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## RESEARCH ARTICLE

## BIOCHEMICAL STUDIES ON THE EFFECT OF GA<sub>3</sub> AND IAA ON BIOCHEMICAL CONSTITUENTS OF VICIA FABA.

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### Abstract

A Field experiment was conducted to study the effect of foliar spraying with plant growth regulators treatments i.e. gibberellic acid (GA<sub>3</sub>) and Indole acetic acid (IAA) at control, 100 ppm GA<sub>3</sub>, 100 ppm IAA and 100 ppm GA<sub>3</sub>+100 ppm IAA on the yield and biochemical constituents of some promising faba bean (*Vicia faba* L.) cultivars i.e. Nubaria 1, Misr3 and Giza 716. Foliar application with GA<sub>3</sub> and IAA treatments had positive effect on the yield of seeds, but using a mixture of both hormones are more effective than the using of a single hormones in case of three studied cultivars.. All the used Plant growth regulators (PGRs) caused significant increased the content of total sugar, protein, total carbohydrates, total nitrogen content and seed yield in all treatments.. All the used Plant growth regulators (PGRs) caused significant increase in the protein and total carbohydrate percentage of the produced seeds resulted from the treated plants. The highest values of protein and carbohydrate were found in Nubaria 1 seed treated by the foliar spraying at 100ppm GA<sub>3</sub>+IAA. The consumed periodate for each glucose unit decrease as foliar application of GA<sub>3</sub> or IAA treatments. Formic acid was slightly decreased by all treatments except when applied GA<sub>3</sub> treatment. All treatments increased contents of P, K, Ca and Mg in seeds, but Na contents was decreased in seeds of vicia faba plants.

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### Introduction:-

In Egypt, among the most important legumes faba bean, which used on a large scale as traditional human diet because it contain high percentage of proteins, so, it is important to increase maximize yield of faba bean.. On the other hand faba bean is important for agriculture because of its high protein content and symbiosis with Rhizobium bacteria. In Egypt mature seeds of faba bean are good sources of protein, starch, cellulose, vitamin C and minerals [1]. Biostimulants are an organic material that has been shown to influence several metabolic processes such as respiration, photosynthesis, and nucleic acid synthesis and ion uptake and when applied in small quantities, enhances plant growth and development. They are mixtures of one or more things such as microorganisms, yeast, seaweed extracts, plant growth promoting Rhizobium bacteria (PGPR) and humic acid trace elements, enzymes and plant hormones [2]. Growth regulators improve growth, yield and its components, photosynthetic pigments, mineral content as well as the seed yield quality of faba bean [3]. Plant growth regulators (PGRs) are known to influence plant growth and development at very low concentrations but inhibit plant growth and development at high concentrations [4].

Moreover, response of plant to PGRs may vary with species, varieties, environmental conditions, physiological and nutritional status, stage of development and endogenous hormonal balance [5]. Plants have the ability to store excessive amounts of exogenously supplied hormones in the form of reversible conjugates which release active hormone when the plants need them during the growth period [6]. One of the important functions of gibberellins is synthesis of the  $\alpha$ -amylase enzyme in the aleurone layer surrounding the endosperm of cereal grains during

germination. This enzyme hydrolysis starch to form simple sugars which are then translocated to growing embryo to provide energy source [7]. It is found that the use of gibberellins lead to increase the seeds yield of (*vicia faba* L.) due to increased seed weight by enhance the rate of photosynthesis process during the period of full seeds [8]. Gibberellic acid has been used to stimulate cell division and elongation [9]. Also, GA<sub>3</sub> plays an important role in alleviating abiotic stress such salinity and induction of nitrogen use efficiency [10]. Sowing fababean Giza-716 cultivars and foliar application of IAA and GA<sub>3</sub> at 100 ppm maximize seed yield and its components due to reducing flower shedding percentage and an escalating yield components [11]. Foliar spraying IAA and GA<sub>3</sub> showed significant effect on plant in the extent of reducing the hunt effect of salinity on the vegetative measurement and some physiological components [12]. Also, found that GA<sub>3</sub> or IAA increase the soluble sugars, soluble protein and amino acids [13]. It is found that IAA treatments had positive effects on growth and dry weight. Also, photosynthetic pigments, total carbohydrate, free amino acids and total phenolic contents in the two cultivars [14]. Found that Soaked faba bean seeds in mutagen nitrous and GA<sub>3</sub> for 24 hours gave a significant increases of growth, seed weight, seed yield and protein yield [15]. The aim of the present study is to investigate the effect of GA<sub>3</sub> and/or IAA on the yield and some biochemical constituent's faba bean plant.

