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Research Article

Effect of different fertilizers on nutrient uptake, some morphological traits and protein and amylose content of rice grain

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Abstract

To examine the effect of S powder macro- and microelements on the nutrient uptake and morphological traits of rice grain protein and amylose content, the present study was carried out in 2014 in a paddy field in Babool city, located in Northern Iran, and all samples were tested at both the Mazandaran Agricultural Research Institute and the chemical laboratory of Pune, India. The results showed that macro elements affected the N, Ca and Na content very significantly. N and P grain content were significantly affected when macro and S powder were applied. K grain content was not influenced by any treatments. Ca and Mg grain content decreased very significantly when S powder and microelements were applied separately or together. Macro- and microelements decreased Na grain content very significantly. Amylose content was also affected by microelements very significantly. The minimum Ca (0.33%), Mg (0.14%), and Na (0.035%) and the maximum N (1.43%), P (0.19%), protein (8.54%), amylose (19.7%) content, germination ability (91.6%), and seedling (8.39 cm) and rootlet (5.30 cm) length were attained when NPK+S+Zn, Cu, and Mn-sulfate fertilizers were applied together. This treatment can be recommended to attain maximum amylose and protein and minimum Ca, Mg, and Na content that is also the best treatment for suitable viability of rice grain.

Keywords: Chemical fertilizers, Seedling, soil fertility, Soil pH, NPK fertilizer, Seed germination, Yield

Introduction

The rice plant is considered one of the most strategic commodities for the world (Peng *et al.,* 2023). It is the key staple food for the world's poorest and undernourished people living in Asia and Africa; this plant is not only linked with global food security but also closely connected with employment, economic growth, currency entry, regional peace, and social stability (Yadev and Kumar, 2018).

Rice production and research have faced unprecedented challenges in recent years (Mobasser *et al*., 2024). Yields and total production have increased for years in some major rice-producing countries, while demand from poor populations is increasing. The two greatest problems faced by the third-world countries, at present, are the exponential increase in population and the basic need for providing adequate food (Bin Rahman and Zhang, 2023). The two greatest problems faced by the third world countries, at present, are the exponential increase in population and the basic need for providing adequate food. The food production in turn can be improved by improving the conditions of arable soil and the correct use of fertilizers. Therefore, sustainable production and increasing yield of rice are necessary for world food security (Peng *et al*., 2023). Iran is one of the major paddy producers in the Middle East. Rice produced in Iran provides about two-thirds of Iran's annual consumption (Jafrodi *et al.,* 2022). The total undercultivated area of paddy production in Iran is more than 700,000 ha (FAO, 2023). More than 75 percent of the rice area is distributed in the two northern provinces of Mazandaran and Gilan. The most important method for protecting and breeding soil fertility and increasing product yield is correctly using suitable kinds of fertilizers. Chemical fertilizers should be able to increase the quantity and quality of agricultural products and provide people and stock healthy food (Malakoti and Tehrani, 1999; Malakoti and Tabatabai, 1997). Deficiencies of nutrients in soil limit the crop growth and can be corrected by application of fertilizers containing the required nutrients (Wild, 2003). For example, there is Zn deficiency in most of the cereals arable in the world (Malakoti *et al*., 2000; Malakoti and Tehrani, 1999). Zn deficiency in rice has been reported in most of the rice producing countries of the world and is recognized as the main micronutrient

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limitation in flooded rice (Moore and Patrick, 1988). It also occurs in seedling rice grown on alkaline soils (Bin Rahman and Zhang, 2023; Mikkelsen and Brandon, 1975). The presence of excess amounts of lime in soil disorders the nutrition uptake and injures plant growth (Somani and Kanthaliya, 2004; Das, 1996). There is a deficiency of nutrients in agronomic soils because of the existence of alkaline soils in Iran (Parvizi and Ronaghi, 2000). Thus, correct use of nutrients and providing of ideal conditions for their availability and uptake are important factors for increasing a plant's yield (Mobasser *et al.*, 2024; Malakoti and Tehrani, 1999).

