Research Article

Effect of L-Tyrosine Application on Anthocyanin Accumulation and Antioxidant Activity in Local Black Rice Variety

Jawad Kadhim Ali[1]*, Ali Adnan Al-Akkam[2], Hasanin S. Oleiwi[3] and Salam A. Khuit[4]

[1]Field Crop Department, Agriculture College, Al-Qasim Green University, Babylon, Iraq.

Abstract | This study aimed to explore the impact of L-Tyrosine foliar application on the accumulation of anthocyanins, phenolic metabolic activity, antioxidant capacity and yield performance in two black rice (Oryza sativa L.) varieties namely Local and Guora. L-Tyrosine was applied at four levels (0, 50, 100 and 150 mg L^{-1}) during three growth stages (50% tillering stage, flower bud stage and grain filling stage). The findings indicated that L-Tyrosine significantly increased the contents of total anthocyanin, phenolics and the enzyme activities (SOD, CAT and POD) in both varieties. The highest values were recorded at 50 mg L^{-1}, resulting in an enhancement of the anthocyanin content by 24-26%, total phenolics by 30-32% and DPPH radical scavenging activity (by about +17-19%) as compared to control. Guaro did better than Local in all cases having a greater pigment concentration, antioxidant activities and yield components. Pairwise correlation of anthocyanin, phenolics and antioxidant traits showed strong positive relationship (r = 0.89–0.94) between these variables, indicating that L-Tyrosine might serve a metabolic stimulator perhaps affecting the TAL and PAL limbs of the phenylpropanoid pathway. The concurrent elevation of both enzymatic and non-enzymatic antioxidant activities demonstrates that an appropriate concentration of L-Tyrosine can promote the synthetic ability of phenolic compounds, enhance antioxidative defense and increase yield consistency. The foliar spraying of 50 mg L^{-1} L-Tyrosine was the best strategy to enhance biochemical and physiological parameters in black rice, especially when applied to Guora which presented the highest metabolic performance among varieties. These results suggested that L-Tyrosine can be a sustainable biofortification reagent for improving the functional and nutritional quality of pigmented rice.

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***Correspondence** | Jawad Kadhim Ali, Field Crop Department, Agriculture College, Al-Qasim Green University, Babylon, Iraq; **Email:** jw50431@agre.uoqasim.edu.iq
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Introduction

Black rice (*Oryza sativa* L.) has caught much attention as a functional cereal crop due to its high anthocyanin content—the bioactive flavonoid pigment that provides a dark-purple color to the pericarp and strong antioxidant activity in black rice (Thilavech *et al.*, 2025). These anthocyanins, specifically cyanidin-3-glucoside and peonidin-3-glucoside are functioning in two ways: protecting plants against oxidative stress and UV radiation, while they have health-promoting properties also for humans because of their antioxidant, anti-inflammatory and anticarcinogenic potential at the same time (Zhao *et al*., 2025; Lee *et al*., 2023).

Recent field studies on legumes and cereals have shown that nutrient management, particularly potassium and nitrogen availability, plays a significant role in determining crop vigor and yield performance under Iraqi agro-climatic conditions. Variable potassium concentrations were reported to enhance mungbean productivity (Al-Wardy *et al*., 2022), while wheat cultivars exhibited differential responses to late planting conditions (Al-Khafagi *et al*., 2020). Similarly, fertilization regimes and irrigation management improved mungbean yield traits in middle Iraq (Khuit *et al*., 2020), and row spacing was found to markedly influence the growth and yield components of wheat (Khuit *et al*., 2021).

Anthocyanin biosynthesis is regulated by the phenylpropanoid-flavonoid pathway, which branches off from the shikimate pathway leading to production of aromatic amino-acids such as phenylalanine and tyrosine. The key enzymes phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase enzyme (TAL) convert these amino acids to p-coumaric acid, a central precursor for flavonoid and anthocyanin biosynthesis pathway (Vogt, 2010; Kumar *et al*., 2022). The expression of these enzymes and the corresponding structural genes, i.e., CHS, DFR, UGT, and ANS can directly regulate the level of pigments in rice pericarp (Mackon *et al*., 2023).

A number of researchers have reported that addition of precursor amino acids can stimulate the formation of secondary metabolites. For instance, exogenous L-phenylalanine applied as a foliar spray exerts stimulatory effects. Foliar applications of L-Phenylalanine enhanced PAL activity and anthocyanin concentration in grapes, leaves of cabbage (Ali & Al-Akkam, 2021) and black rice. Similarly, L-Tyrosine has a metabolic proximity to TAL-pathway and results in the upregulation of anthocyanin accumulation in ornamental species including Viola wittrockiana (Wang *et al*., 2022). This indicates that tyrosine may act as both a metabolic precursor and a signaling molecule enhancing anthocyanin accumulation. Although amino acid precursors on phenolic metabolism have been established, information on the direct effect of L-Tyrosine on anthocyanin biosynthesis and antioxidant activity in rice including local genotypes adapted to Iraq exhibiting environment is scant. Thus, the objective of this study was to investigate the influence of exogenous foliar application L-Tyrosine on anthocyanin accumulation and antioxidant capacity in local black rice variety at field levels. Results will offer a perspective of biochemical modulation of anthocyanin metabolism as well as the effective potential to improve functional and nutritional quality in black rice.

Materials and Methods

Experimental site and design

The rice experiment was established on 17 June 2024 with transplanting. The nursery was ready before time, seedlings having attained the desired age were transplanted to the main field after ploughing operation and leveling and a few early puddles have been taken in order to establish good system of root. The two cultivars were transplanted into experimental plots arranged with the used design keeping the recommended distance between seedlings for respective cultivar. Irrigation was based on crop needs under set semi arid climate, hot temperatures and fluctuating relative humidity during the growing season. Under such a situation, the field required to be monitored on regular basis to ensure an adequate flooding of water. Standard field practices were carried out, such as weeding and pest control and fertilization based on technical recommendations. The plants grew until late maturity, when they were sampled on 15th November of multrier year, upon which the assessed traits were measured.Al-Mishkhab, Najaf Governorate, Iraq. A split-plot arrangement within a Randomized Complete Block Design (RCBD) with three replications was used.