### Materials and methods:-

The present investigation was carried out as a field experiment in the Agronomy farm Ghazala, Zagazig, Sharkia Governorate at 2014 - 2015 season. A complete randomized block design was applied. Each treatment was replicated three times. Three varieties from *vicia faba* were used. Nubaria-1, Misr3, and Giza716. Foliar hormones were applied after 25 days from sowing data at rate 100 ppm GA<sub>3</sub>, 100 ppm IAA and 100 ppm GA<sub>3</sub>+100 ppm IAA.

At full maturity, the yield was determined and samples were taken and stored until chemical analysis.

### Method of analysis:-

- ❖ Soluble nitrogen was determined according to the method of [16].
- ❖ Total nitrogen was determined by the method of [17].
- ❖ Amino acid composition of seed protein was analyzed after hydrolysis with HCl (6N) according the method [18].
- ❖ Carbohydrate fractions were determined as described by [19].
- ❖ Starch was isolated from seeds by the method [20].
- ❖ Phosphorus was determined in the acid digestion according to the method of [21].
- ❖ K, Na, Ca and mg contents of seeds were determined according to the methods applied [22].
- ❖

### Results and discussion:-

Data presented in table (1) show the effect of treating *vicia faba* plants with GA<sub>3</sub> and / or IAA on the yield expressed as the straw or seeds per plant or weight of 100 seeds. It is clear that there were positive increase in *vicia faba* yield in response to different treatments application in case of three studied cultivars. The most increase was obtained at the 100 ppm GA<sub>3</sub>+IAA treatment. The obtained results indicated from the same table that the results of straw yield followed nearly the same trend of seeds. The highest straw yield was obtained in Misr3 variety by application of 100 ppm GA<sub>3</sub>+IAA. These results are in full agreement with those obtained by Ibrahim et al [3] and Kandil et al [24] who found that foliar application of IAA and GA<sub>3</sub> at 100 ppm maximize seed yield and its components due to reducing flower, shedding percentage and an escalating yield components. Also, the data in the same table demonstrate that the weight of 100 seeds was increased by all used growth regulators in all varieties in comparison with other treatments. Similar results were obtained by Hamdia [13] and Kandil et al [11] who found that sowing faba bean Giza716 cultivars and foliar application of IAA and GA<sub>3</sub> at 100 ppm maximize seed yield and its components.

Data in Table (1), also show that Straw yield, (g) per plot, (g) per plant, weight of seeds per (g) per plot and (g) per plant and seed index were significantly increased with different foliar application treatments. Furthermore, the highest values of seed yield were obtained from the application of 100 ppm GA<sub>3</sub> + IAA treatment in all cultivars. It is worthy to mention that, seed of Nubaria 1 cultivar at control treatment was more pronounced than that of Misr 3 and Giza 617 cultivars. (Table 1) indicated that there was a significant difference between *Vicia faba* L seed yield i.e. Nubaria 1, Misr3 and Giza 617 cultivars. Where found that the highest seed yield in Nubaria 1 at 100 ppm GA<sub>3</sub>+IAA, but the lowest seed yield in Giza 617 at 100 ppm IAA. Meanwhile, response of Nubaria 1 cultivar to all

applied treatments was more effective than Misr 3 and Giza 617 cultivars. The increase in the yield could be a reflection of the promotive effect of growth regulators on plant growth.

