The Effect of Cytokines And Gibberellins on the Multiplication of Single Nodal Explants of Finger lemon (*Citrus Australasica*) Regulate of Yellowing And leaf Abscission

Noor ALhuda Dawood Salman[1]* , Ayad Assi Obaid [2 *]
[2]Department of Horticulture and Landscape, College of Agriculture, University of Diyala. Iraq
*Corresponding autho's email:
nouralhudadawoodsalman@gmail.com,
ayadassi73@gmail.com

Abstract
The Experiment was conducted in the Plant Tissue Culture in vitro of the Department of Horticulture and Garden Engineering - College of Agriculture - University of Diyala for the year 2024-2025. In the Initiation stage, the effect of different concentrations of BA (0, 0.5, 1, 1.5, 2) mg. L^{-1} was studied, and the effect of Kin concentrations (0, 2, 4, 6, 8) mg. L^{-1} was studied. During the multiplication stage, the effect of different concentrations of GA3 (0.5, 1.0, 1.5, 2.0, 2.5, 10.0) mg. L^{-1} added to MS medium was studied, with the aim of developing a successful program for the propagation of three varieties of Finger lemon *Citrus australasic* outside in vitro, as the concentrations (0.5, 1.5, 2 mg. L^{-1} Concentrations (0.5, 1.5, 2) mg. L^{-1} gave the highest regeneration rates and the largest number of shoots, with the green variety giving the highest regeneration rate. gave the highest shoot length and the highest average number of leaf.while the concentration of 4 mg.L^{-1} gave the highest regeneration rate, the largest number of shoots and shoots length and the lowest percentage of yellowing and leaf Abscission. The concentration of 1.5 and 10 gave the largest number of shoots, while the concentrations of (1.0, 1.5, 2.0) mg. L^{-1} gave the largest average number of leaves and the black variety outperformed the rest of the varieties.

Keywords: Finger lemon, micropropagation, $GA_{3.}$

Introduction

Finger lemon (*Citrus australasica* F. Muell), formerly known as Microcitrus australasica F. Muell Swingle, also known as finger lemon, belongs to the Rutaceae family (2:12). The trees grow mainly in their native subtropical rainforests of southeast Queensland, which extends from the Tambourine Mountains to the Richmond River in northeastern New South Wales. They are now cultivated in almost all tropical and subtropical climates, including southeastern New Guinea. They were introduced into Australia, the European Union, Asia, the United Kingdom, Canada, Japan, France, and China in the mid-20th century (3:18). They are small, thorny, vigorous trees or shrubs, reaching 6 m in height, with thorns up to 25 mm long. The buds are purple, and the flowers are small, white or pale pink. Finger lemons flower year-round, beginning at 2-3 years and continuing for 15- 16 years, the fruit is cylindrical and can reach 12 cm in length. One of the most unique characteristics of the fruit is the oval or spherical pulp vesicles, also known as pearls. All trees prefer well-drained soil, with a pH between 5 - 6.5) (8; 5: 18, 16). Plants are a rich source of vitamins, including vitamin C, citric acid, and vitamin E, and a rich source of essential minerals such as calcium, magnesium, phosphorus, and potassium. They also contain levels of phenolic compounds, anthocyanins, sugars, and carotenoids (17; 1). Establishing an effective protocol for

in vitro propagation is crucial for its success, but currently there is no effective tissue culture protocol for this species. Initial experiments to develop finger lemon production failed due to excessive leaf drop caused by increased ethylene production in culture containers (13). It can be said that plant tissue culture cannot be successful without the use of plant growth regulators, which are effective organic compounds in very small quantities that play a fundamental role in regulating growth processes in plants and are most commonly used in tissue cultures. Plant cytokinins are involved (6).

