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EFFECT OF CULTIVAR AND SPRAYING WITH THE AMINO ACID PHENYLALANINE ON THE YIELD OF BLACK RICE GRAINS AND THEIR CONTENT OF SOME ANTHOCYANIN PIGMENTS

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Abstract

The two field experiments were conducted during the 2019 and 2020 agricultural seasons at the Najaf Research Department/Rice Research Station in Al-Mashkhab. In the first season, they were planted with Goura, Local and Chakhao rice cultivar (one plate for each cultivar) and by 100 plants per cultivar/ plot .The selection intensity of 10% was used to select plants with the highest total anthocyanin pigment content In order to study the effect of spraying the amino acid phenylalanine in improving the grain yield of black rice and its content of some anthocyanin pigments, it was planted in the second season with the origin cultivar under the influence of four levels of spraying the amino acid phenylalanine. The selected Goura genotype was significantly excelled by giving the highest averages in plant height, weight of 1000 grains and total grain yield, which were 118.47 cm, 23.44 g and 6.70 tons.ha^{-1}, respectively. Whereas, the local selected genotype was significantly excelled by giving the highest averages in the leaves content of total chlorophyll pigments SPAD, total anthocyanins, Cyanidin 3-O-glucoside, Peonidin 3-O-glucoside, Cyanidin, Delphinidin, Peonidin and Malvidin, which amounted to 39.13 SPAD and 17.79% and 61.67%, 57.81, 29.92, 7.89, 5.82, 4.34, 1.40, 1.36, 61.17 mg.kg^{-1} dry weight, respectively. The spraying of the amino acid phenylalanine at a concentration of 50 mg.l-1 was significantly excelled by giving the highest averages in the indicators of vegetative growth and yield, its components and active compounds.

Key words: amino acid, phenylalanine, anthocyanin pigments, black rice.

Introduction

The rice plant, whose scientific name is *Oryza sativa L*. belongs to the Poaceae family, is one of the important grain crops in Iraq for its high nutritional value as a source of energy, protein, and carbohydrates. It comes in second place after the wheat crop in its economic importance and its role in food security in Iraq and the world. Half of the world's population depends on rice as basic food, and it is the main source of energy for them, and it enters the food of another 25% of the world's population as well, and it is the most important grain crop that is directly eaten. In view of the increase in the incidence of chronic diseases and addiction, the global health community awareness has increased, researchers have directed their attention to searching for ways to increase the concentrations of antioxidant compounds in the grains and fruits of dual-purpose plants in food and medical use, especially those plants that are included in the daily food of most countries of the world. This list is of rice cultivar (Sompong et al., 2011), Especially black rice, which contains

good concentrations of anthocyanin pigment group, which has shown an important role in increasing the immune system effects of patients with amnesia, diabetes, heart disease, and cancer (Park et al., 2008), the accumulation of anthocyanins in black rice cultivars (0.262-2.539 mg. g^{-1} dry weight). The interest in this cultivar of rice has increased in the past few years because it is rich in protein and nutrients and the presence of the antioxidant anthocyanin pigments and the percentage of anthocyanins of the type Cyaniding-3-O.glucoside and Peonidin-3-O.gluciside in the grain of rice cultivars It constitutes 90% of the total amount of pigment in grain (Chang et al., 2010). While (Kang et al., 2011) and (Frank et al., 2012) showed that there is a wide variation in the physical and chemical traits of black rice cultivars.(Chen et al., 2012) indicated in their study on four groups of rice, which are the white rice group, which includes the Nakateshinsenbon cultivar, and the red rice group, which includes the cultivars Benisavasa, Tcukushiakamochi, Beniroman, and Tohposhi. The black rice group includes Okunomurasaki, Chinakuromai and Asamyrasaki cultivars, and the green rice group includes Akunemochi. There are significant differences between the cultivated cultivars in the grain content of total anthocyanin pigment. Asamurasakilk from the black rice group gave the highest average of 473.7 mg.100 g^{-1} dry weight by measurement. With the white rice cultivar, Nakateshinsenbon gave the lowest mean of 79.5 mg.100 g^{-1} dry weight.It was reported (Vichit and Saewan, 2015) in their study on the cultivation of two types of black and red rice, where the genotype Niaw dum 2 gave the highest average of total anthocyanin pigment content of 4.64 $mg.L^{-1}$ compared to the genotype Mun pu 3 which gave the lowest average of 0.03 $mg.L^{-1}$.

