shoots and average number of while The black variety outperformed the other varieties by giving the highest number and length of shoots, number of leaves and the lowest percentage of yellowing and leaves drop, As for BA gave the highest concentration of 1.0 mg L^{-1} length of shoots, while the concentration of 2.0 mg L^{-1} gave the highest number of leaves, and the concentration of 3.0 mg L^{-1}, it gave the highest amount of callus formation,. As for the varieties, the black variety outperformed by giving the highest percentage of shoots, shoot length, number of leaves and the lowest percentage of yellowing and leaves drop, As for callus formation, the green variety gave the largest amount of callus formation.

Keywords: Finger Lime, micropropagation, Cytokinin , IAA, in vitro

Introduction

Finger limes are cylindrical fruits belonging to the citrus genus and their taste like lemon but are generally less sour. They are native to the Australian rainforests, and trees were found growing wild in coastal regions of Western Australia and were planted in the Mediterranean region and moved to the rest of the world (1) Initial experiments to develop successful and effective protocol for the micropropagation of finger lime failed due to excessive leaves drop and thus the death of the plants, which is caused by the increase in the production of ethylene and phenolic substances in the vitro culture vessels on the one hand, and on the other hand, internal hormones play an important role in regulating the growth and development of the plant and any deficiency in their levels leads to leaves drop (2) (3). Plant tissue culture cannot be successful without the use of plant growth regulators, which are organic compounds and in very small quantities are effective and play a fundamental role in regulating the growth and development of the plant. The most commonly used are auxins and cytokinins because they It plays a major role in stimulating cell division and expansion, as well as encouraging vegetative growth in the plant, encouraging cell division and breaking the apical dominance, and stimulating the growth of lateral shoots and axillary buds of the plant (4 and 5), and in a study conducted by (2) when multiplied the tips of the shoots of the Finger Lime(*Citrus australasica* var. Tasty Green finger lime) in a full MS medium and half the strength of the salts and adding BA or Kin to the medium increased the emergence rate, number and length of the shoots after 6 weeks of culturing

Materials and methods

The Experiment was conducted in the Plant Tissue Culture Laboratory of the Department of Horticulture and Garden Desgin at the College of Agriculture, University of Diyala in 2024

* Explant preparation and inoculation

The nodal explant were sterilized with sodium hypochlorite solution obtained from a commercial solution containing 6% Sodium Hypochlorite NaOCl at a concentration of 2%, then the volume was completed to 100 ml with sterile distilled water for 15 minutes, after which the parts were washed with distilled water four times in a row to remove any trace of the sterile solution to avoid damage to plant tissues. In an experiment conducted by (6), this method was effective in reducing contamination and not causing damage to the treated explant, and the nodal were grown on MS medium (7) which contains some modifications in it including (Thiamine, Nicotinic acid and Pyridoxine HCl at a concentration of 1 mg L^{-1} + 100 mg L^{-1}

myo-Inositol + 40 mg L^{-1} adenine sulfate + 400 mg L^{-1} PVP + 0.2 mg L^{-1} GA_3 with Kin added at different concentrations (0, 2, 4, 6, 8) mg L^{-1} and the data were taken after 6 weeks of cultivation.

* **Plant growth regulators on shoot regeneration and Multiplication stage**:

Based on the results obtained from the emergence experiments, the shoots resulting from the node explants were used without cutting and with a length of approximately 1 cm and were grown on MS medium with the best concentration of 4 Kin mg L^{-1} In combination with different concentrations of IAA (0.0, 0.1, 0.2, 0.4) mg L^{-1} or BA at concentrations (0.0, 1.0, 2.0, 3.0, 4.0) mg L^{-1}, one explant was planted with ten replicates for each treatment and the results were taken after 6 weeks, and the plants were incubated in a growth chamber at a temperature of 25 ± 1 °C and a light intensity of 3000 lux for 16 hours of light and 8 hours of darkness. The experiments were arranged according to a completely randomized design (CRD) and the data were analyzed according to Duncan's multiple range test at 5% level using SAS program (8).

