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Effect of antioxidants and Kin in the Induction of shoots explants of the Australian finger lime (*Citrus australasica*) in vitro

Noor ALhuda Dawood Salman and Ayad Assi Obaid

Department of Horticulture and Landscape, College of Agriculture, University of Diyala, Iraq

Emails: nouralhudadawoodsalman@gmail.com , ayadassi73@gmail.com

Abstract

Finger lime is a small-leaved citrus fruit native to Australia and increasingly increasingly popular worldwide, Finger lime is monoembryonic, initial studies to develop a tissue culture protocol for its propagation were unsuccessful due to complete leaf abscission during the culture Initiation stage, In this study, the effect of different concentrations of sodium hypochlorite (0, 15, 20, 25, 35, 45)% was studied to sterilize the nodal segment, and shoot tip, as the concentration of 20% for 15 minutes gave the lowest contamination rate and the best response rate, and shoot tip gave the lowest contamination rate and the single nodes gave the best response rate, while the black variety gave the lowest contamination rate and the highest response rate. The effect of different concentrations of Kin (0, 2, 4, 6, 8) mg L^{-1} with the addition of 400 mg $^{L-1}$ PVP or 1.5 mg L^{-1} of activated charcoal was studied, as the concentration of 4 mg L^{-1} of Kin gave the highest number of shoots, the longest length of shoots and the highest number of leaves, and PVP gave the highest response rate and the highest number of shoots The longest shoot length, the largest number of leaves, and the lowest percentage of yellowing and leaf abscission, while the black variety gave the highest response percentage, the highest number of shoots, the longest shoot length, the largest number of leaves, and the lowest percentage of yellowing and leaf abscission.

Keywords: *Citrus australasica*, BA, Kin, PVP, AC, Nodal segments, Tissue Culture

Introduction

Finger lime (*Citrus australasica*) is a species of Australian citrus and belongs to the family Rutaceae. Finger lime is native to eastern Australia and southeastern New Guinea. The trees growing mainly in southeastern Queensland and northeastern South Wales. It is known for its distinctive finger-like shape and separate pulp vesicles, which resemble Finger lime, earning it the name "citrus Finger " (Cai et al., 2022). It is a small, thorny, vigorous tree or shrub that grows to 6 m tall and has spines up to 25 mm long. The leaves are small, opposite, borne on very short petioles, often glossy and always without auricles, 5–1 cm long, 25–3 mm wide with a serrated apex and a conical base, rich in aromatic oil glands and small axillary spines. The Shoot are purple in colour. The flowers are small, generally white or pale pink, not very fragrant. The calyx is concave and free and contains many With free stamens and yellow anthers, finger lime flowers year-round (Hawkeswood, 2017: Delort and Yuan, 2018: Chandrika Ramadugu et al., 2017) and has recently attracted increased interest due to its potent antioxidant capacity and is beneficial for cell protection (Qi et al., 2023). Conventional propagation of Australian citrus faces problems of incompatibility during grafting and budding, its monoembryonic nature, and the long time required for flowering and fruit bearing. On the other hand, micropropagation is an effective alternative to conventional propagation for many commercial crops including citrus fruits and establishing an effective protocol for propagating Finger lime lemons in the laboratory is essential to facilitate successful studies in genetic transformation. In addition, plant tissue culture is used to produce new varieties and seedless varieties, and produce disease-free seedlings under controlled conditions (Delort and Yuan, 2018: Chamandoosti, 2020). Activated charcoal is used in plant tissue culture to improve growth and development, including reducing the phenomenon of browning, improving bud multiplication and seedling growth in the dark, stem elongation, bulb formation, artificial seed production, protoplast culture, and improving rooting...etc. (Al-Kanani, 1987 and Thomas, 2008). Polyvinyl pyrrolidone (PVP) is a synthetic polymer that was discovered in 1938 by the chemist Walter Reppe and is obtained by N-vinylpyrrolidone polymerization has many properties including being inert, non-toxic, water-soluble, heat-resistant, and pH-stable (Kurakula and Rao, 2020) .

