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# 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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## 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

## 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 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 ($O_2\cdot-$), Hydroxyl radical (OH), and Hydrogen Peroxide ($H_2O_2$) 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 $O_2\cdot-$ to less toxic $H_2O_2$, which is further converted into $H_2O$ 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 $O_2$•− 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 ($H_2O_2$) solution with continuous agitation for 15 min and then rinsed three times with $dH_2O$. 30-day-old 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.

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

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

### Evaluation of Agronomic Traits

**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.

| Parameters | Treatments | JP-5 | SB | ANOVA (p-value) |
|---|---|---|---|---|
| Plant Height (cm) | Control | 71.17±3.7 (100%) | 59.03±7.64 (100%) | Ns |
| | Treatment | 73.17±3.47 (2.81%) | 44.5±4.97 (-24.62%) | |
| Panicle Length (cm) | Control | 23.17±2.01 (100%) | 27.33±1.43 (100%) | Ns |
| | Treatment | 19.97±1.32 (-13.81%) | 21.93±1.18 (-19.76%) | |
| Days To Tillering | Control | 45±0 (100%) | 60±0 (100%) | ** |
| | Treatment | 46.33±0.47 (2.96%) | 61.67±0.47 (2.78%) | |
| Chlorophyll at Tillering | Control | 24.73±1.77 (100%) | 24.11±1.5 (100%) | Ns |
| | Treatment | 21.9±0.42 (-11.43%) | 22.05±0.85 (-8.52%) | |
| Days To Booting | Control | 65±0 (100%) | 85±0 (100%) | *** |
| | Treatment | 66.33±0.47 (2.05%) | 86.33±0.47 (1.57%) | |
| Chlorophyll at Booting | Control | 31.31±1.4 (100%) | 33.37±3.74 (100%) | ns |
| | Treatment | 27.21±0.47 (-13.09%) | 27.77±0.68 (-16.78%) | |
| Days To Heading | Control | 70±0 (100%) | 90±0 (100%) | ** |
| | Treatment | 71.33±0.47 (1.9%) | 91.33±0.47 (1.48%) | |
| Chlorophyll at Heading | Control | 34.03±0.34 (100%) | 34.27±1.51 (100%) | ns |
| | Treatment | 30.28±0.53 (-11%) | 31.11±0.51 (-9.2%) | |
| Days To Anthesis | Control | 80±0 (100%) | 102±0 (100%) | ** |
| | Treatment | 80.67±0.47 (0.83%) | 102.67±0.47 (0.65%) | |
| Chlorophyll at Anthesis | Control | 33.27±1.59 (100%) | 33.1±1.75 (100%) | * |
| | Treatment | 33.1±0.45 (-0.53%) | 33.32±0.36 (0.67%) | |
| Days To Maturation | Control | 115±0 (100%) | 165±0 (100%) | *** |
| | Treatment | 116.33±0.47 (1.16%) | 166.33±0.47 (0.81%) | |
| Flag Leaf Area at Heading ($cm^2$) | Control | 11.62±1.09 (100%) | 13.46±0.36 (100%) | ns |
| | Treatment | 11.09±0.53 (-4.56%) | 13.21±0.25 (-1.91%) | |
| Flag Leaf Area at Anthesis ($cm^2$) | Control | 13.3±1.68 (100%) | 13.49±1.42 (100%) | ns |
| | Treatment | 12.7±0.28 (-4.51%) | 13.92±0.11 (3.21%) | |
| Tillers per Plant | Control | 3±0 (100%) | 4.67±1.25 (100%) | ns |
| | Treatment | 2.33±0.47 (-22.22%) | 5±0.82 (7.14%) | |
| Spikelets per Plant | Control | 23.33±1.89 (100%) | 36.67±6.24 (100%) | ns |
| | Treatment | 17.33±0.47 (-25.71%) | 30.67±3.09 (-16.36%) | |
| Biological Yield (g) | Control | 1.47±0.34 (100%) | 1.88±0.15 (100%) | ns |
| | Treatment | 1.42±0.14 (-3.62%) | 1.2±0.14 (-36.11%) | |
| Grain Yield (g) | Control | 3.84±0.12 (100%) | 3.27±0.09 (100%) | ns |
| | Treatment | 3.41±0.13 (-11.21%) | 3.07±0.11 (-6.01%) | |
| Thousand Grain Weight (g) | Control | 19.18±0.61 (100%) | 16.35±0.46 (100%) | ns |
| | Treatment | 17.03±0.65 (-11.21%) | 15.37±0.56 (-6.01%) | |
| **Stress tolerance indices** | | | | |
| Stress Susceptibility index | Control | 0±0 | 0±0 | ** |
| | Treatment | -0.87±0.03 (-0.87%) | -0.79±0.03 (-0.79%) | |
| Tolerance Index | Control | 0±0 | 0±0 | *** |
| | Treatment | 8.1±0.13 (8.1%) | 6.74±0.11 (6.74%) | |
| Mean Productivity index | Control | 0±0 | 0±0 | *** |
| | Treatment | 7.46±0.07 (7.46%) | 6.44±0.05 (6.44%) | |
| Geometric Mean Productivity | Control | 0±0 | 0±0 | ** |
| | Treatment | 6.26±0.12 (6.26%) | 5.49±0.1 (5.49%) | |
| Stress Tolerance Index | Control | 0±0 | 0±0 | ** |
| | Treatment | 0.09±0 (0.09%) | 0.06±0 (0.06%) | |
| Yield Index | Control | 0±0 | 0±0 | ** |
| | Treatment | 0.18±0 (0.18%) | 0.16±0.01 (0.16%) | |
| Yield Stability Index | Control | 0±0 | 0±0 | ** |
| | Treatment | 0.3±0.01 (0.3%) | 0.31±0.01 (0.31%) | |
| Harmonic Mean | Control | 0±0 | 0±0 | ** |
| | Treatment | 5.25±0.16 (5.25%) | 4.68±0.13 (4.68%) | |

