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Effect of Tryptophan and Glutamic Acid on Morphological Traits of Iranian and Afghan Saffron

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ABSTRACT

In order to investigate the effect of amino acids tryptophan and glutamic acid on the morphological traits of saffron medicinal plant, a factorial experiment was conducted in the form of a randomized complete block design in three replications in 2018 in the research farm of Zanjan University. Experimental treatments include three genotypes (Iranian, Afghani 1 and Afghani 2) as the main treatment and amino acid tryptophan at two levels (1 and 2 mM) and glutamic acid at two levels (1 and 2 mM) as secondary treatments. They were considered as witnesses. The results showed that tryptophan and glutamic acid treatments had a significant effect ($P \leq 0.01$) on most of the investigated traits including number of flowers, dry weight of flowers, vegetative body. The highest content of number of flowers and dry weight of flowers were observed, respectively, 34.6 and 37.36 mg of dry weight. Also, the performance traits of flower fresh weight, stigma dry weight, showed significant difference ($P \leq 0.05$) under the treatment of two amino acids. The maximum yield of phenol and flavonoid in 2 mM tryptophan concentration was 0.35 and 0.026 mg/g/ha, respectively, and the lowest yield in 1 mM glutamic treatment was 0.34 and 0.02 mg/g/ha, respectively.

In total, different levels of tryptophan and glutamic acid can have an effective role in improving the morphological traits and production of this product. The use of 1 mM glutamic acid to produce the maximum vegetative body of the plant, the treatment of 1 and 2 mM of both amino acids to improve physiological indicators and 1 mM of glutamic acid for the performance of secondary metabolites is desirable and recommended for the purpose.

Keywords- Crocin, Glutamic acid, Number of flowers, Saffron, Tryptophan.

I. INTRODUCTION

Saffron (*Crocus sativus*) is a perennial plant belonging to the Iridaceae family. Its leaves are 6 to 10 standing, saffron flowers are 1 to 2 purples in color and relatively large (Wendelbo and Mathew, 1975). Saffron is a triploid vegetable that is sterile with 24 chromosomes. The saffron plant has an underground stem and a solid bulb called corm, and its propagation is done only by cultivating the corm and creating a new daughter corm from the mother corm, due to its sterility. Gresta et al., 2009). Saffron is the most valuable agricultural and medicinal product in the world (Koocheki et al., 2011).

Its stigma is used in the food industry due to its colored and aromatic substances. Saffron is one of the agricultural products of Iran and Afghanistan. which is important in improving the economic and social status of saffron growers even in dry and low-rain regions (Maqsoodi, 2009). Saffron is widely used in traditional medicine and pharmaceutical industries. The dried red stigma of saffron is not only a spice but also a very popular medicinal plant in traditional medicine. which is used against muscle cramps, asthma, menstrual disorders, liver disease, cancer treatment and strengthening the digestive system (Ferrara et al., 2014). Currently, saffron is the most expensive spice in the world and Iran is the most important producer of this product. The importance of saffron in Iran can be examined from various aspects of the lack of water (compared to other agricultural products), social and political, job creation in terms of export development. In saffron genotypes, the observed differences are in different appearance traits. Paying attention to the amount of diversity is important in plant breeding.

Because the appropriate figures are provided. This diversity comes from the influence of environmental and genetic factors (Farshad Far, 2016). The genotypes of cultivated saffron from the point of view of genetic diversity have been done by different researchers with different morphological and molecular methods, in which less protein has been used for diversity. Most of the SDS PAGE studies on saffron have been conducted with the aim of investigating a specific protein in this plant, and less have been used to investigate genetic diversity. Considering the economic importance of saffron as well as the wide genetic diversity of the genus Crocus in Iran, it is necessary to investigate this diversity and compare the similarity and difference between different species of this genus with *C. sativus* and also different genotypes.

Saffron is a mutational phenomenon, and the morphological diversity of genotypes does not follow it. The environmental conditions of the flowering time and the length of the flowering period may have an effect. By using different molecular markers, no genetic variation has been observed between the genotypes of *C. sativus* (Fluch et al., 2009). Which is cultivated in some countries including Iran, Greece, Morocco, Spain, France, India, China, Pakistan and Afghanistan (Izadpanah F. A Kalantari et al., 2015). Iran is known as the largest and most important saffron producing country in the world (Rezvani Moghaddam, 2015). Saffron contains more than 150 volatile and fragrant substances. It also has a number of active and inactive carotenoid compounds including zeaxanthin, lycopene and various types of alpha and beta carotenes, and it has been proven that this plant is a rich source of phenolic contents with antioxidant activity (Goli et al., 2002).

The saffron stigma contains large amounts (over 8% of dry weight) of the apo coronoid crostin and crocin (glycosylated forms of crostin. Crocin is responsible for the red color of the stigma, picrocrocin is responsible for the bitter taste, and safranal is responsible for the spicy aroma of saffron (Caballero et al., 2007). Amino acids directly and indirectly affect the physiological activities, growth and development of plants (Faten et al., 2010). Amino acids are the building blocks of proteins that perform structural, metabolic and transport functions in plants (Liu et al., 2008). Amino acids are the precursors of plant hormones and other growth substances. Amino acids increase the productivity of plant metabolism and increase product quality and yield. Increasing the plant's tolerance and improving it in biological stress, facilitating the absorption of nutrients, transferring the use and increasing the quality characteristics of the product (Calvo et al., 2014). Increasing the process of plant respiration, photosynthesis, protein synthesis, enhances plant growth and performance (Davies, 2010). Foliar spraying of radish plants with compounds containing amino acids increases the concentration of nitrogen in the branches. The use of amino acid increased potato vegetative growth, increased total nitrogen, phosphorus and potassium in strawberry shoots, as well as yield, weight, TSS, vitamin C and total sugar concentration of fruits (Ahmadi, 2018).

