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Impact of Zinc Application on Morphological and Biophysical Parameters of Rice Genotypes in Pot Experiment

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Authors' contributions

This work was carried out in collaboration between all authors. Study design, statistical analysis, protocol and first draft of manuscript was done by author VD. Analysis of study and literature was managed by authors RVK and NGH. All authors read and approved the final manuscript.

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ABSTRACT

Experiment was conducted during *Kharif* -2015 at ARS, Mugad, UAS, Dharwad (Karnataka), to study Impact of application of $ZnSO_4$ on Morphological, Biophysical traits and seed zinc content of rice genotypes raised in pots. Factorial randomized block design consist of 20 genotypes with combination of two treatments *viz.,* T1: Control (no zinc); T2: Soil application of $ZnSO_4$ (20 kg ha^{-1} with three replications. Application of zinc significantly increased the plant height (91.5), leaf area (1.812 cm^{-2} $plant^{-1}$), number of tillers (20.2 $plant^{-1}$), total dry weight (52.1 g $plant^{-1}$), photosynthetic rate (13.1 µmol CO_2 m^{-2} s^{-1}), stomatal conductance (0.27 (µmol m^2 s^{-1}), transpiration rate (3.76 mmol H_2O m^{-2} s^{-1}) and seed zinc content (24.4 ppm). Similarly, genotypes also differed significantly. Whereas; interaction did not differed significantly with all these parameters.

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From the current study, it can be concluded that amending the soil with Zn fertilizer @ 20 kg ha^{-1} resulted in enhancement of morphological traits and biophysical parameters and seed zinc content of rice.

Keywords: Fertilizer; parameters; soil; treatments; yield; $ZnSO_4$.

1. INTRODUCTION

Zinc (Zn) is a vital micronutrient for plants growth and ii act as cofactor for many enzymes which are involved in various physiological and biochemical functions of crop plants. There are many enzymes which require zinc as cofactor to carry their functions *viz.*, Copper- zinc super oxide dismutase (Cu/Zinc SOD), Peroxidase, Carbonic anhydrase and Catalase that plays an important role in protecting crop versus oxidative harmcatalyzed by reactive oxygen species [1]. Deficiency of zinc disrupts the process auxin synthesis, photosystem II activity, stability of biological membranes, carboxylation and for the synthesis of nucleic acids, proteins and lipids which required for overall growth of the crop [2].

Lack of zinc is one of the critical global health problem, affecting nearly one-third of world population [3]. Low dietary Zn intake is considered to be the major reason for the widespread occurrence of deficiency of Zn in human. In South and Southeast Asia, over half a billion people are estimated to be at menace from inadequate Zn intake and also Zn deficiency regions are found the presence of most childhood infectious diseases [4].

Rice is the important staple food of Asia, is inherently very low in Zn and its high consumption relative to other foods contributes to high incidence of Zn deficiency in human populations throughout Asia [4,5]. On average, the grain comprises 80 per cent starch, 7.5 per cent protein, 0.5 per cent ash, and 12 per cent water. The average adult in China and India ingests about 300g of raw rice per day, and annual consumption is 62–190 kg $year^{-1}$. The daily Zn requirement is 15 mg for both adults and children that are 4 and older, but this cannot be achieved through a typical rice-based vegetarian diet. Rice does not provide sufficient mineral requirements for human need even though rice is the dominant source of protein and essential nutrients major part of the world population [6].

Raising the Zn concentration of rice appears to be one of the most economic means to overcome this malnutrition problem among low income rice consumers. In addition, sowing seeds containing high Zn concentration has a potential to benefit crop growth and yield by improving germination and seedling vigor, especially in Zn deficient soils [7,8]. Ideally, once the rice is enriched with essential nutrients, the farmer can cultivate the variety without any supplementary input to produce nutrient enriched rice genotypes in a sustainable way so that the product reaches the malnourished inhabitants in India. Therefore, the objective of this study is to assess the impact of zinc application on morphological, biophysical and zinc content in seed.

