



Anti-oxidative enzymes in relation to iron toxicity caused by differential soil iron content in winter rice (*Oryza sativa*) of Assam, India

MADHUSMITA BARUAH¹, PRIYANKA DAS^{1*}, NILAY BORA¹, SUNAYANA RATHI¹ and BORSHA NEOG¹

Assam Agricultural University, Jorhat, Assam 785 013, India

Received: 14 August 2023; Accepted: 25 March 2025

Keywords: Iron content, Oxidative enzymes, Specific activity, Total activity

For winter rice (*Oryza sativa* L.), iron toxicity is a major problem affecting yield of rice. Baruah and Bharali (2015) stated about large inconsistency on iron toxicity with regard to soil iron content ranging from 20–5000 mg/kg and leaf iron content ranging from 300–2000 mg/kg, occurrence of toxicity from 2 weeks after transplanting to the late reproductive phase, distribution of toxicity symptoms and observed yield loss. Iron is a significant component of heme proteins, which includes cytochromes, catalase, peroxidase, Fe-S proteins like ferredoxin, aconitase and superoxide dismutase. Plant cells use antioxidative enzymes like peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) to protect the tissues from oxidative damage caused by iron. POD is an important antioxidant enzyme that responds to environmental stress. It reduces the harmful effects of H₂O₂ and degree of oxidative stress. In aerobic cells, SOD is the antioxidant enzyme that lowers reactive oxygen species. SOD activity determines the concentration of O₂ and H₂O₂. O₂ and H₂O₂ are produced through the dismutation of superoxide radical by the metal cofactors in their oxidized form (Mⁿ⁺¹). To overcome this, plant cells develop enzymatic mechanism to lessen the harmful effects by Fenton reaction. For enzymes having tetrameric heme, CATs have the ability to directly dismutate H₂O₂ into H₂O and O₂. Heavy metal stress causes a significant increase in CAT activity. Iron tolerance in rice is also influenced by CAT activity (Saikia and Baruah 2012). Mirkhamidova and Shakhmurova (2023) determined the activity of some antioxidant enzymes (CAT and POD) during the germination periods of cereals and legumes. Talubaghi *et al.* (2023) reported the effect of salt stress on rice cultivars' development, physiological and biochemical parameters and antioxidative enzyme (CAT and POD) activity. They suggested that the antioxidant enzyme activity can function

as an efficient defence mechanism against salinity stress. As a result, Binam cultivar which has a strong antioxidant defence system may be more tolerant to salinity stress that increases its yield compared to other cultivars.

Considering the availability of limited information on the soil iron content and its relationship with the activities of few iron containing antioxidative enzymes, the present investigation was conducted to know the activities of a few oxidative enzymes related to iron metabolism as influenced by soil DTPA (diethylene triamine penta acetic acid) extractable iron content. Baruah *et al.* (2022) have already reported about iron biofortification in rice.

The study was conducted at Assam Agricultural University, Jorhat, Assam. Rice varieties, Ranjit (susceptible), Mahsuri (tolerant) and Kajoli chakua (traditional chakua variety), were grown during 2019. To the plastic pots (24 cm diameter and 30 cm depth) of 15 kg capacity, 6 kg bulk surface clay soil collected from 0–20 cm depth was added. Recommended doses of NPK fertilizer were added to each pot. 1 L solution of 50 ppm ferrous sulphate (FeSO₄·7H₂O) was added to the soils of treated plants, two days before transplanting. However, the pots kept as control were treated with same volume of distilled water only. In each pot, three seedlings (30 days old) of same variety were transplanted. Each variety was replicated thrice. From the beginning of the experiment up to grain filling stage, soil of the pots was kept submerged up to 5 cm depth with distilled water. The characteristics of the representative soil samples of experimental pots in terms of pH, organic carbon, cation exchange capacity and diethylene triamine penta acetate (DTPA) extractable iron before planting were 5.10 and 4.80, 9.57 g/kg and 11.26 g/kg, 13.13 [cmol (p⁺)/kg] and 12.66 [cmol (p⁺)/kg] and 159.40 ± 0.51 mg/kg and 182.35 ± 0.57 mg/kg for control and treated, respectively.

The activity of enzyme POD was determined according to the method of Rangwala *et al.* (2018). One unit of total POD activity was defined as an increase in optical density by 1.0 under standard conditions (25°C, pH 6.0). The activity of enzyme SOD was determined according to the method

¹Assam Agricultural University, Jorhat, Assam.