In adverse environmental conditions, amino acid production is reduced or stopped. The aromatic amino acid phenylalanine and tryptophan in plants are not only essential components for protein synthesis, but also as biosynthetic precursors they promote the production of a wide range of plant secondary metabolites (Tzin and Galili, 2010). Also, studies showed that tryptophan plays a role as an important precursor in the biosynthesis of auxin (indole acetic acid) (Ramaiah et al., 2003). Foliar spraying with tryptophan at a concentration of 100 mg/liter resulted in the highest growth parameters including plant height, flower dry weight, and the most components of plant yield and plant seeds, as well as chlorophyll (a+b) content of *Hibiscus sabdariffa* L. had (Gendy and Nosir, 2016). Glutamic acid is one of the amino acids that play a role in seed germination and as a precursor in the synthesis of chlorophyll, as well as in the synthesis of other amino acids, and prevent the blocking of air holes due to adverse environmental conditions. Tryptophan is a precursor for the biosynthesis of auxin hormone, which is one of the most basic factors of plant metabolism (Asadi, 2020).

II. MATERIALS AND METHODS

In order to investigate the effect of tryptophan and glutamic acid on the morphological and phytochemical traits of Iranian and Afghan saffron in the crop year of 2018 and 2019 in the form of a factorial experiment in the form of a randomized complete block design in three replications in the research farm of Zanjan University with a latitude of 35 degrees and 25 minutes and Longitude 47 and 10 minutes with an approximate height of 1663 meters above sea level. The test treatments included the amino acid glutamic acid and tryptophan at the level of 1 and 2 mM. At first, saffron seeds were planted, sorted, measured, and treated with amino acid glutamic acid and tryptophan each at two levels (1 and 2 mM), immersed in different concentrations. In order to sample the soil of the test site from a depth of 030 cm, it was transferred to the soil science laboratory of Zanjan University in order to determine the physical, chemical and texture characteristics of the soil. The physical and chemical properties of farm soil are listed in Table 1. Preparing the farm land, plowing and disking operations, leveling the land, removing stones and clods, weeds and plotting it 5 meters long and 2 meters wide, the distance between the rows 50 cm, the distance between the stumps 20 cm and planting depth the tubers were planted between 15 and 20 cm of soil.

Table 1: Physical and chemical criteria of soil in the cultivation area

Organic material (%)	EC ($dS.m^{-1}$)	PH	Texture of soil			Total N	P ($mg. kg^{-1}$)	K ($mg.kg^{-1}$)
			Sand (%)	Silty (%)	Clay (%)			
1.18	0.96	7.6	40	27	33	0.06	22	174

In each plot, 10 rows with a length of 2 meters with a density of 100 tubers were cultivated with three replications with 45 experimental units. The first irrigation was done after cultivation. Then the cell breaking operation took place. Irrigation was done in two stages during the growing season. Of course, due to the climatic conditions of the region, the second irrigation was done after collecting the flowers. Weed control and weeding was carried out manually. No chemical pesticides and herbicides were used during the experiment. The appearance of saffron flowers occurred on the first of November and flowering continued for 30-50 days after the appearance of the first flowers. The flowers were collected by hand once a day and in order to investigate the effect of the treatments on different traits, samples were taken at the flowering stage. The flowers that appeared daily (between 6 and 8 in the morning) were collected, counted and transferred to the laboratory to measure the wet and dry weight of the stigma, and the flower head was separated from the stigmas, and then the stigmas were placed in the oven (at a temperature of 40 degrees Celsius for 24 hours) dry and their dry weight was weighed with a sensitive scale with an accuracy of 0.0001 g. In order to calculate the length of flower and stigma, the flowers harvested from each replicate were randomly selected daily and measured in centimeters using a ruler. And finally, the average length of flowers and stigmas during the flowering period was considered as the average length of flowers and stigmas for each plot.

In order to analyze the statistical data obtained from the experiment, SAS 9.1.3 Portable software was used. Means were compared with each other using Duncan's test at a probability level of 5%. Excel software was used to draw graphs and figures.

III. RESULTS AND DISCUSSION

The results of analysis of variance (Tables 2 and 3) showed that glutamic acid and tryptophan treatment had a significant effect on the studied traits. So that the attributes of number of flowers, dry weight of flowers, total phenol, antioxidant activity and crocin were significant in the simple and reciprocal effects of glutamic acid and tryptophan treatment at the possible level of 1%. In the characteristics of corm fresh weight, flower fresh weight, and stigma dry weight, with the simple and reciprocal effects of glutamic acid and tryptophan treatment, they were significant at the possible level of 5%. Therefore, the traits of stigma length, flower length in simple effects were not affected by glutamic acid and tryptophan treatment. The results of analyzing the variance of the data of the morphological traits and also the performance of these traits per hectare (Table 2) showed that glutamic acid and tryptophan treatment had a significant effect on the studied traits. So that the traits of number of flowers, dry weight of flowers, total phenol, antioxidant activity and crocin in the simple and reciprocal effects of glutamic acid and tryptophan treatment at the probability level of 1%, as well as the traits of corm fresh weight, flower fresh weight, stigma dry weight, with simple effects and the interaction of glutamic acid and tryptophan treatment was significant at the possible level of 5%.

Table 2: Analysis of variance of morphological traits

S.O. V	(df)	Number of flowers	Fresh flower weight (mg)	Dry weight flower (mg)	Flower length (cm)	Stigma length (cm)	Fresh stigma weight (mg)	Dry stigma weight (mg)
Replication	2	$0/28^{ns}$	$184/98^{ns}$	$1/13^{ns}$	$0/00^{ns}$	$0/16^{*}$	$6/66^{ns}$	$0/92^{**}$
Genotype	2	$4117/08^{**}$	$2183/54^{*}$	$78/93^{**}$	$0/10^{ns}$	$0/02^{ns}$	$2/35^{ns}$	$0/30^{*}$
Main plot error	4	$0/68^{ns}$	$1316/39^{*}$	$12/72^{ns}$	$0/04^{ns}$	$0/06^{ns}$	$30/82^{*}$	$0/39^{*}$
Treatment	4	$61/64^{*}$	$204/19^{ns}$	$17/63^{ns}$	$0/04^{ns}$	$0/02^{ns}$	$15/32^{ns}$	$0/30^{*}$
Genotype and Treatment	8	$54/72^{**}$	$719/26^{ns}$	$12/21^{ns}$	$0/13^{ns}$	$0/04^{ns}$	$11/86^{ns}$	$0/08^{ns}$
Sub-plot error	24	16/25	472/94	8/40	0/07	0/03	7/72	0/09
(C.V)		20/20	7/59	8/15	5/26	6/36	13/50	8/18

ns* and **: represent non-significant and significant at 5 % and 1 % level, respectively.

