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Effect of zinc management under elevated CO_2 and temperature on growth and yield of rice (*Oryza sativa* L.)

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Abstract
A pot culture experiment was carried out out in the phytotron facility at ICAR-Indian Agricultural Research Institute, Pusa, New Delhi, where the effect of environmental conditions and zinc management strategies (Treatments) on the growth and yield of zinc-efficient rice genotype under zinc-deficient condition wase evaluated. The experiment was laid out using factorial CRD design with three replications. Plant height, leaf greenness, shoot mass, root mass and grain yield were reported significantly higher under elevated CO_2 and temperature condition than ambient CO_2 and temperature condition. All the treatments showed significantly higher results over the control irrespective of the environmental conditions. Best results obtained in the treatment T_8, T_2 and T_6 which were statistically at par majority of the cases and signifies integrated use of organic acid, zinc sulphate and FYM may effectively manage zinc deficiency in rice field under climate change scenario.

Keywords: Zinc management, zinc-efficient rice, elevated CO_2 and temperature

Introduction
It is being projected that ambient atmospheric CO_2 is expected to increase from current 400 μmol mol^{-1} to 540-958 μmol mol^{-1} by 2100 and consequently the mean average surface air temperature is expected to increase by 3 to 4 °C during this period (IPCC, 2013) [9]. The increased atmospheric CO_2 content coupled with the soil atmosphere CO_2 will dissolve the $CaCO_3$ and $MgCO_3$ thereby increasing the carbonate and bicarbonate ion activity (Emmerich, 2003) [5] of the soil. The formation of Zn carbonates may reduce Zn solubility at either high (in a calcareous soil) or low redox potential (Eh) (in any soil) due to the increase in partial pressure of CO_2 rising from organic matter decomposes (Kirk and Bajita, 1995) [10]. Also, high bicarbonate concentrations in soil solution hinder root growth and cause root leakage of Zn-inefficient rice genotypes (Rose *et al*., 2011) [17].
Rice (*Oryza sativa* L.) is one of the most important global staple food crops feeding more than half of the world population. India is one of the principal rice producing and consuming countries in the world. It may be possible to improve crop yields on Zn-deficient soils by exploiting genotypic differences in Zn uptake and tissue use-efficiency that exists within crop species and genotypes (Rengel, 2001; Cakmak, 2002; Hacisalihoglu and Kochian, 2003; Alloway, 2004) [16, 2, 6, 1]. For example, in rice, Zn uptake efficiency correlates with exudation rates of low-molecular weight organic anions, and thus a substantial proportion of the phenotypic variation in Zn uptake efficiency is under genetic control (Hoffland *et al*., 2006; Wissuwa *et al*., 2006) [8, 21]. The expression of high zinc efficiency in rice is related to enhanced uptake and translocation capacity of Zn into shoots and higher amounts of physiologically active Zn in leaf tissues (Cakmak and Marschner, 1998) [3].

Materials and Methods
Present investigation was carried out in the phytotron chamber facility of ICAR-Indian Agricultural Research Institute, New Delhi. Zn-efficient rice genotype and Zn-deficient soil was

used along with eight treatments under ambient and elevated CO_2 and temperature condition. Ambient CO_2 level was maintained at 410 ±10 µmol mol^{-1} and elevated CO_2 level was maintained at 610 ±10 µmol mol^{-1}; whereas ambient temperature was maintained at 30^{0}C during the light hours & 22 °C during the dark hours and elevated temperature was maintained at 35 °C during the light hours & 22^{0}C during the dark hours. The soil used was silt loam in nature, slightly alkaline in pH (7.80) with low available soil nitrogen (198.3 kg ha^{-1}), medium in phosphorous (15.65 kg ha^{-1}), medium in available potassium (224.6 kg ha^{-1}), sufficient in DTPA extractable micronutrients (Mn, Fe and Cu) and deficient in Zn (0.40 mg kg^{-1}). Organic carbon content of the soils was in low range (3.12 g kg^{-1}). Alternate wetting and drying condition were followed during the entire growing period. The different growth attributes and yield were recorded.

