RESEARCH ARTICLE

Nitric Oxide-Mediated Modulation of Functional Iron Status in Iron-Deficient Maize Plants

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Abstract

Nitric oxide is reported to alleviate Fe-deficiency effects, possibly by enhancing the functional Fe status of plants. Study describes changes in leaf tissue Fe status and consequent modulation of oxidative stress and antioxidant defense in Fe-deficient maize (*Zea mays* L.) plants supplied with NO. Supply of sodium nitroprusside (SNP), but not of sodium ferrocyanide (SF), caused regreening of leaves, syntheses of chlorophylls and carotenoids and increased activities of hydrogen peroxide-scavenging heme-Fe enzymes and lipid peroxidation, decreased SOD activity and hydrogen peroxide concentration. Though SNP or SF appears to donate Fe and increase leaf active Fe, the later did not induce increases in chlorophyll and carotenoids, and therefore NO appears to have a role in Fe nutrition irrespective of total or active Fe status of plants.

Keywords: Ascorbate peroxidase, Catalase, Functional Iron, Lipid peroxidation, Nitric Oxide, *Zea mays.*

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Introduction

Iron is an essential micronutrient for all forms of life (Barberon *et al*., 2014). It is an integral constituent of several proteins required for various vital activities such as respiration, photosynthesis and cell division (Broadley *et al*., 2012). Fe present in the earth's crust is insoluble and therefore it is largely unavailable to plants (Broadley *et al*., 2012). Under deficient availability of Fe, plants exhibit typical interveinal chlorosis in young and emerging leaves (Tewari *et al*., 2005). Fe concentration of plants may not always be an index of Fe nutritional status (Mehrotra *et al*., 1990). Mild acids and chelants extractable Fe has been reported to show a good negative correlation with the extent of chlorosis and is referred as active Fe (Abadia *et al*., 1984). HCl (1 N) extractable active Fe may represent functional Fe (Mehrotra *et al*., 1985). However, it has limited practical applications (Mehrotra *et al*., 1990). The poorly characterized functional Fe in plant tissues is often referred to as labile iron pool (LIP) (Graziano and Lamattina, 2005). Most of the Fe in plant leaves is accumulated in the chloroplasts and therefore they are highly sensitive to low Fe supply (Buet and Simontacchi, 2015). After acquisition from soils Fe^{3+} is required to be reduced to Fe^{2+} before reaching to its final destination. Reduction Fe is mediated by a plasma membrane-bound enzyme, the ferric-chelate reductase (Guerinot and Yi, 1994). *In vivo* reduction of Fe^{3+} may also be aided by superoxide anion radicals ($O_2^{\cdot-}$) (Brüggemann *et al*., 1993), indicating that a shift in redox state of apoplast might affect Fe^{3+} reduction.

Iron is a cofactor of various antioxidant enzymes such as catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and Fe-superoxide dismutase (Fe-SOD). Apart from antioxidant role, Fe may generate pro-oxidant radical because ferrous ion (Fe^{2+}) catalyzes Fenton reaction and in this process it generates the highly deleterious hydroxyl ($OH^{\cdot}$) radical (Halliwell, 2006). Fe-deficient plants often exhibit decreased heme-Fe-enzyme (CAT, POD and APX) activities and less lipid peroxidation (Tewari *et al*., 2013a; Tewari *et al*., 2015). Despite increased production of $O_2^{\cdot-}$ and induction of SOD activity leading to increased H_2O_2 concentration in Fe-deficient plants, they are protected from the damages because of poor functional Fe status (Iturbe-Ormaetxe *et al*., 1995; Ranieri *et al*., 2001; Tewari *et al*., 2005).

Nitric oxide (NO) is signaling molecule of plants and it has been implicated in disease resistance, stomatal closure and Fe-homeostasis (Arasimowicz and Floryszak-Wieczorek, 2007).

