Response of Kumquat (*Fortunella margarita*) Transplants to Foliar Spray with Bio-stimulator and GA3

Th. H. R. Al-Falahy*

Department of Horticulture, College of Agriculture, University of Anbar, Iraq

*Corresponding author's e-mail: ag.thamer.hameed@uoranbar.edu.iq.

Abstract. The experiment was conducted in one of the private orchards at Ramadi district, Anbar governorate for two seasons (2019 and 2020) with the objective to observe the effect of spraying kumquat transplants with age 2 years by bio-stimulator "Grow Amine" and gibberellic acid (GA3) on some vegetative characteristics and leaf mineral content. Bio-stimulator spray included control (distilled water only), 0.5, 0.75, and 1 gm.l⁻¹, on the other hand, spraying GA3 included, spray with distilled water served as (control), 15 and 20 mg.l⁻¹. Spray bio-stimulator and GA3 had a significant effect in most of the vegetative traits and N, P, K leaf content compared to the control, bio-stimulator with 1 gm.l⁻¹ and GA3 with 20 mg.l⁻¹ had achieved the highest value for all parameters.

1. Introduction

The kumquat, *Fortunella margarita* (Lour) Swingle or what is called Japanese orange, is the smallest citrus tree in size, which reaches a height of 2.5 meters, and it is one of the species of the genus *Fortunella* and developed by the scientist Swingle, its origins back to India, China, Southeast Asia and Japan [1], and it is a perennial evergreen tree that tolerates low temperatures, but its thermal needs for growth are high and therefore it is late flowering (late spring to summer from June to July depending on the climate) and is considered one of the most types of citrus fruits adapted to various environmental conditions as it tolerates high temperatures as well as cold and frost, some of its varieties tolerate up to (-7 °C). The kumquat tree differs from other types of citrus fruits in cold regions, as it enters deep dormancy during the winter period, and during cold periods the trees stop growing quickly and become inactive, which enables them to withstand the cold weather, but their growth improves better in warm areas which may give trees ability to produce two crops per year, and thus kumquat is more resistant to cold compared to oranges and tangerines [2]. The fruits are small and weigh between 8-10 g and reach a length of 2-2.5 cm, they are rich in vitamin C, while orange fruits give 50 mg of vitamin C per 100 g, and kumquat gives 150 mg of vitamin C per 100 g of fruits [1]. Amino acids are considered to be the precursor and component of proteins [3], which are important to stimulate growth and cell division; they act as buffers which help to maintain a favorable pH value within the plant cell, since they contain both acid and basic groups [4]. Studies have been proved that amino acids, directly and indirectly, affect the physiological activities which affecting plant growth and development in addition to having positive effects on plant growth by mitigating the damage caused by abiotic stresses [5]. Results revealed that using mixture of amino acids gave the highest significant increase in most of studied parameters of olive and washington navel orange.
diameter, branch numbers, leaves area and mineral content) [6,7]. Gibberellins are the second group of plant hormones that have been discovered after auxins. Gibberellins have biological activity in the growth and development of plants, including stem elongation, germination and transition from vegetative growth to flowering [8,9]. Researches demonstrated that foliar spray of GA3 at 20 and 30 mg.l\(^{-1}\) has a significant increase in vegetative growth and leaves mineral content [10]. Very little is known about growth and development of kumquat (Fortunella margarita) in Iraq, so the scope of the current research is to study the effect of amino acids as bio-stimulator and GA3 and their combination on some vegetative traits and leaf mineral content.