Results and Discussion

***Effect of Finger Lime varieties, Kin concentrations and their interaction on the emergence of single node explants**

The results in Table (1) and Figure (1) showed that the red variety outperformed the rest of the varieties by giving the highest response rate of 96 (%), and the black variety outperformed by giving the largest number and length of shoots and number of leaves of 1.560 (shoot node explant^{-1}) and 0.832 (cm) and 7.480 (leaves shoot^{-1}) respectively, and the green variety gave the largest amount of yellowing and leaves drop of 50.00 (%), and as for the effect of concentrations, the concentration of 4 mg L^{-1} outperformed by giving the largest response rate of number and length of shoots and number of leaves and the lowest yellowing rate of 100 (%) and 1.867 (shoot node explant^{-1}) and 1.020 (cm) and 7.733 (leaves shoot^{-1}) and 16.67 (%) Respectively, as for the interaction between varieties and concentrations, the black variety with a concentration of 4 mg L^{-1} gave the highest response rate, the largest number and length of shoots, and the lowest yellowing rate, which amounted to (%) 100 and 2.400 (shoot node explant^{-1}) and 1.150 (cm) and 0.00 (%), and the black variety with a concentration of 2 mg L^{-1} gave the largest amount of the number of leaves, which amounted to 9.00 (leaves shoot^{-1}).

Table (1) shows the effect of varieties and concentrations of Kin and the interaction between them on the response rate, average number of shoots (shoot node explant^{-1}), shoot length (cm), number of leaves (leaves shoot^{-1}), and yellowing rate (%) of Finger Lime after 6 weeks of cultivation on MS medium.

Traits / Treatments	response %	Shoots number	Shoots length	Leaves number	Yellowing (%) percentage
Effect of varieties					
Green	88.00 A	1.020 B	0.642 B	5.300 C	50.00 A
Red	96.00 A	1.100 B	0.660 B	6.580 AB	38.00 A
Black	94.00 A	1.560 A	0.832 A	7.480 A	20.00 B
Effect of Kin mg L^{-1}					
Control	80.00 B	1.100 B	0.503 C	5.300 C	46.67 AB
2	96.67 A	1.167 B	0.750 B	7.367 AB	33.33 BC
4	100.00	1.867	1.020	7.733	16.67

		A	A	A	A	C
6		90.00 AB	1.067 B	0.637 BC	5.733 C	30.00 BC
8		96.67 A	0.933 B	0.647 BC	6.133 BC	53.33 A
Effect of varieties X Kin mg L^{-1}						
control	Green	60.00 b	0.600 c	0.350 e	3.500 e	50.00 a
	Red	90.00 a	0.900 bc	0.450 de	4.800 de	50.00 a
	Black	90.00 a	1.800 ab	0.710 bcde	7.600 abcd	40.00 ab
2	Green	90.00 a	1.000 bc	0.600 cde	5.700 cde	50.00 a
	Red	100.00 a	1.100 bc	0.780 bcde	7.400 abcd	50.00 a
	Black	100.00 a	1.400 bc	0.870 abc	9.000 a	00.00 c
4	Green	100.00 a	1.600 ab	0.990 ab	6.300 abcde	40.00 ab
	Red	100.00 a	1.600 ab	0.920 abc	8.200 abc	10.00 bc
	Black	100.00 a	2.400 a	1.150 a	8.700 ab	0.00 c
6	Green	90.00 a	1.000 bc	0.550 cde	4.900 de	50.00 a
	Red	90.00 a	0.900 bc	0.580 cde	5.500 cde	50.00 a
	Black	90.00 a	1.300 bc	0.780 bcde	6.800 abcd	10.00 c
8	Green	100.00 a	0.900 bc	0.720 bcde	6.100 bcde	60.00 a
	Red	100.00 a	1.000 bc	0.570 cde	7.000 abcd	50.00 a
	Black	90.00	0.900	0.650	5.300	50.00

		a	bc	bcde	de	a

*Coefficients with similar letters for each factor or the interaction between them are not significantly different according to Duncan's multiple range test at the 0.05 probability level.