Materials and method

The experiment was conducted in the plant tissue culture laboratory in the building of the Department of Horticulture and Garden Engineering - College of Agriculture - University of Diyala, to induce the development of Explants of three varieties of *Citrus australasic* (green, red and black) *in vitro*.

***The effect of sodium hypochlorite concentrations in sterilizing the Explant parts of *Citrus australasic* varieties.**

The mature shoots (tender shoot) 10 cm long were collected from well-grown mother seedlings at the beginning of spring (beginning of growth) and transferred to in vitro, left under running water for 30 minutes, then washed with water and liquid soap to eliminate dust and suspended materials and transferred to the laminar air flow table for the purpose of surface

sterilization, which was done by immersing the plant parts in different concentrations of commercial bleach (FAS) containing 6% sodium hypochlorite (Naocl), which was diluted with sterile distilled water and the volume was completed to 100 ml for each concentration, and the concentrations used were (0, 15, 20, 25, 35, 45) % for 15 minutes with the addition of abscissions of Tween-20 and cut to the ends of shoots and one or two single nodes with a length of 2-1 cm and used 10 repetitions for each glass at a rate of one part for each repetition, and the percentage of contamination and the percentage of emergence (%) were recorded after 4 weeks of cultivation.

***Effect of varieties, Kin concentrations, addition of activated charcoal and PVP and their interaction on the emergence of cultures on MS medium**

During this stage, the response of individual nodes of the three varieties of Finger lime (green, red, black) was studied, in addition to studying the effect of adding different concentrations of Kin (0, 2, 4, 6, 8) mg L^{-1} + 0.2 mg L^{-1} GA3 + 40 mg L^{-1} adenine sulfate+ 6 g L^{-1} Agar to MS medium on the growth of lateral Shoot. The effect of adding activated charcoal at a concentration of 1.5 mg L^{-1} and PVP at a concentration of 400 mg L^{-1} was studied, and the interaction between varieties and Kin concentrations was studied. One node was planted in each glass with ten repetitions, and the results were taken after 6 weeks

Statistical analysis

All experiments were designed as factorial experiments (with two or three factors) according to the Completely Randomized Design (CRD), and the data of each experiment were analyzed separately using the ready-made SAS (SAS, 2003) and the averages were compared according to Duncan's multiple range test at a probability level of 5% for all treatments

Results and discussion

1-The effect of sodium hypochlorite concentrations in sterilizing the explant of Finger lime varieties

A- The effect of varieties and sodium hypochlorite concentrations on the percentage of contamination (%)

The results shown in Table (1) and Figure (1) that the best concentration of sodium hypochlorite (20 ,45%) gave the lowest percentage of contamination, reaching 28.33%, while Shoot tip excelled by giving the lowest percentage of contamination, reaching 28.89%. As for the varieties, they did not give significant differences in the percentage of contamination. As for the interaction between concentrations and explant, the concentration of 20% and Shoot tip gave the lowest percentage of contamination, reaching 13.33%. As for the interaction between varieties and concentrations, the concentration of 20 and 45% of sodium hypochlorite and the three varieties gave the lowest percentage of contamination, reaching 25 and 30%, while the concentration of 25% and the red variety gave a percentage of contamination, reaching 35%, while the interaction between varieties The explant gave the green, red, black and Shoot tip the lowest contamination rate (25, 33.33 and 28.33%). As for the interaction between the varieties, explant and concentrations, the green, black, Shoot tip and concentration 20% gave the lowest contamination rate (10%).

Table (1) The effect of varieties and sodium hypochlorite concentrations on the contamination rate (%) of the explant grown on MS medium after 7 days of cultivation.