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Being a free radical, NO can react with transition metal ions, specially heme-iron and Fe-S centres of proteins (Stamler *et al*., 1992). Formation of Fe^{2-}-NO complexes with thiols are referred as dinitroso-iron complexes (DNICs) (Graziano and Lamattina, 2005; Vanin *et al*., 2004). The DNICs have been implicated as cellular reservoirs of Fe, thiols and NO and are capable of producing a particular component depending on the cellular requirements (Graziano and Lamattina, 2005). While NO-induced release of Fe from ferritin by mobilization of the ferritin-bound Fe is debated (Graziano and Lamattina, 2005), a high concentration of GSH is reported to trigger release of Fe from ferritin in animal cell cultures (Watts and Richardson, 2001).

Application of NO has been reported to prevent interveinal chlorosis in maize plants supplied with deficient Fe (Graziano *et al*., 2002). Up-regulation of various Fe-responsive genes viz., *AtFIT, AtbHLH38, AtbHLH39, AtFRO2, AtIRT1, AtNAS1, AtNAS2, AtFRD, AtMYB72* by NO have been reported (Garcia *et al*., 2010). Considering chloroplastic pigments and activities of heme-Fe enzymes APX, CAT and POD as indices of functional Fe (Tewari *et al*., 2005, 2015), we suggest that NO-mediated increase in the availability of cellular Fe alleviate Fe-deficiency effects and modulate oxidative status and antioxidant responses of Fe-deficient plants. We investigated the involvement of NO in the modulation of oxidative damage and activities of antioxidant enzymes- SOD, APX, CAT and POD and their isoforms in Fe-deficient maize plants for which information is unavailable. Moreover, in present work, we considered the presence of an atom of Fe in NO supplying compound, sodium nitroprusside (SNP), and its non-NO supplying analog, sodium ferrocyanide (SF). Thus Fe status (20 µM) of Fe-deficient plants was maintained by supplying 20 µM Fe-EDTA to Fe-deficient control plants,

10 µM Fe-EDTA along with 10 µM SNP to Fe-deficient NO-treated plants and 10 µM Fe-EDTA along with 10 µM SF to Fe-deficient NO-untreated plants.

Materials and Methods

Plant material and treatments

Maize (*Zea mays* L. cv. GSF-2) plants were grown in plastic pots (with a drainage hole in the center covered with glass wool and inverted watch glass), containing purified white silica sand (5 kg) in a glasshouse. The composition of complete nutrient solution (Hewitt, 1966) was: 4.0 mM KNO_3, 4.0 mM Ca $(NO_3)_2$, 2.0 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.1 mM NaCl, 0.1 mM Fe-K_2EDTA, 10.0 µM $MnSO_4$, 1.0 µM $CuSO_4$, 2.0 µM $ZnSO_4$, 33.0 µM H_3BO_3, 0.2 µM Na_2MoO_4, 0.1 µM $CoSO_4$ and 0.1 µM $NiSO_4$. Salt solutions supplying macronutrients were purified against Fe by calcium carbonate-phosphate adsorption method (Hewitt, 1966). Plants were grown for 15 days either under Fe-sufficient (100 µM Fe-EDTA; for lot 1) or Fe-deficient (20 µM Fe-EDTA; for lots 2-4) conditions and subsequently the pots were flushed with deionized water and grouped into 4 lots with 4 pots each. Whereas, plants in lot 1 continued to receive complete nutrient solution (Fe sufficient control), those in lot 2 were supplied nutrient solution deficient in Fe (20 µM Fe-EDTA). Plants in the lots 3 was supplied 10 µM sodium nitroprusside (SNP, a NO donor), along with Fe-deficient (10 µM Fe-EDTA) nutrient solution. Since SNP contains an atom of Fe, respective Fe-controls for SNP treatment was maintained in the lot 4 which received 10 µM sodium ferrocyanide (SF, an analog of SNP having equivalent amount of Fe in the coordination complex and incapable of donating NO) along with Fe-deficient nutrient solution. Plants were irrigated daily with nutrient solution along with respective treatments until full saturation of sands which was characterized by trickling down of excess nutrient solution through drainage hole. Studies were made with young expanded leaves of plants, after 20 days of differential treatments (35 days of growth).

Visual observations and dry matter yield

The visible effects were recorded day to day. Finally, 20 days after initiating the differential treatments (DAT), plants were harvested and dried in an oven at 80°C for 48 h and weighed.

