Research Article

Evaluating the Impact of Copper-Induced Oxidative Stress on Growth and Nutrient Profiles in JP-5 and Super Basmati Rice Cultivars

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Introduction

Rice (Oryza sativa L.) is a well-known cereal crop that is widely cultivated throughout the world [27]. It is the second most consumed cereal and is enriched with several nutritional components including proteins, carbohydrates, phenolics, and antioxidants concentrated in its starchy endosperm [10]. However, recent literature regarding the contamination of paddy fields with heavy metal stress around the world has raised alarming concerns [21,58]. One such heavy metal well known for its toxicity in rice crops is Copper (Cu), which is released into the ecological environment due to various factors including Cu parent materials, mining, consumption of wastewater, and Cu-based agrochemicals [28,39]. It is responsible for adversely affecting the growth and development of rice by hindering biochemical and physiological processes, including respiration, nitrogen metabolism, photosynthesis, protein metabolism, mineral uptake, and oxidative stress responses [16,17]. Cu is also reported to

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Abstract

The rapidly increasing concentration of Copper (Cu) metal in agricultural soils around the world is alarming for food security and sustainable production of crops. Cu being a naturally hydrophilic metal is easily taken up by crops through roots and translocated to upper parts. Rice (Oryza sativa L.) is one of the most consumed cereal crops around the world. The incidence of Cu toxicity in rice is well-known for hindering crop biomass and overall productivity. Therefore, it is important to study Cu stress in rice and identify Cu-tolerant cultivars. For that purpose, two rice cultivars (JP-5 and Super Basmati) were grown in paddy conditions under 100 mg/kg Cu stress in a completely randomized design. Both cultivars were then examined for agronomic production, antioxidant defense, nutritional composition, and germination indices. It was reported that JP-5 accumulated a lesser concentration of copper in roots (0.08 mg/kg), and grains (0.05 mg/kg) as compared to SB (0.20 mg/kg in roots and 0.05 mg/kg) under Cu stress. SB showed better response to agronomic parameters whereas JP-5 showed better germination rate and stress tolerance index. under Cu stress, JP-5 also showed higher SOD, POD, GPX, and APX in both root and leaf tissues compared to SB. The sugar and starch content of SB was more affected by Cu stress. Overall, JP-5 proved to be more tolerant against Cu stress with a higher stress tolerance index and lesser accumulation of Cu. These findings are thus very useful for further studies related to enhanced growth and yield of widely cultivated rice cultivars under heavy metal stress.

Keywords: Cu toxicity; Rice; Antioxidant defense mechanism; Agronomy; Nutritional profiling; Tolerance

affect seed germination, nutritional profile, oxidative homeostasis, and normal physiology of cells thus leading to an acute reduction in the overall productivity of the crop [15,40]. The excessive concentration of Cu in rice tissues is reported to induce the oxidative burst, which results in an overabundance of numerous Reactive Oxygen Species (ROS) that are inimical to plant physiology. The prominent ROS including superoxide radicals $(O_2, -)$, Hydroxyl radical (OH), and Hydrogen Peroxide (H_2, O_2) further damage the plasma membrane, trigger oxidative stress, and disrupt metabolism as well as physiological responses [45]. Plants have an in-built antioxidant defense system to counteract oxidative damage caused by heavy metals. This includes various enzymes to prevent oxidative damage, such as superoxide dismutase (SOD) protein, which catalyzes the dismutation process of highly toxic O_2 - to less toxic H_2O_2 , which is further converted into H₂O by several enzymes including Peroxidases (POD), Guai-

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acol Peroxidase (GPX), Catalases (CAT), and Ascorbate Peroxidase (APX), along with non-enzymatic metabolite Glutathione (GSH), which is a low molecular weight antioxidant [9,26]. SOD is also responsible for converting $O_2^{\bullet-}$ into H_2O_2 that is further converted into H_2O via APX, GPX, POD, and CAT enzymes [8]. Besides, GSH also function as a potent non-enzymatic antioxidants to directly scavenge the production of ROS [4].

Recent literature has vastly reported the excessive concentration of Cu in various regions of Pakistan, ranging from less than 6 to 412 mg/kg, which was way above the permissible limit of Cu in soils set by World Health Organization (W.H.O), i.e., 36 mg/kg [53,55]. Such extensively high concentration of Cu affects food safety, thus threatening human health [57]. One of the most important ways to tackle heavy metal stress is to identify and develop heavy metal tolerant cultivars via holistic assessment of plant response under stress conditions [6,43]. Therefore, it is imperative to identify and develop rice cultivars tolerant to Cu contamination. Current study thus aims to understand the antioxidant defense mechanism of two rice cultivars in response to agronomic alteration triggered by Cu stress and to compare the bioaccumulation and uptake of copper in both rice cultivars to assess their respective tolerance to Cu stress. Moreover, it also aims to provide a comprehensive profile of nutrient imbalance under exceeded level of Cu as well as the impact of Cu stress on the germination pattern of rice seeds.

Materials and Methods

Plant Material and Experimental Layout

The healthy and equal sized seeds of two highly consumed rice (Oryza sativa L.) cultivars (JP-5 and super basmati) sourced from the Pakistan Agricultural Research Center (PARC), Pakistan, were grown in paddy soil. Selection of cultivars was accomplished by considering the tolerance capacity and quality of grains [14,19]. The seeds underwent surface sterilization in a 20% Hydrogen Peroxide (H₂O₂) solution with continuous agitation for 15 min and then rinsed three times with dH₂O. 30-dayold seedlings grown in paddy soil were shifted into equal sized pots containing air dried, sifted, and sterilized mixture of sand and soil (5:1), respectively. Each pot containing 6-7 plant seedlings was saturated, maintaining a 1-3 cm water layer above the soil surface throughout the growth phase. After 7 days of transplantation, Cu stress was induced by applying 100mg/kg of Cu as copper sulfate. Cu concentration was kept higher than the WHO permissible limit of the heavy metal levels in soil [56]. The experiment was performed with three replications in a completely randomized experimental design.

After harvesting, various agronomic traits including Panicle Length (PL), Plant Height (PH), Spikelets Per Panicle (Sp/P), Grain Yield (GY), Tillers per Plant (T/P), Biological Yield (BY), Thousand-Grain Weight (TGW) (the weight of thousand unhusked rice grains), and panicle per plant (P/P) were recorded following the method proposed by Abedin et al., [1]. The length and width of the flag leaf were measured to determine the flag leaf area (FLA) during the heading and anthesis stages [22]. Using a SPAD-502 device, the amount of chlorophyll in leaves was measured at several growth phases, including tillering, booting, heading, and anthesis. To calculate the germination index of grains before sowing, well sterilized seeds were grown in petri plates on Whatman filter paper under control and Cu stress conditions for two weeks. Various germination parameters were recorded according to Hayat et al., [19].

Evaluation of Cu uptake and Accumulation

The SE [44] method for the digestion of samples in acid, was followed to measure the accumulation of Cu metal in the soil, and plant tissues including root, leaf and most importantly, grains. An FAAS-AA7000 Shimadzu flame atom absorption spectrophotometer was used to measure the filtrate's Cu concentration. Furthermore, to assess the transport of Cu from soil to root, leaf, and ultimately grains, Translocation Factor (TF), Biological Concentration Factor (BCF), and Biological Accumulation Factor (BAF) were calculated. BAF was calculated following the method of Zhuang et al., [59], while TF and BCF were measured according to Soares et al., [46], respectively.

Analysis of Stress Tolerance Indices

To reveal the extent of tolerance, Tolerance Index (TOL), Stress Susceptibility Index (SSI), Stress Tolerance Index (STI), Mean Productivity index (MP), Geometric Mean Productivity (GMP), Harmonic Mean (HM) of both cultivars were calculated according to Mahdavi et al., [34] method. Moreover, F. Khan and Mohammad, [29] method was followed for evaluation of Yield Stability Index (YSI), and Yield Index (YI).

Oxidative Stress Markers

MDA and H_2O_2 analysis: The peroxidation level of lipid was assessed by measuring Malondialdehyde (MDA) content following Heath and Packer, [20] method. For Hydrogen Peroxide (H_2O_2) estimation, Velikova et al., [50] method was followed.