Materials and Methods

1-Effect of varieties, BA concentrations, and their interaction on the development of explants on MS medium

The nodal explants (of the three Finger lemon varieties: green, red, and black) were sterilized with a 20% sodium hypochlorite solution (NaOCl) for 15 minutes (13). The Explant were then washed with distilled water four times to remove the sterile solution and avoid damage to plant tissue. During this stage, the response of single nodal explants and the effect of different concentrations of BA (0, 0.5, 1, 1.5, and 2) mg. L^{-1} to MS medium (9) on the growth of lateral shoots and the interaction between varieties and BA were studied. One nodal explant was planted in each tubs , and results were taken after 6 weeks, response rate (%), number of shoots (nodal $shoot^{-1}$), and length shoots (cm). the number of leaf (leaf $shoot^{-1}$) and rate of yellowing and leaf Abscission (%)

2- The effect of varieties, Kin concentrations, and their interaction on the development of explants on MS

medium

During this stage, the response of individual nodal explants of the three Finger Lemon varieties was studied. The effect of adding different concentrations of Kin (0, 2, 4, 6, and 8) $mg.L^{-1}$ to MS medium on the growth of lateral shoots was also studied, as was the interaction between varieties and Kin concentrations. One nodal explant was planted in each tubs, and results were taken after 6 weeks. These included the growth response of the nodal explants (%), the number of shootes (shoot nodal $^{-1}$), the length of the shoots (cm), the number of leaf (leaf $shoots^{-1}$), and the rate of yellowing and leaf Abscission (%).

3- Effect of varieties and GA3 concentrations on the multiplication of single nodal explants of Finger lemon.

Based on the results obtained from the emergence experiments, the shoots produced from the nodal explants were used without pruning and approximately 1 cm long. They were cultivated on MS medium with the best concentration of 2 BA + 4 Kin $mg.L^{-1}$, mixed with different GA3 concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 10.0) mg. L^{-1} (15). One explant was cultivated with 10 replicates for each treatment. The results were taken after 6 weeks. The explants were incubated in a growth chamber at 25 ± 1°C and a light intensity of 3000 lux for 16 hours of light and 8 hours of darkness. All experiments were arranged as factorial experiments (with two factors) according to a completely randomized design (CRD). The data were analyzed using the ready-made SAS (19) and the means were compared according to Duncan's multiple range test at a 5% probability level for all coefficients

Results and Discussion

3-Effect of varieties, BA concentrations, and their interaction on the development of single nodal explants of Finger lemon.

The results in Table (1) and

Figure (1) show that there were no significant differences in all traits regarding the effect of varieties. As for the effect of BA concentrations, the concentrations of 0.5, 1.5, and 2 mg. L^{-1} gave the highest response rate of 83.33% and the highest number of shootes, reaching 1.133, 1.100, and 1.133 (nodal explant. $shoot^{-1}$), respectively. As for shoot length and leaf number, the concentration of 1.5 mg L^{-1} gave no significant differences from the other concentrations, with the exception of the control treatment, which gave the lowest values of 0.356 (cm) and 3.067 (leaf . $shoot^{-1}$), respectively. As for yellowing and leaf Abscission , there were no significant differences between them. The results are shown in the same table for the two-way interaction, as the concentration gave 0.5 mg. L^{-1} and the green variety had the highest response rate, reaching 100%. As for the number of shootes, 0.5 mg. L^{-1}, the green variety, and 2 mg. L^{-1} and the black variety produced the highest number of shootes, reaching 1,400 (nodal .explant $shoot^{-1}$) for each concentration. No significant differences were observed for shoot length. The highest number of leaf was recorded at 1.5 mg. L^{-1} and the red variety, reaching 6.00 (leaf .$shoot^{-1}$). No significant differences were observed for yellowing and leaf Abscission , as leaf Abscission occurred in all varieties and at all concentrations.

Table 1: The interaction effect between Finger Lemon and BA concentrations. After 6 weeks cultivation on MS medium.

Traits / Treatments		Regeneration (%)	Shoots Number	Shoots length	Leaf number	yellowing and leaf Abscission (%) n
Effect of varieties						
Green		78.00 A	0.940 A	0.510 A	3.840 A	76.00 A
Red		74.00 A	0.980 A	0.524 A	4.400 A	68.00 A
Black		76.00 A	1.060 A	0.534 A	4.440 A	60.00 A
Effect of BA mg L^{-1}						
0		56.67 B	0.667 B	0.356 B	3.067 B	80.00 A
0.5		83.33 A	1.133 A	0.550 AB	4.367 AB	60.00 A
1		73.33 AB	0.933 AB	0.470 AB	4.200 AB	80.00 A
1.5		83.33 A	1.100 A	0.670 A	5.267 A	63.33 A
2		83.33 A	1.133 A	0.567 AB	4.233 AB	56.67 A
Effect of varieties X BA mg L^{-1}						
0	Green	50.00 b	0.500 C	0.330 a	2.600 b	90.00 a

