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Effectiveness of the bacterial biofertilizer (Azotobacter), Gibberellic acid and kinetin solution in metabolic some the physiological processes of *Psidium guajva* L. seedlings

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Abstract

The experiment was carried out in the College of Agriculture - University of Karbala at the beginning of September of the year 2022 according to a randomized complete block design to study the effect of the bacterial biofertilizer Azotobacter and a solution of gibberellic acid (0, 150 and 300 mg. L^{-1}), and kinetin (0, 25 and 50 mg. L^{-1}) in changes of metabolic some physiological processes of *Psidium guajava* L. seedlings. The results showed that the single effect of the Azotobacter was superior in all studied characteristics, except for the relative moisture content compared to the control treatment, as for the gibberellic acid solution, the concentration (300 mg.L^{-1}) was superior by giving it the highest significant increase in all studied traits except for the relative moisture content compared control treatment, while the treatment of seeds with chitin solution excelled, and the concentration (50 mg.L^{-1}) recorded the highest significant difference in all studied traits except for the characteristic of the degree of stability of cell membranes.

Keywords: Guava, Azotobacter, Gibberellic acid and Kinetn.

Introduction

The guava tree *Psidium guajava* L. belongs to the Myrtaceae family, which includes several genera of perennial shrubs, including guava, it grows in tropical and subtropical regions of the world [1], the guava tree is cultivated in a number of countries for its nutritional or medicinal importance, it is an important fruit in tropical regions such as India, Indonesia, Pakistan, Bangladesh, South and Central America, including Brazil and Mexico, its global production is estimated at about 40 million tons annually. India ranks first in global production, followed by China, Kenya and Brazil. In the Arab world, it is grown in Egypt, Sudan, Palestine and Algeria. It can be said that its cultivation is good in areas that rise (0-2000 meters) above the level sea surface, provided it is free of frost [2].

Azotobacter is considered a group of bacteria that stimulate plant growth, if some studies indicate that the process of inoculating the soil with this type of bacteria can reduce the use of nitrogen fertilizer by 50% [3], this is through its role in the process of fixing atmospheric nitrogen in the soil, which improves soil fertility, increases plant growth, and thus increases production [4].

Plant growth regulators have an important role in regulating most of the biological and physiological activities in plants, gibberellic acid is one of the basic plant growth regulators that are widely used in agriculture due to its physiological effects within plant tissues [5], it also has a significant effect on the speed of division of cambium cells, which stimulates the elongation of plant stems [6], it also works to delay aging by slowing the demolition of chlorophyll and RNA and helps in building them, as well as stimulates the process of mitotic division of cells [7].

kinetin belongs to the group of cytokinins, which is one of the plant growth regulators, as it has an important role in breaking the dormancy of the lateral buds of fruit trees. In addition, it works to reduce apical dominance and thus reduces plant height. Which, in turn, keeps chlorophyll from being broken down [8], and is also included in the synthesis of tRNA because it contains amino acids such as serine and tyrosine [9].

Materials and Methods

The experiment was carried out in the Shade House of the Department of Horticulture and Landscape Engineering - College of Agriculture - University of Karbala, Al-Hussainiya district, located at the intersection of longitude 44° 06^{-} $58^{=}$ east and latitude 32° 32^{-} $17^{=}$ north, which rises 29 m above sea level for the fall season of 2022, to study the effect of Azotobacter biofertilizer, gibberellic acid and kinetin solution in some physiological characteristics of *Psidium guajava* L. seedlings.

The factorial experiment was carried out with three factors with randomized complete block design (R C B D), where the first factor represents the biological fertilizer (without an inoculant, with an inoculant). Azotobacter bacteria were prepared in the postgraduate laboratory of the Plant Protection Department of the College of Agriculture, University of Karbala. The soil was inoculated by adding 25 ml of Azotobacter per bag. With a bacterial density of (10^8) and by injection method using a syringe designated for this purpose, the second factor is a solution of gibberellic acid in three concentrations (0, 150 and 300 mg. L^{-1}), and the third factor is a solution of kinetin in three concentrations (0, 25 and 50 mg. L^{-1}), the seeds were soaked for 24 hours and then planted in bags, thus, the experiment becomes factorial with three factors (2 x 3 x 3), and with the number of transactions (18) transactions and (54) experimental units, and each experimental unit consisting of (8) observations. the data was statistically analyzed for all the studied traits according to the design of the experiment using the electronic calculator and the SAS program for statistical analysis (2012), and the arithmetic means of the coefficients were compared statistically according to Duncan's Multiple Range Test at the 0.05 probability level.