Materials and methods

The experimental land was Tillage by two perpendicular Tillage with a Moldboard plows, then the smoothing and leveling process was conducted. Three samples were taken from each depth of the field soil 0-30 cm.They were air-dried, then crushed, mixed well, and sieved for the purpose of preparing them for analysis in the laboratories of the Soil and Water Resources Department at the College of Agriculture, Al-Qasim Green University to determine some of the physical and chemical properties as in Table (1)They were air-dried, then crushed, mixed well, and sieved for the purpose of preparing them for analysis in the laboratories of the Soil and Water Resources Department at the College of Agriculture, Al-Qasim Green University to determine some of the physical and chemical properties as in Table (1).

Table (1) Some physical and chemical properties of field soil

Traits		values	units
Soil texture components	sand	222	$g.Kg^{-1}$
	silt	474	$g.Kg^{-1}$
	Clay	304	$g.Kg^{-1}$
silty clay loam			
bulk density		1.29	Mg.m-3
Organic matter		1.60	$g.Kg^{-1}$
availability nitrogen		0.44	%
availability phosphorous		0.61	%

availability potassium	**0.52**	**%**
electrical conduction EC	**3.21**	**DS.m^{-1}**
pH	**7.78**	**-**

Experiment plants were fertilized for all treatments according to the recommendation of (Hassan, 2001) by adding 140 kg N.ha^{-1} nitrogen fertilizer, 46 kg P_2O_5.ha^{-1} phosphate fertilizer and 50 kg K_2O ha^{-1} potassium fertilizer. Phosphate fertilizer (Super Triple Calcium Phosphate) was added. Ca3Po4, 46% P_2O_5) by mixing it with the soil before planting ,Nitrogen fertilizer (urea 46%) was added in two batches, the first 10 days after planting and the second a month after the first batch .As for the potassium fertilizer (potassium sulfate, 50% K2O) added after 10 days of planting, The weeds growing in the experimental plots was manually removed three times. The first weeding was conducted 10 days after planting, the second 15 days after the first weeding, and the third 15 days after the second weeding and cut off irrigation 15 days before harvest. In order to measure the grain content of total anthocyanin pigments and their compounds, the selected cultivars were grown with the original cultivar under the influence of four levels of phenylalanine spraying. The amino acid treatments occupied the main plates 50, 100 and 150 mg.L^{-1} as well as the control treatment (spraying distilled water only). The first spraying was done three times, the first in the Tilliring stage after 50 days of planting and the second in the Heading stage after 75 days from planting, and the third in the stage of ripening after 100 days of planting, The sub plot occupied the selected genotypes with the original so that the number of cultivated genotypes was 6 genotypes, thus bringing the number of experimental units to 72 experiment units. The plants of the experimental unit were distributed on the dimensions of 2 x 3 m2 where they contained eight lines, as well as two guard lines for each experimental unit, the length of each of them was 2 m, and the distance between one line and another was 30 cm, and between one plant and another 20 cm (Ahmed et al., 2017).The cultivar Goura was harvested on November 20 for the two experiment seasons in 2019 and 2020, and the other two cultivars were harvested on December 1 and 10 for the experiment season 2019 and 2020 respectively. The water was dried from the plots when the plants reached the stage of physiological maturity by turning the dahlias to a yellow color tinged with black, and the leaves and stems withered, and the moisture content of the grains began to decrease .A selection intensity of 10% was used to select plants with the largest content of total anthocyanin, which was measured according to the (Giusti and Wrolstad, 2001) method. After data collection, the ready-made statistical program GenStat V.20 was used according to the method approved by (Al-Asadi, 2019).

Studied traits

1- plant height (cm)

2- Leaves content of total chlorophyll pigment (SPAD)

The measurement was made in the unit SPAD based on the method used by (Jemison and Williams, 2006).

