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Application of Excess Nitrogen, Phosphorus, and Potassium Fertilizers Leads to Lowering of Grain Iron Content in High-Yielding Tropical Rice

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Influences of nitrogen (N), phosphorus (P), and potassium (K) fertilizer doses were assessed on iron (Fe) accumulation in leaves and grains of three high-yielding rice cultivars differing in grain Fe concentration. Effects of these treatments were also measured on grain yield, leaf area, and plant biomass of the cultivars. Nitrogen, P, and K applications improved plant biomass and grain yield of all cultivars. Among the nutrients, N was most effective in increasing leaf Fe concentration, followed by P and K in all three rice cultivars. Sharbati accumulated the greatest concentration of leaf Fe followed by IR-64 and Lalat. However, greater doses of these nutrients adversely affected grain yield and Fe content of leaf and grain. Application of excess N, P, and K fertilizers may, thus, sometimes results in lowering of grain Fe content in rice. Judicious application of the elements is recommended for prevention of Fe-induced malnutrition.

Keywords Iron, nitrogen, phosphorus, potassium, rice

Introduction

As a valuable staple food for half of the world's population, rice provides proteins, carbohydrates, and minerals. Rice and other cereal crops, however, are deficient in micronutrients, especially in iron (Fe) and zinc (Zn), which may endanger human health (Welch and Graham 1999; Cakmak 2008). Iron deficiency affects more than one third of the population worldwide, and it contributes to about 800,000 deaths annually (Welch, Combs, and Duxbury 1997; Mayer, Pfeiffer, and Beyer 2008). Traditional food fortification or medication cannot thoroughly solve this problem because they only have a temporary role in malnutrition but expensive costs. However, the micronutrient biofortification of staple food crops is regarded as an effective way to solve this problem (Welch and Graham 2004). Various approaches have been proposed to increase micronutrient accumulation in foods to meet human health requirements (Bouis 2002; Poletti et al. 2004; Brinch-Pedersen et al. 2007). One promising approach is to increase the concentration and bioavailability of micronutrients in cereal grains. This approach includes application of

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micronutrient fertilizer to soil or foliage; the use of intercropping or other cropping systems to increase micronutrient availability; and biofortification, that is, the increase in nutrient status of crop plants via conventional breeding or genetic engineering (Rengel, Batten, and Crowley 1999; Ortiz-Monasterio et al. 2007; Cakmak 2008). Obtaining micronutrient-enriched grains requires the consideration of many factors, including bioavailability, grain quality, and, most important, grain yield. Genotypes of grain crops that combine the traits of stable, high micronutrient status and high grain yield are required because biofortified genotypes that yield less grain than conventional genotypes will not be adopted by farmers. Achieving acceptable yields of biofortified genotypes requires management of major elements, nitrogen (N), phosphorus (P), and potassium (K) along with other nutrients. Large quantities of N, P, and K fertilizers are commonly used to obtain good yields in many crops, including rice. Although the effect of N fertilization on micronutrient density has seldom been studied, several publications suggest that management of N fertilizer could affect grain micronutrient status. Morgounov et al. (2007), for example, found a strong positive relationship among Fe, Zn, and protein content (r^2 0.65; r^2 0.68, respectively) of grain from 25 spring wheat varieties grown under field conditions. Other research also indicates a potentially positive effect of N fertilization on micronutrient density i will n wheat grain (Kimball et al. 2001; Yue et al. 2007). No research, however, has been published on how N, P, and K application affects the concentration of micronutrients in the in rice grain. The aim of this work was to quantify the effects of N, P, and K fertilization rates on Fe density in rice grain apart from its effect on grain yield, leaf area, and plant biomass of three cultivars differing in grain Fe content. In addition, the article considers the possible mechanisms and agronomic implications for the effects of N, P, and K fertilization on Fe density in rice grain.

