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# Iron treatment enhances the levels of reduced glutathione, oxidized glutathione and glutathione reductase activity in Rice (*Oryza sativa* L.)

**Ritu Saini, Harnek Singh Saini and Anjali Dahiya**

**Abstract**
The six rice varieties (Govind, Super, HKR120, Pusa1121, HBC19 and Palman579) differing in grain iron concentration (35- 400 µg/g) were evaluated for changes in reduced (GSH), oxidized glutathione (GSSG) content and glutathione reductase (GR) activity in root and shoot tissues in response to varied iron concentrations at vegetative and reproductive stages. Plants were grown in pots in the net house and treated twice with Yoshida solution containing different iron concentrations (0, 0.1 mM, 0.5 mM EDTA-Fe II). Both reduced and oxidized glutathione content and activity of glutathione reductase invariably increased with increasing iron treatment in both roots and shoots. The GR activity remained significantly low in the low grain Fe content varieties, Govind and Super, as compared to medium, HKR120 and PUSA1121 and high Fe varieties, HBC19 and Palman579. A significant positive correlation was observed between the per cent change in glutathione content and GR activity.

**Keywords:** Rice, iron, glutathione content, GR, grains

## Introduction
Plants are the primary source of nutrients for human nutrition on a global basis. Staple seed crops, such as rice, supply the majority of daily dietary nutrients for billions of people. However, rice has a low density of mineral nutrients, and for those whose diets are high in staple foods, micronutrient malnutrition is widespread (Grusak and DellaPenna, 1999) [8]. Iron (Fe) is an essential microelement that is involved in various important processes in plant cells. Through the redox status change between the ferrous ($Fe^{2+}$) and ferric ($Fe^{3+}$) form, Fe functions as an electron donor or acceptor, which is crucial in the processes of respiration and photosynthesis (Kobayashi & Nishizawa, 2012) [14]. The relationship between decrease iron availability in the nutrient media and the possible onset of oxidative stress is becoming more evident, because of the dual role played by iron in cell metabolism as either an antioxidant or a pro-oxidant factor. In fact, iron is a constituent or a cofactor of many antioxidant enzymes but, on the other hand, it can act as pro-oxidant because it catalysis free radical generation through the Fenton's reaction (Minotti and Aust, 1987) [21]. Ascorbate and glutathione play decisive roles in cell redox homeostasis, antioxidant defense, and plant development in normal metabolism and under oxidative stress (Gill and Tuteja, 2010; Mhamdi *et al.*, 2010) [6, 20]. Glutathione, an important antioxidant involved in cellular defence against toxicants, was shown to increase under metal stress (Sun *et al*., 2007; Anjum *et al*., 2014) [34, 1]. Glutathione occurs abundantly in reduced form (GSH) in plant tissues and provides a substrate for multiple cellular reactions that yield GSSG (i.e., two glutathione molecules linked by a disulfide bond). The balance between the GSH and GSSG has been reported to be a central component in maintaining cellular redox state (Foyer and Noctor, 2005) [4]. Glutathione reductase (GR), the key enzyme for maintaining the GSH pool in a reduced state (Rennenberg, 1982) [28], is a disulfide oxido-reductase flavoprotein, dependent on NADPH as electron donor. GR catalyzes the last and rate-limiting step of the Halliwell-Asada enzymatic pathway. The NADPH-dependent GSSG reduction is catalyzed by GR, a flavoenzyme found in the chloroplast, cytosol and mitochondria. The elevated levels of GR activity could increase the GSH/GSSG ratio, which is required for ascorbate regeneration. Glutathione is a redox buffer that protects cell against reactive oxygen species (ROS), which accumulate in response to heavy metal stress (Mullineaux, 2005) [23]. It functions through the ascorbate–GSH-cycle (AGC) and glutathione S-transferase (GST) based detoxification mechanisms (Labrou *et al.,* 2015) [17]. Elevated GSH level has been correlated with increased plant resistance to oxidative damage and a high ratio of GSH/GSSG is required for efficient removal of ROS generated under abiotic stresses (Kocsy *et al.,* 2004) [15].

The objective of this work is to investigate the effect of iron treatments on shoots and roots of six rice varieties by using antioxidant component (GSH and GSSG) content and GR activity.

## Materials and methods

### Plant material

The experimental material comprised of fifteen rice varieties *viz*. Palman579, HKR95-157, HKR95-130, HBC19, HKR47, PS 4, HKR120, PUSA1121, IR64, IR72, Jaya, Govind, Azucena, PAU201 and Super. Seeds of the ten rice varieties *i.e* HBC19, HKR47, PS4, IR64, Jaya, Govind, HKR120, Azucena, PAU201 and PUSA1121 were procured from Department of Genetics and Plant breeding, Rice Research Station, Kaul and seeds of Palman579, HKR95-157, HKR95-130, IR72 and Super were obtained from Department of Molecular Biology, Biotechnology and Bioinformatics, CCS HAU (Haryana).

### Raising of crops and treatments

Grain iron (Fe) content of fifteen rice varieties was analyzed by method of and based on the data, six rice varieties, two each with low grain iron (Govind, Super), medium grain iron (HKR120, PUSA1121) and high grain iron (HBC19, Palman579) content were selected for this study. The crop was raised during kharif seasons of 2013-2014 and 2014-2015 in net house of Department of Chemistry and Biochemistry, CCS HAU, Hisar. Seeds of all rice varieties were sown directly in pots at 2-3 cm depth in light textured (loamy) soil with standard conventional cultivation practices and the pots were divided in three sets after 20 days of sowing and following treatment were given: One set was given Yoshida nutrient medium without Fe (0 mM EDTA-Fe(II)). Second set was given Yoshida nutrient medium with 0.1mM EDTA-Fe(II)) concentration. Third set was given Yoshida nutrient medium with high Fe concentration (0.5 mM EDTA-Fe (II)).