Main plots: Two black rice varieties (Local and Guora).

Subplots: Four concentrations of L-Tyrosine (0, 50, 100, and 150 mg L^{-1}).

Each subplot measured 2 × 3 m and contained eight rows spaced at 30 cm with 20 cm between hills. Treatments were randomly assigned within each block following standard split-plot randomization procedures, where varieties were allocated to main plots and L-Tyrosine levels were randomized within each main plot.

Soil and water analysis

Composite soil samples (0–30 cm) were collected before transplanting, air-dried, sieved (2 mm), and analyzed for physical and chemical properties. The irrigation water was also analyzed for chemical composition (Tables 1 and Table 2).

Table 1: *Physical and chemical characteristics of the experimental soil (0–30 cm depth)*

Traits	Values	Units
Soil texture	Silty clay loam	—
Sand	202	g kg^{-1}
Silt	460	g kg^{-1}
Clay	328	g kg^{-1}
Bulk density	1.30	Mg m^{-3}
CaCo3	28	%
CaSo4	1.2	%
Organic matter	1.62	g kg^{-1}
Available N	52.3	mg.kg^{-1}
Available P	18.7	mg.kg^{-1}
Available K	203	mg.kg^{-1}
EC	3.20	dS m^{-1}
pH	7.62	—

The soil showed moderate salinity and slightly alkaline reaction, similar to Ali & Al-Akkam (2021).

Table 2: *Chemical composition of irrigation water*

Parameter	Value	Unit
pH	7.7	—
EC	1.66	dS m^{-1}
Ca^{2+}	2.64	mmol L^{-1}
Na^{+}	6.38	mmol L^{-1}
Mg^{2+}	3.19	mmol L^{-1}
K^{+}	0.11	mmol L^{-1}
NO_3^{-}	5.62	mmol L^{-1}

Crop management

Standard agronomic practices for rice cultivation were followed. Fertilization rates: 140 kg N ha^{-1} (urea), 46 kg P_2O_5 ha^{-1} (triple superphosphate), 50 kg K_2O ha^{-1} (potassium sulfate). Phosphate fertilizer was incorporated before transplanting; nitrogen was split into two equal doses (10 and 40 days after transplanting); potassium was applied 10 days after transplanting.

L-Tyrosine foliar application

L-Tyrosine solutions were prepared at concentrations of 0, 50, 100, and 150 mg L^{-1} using distilled water as solvent. A non-ionic surfactant (10 mL per 18 L) was added to improve foliar coverage and absorption efficiency. Spraying was carried out using a backpack sprayer (18 L capacity) during the evening hours to minimize evaporation losses.

Foliar applications were performed at three key physiological stages of rice growth:

- 50% tillering stage
- 50% flowering stage
- Grain-filling stage

A non-ionic surfactant (10 mL per 18 L solution) was added to all spray treatments, including the control (0 mg L^{-1}), in order to eliminate any potential surfactant-related *effects and ensure that differences among treatments were attributable solely to L-Tyrosine.*

- Growth and yield parameters
- Number of tillers per plant
- Number of panicles per plant
- 1000-grain weight (g)
- Grain yield (t ha^{-1})

Measurements were taken according to Omar and Fadala (2025), rice evaluation procedures.

Biochemical determinations

Extraction: Grain samples were oven-dried (60 °C, 48 h), ground, and extracted with methanol : 1 N HCl (85:15 v/v) at 4 °C for 24 h. Filtrates were stored at 4 °C until analysis.

Total phenolics: Folin–Ciocalteu method (Liu *et al*., 2011).

Total flavonoids: $AlCl_3$ colorimetric assay (Chang *et al*., 2002).

Total anthocyanins: pH-differential method (Giusti & Wrolstad, 2001).

Individual anthocyanins: HPLC (Shimadzu LC-10AV, C18 column, 520 nm).

Individual anthocyanins were quantified using a Shimadzu LC-10AV HPLC system equipped with a C18 reversed-phase column (250 × 4.6 mm, 5 µm). The mobile phase consisted of solvent A: water containing 5% formic acid, and solvent B: acetonitrile. A linear gradient was applied as follows: 10% B (0–5 min), 10–25% B (5–20 min), 25–35% B (20–30 min), followed by re-equilibration to initial conditions. The flow rate was 1.0 mL min^{-1}, the injection volume was

20 µL, and the column temperature was maintained at 30 °C.

Detection was performed at 520 nm. Anthocyanin standards (cyanidin-3-glucoside and peonidin-3-glucoside) were used to generate external calibration curves (R^2 > 0.998). Limits of detection (LOD) and quantification (LOQ) were 0.15 mg L^{-1} and 0.50 mg L^{-1}, respectively. Retention times for major anthocyanins ranged from 18.2 to 23.7 minutes.

DPPH radical scavenging activity: 0.04 % DPPH in methanol, 516 nm (Musa *et al*., 2011).DPPH radical scavenging activity was measured using 0.04% DPPH prepared in methanol, and absorbance was recorded at 517 nm following Musa *et al.* (2011).
Total antioxidant capacity: Phosphomolybdenum assay (695 nm) (Prieto *et al*., 1999).
Total soluble carbohydrates: Phenol–sulfuric acid method (490 nm) (Dubois *et al*., 1956).
Total protein: Micro-Kjeldahl method (N × 6.25).