Data in Table (2) showed that, insoluble and total nitrogen fractions contents in seeds were increased by all treatments, whereas soluble nitrogen decreased. For the cultivar Nubaria 1a gradual decrease in the soluble nitrogen accompanied by a parallel increase in the insoluble one occurred as results of applied GA<sub>3</sub> and IAA and both hormone at 100ppm treatments. This change was reflected on the soluble N/ insoluble N ratio which applied 100ppm GA<sub>3</sub>+IAA. This trend was observed with the all cultivars; Misr3 and Giza 716. The soluble and insoluble nitrogen fractions remained nearly the same in these two cultivars when treated with all treatments. Hence, the variations in the sol. / insol. N were slight and did not manifest a clear trend. The highest response of Nubaria-1 cultivar to GA<sub>3</sub>+IAA treatments is well connected to its high productivity. So it can be concluded that growth hormones application and accumulation favorably affected protein synthesis in plant seeds at the combination of GA<sub>3</sub> and IAA. These results were supported with those obtained by Gherroucha et al [12] who reported that spraying with IAA and GA<sub>3</sub> caused increase the protein and amino acids. Also, Kandil et al [11] found that sowing faba bean Giza 716 cultivars and foliar application of IAA and GA<sub>3</sub> at 100 ppm maximize seed yield and its components.

Data in table (3) show the differences in the amino acid composition as affected by growth hormones (GA<sub>3</sub> and / or IAA) treatments seems to be trivial within every cultivar. It can be easily seen that, the foliar spray of GA<sub>3</sub>+IAA treatment more affected on the content of amino acid in protein than , GA<sub>3</sub> or IAA alone . Moreover, GA<sub>3</sub> or IAA alone recorded a little increase in the content of total amino acid in protein of vicia faba seeds especially in Nubaria 1 cultivars. These results were supported with those obtained by Hamdia [13] who found that GA<sub>3</sub> or IAA caused increase the protein and amino acids content. As shown in (Table3) total amino acids percentage were affected by different treatments. There is also significant differences between the interaction between varieties and treatments . Results in (Table3) indicated that there were significant differences between Vicia faba L. varieties, the highest value of amino acids percentage was found at 100ppm GA<sub>3</sub>+IAA treatment in Nubaria 1 cultivars which reach to 22.18 g/100 g compared to 19.55 mg and 18.63g for Misr 3 and Giza 617 respectively at the same treatment. While the lowest value of protein content were found at 100ppm IAA treatment in Giza 617 which reach to 17.08 g compared to 18.05g and 19.19g for Misr3 and Nubaria 1 respectively at the same treatment.

In general amino acids composition of vicia faba seed protein seems to be relatively rich in some essential amino acids. Lysine and methionine amino acid scoring pattern in case of three cultivars; Nubaria-1, Misr-3 and Giza 716 respectively. These data may refer to a relatively good nutritional vegetable protein quality, moreover GA<sub>3</sub> and /or IAA treatments with it the recommended levels (100 ppm) did not deteriorate the good quality of that protein Hence that protein may be recommended for nutritional application without fear of the hormones treatments Also, from the same table, it can be noticed that all treatments generally improved the contents of some amino acid composition which are important in some bioprocesses. This increase may be due to the increase in amino acyl transferase RNA synthesis and to the increase in content and activity of enzymes.

It can be generally observed in table (4) that there was a decreasing effect of growth hormone treatment on the reducing sugars contents of vicia faba seeds met by a parallel increasing effect on the non-reducing sugar. This relationship was especially evident in case of Nubaria 1. This may lead to a conclusion that growth hormones (GA<sub>3</sub> and /or IAA) might have affected the synthesis of the oligosaccharides. The total sugar content was also increased by all treatments (GA<sub>3</sub> and /or IAA) revealing that the decrease in the reducing sugar content was not because of GA<sub>3</sub> or IAA inhibitory effect on the synthesis of monosaccharides but because of GA<sub>3</sub> and IAA stimulating effect on oligosaccharides synthesis. A similar effect might be concluded for GA<sub>3</sub> and IAA on the synthesis of polysaccharides since there were generally pronounced increase in the insoluble carbohydrate and starch contents of vicia faba seeds of plants treated with GA<sub>3</sub> and /or IAA regardless of the cultivar, these increases resulted in enhancing the total carbohydrates content of vicia faba seeds of growth hormones treated plants. Comparing the cultivars shows that both Nubaria 1 and Misr 3 were at the same highly responsive level. The 100 ppm GA<sub>3</sub>+ 100 ppm IAA induced the highest increases in starch and total carbohydrates, but the 100 ppm IAA level caused a least effect when compared with other treatments similar results were shown by Ibrahim et al [3] and Kandil et al [11], who found that the foliar application of IAA and GA<sub>3</sub> at 100 ppm maximize seed yield and its components, and photosynthetic pigments. Also, Hamdia [13] found that phytohormonal treatments with GA<sub>3</sub> or IAA caused increase the sugars and protein contents in vicia faba seeds.