### Reduced and Oxidized Glutathione content

### Extraction

One gram each of shoot and root tissues from control and treated plants was ground in 5 ml of chilled 0.8 N $HClO_4$ and centrifuged at 10,000 rpm for 25 min. The clear supernatant was decanted carefully and was used for the estimation of glutathione.

### Estimation

Glutathione was estimated by the method of Griffith (1980). The reaction mixture (2.5 ml) consisted of 2.1 ml of 125 mM phosphate buffer (pH 7.5) containing 6.3 mM EDTA, 0.1 ml of 2.5 mM NADPH, 0.1 ml of 6 mM 5,5'-dithiobis (2-nitrobenzoic acid) and 0.5 units of glutathione reductase enzyme and 0.1 ml of supernatant neutralized with sodium bicarbonate. Increase in absorbance was recorded at 412 nm for 3 min. Oxidized glutathione (GSSG) was determined by incubating 1.5 ml of 0.5 M potassium phosphate buffer (pH 7.5), 0.2 ml of 4-vinylpyridine and 1 ml supernatant for 1 hour to make GSH nonreactive. The same procedure as for total glutathione was now used to measure GSSG. GSH was measured by subtracting GSSG from the total glutathione content. The standard curve was prepared with 1-50 µmol GSSG.

### Glutathione reductase (EC 1.6.4.2)

Method of Halliwell and Foyer (1978) [9] was followed for measuring GR activity. The reaction mixture consisted of 2 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of 5 mM oxidized glutathione (GSSG), 0.1 ml of 3.5 mM NADPH and 0.1 ml enzyme extract in final volume of 2.3 ml. The decrease in absorbance at 340 nm due to oxidation of NADPH was monitored. Non-enzymatic oxidation of NADPH was recorded and subtracted from it. An extinction coefficient of 6.22 $mM^{-1}$ $cm^{-1}$ for NADPH was used to calculate the amount of NADPH oxidized which corresponded to GR activity. One enzyme unit was defined as amount of enzyme required to oxidize 0.1 µmole of NADPH oxidized per min.

The data obtained in the present investigation was subjected to analysis of variance (ANOVA) technique and thus analyzed according to two/three factorial completely randomized designs. The critical difference value at 5% level was used for making comparison among various rice varieties grown under different iron treatments and at all the stages**.** OP stat was used to analyze experimental results statistically.

## Results

### Reduced glutathione content (GSH)

Reduced glutathione content varied from 5.35 to 12.14 nmoles $g^{-1}$ f.wt. and 4.43 to 9.69 nmoles $g^{-1}$ f.wt., respectively in the shoots of six rice varieties grown under different Fe treatments at vegetative and reproductive stages (Fig. 1.1; A, B). Rice varieties with high and medium grain Fe had substantially higher level of GSH content as compared to varieties with low grain Fe. GSH content increased with increasing iron concentration and the increase was more pronounced in medium- and high- iron varieties. At vegetative stage, maximum increase in GSH was observed in PUSA1121 (72.36%) while minimum in Govind (26.38%) and Super (29.67%) at 0.5 mM iron concentration (Fig. 1.1 A). At reproductive stage, 0.1 and 0.5 mM Fe treatments enhanced the GSH content in shoots of six rice varieties by 6.35% to 67.62% (Fig. 1.1 B).

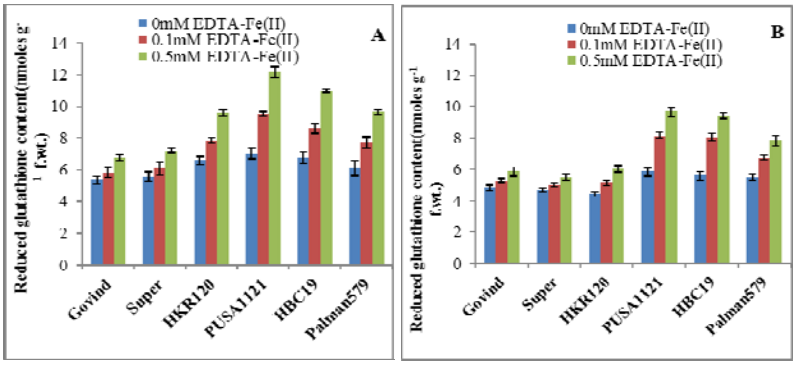


**Fig 1.1:** Variation in reduced glutathione (GSH) content in response to iron treatments in shoots of six rice varieties at vegetative (A) and reproductive (B) stages [The bars denote ± SE]

Similarly, high GSH content was observed in the roots of iron-rich rice varieties (HBC19 and Palman579) at both the stages. GSH content increased with increase in Fe concentrations in all the rice varieties with maximum increment of 67.42% in HBC19 followed by Palman579 (63.73%), PUSA1121 (50.91%), HKR120 (41.21%), Govind (26.32%) and Super (24.25%) (Fig. 1.2 A). Likewise at reproductive stage, maximum increase in GSH content was shown by HBC19 (64.59%) followed by Palman579 (58.50%), PUSA1121 (42.86%), HKR120 (39.73%), Super (19.14%) and Govind (18.12%) (Fig. 1.2 B). Reduced glutathione content was significantly less at reproductive stage as compared to vegetative stage in all six varieties and at all the three treatments.

**Oxidized glutathione content (GSSG)**

As shown in Fig. 1.3 (A and B), oxidized glutathione (GSSG) content in shoots was significantly higher in low-Fe rice varieties at both vegetative and reproductive stages under all the Fe treatments. The minimum increment in GSSH content was 26.76% in PUSA1121 while maximum in Govind (66.68%) (Fig. 1.3 A).

