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Using Of Phenylalanine To Increase The Anthocyanin Compounds Content Of The Local Variety Of Black Rice And It's Oxidative Activity

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Abstract *A field experiment was conducted during the summer season of 2021 at the rice research station in Al-Mishkhab, with the aim of improving the proportions of active compounds with a medical effect among the genotypes used for black rice.The experiment was conducted according to a completely randomized block design (RCBD), in a split-plot arrangement with three replicates. The amino acid Phenylalanine treatments were randomly distributed in the main plots, 50, 100, and 150 mg.L^{-1}, in addition to the comparison treatment (spraying distilled water only)In the sub plot, the genotypes of Goura, Local and Chakhao black rice were distributed.The results obtained showed that the Goura genotype was significantly excelled, giving the highest averages in the number of branches per plant, the number of total panicle, and the weight of 1000 grain .The total grain yield and the percentage of free radical suppression amounted to 114.77 cm^2, 32.34 cm^2, 16.96 tillers .plant^{-1}, 15.12 panicle.plant^{-1}, 22.83 g, 5.86 tons.ha^{-1}, and 65.59%, respectively. While the Local genotype gave the highest averages for total anthocyanins, Cyanidin 3-O-glucoside, Peonidin 3-O-glucoside, Cyanidin, Delphinidin, Peonidin, and Malvidin. The total antioxidant and carbohydrate capacity amounted to 53.32 mg.kg-1 dry weight, 27.88 mg.kg^{-1} dry weight, 7.11 mg.kg^{-1} dry weight, 5.34 mg.kg^{-1} dry weight, and 3.84 mg. kg^{-1} dry weight, 1.28 mg.kg^{-1} dry weight, 1.24 mg.kg^{-1} dry weight, 60.06 mg.kg^{-1} dry weight, and 54.68%, respectively.The results also showed the significantly excelled of spraying the amino acid Phenylalanine at a concentration of 50 mg.L^{-1} by giving the highest averages for the yield traits, its components, and all the active compounds.*

Keywords: *black rice, active compounds, anthocyanin pigments*

INTRODUCTION

The black color in the rice grain is due to the presence of a high percentage of anthocyanin pigments, which are flavonoid compounds that play the role of antioxidants in the plant and animal biological system. Black rice has several names, including purple, heavenly, imperial, precious, and king [1 , 2] showed that the antioxidant activity of black rice is two times stronger than that of black grapes and blackberries.After increasing global health community awareness, researchers turned their attention to searching for ways to increase the concentrations of antioxidant compounds in the grains and fruits of plants with both nutritional and medicinal uses, especially those plants that are included in the nutritional program of the daily popular diet in most countries of the world. This list may be topped by black rice of its varieties. Which contains good concentrations of anthocyanin pigment group [3]. The production capacity of any variety, regardless of its specifications, is dependent on the service processes followed in accordance with the correct scientific principles. For the purpose of increasing the productivity of the grain and its content of some secondary metabolic compounds with biological and medical effectiveness, interest has recently increased in some approved varieties of rice, especially the colored varieties for their important nutritional and medical benefits [4]The high content of anthocyanins in rice varieties helps prevent the harmful effects of free radicals, as they act as antioxidants that prevent carcinogenesis, enhance blood circulation, delay tissue aging, reduce cholesterol and blood sugar levels, positively affect the function of the pituitary gland, and prevent the breakdown of platelets [5]. Eating black rice grains helps control blood sugar levels and prevent dementia [6]. showed that consuming black rice in the daily diet of people with asthma, bronchoconstriction, and chest allergies makes them feel comfortable because it fights free radicals inside the body, especially those that cause inflammation within the bronchial tubes and are responsible for the mucous secretions associated with asthma patients, and this helps to act as a factor. It helps in relieving asthma attacks. It also explained that it reduces the symptoms of allergic skin infections, swelling, and skin allergies, preventing them from occurring in the future, and prevents the secretion of the amino acid histamine responsible for triggering allergic symptoms, while [7] explained that consumption of

black rice flour can increase Inhibiting some cancers, including skin, breast, prostate, and blood cancer (leukemia), by inducing programmed cell death of affected cells .[8] noted that consuming black rice is a good source of phenols in a healthy diet. Therefore, it is very beneficial for liver health and preventing cirrhosis. It also maintains eye health, improves vision, and protects it from retinal disease problems because it contains antioxidant vitamins, including vitamin E. Black rice protects against osteoporosis, promotes hair growth, and protects and reduces hair loss because it contains biotin and vitamin B. Black rice is used in preparing pastries, biscuits, snacks, and various food drinks, and decorating the table with a wide spectrum of different food colorings, whose colors range from pink to black, and thus provides an alternative.

MATERIALS AND METHODS

A field experiment was conducted with the aim of improving the proportions of active compounds with a medical effect among the genotypes used for black rice. The experimental land was plowed with two perpendicular plows using Mold-board plows, then the process of smoothing and leveling was conducted. Three samples were taken from each depth of the field soil, 0-30 cm, and dried air-dried. Then grind it, mix it well, and sift it for the purpose of preparing it for analysis. Some physical and chemical traits were measured in Table (1), and the chemical analysis of irrigation water was as in Table (2). The experimental plants were fertilized for all treatments according to the recommendation of [9] by adding nitrogen fertilizer 140 kg N.ha-1, phosphate fertilizer 46 kg $P_2O_5.ha^{-1}$ and potassium fertilizer 50 kg K2O.ha-1. Phosphate fertilizer (triple calcium superphosphate) was added. Ca_3P_o4, 46% P_2O_5) by mixing it with the soil before the transplantation process, and nitrogen fertilizer (urea fertilizer 46%) was added in two batches, the first 10 days after transplantation and the second one month after the first batch. As for potassium fertilizer (potassium sulphate, 50% K2O) was added. After 10 days of transplantation, the growing bushes in the experimental panels were manually uprooted three times. The first weeding was carried out 10 days after transplantation, the second 15 days after the first weeding, and the third 15 days after the second weeding, and irrigation was cut off 15 days before harvest.