*Corresponding author email: priyanka.das@aau.ac.in

of Asthir *et al.* (2012). One unit of total SOD activity was defined as change in $\Delta A/\text{min/g}$ fresh weight. The activity of enzyme CAT was determined according to the method of Alhasnawi *et al.* (2016). One unit of total activity of CAT decomposes $1.0 \mu\text{mol}$ of H_2O_2 per min at pH 7.0 at 25°C , while the H_2O_2 concentration falls from 10.3 mM to 9.2 mM . The rate of disappearance of H_2O_2 was followed by observing the rate of decrease in the absorbance at 240 nm . Total soluble protein content (required to estimate the specific activity) was determined according to the method of Aiyyswaraya *et al.* (2017). The mean data of three varieties were analysed statistically by using paired t-test in MS-Excel for comparison between control and treated. Within the range of soil available iron content ($159.40\text{--}182.35 \text{ mg/kg}$), iron toxicity was not observed in the present study.

The specific activity of POD in leaves was found to be non-significantly higher in treated than the control, at all the three stages (Table 1). Rossatto *et al.* (2017) reported the specific activity of POD in leaves of rice in both control and salt (NaCl) stressed condition. At 10 days after germination, it was found to be higher ($2.4 \mu\text{mol}/\text{min}/\text{mg}$ protein) in stressed condition than in control ($1.2 \mu\text{mol}/\text{min}/\text{mg}$ protein). However, at 15 and 20 days after germination, the same was observed to be higher in control than in stressed condition. Poli *et al.* (2018) observed higher specific activity of antioxidative enzymes (SOD, POD and CAT) in rice varieties grown in soil of low P content (stressed condition) than in soils containing normal levels of P. Mirkhamidova and Shakhmurova (2023) found that the highest activity of POD enzyme was found on the 1st day in wheat ($1,158 \mu\text{mol}/\text{min}/\text{g}$ mass).

The specific activity of POD in brown rice at grain filling stage (Table 1) was found to be significantly higher in treated plants which ranged from 2.50 ± 0.01 to 2.99 ± 0.01 units/mg protein (control) and 2.89 ± 0.03 to 3.61 ± 0.05 units/mg protein (treated). The highest was found in Kajoli chakua (traditional chakua variety) for control and Ranjit (susceptible) for treated and for both, the least was detected in Mahsuri (tolerant), respectively. At harvesting stage, it was found to be non-significant.

It was found that the specific activity was higher in leaves at both the stages, grain filling and harvesting. This might be due to detection of comparatively higher amount of iron in leaves, together with lower protein content of the leaves. The amount of active POD/mg soluble protein is higher in leaves than in brown rice.

The specific activity of SOD in leaves was found to be significantly higher in treated than in control only at grain filling stage (Table 1) which ranged from 1.85 ± 0.21 to 3.12 ± 0.20 units/mg protein (control) and 2.63 ± 0.30 to 4.05 ± 0.32 units/mg protein (treated). It was found to be the highest in Ranjit followed by Kajoli chakua and Mahsuri for treated, whereas in control, the same was found to be the highest for Kajoli chakua followed by Ranjit and Mahsuri.

Rossatto *et al.* (2017) also reported the specific activity of SOD in leaves of rice. At 10 and 15 days after

germination, it was found to be higher in stressed condition than in control. However, at 20 days after germination, the same was observed to be higher in control than in stressed condition. Alvarez *et al.* (2019) studied the induced response to antioxidative enzymes (SOD) in rice under stress due to lead and nickel. They found that rice plants with NiCl_2 treatments grew less than those treated with PbAc_2 at all concentrations ($0, 50, 100$ and 300 mg/kg) used compared to the control.

The specific activity of SOD in brown rice at grain filling stage (Table 1) ranged from 0.57 ± 0.03 to 1.72 ± 0.01 units/mg protein in control and 1.27 ± 0.03 to 1.97 ± 0.02 units/mg protein in treated plants. At harvesting stage, the same ranged from 0.25 ± 0.01 to 0.98 ± 0.02 units/mg protein in control and 1.28 ± 0.02 to 2.20 ± 0.03 units/mg protein in treated plants.

Detection of higher level of specific activity of SOD in leaves than in brown rice at both grain filling and harvesting stages indicated higher amount of active SOD per mg protein of the SOD extract in leaves. The specific activity of CAT extract in leaves was found to be significantly higher in treated than in control only at maximum tillering stage (Table 1) which ranged from 53.71 ± 0.29 to 71.62 ± 0.32 units/mg protein (control) and 59.75 ± 0.26 to 80.16 ± 0.40 units/mg protein (treated). The highest was found in Ranjit followed by Kajoli chakua and Mahsuri. As in total activity, the highest specific activity was detected at maximum tillering stage followed by grain filling stage and harvesting stage.