Enzymatic Antioxidants Assay

Fresh leaves were crushed in 0.05M buffered potassium phosphate (PPB) (pH 7.8) in order to prepare the extract, and the mixture was then centrifuged at 10,000 rpm for 20 minutes. In preparation for further analysis, the supernatant was obtained and kept at 4°C. The Nitro-Blue Tetrazolium chloride (NBT) technique was used to photochemically assess the activity of SOD (EC 1.15.1.1) [11]. For CAT (EC 1.11.1.6) activity, the method of Aebi, [3] was followed. The activity of POD (EC 1.11.1.7) was assessed using method proposed by Lundquist and Josefsson [33]. Similarly, activity of APX (EC 1.11.1.11) and GPX (EC 1.11.1.9) was estimated following Nakano and Asada, [38] and Nagalakshmi and Prasad, [37] described method.

Non-Enzymatic Antioxidants Assay

Total Antioxidant Capacity (TAC) and Total Reducing Power (TPC) were measured according to the method reported by Prieto et al., and Kumar et al., [31,42]. The estimation of reduced Glutathione (GSH), oxidized Glutathione (GSSG), and Total Glutathione (TG) was performed according to Anderso, [7] method.

Determination of Carbohydrate, Starch, and Protein

The nutritional profile of harvested grains was measured in the form of carbohydrate, protein, and starch content. Anthrone method was used for estimation of total soluble sugars Blanche et al., [12]. Non-reducing sugar was measured by method proposed by Malhotra and Sarkar, [35], while reducing sugar was calculated by subtracting the value of non-reducing sugar from total sugar content. For estimation of starch content, Mukhopadhyay et al., [36] method was followed. Method reported by Peterson, [41] was used for the estimation of the grain protein content (mg/g).

Statistical Analysis

Various tools were employed for statistical analyses. F-test (one-way ANOVA) was performed using the statistical software XLStat 2024. Correlation analysis between varieties and treatments was conducted using IBM SPSS Statistics (v25), and the results were visualized using the GGally package in RStudio.

Results

Evaluation of Germination Index

Copper (Cu) stress induced reduction in Germination Percentage (GP), Root Length (RL), Shoot Length (SL), seedling dry weight and water uptake percentage as compared to control in both cultivars (Table 1). SB showed 53.32% while JP-5 exhibited 42.86% reduction in GP under Cu stress. Similarly, Cu stress significantly reduced SL and RL in both cultivars where JP-5 showed 61.11% decrease in SL and 90.85% decrease in RL while SB exhibited 65.2% decrease in SL and 83.94% reduction in RL compared to control. An equal reduction in GI was observed in both cultivars under Cu stress. Regarding MGT, Cu stress induced significant extension of duration in both cultivars at equal rates. WUP showed lesser reduction in JP-5 (8.08%) compared to SB (15.55%) under Cu stress. DW was reduced in both cultivars with JP-5 showing 10.11% reduction more than that observed in SB (8.72%). FW was reduced by 8.43% in SB however, JP-5 exceptionally showed elevation of FW by 10.26% under Cu stress (Figure 1).

Table 1: ANOVA (p-Table) for the germination indices of JP-5 and SB

 rice cultivars under copper and control conditions.

Param- eters	Treat- ments	JP-5	SB	ANOVA (p-value)	
Shoot	Control	4.8±0.22 (100%)	5.38±0.76 (100%)		
length (cm)	Treatment	1.87±0.33(-61.11%)	1.87±0.37(-65.28%)	ns	
Root	Control	5.47±0.37 (100%	6.43±0.45 (100%)	*	
length (cm)	Treatment	0.5±0.08(-90.85%)	1.03±0.12(-83.94%)		
Dry	Control	29.67±2.36 (100%	26.33±2.05 (100%)		
weight (mg)	Treatment	26.67±4.99(-10.11%)	24.04±0.81(-8.72%)	ns	
Fresh	Control	39±2.94 (100%	35.67±0.94 (100%)		
Weight (mg)	Treatment	43±4.24(10.26%)	32.66±0.18(-8.43%)	ns	
Water	Control	41.89±4.74 (100%	35.53±4.76 (100%)		
Uptake Per- centage (%)	Treatment	38.5±6.35(-8.08%)	30.01±0.48(-15.55%)	ns	
Seed	Control	9.71±0.08 (100%	14.4±0.08 (100%)	**	
Vigor	Treatment	4.79±0.14(-50.64%)	4.97±0.14(-65.46%)		
Germi-	Control	11.43±0.08 (100%	10.57±0.08 (100%)		
nation Index	Treatment	4.5±0.08(-60.63%)	4.19±0.16(-60.33%)	**	
Mean	Control	12.64±0.54 (100%	11.38±0.08 (100%)		
Germi- nation Time	Treatment	4.84±0.08(-61.7%)	4.34±0.08(-61.83%)	**	
Germi-	Control	93.33±0.08 (100%	100±0.08 (100%)		
nation Percent- age	Treatment	53.33±0.08(-42.86%)	46.68±0.08(-53.32%)	***	
Relative	Control	0±0 (100%	0±0 (100%)		
Injury Rate	Treatment	0.42±0.01(419900%)	0.57±0.02(573233.3%)	***	

Evaluation of Agronomic Traits

Cu contamination showed a notable influence on agronomic traits of both cultivars (Table 2). PH was observed to be increas-**Table 2:** ANOVA (p-Table) for the agronomic traits and stress tolerance indices of JP-5 and SB rice cultivars under control and copper conditions.