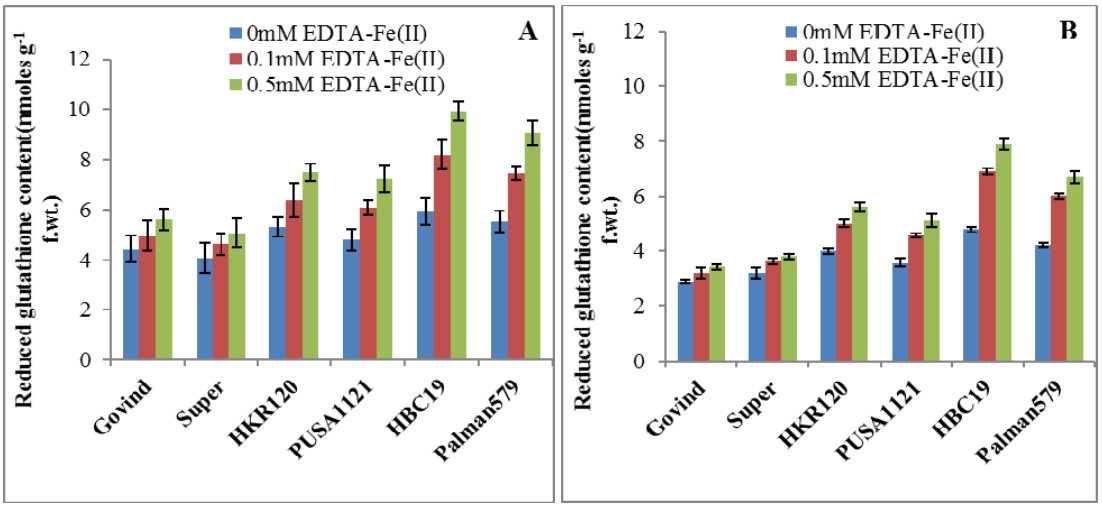


**Fig 1.2:** Variation in reduced glutathione (GSH) content in response to iron treatments in roots of six rice varieties at vegetative (A) and reproductive (B) stages [The bars denote ± SE]

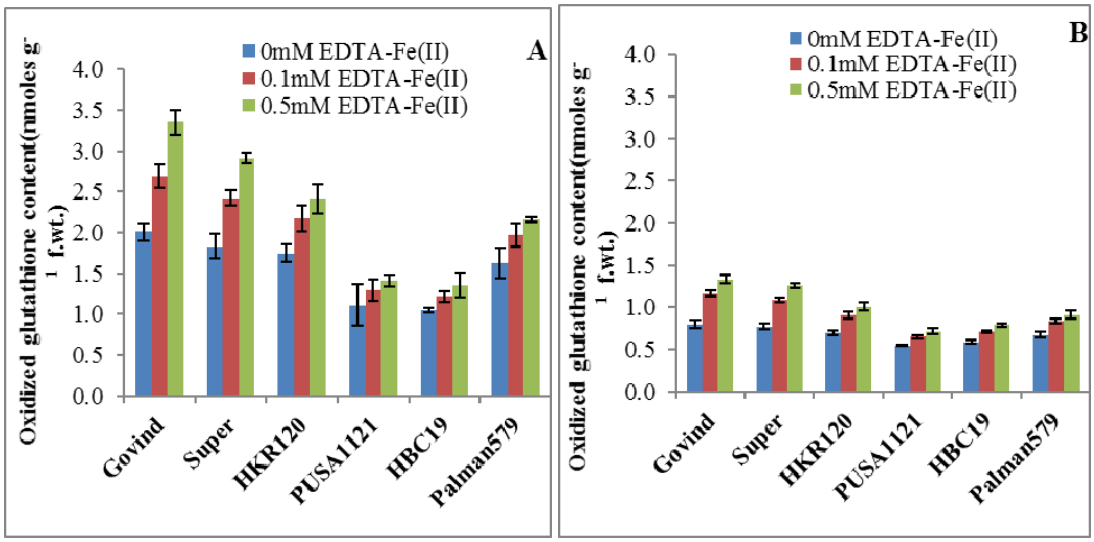


**Fig 1.3:** Variation in oxidized glutathione (GSSG) content in response to iron treatments in shoots of six rice varieties at vegetative (A) and reproductive (B) stages [The bars denote ± SE]

Similar results were observed at reproductive stage. GSSG content in shoots of six rice varieties varied from 0.65 to 1.16 and 0.72 to 1.34 nmoles $g^{-1}$ f.wt. at 0.1 mM and 0.5 mM iron treatments, respectively. The highest GSSG increase of 67.20% in Govind and lowest (30.55 %) in PUSA1121 was observed at 0.5 mM iron treatment.

GSSG content was significantly lower in roots of Palman579 (0.73 nmoles $g^{-1}$ f.wt.) and HBC19 (0.77 nmoles $g^{-1}$ f.wt) whereas higher GSSG levels were observed in Govind (1.38 nmol $g^{-1}$ f.wt.) and Super (1.25 nmoles $g^{-1}$ f.wt.) at vegetative stage at 0mM iron condition conditions (Fig. 1.4 A). GSSG content increased in all rice varieties with increasing Fe concentrations; however, the magnitude of increase was more in Super (59.01%) and Govind (54.84%) as compared to Palman579 (24.83%) and HBC19 (26.03%) at 0.5 mM iron treatment. A similar trend of change in GSSG levels was observed at reproductive stage (Fig. 1.4 B) where maximum increase was observed in the root tissues of Super (60.83%) and Govind (55.57%) followed by HKR120 (43.95%), PUSA1121 (39.81%), HBC19 (33.90%) and Palman579 (30.25%) at 0.5 mM Fe treatment.