2. MATERIALS AND METHODS

To evaluate growth, biophysical traits, yield and grain zinc content of rice genotypes, the experiment was carried in pots during *kharif* 2015 in ARS, Mugad, UAS, Dharwad, rice genotypes were sown with and without zinc to the soil. Polythene bags having dimensions of 100×25 cm were filled with soils of paddy fields. Experiment was laid out in Factorial randomized concept with 20 rice genotypes with the two treatments *viz.,* T_1: control and T_2: Soil application of $ZnSO_4$ (20 kg ha^{-1}), with three replications. The soils of this tract are categorized as Laterites and low productivity because of low water and nutrient holding capacities. Soil texture is characterised under silty-clay-loam with pH of 6.7. The soil iron (12.82 mg kg^{-1}) and zinc contents in the soil (0.48 mg kg^{-1}) were lower.

Each genotype from each pot of two treatments was used to study morphological traits. Plant height was recorded from each pot and measured in centimetre (cm). Tillers were counted from each plant and expressed as number of tillers $plant^{-1}$. Leaves were separated from each sample and then weighed separately for recording leaf and stem weight. The sample was air dried and then placed into oven at 70 C^0 for recording total dry weight (g $plant^{-1}$). Length and breadth of fully open leaf were measured, and leaf area was calculated by formula; Leaf

Area = Leaf length x breadth x 0.71 [9] and expressed in cm^2.

Measurements of rate of photosynthesis (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}) and rate of transpiration (μmol m^{-2} s^{-1}) were made on the top third fully expanded leaf by using portable photosynthesis system (LI-6400 LICOR, Nebraska, Lincoln USA,). These measurements were made between 10.00 AM to 12.00 noon on all the sampling dates at harvest.

Before estimation of zinc concentration in seed, 0.50g seed samples were pre-digested with 10 ml concentrated HNO_3 and incubated in a digestion hood overnight. The next day, samples were wet digested (HNO_3: $HClO_4$; 4:1) and in the extracts, zinc concentration was measured by using atomic absorption spectrophotometer GBC Avanta Ver 2.02 Model. Zinc content was expressed in parts per million (ppm).

Fisher's method of analysis of variance was applied to the analysis and interpretation of the experimental data as suggested by [10]. The level of significance used in 'F' and 't' test was $P \leq 0.05$ for field experiments. Critical difference (CD) values were calculated at 5 per cent level, wherever 'F' test was significant.

3. RESULTS AND DISCUSSION

3.1 Morphological Characters

The data on height of the plant (cm) number of tillers ($plant^{-1}$), leaf area (cm^2) and total dry weight (g $plant^{-1}$) of rice genotypes by the application of zinc sulphate ($ZnSO_4$) and their interactions are showed in Table 1. Significant differences for plant height, number of tillers, leaf area and total dry weight with zinc application resulted among the treatments and genotypes at harvest. Contrary, their interaction did not differ significantly.

The height of the plant was significantly increased due to supplement of zinc to soil (91.50) over control (83.00) at harvest. Among the genotypes, Laldodki recorded significantly higher plant height (110), Whereas, BPT-5204 resulted in lower plant height (63.7) followed by Improved Chitimutayalu (66.8) and Chandibatta (73.8). SIRI-1253 resulted in the significantly maximum number of tillers (24.7) higher leaf area (1,960) and total dry weight (63.7). However, Kempunellu resulted in the lowest leaf area (1,488), a minimum number of tillers and lowest dry weight was found with Ambemohar-2 (15.0 and 35.5, respectively) at harvest . However, the interaction between zinc treatments and genotypes was not significantly.

The increase in plant height and a number of tillers by soil application of $ZnSO_4$, which might be due to the supply of zinc which involved in the enzymatic activity and auxin metabolism in crops [11]. The results are also in agreement with those obtained by Khan et al. [12], Hafeez et al. [13]. During vegetative growth, leaf is the most actively growing organ. Early effects of variation in environment or treatment appear first on leaf either in the form of leaf expansion or elongation. One of the means of knowing it is by simple leaf area measurement at a given time. It was observed that the leaf area increased with application of zinc. Similarly, Nawab et al. [14] observed an increase in leaf area in wheat due to zinc application. Further, Increase in leaf area was responsible for increased shoot weight (TDM) and improves the active period of the photosynthetic area which eventually leads for overall growth of plant in terms of dry matter production. Higher total dry matter production (TDMP) is more important for getting higher yields in any crop is and it's partitioning into various plant parts, coupled with maximum translocation of photosynthates to the sink. TDMP is a result of dry matter accumulation in individual plant parts which depends on nutrient concentration in individual plant parts. The total dry matter production of rice found 9.8 per cent improvement by application of zinc over control at harvest in the pot experiment (Table 1).