Plant Height (cm) Control 71.1743.7 (100%) 50.037.64 (100%) Ns Panicle Control 23.1742.01 (100%) 27.331.43 (100%) Ns Panicle Control 23.1742.01 (100%) 27.331.43 (100%) Ns Length (cm) Treatment (-13.81%) (-19.76%) Ns Days To Til- Control 45.40 (100%) 6.610 (100%) extent Chlorophyll Treatment 24.311.71 (100%) 24.111.51 (100%) extent Days To Control 65.310.47 (2.5%) 86.330.47 (1.5%) extent Days To Control 65.310.47 (2.5%) 85.330.47 (1.07%) extent Days To Control 70.01 (00%) 90.01 (100%) extent Days To Control 70.21 (00%) 90.21 (100%) extent Atthesis Treatment 70.32 (100%) 33.21 ±7.5 (100%) extent Atthesis Treatment 33.21 ±5.1 (00%) 33.21 ±5.1 (00%) extent Atthesis Treatment 33.21 ±0.1 (3.31 ± 7.5 (100%) extent	Parameters	Treat- ments	JP-5	SB	ANO- VA (p- value)
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at Heading Treatment 30.2840.53 (-11%) 31.1140.51 (-9.2%) Tris Days To Anthesis Treatment 80.67±0.47 (0.83%) 102.67±0.47 *** Chlorophyll Control 33.27±1.59 (100%) 33.1±1.75 (100%) *** dt Anthesis Treatment 33.1±0.45 (-0.53%) 33.3±0.36 (0.67%) **** Maturation Treatment 116.3±0.47 (1.16%) (0.81%) ***** Flag Leaf Control 13.2±1.09 (100%) 13.4±0.42 (100%) Acfeuas 6 (100%) Area at Incertains 11.09±0.53 (-4.56%) (-1.91%) ns Flag Leaf Control 13.3±1.68 (100%) 13.49±1.42 (100%) Acfeuas 6 (100%) Area at Incertains Incertains Ins ns ns (Cm ⁷) Incertains 13.2±0.47 (22.22%) 5±0.82 (7.14%) ns Pilant Treatment 12.7±0.28 (-4.51%) 13.9±1.42 (100%) ns Spikelets Control 3±0 (100%) 4.67±1.25 (100%) ns Pilant Treatment <	Chlorophyll	Control	34.03±0.34 (100%)	34.27±1.51 (100%)	ns
Days To Anthesis Control 80±0 (100%) 102±0 (100%) *** Anthesis Treatment 80.67±0.47 (0.83%) (0.65%) *** Chlorophyll Control 33.27±1.59 (100%) 33.1±1.75 (100%) * at Anthesis Treatment 33.1±0.45 (-0.53%) 33.32±0.36 (0.67%) **** Days To Control 115±0 (100%) 165.50 (100%) **** Maturation Treatment 116.33±0.47 (1.16%) (0.81%) **** Flag Leaf Control 11.62±1.09 (100%) 13.49±1.42 (100%) Area at Area at Incertain 11.09±0.53 (-4.56%) (-1.91%) rs (cm ²) Control 13.3±1.68 (100%) 13.49±1.42 (100%) Area at Arthesis Treatment 12.7±0.28 (-4.51%) 13.92±0.11 (3.21%) ns Spikelets Control 3.40 (100%) 4.67±1.25 (100%) ns per Plant Treatment 17.33±0.47 30.67±3.09 ns Spikelets Control 3.4±0.13 (10.21%) 3.62±0.15 (100%)	at Heading	Treatment	30.28±0.53 (-11%)	31.11±0.51 (-9.2%)	115
Anthesis Treatment 80.67±0.47 (0.83%) 102.67±0.47 (0.65%) 102.67±0.47 (0.65%) Chlorophyll Control 33.27±1.59 (100%) 33.1±1.75 (100%) * at Anthesis Treatment 33.1±0.45 (-0.53%) 33.32±0.36 (0.67%) * Days To Control 115±0 (100%) 165±0 (100%) **** Maturation Treatment 116.2±1.09 (100%) 13.46±0.36 (100%) **** Heading Treatment 11.09±0.53 (-4.56%) (-1.91%) **** (cm ²) I 13.2±0.25 (100%) ns **** Anthesis Treatment 12.7±0.28 (-4.51%) 13.92±0.11 (3.21%) ns Spikelets Control 33.04.7 (-22.23%) 5±0.82 (7.14%) ns Spikelets Control 23.3±0.47 (-22.23%) 5±0.82 (7.14%) ns Spikelets Control 3.4±0.12 (100%) 3.67±3.09 ns Grain Yield Control 3.4±0.41 (-36.25%) 1.2±0.14 (-36.15%) ns Grain Yield Control 3.4±0.21 (100%) 3.27±0.09 (100%)<	Days To	Control	80±0 (100%)	102±0 (100%)	**
$\begin{array}{c ccccc} Colorophyll Control 33.27\pm 1.59 (100%) 33.11.75 (100%) at Anthesis Treatment 33.1\pm 0.45 (-0.53%) 33.32\pm 0.36 (0.67\%) Treatment 33.1\pm 0.45 (-0.53\%) 33.32\pm 0.36 (0.67\%) Treatment 115\pm 0 (100%) 165\pm 0 (100\%) Treatment 116.33\pm 0.47 (1.16\%) 166.33\pm 0.47 (0.81\%) Treatment 116.33\pm 0.47 (1.16\%) (0.81\%) Treatment 116.33\pm 0.47 (1.16\%) (0.81\%) Treatment 116.33\pm 0.47 (1.16\%) (1.321\pm 0.25 (-1.91\%) Treatment 11.09\pm 0.53 (-4.56\%) (-1.91\%) Treatment 12.7\pm 0.28 (-4.51\%) 13.92\pm 0.11 (3.21\%) Treatment 2.33\pm 0.47 (-22.22\%) 5\pm 0.82 (7.14\%) Treatment (-25.71\%) (-16.36\%) Treatment 2.33\pm 0.47 (30.67\pm 0.24 (100\%) Treatment 2.33\pm 0.47 (30.67\pm 0.24 (100\%) Treatment 2.33\pm 0.47 (30.67\pm 0.24 (100\%) Treatment 1.42\pm 0.14 (-3.62\%) 1.2\pm 0.14 (-3.611\%) Treatment 3.41\pm 0.13 (-11.21\%) (-6.01\%) Treatment 3.41\pm 0.13 (-11.21\%) (-6.01\%) Treatment 3.41\pm 0.13 (-11.21\%) (-6.01\%) Treatment 1.42\pm 0.14 (-3.62\%) 1.2\pm 0.41 (100\%) Treatment 3.41\pm 0.13 (-11.21\%) (-6.01\%) Treatment 4.14\pm 0.13 (-11.21\%) (-6.01\%) Treatment 4.14\pm 0.13 (-0.75\%) (-0.79\pm 0.03 (-0.79\%) Treatment 5.2\pm 0.12 (-2.65\%) 5.49\pm 0.1 (5.49\%) Treatment 5.4\pm 0.13 (0.00\%) (0.05\pm 0.00\%) Treatment 5.4\pm 0.01 (0.00\%) (-0.05\pm 0.45\%) Treatment 5.2\pm 0.12 (-0.26\%) 5.49\pm 0.1 (5.49\%) Treatment 6.26\pm 0.12 (6.26\%) 5.49\pm 0.1 (5.49\%) Treatment 6.26\pm 0.12 (6.26\%) 5.49\pm 0.1 (5.49\%) Treatment 0.18\pm 0.01 (0.30\%) 0.06\pm 0.00\%$ Treatment 0.18\pm 0.01 (0.30\%) 0.06\pm 0.010\% Treatment 0.18\pm 0.01 (0.30\%) 0.06\pm 0.010\% Treatment 0.18\pm 0.01 (0.30\%) 0.3\pm 0.01 (0.31\%) Treatment 0.18\pm 0.01 (0.30\%) 0.3\pm 0.01 (0.31\%) Treatment 0.18\pm 0.01 (0.30\%) 0.3\pm 0.01 (0.31\%) Treatmen	Anthesis	Treatment	80.67±0.47 (0.83%)	102.07±0.47	
at Anthesis Treatment 33.12to.45 (-0.53%) 33.32to.36 (0.67%) *** Days To Maturation Control 115±0 (100%) 165±0 (100%) **** Maturation Treatment 116.33±0.47 (1.16%) (0.81%) **** Flag Leaf Control 11.62±1.09 (100%) 13.46±0.36 (100%) Area at Heading Treatment 11.09±0.53 (-4.56%) (1.321±0.25) ns (cm²) Control 13.3±1.68 (100%) 13.49±1.42 (100%) Area at Anthesis Treatment 12.7±0.28 (-4.51%) 13.92±0.11 (3.21%) ns Spikelets Control 23.3±0.47 (-22.22%) 5±0.82 (7.14%) ns Spikelets Control 23.3±0.47 (-22.22%) 5±0.82 (7.14%) ns Spikelets Control 23.3±0.47 (-22.22%) 5±0.82 (7.14%) ns grain Yield Control 1.7.33±0.47 30.67±3.09 ns grain Yield Control 1.47±0.34 (100%) 3.27±0.09 (100%) ns (g) Treatment 3.41±0.13 (-11.21%) 3.07±0.11	Chlorophyll	Control	33.27±1.59 (100%)	33.1±1.75 (100%)	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	at Anthesis	Treatment	33.1±0.45 (-0.53%)	33.32±0.36 (0.67%)	*
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Naturation Institution Institution (0.81%) Flag Leaf Control 11.62±1.09 (100%) 13.46±0.36 (100%) Area at Institution Institution Institution Heading Treatment 11.09±0.53 (-4.56%) Institution Flag Leaf Control 13.3±1.68 (100%) 13.49±1.42 (100%) Area at Anthesis Irreatment 12.7±0.28 (-4.51%) 13.92±0.11 (3.21%) Grave at Irreatment 2.33±0.47 (-22.22%) 5±0.82 (7.14%) ns Spikelets Control 23.33±1.89 (100%) 36.67±6.24 (100%) ns Plant Treatment 1.42±0.14 (-3.62%) 1.2±0.14 (-3.611%) ns Spikelets Control 1.47±0.34 (100%) 3.82±0.15 (100%) ns Grain Yield Control 1.47±0.34 (100%) 3.27±0.09 (100%) ns Grain Yield Control 1.918±0.61 (100%) 16.35±0.46 (100%) ns Grain Yield Control 0.918±0.61 (100%) 16.35±0.46 (100%) ns Treatment 0.70.310.65	Days IO	Treatment	116 33+0 47 (1 16%)	166.33±0.47	* * *
Flag Leaf Control 11.62±1.09 (100%) 13.46±0.36 (100%) Area at Treatment 11.09±0.53 (-4.56%) 13.21±0.25 (-1.91%) ns (cm²) (-1.91%) (-1.91%) (-1.91%) ns Area at Image: Control 13.3±1.68 (100%) 13.49±1.42 (100%) Area at Anthesis Treatment 12.7±0.28 (-4.51%) 13.92±0.11 (3.21%) ns (cm²) Image: Control 3±0 (100%) 4.67±1.25 (100%) ns Spikelets Control 2.33±0.47 (-22.22%) 5±0.82 (7.14%) ns Spikelets Control 1.47±0.34 (100%) 3.6.67±6.24 (100%) ns Spikelets Control 1.47±0.34 (100%) 1.8±0.15 (100%) ns grain Yield Control 1.47±0.34 (100%) 3.27±0.109 (100%) ns freatment 1.42±0.13 (-11.21%) 3.07±0.11 (-6.01%) ns Thousand Control 19.18±0.61 (100%) 16.35±0.46 (100%) ns Grain Treatment 17.03±0.65 15.37±0.56 ns Stress tole=moce indices Stress tole=moce indices stress tole=moce indices <td< td=""><td>Iviaturation</td><td>incutinent</td><td>110.0010.47 (1.1070)</td><td>(0.81%)</td><td></td></td<>	Iviaturation	incutinent	110.0010.47 (1.1070)	(0.81%)	
Area at Heading (cm ²) Treatment 11.09 \pm 0.53 (-4.56%) 13.21 \pm 0.25 (-1.91%) ns Flag Leaf Anthesis Control 13.3 \pm 1.68 (100%) 13.49 \pm 1.42 (100%) Area at Anthesis Treatment 12.7 \pm 0.28 (-4.51%) 13.92 \pm 0.11 (3.21%) ns (cm ²) Treatment 2.33 \pm 0 (100%) 4.67 \pm 1.25 (100%) ns Plant Treatment 2.33 \pm 0.47 (-22.22%) 5 \pm 0.82 (7.14%) ns Spikelets Control 2.33 \pm 0.47 (-22.22%) 5 \pm 0.82 (7.14%) ns Spikelets Control 1.47 \pm 0.34 (100%) 3.667 \pm 3.00%) ns grain Yield Control 1.47 \pm 0.34 (100%) 1.88 \pm 0.15 (100%) ns Grain Yield Control 1.42 \pm 0.14 (-3.62%) 1.2 \pm 0.14 (-36.11%) ns Thousand Control 19.18 \pm 0.61 (100%) 16.35 \pm 0.46 (100%) ns Grain Yield Control 19.18 \pm 0.61 (100%) 16.35 \pm 0.46 (100%) ns Stress tole=	Flag Leaf	Control	11.62±1.09 (100%)	13.46±0.36 (100%)	
