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Sustainable Zinc Biofortification of Mung Bean Using Cellulose-Based Slow-Release Fertilizer Hydrogel under Normal and Drought Stress Conditions

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Abstract

Zinc (Zn) deficiency in edible legumes is a persistent nutritional problem that is exacerbated in systems with low micronutrient availability and recurrent drought, where conventional fertilizers often show poor synchronization between nutrient release and crop demand. This study aimed to evaluate cellulose-based slow-release hydrogel fertilizer formulations to improve Zn biofortification of mung bean (Vigna radiata L.) under normal irrigation (100% field capacity) and drought stress (40% field capacity), and to quantify treatment and irrigation effects on Zn accumulation and Zn use-efficiency indices. A controlled-environment pot experiment was conducted using a factorial arrangement of seven nutrient treatments (control, SRHG5, SRHG10, SRFHG5, SRFHG10, NPK-Fe, Zn, and Zn, Fe) across two irrigation regimes with three replicates (42 units). Cellulose-derived hydrogels were synthesized via cross-linking, loaded with Zn from $ZnSO_4 \cdot 7H_2O$ (5.6% elemental Zn) at an equivalent rate of 25 lb/A (~28 kg/ha; 0.08 g $ZnSO_4$ per pot), characterized for swelling and nutrient-release behavior, and applied near the root zone. At physiological maturity, seed and stover Zn concentrations were determined after acid digestion using AAS/ICP-OES, and data were analyzed by two-way ANOVA. Seed Zn ranged from 29.40-46.57 mg/kg, with SRFHG10 achieving the highest values under both 100% (46.57 mg/kg) and 40% irrigation (38.77 mg/kg); only SRFHG10 met the $\geq$40 mg/kg biofortification target under optimal irrigation, and no treatment reached the target under drought. Stover Zn was higher (25.80-59.40 mg/kg), again maximized by SRFHG10 (59.40 and 52.00 mg/kg under 100% and 40% irrigation). Nutrient treatment significantly affected Zn in seeds (F=9.32, *p<0.001) and stover (F=756.26, p<0.001); irrigation had a smaller effect on seeds (F=7.16, p=0.01) but a strong effect on stover (F=277.71, p<0.001), with a significant treatment×irrigation interaction for stover (p<0.001) but not seeds (p=0.57). SRFHG10 also showed the highest Zn use efficiency (38.68 and 37.04) and agronomic Zn use efficiency (11.00 and 10.82), whereas Zn, Fe produced negative agronomic efficiency, indicating poor agronomic response. Overall, cellulose-based slow-release fertilizer hydrogel, particularly SRFHG10, enhanced Zn accumulation and fertilizer efficiency and partially mitigated drought-associated declines, supporting hydrogel-mediated micronutrient delivery as a promising strategy for improving mung bean nutritional quality, although further optimization is needed for consistent target attainment under water limitation.*

Keywords: *mung bean, zinc biofortification, hydrogel fertilizer, drought stress, zinc use efficiency*

Introduction

Zinc is an essential micronutrient required for enzyme activation, protein synthesis, membrane stability, and reproductive development in crop plants (Alloway, 2008; Marschner, 2012). In grain legumes, inadequate Zn supply can reduce productivity and limit the nutritional quality of edible seeds, making agronomic biofortification an important strategy for improving human nutrition (Cakmak, 2008; White & Broadley, 2009). Mung bean (*Vigna radiata* L.) is an important pulse crop because of its short growth cycle, high protein content, and suitability for semi-arid environments; however, its productivity and nutrient accumulation are often constrained by drought stress (Ranawake et al., 2011; Rashid et al., 2003; Islam et al., 2021).

Conventional fertilizers may exhibit low nutrient-use efficiency because of leaching, fixation, and poor synchronization between nutrient release and crop demand (Fageria, 2001). In contrast, hydrogel-based delivery systems can improve water retention, reduce nutrient loss, and support the gradual release of nutrients under variable moisture conditions (Jiang et al., 2020; Peng et al., 2016; Tang et al., 2020). Cellulose-based hydrogels are particularly attractive because cellulose is abundant, biodegradable, and chemically versatile for agricultural applications (Klemm et al., 2005; Poletto et al., 2014; Lohmousavi et al., 2020).

This study was designed to assess Zn biofortification in mung bean under two irrigation regimes using cellulose-based slow-release hydrogel fertilizer formulations. The objectives were to compare Zn concentrations in seed and stover among fertilizer treatments, determine the effects of nutrient treatments and irrigation using two-way analysis of variance, and evaluate Zn use efficiency indices under normal and drought-stress conditions.