Results and Discussion

Plant height

Plant height at all the crop growth stages was significantly affected by treatments and environmental conditions but their interaction is found to be insignificant (Table 1). Plant height at tillering, flowering and harvest was higher under elevated CO_2 and temperature (39.9, 79.7 and 86.0 cm respectively at tillering, flowering and at harvest) than under ambient CO_2 and temperature (38.1,77.21 and 83.5 cm respectively at tillering, flowering and at harvest). These findings align with previous research indicating that elevated CO_2 and temperature conditions enhance plant growth by increasing photosynthetic efficiency and nutrient uptake (Zhao *et al*. 2020; Wang *et al*. 2022 and Usui *et al*., 2023) [24, 20, 19]. Under ambient CO_2 and temperature condition among the treatments, T_8 recorded the highest plant height (39.7,80.0 and 85.7 cm respectively at tillering, flowering and at harvest), followed by T_2 (39.3,79.3 and 85.3 cm respectively at tillering, flowering and at harvest). The lowest mean plant height was observed in the control treatment, T_1 (34.3,73.0 and 79.0 cm). Similar results also reported under elevated CO_2 and temperature condition where T_8 achieved the highest plant height (41.3, 81.7 and 87.7 cm respectively at tillering, flowering and at harvest) followed by T_2 (41.0,79.3 and 87.0 cm respectively at tillering, flowering and at harvest), while the control (T_1) had the lowest (37.0, 76.3 and 82.3 cm respectively at tillering, flowering and at harvest). Treatments involving citric acid and zinc sulphate showed the most significant improvement in plant height, indicating their effectiveness in promoting growth in Zn-deficient soils under both the environmental conditions. The effectiveness of T_8 can be attributed to the chelating properties of citric acid, which improves zinc solubility and uptake by plants (Liu *et al*., 2021) [12]. Citric acid and $ZnSO_4$ application significantly enhance plant growth in Zn-deficient soils, consistent with earlier studies showing that organic acids improve Zn bioavailability and uptake (Hakeem *et al*., 2023) [7]. Despite the significant influence of treatments and environmental conditions on plant height, their insignificant interaction indicating that the effect of Zn supplementation is relatively stable under different CO_2 and temperature conditions. This suggests that integrating Zn fertilizers with organic amendments like citric acid can serve as a reliable strategy for improving rice growth in Zn-deficient soils, regardless of future climate change scenarios.

Leaf greenness

Chlorophyll content, as indicated by SPAD readings, is a key indicator of photosynthetic efficiency and plant health and it is found that at both the tillering and flowering stage leaf greenness was significantly influenced by treatments and environmental conditions, however, the interaction between environment and treatment was not significant (Table 2). Elevated CO_2 & temperature resulted in higher SPAD readings at tillering and flowering stage (32.90 and 34.81 respectively at tillering and flowering) compared to ambient conditions (32.17 and 33.94 respectively at tillering and flowering). Under ambient CO_2 & temperature T_8 recorded the highest SPAD readings (33.25 and 35.32 respectively at tillering and flowering), while T_1 exhibited the lowest (30.56 and 31.75 respectively at tillering and flowering) similar results are found under elevated CO_2 & temperature where T_8 recorded the highest SPAD readings (33.26 and 35.74 respectively at tillering and flowering), while T_1 exhibited the lowest (31.43 and 32.65 respectively at tillering and flowering). Treatments incorporating citric acid and zinc sulfate consistently outperformed others, emphasizing their positive impact on leaf greenness and chlorophyll content under both the environmental conditions. The non-significant interaction between environment and treatment indicates that treatment performance was consistent across environmental conditions, corroborating findings. While the interaction between environmental conditions and treatments was not significant, indicating that the effect of Zn management strategies on chlorophyll content was consistent across environmental scenarios, the overall improvement in SPAD readings under elevated CO_2 and temperature suggests that climate change could potentially enhance the effectiveness of these treatments. These findings align with research showing that elevated CO_2 and temperature can stimulate plant growth and nutrient uptake, enhancing the overall health of plants under Zn-deficient conditions (Shahzad, Keller & Krämer, 2022) [18].

