

Research Article

## Influence of IBA (Indole-3-butyric acid) dipping and BA (Benzyle adenine)-GA<sub>3</sub> (Gibberellic acid) spraying on vegetative and biochemical traits of sour orange cuttings

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### Abstract

Sour oranges (*Citrus aurantium* L.) are propagated in two ways: by seed and by semi-hardwood cuttings. Producing sour orange seedlings from seed is time-consuming and expensive. Semi-hardwood cuttings offer a faster method of propagating sour oranges, reducing both time and costs. However, they are considered relatively difficult to root. The present investigation aimed to improve the growth and development of cuttings more quickly and at a lower cost by using growth regulators IBA (Indole-3-butyric acid), BA (Benzyle adenine), and GA<sub>3</sub> (Gibberellic acid). The greenhouse experiment was conducted during the 2023-2024 season. A completely randomized design (C.R.D.) was used as a factorial experiment with three factors. The first factor was dipping the tips of sour orange cuttings in an IBA solution at 200 ppm for 2 periods (0, 10) minutes before planting. The second factor was the sprayed growth regulator (BA) at three levels (0, 0.5, 1 ppm). Third factor was sprayed by (GA<sub>3</sub>) at (0.50, 100) ppm. The investigation was performed in three replicates, by six cuttings per experimental unit. The results showed that dipping Sour orange cuttings in IBA was more effective in plant height (52.48 cm), leaf area (31.16 cm<sup>2</sup>), chlorophyll content (32.42 spad), carbohydrate (36.74%), nitrogen (1.39%), and phosphorus (0.48%) compared to the control. Spraying by BA at 1 ppm and GA<sub>3</sub> at 100 ppm had a significant impact on the studied parameters (plant height, leaf area, chlorophyll content, carbohydrate, nitrogen and phosphorus). Double and triple interactions had a significant effect on most of the studied characters.

**Keywords:** BA, Cuttings, Dipping, GA<sub>3</sub>, IBA (Indole-3-butyric acid), Propagation, Sour orange

### INTRODUCTION

Sour orange (*Citrus aurantium* L.) belongs to the Citrus genus and Rutaceae family. It is preferred as a rootstock for citrus grafting due to its resistance to many fungal and viral diseases and its tolerance to environmental conditions and soil stress. Seedlings are produced in two ways: sexually by seed and vegetatively by semi-hardwood cuttings. Sexual propagation by seeds is a slow and expensive method. Vegetative

propagation by semi-hardwood cuttings offers a faster process, reducing both time and costs (Radha and Mathew, 2007).

Semi-hardwood cuttings are described as somewhat difficult to root. Therefore, treating cuttings with growth stimulants before planting is necessary to improve rooting and increase growth. One technique used to stimulate seedling growth and regulate its physiological activities is spraying it by growth regulators (Alexander, 1985). Growth regulators are substances that work in very

small quantities to control growth by enhancing, modifying, or replacing the action of natural hormones responsible for regulating physiological functions in plants (Rademacher, 2015).

Dipping of cuttings bases in indole-butyric acid (IBA) before planting is a safe, non-toxic, and more effective method than other auxins for rooting cuttings (Radha and Mathew, 2007). Foliar application of growth regulators benzyl adenine (BA) and gibberellic acid (GA3) improves seedling growth and facilitates transplanting to its permanent location or grafting (Bisht *et al.*, 2018; Davies, 1995).

Stimulating the growth of fruit seedlings, especially citrus, is closely linked to sustainable, pollution-free fertilization. Nutrient deficiencies can significantly inhibit healthy growth and productivity (Abdulkadhim, 2024; Abdulkadhim *et al.*, 2024; Abdulkadhim and Hashem, 2025). Some studies have appear that spraying with growth promoters significantly improves seedling growth. Hashim *et al.* (2021) demonstrated that spraying growth regulator 2,4-D at a dose of 3 ml/L on sour orange trees significantly affected on chlorophyll, leaf area, nitrogen and phosphorus content. Hasan *et al.* (2022) achieved significant improvements in pomegranate tree growth when sprayed with the growth regulators Floratone and Ultra keIP40.

This investigation aimed to enhance the growth and development of sour orange cuttings more quickly and at a lower cost by using IBA, BA, and GA3. Furthermore, determining the importance of duple and triple interactions between the experimental agents. In addition, determining the importance of bilateral and tertiary interactions between experimental factors.

