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RESEARCH ARTICLE

Nutrient use efficiency of rice genotypes under iron-toxic lowland soil influenced by high potassic fertilizer and foliar application of kinetin

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Abstract

Improving global rice yield productivity under low-input conditions is the main challenge, especially in iron-toxic lowland acid soils. With India's irregular rainfall patterns and continual environmental anomalies, particularly in Odisha, the identification of climate-smart management practices that can withstand iron toxicity is critical. In this context, an experiment was conducted to develop effective nutrient use efficiency and nutrient management practices under iron-toxic lowland rice in lateritic acid soils of Central Farm, Odisha University of Agriculture & Technology, Odisha, with high-level use of potassic fertilizer along with the foliar application of Kinetin and five genotypes suitably fitted in a split-plot design. The results showed that the mean average performance of the genotypes was significantly increased at K120 and K100 levels along with Kinetin. At K levels of K100+Kn, the nutrient use efficiency was highest for nitrogen **(**68.60**)** and phosphorus (137.20). As regards potassium use efficiency in terms of AKR (100.81%), K40+Kn had the highest value of KGPE (935.72). The mean performance of the genotype in terms of total nutrient uptake in response to iron toxicity to different doses of K application showed a significant gradual increase with increasing K levels from K0-Kn to K120+Kn, and Hiranmayee had the highest total K uptake of 121.83 kg/ha. Total K uptake at K100+Kn was much higher than other doses, including control. These results suggest that high doses of potassium and foliar spray of Kinetin can alleviate the deleterious effects of iron toxicity in rice plants by enhancing physiological growth and nutrient uptake.

Keywords

iron toxicity; kinetin (Kn); nutrient use efficiency; potassium; rice

Introduction

One of the major staple foods grown in India and around the world is rice (*Oryza sativa* L.). However, rice agro-ecosystems are hampered by a variety of abiotic stresses and climate change-related limitations, achieving this goal even more challenging. One of the most worrying abiotic stresses that negatively affects rice growth and productivity is mineral toxicity, especially iron (Fe) (1,2). Fe toxicity was only a problem on very acidic soils until recently. However, as time has gone on and as a result of the detrimental effects of climate change, Fe toxicity has spread to 18% of the world's cultivable land for rice (3). Fe poisoning negatively impacts over 11.7 million

hectares of land in India alone (4). Iron toxicity is a multifaceted nutritional disorder, and it has been suggested that shortages in some nutrients, particularly Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), and Zinc (Zn), may impact the condition's incidence in rice (5).

The appearance of iron toxicity symptoms in rice involves an excessive uptake of ferrous ion ($Fe²⁺$) by the rice roots and its acropetal translocation into the leaves, where an elevated production of toxic oxygen radicals can damage cell structural components and impair physiological processes. Nutrient imbalances deficiencies are contributing factors to the current stalling yield levels (6). This issue indicates that there is a significant opportunity to improve yield levels and nutrient use efficiency by taking into account all essential plant nutrients in fertilizer products and fertilization techniques. Applying balanced levels of the most limiting nutrients will be essential to achieving these benefits and maximizing output while minimizing nutrient losses; in other words, fertilization must be precisely tailored to the crop's needs and the chemical conditions of the local soil (7). Iron toxicity can be reduced by using iron-tolerant rice genotypes and through soil, water, and nutrient management practices, i.e., balance the use of fertilizers with sufficient Potassium (K) fertilizer.

Plant nutrient availability and crop yield are known to be enhanced by several soil and environmental parameters under the soil-plant biological system. In this particular situation, the organisms that comprise the soil microbial population of the rhizosphere may have the greatest influence (8). Complex interactions take place in the soil surrounding plant roots, involving the roots, soil, microbes, and root exudates (9). Plants rely on the ability of their roots to establish a relationship with rhizospheric microorganisms using signaling pathways (10). Different rice genotypes were found to have higher yields, which suggests that plants with better nutrition uptake through roots are more resistant to iron. One of the solutions to address this problem is the use of nutrients related to increasing the fertility percentage and flowers and panicles, especially by the use of high potassium. This element has an appositive correlation with increasing the fertility percentage and flowers. Besides, the plant's need for this element may exceed all other nutrients in some stages of plant growth (11). On the other hand, the growth regulator kinetin (Kn) also activates cell division, reduces apical dominance, encourages the growth of lateral branches, and encourages the plant towards increasing reproductive parts, and this is reflected in an increase in yield (12). When compared to other crop species, rice is highly vulnerable to iron toxicity, and since this has been observed in the lowland acidic soils, it has been decided to study this nutrient use efficiency in rice in detail along with the management strategies of high potassic fertilizer use along with the foliar application of kinetin.