studied Traits**:** Dry weight of shoots (gm), Relative water content (%), Cell membrane stability index or damage index (%), and Estimation of the content of fresh leaves of total chlorophyll (mg. gm^{-1} wet weight).

Results and Discussion

Dry weight of shoots (gm)

The statistical data in Table (1) indicate that soil pollination Azotobacter recorded the highest significant increase in the dry weight of the shoot at a rate of (2.339 g) and an increase rate of (11.540%) compared to the control treatment that gave the lowest rate of (2.097 g), the data of the same table also showed that soaking guava seeds with different concentrations of gibberellic acid produced seedlings that recorded the highest dry weight of the shoot, especially at the concentration (300 mg. L^{-1}) at a rate of (2.356 g) and an increase of (10.818%) compared to the control treatment that gave the lowest rate (2.126 g), the data of the table also shows that there are significant differences between the used concentrations of kinetin, the concentration (50 mg. L^{-1}) recorded the highest significant increase in this characteristic with an average of (2.443 g) and an increase rate of (28.646%) compared With control treatment, which recorded the lowest, the average dry weight of the shoots was (1.899 g).

The results of the binary interaction table between the bacterial biofertilizer and gibberellic acid indicate that there are significant differences in this characteristic, as the seeds that were soaked with a gibberellic acid solution at a concentration of (300 mg.L^{-1}) and bacterial fertilized with the bacterial fertilizer produced seedlings that were distinguished by giving them the highest rate of (2.495 g), an increase (26.521%) compared to the control treatment whose seedlings produced the lowest rate of (1.972 g).

As shown in Table (1) the inoculation of the soil with the bacterial biofertilizer and the seed treatment with a concentration of (50 mg. L^{-1}) of kinetin gave the highest rate of (2.601 gm), with an increase rate of (48.713%) over the control treatment whose seedlings recorded the lowest rate (1.749 gm).

It is clear from the data of the binary interaction table between gibberellic acid and kinetin that there was a significant increase in the dry weight of the shoot, especially when the seeds were soaked at a concentration of (300 mg. l^{-1}) of gibberellic acid with (50 mg. l^{-1}) of kinetin at a rate of (2.597 g), with an increase rate of (57.013%) compared to the control treatment, which produced seedlings that were characterized by the lowest dry weight of the shoot which averaged (1.654 g).

Among the results of the triple overlap of the studied factors, we notice that there are differences that reached a significant level between the treatments, as the soil inoculation with the bacterial biofertilizer with the treatment of seeds with a concentration of (300 mg. l^{-1}) of gibberellic acid and a concentration of (50 mg. l^{-1}) of kinetin has achieved the highest average in the studied trait was (2.906 gm) with an increase rate of (126.67 %) compared With control treatment which recorded the lowest dry weight of the shoot at a rate of (1.282 %).

By reviewing the results of the table, we note that Azotobacter bacteria had a significant effect on the studied trait due to the increased availability of nutrients that helped the roots of the seedlings to increase their absorption and transmission, and then the increase in the concentration of these elements in the leaves, which reflected positively on this trait [10]. As for gibberellic acid, it has a significant effect, as high concentrations of gibberellic acid led to a significant increase in the dry weight of the stem. The reason for this is the physiological role that gibberellic acid plays in transforming processed food compounds towards growth and construction sites. As for kinetin, it had a significant effect on the studied trait. [11, 12]

Table (1): Effect of Azotobacter, gibberellic acid solution and kinetin and interaction factors on shoot dry weight (g)