Varieties	explant	sodium hypochlorite						Varieties X explant
		0	15	20	25	35	45	
Green	Shoot tip	40.00 b-e	30.00 cde	10.00 e	20.00 de	30.00 cde	20.00 de	25.00 B
	nodal segment	60.00 abc	60.00 abc	50.00 a-d	70.00 ab	40.00 b-e	40.00 b-e	55.00 A
Red	Shoot tip	60.00 abc	40.00 b-e	20.00 de	20.00 de	30.00 cde	30.00 cde	33.33 B

	nodal segment	80.00 a	60.00 abc	40.00 b-e	50.00 a-d	70.00 ab	20.00 de	53.33 A
Black	Shoot tip	40.00 b-e	40.00 b-e	10.00 e	30.00 cde	30.00 cde	20.00 de	28.33 B
	nodal segment	80.00 a	50.00 a-d	40.00 b-e	50.00 a-d	30.00 cde	40.00 b-e	48.33 A
								Effect of Varieties
Varieties	Green	50.00 abc	50.00 abc	30.00 c	45.00 bc	35.00 c	30.00 c	40.00 A
	Red	70.00 a	50.00 abc	30.00 c	35.00 c	50.00 abc	25.00 c	43.33 A
	Black	60.00 ab	45.00 bc	25.00 c	40.00 bc	30.00 c	30.00 c	38.33 A
								Effect of explant
explant parts	Shoot tip	46.67 bc	36.67 cd	13.33 e	23.33 de	30.00 cde	23.33 de	28.89 B
	nodal segment	73.33 a	60.00 ab	43.33 bc	56.67 ab	46.67 bc	33.33 cd	52.22 A
Effect of concentrations		60.00 A	48.33 AB	28.33 C	40.00 B C	38.33 B C	28.33 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.

B_ Effect of varieties and sodium hypochlorite concentrations on the response rate (%)
It is clear from the results shown in Table (2) and Figure (1) that the best concentration of sodium hypochlorite (20%) gave the highest response rate of 56.67%, while single nodes excelled by giving the highest response rate of 59.44%, and the black variety gave the highest response rate of 42.50%. As for the interaction between varieties and plant parts, the black variety and single nodes gave the highest response rate of 68.33%, and as for the interaction between varieties and concentrations, the 20% concentration of sodium hypochlorite and the green variety gave the highest response rate of 65%, while the interaction between plant parts and concentrations gave the 20% concentration and single nodes the highest response rate of 83.33%.

Table (2) The effect of varieties and sodium hypochlorite concentrations on the response rate (%) of explant grown on MS medium after 4 weeks of cultivation

Varieties	explant	sodium hypochlorite						Varieties **X** explant
		0	15	20	25	35	45	
Green	Shoot tip	20.00 def	30.00 c-f	40.00 b-e	20.00 def	0.00 f	0.00 f	18.33 C

	nodal segment	50.00 bcd	60.00 abc	90.00 a	40.00 b-e	50.00 cde	60.00 abc	58.33 AB
Red	Shoot tip	20.00 def	20.00 def	30.00 c-f	10.00 ef	0.00 f	0.00 f	13.33 C
	nodal segment	40.00 b-e	40.00 b-e	70.00 ab	60.00 abc	50.00 bcd	50.00 bcd	51.67 B
Black	Shoot tip	10.00 ef	20.00 def	20.00 def	30.00 c-f	10.00 ef	10.00 ef	16.67 C
	nodal segment	50.00 bcd	50.00 bcd	90.00 a	70.00 ab	60.00 abc	90.00 a	68.33 A
								Effect of Varieties
Varieties	Green	35.00 bcd	45.00 a- d	65.00 a	30.00 cd	25.00 d	30.00 cd	38.33 A B
	Red	30.00 cd	30.00 cd	50.00 abc	35.00 bcd	25.00 d	25.00 d	32.50 B
	Black	30.00 cd	35.00 bcd	55.00 ab	50.00 abc	35.00 bcd	50.00 abc	42.50 A
								Effect of explant
explant	Shoot tip	16.67 de	23.33 d	30.00 d	20.00 de	3.333 e	3.333 e	16.11 B
	nodal segment	46.67 c	50.00 bc	83.33 a	56.67 bc	53.33 bc	66.67 b	59.44 A
Effect of concentrations		31.67 B	36.67 B	56.67 A	38.33 B	28.33 B	35.00 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 0.05 probability level.