Protein content in enzymatic extracts was quantified using the Bradford method with bovine serum albumin as the standard. Enzyme activity was expressed on a protein basis:

- One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction at 560 nm.
- One unit of CAT activity was defined as the amount of enzyme decomposing 1 µmol of H_2O_2 per minute at 240 nm.
- One unit of POD activity was defined as the change of 0.01 absorbance units per minute at 470 nm during guaiacol oxidation.
- All assays were performed in triplicate (n = 3).

Statistical analysis

Data were analyzed using SAS v9.4 following the RCBD-split plot design. Mean comparisons were carried out using LSD at $p \le 0.05$, and figures were generated with GraphPad Prism 9.

Results and Discussion

Effect of L-Tyrosine on growth and yield traits of local black rice

Foliar application of L-Tyrosine improved vigour and yield in Local and Guora black rice (*Oryza sativa*. L.) varieties. According to Table 3, increasing the L-Tyrosine concentration from 0 to 50 mg L^{-1} significantly enhanced the number of tillers and panicles per plant, 1000-grain weight as well as grain yield in both genotypes. The highest response was consistently found at 50 mg L^{-1}, whereby the Local variety attained tillers of 19.4 $plant^{-1}$, panicles up to 17.2 $plant^{-1}$, grain mass of 28.1 g and yielding capacity of 5.21 t ha^{-1} whereas the Guora variety slightly surpassed it by producing tillers ranging from (20.2-plant ter)(the exponent -1), These rises would be greater than ~15–18% as compared to the control (0 mg L^{-1}) in both cultivars.

The decline at higher concentrations (100–150 mg L^{-1}) may reflect a possible feedback inhibition or reduced enzymatic efficiency once precursor levels exceed the optimal physiological range. These mechanisms remain hypothetical in the present context because no enzymatic or transcriptomic measurements were performed. Future studies incorporating detailed enzyme assays or pathway-level gene expression analyses are recommended to validate these interpretations.

Table 3: *Effect of L-Tyrosine concentration on growth and yield traits of local black rice*

Variety	L-Tyrosine (mg L^{-1})	Tillers $plant^{-1}$	Panicles $plant^{-1}$	1000-grain weight (g)	Grain yield (t ha^{-1})
Local black rice	0 (control)	16.8 c	14.9 c	25.6 c	4.51 c
	50	19.4 a	17.2 a	28.1 a	5.21 a
	100	18.6 ab	16.4 ab	27.3 ab	5.03 ab
	150	17.1 bc	15.8 b	26.0 bc	4.66 bc
Guora black rice	0 (control)	17.3 c	15.2 c	26.1 c	4.68 c
	50	20.2 a	17.9 a	28.9 a	5.42 a
	100	19.1 ab	16.8 ab	27.8 ab	5.17 ab
	150	17.7 bc	16.1 b	26.5 bc	4.82 bc
LSD (0.05)	—	0.81	0.63	0.52	0.28

Means followed by different letters differ significantly at p ≤ 0.05 (LSD test).

The improvement in plant vigor and productivity may be attributed to L-Tyrosine as a precursor of the shikimate–phenylpropanoid pathway that was substrates for tyrosine ammonia-lyase (TAL) for p-coumaric acid biosynthesis – the central intermediate of phenolic and flavonoid synthesis (Vogt, 2010; Kumar *et al*., 2022). The corresponding phenolic compounds control the transport of auxins, biosynthesis of lignin and antioxidative potential to enhance vegetative growth or reproductive efficiency as a whole.

Previous anatomical and physiological investigations have emphasized that internal structural features and seed activation processes can greatly influence plant growth efficiency and yield formation across several crops (Al-Hadeethi *et al*., 2020; Musa *et al*., 2024). Moreover, foliar spraying of micronutrients such as zinc and potassium humate has been shown to enhance metabolic activity and yield performance in vegetable crops (Mahmoud *et al*., 2025). Hydrological studies on gypsiferous soils further demonstrated that gypsum content modifies soil–water relations and consequently affects wheat growth under field conditions (Dahham *et al*., 2025).

As noted by the results in Table 3, the Guora variety excels in the basic components of growth yield, which reflects the quality of the variety in the conditions of Iraq. The most important component that is reflected in the grain yield is the number of vines, which directly affects the weight of the grains and the total yield later on. This reflects the ability of the Guora variety to convert the products of photosynthesis from the vegetative mass to the vines (grains), which is the winning deal in any field crop grown. Al-Khafagi *et al*. (2020).The better response of Guora may reflect a genotype-dependent metabolic efficiency, The enhanced biochemical response observed in both varieties may be associated with increased flux through the phenylpropanoid pathway. Although gene expression was not measured in this study, previous reports suggest that precursor amino acids such as L-Tyrosine can influence the activity of enzymes related to TAL/PAL-mediated pathways. Therefore, the improvements noted in phenolic and antioxidant traits in the present work should be interpreted as a functional response rather than direct evidence of transcriptional upregulation. The fact that guora had slightly higher grain weight and yield under L-Tyrosine indicated more assimilate translocation and photo-protective effects, in general. Furthermore, L-Tyrosine application probably promoted nitrogen assimilation and antioxidant enzyme activities,and thus preserve stomatal structure as well as postpone leaf senescence under field conditions with the stress (Lee *et al*., 2023; Mackon *et al*., 2023). These physiological effects served to achieve a more source–sink balance, which appeared to be the cause of significantly higher grain yield.

The present result is in line with those of Ali and Al-Akkam (2021) who found that an equivalent maximum response was obtained with 50 mg L^{-1} phenylalanine in black rice suggesting both amino acids act as metabolic inducers operating related to the PAL/TAL-mediated phenylpropanoid frame work. Similarly, Thilavech *et al*. (2025) and Zhao *et al*. (2025) emphasized that the phenolic metabolism is stimulated to confer resilience and productivity in colored rice, as a result of the antioxidant protection and increased carbon–nitrogen efficiency. Taken together, the results of this study show that for both cultivars 50 mg L^{-1} L-Tyrosine is the most responsive concentration (with slightly increased productivity in Guora). The enhancement is due to synergistic increase in the physiological growth and biochemical metabolism, consistent with an supplementation of L-Tyrosine into key phenolic/antioxidant pathways of plant performance.