Microelements are involved in the plant body as enzymes that play roles in protein and amylose production, especially Zn. S powder and Zn, Cu, and Mn-sulfate fertilizers can lower soil pH under calcareous soils and increase nutrient uptake like P, Ca, Mg, and Na by the rice plant (Qasempour Alamdari, 2004). Rice grain quality improvement has always been one of the top priorities in rice breeding programs. Similar trends will also continue in the future, as rice quality is a primary determinant of its market price and consumer acceptance (Bin Rahman and Zhang, 2023). Hence, it is thought of interest to study the effect of N, P, and K fertilizers and sulfur as powder and Zn, Cu, and Mn fertilizers as microelements on protein and amylose amount and uptake of nutrients by the rice grain and germination ability of nutrient-bearing seed.

Materials and methods

In order to study the effect of S powder macro- and microelements on nutrient uptake and germination ability of rice grain (*Taroom* rice variety), a split-split plot design in a randomized complete block was carried out with three replications in this research. This experiment was carried out in a paddy field in Babol City, located in northern Iran, and all samples were tested at both the Mazandaran Agricultural Research Institute and the chemical laboratory of Pune University, India, in 2014. Chemical fertilizers have been chosen as factors at different levels; these are NPK fertilizers as the main plot (control, NPK), sulfur powder as the subplot (control, S), and micronutrients as sub-subplot (control, Zn sulfate, Cu sulfate, Mn sulfate, and Zn, Cu, Mn sulfate). N and P fertilizers were added into the nursery (10 sq m) as urea (1 kg) and superphosphate (0.5 kg). All chemical fertilizers have been added to the soil before transplanting. The experimental field was leveled and divided into 60 plots, and macro- and microelements and sulfur powder were applied to the plot area as recommended by routine soil analysis (Malakoti and Gheybi, 1997). N, P, and K fertilizers were urea (50 kg ha^{-1}), triple superphosphate (50 kg ha^{-1}), and potassium sulfate (25 kg ha^{-1}), respectively. Zn, Cu, and Mn fertilizer were applied as Zn sulfate (30 kg ha^{-1}), Cu sulfate (10 kg ha^{-1}), and Mn sulfate (20 kg ha^{-1}), respectively. S powder has also been chosen at the rate of 100 kg ha^{-1}. The Taroom rice variety, which belongs to the low-yield variety (Basmati varieties), has been transplanted in this study. Afterward, the rice seedling was transplanted when it was at 15 cm height. The distance of the transplanting hill was 25 x 25 cm, and there were 4 seedlings in each hill (Ghasempour and Khodabandeh, 2004). All agronomic practices, such as nursery preparation, transplanting method, care operation, and harvesting methods, have been done as optimum techniques of agronomic engineering (Qasempour Alamdari and Khodabandeh, 2004).

Determination of N, P, and K content in the grain: After reaching physiological maturity, the plants were harvested within the central 2 meters of each plot to avoid contamination from the adjacent plot. The grains were separated by the combine machine. The grain humidity was measured and brought to the level of 14% humidity. To determine the N, P, and K concentrations in the rice grains, the following methods have been done: one gram of each sample was powdered and burnt in the heater (dish) at about 500-550°C for 5-6 hours, which changed it to ash. To the ash some HCl was added, and after one hour distilled water was also added to make the volume of the solution about 100 cc. N, P, and K content of this solution was also obtained using the Kjeldahl system (KJELTEC auto tecator 1030 analyzer, Sweden), spectrophotometer (Pharmacia-LKB-Novaspec 2, England), and flame photometer (410 Corning, England), respectively (Qasempour Alamdari and Khodabandeh, 2004).