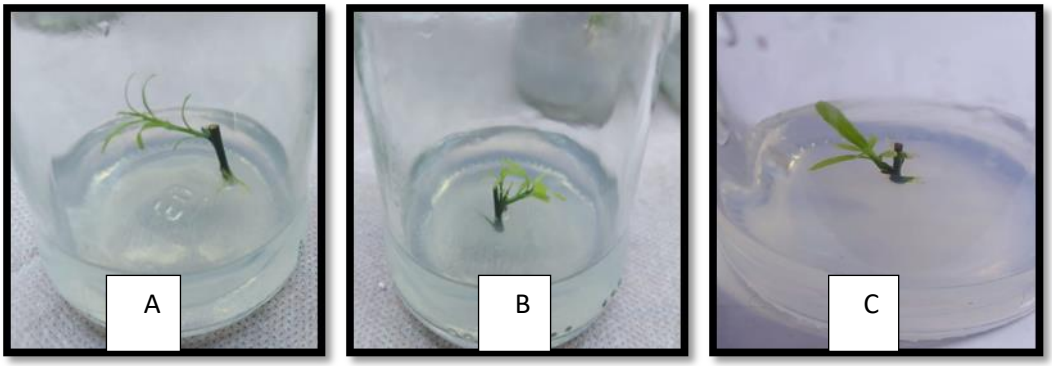

Figure (1): Shows the emergence of single node explants of three varieties of Finger Limeat the best concentration of 4 mg L^{-1} of Kin on MS medium after 6 weeks of cultivation,(A) Green, (B) Red, (C) Black.

***Effect of Finger Lime varieties, IAA concentrations and their interaction on shoot multiplication**

The results in Table (2) showed that the black variety outperformed the rest of the varieties by giving the largest number and length of shoots and the number of leaves, which reached 1.950 (shoot node explant-1), 1.547 (cm) and 10.95 (leaves $shoot^{-1}$), respectively. The green variety gave the largest amount of yellowing and leaves drop, which reached 4.025 (%). As for the effect of concentrations, it did not significantly affect the number of shoots, noting that the comparison treatment gave the largest amount of leaves, which reached 1.967 (shoot node explant-1). The longest length of shoots and the largest number of leaves in the comparison treatment reached 1.683 (cm) and 11.20 (leaves $shoot^{-1}$), respectively. As for the concentration of 0.1 mg L^{-1}, it gave the largest percentage of yellowing and leaves drop, which reached 3.000 (%). As for the interaction between varieties and concentrations The black variety with a concentration of 0.1 mg L^{-1} gave the highest number of shoots, reaching 2,100 (shoot node explant-1), and the comparison treatment and the black variety gave the highest shoot length and the highest number of leaves, reaching 2,200 (cm) and 12.70 (leaves $shoot^{-1}$), and the comparison treatment and the green variety gave the highest amount of yellowing and leaves drop, reaching 5,200.

Table (2) shows the effect of varieties, IAA concentrations and their interaction on the average number of shoots (shoot node $explant^{-1}$), shoot length (cm), number of leaves (leaves $shoot^{-1}$) and yellowing percentage (%) of Finger Lime after 6 weeks of cultivation on MS medium

Traits / Treatment	Shoots number	Shoots length	Leaves number	Yellowing (%) percentage
Effect of varieties				
Green	1.500 B	1.142 B	8.500 B	4.025 A
Red	1.750 AB	1.225 AB	9.250 AB	2.575 B
Black	1.950 A	1.547 A	10.95 A	1.125 C
Effect of IAA mg L^{-1}				