Materials and Methods

Plant Materials and Experimental Site

Three *indica* rice (*Oryza sativa* L.) cultivars differing in grain Fe concentration [Sharbati (high, 26 mg kg^{-1} Fe in brown rice), IR-64 (medium, 12 mg kg^{-1} Fe in brown rice), and Lalat (low, 7 mg kg^{-1} Fe in brown rice)] were grown in irrigated open field conditions at the research farm of the Central Rice Research Institute, Cuttack, Orissa, India (latitude 20° N, longitude 86° E) during the dry seasons of 2008 and 2009. Three independent randomized block designs (RBD; four treatments, three replicates, and three varieties) were made for different doses of N, P, and K applications. The normal dose of N, phosphorus pentoxide (P_2O_5), and potassium chloride (KCl) application was in the ratio of 80:40:40 kg ha^{-1}. For each RBD design, the level of one macronutrient was varied while keeping other two normal. The soil of the experimental area was a sandy loam type. The field was plowed, puddled, levelled, and bunded before transplantation, and each bunded plot was separated by 0.5-m drain to avoid mixing of fertilizers with those from nearby plots. The water level in the field was maintained at 5 ± 2 cm. Seedlings were raised in a nursery bed for 30 days before transplantation to 3-m × 3-m plots at a hill spacing of 20 cm × 15 cm with one seedling per hill. Nitrogen was applied as urea with four different doses (0, 40, 80, 120 kg ha^{-1}). Similarly, P and K were applied as P_2O_5 and KCl, respectively, with four different doses (0, 20, 40, and 80 kg ha^{-1}). Nitrogen was applied in three split doses: 50% as basal, 25% at maximum tillering stage, and the rest at panicle initiation stage. Phosphorus and K fertilizers were applied as basal only at the time of puddling.

Sampling

Seedlings with uniform growth and development were transplanted to different plots. At the tillering stage, the main shoots of the plants were tagged. Laminas of the uppermost mature leaves of the main shoots were excised on 25, 50, 75, and 100 days after transplanting. The material was first washed with normal water followed by doubly distilled water, 1 N hydrochloric acid (HCl), and finally again with doubly distilled water. The samples were dried in a hot-air oven at 60 °C for 8 h. A similar method of processing was adopted for the grains at maturity.

Morphological Measurements

For each cultivar, panicle length (neck node to the tip of the uppermost primary branch) and percentage of filled grains were measured in the main shoot at maturity. Panicle dry weight of the main shoot and total dry biomass hill^{-1} were measured by keeping the materials at 60 °C for 8 h in a hot-air oven. Flag leaf area of the main shoot was calculated according to Yoshida (1981) by multiplying leaf length with width at half height by a factor of 0.69:

$$\text{Leaf area (cm}^2\text{)} = \text{K} \times \text{length (cm)} \times \text{width (cm)}$$

where K is the correction factor (0.69).

The correction factor used for rice leaves ranges from 0.67 to 0.80, depending, on variety and growth stage.

Chemical Analyses of Sample

Completely dried plant parts were digested in a diacid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) (15:2). Two g of the dehusked grains and 500 mg of dried leaf sample were immersed overnight in 15 mL concentrated HNO_3 in a conical flask. On the following day, 2 mL $HClO_4$ was added and the sample was digested on a hot plate at 60 ^{o}C for 2 h, followed by further digestion at 90 ^{o}C until the white fumes of $HClO_4$ effervesced out. The leftover liquid was transferred to a 50-mL volumetric flask and diluted with doubly distilled water. The solution was filtered with Whatman No. 41 filter paper and used for estimation of Fe in an atomic absorption spectrophotometer (GBC, Avanta).

Results

Nitrogen Nutrition

The weight, length, and percentage of fertile grains on the panicle at maturity improved with increase of N application in the soil up to the level of 80 kg ha^{-1} and declined thereafter at 120 kg ha^{-1} (Table 1). Rice cultivar Lalat was found to be most responsive to N application in increasing panicle weight and length, and the response was lowest in IR-64. The response of Sharbati to N application in filled grain percentage of panicle was more than that of Lalat and IR-64. The Fe concentration of the grains also followed a trend similar to that of the panicle length with rise of N application in the soil; the response was greatest in Sharbati and lowest in Lalat. Leaf area of the cultivars increased with increase in the level of N in the growing medium; the response of N on leaf area was maximum in Lalat and minimum in IR-64. Total dry biomass, on the other hand, showed an opposite trend with IR-64 showing the greatest accumulation of biomass followed by Lalat and