Determination of Ca, Mg, and Na contents in the grain: To determine the Ca, Mg, and Na content of the grain, 1 gm of dried powdered sample was digested with a mixture of 5 ml each of HCl, HNO_3, and $HClO_4$ using heat for a period of 30 minutes until the disappearance of white fumes. After cooling, 100 ml of distilled water was added and filtered (Qasempour Alamdari and Khodabandeh, 2004). Next, 5 ml of each sample was chosen and diluted with 100 ml of distilled water. The clear solution obtained was analyzed for Ca, Mg, and Na using ICPAES (Inductively Coupled Plasma-Atomic Emission Spectrometer).

Determination of protein and amylose contents in the grain: Protein content was measured by following the formulation (protein % = N% × 5.95). Amylose content was measured by chromatography method (Kobayashi *et al.*, 1985). Data were analyzed by the analysis of variance technique (ANOVA), and the mean differences were adjudged by Duncan' new multiple range tests (DNMRT) (Gomez and Gomez, 1984). All computations and analyses were carried out using the SAS software package.

Determination of seed germination ability, seedling and rootlet length: After harvesting, the rice grains, which were enriched with nutrients, were cultivated again under indoor conditions to determine the germination ability, the seedling height, and the rootlet length.

Results and discussions

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N, P, K, Ca, Mg, and Na contents in the grain: The results showed that N content was affected by NPK fertilizers significantly. It was also significantly affected by S powder, micronutrient fertilizers, and the interaction effect of NPK with micronutrient fertilizers. NPK fertilizers and S powder affected the P content significantly and very significantly, respectively. The K content of the rice grain was not significantly affected by any of the applied fertilizers (Table Analysis of variance tables were not imported). It was found that N and P uptake by the rice grain increased significantly using NPK, S, and micronutrient fertilizers compared to the control plot. That is because of lowering soil pH. The maximum N (1.43%) and P (0.19%) contents in the grain were attained when NPK, S, Zn, Cu, and Mn-sulfate fertilizers were applied together (Table 1). As can be seen in Table 2, N and P contents increased only with NPK fertilizers compared to the control and main effects of other treatments. Its maximum value was also attained by the interaction effect between NPK, S, and Zn, Cu, and Mn fertilizers. The average removal of P element in modern irrigated rice in the grain is 0.2% (Dobermann and Fairhurst, 2000). In the present study, the maximum P content in the grain is 0.19%, which was not reached to the average range in the grain. But it increased compared to the control plot (0.163%). It might be precipitated on the soil mineral as Ca-phosphate because of the presence of calcareous soil in this area.