3- The weight of 1000 grains (g)

4- Total grain yield (tons.ha^{-1})

5- Determination of total anthocyanins (mg.kg^{-1} dry weight)

The method approved by (Kim et al., 2007) was followed by taking 5 g of grains for each treatment and after drying them in an electric oven at a temperature of 60 °C for 48 hours, then milled the grain samples and taking the permeable rice bran through a 60-mash filter. Samples were extracted by adding 30 cm3 of methanol acidified with 0.1 N hydrochloric acid (85 methanol: 15 v/v hydrochloric acid), then the extract was filtered using filter paper with a permeability of 0.45 µm, and a rotary evaporator was used at a temperature of 60 °C to reduce the volume of the extract. to 5 cm3 solvent. Then the extracts were placed in labeled test tubes and kept in the refrigerator at a temperature of 4°C until used in the estimation of some groups of active compounds. The optical absorption method was adopted by changing the acidity of the plant sample extract (Giusti and Wrolstad, 2001).

6- Determination of some anthocyanins compounds (mg.kg^{-1} dry weight)

Some of the anthocyanin compounds that included Cyanidin 3-O-glucoside, Peonidin 3-O-glucoside, Delphinidin, Cyanidin, Peonidin and Malvidin in the three cultivars were estimated using a high-performance liquid chromatography-liquid separator (HPLC) according to the method approved by (Kim et al., 2007).The anthocyanins were determined at the UV detector wavelength of 520 nm using a detection column identified with number C18 (50 mm × 2 mm I.D.; 3 µm). The mobile phase is composed of two solvents, first A (0.1% Trifluroacetic acid (TFA)), and second solvent B (4% acetonitrile). The separation conditions were as follows: 12 min 0-100% solvent B,Standard compounds of anthocyanin pigment were used and each of them was dissolved using methanol acidified with 0.1 N hydrochloric acid (85 methanol: 15 v/v HCl) to make a concentration of 1 mg. -

Compound concentration in the sample (mg.kg^{-1} dry weight) = sample package area / standard package area x standard sample concentration x number of dilutions

Results and discussion

Table (2, 3, 4 and 5) shows a significant effect of the study factors and the interaction between them, where the selected Goura cultivar was significantly excelled and gave the highest average plant height, the weight of 1000 grains and the total grain yield was 118.47 cm, 23.44 g and 6.70 tons. ha^{-1} compared to the lowest average was 104.38 cm, 17.59 g and 3.69 ton.ha^{-1} for Chakhao-origin, Goura-origin, and Local-origin, respectively.on the other hand, the cultivar Goura gave the lowest average leaf content of the total chlorophyll pigments, which amounted to 33.43 SPAD, in comparison with the selected Local cultivar, which gave the highest average of 39.13 SPAD. The reason for the different cultivars may be due to the different genotypes in the competition for solar radiation, where they are newly introduced genotypes grown for the first time in this environment (Al-Issawi, 2004, Al Silawi, 2011 and Al-Fatlawi, 2020).The reason for the difference in varieties may be due to the difference in genotypes in response to the amount and intensity of rays falling on the surfaces of photosynthesis (Al-Aboudi et al., 2016 and Al-Amri, 2014).The spraying of phenylalanine concentrations also had a significant effect on these traits, the concentration 50 mg.L^{-1} giving the best averages for the above traits amounted to 114.11 cm and 40.76 SPAD and 21.79 g and 6.13 tons.ha-1, compared with the spraying concentration of 0.0 mg.L^{-1}, which gave

the lowest average of 105.31 cm, 34.10 SPAD, 20.58 g, and 4.11 tons.ha^{-1}, respectively ,The spraying concentration of phenylalanine acid 50 mg.L^{-1} excelled in all indicators of vegetative growth may be due to the fact that the acid promotes plant growth during the vegetative and flowering stages and as a result of the application of the acid associated with its role as the building blocks of proteins and stimulating a number of additional vital functions in regulating the construction of food compounds and the transport of nutrients and storage of nitrogen.Moreover, it can serve as a source of carbon, energy, and synthesis of other organic compounds such as proteins, amines, purines, alkaloids, vitamins, enzymes, terpenoids, etc. (Reham et al., 2016 and AL-Mohammad and AL-Teay, 2019).