Table 1

Effect of nitrogen application (0, 40, 80 and 120 kg ha^{-1}) on panicle length (cm), panicle dry weight (g), filled grains (%), total dry biomass (g), grain Fe content ($\mu g\ g^{-1}$ dry wt.) and leaf area (cm^2) in three *indica* rice cultivars (Sharbati, Lalat and IR-64) at maturity, grown in open field conditions

	Panicle length			Panicle dry weight			Filled grain %			Total dry biomass			Grain Fe content			Leaf area		
N treatments	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64
0	25.0d	27.6c	24.1d	2.5d	2.9c	2.1d	80.3b	62.7b	75.8a	27.8d	37.7d	43.7d	7.9c	6.6c	7.4d	1.7d	2.3d	1.3d
40	26.2c	29.7b	25.5c	2.8c	3.4b	2.5c	87.7a	66.3a	78.6a	43.3c	50.0c	58.1c	9.5b	7.2b	9.2b	2.1c	2.7c	1.9c
80	27.9a	30.5a	27.4a	3.3a	3.6a	2.9a	89.3a	69.5a	79.4a	70.8a	81.8a	88.6a	11.6a	8.3a	10.9a	2.5b	3.3b	2.2b
120	27.4b	30.2a	26.5b	3.1b	3.4b	2.7b	79.7b	60.3b	75.4a	59.7b	56.0b	68.0b	9.2b	7.4b	8.6c	3.1a	3.9a	2.4a

Means values (n = 3) in a column followed by a common letter are not significantly different ($P < 0.05$) by Duncan's multiple range test (DMRT). The CV % and 5 % LSD of panicle length, panicle dry weight, filled grain %, total dry biomass, grain Fe content and leaf area are 0.8, 3.3, 3.8, 4.1, 3.9, 4.1 and 0.38, 0.16, 4.89, 3.96, 0.57, 0.17 respectively.

Sharbati. In all the cultivars, maximum biomass accumulation was observed with 80 kg N ha^{-1} followed by 120, 40, and 0 kg N ha^{-1}, respectively (Table 1).

Phosphorus Nutrition

Panicle length and weight increased marginally up to the level of 20 kg ha^{-1} of P application in the soil in all the three cultivars and remained stable thereafter (Table 2). Increase of P application was not effective on panicle length or weight; the response was much lower compared to that of N. Panicle growth was greatest in Lalat and lowest in Sharbati at all P levels. In contrast, filled grain percentage and Fe concentration of the grains were greater in Sharbati and IR-64 than in Lalat. Iron concentration in grains of all the cultivars increased with increase in the level of P application in the soil up to 40 kg ha^{-1} and declined at 80 kg ha^{-1}. Among the cultivars, the concentration was high in Sharbati and low in Lalat (Table 2).

Potassium Nutrition

Increase of K level in the soil did not improve panicle weight as much as that of N. Similar to N, K application was more effective on panicle weight and length of Lalat (Table 3). However, unlike N, the response of Sharbati to K application was less than that of IR-64. In all the cultivars, K application improved panicle weight and length marginally up to the level of 20 kg ha^{-1}, and there was no improvement of growth beyond this level. The grain Fe concentration increased with increase of K application up to 20 kg ha^{-1} and declined thereafter in all cultivars. Among the cultivars, the concentration was greatest in Sharbati and lowest in Lalat. The filled grain percentage of the cultivars was greatest in Sharbati, followed by IR-64 and Lalat. Increase in the level of K application did not have any significant influence on grain filling. Compared to IR-64 or Sharbati, leaf area was high in Lalat, and increases in the level of K application had marginal influence on leaf area of the cultivars.

Effects of Nitrogen, Phosphorus, and Potassium Applications on Leaf Iron Concentration

The concentration of Fe in the leaf remained almost unchanged during the first 75 days of crop growth with all the four levels of N application and increased significantly after 100 days of transplanting (Table 4). The leaf Fe concentration increased gradually with the increase in N application up to 80 kg ha^{-1} and thereafter declined at 120 kg ha^{-1} N. Among the cultivars, the concentration of Fe was greatest in Sharbati, followed by IR-64 and Lalat. However, up to 25 days after transplanting, leaf Fe concentration was greatest with 120 kg N ha^{-1} in all the cultivars, which started declining, and at 50 days after transplanting there was little difference among the N doses. Effect of 80 kg N ha^{-1} surpassed that of 120 kg at 75days after transplanting and attained the greatest value at 100 days after transplanting. Similar to N, K application also improved Fe concentration of the leaf.

