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Effect of zinc and iron biofortification on growth and quality parameters of greengram (*Vigna radiata* L. wilczek) under medium black calcareous soil

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Abstract

The research was performed at the Instructional Farm, Department of Agronomy, Junagadh Agricultural University, in the summer season year of 2024 using a randomized block design (RBD) consisting eight treatments with three replications. The soil of experimental area was low in nitrogen, medium in phosphorus and high in potassium content having pH 7.9 and EC 0.33 dSm⁻¹. The treatments involved recommended dose of fertilizer (RDF) along with foliar sprays of ZnSO₄ and FeSO₄ either singly or in combination at critical growth stages (flowering and pod formation). Among various treatments application of RDF 20:40 NP kg/ha along with 0.5% ZnSO₄ and 0.5% FeSO₄ each at flowering and pod stages recorded significantly higher values of growth parameters viz., plant height, number of branches, dry matter accumulation and quality parameters viz., protein content and protein yield. The dual foliar application of zinc and iron significantly boosts growth and quality performance and productivity of green gram.

Keywords: Biofortification, Greengram, Growth parameters, Iron, Quality, Zinc

Introduction

Green gram contributes significantly to nutritional security due to its rich protein content, essential nutrients and dietary fiber. It serves as a valuable plant-based protein source, providing nutrients essential for health and well-being. Green gram is used as green manure crop which helps to prevent soil erosion. By symbiotic nitrogen fixation, green gram also improves soil fertility. The production of green gram relies on several factors including soil, nutrition, soil moisture, plant protection measures, etc. It is essential to pay great attention to the nutrition of green gram for enhancing its productivity. In India during 2023-24, about 15.93 lakh ha area was covered under green gram as against 15.57 lakh ha during the same period in 2023-24. According to Anonymous, (2024)^[1], in duration of summer season it is grown in 47.45 thousand hectares area producing 54.69 thousand tonnes, yielding 1152.53 kg/ha. Mungbean have composition of protein (24.5%) which contains of tryptophan (60 mg/g) and lysine (460 mg/g) amino acid, and also contains vitamin riboflavin (0.21mg/100g) having mineral sources (Gopalan *et al.*, 1995)^[2].

In recent era, the term biofortification indicates an enhanced in the nutrient content of crops with the help of traditional breeding, genetic engineering for crop improvement with several type of agronomical parameters and management practices. Biofortification play fundamental roles towards leguminous crop specially pulse grain in essential nutrients during the crop growth and development stage. It is a new approach for public health to providing grain, pulses and vitamin A with removing iron and zinc deficiencies more specifically in poor countries. In human being also iron and zinc play an important role as these are indispensable micronutrients crucial for sustaining human health. Iron, integral to hemoglobin and myoglobin, facilitates oxygen transport in the blood, supporting cellular respiration and energy production. Naik and Kumar, (2020)^[3] suggested that zinc is a structural and having catalytic functions of enzymes serve as protein sites. During pregnancy, iron becomes even more vital for fetal growth and development. Meanwhile, zinc serves as a pivotal element in immune response and enzyme

activity, essential for growth, cellular differentiation, and sensory functions like taste and smell. Additionally, zinc contributes to antioxidant defense, mitigating the risk of chronic disease. It is a sustainable agricultural practice and it can reach to the undernourished populations in remote areas (Jangir *et al.*, 2017) ^[4]. Agronomic biofortification specifically refers to enhancing the nutrient content of crops through agronomic practices such as soil management, fertilization, irrigation, and plant nutrient uptake optimization.

Unlike conventional breeding or genetic engineering, agronomic biofortification focuses on improving nutrient uptake and availability in plants through agricultural practices. Liu *et al.* (2017) ^[5] using the DALYs (daily-adjusted life years) equation to assess the health issue in relation to zinc deficiency and the force of agronomic biofortification with zinc in children. Considering the above facts, investigation was conducted to know the role of application of zinc and iron through foliar spray on physiological parameters of plant.