	Red	60.00 ab	0.700 Bc	0.310 a	2.800 b	80.00 a
	Black	60.00 ab	0.800 Abc	0.430 a	3.800 ab	80.00 A
0.5	Green	100 a	1.400 A	0.610 a	4.400 ab	60.00 ab
	Red	70.00 ab	1.000 Abc	0.550 a	4.300 ab	60.00 ab
	Black	80.00 ab	1.000 Abc	0.490 a	4.400 ab	60.00 ab
1	Green	80.00 ab	1.000 Abc	0.510 a	4.000 ab	80.00 A
	Red	70.00 ab	0.800 Abc	0.470 a	4.400 ab	70.00 ab
	Black	70.00 ab	1.000 Abc	0.430 a	4.200 ab	80.00 a
1.5	Green	80.00 ab	1.000 Abc	0.640 a	4.500 ab	70.00 ab
	Red	90.00 ab	1.200 Ab	0.740 a	6.000 a	70.00 ab
	Black	80.00 ab	1.100 Abc	0.630 a	5.300 ab	50.00 ab
2	Green	80.00 ab	0.800 Abc	0.460 a	3.700 ab	80.00 a
	Red	80.00 ab	1.200 Ab	0.550 a	4.500 ab	60.00 ab
	Black	90.00 ab	1.400 A	0.534 a	4.500 ab	30.00 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.

2-The effect of varieties, Kin concentrations, and their interaction on the development of single nodal explants in Finger Lemon.

The results in Table(2) and Figure (1) showed that the black variety outperformed the other varieties, giving the highest response rate of 98%, with a total number of shootes of 1,320 (shoot. nodal explant^{-1}), shoot length of 0.696 cm, number of leaf of 6,040 (leaf .shoot^{-1}), and the lowest percentage of yellowing and leaf Abscission of 52.0%. The green and red varieties gave the highest amount of yellowing and leaf Abscission of 76.68% for each variety. Regarding the effect of concentrations, the 4 mg. L^{-1} concentration gave the highest response rate, the highest number of shootes, and the longest shoot length of 96.67%, 1.767 (shoot. nodal explant^{-1}), 0.720 cm. The (2,4) mg. L^{-1} concentration gave the highest number leaf of 6,030. 6,500 (leaf .shoot^{-1}) respectively. As for the interaction between the varieties and concentrations, all varieties and concentrations yielded response rates ranging between 80, 90, and 100%. The black and red varieties, with a concentration of 4 mg. L^{-1}, produced

the largest number of shootes, reaching 1,900 and 2,200 (shoot. nodal explant-1) for each variety. Regarding shoot length, the concentrations of 2 and 4 mg L^{-1} and the black variety produced the highest shoot length, reaching 1,020 and 1.00 (cm) for each concentration. Regarding the number of leaf, the concentration of 2 mg. L^{-1} and the black variety produced the largest number of leaf, reaching 8,800 (shoot .nodal explant^{-1}). The green variety, the control treatment, and the concentration of 6 mg. L^{-1} produced the largest amount of yellowing and leaf Abscission , reaching 90%.

Table 2: The interaction effect between Finger Lemon and Kin concentrations. After 6 weeks cultivation on MS medium.

Traits / Treatments		Regeneration (%)	Shoots number	Shoots length	Leaf number	yellowing and leaf Abscission (%) n
Effect of varieties						
Green		78.00 B	0.680 C	0.372 B	3.460 B	76.00 A
Red		84.00 B	0.980 B	0.500 B	4.500 B	68.00 A
Black		98.00 A	1.320 A	0.696 A	6.040 A	52.00 B
Effect of Kin mg L^{-1}						
Control		73.33 B	0.500 C	0.267 C	3.033 B	83.33 A
2		90.00 AB	1.200 B	0.656 AB	6.033 A	60.00 B
4		96.67 A	1.767 A	0.720 A	6.500 A	33.33 C
6		90.00 AB	0.733 C	0.457 BC	4.267 B	83.33 A
8		83.33 AB	0.767 C	0.513 AB	3.500 B	66.67 AB
Effect of varieties X Kin mg L^{-1}						
control	Green	50.00 b	0.400 d	0.19 c	2.200 d	90.00 a
	Red	70.00 ab	0.400 d	0.240 c	2.700 d	80.00 ab
	Black	100.0 a	0.700 cd	0.370 bc	4.200 bcd	80.00 ab
2	Green	90.00 a	0.900 cd	0.430 bc	4.000 cd	70.00 abc
	Red	80.00 ab	1.000 cd	0.520 bc	5.300 bcd	70.00 abc
	Black	100.0 a	1.700 ab	1.020 a	8.800 a	40.00 cd
4	Green	90.00	1.200	0.430	5.500	50.00

