

## A CONSEQUENT STUDY TO DIMINISH ANTI-NUTRITIONAL FACTORS (VICINE AND CONVICINE) CONTENT AND PHYSIOLOGICAL RESPONSE OF SOME EGYPTIAN FABA BEAN CULTIVARS TREATED WITH GA<sub>3</sub>

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### ABSTRACT

Two pot experiments were carried out during winter seasons 2002 and 2003 at the green house of the experimental station of Botany department, National Research Center, Dokki, Giza, Egypt. The experiments aimed to study the effect of foliar spraying 100 ppm GA<sub>3</sub> on 5 varieties of *Vicia faba* plants (Giza 40, 429, 461, 643 and 674) on growth, photosynthetic pigments, yield, yield components, the contents of toxic constituents (vicine and convicine) in green pods and dry seeds (yielded seeds) at harvest. The contents of protein and amino acids composition of two varieties (Giza 429 and Giza 674) were focused.

The results revealed that foliar spraying of 100 ppm GA<sub>3</sub> enhanced all the morphological criteria (number of leaflets, number of leaves, number of flowers, leaf area per plant, fresh and dry weight of shoots as well as the yield and its components (plant height, plant weight, number of branches, number of pods, number of seeds and 100-seeds weight in all varieties of *Vicia faba* plant.

The different varieties of *Vicia faba* treated plants showed an increase in chlorophyll a, chlorophyll b, carotenoides and consequently total pigments compared with untreated plants.

Marked reductions were observed regarding the toxic glucosides (vicine and convicine) which are the main purpose of the presented work, in response to 100 ppm GA<sub>3</sub> of all varieties especially Giza 429 and Giza 674 which showed a marked reduction in green pods and dry seeds. This reduction was increased when the yielded seeds of all varieties re-treated by soaking in 100 ppm GA<sub>3</sub> solution. This reduction reached nearly to 53 and 58% in Giza 674 and Giza 429, respectively. Moreover, the amino acids composition of these two varieties increased markedly, except for the sulphur amino acids (cystin and methionine) of Giza 674 in response to GA<sub>3</sub> treatment which improved the quality of seeds. Also, the protein content in *Vicia faba* seeds of the two markedly responded varieties recorded the highest values under the effect of GA<sub>3</sub> foliar spraying.

**Key words:** Gibberellic acid, *Vicia faba*, growth, yield, pigments, protein, glucosides, vicine, convicine.

### INTRODUCTION

Faba bean (*Vicia faba*, L.) seeds are a good source of calories. The seed contain nutritional factors nearly 58% carbohydrate, 24% protein, 3% ash and 0.9% lipid. It is also rich in phosphorus, iron, potassium and vitamin B complex (FAO, 1982). Also, the immature seeds (green seeds) are a much better source of vitamin A and C. Moreover, it contains most of the amino acids necessary for human and animal nutrition and low sulphur amino acids concentration (Smartt, 1976).

Two major pyrimidinone glucosides; vicine [(2, 6 diamino - 4, 5 dihydroxy pyrimidine, 5-β- glucopyranoside)] and convicine [2, 4, 5-

trihydroxy - 6 - aminopyrimidine, 5- ( $\beta$ -D glucopyranoside)]; are present in faba bean (*Vicia faba*, L.) (Ramsay and Griffiths, 1996). These glucosides hamper the development of faba bean as a worldwide food and feed crop where it is the main cause of favism (Jamalian *et al.*, 1977) to the people who inherited the absence of the enzyme glucose-6-phosphate dehydrogenase (G6PD) in their red blood cells (Corchia *et al.*, 1995). More than 100 million people worldwide are genetically deficient in G6PD (Beutler *et al.*, 1996). In Egypt, favism represents about 26% especially among the males (Mager *et al.*, 1980). The enzyme G6PD functions to maintain plentiful supplies of reduced glutathione. Where in their absence the aglycone of vicine (divicine) and convicine (isouramil) in vulnerable people cause acute hemolytic either by direct action on the red blood cell membranes or by producing hydrogen peroxide which in turn breakdown red cell membrane as the result of a reduction in the reduced glutathione which normally block the destruction of red cell membrane by these mentioned oxidants (Chevion *et al.*, 1982).