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Number of flowers:

Based on the results of comparing the average data, it showed that the treatments of amino acid glutamic acid and tryptophan increased the number of flowers in this plant. Thus, the highest average number of flowers (33.66) was recorded in the treatment of the interaction effects of 2 mM Glutamic acid, and the lowest number of flowers was related to the control treatment. The number of flowers in other treatments was significantly higher than the control (Figure 1). In the studies conducted, amino acid tryptophan treatment increased the growth and development of the number of flowers in the plant. The use of amino acid not only increases growth, but also increases the quality and quantity of the product (Bilal et al., 2016).

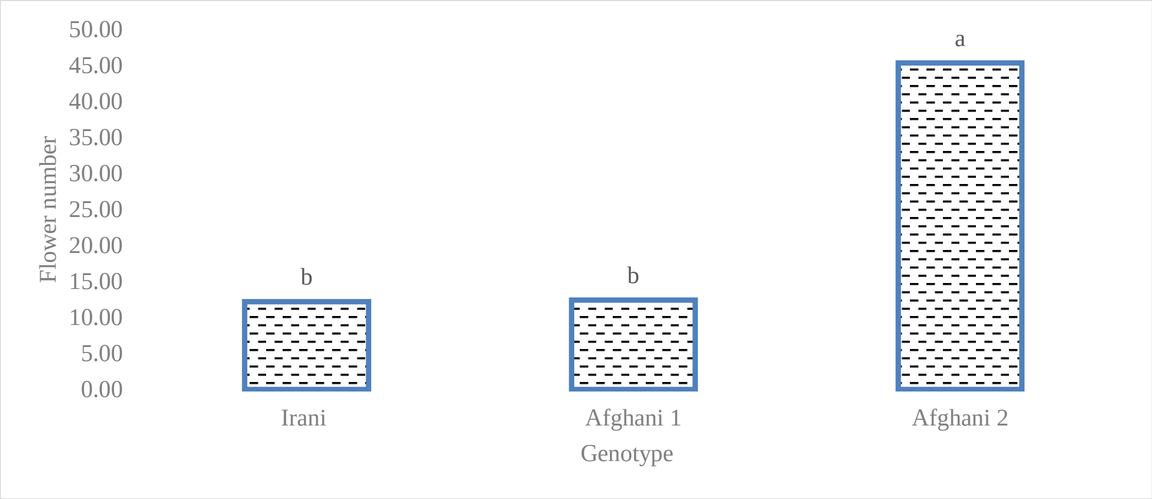

Figure 1: Simple effect of glutamic acid and tryptophan on the number of saffron flowers genotype

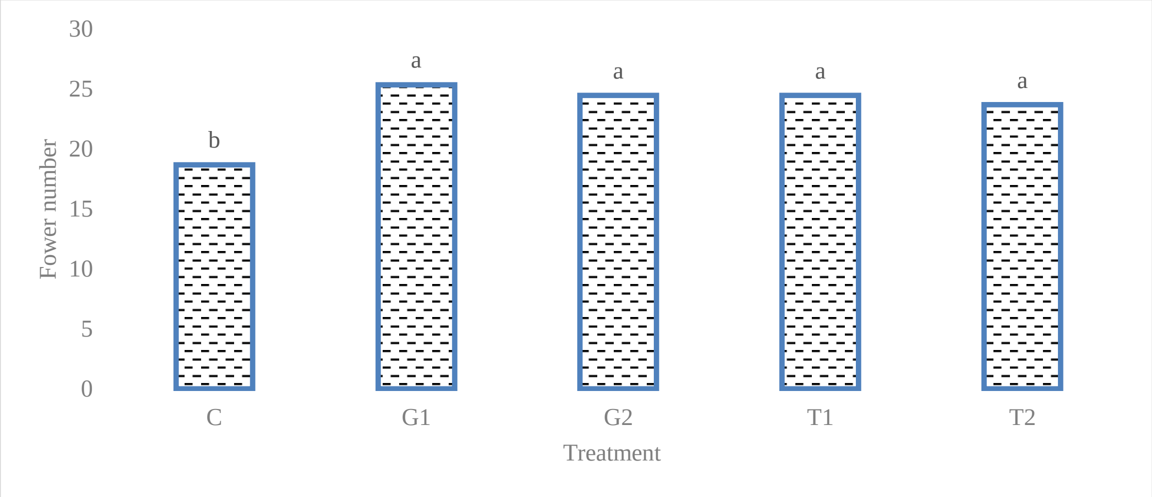

Figure 2: Simple effects of glutamic acid and tryptophan on the number of saffron flowers Treatment

Flower fresh weight:

Based on the results of analysis of variance (Table 2), it showed a completely significant difference on the trait of flower fresh weight in different genotypes. A low percentage of variation and a high-test error indicate the insignificant effect of the environment on this trait. Figure 3. The results of the average comparison between genotypes indicated that Afghani genotype 1 with a weight of (295.53) mg per plot row was the best genotype in terms of this trait. Afghan 2 and Iranian genotypes with lower weight in each row of plots were placed in the second group. Glutamic acid and tryptophan treatments significantly affect the fresh weight of saffron flowers. It increased the fresh weight of flowers compared to the control. So that the highest flower fresh weight (291.52) mg was observed due to the treatment of glutamic acid and 2 mM tryptophan. Glutamic acid and tryptophan level treatments of 1 mM showed the lowest fresh weight of flowers in control treatment. In this research, comparing the average, it showed that the amino acid glutamic acid and tryptophan 2 mM had the greatest effect, which caused an increase in the fresh weight of the flower and plant yield. It has been reported in many studies that foliar spraying with amino acid increases the growth and development of plants (Faten et al., 2010).