Biofertilizer Azotobacter	gibberellic acid concentration (mg. L^{-1})	Kinetine concentration (mg. L^{-1})			Interaction between Azotobacter and gibberellic acid	Azotobacter average
		0	25	50		
Without Azotobacter	0	1.282 c	2.280 ab	2.354 ab	1.972 b	2.097 b
	150	1.836 bc	2.252 ab	2.218 b	2.102 b	
	300	2.130 b	2.234 b	2.288 ab	2.217 ab	
Biofertilizer	0	2.027 b	2.338 ab	2.478 ab	2.881 ab	2.339 a
	150	2.008 b	2.294 ab	2.419 ab	2.240 ab	
	300	2.115 b	2.465 ab	2.906 a	2.495 a	
kinetine average		1.899 b	2.311 a	2.443 a		
Interaction between Azotobacter and gibberellic acid	Without Azotobacter	1.749 c	2.555 ab	2.286 ab	gibberellic acid averages	
	Azotobacter	2.050 bc	2.366 ab	2.601 a		
Interaction between gibberellic acid and Kinetine	0	1.654 d	2.309 abc	2.416 ab	2.126 b	
	150	1.922 cd	2.273 abc	2.318 abc	2.171 ab	
	300	2.122 bc	2.350 abc	2.597	2.356	

*Means that share the same letters for the single factors and their interactions do not differ significantly between them according to Duncan's polynomial test at the 0.05 probability level.

Relative water content (%)

Statistical analysis table and comparison of averages (2) for individual treatments shows that there is no significant effect of each of the bacterial biofertilizer and gibberellic acid on the relative moisture content of guava seedling leaves, it is also clear from the same table that the treatment of soaking the seeds with a concentration of (50 mg. l^{-1}) of kinetin recorded the highest significant increase in the relative moisture content of the leaves at a rate of (71.063 %) with an increase of (8.34 %) compared with control treatment which gave the lowest seedlings the relative moisture content of the leaves was (65.591%).

From the table of binary interactions, it is clear that there are no significant differences between the two interaction treatments between the bacterial biofertilizer and gibberellic acid in the characteristic of the relative moisture content of the leaves.

bacterial yielded the highest relative moisture content at a rate of (73.052%), with an increase rate of (9.80%), compared to the lowest rate of leaf seedlings of the Control treatment, which amounted to (66.529%).

From the table of binary interactions, it is clear that there are no significant differences between the two interaction treatments between the bacterial biofertilizer and gibberellic acid in the characteristic of the relative moisture content of the leaves.

It is noted from the same table that the bilateral interference between gibberellic acid and kinetin has led to the production of seedlings that were characterized by a high relative moisture content when the seeds were soaked in a concentration (300 mg. l^{-1}) of gibberellic acid and a concentration of (50 mg. l^{-1}) of kinetin together at a rate of It reached (72.752%) with an increase rate of (15.29 %) compared to the control treatment which produced seedlings whose leaves were characterized by a low relative moisture content with an average of (63.103 %).

And from reviewing the results of the triple interaction of the studied factors, it is clear that the interaction of the bacterial biofertilizer with gibberellic acid at a concentration of (300 mg.l-1) and kinetin at a concentration of (50 mg.l-1) has recorded the highest significant mean in the relative moisture content of guava seedlings, which amounted to (76.317). %) with an increase rate of (22.308%) compared Control comparison treatment, which gave the lowest relative moisture content in the leaves of its seedlings at an average of (62.397%).

Through the results of the table above, it is clear that Azotobacter had a significant effect on the mentioned trait. Through its positive effect on physiological processes, such as increasing the efficiency of photosynthesis in the leaves and their output of carbohydrates and proteins, and increasing the number and area of leaves, which positively affects the studied traits [11]. Also, gibberellic acid has a significant effect on the studied, and the reason for that is the physiological role that gibberellic acid plays in transforming manufactured food compounds towards growth and construction sites. Gibberellic acid also delays leaf aging. In addition, gibberellic acid plays an important role in reducing the harmful effect of abscisic acid. As for kinetin, it had a significant effect. This may be attributed to the important role of kinetin in cell division, differentiation, growth of lateral buds, increase in leaf area, delay aging and development of chloroplasts, and it also It helps in cell division and preserves chlorophyll pigment as a result of its ability to draw nutrients from the soil into the developing leaves and tops to encourage the formation of chlorophyll and prevent its loss, thus the leaves retain their greenness for as long as possible, in addition to increasing the number of leaves, the number of branches, and the dry weight of the stem [13 , 14].