## MATERIALS AND METHODS

### Study area

A greenhouse experiment (temperature 25°C, humidity 75%, pot soil type: sandy loam) was conducted during the 2023-2024 agricultural season on sour orange cuttings. 350 cuttings (10 cm long) were taken from healthy, disease-free, and pest-free sour orange trees. The cuttings were slanted at the top and flat at the base. Factorial experiment was used with three factors (2x3x3) and three replicates, by six cuttings per experimental unit. The first factor was dipping the tips of sour orange cuttings in an auxin solution (IBA) at 200 ppm for 2 periods (0,10) minutes before planting. The cuttings were planted in pots filled with sandy loam soil, on 11/15/2023. Second factor was spraying of cytokinin (BA) at (0, 0.5, 1), on 4/7/2024. Third factor was spraying of GA3 at (0.50, 100) ppm, on 4/14/2024 (one week after spraying BA). Two weeks later, the spraying process was repeated using the same mechanism and method mentioned above. Seedling leaves were sprayed in the early morning until thoroughly moist with

a 15-litre hand sprayer, using the spreading material Tween-20 (0.01% v/v) (LAWLOR *et al.*, 2004). A completely randomized design (CRD) was used as a factorial experiment to analyze the results.

### Studied indicators

#### Vegetative indicators

Average plant height (cm): It was taken using a measuring tape from the beginning of the main bud growth to the top of the plant. Average leaf area cm<sup>2</sup>: This was calculated by using leaf dry weight and discs of a specific area (6 cm<sup>2</sup>). The whole leaves and cut discs whose area was to be measured were dried at 71°C for 48 hours in an electric oven until the dry weight was constant. Calculations were executed by the next equation executed calculations:

Average leaf area (cm<sup>2</sup>) = dry leaf weight (g) × area of the cut part (cm<sup>2</sup>) / dry weight average of the cut area (g)

#### Biochemical indicators and ions absorption

**Chlorophyll content (SPAD Unit):** It was measured using a chlorophyll content meter type SPAD-503 (Ranganna, 1977). Soluble carbohydrate content of leaves (mg kg<sup>-1</sup> dry weight): determined utilizing the procedure of Joslyn (1970).

Nitrogen percentage (N %) was determined using the Kjeldahl method with a microkjeldahl apparatus (Novozamsky *et al.*, 1974). Phosphorus percentage (P%): it was determined using a soft digestion procedure; the colour was modified with AsA (ascorbic acid) and 6Mo<sub>7</sub>O<sub>24</sub>(NH<sub>4</sub>) (Ammonium molybdate). Calculations were made by Spectro - spectro-photometer device at 620 nm wavelengths. (Johns, 1970).

#### Data analysis

Data were analyzed graphically using the GENE STAT 2010 (Version 12) software. A completely randomized design (CRD) was used as a factorial experiment to analyze the data. Differences between coefficients were compared to determine the significance status by using LSD (least significant difference) with a probability < 5% (Al-Rawi and Khalafalla, 2000).

## RESULTS AND DISCUSSION

#### Vegetative indicators

The results of Table 1 can be summarized as follows: dipping Sour orange cuttings in IBA for 10 minutes (IBA 10 min) resulted in a positive increase in seedling height (52.48 cm) and leaf area (31.16 cm<sup>2</sup>). The IBA at 0-minute treatment resulted in the lowest rates of seedling height (50.14 cm) and leaf area (30.45 cm<sup>2</sup>). Also, the results of the Tables mentioned above show that spraying with (BA) at 1 ppm had a significant increase in seedling height (53.60 cm) and leaf (31.79 cm<sup>2</sup>),