The vicine and convicine contents were highest in fresh green cotyledons and gradually declined till a constant level when the dry matter of seeds was around 40% (Burbano *et al.*, 1995 and Nestorowicz *et al.*, 1996). Different methods listed by Hussein *et al.* (1986) and Jaddou (1988) for the elimination of vicine and convicine from faba bean seeds. The most potent effect was that soaking the seeds for 48 hours at 40°C when acetic acid (1%) was changed every 24 hours, since it resulted in reducing total vicine and convicine of the beans by 90%. Moreover, Donath and Kujawa (1991) and McKay (1992) used the microbial (*Lactobacillus plantarum* and *Streptococcus faeciens*) enzymes for detoxification of faba bean which eliminated the glycosides from the faba bean extract. Also, Chevion and Navok (1983) found that treating the seeds of *Vicia faba* with 25% acetic acid followed by enzymatic hydrolysis betaglucosidase reduced the content of favism causative agents approximately 0.5% of the wet weight of seeds.

Gibberellins are natural bioregulators. Extensive literature has been developed about the effect of GA<sub>3</sub> on the physiology of certain economic crops. Recently, Singh *et al.* (1999); Zaghlool (2002); Balraj *et al.* (2002) and Sadak (2005) found that GA<sub>3</sub> treatments increased the plant height, stem diameter, number of branches, number of leaves, leaf area and fresh and dry weights of shoot of different plants. Also, Rabie (1996) on wheat; Ramadan (1998) on *Vicia faba*; Mousa *et al.* (2001) on *Nigella sativa*; Kaushal and Rana (2003) on saffron; Bekheta (2004) on wheat and Sadak (2005) on *Hibiscus sabdariffa* reported that GA<sub>3</sub> treatments improved the yield in several ways.

Shaddad and Heikal (1982); Narendra *et al.* (1988) and Ramadan (1998) showed that all photosynthetic pigments (chl. a, chl. b and carotenoides) increased markedly as a result of GA<sub>3</sub> foliar application of kidney bean, tomato and *Vicia faba* plants, respectively.

It is recorded that GA<sub>3</sub> act as a regulators for the synthesis as accumulation of seed protein. Thus, Shady *et al.* (1984) found that seed total protein as well as total soluble-N, amino-N and free amino acids were increased in response to foliar spraying of faba bean plants with various GA<sub>3</sub> concentrations. Moreover, the amino acids (glutamic acid, aspartic acid,

glycine, threonine and arginine increased in mature seeds, whereas leucine and isoleucine were decreased due to GA<sub>3</sub> application. Similar results were obtained by Hussein *et al.* (1983) on *Vicia faba* plants. In this regard, it should not neglect the role of gibberellins (GA<sub>3</sub>) on controlling the molecular activities regarding the RNA synthesis and in particular during the transcription phase (Mostafa, 1978).

The present work was carried out to investigate the possible effect of GA<sub>3</sub> on 5 cultivars of *Vicia faba* plants (Giza 40, 429, 461, 643 and 674) on growth, photosynthetic pigments, yield, yield components, the contents of toxic constituents (vicine and convicine) in green pods and dry seeds (yielded seeds) at harvest. The contents of protein and amino acids composition were also considered.

## MATERIAL AND METHODS

Two pot experiments were conducted during winter seasons 2002 and 2003 at the green house of National Research Center, Dokki, Giza, Egypt to study the effect of foliar spraying of 5 cultivars of *Vicia faba* plants (Giza 40, 429, 461, 643 and 674) with 100 ppm GA<sub>3</sub> solution to investigate the effect of the growth regulator on growth, photosynthetic pigments, yield, yield components as well as the contents of toxic constituents (vicine and convicine) in green pods and dry seeds (yielded seeds) at harvest. The use of 100 ppm GA<sub>3</sub> was a result of pre-experiment which used a different concentrations of GA<sub>3</sub> (50, 100 and 200 ppm) in two successive seasons (2000 and 2001) on *Vicia faba* var. Giza 2, since when determined the contents of vicine and convicine in the yielded seeds, the maximum reduction was observed with 100 ppm GA<sub>3</sub>.