Tillering

The tillering was analyzed based on treatments and environmental conditions (Table 2). The mean number of tillers under elevated CO_2 and temperature (3.79) was slightly higher than under ambient conditions (3.63). The increase in tillering under elevated CO_2 and temperature was not statistically significant, similarly, treatments and their interaction with the environmental conditions did not show statistically significant differences in tillering. The lack of significant differences in tillering under varying environmental conditions is consistent with prior research suggesting that while elevated CO_2 can enhance photosynthesis and biomass accumulation, its effects on tillering may be limited in Zn-deficient conditions due to constraints in nutrient availability (Myers *et al*., 2023) [15]. In Zn-deficient soils, reduced Zn availability may counteract the potential benefits of elevated CO_2, leading to negligible differences in tillering between environmental treatments. Environmental conditions, particularly temperature, have been shown to affect tillering dynamics in rice, with elevated temperatures potentially suppressing tiller formation due to altered carbohydrate partitioning and hormonal balance (Usui *et al*., 2023) [19]. However, in this study, the interaction treatments and environmental factors did not significantly impact tillering, suggesting that the intrinsic Zn efficiency of the genotype under investigation may have buffered potential environmental effects.

Shoot biomass

Shoot mass of Zn-efficient rice plants under Zn-deficient soil conditions was significantly influenced by zinc management strategies and environmental conditions (Table 3). The mean shoot mass was higher under elevated CO_2 & temperature (31.31

g pot⁻¹) compared to ambient conditions (28.65 g pot⁻¹). This finding is consistent with the research that indicates elevated CO_2 and higher temperatures can enhance plant biomass accumulation by improving photosynthesis and nutrient uptake efficiency (Zhao *et al*., 2023; Liu *et al*., 2023) [22, 11]. Under ambient CO_2 & temperature, T_6 achieved the highest shoot mass (30.10 g pot^{-1}), while T_1 had the lowest (26.38 g pot^{-1}). Similar results are found under elevated CO_2 & temperature, where T_6 achieved the highest grain yield (33.05 g pot^{-1}), while T_1 had the lowest (28.18 g pot^{-1}). These results are in line with previous studies that emphasize the importance of zinc as a crucial micronutrient for plant growth, as it contributes to various physiological processes such as enzyme activation, protein synthesis, and stress tolerance (Marschner, 2022; Muneer *et al*., 2023) [13, 14]. Treatments incorporating citric acid and zinc sulphate showed superior performance compared to others, reflecting their effectiveness in improving shoot mass under Zn-deficient conditions under both the environmental conditions.

Root biomass

Root mass of Zn-efficient rice plants under Zn-deficient soil conditions was significantly influenced by zinc management strategies and environmental conditions (Table 3). The mean root mass was higher under elevated CO_2 and temperature (6.93 g pot^{-1}) compared to ambient conditions (6.38 g pot^{-1}). This finding aligns with previous studies that have demonstrated the potential for elevated CO_2 and temperature to enhance root biomass, primarily by improving root architecture and nutrient uptake efficiency (Zhao *et al*., 2023) [22]. Under ambient CO_2 and temperature, T_6 achieved the highest shoot mass (6.82 g pot^{-1}), while T_1 had the lowest (5.75 g pot^{-1}). Similar results are found under elevated CO_2 & temperature, where T_6 achieved the highest grain yield (7.49 g pot^{-1}), while T_1 had the lowest (5.77 g pot^{-1}). Citric acid has been shown to increase the bioavailability of zinc in the soil, which is particularly important in Zn-deficient conditions (Muneer *et al*., 2023) [14]. Zinc is vital for various physiological processes, including root development, and its proper management can lead to improved root mass (Marschner, 2022) [13]. Treatments incorporating citric acid and zinc sulphate showed better result compared to others, reflecting their effectiveness in improving root mass under Zn-deficient conditions under both the environmental conditions. Liu *et al*., (2023) [11] highlighted the role of elevated CO_2 in improving nutrient acquisition by roots, which may explain the increase in root mass under these conditions. Furthermore, higher temperatures could accelerate metabolic processes in plants, thus contributing to improved root growth (Cheng *et al*., 2023) [4].