$\begin{array}{c} \mbox{Heading} & \mbox{Ireatment} & \mbox{II.09\pm0.53} (-4.56\%) & (-1.91\%) & (-1.9$	Area at	T	44 00:0 52 (4 5 60()	13.21±0.25	ns
$\begin{array}{ cm' \\ Flag Leaf \\ Flag Leaf \\ Area at \\ Anthesis \\ Treatment \\ 12.7\pm0.28 (-4.51\%) \\ 13.92\pm0.11 (3.21\%) \\ (cm') \\$	Heading	Ireatment	11.09±0.53 (-4.56%)	(-1.91%)	
PriorControl13.3±1.88 (100%)13.49±1.42 (100%)Area at AnthesisTreatment12.7±0.28 (-4.51%)13.92±0.11 (3.21%)ns(cm²)Treatment2.3±0.47 (-22.22%)5±0.82 (7.14%)nsPlantTreatment2.33±0.47 (-22.22%)5±0.82 (7.14%)nsSpikeletsControl23.33±1.89 (100%)36.67±6.24 (100%)nsper PlantTreatment(-25.71%)(-16.36%)nsBiologicalControl1.47±0.34 (100%)1.88±0.15 (100%)nsGrain YieldControl3.84±0.12 (100%)3.27±0.09 (100%)nsGrain YieldControl19.18±0.61 (100%)3.07±0.11 (-6.01%)nsThousandControl19.18±0.61 (100%)16.35±0.46 (100%)nsGrainTreatment17.03±0.6515.37±0.56nsWeight (g)Treatment-0.87±0.03 (-0.87%)-0.79±0.03 (-0.79%)***ToleranceControl0±00±0***IndexTreatment7.46±0.07 (7.46%)6.44±0.05 (6.44%)***MeanControl0±00±0***Mean Pro- ductivityTreatment6.26±0.12 (6.26%)5.49±0.1 (5.49%)***Stress Toler- control0±00±0***Mean Pro- ductivityTreatment0.3±0.01 (0.3%)0.06±0 (0.06%)***Yield IndexTreatment0.9±0 (0.09%)0.06±0 (0.06%)***Yield Stabil- Vield Stabil-Control0±00±0***Yiel	(cm ²)	Control	12 2+1 69 (100%)	12 40+1 42 (100%)	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Aroa at	Control	13.3±1.08 (100%)	13.49±1.42 (100%)	
$\begin{array}{c} \mbox{Altrices} & \mbox{Investment} & \$	Area at	Treatment	12 7+0 28 (-4 51%)	13 92+0 11 (3 21%)	ns
$\begin{array}{c} \mbox{Chirol} & 3\pm 0 (100\%) & 4.67\pm 1.25 (100\%) \\ \mbox{Plant} & Treatment & 2.33\pm 0.47 (-22.22\%) & 5\pm 0.82 (7.14\%) \\ \mbox{Presented} & Treatment & 2.33\pm 1.89 (100\%) & 36.67\pm 6.24 (100\%) \\ \mbox{Spikelets} & Control & 23.33\pm 1.89 (100\%) & 36.67\pm 6.24 (100\%) \\ \mbox{Per Plant} & Treatment & 17.33\pm 0.47 & 30.67\pm 3.09 \\ \mbox{Treatment} & 1.7.33\pm 0.47 & 30.67\pm 3.09 \\ \mbox{Treatment} & 1.42\pm 0.14 (-3.62\%) & 1.2\pm 0.14 (-36.11\%) \\ \mbox{Spikeletg} & Treatment & 1.42\pm 0.14 (-3.62\%) & 1.2\pm 0.14 (-36.11\%) \\ \mbox{Srield} & Control & 3.84\pm 0.12 (100\%) & 3.27\pm 0.09 (100\%) \\ \mbox{(g)} & Treatment & 3.41\pm 0.13 (-11.21\%) & 3.07\pm 0.11 (-6.01\%) \\ \mbox{Thousand} & Control & 19.18\pm 0.61 (100\%) & 16.35\pm 0.46 (100\%) \\ \mbox{Grain} & Treatment & (-11.21\%) & (-6.01\%) \\ \mbox{Stress tolerance indices} \\ \mbox{Stress tolerance indices} \\ \mbox{Stress Sus} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{ceptibility} & Treatment & -0.87\pm 0.03 (-0.87\%) & -0.79\pm 0.03 (-0.79\%) \\ \mbox{Index} & Treatment & 8.1\pm 0.13 (8.1\%) & 6.74\pm 0.11 (6.74\%) \\ \mbox{Mean} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Productivity} & Treatment & 7.46\pm 0.07 (7.46\%) & 6.44\pm 0.05 (6.44\%) \\ \mbox{Geometric} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Mean Pro-} & Treatment & 6.26\pm 0.12 (6.26\%) & 5.49\pm 0.1 (5.49\%) \\ \mbox{Vield Index} & Treatment & 0.09\pm 0 (0.09\%) & 0.06\pm 0 (0.06\%) \\ \mbox{Vield Index} & Treatment & 0.18\pm 0 (0.18\%) & 0.16\pm 0.01 (0.16\%) \\ \mbox{Vield Stabil-} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Vield Stabil-} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Vield Stabil-} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Vield Stabil-} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Vield Stabil-} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Vield Stabil-} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Mean} & Treatment & 0.3\pm 0.01 (0.3\%) & 0.31\pm 0.01 (0.31\%) \\ \mbox{Harmonic} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Mean} & Treatment & 0.3\pm 0.01 (0.3\%) & 0.31\pm 0.01 (0.3\%) \\ \mbox{Harmonic} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Mean} & Treatment & 0.3\pm 0.01 (0.3\%) & 0.31\pm 0.01 (0.3\%) \\ \mbox{Harmonic} & Control & 0\pm 0 & 0\pm 0 \\ \mbox{Harmonic} & Control & 0\pm 0 & 0\pm 0 \\ \mb$	(cm ²)	neutinent	12.7 20.20 (1.9170)	10.0220.11 (0.21/0)	
Plant Treatment 2.33 ± 0.47 (- 22.22%) 5 ± 0.82 (7.14%) ns Spikelets per Plant Control 23.33 ± 1.89 (100%) 36.67 ± 6.24 (100%) ns Biological Control 17.33 ± 0.47 30.67 ± 3.09 ns Spikelets per Plant Treatment (-25.71%) (-16.36%) ns Biological Control 1.47 ± 0.34 (100%) 1.88 ± 0.15 (100%) ns Grain Yield Control 3.84 ± 0.12 (100%) 3.27 ± 0.09 (100%) ns (g) Treatment 3.41 ± 0.13 (-11.21%) 3.07 ± 0.11 (-6.01%) ns Thousand Control 19.18 ± 0.61 (100%) 16.35 ± 0.46 (100%) ms Grain Treatment 17.03 ± 0.65 15.37 ± 0.56 ns Weight (g) Treatment (-11.21%) (-6.01%) *** Stress Sus- Control 0 ± 0 0 ± 0 *** index Treatment -0.87 ± 0.03 (-0.87%) -0.79 ± 0.03 (-0.79%) *** Index Treatment 0.40 0 ± 0 ***	Tillers per	Control	3±0 (100%)	4.67±1.25 (100%)	
	Plant	Treatment	2.33±0.47 (-22.22%)	5±0.82 (7.14%)	ns
	Snikelets	Control	23.33±1.89 (100%)	36.67±6.24 (100%)	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	ner Plant	Treatment	17.33±0.47	30.67±3.09	ns
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Diala a inal	Control	(-25.71%)	(-16.36%)	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Biological	Control	$1.4/\pm0.34(100\%)$	$1.88\pm0.15(100\%)$	ns
	Grain Yield	Control	3 84+0 12 (100%)	3 27+0 09 (100%)	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	(g)	Treatment	3.41±0.13 (-11.21%)	3.07±0.11 (-6.01%)	ns
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Thousand	Control	19.18±0.61 (100%)	16.35±0.46 (100%)	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Grain	Tuestant	17.03±0.65	15.37±0.56	ns
Stress tolerance indicesStress Sus- ceptibility indexControl 0 ± 0 0 ± 0 Treatment $-0.87\pm 0.03 (-0.87\%)$ $-0.79\pm 0.03 (-0.79\%)$ ***Tolerance IndexControl 0 ± 0 0 ± 0 ***IndexTreatment $8.1\pm 0.13 (8.1\%)$ $6.74\pm 0.11 (6.74\%)$ ***MeanControl 0 ± 0 0 ± 0 ***Productivity indexTreatment $7.46\pm 0.07 (7.46\%)$ $6.44\pm 0.05 (6.44\%)$ ***GeometricControl 0 ± 0 0 ± 0 ***Mean Pro- ductivityTreatment $6.26\pm 0.12 (6.26\%)$ $5.49\pm 0.1 (5.49\%)$ ***Stress Toler- ance IndexControl 0 ± 0 0 ± 0 **Yield IndexTreatment $0.09\pm 0 (0.09\%)$ $0.06\pm 0 (0.06\%)$ **Yield Stabil- ity IndexControl 0 ± 0 0 ± 0 **Yield Stabil- KeanControl 0 ± 0 0 ± 0 **MeanTreatment $0.3\pm 0.01 (0.3\%)$ $0.31\pm 0.01 (0.31\%)$ **Harmonic MeanControl 0 ± 0 0 ± 0 **	Weight (g)	freatment	(-11.21%)	(-6.01%)	
$\begin{array}{c cccc} Stress Sus- \\ ceptibility \\ index \\ Treatment \\ \hline 0.87\pm 0.03 (-0.87\%) \\ index \\ \hline Treatment \\ \hline 0.87\pm 0.03 (-0.87\%) \\ \hline 0.79\pm 0.03 (-0.79\%) \\ \hline 0.79\pm 0.03 (-0.79\%) \\ \hline \end{array} \\ \begin{array}{c} *** \\ \hline \end{array} \\ \hline \end{array} \\ \hline \end{array} \\ \begin{array}{c} *** \\ \hline \end{array} $ \\ \hline \end{array} \\ \hline \\ \hline \end{array} \\ \hline \\ \\ \\ \hline \end{array} \\ \\ \\ \hline \end{array} \\ \\ \\ \\ \hline \\ \hline \\ \\ \\ \\ \hline \end{array} \\ \\ \\ \\ \hline \\ \hline \\ \\ \\ \\ \hline \\ \\ \\ \\ \hline \\ \\ \\ \\	Stress tolera	nce indices			
$\begin{array}{c c} ceptibility\\index & Treatment\\index & Treatment\\ \hline control & 0\pm 0 & 0\pm 0 & 0\pm 0 & \\ \hline control & 0\pm 0 & 0\pm 0 & \\ \hline control & 0\pm 0 & 0\pm 0 & \\ \hline reatment & 8.1\pm 0.13 (8.1\%) & 6.74\pm 0.11 (6.74\%) & \\ \hline Mean & Control & 0\pm 0 & 0\pm 0 & \\ \hline Productivity & \\ \hline rreatment & 7.46\pm 0.07 (7.46\%) & 6.44\pm 0.05 (6.44\%) & \\ \hline rreatment & 7.46\pm 0.07 (7.46\%) & 6.44\pm 0.05 (6.44\%) & \\ \hline mean & Pro- & \\ \hline ductivity & \\ \hline Stress Toler- & Control & 0\pm 0 & 0\pm 0 & \\ \hline wean & Treatment & 0.09\pm 0 (0.09\%) & 0.06\pm 0 (0.06\%) & \\ \hline Yield Index & Treatment & 0.18\pm 0 (0.18\%) & 0.16\pm 0.01 (0.16\%) & \\ \hline Yield Stabil- & \\ \hline ity Index & Treatment & 0.3\pm 0.01 (0.3\%) & \\ \hline Harmonic & Control & 0\pm 0 & 0\pm 0 & \\ \hline Mean & Treatment & 0.525\pm 0.16 (5.25\%) & 4.68\pm 0.13 (4.68\%) & \\ \hline \end{array}$	Stress Sus-	Control	0±0	0±0	
$\begin{array}{ c c c c c c c } \hline \mbox{index} & \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $	ceptibility	Treatment	-0.87±0.03 (-0.87%)	-0.79±0.03 (-0.79%)	**
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	index	Control	0+0	0+0	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Index	Troatmont	0±0 8 1+0 12 (8 1%)	0±0 6 7/+0 11 (6 7/%)	***
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	Mean	Treatment	5.25±0.16 (5.25%)	4.68±0.13 (4.68%)	-11 - 12 -