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

Cu contamination showed a notable influence on agronomic traits of both cultivars (Table 2). PH was observed to be increas-

**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** | | | | |
| Superoxide Dismutase (Unit/g/FW) | Control | 323.04±0.09 (100%) | 313.68±0.19 (100%) | *** |
| | Treatment | 329.37±0.46(1.96%) | 316.7±0.77(0.96%) | |
| Peroxidase (Unit/g/FW) | Control | 0.06±0 (100%) | 0.06±0 (100%) | ns |
| | 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%) | |
| Ascorbate Peroxidase (Unit/min/g/ FW) | Control | 40.26±0.09 (100%) | 42.85±0.09 (100%) | *** |
| | Treatment | 47.8±0.74(18.71%) | 48.32±0.51(12.77%) | |
| Glutathione Peroxidase (µ mg$^{-1}$ protein) | Control | 28.14±0.08 (100%) | 30.6±0.48 (100%) | *** |
| | Treatment | 34.86±0.86(23.88%) | 37.29±0.42(21.87%) | |
| Oxidized Glutathione (mmol/g FW) | Control | 88.13±0.08 (100%) | 86.22±0.08 (100%) | *** |
| | Treatment | 93.71±0.86(6.33%) | 91.18±0.38(5.75%) | |
| Reduced Glutathione (mmol/g FW) | Control | 18.28±0 (100%) | 15.31±0.14 (100%) | *** |
| | Treatment | 23.65±0.87(29.33%) | 43.47±0.49(183.95%) | |
| Total Glutathione (mmol/g FW) | Control | 106.42±0.08 (100%) | 101.53±0.08 (100%) | *** |
| | Treatment | 117.36±1.26(10.28%) | 134.65±0.57(32.62%) | |
| Malondialdehyde (mmol/g FW) | Control | 3.32±0.01 (100%) | 4.64±0 (100%) | *** |
| | Treatment | 4.63±0.2(39.46%) | 6.14±0.22(32.3%) | |
| Hydrogen Peroxide (µmol g/FW) | Control | 101.69±0.09 (100%) | 110.28±0.07 (100%) | *** |
| | Treatment | 116.37±0.52(14.44%) | 122.73±0.87(11.29%) | |
| **Leaf Antioxidants** | | | | |
| Superoxide Dismutase (Unit/g/FW) | Control | 318.74±0.09 (100%) | 305.77±0.05 (100%) | *** |
| | Treatment | 328.78±0.07(3.15%) | 308.71±0.44(0.96%) | |
| Peroxidase (Unit/g/FW) | Control | 0.04±0 (100%) | 0.05±0 (100%) | ns |
| | Treatment | 0.07±0.01(61.54%) | 0.08±0(64.29%) | |
| Catalase (Unit/g/FW) | Control | 0.66±0.01 (100%) | 0.72±0.01 (100%) | *** |
| | Treatment | 0.81±0.01(22.84%) | 0.77±0.01(6.94%) | |
| Ascorbate Peroxidase (Unit/min/g/ FW) | Control | 35.86±0.13 (100%) | 37.09±4.11 (100%) | *** |
| | Treatment | 44.2±0.4(23.27%) | 37.79±0.15(1.88%) | |
| Glutathione Peroxidase (µ mg$^{-1}$ protein) | Control | 21.35±0.46 (100%) | 23.28±0.48 (100%) | * |
| | Treatment | 25.7±0.2(20.36%) | 29.25±0.49(25.66%) | |
| Oxidized Glutathione (mmol/g FW) | Control | 85.87±0.09 (100%) | 84.82±0.08 (100%) | *** |
| | Treatment | 89.54±0.09(4.27%) | 88.67±0.86(4.54%) | |
| Reduced Glutathione (mmol/g FW) | Control | 14.94±0.03 (100%) | 16.79±0.11 (100%) | *** |
| | Treatment | 17.57±0.08(17.58%) | 38.34±0.36(128.4%) | |
| Total Glutathione (mmol/g FW) | 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 FW) | 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 Peroxide (µmol g/FW) | Control | 122.76±0.86 (100%) | 135.76±0.04 (100%) | *** |
| | 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 ≤ 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%) | |
| Reducing sugar | Control | 0.1±0.03 (100%) | 0.07±0.01 (100%) | ns |
| | Treatment | 0.11±0.01(6.45%) | 0.04±0(-38.1%) | |
| 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%) | |
| Starch | Control | 2.37±0.09 (100%) | 1.93±0.07 (100%) | ** |
| | 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%) | |
| Total antioxidant capacity | Control | 11.75±0.08 (100%) | 5.31±0.09 (100%) | *** |
| | Treatment | 12.33±0.01(4.94%) | 6.89±0.02(29.67%) | |

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

### $H_2O_2$ 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 $H_2O_2$ 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 $H_2O_2$ in leaves and with CAT, GSH, MDA, and $H_2O_2$ in roots.

### Nutritional Profiling

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 μM Cu is enough for reducing 50% of wheat growth (*Triticum aestivum* L.) whereas Wheeler et al., [54] reported that only 0.5 μM 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.

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