Total and active iron

Total Fe was estimated in leaf tissue digest in a mixture of HNO_3: $HClO_4$ (10:1 [v/v]). For the determination of active Fe, 1 N HCl extracts were prepared by homogenizing fresh leaf material in the proportion of 1 g tissue per 10 ml 1 N HCl, following the procedure of Mehrotra *et al*. (1985). The extracts were filtered through Whatman 1 filter paper, and digested in HNO_3: $HClO_4$ (10:1 [v/v]). Fe was quantified as described earlier (Tewari *et al*., 2015).

Nitric oxide concentration

Nitric oxide was determined after Zhou *et al*. (2005). Leaves (0.5 g) were homogenized in 2 ml of 50 mM cool acetic acid buffer (pH 3.6, containing 4% zinc diacetate). The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C. The supernatants were treated with 0.05 g of activated charcoal. The suspension was vortexed and filtered. The mixture of 0.5 ml of filtrate and 0.5 ml of the Greiss reagent was incubated at room temperature for 30 min. Absorbance was determined at 540 nm. NO concentration was calculated by comparison with a standard curve using $NaNO_2$.

Chloroplastic pigments, hydrogen peroxide and lipid peroxidation

Chlorophylls and carotenoids were measured in 80% (v/v) acetone extract of young expanded leaves following the method of Lichtenthaler (1987). Concentrations of peroxides, and thiobarbituric acid reactive substance (TBARS) were measured in the acetone extracts by the method of Brennan and Frenkel (1977), and in the trichloroacetic acid extract by the method of Heath and Packer (1968), respectively.

Enzyme activities and protein determination

For the assay of enzyme activities leaves were homogenized in grinding medium containing 100 mM potassium phosphate buffer (pH 7.0), 2 % PVP and 0.5 mM phenylmethylsulphonyl fluoride. The grinding medium also contained 1 mM ascorbic acid for extraction of APX. Enzyme activities were assayed in the leaf homogenate cleared by centrifugation at 22,000×g for 10 min at 2°C. Concentration of soluble proteins was measured by the method of Lowry *et al*. (1951). The reaction mixtures of different enzymes were: SOD (EC 1.15.1.1), in 5 ml: 25 mM phosphate buffer pH 7.8, 0.1 mM EDTA, 65 µM *p*-nitro blue tetrazolium chloride (NBT), 2 µM riboflavin, 300 µl enzyme extract, and 15 µl *N,N,N′,N′*-tetramethylethylenediamine (TEMED) and the reaction mixture was exposed to light of 350 µmol m^{-2} s^{-1} for 15 min. The change in absorbance was recorded at 560 nm [modified from Beauchamp and Fridovich (1971)]. The enzyme activity was expressed as unit min^{-1} (mg protein)$^{-1}$. Fresh matter equivalent of enzyme corresponding to 50% inhibition in formazan formation was considered as one enzyme unit; Catalase (EC 1.11.1.6), 0.5 µmoles H_2O_2 in 10 ml 100 mM phosphate buffer pH 7.0, H_2O_2 decomposed after 10 min reaction was assayed by titrating the reaction mixture with 0.1 N $KMnO_4$ (Sharma *et al*., 2004). CAT activity was expressed as unit [µmol H_2O_2 decomposed min^{-1}] (mg protein)$^{-1}$; Peroxidase (EC 1.11.1.7), 5 ml 100 mM phosphate buffer pH 6.5, 1 ml 0.5% *p*-phenylenediamine, 1 ml 0.01 % H_2O_2 and 1 ml tissue extract, change in absorbance after 5 min was monitored at 485 nm (Sharma *et al*., 2004). The activity of the enzyme has been expressed as unit (mg protein)$^{-1}$. The enzyme unit is defined as ΔA_{485} of 0.01 between the blank and the sample min^{-1} of reaction time, calculated using purified horse radish peroxidase; Ascorbate peroxidase (EC 1.11.1.11), in 3 ml: 50 mM phosphate buffer pH 7.0, 0.5 mM ascorbic acid, 0.1 mM H_2O_2, 0.1 mM EDTA and suitable quantity of enzyme extract, blanks were run and the changes in absorbance every 15 s were read at 290 nm (Nakano and Asada, 1981). The activity of APX was calculated in terms of unit [µmol ascorbate oxidized min^{-1}] (mg protein)$^{-1}$.

