



## Maximizing the quality and productivity of two faba bean cultivars via foliar application of L-glutamic acid

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**Abstract:** A potentially unique method for controlling and/or altering physiological processes is provided by biostimulants to stimulate plants growth, quality and quantity of different crops. So, this trial was conducted to investigate the physiological role of L-glutamic acid (L-GLU) at 50, 75, 100 mg/L on growth, quality and quantity the yielded seeds of two cultivars of faba bean (Giza 843 and Nubaria 1). Results show that the growth, seeds weight/plant and biochemical composition of leaf tissues (IAA, total carbohydrate content, total soluble carbohydrate, and free amino acids) of cultivar Nubaria 1 was significantly higher than that of cultivar Giza 843 under control treatment. Whereas, yielded seeds of cultivar Giza 843 was characterized by higher carbohydrate content, protein content and phenolic compound than that of cultivar Nubaria 1 under control treatment. All applied treatments (L-GLU at 50, 75 and 100 mg/L) caused significant increases in most cases as vegetative growth parameters, photosynthetic pigments, seed yield /plant, biochemical composition of leaf tissues at 75 days after sowing as well as biochemical composition of the yielded seeds of two faba bean cultivars. L-GLU at 75 mg/L was the most significant treatment. Where, L-GLU at 75mg/L significantly increased shoot dry weight of cultivar Giza 846 and cultivar Nubaria 1 by 69.53% and 41.42% respectively relative to corresponding controls. Likewise, it significantly increased seeds weight/plant of Giza 843 from 27.45 g to 46.40g (1.69 times) and seeds weight /plant of Nubaria 1 from 33.30g to 69.69 g (2 times). although cultivar Nubaria 1 characterized by higher vicine content (514.43 mg/100g) thancultivar Giza 843 (381.94mg/100g) under control treatment but the highest decreases appeared in cultivar Nubaria 1 due to L- GLU treatments was more pronounced than that occurred in cultivar Giza 843. It could be concluded that L- GLU treatments especially at 75 mg/L could be used as promise treatment to increase the quality and productivity of faba bean plants.

### 1. Introduction

To ensure good crop yields, a lot of chemical fertilizer is applied, which has negative effects on the environment and agricultural products. Therefore, finding sustainable horticulture methods is necessary to combat chemical-based agriculture.

Biostimulants provide a potentially innovative method for controlling and/or altering physiological processes in order to promote plant development, minimize stress-related constraints, increase yield, and reduce the need for fertilizers (Du Jardin, 2015). Such products are environmentally friendly, and encourage sustainable crop productions (Radkowski and Radkowska, 2013; Du Jardin, 2015). Their use in agriculture decreased the quantity of chemicals used (Radkowski and Radkowska, 2013). One type of these biostimulants preparation are depended on amino acids (Ertani *et al.*, 2013;

Chojnacka *et al.*, 2014; Subbarao *et al.*, 2015, Colla *et al.*, 2015; Nardi *et al.*, 2016). It is widely recognized that amino acids are organic nitrogen compounds that used as the basis units of peptide compounds, enzymes and proteins (Shokunbi *et al.*, 2012). In addition, they serve as the building blocks for other vital elements of living organisms like coenzymes, vitamins, nucleotides (bases of purine and pyrimidine), and plant growth regulators (Calvo *et al.*, 2014; Rouphael and Colla 2018; Souri and Hatamian, 2019). In addition, amino acids promote plant development, increase the availability of nutrient, and improve the quality of the plants (Rouphael and Colla, 2018). They serve as regulators of nitrogen uptake (Miller *et al.*, 2007), root development (Calvo *et al.*, 2014; Weiland *et al.*, 2016; Halpern *et al.*, 2015), and antioxidant metabolism (Ertani *et al.*, 2013; Calvo *et al.* 2014; Halpern *et al.*, 2015; Teixeira *et al.*, 2017). In this context, amino acids application via soaking of seeds or foliar application results in enhanced plant development and productivity, because these substances are able to serve as signals of a variety of important biochemical processes occurring in plants, (Calvo *et al.*, 2014; El-Awadi *et al.*, 2019; Sadak *et al.*, 2014; 2023). Moreover, application of amino acids as bio-regulators can decrease the consumption of fertilizer and enhance productivity of different crops by increasing uptake of minerals and consumption of nutrients efficiency (Vernieri *et al.*, 2005) as well as purification agent of water when associated to Cellulose based polyurethane (Abu Rub *et al.*, 2023). Amino acids act as a good source of nitrogen for plant uptake and utilization to stimulate plant root system and aboveground plant parts (Shehata *et al.*, 2011; Souri and Hatamian, 2019; Noroozlo *et al.* 2019). It has been demonstrated that using amino acids to partially substitute nitrate has positive impacts on plant growth and yield (Sadak *et al.*, 2015; Souri *et al.*, 2017) and considered as a safe method through reducing inorganic fertilization. Generally, amino acids treatment is one of the most modern agricultural techniques to enhance plant development, and quality properties of the yield (Sadak *et al.*, 2015; Souri and Hatamian, 2019; El-Metwally *et al.*, 2022).