Table 3: ANOVA (p-Table) for the root and leaf antioxidants of JP-5 and SB rice cultivars under control and copper conditions.

Parameters	Treatments	JP-5	SB	ANOVA (p-value)	
Root Antioxidants		1	1		
	Control	323.04±0.09 (100%)	313.68±0.19 (100%)	***	
Superoxide Dismutase (Unit/g/FW)	Treatment	329.37±0.46(1.96%)	316.7±0.77(0.96%)		
Derevidese (Upit/g/EW/)	Control	0.06±0 (100%)	0.06±0 (100%)	ns	
Peroxidase (Unit/g/FW)	Treatment	0.06±0(11.76%)	0.05±0(-21.05%)		
Catalase (Unit/g/FW)	Control	0.57±0.01 (100%)	0.62±0.01 (100%)	**	
	Treatment	0.62±0.01(8.77%)	0.69±0(10.75%)		
	Control	40.26±0.09 (100%)	42.85±0.09 (100%)	***	
Ascorbate Peroxidase (Unit/min/g/ FW)	Treatment	47.8±0.74(18.71%)	48.32±0.51(12.77%)		
Clutathiana Dagauidana (u. ma-1 anatain)	Control	28.14±0.08 (100%)	30.6±0.48 (100%)	de ste de	
Giutathione Peroxidase (µ mg - protein)	Treatment	34.86±0.86(23.88%)	37.29±0.42(21.87%)		
Quidined Clutethians (new cl/c EVA)	Control	88.13±0.08 (100%)	86.22±0.08 (100%)	***	
Oxidized Glutathione (mmol/g FW)	Treatment	93.71±0.86(6.33%)	91.18±0.38(5.75%)		
Deduced Clutethians (mercel/a 514()	Control	18.28±0 (100%)	15.31±0.14 (100%)	***	
Reduced Glutathione (mmol/g FW)	Treatment	23.65±0.87(29.33%)	43.47±0.49(183.95%)		
	Control	106.42±0.08 (100%)	101.53±0.08 (100%)	***	
iotal Glutathione (mmol/g FW)	Treatment	117.36±1.26(10.28%)	134.65±0.57(32.62%)		
Malandialdahuda (mmal/a FM/)	Control	3.32±0.01 (100%)	4.64±0 (100%)	***	
Maionalaidenyde (mmol/g FW)	Treatment	4.63±0.2(39.46%)	6.14±0.22(32.3%)		
	Control	101.69±0.09 (100%)	110.28±0.07 (100%)	***	
Hydrogen Peroxide (µmoi g/Fw)	Treatment	116.37±0.52(14.44%)	122.73±0.87(11.29%)		
Leaf Antioxidants					
Superavida Disputaça (Upit/a/EMI)	Control	318.74±0.09 (100%)	305.77±0.05 (100%)	***	
Superoxide Districtase (Offit/g/FW)	Treatment	328.78±0.07(3.15%)	308.71±0.44(0.96%)		
Derevidees (Unit/c/ENA)	Control	0.04±0 (100%)	0.05±0 (100%)		
Peroxidase (Onit/g/FW)	Treatment	0.07±0.01(61.54%)	0.08±0(64.29%)	115	
Catalaso (Unit/g/EW)	Control	0.66±0.01 (100%)	0.72±0.01 (100%)	***	
	Treatment	0.81±0.01(22.84%)	0.77±0.01(6.94%)		
Ascorbato Porovidaso (Unit/min/g/EW/)	Control	35.86±0.13 (100%)	37.09±4.11 (100%)	***	
Ascorbate Peroxidase (Onit/Init/g/ 1 W)	Treatment	44.2±0.4(23.27%)	37.79±0.15(1.88%)		
Glutathione Perovidase (11 mg ⁻¹ protein)	Control	21.35±0.46 (100%)	23.28±0.48 (100%)	*	
Glutathione Peroxidase (µ mg ⁻¹ protein)	Treatment	25.7±0.2(20.36%)	29.25±0.49(25.66%)		
Ovidized Glutathione (mmol/g EW)	Control	85.87±0.09 (100%)	84.82±0.08 (100%)	***	
Oxidized Glutatilione (Initiol/g I W)	Treatment	89.54±0.09(4.27%)	88.67±0.86(4.54%)		
Reduced Glutathione (mmol/g EW)	Control	14.94±0.03 (100%)	16.79±0.11 (100%)	***	
Reduced Glutathione (mmol/g FW)	Treatment	17.57±0.08(17.58%)	38.34±0.36(128.4%)		
Total Glutathione (mmol/g EW/)	Control	100.81±0.09 (100%)	101.61±0.04 (100%)	***	
	Treatment	107.11±0.09(6.24%)	127.01±0.9(25%)		
Malondialdehyde (mmol/g EW)	Control	4.96±0.12 (100%)	6.08±0.12 (100%)	***	
	Treatment	6.34±0.08(27.76%)	8.19±0.24(34.58%)		
Hydrogen Perovide (umol g/EW/)	Control	122.76±0.86 (100%)	135.76±0.04 (100%)	***	
nyurugen Peroxide (µmol g/FW)	Treatment	129.56±0.08(5.54%)	143.47±0.53(5.68%)		

