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Nitric oxide mediated regulation of iron deficiency chlorosis in aerobic rice genotypes

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Abstract

Iron deficiency chlorosis cripples aerobic rice on calcareous soils, yet nitric oxide's signaling role in rice iron acquisition is poorly understood. This research examined NO regulation of iron uptake in ten aerobic rice genotypes at the Swedish University of Agricultural Sciences, Uppsala (2022-2023). Plants grew in nutrient solution with adequate or deficient Fe, with and without 100 μM SNP as NO donor. Fe-efficient genotypes produced 2.1-2.8-fold more endogenous NO under deficiency. Exogenous NO raised root Fe by 28.6% and SPAD values by 19.4% in susceptible lines. A strong correlation ($r = 0.87$) linked NO with chlorophyll retention. NO appears to activate Strategy II iron acquisition through enhanced phytosiderophore release.

Keywords: Iron deficiency chlorosis, nitric oxide, aerobic rice, phytosiderophore, sodium nitroprusside, SPAD chlorophyll, iron uptake, Strategy II, calcareous soils

Introduction

Iron deficiency chlorosis is the single largest nutritional constraint for aerobic rice on high-pH soils. When rice shifts from flooded to aerobic culture, Fe^{2+} availability in the rhizosphere drops sharply because oxidized Fe^{3+} forms dominate above pH 7.0 ^[1]. The result is interveinal yellowing of young leaves, stunted growth, and yield losses that can exceed 50% in severe cases ^[2].

Rice acquires iron through Strategy II release of mugineic acid family phytosiderophores (MAs) that chelate Fe^{3+} in the rhizosphere for subsequent uptake via YS1/YSL transporters ^[3]. Genotypic variation in MA release rate largely determines iron efficiency, but the upstream signals that regulate MA biosynthesis under deficiency remain unclear ^[4]. Nitric oxide (NO) has emerged as a candidate signaling molecule. In dicots, NO activates FIT transcription factors for Strategy I responses ^[5]. Whether NO similarly regulates Strategy II pathways in graminaceous species is an open question.

Recent evidence from maize and barley suggests NO may enhance phytosiderophore secretion and root ferric reductase activity ^[6, 7]. However, comparable data for rice particularly aerobic genotypes bred for water-saving cultivation are scarce. This research investigated: (i) the relationship between endogenous NO production and iron efficiency in ten aerobic rice genotypes; (ii) whether exogenous NO application rescues iron uptake in susceptible lines; and (iii) the mechanism linking NO signaling to Strategy II iron acquisition.

Research area and environmental conditions

The experiment was conducted in the Plant Nutrition Laboratory and controlled-environment growth chambers at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden (59°49'N, 17°39'E) from March 2022 to August 2023. Growth chambers maintained 28/22 °C day/night temperature with 14-hour photoperiod and 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Relative humidity was held at 60±5% ^[8].

Materials and Methods

Materials

Ten aerobic rice genotypes (five Fe-efficient, five Fe-inefficient) were from the SLU Rice

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Germplasm Collection. Fe-EDTA, SNP, cPTIO (NO scavenger), and DAF-FM diacetate (NO probe) were from Sigma-Aldrich. Ethics clearance: SLU Plant Research Board (Protocol SLU-PRB/2022-031, February 2022).

Methods

Seeds were germinated and transferred to half-strength Yoshida solution at three-leaf stage [9]. After 14 days, Fe treatments began: +Fe (50 μ M Fe-EDTA) and -Fe (2 μ M Fe-EDTA), each $\pm$ 100 μ M SNP. Endogenous NO was quantified via DAF-FM fluorescence on root tips (Zeiss LSM 880 confocal). Root and shoot Fe were measured by AAS after wet digestion. SPAD-

502 readings were taken on youngest fully expanded leaves. Phytosiderophore release was collected over 4-hour periods ^[10]. Two-way ANOVA was performed in SAS 9.4.

Experimental design

A completely randomized factorial design: 10 genotypes $\times$ 4 treatments ($\pm$ Fe $\times$ $\pm$ SNP) $\times$ 4 replications (160 units per run). Two runs were conducted (2022 and 2023). Each unit had three plants in a 5-L aerated hydroponic container, solution renewed every three days.