L-glutamic acid (L-Glu) is a vital amino acid that is crucial for the growth and development of plants (Qiu *et al.*, 2020). It shows great potential regarding agricultural use due to its biocompatible and biodegradable properties (Sung 2015; Ghani *et al.*, 2023) and acts as a central molecule in the metabolism of higher plants (Forde and Lea, 2007). L-Glu also is a common precursor of many organic substances as protein amino acids (proline; arginine, ornithine and histidine), non-protein amino acid (g-aminobutyric acid), antioxidant tripeptide (glutathione), and chlorophyll (Brosnan and Brosnan, 2013; Reiner and Levitz, 2018; Qiu *et al.*, 2020). The metabolism of nitrogen is affected by L-Glu, which affects how plants assimilate nitrogen (Ghani *et al.*, 2023) and is implicated to all aspects of a plant's life cycle (Brosnan and Brosnan, 2013; Toyota *et al.*, 2018, Reiner and Levitz, 2018). Under normal conditions, it has been reported that L-Glu has a unique signaling role in a variety of physiological processes. These processes involved germination of seed (Kong *et al.*, 2015; Souri, 2015; Rosa *et al.*, 2023); root architecture (Kong *et al.*, 2015; Souri, 2015; López-Bucio *et al.*, 2019; Rosa *et al.*, 2023); and growth of pollen tube (Wudick *et al.* 2018). It has been demonstrated that L-Glu participates in various crucial biological functions, such as the removal of reactive oxygen species (ROS) via enhancing the activity of antioxidant enzymes (Asgher *et al.*, 2022), chlorophyll synthesis (Lv *et al.*, 2009), controlling stomatal movement (Dinu *et al.*, 2011), and tricarboxylic acid cycle (TCA) metabolism (Forde and Lea 2007).

Faba bean (*Vicia faba* L.) is a highly valued strategic crop in Egypt, providing food security, income generation, crop rotation benefits, and climate change resilience. Its continued cultivation and improvement are critical for the sustainable development of Egyptian agriculture and food systems (Abdeen and Hefni, 2023). Faba bean is an important source of protein and essential nutrients, and is a staple food for millions of Egyptians. It is mainly cultivated in the Mediterranean region and used as

both human food and animal feed (Saldanha do Carmo *et al.*, 2020). It was mentioned by Fouda *et al.* (2022) that a sustainable nitrogen supply for crops and soils is provided by faba beans. Where, faba bean crop can deliver 100 to 200 kg N per hectare to the soil (Jensen *et al.*, 2010). Seeds of faba beans are characterized by high levels of protein (~25%–30%), and carbohydrate (~55%), suitable level of minerals (magnesium, calcium, and iron) and vitamins (riboflavin, thiamine, and pyridoxine), and low level of fat (<1%) (Martineau *et al.*, 2022).

This investigation aimed to study the physiological role of L-glutamic acid on growth and productivity of two cultivars of faba bean.

## 2. Methodology

### 2.1 Experimental design

Two field experiments were carried out in a private farm at Sharkia Governorate during the winter seasons of 2021/2022 and 2022/2023 to study effect of foliar spraying with L-Glu on both quality and economic characters of *Vicia faba* L- plants. Seeds of two cultivars of *Vicia faba* L. (*cv.* Giza 843 and *cv.* Nubaria 1) were obtained from Agricultural Research Center, Giza, Egypt.

The physical and chemical characteristics of studied soil (Table, 1) were carried according to Cottenie *et al.* (1982).

In the middle of November during the two growing seasons, faba bean seeds were sown in hills 20 cm apart on both sides of the ridge. All of the established cultivation procedures were followed, including nursery growing, main field preparation, applying fertilization, watering, weeding, and protection of plants. After 15 days from sowing, thinning was done to leave two plants per hill. The experiments were laid out in a randomized complete block design with four replicates for each treatment. 10.5 m<sup>2</sup> (1/400 fed) was the size of the trial unit. 3 m long, 3.5 m wide, with ridges spaced 60 cm apart.

However, during the two experimental seasons, foliar application with three concentrations of L-glutamic acid (50, 75 and 100 mgL<sup>-1</sup>) were applied twice after 45 and 60 days from sowing date, respectively.

**Table 1:** Characteristics of the empirical soil in Sharkia governorate, Egypt (Collective data of two seasons)

Physical characteristics					
Texture	Clay	Silt	Sand	EC(ds .m <sup>-1</sup> )	pH
	(%)				
Clay	50.2	38.3	11.6	0.46	7.49
chemical characteristics					
Cations (meq .l <sup>-1</sup> )			Anions (meq .l <sup>-1</sup> )		
Ca	Mg	Na	SO <sub>4</sub>	CL	HCO <sub>3</sub>
2.19	0.68	1.15	3.36	1.18	1.40
Macronutrient			Micronutrient (mg .kg <sup>-1</sup> )		
N (meq .l <sup>-1</sup> )	P (ppm)	K (ppm)	Zn	Fe	Mn
47.2	26.5	386	1.16	5.9	0.30

### 2.2 Collected data

After 75 days of sowing during vegetative growth parameters, plant samples were collected for determination of some morphological characteristics (shoot height (cm), number of leaves and branches/plant, fresh and dry weight of plant (g)). Dry weight was measured after drying plant sample

in oven for 48h at 50°C. Fresh leaves were collected for determination photosynthetic pigments, and indole acetic acid (IAA). While, dry leaves were used to determine total carbohydrate, total soluble carbohydrate, free amino acids, and proline content.