Table (1) Some physical and chemical traits of the field soil in which the experiment was conducted

units		**values**	**traits**	
silty clay loam	gm.kg-1	222	Sand	**Soil texture components**
	gm.kg-1	474	Silt	
	gm.kg-1	304	Clay	
Mg.m-3		1.29	Bulk density	
gm.kg-1		1.60	Organic matter	
%		0.44	available nitrogen	
%		0.69	available phosphorus	
%		0.57	available potassium	
$DS.m^2$		3.21	Electrical conductivity EC	
---		7.78	pH	

Table (2) Chemical analysis of irrigation water used in the experiment

NO_3	K	Mg	Na	Ca	EC	pH	traits
5.62	0.11	3.19	6.38	2.64	1.66	7.7	Value
mmoL.L-1	mmoL.L-1	mmoL.L-1	mmoL.L-1	mmoL.L-1	DS.m2	-	Unit

The experimental panels were planted with genotypes of Goura, Local, and Chakhao black rice, as the first brown genotype was introduced from the United Kingdom for the first time to Iraq in 2018, and the black genotype Local was first introduced to Iraq in 2001 from Vietnam. The genotype of Chakhao, which is also black, was introduced from India for the first time in 2019. Seeds for the three varieties were sown on 6/17/2021. In order to evaluate the performance of the introduced varieties and compare them with the local variety under the influence of four levels of spraying the amino acid phenylalanine, a randomized complete block design (RCBD) was used according to the split-plate arrangement and with three replicates, where the

main panels were occupied by amino acid treatments of 50, 100, and 150 mg.L-1, in addition to the treatment Comparison (spraying distilled water only) as the spraying process was conducted three times, the first in the tillering stage after 50 days of planting, the second in the heading stage after 75 days of planting, and the third in the ripening stage after 100 days of planting using an 18 capacity backpack sprinkler. liters, and spraying was done at sunset to give sufficient time for the spray solution to contact the foliage. A dispersing agent (cleaning solution 10 ml/spray) was also added to break the surface tension of the solution and create complete and homogeneous wetness and to increase its efficiency in absorption and make the most of the amino acid. The secondary panels were occupied by the genotype s so that The number of genotypes planted is 3 genotypes, and thus the number of experimental units becomes 36 experimental units. The plants of the experimental unit were distributed on dimensions of 2 x 3 m2, as it contained eight lines in addition to two guard lines for each experimental unit, each of which was 2 m long. The distance between one line and another was 30 cm and between one plant and another was 20 cm [10], leaving a space between. The main plote are 1 m and between Miqra and the last 2 m. The Goura variety was harvested on November 20, 2021, and the other two varieties on December 1 and 10 in the same year, sequentially.

Studied traits

First: The traits of the product and its components

1- The number of tillers in the plant (leaves.plant^{-1})
2- The number of panicle per plant (panicle.plant^{-1})
3- Weight of 1000 grain (g)
4- Total grain yield (tons.ha^{-1})

Second: Indicators for estimating some active compounds in grains

Preparation of extract

A certain amount of grains, 25-30 g for each treatment, was dried in an electric oven at a temperature of 60 °C for 48 hours according to the method approved by [11] and described by [12] in grinding samples. The dried samples were ground using an electric grinder. The permeable rice flour was taken through a sieve with a diameter of 250 µm, then 5 g of rice flour was taken for each treatment, and mixed with 30 cm3 of methanol acidified with 1 N hydrochloric acid (85 methanol: 15 hydrochloric acid HCL v/v), then shaken. The mixture was placed on a shaking device at 4°C for 24 hours, after which the extract was filtered using Whatman No. Marked and stored at 4°C until used to estimate some groups of active compounds.

1- Estimation of total phenols (mg.kg^{-1} dry weight) Total Phenols Compounds

The method used by [13] was adopted by taking 0.5 ml of black rice extract for each treatment and placing each in a test tube. Then 3 ml of Folin-Ciocatleu reagent diluted in a ratio (1 reagent: 10 distilled water) was added. 2.5 ml of sodium carbonate (Na2CO3 Sodium carbonate, concentration 0.2% (w/v), then mixed well and left the test tubes for three minutes at room temperature, then took an optical absorption reading using a spectrophotometer, type Optima UV-3000, at a wavelength of 765 nm. The optical absorption readings were calibrated with concentrations of gallic acid (C7H6O5) on the basis of milligrams of gallic acid.L^{-1}, then the phenols content in black rice was proportioned to (mg.kg^{-1} dry weight). The standard curve was prepared using the concentrations 50, 100, 150, 250, and 500 mg of acid, each of which was dissolved in a liter of distilled water. 0.5 ml of the prepared acid concentrations were taken and the reagent and sodium carbonate were added to it as in the plant model, except for the blank model, to which nothing was added. Then the relationship between concentration and absorbance was drawn. The straight line equation was extracted for use in calibration and the Determination Coefficient (R2) to determine the degree of reliability of the relationship between concentration and absorbance [1] [14] as in Figure (1).

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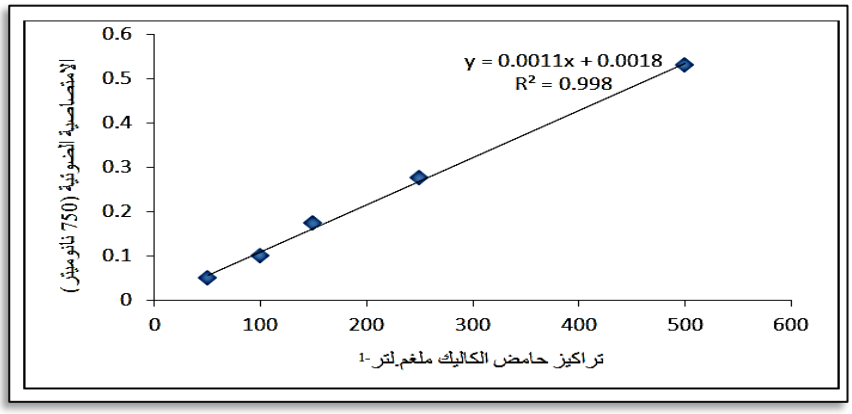

Figure (1) Standard curve for gallic acid concentrations.