2. Materials and Methods
The present study was conducted during two successive seasons of 2019 and 2020 in one of the private orchards at Ramadi district, Anbar Governorate, Iraq to study the effect of spraying with bio-stimulant under the trade name "Grow Amine" and Gibberellic Acid "GA3" on the growth of kumquat transplants "oval kumquat".108 transplants were selected healthy and uniform as much as possible with age 2 years budded on Citrus jambhiri rootstock and cultivated in a black plastic pot with 30 cm diameter filled with 7 kg sand soil mixed with 3kg cattle manure. Transplants received the normal horticulture practices applied in the commercial orchard except for the tested treatments. Transplants were sprayed with Deltacide pesticide at a concentration of 1ml.l\(^{-1}\) to control the citrus leaf miner and aphids after the infection was noticed, spray continued every two weeks during March and April for the two seasons respectively, irrigation was done as needed besides to sprinkle irrigation during the hot summer months, during June and July plastic nets (saran) with a permeability of 50% of the sun's radiation were used to protect the transplants from the expected heat waves during these months, transplants were fertilized with NPK fertilizer (15:15:15) during March, April and May, with an interval of two weeks between one addition and another, at a rate of 10 g per transplant [11]. Some physical and chemical properties of the soil are presented (Table 1).

Table 1) Some physio-chemical characteristics of the experimental soils.

| Texture Grade | Available nutrients mg.kg\(^{-1}\) soil | pH | EC(1:1) ds.m\(^{-1}\) | CEC C.mol.1\(^{-1}\) | O.M g.kg\(^{-1}\) soil | Sand g.kg\(^{-1}\) soil | Loam g.kg\(^{-1}\) soil | Clay g.kg\(^{-1}\) soil | N | P | K | Total CaCO3 g.kg\(^{-1}\) soil | Fe g.kg\(^{-1}\) soil | Zn g.kg\(^{-1}\) soil |
|---------------|--------------------------------------|----|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|---|---|---|----------------------|----------------------|----------------------|
| Clay Loam     |                                      | 7.4| 2.17                | 26.40                | 12                   | 43.4                 | 28.0                 | 28.6                 | 68.5| 13.6| 222.7| 186.0                | 3.22                 | 1.56                 |

Two factors were used in this experiment, first factor is spraying with the bio-stimulant "Grow Amine" containing 15 amino acids with a percentage of 80% (Alanine, Arginine, Asparagine, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Phenylalanine, Proline, Serine, Threonine, Tyrosine and Valine) in addition to 14% organic nitrogen, spray was carried out with four levels (0, 0.50, 0.75 and 1g.l\(^{-1}\)) denoted by the symbol A\(_0\), A\(_{0.50}\), A\(_{0.75}\), and A\(_1\), respectively, the second factor included spraying with GA3 (Assay 90%) in three levels (0, 15 and 20 mg.l\(^{-1}\)) symbolized by the symbol G\(_0\), G\(_{15}\), and G\(_{20}\) respectively. transplants were sprayed during the first week of April and May with the cessation of spraying treatments during June and July, spraying operations resumed during September and October for both seasons respectively, spraying was carried out early in the morning till run- off with 0.1% tween 20 as a wetting agent, and then the following traits were measured:

2.1 Increment in leaves number (leaf.transplant\(^{-1}\)): It was measured by calculating leaves number at the beginning and the end of the experiment, the difference between them represents the increment in leaves number for both seasons.
2.2 Leaves area (dm$^2$): It was measured by taking 10 leaves (fifth –sixth node) from the apex of the branch [12], leaves area were calculated according to [13].

2.3 Increment of transplant length (cm): It was measured by using tape measure at the beginning and the end of the experiment, the difference between the two readings represents the increment of transplant length.

2.4 Secondary branches number (branch. transplant$^{-1}$): It was measured at the end of the two seasons of the experiment.

2.5 Secondary branches length (cm): It was measured by using tape measure at the end of the experiment for both seasons.

2.6 Rootstock and bud stem diameter increment (mm): It was measured at the beginning and the end of the experiment for both seasons by using caliper vernier [14].

2.7 Chlorophyll content (mg.100g$^{-1}$ fresh weight): To quantify chlorophyll content in leaves, sample of 10 fully expanded leaves were taken from (6-8 node) from the apex of the branches, using methanol as a solvent to extract chlorophyll, the amount of chlorophyll extracted were estimated according to [15].