**Table 1.** Influence of IBA dipping and BA–GA3 spraying on seedling height (cm) of sour orange cuttings

Dipping time IBA	BA	GA3			IAB×BA
		0	50	100	
0 min.	0	43.72	48.57	49.73	47.34
	0.5 ml. L <sup>-1</sup>	46.80	50.48	53.41	50.23
	1 ml. L <sup>-1</sup>	50.19	53.62	54.76	52.86
	0	47.17	50.04	52.24	49.82
10 min.	0.5 ml. L <sup>-1</sup>	50.78	53.40	55.64	53.27
	1 ml. L <sup>-1</sup>	51.73	53.89	57.41	54.34
L.S.D <0.05		2.000			1.15
IBA					
IBA×GA3	0	46.90	50.89	52.63	50.14
	10 min	49.89	52.44	55.10	52.48
L.S.D <0.05		1.16			0.67
BA					
BA×GA3	0	45.44	49.31	50.99	48.58
	0.5 ml. L <sup>-1</sup>	48.79	51.94	54.53	51.75
	1 ml. L <sup>-1</sup>	50.96	53.76	56.09	53.60
L.S.D <0.05		N.S			0.82
GA3		48.40	51.67	53.87	
L.S.D <0.05		0.82			

compared to other treatments. Spraying with (GA<sub>3</sub> at 100 ppm) had a significant impact on the seedling height (53.87 cm), and leaf area (31.98 cm<sup>2</sup>).

Two-way interactions (binary) led to a significant increase in seedling length. and leaf area. The binary interactions (IBA 10 min + BA 1 ppm) achieved the highest percentages in seedling height (54.34 cm) and leaf area (31.80 cm<sup>2</sup>). The binary interactions (IBA 10 min + GA<sub>3</sub>100 ppm) achieved the highest percentages in seedling height (55.10 cm) and leaf area (32.29 cm<sup>2</sup>) compared to other treatments. The interaction (BA1ppm + GA<sub>3</sub>100 ppm) achieved the highest value in leaf area (32.77 cm<sup>2</sup>), compared to other interactions. The dual interaction (BA + GA<sub>3</sub>) did not have a significant impact on seedling length. The triple interaction (IBA 10 min + BA 1 ppm + GA<sub>3</sub>100 ppm) had a significant impact on seedling length (57.41 cm) and leaf area (32.70 cm<sup>2</sup>).

The increase in seedling length and leaf area is due to the importance of growth regulators (IBA, BA, and GA<sub>3</sub>) in stimulating plant cell growth. Auxins and cytokinins promote cell elongation and division by enhancing physiological processes within plant cells. These physiological changes positively influenced plant height and leaf area. Likewise, gibberellins elongate cells, increase cell membrane formation, and regulate its permeability (Bisht *et al.*, 2018; Davies, 1995). Hassan *et al.* (2022) found that Foliar applications by growth regulators (FLORATONE and ULTRA KELP40) on pomegranate trees contributed positively to seedling length and leaf area.

#### Biochemical indicators and ions absorption

Findings in Tables 1, 2, 3 and 4 indicated that dipping of Sour orange cuttings in IBA for 10 minutes (IBA 10 min) had a significant impact in the chlorophyll content (32.42 spad unit), carbohydrate 936.74 mg kg<sup>-1</sup> dw), nitrogen (1.34%), and phosphorus (0.48%). While the zero-minute treatment (IBA 0 min) showed a negative decrease (30.54 spad units, 33.48 mg kg<sup>-1</sup> dw, 1.23%, 0.47%), respectively. Spraying with (BA1 ppm) had a significant increase on chlorophyll content (34.34 spad unit), carbohydrate ( 37.14 mg kg<sup>-1</sup> dw), nitrogen (1.36%), and phosphorus (0.52%). Spraying with GA<sub>3</sub> (100 ppm) resulted in a significant increase in chlorophyll content (32.58 spad units), carbohydrates (36.97 mg kg<sup>-1</sup> dw), nitrogen (91.33%), and phosphorus (0.51%), outperforming the other interactions.

The binary interactions recorded a significant impact on biochemical indicators and ion absorption. Immersing the cuttings for ten minutes in IBA with spraying the seedling with BA at 1ppm (IBA 10 min + BA 1ppm) had significant impact of chlorophyll content (37.27 spad unit), carbohydrate (39.18 mg kg<sup>-1</sup> dw) , nitrogen (1.41%), and phosphorus ( 0.51% ), outperforming the other interactions. The two-way interactions (IBA 10 min + GA<sub>3</sub>100 ppm) and (BA1 ppm + GA<sub>3</sub>100 ppm) showed no Significant difference in chlorophyll. Moreover, the dual interaction (IBA 10 min + GA<sub>3</sub>100 ppm) had a significant impact on carbohydrate (38.69 mg kg<sup>-1</sup> dw) and nitrogen (1.39%). The two-way interaction (BA1 ppm + GA<sub>3</sub>100 ppm) had a significant impact on carbohydrate (39.95mg kg<sup>-1</sup> dw) and phosphorus