3.2 Bio-physical Characters

Application of zinc increased the photosynthetic rate (13.1 μmol CO_2 m^{-2} s^{-1}), stomatal conductance (0.27 μmol m^2 s^{-1}) and transpiration rate (3.76 mmol H_2O m^{-2} s^{-1}) over the control are presented in (Table 2). Among the genotypes, SIRI-1253 found a significantly higher photosynthetic rate (15.6 μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (0.32 0.27 μmol m^2 s^{-1}), whereas with respect to transpiration rate Chandibatta recorded significantly higher transpiration rate (4.56 mmol H_2O m^{-2} s^{-1}). Zn-deficiency depressed plant leaf photosynthetic capacity may be associated to decrease in intercellular CO_2 concentration and function of stomata [15]. Stomatal closure allows plants to limit transpiration, but it also limits CO_2 absorption, which leads to a decreased

Table 1. Impact of Zn application on morphological parameters of rice genotypes at harvest

Genotypes	Plant Height (cm)		Mean	Leaf Area (cm^{-1} plant)		Mean	Number of tillers $plant^{-1}$		Mean	Total Dry Wight (g $plant^{-1}$)		Mean
	T_1	T_2		T_1	T_2		T_1	T_2		T_1	T_2	
Ambemohar 1	66.2	73.9	**70.0**	1,603	1,843	**1,723**	18.4	20.5	**19.5**	45.3	52.8	**49.1**
Koorigenellu	103	113	**108**	1,440	1,622	**1,532**	15.0	17.0	**16.0**	35.6	42.0	**38.8**
Dambersali	95.4	107	**101**	1,463	1,668	**1,566**	17.2	19.7	**18.4**	44.4	50.1	**47.3**
Kempunellu	72.4	85.5	**78.9**	1,386	1,589	**1,488**	14.5	16.7	**15.6**	36.0	38.9	**37.4**
Dodda Batta	100	107	**104**	1,689	1,867	**1,779**	19.7	21.0	**20.3**	50.1	55.2	**52.6**
Ambemohar 2	69.2	76.4	**72.8**	1,432	1,612	**1,522**	14.4	15.6	**15.0**	33.7	37.4	**35.6**
Dodigya	87.3	99.7	**93.5**	1,668	1,859	**1,764**	19.0	21.3	**20.2**	51.0	55.8	**53.4**
Laldodki	104	116	**110**	1,715	1,845	**1,781**	18.6	20.5	**19.6**	48.5	53.5	**51.0**
Budda	77.5	89.1	**83.3**	1,647	1,858	**1,753**	19.4	21.7	**20.6**	51.8	56.9	**54.3**
Wari M. S.	93.1	100	**96.6**	1,826	2,018	**1,923**	20.3	22.2	**21.3**	56.0	60.6	**58.3**
Champakali	71.7	77.2	**74.5**	1,641	1,746	**1,694**	16.8	18.0	**17.4**	42.3	46.6	**44.4**
Improved chitimutayalu	63.5	70.0	**66.8**	1,408	1,603	**1,506**	15.2	16.8	**16.0**	37.3	40.4	**38.8**
Karibatta	84.2	94.1	**89.1**	1,576	1,725	**1,651**	15.4	17.4	**16.4**	39.1	43.5	**41.3**
Chandibatta	69.9	77.7	**73.8**	1,793	2,038	**1,916**	21.6	23.8	**22.7**	56.3	63.0	**59.6**
Halga	92.3	97.4	**94.8**	1,753	1,872	**1,813**	20.1	21.6	**20.9**	53.5	58.7	**56.1**
Siri1253	76.8	82.1	**79.4**	1,907	2,011	**1,960**	24.2	25.3	**24.7**	61.0	66.5	**63.7**
Kalanamak	95.3	103	**99.2**	1,680	1,806	**1,743**	18.3	19.3	**18.8**	44.4	47.8	**46.1**
Hugibatta-1	93.1	101	**97.3**	1,602	1,711	**1,657**	16.4	17.5	**16.9**	41.2	44.6	**42.9**
MTU1001	83.9	90.6	**87.2**	1,835	2,050	**1,943**	21.7	24.0	**22.9**	59.4	64.9	**62.1**
BPT5204	60.2	67.1	**63.7**	1,690	1,900	**1,795**	21.4	23.7	**22.6**	54.5	62.8	**58.6**
Mean	**83.0**	**91.5**	87.3	**1,638**	**1,812**	1,725	**18.4**	**20.2**	19.3	**47.1**	**52.1**	49.6
	S.Em. ±		**C.D. @ 5 %**	**S.Em. ±**		**C.D. @ 5 %**	**S.Em. ±**		**C.D. @ 5 %**	**S.Em. ±**	**C.D. @ 5 %**	
Genotypes (G)	**3.52**		**9.92**	**89.07**		**250.8**	**0.88**		**2.48**	**2.32**	**6.53**	
Treatments (T)	**1.11**		**3.14**	**28.17**		**79.31**	**0.28**		**0.79**	**0.73**	**2.06**	
G x T	**4.98**		**NS**	**126.0**		**NS**	**1.25**		**NS**	**3.28**	**NS**	

