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RESEARCH ARTICLE

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF THREE LOWLAND RICE (*Oryza sativa* L.) VARIETIES TO IRON TOXICITY UNDER SEMI-CONTROLLED CONDITIONS

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ABSTRACT

Iron toxicity is one of the major abiotic constraints limiting rice production in tropical hydromorphic lowland soils. Excessive accumulation of ferrous iron (Fe^{2+}) induces oxidative stress, leading to physiological and biochemical disturbances that ultimately reduce plant growth and productivity. This study aimed to evaluate the physiological and biochemical responses of three lowland rice varieties (DANANE, NERICA L19, and WITA 9) in order to identify genotypes with superior tolerance to iron toxicity. The experiment was conducted under semi-controlled conditions using a randomized complete block design with three replications in a 3 × 5 factorial arrangement. The rice varieties were exposed to five Fe^{2+} concentrations (Control, 150, 300, 600, and 900 ppm). Chlorophyll contents, antioxidant enzyme activities (catalase, peroxidase, and ascorbate peroxidase), stress tolerance indices (STI_s), and the chlorophyll stability index (CSI) were determined. Data were analyzed using two-way analysis of variance (ANOVA), Pearson's correlation analysis, and principal component analysis (PCA). Increasing Fe^{2+} concentrations resulted in a progressive decline in chlorophyll contents and CSI, whereas antioxidant enzyme responses varied according to genotype and stress intensity. NERICA L19 exhibited the highest total chlorophyll content (1.50 mg g^{-1} FW), the highest CSI value (58.53), and the highest STI_{POD} and STI_{APX} values under 900 ppm Fe^{2+}. DANANE showed an intermediate response, whereas WITA 9 was the most susceptible variety. Multivariate analyses confirmed the varietal differences and clearly discriminated treatments according to Fe^{2+} concentrations. Overall, the results indicate that NERICA L19 possesses superior adaptive capacity to iron toxicity and represents a promising genotype for breeding programs aimed at improving iron toxicity tolerance in rice.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most widely cultivated and consumed cereal crops, providing food security for more than half of the global population. With an annual production exceeding 520 million tonnes of milled rice, it represents the primary source of dietary energy for approximately 3.5 billion people, particularly in Asia, Africa, and Latin America (1). Beyond its nutritional importance, rice plays a pivotal economic and social role by sustaining the livelihoods of hundreds of millions of farmers and contributing to the stability of food systems in many developing countries. Global demand for rice is expected to continue increasing over the coming decades due to population growth, highlighting the urgent need to sustainably improve rice productivity. However, achieving this goal remains challenging because rice production is severely constrained by numerous biotic and abiotic stresses that adversely affect plant growth and grain yield. Among the abiotic constraints, drought, salinity, flooding, and mineral toxicities are recognized as major factors limiting rice production, particularly in tropical and subtropical regions (2). In lowland rice ecosystems, iron toxicity is considered one of the most severe abiotic constraints, affecting nearly 18% of the world's rice-growing areas (3). Under flooded conditions, the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) markedly increases iron availability in the rhizosphere, promoting excessive uptake by rice roots (4). When present at toxic concentrations, Fe^{2+} disrupts several physiological and biochemical processes, resulting in leaf bronzing, chlorophyll degradation, reduced photosynthetic activity, and ultimately substantial yield losses (5). At the cellular level, excessive Fe^{2+} stimulates the production of

reactive oxygen species (ROS), leading to oxidative damage to membrane lipids, proteins, and nucleic acids (6). This constraint is particularly critical in the humid tropical regions where lowland rice cultivation represents a major opportunity for increasing rice production. In Côte d'Ivoire, the inland valleys of the western region constitute an important resource for rice cultivation owing to their favorable hydrological conditions and soil characteristics. However, these ecosystems are frequently affected by iron toxicity, which considerably limits rice productivity and compromises the sustainable exploitation of their agricultural potential (2). To cope with iron toxicity, rice plants have evolved a range of defense mechanisms to mitigate Fe^{2+}-induced oxidative stress. Among these mechanisms, the enzymatic antioxidant system, mainly composed of catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), plays a crucial role in scavenging reactive oxygen species and protecting cellular structures from oxidative damage. The efficiency of these antioxidant enzymes varies among genotypes and is considered one of the major determinants of varietal tolerance to iron toxicity (7). Although considerable progress has been made in understanding the physiological and biochemical mechanisms underlying rice responses to iron toxicity, studies simultaneously comparing these responses among rice varieties cultivated in Côte d'Ivoire remain scarce. A better understanding of these adaptive mechanisms is essential for identifying genotypes suitable for iron-toxic lowland environments and for supporting rice breeding programs aimed at improving iron toxicity tolerance. Therefore, the present study aimed to evaluate the physiological and biochemical responses of three rice varieties exposed to different Fe^{2+} concentrations in order to identify genotypes with superior tolerance to iron toxicity.