		a	bc	bc	bcd	bc
	Red	100.0 a	1.900 a	0.730 ab	6.500 abc	40.00 cd
	Black	100.0 a	2.200 a	1.000 a	7.500 ab	10.00 D
6	Green	80.00 ab	0.400 d	0.330 bc	2.900 d	90.00 a
	Red	90.00 a	0.800 cd	0.510 bc	4.700 bcd	80.00 ab
	Black	100.0 a	1.000 cd	0.530 bc	5.200 bcd	80.00 ab
8	Green	80.00 ab	0.500 d	0.480 bc	2.700 d	80.00 ab
	Red	80.00 ab	0.800 cd	0.500 bc	3.300 cd	70.00 abc
	Black	90.00 a	1.000 cd	0.560 bc	4.500 bcd	50.00 bc

*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

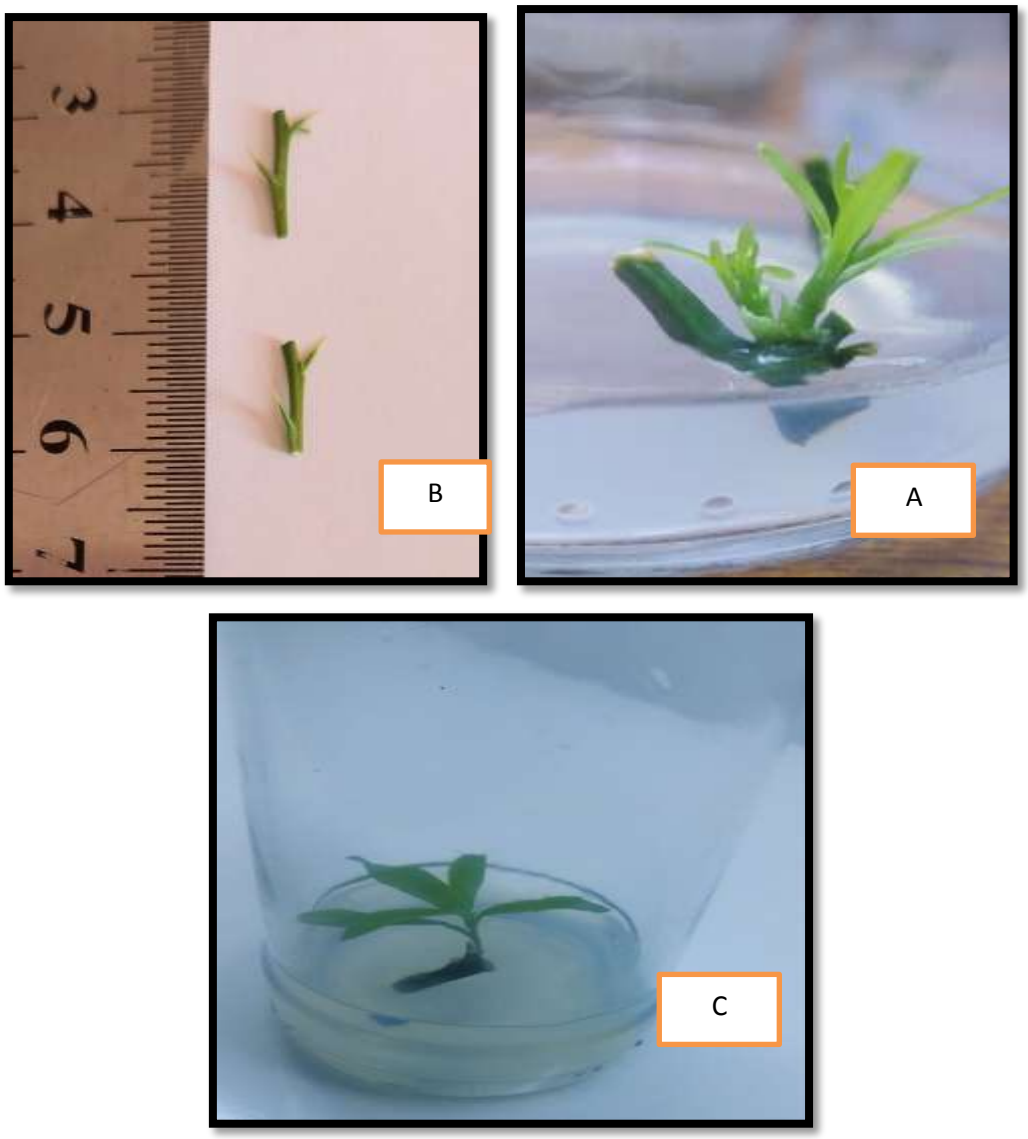

B
A
C

Figure (1): A. Nodal expants (B). Emergence of single nodal expants at the best concentration of 0.5 mg. L^{-1} of BA (C). Emergence of single nodal expants at the best concentration of 4 mg. L^{-1} of KIN on MS medium (D). Yellowing and leaf Abscission n in the green variety (E). Yellowing and leaf Abscission n in the black variety after 6 weeks of cultivation.

3-The effect of varieties, GA3 concentrations, and their interaction on themultiplication of single nodal explants of Finger lemon.

The results in Table (3) and Figure (1) showed that the black variety outperformed the other varieties, producing the largest number of shootes and leaf, reaching (shoot .nodal explant^{-1}) and 25.93 (leaf .shoot^{-1}), respectively. The green