At harvest time, ten plants from each treatment were chosen randomly to measure the number of pods/plant, number of seeds /pod, and weight of seeds/plant. The yielded dry seeds were used to determine total carbohydrate, starch, protein content, phenolic content, and vicine content.

### 2.3 Biochemical analysis

Photosynthetic pigments were determined as the method reported by [Li and Chen \(2015\)](#). Indole acetic acid content was determined according to [Gusmiaty et al. \(2019\)](#). Total carbohydrate content was estimated according to [Albalasmeh et al. \(2013\)](#). Total soluble carbohydrate was estimated by the method of [Mecozzi \(2005\)](#). Starch content was determined as the method of [Chow and Landhausser \(2004\)](#). Free amino acid was determined by method of [Tamayo and Bonjoch \(2001\)](#). Proline content was determined by method of [Kalsoom et al. \(2016\)](#). Total protein was determined according to the method of [Pedrol and Tamayo \(2001\)](#). Total phenolic content was determined by method of [Gonza'lez et al. \(2003\)](#). Vicine content was determined according to [Ramasay and Griffiths \(1996\)](#).

### 2.4 Genetical analysis

DNA of *faba bean* under study was isolated from fresh and young leaves using plantgenomic DNA extraction kit according to [Abdalla et al. \(2020\)](#). A total of 10 ISSR primers were tried, but 6 ISSR primers ((AG)<sub>8</sub> T , (GA)<sub>8</sub> T, (GA)<sub>8</sub> YC, (AC)<sub>8</sub> YG, (AG)<sub>8</sub> GYC and (CA)<sub>6</sub> GT ) with positive results were used in this study. However, the primers were chosen upon the production of distinct and reproducible bands in PCR reactions. Moreover, PCR procedures were done as described by [Abdalla et al. \(2020\)](#).

### 2.5. Statistical analysis

Analysis of variance was used to statistically examine the average of two seasons' value of data. According to [Silva and Azevedo \(2016\)](#), the differences between means were evaluated by the least significant differences (LSD) at 5%.

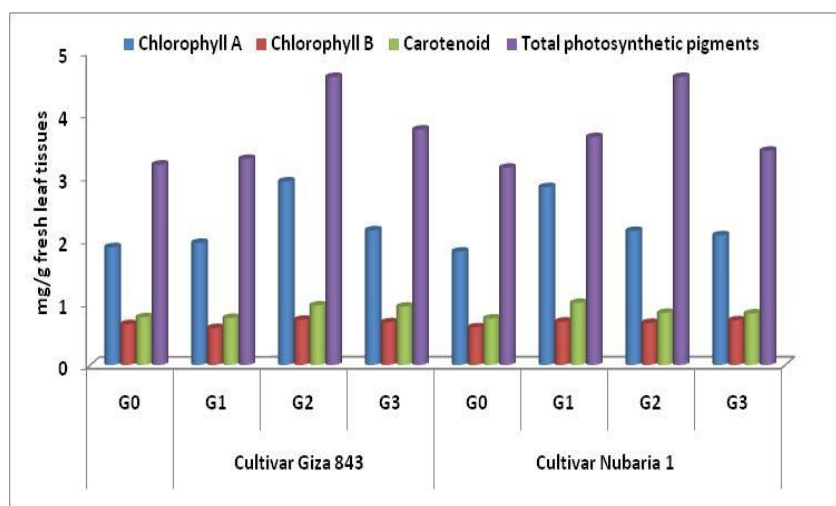
## 3. Results and Discussion

Under control treatment, it was noted from [Table 2 and Figure 1](#) that the growth of cultivar Nubaria 1 was significantly higher than the growth of cultivar Giza 843 under the same conditions. Since, cultivar Nubaria 1 was characterized by higher shoot length, fresh and dry weight of shoot than that of cultivar Giza 843. Regarding L-GLU application, it was noted that all applied levels of L-GLU (50,75 and 100 mg/L) caused significant increases in shoot height, number of leaves/plant, fresh and dry weight of shoot of two cultivars relative to corresponding controls. While, branches number/plant was significantly increased only by L-GLU at 75mg/L. It is clear that L-GLU at 75mg/L was the most pronounced treatments in both cultivars. Where, L-GLU at 75mg/L significantly increased shoot dry weight of cultivar Giza 846 and cultivar Nubaria 1 by 69.53% and 41.42% respectively relative to corresponding controls. These results indicate that response of cultivar Giza 846 to 75mg/L was more effective than cultivar Nubaria 1.