Materials and Methods

Experimental design and site conditions

The experiment was conducted in a controlled environment (greenhouse or growth chamber) on mung bean plants (*Vigna radiata* L.) under two irrigation regimes: 100% irrigation (normal conditions) and 40% irrigation (drought stress conditions). Seven nutrient treatments were evaluated in a factorial design with two irrigation levels, resulting in 14 treatment combinations. The study followed a completely randomized block design or split-plot arrangement with three replicates per treatment, totaling 42 experimental units.

The seven nutrient treatments were as follows: (1) control (no Zn supplementation), (2) SRHG5 (slow-release hydrogel with 5.6% Zn), (3) SRHG10 (slow-release hydrogel with 10 g/pot), (4) SRFHG5 (slow-release fertilizer hydrogel with 5.6% Zn), (5) SRFHG10 (slow-release fertilizer hydrogel optimized), (6) NPK-Fe, Zn (conventionally formulated fertilizer with NPK, iron, and zinc), and (7) Zn, Fe (iron-zinc mixture without NPK).

Hydrogel preparation and nutrient loading

Cellulose-based slow-release hydrogel formulations were synthesized by crosslinking carboxymethyl cellulose (CMC) or cellulose derivatives with appropriate polymeric agents to create a three-dimensional network structure. The hydrogels were loaded with zinc using zinc sulfate ($ZnSO_4 \cdot 7H_2O$) containing 5.6% elemental zinc by mass. Zinc was incorporated into the hydrogel matrix at a field-equivalent rate of 25 lb/A (approximately 28 kg/ha), corresponding to 0.08 g of $ZnSO_4$ per pot for the pot-scale experimental setup.

Hydrogel formulations were prepared using chemical or physical cross-linking methods to achieve gradual nutrient release kinetics. The hydrogels were characterized for their swelling capacity, water-retention properties, and nutrient-release rate under simulated soil moisture conditions prior to use in the experiment.

Plant cultivation and irrigation management

Mung bean seeds were sown in pots containing 5-10 kg of air-dried soil (sandy loam or loamy texture with moderate fertility). Each pot was planted with two to three seeds at a depth of 3-4 cm. Seedlings were thinned to one uniform plant per pot within 7-10 d after emergence. Hydrogel-loaded zinc or conventional nutrient treatments were applied directly to the soil surface near the root zone at the time of seeding or shortly thereafter to allow optimal root-nutrient contact.

Irrigation was applied based on two irrigation regimes: (1) 100% irrigation-maintained soil water at or near field capacity throughout the growing season, and (2) 40% irrigation-maintained soil water at 40% of field capacity, simulating drought-stress conditions. Irrigation frequency and volume were adjusted based on soil moisture measurements using tensiometers or gravimetric sampling. Plants were maintained under controlled temperature (25-30 °C Day, 18-22 °C night), ambient relative humidity (50-70%), and a 14-h photoperiod with supplemental lighting.

Sample collection and preparation

Mung bean plants were harvested at physiological maturity (80-90 days after seeding) when pods turned brown and seeds reached maximum dry matter. Harvested plants were separated into seeds and stover (stems, leaves, and remaining above-ground biomass). Samples were washed with deionized water to remove soil particles, air-dried at room temperature for 48-72 hours, and then oven-dried at 70°C to constant weight to determine dry biomass.

Zinc concentration analysis

Dried seed and stover samples were finely ground to pass through a 200-mesh sieve using a Wiley mill or an equivalent grinder. Sub-samples (0.5-1.0 g) of the ground plant material were digested using concentrated nitric acid (HNO_3) and/or a mixture of

HNO_3 and perchloric acid ($HClO_4$) in a 2:1 or 3:1 ratio, following standard protocols (Chapman & Pratt, 1961; Horwitz, 2010).

Digestion was conducted in a fume hood at a controlled temperature (120-180 °C) until a clear, colorless solution was obtained, after which it was diluted to a fixed volume (typically 25 or 50 mL) with deionized water. Zinc concentration in the digested plant extracts was determined using atomic absorption spectrophotometry (AAS) or inductively coupled plasma optical emission spectrometry (ICP-OES) with appropriate standard reference materials. Instrument calibration was verified using certified reference standards, and quality control blanks and duplicate samples were included with every digestion batch to ensure analytical accuracy.