Table (2): Effect of cultivar and spraying with the amino acid phenylalanine and the interaction between them on plant height (cm)

Cultivars		Phenylalanine spraying concentrations (mg.L^{-1})				average cultivar effect
		00	**50**	**100**	**150**	
Guora	Origin	103.80	117.60	112.80	110.07	111.07
	selected	109.80	119.47	118.87	125.73	118.47
Local	Origin	102.13	110.13	107.07	105.20	106.13
	selected	109.13	117.53	108.13	110.67	111.37
Chakhao	Origin	99.20	109.33	102.53	106.47	104.38
	selected	107.80	110.60	106.93	112.87	109.55
Average effect of Phenylalanine		105.31	114.11	109.39	111.84	
L.S.D 0.05	Cultivars		Phenylalanine		Interaction	
	2.06		3.50		4.12	

Table (3): Effect of cultivar and spraying with the amino acid phenylalanine and the interaction between them on the total chlorophyll pigment content (SPAD) in leaves

Cultivars		**Phenylalanine spraying concentrations (mg.L^{-1})**				**average cultivar effect**
		00	**50**	**100**	**150**	
Guora	**Origin**	**28.33**	**38.39**	**34.27**	**32.20**	**33.43**
	selected	**33.73**	**41.67**	**37.07**	**32.60**	**36.27**
Local	**Origin**	**32.53**	**41.60**	**40.73**	**35.80**	**37.67**
	selected	**34.80**	**42.13**	**41.80**	**37.80**	**39.13**
Chakhao	**Origin**	**36.07**	**40.00**	**34.87**	**35.80**	**36.69**
	selected	**39.13**	**40.20**	**38.27**	**36.27**	**38.47**

Average effect of Phenylalanine		**34.10**	**40.76**	**37.84**	**35.08**	
L.S.D 0.05	**Cultivars**		**Phenylalanine**		**Interaction**	
	0.83		**0.52**		**1.67**	

Table (4): Effect of the cultivar and spraying with the amino acid Phenylalanine and the interaction between them on the weight of 1000 grains (g)

Cultivars		**Phenylalanine spraying concentrations ($mg.L^{-1}$)**				**average cultivar effect**
		00	**50**	**100**	**150**	
Guora	**Origin**	**22.95**	**20.82**	**23.55**	**21.58**	**22.22**
	selected	**23.12**	**23.57**	**23.92**	**23.15**	**23.44**
Local	**Origin**	**17.08**	**16.71**	**18.71**	**17.88**	**17.59**
	selected	**18.56**	**22.43**	**16.20**	**17.12**	**18.58**
Chakhao	**Origin**	**20.00**	**22.55**	**20.78**	**22.85**	**21.54**
	selected	**21.81**	**24.65**	**21.54**	**20.99**	**22.25**
Average effect of Phenylalanine		**20.58**	**21.79**	**20.78**	**20.59**	
L.S.D 0.05	**Cultivars**		**Phenylalanine**		**Interaction**	
	0.57		**0.71**		**1.14**	

Table (5): Effect of cultivar and spraying with the amino acid Phenylalanine and the interaction between them on the total grain yield ($ton.ha^{-1}$)

Cultivars		**Phenylalanine spraying concentrations ($mg.L^{-1}$)**				**average cultivar effect**
		00	**50**	**100**	**150**	
Guora	**Origin**	**3.00**	**6.06**	**6.00**	**5.01**	**5.02**
	selected	**4.39**	**8.99**	**6.56**	**6.85**	**6.70**
Local	**Origin**	**3.25**	**4.78**	**3.65**	**3.07**	**3.69**
	selected	**4.47**	**5.64**	**4.93**	**3.50**	**4.63**
Chakhao	**Origin**	**4.61**	**5.13**	**4.50**	**4.31**	**4.64**
	selected	**4.96**	**6.21**	**5.36**	**4.73**	**5.32**
Average effect of Phenylalanine		**4.11**	**6.13**	**5.17**	**4.58**	
L.S.D 0.05	**Cultivars**		**Phenylalanine**		**Interaction**	
	0.25		**0.33**		**0.51**	

The tables show that there are significant differences between the interaction between the cultivar and the spraying of the amino acid Phenylalanine, where the interaction treatment between the selected Guora cultivar and the spraying concentration 150 mg.L^{-1}, which gave the highest average for the above traits reached 125.73 cm, and the local selected with the spraying concentration 50 mg.L^{-1} It was 42.13 SPAD and Chakhao selected with spraying concentration of 50 mg.L^{-1} was 24.65 g, Guora selected with a spraying concentration of 50 mg.L^{-1} of 8.99 tons.ha^{-1} in comparison with the lowest average for the mentioned traits that reached 99.20 cm for the interaction treatment of the Chakhao origin cultivar with a spraying concentration of 0.0 mg.L^{-1} and 28.33 SPAD for the interaction treatment of the cultivar Guora origin with a spraying concentration of 0.0 mg.L^{-1} and 16.20 g for the interaction treatment of the selected Local cultivar with the spraying concentration of 100 mg.L^{-1} and 3 .00 ton.ha^{-1} for interaction treatment of selected Guora cultivar with a concentration of 0.0 mg.L^{-1} respectively.