**Table 2.** Effect of L-glutamic on some vegetative growth parameters of faba bean plants at 75 days after sowing

Treatments	Shoot height (cm)	Branches number / plant	Leaves number/plant	Fresh weight shoots (g)	Dry weight shoots (g)
<b>Cultivar Giza 843</b>					
<b>G0</b>	34.00	2.33	62.67	10.45	1.28
<b>G1</b>	43.67	2.33	81.34	14.99	1.88
<b>G2</b>	55.33	3.67	97.34	18.23	2.17
<b>G3</b>	48.67	2.67	74.34	17.07	2.11
<b>Cultivar Nubaria 1</b>					
<b>G0</b>	38.67	2.33	62.00	13.17	1.69
<b>G1</b>	41.00	2.33	85.66	14.700	1.78
<b>G2</b>	50.33	3.67	109.33	19.14	2.39
<b>G3</b>	44.67	2.33	95.34	15.64	1.87
<b>LSD at 5%</b>	<b>3.35</b>	<b>1.172</b>	<b>4.299</b>	<b>1.457</b>	<b>0.177</b>

G0 (control), G1 (50mg/L), G2 (75mg/L), G3 (100mg/L)

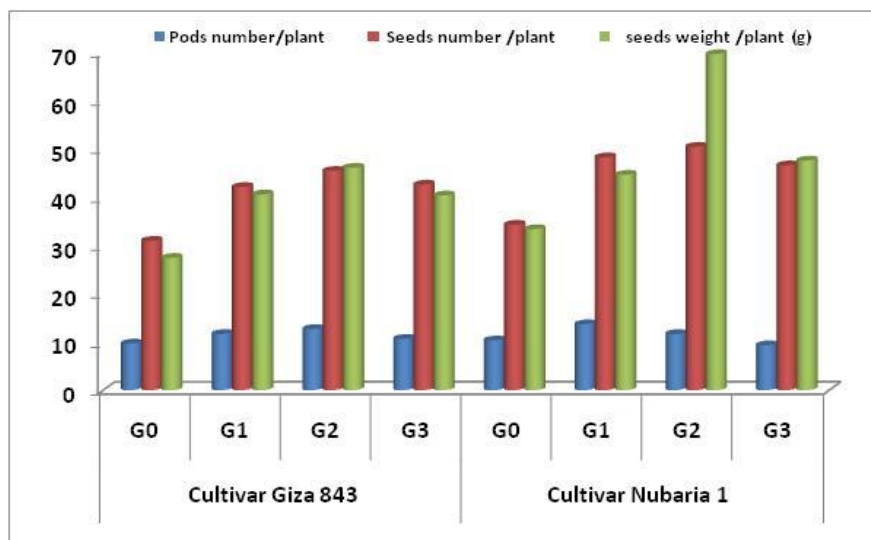


**Figure 1.** Effect of L-glutamic on photosynthetic pigments of two cultivars of faba bean plants at 75 days after sowing (LSD at 5% is 0.53 for chlorophyll A; 0.12 for chlorophyll B; 0.18 for carotenoid; 0.64 for total photosynthetic pigments) G0 (control), G1 (50mg/L), G2 (75mg/L), G3 (100mg/L)

Regarding photosynthetic pigments, it was noted non-significant differences between photosynthetic pigments of two cultivars grown under control treatment (Figure 2). Meanwhile, all applied treatments (L-GLU at 50, 75, 100 mg/L) caused marked increases in chlorophyll A, chlorophyll B, carotenoid and consequently total photosynthetic pigments of both cultivars relative to corresponding controls. The most pronounced treatment was L-GLU at 75mg/L, since it significantly increased total photosynthetic pigments of cultivar Giza 843 from 3.20 to 4.62 mg/g i.e. by 44.73 % relative to control, and significantly increased total photosynthetic pigments of cultivar Nubaria 1 from 3.15 to 4.64 mg/g i.e. by 47.30 % relative to control. These results indicate that there is slight difference response of photosynthetic pigments of cultivar Nubaria 1 to L-GLU at 75mg/L over cultivar Giza 846.

It was noted from Figure (2) that seed number /plant and seeds weight/plant of cultivar Nubaria 1 showed more significant values than those of cultivar Giza 843 under control treatment. Whereas, pods number/plant showed non-significant difference between two cultivars under control treatment. All applied treatments (L-GLU at 50, 75, 100 mg/L) caused marked increases in pods weight/plant,

whereas, L-GLU at 75 mg/L showed significant increases in both cultivars. Meanwhile, all applied treatment caused significant increases in seeds number /plant and seeds weight /plant. L-GLU at 75 mg/L was the most significant treatments. Since, it increased seeds weight/plant of Giza 843 from 27.45 g to 46.40g (1.69 times) and seeds weight /plant of Nubaria 1 from 33.30g to 69.69 g (2 times). This indicates that response of seed number /plant and seeds weight/plant of cultivar Nubaria 1 was more effective than cultivar Giza 843 regarding seed yield.