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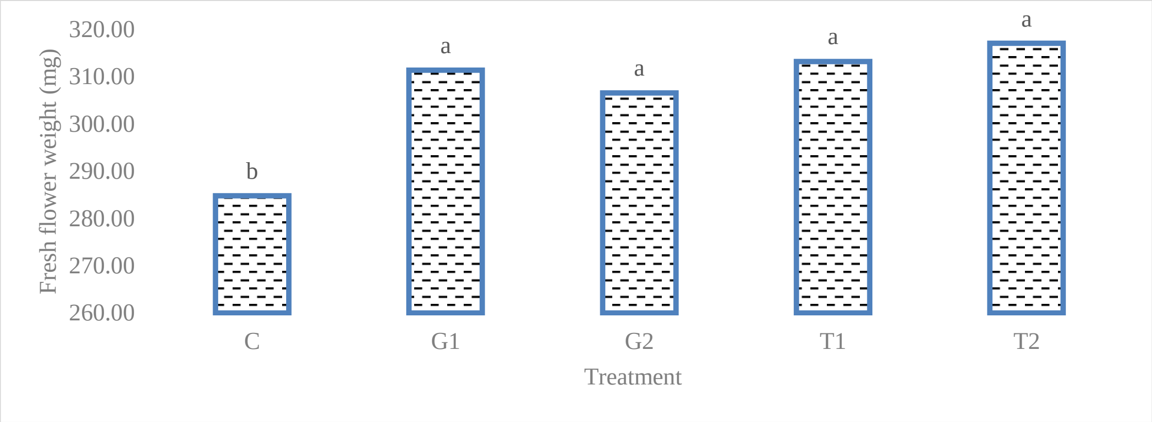

Figure 3: Simple effects of glutamic acid and tryptophan treatments on saffron genotypes

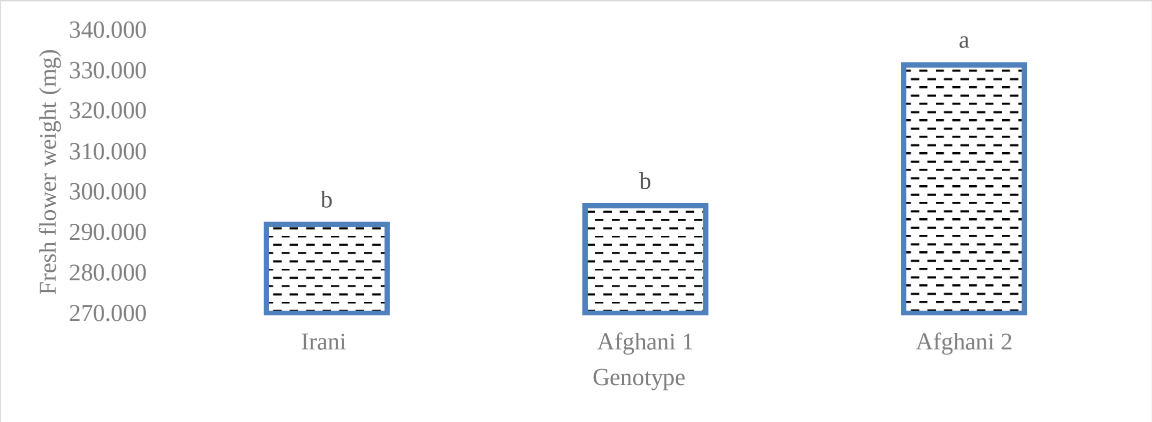

Figure 4: Simple effects of glutamic acid and tryptophan treatments on fresh flower saffron

Dry weight of flower:

Based on the results of variance analysis (Table 2), it was found that the difference between genotypes for flower dry weight trait is significant at the 1% probability level. But the percentage of phenotypic and genetic variation was low for this experiment. This indicates low diversity in terms of flower dry weight. The results of the average comparison between different genotypes indicated that the Afghani genotype 1 with a weight of (37.584) mg for each flower took the highest amount and was placed in group a (Figure 5). These results show the positive effect of different treatment levels of glutamic acid and tryptophan and their effect on dry flower weight. The highest average dry weight of flowers was obtained with (37.364) mg in the simple effects treatment of 2 mM tryptophan level. The lowest dry flower weight is 34.397 and 34.36 mg respectively in the control treatment. The highest level of each treatment of glutamic acid and tryptophan improved the dry flower weight compared to the control.

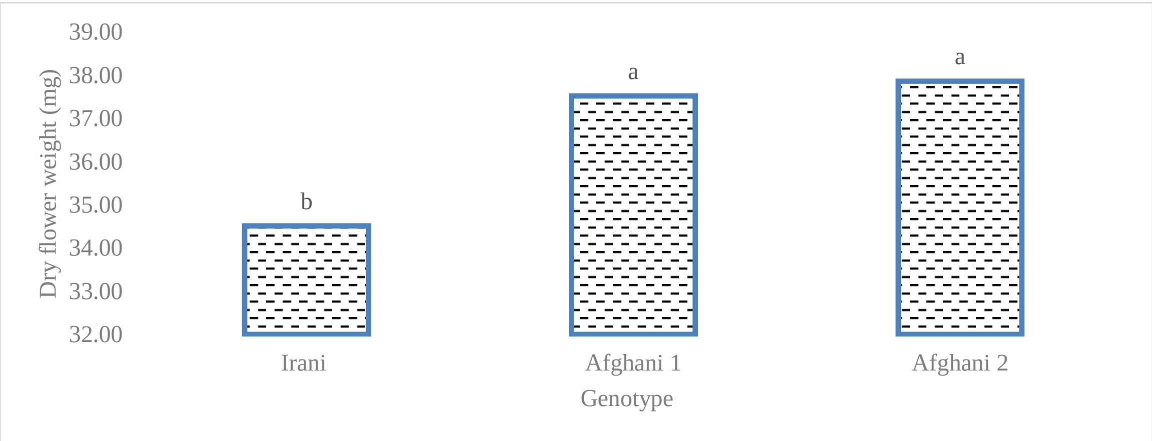

Figure 5: Simple effects of glutamic acid and tryptophan genotype on saffron genotypes