ns = non-significant, *= P < 0.05, ** = P < 0.01, *** = P < 0.001

ing in JP-5 (2.81%) while decreasing in SB (24.62%) under Cu stress. On contrary, T/P was decreased in JP-5 (22.22%) while increased in SB (7.14%) under Cu stress. However, a decline in GY was recorded in both cultivars with JP-5 showing minorly lesser GY value (11.21%) compared to SB (6.01%) under Cu stress. Similarly, chlorophyll content was reduced in both cultivars under Cu stress. Different values of tolerance indices were observed in both cultivars under Cu stress (Figure 2). A significant increase in STI, YI, YSI, MP, and HM was observed in both rice cultivars. JP-5 showed higher values of tolerance indices as compared to SB indicating that JP-5 is a more tolerant cultivar under Cu stress. A Stress Susceptibility Index (SSI) of \leq 1 indicates greater tolerance. JP-5 having a higher negative SSI value compared to SB, is a more stress-tolerant cultivar. A significant difference in the grain yield of both cultivars was recorded in Cu treated plants compared to control. JP-5 showed higher TOL (8.1%) value as compared to SB (6.74%). A higher TOL value indicates a greater reduction in grain yield. Hence, Based on TOL, SB had higher grain yield than JP-5.

Cu Accumulation and Translocation

Cu stress significantly increased the accumulation of this metal in soil, roots, leaves, and grains of both cultivars. Roots and grains of SB showed more Cu accumulation as compared to JP-5. Similarly, there was an increase in the Translocation Factor (TF) of both cultivars under Cu stress. Bioconcentration Factor (BCF) was notably higher in SB, whereas Bioaccumulation Factor (BAF) was found to be maximum in JP-5 as compared to SB (Figure 3).

Table 4: ANOVA (p-Table) for the nutritional profile of JP-5 and SB rice cultivars under copper and control conditions.

Parameters	Treatments	JP-5	SB	ANOVA (p-value)
Total Sugar	Control	22.19±0.08 (100%)	7.53±0.09 (100%)	***
	Treatment	19.72±0.08(-11.14%)	5.12±0.02(-31.98%)	
De la deserver	Control	0.1±0.03 (100%)	0.07±0.01 (100%)	
Reducing sugar	Treatment	0.11±0.01(6.45%)	0.04±0(-38.1%)	ns
Non reducing sugar	Control	22.09±0.1 (100%)	7.46±0.08 (100%)	***
	Treatment	19.61±0.08(-11.23%)	5.08±0.02(-31.92%)	
Cha ach	Control	2.37±0.09 (100%)	1.93±0.07 (100%)	ىلە بى <i>ل</i>
Starch	Treatment	4.07±0.02(71.49%)	2.13±0.02(10.17%)	**
Proteins	Control	14.1±0.07 (100%)	10.19±0.09 (100%)	**
	Treatment	12.87±0.02(-8.7%)	10.02±0.01(-1.64%)	
Total phenol content	Control	3.54±0.08 (100%)	4.34±0.08 (100%)	***
	Treatment	2.31±0.02(-34.68%)	3.46±0.01(-20.35%)	
	Control	11.75±0.08 (100%)	5.31±0.09 (100%)	at at at
lotal antioxidant capacity	Treatment	12.33±0.01(4.94%)	6.89±0.02(29.67%)	***

ns = non-significant, ** = P < 0.01, *** = P < 0.001

H₂O₂ and MDA Content

The accumulation of H_2O_2 content was reported in the root and leaf of both cultivars under Cu stress compared to control (Table 3). Leaf of both cultivars showed a similar increase in H_2O_2 i.e., JP-5 (5.54%) and SB (5.68%), while a higher H_2O_2 content was measured in the roots of JP-5 (14.44%) relative to SB (11.29%), respectively.

MDA content was also elevated under Cu stress in both cultivars (Table 3). The leaf of SB showed maximum MDA (34.58%) than the leaf of JP-5 (27.76%) whereas the root of JP-5 showed higher level of MDA (39.46%) compared to the root of SB (32.3%).

Activity of Enzymatic Antioxidants

Cu stress triggered a remarkable increase in the activity of SOD enzyme when compared to control in both cultivars. Among cultivars, JP-5 exhibited more increase in SOD level (1.96% in roots and 3.15% in leaf) compared to SB (0.96% in root and leaf) demonstrating that JP-5 is more tolerant cultivar against Cu stress. Prominent differences were observed in the level of POD in both cultivars with JP-5 showing a considerable increase in both parts (11.76% in root and 61.54% in leaf) whereas SB showed a decline in the root (21.05%) and increase in the leaf (64.29%) under Cu stress. (Table 3).

The CAT activity was increased in both cultivars under Cu stress where the roots of SB presented more increase (10.75%) than the roots of JP-5 (8.77%) whereas the leaf of JP-5 showed more increase (22.84%) than the leaf of SB (6.94%). Similarly, GPX and APX were reported to increase in both cultivars under Cu stress. Maximum elevation in APX was observed in JP-5 (18.71% in roots, 23.27% in leaf) compared to SB (12.77% in roots, 1.88% in leaf) while GPX was observed to be maximum in the leaf of SB (25.66%).

Activity of Non-Enzymatic Antioxidants

Compared with control, the level of GSH+GSSG, GSH, and GSSG fluctuated significantly under Cu stress in both cultivars. Increase in GSSG occurred at equal rate in the roots and leaf of both JP-5 and SB, but the GSH presented a highly significant increase in SB (183.95% in roots, 128.4% in leaf) as compared to JP-5 (29.33% in roots, 17.58% in leaf). TG was also higher in SB (32.62% in roots, 25% in leaf) as compared to JP-5 (10.28% in roots, 6.64% in leaf) (Table 3). Increase in TAC was observed in both JP-5 and SB with highest value reported in SB i-e.,

29.67%. Decline in TPC was observed in both cultivars, where JP-5 showed more reduction (34.68%) compared to SB (20.35%) under Cu stress (Table 4).

Pearson correlation analysis indicated that H₂O₂ in leaves

showed a positive correlation with MDA, POD, GPX, and GSH

in leaves while MDA, CAT, APX, GPX, and GSH in roots. Similarly,

GSH in leaves was positively correlated to GSH in roots. SOD in

roots also showed a significantly positive correlation with POD, CAT, SOD and GSSG in leaves as well as APX, GPX, and GSSG

in roots (Figure 4). On the other hand, SOD in roots showed a

negative correlation with GSH, TG, MDA, and H₂O₂ in leaves and

Nutritional Profiling

with CAT, GSH, MDA, and H₂O₂ in roots.