MATERIALS AND METHODS

Plant material: The plant material consisted of three rice (Oryza sativa L.) varieties, namely NERICA L19, WITA 9, and DANANE. Seeds of the NERICA L19 and WITA 9 varieties were supplied by the National Center for Agronomic Research (CNRA, Côte d'Ivoire), whereas seeds of the DANANE variety were collected from a rice farmer in the DANANE Department, western Côte d'Ivoire.

Experimental design and crop management: The experiment was conducted under semi-controlled conditions using a completely randomized design (CRD) with three replicates in a 3 × 5 factorial arrangement, comprising three rice varieties (NERICA L19, WITA 9, and DANANE) and five ferrous iron (Fe^{2+}) concentrations (Control, 150, 300, 600, and 900 ppm). Each variety × Fe^{2+} concentration combination consisted of 10 pots, giving a total of 450 experimental units (3 varieties × 5 Fe^{2+} concentrations × 10 pots × 3 replicates). Before establishing the experiment, the chemical properties of the soil used as the growth substrate were analyzed. The soil was collected from a lowland rice field to determine its initial nitrogen (N), phosphorus (P), potassium (K), and iron (Fe) contents. The initial soil Fe concentration, estimated at 115 ppm, was used as the reference for preparing the Fe^{2+} solutions corresponding to the four iron treatments (150, 300, 600, and 900 ppm). Plastic pots (847 cm^3) were filled with 1.15 kg of soil and randomly arranged with a spacing of 20 cm between adjacent pots to minimize interference among experimental units. Two days before sowing, 400 mL of the Fe^{2+} solution corresponding to each treatment was applied to each pot. A basal fertilizer consisting of 0.5 g of NPK (15-15-15) per pot was subsequently incorporated to ensure adequate mineral nutrition. Two rice seeds were sown at a depth of approximately 3 cm in each pot. Twenty days after sowing, an additional 0.5 g of urea per pot was applied to promote vegetative growth.

Sample collection: Leaf samples intended for physiological and biochemical analyses were collected 60 days after sowing (DAS), a growth stage at which iron toxicity symptoms are generally well expressed (8). Immediately after sampling, the leaves were rinsed with distilled water, carefully labeled, and promptly transported to the laboratory for subsequent analyses.

Physiological analyses

Determination of chlorophyll content: The contents of chlorophyll a, chlorophyll b, and total chlorophyll were determined according to the modified method of Arnon (9). Approximately 0.5 g of fresh leaf tissue was homogenized in 10 mL of 80% (v/v) acetone and the homogenate was centrifuged. The absorbance of the supernatant was measured at 663 and 645 nm using a UV-Vis spectrophotometer. Chlorophyll contents were calculated using the following equations:

$$\text{Chla} = \frac{(12.7 \times A_{663} - 2.69 \times A_{645}) \times V}{1000 \times m}$$

$$\text{Chl b} = \frac{(22.9 \times A_{645} - 4.68 \times A_{663}) \times V}{1000 \times m}$$

$$\text{Total chl} = \frac{(20.2 \times A_{645} + 8.02 \times A_{663}) \times V}{1000 \times m}$$

where Chl a, Chl b and Total Chl represent chlorophyll a, chlorophyll b and total chlorophyll contents (mg g^{-1}FW), respectively; A_{663} and A_{645} are the absorbance values measured at 663 and 645 nm, respectively; V is the extract volume (mL); and m is the fresh weight (g) of the sample.

Chlorophyll Stability Index (CSI): The chlorophyll stability index (CSI), an indicator of plant physiological tolerance to oxidative stress affecting chlorophyll pigments, was calculated using the following equation (10, 11):

$$\text{CSI (\%)} = \frac{\text{Total chlorophyll under stress}}{\text{Total chlorophyll under control}} \times 100$$

Biochemical analyses

Preparation of enzyme extracts: The activities of catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were determined using enzyme extracts prepared from fresh leaf tissues. Briefly, 1 g of fresh leaves was homogenized in 5 mL of 100 mM potassium phosphate buffer (pH 7.0). The homogenate was then centrifuged at 5,000 rpm for 10 min, and the resulting supernatant was used for the subsequent enzyme activity assays.