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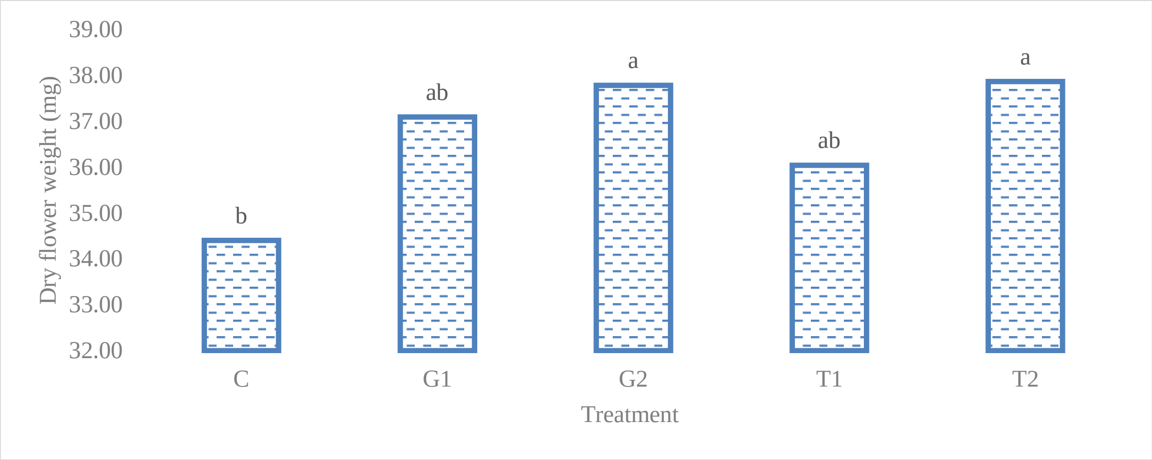

Figure 6: Simple effects of glutamic acid and tryptophan treatments on dry flower saffron

Stigma length:

The result of analysis of variance (Table 2) showed that the stigma length of different genotypes had no statistically significant difference. The coefficient of phenotypic and genotypic variation is low, which indicates low diversity in terms of stigma length. Figures (6 and 7) comparing the average length of the stigma showed that the Afghani genotype 1 with a length of (5.17) cm had the highest amount and there was no significant difference with the Afghani 2 and Iranian genotypes. The results of the average comparison (Figure 7) of the treatments showed that the effect of 1- and 2-mM glutamic acid increased the length of the stigma compared to the control and other treatments. Therefore, the diversity for this trait in the test was low and it did not show a relationship with yield, so it cannot be a suitable trait for saffron breeding.

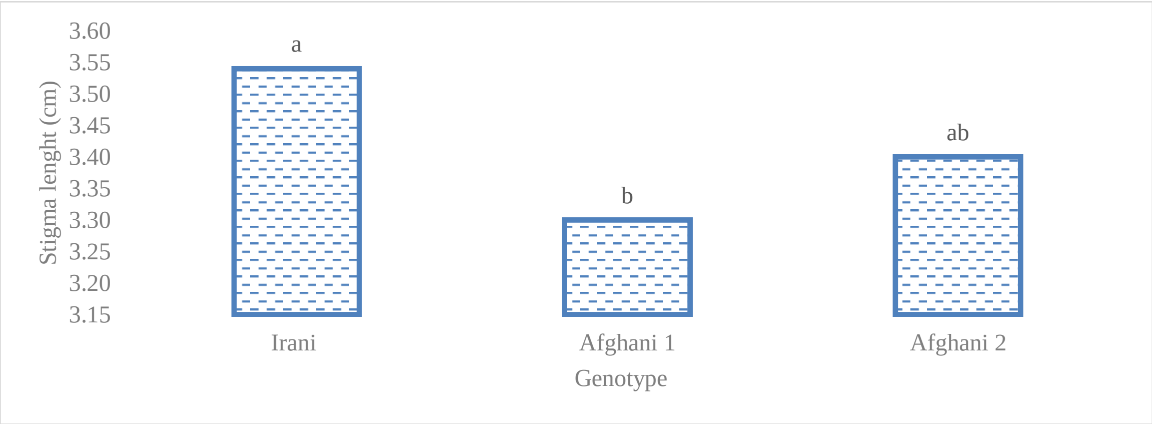

Figure 7: Simple effects of glutamic acid and tryptophan genotype on saffron genotypes

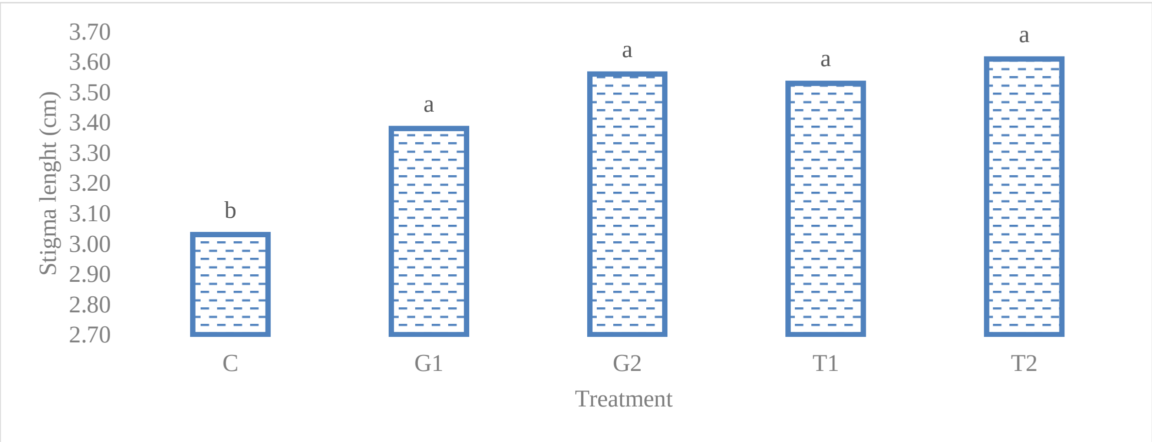

Figure 8: Simple effects of glutamic acid and tryptophan treatments on length flower saffron

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Stigma wet weight:

The results of analysis of variance (Table 2) of the observations indicated that there are no significant differences among the genotypes. The percentage of phenotypic and genetic variation coefficient is medium, which indicates the absence of high variation in terms of stigma weight trait. The results of the average comparison (Figure 9) between the genotypes showed that the Afghani genotype 1 had the highest stigma weight (20.815 mg). In this respect, they have a significant difference only with the Afghani 2 genotype and the Iranian genotype. The results of the average comparison (Figure 9) of the treatments, despite the fact that the treatment of glutamic acid and tryptophan significantly had a positive effect on the fresh weight of the stigma. But while the control showed the highest weight in the growth level compared to the treatments and there was a significant difference in this respect. The comparison of this trait with flower dry weight and stigma dry weight were positive and significant, but it did not show a significant relationship with yield in the unit area.