Experimental site

The field experiment was carried out during the wet season of 2022 and 2023 at Central Experimental Farm, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha (20° 15' N latitude, 85° 52'E longitude, elevation 25.9 m above mean sea level), which belongs to the east and south eastern coastal plain agro climatic zone of Odisha and falls under the east coastal plain and hills zone of humid tropics of India. The experimental plot soil was sandy loam, medium fertile, non-saline (EC-0.120 dSm^{-1}) inceptisol, deep with adequate drainage and was low available nitrogen, phosphorus, and potassium soil [N (Urea), $P_2O_5(DAP)$, K_2O (MOP) of 190, 10.4 and 59.67 kg ha $^{-1}$ respectively], pH 4.55, organic carbon (0.54%) and DTPA (Diethylenetriaminepentaacetic acid) extractable iron (376.88 ppm). The experiment was conducted during the period of which Day/night temperature was 31.7° C/ 23.0°C, relative humidity 7 hr/14 hr-90.4%/ 70.6%, Bright sunshine hours-4.4 hours, annual rainfall-1821 mm, of which 80% is received between June to October in about 65 to 75 rainy days was recorded. The climate is classified as hot and moist subhumid with a crop growing period of around 138 days in a year.

Experimental design

Five genotypes viz; Lalat(G1), Manaswini (G2), Hiranmayee (G3), Pratikshya (G4), and Tejaswini(G5) were selected as per screening performance (Our earlier Finding of *Kharif* 2022 reported in this journal, PST-2024) for further study in the same iron-rich soil during *Kharif* 2023 and trialed in a split-plot design having five levels of potassic fertilizer [0 (K0), 40(K40), 80(K80), 100(K100), 120(K120) kg K2O ha⁻¹] along with recommended doses of N (80 kg ha^{-1}) applied in 2 split and P (40 kg P_2O_5 ha⁻¹) and was replicated thrice. The potassium was applied in 3 split doses of 25%, 50%, and 25% of the K requirement. Further, at the tillering stage, the crop was foliar sprayed twice with kinetin (dissolved in 1M KOH) with a concentration of 200 mg/L in a gap of 10 days to the first spray except for control.

Nutrient analysis

Grain and straw from rice cultivars were collected after crop harvesting. Then, the samples were processed for the analysis of nutrients. The samples were grounded, and the powered samples (0.5gm) were taken for pre-digestion in 15 ml of concentrated nitric acid overnight (13). Then the samples were digested in a diacid mixture (HNO₃: HClO₄ = 3:2). After filtering the contents with Whatman No. 42, the contents were moved to a 50 ml volumetric flask, and double-distilled water was added to bring the volume up to 50 ml. After that, the samples were analyzed for Fe, Ca, Mg, and Zn in Inductively coupled plasma optical emission spectroscopy (14) [ICP-OES, PerkinElmer, Model- Avio 200], K in flame photometer (15) [Systronics flame photometers 128] and P in a spectrophotometer (16) (PerkinElmer, Model- Lambda 365). The nitrogen (N) content of the processed plant sample was assessed using the Kjeldahl digestion method (Kelplus supra-LX VA), as detailed in AOAC (Association of Official Analytical Chemists1960).

Estimation of C, H, N, S of grain was analyzed by CHNS-O₂ analyzer (make-Elementar, model-UNICUBE,070721) by instantaneous oxidation of the sample by "Flash combustion" which was then detected with the help of thermal conductivity detector. The nutrient use efficiency was calculated by using the formula (17) presented in Table 1.