2- Estimation of total flavonoids (mg.kg^{-1} dry weight) Total Flavonoids Compounds

It followed the method used by [13] by taking 0.5 ml of black rice extract for each treatment and placing each in test tubes, then adding 4.5 ml of methanol (CH3OH) to each, then mixing well, then adding 5 ml of aluminum chloride (AlCl3) concentration of 0.01 mol.L^{-1}, then the test tubes were left for ten minutes at room temperature, after which the optical absorption reading was taken at the wavelength of 400 nm. The standard curve was prepared using Rutin (C27H30O16). Concentrations were prepared as 5, 10, 20, 30, and 40 mg.L^{-1} The relationship between concentration and absorbance was drawn and the straight line equation was used to calibrate the readings and then attributed to mg.kg-1 dry weight [15] as in Figure (2).

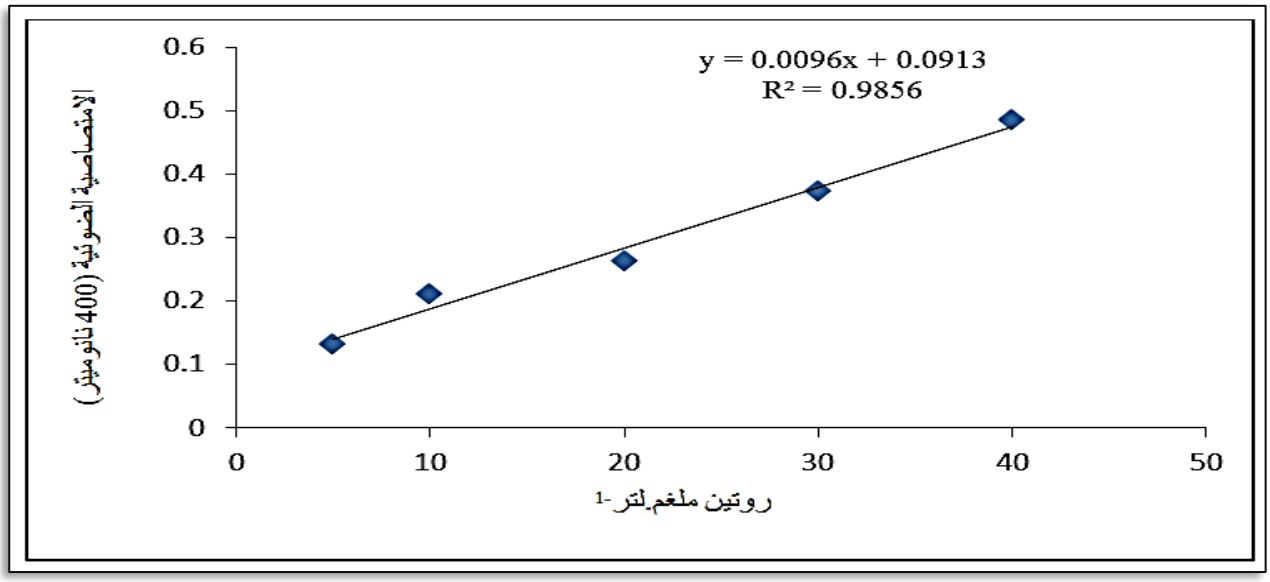

Figure (2) Standard curve for protein concentrations

3- Estimation of total anthocyanins (mg.kg^{-1} dry weight) Total Anthocyanins Compounds

The photoabsorption method was adopted by changing the pH of the plant sample extract [16], by preparing two standard solutions. The first standard solution (Buffer 1 pH 1.0) was prepared by dissolving 1.5 g of potassium chloride (KCl) in 100 ml of distilled water to obtain On the KCl solution (N 0.2), take 4.25 ml of hydrochloric acid (HCl) concentration (37%) and add 245 ml of distilled water to it to obtain a solution (N 0.2), then take 75 ml of KCl solution (0.2 N) and mix With 201 ml of HCl (N0.2) to obtain 276 ml of the first standard solution (Buffer 1 pH 1.0), and prepare the second standard solution (Buffer 2 pH 4.5) by dissolving 12.3 g of sodium acetate (C2H3NaO2) in 150 ml of water. Distilled, then take 8.5 ml of hydrochloric acid HCl concentration (37%) and add 91 ml of distilled water to it, then take 150 ml of sodium acetate solution and mix with 100 ml of hydrochloric acid HCl solution and add 120 ml of distilled water to produce 370 ml of The second standard solution is Buffer 2 pH 4.5.

Take 1 ml of the extract of each treatment and put it in a test tube and add to each of them 5 ml of Buffer 1 pH 1.0 solution. Then I took another set of test tubes and put 1 ml of the extract of each treatment in it and added 5 ml of Buffer 2 pH 1.0 solution. 4.5) Then I left the test tubes for 15 minutes, after which I took optical absorption readings at the wavelength of 510 nm for both test tubes (for each treatment). Then I placed all the test tubes in a dark container for 60 minutes, after which I took optical absorption readings at the wavelength of 700 nm for both tubes. The test was applied and the equation adopted from (Giusti and Wrolstad, 2001) was applied to estimate the content of total anthocyanins as follows:

$$\text{Anthocyanin Pigment (mg/L)} = \frac{1000\times DF\times MW\times A}{\times 1\varepsilon}$$

$A = (O.D_{510} - O.D_{700})*pH_{1.0} - (O.D_{510} - O.D_{700})*pH_{4.5}$

DF= Dillution factor

MW= 449.2

ε= 26900

Due to the increase in the concentration of anthocyanins, the reading was beyond the device's capabilities, which led to taking 1/4 of the sample volume imposed by the equation, so V=1 and its volume equals 0.25 ml.