Peroxidase (POD) activity assay: Peroxidase (POD) activity was determined using a guaiacol-based method adapted from Chance and Maehly (12). The reaction mixture consisted of 2.5 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.3 mL of 20 mM guaiacol, 0.1 mL of enzyme extract, and 0.1 mL of

20 mM H_2O_2. The increase in absorbance was monitored at 470 nm for 1 min using a spectrophotometer. POD activity, expressed as µmol H_2O_2 min^{-1} g^{-1} FW, was calculated using the following equation:

$$POD = \frac{\Delta A_{470}/min \times V_{extract}}{26.6 \times V_{sample} \times m}$$

where ΔA_{470} min^{-1} is the change in absorbance per minute at 470 nm; $V_{extract}$ is the total reaction mixture volume (mL); 26.6 is the molar extinction coefficient of tetraguaiacol ($mM^{-1}cm^{-1}$); V_{sample} is the volume of enzyme extract used (mL); and m is the fresh weight (g) of the sample.

Catalase (CAT) activity assay: Catalase (CAT) activity was determined using a modified method of Sinha (13) based on the decomposition of hydrogen peroxide (H_2O_2). The reaction mixture consisted of 1.9 mL of 100 mM potassium phosphate buffer (pH 7.0), 1.0 mL of 15 mM H_2O_2 solution, and 0.1 mL of enzyme extract. After incubation for 1 min at room temperature, the reaction was terminated by adding 2 mL of dichromate–acetic acid reagent. The reaction mixtures were then heated in a boiling water bath (100 °C) for 10 min, cooled to room temperature, and the absorbance was measured at 570 nm using a spectrophotometer. A standard curve prepared from known H_2O_2 concentrations was used to determine the amount of residual H_2O_2. Catalase activity was calculated from the amount of H_2O_2 decomposed during the incubation period and expressed as µmol H_2O_2 min^{-1} g^{-1} fresh weight (FW).

$$CAT = \frac{\Delta A \times V_{extract}}{m \times t \times V_{sample}}$$

where ΔA is the difference between the absorbance of the blank and that of the sample; $V_{extract}$ is the total extraction volume (mL); m is the fresh weight (g); V_{sample} is the volume of enzyme extract used in the assay (mL); and t is the incubation time (min).

Ascorbate peroxidase (APX) activity assay: Ascorbate peroxidase (APX) activity was determined according to the method described by Nakano and Asada (14). The reaction mixture consisted of 2.7 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 0.5 mM ascorbic acid, 0.1 mL of enzyme extract, and 0.1 mL of 0.1 mM H_2O_2, which was added last to initiate the reaction. The decrease in absorbance was monitored immediately at 290 nm for 1 min using a spectrophotometer. APX activity was calculated using the following equation:

$$APX = \frac{\Delta A_{290}/min \times V_{extract}}{2.8 \times V_{sample} \times m}$$

where ΔA_{290} min^{-1} is the decrease in absorbance per minute at 290 nm; $V_{extract}$ is the total extraction volume (mL); 2.8 is the molar extinction coefficient of ascorbate (mM^{-1} cm^{-1}); V_{sample} is the volume of enzyme extract used in the assay (mL); and m is the fresh weight (g) of the sample. APX activity was expressed as µmol of ascorbate oxidized min^{-1} g^{-1} FW.

Stress tolerance indices (STI_s): Stress tolerance indices (STI_s) were calculated to assess the relative response of antioxidant enzyme activities under iron toxicity according to the approach proposed by **Fernandez (15)**. Separate STIs were calculated for catalase (STI_{CAT}), peroxidase (STI_{POD}), and ascorbate peroxidase (STI_{APX}) using the following equation:

$$STI_S = \frac{A_{stress}}{A_{control}}$$

Where A_{stress} is the enzyme activity measured under a given Fe^{2+} concentration, and $A_{control}$ is the corresponding enzyme activity measured in the control treatment.

Statistical analysis: Data were analyzed using a two-way analysis of variance (ANOVA) to assess the effects of rice variety, Fe^{2+} concentration, and their interaction on the measured physiological and biochemical traits. When significant differences were detected ($p < 0.05$), means were separated using Tukey's honestly significant difference (HSD) test. Pearson's correlation analysis was performed to examine relationships among the measured variables. Principal component analysis (PCA) was subsequently conducted to explore the multivariate relationships among traits and to discriminate treatments according to their responses to iron toxicity. All statistical analyses were performed using R software (version 4.5.2) within the Positron integrated development environment (Posit Software, PBC, Boston, MA, USA).