Table (6, 7 and 8) showed a significant effect of the study factors and the interaction between them on the grain content of total anthocyanin, Cyanidin 3-O-glucoside and Peonidin 3-O-glucoside, The selected Local cultivar significantly excelled and gave the highest average for the above traits which amounted to 57.81, 29.92 and 7.89 mg.kg^{-1} dry weight respectively, compared to the lowest average for the mentioned traits which amounted to 43.85, 23.80 and 5.62 mg.kg^{-1} dry weight for the cultivar Guora respectively, The reason for this is due to the varying response of genotypes to environmental conditions and their ability to produce some effective compounds of biological and medical benefit, especially antioxidant compounds, including plant pigments. The pigmented and colored cultivars sometimes contain more than the contents of the active compounds of the non-pigmented or colored rice, where the genetic factor has an important role in the variation of the cultivars in the production of those compounds that fall under their classification with the phenolic compounds. The selected local genotype excelled in the grain content of total anthocyanins pigments may be due to its genetic ability to excelled in anthocyanin compounds, especially Cyanidin 3-O-glucoside and Peonidin 3-O-glucoside, and its adaptation to the environmental conditions in which it was cultivated, as it was introduced to Iraq in 2001.Phenylalanine concentrations of the amino acid spraying also had a significant effect on this trait, where the concentration of 50 mg.L^{-1} gave the best averages for the studied traits, which were 57.14, 29.20, and 7.99 mg.kg^{-1} dry weight compared to the concentration of 0.0 mg.L^{-1}, which It gave the lowest average of 46.37, 25.57 and 5.28 mg.kg^{-1} dry weight, respectively, The effect of foliar spraying of phenylalanine acid on the accumulation of anthocyanin pigments, in general, may be limited by conditions, including acid concentration, plant type, plant age, and nitrogen abundance. The increase in the concentrations of some anthocyanin compounds logically reflects on the increase in the accumulation of total anthocyanins, like phenylalanine, in addition to its role in protein synthesis, is a primary initiator for natural products of anthocyanins and other plant pigments, alkaloids, and hormones,Phenylalanine is a product of the Shikimic acid pathway. It is one of the essential amino acids in the synthesis of phenolic compounds and their derivatives, including the pigments of total anthocyanins and their compounds.Accordingly, providing the plant with what it needs of this acid leads to an increase in anthocyanin compounds as they are derived from

flavonoid compounds and spraying Phenylalanine on black rice increases its concentration inside the plant and thus accelerates the appearance of the anthocyanin pigment and increases its concentration (Portu et al., 2017, AL-Duraid et al., 2019).The tables show that there are significant differences in the interaction between the cultivar and the phenylalanine spraying, The interaction treatment between selected Chakhao cultivar and spraying concentration 50 mg.L^{-1} was excelled, which gave the highest average of 72.07 mg.kg^{-1} dry weight, and the interaction treatment between selected Local cultivar and spraying concentration 50 mg.L^{-1} which gave an average of 33.83 mg. Kg^{-1} dry weight and the interaction treatment between selected Chakhao cultivar and spraying concentration 50 mg.L^{-1}, which gave an average of 11.55 mg.kg^{-1} dry weight, compared with the lowest average of 39.05 mg.kg-1 dry weight for the interaction treatment of Guora origin with spraying concentration of 50 mg. L^{-1} and 19.52 mg.kg^{-1} dry weight for the interference treatment of Guora origin cultivar with spraying concentration of 100 mg.L^{-1} and 2.62 mg.kg^{-1} dry weight for the interaction treatment of Chakhao origin with spraying concentration of 0.0 mg.L^{-1} for the studied traits respectively.

