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Effect of iron management on chlorophyll content and amylose content of rice

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Abstract
The field study was carried out at the College farm in Navsari Agriculture University, Navsari during *kharif* season of the year 2020 and 2021 to study the effect of iron management on chlorophyll content and amylose content of rice under South Gujarat condition. Total twelve treatments of different iron fertilizers along with *Arbuscular mycorrhiza* (AM) and *Pseudomonas fluorescens* biofertilizers replicated three times in Randomized Block Design. Among the different treatments, the application of $FeSO_4$ @ 50 kg ha^{-1} + AM + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1} gave significantly higher content of chlorophyll content and amylose content of rice during both the individual years and pooled analysis.

Keywords: Iron management, arbuscular mycorrhiza, pseudomonas fluorescens and chlorophyll content, amylose content

Introduction

Micronutrients are essential for the proper growth and development of the human body, and their deficiency hurts health, contributing to low productivity and a vicious cycle of malnutrition, underdevelopment, and poverty. Micronutrient deficiency is a public health issue that affects more than one-quarter of the world's population. (Gonmei and Toteja, 2018) [5]. Micronutrients play a significant role in plant growth and metabolic processes associated with photosynthesis, chlorophyll formation, cell wall development, respiration, water absorption, xylem permeability, resistance to plant diseases, enzyme activities involved in the synthesis of primary and secondary metabolites with nitrogen fixation and reduction (Adhikary *et al*. 2010 and Vitti *et al*.2014) [1, 7]. Some common farming practices, such as liming acid soils, contribute to the widespread occurrence of micronutrient deficiencies in crops by reducing the availability of micronutrient in the soil. Major issues in alleviating micronutrient deficiencies include difficulty identifying field crop symptoms, variation in soil micronutrient status, soil pH, and intensity, and seasonal fluctuations in the region's levels and temperature regimes, as well as insufficient facilities and field tests to validate soil and plant micronutrients (Das, 2014) [4].

Rice is a supreme commodity to mankind because rice is truly life, culture, tradition, and a means of livelihood for millions. Rice is one of the most important staple foods and a significant source of daily caloric intake for more than half of the world's population. It has been estimated that rice yields have to be increased by an additional 100 million tons to feed 9.1 billion people by 2050 (Jaggard *et al*., 2010) [6]. Rice farming is the largest single use of land for producing food and nearly all (90%) of rice is produced in Asia. Rice feeds almost 50–58% of the world's population, hence it can be considered a global grain (Zeng *et al.*, 2010) [8], but it is considered deficient in micronutrients especially iron (Bouis and Welch, 2010) [3]. Therefore, even a small increase in the nutritive value of rice can be highly significant for human nutrition (Zhang *et al*., 2012) [9].

The iron content of rice is primarily affected by iron absorption from the soil, as well as iron transport and accumulation in rice. Although it is only required in trace amounts by plants, Iron (Fe) is involved in a wide range of important compounds and physiological processes. Iron is used in the production of chlorophyll and is required for certain enzyme functions. The role of Fe in chlorophyll synthesis is responsible for the chlorosis (yellowing) associated with Fe deficiency. Iron is found in iron-containing (heme) proteins found in plants, such as the cytochromes. Cytochromes are found in chloroplasts and mitochondrial electron transfer systems. Iron is also linked to non-heme proteins such as ferredoxin. Iron is the third among the most limiting nutrients for plant growth due to the low solubility of the oxidized ferric form in aerobic environments (Zuo and Zhang, 2011) [10].

The combination of types of microorganisms used includes *Arbuscular mycorrhiza* and *Pseudomonas fluorescens* are P solvents, decomposers of organic matter, and producing plant growth regulators. Iron (Fe) is useful for spurring plant growth, especially in the vegetative phase, and plays a role in the formation of chlorophyll. Low chlorophyll content under control condition was reported as a result of lower chlorophyll synthesis, low rate of the PSII reaction centre, reduce the activity of carbonic anhydrase and nitrate reductase, an imbalance in the ion flux inside plants, affect membrane stability index. Oxidative stress could happen due to low iron (Fe) content may leading to deterioration in chloroplast structure and consequently decrease in chlorophyll content.
The most important criterion of milled rice grain quality is amylose content, which is an indicator of the amylose/amylopectin ratio. It is commonly expressed as a percentage of milled rice dry weight rather than as a percentage of starch basis. The amylose content of rice has a significant impact on its cooking and eating characteristics. Low amylose content promotes stickiness and softness in rice grains, whereas high amylose content promotes brokenness and hardness. The consistent supply of nitrogen throughout the rice growing season promoted protein storage and decreased amylose content in rice grain.