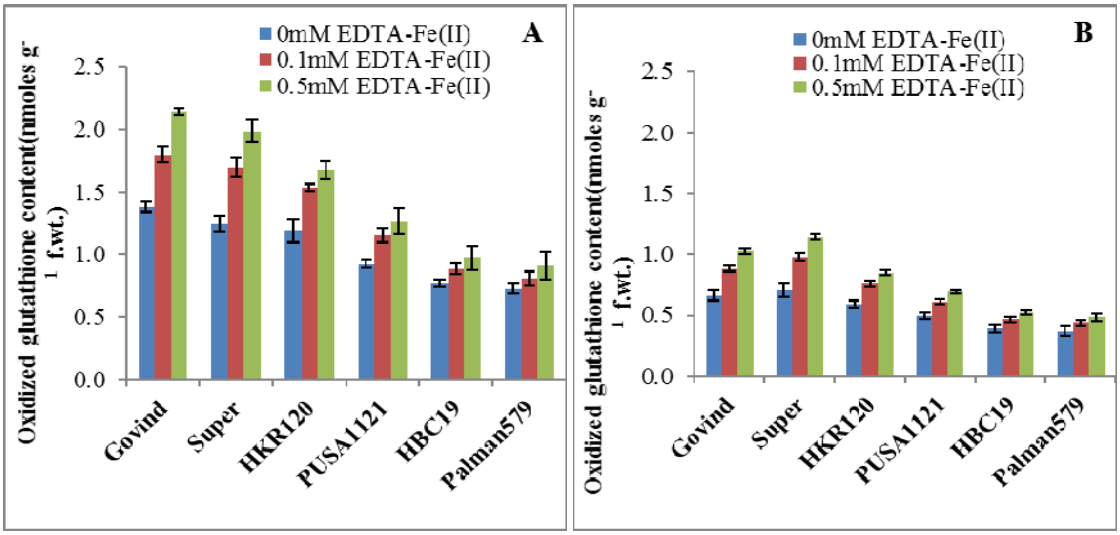


**Fig 1.4:** Variation in oxidized glutathione (GSSG) content in response to iron treatments in roots of six rice varieties at vegetative (A) and reproductive (B) stages [The bars denote ± SE]

**Glutathione reductase (GR)**

The activity of GR was lowest in both the tissues of Govind and Super rice varieties at both the vegetative and reproductive stages under different Fe treatments. In the shoot tissues, glutathione reductase activity showed a progressive increase with Fe treatment in all rice varieties but increase was relatively more in medium- and high- Fe rice varieties (Fig. 1.5 A and B). At vegetative stage, the enzyme activity was significantly higher (15.85- 20.44 units $g^{-1}$ f.wt.) in HKR120, PUSA1121, HBC19 and Palman579 as compared to Govind (13.61 units $g^{-1}$ f.wt.) and Super (12.68 units $g^{-1}$ f.wt.) under control conditions.

Maximum increase of 34.16% was observed in PUSA1121 at 0.1 mM Fe treatment followed by HBC19 (30.27%), Palman579 (27.03%), HKR120 (20.51%), Super (13.21%) and Govind (11.87%). Similarly, at 0.5 mM Fe treatment, increase in GR activity ranged from 34.85 (Govind) to 72.47% (PUSA1121). At reproductive stage, GR activity varied between 12.23-18.38 units $g^{-1}$ f.wt. under control (no additional iron in soil) conditions. The enhancement in GR activity was between 21.04% (Super) to 40.95% in (PUSA1121) at 0.1 mM Fe concentration and it further increased to 36.44% and 70.89%, respectively at 0.5 mM Fe treatment (Fig. 1.5 B).

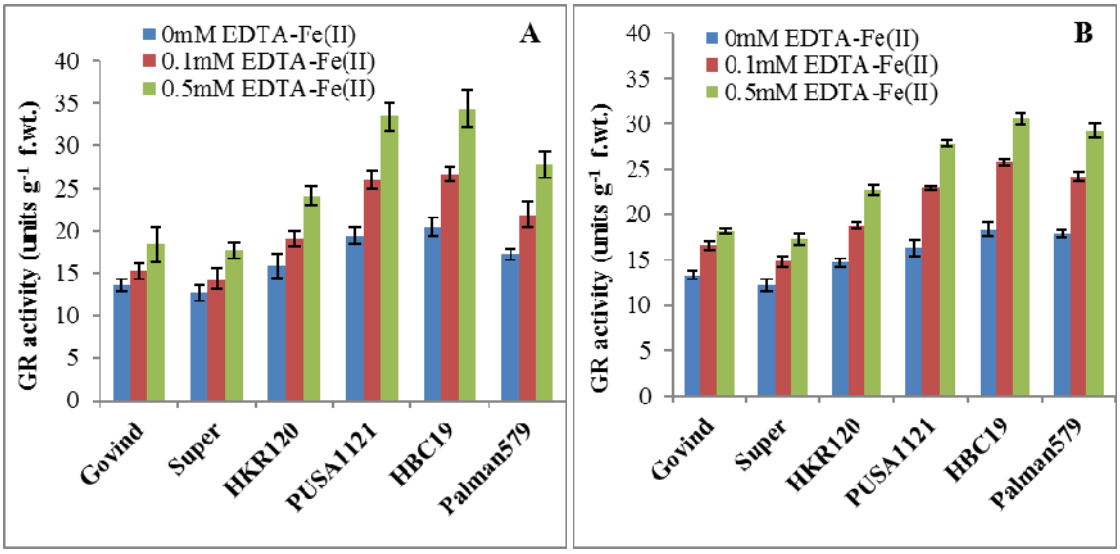


**Fig 1.5:** Variation in glutathione reductase (GR) activity in response to iron treatments in shoots of six rice varieties at vegetative (A) and reproductive (B) stages [The bars denote ± SE]