A homogenous lot of the 5 varieties of *Vicia faba* seeds secured from Agricultural Research Center, Giza, Egypt, were sown in pots (30 cm in diameter) containing equal amounts of homogenous clay and sand (2:1). The soil contained a phosphorus fertilizer which was applied in the form of Triple-superphosphate. Irrigation was carried out according to the usual practice by adding equal amount of water. After 17<sup>th</sup> day, thinning was performed where 5 uniform seedlings left in each pot for experimentation. Each group of each variety was divided into two subgroups (10 pots), one sprayed with H<sub>2</sub>O (untreated) and the other sprayed with 100 ppm GA<sub>3</sub> (treated) two times at 30 and 40 days from sowing for each variety. The experiments were distributed in a complete randomized design with 10 replicates.

Throughout the two successive seasons, growth measurements (plant height, number of nodes/plant, number of branches/plant, number of leaflet/plant, number of leaves/plant, number of flowers/plant, leaf area/plant, plant fresh weight and plant dry weight) and pigments (chlorophyll a, chlorophyll b, and carotenoides) were undertaken after 50 days from planting. During harvest, green seeds (green pods) were collected for the determination of vicine and convicine content. At the end of the experiment, 10 plants were chosen at random to obtain the data of yield parameters (plant height, plant weight, number of branches, number of pods/plant, number of seeds/plant and 100-seed weight). The yielded seeds were kept

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dry for the purpose of determining vicine and convicine content for the 5 tested varieties and protein % and amino acids content for the most affected varieties (Giza 429 and 674) with GA<sub>3</sub> application.

The yielded seeds of all cultivars were shaken mechanically for about 12 hours on 100 ppm GA<sub>3</sub>, and then the beans were placed on absorbent paper, freeze, dried and finally ground to determine the content of vicine and convicine.

### **Chemical analysis:**

#### **Photosynthetic pigments:**

The plant pigments (Chlorophyll a, chlorophyll b and carotenoids) were determined according to the spectrophotometric method as recommended by Metzner *et al.* (1965).

#### **Isolation and Quantitative Estimation of Vicine and Convicine**

The separation and quantitative estimation of faba bean vicine and convicine were performed by a high pressure liquid-chromatography (HPLC) according to the reversed phase method described by Marquardt and Frohlich (1981).

#### **The identification and quantitative determination of amino acids composition:**

Amino acids of yielded seeds for the most affected varieties (Giza 429 and 674) were carried out by Amino Acid Analyzer LC3000, flow rate 0.2 ml/minute, pressure of buffer from 0-50 bar, pressure of reagent from 0-150 bar and reaction temperature 123°C.

#### **Protein percentage:**

The protein content of the most affected varieties (Giza 429 and 674) were determined according to the method of A.O.A.C. (1990).

### **Statistical analysis:**

Data were statistically analyzed by performing a combined analysis for the two growing seasons. The means were compared by L.S.D. test at significant probability level of 0.05 according to Snedecor and Cochran (1980).

## **RESULTS AND DISCUSSION**

### **Growth responses:**

Data presented in Table (1) indicated that foliar spraying with 100 ppm GA<sub>3</sub> increased significantly ( $P < 0.05$ ) growth parameters (plant height, number of nodes/plant, number of branches/plant, number of leaflet/plant, number of leaves/plant, number of flowers/plant, leaf area/plant, plant fresh weight and plant dry weight) of all varieties of *Vicia faba* plant (Giza 40, 429, 461, 643 and 674) as compared with the control. In accordance with these results, Misra (1995) indicated that the application of 10-500 mg GA<sub>3</sub>/l increased the number of nodes, branches, green leaves, total leaf area, specific fresh weight and leaf area index of *Pogostemon cablin*. Also, Ramadan (1998) on *Vicia faba* plants var. Giza 2 and Zaghlool (2002) on

mungbean found that GA<sub>3</sub> increased significantly shoot length, number of nodes, number of leaves, leaf area and fresh and dry weights of shoot.