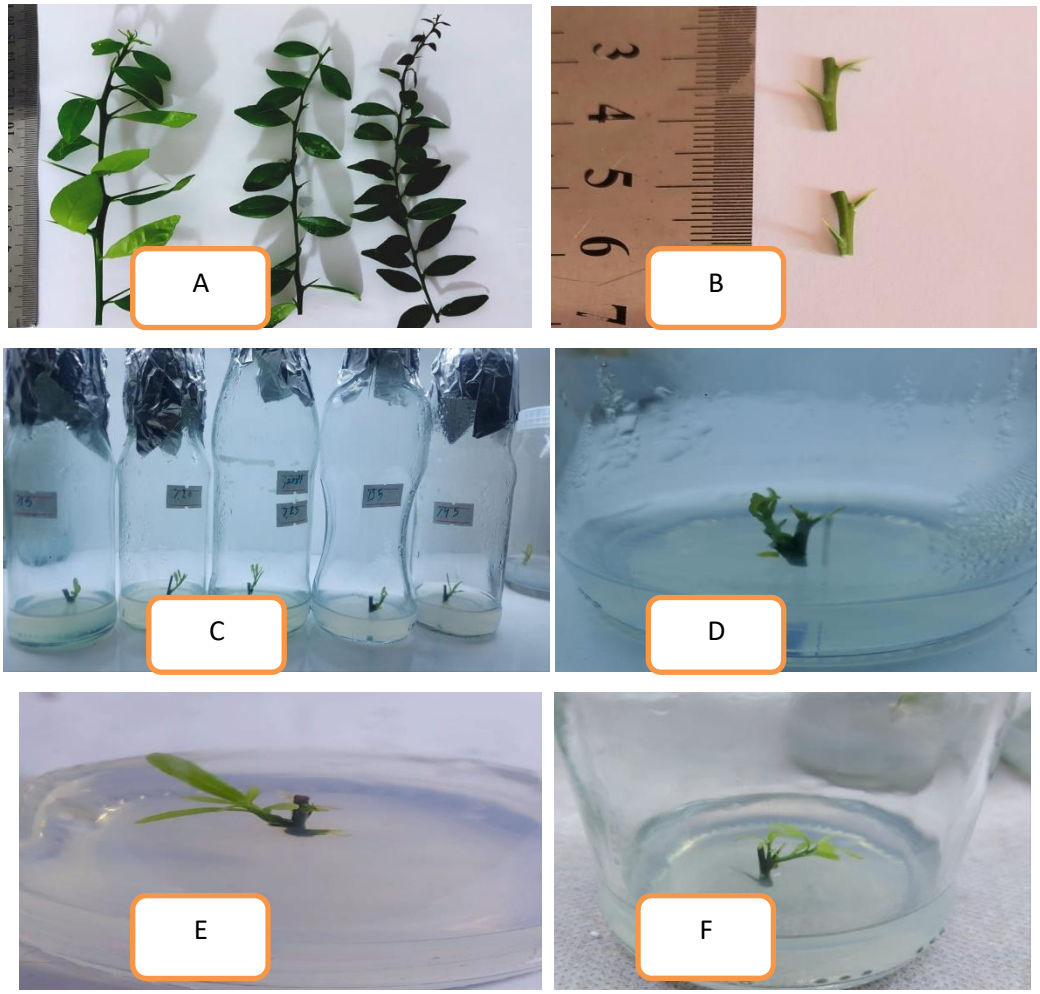

Figure (1) A- The shoots of the three varieties, B- The effect of the sterilizing substance of sodium hypochlorite solution on three varieties of Finger lime, C- The tips of the shoots and single nodes, D- The green variety, F- The red variety, and F- The black variety.

2- The effect of varieties, Kin concentrations, antioxidants and their interaction on the response rate (%) of single nodes.