Rossatto *et al.* (2017) reported the specific activity of catalase in leaves of rice at 10, 15 and 20 days after germination to be higher in stressed condition than in control. Mirkhamidova and Shakhmurova (2023) found that the highest activity of the CAT enzyme during the germination period of cereal plant was determined on the 5th day of the germination period.

The specific activity of catalase in brown rice at both the stages (Table 1) were found to be non-significant irrespective of the iron content of soil. Detection of higher level of specific activity of catalase in leaves than in brown rice at both grain filling and harvesting stages indicated higher amount of active catalase/mg protein of the catalase extract in leaves.

SUMMARY

A study was conducted at Assam Agricultural University, Jorhat, Assam to know the effect of differential soil iron content on plant (leaves and grain) iron content together with the activities of some anti-oxidative enzymes, viz. peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) in a few popular rice varieties of Assam. The result of the pot experiment under rain protected condition revealed that application of iron fertilizer to soil (1 L of 50 ppm $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ to 6 kg soil) of initial soil iron content 159.40 mg/kg before planting led to increase in leaf iron content from $39.16 \text{ mg}/100 \text{ g}$ (control) to $45.36 \text{ mg}/100 \text{ g}$ (treated) at harvesting stage. However, no

Table 1 Specific activity (units/mg protein) of the anti-oxidative enzyme extracts and iron content (mg/100 g, dry weight basis) at different growth stages