Phosphorus nutrition also improved the concentration of Fe in the leaf, and the pattern of improvement was similar to that of N and K (Table 4). However, unlike K, P application at 40 kg ha^{-1} always gave the greatest Fe content of the leaf throughout the growing period in all three cultivars. The impact of P application on increasing leaf Fe concentration was greater than that of K and lower than that of N.

Potassium application also influenced leaf Fe concentration. Like N, effect of K application on leaf Fe content changed over the growing period. During the early stage of

Table 2

Effect of phosphorus application (0, 20, 40 and 80 kg ha^{-1}) on panicle length (cm), panicle dry weight (g), filled grains (%), total dry biomass (g), grain Fe content ($\mu g\ g^{-1}$ dry wt.) and leaf area (cm^2) in three *indica* rice cultivars (Sharbati, Lalat and IR-64) at maturity, grown in open field conditions

	Panicle length			Panicle dry weight			Filled grain %			Total dry biomass			Grain Fe content			Leaf area		
P treatments	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64
0	25.5b	27.0b	24.8b	2.4a	2.6a	2.6a	74.9c	63.1b	70.2d	43.8d	40.0d	46.3c	9.0c	8.2c	8.6b	2.1b	2.7c	1.8c
20	27.1a	29.3a	26.0a	2.8a	3.1a	3.0a	84.8a	69.8a	79.7a	53.0c	59.5c	77.0a	9.5b	8.8b	8.9b	2.2b	2.8c	2.0b
40	26.5a	29.4a	26.3a	2.6a	3.0a	2.8a	83.8a	63.1b	76.9b	73.5a	75.5a	62.8b	11.1a	9.5a	10.5a	2.5a	3.3b	2.4a
80	26.1b	29.3a	25.6a	2.5a	2.8a	2.6a	80.1b	60.5c	74.0c	60.5b	67.7b	58.5b	9.1c	8.2c	8.7b	2.5a	3.6a	2.4a

Means values ($n = 3$) in a column followed by a common letter are not significantly different ($P < 0.05$) by Duncan's multiple range test (DMRT). The CV % and 5% LSD of panicle length, panicle dry weight, filled grain %, total dry biomass, grain Fe content and leaf area are 1.6, 1.9, 1.8, 4.6, 2.3, 3.9 and 0.73, 0.86, 2.22, 4.62, 0.35, 0.16 respectively.

Table 3

Effect of potassium application (0, 20, 40 and 80 kg ha^{-1}) on panicle length (cm), panicle dry weight (g), filled grains (%), total dry biomass (g), grain Fe content ($\mu g\ g^{-1}$ dry wt.) and leaf area (cm^2) in three *indica* rice cultivars (Sharbati, Lalat and IR-64) at maturity, grown in open field conditions

	Panicle length			Panicle dry weight			Filled grain %			Total dry biomass			Grain Fe content			Leaf area		
K treatments	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64	Sharbati	Lalat	IR64
0	25.7a	29.9b	25.3a	2.2a	2.9a	2.7a	74.1a	56.4a	76.7a	46.2c	61.7c	52.7c	9.2c	8.1c	8.6d	2.3a	3.6b	2.1b
20	26.3a	31.6a	26.4a	2.5a	3.4a	3.1a	76.5a	61.2a	77.1a	66.3b	74.3b	65.0b	11.0a	9.4a	10.0a	2.4a	4.0a	2.3a
40	25.5a	30.8a	25.9a	2.4a	3.3a	2.8a	74.4a	60.2a	80.5a	73.8a	78.2a	72.8a	9.9b	9.1a	9.5b	2.3a	3.6b	2.2a
80	25.0a	30.1a	25.7a	2.2a	3.1a	2.7a	71.9a	57.5a	72.5a	67.8b	72.3b	66.0b	9.1c	8.9b	9.0c	2.2b	3.5b	2.0b

Means values (n = 3) in a column followed by a common letter are not significantly different ($P < 0.05$) by Duncan's multiple range test (DMRT). The CV % and 5 % LSD of panicle length, panicle dry weight, filled grain %, total dry biomass, grain Fe content and leaf area are 3.2, 13.1, 7.9, 3.2, 2.5, 3.6 and 1.46, 0.61, 9.32, 3.53, 0.39, 0.17 respectively.