Materials and Methods

A field experiment was performed at the Instructional Farm, Department of Agronomy, Junagadh Agricultural University, during summer 2024. The experimental soil has medium black calcareous with pH 7.9 and EC 0.33 dSm⁻¹, low nitrogen (274 kg/ha), medium phosphorus (29 kg/ha), and high potassium content (257 kg/ha). The experiment was laid out in RBD with eight treatments: T₁ - Absolute control, T₂ - RDF (20:40 NP kg/ha) + water spray, T₃ - RDF + 0.5% ZnSO₄ at flowering, T₄ - RDF + 0.5% ZnSO₄ at flowering + pod formation, T₅ - RDF + 0.5% FeSO₄ at flowering, T₆ - RDF + 0.5% FeSO₄ at flowering + pod formation, T₇ - RDF + 0.5% ZnSO₄ + 0.5% FeSO₄ at flowering, T₈ - RDF + 0.5% ZnSO₄ + 0.5% FeSO₄ at flowering + pod formation which replicated thrice. The crop was sown on 21st February during summer season 2024. The seed rate of 20 kg/ha was sown with row spacing of 45 cm and plant to plant spacing of 10 cm maintained by thinning during crop season. The basal application of fertilizer N 20 kg/ha and P₂O₅ 40 kg/ha as per treatments were applied with urea and diammonium phosphate. The foliar application of 0.5% ZnSO₄ and 0.5% FeSO₄ each were applied at flowering and pod formation stages. The application of ZnSO₄ and FeSO₄ were combined with 0.25% lime and 0.1% citric acid, respectively. The observations on five randomly 5 tagged plants from each treatments for plant height were recorded at 30, 60 DAS and harvest. The dry matter was taken from observation line. According to A.O.A.C., 1995^[6], the protein content in seed was calculated by multiplying per cent

nitrogen in seed with a constant factor 6.25 (A.O.A.C., 1995) ^[6]. Protein yield was calculated by using the following formula.

$$\text{Protein yield (kg/ha)} = \frac{\text{Seed protein content (\%)} \times \text{Seed yield (kg/ha)}}{100}$$

Experimental data collected and it was subjected to statistical analysis by using Fishers method of Analysis of Variance (ANOVA) which is also explained by Gomez and Gomez (2010) ^[7]. Critical Difference (CD) value was calculated whenever the 'F' test was found significant at 5 % level.

Results and Discussion

Growth parameters

Plant height at 30 days

With the progression of crop season, plant height increased up to physiological maturity (Table 1). Plant height at 30 DAS showed non-significant results while numerically higher value of plant height (15.56 cm) was observed with application of RDF 20:40 kg NP/ha + ZnSO₄ (0.5%) + FeSO₄ (0.5%) spray at flowering and pod formation stage (T₈). The treatment T₁ (Absolute control) produced lowest plant height (14.62 cm).

Plant height at 60 days

Further glance of data presented in Table 1 revealed that plant height at 60 DAS significantly affected by application of zinc and iron. Data revealed that highest height at 60 DAS (39.07cm) was found in treatment RDF + 0.5 percent of ZnSO₄ + 0.5 percent of FeSO₄ foliar spray at flowering and pod formation stage (T₈) which was statistically at par with RDF+ 0.5% ZnSO₄ at flowering and pod formation stage (T₄), RDF + 0.5 percent of FeSO₄ foliar spray at flowering and pod formation stage (T₆), RDF + 0.5 percent of ZnSO₄+ 0.5 percent of FeSO₄ at flowering and pod formation stage (T₇) respectively.

Plant height at harvest

At harvest significantly higher plant height (45.29 cm) was observed with the foliar application of 0.5% ZnSO₄ + 0.5% FeSO₄ and recommended dose of fertilizer 20:40 NP/ha applied during flowering and pod formation stage (T₈) which remained statistically at par with RDF + 0.5% ZnSO₄ at flowering and pod formation stage (T₄), RDF + 0.5 percent FeSO₄ foliar spray at flowering and pod formation stage (T₆), RDF + 0.5 percent ZnSO₄ + 0.5 percent FeSO₄ at flowering and pod formation stage (T₇) respectively.