Grain yield

Grain yield of Zn-efficient rice plants under Zn-deficient soil conditions was significantly influenced by zinc management strategies and environmental conditions (Table 3). The mean grain yield was higher under elevated CO_2 & temperature (20.00 g pot^{-1}) compared to ambient conditions (18.55 g pot^{-1}), which aligns with findings that higher atmospheric CO_2 increases photosynthetic activity, biomass production, and nutrient use efficiency (Zhao *et al*., 2020; and Wang *et al*., 2022) [24, 20]. Under ambient CO_2 & temperature, T_6 achieved the highest grain yield (19.90 g pot^{-1}), while T_1 had the lowest (16.80 g pot^{-1}). Similar results are found under elevated CO_2 & temperature, where T_6 achieved the highest grain yield (21.54 g pot^{-1}), while T_1 had the lowest (17.66 g pot^{-1}). T_6 outperformed other treatments, likely due to the combined effect of CA and FYM in improving zinc availability, enhancing nutrient uptake, and supporting reproductive growth (Liu *et al*., 2021) [12]. Similarly, T_8 leveraged the chelating properties of citric acid to maintain adequate zinc availability throughout the growing season. Treatments incorporating citric acid and zinc sulphate showed superior performance compared to others, reflecting their effectiveness in improving grain yield under Zn-deficient conditions under both the environmental conditions.

Table 1: Effect of zinc management strategies and environmental conditions on plant height at different growth stages of rice plant under ambient and elevated CO_2 & temperature conditions

Treatments	Plant height at tillering (cm)			Plant height at flowering (cm)			Plant height at harvest (cm)		
	Ambient	Elevated	Mean	Ambient	Elevated	Mean	Ambient	Elevated	Mean
T_1: (Control)	34.33	37.00	35.67	73.00	76.33	74.67	79.00	82.33	80.67
T_2: (12.5 mg/kg $ZnSO_4$)	39.33	41.00	40.17	79.33	81.33	80.33	85.33	87.00	86.17
T_3: (Zinc solubilizer biofertilizer)	37.00	40.00	38.50	76.00	79.67	77.84	83.00	86.33	84.67
T_4: (1.25 g/kg FYM)	37.67	39.67	38.67	76.33	79.00	77.67	82.00	85.67	83.84
T_5: (1.25 g/kg FYM + 2.5 mg/kg OA)	38.67	40.33	39.50	77.33	80.33	78.83	83.67	86.67	85.17
T_6: (1.25 g/kg FYM + 2.5 mg/kg CA)	39.33	41.00	40.17	78.33	80.67	79.50	85.00	87.00	86.00
T_7: (5 mg/kg OA)	38.33	39.00	38.67	77.33	78.67	78.00	84.33	85.33	84.83
T_8: (10 mg/kg CA)	39.67	41.33	40.50	80.00	81.67	80.84	85.00	87.67	86.34
Mean	38.04	39.92	38.98	77.21	79.71	78.46	83.42	86.00	84.71
LSD at 5%	Environment (E) = 0.91 Treatment (T) = 1.83 E * T = 2.58 (NS)			Environment (E) = 0.98 Treatment (T) = 1.96 E * T = 2.77 (NS)			Environment (E) = 0.77 Treatment (T) = 1.55 E * T = 2.19 (NS)		

(OA: oxalic acid; CA: citric acid; LSD: least significance difference)

Table 2: Effect of zinc management strategies and environmental conditions on tillering and leaf greenness of rice plant under ambient and elevated CO_2 & temperature conditions