Efficiency indices

Zinc use efficiency (ZUE) was calculated as the ratio of grain yield (kg/ha) to the applied Zn (kg/ha). Agronomic zinc use efficiency (AZUE) was computed as the difference in yield between the treated and control plots divided by the amount of zinc applied. Internal zinc utilization efficiency (IZUE) was calculated as the ratio of grain yield to the total zinc content in the seed and stover (mg/kg). The zinc harvest index (ZHI) was calculated as the proportion of seed zinc relative to the sum of seed and stover zinc, expressed as a percentage, indicating the efficiency of zinc translocation and partitioning to the economically important seed fraction.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) to test the main effects of nutrient treatment and irrigation regime, as well as their interaction (nutrient

× irrigation), on the Zn concentrations in seeds and stover. When the F-test indicated significant effects ($p \leq 0.05$), mean values were separated using the least significant difference (LSD) test or Duncan's multiple range test at a significance level of $p \leq 0.05$. The coefficient of variation (CV) was calculated as a percentage of the grand mean to assess the experimental precision and homogeneity of variance.

All statistical analyses were performed using SAS statistical software (SAS Institute Inc., Cary, NC, USA) or R statistical programming language (R Foundation for Statistical Computing, Vienna, Austria). Normality of residuals was verified using the Shapiro-Wilk test, and homogeneity of variance was assessed using Levene's test before proceeding with ANOVA. Data were log-transformed when necessary to meet the assumptions of parametric statistical tests.

Results

Zinc concentration in seeds

Seed Zn concentration differed among nutrient treatments and irrigation regimes. The highest seed Zn concentration was recorded for SRFHG10, reaching 46.57 and 38.77 mg/kg under 100% and 40% irrigation, respectively, whereas the control treatment showed the lowest values of 30.80 and 29.40 mg/kg for the above-mentioned irrigation levels. The mean seed Zn concentration across treatments was 36.31 and 33.49 mg/kg under normal and drought stress irrigation, respectively (Figure 1).

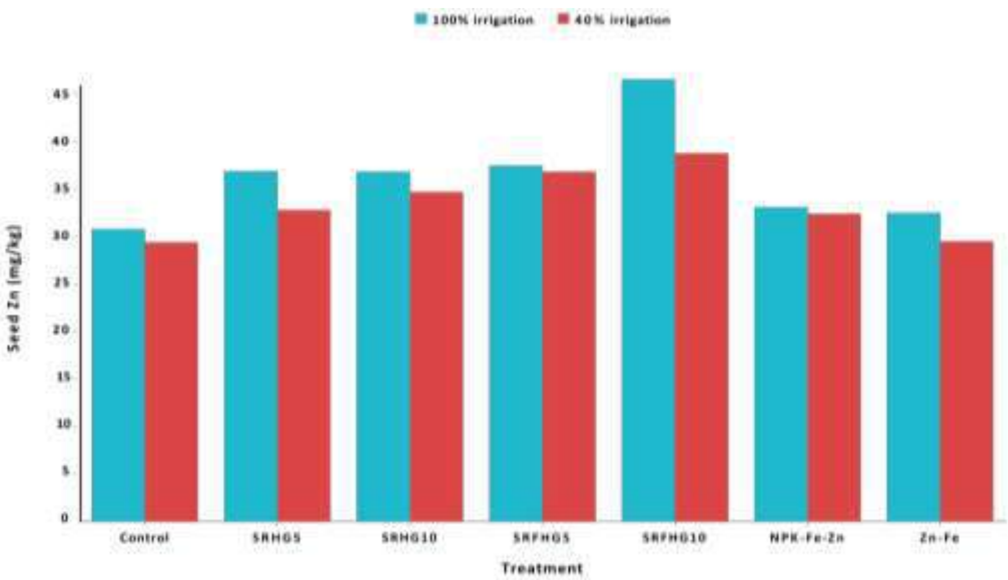

Figure 1. Seed Zn concentration (mg/kg) of mung beans under 100% and 40% irrigation across fertilizer treatments. Bars represent treatment means. SRFHG10 produced the highest seed Zn concentration under both irrigation regimes.

Two-way ANOVA showed that nutrient treatment significantly affected seed Zn concentration ($F = 9.32$, $p < 0.001$), whereas irrigation had a smaller but significant effect ($F = 7.16$, $p = 0.01$). The treatment × irrigation interaction was not significant ($F = 0.82$, $p = 0.57$), indicating that the relative performance of nutrient treatments for seed Zn concentration was broadly consistent across irrigation regimes.

Zinc concentration in stover

Stover Zn concentration was higher than seed Zn concentration across all treatments. The highest stover Zn concentration was obtained with SRFHG10, with values of 59.40 mg/kg under 100% irrigation and 52.00 mg/kg under 40% irrigation, while the control treatment showed the lowest values of 28.90 and 25.80 mg/kg, respectively. Mean stover Zn concentration was 41.24 mg/kg under normal irrigation and 36.10 mg/kg under drought stress (Figure 2).