The results in Table (3) show that Kin concentrations did not give significant differences in the response rate, while PVP gave the highest response rate of 83.33%. As for the effect of the varieties, the black variety gave the highest response rate of 86%. As for the interaction between the concentrations and antioxidants, no significant differences were observed. As for the interaction between the varieties and concentrations, the concentration of 4 mg L^{-1} and the black variety gave the highest response rate of 90%. As for the interaction between the varieties and antioxidants, the black variety and PVP gave the highest response rate of 90%. No significant differences were observed for the interaction between the varieties, concentrations and antioxidants.

Table (3) The effect of the varieties, Kin concentrations and antioxidants on the survival rate (%) of single nodes Finger lime after 6 weeks of cultivation on the modified MS medium.

Varieties	antioxidants	Kin mg L^{-1}					Varieties **X** antioxidants
		0	2	4	6	8	
Green	AC	70.00 a	70.00 a	70.00 a	80.00 a	70.00 a	72.00 C

	PVP	70.00 a	80.00 a	70.00 a	80.00 a	80.00 a	78.00 BC
Red	AC	80.00 a	80.00 a	70.00 a	70.00 a	70.00 a	76.00 BC
	PVP	80.00 a	80.00 a	80.00 a	90.00 a	80.00 a	82.00 AB
Black	AC	80.00 a	80.00 a	80.00 a	80.00 a	80.00 a	82.00 AB
	PVP	90.00 a	90.00 a	90.00 a	90.00 a	90.00 a	90.00 A
							Effect of Varieties
Varieties	Green	70.00 b	75.00 ab	75.00 ab	80.00 ab	75.00 ab	75.00 B
	Red	80.00 ab	80.00 ab	80.00 ab	80.00 ab	75.00 ab	79.00 B
	Black	85.00 ab	85.00 ab	90.00 a	85.00 ab	85.00 ab	86.00 A
							Effect of antioxidants
antioxidants	AC	76.67 a	76.67 a	80.00 a	76.67 a	73.33 a	76.67 B
	PVP	80.00 a	83.33 a	83.33 a	86.67 a	83.33 a	83.33 A
Effect of concentrations Kin		78.33 A	80.00 A	81.67 A	81.67 A	78.33 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.

3- Effect of varieties, Kin concentrations, antioxidants and their interaction on the average number of shoots

The results in Table (4) show that the best percentage of the number of shoots was at concentrations of 4 mg L^{-1} Kin, which amounted to 1.367 (node $Shoot^{-1}$), while PVP gave the largest number of shoots, which amounted to 1.153 (node $Shoot^{-1}$). As for the effect of varieties, the black variety gave the largest number of shoots, which amounted to 1.280 (node $Shoot^{-1}$). As for the interaction between concentrations and antioxidants, the concentration of 4 mg L^{-1} and PVP gave the largest number of shoots, which amounted to 1.553 (node $Shoot^{-1}$). As for the interaction between varieties and concentrations, the concentration of 4 mg L^{-1} and the black variety gave the largest number of shoots, which amounted to 1.800 (node $Shoot^{-1}$). As for the interaction between varieties and antioxidants, the black variety and PVP gave the largest number of shoots. It reached 1.440 (node $Shoot^{-1}$) and the interaction between varieties, concentrations and antioxidants gave the black variety and the concentration of 4 mg L^{-1} and PVP the largest number of shoots, reaching 2.00 (node $Shoot^{-1}$).

Table (4) The effect of varieties, Kin concentrations and antioxidants on the average number shoots for single nodes (node Shoot $^{-1}$) of Finger lime after 6 weeks of cultivation on the modified MS medium.