The increase in growth and development of faba bean plants in response to GA<sub>3</sub> treatment may be attributed to the stimulatory effect of GA<sub>3</sub> on cell elongation and / or cell division (Nair *et al.*, 2002). Previously, Weaver (1972) reported that the application of GA<sub>3</sub> produced a pronounced increase in cell division in the sub-apical meristem and the stem of plants became much longer as a result of both greater number of cells formed and the increase in the elongation of the individual cells. Moreover, De La Guardia and Benlloch (1980) indicated that the stimulatory effect of GA<sub>3</sub> on stem growth was due to the stimulation of cell wall extensibility, membrane permeability, enzymatic activity and mobilization of sugars.

**Table (1): Effect of spraying GA<sub>3</sub> on the growth parameters of different varieties of *Vicia faba* plants.**

Variety	GA <sub>3</sub> (100 ppm)	Plant height (cm)	No. of nodes / plant	No. of branches / plant	No. of leaflet s/plant	No. of leaves / plant	No. of flowers / plant	Leaf area (cm <sup>2</sup> / plant)	Plant fresh weight (g)	Plant Dry weight (g)
Giza 40	Untreated	37.25	11.25	1.00	68.00	19.00	9.75	590.38	22.95	7.12
	Treated	56.00	14.60	2.00	71.00	22.00	18.00	718.92	24.21	9.01
Giza 429	Untreated	46.00	15.00	1.60	56.00	19.00	8.33	650.17	25.37	7.06
	Treated	56.75	17.00	2.30	84.25	23.25	14.00	724.98	29.69	8.87
Giza 461	Untreated	51.83	14.20	1.70	63.33	17.00	10.33	481.06	25.35	7.80
	Treated	80.75	14.50	2.80	66.00	18.00	17.66	561.96	33.41	9.03
Giza 643	Untreated	41.00	14.80	1.20	59.00	17.00	16.33	445.89	18.09	7.94
	Treated	52.75	15.60	2.00	67.75	18.00	20.16	542.13	22.48	8.55
Giza 674	Untreated	37.00	15.00	1.00	57.30	17.31	9.00	547.66	22.15	6.05
	Treated	48.00	16.33	2.10	82.25	19.63	14.00	620.88	29.00	9.87
L.S.D (0.05)		3.63	1.81	0.53	4.45	2.72	2.18	34.57	3.45	1.27

#### Yield and its components:

It has been found in the present work (Table 2) that yield parameters (Number of pods/plant, number of seeds/plant and 100-seed weight) increased significantly ( $P < 0.05$ ) in response to 100 ppm GA<sub>3</sub> as compared to the untreated plants of all tested faba bean varieties. These results are supported by several workers who found that gibberellins improve yield and yield components of different plants (Clements *et al.* 1995; Rabie, 1996; Ramadan 1998; Mousa *et al.*, 2001; Bekheta, 2004; Kaushal and Rana, 2003 and Sadak, 2005). The increase of yield may be a reflection of GA<sub>3</sub> beneficial effect on vegetative growth, number of flowers and pods and leaf area as well as photosynthetic pigments, which in turn could lead to an increase in photosynthetic-assimilate production translocated to reproductive organs during maturation of seeds.

In this regard, Nair *et al.* (2002) indicated that GA<sub>3</sub> increased the photosynthetic rate, promoted the translocation of assimilates from the leaves to the fruits, increased the number of pods and their weights per plant in *Arachis hypogaea* and gerbera plants, respectively.

The possible explanation for the promoting effect of GA<sub>3</sub> on photosynthetic pigments in the present work is that GA<sub>3</sub> may retard the chlorophyll destruction and/or induces chlorophyll biosynthesis (Misra, 1995).

Thus, the increments in chlorophyll content due to GA<sub>3</sub> treatments may be due to the effect of GA<sub>3</sub> on endogenous cytokinin (Khafagy and Moussa, 1981) which not only induced a higher accumulation of chlorophylls and protochlorophellides, but also changed the amount of *in vivo* chlorophyll forms which accelerate photosynthesis in plants (Niето and Frankenberger, 1990).

Table (2): Effect of spraying GA<sub>3</sub> on certain morphological characters and the yield and its components of different varieties of *Vicia faba* plants.