Table (6): Effect of cultivar and spraying with the amino acid phenylalanine and the interaction between them on the grain content of total anthocyanins pigments (mg.kg^{-1} dry weight)

Cultivars		Phenylalanine spraying concentrations (mg.L^{-1})				average cultivar effect
		00	50	100	150	
Guora	Origin	41.66	39.05	50.41	44.26	43.85
	selected	49.76	52.55	54.40	50.58	51.82
Local	Origin	44.21	51.65	56.33	43.17	48.84
	selected	58.47	59.38	57.11	56.29	57.81
Chakhao	Origin	41.36	68.12	48.26	48.96	51.68
	selected	42.73	72.07	49.08	51.55	53.86
Average effect of Phenylalanine		46.37	57.14	52.60	49.14	
L.S.D 0.05	Cultivars		Phenylalanine		Interaction	
	2.049		2.295		4.099	

Table (7): Effect of cultivar and spraying with the amino acid Phenylalanine and the interaction between them on Cyanidin 3-O-glucoside (mg.kg^{-1} dry weight)

Cultivars		Phenylalanine spraying concentrations (mg.L^{-1})				average cultivar effect
		00	50	100	150	
Guora	Origin	22.97	25.37	19.52	27.35	23.80

	selected	22.65	33.01	20.68	27.03	27.09
Local	Origin	20.27	30.20	24.73	28.16	25.84
	selected	28.48	33.83	28.64	28.72	29.92
Chakhao	Origin	26.95	25.38	31.26	24.09	26.92
	selected	28.19	27.39	32.18	26.85	28.65
Average effect of Phenylalanine		25.75	29.20	26.17	27.03	
L.S.D 0.05	Cultivars		Phenylalanine		Interaction	
	1.042		0.514		2.083	

Table (8): Effect of cultivar and spraying with the amino acid Phenylalanine and the interaction between them on Peonidin 3-O-glucoside (mg.kg^{-1} dry weight)

Cultivars		Phenylalanine spraying concentrations (mg.L^{-1})				average cultivar effect
		00	50	100	150	
Guora	Origin	4.61	4.64	8.16	5.08	5.62
	selected	6.49	6.61	11.20	6.32	7.65
Local	Origin	6.13	6.53	8.71	4.00	6.34
	selected	8.02	7.62	10.38	5.55	7.89
Chakhao	Origin	2.62	10.99	2.84	6.32	5.69
	selected	3.80	11.55	3.44	7.01	6.45
Average effect of Phenylalanine		5.28	7.99	7.45	5.72	
L.S.D 0.05	Cultivars		Phenylalanine		Interaction	
	0.511		0.749		1.022	

Table (9, 10, 11 and 12) shows a significant effect of the study factors and the interaction between them in Cyanidin, Peonidin, Delphinidin, and Malvidin, The selected Local cultivar significantly excelled and gave the highest average for the above traits which amounted to 5.82, 4.34, 1.40, and 1.36 mg.kg^{-1} dry weight respectively, compared with the lowest mean for the mentioned traits which were 4.28, 2.61, 0.75 and 0.81 mg.g $^{-1}$ kg dry weight of Guora origin cultivar respectively, The reason for this is due to the different responses of the genotypes used to the local environmental conditions because these genotypes were recently introduced to Iraq, namely Guora from the United Kingdom and Chackhao from India, compared to the excelled local genotype as a result of its adaptation to local environmental conditions (Ahmed et al., 2017).As a result of the increase in the chlorophyll pigment in the leaves, the pace of biosynthesis of primary metabolic compounds increased, including total soluble carbohydrates, and thus the content of secondary metabolites increased as they are intermediate compounds, especially those with antioxidant activity (Al-Asadi, 2018).on the other hand, the increase of secondary metabolisms such as

terpenoids, phenols, and alkaloids is derived from the metabolism of carbohydrates, lipids, and amino acids, and phenylalanine and its other form of the amino acid Tyrosine contribute to the metabolism of tryptophan, which has an indirect role in growth through auxin metabolism by alternative pathways to manufacture IAA in plants, starting with tryptophan as a starting material, and therefore when it is available, the concentration of IAA auxin increases in plant tissues (Al-Asadi and Al-Khikani, 2019).The same table shows that there are significant differences in the interaction between the cultivar and phenylalanine spraying, The interaction treatment between the local cultivar and the spraying concentration of 50 mg.L^{-1} was excelled, which gave the highest average for the above traits which was 10.08 mg.kg^{-1} dry weight and Chakhao selected with the spraying concentration of 50 mg.L^{-1} was 6.33 mg.kg^{-1} dry weight and Chakhao selected with spraying concentration 50 mg.L^{-1} was 1.97 mg.kg^{-1} dry weight and local selected with spraying concentration 50 mg.L^{-1} was 1.92 mg.kg^{-1} dry weight compared with the lowest average for the mentioned traits was 2.95 mg.kg-1 dry weight for the interaction treatment of the cultivar Guora selected with spraying concentration 0.0 mg.L^{-1} and 1.71 mg.kg^{-1} dry weight for the interaction treatment of the cultivar, Guora with spraying concentration of 0.0 mg.l-1 and 0.58 mg.kg^{-1} dry weight for the intervention treatment of Guora origin with the spraying concentration of 50 mg.L^{-1} and 0.67 mg.kg^{-1} dry weight for the interaction treatment The cultivar Guora origin with the spraying concentration of 0.0 mg.l-1 for the studied traits respectively.