Statistical relationship among growth, yield, and bio-chemical traits

A detailed correlation and multivariate analysis were conducted to examine the relationships among growth, yield, and biochemical parameters of both black rice varieties (Local and Guora) under different L-Tyrosine treatments (Table 4 and Figure 1). High positive correlations were found between vegetative growth (tillers and panicles per plant) and yield components such as 1000-grain weight and grain yield with correlation coefficients of $r = 0.78$ to 0.88 ($p \leq 0.01$ in both the variety). The strongest relationships were tillering with yield ($r = 0.88$), and weight of 1000 kernel with yield ($r = 0.86$), indicating that these traits are the most reliable from a morphological point of view to evaluate growth and productivity under L-Tyrosine application. Biochemical traits such as total anthocyanins, TPC, and antioxidant indicators (DPPH and TAC) also showed significant positive correlations with grain yield ($r = 0.84$–0.92). This indicates that L-Tyrosine improves both morphological and metabolic responses whos biological relevance in oxidative homeostasis is sustained. Additionally, the high correlation between anthocyanin content and antioxidant activity ($r = 0.93$) suggests that biosynthesis of phenolic compounds directly participates in increasing yield by protecting the photosynthetically active tissue and enhancing assimilate transport.

Table 4: *Correlation matrix among growth, yield, and biochemical traits of two black rice varieties under L-Tyrosine treatments*

Traits	Tillers	Panicles	1000-grain weight	Grain yield	Anthocyanin	Total phenolics	DPPH	TAC
Tillers	1.00	0.85**	0.80**	0.88**	0.79**	0.74**	0.72**	0.70**
Panicles		1.00	0.82**	0.86**	0.77**	0.73**	0.75**	0.74**
1000-grain weight			1.00	0.86**	0.84**	0.81**	0.83**	0.82**
Grain yield				1.00	0.89**	0.86**	0.87**	0.85**
Anthocyanin					1.00	0.90**	0.93**	0.91**
Total phenolics						1.00	0.88**	0.87**
DPPH							1.00	0.89**
TAC								1.00

Table 5: *Comparative effect of L-Tyrosine concentration on total anthocyanin content and antioxidant activity in Local and Guora black rice varieties*

Variety	L-Tyrosine (mg L^{-1})	Total anthocyanin (mg C3G kg^{-1} DW)	DPPH radical scavenging Activity (%)	TAC (mg AAE g^{-1} DW)
Local black rice	0 (control)	47.2 c	60.4 c	22.4 c
	50	58.7 a	71.3 a	25.8 a
	100	55.6 ab	69.4 ab	24.7 ab
	150	50.8 bc	64.7 bc	23.1 bc
Guora black rice	0 (control)	48.1 c	61.2 c	22.9 c
	50	60.3 a	72.4 a	26.2 a
	100	56.4 ab	70.1 ab	25.0 ab
	150	51.6 bc	65.8 bc	23.5 bc
LSD (0.05)	—	1.64	1.83	0.48