Native PAGE and in gel localization of enzymes

Polyacrylamide gel electrophoresis was performed to separate the enzyme isoforms on a discontinuous non-denaturing gel system. Native gels with a concentration of 7.5 % (for CAT) and 10 % (for APX and SOD) were prepared and run at constant current (30 mA) at 4°C. In gel activities of CAT, APX and SOD were localized as described earlier (Tewari *et al*., 2013).

Statistical analysis

The data were analyzed by analysis of variance (ANOVA) and comparisons among the means were tested for significance by Fisher LSD method using Sigma-stat software. Means followed by different alphabets are statistically significant at $P \leq 0.05$.

Results and Discussion

NO supply induces re-greening and increases in chloroplastic pigments in the chlorotic leaves of Fe-deficient maize

Iron deficient maize plants supplied with 10 µM SNP showed perceptible re-greening of chlorotic leaves. SF, a non-NO-supplying

Table 1: Dry matter yield (30 DAT), NO and Fe concentration (20 DAT) in Fe-sufficient (100 µM Fe-EDTA), Fe-deficient (20 µM Fe-EDTA) controls and Fe-deficient (10 µM Fe-EDTA) plants supplied 10 µM SNP, or 10 µM of sodium ferrocyanide in the growing medium with nutrient solution. Data are mean of experimental replicates (n=6). Values in the same rows carrying different letters are significantly different at P ≤ 5% by Fisher LSD method.

	Fe-EDTA (µM)		*Fe-EDTA (10 µM)*		
			SNP (µM)	*SF(µM)*	
Parameters	*100*	*20*	*10*	*10*	*LSD 0.05*
Dry weight (g plant^{-1})	30.09a (±1.25)	18.40b (±0.53)	21.57c (±0.36)	17.57b (±0.25)	2.10
Total Fe (µg g^{-1} DW)	341.80a (±30.92)	269.45ac (±33.00)	180.31b (±37.00)	201.97bc (±13.91)	88.60
Active Fe (µg g^{-1} DW)	113.91a (±10.88)	40.58b (±16.55)	122.44a (±19.90)	120.79a (±20.26)	51.07
NO (nmol g^{-1} FW)	45.85a (±2.66)	9.04c (±2.15)	35.16b (±1.59)	10.65c (±2.68)	6.83

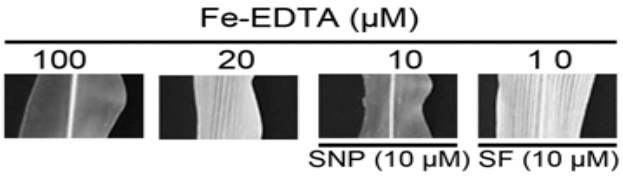

Fig. 1 : Visible effect of NO treatment in the leaf of Fe-deficient maize plants grown in sand culture. Leaves of maize plants showing the effect of NO (SNP) supply: Fe-sufficient control plants (100 µM Fe-EDTA), Fe-deficient control plants (20 µM Fe-EDTA), Fe-deficient (10 µM Fe-EDTA) plants treated with 10 µM SNP or 10 µM SF.

analog of SNP, did not induce re-greening of chlorotic leaves (Fig. 1). Apart from re-greening of chlorotic leaves, supply of SNP increased growth and dry matter accumulation in the Fe-deficient plants (Table 1). Presence of Fe is important for the development of chloroplast and chlorophyll biosynthesis. Thus chlorophyll concentration of leaves may be considered as a primary index of functional Fe status. Alleviation of chlorosis (Fig. 1) along with increase in chlorophyll concentration on treating Fe-deficient plants with NO producer, SNP (Table 2), agrees with the observations made by Graziano *et al*. (2002) and Graziano and Lamattina (2007). Fe-deficient plants treated with non-NO producing analog, 10 µM SF, did not show any recovery from Fe deficiency effects. It suggests that the observed recovery of Fe deficiency was indeed caused by NO. Graziano *et al*. (2002) found that the effect of SNP on the recovery of plants from Fe deficiency effects was comparable to that of GSNO- and gaseous NO-treated plants.