Table 4

Temporal changes in total leaf Fe content ($\mu g\ g^{-1}$ dry wt.) of mature top leaves at 25, 50, 75 and 100 days after transplanting of three *indica* rice cultivars, Sharbati, Lalat and IR-64 grown in open field condition with four levels of N (0, 40, 80 and 120 kg ha^{-1}), P (0, 20, 40 and 80 kg ha^{-1}) and K (0, 20, 40 and 80 kg ha^{-1})

		N treatment				P treatment				K treatment			
Cultivars	Days after transplanting	0	40	80	120	0	20	40	80	0	20	40	80
Sharbati	25	192.4d	284.3c	355.0b	503.9a	221.3d	308.4b	373.2a	274.4c	211.5d	315.5b	347.5a	261.5c
Lalat		185.3d	252.0c	321.0b	445.8a	187.0c	255.3b	322.2a	189.8c	162.6d	227.9b	308.8a	202.7c
IR-64		174.6d	279.7c	340.2b	482.9a	207.3d	286.3b	359.0a	253.0c	184.0d	252.4b	321.3a	239.1c
Sharbati	50	153.4b	170.7a	187.0a	189.0a	117.9c	217.8b	246.0a	205.7b	168.0d	237.13b	262.3a	214.7c
Lalat		117.4c	138.3b	160.3a	166.7a	85.7c	125.9b	201.1a	120.2b	131.2d	185.0b	228.1a	156.6c
IR-64		130.0c	143.9b	177.2a	177.3a	103.7c	191.0b	232.3a	182.7b	154.0d	214.1b	246.7a	182.3c
Sharbati	75	186.0c	254.6a	261.3a	236.3b	200.0c	272.5b	322.7a	277.1b	215.3b	265.3a	263.7a	224.3b
Lalat		157.8b	193.7a	211.3a	174.3b	153.4c	227.8b	262.7a	214.3b	155.0c	235.3a	229.7a	169.3b
IR-64		174.0c	230.9a	241.5a	202.1b	177.0c	257.8b	295.3a	244.3b	196.1b	250.5a	247.0a	189.7b
Sharbati	100	618.4d	940.3c	1240.2a	1131.8b	869.3d	926.6c	1136.4a	1052.8b	662.9c	857.3a	774.0b	634.3d
Lalat		356.3d	666.0c	1033.0a	972.9b	801.0c	871.3b	978.3a	809.0c	562.7c	646.3a	610.3b	509.7d
IR-64		515.3d	895.0c	1133.3a	1063.7b	836.8d	910.4c	1042.0a	948.0b	633.7c	810.0a	686.7b	603.7d

Mean values ($n = 3$) in a row within a treatment followed by a common letter are not significantly different ($P < 0.05$) by Duncan's multiple range test (DMRT). The CV % and 5 % LSD of N treatment, P treatment and K treatment are 3.6, 3.0, 2.2 and 22.8, 19.8, 11.8 respectively.

growth (i.e., up to 50 days after transplanting) leaf Fe content was greatest with 40 kg ha^{-1}, which started declining and became similar to that of 20 kg ha^{-1} at 75 days after transplanting. After 100 days of transplanting, adverse affect of K on Fe absorption became apparent, and 20 kg K ha^{-1} gave the greatest Fe accumulation. Among the cultivars, the concentration was high in Sharbati and low in Lalat. However, K application could not increase the concentration leaf Fe as much as that of N, and in fact there was decline in Fe content at 40 kg ha^{-1} and beyond (Table 4).

Statistical Analysis

The experiments were conducted in a RCB design. Results were presented as the means of three independent replications. Mean comparisons were made by Duncan's multiple-range test (DMRT). Variation among the cultivars and different levels of N, P, and K with respect to various parameters (viz., panicle dry weight, filled grain, panicle length, total grain Fe concentration, leaf area, and total dry mass at maturity) were found to be significant.

The correlation between grain Fe concentration and grain filling percentage is presented in Figure 1. Significant positive correlations existed between grain Fe and grain filling percentage when measured at different levels of N, P, and K application (Figure 1).