T_1 – Control, T_2 – Soil application of $ZnSO_4$ @ 20 kg ha^{-1}, C.D- Critical difference, S.Em.- Standard Error of Mean, NS-Non Significant

Table 2. Impact of Zn application on Biophysical parameters of rice genotypes at harvest and seed zinc content of rice genotypes

Genotypes	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})		Mean	Stomatal conductance (μmol m^2 s^{-1})		Mean	Transpiration rate (mmol H_2O m^{-2} s^{-1})		Mean	Seed Zn (ppm)		Mean
	T_1	T_2		T_1	T_2		T_1	T_2		T_1	T_2	
Ambemohar 1	11.0	13.0	**12.0**	0.23	0.26	**0.24**	3.08	3.60	**3.34**	26.6	32.1	**29.4**
Koorigenellu	9.71	10.8	**10.2**	0.20	0.23	**0.21**	2.39	2.70	**2.55**	30.1	36.0	**33.1**
Dambersali	11.1	12.7	**11.9**	0.22	0.25	**0.24**	3.13	3.60	**3.36**	29.0	33.8	**31.4**
Kempunellu	9.12	10.4	**9.7**	0.18	0.21	**0.20**	2.28	2.60	**2.44**	21.3	23.6	**22.5**
Dodda Batta	9.61	10.5	**10.1**	0.26	0.28	**0.27**	3.70	4.10	**3.90**	26.9	29.7	**28.3**
Ambemohar 2	9.40	10.4	**9.9**	0.19	0.21	**0.20**	2.43	2.60	**2.52**	27.8	31.4	**29.6**
Dodigya	13.2	14.5	**13.8**	0.26	0.28	**0.27**	3.74	4.10	**3.92**	17.0	19.0	**18.0**
Laldodki	12.7	13.5	**13.1**	0.24	0.26	**0.25**	3.60	4.00	**3.80**	22.2	25.2	**23.7**
Budda	13.1	14.9	**14.0**	0.24	0.29	**0.27**	3.72	4.30	**4.01**	23.2	25.8	**24.5**
Wari M. S.	14.0	15.5	**14.7**	0.29	0.31	**0.30**	4.14	4.50	**4.32**	22.8	24.4	**23.6**
Champakali	11.2	11.9	**11.5**	0.22	0.24	**0.23**	3.07	3.50	**3.29**	24.7	27.5	**26.1**
Improved chitimutayalu	9.3	10.5	**9.9**	0.19	0.22	**0.20**	2.37	2.60	**2.48**	24.4	26.6	**25.5**
Karibatta	10.0	10.9	**10.4**	0.21	0.23	**0.22**	3.01	3.20	**3.10**	17.6	19.7	**18.6**
Chandibatta	13.9	15.9	**14.9**	0.29	0.32	**0.31**	4.52	4.60	**4.56**	19.6	22.3	**21.0**
Halga	13.9	15.2	**14.5**	0.26	0.30	**0.28**	3.81	4.40	**4.11**	13.5	14.7	**14.1**
Siri1253	15.0	16.2	**15.6**	0.31	0.33	**0.32**	4.23	4.70	**4.46**	15.2	16.8	**16.0**
Kalanamak	10.9	12.1	**11.5**	0.22	0.24	**0.23**	3.23	3.50	**3.37**	14.4	15.6	**15.0**
Hugibatta-1	10.7	11.4	**11.0**	0.21	0.23	**0.22**	3.09	3.30	**3.20**	14.5	15.5	**15.0**
MTU1001	14.5	16.2	**15.4**	0.30	0.33	**0.31**	4.22	4.70	**4.46**	16.7	18.5	**17.6**
BPT5204	13.7	15.7	**14.7**	0.27	0.32	**0.29**	4.05	4.60	**4.32**	24.3	28.8	**26.5**
Mean	**11.8**	**13.1**	**12.5**	**0.24**	**0.27**	**0.25**	**3.39**	**3.76**	**3.58**	**21.6**	**24.4**	**23.0**
	S.Em. ±		**C.D. @ 5 %**	**S.Em. ±**		**C.D. @ 5 %**	**S.Em. ±**		**C.D. @ 5 %**	**S.Em. ±**		**C.D. @ 5 %**
Genotypes (G)	**0.99**		**2.79**	**0.014**		**0.041**	**0.208**		**0.587**	**1.14**		**3.22**
Treatments (T)	**0.31**		**0.88**	**0.005**		**0.013**	**0.066**		**0.185**	**0.36**		**1.02**
G x T	**1.39**		**NS**	**0.020**		**NS**	**0.295**		**NS**	**1.62**		**NS**