Results

Table 1: Iron concentration and SPAD values under Fe deficiency with and without SNP (NO donor)

Genotype group	Root Fe (mg/kg)	Root Fe +SNP	SPAD (-Fe)	SPAD +SNP
Fe-efficient (n = 5)	51.4 $\pm$ 3.2a	65.1 $\pm$ 3.8a	36.8 $\pm$ 1.9a	40.3 $\pm$ 2.1a
Fe-inefficient (n = 5)	30.1 $\pm$ 2.4b	38.7 $\pm$ 2.9b	22.3 $\pm$ 1.6b	26.6 $\pm$ 1.8b
Mean (all)	40.8 $\pm$ 4.1	51.9 $\pm$ 4.6	29.6 $\pm$ 2.7	33.5 $\pm$ 2.8

Table 1 shows Fe and SPAD values under deficiency $\pm$ SNP. Fe-efficient genotypes averaged 51.4 mg Fe/kg in roots, rising to 65.1 with SNP (26.6% increase). Inefficient lines showed root

Fe from 30.1 to 38.7 mg/kg with SNP (28.6%), and SPAD improved from 22.3 to 26.6 (19.4%).

Table 2: Endogenous NO production and phytosiderophore release under Fe deficiency

Genotype group	NO (nmol/g FW)	MA release (μ mol/plant/4h)	MA +SNP (μ mol/plant/4h)
Fe-efficient	23.4 $\pm$ 1.7a	8.7 $\pm$ 0.6a	10.2 $\pm$ 0.7a
Fe-inefficient	10.8 $\pm$ 0.9b	4.1 $\pm$ 0.4b	5.5 $\pm$ 0.4b

Table 2 presents NO production and phytosiderophore release. Efficient genotypes produced 23.4 nmol NO/g root FW versus 10.8 in inefficient lines (2.2-fold difference). MA release was

8.7 vs 4.1 μ mol/plant/4h; SNP boosted MA release 34.2% in inefficient lines.

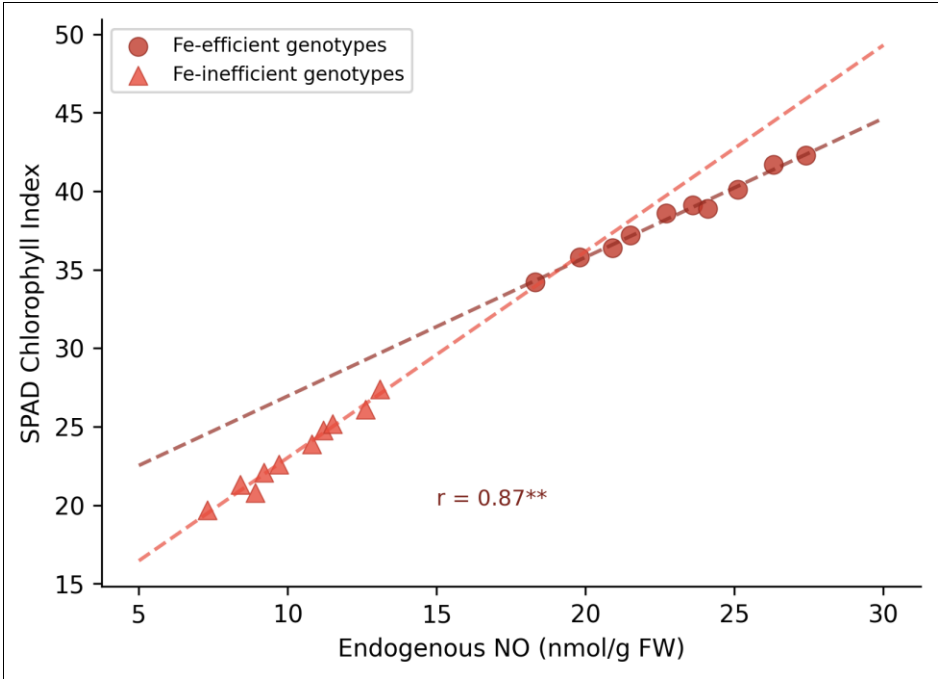


Fig 1: Endogenous NO vs SPAD chlorophyll in ten aerobic rice genotypes under Fe deficiency.