Control		1.867 A	1.683 A	11.20 A	2.867 A
0.1		1.733 A	1.330 AB	9.967 AB	3.000 A
0.2		1.633 A	1.203 B	8.433 B	2.233 A
0.4		1.700 A	1.003 B	8.667 B	2.200 A
Effect of varieties X IAA mg L^{-1}					
control	Green	1.800 ab	1.380 bc	10.90 ab	5.200 a
	Red	1.800 ab	1.470 bc	10.90 abc	2.400 bc
	Black	2.000 ab	2.200 a	12.70 a	1.000 c
0.1	Green	1.300 b	1.050 bc	8.300 bc	3.200 abc
	Red	1.800 ab	1.240 bc	9.800 abc	3.700 bc
	Black	2.100 a	1.700 ab	11.80 ab	2.100 bc
0.2	Green	1.600 ab	1.250 bc	8.900 abc	4.000 ab
	Red	1.600 ab	1.190 bc	7.800 bc	1.900 bc
	Black	1.700 ab	1.170 bc	8.600 abc	0.800 c
0.4	Green	1.300 b	0.890 c	5.900 c	3.700 ab
	Red	1.800 ab	1.000 bc	9.400 abc	2.300 bc
	Black	2.000 ab	1.120 bc	10.70 ab	0.600 c

*Coefficients with similar letters for each factor or the interaction between them are not significantly different according to Duncan's multiple range test at the 0.05 probability level.

***Effect of Finger Lime varieties, BA concentrations and their interaction on shoot multiplication**

The results in Table (3) and Figure (2) showed that the black variety was superior to the rest of the varieties by giving the largest number and length of shoots and the number of leaves, which reached 4.340 (shoot node explant^{-1}), 1.766 (cm) and 20.96 (shoot leaves^{-1}), respectively. The green variety gave the largest amount of yellowing and leaves drop, which reached 5.780 (%) and the largest amount of callus, which reached 60.00 (%). As for the effect of the concentrations, they did not significantly affect the number of shoots, the percentage of yellowing and leaves drop, while the concentration of 1 mg L^{-1} gave the highest length of shoots, 1.730 (cm), and the concentration of 2 mg L^{-1} gave the largest number of leaves, which reached 18.57 (shoot leaves^{-1}), while the concentration of 3 mg L^{-1} gave the largest percentage of callus formation, which reached 26.67 (%). As for the interaction between the varieties and concentrations, the black variety with a concentration of 2 mg L^{-1} gave the largest number of shoots, the highest length of shoots, and the largest amount of leaves, which reached 5,400 (shoot node explant^{-1}), 2,300 (cm), and 28.10 (leaves shoot^{-1}). The green variety with a concentration of 4 mg L-1 gave the largest amount of yellowing and leaves drop, which reached 6,800 (%), and the largest amount of callus was found in the green variety with a concentration of 2 mg L^{-1}, which reached 80 (%).

Table 3 shows the effect of varieties, BA concentrations and their interaction on the average number of shoots (shoot node explant $^{-1}$), shoot length (cm), number of leaves (leaves shoot^{-1}) and yellowing percentage (%) of Finger Lime after 6 weeks of cultivation on medium.

Traits / Treatments		Shoots number	Shoots length	Leaves number	Yellowing (%) percentage	Callus (%) percentage
Effect of varieties						
Green		3.200 B	1.490 AB	13.60 B	5.780 A	60.00 A
Red		2.740 B	1.246 B	13.74 B	3.200 B	0.00 B
Black		4.340 A	1.766 A	20.96 A	1.400 C	0.00 B
Effect of BA mg L^{-1}						
Control		3.066 A	1.460 AB	14.63 B	3.167 A	10.00 B
1		3.233 A	1.730 A	16.73 AB	3.567 A	23.33 AB
2		3.700 A	1.523 AB	18.57 A	3.200 A	23.33 AB
3		3.433 A	1.240 B	15.87 AB	3.667 A	26.67 A
4		3.700 A	1.550 AB	14.70 B	3.700 A	16.67 AB
Effect of varieties X BA mg L^{-1}						
control	Green	2.400 c	1.380 c	8.900 e	4.800 abcde	30.00 c