Ca and Mg contents were very significantly affected by S powder, micronutrient fertilizers, the interaction effect of NPK and S, the interaction effect of NPK and micronutrients, the interaction effect of S and micronutrients, and the interaction effect of NPK, S, and micronutrient fertilizers. Micronutrient fertilizers, the interaction effect between NPK and micronutrients, and the interaction effect between S powder and micronutrients affected the Na content very significantly (Table 1). The experimental soil pH is 7.62, which can be categorized as calcareous soil, indicating a sufficient amount of lime ($CaCO_3$) present in the soil (16.7%). Calcareous soils typically have pH > 7.2 (Das, 1996; Biswas and Mukherjee, 2003). Perhaps the alkaline pH (7.62) of the sample analyzed is due to the hydrolysis of lime that releases OH^- ions (Das, 1996). The experimental soil is marly (limey) with sand-sized carbonate particles that have been transported from the Alborz Mountains located in northern Iran. The experimental soil does not belong to excess lime soil but has adequate Ca^{2+} available in the form of $CaCO_3$. Ca levels in calcareous soils vary from less than 1% to more than 25% (Havlin *et al.*, 2003); That in the experimental soil is 6.6% if all the Ca is presumed to be present as $CaCO_3$. Average removal of Ca and Mg in modern irrigated rice in the grain is 0.05 and 0.15%, respectively (Dobermann and Fairhurst, 2000). Ca and Mg availability are excessive at the present experimental soil under pH 7.62. It can be seen in Table 1.a. that Ca (0.65%) and Mg (0.41%) content were more than the average range in the untreated grains (control plot). It was found that Ca, Mg, and Na contents decreased significantly using NPK, S powder, and Zn, Cu, and Mn sulfate fertilizers compared to the control plot. Table 1. shows that the minimum Ca (0.31%), Mg (0.17%), and Na (0.035%) contents in the grain were attained when NPK, S, and Zn, Cu, and Mn sulfate fertilizers were applied together. Zhao *et al.* (2023), with the review Inhibitory effect of exogenous mineral elements (Si, P, Zn, Ca, Mn, Se, Fe, and S) on rice Cd accumulation, reported that on-farm application of mineral elements reduced rice Cd accumulation, root Cd accumulation, and soil Cd bioavailability by 31.97%, 19.89%, and 20.27%, respectively. They stated that Fe fertilizer had the strongest inhibitory effect on Cd concentration in rice grains (43.03%), followed by P (38.45%) (Zhao *et al*., 2023). Table 2 shows that Ca and Na contents decreased by only NPK fertilizers compared to the control and main effects of other treatments. Ca, Mg, and Na content reached the minimum values by interaction effect between NPK, S, and Zn, Cu, and Mn-sulfate fertilizers. Ca and Mg content in the grain were decreased, which might be pointed to the lowering soil pH using S powder and sulfate fertilizers. Application of S powder and sulfate fertilizers also created more availability of the P element for the rice plant, which can also be pointed to the lowering soil pH. Thus, the addition of sulfur powder and sulfate fertilizers to the paddy field increases nutrient uptake like N and P. It also significantly decreases Ca, Mg, and Na uptake by the rice grain using S powder and sulfate fertilizers under the calcareous soil conditions. It was found that increasing P uptake and Ca and Mg contents have been decreased in the rice grain, which can be the result of the lowering soil pH. The relationship between P uptake and Ca and Mg uptake by the rice grain has been shown in Figures 1 and 2. Accordingly, the use of NPK + S + Zn, Cu, Mn sulfates could be defined as the best treatment in the present work, which increased nutrient uptake like N and P and decreased Ca, Mg, and Na uptake by the rice grain by lowering soil pH.

Protein and amylose contents in the grain: Micronutrients are involved in enzyme reactions and the constitution of hormonal growth in healthy plants and are therefore crucial also in rice cultivation (Benton, 1999). Rice is important for people's nourishment, so it seems that using micronutrients can increase rice yield and its quality too (Ziaeeyan and Malakoti, 1997). Starch is the most abundant substance in the rice grain, which includes amylose and amylopectin. One of the factors that affects the cooking quality of rice grain is the amylose content in the grain. If the amylose content is 15-25%, the rice grain will be smooth, soft, and tasty after cooking (Ghasempour and Khodabandeh, 2004). Table 1.a. shows that micronutrient fertilizers (Zn, Cu, and Mn sulfate) affected the amylose content of the rice grain very significantly. It can be seen in Table 1 that amylose content was significantly increased when Zn

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Table 1. Mean comparison of S powder, macro and microelement treatments on the measured characteristics