2.8 Total carbohydrate content in branches (%): The total carbohydrate concentration were measured by using the colorimetric reaction between phenol and sulphuric acid, using spectrophotometer at 485nm according to [16].

2.9 Leaf mineral content (%): Leaf samples were collected for chemical analysis in the 1st week of November of both seasons. Each sample consists of 20 leaves/transplant. Leaves were washed several times with tap water, rinsed with distilled water, and then dried at 70 c° until constant weight, ground, and digested according to [17].Nitrogen, Phosphorus, and Potassium were estimated by the methods mentioned by [18].

Statistical analysis: Randomized Complete Block Design (RCBD) was used as the experimental design with three replicates, each replicate represented by three transplants, obtained data were analyzed by analysis of variance (ANOVA) using statistical package software GenStat, Differences between treatments were made by F-test and the least significant differences at $P= 5\%$ according to [19].

3. Results and Discussion

3.1. Increment in leaves number (leaf. transplant$^{-1}$)

Data presented in table2 shows that the results took similar trend during the two studied seasons, significant difference was detected in leaves number, A$_1$ (1g.1$^{-1}$) gave the highest leaves number which recorded (398.56 and 287.67 leaf. transplant$^{-1}$) for two seasons respectively, compared to (A$_0$), on the other hand, G$_{20}$ (20 mg.1$^{-1}$) gave the highest value (363.75 and 274.92 leaf. transplant$^{-1}$) for two seasons respectively, compared to the fewer value were observed in (G$_0$). The interaction between two factors were also significantly different among treatments, the highest value (416.33 leaf. transplant$^{-1}$) were noticed in A$_1$G$_{20}$ for 2019 season while (327.33 leaf. transplant$^{-1}$) were observed in A$_0$G$_{20}$ for 2020 season, while the lowest value (323.67 and 234.67 leaf. transplant$^{-1}$) was obtained in control treatment (A$_0$G$_0$) for two seasons respectively.

3.2. Leaves area (dm$^2$)

From the results shown in table2. It appears that the maximum value of leaves area was in A$_1$ spray treatment which reached (56.77 and 54.51 dm$^2$) for two seasons respectively, compared to the minimum value which given by A$_0$. Regarding (G) treatment, the highest leaves area (49.63 and 47.65 dm$^2$) for two seasons respectively was observed in G$_{20}$ treatment, while the lowest value was noticed in G$_0$. According to the statistical analysis, the highest interaction value (59.44 and 57.40 dm$^2$) was noticed in A$_1$G$_{20}$ treatment while A$_0$G$_0$ gave the lowest value.
3.3. Increment of transplant length (cm)

It was observed from results shown in table2, that there were increase in transplant length when transplant sprayed with Bio-Stimulator, the highest value has been registered when sprayed with A₁ (23.22 and 22.77 cm) for two seasons respectively, while the lowest values was observed in A₀. Similarly, transplants sprayed with GA3 showed a significant difference, G₂₀ had achieved the highest value (18.68 and 18.67cm) for two seasons respectively, while the lowest value was observed in G₀. The interaction between treatments taking the same trend, the highest value has been achieved in A₁G₂₀ (23.33 and 25.0 cm) for two seasons respectively, compared to A₀G₀ treatment.