Soil microbial population analysis

Bacteria, actinomycetes, and fungi cultivation methods and growth/ colonies were determined by modified standard serial plate count method (18) using viz. Nutrient Agar for bacteria, Potato Dextrose Agar (PDA) for fungi, Actinomycetes Isolation Agar for Actinomycetes. Preparation of the three media was carried out aseptically by pouring and solidification of the media. Serial dilution of the provided soil was done using sterile normal saline solution (NSS) followed by aseptic inoculation on the media by using the spread plate technique. The Plates were then kept for incubation at the respective optimal temperature (28±2°C) for the required period (24 hrs for bacteria, 48 hrs for Fungi, and 5 days for actinomycetes growth). Then, these were counted as colony forming units/g (CFU/g) fresh weight of the soil. The microbial population was exposed as several colonies forming units per gram of the soil.

Table 1. Nutrient use efficiency

Statistical analysis

The data were analyzed statistically in a split-plot design, which was outlined (19). The correlation coefficients were estimated with the help of the Microsoft Office Excel 2019 unit operating system. The Statistical analysis of rice cultivars was calculated using R Software (R.4.3.3).

Results

Effect of potassium doses and kinetin on nutrient use efficiency of rice grown under iron toxic soil

The statistical analysis of nutrient use efficiency in response to iron toxicity is presented in Table 2. The mean average performance of the genotypes was significantly increased at K120 and K100 levels along with Kn. At K levels of K100+Kn, the nutrient use efficiency was highest for nitrogen (68.80) and phosphorus (137.20) except KHI (5.18), where the effect of K120+Kn was highest. As regards potassium use efficiency in terms of AKR (100.81%), K40+Kn had the highest value of KGPE (934.72) except KAE and KPE with a value of 12.83, 14.73 at K100+Kn, respectively. The mean performance of the genotype in terms of total nutrient uptake in response to iron toxicity to different doses of K application showed a significant gradual increase with increasing K levels from K0-Kn to K120+Kn, and Hiranmayee (G3) had the highest total K

Table 2. Variation of treatments among nutrient use efficiency

Treatments	NUE	PUE	KUE	NHI	PHI	KHI	AKR
Genotypes							
G1	39.48	78.96	44.73	48.69	61.09	3.33	73.38
G ₂	56.72	113.44	62.48	60.39	74.82	5.16	94.95
G ₃	69.47	138.93	79.93	60.44	65.25	3.60	74.68
G4	66.97	133.94	75.41	67.47	69.67	2.92	95.56
G5	73.36	146.72	81.62	58.60	71.12	5.30	84.49
Levels of potassium with kinetin							
K0+Kn	52.57	105.14	$\overline{}$	77.37	67.56	4.07	$\overline{}$
K40+Kn	57.93	115.87	115.87	59.43	70.26	2.81	100.81
K80+Kn	59.98	119.96	59.98	56.18	69.29	3.52	77.27
K100+Kn	68.60	137.20	54.88	49.45	69.44	4.72	88.30
K120+Kn	66.91	133.82	44.61	53.16	65.41	5.18	72.07
G				$***$	$***$	$***$	
K	\blacksquare			$***$	$***$	$***$	
$G^{\star}K$	\blacksquare			$***$	$***$	$***$	\blacksquare
Genotype	KAE	KGPE	KPE	N uptake	P Uptake	K Uptake	Fe uptake
G1	9.74	545.94	12.83	114.22	18.98	95.14	2.81
G ₂	6.57	203.69	7.40	142.10	24.98	118.68	4.04
G ₃	12.67	563.75	17.00	149.64	27.92	121.83	4.36
G4	11.06	615.32	12.04	145.40	27.37	114.25	2.53
G ₅	10.63	419.78	13.48	133.85	32.32	120.34	4.89
Levels of potassium with kinetin							
K0+Kn	\overline{a}		\mathbf{r}	71.83	15.38	58.64	4.23
K40+Kn	10.73	934.72	12.47	110.44	23.02	98.96	3.82
K80+Kn	7.41	386.51	9.75	131.00	26.32	120.44	3.78
K100+Kn	12.83	313.54	14.73	192.94	32.73	146.88	3.35
K120+Kn	9.56	244.01	13.25	179.00	34.12	145.10	3.43
G	$\overline{}$			$***$	$***$	$***$	***
Κ				$***$	$***$	***	***
G*K	\blacksquare	\blacksquare		$***$	$***$	\star	$***$