Varieties	antioxidants	Kin mg L^{-1}					Varieties **X** antioxidants
		0	2	4	6	8	
Green	AC	0.800 ef	0.700 f	0.900 def	0.800 ef	0.700 f	0.780 C
	PVP	0.800 ef	1.000 c-f	1.200 b-f	0.800 ef	0.900 def	0.940 B C
Red	AC	0.800 ef	0.900 def	1.100 c-f	0.700 f	0.900 def	0.880 C
	PVP	1.000 c-f	1.000 c-f	1.400 bcd	1.100 c-f	0.900 def	1.080 B
Black	AC	0.900 def	1.300 b-e	1.600 ab	1.000 c-f	0.800 ef	1.120 B
	PVP	1.100 c-f	1.500 bc	2.000 a	1.500 bc	1.100 c-f	1.440 A
							Effect of Varieties
Varieties	Green	0.800 d	0.850 d	1.050 cd	0.800 d	0.800 d	0.860 B
	Red	0.900 d	0.950 cd	1.250 bc	0.900 d	0.900 d	0.980 B
	Black	1.000 cd	1.400 b	1.800 a	1.250 bc	0.950 cd	1.280 A
							Effect of antioxidants
antioxidants	AC	0.833 c	0.967 bc	1.200 b	0.833 c	0.800 c	0.927 B
	PVP	0.967 bc	1.167 b	1.533 a	1.133 b	0.967 bc	1.153 A
Effect of concentrations Kin		0.900 B	1.067 B	1.367 A	0.983 B	0.883 B	0.927 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 0.05 probability level.

4- Effect of varieties, Kin concentrations, antioxidants and their interaction on the average Shoot lengths

The results in Table (5) show that the concentrations of 4 mg L^{-1} Kin gave the highest Shoot lengths of 0.855 (cm), while PVP gave the highest Shoot lengths of 0.737 (cm). As for the effect of varieties, the black variety gave the highest Shoot lengths of 0.770 (cm). As for the interaction between concentrations and antioxidants, the concentration of 4 mg L^{-1} and PVP gave the highest Shoot lengths of 0.950 (cm). As for the interaction between varieties and concentrations, the concentration of 4 mg L-1 and the black variety gave the highest Shoot lengths of 0.980 (cm). As for the interaction between varieties and antioxidants, the black variety and PVP gave the highest Shoot lengths of 0.872 (cm). The interaction between varieties, concentrations and antioxidants gave the black variety and concentrations of 2 and 4 mg L^{-1} and PVP the highest Shoot lengths, reaching 1.040 (cm) for each concentration.

Table (5) The effect of varieties, Kin concentrations and antioxidants on the average length shoots of single nodes (cm) of Finger lime after 6 weeks of cultivation on the modified MS medium.

Varieties	antioxidants	Kin mg L^{-1}					Varieties X antioxidants
		0	2	4	6	8	
Green	AC	0.510 cde	0.520 cde	0.580 b-e	0.510 cde	0.400 e	0.504 C
	PVP	0.500 cde	0.630 a-e	0.800 a-e	0.550 b-e	0.640 a-e	0.624 B C
Red	AC	0.560 b-e	0.690 a-e	0.840 a-d	0.470 ed	0.540 b-e	0.620 B C
	PVP	0.750 a-e	0.710 a-e	0.950 ab	0.630 a-e	0.530 b-e	0.7140 B
Black	AC	0.540 b-e	0.720 a-e	0.920 abc	0.650 a-e	0.510 cde	0.668 B C
	PVP	0.680 a-e	1.040 a	1.040 a	0.910 abc	0.690 a-e	0.872 A
							Effect of Varieties
Varieties	Green	0.505 d	0.575 d	0.690 bcd	0.530 d	0.520 d	0.5640 B
	Red	0.655 bcd	0.700 bcd	0.895 ab	0.550 d	0.535 d	0.667 A B
	Black	0.610 cd	0.880 abc	0.980 a	0.780 a-d	0.600 d	0.770 A
							Effect of antioxidants
antioxidants	AC	0.537 c	0.643 bc	0.780 ab	0.543 c	0.483 c	0.5973 B
		0.643 bc	0.793 ab	0.930 a	0.697	0.620 bc	0.7367

	PVP				bc		A
Effect of concentrations Kin		0.590 B C	0.718 B A	0.855 A	0.620 BC	0.557 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

5-Effect of varieties, Kin concentrations, antioxidants and their interaction on the average number of leaves