Variety	GA <sub>3</sub> (100 ppm)	Plant height (cm)	Plant weight (g)	No. of branches/plant	No. of pods/plant	No. of seeds/pod	100-seed weight
Giza 40	Untreated	72.75	47.81	2.83	7.35	20.83	83.75
	Treated	85.50	56.23	3.95	8.13	25.60	97.97
Giza 429	Untreated	72.95	40.80	2.00	6.63	20.32	72.18
	Treated	87.75	58.37	2.80	7.82	24.27	89.77
Giza 461	Untreated	83.92	54.62	3.15	8.65	24.15	80.42
	Treated	92.17	63.80	3.85	9.47	27.82	93.15
Giza 643	Untreated	85.84	61.39	2.79	8.21	23.05	87.24
	Treated	99.03	69.14	3.52	9.75	29.70	99.38
Giza 674	Untreated	63.33	36.60	2.37	6.02	18.70	61.92
	Treated	89.40	46.70	3.41	8.31	22.30	79.18
L.S.D (0.05)		3.63	3.45	0.27	1.18	2.10	5.82

#### Photosynthetic pigments:

The present investigation (Table 3) showed that all varieties of *Vicia faba* plants are responses to GA<sub>3</sub> treatment which showed that the chlorophyll a, chlorophyll b and carotenoids increased significantly ( $P < 0.05$ ) in response to foliar spraying of 100 ppm GA<sub>3</sub> with more clearance in the variety Giza 674. Similar promoting effect of GA<sub>3</sub> on photosynthetic pigments had been observed by Misra (1995) on *Pogostemon cablin*; Singh (1996) on rice; Ramadan (1998) on *Vicia faba*; Singh *et al.* (1999) on *Mentha spicata* and Sadak (2005) on *Hibiscus sabdarifa*.

Table (3): Effect of spraying GA<sub>3</sub> on photosynthetic pigments (mg/gm fresh weight) of different varieties of *Vicia faba* plants.

Variety	GA <sub>3</sub> (100 ppm)	Chlorophyll A	Chlorophyll B	Carotenoides	Total chlorophyll	Total pigments
Giza 40	Untreated	3.096	2.107	0.904	5.203	6.107
	Treated	4.020	2.973	1.527	6.996	8.520
Giza 429	Untreated	3.737	2.037	0.958	5.774	6.732
	Treated	5.074	3.205	1.623	8.279	9.902
Giza 461	Untreated	3.418	2.414	1.824	5.832	7.626
	Treated	5.941	2.716	1.239	8.657	9.896
Giza 643	Untreated	3.234	2.067	0.931	5.301	6.232
	Treated	4.582	2.206	1.356	6.788	8.144
Giza 674	Untreated	4.223	2.878	1.763	7.101	8.864
	Treated	5.603	3.637	2.375	9.240	11.615
L.S.D (0.05)		0.36	0.11	0.21	1.45	2.00

**Vicine and convicine:**

The main purpose of this work was not only improving the productivity, but also looking for a devised tool to reduce or eliminate the toxic causative agents causing favism found in legumes especially all *Vicia faba* cultivars. Since, no single variety has yet been identified as free from these glucosides (Arbid and Marquardt, 1985 and Hussein *et al.*, 1986). The reduction of these compounds in *Vicia faba* will have benefits for human nutrition through the elimination of favism and still considered a very important research topic.

The data recorded in Tables (4 & 5) indicated that foliar spraying with 100 ppm GA<sub>3</sub> decreased significantly the content of vicine and convicine in all tested varieties especially Giza 429 and 674. The maximum decrease (percentage) was observed in green pods which reached 43.94% (Giza 429) and followed by Giza 674 with reduction percentage of 40.98. Meanwhile, the reduction in the content of vicine and convicine in dry yielded seeds (Table 5) reached 33.67 and 32.81% for the variety Giza 674 and Giza 429, respectively, which shows the high response to GA<sub>3</sub> treatment. The yielded seeds of the 5 tested varieties were re-treated with soaking in 100 ppm GA<sub>3</sub> for further monitoring of vicine and convicine contents. The results (Table 6 and Fig. 1) showed clearly that the content of toxic constituents more reduced in general for all tested varieties and in particular the two varieties Giza 429 and Giza 674 with reduction percentages of 58.49 and 53.37%, respectively.

Table (4): Effect of spraying GA<sub>3</sub> on vicine and convicine content (mg/g dry weight) of different varieties of *Vicia faba* green pods.