(*** Significant at p value ≤ 0.001, * Significant at p value ≤ 0.05) (G1-Lalat, G2-Manaswini, G3- Hiranmayee, G4-Pratishya, G5-Tejaswini, K0-Kn = Control, K40+Kn = 40 kg K₂O ha⁻¹ with kinetin, K80+Kn= 80 Kg K₂O ha⁻¹ with kinetin, K100+Kn = 100 kg K₂O ha⁻¹ with kinetin, K120+Kn= 120 kg K₂O ha⁻¹ with kinetin)

uptake of 121.83 kg/ha. Total K uptake at K100+Kn was much higher than other doses, including control. On the contrary, Tejaswini (G5) had the highest mean Fe uptake (4.89 kg/ha) among genotypes and the lowest at K100+Kn (3.35 kg/ha). Iron content was negatively correlated with total K uptake. Total N uptake was found to be higher by K100 (192.94 kg/ha), but P uptake (34.12 kg/ha) was found to be highest at K120+Kn. Among genotypes, Pratikshya (2.53) and Lalat (2.81) were found to lower the uptake of iron to different doses of $K₂O+Kn$ and can be considered as tolerant genotypes to Fe toxicity.

Effect of potassium doses and kinetin on microbial population in iron toxic soil

Microbial activity in the rice root rhizosphere study (Table 3) revealed that the initial microbial population was gradually increased towards increasing doses of K levels with Kn in soil substantially over control, and thereafter, the microbial population decreased at the harvest stage. An increased number of bacteria was noted to be higher than that of fungi and actinomycetes. But in comparison to different doses of potassic fertilizer, at K120+Kn bacteria (86.40 CFU/g x 10⁴), actinomycetes (9.60 CFU/g x 10⁴) and

fungus (17.20 CFU/g \times 10⁴) population growth occurred rapidly and was found significant (p<0.001) within the treatments. Varietal preference for microbial growth and their interaction with treatments was also found to be significant (p<0.001).

Effect of Potassium doses and kinetin on nutrient content of rice straw grown under iron toxic soil

Among the five tested K levels, with or without Kn, a significant difference was observed in the micro and macronutrient content of paddy straw regardless of genotype (Table 4). A drastic increase in N %, P %, and K% was evident in the increase in K levels from K0 to K120+Kn. However, an exception was observed in iron, where the micronutrient concentrations decrease with an increase in K doses. However, the Ca, Mg, and Zn concentrations in paddy straw did not follow the uniform pattern of accumulation to K levels. As regards genotypes' response to K levels +Kn, the variation was observed in the accumulation of nutrients in straw. However, Tejaswini showed the highest accumulation of K (1.54%), Fe (638.92 ppm), Zn (251.32 ppm), and Mg (0.05%). The two-way interaction was found to be highly significant at p<0.001.

Table 3. Variation of treatments to microbial populations

(*** Significant at p value ≤ 0.001, * Significant at p value ≤ 0.05) (G1-Lalat, G2-Manaswini, G3- Hiranmayee, G4-Pratishya, G5-Tejaswini, K0-Kn = Control, K40+Kn = 40 kg K₂O ha⁻¹ with kinetin, K80+Kn= 80 Kg K₂O ha⁻¹ with kinetin, K100+Kn = 100 kg K₂O ha⁻¹ with kinetin, K120+Kn= 120 kg K₂O ha⁻¹ with kinetin)

Table 4. Variation among treatments to nutrient status in straw

(*** Significant at p value ≤ 0.001.) (G1-Lalat, G2-Manaswini, G3- Hiranmayee, G4-Pratishya, G5-Tejaswini, K0-Kn = Control, K40+Kn = 40 kg K₂O ha⁻¹ with kinetin, K80+Kn= 80 Kg K2O ha⁻¹ with kinetin, K100+Kn = 100 kg K2O ha⁻¹ with kinetin, K120+Kn= 120 kg K2O ha⁻¹ with kinetin)