**Table 2.** Influence of IBA dipping and BA–GA3 spraying on leaf area (cm<sup>2</sup>) of sour orange cuttings

Dipping time IBA	BA	GA3			IAB×BA
		0	50	100	
0 min.	0	27.61	28.96	30.23	28.93
	0.5 ml. L <sup>-1</sup>	29.13	30.80	31.95	30.63
	1 ml. L <sup>-1</sup>	29.93	32.57	32.83	31.78
10 min.	0	28.50	30.50	31.60	30.20
	0.5 ml. L <sup>-1</sup>	30.50	31.40	32.57	31.49
	1 ml. L <sup>-1</sup>	30.80	31.90	32.70	31.80
L.S.D <0.05		0.54			0.31
					IBA
IBA×GA3	0	28.89	30.78	31.67	30.45
	10min	29.93	31.27	32.29	31.16
L.S.D <0.05		0.31			0.17
					BA
BA×GA3	0	28.06	29.73	30.91	39.57
	0.5 ml. L <sup>-1</sup>	29.82	31.10	32.26	31.06
	1 ml. L <sup>-1</sup>	30.37	32.23	32.77	31.79
L.S.D <0.05		0.38			0.22
GA3		29.41	31.02	31.98	
L.S.D <0.05		0.22			

**Table 3.** Influence of IBA dipping and BA–GA3 spraying on chlorophyll (spad unit) of sour orange cuttings

Dipping time IBA	BA	GA3			IAB×BA
		0	50	100	
0	0	22.36	25.74	25.88	24.66
	0.5 ml. L <sup>-1</sup>	26.70	30.15	31.33	29.39
	1 ml. L <sup>-1</sup>	27.77	32.04	34.00	31.40
10 min	0	24.00	28.10	29.93	27.34
	0.5 ml. L <sup>-1</sup>	30.40	33.00	34.50	32.63
	1 ml. L <sup>-1</sup>	35.37	36.99	39.46	37.27
L.S.D <0.05		1.13			0.65
					IBA
IBA×GA <sub>3</sub>	0	25.61	29.31	30.54	28.49
	10 min	29.92	32.70	34.63	32.42
L.S.D <0.05		N. S			0.38
					BA
BA×GA <sub>3</sub>	0	23.18	26.92	27.91	26.00
	0.5 ml. L <sup>-1</sup>	28.55	31.58	32.92	31.01
	1 ml L <sup>-1</sup>	31.57	34.52	36.93	34.34
L.S.D< 0.05		N.S			0.46
GA3		27.77	31.00	32.58	
L.S.D< 0.05		0.46			

**Table 4.** Influence of IBA dipping and BA–GA3 spraying on carbohydrate (mg kg<sup>-1</sup> dw) of sour orange cuttings

Dipping time IBA	BA	GA3			IAB×BA
		0	50	100	
	0	30.36	30.10	32.37	30.94
0	0.5 ml. L <sup>-1</sup>	32.50	35.04	35.65	34.40
	1 ml. L <sup>-1</sup>	32.22	35.37	37.73	35.11
	0	31.23	33.13	33.80	32.72
10 min	0.5 ml. L <sup>-1</sup>	35.04	39.77	40.12	38.31
	1 ml. L <sup>-1</sup>	36.12	39.27	42.16	39.18
L.S.D <0.05		0.46			0.27
IBA					
IBA×GA <sub>3</sub>	0	31.69	33.50	35.25	33.48
	10 min	34.13	37.39	38.69	36.74
L.S.D <0.05		0.27			0.15
BA					
BA×GA <sub>3</sub>	0	30.80	31.62	33.08	31.83
	0.5 ml. L <sup>-1</sup>	33.77	37.40	37.89	36.35
	1 ml L <sup>-1</sup>	34.17	37.32	39.95	37.14
L.S.D < 0.05		0.33			0.19
GA3		32.91	35.45	36.97	
L.S.D < 0.05		0.19			

