



## Effect of Some Regulating Substances on the Vegetative Parameters of Flame Seedless Grapes Cleft Grafted on Three Nematode Resistant Rootstocks

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### Abstract

Grapes, the second next to Citrus, are one of Egypt's most popular fruits. Egypt is the world's 14<sup>th</sup> largest grape producers. The Flame seedless grape is a popular and early-season cultivar and one of the most prevalent grape kinds. Because the grape is nematode-sensitive, grafting on nematode-resistant rootstocks could be an effective way to minimize worm infection. The purpose of this study is to investigate the effect of IBA, Kinetin in graft union, and some grapevine rootstocks, such as Harmony, Freedom, and Salt Creek, on the percentage of Flame seedless grapevine survival and vegetative growth parameters when grafted using the cleft grafting method on the 15<sup>th</sup> February grafting date. When the Flame seedless grapes were treated at the graft union of the Freedom rootstock with a high level of indole-3-butyric acid (IBA, 1000 ppm) and a low level of Kinetin (250 ppm), they had a significantly higher survival percentage, shoot length, shoot diameter, L/D ratio, number of leaves per shoot, leaf area, fresh weight of leaves, fresh weight root system, dry weight leaves and shoots, and dry weight root system in both seasons. It can be concluded that grafting on Freedom rootstock and using a low amount of Kinetin (250 ppm) are the most effective treatments for getting the highest percentage of survival and vegetative development parameters in Flame seedless grapevine.

**Key words:** Grape; Grafting; IBA; Kinetin; Flame seedless; Rootstock; Salt Creek; Freedom; Harmony.

### 1. Introduction

Grapes (*Vitis vinifera* L.) are one of the world's most ancient and important perennial crops [1]. All edible varieties of the grape are nematode-sensitive. On grapevines, nematodes produce major difficulties such as root gall formation, overall weakness, lower production, and viral agent transmission. As a result, grafting on nematode-resistant rootstocks could be an effective way to avoid worm infestation. In the last few decades, grafting has become a common means of propagating promising grape cultivars. Some nematode-resistant grape rootstocks, such as Freedom, Harmony, Salt Creek, SO4, Richter, and others, have been enrolled in Egypt to address this issue.

One of the most popular grape varieties is the Flame seedless grape, which is a variety that blooms early in the season [2]. As a result, the grafting procedure is crucial, as it involves complex

biochemical and structural changes during the adhesion of the two grafted partners, callus formation, and the generation of a functional vascular system [3, 4, 5]. Grafting success is influenced by plant genetics, growth features, and physiological and biochemical parameters [6]. Plant growth regulators such as auxins and cytokinins have been shown to drive the production and proliferation of callus and new vascular tissue by boosting cell division and/or cell development, according to several authors [7, 8, 9, 10].

Several elements influence the establishment of graft unions. The rootstock scion pairings, propagation strategies, environmental conditions, pest and disease pressure, rootstock activity, and the application of plant growth regulators are among the most critical [4]. The purpose of this study is to see how IBA, Kinetin in graft union, and some grapevine rootstocks, such as Harmony, Freedom, and Salt Creek, affect the percentage of Flame seedless

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grapevine survival and vegetative growth parameters when grafted using the cleft grafting method on the 15<sup>th</sup> of February grafting date.

## 2. Experimental

The current study was conducted in a private vineyard in Sadat city, Menofia Governorate, Egypt, over two seasons in 2020 and 2021 to investigate the influence of IBA, Kinetin in graft union, and some grapevine rootstocks, namely Harmony, Freedom, and Salt Creek, using the cleft grafting method at the 15<sup>th</sup> of February grafting date on the percentage of Flame seedless grapevine survival, vegetative growth parameters, and congeniality.

### 2.1. Preparing of the rootstocks and cultivars

The samples were sliced into 20 cm lengths with a diameter of 1 cm, each containing three nodes (eyes). These cuttings were taken on February 15<sup>th</sup> from canes that were free of insects and disease infection.

### 2.2. The grafting procedure

After that, cleft grafting procedures are used. Both the scion and rootstock's cut grafting surfaces were immersed in either indole-3-butyric acid (IBA) or kinetin (Ki) for 20 seconds. According to Köse and Güleriyüz [11], the concentrations of IBA and Ki were used at 250, 500, and 1000 mg/liter and then dipped in polyethylene ribbon and waxed with grafting wax. The bases of grafts were dipped in each concentration of indole butyric acid (IBA) and kinetin for 10 seconds before being placed in a callus formation box containing moist peat moss, sand, and sawdust medium (1:1:1), covering the graft tips by 3 cm.