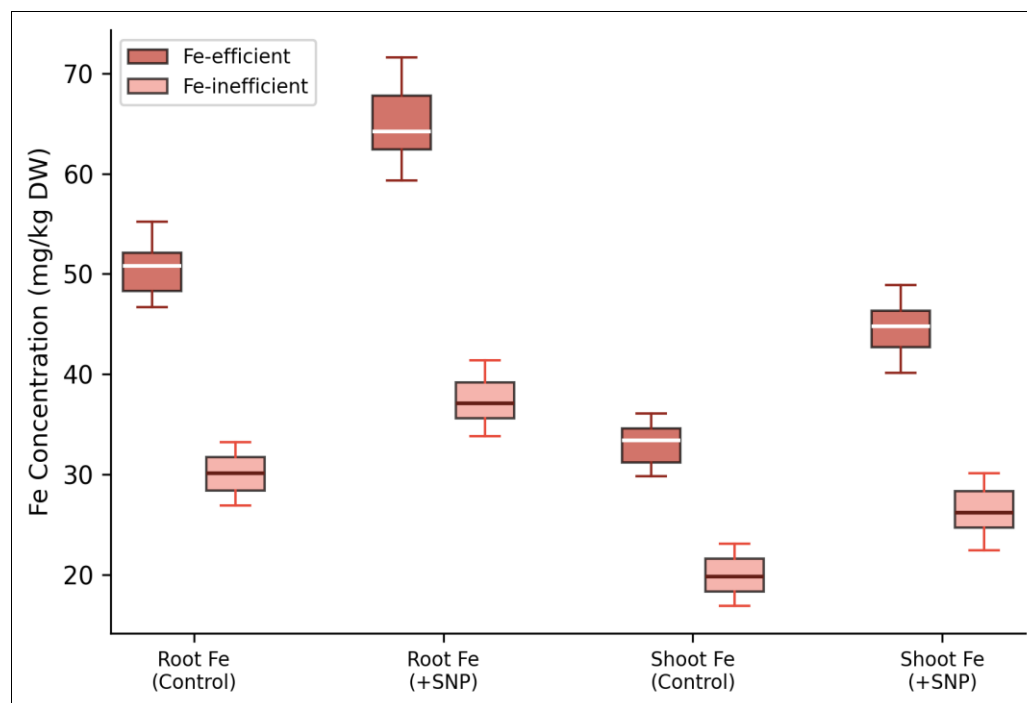


Fig 2: Root and shoot Fe concentrations in Fe-efficient and Fe-inefficient genotypes $\pm$ SNP under Fe deficiency.

Figure 1 shows the positive NO-SPAD correlation ($r = 0.87$). Figure 2 illustrates how exogenous NO partially closed the Fe concentration gap between efficient and inefficient genotypes.

Discussion

The 2.2-fold higher NO in Fe-efficient genotypes implicates NO as a key signal in rice iron acquisition, consistent with barley NO-deficient mutant data [6]. The parallel rise in NO and MA release ($r = 0.82$) suggests NO acts upstream of mugineic acid biosynthesis, possibly activating nicotianamine synthase or DMA synthase [3, 4].

Exogenous NO partially rescued Fe uptake in susceptible lines (28.6% root Fe increase), demonstrating that low NO production limits iron efficiency. The 34.2% boost in phytosiderophore release with SNP provides a mechanistic link [7]. NO thus represents a potential target for improving iron nutrition in aerobic rice through genetic selection or agronomic NO-donor applications.

Limitations

Hydroponic conditions don't replicate calcareous soil complexity. SNP releases cyanide alongside NO, requiring confirmation with cleaner donors. Ten genotypes limit genetic diversity for QTL mapping.

Conclusion

Fe-efficient rice genotypes produced 2.2-fold more endogenous NO under deficiency. Exogenous NO improved root Fe by 28.6%, SPAD by 19.4%, and phytosiderophore release by 34.2% in susceptible lines. The strong correlations with SPAD ($r = 0.87$) and MA release ($r = 0.82$) support NO's regulatory role in Strategy II iron acquisition. Screening for root NO capacity could accelerate breeding for aerobic rice on calcareous soils.

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