**Table 5.** Influence of IBA dipping and BA–GA3 spraying on nitrogen (%) of sour orange cuttings

Dipping time IBA	BA	GA3			IAB×BA
		0	50	100	
	0	1.10	1.13	1.17	1.13
0	0.5 ml. L <sup>-1</sup>	1.22	1.25	1.30	1.26
	1 ml. L <sup>-1</sup>	1.27	1.32	1.35	1.31
10min	0	1.20	1.29	1.31	1.27
	0.5 ml. L <sup>-1</sup>	1.30	1.35	1.41	1.35
	1 ml. L <sup>-1</sup>	1.35	1.41	1.46	1.41
L.S.D < 0.05		N.S			0.012
IBA					
IBA×GA3	0	1.20	1.23	1.27	1.23
	10 min	1.28	1.35	1.39	1.34
L.S.D < 0.05		0.012			0.007
BA					
BA×GA3	0	1.15	1.21	1.24	1.20
	0.5 ml. L <sup>-1</sup>	1.26	1.30	1.36	1.31
	1 ml. L <sup>-1</sup>	1.31	1.36	1.41	1.36
L.S.D <0.05		N.S			0.009
GA3		1.24	1.29	1.33	
L.S.D <0.05		0.009			

**Table 6.** Influence of IBA dipping and BA–GA3 spraying on phosphorous (%) of sour orange cuttings

Dipping time IBA	BA	GA3			IAB×BA
		0	50	100	
0	0	0.40	0.45	0.47	0.44
	0.5 ml. L <sup>-1</sup>	0.42	0.48	0.51	0.47
	1 ml. L <sup>-1</sup>	0.48	0.51	0.54	0.51
10min	0	0.38	0.43	0.48	0.43
	0.5 ml. L <sup>-1</sup>	0.44	0.50	0.51	0.48
	1 ml. L <sup>-1</sup>	0.51	0.52	0.55	0.51
L.S.D < 0.05		N.S			0.011
IBA					
IBA×GA3	0	0.43	0.48	0.51	0.47
	10 min	0.44	0.48	0.51	0.48
L.S.D < 0.05		N .S			0.006
BA					
BA×GA3	0	0.39	0.44	0.48	0.44
	0.5 ml. L <sup>-1</sup>	0.43	0.49	0.51	0.48
	1 ml. L <sup>-1</sup>	0.50	0.52	0.55	0.52
L.S.D <0.05		0.015			0.008
GA3		0.44	0.48	0.51	
L.S.D <0.05		0.008			

(0.55%), outperforming the other interactions. But the same interaction above (BA1ppm + GA<sub>3</sub>100 ppm) showed no Significant difference in nitrogen percentage rate. The triple interaction in Tables 1 and 2 (IBA 10 min + BA 1 ppm + GA 3100 ppm) showed a significant difference in chlorophyll content (39.46 spad units) and carbohydrate content (42.16 mg kg<sup>-1</sup> dw), outperforming the other interactions. Tables 3 and 4 indicated that there was no significant difference in the triple interactions for nitrogen and phosphorus percentages.

Plant growth regulators (IBA, BA, and GA<sub>3</sub>) improve plant growth by mobilising stored nutrients to vegetative parts, leading to increased carbohydrate and nutrient levels in those parts. Plant growth regulators increased various physiological processes, such as the production of proteins from amino acids, which enter into many compounds, including the formation of chlorophyll, which helps raise the effectiveness of the photosynthesis process (Abdul Qader *et al.*, 1982; Benton and Vernon, 1990; Taiz and Zeiger, 2010). These findings are consistent with Hashim *et al.* (2021), who found that spraying orange trees with the growth regulator 2,4-D at a rate of 3 ppm increased leaf area, chlorophyll index, and nitrogen and phosphorus levels.

## Conclusion

The present study concluded that dipping sour orange cuttings (*Citrus aurantium* L.) in IBA for 10 min and spraying the seedlings with BA and GA3 are important

for accelerating growth and development, increasing growth rate, and reducing cost by improving Vegetative and Biochemical indicators. These indicators may serve as an index for the use of growth regulators (IBA, BA, and GA3) as a necessary step to stimulate the rapid growth of sour orange cuttings, which are reported to be difficult to root in future studies. Therefore, it is recommended to test these findings on other difficult-to-root citrus varieties. Further studies can also be made on the growth regulators used in the current study, given their importance in reducing pollution and increasing plant growth.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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