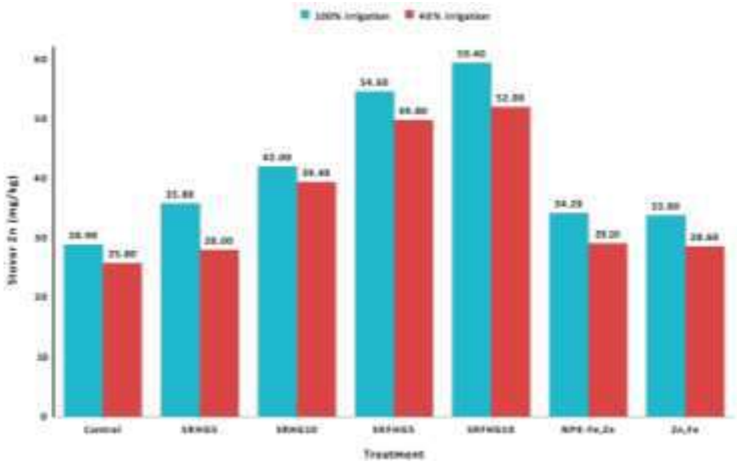

Figure 2. Stover zinc concentration (mg/kg) of mung bean under 100% and 40% irrigation across fertilizer treatments. The bars represent the treatment means. SRFHG10 produced the highest stover Zn concentration, and the reduction under drought stress remained consistent across treatments.

Nutrient treatment had a highly significant effect on stover Zn concentration ($F = 756.26$, $p < 0.001$), irrigation also had a highly significant effect ($F = 277.71$, $p < 0.001$), and the treatment × irrigation interaction was also significant ($F = 5.73$, $p < 0.001$). The coefficient of variation was lower for the stover Zn concentration (2.59%) than for the seed Zn concentration (9.80%), indicating greater precision in the stover measurements.

Comparative Zn biofortification response

A comparative analysis of Zn biofortification showed greater Zn accumulation in stover than in seeds across all fertilizer treatments. The SRFHG10 treatment produced the highest mean Zn concentration in both stover (55.70 mg/kg) and seeds (42.67 mg/kg), suggesting superior Zn uptake and translocation efficiency. In contrast, the control treatment yielded the lowest Zn values, confirming that external Zn supplementation was necessary to improve Zn enrichment in mung bean tissue.

Higher Zn retention in stover than in seeds suggests restricted remobilization of Zn to reproductive tissues, which is consistent with the known physiological limits to Zn transport in edible crops (White & Broadley, 2011). The stronger irrigation effect observed in stover than in seeds also suggests that water availability influenced Zn uptake and storage in vegetative tissue more strongly than final Zn partitioning to seed (Marschner, 2012; White & Broadley, 2009).

Zinc efficiency indices

Zinc use efficiency (ZUE) was highest in SRFHG10 under both irrigation regimes, with values of 38.68 under 100% irrigation and 37.04 under 40% irrigation. Agronomic zinc use efficiency (AZUE) also reached its highest values in SRFHG10, at 11.00 and 10.82 under 100% and 40% irrigation, respectively. In contrast, the Zn and Fe treatments showed negative AZUE values under both irrigation regimes, indicating weak agronomic performance relative to the control.

Internal zinc utilization efficiency (IZUE) ranged from 10.22 to 12.23 under 100% irrigation and from 11.42 to 12.87 under 40% irrigation. The zinc harvest index (ZHI) ranged from 40.7% to 50.8% under 100% irrigation and from 42.5% to 53.9% under 40% irrigation. Based on the target seed Zn concentration of 40 mg/kg, only SRFHG10 achieved the desired biofortification threshold under 100% irrigation.

Discussion

The present findings indicate that cellulose-based SRFHG improved Zn accumulation in both seed and stover relative to non-hydrogel or less effective treatments. The advantage of SRFHG10 under both irrigation regimes is in line with the general role of controlled-release and water-retentive hydrogel systems in improving nutrient availability in the rhizosphere (Jiang et al., 2020; Peng et al., 2016; Tang et al., 2020). The biological importance of enhanced seed Zn concentration is substantial because Zn is essential for crop metabolism and is also a key micronutrient in human diets (Alloway, 2008; Cakmak, 2008).

Although Zn concentration improved in both plant fractions, the stover consistently accumulated more Zn than the seed. This pattern supports previous explanations that physiological barriers limit complete Zn remobilization to seeds, even when the external Zn supply is increased (Waters & Sankaran, 2011; White & Broadley, 2011). The strong irrigation response, especially in the stover, further supports the view that drought restricts nutrient mobility, uptake, and transport in mung bean and related crops (Ranawake et al., 2011; Rashid et al., 2003; Islam et al., 2021).