### 2.3. Planting of the successful grafts

Each graft was planted in a peat moss and sand mixture (2:1v/v) in a 19.5×11.5×9.5 cm black polyethylene bag. The polyethylene bags were covered with 60 micron plastic tubes until the end of April, when the cover was removed and normal irrigation and management practices resumed.

#### 2.3.1. Survival percentage

After one month of transplanting, the survival percentage (the percentage of sprouting grafts with rooted rootstocks) was calculated.

#### 2.3.2. Vegetative growth parameters of the scion

The vegetative growth parameters of the scion were measured after six months of transplanting, including shoot length (cm), shoot diameter (cm), L/D ratio, number of leaves per shoot, fresh weight (g) leaves and shoots, fresh weight (g) root system, dry weight (g) leaves and shoots, and dry weight (g) root system. According to Sorial *et al.* [12], the leaf area (cm<sup>2</sup>) was determined using the following equation: leaf area = 3.14 square diameter /4.

## 3. Statistical Analysis

M.statistic (M.S.) software was used to perform statistical analysis on all data according to Snedecor and Cochran [13]. The comparison among the means of the different treatments was determined. The least significant differences test was used to compare the treatment means at the (0.05) level of significance.

## 4. Results

### 4.1. Survival percentage

The data in Table 1 shows the effect of IBA, Kinetin, and different rootstocks on Flame seedless grape survival percentages in the 2020 and 2021 seasons. The survival percentage varied significantly according to rootstocks and treatments. In comparison to the control, the treated rootstocks at graft union with IBA at a high level of 1000 ppm exhibited a highly significant survival percentage of Flame seedless grapes in both seasons. In both seasons, treated Flame seedless grapes with a high level of IBA (1000 ppm) had a significantly higher survival percentage at the graft union of Freedom rootstock by 75 and 80%, respectively, followed by Salt Creek (70% and 75%) and Harmony (55% and 60%). Interestingly, the treatment with Kinetin at a low level gave in contrast to the control, the treated rootstocks at graft union with Kinetin at a low level of 250 ppm resulted in a highly significant survival percentage of Flame seedless grapes in both seasons. The Flame seedless grapes showed a significantly higher survival percentage (95%) in both seasons when they were treated with a low level of Kinetin (250 ppm) at the graft union of the Freedom rootstock, followed by Salt creek rootstock (90% and 91%) and Harmony rootstock (80% and 85%) in the 2020 and 2021 seasons, respectively, compared to the control. Interestingly, the treated rootstocks at graft

Table (1): Effect of IBA, Kinetin and different rootstocks on survival percentage (%) of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
<b>Freedom</b>	65.00 G	35.00 M	45.00 J	75.00 E	95.00 A	83.33 C	65.00 G
<b>Harmony</b>	40.00 K	15.00 O	36.67 L	55.00 I	80.00 D	75.00 E	45.00 J
<b>Salt creek</b>	55.00 I	25.00 N	40.00 K	70.00 F	90.00 B	80.00 D	60.00 H
<b>2021</b>							
<b>Freedom</b>	75.00 E	33.33J	60.00 F	80.00 D	95.00 A	85.00 C	60.00 F
<b>Harmony</b>	35.00 I	20.00L	40.00 H	60.00 F	85.00 C	80.00 D	40.00 H
<b>Salt creek</b>	40.00H	25.00K	55.00 G	75.00 E	91.00 B	80.00D	55.00 G

a,b,c,d and e means at the same column with different superscript are significantly ( $P < 0.05$ ) different. \*IBA; indole-3-butyric acid

union with Kinetin at a low level of 250 ppm resulted in a highly significant survival percentage of Flame seedless grapes compared to the IBA treatments and the control in both seasons.