4- Determination of some anthocyanin compounds (mg.kg-1 dry weight)

Some anthocyanin compounds, including Cyanidin 3-O-glucoside, Peonidin 3-O-glucoside, Delphinidin, Cyanidin, Peonidin, and Malvidin, were estimated in the grains of the three varieties. 1 gram of grain samples were ground and then soaked with 60% methanol diluted with 60% distilled water. 50:50 and acidified with 0.1% HCL hydrochloric acid. Extraction was performed twice using continuous shaking for two hours at a temperature of 25 degrees Celsius using 20 ml of water and a methanol solution acidified with HCL 1% sulfuric acid. The samples were incubated in an ultrasonic bath for two hours, after They were centrifuged for 20 minutes at a speed of 3200 rpm-1, the samples were filtered using filter paper with a permeability of 0.45 micrometers, then the ethanol solvent was removed by vacuum drying to make the final volume 1 ml, using a high-performance liquid chromatography device. High Performance Liquid Chromatography HPLC (High Performance Liquid Chromatography) equipped by Shimadzu Company, type 10AV-LC, specialized in measuring variable wave spectra, spectrophotometer 10A- SPD-UV, with the following specifications

Model component

High pressure dual graduated pump P 6.1 L

DAD 2.1 L Diode Detector

Sample loop (20 µl) and D 1357 injector

System analysis and control software Claritychrom, V 7.4.2.104

Sample	Component
P 6.1 L	High pressure dual graduated pump
DAD 2.1 L	Diode detector
D 1357	Sample loop (20 µl) and injector
Claritychrom, V 7.4.2.104	System analysis and control programs

Appreciation

A high-performance liquid chromatography (HPLC) device was used according to the method approved by [17] as follows:

1. Anthocyanin compounds were determined at a UV detector wavelength of 520 nm.
2. Use the detection column identified by the number C18, measuring (50 mm x 2 mm I.D.; 3 µm)
3. The Mobile Phase consists of two solvents, the first A (0.2% of Trifluoroacetic acid (TFA) in water), and the second solvent B (Acetonitrile concentration 4%).
4. The circumstances of dismissal were as follows:

minutes 0 %10
minutes 20 %15
Minutes 30 ---------- %18

Minutes 50 ---------- %35
Minutes 60 ---------- %90

At 60-65 minutes, leveling and neutralization occurred between solvent A and B

5. The separation speed was 1 ml/min.
6. The standard compounds Cyanidin 3-O-glucoside, Peonidin 3-O-glucoside, Delphinidin, Cyanidin, Peonidin, and Malvidin were used. Each of them was dissolved using methanol acidified with 0.1 N hydrochloric acid (85 methanol:15 hydrochloric acid v/v) to make a concentration of 1 mg. cm3, as shown in Figures (3,4,5.(
7. The concentration of anthocyanins was calculated according to [17]as follows-:

Concentration of the compound in the sample (mg.kg-1 dry weight) = area of sample package / area of standard package x concentration of standard sample x number of dilutions

Table (3) Results of estimating the concentrations of some anthocyanin compounds using a high-performance liquid chromatography device for some parameters

concentration mg.kg^{-1} dry weight	Appearance time	Dilution factor	Standard sample concentration mg.L-1	Standard band area	Sample band area	treatments
						Cyanidin 3-O-glucoside
23.36	2.59	0.2	25	126321	590217	× 100 Phenylalanine Goura
26.07	2.59	0.2	25	126321	658749	Local × 100 Phenylalanine
						Peonidin 3-O-glucoside
11.29	3.75	0.2	25	110492	249390	× 100 Phenylalanine Goura
10.37	3.75	0.2	25	110492	229125	Local × 100 Phenylalanine
						Delphinidin
3.81	4.58	0.2	25	137649	104946	× 100 Phenylalanine Goura
4.34	4.58	0.2	25	137649	119588	Local × 100 Phenylalanine
						Cyanidin
3.70	5.94	0.2	25	127015	93931	× 100 Phenylalanine Goura
3.39	5.94	0.2	25	127015	86059	Local × 100 Phenylalanine
						Peonidin
1.61	7.42	0.2	25	137676	44405	× 100 Phenylalanine Goura
1.61	7.42	0.2	25	137676	44405	Local × 100 Phenylalanine
						Malvidin
1.20	8.49	0.2	25	146793	35305	× 100 Phenylalanine Goura
1.38	8.49	0.2	25	146793	40532	Local × 100 Phenylalanine

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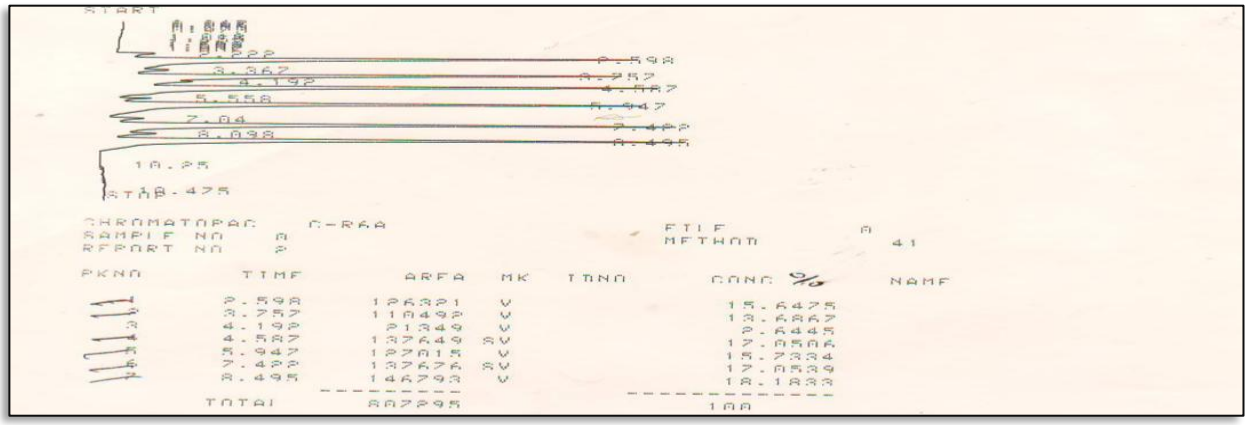

Figure (3): Black rice content of anthocyanin compounds for the control treatment