In roots also, high GR activity was observed in rice varieties with medium and high grain iron content as compared to iron-poor varieties, Govind and Super, grown under different Fe conditions at both the developmental stages. The GR activity in root tissues at vegetative stage varied from 3.83 to 5.35 units $g^{-1}$ f.wt. under control conditions. The activity increased with increasing iron treatments in all the six varieties; the increase was in the range of 11.23 (Govind) to 38.74% (HBC19) at 0.1 mM Fe concentration and 39.99 (Govind) to 75.69% (Palman579) at 0.5 mM Fe concentration. Maximum increase was observed in HBC19 and Palman579 while minimum in Govind (Fig. 1.6 A). At reproductive stage also, at 0.1 mM Fe treatment, maximum increase in GR activity was observed in HBC19 (38.47%) and Palman579 (35.65%) in comparison to 16.24% and 18.42% increase in Super and Govind, respectively. Similar observations were made at 0.5 mM Fe treatment (Fig. 1.6 B).

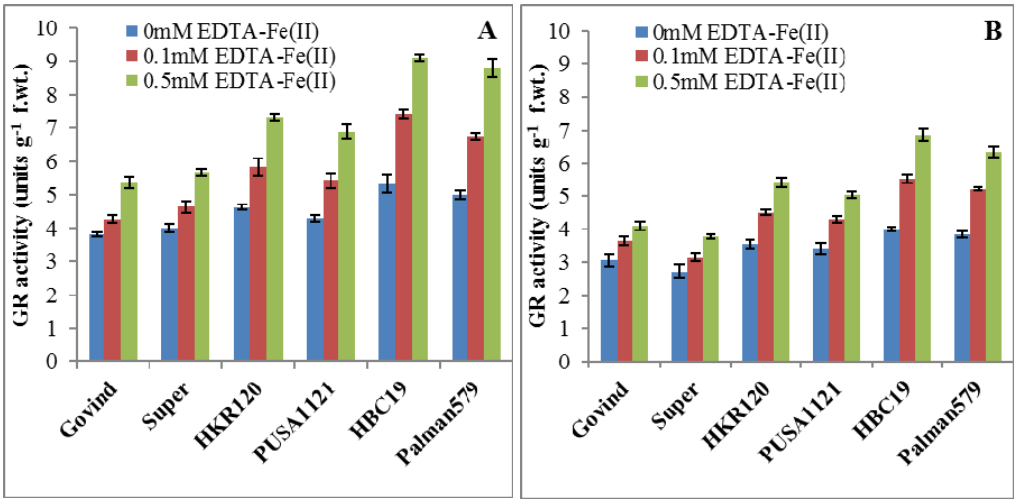


**Fig 1.6:** Variation in glutathione reductase (GR) activity in response to iron treatments in roots of six rice varieties at vegetative (A) and reproductive (B) stages [The bars denote ± SE]

**Correlation between per cent change in GSH content and GR activity**

Fig. 1.7 and 1.8 depict that a significant positive correlation (at p≤0.5) existed between the per cent change in reduced glutathione content and GR activity in shoots and roots of six rice varieties, both at vegetative and reproductive stages. In shoots, significant positive correlations ($R^2$) of 0.986 and 0.936 between per cent change in GSH and GR activity were observed at 0.1 and at 0.5 mM Fe treatments, respectively at vegetative stage (Fig. 1.7 A and C). Likewise, at reproductive stage higher correlation ($R^2$ = 0.989) was noticed at 0.1 mM Fe treatment (Fig. 1.7 C).