Table (2): Effect of Azotobacter, gibberellic acid solution and kinetin and interaction factors on the relative moisture content (%)

Biofertilizer Azotobacter	gibberellic acid concentration (mg. L^{-1})	Kinetine concentration (mg. L^{-1})			Interaction between Azotobacter and gibberellic acid	Azotobacter average
		0	25	50		
Without Azotobacter	0	63.809 bc	68.408 abc	69.913 abc	67.377 a	67.818 a
	150	66.856 abc	65.862 abc	68.120 abc	66.946 a	
	300	68.923 abc	69.281 abc	69.187 abc	69.131 a	
Azotobacter	0	62.397 c	65.063 bc	68.405 abc	65.289 a	68.350 a
	150	67.864 abc	67.491 abc	74.433 ab	69.929 a	
	300	63.696 bc	69.482 abc	76.317 a	69.832 a	
kinetine average		65.591 b	67.598 ab	71.063 a		
Interaction between Azotobacter r and gibberellic acid	Without Azotobacter	66.529 b	67.850 ab	69.074 ab	gibberellic acid averages	
	Azotobacter	64.652 b	67.345 ab	73.052 a		
Interaction between gibberellic acid and Kinetine	0	63.103 b	66.736 ab	69.159 ab	66.333 a	
	150	67.360 ab	66.676 ab	71.277 a	68.438 a	
	300	66.310 ab	69.382 ab	72.752 a	69.481 a	

*Means that share the same letters for the single factors and their interactions do not differ significantly between them according to Duncan's polynomial test at the 0.05 probability level

Cell membrane stability index or damage index (%)

It is clear from the data of the statistical analysis table (3) that the single effect of the bacterial inoculum led to the presence of significant differences in the stability of the membranes of the cells of the leaves of guava seedlings, where the percentage of leaves ripening decreased to the lowest rate of (58.780%) compared with comparison treatment, whose seedlings gave the highest rate of leaf membranes ripening amounted to (60.345%) with a decrease of (2.662%).The same table also shows that the treatment of guava seeds with different concentrations of gibberellic acid significantly affected the rate of this characteristic, as the concentration (300 mg. L^{-1}) of gibberellic acid solution recorded the lowest rate of exudation, at a rate of (58.631%) with a decrease rate of (4.795%) compared to the control treatment, whose seedlings were characterized by the highest rate of maturity at a rate of (61.584%). It is also noted from the table that the treatment of seeds before sowing with different concentrations of kinetin did not reach the extent significant effect of the characteristic stability of the membranes of the cells of the leaves of guava seedlings.

The table also showed that the interaction between the bacterial biofertilizer and gibberellic acid led to a significant effect on the same trait it reached (56.741%) while the comparison Control achieved the highest degree with an average of (63.503%). It is noted from the data of the bilateral interaction between the studied factors that the decrease in the rate of exudation of the membranes reached a significant level in the interaction treatment between the bacterial inoculum and the concentration (0 mg. L^{-1}) of the kinetin at a rate of (57.356%) compared to the control treatment which recorded the highest rate. reached (61.680%). The treatment of the binary interaction between gibberellic acid and kinetin achieved a significant effect in the rate of this trait, as the seeds that were treated with a concentration of (300 mg. l^{-1}) of gibberellic acid and a concentration of (0 mg. l^{-1}) of kinetin excelled by giving them seedlings that were characterized by the lowest percentage maturity at a rate of (56.819%) compared to the control treatment which produced seedlings whose leaves were characterized by the highest rate of maturity at (63.524%).

From the table of the results of the triple interaction of the studied factors, it is clear that the inoculation of the soil with the bacterial biofertilizer and the treatment of the seeds with a concentration of (300 mg. L^{-1}) of gibberellic acid and a concentration of (0 mg. L^{-1}) of kinetin gave seedlings that were characterized by the lowest maturity rate. Its rate is (55.709%) while the control treatment produced shells with a high rate of (67.671%) for this characteristic.