#### 4.2. Shoot length

The effect of IBA, Kinetin, and different rootstocks on the shoot length of Flame seedless grape is shown in Table 2. In both seasons, the treated rootstocks at graft union with IBA at a high level of 1000 ppm had significantly higher shoot length values of Flame seedless grapes than the control. The Flame seedless grapes had significantly higher shoot length values (97.25 and 98.05 cm, respectively) when they were treated with a high level of IBA (1000 ppm) at the graft union of the Freedom rootstock in both seasons. In contrast, the treated rootstocks at graft union with Kinetin at a low level of 250 ppm resulted in highly significant shoot length values of Flame seedless grapes in both seasons compared to the control and IBA levels. When the Flame seedless grapes were treated with a low level of Kinetin (250 ppm) at the graft union of the Freedom rootstock, they had significantly higher shoot length values (119.87 and 121.79 cm, respectively), followed by Salt Creek (113.76 and 114.97 cm) and Harmony (101.20 and 102.13 cm), respectively, in the 2020 and 2021 seasons compared to the control.

#### 4.3. Shoot diameter

The effect of IBA, Kinetin, and different rootstocks on the shoot diameter of Flame seedless grape is shown in Table 3. In both seasons, the treated rootstocks with high levels of IBA (1000 ppm) or low levels of Kinetin (250 ppm) showed significantly higher shoot diameters of Flame seedless grapes than the control. The Flame seedless grapes had significantly higher shoot diameters when treated with a high level of IBA (1000 ppm) and a low level (250 ppm) of Kinetin at the graft union of the Freedom rootstock (0.63 and 0.63 cm and 0.65 and 0.66 cm, respectively) in the 2020 and 2021 seasons, compared to the control.

#### 4.4. Number of leaves per shoot

Table 4 shows how IBA, Kinetin, and different rootstocks effect of the number of leaves per shoot of Flame seedless grape in the 2020 and 2021 seasons. The treated rootstocks with IBA at a high level of 1000 ppm had significantly higher leaves per shoot of Flame seedless grapes than the control in both seasons. When graft union was treated with Kinetin at a low level of 250 ppm, the treated rootstocks yielded significantly higher leaves per shoot of Flame seedless grapes than the control and IBA treated levels. In both seasons, Flame seedless grapes treated with a low level (250 ppm) of Kinetin at the graft union of the Freedom rootstock had considerably more leaves per shoot (57.57 and 59.09), followed by Salt Creek (53.75 and 55.37) and Harmony (49.72

and 50.54) in the 2020 and 2021 seasons, the control. respectively, than those treated with IBA levels and

Table (2): Effect of IBA, Kinetin and different rootstocks on shoot length (cm) of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
<b>Freedom</b>	72.63J	70.37K	83.57H	97.25F	119.87 A	110.55C	86.97G
<b>Harmony</b>	59.18M	57.75M	72.63J	80.72I	101.20D	99.13E	72.63J
<b>Salt creek</b>	67.59L	66.90L	80.07I	86.72G	113.76 B	101.20D	80.07I
<b>2021</b>							
<b>Freedom</b>	73.95J	70.08K	86.72G	98.05E	121.79A	112.71C	93.49F
<b>Harmony</b>	54.50N	59.08M	73.95J	83.57I	102.13D	98.05E	73.95J
<b>Salt creek</b>	66.90L	60.69M	84.59HI	85.86HG	114.97B	102.13D	83.57I

a,b,c,d and e means at the same column with different superscript are significantly ( $P<0.05$ ) different. \*IBA; indole-3-butyric acid

Table (3): Effect of IBA, Kinetin and different rootstocks on shoot diameter (cm) of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
<b>Freedom</b>	0.60A-C	0.44B-D	0.54A-C	0.63AB	0.65A	0.59A-C	0.53BAC
<b>Harmony</b>	0.48A-D	0.33D	0.47A-C	0.51A-D	0.52A-D	0.55A-C	0.48A-C
<b>Salt creek</b>	0.54A-C	0.42CD	0.49A-C	0.59A-C	0.60A-C	0.48A-D	0.51A-D
<b>2021</b>							
<b>Freedom</b>	0.63A-C	0.48A-C	0.57A-C	0.63AB	0.66A	0.60AB	0.55A-C
<b>Harmony</b>	0.49A-C	0.36C	0.52A-C	0.48A-C	0.53A-C	0.57AB	0.47A-C
<b>Salt creek</b>	0.51A-C	0.44BC	0.45BC	0.55A-C	0.60AB	0.49A-C	0.52A-C

a,b,c,d and e means at the same column with different superscript are significantly ( $P<0.05$ ) different. \*IBA; indole-3-butyric acid