T_1 – Control, T_2 – Soil application of $ZnSO_4$ @ 20 kg ha^{-1}, C.D- Critical difference, S.Em.- Standard Error of Mean, NS-Non Significant

photosynthetic activity. Sharma et al. [16] reported a significant influence of Zinc in the regulation of the stomatal aperture, which is accounted for possible role of Zn in maintaining a high K content in guard cells. A decrease in carbonic anhydrase activity due to Zn deficiency may also contribute to the reduced net photosynthetic rate, P_N [17]. In addition, the accumulation of saccharides in leaves may be an important factor for the reducing photosynthesis activity under Zn-deficiency [18].

It also suggests that Zn deficiency cause lesions in metabolic pathways regulating photochemical activity and/or carbon reduction lead to the limited photosynthesis. Similar result was found safflower *(Carthamus tinctorius* L.) [19] and *(Zea mays* L.) [20]. Reductions in light and dark reactions of photosynthesis might be expected because Zn deficiency decreases RNA and protein synthesis [21].

Seed zinc content presented in Table 2, higher seed zinc content (24.4) were found with application of zinc over the control. Similar results were found by Jan et al. [22] in rice. Application of Zinc increased the zinc in soil which leads to the favourable conditions for root development and increased the amount of absorption which ultimately enhance the seed zinc content of rice [23]. Grain Zn content enhanced by the application of zinc was also reported by earlier [24].

Similarly, genotypes found a significant difference, Koorigenellu foud higher seed zinc content (33.1) whereas; Halga resulted in significantly lower seed zinc content (14.1). Low and high affinity transporters in roots are varied with genotypes this the main reason for genotypic variation [25]. Ramesh et al. [26] reported that expression of zinc transporters could increase plant zinc uptake. In conclusion, basal application of Zn fertilizer @ 20 kg ha^{-1} was found as the most suitable dose for overall development of shoot growth, biophysical parameters and seed zinc content of rice crop.

4. CONCLUSION

Zn deficiency in cereal plants, including rice, is a well-known problem that causes reduced agricultural productivity all over the world. Zn deficiency can be mitigated by breeding staple genotypes with zinc efficient. Moreover, food production can be maximised by ameliorating Zn deficiency. Genotypes vary considerably in terms of uptake and translocation of zinc to both shoot and seed. Eventually, necessary trait from human nutrition and productivity it is the seed zinc content. Therefore, the experiment was conducted to screen the genotypes for morphological traits and bio-physical attributes. From this study, it confirmed zinc is vital nutrient for enhancement of morphological traits, biophysical parameters and seed zinc content of rice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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