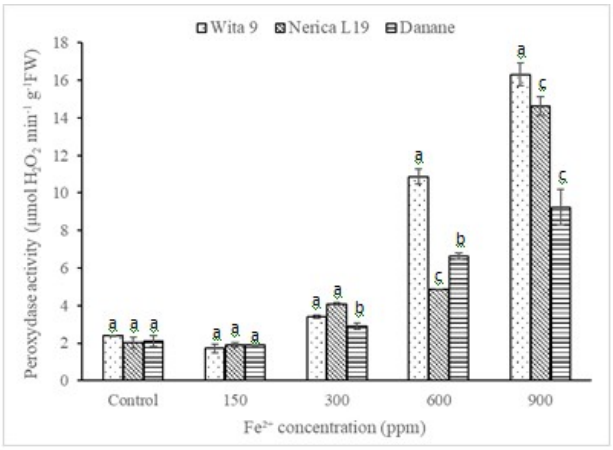
RESULTS

Effect of ferrous iron concentrations on the physiological parameters of the rice varieties DANANE, NERICA L19, and WITA 9: Analysis of variance revealed highly significant ($p < 0.001$) effects of Fe^{2+} concentration, variety, and their interaction on chlorophyll a, chlorophyll b, total chlorophyll contents, and the chlorophyll stability index (CSI) (Table 1). Increasing Fe^{2+} concentrations resulted in a progressive decline in chlorophyll a, chlorophyll b, and total chlorophyll contents in all three rice varieties. However, the magnitude of this reduction differed among varieties. From 150 ppm onward, WITA 9 exhibited the lowest chlorophyll contents, whereas DANANE and NERICA L19 showed statistically similar values. This trend persisted at 300 and 600 ppm, where NERICA L19 maintained the highest total chlorophyll content, followed by DANANE, while WITA 9 consistently recorded the lowest values. At 900 ppm, chlorophyll contents markedly decreased in all three varieties; nevertheless, NERICA L19 retained the highest total chlorophyll content (1.50 mg g^{-1} FW), whereas WITA 9 exhibited the lowest value (1.42 mg g^{-1} FW). The chlorophyll stability index (CSI) also decreased progressively with increasing Fe^{2+} concentrations. NERICA L19 consistently recorded the highest CSI values between 150 and 600 ppm, whereas no significant differences among varieties were observed at 900 ppm.

Effect of Fe^{2+} concentrations on antioxidant enzyme activities

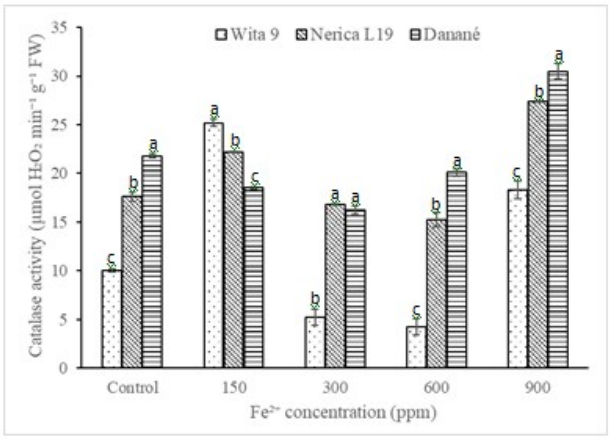
Peroxidase (POD) activity: Analysis of variance revealed highly significant ($p < 0.001$) effects of Fe^{2+} concentration, variety, and their interaction on peroxidase (POD) activity (Figure 1). Under the control treatment and at 150 ppm, all three rice varieties exhibited low POD activities, with no significant differences among them. From 300 ppm onward, POD activity increased in all varieties, although the magnitude of the increase differed among genotypes. At this

concentration, WITA 9 and NERICA L19 exhibited significantly higher POD activities than DANANE.



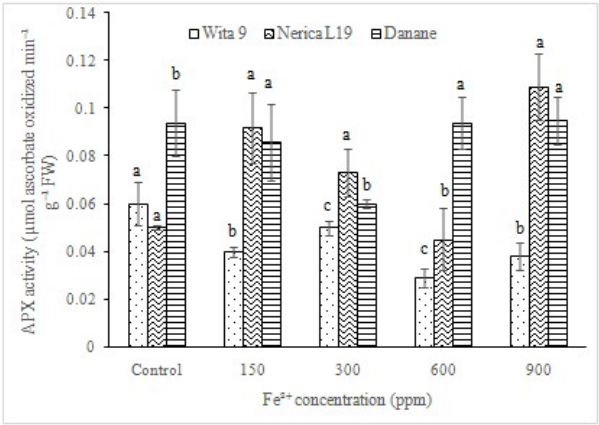
Bars represent the mean ± SD. Within each Fe^{2+} concentration, bars sharing the same lowercase letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

Figure 1. Peroxidase activity (POD) of the rice varieties DANANE, NERICA L19 and WITA 9 under different Fe^{2+} concentrations