variety produced the longest shoot length, reaching 1.518 cm. The green and red varieties produced the largest amount of yellowing and leaf Abscission , reaching 25.93 and 25% for each variety. As for the effect of concentrations, the concentration of 1.5 and 10 mg. L^{-1} produced the largest number of shootes, reaching 4.00 (shoot . nodal explant^{-1}). As for the shoot length trait, no significant differences were observed between the concentrations. The concentration of 1, 1.5, and 2 mg. L^{-1} produced the largest number of leaf, reaching 21.40 and 23.37. 22.17 (leaf .shoot^{-1}) respectively. As for the interaction

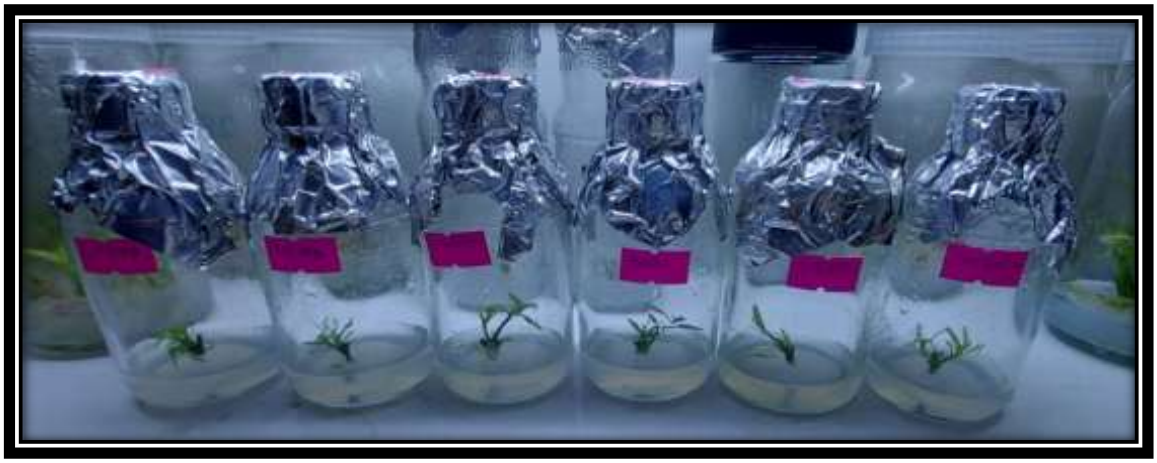
between the varieties and concentrations, the black variety with a concentration of 1.5 mg. L^{-1} gave the largest number of shootes, reaching 5,700 (shoot .nodal explant^{-1}). As for the shoot length trait, the concentration of 2 mg. L^{-1} gave the green variety the highest shoot length, reaching 1,970 (cm). As for the number of leaf, the concentration of 1.5 mg. L^{-1} and the black variety gave the largest amount, reaching 30.40 (shoot. nodal explant^{-1}). The green variety with a concentration of 0.5 mg. L^{-1} gave the largest amount of yellowing and leaf Abscission , reaching 60.00 (%).