Effects of NO do not relate with total and active tissue Fe concentrations

Total Fe concentration (Table 1) of plants did not show any relation with chloroplastic pigments, as could be expected in view of several reports of poor relationship between total Fe concentration and the extent of chlorosis or chlorophyll concentration in the leaves of Fe-deficient plants, both in field as well as controlled culture conditions (Abadía, 1992; González-Vallejo *et al*., 2000). Though active (1N HCl extractable) Fe concentration of plants was also not related to the level of chlorosis or chlorophyll concentration (Table 2), it was related to supply of both SNP and SF in Fe-deficient plants, indicating that both complexes contributed to the "active" Fe in the plants. Use of SNP as NO donor has so far been made with a belief that it does not contribute Fe to the biological system (Kim and Ponka, 2002; Wang *et al*., 2006). Since the active Fe in plants supplied SNP or SF does not relate with chlorosis or chlorophyll concentration, it does not appear to represent the functional Fe. There are reports of unsatisfactory relationship between active Fe and chlorophyll concentration in leaves of plants having variable Fe status (Manzanares *et al*., 1990; Mehrotra *et al*., 1990). Irrespective of the active Fe concentration, alleviation of Fe deficiency effects was observed when Fe-deficient plants were supplied SNP and where nitrite concentration of the leaves was improved to a level

Table 2: Total chlorophyll, carotenoids concentration, Chl a/b and Car/Chl ratio in Fe-sufficient (100 µM Fe-EDTA), Fe-deficient (20 µM Fe-EDTA) controls and Fe-deficient (10 µM Fe-EDTA) plants supplied 10 µM SNP, or 10 µM of SF in the growing medium with nutrient solution. Data are mean of experimental replicates (n=6). Values in the same rows carrying different letters are significantly different at P ≤ 5% by Fisher LSD method.

	Fe-EDTA (µM)		*Fe-EDTA (10 µM)*		
			SNP (µM)	*SF(µM)*	
Parameters	*100*	*20*	*10*	*10*	*LSD 0.05*
Total chlorophyll (mg g^{-1} FW)	2.17a (±0.028)	0.64c (±0.008)	0.99b (±0.009)	0.36d (±0.010)	0.048
Carotenoids (mg g^{-1} FW)	0.30a (±0.007)	0.19b (±0.007)	0.18b (±0.009)	0.10c (±0.006)	0.023
Chl. a/b ratio	1.94a (±0.010)	1.64b (±0.0004)	2.25a (±0.010)	1.03c (±0.041)	0.064
Chl/Car. ratio	0.14b (±0.005)	0.30a (±0.015)	0.18b (±0.011)	0.27a (±0.026)	0.048

Table 3: H_2O_2 and lipid peroxidation in Fe-sufficient (100 µM Fe-EDTA), Fe-deficient (20 µM Fe-EDTA) controls and Fe-deficient (10 µM Fe-EDTA) plants supplied 10 µM SNP, or 10 µM of SF in the growing medium with nutrient solution. Data are mean of experimental replicates (n=6). Values in the same rows carrying different letters are significantly different at P ≤ 5% by Fisher LSD method.

	Fe-EDTA (µM)		*Fe-EDTA (10 µM)*		
			SNP (µM)	*SF(µM)*	
Parameters	*100*	*20*	*10*	*10*	*LSD 0.05*
H_2O_2 (µmol g^{-1} FW)	3.25b (± 0.16)	4.23a (± 0.27)	3.20b (± 0.32)	4.82a (± 0.21)	0.74
Lipid peroxidation (mg g^{-1} FW)	21.07a (± 0.60)	14.79c (± 0.17)	17.04b (± 0.86)	14.19c (± 0.43)	1.69

comparable to that of controls (Table 1). Lack of relation between tissue Fe status and chlorophyll concentration is largely attributable to much of tissue Fe exists in the apoplast in an insoluble form (Graziano and Lamattina, 2005). Fe (III)-chelate reductase-mediated reduction and transport of the apoplastic Fe across the plasma membrane is known to be regulated by NO-mediated physiological processes and NO concentration (Beligni and Lamattina, 2000). Reduction of Fe^{3+} to Fe^{2+} and its uptake by the cells is likely to be facilitated by increased NO concentration in the cellular environment (Graziano *et al*., 2002).