Table (4): Effect of IBA, Kinetin and different rootstocks on No. of leaves per shoot of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
Freedom	34.74GH	42.19E	44.35D	52.81B	57.57A	56.91A	44.35D
Harmony	24.46K	32.42I	33.40HI	46.08D	49.72C	45.54D	29.43J
Salt creek	28.02J	36.25G	38.65F	50.10 C	53.75B	52.81B	38.65F
<b>2021</b>							
Freedom	35.65IH	42.63F	45.07DE	53.75B	59.09A	55.37B	44.59E
Harmony	25.76L	33.65J	34.52IJ	46.55D	50.54C	45.78DE	29.74K
Salt creek	28.34	36.81H	39.72G	51.56C	55.37B	53.75B	39.72G

a,b,c,d and e means at the same column with different superscript are significantly ( $P < 0.05$ ) different. \*IBA; indole-3-butyric acid

#### 4.5. Leaf area ( $cm^2$ )

The effect of IBA, Kinetin, and different rootstocks on the leaf area ( $cm^2$ ) of Flame seedless grape in the 2020 and 2021 seasons was demonstrated in Table 5. The treated rootstocks with 1000 ppm of IBA had significantly greater grape leaf area of Flame seedless than the control group. The Flame seedless grapes that treated rootstocks with a low level of 250 ppm had a significantly higher area of leaf area in both seasons than those treated with IBA levels and the control. Grafting Flame seedless grapes on Freedom rootstock and treated with a low level of Kinetin (250 ppm) resulted in significantly higher leaf area in both seasons (144.45 and 148.75  $cm^2$ , respectively) than those treated with IBA levels and the control group, followed by Salt Creek (142.0 and 143.14  $cm^2$ ) and Harmony (112.56 and 118.48  $cm^2$ ) in the 2020 and 2021 seasons, respectively.

#### 4.6. Fresh weight of leaves (g) and Shoots

Table 6 illustrates the effect of IBA, Kinetin, and different rootstocks on the fresh weight of Flame seedless grape leaves and shoots in the 2020 and 2021 seasons. The fresh weight of Flame seedless grape leaves and shoots was significantly higher in the treated rootstocks with IBA (1000 ppm) than in the control. Furthermore, when the grafted union of rootstocks was impeded with Kinetin at a low level of

250 ppm, had a significantly higher fresh weight of leaves and shoots of Flame seedless grapes than those treated with IBA levels and the control in both seasons. Grafting using Freedom rootstock and treating the graft union of the Freedom rootstock with a low level of Kinetin (250 ppm) resulted in significantly higher fresh weights of leaves and shoots in both seasons (18.74 and 19.41 g, respectively) than those treated with IBA levels and the control group, followed by Salt Creek (17.25 and 17.41 g) and Harmony (15.45 and 15.95 g), respectively.

#### 4.7. Fresh weight for the root system

The effect of IBA, Kinetin, and different rootstocks on the fresh weight of the root system of Flame seedless grape in the 2020 and 2021 seasons illustrated in Table 7. The dipped grafted union rootstocks with a high level (1000 ppm) of IBA or a low level (250 ppm) of Kinetin had a considerably higher fresh weight root system for the Flame seedless grape than the control in both seasons. The freedom rootstock gave a higher significantly fresh weight of the root system when treated graft union with a low level of Kinetin (250 ppm) (12.55 and 13.01g) respectively than those treated with IBA levels and control group.

#### 4.8. Dry weight of leaves and shoots

The effect of IBA, Kinetin, and various rootstocks on the dry weight of Flame seedless grape leaves and shoots for the 2020 and 2021 seasons is shown in Table 8. The treated rootstocks with a high level of IBA had a significantly higher dry weight of leaves and shoots of Flame seedless grapes than the control in both seasons. The same trend was observed when treated rootstocks with a low level of Kinetin (250 ppm). In both seasons, the dry weight of the Flame seedless grapes' leaves and shoots decreased

dramatically when a high level of IBA was applied at the graft union of the freedom rootstock (6.56 and 6.87 g), (5.47 and 5.72 g) for Salt creek and (4.09 and 4.31 g) for Harmony in comparison with those treated with a low level of Kinetin: Freedom (9.77 and 9.54 g), Salt creek (8.81 and 8.23 g) and (7.57 and 7.15 g) for Harmony in both seasons, respectively.