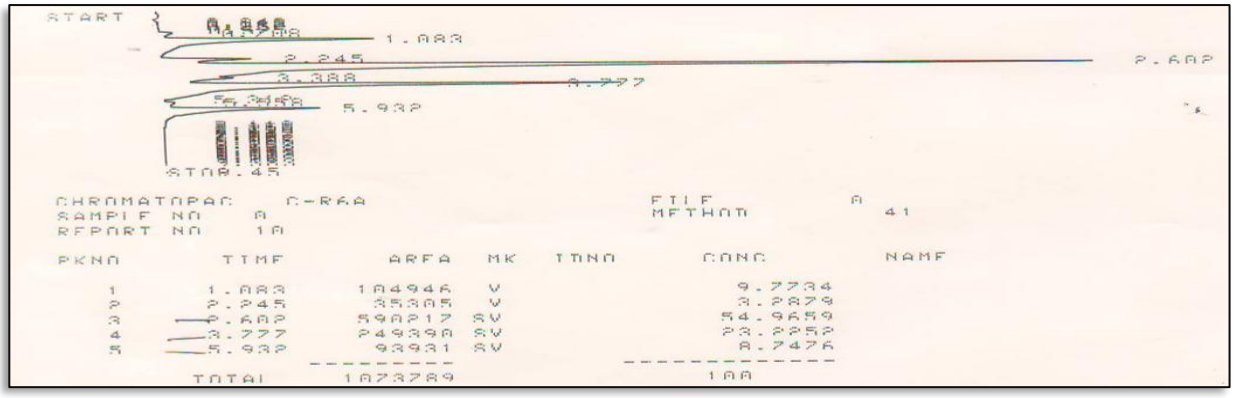

Figure No. (4) Chart of black rice content of anthocyanin compounds for the interaction treatment between spraying Phanelalanine 100 mg.L-1 with the Goura genotype.

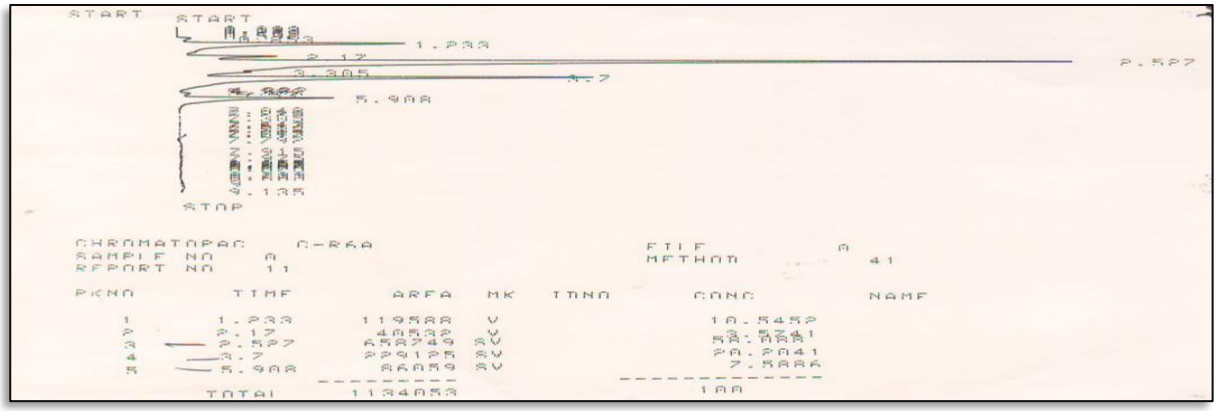

Figure (5) Figure of black rice content of anthocyanin compounds for the interaction treatment between spraying Phanelalanine 100 mg.L-1 with the local genotype.

5- Estimating the percentage of free radical suppression (%) Radical Scavenging Activity

The method followed by [18] was adopted, following the method for estimating the percentage of free radical suppression described by [19] as follows:

1. Dissolve 40 mg of Diphenyl-1-picrylhydrazyl (DPPH) in 100 ml of methanol with continuous stirring. After complete dissolution, other amounts of methanol are gradually added while simultaneously taking the optical absorption of the detector at the wavelength of 516 nm, until the optical absorption of the detector becomes 0.7 ± 0.01. Nanometer.

2. Take 100 microliters of seed extract, add 1 ml of DPPH reagent, and leave it for 24 hours at laboratory temperature.

3. The optical absorption reading of the samples was taken at the wavelength of 516 nm and the following equation was applied:

$$\text{Oxidative activity DPPH (\%)} = \frac{\textit{photoabsorbance of the blank sample} - \textit{photoabsorbance of the plant sample}}{\textit{Optical absorption of the blank sample}} \times 100$$

6- Estimation of the total antioxidant capacity (mg.kg-1 dry weight)

The method for measuring the intensity of the green color of the complex compound consisting of Molybdenum Phosphate in acidic media was adopted, which was described by [20]. 0.1 ml of the seed extract was taken and placed in an Eppendorf tube, and 1 ml of the reagent was added to it (which was prepared from taking 58.8 ml of sulfuric acid and dissolved in it 459.1 mg sodium phosphate and 78.4 mg ammonium molybdate), after which the samples were placed in a water bath at a temperature of 95°C for 90 minutes, then the samples were cooled to room temperature. The intensity of the color formed was measured at the wavelength of 695 nm, then it was calibrated. Readings with the standard curve for ascorbic acid, Figure (6).

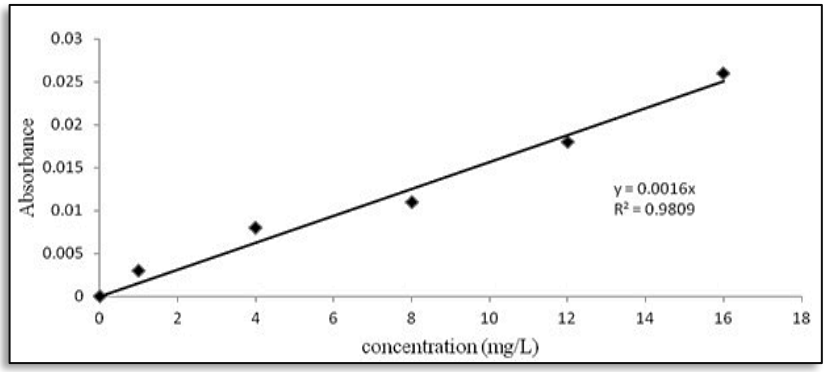

Figure (6) Standard curve of ascorbic acid (µg.ml).