Table 1: Effect of different treatments on plant height and number of branches per plant at harvest of summer green gram

	Treatments	Plant height (cm)			Branches per plant at harvest
		30 DAS	60 DAS	At harvest	
T ₁	Absolute control	14.62	30.80	36.59	3.77
T ₂	RDF + water spray	15.14	32.50	38.54	4.13
T ₃	RDF + 0.5 percent ZnSO ₄ spray at flowering stage	15.21	34.30	39.93	4.34
T ₄	RDF + 0.5 percent ZnSO ₄ spray at flowering + pod formation stage	15.24	35.70	41.55	4.60
T ₅	RDF + 0.5 percent FeSO ₄ spray at flowering stage	15.36	33.63	40.81	4.47
T ₆	RDF + 0.5 percent FeSO ₄ spray at flowering + pod formation stage	15.37	35.27	42.28	4.73
T ₇	RDF + 0.5 percent ZnSO ₄ + 0.5 percent FeSO ₄ spray at flowering stage	15.45	38.47	43.09	5.02
T ₈	RDF + 0.5 percent ZnSO ₄ + 0.5 percent FeSO ₄ spray at flowering + pod formation stage	15.56	39.07	45.29	5.21
	S.E.m.±	0.76	1.65	1.58	0.25
	CD (P=0.05)	NS	5.02	4.81	0.76
	CV (%)	8.67	8.21	6.07	9.62

At 60 Days after sowing and at harvest stage plant height recorded significantly higher with the use of foliar applications of Zn and Fe along with 20:40 kg NP/ha. These outcomes might be due to the consequence of Zn and Fe foliar spray being directly absorbed by crop shoots and leaves, which increases plant metabolism. An efficiency of zinc to bio-synthesize plant growth promoting regulators such as auxins which actively participated in the plant cells enlargement and elongation, and boosts the meristematic activity of crops and ultimately the plant height. Increased iron availability to plants might have promoted their enzymatic and metabolic processes, boosting their development. These results are supported with Pal *et al.* (2020)^[8], Ramzan *et al.* (2020)^[9], Kavva *et al.* (2021)^[10] and Boradkar *et al.* (2023)^[11].

Number of branches per plant at harvest

Our research data on number of branches per plant were observed at harvest presented in Table 1. The number of branches observed at harvest showed significant difference with the application of zinc and iron at different stages. The application of RDF 20:40 NP kg/ha with 0.5 percent ZnSO₄ and 0.5 percent FeSO₄ foliar spray at flowering and pod formation stage (T₈) produced significantly higher number of branches (5.21/plant) over control. Although, this treatment remained at par with all other treatments except T₂ and T₃. The percent increase in number of branches per plant was 38.19% with the application of RDF + 0.5 percent ZnSO₄ and 0.5 percent FeSO₄ each at flowering and pod formation stages over control.

The increase in number of branches could be seen because iron participates in protein, chlorophyll, and chloroplast development, as well as the production of starch, which explains that iron treatment results in a higher number of branches.

According to Balai *et al.* (2017)^[12], the increase in branches were observed because of the increased availability of zinc, which boosts metabolic and enzymatic activity. In comparison to foliar sprays of zinc and iron alone, both combined tended to produce more number of branches. This might be because of the application of additional nutrients, which aided in improved photosynthesis and photosynthates partitioning to produce attributing characteristics that led to a larger sink size. The results are in support with the findings of Ram *et al.* (2013)^[13] and Balai *et al.* (2017)^[12].

Dry matter accumulation per plant

The data of dry matter recorded at 30, 60 DAS and at harvest are shown in Table 2. The dry matter accumulation at 30 DAS showed non-significant results with the application of RDF and foliar spray of Zn and Fe at different stages. Dry matter production data recorded at 60 DAS and at harvest was significantly influenced with the application of zinc and iron at different stages. Our result revealed that the significantly higher dry matter recorded per plant at 60 DAS (30.81 g/plant) was recorded with application of RDF 20:40 kg NP/ha with 0.5 percent ZnSO₄ and 0.5 percent FeSO₄ foliar spray at flowering and pod formation stage (T₈).