In comparison with control, total soluble, non-reducing, and reducing sugar content varied significantly in the grains of both rice cultivars under Cu stress (Table 4). Maximum sugar content was recorded in JP-5 compared with SB in control. Under Cu stress, reducing, non-reducing and total sugars were significantly reduced (38.1%, 31.92%, 31.98%) in SB. In JP-5 cultivar, only reducing sugar was increased (6.45%), while the non-reducing and total sugar were reduced (11.23%, 11.14%), respectively. Similarly, a reduction in protein content was recorded in both SB (1.64%) and JP-5 (8.7%) under Cu stress (Figure 5). On contrary, starch content raised in both cultivars under Cu stress where JP-5 showed more increase (71.49%) compared to SB (10.17%).

Discussion

Current study showed a comparative study of two rice cultivars under copper stress to assess their tolerance mechanism better and identify Cu-tolerant cultivar. Taylor and Foy [49] found that 30 μ M Cu is enough for reducing 50% of wheat growth (*Triticum aestivum* L.) whereas Wheeler et al., [54] reported that only 0.5 μ M Cu can reduce 50% of wheat growth. Previous studies also reported that growth of young sweet potato plant inhibited significantly by increasing Cu concentrations [30].

Previous studies confirmed that seed germination of date palm as well as Arabidopsis and cucumber drastically reduced by increasing Cu levels [32]. In our experiment seed germination was reduced in both cultivars. There was a notable decline in the germination rate of the rice seeds as the concentration of copper increased [5]. When exposed to copper sulfate, Lens culinaris exhibited lower percentages of germination, seedling growth, dry weight, and root/shoot ratio compared to control [25]. A significant reduction in dry weight, root and shoot length were observed in both cultivars with JP-5 showing more reduction under Cu stress. The fresh weight of rice seedlings reduced under high concentration of Cu [5]. In our experiment SB showed reduction in fresh weight while JP-5 contradicted with previous studies by showing an elevation in fresh weight.

It was reported that grain yield of rice significantly decreased by increasing Cu levels in soil [24]. The main factor contributing to the reduction in yield was the decrease in both panicles and spikelets per panicle, due to reduction in tillering under cadmium treatments [23]. A considerable reduction was observed in plant height, tillers per plant, and grain yield under Cu stress where JP-5 showed low yield due to greater reduction in panicles and spikelets per panicle. It was also reported that increasing soil Cu levels significantly affected the plant height at tillering stage [48]. In our experiment SB showed a notable reduction in plant height while JP-5 showed an elevation in plant height under Cu stress. Excessive copper exposure to plants results in a notable decrease in grain yield and biomass [2]. Grain yield declined in both cultivars under Cu stress, but JP-5 showed more reduction compared to SB.

Previous studies has demonstrated that heavy metals including Cu were translocated and accumulated in edible parts of crops and caused an adverse effects when plants were grown in contaminated soil [18]. Our study showed that Cu stress significantly increased Cu concentration in leaf, root, and grains of both SB and JP-5. SB showed more Cu accumulation in the roots and grains as compared to the roots and grains of JP-5.

Increase in the levels of MDA and H_2O_2 content has been observed in rice under Cu stress [13]. Our results correlate with previous studies as both MDA and H_2O_2 content elevated in both cultivars under Cu stress where the roots of JP-5 showed more elevation than roots of SB. Previous studies also revealed increase in levels of SOD, POD, CAT, APX, and GPX in young seedlings of Paulownia fortune under heavy metal stress [52]. In our experiment, the application of Cu stress also resulted in a considerable increase in total activity of enzymatic antioxidants of both rice cultivars where JP-5 showed more proliferation in SOD and CAT activity relative to SB.

The glutathione level was reduced by increasing heavy metal concentrations [47]. Our findings contradicted those of prior studies as an increase in the level of glutathione was observed under Cu stress in both cultivars where SB showed higher values of GSH compared to JP-5. SB also showed higher levels of TG in the roots and leaf compared to JP-5. Protein and carbohydrate contents of wheat were reduced significantly under Cu and Zn stress [51]. Current experiment correlates with previous studies as the carbohydrate concentration decreased in both rice cultivars under Cu stress compared to control. Protein content was also observed to be decreasing but starch increased significantly in both cultivars under Cu stress.

Conclusion

The increasing concentration of Copper (Cu) in the agroecological zones around the world is damaging the production and yield of rice (*Oryza sativa* L.) on a larger scale. One of the very few ways to tackle this damage is to identify and develop the tolerant rice cultivars against Cu stress. Current study therefore was carried out to assess the tolerance of two widely cultivated rice cultivars against Cu toxicity on multiple scales including germination pattern, agronomic traits, determining phytochemical and antioxidant homeostasis via spectrophotometry and most importantly analysis of the bioaccumulation and translocation of Cu metal from soil to grains. It was reported that JP-5 showed lesser damage to germination rate, higher number of panicles and spikelets per panicle, higher SOD levels and greater stress tolerance indices compared to SB under Cu stress. On the other hand, SB showed higher accumulation of Cu in soil, roots, and grains, and eventually higher translocation of Cu from soil to root and subsequently to the grains. All these parameters suggest that SB exhibited more damage on multiple levels compared to JP-5 and is thus more susceptible to Cu stress. JP-5 proved to be a more tolerant cultivar against Cu stress and is thus recommended to be grown in Cu-contaminated soils. It is also recommended for future breeders to grow JP-5 in Cu contaminated areas for enhancing food security and attaining sustainable food production.

Author Statements

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Author Contributions

UMQ provided rational for the study and supervised it, AF performed research work and article writing, MA performed statistical analysis and visualization, AH, MAN, and ZS helped in methodology, SB helped in writing and proof reading.

Data Availability

The data is made available in the article in tabular as well as graphical form.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- 1. Abedin MJ, Cotter-Howells J, Meharg AA. Arsenic uptake and accumulation in rice (Oryza sativa L.) irrigated with contaminated water. Plant and Soil. 2002; 240: 311-319.
- Adrees M, Ali S, Rizwan M, Ibrahim M, Abbas F, Farid M, et al. The effect of excess copper on growth and physiology of important food crops: a review. 2015; 22: 8148-8162.
- Aebi H. Catalase in vitro Methods in enzymology. Elsevier 1984; 105: 121-126.
- 4. Ahmad P, Jaleel CA, Azooz M, Nabi G. Generation of ROS and non-enzymatic antioxidants during abiotic stress in plants. Botany Research International. 2009; 2: 11-20.
- Ahsan N, Lee DG, Lee SH, Kang KY, Lee JJ, Kim PJ, et al. Excess copper induced physiological and proteomic changes in germinating rice seeds. 2007; 67: 1182-1193.
- Anas M, Saeed M, Elahi M, Naeem K, Shafique MA, Quraishi UM. Histological and ionomics assessment to elucidate tolerance mechanisms of nickel-tolerant and sensitive cultivars of bread wheat (Triticum aestivum L.). Plant Stress. 2023; 10: 100277.
- Anderson ME. Determination of glutathione and glutathione disulfide in biological samples Methods in Enzymology. Academic Press. 1985; 113: 548-555.