The results in Table (6) show that the best average number of leaves was at concentrations of 4 mg L^{-1} Kin, which amounted to 7.317 (leaves $shoot^{-1}$), while PVP gave the largest number of leaves, which amounted to 6.893 (leaves $shoot^{-1}$). As for the effect of varieties, the black variety gave the largest number of leaves, which amounted to 7.482 (leaves $shoot^{-1}$). As for the interaction between concentrations and antioxidants, the concentration of 4 mg L^{-1} and PVP gave the largest number of leaves, which amounted to 8.300 (leaves $shoot^{-1}$). As for the interaction between varieties and concentrations, the concentration of 4 mg L^{-1} and the black variety gave the largest number of leaves, which amounted to 9.250 (leaves $shoot^{-1}$). As for the interaction between varieties and antioxidants, the black variety and PVP gave the largest number of leaves, which amounted to 8.600 (leaves $shoot^{-1}$). The interaction between varieties, concentrations and antioxidants gave the black variety and the concentration of 4 mg L^{-1} and PVP the largest number of leaves, reaching 10.80 (leaves $shoot^{-1}$), which did not differ significantly from the concentration of 2 mg L^{-1}, which gave a number of leaves reaching 9.600 (leaves $shoot^{-1}$).

Table(6) The effect of varieties, Kin concentrations and antioxidants on the average number of leaves (leaves $shoot^{-1}$) for Finger lime after 6 weeks of cultivation on the MS medium.

Varieties	antioxidants	Kin mg L^{-1}					Varieties **X** antioxidants
		0	2	4	6	8	
Green	AC	4.800 efg	4.800 efg	5.600 d-g	4.700 fg	4.200 g	4.820 C
	PVP	5.200 d-g	5.900 c-g	6.300 c-g	5.800 d-g	5.100 d-g	5.660 B C
Red	AC	5.400 d-g	5.500 d-g	5.700 d-g	4.400 g	5.800 d-g	5.360 B C
	PVP	5.400 d-g	6.300 c-g	7.800 bcd	6.300 c-g	6.300 c-g	6.420 B
Black	AC	6.200 c-g	5.720 d-g	7.700 b-e	5.700 d-g	6.600 c-g	6.384 B
	PVP	7.500 b-f	9.600 ab	10.80 a	8.700 abc	6.400 c-g	8.600 A
							Effect of Varieties
	Green	5.000 ed	5.350 cde	5.950 b-e	5.250 cde	4.650 e	5.240 B
	Red	5.400 cde	5.900	6.750 bcd	5.350	6.050	5.890

Varieties			b-e		cde	b-e	B
	Black	6.850 bcd	7.660 ab	9.250 a	7.200 bc	6.500 b-e	7.492 A
							Effect of antioxidants
antioxidants	AC	5.467 cd	5.340 d	6.333 bc	4.933 d	5.533 cd	5.521 B
	PVP	6.033 bcd	7.267 ab	8.300 a	6.933 abc	5.933 bcd	6.893 A
Effect of concentrations Kin		5.750 B	6.303 B	7.317 A	5.933 B	5.733 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 0.05 probability level

6- The effect of varieties, Kin concentrations, antioxidants and their interaction on the rate of yellowing and leaf abscission.

The results in Table (7) show that there were no significant differences between the concentrations to reduce the percentage of yellowing and leaf abscission , while PVP reduced the percentage of yellowing and leaf abscission. As for the effect of the varieties, the black variety gave the lowest percentage of yellowing and leaf abscission, reaching 19%. As for the interaction between the concentrations and antioxidants, the comparison treatment and concentration 2 mg L^{-1} and activated charcoal gave the highest percentage of yellowing and leaf abscission, reaching 43.33%. As for the interaction between the varieties and concentrations, the comparison treatment and concentrations (2 and 8) mg L^{-1} and the green and red varieties gave the highest percentage of yellowing and leaf abscission, reaching 45, 40, and 50% respectively. As for the interaction between the varieties and antioxidants, the green and red varieties and activated charcoal gave the highest percentage of yellowing and leaf abscission, reaching (52 and 42)%. As for the interaction between the varieties and concentrations Antioxidants, the green variety and concentration 8 mg L^{-1} and activated charcoal gave the highest percentage of yellowing and leaf abscission, reaching 60%, while the black variety and all concentrations and activated charcoal gave the lowest percentage of yellowing and leaf abscission, reaching 10%.