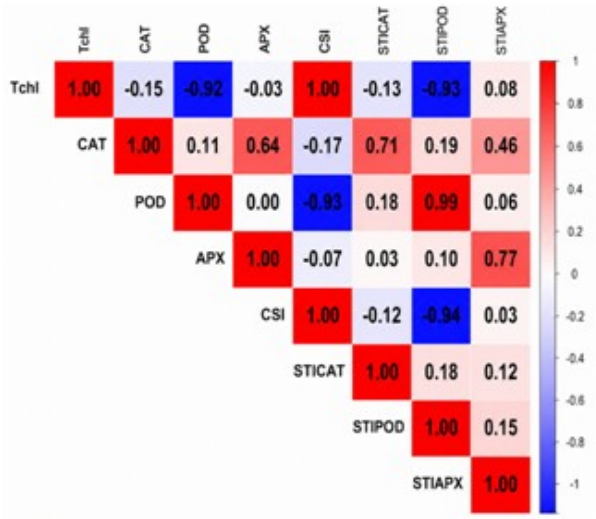
Bars represent the mean ± SD. Within each Fe^{2+} concentration, bars sharing the same lowercase letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

Figure 2. Catalase (CAT) activity of the rice varieties DANANE, NERICA L19 and WITA 9 under different Fe^{2+} concentrations



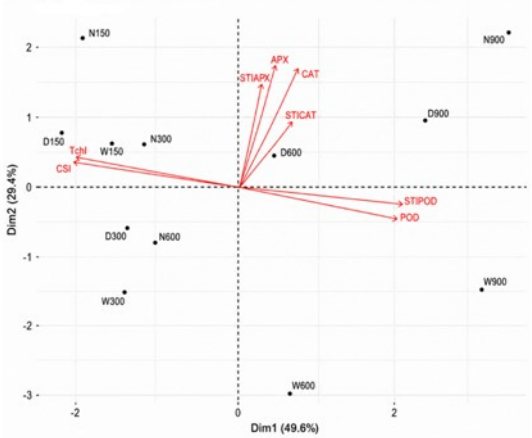
Bars represent the mean ± standard deviation (SD, n = 3). Within each Fe^{2+} concentration, bars sharing the same lowercase letter are not significantly different according to Tukey's honestly significant difference (HSD) test (**p**< 0.05).

Figure 3. Ascorbate peroxidase (APX) activity of the rice varieties WITA 9, NERICA L19 and DANANE under different Fe^{2+} concentrations



TChl: total chlorophyll content; CSI: chlorophyll stability index; CAT: catalase activity; POD: peroxidase activity; APX: ascorbate peroxidase activity; STI_{CAT}: catalase stress tolerance index; STI_{POD}: peroxidase stress tolerance index; STIAPX: ascorbate peroxidase stress tolerance index.

Figure 4. Pearson correlation matrix of physiological and biochemical parameters measured in rice varieties exposed to different Fe^{2+} concentrations



TChl, total chlorophyll content; CAT, catalase activity; POD, peroxidase activity; APX, ascorbate peroxidase activity; CSI, chlorophyll stability index; STICAT, STIPOD, and STIAPX, stress tolerance indices of catalase, peroxidase, and ascorbate peroxidase, respectively. D, N, and W denote the rice varieties DANANE, NERICA L19, and WITA 9, respectively. 150, 300, 600, and 900 indicate and the applied Fe^{2+} concentrations (ppm), respectively.

Figure 5. Principal component analysis (PCA) of physiological and biochemical parameters involved in the responses of rice varieties to different Fe^{2+} concentrations

This difference became more pronounced at 600 and 900 ppm, where the highest POD activities were recorded in WITA 9 (10.87 and 16.31 µmol H_2O_2 min^{-1} g^{-1} FW), followed by NERICA L19 (4.88 and 14.62 µmol H_2O_2 min^{-1} g^{-1} FW), whereas DANANE maintained the lowest activities (6.63 and 9.23 µmol H_2O_2 min^{-1} g^{-1} FW). Overall, POD activity increased with increasing Fe^{2+} concentrations, with a greater increase observed in WITA 9 and NERICA L19 than in DANANE.