Treatments	N %	P %	K %	Ca %	Mg %	Na %	Protein %	Amylose %	Seed germination ability/%	Seedling length/cm	Rootlet length/cm
Control	1.25 e-h	0.163 ef	0.3 a	0.65 q	0.41 k	0.059 o	7.43 f-n	17.99 d-k	85.0 i-l	6.13 m-q	3.85 e-q
Zn	1.25 e-h	0.163 ef	0.3 a	0.64 pq	0.26 e-i	0.067 no	7.47 f-l	19.3 abcd	87.0 g-k	6.09 p-r	3.90 d-o
Cu	1.25 e-h	0.166 d-f	0.3 a	0.63 op	0.35 j	0.04 c-g	7.47 f-l	18.2 d-h	87.3 f-j	6.73 j-n	3.30 pq
Mn	1.26 d-h	0.166 d-f	0.3 a	0.62 no	0.27 f-i	0.058 k-m	7.49 e-k	17.6 e-m	86.6 h-l	5.69 qr	3.88 e-p
Zn,Cu,Mn	1.25 e-h	0.166 d-f	0.31 a	0.6 lmn	0.25 d-h	0.068 m	7.47 f-l	18.6 b-e	87.3 f-j	6.58 l-p	4.03 d-l
S	1.26 d-h	0.173 b-e	0.3 a	0.6 lmn	0.24 c-g	0.06 m-o	7.49 e-k	18.7 bcd	88.6 d-h	6.71 k-o	4.23 c-f
S+Zn	1.26 d-h	0.176 b-d	0.31 a	0.61 mno	0.21 a-d	0.048 g-k	7.47 f-l	20.1 a	87.6 f-i	7.19 f-k	4.15 c-i
S+CU	1.25 e-h	0.173 b-e	0.3 a	0.62 no	0.25 d-h	0.042 d-h	7.47 f-l	18.29 c-g	87.3 f-j	7.09 g-l	4.16 c-h
S+MN	1.26 d-h	0.173 b-e	0.3 a	0.52 i	0.23 b-f	0.051 h-m	7.53 d-j	18.16 d-i	87.3 f-j	7.39 d-i	4.09 d-k
S+ZN,Cu,Mn	1.25 e-h	0.176 b-d	0.3 a	0.55 j	0.2 abc	0.058 k-m	7.45 f-m	18.7 bcd	88.0 bcd	6.85 h-m	4.01 d-m
NPK	1.27 d-g	0.173 b-e	0.31 a	0.58 k	0.26 e-i	0.047 f-j	7.55 d-i	17.9 d-l	89.3 b-g	7.4 g-h	4.23 c-f
NPK+Zn	1.31 c-e	0.173 b-e	0.3 a	0.52 i	0.22 b-e	0.048 g-k	7.83 c-f	19.3 abcd	88.0 bcd	7.53 c-g	4.40 bcd
NPK+Cu	1.31 c-e	0.176 b-d	0.3 a	0.44 gh	0.25 hi	0.042 d-h	7.79 d-g	18.1 d-j	88.0 bcd	7.28 e-j	4.13 c-j
NPK+Mn	1.31 c-e	0.173 b-e	0.3 a	0.45 h	0.23 b-f	0.036 b-e	7.83 d-f	17.6 e-m	89.3 b-g	7.74 cde	4.15 c-i
NPK+Zn,Cu,Mn	1.34 c	0.176 b-d	0.31 a	0.42 fg	0.19 ab	0.056 i-m	7.97 b-d	18.5 b-f	89.6 a-f	7.56 c-f	4.21 c-g
NPK+S	1.29 c-f	0.18 a-c	0.3 a	0.41 ef	0.24 c-g	0.057 j-m	7.69 d-h	18.1 d-j	91.3 ab	7.83 cd	4.50 bc
NPK+S+Zn	1.33 cd	0.183 ab	0.3 a	0.33 b	0.19 ab	0.035 a	7.95 c-e	19.6 abc	90.6 a-d	7.4 d-h	4.00 d-n
NPK+S+Cu	1.35 b	0.176 b-d	0.3 a	0.38 d	0.22 b-e	0.045 e-h	8.03 abc	18.3 i-l	90.3 a-e	7.86 bc	4.24 cde
NPK+S+MN	1.35 b	0.176 b-d	0.3 a	0.35 c	0.2 abc	0.04 c-g	8.4 ab	18.6 b-e	91.0 abc	8.16 ab	5.16 ab
NPK+S+Zn,Cu,Mn	1.43 a	0.190 a	0.31 a	0.31 a	0.17 a	0.035 a	8.54 a	19.7 ab	91.6 a	8.39 a	5.30 a

A given means per each column with the same letters, have not significant difference, statistically (P<0.05).