Table (2) Effect of Bio-Stimulator and GA3 foliar spray on increment in leaves number, leaves area and increment of transplant length of Kumquat transplants during 2019 and 2020 seasons.

| Season   | 2019 | 2020 | GA3 |
|----------|------|------|-----|
| A₀       | 323.67 | 338.67 | 345.00 | 335.78 | 234.67 | 249.67 | 256.67 | 256.67 | 247.00 |
| A₀.50    | 330.67 | 344.00 | 343.33 | 339.33 | 242.33 | 255.67 | 254.33 | 250.78 |
| A₀.75    | 345.33 | 348.33 | 350.33 | 348.00 | 256.33 | 259.33 | 327.33 | 281.00 |
| A₁       | 385.67 | 393.67 | 416.33 | 398.56 | 297.00 | 304.67 | 261.33 | 287.67 |
| Mean     | 346.33 | 356.17 | 363.75 | 257.58 | 267.33 | 274.92 |       |       |
| LSD 5%   | A 0.56 | A 0.98 | A 0.32 | A 0.98 | A 0.56 | A 0.98 | A 0.32 | A 0.98 |

3.4. Secondary branches number (branch. transplant⁻¹)

Branches number was increased with all sprayed substances when compared with unsprayed treatment. As shown in table 3, branches number recorded the highest value with A₁ which gave (16.96 and 14.55 branch) for both seasons respectively, while the minimum value (10.88 and 10.66 branch) obtained by A₀, the same trend was found when sprayed transplant with GA3 which registered (14.91 and 13.69 branch) for both seasons respectively when treated with G20 compared with G0. Interaction treatment A₁G₂₀ achieved the highest value for the first season which reached (17.55 branch) and A₀.75 G₂₀ for the second season which gave(15.50 branch) compared to the lowest value at A₀G₀.

3.5. Secondary branches length (cm)

The data of both seasons shown in table 3. Indicated that A₀.75 achieved the highest value of branch length (15.38 cm) for the first season and A₁ for the second season which gave (15.11 cm) respectively compared to A₀. As for the effect of GA3, the data of both seasons showed that G₂₀ recorded the highest value (14.16 and 13.14 cm) compared to G₀. Similarly, A₁G₂₀ recorded the highest value (16.33 and 15.83 cm) for both seasons respectively compared to the lowest value (A₀G₀).

LSD = Least significant difference at 5% probability
3.6. Rootstock stem diameter (mm): Data in table 3. Clearly showed that, rootstock stem diameter was increased gradually and significantly with increasing Bio-Stimulator levels in two seasons. \( A_1 \) treatment caused stimulatory effects which gave (9.86 and 9.34 mm) for two seasons respectively compared to the lowest value in \( A_0 \) (6.78 and 6.70 mm) for two seasons respectively. Similarly, \( G_{20} \) registered the highest value for stem diameter which reached (8.52 and 8.35 mm) for two seasons respectively compared to the minimum value in \( G_0 \) (7.97 and 7.54 mm) respectively. On the other hand, the interaction between two factors indicated that \( A_1G_{20} \) recorded the highest value (10.0 and 9.80 mm) respectively compared to the lowest value in \( A_0G_0 \) which gave (6.32 and 6.10 mm).

Table (3) Effect of Bio-stimulator and GA3 foliar spray on secondary branches number, secondary branches length and increment in rootstock stem diameter of Kumquat transplants during 2019 and 2020 seasons.

| Season | 2019 | 2020 |
|--------|------|------|
|        | GA3  | GA3  |
| A_0    | 10.16| 10.37|
| A_0.50 | 11.83| 11.43|
| A_0.75 | 14.33| 15.40|
| A_1    | 14.66| 14.16|
| Mean   | 12.75| 12.27|
| LSD    | 0.53 | 0.26 |

3.7. Bud stem diameter (mm)

Data in table 4 Proved that bud stem diameter was significantly increased by using Bio-Stimulator and GA3 as compared with control in both seasons. \( A_1 \) gave the superior value (7.73 and 7.34 mm) compared to the lowest value in \( A_0 \). Similarly, \( G_{20} \) gave the highest value (6.68 and 6.67 mm) compared to the fewest value in \( G_0 \). Meanwhile, the interaction treatment \( A_1G_{20} \) gave the highest value for two seasons compared to \( A_0G_0 \).