NO downregulates SOD activity but upregulates activities of heme-Fe enzymes in Fe-deficient maize

Supply of SNP, but not of SF**,** reversed Fe deficiency effects. SNP-treated plants exhibited decreased SOD activity and enhanced activities of heme-Fe enzymes (CAT, POD and APX) (Table 4, Fig. 2) along with accumulation of MDA and decreased H_2O_2 concentration (Table 3). Since the H_2O_2-scavanging heme enzymes - CAT, POD and APX constitutively contain heme-Fe, several workers have suggested CAT, POD (Mehrotra *et al*., 1990; Broadley *et al*., 2012) and APX (Iturbe-Ormaetxe *et al*., 1995; Tewari *et al*., 2005) as indices of Fe status of plants. Moreover, expression of APX is also reported to be regulated by Fe (Ishikawa *et al*., 2003; Fourcroy *et al*., 2004).

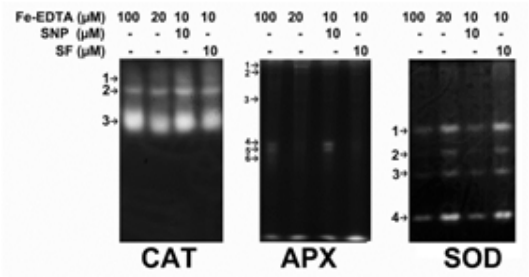

Fig. 2: Activity of CAT, APX and SOD on native gel in the young expanded leaves of maize plants grown in sand culture with sufficient (100 µM Fe-EDTA) and deficient (20 µM Fe-EDTA) Fe supply and Fe-deficient (10 µM Fe-EDTA) plants treated with 10 µM SNP or 10 µM sodium ferrocyanide (SF). Data at 20 days after differential treatments (DAT).

This is quite comprehensible as both heme and chlorophyll possess tetrapyrrole ring and have been shown to share a common pathway, which is adversely affected by Fe limitation. In gel activity of CAT and APX also exhibited similar effect as those obtained in spectrophotometric assay. CAT isoforms 2 and 3 and APX isoforms 4 and 5 were particularly up-regulated by NO treatment. Activity of SOD, increased by Fe deficiency, is depressed by supplying SNP but not affected significantly by supply of SF to the Fe-deficient plants (Table 4, Fig. 2). High SOD activity in Fe-deficient maize plants has been shown to be related with $O_2^{\cdot-}$ and H_2O_2 concentration (Tewari *et al*., 2005). Decreased H_2O2 concentration alongwith SOD activity and down regulation of all four existing SOD isoforms in SNP-supplied Fe deficient plants (but not in the plants supplied with SF), suggests a decrease in $O_2^{\cdot-}$ accumulation in these plants, which may be attributed to consumption of $O_2^{\cdot-}$ in the condensation reaction with NO to form peroxynitrite ($ONOO^-$) (Halliwell, 2006). $ONOO^-$ is a strong oxidant and therefore highly cytotoxic and mediate apoptosis in animal cells. Peroxynitrite is suggested to be relatively non-toxic for plants (Delledonne *et al*., 2001; Delledonne, 2005).