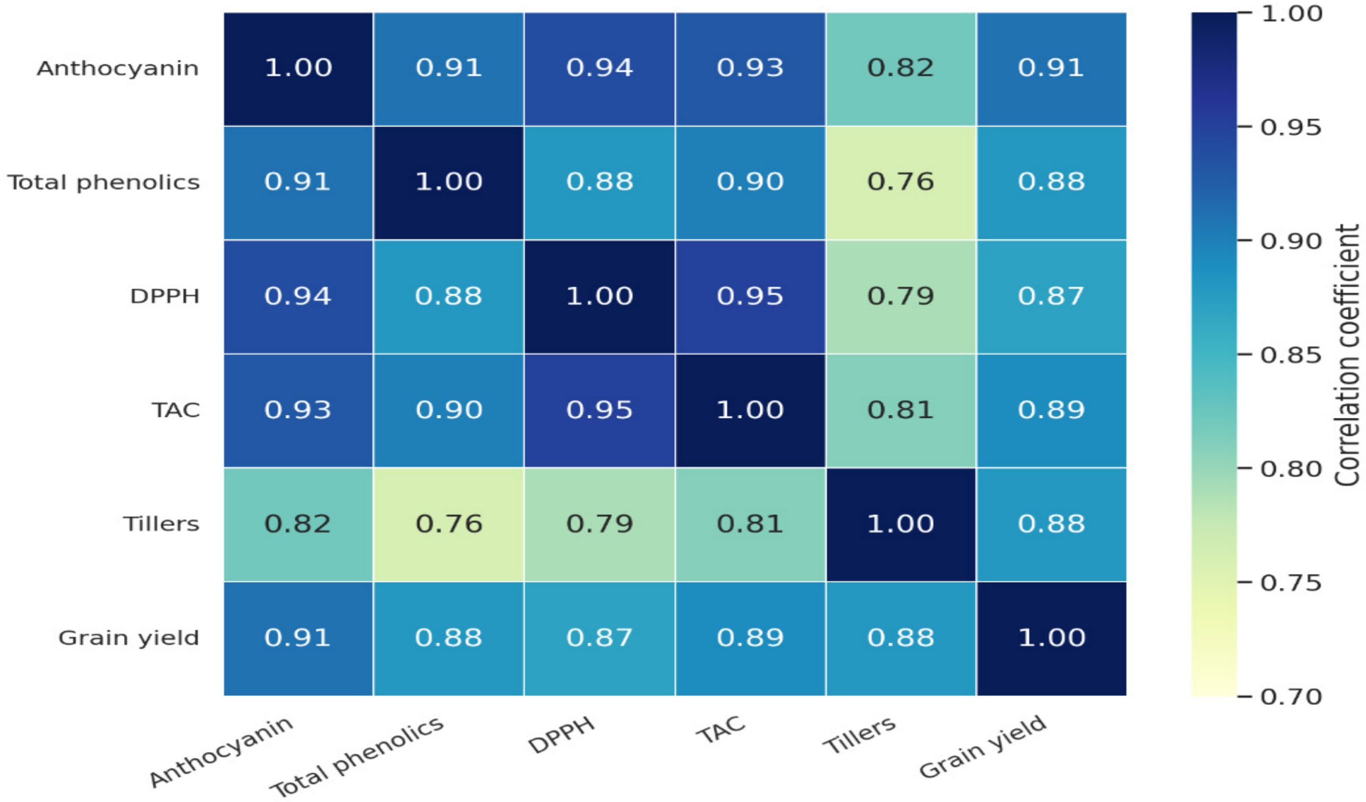

Figure 1: *Heatmap showing correlation coefficients among growth, yield, and biochemical traits*
(Figure displays the correlation heatmap where darker violet tones denote stronger positive correlations (r > 0.9).)

The first axis of the PCA explained 89.4% variance in trait co-variation. PC1 and PC2 depicted 65.8% and 23.6% of variations, respectively. PC1 positively entered growth and yield characters, while PC2 traits had an effect mainly on biochemical traits suggesting a metabolic-physiological crosstalk built up under the influence of L-Tyrosine. Both Local and Guora with LT 50 mg L -1 treatments were located toward the positive direction of PC1 and PC2 on the biplot, suggesting a set composite response of growth vigor vs. roots biochemical capacity. Plants of Guora had higher loading on PC1, which indicates that they derived more advantage from L-Tyrosine supply, whereas the position of Local type along the PC2 hinted towards a greater conversion of biochemical fortification into reduction in yield. This fact confirms that less enzymatic activity is driving the biochemical advancement of Local variety, possibly PAL, TAL, and UGT activity. Therefore, the statistical evidence shows that L-Tyrosine treatment, mainly 50 mg L $^{-1}$, benefits from physiological and root biochemical performance, resulting in increased yield and antioxidant potential. The strong inter-correlation and PCA model also highlight that yield improvement is achieved through the synchronized strategy of growth structures, phenolic metabolism, and oxidative defense.

Comparative Effect of L-Tyrosine on anthocyanin accumulation and antioxidant activity in local and guora black rice varieties

Results in Table 5 showed that the total anthocyanin content and antioxidant activity increased significantly in both black rice varieties by L-Tyrosine foliar application. Enhancements were concentration-dependent up to 50 mg L^{-1}, after which a slight decline indicated the optimal level for secondary metabolism. Total anthocyanin content in the Local black rice increased gradually from 47.2 mg C3G/kg DW in the control to a peak of 58.7 mg kg^{-1} DW at 50 mg L^{-1}, corresponding to a 24% increase. The same trend was observed in the percentage of DPPH radical scavenging activity and total antioxidant capacity from 60.4% to 71.3% and 22.4 to 25.8 mg AAE g^{-1} DW, respectively. Nevertheless, the values reduced slightly at the highest concentrations which could be translated to negative feedback in the phenylpropanoid pathway where excess substrate inhibits the activity of enzymes.

The Guora variety had an analogous pattern with but slightly higher values at all the concentrations tested. The maximum observed anthocyanin content was 60.3 mg C3G kg^{-1} DW at 50 mg L^{-1}, 72.4% DPPH activity, and 26.2 mg AAE g^{-1} TAC, both outperforming the local genotype by just about 3–5%. This difference suggests that Guora possesses a higher genetic capacity for L-Tyrosine-induced activation of TAL and PAL enzymes, thereby improving phenolic and anthocyanin biosynthesis. The difference in anthocyanin and antioxidant indices concerning L-Tyrosine treatments indicates a dual biochemical role : 1. precursory substrate in the TAL pathway, which converts Tyrosine into p-Coumaric acid – an essential intermediate in flavonoid and anthocyanin biosynthesis, 2. a metabolic inducer, stimulating the transcriptional upregulation of genes of structural enzymes, such as CHS, DFR, ANS, UGT, and regulatory factors, such as MYB and bHLH – promoting the pigment accumulation. In addition, the better performance of Guora can be linked to higher antioxidant enzyme activities, positively influencing the cellular redox balance and the stability of pigment molecules. The genotype-dependent L-Tyrosine response was also identified by Mackon and Thilavech the anthocyanin content and the antioxidant strength relationship among black rice and purple barley genotypes. The co-increase in the anthocyanin, DPPH, and TAC values demonstrates that anthocyanin compounds have a controlling function in determining the antioxidant potential. The correlation reflects a functional link between the specific secondary metabolite accumulation and oxidative protection. The reduction movement at larger concentrations might be accountable for either the excessive substrate feedback inhibition of TAL activity or limited cofactor requirement for the flavonoid synthesis. Such findings suggest that the 50 mg L^{-1} L-Tyrosine represents the most optimal foliar dose for the pharmacological stimulation of anthocyanin biosynthesis and antioxidant propensities in both black rice varieties, with Guora standing for merely slightly more metabolic efficiency and potential for bio fortification. This extra-quantitative integrated level of the metabolic response opposes that L-Tyrosine could represent an efficient metabolic bioactivator for the agent definition of both biologically valuable and technological anthocyanins, thus.

Statistical relationship between anthocyanin content and antioxidant activity in local and guora black rice

Statistical analysis

The correlation analysis between anthocyanin content and antioxidant parameters (DPPH and TAC) revealed a highly significant positive association ($p \leq 0.01$) for both rice varieties (Table 6). orrelation coefficients between anthocyanin and DPPH were r = 0.94 (Local) and r = 0.96 (Guora), while those with TAC were r = 0.93 and 0.95, respectively.