Effect of potassium doses and kinetin on nutrient content of rice grain grown under iron toxic soil

Data from **Table 5** presented the interaction effect of 5 potassium levels with Kn and five genotype treatments under iron toxicity on quality attributes of rice grain, which showed a significant increase in all the quality attributes. C (Carbon), H (Hydrogen), N (Nitrogen), P (Phosphorus), K (Potassium), and S (Sulphur) content increases with increasing doses with K+Kn, and the variation in genotypes was quite prominent in Pratikshya (N, P, H), Manaswini (P,

K), Lalat (S). However, the iron content decreased with increased doses of K with kn and was found more in Tejaswini (G5). The mean average performance of genotypes was significant with K levels and their interactions with grain protein and grain carbohydrate concentration. Tejaswini was found to have more protein content, whereas Pratikshya had more carbohydrate accumulation in grain. However, as regards K nutrition in iron-toxic soil, the application of K120+Kn was found to be more prominent in their mean two-way ANOVA.

Table-5 Variation among treatments to grain quality attributes

(*** Significant at p value ≤ 0.001, * Significant at p value ≤ 0.05) (G1-Lalat, G2-Manaswini, G3- Hiranmayee, G4-Pratishya, G5-Tejaswini, K0-Kn = Control, K40+Kn $=$ 40 kg K₂O ha⁻¹ with kinetin, K80+Kn= 80 Kg K₂O ha⁻¹ with kinetin, K100+Kn = 100 kg K₂O ha⁻¹ with kinetin) k120+Kn= 120 kg K₂O ha⁻¹ with kinetin)

Relationship between soil microorganisms and nutrient uptake by rice plant

The correlation coefficient between soil microorganisms and nutrient uptake is presented in Table 6. The soil microorganisms were positively correlated with N, P, and K uptake but negatively correlated with Fe uptake. K uptake was found to be strongly correlated with N and P uptake but negatively and highly correlated with iron uptake.

Discussion

Effect of potassium doses and kinetin on nutrient use efficiency of rice grown under iron toxic soil

The identical five genotypes were studied again in lowland iron toxic conditions using varying K fertilizer levels and two foliar sprays of kinetin at a rate of 200 mg/L, excluding the control. The results showed a significant increase in the genotypes' overall growth, yield, and physiological traits. On the other hand, elevated iron concentration was harmful and harmed growth in the control group, supporting K's beneficial interaction with Kn to reduce iron toxicity. This explains the inconsistent relationship between nutrient use efficiency and biomass (especially at no K and Kn) and underlies that those two parameters could effectively correlate under lower stress intensity. Moreover, our results indicate that NUE, PUE, KUE, seed carbohydrate, and harvested protein could be effectively used to differentiate rice genotypes depending on their Fe uptake under levels of K and Kn application. The physiological response to kinetin that was seen in this study may have resulted from an increase in endogenous cytokinin levels that were maybe stored as conjugates for potential use at a later stage of growth (20).

Table 6. Correlation coefficient between soil microorganism and total nutrient uptake by plant

* Signifies correlated at p value ≤ 0.05, ** signifies correlated at p value ≤ 0.01

Effect of potassium doses and kinetin on nutrient content of rice grain grown under iron toxic soil

Put another way, at the maturity stage, a higher percentage of dry matter is delivered to the ears in rice genotypes with significant sink capacities, which may be controlled by kinetin. Furthermore, several enzymes in the rice plant are co-activated by potassium, including some that transfer enzymes. Increases in carbohydrate and protein content in grains are, therefore, tied to starch production, which may be attributed to the presence of potassium and kinetin. These findings are in agreement with other scientists (21,22,23). The continuous supply of K to rice during its various stages is more beneficial and increases the translocation of carbohydrates from stem, leaf, and other storage organs to grains, leading to high sink capacity. Kinetin is involved in controlling development events like cell division, cell elongation, and protein synthesis, and consequently, higher grain yield.