Table 2. Mean comparison of main and interaction effects of S powder macro and microelements on the measured characteristics at the present study

Treatments	N %	P %	K %	Ca %	Mg %	Na %	Protein %	Amylose %	Seed germination ability/%	Seedling length/cm	Rootlet length/cm
Control	1.25	0.163	0	0.65	4.1	0.059	7.43	17.99	85	6.13	3.85
Zn,Cu,Mn	1.25	0.166	0.31	0.6	2.5	0.068	7.47	18.6	87.3	6.58	4.03
S	1.26	0.173	0.3	0.6	2.4	0.06	7.49	18.7	88.6	6.71	4.23
NPK	1.27	0.173	0.31	0.58	2.6	0.047	7.55	17.9	89.3	7.4	4.23
S+Zn,Cu,Mn	1.25	0.176	0.3	0.55	2.0	0.058	7.45	18.7	88.0	6.85	4.01
NPK+Zn,Cu,Mn	1.34	0.176	0.31	0.42	1.9	0.056	7.97	18.5	89.6	7.56	4.21
NPK+S	1.26	0.173	0.3	0.6	2.4	0.06	7.49	18.7	88.6	6.71	4.23
NPK+S+Zn,Cu,Mn	1.43	0.190	0.31	0.31	1.7	0.035	8.54	19.7	91.6	8.39	5.30

A given means per each column with the same letters, have not significant difference, statistically (p<0.05).

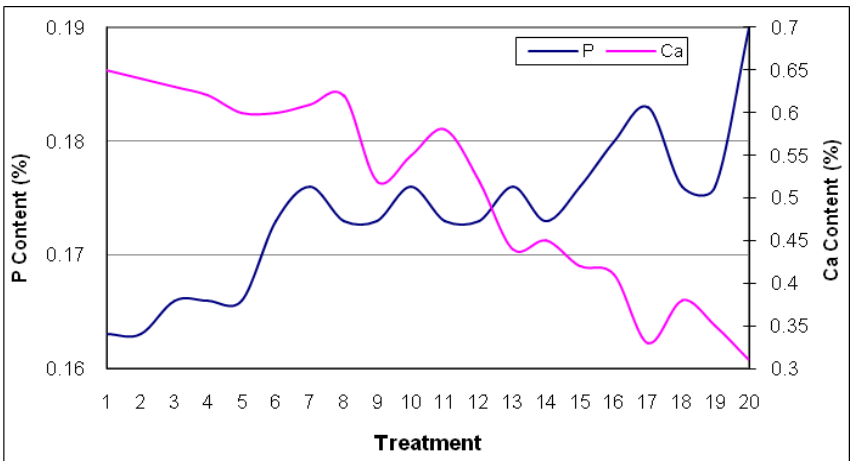


Figure 1. Relationship between P and Ca content in the rice grain under chemical fertilizers treatments

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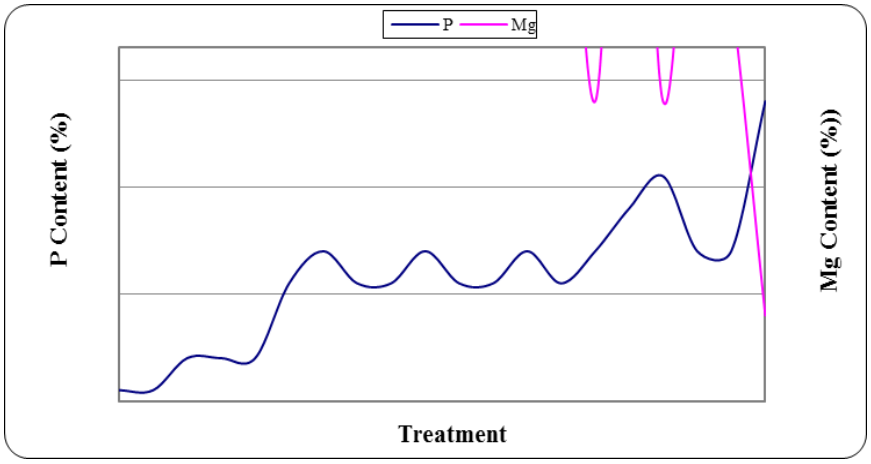


Figure 2. Relationship between P and Mg content in the rice grain under chemical fertilizers treatments