**Figure 2.** Effect of L-glutamic on seed yield of two cultivars of faba bean plants (LSD at 5% is 2.71 for pod number/plant; 1.89 for seed number/plant; 3.4 for weight of seeds/plant) **G0** (control), **G1** (50mg/L), **G2** (75mg/L), **G3** (100mg/L)

Regarding biochemical composition of leaf tissues at 75 days after sowing, it was noted that leaf tissues cultivar Nubaria 1 was characterized by higher significant values of IAA, total carbohydrate content, total soluble carbohydrate, and free amino acids than cultivar Giza 843 (Table 3) as grown under control treatment. All applied treatments (L- GLU at 50, 75 and 100 mg/L) caused significant increases in IAA, total carbohydrate content, total soluble carbohydrate, proline, and free amino acids of two cultivars relative to corresponding controls. L- GLU 75 mg/L was the most pronounced treatment of both cultivars.

**Table 3.** Effect of L-glutamic on some biochemical constituents of leaf tissues of two cultivars of faba bean plants at 75 days after sowing

Treatments	Indole acetic acid (mg/100g fresh leaf tissues)	Total carbohydrate (%)	Total soluble carbohydrate (%)	Proline (mg/g)	Free amino acids (mg/100g)
<b>Cultivar Giza 843</b>					
<b>G0</b>	32.01	19.89	2.26	29.35	217.77
<b>G1</b>	41.02	21.58	2.87	36.00	272.50
<b>G2</b>	51.23	26.00	3.67	47.26	310.98
<b>G3</b>	49.15	23.52	3.35	39.30	298.10
<b>Cultivar Nubaria 1</b>					
<b>G0</b>	35.23	20.81	3.18	25.39	274.85
<b>G1</b>	52.03	24.46	4.13	36.90	296.10
<b>G2</b>	58.59	26.66	5.08	43.46	320.99
<b>G3</b>	51.25	25.55	4.79	40.45	314.95
<b>LSD at 5%</b>	<b>1.03</b>	<b>0.25</b>	<b>0.10</b>	<b>1.14</b>	<b>1.79</b>

**G0** (control), **G1** (50mg/L), **G2** (75mg/L), **G3** (100mg/L)

Since, it significantly increased IAA, total carbohydrate content, total soluble carbohydrate, proline, and free amino acids of cultivar Giza 843 by 60.04, 30.72, 62.39, 61.02, 42.80 % respectively relative to control. Whereas, it significantly increased IAA, total carbohydrate content, total soluble carbohydrate, proline, and free amino acids of cultivar Nubaria 1 by (66.31, 28.11, 59.79, 18.26, and 19.79 %), respectively relative to control. This indicates that response of cultivar Giza 843 was more effective than cultivar Nubaria 1 regarding total carbohydrate content, total soluble carbohydrate, proline, and free amino acids. Regarding chemical composition of the yielded seeds, under control treatment, it was noted that cultivar Giza 843 was characterized by higher carbohydrate content, protein content and phenolic compound than cultivar Nubaria 1 (Table 4). On the other hand, cultivar Nubaria 1 was characterized by antinutritional compound (vicine) than cultivar Giza 843. All applied treatments (L- GLU at 50, 75, 100 mg/L) caused significant increases in total carbohydrate content, starch, protein content, and phenolic compound accompanied by significant decreases in vicine content of both cultivars. L- GLU at 75mg/L was the most pronounced treatment of both cultivars. Since, it significantly increased total carbohydrate content, starch, protein content, and phenolic compound of cultivar Giza 843 by 7.11, 26.71, 16.86, and 38.54% relative to control. Similarly, it significantly increased total carbohydrate content, starch, protein content, and phenolic of cultivar Nubaria 1 by 8.20,22.46,15.06, and 50.60% relative to control. On the other hand, L- GLU at 75mg/L significantly decreased vicine content of cultivar Giza 843 by 3.87% and by 6.57%in cultivar Nubaria 1 relative to corresponding controls. These results indicate that response of cultivar Giza 843 was more effective than cultivar Nubaria 1 regarding, starch and protein content. Whereas, response of cultivar Nubaria 1 was more effective than cultivar Giza 843 regarding carbohydrate content and phenolic compound. Regarding vicine content, although cultivar Nubaria 1 characterized by higher vicine content (514.43 mg/100g) than cultivar Giza 843 (381.94mg/100g) under control treatment but the decreases appeared in cultivar Nubaria 1 to L- GLU treatments was more pronounced than that occurred in cultivar Giza 843.