Enzyme	Variety/ Stage	Leaves						Brown rice					
		Maximum tillering stage			Grain filling stage			Harvesting stage			Grain filling stage		
		Control	Treated		Control	Treated		Control	Treated		Control	Treated	
POD	Ranjit	50.34±1.10 (21.14±0.04)	78.09±2.63 (21.86±0.11)	53.04±1.23 (21.74±0.03)	59.36±1.24 (21.96±0.02)	38.37±1.25 (22.64±0.04)	55.49±1.19 (23.30±0.03)	2.91±0.02 (19.29±0.31)	3.61±0.05 (20.21±0.24)	2.10±0.01 (20.76±0.12)	2.10±0.01 (20.76±0.12)	3.84±0.04 (21.76±0.32)	
	Mahsuri	49.59±1.05 (22.81±0.01)	57.80±1.36 (23.12±0.07)	49.92±2.20 (22.46±0.03)	60.52±1.31 (23.00±0.20)	42.15±1.13 (24.02±0.03)	43.88±1.10 (22.82±0.03)	2.50±0.01 (20.04±0.51)	2.89±0.03 (20.86±0.22)	2.21±0.02 (21.79±0.33)	2.21±0.02 (21.79±0.33)	2.03±0.02 (22.10±0.14)	
	Kajoli chakua	48.47±1.36 (22.30±0.08)	65.14±1.15 (22.80±0.04)	54.28±1.41 (22.80±0.05)	55.55±1.37 (22.22±0.01)	36.33±1.24 (23.62±0.05)	44.70±1.16 (24.14±0.04)	2.99±0.01 (20.06±0.44)	3.30±0.01 (20.47±0.52)	1.90±0.01 (21.02±0.42)	1.90±0.01 (21.02±0.42)	2.32±0.05 (21.65±0.61)	
	Mean	49.46±0.94 (22.08±0.85)	67.01±10.27 (22.59±0.65)	52.41±2.24 (22.33±0.54)	58.47±2.60 (22.39±0.54)	38.95±2.95 (23.42±0.71)	48.02±6.47 (23.42±0.66)	2.80±0.26 (19.81±0.41)	3.26±0.36 (20.53±0.30)	2.07±0.15 (21.19±0.53)	2.07±0.15 (21.19±0.53)	2.73±0.97 (21.50±0.35)	
	P value	0.09 ^{NS} (0.05 [*])		0.15 ^{NS} (0.87 ^{NS})		0.17 ^{NS} (0.99 ^{NS})		0.05 [*] (0.04 [*])		0.36 ^{NS} (0.60 ^{NS})			
SOD	Ranjit	2.34±0.22 (0.75±0.05)	2.60±0.31 (1.25±0.01)	3.04±0.20 (1.25±0.03)	4.05±0.32 (1.50±0.01)	2.45±0.21 (1.25±0.02)	4.05±0.32 (1.50±0.01)	1.72±0.01 (1.50±0.02)	1.92±0.02 (1.00±0.05)	0.98±0.02 (0.75±0.01)	0.98±0.02 (0.75±0.01)	2.20±0.03 (1.50±0.03)	
	Mahsuri	1.61±0.19 (0.50±0.03)	2.50±0.24 (1.00±0.10)	1.85±0.21 (0.50±0.02)	2.63±0.30 (1.00±0.05)	0.55±0.22 (0.25±0.01)	1.04±0.21 (0.50±0.03)	0.57±0.03 (0.50±0.01)	1.27±0.03 (1.25±0.03)	0.25±0.01 (0.25±0.03)	0.25±0.01 (0.25±0.03)	1.63±0.02 (1.50±0.01)	
	Kajoli chakua	1.13±0.20 (0.50±0.01)	1.59±0.22 (0.75±0.03)	3.12±0.20 (1.00±0.05)	3.65±0.22 (1.50±0.03)	1.87±0.30 (0.75±0.03)	3.28±0.20 (1.25±0.03)	0.88±0.01 (0.75±0.02)	1.97±0.02 (1.50±0.01)	0.83±0.04 (1.00±0.02)	0.83±0.04 (1.00±0.02)	1.28±0.02 (1.25±0.02)	
	Mean	1.69±0.60 (0.58±0.14)	2.23±0.55 (1.00±0.25)	2.67±0.71 (0.91±0.38)	3.44±0.73 (1.33±0.28)	1.62±0.97 (0.75±0.50)	2.79±1.56 (1.08±0.52)	1.05±0.59 (0.91±0.52)	1.72±0.39 (1.25±0.25)	0.68±0.38 (0.66±0.38)	0.68±0.38 (0.66±0.38)	1.70±0.02 (1.41±0.14)	
	P value	0.10 ^{NS} (0.03 [*])		0.03 [*] (0.03 [*])		0.07 ^{NS} (0.05 [*])		0.12 ^{NS} (0.50 ^{NS})		0.07 ^{NS} (0.12 ^{NS})			
CAT	Ranjit	71.62±0.32 (88.81±0.24)	80.16±0.40 (106.62±0.21)	72.88±0.22 (107.87±0.52)	86.74±0.31 (114.50±0.48)	50.23±0.25 (80.37±0.44)	51.02±0.34 (84.18±0.22)	7.70±0.05 (127.75±0.79)	8.50±0.04 (128.93±0.43)	4.33±0.03 (104.31±0.26)	4.33±0.03 (104.31±0.26)	4.76±0.04 (119.00±0.10)	
	Mahsuri	53.71±0.29 (79.50±0.69)	59.75±0.26 (92.62±0.32)	56.00±0.28 (84.56±0.23)	57.91±0.30 (80.50±0.09)	45.51±0.22 (74.18±0.14)	45.73±0.26 (76.37±0.18)	7.48±0.09 (122.81±0.21)	7.64±0.07 (128.12±0.40)	4.01±0.03 (90.87±0.05)	4.01±0.03 (90.87±0.05)	4.19±0.02 (98.25±0.34)	
	Kajoli chakua	68.78±0.20 (109.37±0.60)	75.34±0.36 (122.06±1.38)	63.69±0.31 (83.43±0.22)	66.32±0.36 (97.50±0.35)	48.27±0.30 (78.68±0.11)	49.81±0.24 (85.18±0.53)	7.73±0.04 (127.68±0.50)	7.97±0.04 (128.93±0.23)	4.12±0.02 (97.25±0.23)	4.12±0.02 (97.25±0.23)	4.34±0.03 (103.25±0.46)	
	Mean	64.70±9.62 (92.56±15.28)	71.75±10.66 (107.10±14.72)	64.19±8.45 (91.95±13.79)	70.32±14.82 (97.50±17.00)	48.00±2.37 (77.74±3.19)	48.85±2.77 (81.91±4.82)	7.63±0.13 (126.08±2.83)	8.03±0.43 (128.66±0.46)	4.15±0.02 (97.47±6.72)	4.15±0.02 (97.47±6.72)	4.43±0.04 (106.83±10.82)	
	P value	0.01 [*] (0.01 [*])		0.25 ^{NS} (0.40 ^{NS})		0.15 ^{NS} (0.08 ^{NS})		0.18 ^{NS} (0.19 ^{NS})		0.07 ^{NS} (0.07 ^{NS})			
Iron*	Ranjit	27.64±0.06	54.17±0.25	58.81±0.26	75.01±0.26	57.58±0.01	69.71±0.19	2.18±0.08	5.92±0.10	4.41±0.55	4.41±0.55	10.39±0.47	
	Mahsuri	16.12±0.20	33.91±0.03	18.39±0.12	36.76±0.12	24.77±0.21	26.75±0.14	1.17±0.04	4.18±0.03	2.52±0.57	2.52±0.57	6.70±0.73	
	Kajoli chakua	27.29±0.36	36.01±0.04	47.05±0.03	62.26±0.07	35.12±0.06	39.62±0.20	1.87±0.14	5.13±0.08	3.46±0.24	3.46±0.24	7.95±0.36	
	Mean	23.68±6.55	41.36±11.14	41.42±20.79	58.01±19.47	39.16±16.77	45.36±22.04	1.74±5.17	5.08±8.70	3.46±1.12	3.46±1.12	8.34±1.87	
	P value	0.07 ^{NS}		0.00 [*]		0.17 ^{NS}		0.00 [*]		0.01 [*]			