Table (9): Effect of cultivar and spraying with the amino acid Phenylalanine and the interaction between them on Delphinidin (mg.kg^{-1} dry weight)

Cultivars		Phenylalanine spraying concentrations (mg.L^{-1})				average cultivar effect
		00	50	100	150	
Guora	Origin	4.21	3.46	3.89	5.56	4.28
	selected	2.95	4.79	4.17	6.85	4.69
Local	Origin	3.93	4.43	4.85	4.15	4.34
	selected	3.68	10.08	4.68	4.85	5.82
Chakhao	Origin	2.97	9.31	4.42	4.13	5.21
	selected	4.22	5.64	4.35	7.66	5.47
Average effect of Phenylalanine		3.66	6.29	4.39	5.53	
L.S.D 0.05	Cultivars		Phenylalanine		Interaction	
	0.409		0.533		0.817	

Table (10): Effect of cultivar and spraying with phenylalanine acid and the interaction between them on Cyanidin (mg.kg^{-1} dry weight)

Cultivars	Phenylalanine spraying concentrations (mg.L^{-1})	average cultivar effect

		00	50	100	150	
Guora	Origin	2.93	1.71	3.36	2.46	2.61
	selected	2.57	3.23	4.44	4.26	3.63
Local	Origin	2.56	4.42	3.38	3.05	3.35
	selected	3.83	4.65	3.62	5.26	4.34
Chakhao	Origin	2.35	5.49	3.21	2.06	3.28
	selected	2.49	6.33	3.75	2.95	3.88
Average effect of Phenylalanine		2.79	4.31	3.63	3.34	
L.S.D 0.05	Cultivars		Phenylalanine		Interaction	
	0.281		0.429		0.563	

Table (11): Effect of cultivar and spraying with the amino acid Phenylalanine and the interaction between them on Peonidin (mg.kg^{-1} dry weight)

Cultivars		Phenylalanine spraying concentrations (mg.L^{-1})				average cultivar effect
		00	50	100	150	
Guora	Origin	0.85	0.58	0.72	0.83	0.75
	selected	0.88	0.78	0.86	1.09	0.90
Local	Origin	0.71	0.98	1.25	1.13	1.02
	selected	0.92	1.63	1.73	1.32	1.40
Chakhao	Origin	0.72	1.71	1.16	1.15	1.18
	selected	0.87	1.97	1.43	1.26	1.38
Average effect of Phenylalanine		0.83	1.28	1.19	1.13	
L.S.D 0.05	Cultivars		Phenylalanine		Interaction	
	0.060		0.059		0.121	

Table (12): Effect of cultivar and spraying with the amino acid Phenylalanine and the interaction between them on Malvidin (mg.kg^{-1} dry weight)

Cultivars		Phenylalanine spraying concentrations (mg.L^{-1})				average cultivar effect
		00	50	100	150	
Guora	Origin	0.67	0.86	0.99	0.74	0.81
	selected	0.74	1.10	1.14	1.04	1.01
Local	Origin	0.73	1.30	1.18	1.26	1.12
	selected	0.78	1.92	1.34	1.39	1.36
Chakhao	Origin	0.84	1.66	1.06	1.09	1.16

	selected	**0.91**	**1.48**	**1.27**	**1.21**	**1.22**
Average effect of Phenylalanine		**0.78**	**1.39**	**1.16**	**1.12**	
L.S.D 0.05	**Cultivars**		**Phenylalanine**		**Interaction**	
	0.065		**0.069**		**0.130**	

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