Variety	GA <sub>3</sub> (100 ppm)	Vicine	Convicine	Vicine + Convicine	Reduction %
Giza 40	Untreated	5.59	1.28	6.87	-
	Treated	4.60	0.48	5.08	26.06
Giza 429	Untreated	7.39	1.28	8.67	-
	Treated	3.92	0.94	4.86	43.94
Giza 461	Untreated	6.56	0.99	7.55	-
	Treated	5.36	0.69	6.05	19.87
Giza 643	Untreated	4.63	0.82	5.46	-
	Treated	4.02	0.60	4.62	15.38
Giza 674	Untreated	5.99	1.77	7.76	-
	Treated	3.90	0.68	4.58	40.98
L.S.D (0.05)		0.52	0.27	-	-

From the above mentioned results it is worthy to point out that, the content of vicine and convicine differs with cultivars of *Vicia faba* plant and type of treatment. This according to the finding results confirmed by Hussein *et al.* (1986) who concluded similar results on 29 varieties of *Vicia faba* grown in Egypt. Also, Mager *et al.* (1980) found that favism is governed not only by genetic factors, but also a number of environmental deterrents such as variation in the amount of toxic principle present in each of the different varieties of faba bean seeds.

Table (5): Effect of spraying GA<sub>3</sub> on vicine and convicine content (mg/g dry weight) of different varieties of *Vicia faba* dry seeds.

Variety	GA <sub>3</sub> (100 ppm)	Vicine	Convicine	Vicine + Convicine	Reduction %
Giza 40	Untreated	2.29	0.69	2.98	-
	Treated	1.82	0.49	2.31	22.48
Giza 429	Untreated	3.04	0.77	3.81	-
	Treated	2.13	0.43	2.56	32.81
Giza 461	Untreated	4.39	0.64	5.03	-
	Treated	3.26	0.50	3.76	25.25
Giza 643	Untreated	2.94	0.63	3.57	-
	Treated	2.15	0.54	2.69	24.65
Giza 674	Untreated	3.19	0.82	4.01	-
	Treated	2.04	0.62	2.66	33.67
L.S.D (0.05)		0.21	0.11	-	-

Table (6): Effect of re-treatment of yielded seeds with 100 ppm GA<sub>3</sub> on vicine and convicine content (mg/g dry weight) of different varieties of *Vicia faba* plants.

Variety	Vicine	Convicine	Vicine + Convicine	Reduction %
Giza 40	1.23	0.52	1.75	41.28
Giza 429	1.26	0.31	1.57	58.79
Giza 461	1.98	0.56	2.54	49.50
Giza 643	1.31	0.54	1.95	48.18
Giza 674	1.54	0.33	1.87	53.37

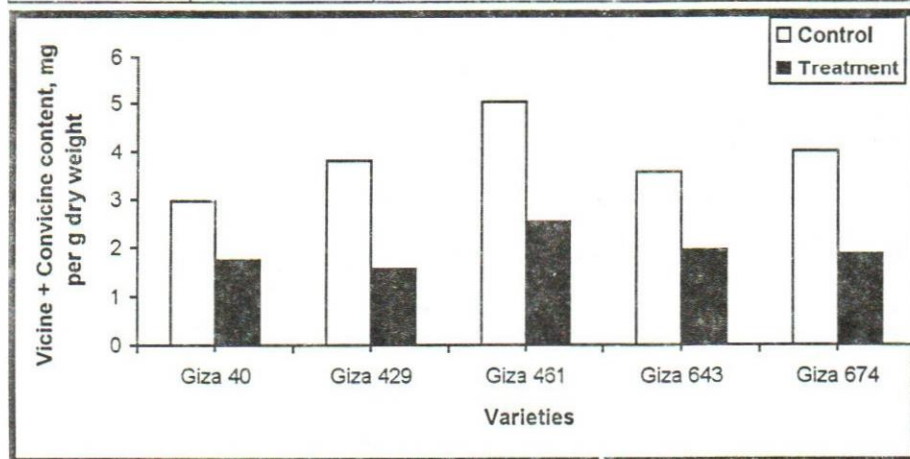


Figure (1): The vicine and convicine content of the tested faba bean seeds after soaking in GA<sub>3</sub>.