7- Estimating the percentage of total soluble carbohydrates (%)

It was determined by the phenol-sulfuric acid method followed by [21]. Samples were taken from the seeds, and after cleaning them well, they were dried using an electric oven at a temperature of 75°C for 48 hours. Then the seeds were ground well, and 10 mg of the dried and ground plant sample was taken from each treatment and placed in a tube. Test and add 10 ml of distilled water free of ions. After homogenizing the mixture, it was placed in a centrifuge at a speed of 1500 rpm for 10 minutes. Then the solution was filtered with filter paper and the filtrate was taken and its volume was completed to 10 ml of distilled water free of ions. 1 ml of it was taken and added. To it, 1 ml of 5% phenol reagent and 5 ml of 80% sulfuric acid were mixed well and cooled to room temperature. 1 ml of each sample was taken for measurement. The absorbance was measured at a wavelength of 490 nm using a UV-Visible Spectrophotometer for each treatment. Carbohydrates were estimated. Total dissolved substances based on the glucose standard curve, which was taken by taking optical density measurement readings of pure glucose concentrations (Figure 7).

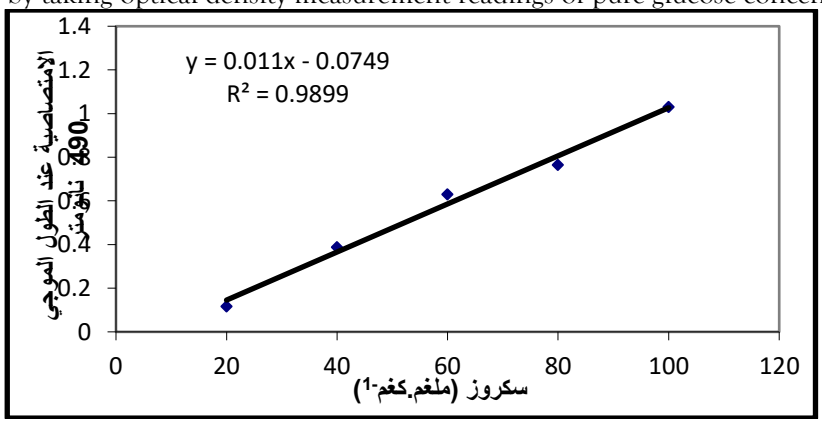

Figure (7) standard curve for glucose.

8- Estimation of the percentage of total proteins (%)

A sample of the grains used in calculating the yield was taken and the percentage of total proteins was estimated. The Micro Kjeldahl device was used to digest and purify the samples with sulfuric acid after collecting the ammonia in the presence of boric acid to estimate the percentage of nitrogen in the samples, then they were converted into proteins according to the equation approved by [22]. as follows:

Protein percentage = Nitrogen percentage x 6.25

statistical analysis

The treatments were distributed in an experiment in a split plot design. The data were analyzed according to a randomized complete block design (RCBD) with three replicatos. The means were compared according to the least significant difference (LSD) test at a probability level of 0.05 [23].

RESULTS AND DISCUSSION

Productivity indicators

Table (4) shows a significant effect of the two study factors and the interaction between them on productive traits, where the Guora genotype gave the highest average for the number of tillers, the number of panicle, the weight of 1000 grains, and the total grain yield of 16.96 panicle.plant^{-1}, 15.12 panicle.plant^{-1}, and 22.83 g and 5.86 tons.ha^{-1} compared to the Local genotype, which gave the lowest average for the mentioned traits, which amounted to 15.11 tillers .plant^{-1}, 12.53 panicle.plant^{-1}, 18.09 g, and 4.16 tons.ha^{-1}, respectively.As for the effect of spraying the amino acid phenylalanine, the spray concentration of 50 mg.l^{-1} gave the highest averages in the mentioned traits, reaching 16.92 tillers .plant^{-1}, 15.01 panicle.plant-1, 21.79 g, and 6.13 tons.ha^{-1}, respectively, while the treatment was given Comparison: The lowest averages for the above traits reached 14.17 panicle.plant-1, 12.16 panicle.plant^{-1}, 20.85 g , and 4.11 tons.ha^{-1}, respectively.The interaction treatment between the Guora genotype and the spray concentration of 50 mg.L^{-1} gave the highest average number of total tillers per plant, reaching 19.16 tillers.plant^{-1}, and the highest average number of panicle per plant, reaching 17.00 panicle.plant^{-1}, respectively.

Table (4): effect of genotypes and concentrations of the amino acid phenylalanine and their interaction on the grain content of productive indicators

Grain yield ((tons.ha-1	Weight of 1000 (grain (g	Number of panicle(pan (icle.plant-1	Number of tillers (tiller.plant-(1	treatments	
5.86	22.83	15.12	16.96	Guora	genotype
4.16	18.09	12.53	15.11	Local	
4.98	21.90	12.61	15.16	Chakhao	
0.25	0.57	0.42	0.47	LSD	
4.11	20.85	12.16	14.17	00	Phenylalanine acid spray (mg.L^{-1})
6.13	21.79	15.01	16.92	50	
5.17	20.78	13.70	16.09	100	
4.58	20.59	12.81	15.80	150	
0.33	0.71	0.39	0.39	LSD	
3.70	23.04	12.77	13.86	00	Guora
7.53	22.20	17.00	19.16	50	
6.28	23.74	15.20	17.41	100	
5.93	22.37	15.50	17.46	150	
3.86	17.82	11.90	14.10	00	Local
5.21	19.57	13.93	15.92	50	
4.29	17.46	13.07	15.43	100	
3.29	17.50	11.53	14.96	150	
4.79	20.91	11.80	14.55	00	Chakhao
5.67	23.60	14.10	15.70	50	

4.93	21.16	12.83	15.43	100	
4.52	21.92	11.40	14.98	150	
0.51	1.14	0.83	0.93	LSD	

The results shown in Tables 4 indicate that there are significant differences in the effect of genotype on yield traits and components. The reason for this is the difference in the response of the genotypes used to the local environmental conditions. This is because these genotypes are newly introduced to Iraq: Guora from the United Kingdom and Chackhao from India, compared to the local genotype. Local [24].The yield and its components for each genotype are determined by three main factors: genetic, environmental, and field practices. Several studies have also shown that the number of grains is the component most closely related to the yield. genotype has an important impact on the accumulation of dry matter through its effect on traits of the yield and its components, and this is demonstrated by the results of the Guora genotype , which achieved the best averages for the yield components with a combined effect of the surrounding environmental conditions [25] [26] .The significant effect of foliar spraying with Phenylalanine acid, concentration of 50 $mg.L^{-1}$, on yield traits and components may be due to the amino acids increasing the production of some plant growth hormones such as Tryptophan, which is the basic building block for the biosynthesis of auxin 3-Indol Acetic Acid (IAA). The amino acid Phenylalanine is also included in the biosynthesis of gibberellins as a basic substance in the construction of terpenes, and the increase of these hormones causes an increase in the pace of the cell division process and the elongation and increase in the size of the cells. This in turn increases the yield indicators and its components such as the number of moieties, or the amino acid Phenylalanine may combine with some proteins to form a complex. It is called Protine-Fluorophenylalanine which stimulates an increase in the number of deltoid, the length of the deltoid and the number of deltoid branches [27] [28].