Low lipid peroxidation (Table 3) in Fe-deficient plants is in agreement with previous observations (Iturbe-Ormaetxe *et al*. 1995; Ranieri *et al*., 2001; Tewari *et al*., 2005). Despite high ROS accumulation in chlorotic leaves of Fe-deficient plants, they were better protected from oxidative damage as compared to green leaves. Decreased TBARS in Fe-deficient plants is attributed to low availability of catalytic (functional) Fe^{2+} that limits $OH^{\cdot}$ formation (Iturbe-Ormaetxe *et al*. 1995, Ranieri *et al*. 2001). Increased functional Fe in SNP-treated Fe-deficient plants may disposed them to oxidative damages as indicated by enhanced lipid peroxidation (Table 3). However, possible production of peroxynitrite cannot be

Table 4: Catalase, Peroxidase, Ascorbate peroxidase and superoxide dismutase activities (20 DAT) in Fe-sufficient (100 µM Fe-EDTA), Fe-deficient (20 µM Fe-EDTA) controls and Fe-deficient (10 µM Fe-EDTA) plants supplied 10 µM SNP or 10 µM of SF in the growing medium with nutrient solution. Data are mean of experimental replicates (n=6). Values in the same rows carrying different letters are significantly different at P ≤ 5% by Fisher LSD method.

	Fe-EDTA (µM)		*Fe-EDTA (10 µM)*		
			SNP (µM)	*SF (µM)*	
Enzymes	*100*	*20*	*10*	*10*	*LSD 0.05*
Superoxide dismutase (Unit mg^{-1} protein)	2.06c (±0.403)	6.82a (±0.238)	5.62b (±0.267)	7.65a (±0.426)	1.015
Catalase (Unit mg^{-1} protein)	135.30a (±10.21)	36.11c (±0.11)	85.92b (±2.01)	38.77c (±0.24)	15.35
Peroxidase (Unit mg^{-1} protein)	18.48a (±0.184)	14.05b (±0.018)	19.38a (±0.140)	16.74b (±0.135)	0.397
Ascorbate peroxidase (Unit mg^{-1} protein)	0.96a (±0.022)	0.39c (±0.017)	1.11a (±0.053)	0.53b (±0.004)	0.089

ruled out as a cause of increased lipid peroxidation the SNP-treated Fe-deficient plants (Miles *et al*., 1996; Jasid *et al*., 2006). Formation of S-nitrosoglutathione in NO treated Fe-deficient plants having high concentration of non-protein thiols, might be responsible for the release of Fe from bound sources as reported by Watts and Rechardson (2001). Release of Fe in a functionally utilizable form by NO (Graziano and Lamattina, 2005) cannot be ruled out.

Conclusion

Total or active tissue Fe has a poor relationship with chloroplastic pigments or other related parameters concerning Fe-nutritional status. Both SNP (NO donor) and SF (non-NO-releasing analogue of SNP), contributed Fe to the 'active Fe' pool, but NO was instrumental in alleviating the Fe deficiency effects. The results suggest that NO probably ameliorated symptoms and other Fe deficiency effect of by improving functional iron leading to increased synthesis of chloroplastic pigments and heme-Fe proteins, and decline in H_2O_2 but at the same time disposed them to oxidative damage (TBARS) (Fig. 3).

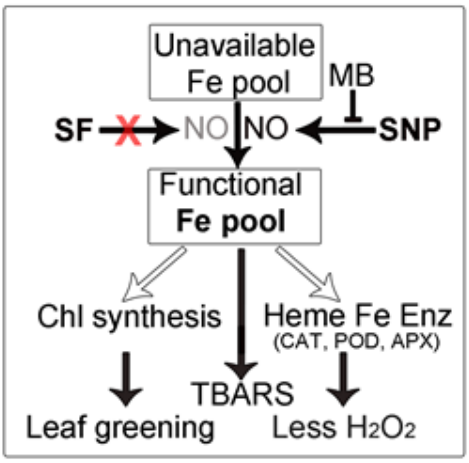

Fig. 3: Schematic model showing possible mechanism of NO-mediated alleviation of Fe-deficiency effects and modulation of antioxidant defense in maize plants. Abbreviations: MB, methylene blue (a NO scavenger); SNP, sodium nitroprusside (a NO producing molecules); SF, sodium ferrocyanide (a non-NO producing analog of SNP); TBARS, thiobarbituric acid reactive substance (lipid peroxidation); Heme Fe enzymes - CAT, POD and APX are catalase, peroxidase and ascorbate peroxidase, respectively.

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