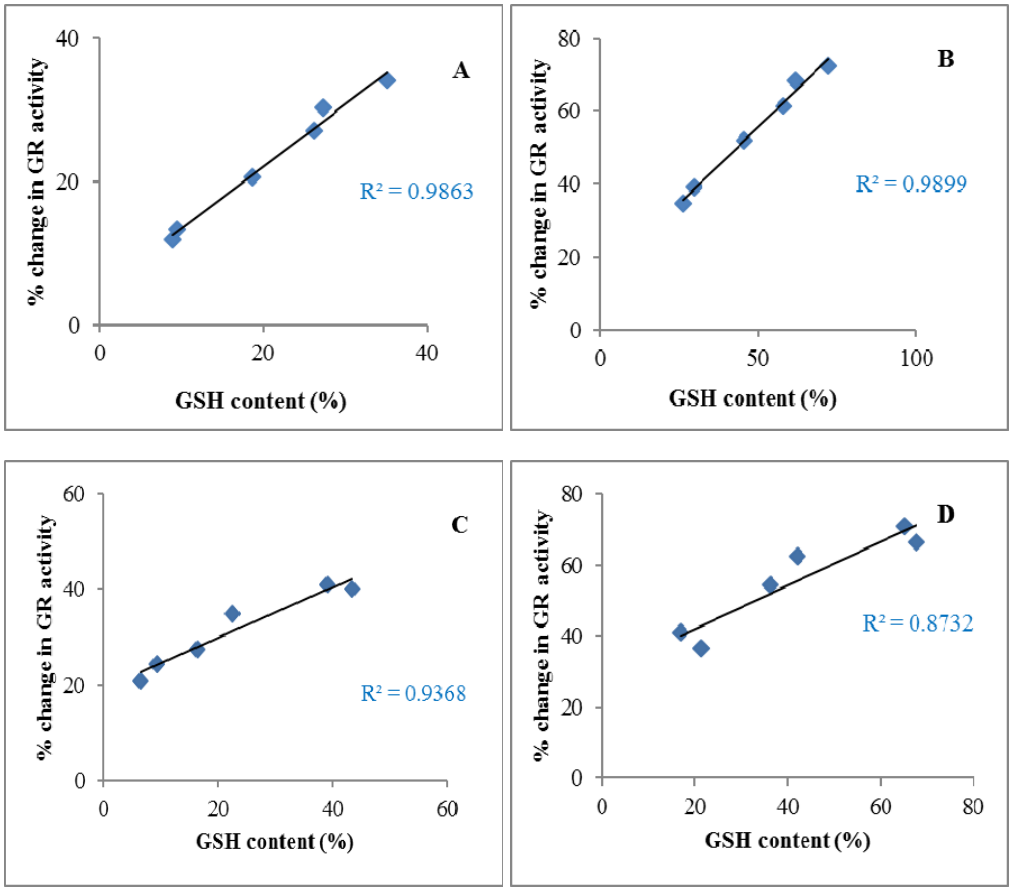


**Fig 1.7:** Correlation between per cent change in GSH content and GR activity in response to iron treatments (A and B, 0.1 mM Fe-EDTA and C and D, 0.5 mM Fe-EDTA) in shoots of six rice varieties at vegetative (A,C) and reproductive (B,D) stages

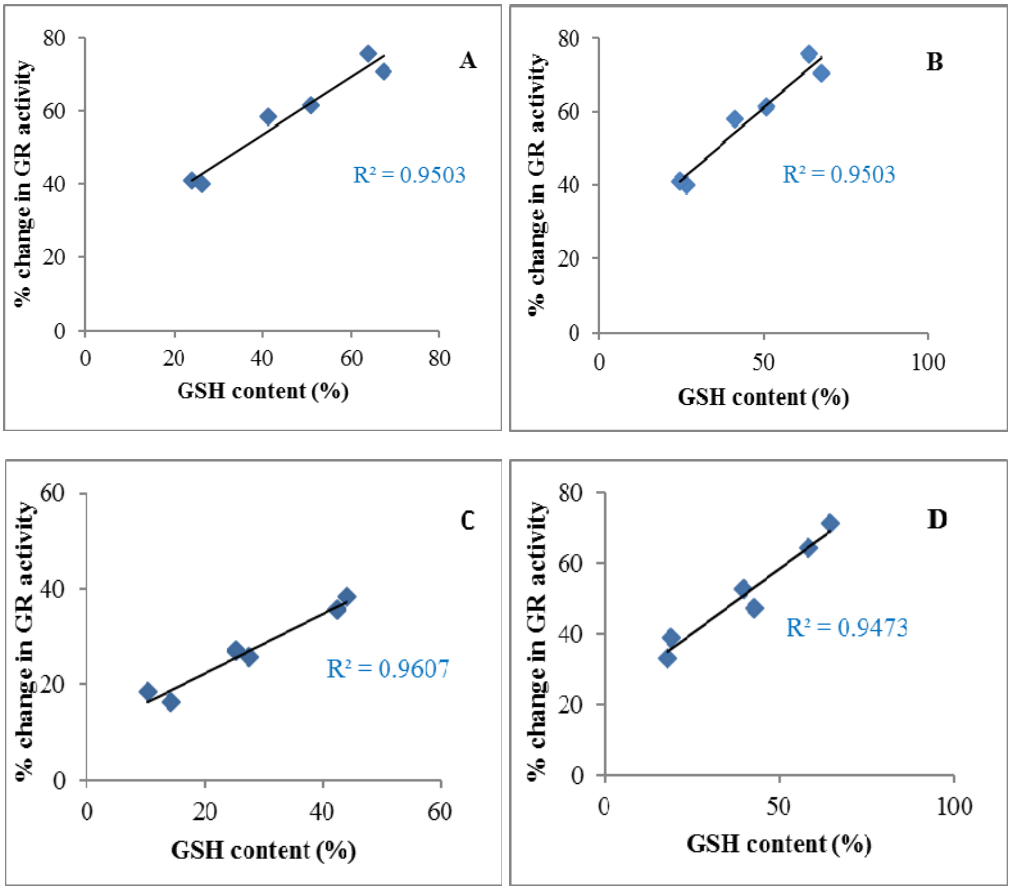


**Fig 1.8:** Correlation between per cent change in GSH content and GR activity in response to iron treatments (A and B, 0.1 mM Fe-EDTA and C and D, 0.5 mM Fe-EDTA) in roots of six rice varieties at vegetative (A,C) and reproductive (B,D) stages

In roots at 0.1 mM Fe concentration, significant correlation ($R^2$=0.950) was observed between per cent change in GSH and GR activity at both vegetative and reproductive stages (Fig. 1.8 A and B). Maximum correlation ($R^2$= 0.960) was observed at reproductive stage at high (0.5 mM) Fe treatment (Fig. 1.8 C).