Table (7) The effect of varieties and Kin concentrations and antioxidants on the rate of yellowing and leaf abscission (%) of Finger lime after 6 weeks of cultivation on the modified MS medium

Varieties	antioxidants	Kin mg L^{-1}					Varieties **X** antioxidants
		0	2	4	6	8	
Green	AC	50.00 ab	50.00 ab	50.00 ab	50.00 ab	60.00 a	52.00 A
	PVP	40.00 abc	30.00 abc	20.00 bc	10.00 c	40.00 abc	28.00 B
Red	AC	50.00 ab	50.00 ab	30.00 abc	40.00 abc	40.00 abc	42.00 A
	PVP	40.00 abc	40.00 abc	10.00 c	20.00 bc	20.00 bc	26.00 B

Black	AC	30.00 abc	30.00 abc	30.00 abc	30.00 abc	20.00 bc	28.00 B
	PVP	10.00 c	10.00 c	10.00 c	10.00 c	10.00 c	10.00 C
							Effect of Varieties
Varieties	Green	45.00 a	40.00 ab	35.00 abc	30.00 abc	50.00 a	40.00 A
	Red	45.00 a	45.00 a	20.00 bc	30.00 abc	30.00 abc	34.00 A
	Black	20.00 bc	20.00 bc	20.00 bc	20.00 bc	15.00 c	19.00 B
							Effect of antioxidants
antioxidants	AC	43.33 a	43.33 a	36.67 ab	40.00 ab	40.00 ab	40.67 A
	PVP	30.00 abc	26.67 abc	13.33 c	13.33 c	23.33 bc	21.33 B
Effect of concentrations Kin		36.67 A	35.00 A	25.00 A	26.67 A	31.67 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

The reason for the variation in the response rate of explant to surface sterilization with sodium hypochlorite is due to the difference in the growth of explant, their degree of maturity, development, and physiological age. These results are consistent with what was reached by Al-Jubori and Al-Amery, (2022), as the concentrations of 35 and 45% gave the lowest percentage of contamination for explant grown in vitro. The reason for the difference in the ability of explant to respond between plant parts and the superiority of single nodes over Shoot tips may be due to the accumulation of nutrients and hormonal materials in their tissues in a larger quantity than the Shoot tips and to their location on the plant organ from which they were taken. This phenomenon is called Topopysis (Mahmoud, 2017). The reason may also be due to the degree of maturity and differentiation of single nodes, which is what Bhalerao and Kumre, 2019, reached, that mature explant parts give a better response because they contain parenchyma tissue, cambium, and transport vessels. They agree The results are consistent with the findings of many studies, including (Al-Khayri and Al Bahrany, 2001, Al-Ani, 2015, and Santiago et al., 2019), and do not agree with studies, including (Chhetri et al., 2021 and Bodade et al., 2022), as they concluded that the growing tips are more responsive than single nodes. As for the reason for the superiority of PVP and reducing the phenomenon of yellowing and leaf abscission, its importance lies in the absorption of many substances, including growth-inhibiting compounds and phenolic secretions, and the absorption of harmful substances present in the cultivation medium, including impurities present in the agar, and hydroxyl methylfurfural, which is produced from sucrose and phenoquinones secreted by the explanted plants during cultivation, on the one hand, and on the other hand, it works to absorb the plant hormones present in the nutritional medium, that the stimulating action of Kin in urging plant cells to enhance division Cells and increase their growth expansion during the stages of emergence, as well as its effect in hindering the breakdown of protein and chlorophyll and stimulating photosynthesis enzymes, which is reflected in increasing the size of the plant cell and thus encouraging division and morphological differentiation (Wu et al., 2021: Wybouw and De Rybe, 2019), and that Kin has an important role in building RNA, proteins and enzymes within plant cells (Al-Asadi and Al-Khaikani, 2019).

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