**Table 4.** Effect of L-glutamic on some biochemical constituents of the yielded seeds of two cultivars of faba bean plants

Treatments	Total carbohydrate (%)	Starch (%)	Protein (%)	Phenolic compound (mg/g)	Vicine% (mg/100g)
<b>Cultivar Giza 843</b>					
<b>G0</b>	47.35	17.63	21.00	53.39	381.94
<b>G1</b>	48.57	18.23	23.34	62.87	373.47
<b>G2</b>	50.72	22.34	24.54	73.97	367.16
<b>G3</b>	49.11	20.66	23.84	65.39	328.35
<b>Cultivar Nubaria 1</b>					
<b>G0</b>	44.75	17.63	20.77	48.55	514.43
<b>G1</b>	46.42	20.33	22.21	60.60	505.68
<b>G2</b>	48.42	21.59	23.90	73.12	480.60
<b>G3</b>	48.25	20.76	22.78	63.93	469.18
<b>LSD at 5%</b>	0.640	0.239	0.460	0.964	3.461

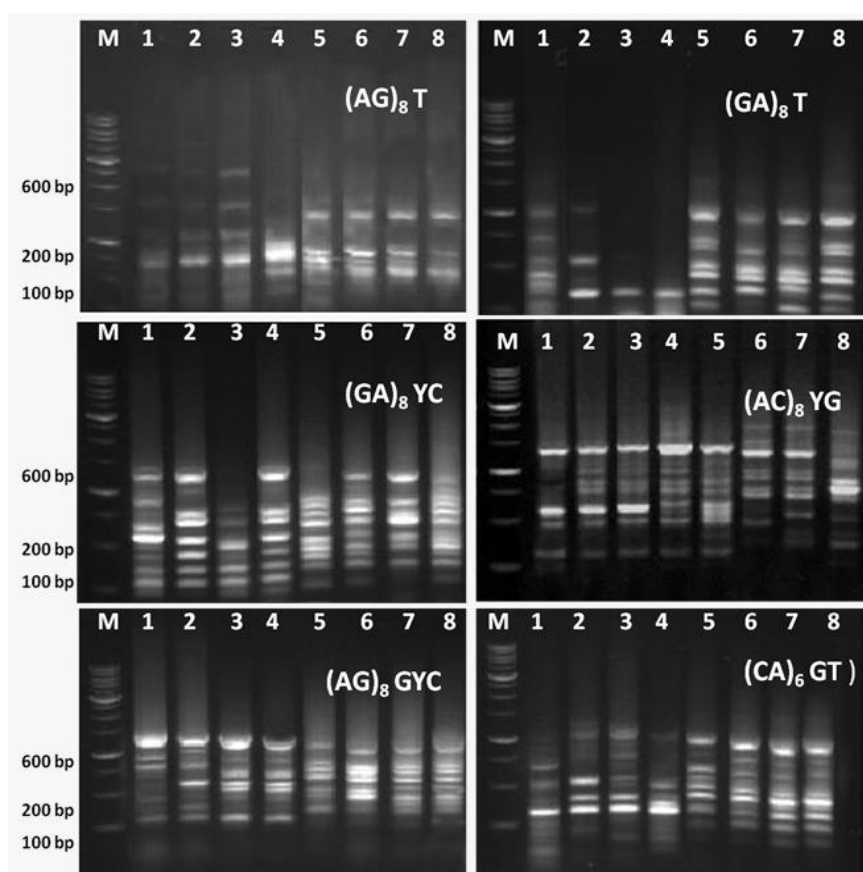
**G0** (control), **G1** (50mg/L), **G2** (75mg/L), **G3** (100mg/L)

Table (5) represent a general idea about the reproducible bands detected using previous six ISSR primers. However, Table 5 draws the attention to number, size, type and conjugative reproducible bands that were detected by each primer separately. Moreover, there were some bands which have the same molecular weight and these called polymorphic bands and this conjunction due to the effect of

the treatments. Table (5) and Figure (3) illustrated the effects of L- GLU on both cultivars under study using 6 ISSR primers. It was noticed that 153 bands (Total bands TB) with molecular weights ranged from 37.75 – 575.08 bp were detected. Moreover, these total band were distributed between 68 polymorphic bands (PB) with an average 11.33, 85 unique bands (UB) with an average 14.17 and there is no any monomorphic bands (MB), so, the highest levels of polymorphism (PB% was 100.00%) were scored with all plant samples under study.

**Table 5.** Effect of L- GLU acid on reproducible DNA fragments of faba bean cultivars using ISSR molecular markers.

Primers	Marker weights (bp)	Amplified bands				PB %
		TB	MB	UB	PB	
(AG) <sub>8</sub> T	37.57 - 387.36	25	-	19	6	100 %
(GA) <sub>8</sub> T	109.47 – 373.46	25	-	15	10	100 %
(GA) <sub>8</sub> YC	175.84 – 421.54	28	-	14	14	100 %
(AC) <sub>8</sub> YG	218.10 – 510.10	26	-	12	14	100 %
(AG) <sub>8</sub> GYC	179.09 – 478.14	26	-	12	14	100 %
(CA) <sub>6</sub> GT	269.07 – 575.08	23	-	13	10	100 %
<b>Total</b>		153	-	85	68	-
Average		25.5	-	14.17	11.33	100 %



**Figure (3):** Effects of L- GLU on both cultivars under study using 6 ISSR primers.

Moreover, every primer detected a different number of bands with different ranges of molecular weights and high ratio of polymorphism as follow:



- 25 bands with molecular weights ranged between (37.57 – 387.36bp) were detected using ((AG)<sub>8</sub> T) primer, and these repressible bands were distributed as 0 (MB), 19 (UB) and 6(PB) with 100.00% polymorphism.
- 25 bands with molecular weights ranged between (109.47 – 373.46bp) and 100.00% polymorphism were detected using (GA)<sub>6</sub> T)primer, and distributed as 0 (MB), 15(UB) and 10(PB).
- 28 bands with molecular weights ranged between (175.84 – 421.54 bp) and 100.00% polymorphism were detected (GA)<sub>8</sub> YC)primer, and distributed as 0(MB), 14(UB) and 14(PB).
- 26 bands with molecular weights ranged between (218.10 – 510.10 bp) and 100.00% polymorphism were detected using (AC)<sub>8</sub> YG)primer, and distributed as 0(MB), 12(UB) and 14(PB).
- 26 bands with molecular weights ranged between (179.09 – 478.14 bp) and 100.00% polymorphism were detected using (AG)<sub>8</sub> GYC) primer, and distributed as 0 (MB), 12 (UB) and 14 (PB).
- At the end there were 23 bands with molecular weights ranged between (269.07 – 575.08 bp) and 100.00% polymorphism were detected using (CA)<sub>8</sub> GT) primer, and distributed as 0 (MB), 13 (UB) and 10 (PB); (Table 5 and Figure 3).

## Discussion

The results of this investigation clearly illustrated the beneficial effect of L- GLU on vegetative growth parameters (Table 2), photosynthetic pigments (Figure 1), seed yield and its components (Figure 2), as well as some chemical composition of leaf tissues at vegetative stage (Table 3), and nutritive value of the yielded seeds (Table 4) of two faba bean cultivars.

Amino acid application as an organic fertilizer and growth-promoting agent provides plants with nutrients and enhances plant quality, which eventually increases agricultural yield and commercial output (Du Jardin, 2015). Therefore, it has become popular in sustainable agriculture (Rouphael and Colla, 2018). In this respect, Thon *et al.* (1981) claimed that amino acids supply plant cells a source of nitrogen that is immediately available and, generally, more easily absorbed by cells than inorganic nitrogen. However, amino acids applied to leaves can serve as both a source of nitrogen for the plant and a signal for several metabolic processes (Teixeira *et al.*, 2017, Santi *et al.*, 2017). Amino acids are essential for protein assimilation since they are necessary for cell development and hence increase fresh and dry matter, which increases plant growth and plant production. The application of L-Glu increased the growth parameters of faba bean may be due to its role in activating physiological and biochemical processes. Noroozlo *et al.* (2019) showed that amino acid contributes to the synthesis of proteins and the production of carbohydrates by manufacturing chlorophyll, promoting photosynthesis process and producing growth-stimulating hormones like gibberellic acid and cytokinin, which boosted cell divisions, cell enlargement, and production of lateral buds. Moreover, the improvements in plant growth and biomass due to L-Glu application might have resulted from decreased the electrolyte leakage and production of reactive oxygen species (Farid *et al.*, 2020). It worthy to mention that glutamine is transformed into  $\alpha$ -ketoglutaric acid, which enters the Krebs cycle and assists in supplying energy and the production of intermediate compounds for different bio-building processes (El-Desouky, 2008). Owing to hormone like activity of amino acids and their role in transmitting signals, they operate as preventive agents against stress conditions and can also improve the control of stomata and gene expression toward better plant growth (Souri, 2017). According to Noroozlo *et al.* (2019),