This close relationship demonstrates that anthocyanin accumulation is directly linked to antioxidant performance, as both parameters are products of the same metabolic route governed by the *L-Tyrosine*-activated phenylpropanoid pathway. The stronger correlation observed in Guora indicates higher efficiency in converting L-Tyrosine into flavonoid derivatives and in maintaining antioxidant defense.

The regression analysis (Figure 2) confirmed the strong linear relationship between anthocyanin content and DPPH activity in both genotypes, with $R^2 = 0.88$ for Local and $R^2 = 0.91$ for *Guora*. The slopes of the regression lines indicate that a unit increase in anthocyanin concentration results in an approximate 0.9% rise in DPPH scavenging efficiency, confirming the functional link between pigment synthesis and antioxidant activity.

Table 6: *Correlation between anthocyanin content and antioxidant activity (DPPH and TAC) in two black rice varieties*

Variety	Parameter Pair	Correlation Coefficient (r)	Coefficient of Determination (R^2)	Significance
Local Black Rice	Anthocyanin ↔ DPPH	0.94	0.88	$p \leq 0.01$
Local Black Rice	Anthocyanin ↔ TAC	0.93	0.86	$p \leq 0.01$
Guora Black Rice	Anthocyanin ↔ DPPH	0.96	0.91	$p \leq 0.01$
Guora Black Rice	Anthocyanin ↔ TAC	0.95	0.90	$p \leq 0.01$

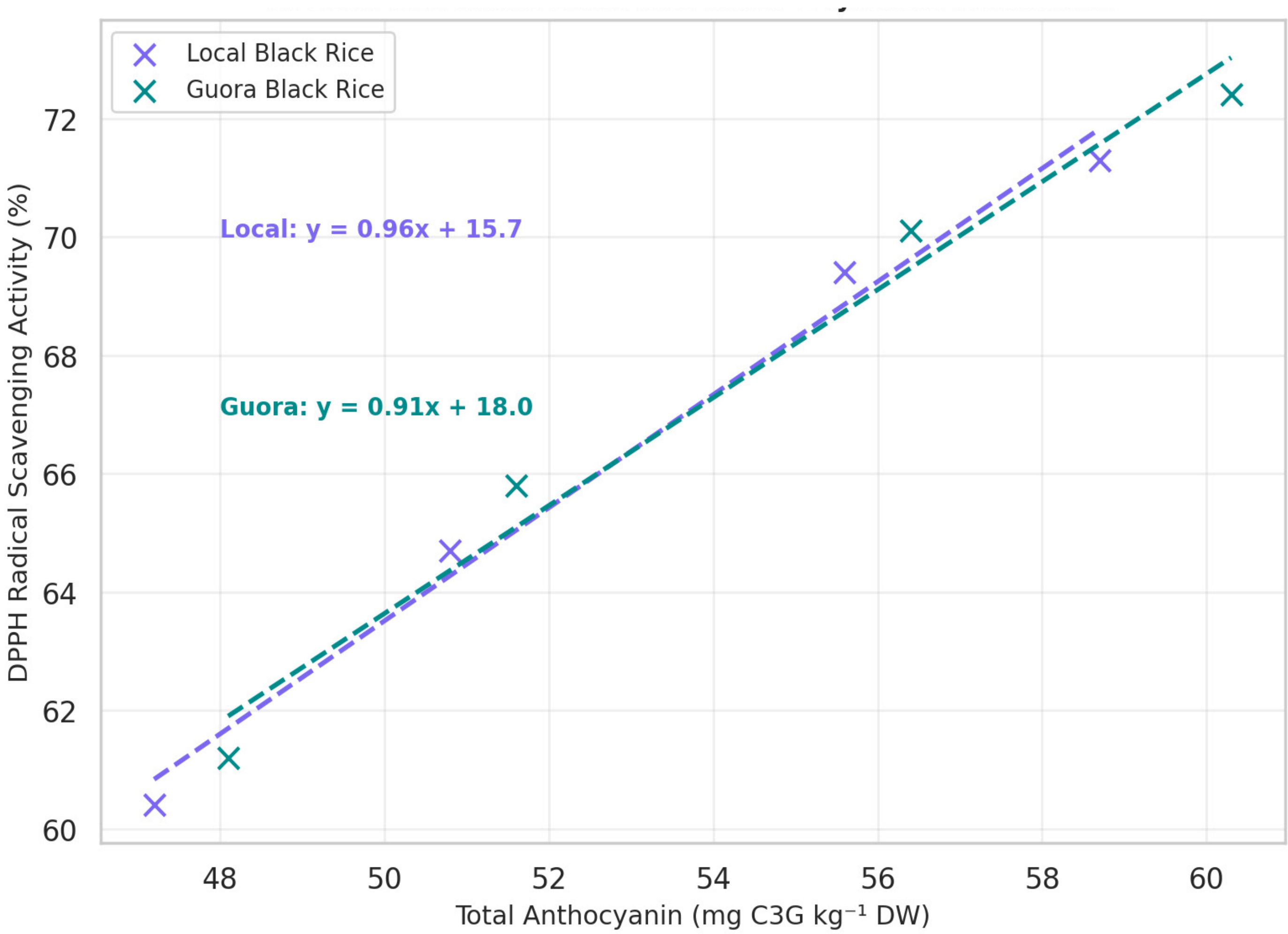

Figure 2: *Linear relationship between total anthocyanin content and DPPH radical scavenging activity in local and guora black rice under different L-Tyrosine concentrations*

Integrated discussion: phenolic compounds, antioxidant mechanisms, and metabolic response to L-Tyrosine

According to Table 7 data, foliar application of L-Tyrosine significantly improved TPC and SOD, CAT, and POD activities in both black rice varieties. Remarkable enhancements were noted at 50 mg L^{-1} and this concentration can, therefore, be considered the most efficient for the induction of phenolic metabolism and enzymatic antioxidant defense system. In Local, the TPC level rose from 2.84 mg GAE g^{-1} DW to 3.72 mg g^{-1} DW at 50 mg L^{-1}, or around 31%. The same positive tendencies were noted for SOD (from 82.1 to 108.3 U mg^{-1} protein), CAT (from 6.9 to 9.4 µmol H_2O_2 min^{-1} mg^{-1} protein), and POD (from 18.4 to 23.8 U mg^{-1} protein). TPC levels in Guora amounted to 3.81 mg g^{-1} DW at 50 mg L^{-1}, L-Tyrosine, meanwhile the SOD, CAT, and POD activities showed increases by 32%, 36%, and 29% respectively compared to the control.