Table (5): Effect of IBA, Kinetin and different rootstocks on leaf area (cm<sup>2</sup>) of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
Freedom	99.59F	92.82H	98.47F	102.02E	144.45A	104.74D	99.59F
Harmony	84.10I	79.82J	84.10I	91.70H	112.56C	100.26F	82.48I
Salt creek	91.70H	82.48I	95.97G	98.60F	142.00B	105.27D	92.82H
<b>2021</b>							
Freedom	100.26G	94.16I	98.60HG	104.70E	148.75A	105.27E	100.26G
Harmony	87.58 J	83.29L	88.90J	92.82I	118.48C	102.02F	84.10L
Salt creek	93.30 I	85.89K	97.02H	99.59G	143.14B	108.40D	93.30I

a,b,c,d and e means at the same column with different superscript are significantly ( $P < 0.05$ ) different. \*IBA; indole-3-butyric acid

Table (6): Effect of IBA, Kinetin and different rootstocks on fresh weight of leaves (g) and Shoots of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
Freedom	9.22H-J	10.49GH	11.26FG	15.29DC	18.74A	16.47BC	12.76EF
Harmony	7.73JK	7.13K	8.83H-K	11.46FG	15.45DC	13.05EF	8.99H-K
Salt creek	8.23JK	8.58I-K	8.95H-K	12.67EF	17.25AB	14.13DE	10.38G-I
<b>2021</b>							

<b>Freedom</b>	10.03G-I	10.85F-H	11.62F-G	15.95BC	19.41A	17.41B	12.67EF
<b>Harmony</b>	7.82J	7.73J	8.58 IJ	11.75E-G	15.95BC	13.34DE	9.22H-J
<b>Salt creek</b>	8.58IJ	8.99IJ	9.22H-J	12.85DE	17.41B	14.50CD	10.85F-H

a,b,c,d and e means at the same column with different superscript are significantly ( $P<0.05$ ) different. \*IBA; indole-3-butyric acid

Table (7): Effect of IBA, Kinetin and different rootstocks on fresh weight root system (g) of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
<b>Freedom</b>	5.84G-I	6.76FG	9.10C-E	10.03B-C	12.55A	11.49AB	10.89A-C
<b>Harmony</b>	3.72J	3.36J	5.77G-I	8.77DE	9.61C-E	8.77DE	8.06EF
<b>Salt creek</b>	4.83H-J	4.20LJ	6.03GH	9.10C-E	10.03B-C	9.10C-E	8.90DE
<b>2021</b>							
<b>Freedom</b>	5.86F-H	6.69EF	9.79B-D	10.89BC	13.01A	11.64AB	11.08BC
<b>Harmony</b>	4.10HI	3.37I	5.84F-H	8.90D	9.79B-D	8.83D	8.16DE
<b>Salt creek</b>	4.71G-I	4.44G-I	6.13FG	9.23CD	10.89BC	9.23CD	8.58D

a,b,c,d and e means at the same column with different superscript are significantly ( $P<0.05$ ) different. \*IBA; indole-3-butyric acid

Table (8): Effect of IBA, Kinetin and different rootstocks on dry weight leaves and shoots (g) of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
<b>Freedom</b>	4.55GK	4.89FK	5.24FJ	6.56DF	9.77A	8.98AB	7.15CE
<b>Harmony</b>	3.30K	3.36JK	3.41JK	4.09IK	7.57B-D	6.40DG	5.47EI
<b>Salt creek</b>	4.09IK	4.33HK	4.55GK	5.47EI	8.81A-C	7.56B-D	6.08DH
<b>2021</b>							
<b>Freedom</b>	5.06FI	5.14FI	5.81DH	6.87CF	9.54A	8.81AB	7.51BD
<b>Harmony</b>	3.36J	3.37J	3.66IJ	4.31HJ	7.15BE	6.28DG	5.46EI

<b>Salt creek</b>	4.18HJ	4.63GJ	4.63GJ	5.72DH	8.23AC	7.15BE	6.44CG
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a,b,c,d and e means at the same column with different superscript are significantly ( $P < 0.05$ ) different. \*IBA; indole-3-butyric acid

Table (9): Effect of IBA, Kinetin and different rootstocks on dry weight root system (g) of Flame seedless grape in 2020 and 2021 seasons.