	Red	2.700 bc	1.240 c	13.80 cde	2.700 cdefg	0.00 b
	Black	4.100 abc	1.760 abc	21.20 b	2.000 efg	0.00 b
1	Green	3.200 bc	2.250 ab	18.00 bcd	5.300 ab	70.00 ab
	Red	2.400 c	1.360 c	12.00 de	4.700 abcdef	0.00 b
	Black	4.100 abc	1.580 abc	20.20 bc	0.700 g	0.00 b
2	Green	2.800 bc	1.020 c	11.00 ed	5.700 abc	70.00 ab
	Red	2.900 bc	1.250 c	16.60 bcd	2.400 defg	0.00 b
	Black	5.400 a	2.300 a	28.10 a	1.500 fg	0.00 b
3	Green	3.700 bc	1.150 c	15.60 bcde	6.300 ab	80.00 a
	Red	2.900 bc	1.030 c	14.50 bcde	2.700 cdefg	0.00 b
	Black	3.700 bc	1.540 bc	17.50 bcd	2.000 efg	0.00 b
4	Green	3.900 abc	1.650 abc	14.50 bcde	6.800 a	50.00 bc
	Red	2.800 bc	1.350 c	11.80 de	3.500 bcdefg	0.00 b
	Black	4.400 ab	1.650	17.80 bcd	0.800 g	0.00 b

*Coefficients with similar letters for each factor or the interaction between them are not significantly different according to Duncan's multiple range test at the 5% probability level.

A

B

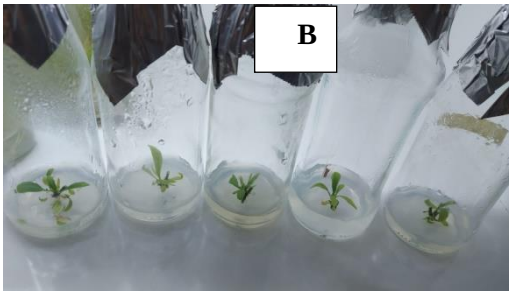

Figure (3) shows the effect of varieties and concentrations of BA + 4 mg L^{-1} of Kin and the interaction between them on the multiplication of single nodules of Finger Lime and their cultivation on MS medium after 6 weeks (A) Green variety, (B) Red variety, (C) Black variety, (D) callus formation.

The significant differences between the varieties in the studied traits during the emergence and multiplication stage are due to the existence of a difference in the growth strength of the seedlings of the three varieties, which is a genetic trait linked to the genetic structure of each variety and accompanies it throughout life when the environmental conditions are suitable for growth. The differences in the values of some of the studied traits may also be due to the difference in the degree of maturity, differentiation and cell tissues of the mother seedlings, and thus the difference in their nutritional and hormonal contents, as the treatments that had a significant superiority in the values of their traits achieved a hormonal and nutritional balance to accelerate the opening of the buds and their growth into shoots. The addition of cytokinins to the nutrient medium leads to an increase in its concentration inside the plant shoots, which leads to a reduction in the role of accumulated auxins responsible for cell elongation and thus a reduction in the length of the shoots (9) or the reason may be that the exchange between cytokinins and auxins in the cultivated plant part may be attributed to the importance of these two regulators in cell division and elongation, and that the effectiveness of cytokinin in multiplication events increases with the presence of auxin in the nutrient medium (10) and that the reason for the yellowing of leaves and their excessive drop is due to the increased production of ethylene inside the culture vessels, which leads to inhibition of cell division on the one hand, and on the other hand, internal hormones play an important role in regulating plant growth and development, and any deficiency in their levels leads to leaves drop (11). As for the reason for callus formation, it may be attributed to an imbalance in the hormonal balance between inside and outside the cell, and thus the requirements of the plant cell cycle are not completed. (3)

Conclusion

It was necessary to add (Thiamine, Nicotinic acid and Pyridoxine HCl at a concentration of 1 mg L^{-1} + 100 mg L^{-1} myo-Inositol + 40 mg L^{-1} adenine sulfate + 400 mg L^{-1} PVP + 0.2 mg L^{-1} GA3) to MS medium for the regeneration and multiplication of single nod explant of Finger Lime. As for kin, the concentration of 4 mg L-1 gave the best emergence rate, while lAA gave the concentration of 0.1 mg L-1 the highest number of shoots, and BA gave the concentration of 4.2 mg L-1 the highest number of shoots. As for the varieties, the Black variety outperformed the rest of the varieties in all characteristics.

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