From reviewing the results of the table, we note that Azotobacter bacteria had a significant effect on the degree of stability of cell membranes. The increased readiness helped the roots of the seedlings to increase their uptake and transmission, and then increase the concentration of these elements in the leaves [10], which positively affected physiological processes such as increasing the efficiency of photosynthesis in the leaves and their output of carbohydrates and proteins and increasing the number and area of leaves [11].

Gibberellic acid also had a significant effect on the studied trait, as high concentrations led to The reason for this is the physiological role that gibberellic acid plays in diverting processed food compounds towards sites of growth and construction. Gibberellic acid also delays leaf aging. In addition, gibberellic acid plays an important role in reducing the harmful effect of abscisic acid [12].

As for kinetin, it was not significantly affected in the studied trait

Table (3): Effect of Azotobacter, gibberellic acid solution and kinetin and in teraction factors in cell membrane stability or damage index(%)

Biofertilizer Azotobacter	**gibberellic acid concentration (mg. L^{-1})**	**Kinetine concentration (mg. L^{-1})**			**Interaction between Azotobacter and gibberellic acid**	**Azotobacte average**
		0	**25**	**50**		
Without Azotobacter	0	67.671 a	62. 061 bc	59.335 c-f	63.503 a	60.345 a
	150	59.444 c-f	57.565 d-g	55.473 g	57.493 c	
	300	57.928 d-g	59.918 cde	63.717 b	58.923 b	
Azotobacte	0	59.376 c-f	60.906 bcd	60.154 b-d	60.145 b	58.780 b
	150	56.985 efg	60.182 b-e	61.197 bcd	58.750 b	
	300	55.709 f-g	56.919 efg	57.595 d-g	56.741 c	
kinetine average		59.518 a	59.591 a	59.578 a		
Interaction between Azotobacte and gibberellic acid	**Without Azotobacte**	61.680 a	59.848 ab	59.508 b	**gibberellic acid averages**	
	Azotobacte	57.356 c	59.335 b	59.649 b		
Interaction between gibberellic acid and Kinetine	0	63.524 a	61.484 ab	59.745 bc	61.584 a	
	150	58.214 cd	58.873 cd	58.335 cd	58.474 b	
	300	56.819 d	58.418 cd	60.656 bc	58.631	

*Means that share the same letters for the single factors and their interactions do not differ significantly between them according to Duncan's polynomial test at the 0.05 probability level.

Estimation of the content of fresh leaves of total chlorophyll (mg. gm^{-1} wet weight)

Through the results of Table (4) it is clear that the bacterial biofertilizer produced seedlings whose leaves were characterized by a high content of total chlorophyll at an average of (0.887 mg. gm^{-1}) while the control treatment recorded the lowest value at a rate of (0.838 mg. gm^{-1}), As for the treatment of seeds before planting with different concentrations of gibberellic acid, the data indicate that there were significant differences in the content of fresh leaves of total chlorophyll, where the concentration (300 mg. L^{-1}) of gibberellic acid gave the highest significant increase, at a rate of (0.899 mg. gm^{-1}) Compared to the control treatment that gave the lowest rate of (0.823 mg. gm^{-1}), it is clear from Table (4) that the seedlings resulting from soaking its seeds with a concentration of (50 mg. l^{-1}) of kinetin were significantly superior in the rate of this characteristic amounting to (0.867 mg. gm^{-1}) with an increase rate of (3.337%) Compared to the control treatment seedlings whose leaves gave the lowest total chlorophyll content (0.839 mg. gm^{-1}).

With regard to the bilateral interactions of the studied factors, the same table showed that the bilateral interaction between the bacterial inoculum and gibberellic acid had a significant effect on the characteristic of the leaf content of total chlorophyll with concentration (300 mg. l^{-1}) of gibberellic acid and the biofertilizer recorded the highest rate of (0.924 mg. gm^{-1}) compared to the control treatment that gave the lowest rate (0.793 mg. gm^{-1}). The interaction between the bacterial biofertilizer and kinetin indicate that there are significant differences, as the concentration (25 mg. l^{-1}) of kinetin achieved the highest rate of (0.934 mg. gm^{-1}) compared to the control that recorded the lowest content of total chlorophyll which averaged (0.828 mg. gm^{-1}), The interaction between gibberellic acid and kinetin had a significant effect in this trait, as the seedlings whose seeds were treated with a concentration of (300 mg. l^{-1}) of gibberellic acid and a concentration of (50 mg. l^{-1}) of kinetin gave the highest significant increase in the chlorophyll content of its leaves the total rate was (0.919 mg. gm^{-1}) with an increase rate of (18.275%) compared to the control treatment which recorded the lowest rate (0.777 mg. gm^{-1}).