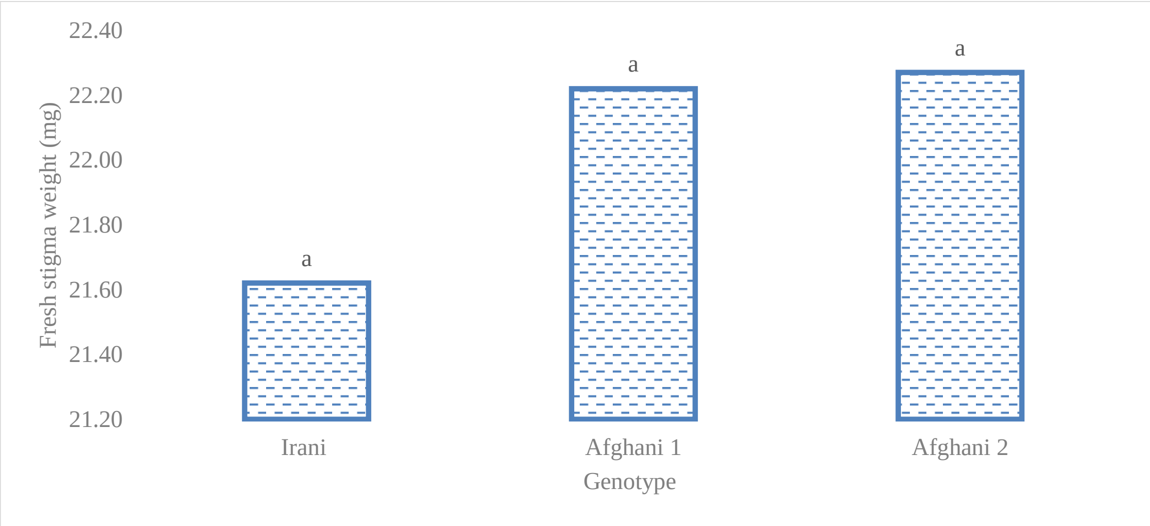

Figure 9: Simple effects of glutamic acid and tryptophan genotype on saffron genotypes

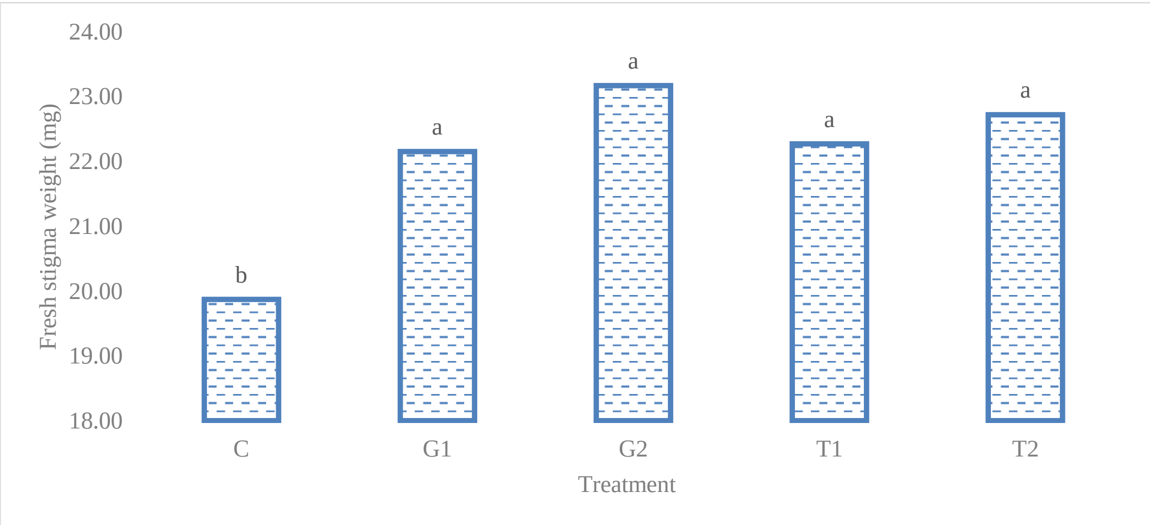

Figure 10: Simple effects of glutamic acid and tryptophan treatments on fresh stigma flower saffron

Stigma dry weight:

The results of analysis of variance (Table 2) revealed the observations. The difference between genotypes was significant at the 5% probability level. Figure (11) is the highest dry weight of the stigma of Afghani 1 genotype. In this respect, there was a significant difference with the Afghani 2 genotype and the Iranian genotype. The average comparison results (Figure 11) showed that the effects of glutamic acid and tryptophan treatment on saffron genotypes were significant at the 5% probability level. Also, the highest yield of stigma dry weight was obtained in the simple effects treatment of 2 mM tryptophan level and the lowest in the 1 mM tryptophan treatment level. Small logs with lower density had lower dry weight.