Several attempts made to detoxify faba beans by different treatments of *Vicia faba* seeds which affected the concentrations of vicine and convicine (favism causative agents). In this concern, Abd Allah *et al.* (1988) found that



soaking faba bean seeds (var. Giza 2) with sodium carbonate and / or acetic acid reduced the toxic glucosides. Moreover, Jamalian (1999) found that soaking the whole seeds with 0.01 M HCl, 0.066 M phosphate buffer or 0.013 M NaOH decreased the content of vicine and convicine to 38% (acid + H<sub>2</sub>O<sub>2</sub>) and 41% (NaOH). On the other hand, others used heat treatments at 100°C and found that such treatments did not significantly reduce the content of toxic constituents in faba bean seeds (Nesterowicz *et al.*, 1996).

Regarding the use of growth bioregulator treatments for reducing these constituents percentage, Gaber *et al.* (2000) indicated that foliar sprayed and seed presoaked *Vicia faba* plants (var. Giza 2) with either abscisic acid or gibberellic acid or coumarin decreased significantly the content of vicine and convicine in the yielded seeds.

The obtained reduction in this investigation regarding the contents of vicine and convicine may be attributed to the effect of these treatments (GA<sub>3</sub>) on the metabolic pathway of vicine and convicine precursor (Orotic acid) formation which responsible for the formation of pyrimidine ring of these glucosides (Brown and Roberts, 1972 and Ramsay and Griffiths, 1993) or may be due to the effect of GA<sub>3</sub> on the contents of beta-glucosidase enzyme which is responsible for the hydrolysis of vicine and convicine. In this concern, Sisini *et al.* (1981) found that beta-glucosidase play a role in regulating the metabolism of possibly haemolytic glucosides (vicine and convicine) during the early stages of seed maturation but rapidly declined during maturation. Similar results were obtained by McKay (1992) and Chevion and Navok (1983).

#### Protein and amino acids composition:

*Vicia faba* plants varieties Giza (429) and Giza (674), which showed the most potent effect in reducing vicine and convicine in response to GA<sub>3</sub> treatments, were selected to the following analysis of its content from protein and amino acids to assure the undesirable effect of such treatment on the seed quality.

The results of HPLC amino acids composition of untreated *Vicia faba* yielded seeds var. Giza 429 and 674 (Table 7) revealed that glutamic acid was the most predominant amino acid in both varieties, being 37.43 and 36.12 mg/gm, followed by proline (14.98 and 15.28) and aspartic acid (11.84 and 12.66), respectively. The foliar spraying of *Vicia faba* plants with GA<sub>3</sub> induced a marked increase in all amino acids either essential, threonine, valin, Leucine, iso-leucine, lysine, phenylalanine and tyrosine, and non-essential amino acids, aspartic, serine, proline glycine, alanine, histidine, arginine, except for sulphur amino acids (cystin and methonine) in case of var. Giza (674). In this regard, Hussein *et al.* (1983) recorded that foliar spraying of GA<sub>3</sub> increased the content of amino acids (Histidine, Leucine, Cystin, Glycine, Aspartic, Glutamic, Threonine and Valin) of *Vicia faba* seeds.

In the meantime, the protein content increased in response to GA<sub>3</sub> treatments for the yielded seeds of the two varieties (Giza 429 & 674). In this respect, Khafagy (1995) found that GA<sub>3</sub> induced an increase in protein % of soybean. Moreover, Sadak (2005) also found that GA<sub>3</sub> application increased

significantly the total soluble nitrogen, protein nitrogen and total nitrogen of *Hibiscus sabdariffa* yielded seeds.