(19.3%), S+Zn (20.1%), NPK+Zn (19.3%), NPK+S+Zn (19.6%), and NPK+S+Zn,Cu,Mn (19.7%) fertilizers were applied compared to the control plot (17.99%). It seems that the Zn element is involved in the amylose constitution in the plant body. The obtained amylose content by these treatments is at the same level, statistically, but the maximum amylose content was attained when S+Zn and NPK+S+Zn, Cu, and Mn sulfate fertilizers were applied. Analysis of variance shows that NPK fertilizers, S powder, and Zn, Cu, and Mn sulfate fertilizers significantly affected the protein content . The suitable protein content in the rice grain is 6-8% (FAO, 1989). Table 1 shows that the protein content in the grain was in the suitable value range of 7.43-8.54%. But the maximum protein content was attained when NPK+S+Zn, Cu, and Mn sulfate fertilizers were applied together. This treatment can be defined as critical level for amylose and also protein content in the grain. It was also observed that protein content was increased when only NPK fertilizers were applied compared to the control plot and the main effects of the other treatments. The protein content was also increased when NPK+S+Zn, Cu, Mn-sulfate fertilizers were applied together compared to the interaction effects of the other treatments (Table 2). Zhao *et al.* (2023) had comparable results.

Seed germination ability, seedling and rootlet length: The results of this study showed that seed germination ability, seedling, and rootlet length were significantly affected by S powder and NPK fertilizers. As can be seen in Table 1, the seed germination ability and seedling and rootlet length increased when all treatments were applied compared to the control plot, except for Zn and Cu fertilizers that decreased seedling and rootlet length, respectively. The maximum seed germination ability (91.6%), seedling length (8.39 cm), and rootlet length (5.30 cm) were obtained when NPK+S+Zn, Cu, and Mn sulfate fertilizers were applied as compared with the control treatment. It was also observed that the seed germination ability, seedling, and rootlet length were increased when only NPK fertilizers were applied compared to the control plot and the main effects of the other treatments. These morphological characteristics of the rice grain were also increased when NPK+S+Zn,Cu,Mn-sulfate fertilizers were applied together compared to interaction effects of the other treatments (Table 2). In fertile soil, the rice plant mainly increases root diameter and reduces root length (Wang *et al.*, 2018). Andrew Bebeley *et al.* (2021) and Mobasser *et al.* (2024) reported that plant growth increased by improving the fertility of fields. It has also been concluded that N, P, protein, and amylose content reached the average amount of modern irrigated rice grain (Dobermann and Fairhurst, 2000) using this treatment. In total, overall, with effective management, rice may use growth factors (nutrients, and light ...) more effectively, leading to enhanced performance (Mobasser *et al*., 2024).

Conclusion

The N, P, amylose, and protein contents in the rice grain were significantly increased using NPK+S+Zn, Cu, and Mn-sulfate fertilizers together compared to the control plot and other treatments. Ca, Mg, and Na contents were also decreased using this treatment under calcareous soils. It has been concluded that morphological characteristics of the rice grain were also increased when NPK+S+Zn,Cu,Mn-sulfate fertilizers were applied together compared to interaction effects of the other treatments. Seed germination ability, seedling, and rootlet length have also been increased by this treatment. Thus, the use of NPK+S+Zn, Cu, and Mn-sulfate fertilizers can be recommended as the best treatment in the present work, which increased nutrient uptake like N, P, protein, and amylose and decreased Ca, Mg, and Na uptake by the rice grain by lowering soil pH. In total, with effective management, rice may use growth factors (nutrients, and light …) more effectively, leading to enhanced performance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

Majid Qasempour Alamdari: Data curation methodology, software, formal analysis, investigation, writing—original draft .Nilimma Rajurkar: Conceptualization, writing—review and editing, validation, writing—original draft, supervision, project administration, and funding acquisition .Ashok Patwardhan: Conceptualization ,Validation. Supervision, project administration, funding acquisition.

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