The results of the triple interaction indicate that there are significant differences between their treatments in the content of guava seedlings leaves of total chlorophyll, the biofertilizer and a concentration of (300 mg. l^{-1}) of gibberellic acid and a concentration of (50 mg. l^{-1}) of kinetin produced seedlings whose leaves were characterized by a high content of total chlorophyll at a rate of (0.926 mg. gm^{-1}) with an increase of up to (24.966 mg. gm^{-1}) compared to seedlings of comparison Control which recorded the lowest rate (0.741 mg. gm^{-1}).

Through the results of the table, it is clear that the addition of Azotobacter bacteria had a significant effect on the trait studied. The increased readiness helped the roots of the seedlings to increase its absorption and transmission, and then increase the concentration of this element in the leaves [10], and the increase in the concentration of nitrogen in the leaves increased the content of the pigment chlorophyll, because nitrogen enters the formation of the pigment chlorophyll because it participates in the formation of porphyrin units that It is involved in the formation of pigment, which leads to an increase in the concentration of chlorophyll in the leaves [11].

It is also clear from the table data that gibberellic acid has a significant effect, as high concentrations of gibberellic acid led to a significant increase in leaves. total chlorophyll content. The reason for this is the physiological role that gibberellic acid plays in diverting processed food compounds towards sites of growth and construction. Gibberellic acid also delays leaf aging. In addition, gibberellic acid plays an important role in reducing the harmful effect of abscisic acid [12].

As for kinetin, it had a significant effect on the studied trait B. This may be attributed to the important role of kinetin in delaying aging and the development of chloroplasts. It also helps in cell division and the preservation of chlorophyll pigment as a result of its ability to withdraw nutrients from the soil to the developing leaves and tops to encourage The formation of chlorophyll and preventing its loss, and thus the leaves retain their greenness for the longest possible period, in addition to increasing the number of leaves, the number of branches, and the dry weight of the stem [13, 14].

Table (4): Effect of Azotobacter biofertilizer, gibberellic acid solution and kinetin and interaction factors in Estimation of the content of fresh leaves of total chlorophyll (mg. gm^{-1} wet weight)

Biofertilizer Azotobacter	gibberellic acid concentration (mg. L^{-1})	Kinetine concentration (mg. L^{-1})			Interaction between Azotobacter and gibberellic acid	Azotobacter average
		0	25	50		
Without Azotobacter	0	0.741 f	0.811 ef	0.829 c-f	0.793 c	0.838 b
	150	0.866 a-e	0.845 b-e	0.833 b-f	0.848 b	
	300	0.878 a-e	0.831 b-f	0.912 a-d	0.874 ab	
Azotobacter	0	0.813 def	0.926 abc	0.817 def	0.852 b	0.887 a
	150	0.840 b-e	0.929 ab	0.888 a-e	0.886 ab	
	300	0.898 a-e	0.947 a	0.926 abc	0.924 a	
kinetine average		**0.839 b**	**0.881 a**	**0.867 ab**		
Interaction between Azotobacterr and gibberellic acid	**Without Azotobacter**	0.858 b	0.829 b	0.828 b	**gibberellic acid averages**	
	Biofertilizer	0.877 b	0.934 a	0.850 b		
Interaction between gibberellic acid and Kinetine	0	0.823 bc	0.869 ab	0.777 c	0.823 b	
	150	0.860 ab	0.887 ab	0.853 ab	0.867 a	
	300	0.919 a	0.889 ab	0.888 ab	0.899 a	

*Means that share the same letters for the single factors and their interactions do not differ significantly between them according to Duncan's polynomial test at the 0.05 probability level.

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