amino acids are useful for enhancing nutrient uptake and, consequently, plant growth, productivity, and quality from the point of view of modern and environmentally friendly agriculture. Numerous studies have shown that applying amino acids externally can improve plant growth and productivity. Since, foliar application of amino acids increased the yield production of crop plants due to increased protein, chlorophyll and photosynthesis rates (Amin *et al.*, 2011; Souri *et al.*, 2017; Basanth and Mahesh, 2018; Souri and Hatamian, 2019). According to Abdallah *et al.* (2015), the promoting impact of amino acids may also be due to their effects on enzymatic activity and the translocation of metabolites from the source (leaves) to the mature peanut seed (sink). The increases of growth parameters, photosynthetic pigment content, and endogenous growth regulators (as IAA) may have contributed to the increased seed yield and its component parts in faba bean plants. This increases in photosynthesis process led to an increase the transfer of photo assimilates from leaves to seeds, which increased their weights and, in turn, the various yield components. Fahimi *et al.* (2016); Souri, *et al.* (2017); Souri and Hatamian (2019) mentioned that application of amino acid lead to higher chlorophyll content of leaves and attributed these increases to the promoting effect of amino acids on biosynthesis of chlorophyll, synchronizing with decreases in degeneration of chlorophyll. They can prevent oxidation, peroxidation and degradation of cell constituents, especially chlorophylls, thus resulted in prolong the life of cell. Accordingly, Sánchez-Pale (2017) reported that L-Glu is linked to higher levels of photosynthetic activity and chlorophyll production. In addition, these increases in photosynthetic pigments may be explained by the involvement of amino acids in starting the metabolic process that produces chlorophyll and succinyl COA, an intermediate step in the Kerb's cycle (Taylor *et al.*, 1982). It was proved by Souri and Hatamian (2019) that L-Glu application not only has a promotive effect on the growth and yield of plants, but they can also affect their nutritional value. So, regarding the beneficial effect of L-Glu on biochemical constituent of leaf and seed of faba bean (Table 3 and 4). Colla and Rouphael (2015); Bulgari *et al.* (2019) mentioned that amino acids can act as hormone precursors. Previously, Maxwell and Kieber (2004); Amin *et al.* (2011); Souri and Hatamian (2019) confirmed the correlation between amino acids and the production of growth regulating substances like IAA and their translocation. Several researches verified a positive effect of exogenous application of amino acid on plants as they boosted production of starch, and polysaccharides in *Vicia faba* L. (Sadak *et al.*, 2014), increased leaf N concentration, and leaf soluble carbohydrates of celery (Shehata *et al.*, 2011), increased content of sugars in *Daucus carota* L roots (Grabowska *et al.*, 2012); significantly increased the amino acids and proteins concentrations in onion plant tissues (Amin *et al.*, 2011), and promote protein concentration of wheat grains (Bafeel *et al.*, 2016). The significant increases in carbohydrate contents due to L-Glu application may be related to the increased photosynthetic output (Taiz and Zeiger, 2013). In addition, in the fruits of *Lycopersicon esculentum* L., application of L-Glu boosted fructose content while leaving glucose and sucrose levels unchanged (Alfosea-Simón *et al.*, 2021). Hafez *et al.* (2012) stated that amino acid fertilizers provide directly absorbable nitrogen to plants, which is usually absorbed faster by plant cells than its inorganic form and contributes to protein synthesis. Likewise, amino acids and amides can be identified the main transport form for organic nitrogen, and they can be metabolized or used directly to synthesize protein and other important compounds (Rentsch *et al.*, 2007). In addition to its inherent importance as an amino acid, plants may metabolize L-Glu and use it to make other amino acids and proteins (Souri, 2015; Alfosea-Simón *et al.*, 2021). According to Taiz and Zeiger (2015), L-Glu takes parts in different metabolic routes in plants, as synthesis of other amino acids like aspartate, proline, arginine, and glutamine. The application of glutamine to *Allium cepa* enhanced the amount of total amino acids, soluble sugars, and phenolic compounds, according to Amin *et al.* (2011). Moreover, Rosa *et al.* (2023) indicated positive

effects of L-Glu on carrot yield and nutritional value of carrot root (protein and total soluble solids). The external application of L-Glu in at the seedling stage enhances the plant's functional traits, such as biomass, lipids, and the amount of soluble protein and starch, and aids in the process of the plant's natural strengthening (Guo *et al.*, 2019).

Regarding the phenolic compounds, Heldt and Piechulla (2010) demonstrated that amino acids are the precursor of phenolic compounds. According to Ampofo and Ngadi (2021), high L-Glu levels (5 and 7 mM) caused the highest levels of the enzymes that initiate the formation of phenylpropanoid compounds (phenylalanine ammonia-lyase and tyrosine ammonia-lyase), which in turn caused increases production of phenolic compounds. According to De Gregorio *et al.* (2023), L-Glu dramatically altered the biosynthesis of lignans, flavanols, phenolic acids and low molecular weight phenolics.

Regarding antinutritional substance (vicine), Bjerg *et al.* (1985) mentioned that the presence of favism-causing chemicals in faba bean seeds appears to be influenced by both environmental and genetic factors. Furthermore, foliar application with certain growth regulators (indole acetic acid and kinetin) (Sadak *et al.*, 2013), benzyladenine (Zaki *et al.*, 2021), lowered the contents of vicine in the yielded faba bean seeds. The decrease in the vicine level may be attributed to the impact of these treatments on metabolic pathway of vicine precursor (orotic acid) production which is responsible for the producing of pyrimidine ring of these hazardous components (Brown and Roberts, 1972).

#### 4. Conclusion

It is worthy to mention that all applied treatments (L-GLU at 50, 75, 100 mg/L) caused significant increases in most cases as vegetative growth parameters, photosynthetic pigments, seed yield /plant, biochemical composition of leaf tissues at 75 days after sowing as well as biochemical composition of the yielded seeds of two faba bean cultivars. L-GLU at 75 mg/L was the most significant treatment. It significantly increased seeds weight/plant of Giza 843 by 1.69 times and seeds weight /plant of Nubaria 1 by 2 times accompanied by significant decreases of vicine content. These decreases in vicine content in cultivar Nubaria 1 was more pronounced than that occurred in cultivar Giza 843. It could be concluded that L- GLU treatments specially at 75 mg/L could be used as promise treatment to increase the quality and productivity of faba bean plants.

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*Compliance with Ethical Standards:* This article does not contain any studies involving human or animal subjects

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