**Discussion**

Glutathione play a key role in protecting plants against ROS-mediated oxidative damage (Smirnoff, 2005) [33] and involved in maintenance of redox state of cells. In the present study, reduced glutathione content increased with Fe treatment in shoots and roots of all rice varieties at both the stages. Reduced glutathione (GSH) contents were higher in shoots and roots of the high (Palman579 and HBC19) and medium (PUSA1121 and HKR120) grain Fe rice varieties than Govind and Super (Fig. 1.1; 1.2). In contrast, oxidized glutathione content (GSSG) was more in Govind and Super than high and medium Fe varieties at both the stages (Fig.1.3; 1.4). Similar to our findings, concomitant increase of glutathione in rice (Kabir *et al*., 2016) [13] and *Bacopa monnieri* (Sinha and Sexana, 2006) [32] plants was observed with increased Fe treatment. Foyer and Noctor (2005) [4] suggested that the GSH/GSSG ratio, an indicative of the cellular redox balance, may be involved in ROS perception. GSH acts as an antioxidant and was involved directly in the reduction of ROS generated during oxidative stress (Shao *et al*., 2008) [30]. Increase in GSH biosynthesis enhanced tolerance towards Cd and Ni in the shoots of various plant species (Freeman *et al*., 2004) [5]. Rahman *et al.* (2016) [27] and Hasanuzzaman *et al.* (2012) [10] observed that the levels of GSH and GSSG increased with increased GR activity under Cd-stress in rice. Thounaojam *et al*. (2012) [35] reported that ascorbate and glutathione (GSH) contents increased in all the Cu treated rice plants as compared to the control. Contrarily, Salama *et al*. (2009) [29] reported that glutathione content in leaf showed marked increase in the flax cultivars under Fe starvation treatments against Fe-sufficient treatments. Glutathione increased under Fe-deficiency in *Borage officinalis* (Mohamed and Aly, 2004) [22], cucumber (Zaharieva, *et al*., 1999) [37] and sugar beet (Zaharieva and Abadia, 2003) [36]. Jucoski *et al.* (2013) [12] reported that the GSH and GSSG contents in Fe-treated plants was higher than in controls, especially in the leaf, indicating an adaptive response of the plants to the oxidative stress caused by high Fe concentration. Sharma and Dubey (2005) [31] reported increase in GSH in rice with increase being higher in roots than in shoots and proposed that it could either be due to direct transport of GSH from shoots to roots or due to its direct increased synthesis in roots under stressed environment. Contrarily, both ascorbate and GSH contents were higher in shoots than roots in the present study, possibly due to higher biosynthesis of ascorbate and glutathione in shoot tissues as a result of higher activity of antioxidative enzymes and ascorbate–glutathione cycle.

GR, a key-enzyme of the ascorbate/glutathione cycle, is essential to maintain cell homeostasis during oxidative stress (Noctor and Foyer, 1998) [24]. In this investigation, GR activity increased with increasing Fe content as compared to control (without Fe) conditions in shoots and roots of all six rice varieties. Concomitant with our results, Jucoski *et al.* (2013) [12] analyzed that the enzymatic activity of GR increased with increasing Fe concentration in young *Eugenia*

*uniflora L.* plants. $FeSO_4$ treatment also resulted in a higher GR activity in rice leaves (Fang *et al*., 2001) [3]. Over-expression of GR in several plant species has not in itself been able to confer marked increase in stress resistance (Noctor *et al.,* 2012; Kornyeyev *et al*., 2005; Ding *et al*., 2009) [25, 16, 2]. GR activity was enhanced 1.4-fold with Fe deficiency in sugar beet (Zaharieva and Abadia, 2003) [36]. Increased GR activity was reported in zinc treated wheat seedlings (Li *et al.,* 2012) [18], lead (Pb) treated *Oryza sativa* (Panda *et al*., 2011) [26], under Cd treatment in *Brassica juncea* (Iqbal *et al*., 2010) [11] and nickel (Ni) treated *Elodea Canadensis* (Maleva *et al*., 2009) [19].

**Conclusion**

In this study, more accumulation of glutathione content (GSH, GSSG) along with the gradual increase in glutathione reductase activity at higher iron treatments especially in high grain iron varieties suggest that a superior ROS scavenging system and greater ability to restrict the damage to cellular membranes may be responsible for the adaptation of these varieties at high iron levels.