Further examination of data pointed out that dry matter production significantly influenced by different treatments at harvest. The data showed that higher dry matter mass (33.76 g/plant) with RDF + 0.5 percent ZnSO₄ + 0.5 percent FeSO₄ foliar application at flowering and pod stage (T₈) which was statistically at par with RDF+ 0.5 percent ZnSO₄ at flowering and pod stage (T₄), RDF+ 0.5 percent FeSO₄ application at flowering stage (T₅), RDF + 0.5 percent FeSO₄ foliar application at flowering and pod stage (T₆) and RDF + 0.5 percent ZnSO₄ + 0.5 percent FeSO₄ at flowering and pod formation stage (T₇) (32.399 g/plant), respectively.

Dry matter accumulation is a crucial growth indicator that measures the proportional impact of various treatments on plant development and, eventually, crop output. Higher dry matter mass accumulated at 60 DAS and at harvest stage was recorded with recommended fertilizers dose 20:40 kg NP/ha and foliar application of zinc sulphate and iron sulphate at flowering and pod formation stages. This increase might be due to beneficial effect of iron. As iron plays an important role in cell elongation, which enhanced plant height and the accumulation of shoot dry matter.

It also has a structural function in chlorophyll and energy transport within the plant leading to more photosynthesis and ultimately increased dry matter production. According to Zeidan *et al.* (2010)^[14], zinc is essential for photosynthesis, meristematic tissue formation, cell division, and tissue growth. The increase in photosynthesis leads to attain higher biomass of plant. These results are in accordance with Kumari *et al.* (2019)^[15], Gahlot *et al.* (2020)^[16], Pal *et al.* (2020)^[8], and Boradkar *et al.* (2023)^[11].

Table 2: Effect of different treatments on dry matter accumulation

	Treatments	Dry matter (g/plant)		
		30 DAS	60 DAS	At harvest
T ₁	Absolute control	3.22	21.94	23.99
T ₂	RDF + water spray	3.84	25.92	27.70
T ₃	RDF + 0.5 percent ZnSO ₄ spray at flowering stage	3.87	26.77	28.86
T ₄	RDF + 0.5 percent ZnSO ₄ spray at flowering + pod stage	3.94	27.54	29.84
T ₅	RDF + 0.5 percent FeSO ₄ spray at flowering stage	4.18	28.32	30.60
T ₆	RDF + 0.5 percent FeSO ₄ spray at flowering + pod formation stage	4.25	29.32	31.90
T ₇	RDF + 0.5 percent ZnSO ₄ + 0.5 percent FeSO ₄ spray at flowering stage	4.32	29.62	32.39
T ₈	RDF + 0.5 percent ZnSO ₄ + 0.5 percent FeSO ₄ spray at flowering + pod formation stage	4.40	30.81	33.76
	S.E.m.±	0.31	1.27	1.41
	CD (P=0.05)	NS	3.87	4.29
	CV (%)	13.65	8.03	8.22

Quality parameters

Protein content parameters

A glance of data presented in Table 3, indicated that different zinc and iron levels exhibited their significant influence on protein content. It was observed from the data result that significantly higher protein content (23.56%) with treatment of

RDF 20:40 NP kg/ha applied as basal dose along with 0.5 percent ZnSO₄ + 0.5 percent FeSO₄ foliar application at flowering and pod formation stages, but it remained statistically at par with treatment (T₆) RDF + 0.5% FeSO₄ spray at flowering + pod formation stage (21.64%) and (T₇) RDF + 0.5 percent ZnSO₄ + 0.5 percent FeSO₄ application at flowering stage

(23.12%), respectively. Whereas significantly lower protein content (19.85%) was recorded under control condition.