Total carbohydrate and polysaccharide contents of the faba bean cultivars were significantly increased in response to different concentrations of GA<sub>3</sub> and/or IAA treatments as compared with those of control plants as show in (Table3).The maximum increases in all parameters were observed at treatment of 100 ppm GA<sub>3</sub> +100ppm IAA in all cultivars. Also data in the same (Table 4) it can be concluded that, the consumed periodate for each glucose unit decreases as foliar application of GA<sub>3</sub>and /or IAA treatments for the seeds under investigation. These results might reveal that the application of growth hormones might increase (1-6) glycosides linkages in the starch molecule. The produced formic acid under the effect of hormones of isolated starch was determined and recorded in (Table 4). It can be noticed that formic acid was slightly decreased by the all treatments except when applied GA<sub>3</sub> treatment. These results may indicate that the numbers of chain in starch molecule were decreased under the effect of hormones treatments. Iodine affinity is an indication to the ratio of amylase to amylopectin in the starch from the results recorded in (Table 4).It can be concluded that foliar application of GA<sub>3</sub> and /or IAA treatments decreased the ratio of amylase to amylopectin in the starch. On the other hand GA<sub>3</sub> treatment increased this ratio. These results are in agreement with those obtained by Hamilton [1] and franciset al, [9].(Table5) shows the relationship between growth hormonal effect and content of phosphorus, potassium, sodium, calcium and magnesium in vicia faba seeds It can be noticed that all applied hormones increased contents of phosphorus, potassium, calcium and magnesium in seeds but sodium content was decreased in seeds. The increase of element accumulation in seeds improves its nutritional values and its cooking quality as stated by Davies [6] and Snyder [24]. Also, these elements are considered co-factors for kinases, phosphorylase and transferases enzymes [25].

**Table 1:-** Effect of GA<sub>3</sub> and IAA on the yield of straw and seeds of vicia faba plant

Treatments	Varieties	Straw yield		Seeds yield		Weight of 100 seeds
		Gm / plant	Gm / plot	Gm / plant	Gm/ plot	
1-control	Nobaria-1	20.62	618.62	15.38	461.41	82.22
2-100 ppm GA <sub>3</sub>		23.55	706.54	17.60	528.06	87.90
3-100 ppm IAA		22.75	682.65	16.52	495.6	86.21
4-100 ppm GA <sub>3</sub> +IAA		24.18	752.40	18.48	536.4	88.41
1-control	Misr-3	23.86	715.82	16.66	511.88	81.56
2-100 ppm GA <sub>3</sub>		24.82	804.64	18.07	556.66	84.91
3-100 ppm IAA		24.64	754.22	17.88	540.81	82.11
4-100 ppm GA <sub>3</sub> +IAA		25.66	811.84	18.88	566.48	85.28
1-control	Giza-716	22.50	675.50	16.15	489.86	75.26
2-100 ppm GA <sub>3</sub>		25.11	753.90	18.48	526.78	79.71
3-100 ppm IAA		24.27	928.10	17.85	501.64	77.81
4-100 ppm GA <sub>3</sub> +IAA		25.76	782.80	18.82	564.60	80.96