Materials and Methods

Location and chemical Properties of soil: A field experiment was conducted at the Navsari Agricultural University farm, Navsari (Gujarat) during *kharif* season 2020 and 2021. Geographically, Navsari Agricultural University campus is situated at 20º 57' North Latitude, 72º 54 East Longitudes and has an altitude of 10 meters above the mean sea level. The soil of the experimental field was fairly levelled and uniform. The soils of South Gujarat are locally known as "Deep black cotton soil", The soil of the experimental field was clayey in texture, low in organic carbon (0.36%, 0.42%) and available nitrogen (204.4, 214.1 kg ha^{-1}), medium in available phosphorus (36.2, 34.7 kg ha^{-1}) and high in available potassium (300.3, 312.4 kg ha^{-1}). The soil was slightly alkaline in reaction (pH- 8.01, 7.98).

Climate and weather condition: Weather prevailed during the course of investigation was quite congenial for the satisfactory growth and development of *kharif* rice crop. Moreover, there was no serious incidence of disease and pests. The maximum temperature ranged between 28.1 to 38.1 ºC and 26.4 to 39.5 ºC, while minimum temperature ranged between 10.7 to 27.9 ºC and 11.1 to 25.8 ºC during 2020-21 and 2021-22, respectively. In *kharif* season, total rainfall of 2310 mm and 1565 mm during 2020-21 and 2021-22 was recorded, respectively. The crop has got optimum duration of sunlight and humidity during the growth period.

Treatment: The treatment consisted of iron management *viz*., M_0 - Control (RDF), M_1 - Fe-EDTA @ 1.5 kg ha^{-1}, M_2 - $FeSO_4$ @ 50 kg ha^{-1}, M_3 - *Arbuscular mycorrhiza* (AM) Seedling treatment + Field application: 2L ha^{-1}, M_4 - *Pseudomonas fluorescens* (P) Seedling treatment + Field application: 2L ha^{-1}, M_5 - AM Seedling treatment + Field application: 2L ha^{-1} + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1}, M_6 - $FeSO_4$ @ 25 kg ha^{-1} + AM Seedling treatment + Field application: 2L ha^{-1}, M_7 - $FeSO_4$ @ 25 kg ha^{-1} + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1}, M_8 - $FeSO_4$ @ 25 kg ha^{-1} + AM + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1}, M_9 - $FeSO_4$ @ 50 kg ha^{-1} + AM Seedling treatment + Field application: 2L ha^{-1}, M_{10} - $FeSO_4$ @ 50 kg ha^{-1} + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1}, M_{11} - $FeSO_4$ @ 50 kg ha^{-1} + AM + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1} to rice in *kharif* season replicated three times in randomized block design. During *rabi* season, only RDF (recommended dose of fertilizers) of sweet corn (100:60:60 NPK kg ha^{-1}) was applied.

Note

1. **Seedling treatment:** Slurry was prepared by mixing AM (250 g ha^{-1}: 3000 IP/g of biofertilizer, 1000 spores $gram^{-1}$) and P. fluorescens (cfu: 108 per ml) at 1L ha^{-1} in 40 L of water and rice seedlings were dipped in the suspension for 15-30 minutes before transplanting.
2. **Field application:** Application of AM (250 g ha^{-1}) and 1L ha^{-1} of *P. fluorescens* by mixing it with FYM.

Chlorophyll content analysis

The chlorophyll content of previously selected 10 plants was measured at harvest by SPAD chlorophyll meter. The average chlorophyll content was worked out and recorded for each plot. The SPAD reading were converted to chlorophyll content in Nano mol mg^{-1} FW of plant.

Amylose content analysis

Amylose content from the rice grain was analyzed in the laboratory by following the simplified colorimetric procedure of Juliano (1978) [11] which was modified by Directorate of Rice Research, Hyderabad.

Result and Discussion

The data on chlorophyll content and amylose content in *kharif* rice as influenced by different iron management practices are presented in Table 1 and Fig 1.