The increase in protein content might be due to zinc is a constituent of several dehydrogenase, proteinase and peptidase enzymes, this might cause an increase in the synthesis of carbohydrates and fats, better uptake of nitrogen, increased expression of Zn transporter proteins, maximum nutrient translocation from source to sink and enhanced protease enzyme which results in higher protein content in seed.

Zinc increased cation exchange capacity (CEC) of roots, which in turn favorably enhanced absorption of essential elements, particularly nitrogen which is important for enhance protein content of seed. Zinc treatment resulted in an increase in protein content. These results are in similar to the findings of Roy *et al.* (2013) in green gram and Raghuwanshi *et al.* (2017) [17] in soybean. Iron plays an essential role in increasing protein yield

by facilitating vital physiological and biochemical processes in plants. Improved iron availability enhances nitrate reductase activity, leading to better nitrogen assimilation and, consequently, higher protein synthesis in seeds. Our findings are supported by Chand *et al.* (2017) [18], Kolekar *et al.* (2018) [19] and Choudhary *et al.* (2014) [20].

Protein Yield: An evident of data (Table 3) concluded that zinc and iron fertilization has significantly increased the protein yield of green gram having significantly higher protein yield has been found by application of treatment T₈ having RDF applied as basal dose along with 0.5 percent ZnSO₄ + 0.5 percent FeSO₄ foliar application at flowering and pod formation stage (321.72 kg/ha) over rest of treatments but remained at par with treatment T₇ (RDF + 0.5 percent ZnSO₄ + 0.5 percent FeSO₄ application at flowering stage).

Table 3: Effect of various treatments on protein content and yield of summer green gram

	Treatments	Protein content (%)	Protein yield (kg/ha)
T ₁	Absolute control	19.85	177.65
T ₂	RDF + water spray	20.44	222.64
T ₃	RDF + 0.5 percent ZnSO ₄ spray at flowering stage	20.44	225.83
T ₄	RDF + 0.5 percent ZnSO ₄ spray at flowering + pod formation stage	21.41	248.81
T ₅	RDF + 0.5 percent FeSO ₄ spray at flowering stage	20.64	231.33
T ₆	RDF + 0.5 percent FeSO ₄ spray at flowering + pod formation stage	21.64	263.82
T ₇	RDF + 0.5 percent ZnSO ₄ + 0.5 percent FeSO ₄ spray at flowering stage	23.12	282.75
T ₈	RDF + 0.5 percent ZnSO ₄ + 0.5 percent FeSO ₄ spray at flowering + pod formation stage	23.56	321.72
	S.E.m. ±	0.80	15.80
	CD (P=0.05)	2.43	47.93
	CV (%)	6.51	11.08

Since protein yield is estimated from their content and seed yield, the higher protein yield resulted from the enhanced seed yield under all treatments. Iron and zinc, two crucial components in the structure of enzymes act in biosynthesis of amino acids and protein synthesis, which ultimately increased protein content leading to increase in protein yield. Since zinc is an essential component of many dehydrogenase, proteinase and peptidase enzymes and is important for the bio-synthesis of numerous amino acids, the stimulating impact of Zn administration on these enzymes might be the cause of this. Similarly, iron plays a crucial role in chlorophyll composition, electron transport, and nitrogen metabolism, all of which are directly linked to protein biosynthesis which are essential for the assimilation of nitrogen into simple amino acids. This biological nitrogen assimilation ultimately contributes to greater protein accumulation in seeds, thereby improving the overall protein yield in green gram. These results are in fully supported with findings of Singh *et al.* (2013) [21], Ali *et al.* (2014) [22], Pal *et al.* (2020) [8] and Boradkar *et al.* (2023) [11].

Conclusion

On the basis of our experimental research study, it may be concluded that soil application of recommended fertilizers dose 20:40:00 kg N: P₂O₅ along with the 0.5 per cent ZnSO₄ and 0.5 per cent FeSO₄ foliar application at flowering and pod formation stage were found effective in improving the growth parameters viz., plant height, number of branches, dry matter accumulation mass and quality parameters such as protein content and yield of summer green gram under the medium black calcareous soil of South Saurashtra Agro-climatic Zone.

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