**Table 2:-Effect of GA3 and IAA on the content of nitrogen fractions of vicia faba (gm / 100 gm)**

Treatments	Varieties	Sol. N	Insol. N	Total N	Sol./ insol. Ratio
1-control 2-100 ppm GA3 3-100 ppm IAA 4-100 ppm GA3+IAA	Nubaria -1	0.46 0.41 0.41 0.43	3.01 3.21 3.11 3.34	3.47 3.62 3.52 3.74	0.153 0.128 0.132 0.129
1-control 2-100 ppm GA3 3-100 ppm IAA 4-100 ppm GA3+IAA	Misr-3	0.42 0.39 0.40 0.40	3.22 3.44 3.38 3.51	3.64 3.83 3.78 3.91	0.130 0.113 0.118 0.114
1-control 2-100 ppm GA3 2-100 ppm IAA 4-100 ppm GA3+IAA	Giza-716	0.45 0.40 0.39 0.41	3.11 3.32 3.28 3.38	3.56 3.72 3.67 3.79	0.144 0.128 0.132 0.129

**Table 3:-Effect of GA3 and IAA on the content of amino acids composition of vicia faba seeds (gm / 100 gm dry weight)**

Treatments	Verities	Alanine	Valine	Glycine	Aspartic	Glutamic	Tyrosine	Phenyl alanine	Proline	Threonine	Methionine	cysteine	Arginine	Lysine	Histamine	T. amino acids
1- control 2-100ppmGA3 3- 100ppmIAA 4- 100ppm GA3+IAA	Nobaria1	0.7	0.5	0.3	1.1	1.7	0.7	1.8	0.8	0.8	1.5	0.8	1.4	1.5	2.7	17.0
		5	5	2	8	8	1	8	9	0	4	8	0	8	8	4
		0.9	0.7	0.4	1.4	2.1	0.8	2.0	0.9	0.8	1.8	1.0	1.4	1.8	3.3	19.9
		1	2	0	0	5	8	5	4	5	8	8	8	6	4	5
1- control 2-100ppmAG3 3- 100ppmIAA 4-100ppmGA3+ IAA	Masr-3	0.8	0.6	0.3	1.3	2.0	0.8	1.9	0.9	0.8	1.8	1.0	1.4	1.8	3.3	19.1
		4	8	6	4	5	4	6	1	2	2	2	4	1	0	9
		0.9	0.9	0.4	1.4	2.2	0.9	2.1	0.9	0.9	1.9	1.1	1.4	1.9	3.4	21.1
		8	1	8	8	6	2	5	8	8	8	8	8	8	2	8
1- control 2-100ppmAG3 3- 100ppmIAA 4-100ppmGA3+ IAA	Masr-3	0.6	0.4	0.3	1.1	1.7	0.6	1.7	0.8	0.7	1.4	0.9	1.3	1.4	2.6	16.0
		8	8	0	2	2	4	9	6	0	0	6	6	4	2	7
		0.8	0.7	0.4	1.3	1.8	0.7	1.8	0.9	0.7	1.8	0.9	1.4	1.7	3.1	18.5
		7	2	2	2	8	6	5	0	4	8	6	0	4	5	9
1- control 2-100ppmAG3 3- 100ppmIAA 4-100ppmGA3+ IAA	Giza-716	0.8	0.6	0.4	1.2	1.8	0.7	1.8	0.8	0.7	1.7	1.0	1.4	1.6	3.0	18.0
		3	8	0	9	0	3	1	8	0	8	2	0	5	8	5
		0.8	0.8	0.4	1.3	2.0	0.8	1.8	0.9	0.8	1.9	0.9	1.4	1.8	3.3	19.5
		9	4	3	6	5	2	9	4	6	0	8	2	3	4	5
1- control 2-100ppmAG3 3- 100ppmIAA 4-100ppmGA3+ IAA	Giza-716	0.5	0.4	0.3	1.1	1.7	0.5	1.7	0.8	0.5	1.4	0.9	1.3	1.4	2.6	15.8
		9	8	6	8	8	4	4	6	6	8	1	2	0	0	0
		0.7	0.6	0.3	1.2	1.8	0.6	1.8	0.8	0.6	1.7	0.9	1.3	1.6	3.0	17.7
		8	6	5	5	2	9	8	6	8	7	6	8	6	1	5
1- control 2-100ppmAG3 3- 100ppmIAA 4-100ppmGA3+ IAA	Giza-716	0.7	0.5	0.3	1.2	1.7	0.6	1.8	0.8	0.6	1.7	0.9	1.3	1.5	2.9	17.0
		4	9	6	2	1	2	8	3	2	1	1	5	8	6	8
		0.8	0.7	0.4	1.2	1.9	0.7	1.8	0.8	0.7	1.7	0.9	1.3	1.7	3.2	18.6
		4	8	4	6	2	6	9	8	5	8	8	9	4	2	3