Table 3: The interaction effect between Finger Lemon and GA_3 concentrations. After 6 weeks cultivation on MS medium.

Traits / Treatment		Shoots number	Shoots length	Leaf number	yellowing and leaf abscission (%)
Effect of varieties					
Green		3.100 B	1.518 A	17.00 B	25.93 A
Red		3.367 B	1.237 B	19.28 B	25.00 A
Black		4.467 A	1.207 B	25.93 A	6.667 B
Effect of GA_3 mg L^{-1}					
0.5+2BA+4Kin		2.967 B	1.180 A	16.73 B	40.00 A
1+2BA+4Kin		3.633 A B	1.497 A	21.40 A	26.67 A B
1.5+2BA+4Kin		4.000 A	1.363 A	23.37 A	20.00 A B
2+2BA+4Kin		3.867 A B	1.393 A	22.17 A	10.00 B
2.5+2BA+4Kin		3.400 A B	1.267 A	20.27 A B	13.33 B
10.0+2BA+4Kin		4.000 A	1.223 A	20.50 A B	16.67 B
Effect of varieties X GA_3 mg L^{-1}					
0.5+2BA+4Kin	Green	3.000 cd	1.380 ab	14.50 de	60.00 A
	Red	3.000 cd	1.070 b	16.50 cde	40.00 ab
	Black	2.900 cd	1.090 b	19.20 cde	20.00 ab
1+2BA+4Kin	Green	2.900 cd	1.610 ab	17.60 cde	40.00 ab

	Red	3.100 bcd	1.350 ab	18.60 cde	20.00 ab
	Black	4.900 ab	1.530 ab	28.00 ab	20.00 ab
1.5+2BA+4Kin	Green	2.800 cd	1.610 ab	18.40 cde	30.00 Ab
	Red	3.500 bcd	1.290 ab	21.30 c-e	30.00 Ab
	Black	5.700 a	1.190 b	30.40 a	0.00 B
2+2BA+4Kin	Green	3.600 bcd	1.970 a	22.10 a-d	10.00 B
	Red	3.500 bcd	1.160 b	19.30 cde	20.00 Ab
	Black	4.500 a-d	1.050 b	25.10 abc	0.00 B
2.5+2BA+4Kin	Green	2.600 d	1.260 b	13.10 e	20.00 ab
	Red	3.000 cd	1.220 b	19.30 cde	20.00 ab
	Black	4.600 abc	1.320 ab	28.50 ab	0.00 B
10.0+2BA+4Kin	Green	3.700 bcd	1.280 ab	6.30 cde	30.00 ab
	Red	4.100 a-d	1.330 ab	20.80 b-e	20.00 ab
	Black	4.200 a-d	1.060 b	24.40 abc	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