Indicators of active compounds

The results in Table (5) indicate that there is a significant effect of the genotypes, amino acid spray, and the interaction between them on the effective antioxidant compounds, where the Chakhao genotype gave the highest average for their total phenolic content, amounting to 98.76 mg.kg-1 dry weight, in comparison with the Local genotype, which gave the lowest average. 95.99 mg.kg-1 dry weight, and the Chakhao genotype gave the highest average content of total flavonoids, amounting to 13.35 mg.kg-1 dry weight, compared to the Guora genotype, while the Local genotype gave the highest average grain content of total anthocyanins, amounting to 53.32 mg.kg. $^{-1}$ dry weight, compared to the Guora genotype, which gave the lowest average, which amounted to 47.84 $mg.kg^{-1}$ dry weight, and the Guora genotype gave the highest average ability to suppress free radicals, amounting to 65.59%, compared to the Chakhao genotype, which gave the lowest average, amounting to 58.73%. The Local genotype had the highest average for total antioxidant capacity and total soluble carbohydrates, amounting to 60.06 mg.kg-1 dry weight and 54.68%, compared to the Chakhao genotype, which gave the lowest average for the above traits, amounting to 57.23 $mg.kg^{-1}$ dry weight and 52.58%, respectively.

Table (5): The effect of genotypes and concentrations of the amino acid Phenylalanine and their interaction on the grains' content of some active compounds

Total proteins (%)	Total soluble carbohydrates (%)	Total antioxidant capacity (mg.kg-1 dry weight	Free radical suppression (%)	Total anthocyanins (mg.kg-1 dry weight	Total flavonoids (mg.kg-1 dry weight	Total phenols (mg.kg-1 dry weight	treatments	
9.98	53.70	57.51	65.59	47.84	10.99	97.01	Guora	genotype

9.74	54.68	60.06	60.39	53.32	12.67	95.99	**Local**	
10.15	52.58	57.23	58.73	52.77	13.35	98.76	**Chakhao**	
0.368	1.615	1.721	1.475	2.049	0.337	1.507	**LSD**	
9.15	50.64	56.92	56.73	46.37	10.79	92.20	**00**	**Phenylalanine acid spray (mg.L-1)**
10.54	58.89	60.47	61.08	57.14	14.00	105.19	**50**	
10.17	51.86	58.19	57.80	52.60	12.75	94.33	**100**	
9.97	53.25	57.50	58.68	49.14	11.82	97.30	**150**	
0.507	1.176	1.936	0.857	2.295	0.411	1.247	**LSD**	
9.35	49.30	54.81	54.58	45.71	9.12	82.98	**00**	**Guora**
10.16	56.87	60.84	59.1	45.80	13.41	113.36	**50**	
8.91	53.13	59.24	57.25	52.41	13.35	83.56	**100**	
11.50	55.52	55.15	55.53	47.42	8.10	108.14	**150**	
8.96	55.20	56.29	55.79	51.34	10.77	100.17	**00**	**Local**
9.84	57.09	62.29	63.46	55.52	14.58	94.73	**50**	
11.04	55.96	62.57	61.91	56.72	12.64	85.91	**100**	
9.13	50.47	59.06	60.39	49.73	12.69	103.15	**150**	
9.14	47.42	59.63	59.80	42.05	12.47	93.44	**00**	**Chakhao**
11.61	62.69	58.26	60.76	70.09	14.00	107.48	**50**	
10.57	46.47	52.75	54.25	48.67	12.26	113.51	**100**	
9.28	53.74	58.29	60.08	50.25	14.67	80.59	**150**	
0.971	3.229	3.441	2.953	4.099	0.674	3.014	**LSD**	

While the Chakhao genotype gave the highest average of total proteins, amounting to 10.15%, compared to the Local genotype, which gave the lowest average, amounting to 9.74%.

As for the effect of spraying the amino acid phenylalanine, the spray concentration of 50 mg.L-1 gave the highest averages in grain content of total phenols, total flavonoids, total anthocyanins, ability to inhibit free radicals, total antioxidant capacity, total soluble carbohydrates, and total proteins, reaching 105.19 mg.kg-1 dry weight. And 14.00 mg.kg-1 dry weight, 57.14 mg.kg^{-1} dry weight, 61.08% and 60.47 mg.kg-1 dry weight, 58.89% and 10.54%, respectively, by measurement with the control treatment 00 mg.L^{-1}, which gave The lowest averages for the mentioned traits.

Some anthocyanin compounds

The results of Table (6) indicate that there is a significant effect of genotypes, amino acid spraying, and the interaction between them on some anthocyanin compounds, where the local genotype gave the highest average for Cyanidin 3-O-glucoside, Peonidin 3-O-glucoside, Delphinidin, Cyanidin, Peonidin, and Malvidin, reaching 27.88. 7.11, 5.34, 3.84, 1.28, and 1.24 mg.kg^{-1} dry weight, respectively, with the lowest averages for the above traits for the Guora genotype reaching 25.45, 6.07, 4.48, 3.12, 0.83, and 0.91 mg.kg^{-1} dry weight, respectively. As for the effect of spraying the amino acid phenylalanine, the spray concentration of 50 mg.L^{-1} gave the highest averages in grain content of some of the anthocyanin compounds above, reaching 29.20, 7.99, 6.29, 4.31, 1.28,