**Table 4:-**Effect of GA<sub>3</sub> and IAA on the content of carbohydrate fractions and starch molecule of vicia faba seed.

Treatments	varieties	Carbohydrate fractions gm. / 100 gm.					Stare molecule			
		R.S	N.R.S	T.S	In.C	T.C	Starch %	Consumed Periodote Per A.G.U	Moles of Formic acid	Iodine Affinity
1-control	Nobaria-1	1.62	2.41	4.03	33.66	37.69	31.04	1.39	1.23	76.0
2-100 ppm GA3		1.61	2.68	4.19	34.16	38.35	31.55	1.69	1.23	77.0
3-100 ppm IAA		1.45	2.58	4.03	34.10	38.13	31.22	1.64	1.19	73.0
4-100 ppm GA3+100ppmIAA		1.48	2.68	4.16	34.46	38.62	31.82	1.31	1.19	73.0
1-control	Misr-3	1.59	2.38	3.97	33.45	37.42	30.86	1.83	1.22	75.0
2-100 ppm GA3		1.44	2.63	4.07	33.82	37.89	31.52	1.66	1.22	77.0
3-100 ppm IAA		1.40	2.59	3.99	33.74	37.73	31.24	1.32	1.19	73.0
4-100ppm GA3+100 ppm IAA		1.40	2.61	4.01	33.92	37.93	31.54	1.29	1.16	73.0
1-control	Giza-716	1.48	2.34	3.82	33.40	37.22	30.64	1.36	1.21	74.0
2-100 ppm GA3		1.48	2.60	4.08	33.69	37.77	31.32	1.52	1.22	76.0
3-100 ppm IAA		1.44	2.55	3.99	33.71	37.70	31.04	1.32	1.18	73.0
4-100 ppm GA3+100 ppm IAA		1.36	2.58	3.94	33.87	37.81	31.48	1.32	1.16	72.0

**Table 5:-**Effect of GA<sub>3</sub> and IAA on the content of some minerals in vicia faba seeds (gm / 100 gm dry weight ).

Treatments	Varieties	Phosphorus	Potassium	Sodium	Calcium	Magnesium
1-Controt	Nobaria-1	0.26	1.74	0.26	0.98	1.14
2- 100 ppm GA <sub>3</sub>		0.28	1.76	0.23	1.13	1.14
3- 100 ppm IAA		0.30	1.79	0.24	1.13	1.21
4- 100 ppm GA <sub>3</sub> +IAA		0.30	1.82	0.23	1.18	1.24
1- Contort	Misr-3	0.24	1.68	0.25	0.88	1.15
2- 100 ppm GA <sub>3</sub>		0.25	1.75	0.23	1.12	1.17
3- 100 ppm IAA		0.25	1.75	0.23	1.14	1.19
4- 100 ppm GA <sub>3</sub> +IAA		0.27	1.78	0.22	1.16	1.21
1- Contort	Giza-716	0.25	1.72	0.24	0.41	1.16
2- 100 ppm GA <sub>3</sub>		0.26	1.78	0.22	1.08	1.18
3- 100 ppm IAA		0.28	1.75	0.22	1.11	1.18
4- 100 ppm GA <sub>3</sub> +IAA		0.30	1.82	0.23	1.16	1.20

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