- Apel K, Hirt H. Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. Annual Review of Plant Biology. 2004; 55: 373-399.
- Azooz MM, Abou-Elhamd MF, Al-Fredan MA. Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat ('Triticum aestivum'cv. Hasaawi) at early growing stage. Australian Journal of Crop Science. 2012; 6: 688-694.
- 10. Basu S, Roychoudhury A, Sanyal S, Sengupta DN. Carbohydrate content and antioxidative potential of the seed of three edible indica rice (Oryza sativa L.) cultivars. Indian journal of biochemistry & biophysics. 2012; 49: 115-123.
- 11. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical biochemistry. 1971; 44: 276-287.
- 12. Blanche C, Lorio Jr P, Sommers R, Hodges J, Nebeker T, Management. Seasonal cambial growth and development of loblolly pine: xylem formation, inner bark chemistry, resin ducts, and resin flow. Forest Ecology. 1992; 49: 151-165.
- 13. Chen LM, Lin CC, Kao CH. Copper toxicity in rice seedlings: changes in antioxidative enzyme activities, H_2O_2 level, and cell wall peroxidase activity in roots. Botanical Bulletin of Academia Sinica. 2000; 41: 99-103.
- 14. Chen J, Wu X, Song J, Xing G, Liang L, Yin Q, et al. Transcriptomic and physiological comparsion of the short-term responses of two Oryza sativa L. varieties to cadmium. Environmental and Experimental Botany. 2021; 181: 104292.
- Cheng S, Fang Z, Cheng X, Wu Y, Mo L, Yan C, et al. Modulation of Antioxidant Attributes and Grain Yield in Fragrant Rice by Exogenous Cu Application. Journal of Plant Growth Regulation. 2023; 42: 1937-1952.
- 16. Drzewiecka K, Mleczek M, Gąsecka M, Magdziak Z, Budka A, Chadzinikolau T, et al. Copper and nickel co-treatment alters metal uptake and stress parameters of Salix purpurea× viminalis. 2017; 216: 125-134.
- 17. Gong Q, Wang L, Dai T, Zhou J, Kang Q, Chen H, et al. Effects of copper on the growth, antioxidant enzymes and photosynthesis of spinach seedlings. 2019; 171: 771-780.
- Hao X, Zhou D, Wang Y, Shi F, Jiang P. Accumulation of Cu, Zn, Pb, and Cd in edible parts of four commonly grown crops in two contaminated soils. International Journal of Phytoremediation. 2011; 13: 289-301.
- Hayat A, Anas M, Shaheen Z, Falak A, Quraishi UM. Comparative morpho-physiological traits, antioxidant defense and nutritional profiling under Cd stress of japonica-indica elite rice (Oryza sativa L.) cultivars. Journal of Crop Science and Biotechnology. 2024; 7: 175-186.
- Heath RL, Packer L. Photoperoxidation in isolated chloroplasts:
 I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry biophysics. 1968; 125: 189-198.
- 21. Hojsak I, Braegger C, Bronsky J, Campoy C, Colomb V, Decsi T, et al. Arsenic in rice: a cause for concern. Journal of pediatric gastroenterology and nutrition. 2015; 60: 142-145.
- 22. Hu J, Wang X, Zhang G, Jiang P, Chen W, Hao Y, et al. QTL mapping for yield-related traits in wheat based on four RIL populations. Theoretical and Applied Genetics. 2020; 133: 917-933.
- Huang DF, Xi LL, Yang LN, Wang ZQ, Yang JC. Comparison of Agronomic and Physiological Traits of Rice Genotypes Differing in Cadmium-Tolerance. Acta Agronomica Sinica. 2008; 34: 809-817.

- 24. Iqbal MZ, Nayab S, Shafiq M. Effects of copper on seed germination and seedling growth performance of Lens culinaris Medik. Journal of Plant Development. 2018a; 25: 85.
- 25. Iqbal MZ, Umm-e-Habiba, Nayab S, Shafiq. Effects of copper on seed germination and seedling growth performance of Lens culinaris Medik. 2018b; 25: 85.
- 26. Ivanova E, Kholodova V, Kuznetsov V. Biological effects of high copper and zinc concentrations and their interaction in rapeseed plants. 2010; 57: 806-814.
- Karim MR, Ishikawa M, Ikeda M, Islam MT. Climate change model predicts 33% rice yield decrease in 2100 in Bangladesh. 2012; 32: 821-830.
- 28. Khan NH, Nafees M. Comparative study of copper accumulation and distribution in soil of selected orchard and non-orchard fields. Agriultural Research Technology. 2017; 7: 1-9.
- 29. Khan F, Mohammad F. Application of stress selection indices for assessment of nitrogen tolerance in wheat (Triticum aestivum L.). J Anim Plant Sci. 2016; 26: 201.
- 30. Kim YH, Lee HS, Kwak SS. Differential responses of sweetpotato peroxidases to heavy metals. Chemosphere. 2010; 81: 79-85.
- 31. Kumar SR, Arumugam T, Ulaganathan V. Genetic diversity in eggplant germplasm by principal component analysis. SABRAO Journal of Breeding Genetics. 2016; 48: 162-171.
- 32. Li W, Khan MA, Yamaguchi S, Kamiya Y. Effects of heavy metals on seed germination and early seedling growth of Arabidopsis thaliana. Plant growth regulation. 2005; 46: 45-50.
- 33. Lundquist I, Josefsson JO. Sensitive method for determination of peroxidase activity in tissue by means of coupled oxidation reaction. Analytical Biochemistry. 1971; 41: 567-577.
- Mahdavi Z, Rashidi V, Yarnia M, Aharizad S, Roustaii M. Evaluation of yield traits and tolerance indices of different wheat genotypes under drought stress conditions. Cereal Research Communications. 2023; 51: 659-669.
- Malhotra S, Sarkar S. Effects of sulphur dioxide on sugar and free amino acid content of pine seedlings. Physiologia plantarum. 1979; 47: 223-228.
- 36. Mukhopadhyay M, Das A, Subba P, Bantawa P, Sarkar B, Ghosh P, et al. Structural, physiological, and biochemical profiling of tea plantlets under zinc stress. Biologia plantarum. 2013; 57: 474-480.
- Nagalakshmi N, Prasad MNV. Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in Scenedesmus bijugatus. Plant Science. 2001; 160: 291-299.
- Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant cell physiology. 1981; 22: 867-880.
- Ngole V, Ekosse G. Copper, nickel and zinc contamination in soils within the precincts of mining and landfilling environments. International Journal of Environmental Science Technology. 2012; 9: 485-494.
- 40. Panhwar QA, Naher UA, Radziah O, Shamshuddin J, Mohd Razi I, Dipti SS, et al. Quality and antioxidant activity of rice grown on alluvial soil amended with Zn, Cu and Mo. South African Journal of Botany. 2015; 98: 77-83.
- 41. Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Analytical biochemistry. 1977; 83: 346-356.

- 42. Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Analytical Biochemistry. 1999; 269: 337-341.
- Saeed M, Masood Quraishi U, Malik RN. Identification of arsenic-tolerant varieties and candidate genes of tolerance in spring wheat (Triticum aestivum L.). Chemosphere. 2022; 308: 136380.
- 44. SE, A. J. M. i. p. e. Chemical analysis. 1986; 285-344.
- 45. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. 2012.
- Soares E, Hamid A, Mangkoedihardjo S. Phytoremediation of zinc polluted soil using sunflower (Helianthus annuus L.). Journal of Phytology. 2021; 13: 9-12.
- Srivastava RK, Pandey P, Rajpoot R, Rani A, Dubey R. Cadmium and lead interactive effects on oxidative stress and antioxidative responses in rice seedlings. Protoplasma. 2014; 251: 1047-1065.
- 48. Su Z, Wang G, Xu L, Zhang J, Liu X. Effects of Cu stress on physiological, biochemical, and spectral properties of wheat at different growth stages. 2019; 12: 147-153.
- Taylor GJ, Foy CD. Differential uptake and toxicity of ionic and chelated copper in Triticum aestivum. Canadian Journal of Botany. 1985; 63: 1271-1275.
- Velikova V, Yordanov I, Edreva A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Science - Plant Sci. 2000; 151: 59-66.
- 51. Vinod K, Awasthi G, Chauchan PK. Cu and Zn tolerance and responses of the biochemical and physiochemical system of wheat. Journal of Stress Physiology Biochemistry. 2012; 8: 203-213.

- 52. Wang Li, Li W, Zhang C, Ke S. Physiological responses and detoxific mechanisms to Pb, Zn, Cu and Cd in young seedlings of Paulownia fortunei. Journal of Environmental Sciences. 2010; 22: 1916-1922.
- Waseem A, Arshad J, Iqbal F, Sajjad A, Mehmood Z, Murtaza G. Pollution Status of Pakistan: A Retrospective Review on Heavy Metal Contamination of Water, Soil, and Vegetables. BioMed Research International. 2014; 2014: 813206.
- 54. Wheeler D, Power I, Edmeades D. Effect of various metal ions on growth of two wheat lines known to differ in aluminium tolerance. Plant soil Environ. 1993; 155: 489-492.
- 55. WHO. Permissible limits of heavy metals in soil and plants. Geneva, Switzerland. 1996a.
- WHO. Permissible limits of heavy metals in soil and plants. Geneva, Switzerland., 1996b.
- 57. Xu J, Yang L, Wang Z, Dong G, Huang J, Wang Y. Toxicity of copper on rice growth and accumulation of copper in rice grain in copper contaminated soil. Chemosphere. 2006; 62: 602-607.
- Zakaria Z, Zulkafflee NS, Mohd Redzuan NA, Selamat J, Ismail MR, Praveena SM, et al. Understanding Potential Heavy Metal Contamination, Absorption, Translocation and Accumulation in Rice and Human Health Risks. Plants. 2021; 10: 1070.
- 59. Zhuang P, McBride MB, Xia H, Li N, Li Z. Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. Science of the total environment. 2009; 407: 1551-1561.