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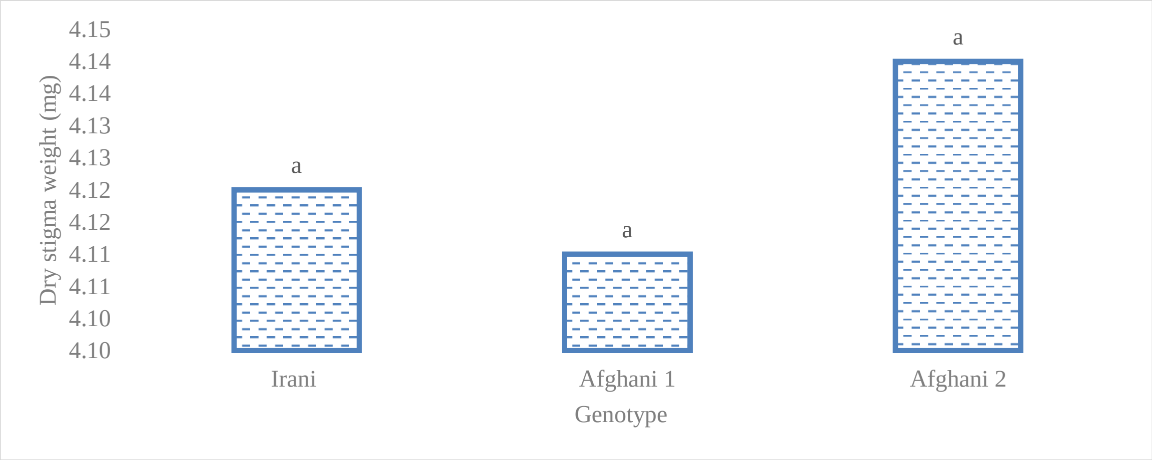

Figure 11: Simple effects of glutamic acid and tryptophan genotype on saffron genotypes

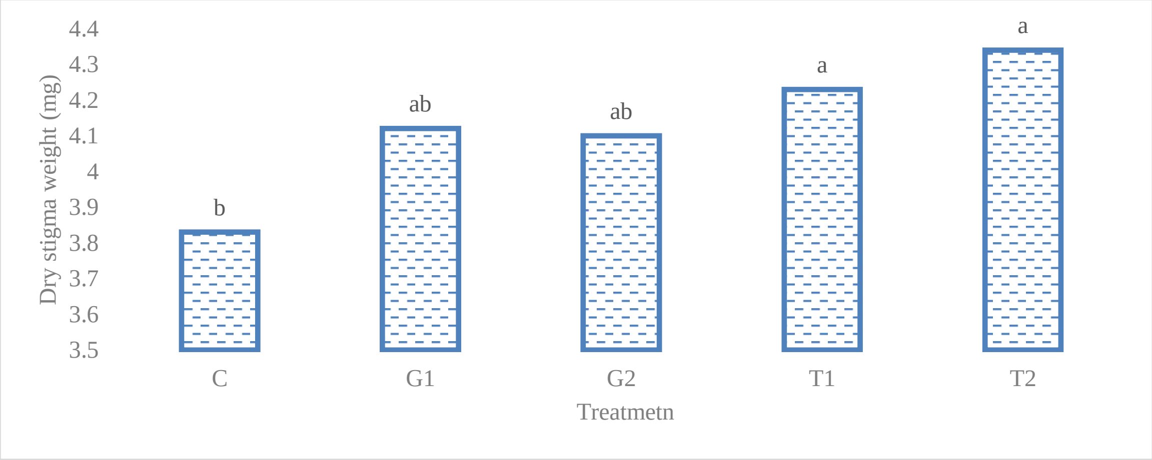

Figure 12: Simple effects of glutamic acid and tryptophan treatments on dry stigma flower saffron

Flower length:

Treatment of glutamic acid and tryptophan significantly affected the flower length in saffron and caused an increase in flower length compared to the control. Thus, the maximum leaf length (5.21) cm was observed in the treatment of the effects of 2 mM glutamic acid. Surface treatments of 1 mM glutamic acid with a length of (5.16) cm and 2 mM tryptophan treatment with a flower length of (5.07) cm was placed in the next categories; the lowest leaf length was (5.05) cm in the treatment of 1 mM tryptophan. became. In this sense, amino acids are biological stimulants that increase the absorption of nutrients and improve plant growth and elongation (Abo sedera et al., 2010).

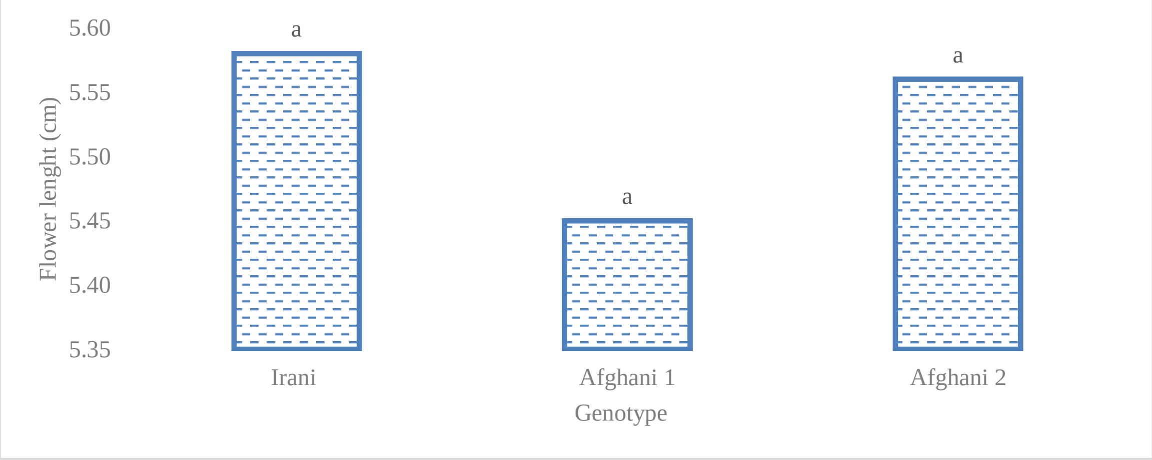

Figure 13: Simple effects of glutamic acid and tryptophan genotype on flower length saffron