1. Chlorophyll content in *kharif* rice

Chlorophyll content in *kharif* rice was significantly higher (1.706, 1.703 and 1.704 Nano mol mg^{-1} FW) with treatment M_{11} ($FeSO_4$ @ 50 kg ha^{-1} + AM + *P. fluorescens* (P) as seedling treatment (S) and field application (F): 2 L ha^{-1}) was remain at par during both individual years and pooled analysis with treatments (M_1) Fe-EDTA @ 1.5 kg ha^{-1}, (M_2) $FeSO_4$ @ 50 kg ha^{-1}, (M_8) FeSO4 @ 25 kg ha^{-1} + AM + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1}, (M_9) $FeSO_4$ @ 50 kg ha^{-1} + AM Seedling treatment + Field application: 2L ha^{-1} and (M_{10}) FeSO4 @ 50 kg ha^{-1} + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1}. The lowest chlorophyll content (1.309, 1.307 and 1.308 Nano mol mg^{-1} FW) in *kharif* rice was found with control (M_0: RDF) during individual years and in pooled.
Iron is an important element as it is required for the formation of chlorophyll in plants. It also plays a role in maintaining the structural integrity of chloroplast, the structure within which chlorophyll is present. Leaf greenness is the measure of chlorophyll content and is used as an important indicator of growth as it determines the photosynthetic activity of the plant. *Arbuscular mycorrhiza* fungi and *P. fluorescens* improve the synthesis of chlorophyll leading to a significant improvement in the concentrations of various metabolites in plants. Mycorrhizal symbiosis induces enhanced

accumulation of anthocyanins and chlorophyll and markedly improved photosynthetic rate, and other gas exchange traits (Birhane *et al*., 2012) [2]. Application of biofertilizers may enhance chlorophyll accumulation by the activity of microflora existing in the sub-surface region of soils.

2. Amylose content in *kharif* rice

Data pertaining to amylose content in rice grain as influenced by various treatments is provided in Table 1 and Fig. 1. As it is evident from data in Table 1, different treatments of iron management imposed their significant effect on amylose content in rice grain during both the years and pooled analysis. Though, application of $FeSO_4$ @ 50 kg ha^{-1} + AM + *P. fluorescens* (P) as seedling treatment (S) and field application (F): 2 L ha^{-1} (M_{11}) registered significantly highest values of amylose content (24.08, 24.51 and 24.29%) in rice during both the years and pooled data, it was found statistically at par with treatments (M_2) $FeSO_4$ @ 50 kg ha^{-1}, (M_8) FeSO4 @ 25 kg ha^{-1} + AM + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1}, (M_9) $FeSO_4$ @ 50 kg ha^{-1} + AM Seedling treatment + Field application: 2L ha^{-1} and (M_{10}) $FeSO_4$ @ 50 kg ha^{-1} + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1} during first year, second year and pooled analysis, respectively.

Table 1: Chlorophyll content and amylose content of *kharif* rice as affected by different iron management treatments

Treatment	Chlorophyll content (Nano mol mg^{-1} FW)			Amylose content (%)		
	2020	2021	Pooled	2020	2021	Pooled
M_0	1.309	1.307	1.308	19.89	19.93	19.91
M_1	1.657	1.653	1.655	21.78	22.25	22.01
M_2	1.660	1.660	1.660	23.05	22.86	22.96
M_3	1.458	1.463	1.461	21.18	21.25	21.21
M_4	1.532	1.500	1.516	21.74	21.84	21.79
M_5	1.552	1.540	1.546	21.97	21.79	21.88
M_6	1.563	1.550	1.557	23.19	22.57	22.88
M_7	1.531	1.517	1.524	22.93	22.89	22.91
M_8	1.694	1.687	1.690	24.01	24.15	24.08
M_9	1.643	1.650	1.647	22.90	23.05	22.97
M_{10}	1.648	1.650	1.649	23.76	23.95	23.85
M_{11}	1.706	1.703	1.704	24.08	24.51	24.29
S.Em ±	0.025	0.025	0.018	0.660	0.751	0.500
CD (P=0.05)	0.07	0.07	0.05	1.94	2.20	1.43
Y x T						
S.Em ±			0.025			0.707
CD (P=0.05)			NS			NS
CV (%)	2.78	2.71	2.75	5.07	5.76	5.43

The lowest amylose content (19.89, 19.93 and 19.91%) was found under the treatment M_0 (RDF) during 2020, 2021 and pooled analysis. Improvement in amylose content of kharif rice resulted from improved concentration of nutrients in the plant tissues by combined application of iron fertilizer along with biofertilizers.

Conclusion

On the basis of two years experimental results, it can be concluded that for getting higher chlorophyll content and amylose content, *kharif rice* crop should be nourished with FeSO4 @ 50 kg ha^{-1} + AM Seedling treatment + Field application: 2L ha^{-1} + *P. fluorescens* Seedling treatment + Field application: 2L ha^{-1}.

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