These increases result from L-Tyrosine activation of the shikimate–phenylpropanoid pathway, where it serves as a substrate for tyrosine ammonia-lyase, forming p-coumaric acid an essential intermediate in phenolic biosynthesis. This enhances the pool of redox-active compounds that improve stress tolerance and regulate metabolic balance. The elevated activity of SOD, CAT, and POD in the present study confirms the plant's physiological response to increased phenolic metabolism. These enzymes are the core of the enzymatic antioxidant system and work through SOD acting by catalyzing the dismutation of superoxide radicals insane into hydrogen peroxide, which is then decomposed by CAT and POD. Therefore, the enzymes' correlation is vital in maintaining cellular redox homeostasis, particularly under oxidative stress such as high irradiance or metabolic activity. Therefore, better growth response indicates an increased plant capacity for the detoxifying reactive oxygen species, thereby enhancing membrane permanency and photosynthesis. In the current findings, the Guora variety had slightly higher values across the board, indicating better genotypic utilization of L-Tyrosine for secondary metabolism. The probable explanation would be the higher expression of genes controlling TAL and PAL or more efficient regeneration of the cofactor NADPH in the Guora genotype enhancing sustained phenolic synthesis and antioxidant enzyme activity. Similar results have been observed in purple rice and barley genotypes, indicating the relationship between amino acid-derived phenolic metabolism and antioxidant profile Figure 3.

Figure 3 The comparative effect of L-Tyrosine concentrations on total phenolics and key antioxidant enzymes in Local and Guora black rice. The graphical trend indicates progressive increment of enzyme activities and phenolic accumulation with L-Tyrosine supplementation up to 50 mg L^{-1}, then a slight decline at 100-150 mg L^{-1}. Guora genotype showed higher reading over the Local genotype with respect to all biochemical traits, demonstrating superior metabolic efficiency in supplying phenolic precursors from L-Tyrosine and effective energizing of enzymatic antioxidant defense reactions. This trend corresponds to the enhancement of the phenylpropanoid shikimate pathway by L-Tyrosine, and increases in phenolics biosynthesis and might induce ROS-sequestering enzymatic antioxidative defense in crop. The decrease in TPC and enzyme activities between 100 mg L^{-1} and 150 mg L -1 reflect a feedback inhibition, which is part of the metabolic regulation where

Table 7: *Effect of L-Tyrosine on total phenolic content (TPC) and antioxidant enzyme activity in Local and Guora black rice varieties*

Variety	L-Tyrosine (mg L^{-1})	Total phenolics (mg GAE g^{-1} DW)	SOD activity (U mg^{-1} protein)	CAT Activity (µmol H_2O_2 min^{-1} mg^{-1} protein)	POD Activity (U mg^{-1} protein)
Local black rice	0 (control)	2.84 c	82.1 c	6.9 c	18.4 c
	50	3.72 a	108.3 a	9.4 a	23.8 a
	100	3.48 ab	101.2 ab	8.7 ab	22.6 ab
	150	3.11 bc	91.6 bc	7.8 bc	20.2 bc
Guora black rice	0 (control)	2.91 c	85.3 c	7.2 c	19.1 c
	50	3.81 a	112.5 a	9.8 a	24.6 a
	100	3.54 ab	104.7 ab	8.9 ab	23.1 ab
	150	3.20 bc	94.8 bc	8.0 bc	21.0 bc
LSD (0.05)	—	0.14	4.6	0.5	0.9