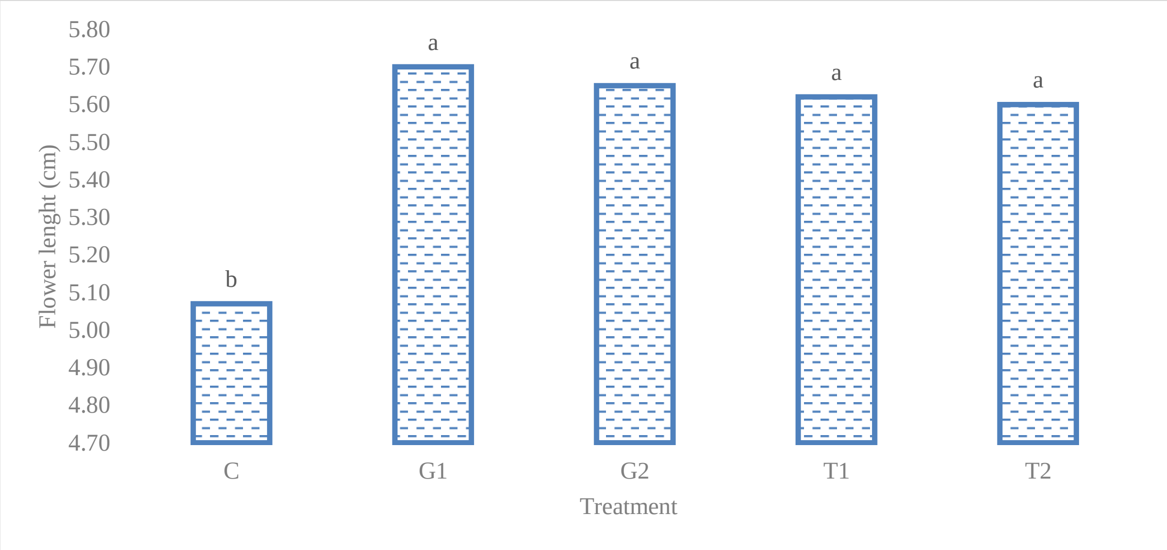

Figure 14: Simple effects of glutamic acid and tryptophan treatments on length flower saffron

Morphological traits:

Amino acids are substances that stimulate metabolism and metabolic processes to increase plant efficiency (Dewic, 2009). Based on the results of analysis of variance (Table 2) and the results of the mean comparison (Figure 1), amino acid 1 mM glutamic acid and tryptophan increased the vegetative growth characteristics and increased the number of saffron plants. Amino acid has increased the number of leaves in plants by accelerating the absorption of water and nutrients and increasing vegetative growth. This result was similar to the results of amino acid effect on mint plants (Dinoo et al., 2009). Therefore, in the first year, the number of flowers in a saffron plant depends on different factors such as weather conditions, farm management, and stresses in the origin areas of the genotypes. The number of flowers was considered the most effective trait in determining yield (Armenia et al., 2014). Reports showed that the use of amino acid foliar spray in different concentrations improved the vegetative growth of *Gladiolus grandiflora*, and increased the quality of flowering Ahmad et al., 2008).

Based on the research done, analysis of variance (Table 2) and the results of simple effects comparing the averages of 2 mM tryptophan amino acid and glutamic acid increased fresh and dry weight of flowers in plants. Because amino acids are biological stimulators that increase the absorption of nutrients, especially nitrogen, increase photosynthesis, and this improves the growth and weight of the plant (Abo Sedera et al., 2010). Among them, the amino acid tryptophan is a precursor for the production of the auxin hormone. which causes an increase in cell elongation and, as a result, an increase in the height and weight of the seedling (Tarek and Hassan, 2014). The results of this research are the same as the findings of researchers such as (Saburi et al., 2014) in evergreen plants, (Golzadeh et al., 2012) in chamomile plants and (Nahed et al., 2010) in sweet plants, increasing the height, volume and Elongation of the root of the plant. In another study, the use of tryptophan amino acid increased the wet and dry weight of nettle (*Utrica pilulifera*) (Wahba et al., 2015). Also, the average comparison (Figure 9) of the simple effects of 2 mM glutamic acid treatment has increased the stigma fresh weight of saffron plant. Therefore, the results of the average comparison (Figure 11) of the simple effects of 2 mM tryptophan amino acid treatment caused an increase in the dry weight of saffron plant stigma. Therefore, amino acid foliar application provides the basis for the absorption of nutrients on plants, which, as a result, increases the weight and volume of the plant (Porcel and Ruiz Lozano, 2004). The use of amino acid not only increases growth, but also increases the quality and quantity of the product (Belal et al., 2016). Amino acid tryptophan and glycine in different concentrations cause cell division and growth, so the height of *Gladiolus grandifloras* can be increased (Hana, 2000). Based on the average comparison results (Figure 7), the treatment of 1 mM glutamic acid increased the stigma length in saffron plants. In thyme plant *(Thymus vulgaris* L), the treatment of amino acid phenylalanine and tryptophan in different concentrations has caused a significant increase in plant height compared to control plants (Ghazal, 2015). In another study, the use of amino acid tryptophan increased fresh and dry weight in a type of nettle (*Urtica pilulifera* L) (Wahba et al., 2015). In a research, Filner (1966) found that some amino acids reduce the growth of tobacco plants. Gamborg (1970) also showed that amino acid glutamine increases dry matter in soybeans. It has been reported in many studies that foliar spraying with amino acid increases the growth and development of plants (Faten et al., 2010). In general, the use of amino acid tryptophan and glutamic acid has increased photosynthetic organic compounds and increased their transfer from leaves to fruits, thereby increasing crop production.

IV. CONCLUSION

The results obtained from this experiment showed that the use of amino acid glutamic acid and tryptophan had a positive and significant effect on the growth, morphological characteristics of saffron plant. The effects of two amino acid levels (glutamic acid and tryptophan) were investigated. They left different effects on the pattern of growth and development of morphological traits. Amino acid concentration of 1 mM glutamic acid and 2 mM tryptophan, in addition to improving growth and development indicators, also increased flower and stigma performance. Amino acid foliar spraying (glutamic acid 1 mM and tryptophan 2 mM) led to increased growth and production of vegetative bodies compared to the concentration of 1 mM tryptophan and 2 mM glutamic acid. Also, according to the results of amino acid, the concentration of 1 mM glutamic acid increased the metabolites although the result of this test, amino acid with a concentration of 2 mM tryptophan and 1 mM glutamic acid had a greater effect on the improvement of morphological indicators. Therefore, amino acid with a concentration of 1 mM glutamic acid and 2 mM tryptophan showed the best effect on the yield of flowers, and a concentration of 1 mM glutamic acid achieved the highest vegetative body yield. In general, it can be said that amino acid is directly and indirectly effective on the morphological activities on the growth and development of plants. Considering the cultivation, both the production and the result of effective substances from the morphological and traits, the application of amino acid in the form of foliar spraying can be effective and recommendable.

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