**References**


1. Anjum N, Aref IM, Duarte AC, Pereira E, Ahmad I, Igbal M. Glutathione and proline can coordinately make plants withstand the joint attack of metalloid and salinity. Front. Plant Sci. 2014; 5:662.
2. Ding HD, Zhang XH, Xu SC, Sun LL, Jiang MY, Zhang AY *et al*. Induction of protection against paraquat-induced oxidative damage by abscisic acid in maize leaves is mediated through mitogen-activated protein kinase. J. Integr. Plant Biol. 2009; 51:961-972.
3. Fang WC, Wang JW, Lin CC, Kao CH. Iron induction of lipid peroxidation and effects on antioxidative enzyme activities in rice leaves. Plant Growth Regulation, 2001; 35:75-80.
4. Foyer CH, Noctor G. Oxidant and antioxidant signaling in plants: a reevaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ. 2005; 28:1066-1071.
5. Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ *et al*. Increased glutathione biosynthesis plays a role in nickel tolerance in Thlaspi nickel hyperaccumulators. Plant Cell, 2004; 16:2176-2191.
6. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 2010; 48:909-930.
7. Griffith OW. Determination of glutathione disulfide using glutathione reductase and 2-vinyl pyridine. Anal. Biochem. 1980; 106:207-212.
8. Grusak MA. Dellapenna D. Improving the nutrient composition of plants to enhance human nutrition and health. Annu. Rev. Plant Physiol. Plant Mol Biol. 1999; 50:133-161.
9. Halliwell B, Foyer CH. Properties and physiological functions of a glutathione reductase purified from spinach leaves by affinity chromatography. Planta, 1978; 139:9-17.
10. Hasanuzzaman M, Hossain MA, Fujita M. Exogenous selenium pretreatment protects rapeseed from cadmium-induced oxidative stress by upregulating antioxidant defense and methylglyoxal detoxification systems. Biol. Trace Elem. Res. 2012; 149:248-261.
11. Iqbal N, Masood A, Nazar R, Syeed S, Khan NA. Photosynthesis, growth and antioxidant metabolism in mustard (*Brassica juncea* L.) cultivars differing in cadmium tolerance. Agricultural sciences in China, 2010; 9(4):519-527.
12. Jucoski GO, Cambraia J, Ribeiro C, Oliveira JA, de Paula SO, Oliva MA. Impact of iron toxicity on oxidative metabolism in young *Eugenia uniflora* L. plants. Acta Physiol. Plant, 2013; 35:1645-1657.
13. Kabir AH, Begum MC, Haque A, Amin R, Swaraz AM, Haider SA *et al*. Genetic variation in Fe toxicity tolerance is associated with the regulation of translocation and chelation of iron along with antioxidant defence in shoots of rice. Functional Plant Biology, 2016. doi: 10.1071/FP16068.
14. Kobayashi T, Nishizawa NK. Iron uptake, translocation, and regulation in higher plants. Annu. Rev. Plant Biol. 2012; 63:131-152.
15. Kocsy G, Kobrehel K, Szalai G, Duviau MP, Buzas Z, Galiba G. Thioredoxin h and glutathione as abiotic stress tolerance markers in maize. Env. Exp. Bot. 2004; 52:101-112.
16. Kornyeyev D, Logan BA, Allen RD, Holaday AS. Field-grown cotton plants with elevated activity of chloroplastic glutathione reductase exhibit no significant alteration of diurnal or seasonal patterns of excitation energy partitioning and $CO_2$ fixation. Field Crops Research, 2005; 94:165-175.
17. Labrou NE, Papageorgiou AC, Pavli O, Flemetakis E. Plant GSTome: structure and functional role in xenome network and plant stress response. Curr. Opin. Biotechnol. 2015; 32:186-194.
18. Li XN, Ma HZ, Jia PX, Wang J, Jia LY, Zhang TG *et al*. Responses of seedling growth and antioxidant activity to excess iron and copper in Triticum aestivum L. Ecotox. Environ. Saf. 2012; 86:47-53.
19. Maleva MG, Nekrasova GF, Malec P, Prasad MNV, Strzałka K. Ecophysiological tolerance of *Elodea canadensis* to nickel exposure. Chemosphere, 2009; 77:393-398.
20. Mhamdi A, Hager J, Chaouch S. Arabidopsis glutathione reductase 1 plays a crucial role in leaf responses to intracellular hydrogen peroxide and in ensuring appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. Plant Physiol. 2010; 153:1144-1160.
21. Minotti G, Aust SD. The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen Peroxide. J. Biol. Chem. 1987; 262:1098-1104.
22. Mohamed AA, Aly AA. Iron deficiency stimulated some enzymes activity, lipid peroxidation and free radicals production in *Borago officinalis* induced *in vitro*. Int. J. Agri. Biol. 2004; 6:179-184.
23. Mullineaux P, Rausch T. Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. Photosynthesis Research, 2005; 86:459-474.
24. Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Mol. Bio. 1998; 49:249-279.
25. Noctor G, Mhamdi A, Chaouch S, Han Y, Neukermans J, Marquez-Garcia B *et al*. Glutathione in plants: an integrated overview. Plant Cell Environ. 2012; 35:454-484.
26. Panda P, Nath S, Thorny CT, Sharma GD, Panda SK. Cadmium stress-induced oxidative stress and role of nitric oxide in rice (*Oryza sativa* L.). Acta Physiol Plant, 2011; 3:1737-1747.

27. Rahman A, Nahar K, Hasanuzzaman M, Fujita M. Manganese-induced cadmium stress tolerance in rice seedlings: Coordinated action of antioxidant defense, glyoxalase system and nutrient homeostasis. Comptes Rendus Biologies, 2016; doi: 10.1016/j.crvi.2016.08.002.
28. Rennenberg H. Glutathione metabolism and possible biological roles in higher plants. Phytochemistry, 1982; 21:2778-2781.
29. Salama Z, Abd El-R, Hossam EL-B S, Dardiri EL- H M. Effect of Fe deficiency on antioxidant system in leaves of three flax cultivars. Not. Bot. Hort. Agrobot. Cluj. 2009; 37(1):122-128.
30. Shao H, Chu L, Jaleel CA, Zhao C. Water-deficit stress-induced anatomical changes in higher plant. C.R. Biologes, 2008; 331:215-225.
31. Sharma P, Dubey RS. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul. 2005; 46:209-221.
32. Sinha S, Saxena R. Effect of iron on lipid peroxidation and enzymatic and non-enzymatic antioxidants and bacoside-A content in medicinal plant Bacopa monnieri L. Chemosphere, 2006; 62(8):1340-1350.
33. Smirnoff N. Ascorbate, tocopherol and carotenoids: metabolism, pathway engineering and functions. In: Smirnoff N, ed. Antioxidants and reactive oxygen species in plants. Oxford: Blackwell Publishing, 2005, 53-86.
34. Sun Q, Ye ZH, Wang XR, Wong MH. Cadmium hyperaccumulation leads to an increase of glutathione rather than phytochelatins in the cadmium hyperaccumulator. J. Plant Physiol. 2007; 164:1489-1498.
35. Thounaojam TC, Panda P, Mazumdar P, Kumar D, Sharma GD, Sahoo L *et al*. Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol. Biochem. 2012; 53:33-39.
36. Zaharieva TB, Abadia J. Iron deficiency enhances the level of ascorbate, glutathione, and related enzymes in sugar beet roots. Protoplasma, 2003; 221:269-275.
37. Zaharieva T, Yamashita K, Matsumoto H. Iron deficiency induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber roots. Plant Cell Physiol. 1999; 40:273-280.