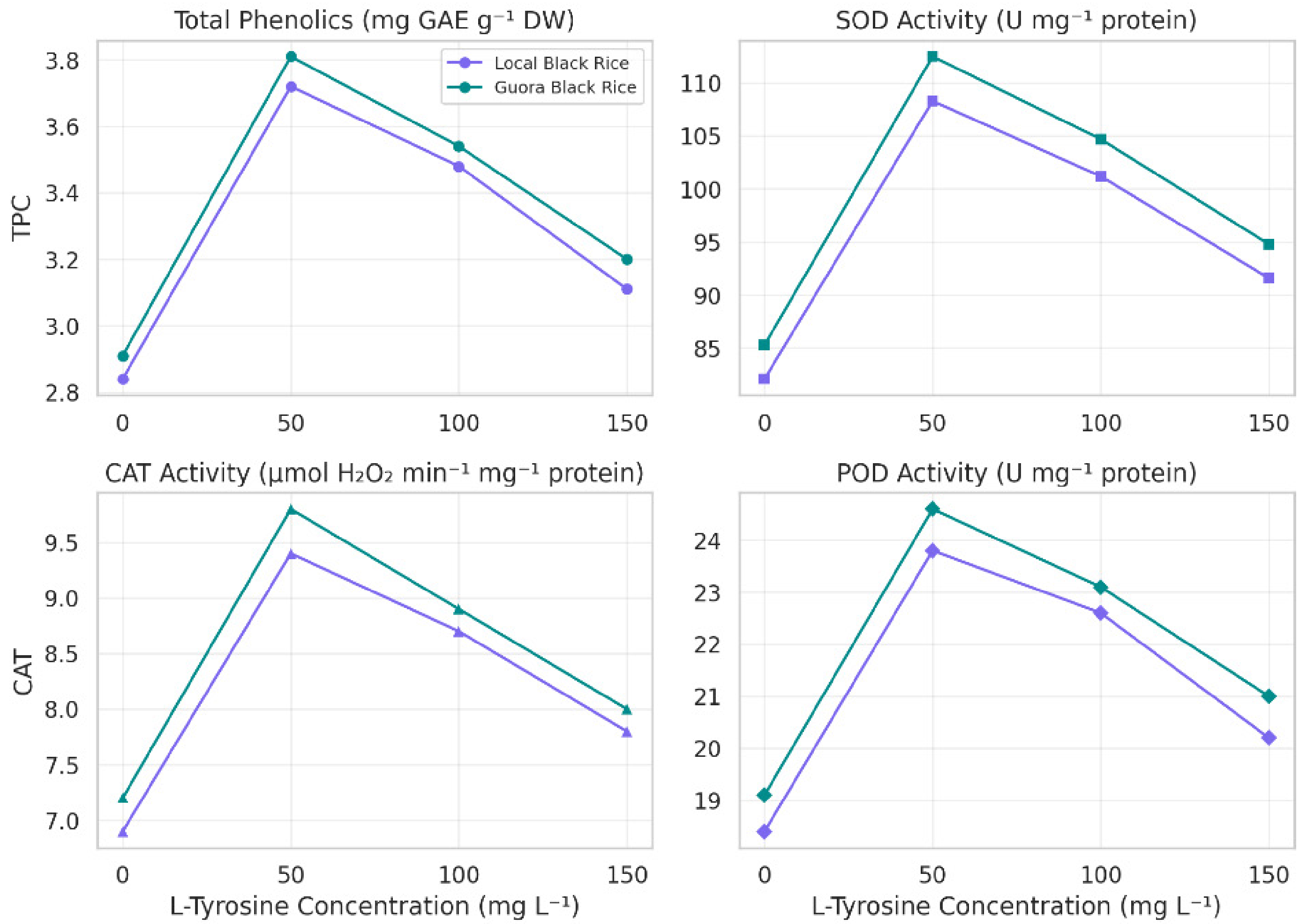

Figure 3: *Effect of L-Tyrosine on phenolic and antioxidant enzyme activities in local and guora black rice varieties*

overabundance of substrate depresses enzyme activity and therefore redox signaling capacity..Our findings showed that there was also prerequisite balance requirement by metabolism for precursor availability as well as enzymatic manipulations processes (for phenolic oriented genes). Finally, L-Tyrosine acts as a a precursor and metabolic signal booster that enhances phytochemical/ enzymatic-mediated antioxidative defence. This co-evolution between enzymatic antioxidants and TPC indicates a joint metabolic selection for an elevated oxidative capacity and to secure grain sterility. This mean that the genotype Guora was more metabolically efficient than the other which makes it better genotype for expression of biofortification, and stress tolerant black rice cultivation.

The results of the maize and citrus studies revealed that the irrigation intervals, mulching techniques and organic amendments significantly increased soil physical quality and crop yield in diverse field situations (Al-Khafagi *et al*., 2025; Hamid *et al*., 2025). Moreover, theoretical approaches in the field of nano-biofertilizers have shown some promising impact in improving soil fertility, nutrient bioavailability and environmental sustainability (Al-Juthery *et al*., 2025). This provides a complementary perspective to thermodynamic analysis of potassium and soil that emphasises the significance of soil–nutrient relationships to the nutrient status in soils and their potential in driving high level plant uptake required for performance maintenance or improvement (Thermo K Study).

Conclusions and Recommendations

The present study demonstrates that leaf application of L-Tyrosine in the form aids biochemical and physiological attributes of black rice genotypes (Var. Local & Guora). The optimal treatment concentration was 50 mg L^{-1}, which could obviously increase the anthocyanin content, total phenolic and antioxidant capacity of cabbage, enhance SOD, CAT, POD activity antioxidant enzymes and then further improve grain yield of cabbage. Guora was more metabolically active indicated by increased anthocyanin and phenolic content, higher antioxidant potential in addition to significantly improved yield than those of Local. LCDs) were prepared, Our chemical data suggest that the synthesis of linkages between primary_AA and secondary_PP pathways are better in G. They tease the possibility that L-Tyrosine as well it doesn't just supplement an amino acid source to roots can stimulate their metabolism from stress resistant whence plausibly for phenolics production and antioxidant defense activity. The association of enzymatic (SOD) and non-enzymatic (DPPH scavenging activity) antioxidant crops with the identified components indicate that it may be biofotified for being transformed into a nutritionally fortified functional pigmented rice. Thus, our results demonstrate that the exogenous L-Tyr supplied at 50 mg L^{-1} could serve as an experimental physiological regulator to increase black rice yield and functional quality products especially in phenolic responsive variety like Guora. These findings may provide information for developing sustainable biofortification practices in a rice ecosystem.

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Novelty Statement

The work provides novel evidence linking L-Tyrosine–induced phenylpropanoid metabolism with coordinated improvements in enzymatic and non-enzymatic antioxidant systems and grain productivity.

Author's Contribution

Jawad Kadhim Ali: Conceptualization, experimental design, field work, data analysis, and manuscript writing.
Ali Adnan Al-Akkam: Methodology, biochemical analysis, and interpretation of results.
Hasanin S. Oleiwi: Statistical analysis and data validation.
Salam A. Khuit: Supervision, critical revision, and final approval of the manuscript.

Generative AI or AI assisted technology statement
The authors declare that no genrative AI was used in the creation of this manuscript.

Conflict of interest
The authors have no conflict of interest.

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