The data represented are the mean of three replications ± Standard deviation; *, Significant @5% level of probability; ^{NS}, Not significant; POD, Paecoxidase; SOD, Superoxide dismutase; CAT, Catalase. The data in the parentheses represent the corresponding values for total activity (Baruah *et al.* 2022).

iron toxicity symptoms were observed which might be due to higher activities of CAT at maximum tillering stage and SOD at grain filling stage. The study revealed that the rice varieties Ranjit, Mahsuri and Kajoli chakua may respond to application of iron fertilization without producing iron toxicity symptom and yield more than 100% iron content in brown rice than that of control.

REFERENCES

- Aiyyswaraya S, Saraswathi R, Ramchander S, Vinoth R, Uma D, Sudhakar D and Robin S. 2017. An insight into total soluble proteins across rice (*Oryza sativa* L.) germplasm accessions. *International Journal of Current Microbiology and Applied Sciences*. **6**(12): 2254–69. DOI: <https://doi.org/10.20546/ijcmas.2017.612.261>
- Alhasnawi A N, Radziah C M Z C, Kadhimi A A, Isahak A, Mohamad A and Yusoff W M W. 2016. Enhancement of antioxidant enzyme activities in rice callus by ascorbic acid under salinity stress. *Biologia plantarum* **60**: 783–87.
- Alvarez S P, Montero D C, Duarte B N D, Tapiia M A M, Arreola J P S, Sanchez E and Ardisana E F H. 2019. Induced response to antioxidative enzymes in rice under stress due to lead and nickel. *Revista mexicana de ciencias agrícolas* **10**(1): 570. DOI: <https://doi.org/10.29312/remexca.v10i1.570>
- Asthir B, Koundal A and Bains N S. 2012. Putrescine modulates antioxidant defense response in wheat under high temperature stress. *Biologia Plantarum* **56**: 757–61.
- Baruah K K and Bharali A. 2015. Physiological basis of iron toxicity and its management in crops. *Recent Advances in Crop Physiology*, pp. 203–24. Singh A L (Ed). Daya Publishing House, New Delhi,
- Baruah M, Das P, Borah N, Chetia S K and Neog B. 2022. Biofortification of iron in rice (*Oryza sativa*) grown in acid soil of Assam, India. *The Indian Journal of Agricultural Sciences* **92**(11): 1399–401.
- Mirkhamidova P and Shakhmurova G. 2023. Determination of the activity of some antioxidant enzymes during the germination periods of cereals and legumes. *E3S Web of Conferences, EDP Sciences* **452**: 01018. DOI: <https://doi.org/10.1051/e3sconf/202345201018>
- Poli Y, Nallamothe V, Balakrishnan D, Ramesh P, Desiraju S, Mangrauthia S K, Voleti S R and Neelamraju S. 2018. Increased catalase activity and maintenance of photosystem II distinguishes high-yield mutants from low yield mutant of rice var. Nagina22 under low-phosphorus stress. *Frontiers in Plant Science* **9**: 1543–57.
- Rangwala T, Bafna A, Vyas N and Gupta R. 2018. Role of soluble silica in alleviating oxidative stress in soybean crop. *Indian Journal of Agricultural Research* **52**(1): 9–15.
- Rossatto T, Amaral M N, Benitez L C, Vighi I L, Braga E J B, Junior A M M, Maia M A C and Pinto L S. 2017. Gene expression and activity of antioxidant enzymes in rice plants, cv. BRS AG, under saline stress. *Physiology and Molecular Biology of Plants* **23**(4): 865–75.
- Saikia T and Baruah K K. 2012. Iron toxicity tolerance in rice (*Oryza sativa*) and its association with anti-oxidative enzyme activity. *Journal of Crop Science* **3**(3): 90–94.
- Talubaghi M J, Daliri M S, Mazloun P, Rameeh V and Mousavi A. 2023. Effect of salt stress on growth, physiological and biochemical parameters and activities of antioxidative enzymes of rice cultivars. *Cereal Research Communications* **51**(2): 403–11. DOI: <http://doi.org/10.1007/s42976-022-00314-w>