Effect of Potassium doses and kinetin on nutrient content of rice straw grown under iron toxic soil

The combined action of kinetin and K fertilizer greatly influenced the uptake of nutrients by rice plants. In general, the order of nutritional absorption was $N > K > P$. It was discovered that rice grain contained more N and P than straw. Straw, on the other hand, had more K than grain. The findings indicated that when the rate of K fertilizers + Kn increases, the mean value of KAE of K falls (Table 4). This finding is consistent with studies by others (24, 25, 26) that showed a decrease in KAE of K with an increase in K rate. The perceived potassium recovery was impacted by the rates of potassium delivery. The findings continuously demonstrated a declining trend with rising K rates. Some scientists (27) found a similar pattern, reporting a decrease in apparent potassium recovery with an increase in K rate. The roles that N and K play in the plant are tightly related. Additionally, K allowed the plant to efficiently utilize N by enabling it to produce organic compounds connected to N absorption. We discovered that the lowland rice plants' shoot N, P, and K contents rose with potassium levels. Stated differently, higher K fertilization led to increased N, P, and K absorption, which was reflected in rice leaf levels of these nutrients (28, 29). However, when the K+Kn application increased, the mean value of the Ca, Mg, Zn, and Fe content decreased.

Effect of potassium doses and kinetin on microbial population in iron toxic soil

The fact that higher doses of K cause rice to have a higher root oxidizing capacity, which oxidizes $Fe²⁺$ to $Fe³⁺$ and excludes this ion from absorption, indicates that higher doses of K inhibit the accumulation of Fe. This was demonstrated by the rise of the microbial population in intensity, which might raise the quantity of soil K that plants could access. Increased K availability from the growing bacterial population aids in the conversion of non -exchangeable K to exchangeable K. A large root surface area increases the likelihood of K uptake and improves plant access to K. Thus; exogenous Kn reacts to low K levels by promoting K absorption.

Relationship between soil microorganisms and nutrient uptake by rice plant

The correlation established among bacteria, fungus, actinomycetes, and nutrient uptake indicated that soil microbial biomass is considered to act both as the agent of biochemical changes in soil and as a repository of plant nutrients such as nitrogen (N) and phosphorus (P) in agricultural ecosystems **(30).** The soil microbial biomass acts as a source and sink of plant nutrients and regulates the functioning of the soil system. The positive correlation among N, P, and K indicates a synergistic effect among these nutrients. When K doses increased from 0 to 120 kg $K₂O$, the uptake of N and P also followed along with K translocation, and a proper source-sink was established from soil to plant. However, as the antagonistic effect was observed between N, P, K, and iron uptake, the translocation of N, P, and K also diminished with a high concentration of Fe at the root rhizosphere zone, which was noted in the control treatment.

Applying the prescribed doses of fertilizers was an attempt to eradicate any form of nutritional stress; this lends more credence to the theory that the effects of kinetin were physiological rather than nutritional. Kinetin, however, has an effect on plant function in combination with or in opposition to other growth regulators; they do not act alone on a plant's physiological processes **(**31**).** Therefore, the observed effect in this study could be the consequence of both kinetin and their interaction with potassium.

Conclusion

Mineral nutrients are one of the most important critical inputs in rice, which necessitates its prudent use in light of its diminishing availability for agriculture, which is exacerbated by climate variability. A management strategy in addition to high doses of potassium (100 kg K_2O ha⁻¹)) and two foliar sprays of Kinetin (200 ppm) alleviated the deleterious effects of iron toxicity in lowland rice plants. As a result of the growing bacterial population's increased K availability, which facilitates the conversion of nonexchangeable K to exchangeable K, our results suggest that NUE, PUE, KUE, seed carbohydrate, and harvested protein may be useful in distinguishing rice genotype based on their Fe uptake under levels of K and Kn application. Exogenous Kn thus increases K absorption in response to low K levels.

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SWAIN ET AL **8**

Authors' contributions

Each author has made an active contribution. Jevelin Swain conducted both the lab and the field. R.K. Panda and R.K. Nayak conceptualized and managed the experiment. The manuscript was prepared and revised by Jevelin Swain and R.K. Panda. R.K. Nayak and M.R Satapathy provided an interpretation and critique of the outcome.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical issues: None.

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