Catalase (CAT) activity: Analysis of variance revealed highly significant ($p < 0.001$) effects of Fe^{2+} concentration, variety, and their interaction on catalase (CAT) activity (Figure 2). Under the control treatment, CAT activity was highest in

Table 1. Chlorophyll a, chlorophyll b, total chlorophyll contents and chlorophyll stability index (CSI) of the rice varieties DANANE NERICA L19 and WITA 9 at 60 days after sowing under different Fe^{2+} concentrations

Treatments (ppm)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Chlorophyll stability index (%)
DC	1.45±0.00^{b}	1.07±0.01^{a}	2.52±0.00^{ab}	-
NC	1.48±0.01^{a}	1.09±0.00^{a}	2.57±0.01^{a}	-
WC	1.43±0.00^{c}	1.07±0.01^{a}	2.49±0.00^{b}	-
D150	1.41±0.00^{a}	0.96±0.01^{b}	2.38±0.00^{a}	94.13±0.35^{a}
N150	1.43±0.00^{a}	0.99±0.00^{b}	2.42±0.00^{a}	94.23±2.20^{a}
W150	1.38±0.00^{b}	0.89±0.02^{c}	2.27±0.00^{b}	91.07±0.76^{c}
D300	1.36±0.00^{a}	0.75±0.00^{e}	2.11±0.00^{b}	83.83±0.10^{b}
N300	1.38±0.00^{a}	0.82±0.00^{d}	2.20±0.00^{a}	85.83±0.45^{a}
W300	1.34±0.01^{b}	0.66±0.01^{f}	2.01±0.01^{c}	80.43±0.23^{c}
D600	1.27±0.01^{a}	0.61±0.02^{g}	1.87±0.01^{b}	74.03±0.12^{b}
N600	1.21±0.01^{a}	0.84±0.00^{d}	2.12±0.01^{a}	82.40±0.30^{a}
W600	1.00±0.01^{b}	0.51±0.00^{h}	1.71±0.01^{c}	68.73±0.32^{c}
D900	1.00±0.00^{a}	0.47±0.01^{h}	1.47±0.00^{ab}	58.10±0.40^{a}
N900	1.00±0.00^{a}	0.51±0.01^{h}	1.50±0.00^{a}	58.53±0.35^{a}
W900	0.99±0.00^{a}	0.43±0.02^{hi}	1.46±0.00^{b}	56.97±0.50^{a}

D, N, and W denote the rice varieties DANANE, NERICA L19, and WITA 9, respectively. Treatments DC, NC, and WC correspond to the control treatment, whereas the numbers 150, 300, 600, and 900 indicate the applied Fe^{2+} concentrations (ppm). Within each Fe^{2+} concentration and for a given parameter, means followed by the same lowercase letter are not significantly different according to Tukey's honestly significant difference (HSD) test at the 5% significance level ($p < 0.05$).

Table 2. Stress tolerance indices of antioxidant enzymes in the rice varieties DANANE, NERICA L19 and WITA 9 under different Fe^{2+} concentrations.

Treatment	STI_{POD}	STI_{CAT}	STI_{APX}
D150	0,91±0,00^{b}	0,85±0,01^{c}	1,05±0,78^{b}
N150	0,95±0,20^{a}	1,27±0,04^{b}	1,83±0,32^{a}
W150	0,72±0,10^{c}	2,51±0,04^{a}	0,67±0,06^{c}
D300	1,40±0,11^{b}	0,74±0,03^{b}	0,68±0,20^{b}
N300	2,04±0,29^{a}	0,96±0,03^{a}	1,45±0,19^{a}
W300	1,44±0,04^{b}	0,52±0,08^{c}	0,83±0,07^{a}
D600	3,30±0,37^{a}	0,92±0,02^{a}	1,00±0,04^{a}
N600	2,44±0,35^{a}	0,86±0,04^{a}	0,90±0,26^{a}
W600	4,57±0,17^{b}	0,41±0,09^{b}	0,49±0,01^{b}
D900	4,50±1,03^{b}	1,40±0,05^{c}	0,94±0,41^{b}
N900	7,28±0,78^{a}	1,56±0,05^{b}	2,16±0,46^{a}
W900	6,85±0,23^{a}	1,82±0,11^{a}	0,63±0,01^{c}

D, N and W denote the rice varieties DANANE, NERICA L19 and WITA 9, respectively. The numbers 150, 300, 600 and 900 indicate the applied Fe^{2+} concentrations (ppm). STI_{CAT}, $STI_{POD}$and STI_{APX} represent the stress tolerance indices of catalase, peroxidase and ascorbate peroxidase. Within each Fe^{2+} concentration and for a given parameter, means followed by the same lowercase letter are not significantly different according to Tukey's honestly significant difference (HSD) test at the 5% significance level (**p**< 0.05).

DANANE, followed by NERICA L19, whereas WITA 9 exhibited the lowest activity. At 150 ppm, CAT activity increased in all three varieties, with a more pronounced increase observed in WITA 9. At the intermediate Fe^{2+} concentrations (300 and 600 ppm), CAT activity markedly decreased in WITA 9, whereas it remained relatively stable in DANANE and NERICA L19. At 900 ppm, CAT activity increased substantially in all three varieties. However, the highest activities were recorded in DANANE (30.51 µmol H_2O_2 min^{-1} g^{-1} FW) and NERICA L19 (27.35 µmol H_2O_2 min^{-1} g^{-1} FW), whereas WITA 9 exhibited a significantly lower activity (18.28 µmol H_2O_2 min^{-1} g^{-1} FW).