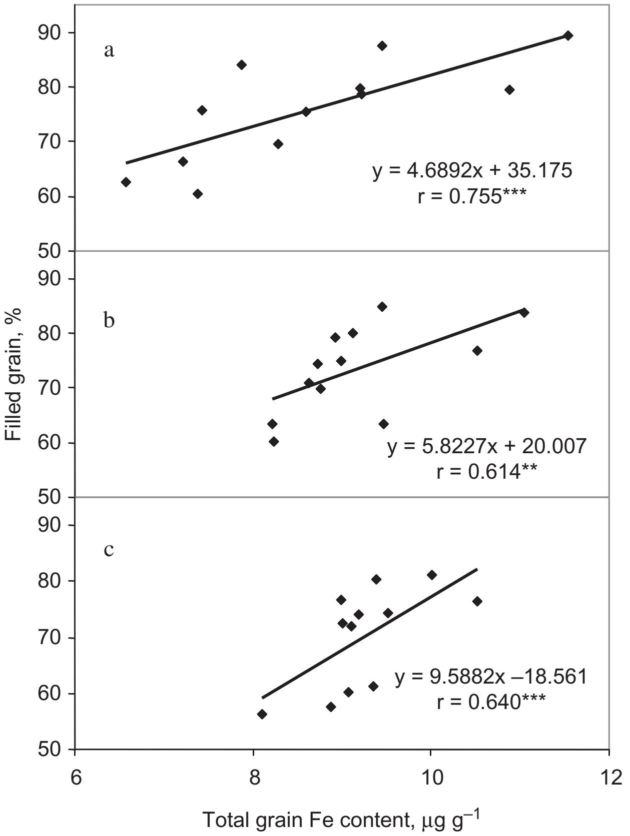

Figure 1. Linear correlations between total grain Fe content and filled grains in three *indica* rice cultivars (Sharbati, Lalat, and IR-64) grown with different doses of N, P, and K (a, b, and c respectively) fertilizers. Asterisks (**, ***) denote correlation coefficients that are significant at the 0.01 and 0.001 levels of probability, respectively (two-tailed, df = 22 (12 × 2 – 2).

Discussion

The results showed that all the three cultivars showed improved grain filling as well as leaf Fe concentration in response to greater doses of N, P, or K application up to a limit, but Sharbati was more responsive compared to the other two cultivars. In general rice plant responds positively to application of these major nutrients in enhancement of grain yield and the yield parameters (Mahato et al. 2007), but the pattern of response in relation to genetic variation of cultivars is not identical. Considerable variation was reported in N-use efficiency of rice cultivars (Haefele et al. 2008; Oikeh et al. 2009). Genotypic variation in uptake of nine elements under chromium stress was elaborated more recently (Zeng et al. 2010). While varietal difference in response pattern to exogenous application of major nutrients is highlighted in these studies, information is scant on the mechanism of interactions among N, P, and K applications and Fe uptake and ultimate effects on grain yield of rice plant in the literature. In this context, the present investigation is a novelty, because it identified the beneficial effects of the application of three major nutrients on Fe content of rice plant and at the same time defined the limits of application of the elements in the rice ecosystem of the eastern part of India. However, the physiological and biochemical mechanism that improved Fe uptake under favorable N, P, or K nutrition remains elusive. It is possible that absorption of N, P, and K by the plant increases biomass and grain yield in rice (Shu and Chung 2006) because the nutrients play positive roles directly or indirectly on leaf photosynthesis to improve primary production. Therefore, a favorable photoassimilate balance of the plant might have helped active uptake of Fe in the rice plant. In our study, grain and leaf Fe concentration increased with increases of N, P, and K application. The correlation between grain Fe concentration and grain filling percentage was positive and significant, when measured at different levels of N, P, and K application (Figure 1). There are several examples where N nutrition has been shown to improve plant biomass and absorption of other important nutrients. In parsley crop, N nutrition improved plant biomass and uptake of various elements, including Fe, from the soil (Chenard, Kopsell, and Kopsell 2005). Similar results were also observed in spinach (Mark, Kopsell, and Kopsell 2007), rice (Hao et al. 2007), and wheat (Shi et al. 2010). In some rice cultivars, Fe concentration of different parts of the grain increased in response to increase in N nutrition (Prom-u-thai and Rerkasem 2003). Rice plants release small molecules called siderophores into the rhizosphere, which bind Fe^{3+} in the form of a ligand. The ligand complex enters into the cell, and Fe^{3+} is reduced into Fe^{2+} inside the cytoplasm. Thus, NPK applications might have contributed to Fe acquisition by enabling the plant to synthesize more photosynthetic assimilates and reducing power ($NADPH+H^+$), which might have helped in the synthesis and release of more siderophores to the rhizosphere of rice and subsequent reduction of Fe^{3+} to a soluble ferrous form. Pearman, Thomas, and Thorne (1979) reported that increase in dry matter due to increase in N application was because of the increased light intercepting area, resulting in more assimilation of photosynthates and increase in filling rate. However, the photosynthetic rates decreased at very high levels of N because the intensity of photosynthetically active radiation was less at the surface of the leaves in the dense crops with additional N. This describes the observed decline in Fe accumulation at very high doses of N (120 Kg ha^{-1}). Decline in micronutrient density in response to very high doses of N was also reported in rice (Hao et al. 2007) and wheat (Shi et al. 2010).