Rootstocks	Experimental treatments						
	Control	IBA*			Kinetin		
		250	500	1000	250	500	1000
<b>2020</b>							
<b>Freedom</b>	2.42DE	2.70DE	3.66A-D	3.82A-D	5.50A	5.16AB	3.48B-D
<b>Harmony</b>	1.93DE	1.41E	2.11DE	2.25DE	3.69A-D	2.63DE	2.25DE
<b>Salt creek</b>	2.26DE	2.02DE	3.25C-E	3.41B-D	4.78A-C	3.11C-E	2.63DE
<b>2021</b>							
<b>Freedom</b>	2.63D-F	2.63D-F	3.72B-E	4.04A-D	5.68A	5.37AB	3.78A-E
<b>Harmony</b>	1.98EF	1.40F	2.42D-F	2.80D-F	3.84A-E	2.80D-F	2.44D-F
<b>Salt creek</b>	2.26D-F	2.18D-F	3.65B-E	3.87A-E	4.98A-C	3.24C-F	2.79D-F

a,b,c,d and e means at the same column with different superscript are significantly ( $P < 0.05$ ) different. \*IBA; indole-3-butyric acid

#### 4.9. Dry weight of root system

The effects of IBA, Kinetin levels, and various rootstocks on the dry weight of the root system of Flame seedless grape leaves and shoots in both seasons are shown in Table 9. In Flame seedless grape, the dry weight of root systems was significantly higher in treated rootstocks with a high level of IBA (1000 ppm) and a low level (250 ppm) of Kinetin than the control in both seasons. Grafting Flame seedless grapes on Freedom rootstock and treated with a low level of Kinetin (250 ppm) had a significantly higher dry weight of leaves and shoots in both seasons (5.16 and 5.37 g) than those treated with IBA levels and the control group, followed by Salt Creek (4.78 and 4.98 g) and Harmony (3.69 and 3.84 g) in the 2020 and 2021 seasons, respectively.

#### 5. Discussion

Plant hormones like auxin are still used in intensive nursery practices in viticulture to provide dense root production and good root development. In general, Kinetin and indole-3-butyric acid (IBA) increased both the start and proliferation of callus at the grafting point as well as the root formation at the basal end of grafted cuttings when compared to the

control, but IBA and 1-Naphthaleneacetic acid (NAA) boosted just the root formation at the basal end of grafted cuttings [14, 15]. As a result of the findings provided in the literature by a significant number of researchers, the use of IBA for the rooting of grapevine rootstock cuttings has been recommended [16].

The grafting process is thought to involve endogenous growth regulators [17]. According to Celik [18], the length of the scion shoot has an effect on the quality of the final grafting process as well as the grafted grapevines. Treatment with a 250 mg/litre application of Kinetin treatment had the best effect on callus rate. Kinetin treatment also resulted in a significant improvement in callus structure, with organisation into dense circular layers. Jamal *et al.*, [19] reported that the highest survival rate was found to be 94.7% in IBA treatment and the lowest survival of 73.3% was found in control. IBA play a crucial role in root and shoot development. Also, Rakibuzzaman *et al.* [20] observed that the stevia cutting survival rate was triggered due to IBA. Results indicated that cytokinins showed different physiological effects than those of auxins. Our findings revealed that hormonal treatment of the graft



union with high levels of IBA (1000 ppm) and low levels of Kinetin (250 ppm) improved graft union survival and scion shoot length.

Auxin (including IBA) application improved graft success rate in grapevines, whereas cytokinin (including Kinetin) treatment considerably improved graft union callus growth [11]. Auxin (IBA) aided the quick growth of callus between the scion and rootstock in grapevines [11]. The creation of a callus at the graft union is a key factor in determining if the scion and rootstock are compatible, which leads to grafting success [18]. Auxins can also cause callogenesis and rhizogenesis to compete [21, 4, 22, 23]. The development of great root formation has been shown to have a negative effect on callus formation, and there is a correlation between root initiation and auxin migration [4]. The use of BAP to treat the graft union increased the activity of POX, which has been reported to be primarily located in the graft union and responsible for lignifications, resulting in improved graft compatibility [24, 25]. Based on the findings of the aforementioned studies, which showed that both IBA and Kinetin have positive effects on growth and callus formation in a variety of fruits and plants, this supports the improvement of grape vegetative measurements as a result of improved root system and graft area in different rootstocks, especially in Flame seedless grapes.

## 6. Conclusions

It could be concluded that the low level of Kinetin (250 ppm) and Freedom rootstock grape are the best treatment and rootstock type for Flame seedless rootstock grafting to get the highest survival percentages, based on these observations followed by application with IBA at high level (1000 ppm).

## 7. Conflicts of interest

The authors declare that there was no conflict of interest in carrying out this work

## 8. Formatting of funding sources

Not applicable

## 9. Acknowledgments

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