C


D

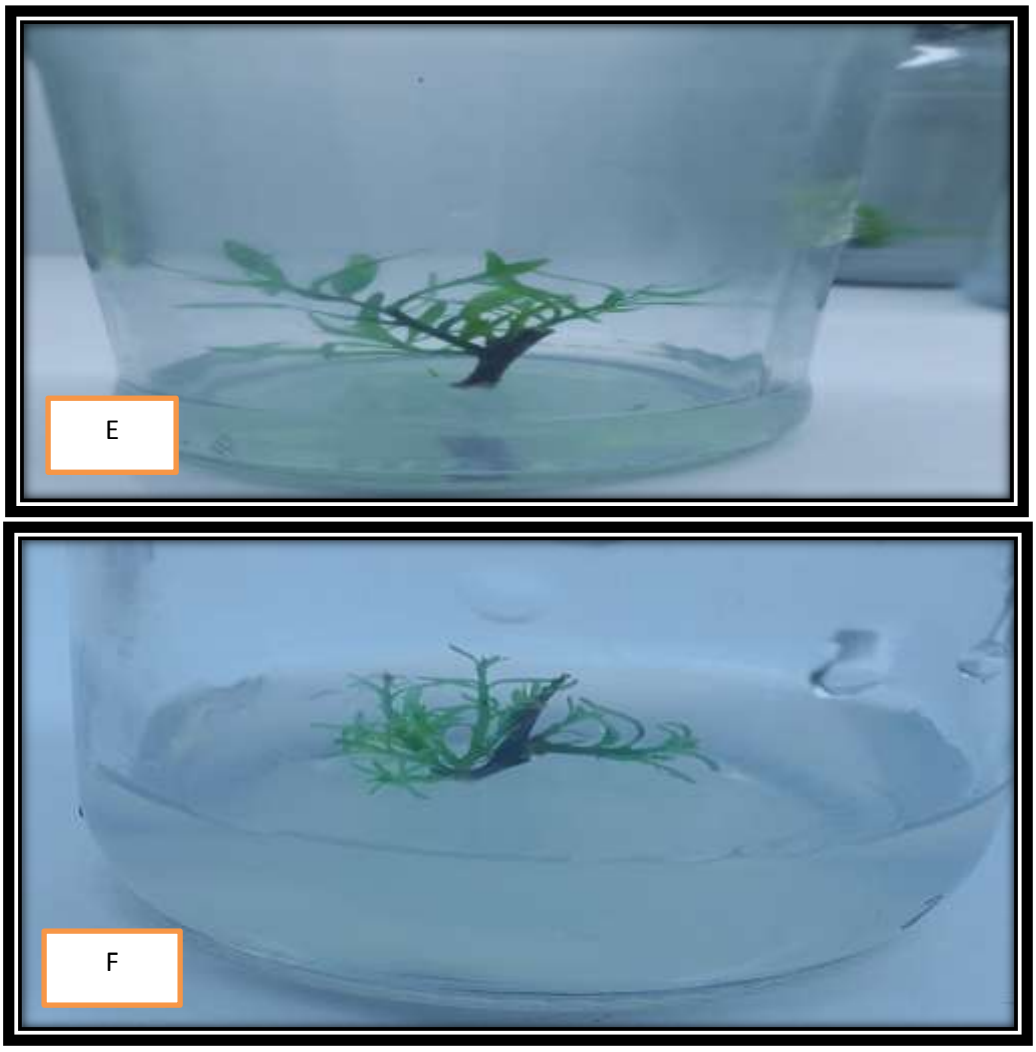


Figure (2): Shows the effect of GA3 concentrations on the three Finger lemon varieties: A- green, B- red, C- black ,D- Single nodal explants of the black variety are multiplied, E- Single nodal explants of the Green variety are multiplied, F- Single nodal explants of the Red variety are multiplied .

It is known that all plants produce ethylene in vitro (9). However, excess ethylene inhibits cell division, and high levels of ethylene lead to leaf Abscission n (4; 10). The addition of growth regulators to the basal medium is important for modifying the growth of cultivated plant explants. Previous studies have shown that propagation of citrus species in vitro requires cytokinins and gibberellins in the culture medium (7; 20). Meanwhile, Abscission n in citrus is a complex phenomenon, and hormonal signals play a major role in its overall regulation. The addition of higher levels of GA_3 improved primary bud regeneration by stimulating the shoots to break dormancy, emerge side by side, produce more leaf, and reduce leaf Abscission. This indicates that the positive function of GA_3 is cell division and organ formation, with its role in cell elongation through interaction with growth factors present in the medium (15)

Conclusion

It was found that adding BA and Kin to the MS medium is necessary for the emergence of Shoot from the nodal explants. The concentration of 2 mg. L^{-}

^{1} BA and 4 mg. L^{-1} Kin gave the best percentages during the Initiation stage. During the multiplication stage, the concentration of 1.5 mg. L^{-1} GA_3 gave the best percentage of Shoots multiplication stage. The addition of high concentrations of GA_3 improved the growth of Shoot and reduced leaf Abscission. Replanting should be done every three weeks to reduce yellowing and leaf Abscission. The black variety outperformed the other varieties .

References

1- Adhikari, B., Dutt, M., and Vashisth, T. (2021). Comparative phytochemical analysis of the fruits of four Florida-grown finger lime (Citrus australasica) selections. *LWT*, *135*, 110003

2-Cai, Q. N., Wang, H. X., Chen, D. J., Ke, X. R., Zhu, Z. X., & Wang, H. F. (2022). The complete chloroplast genome sequence of a Citrus australasica cultivar (Rutaceae) . *Mitochondrial DNA Part B*, *7*(1), 54-55.