**Table (7): Amino acids concentrations (mg/g d.wt.) in the yield of faba bean seeds (var. Giza 429 and 674) as affected by 100 ppm GA<sub>3</sub>.**

Amino acid	Variety Giza 674		Variety Giza 429	
	Control	Spraying	Control	Spraying
Aspartic	11.84	12.81	12.76	18.42
Threonine	4.33	5.04	4.77	6.79
Serine	7.16	8.17	7.62	9.53
Glutamic	37.34	39.57	36.12	51.78
Proline	14.98	16.33	15.28	22.75
Glycine	6.79	7.67	7.29	11.17
Alanine	6.63	7.37	7.25	10.39
Cystin	2.13	2.06	1.83	3.13
Valin	4.39	4.91	4.76	8.32
Methionine	1.25	0.45	0.33	2.72
Leucine	3.20	3.59	3.36	5.70
Iso-leucine	8.35	9.35	8.73	13.02
Phenylalanine	3.79	4.22	3.83	5.61
Tyrosine	4.62	4.94	4.96	7.62
Histidine	3.08	3.58	3.44	4.48
Lysine	2.15	2.57	2.59	4.53
NH <sub>4</sub> <sup>+</sup>	10.09	11.85	11.65	16.44
Arginine	12.65	12.67	12.98	18.92
Protein %	27.18	30.22	25.31	29.37

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دراسة تتبعية لتقليل محتوى المواد الضارة غذائياً (الفيسين والكوفيسين)  
والاستجابة الفسيولوجية لبعض أصناف الفول البلدي المصرية المعاملة بحمض  
الجبريليك

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أجريت تجربتي أصص خلال الموسمين الشتويين لعامي ٢٠٠٢ ، ٢٠٠٣ في صوبة قسم  
النبات بالمركز القومي للبحوث ، الدقي - الجيزة - مصر. وكان الهدف من التجارب هو دراسة  
تأثير المعاملة برش بذور ٥ أصناف من الفول البلدي (جيزة ٤٠ ، ٤٢٩ ، ٤٦١ ، ٦٤٣ ، ٦٧٤)  
بمحلول ١٠٠ جزء في المليون حمض جبريليك على النمو ، الصبغات ، المحصول ، مكونات  
المحصول ، ومحتوي القرون الخضراء من المواد السامة (الفيسين والكوفيسين) وكذلك البذور  
الجافة الناتجة عند الحصاد. وقد تم التركيز على محتوى بذور الصنفين جيزة ٤٢٩ ، ٦٧٤ من  
الأحماض الأمينية ونسبة البروتين.

أوضحت النتائج أن معاملة الرش بحمض الجبريليك (١٠٠ جزء في المليون) قد حسنت  
جميع القياسات المورفولوجية (طول النبات ، عدد العقد ، عدد الأفرع ، عدد الوريقات ، عدد  
الأوراق ، عدد الأزهار ، مساحة الأوراق/نبات ، والوزن الطازج والجاف للمجموع الخضري)  
وكذلك بالنسبة للمحصول ومكوناته (طول النبات ، وزن النبات ، عدد الأفرع ، عدد القرون ، عدد  
البذور ، وزن ١٠٠- بذرة) في كل الأصناف المختبرة. هذا بالإضافة إلى تحسن محتوى النباتات  
من الصبغات الضوئية مقارنة بالنباتات غير المعاملة.

كما لوحظ نقصاً في نسبة الجليكوسيدات الضارة (الفيسين والكوفيسين) والتي كانت هي  
الهدف الأساسي لهذه التجربة استجابة للمعاملة بحمض الجبريليك في كل الأصناف المختبرة عامة  
وفي الصنفين جيزة ٤٢٩ ، ٦٧٤ بصفة خاصة حيث أظهروا نقصاً كبيراً في هذه المواد بكل من  
القرون الخضراء والبذور الجافة. وازداد النقص في محتوى الفول من تلك المواد عند إعادة معاملة  
البذور الناتجة بالنقع في حمض الجبريليك (١٠٠ جزء في المليون) في جميع الأصناف المختبرة.  
وقد وصل هذا النقص إلى أكبر قيمه (٥٣% ، ٥٨%) في الصنفين جيزة ٦٧٤ ، جيزة ٤٢٩ على  
التوالي.

بالإضافة إلى ذلك زادت جميع الأحماض الأمينية للصنفين جيزة ٤٢٩ ، ٦٧٤ فيما عدا  
السيستين والميثيونين (الأحماض الكبريتية) ، علاوة على زيادة المحتوى البروتيني للبذور الناتجة  
من كلا الصنفين نتيجة لرش النبات بحمض الجبريليك ، مما يدل على تحسن جودة البذور الناتجة.