The superior ZUE and AZUE values of SRFHG10 and SRFHG5 suggest that hydrogel-mediated Zn delivery improved nutrient use efficiency under both normal and moisture-stressed conditions. The negative AZUE values in the Zn and Fe treatments may indicate nutrient antagonism or ineffective Zn availability, which agrees with the broader concept of nutrient interaction effects in crop plants (Fageria, 2001; White & Broadley, 2009). Overall, these findings support the use of hydrogel-based micronutrient delivery as a practical strategy for improving Zn biofortification in mung beans, although further validation across seasons, soils, and genotypes is still needed.

Conclusion

Cellulose-based slow-release hydrogel fertilizer substantially improved Zn biofortification of mung bean under both normal and drought-stress irrigation regimes. Among the evaluated treatments, SRFHG10 produced the highest Zn concentration in seeds and stover and showed the strongest Zn use efficiency. However, drought stress reduced seed Zn concentration and prevented most treatments from reaching the biofortification target of 40 mg/kg. These results demonstrate that hydrogel-mediated Zn delivery is a promising approach for improving micronutrient enrichment in mung bean; however, further optimization is required to achieve stable performance under water-limited conditions.

Declarations

Conflict of Interest: The authors declare no conflict of interest

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Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable requests.

Ethics approval: Not applicable for a plant-based pot experiment, unless required by the host institution.

Tables

Table 1. Nutrient sources and hydrogel loading were used for mung bean cultivation.

Nutrient source	Product (%)	Field rate (lb/A)	Amount added to

			hydrogel (g/pot)
ZnSO4	5.6% Zn	25	0.08

Table 2. Zinc concentration in mung bean seeds under normal and drought irrigation conditions.

Treatment	100% irrigation	40% irrigation	Mean
Control	30.80	29.40	30.10 g
SRHG5	36.90	32.80	34.85 d
SRHG10	36.83	34.70	35.77 c
SRFHG5	37.47	36.83	37.15 b
SRFHG10	46.57	38.77	42.67 a
NPK-Fe,Zn	33.10	32.40	32.75 e
Zn,Fe	32.50	29.50	31.00 f
Mean	36.31	33.49	-

Means followed by different letters differ significantly at $p \leq 0.05$.

Table 3. Zinc concentration in mung bean stover under normal and drought irrigation conditions.

Treatment	100% irrigation	40% irrigation	Mean
Control	28.90	25.80	27.35 g
SRHG5	35.80	28.00	31.90 d

SRHG10	42.00	39.40	40.70 c
SRFHG5	54.60	49.80	52.20 b
SRFHG10	59.40	52.00	55.70 a
NPK-Fe,Zn	34.20	29.10	31.65 e
Zn,Fe	33.80	28.60	31.20 f
Mean	41.24	36.10	-

Means followed by different letters differ significantly at $p \leq 0.05$.

Table 4. Two-way ANOVA summary for zinc concentration in seeds and stover

Variable	Source of variation	df	SS	MS	F-value	p-value
Seed Zn	Nutrient	6	654.058	109.010	9.32	0.00
Seed Zn	Irrigation	1	83.726	83.726	7.16	0.01
Seed Zn	N × I	6	57.352	9.559	0.82	0.57
Stover Zn	Nutrient	6	4537.556	756.259	756.26	0.00
Stover Zn	Irrigation	1	277.714	277.714	277.71	0.00
Stover Zn	N × I	6	34.376	5.729	5.73	0.00

Note: The error mean square was 11.695 for seed Zn and 1.000 for stover Zn; the CV was 9.80% and 2.59%, respectively.

Table 5. Zinc efficiency indices under normal and drought irrigation conditions.

Treatment	ZUE 100%	ZUE 40%	AZUE 100%	AZUE 40%	IZUE 100%	IZUE 40%	ZHI 100%	ZHI 40%
SRHG5	28.75	27.39	1.07	1.18	11.07	12.61	50.8	53.9
SRHG10	34.43	34.07	6.75	7.86	12.23	12.87	46.7	46.8
SRFHG5	38.36	35.86	10.68	9.64	11.66	11.59	40.7	42.5
SRFHG10	38.68	37.04	11.00	10.82	10.22	11.42	43.9	42.7
NPK-Fe,Zn	28.54	26.61	0.86	0.39	11.87	12.11	49.2	52.7
Zn,Fe	26.55	25.18	-1.14	-1.04	11.21	12.13	49.0	50.8

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