3-Chandrika Ramadugu, C. R., Razi, M. F., Keremane, M. L., Scora, R. W., & Roose, M. L. (2017). Systematic classification, distribution and botany. In *The lime: botany, production and uses* (pp. 12-36). Wallingford UK: CABI.

4-Constabel F, Kurz WG, Chatson KB, Kirkpatrick JW (1977) Partial synchrony in soybean cell suspension cultures induced by ethylene. Exp Cell Res 105:263–268.

5-Delort, E., and Yuan, Y. M (2018). Finger lime/the Australian Finger—*Citrus australasica*. In *Exotic fruits* (pp. 203-210). Academic Press.

6-George , E.F. ; M.A. Hall and G.J. Deklerk (2008). Plant propagation by tissue culture . Vol. 1 . The Background . 3rd Edition. Published by Springer , The Netherlands.

7-Germana, M. A., Micheli, M., Chiancone, B., Macaluso, L., and Standardi, A. (2011). Organogenesis and encapsulation of in vitro-derived propagules of Carrizo citrange [*Citrus sinensis* (L.) Osb.× *Poncirius trifoliata* (L.) Raf]. Plant Cell, Tissue and Organ Culture (PCTOC), 106, 299-307.

8- Hawkeswood, T. J. (2017). A review of some publications concerning Citrus (Microcitrus) australasica F. Muell.(Rutaceae) in Australia and South-east Asia (mostly Thailand). *Calodema*, *581*, 1-14

9-Iqbal, N., Khan, N. A., Ferrante, A., Trivellini, A., Francini, A., and Khan, M. I. R. (2017). Ethylene role in plant growth, development and senescence: interaction with other phytohormones. Frontiers in plant science, 8, 475.

10-Jackson MB and Osborne DJ (1970) Ethylene, the natural regulator of leaf Abscission n. Nature 225:1019–1022.

11-Johnson, J. B., Batley, R., Manson, D., White, S., & Naiker, M. (2022). Volatile compounds, phenolic acid profiles and phytochemical content of five Australian finger lime (Citrus australasica) cultivars. *LWT*, *154*, 112640

12-Mabberley, D. J. (1998). Australian Citreae with notes on other Aurantioideae (Rutaceae). *Telopea*, *7*(4), 333-344

13-Mahmoud, L. M., Grosser, J. W., and Dutt, M. (2020). Silver compounds regulate leaf Abscission and improve *in vitro* regeneration from mature tissues of Australian finger lime (*Citrus australasica*). Plant Cell, Tissue and Organ Culture

(PCTOC), 141, 455-464.

14-Murashige T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant,15:473-497 .

15-Murugan, T, Prakash Awasthi, O, Kumar Singh, S, Kumar Dubey, A, and Kumar, K (2022). Modified protocol to overcome *in vitro* recalcitrance of premature micro-shoots Abscission n by silver supplementation in Kinnow mandarin (*Citrus nobilis* L.× *Citrus deliciosa* T.). Indian Journal of Biochemistry and Biophysics (IJBB), 59(6), 687-695.

16-Nastasi, J. R., Perry, K. R., Abbott, J. A., King, J. M., & Hoffman, E. W. (2024). Physical, colour, and mechanical properties of pearls (citrus caviar) from three finger lime (Citrus australasica) varieties: implications for economic value, quality grading, and benchmarking. Food Biophysics, 19(3), 784-794.

17-Qi, Y., Liu, H., Agar, O. T., Imran, A., Serrano, T., de Souza, P., ... & Suleria, H. A. (2023). Phytochemicals in finger lime and their potential health benefits: A review

18-**Rennie, S. (2017)**. Cultivation of Australian finger lime (*Citrus australasica*). Australian native plants: cultivation and uses in the health and food industries, 81-87.

19-SAS, Copyright(2002), Institute Inc. Cary, Nc 27513, USA.

20-Singh, S., Ray, B. K., Bhattacharyya, S., and Deka, P. C. (1994*).* *In vitro* propagation of Citrus reticulata Blanco and Citrus limon Burm. f. HortScience, 29(3), 214-216.