3.8. Chlorophyll content (mg.100gm\(^{-1}\) fresh weight)

The analysis of variance showed that foliar application of bio-stimulant, GA3 and their interaction significantly increased chlorophyll content. According to the data given in table 4, \( A_1 \) gave increasing
in chlorophyll content (309.61 and 303.18 mg.100g\(^{-1}\) fresh weight) compared to the minimum value in A\(_0\) (281.52 and 276.17 mg.100g\(^{-1}\) fresh weight). Likewise, G\(_{20}\) gave the highest value (297.73 and 298.44 mg.100g\(^{-1}\) fresh weight) compared to the lowest value in G\(_0\). The interaction between treatments appeared superiority of A\(_1\)G\(_{20}\) in the first season and A\(_{0.75}\)G\(_{20}\) in the second season.

3.9. Total carbohydrate content in branches (%)

The data in table4. Indicate that spraying with amino acids and GA3 in a single way led to a significant increase in the percentage of carbohydrates in branches. A\(_1\) gave the highest value which reached (11.49 and 11.87 \%) for both seasons respectively, on the other hand, G\(_{20}\) achieved the superior value on carbohydrate percentage which gave (11.57 and 11.78 \%) for both seasons respectively, while non-significant difference was noticed in combination between studied factors.

### Table 4

Effect of Bio-stimulator and GA3 foliar spray on bud stem diameter increment, chlorophyll and Total carbohydrate content of Kumquat transplants during 2019 and 2020 seasons.

| Amino acids | 2019 | 2020 |
|-------------|------|------|
| GA3         |      |      |
| G0          | 1.68 | 1.75 |
| G\(_{15}\) | 1.74 | 1.79 |
| G\(_{20}\) | 1.83 | 1.86 |
| Mean        | 1.75 | 1.79 |
| A\(_0\)     | 1.68 | 1.74 |
| A\(_{0.50}\)| 1.74 | 1.79 |
| A\(_{0.75}\)| 1.83 | 1.86 |
| A\(_1\)     | 1.88 | 1.92 |
| Mean        | 1.75 | 1.80 |
| LSD 5%      | G    | G    |
|             | 0.03 | 0.03 |

### 3.10. Leaf mineral content (%)

Application A\(_{0.75}\), G\(_{20}\) in single or in combination resulted in the highest Nitrogen content (1.86 and 1.87\%) for A\(_{0.75}\) and (1.86 and 1.88\%) for G\(_{20}\) for both seasons respectively, the combination between them achieved the highest value (1.91 and 1.94\%) for both seasons respectively. In the same context, A\(_{0.75}\), G\(_{20}\), and the combination between them achieved the superiority for Phosphorus and Potassium compared to control. Table5. Effect of amino acids and GA3 foliar spray on N, P and K leaves content of Kumquat transplants during 2019 and 2020 seasons.

LSD = Least significant difference at 5% probability.

### Table 5

Effect of amino acids and GA3 foliar spray on N, P and K leaves content of Kumquat transplants during 2019 and 2020 seasons.

| Amino acids | 2019 | 2020 |
|-------------|------|------|
| GA3         |      |      |
| N (\%)      |      |      |
| G0          | 1.68 | 1.74 |
| G\(_{15}\) | 1.74 | 1.79 |
| G\(_{20}\) | 1.83 | 1.86 |
| Mean        | 1.75 | 1.79 |
| A\(_0\)     | 1.68 | 1.74 |
| A\(_{0.50}\)| 1.74 | 1.79 |
| A\(_{0.75}\)| 1.83 | 1.86 |
| A\(_1\)     | 1.88 | 1.92 |
| Mean        | 1.75 | 1.80 |
| LSD 5%      | G    | G    |
|             | 0.03 | 0.03 |