Treatments	Number of tillers per plant			SPAD readings at tillering			SPAD readings at flowering		
	Ambient	Elevated	Mean	Ambient	Elevated	Mean	Ambient	Elevated	Mean
T_1: (Control)	3.33	3.67	3.50	30.56	31.43	31.00	31.75	32.65	32.20
T_2: (12.5 mg/kg $ZnSO_4$)	3.67	4.33	4.00	32.61	33.22	32.92	34.71	35.39	35.05
T_3: (Zinc solubilizer biofertilizer)	3.67	4.00	3.83	31.43	32.51	31.97	33.20	34.26	33.73
T_4: (1.25 g/kg FYM)	3.33	3.67	3.50	31.30	32.32	31.81	32.82	34.05	33.44
T_5: (1.25 g/kg FYM + 2.5 mg/kg OA)	4.00	3.33	3.67	32.43	33.45	32.94	34.34	35.43	34.89
T_6: (1.25 g/kg FYM + 2.5 mg/kg CA)	4.00	4.00	4.00	33.15	33.82	33.49	35.20	35.82	35.51
T_7: (5 mg/kg OA)	3.67	3.67	3.67	32.60	33.13	32.86	34.21	35.13	34.67
T_8: (10 mg/kg CA)	3.33	3.67	3.50	33.25	33.26	33.26	35.32	35.74	35.53
Mean	3.63	3.79	3.71	32.17	32.90	32.53	33.94	34.81	34.38
LSD at 5%	Environment(E) =0.39(NS) Treatment(T)= 0.79(NS) E * T = 1.12 (NS)			Environment (E) = 0.36 Treatment (T) = 0.72 E * T = 1.02 (NS)			Environment (E) = 0.31 Treatment (T) = 0.62 E * T = 0.88 (NS)		

(OA: oxalic acid; CA: citric acid; LSD: least significance difference)

Table 3: Effect of zinc management strategies and environmental conditions on shoot biomass, root biomass and grain yield of rice plant under ambient and elevated CO_2 & temperature conditions

Treatments	Shoot biomass (g pot^{-1})			Root biomass (g pot^{-1})			Grain yield (g pot^{-1})		
	Ambient	Elevated	Mean	Ambient	Elevated	Mean	Ambient	Elevated	Mean
T_1: (Control)	26.38	28.18	27.28	5.75	5.77	5.76	16.80	17.66	17.23
T_2: (12.5 mg/kg $ZnSO_4$)	29.94	32.73	31.34	6.62	7.25	6.94	19.67	21.30	20.49
T_3: (Zinc solubilizer biofertilizer)	27.68	30.28	28.98	6.18	6.73	6.46	17.71	19.13	18.42
T_4: (1.25 g/kg FYM)	27.51	29.86	28.69	6.22	6.85	6.54	17.62	19.02	18.32
T_5: (1.25 g/kg FYM + 2.5 mg/kg OA)	28.98	31.86	30.42	6.46	7.09	6.78	18.90	20.44	19.67
T_6: (1.25 g/kg FYM + 2.5 mg/kg CA)	30.10	33.05	31.58	6.82	7.49	7.16	19.90	21.54	20.72
T_7: (5 mg/kg OA)	28.80	31.70	30.25	6.29	6.92	6.61	18.24	19.73	18.99
T_8: (10 mg/kg CA)	29.84	32.80	31.32	6.71	7.34	7.03	19.53	21.16	20.35
Mean	28.65	31.31	29.98	6.38	6.93	6.66	18.55	20.00	19.27
LSD at 5%	Environment (E) = 0.32 Treatment (T) = 0.65 E * T = 0.91 (NS)			Environment (E) = 0.10 Treatment (T) = 0.20 E * T = 0.28 (NS)			Environment (E) = 0.24 Treatment (T) = 0.47 E * T = 0.67 (NS)		

(OA: oxalic acid; CA: citric acid; LSD: least significance difference)

Conclusion

The elevated CO_2 and temperature condition, which is becoming more prevalent due to climate change, significantly influence growth parameters and grain yield of rice plant grown in Zn-deficient soils. Application of citric acid combining with FYM and zinc sulphate while adopting zinc-efficient rice genotype may efficiently manage the zinc requirement of rice plant in zinc-deficient soils under climate changing scenario. The study demonstrated that the elevated CO_2 and temperature condition generally enhanced plant height, leaf greenness, shoot biomass, root biomass, and grain yield. Moreover, zinc management strategies, particularly those incorporating citric acid and zinc sulphate, were found to improve plant growth and productivity under both ambient and elevated CO_2 and temperature conditions. The results underscore the importance of integrated zinc management strategies in sustaining rice productivity in the face of changing climatic conditions, highlighting the potential for using organic amendments like citric acid in mitigating nutrient deficiencies and supporting sustainable agricultural practices.

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