The rice cultivars used in our experiment might differ in the capacity for Fe acquisition because of differences in either siderophore formation or capacity for reduction of the absorbed Fe^{3+}. There are several steps during uptake and transport of Fe in plants that might be affected by N nutrition. By affecting root growth and stimulating root exudation of organic compounds (Marschner 1995; Paterson et al. 2006), N may influence the mobility and root uptake of Fe from soils. In maize, increasing N application was effective in enhancing carbon (C) partitioning into roots and promoting exudation of C-containing compounds from roots into rhizosphere (Liljeroth, Kuikman, and Vanveen 1994). The expression level of Fe transporter proteins located on the root cell membranes such as ZIP (ZRT, IRT-like Protein) family transporter proteins (Grotz and Guerinot 2006) might also be affected by the plant N nutritional status.

Increasing N application has been reported to promote protein synthesis in wheat grains (Hao et al. 2007). Excess of Fe in the cell is stored in the ferritin protein, containing 24 polypeptide subunits (Harrison and Arosio 1996). Any increase in protein synthesis due to greater production of photoassimilates may positively influence Fe assimilation in ferritin protein. Moreover, polypeptides contain N, and a greater application of N might also lead to synthesis of more polypeptide, which will ultimately bind more Fe (Theil 1987).

Similarly, P is a major constituent of large number of biomolecules including adenosine-5'-triphosphate (ATP), the energy currency of the cell (Fujino 1967). Iron acquisition from the soil is an active process and needs energy in the form of ATP. Under the conditions of sufficient P nutrition, such energy-requiring process and synthesis of proteins will be promoted. Moreover, plant ferritin mineral core has high phosphate content, and high P is thus expected to positively influence the formation of ferritin molecule (Wade et al. 1993; Briat et al. 1999). On the other hand, at a very high P level, Fe becomes immobilized because of precipitation as insoluble phosphate in the vein (Ajakaiye 1979), rendering it unavailable for assimilation.

Inhibition of Fe accumulation at greater doses of K is evident from the fact that K in greater doses increases the root oxidizing power of rice, which results in oxidation of Fe^{2+} to Fe^{3+} and exclusion of this ion from uptake. This was evident from the increase in intensity of the Fe oxide coating on rice roots at greater levels of K application (Trolldenier 1988; Yamauchi 1989). Inhibition of Fe uptake by an excess of K was also reported by Li, Yang, and Luo (2001) in rice. Similarly, rice plants grown with low K had low root oxidizing capacity and absorbed more Fe than plants grown with greater K. Adequate K may also enhance Fe-excluding powers of rice roots (Tanaka and Tadano 1972; Yang et al. 1997).

Rice is a fertilizer responsive crop; increments in fertilizer application may thus give rise to better yield but at the cost of decline in Fe accumulation. Hence, judicious application of major elements such N, P, and K is very important for proper uptake and accumulation of micronutrients such as Fe in the grain. Positive effects of N nutrition on uptake and accumulation of Fe and a very close relationship between the concentrations of grain Fe and grain filling are relevant for further research.

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