Ascorbate peroxidase (APX) activity: Analysis of variance revealed highly significant ($p < 0.001$) effects of Fe^{2+} concentration, variety, and their interaction on ascorbate peroxidase (APX) activity (Figure 3). At 150 ppm, NERICA L19 and DANANE exhibited statistically similar APX activities, both significantly higher than that of WITA 9. At 300 ppm, APX activity differed significantly among the three varieties, with NERICA L19 recording the highest activity, followed by DANANE and WITA 9. However, at 600 ppm, DANANE exhibited the highest APX activity, followed by NERICA L19, whereas WITA 9 showed the lowest activity. At 900 ppm, NERICA L19 recorded the highest APX activity (0.109 µmol ascorbate oxidized min^{-1} g^{-1} FW), which was not significantly different from that of DANANE (0.095 µmol ascorbate oxidized min^{-1} g^{-1} FW), whereas WITA 9 exhibited a significantly lower activity (0.038 µmol ascorbate oxidized min^{-1} g^{-1} FW).

Stress tolerance indices: Table 2 presents the stress tolerance indices for catalase (STI_{CAT}), peroxidase (STI_{POD}), and ascorbate peroxidase (STI_{APX}) of the three rice varieties exposed to different Fe^{2+} concentrations. Analysis of variance revealed highly significant ($p < 0.001$) effects of Fe^{2+} concentration, variety, and their interaction on all stress tolerance indices. At 150 ppm, WITA 9 recorded the highest STI_{CAT} value (2.51), whereas NERICA L19 exhibited the highest STI_{POD} (0.95) and STI_{APX} (1.83) values. At 300 ppm, NERICA L19 maintained the highest STI_{CAT} (0.96), STI_{POD}, and STI_{APX} (1.45) values. At 600 ppm, STI_{POD} increased markedly in WITA 9 (4.57), whereas DANANE and NERICA L19 exhibited higher STI_{CAT} and STI_{APX} values, with no significant differences between the two varieties. At the highest Fe^{2+} concentration (900 ppm), WITA 9 recorded the highest STI_{CAT} value (1.82), whereas NERICA L19 exhibited the highest STI_{POD} (7.28) and STI_{APX} (2.16) values.

Relationships between physiological and biochemical parameters under iron toxicity

Pearson's correlation analysis: Pearson's correlation analysis revealed several significant relationships among the physiological and biochemical parameters evaluated (Figure 4). A very strong positive correlation was observed between total chlorophyll (TChl) and the chlorophyll stability index (CSI) (r = 1.00). In contrast, TChl was strongly and negatively correlated with peroxidase (POD) activity (r = −0.92) and STI_{POD} (r = −0.93). Furthermore, catalase (CAT) activity was positively correlated with ascorbate peroxidase (APX) activity (r = 0.64) and STI_{CAT} (r = 0.71). A moderate positive correlation was also observed between CAT and STI_{APX} (r = 0.46). In addition, POD activity showed a very strong positive correlation with STI_{POD} (r = 0.99), whereas CSI was strongly and negatively correlated with both POD activity (r = −0.93) and STI_{POD} (r = −0.94). Finally, APX activity was strongly and positively correlated with STI_{APX} (r = 0.77).

Principal component analysis (PCA): Principal component analysis (PCA) performed using the physiological and biochemical parameters measured in the three rice varieties exposed to different Fe^{2+} concentrations showed that the first two principal components explained 79.0% of the total variance, with 49.6% explained by PC1 and 29.4% by PC2 (Figure 5). PC1 separated total chlorophyll (TChl) and the chlorophyll stability index (CSI), which were projected on the negative side of the axis, from peroxidase (POD), STI_{POD}, catalase (CAT), ascorbate peroxidase (APX), STI_{CAT}, and STIAPX, which were located on the positive side. PC2 mainly discriminated APX, CAT, STI_{CAT}, and STI_{APX}, positioned in the upper part of the factor plane, from POD and STI_{POD}, which were located in the lower part. The projection of the treatments onto the PCA biplot revealed a distinct distribution according to Fe^{2+} concentration and rice variety (Figure 5). Treatments D150, W150, N150, and N300 were projected on the left side of the factor plane, in close proximity to TChl and CSI. Treatments D300, N600, and W300 were located in the lower-left quadrant. Treatments D600, D900, N900, W600, and W900 were distributed on the right side of the biplot in association with the enzymatic variables. Among these treatments, N900 and D900 were projected in the upper-right quadrant, whereas W600 and W900 were located in the lower-right quadrant.