| P (\%)      |      |      |
| A\(_0\)     | 0.13 | 0.14 |
| A\(_{0.50}\)| 0.16 | 0.17 |
| A\(_{0.75}\)| 0.19 | 0.20 |
| A\(_1\)     | 0.20 | 0.23 |
| Mean        | 0.17 | 0.21 |
| LSD 5%      | A    | A    |
|             | 0.02 | 0.03 |

| K (\%)      |      |      |
| A\(_0\)     | 1.21 | 1.25 |
| A\(_{0.50}\)| 1.24 | 1.28 |
| A\(_{0.75}\)| 1.29 | 1.34 |
| A\(_1\)     | 1.28 | 1.32 |
| Mean        | 1.26 | 1.30 |
| LSD 5%      | A    | A    |
|             | 0.02 | 0.02 |
According to the results, the role of the "Grow Amine" bio-stimulator in improving and increasing the vegetative growth characteristics, mineral and carbohydrate content of the transplants may be attributed to the vital role that the bio-stimulator components play in the growth of kumquat transplants. The bio-stimulator has played its positive role because it contains amino acids and nitrogen, which play a great role in stimulating physiological and biochemical processes, in which amino acids participate as protein-building units and stimulate carbon metabolism and carbohydrate building, it also contributes to building and encouraging the action of a number of enzymes and co-enzymes, which result in improving plant growth [20], in addition to its role as a vital catalyst because it has a positive effect on plant growth as well as its effective role in reducing the impact of environmental stress [5], in addition, nitrogen contributes directly and indirectly to the synthesis of hormones like auxins which play an important role in cell elongation and cellular expansion and this effect is due to auxin stimulation of enzymes that degrade some components of the cell neighbor in addition to the important role of cytokinins in increasing cell division in apical meristemes and vascular cambium and addition New cells in the plant leading to increased growth [21, 22]. Also, an increase kumquat leaves nutrients may be attributed to the fact that spraying with bio-stimulant has improved the characteristics of vegetative growth, therefore it is expected that the increase in the characteristics of vegetative growth leads to an increase in root growth because there is a relationship between vegetative and root growth consequently, increase the efficiency of roots in absorbing nutrients. Our results are in line with those obtained by [23] who showed that bio-stimulants treatments had a promotional effect on the growth and nutritional status of Washington navel orange. Effect of gibberellic acid in improving vegetative growth may be due to its role in increasing cells size through its contribution to increasing the flexibility of cell walls or to the role of auxin-induced by gibberellin in cell growth and its importance in stimulating genetic reproduction and then the process of translation and building of RNA and protein on the other hand. Auxins induced by gibberellins stimulate the plasticity of cell walls by breaking cell wall bonds and re-arranging them in new sites under the influence of turgor pressure, which contributes to an increase in cell size [24, 25, 26]. The reason for the increase in the chlorophyll content in the leaves as a result of spraying gibberellin may be due to the role of gibberellic acid in conservation the pigment from breakdown or oxidation by the action of the chlorophyllase enzyme, furthermore, increasing the absorption of elements some of which like nitrogen contribute to building the chlorophyll molecule, on the other hand, gibberellic acid is one the hormones that delay senescence [21]. Thus, the increase in plant height and the number of branches results in an increase in the number of leaves and for the two seasons of research, as the increase in the number of leaves is accompanied by an increase in the leaves area, thus an increase in the efficiency of photosynthesis, which results in an increase in the activity of the roots and absorption a greater amount of nutrients from the soil and this may explain the increase in the content of elements in the leaves. These results are in agreement with [27] on mango trees and [28, 10] on Washington navel orange and olive transplants.

4. Conclusion
From this study it can be concluded that foliar application of amino acids as Bio-Stimulant in (1gm.l⁻¹), GA3 in (20 mg.l⁻¹) and combination between them resulted in increasing in vegetative growth characteristics, while amino acids in (0.75gm.l⁻¹) and GA3 in (20 mg.l⁻¹) achieved the highest percentage for Phosphorus and Potassium compared to control.

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