and 1.39 mg.kg^{-1} dry weight, respectively, when measured with the least The averages for the spray concentration of 00 mg.L^{-1} for the above traits reached 25.75, 5.28, 3.66, 2.79, 0.83, and 0.78 mg.kg^{-1} dry weight, respectively. The interaction treatment between the Local genotype and the spray concentration of 50 mg.L^{-1} gave the highest average for Cyanidin 3-O-glucoside, amounting to 32.02 mg.kg^{-1} dry weight, compared with the lowest average for the Guora genotype and the spray concentration of 100 mg.L^{-1}, and it gave Treatment of interaction between the Chakhao genotype and the spray concentration of 50 mg.L^{-1}. The highest average for Peonidin 3-O-glucoside reached 11.27 mg.kg^{-1} dry weight compared to the lowest average for the Chakhao genotype and the spray concentration of 100 mg.L^{-1}. The treatment was given The interaction between the Chakhao genotype and the spray concentration of 50 mg.L^{-1}. The highest average for Delphinidin reached 7.47 mg.kg-1 dry weight compared to the lowest average for the Guora genotype and the spray concentration of 00 mg.L^{-1}.

Table (6): The effect of genotypes and concentrations of the amino acid Phenylalanine and their interaction on the grain content of some anthocyanin compounds (mg.kg^{-1} dry weight)

Malvidin	Peonidin	Cyanidin	Delphinidin	Peonidin 3-O-glucoside	Cyanidin 3-O-glucoside	treatments	
0.91	0.83	3.12	4.48	6.07	25.45	Guora	genotype
1.24	1.28	3.84	5.34	7.11	27.88	Local	
1.19	1.21	3.58	5.08	6.63	27.78	Chakhao	
0.065	0.060	0.281	0.409	0.511	1.042	LSD	
0.78	0.83	2.79	3.66	5.28	25.75	00	Phenylalanine acid spray (mg.L-1)
1.39	1.28	4.31	6.29	7.99	29.20	50	
1.16	1.19	3.63	4.39	7.45	26.17	100	
1.12	1.13	3.34	5.53	5.72	27.03	150	
0.069	0.059	0.429	0.533	0.749	0.514	LSD	
0.71	0.87	2.75	3.58	5.55	22.81	00	Guora
0.98	0.68	2.47	4.12	5.62	29.19	50	
1.07	0.79	3.90	4.03	9.68	20.10	100	
0.89	0.96	3.36	6.20	5.70	27.19	150	
0.75	0.82	3.19	3.80	7.07	24.37	00	Local
1.61	1.31	4.53	7.25	7.08	32.02	50	
1.26	1.49	3.50	4.76	9.54	26.68	100	
1.32	1.22	4.15	4.50	4.77	28.44	150	
0.87	0.79	2.42	3.59	3.21	27.57	00	Chakhao
1.57	1.84	5.91	7.47	11.27	26.38	50	
1.16	1.29	3.48	4.38	3.14	31.72	100	
1.15	1.21	2.50	5.89	6.66	25.47	150	
0.130	0.121	0.563	0.817	1.022	2.083	LSD	

The interaction treatment between the Chakhao genotype and the spray concentration of 50 $mg.L^{-1}$ gave the highest average for Cyanidin, amounting to 5.91 $mg.kg^{-1}$ dry weight. Compared with the lowest average for the Chakhao genotype and the spray concentration of 00 $mg.L^{-1}$, the interaction treatment between the Chakhao genotype gave The spray concentration was 50 $mg.L^{-1,}$ the highest average for Peonidin was 1.84 $mg.kg^{-1}$ dry weight, compared to the lowest average for the Guora genotype and the spray concentration was 50 $mg.L^{-1}$, while the interaction treatment between the Local genotype and the spray concentration gave 50 mg. L^{-1} The highest average for Malvidin reached 1.61 $mg.kg^{-1}$ dry weight, with the lowest average for the Guora genotype and the spray concentration of 00 $mg.L^{-1}$.The results of Tables 5 and 6 show that there is a significant effect of genotypes on these traits. The reason for this is due to the variation in the response of genotypes to environmental conditions and their ability to produce some active compounds of biological and medical benefit, especially antioxidant compounds, including plant dyes. Colored genotypes sometimes contain Many times the contents of active compounds in uncolored rice, as the genetic factor plays an important role in the variation in genotypes in the production of those compounds that are classified with phenolic compounds [29].Perhaps the superiority of the Local genotype in grain content of total anthocyanin pigments (Table 5) may be attributed to its genetic ability to excel in its content of concentrations of anthocyanin compounds, especially Cyanidin 3-O-glucoside and Peonidin 3-O-glucoside (Table 6), and its adaptation to environmental conditions. Implanted by [30] [31].

The results of Tables 5 and 6 also indicate that there is a significant effect of the effect of spraying with the amino acid Phenylalanine on the indicators of active compounds, as this acid is one of the basic amino acids in the construction of phenolic compounds and their derivatives, from which all polyphenolic compounds (flavonoids) are produced by 60% and phenolic acids by 30%. % and 10% are used in the production of anthocyanin compounds. Its effect on the accumulation of anthocyanin pigments in general may be restricted by conditions, including acid concentration, plant type, plant age stage, and nitrogen abundance, in addition to its role in protein synthesis. It is considered an initial precursor to the natural products of anthocyanins, other plant pigments, alkaloids, and hormones[32]. Accordingly, Spraying Phenylalanine on black rice increases its concentration inside the plant and thus accelerates the appearance of the anthocyanin pigment and increases its concentration[33], and these results are consistent with what was found by [34] [31].

Black rice plants produce active compounds to provide the largest possible amount of natural antioxidants. As a result of the increase in chlorophyll pigment in the leaves, the process of biosynthesis of primary metabolic compounds, including total dissolved carbohydrates, increases. Thus, the content of secondary metabolic compounds increases as intermediate compounds, especially those with antioxidant activity [35]. On the other hand, an increase in secondary plant products such as terpenoids, phenols, and alkaloids are derived from the metabolism of carbohydrates, fats, and amino acids. Phenylalanine and its other form, the amino acid Tyrosine, contribute to tryptophan metabolism, which has an indirect role in growth through auxin metabolism through alternative pathways for IAA synthesis in plants, starting with tryptophan. As a starting material, then when tryptophan is available, the concentration of auxin (IAA) increases in plant tissues [36].

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