DISCUSSION

The results of the present study demonstrated that increasing Fe^{2+} concentrations significantly affected the physiological and biochemical responses of the three rice varieties. The varietal differences observed indicate that the response to iron toxicity depends largely on the ability of each genotype to maintain physiological functions while activating appropriate defense mechanisms. This response is primarily associated with excessive Fe^{2+} accumulation in plant tissues, which promotes the generation of reactive oxygen species (ROS) through the Fenton reaction, thereby inducing oxidative stress (5). The progressive decline in chlorophyll contents with increasing Fe^{2+} concentrations indicates impairment of the photosynthetic apparatus. Excess iron is known to induce oxidative damage to chloroplasts and photosynthetic pigments, resulting in reduced photosynthetic efficiency (16, 17). Likewise, the gradual decrease in the chlorophyll stability index (CSI) further highlights the susceptibility of the photosynthetic machinery to iron toxicity. Among the three varieties, NERICA L19 maintained higher chlorophyll contents than DANANE and WITA 9, suggesting a greater capacity to preserve photosynthetic integrity under Fe^{2+} stress. The activities of antioxidant enzymes were also markedly influenced by increasing Fe^{2+} concentrations. The enhanced activities of catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) under high Fe^{2+} levels indicate activation of the antioxidant defense system in response to oxidative stress (2). These enzymes play a key role in scavenging hydrogen peroxide (H_2O_2) and limiting oxidative damage to cellular components (6). However, the magnitude of this enzymatic response differed among varieties. NERICA L19 and DANANE maintained higher and more stable antioxidant enzyme activities than WITA 9, indicating a more effective biochemical response to iron-induced oxidative stress. Stress tolerance indices further highlighted varietal differences in antioxidant responses. The higher STI_{POD} and STI_{APX} values recorded in NERICA L19 indicate a greater capacity to maintain or enhance the activities of these enzymes under iron stress. In contrast, WITA 9 exhibited lower STI_{APX} values, suggesting a weaker antioxidant response. Similar trends have been reported in rice, where tolerant genotypes generally display a more efficient activation of antioxidant defense systems together with better preservation of chlorophyll pigments under iron toxicity (18,19).

The multivariate analyses supported these findings. Pearson's correlation analysis revealed strong negative relationships between TChl, CSI, and POD or STI_{POD}, whereas positive correlations were observed between CAT, APX, and their corresponding stress tolerance indices. Principal component analysis further discriminated the physiological and biochemical variables and clearly separated treatments according to Fe^{2+} concentration and rice variety. Similar multivariate approaches have been successfully used to distinguish rice genotypes according to their tolerance to abiotic stresses, including iron toxicity (6,7). Overall, the present study demonstrated contrasting responses of the three rice varieties to iron toxicity. NERICA L19 was characterized by better maintenance of chlorophyll pigments, higher STI_{POD} and STI_{APX} values, and a stronger antioxidant enzymatic response. DANANE exhibited an intermediate response, whereas WITA 9 was the most susceptible variety under high Fe^{2+} concentrations. These findings indicate that tolerance to iron toxicity is associated with the combined ability to preserve photosynthetic performance and activate antioxidant defense mechanisms that mitigate oxidative stress.

CONCLUSION

This study demonstrated that increasing Fe^{2+} concentrations induced substantial physiological and biochemical changes in the three rice varieties evaluated. Iron toxicity resulted in a progressive decline in chlorophyll contents and the chlorophyll stability index (CSI), accompanied by changes in the activities of the antioxidant enzymes catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). The stress tolerance indices, Pearson's correlation analysis, and principal component analysis consistently revealed distinct varietal responses to iron toxicity.Among the genotypes investigated, NERICA L19 exhibited the greatest tolerance to iron toxicity, as evidenced by better maintenance of chlorophyll pigments, higher STI_{POD}

and STI_{APX} values, and a more effective antioxidant response. DANANE displayed an intermediate level of tolerance, whereas WITA 9 was identified as the most susceptible variety under high Fe^{2+} concentrations. These findings demonstrate that integrating physiological traits, antioxidant enzyme activities, and stress tolerance indices provides a robust approach for characterizing varietal responses to iron toxicity in rice. NERICA L19 therefore represents a promising genetic resource for rice breeding programs aimed at improving tolerance to iron toxicity and may be recommended for cultivation in iron-toxic lowland environments. Nevertheless, further field-based evaluations and molecular investigations are required to validate these findings and to gain deeper insights into the mechanisms underlying iron toxicity tolerance.

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