Advanced coating on *Zea mays* seeds using modified hydroxyapatite nanoparticles as a plant nutrient delivery system for enhanced plant growth

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The deficiency of nutrients during the early plant growth stages diminishes plant development, leading ultimately to lower crop yields. In this context, nanotechnology has been used to develop advanced seed coatings with sustainable and precise release properties to deliver supplements to the plant effectively. Zinc-doped hydroxyapatite–urea nanoparticles were synthesized and incorporated into a seed coating. This system enhanced plant growth, yield and root–shoot nutrient content in *Zea mays* seeds. The nanohybrid is futuristic as a macro–micro plant nutrient delivery agent and opens up new opportunities to explore the suitability of metal-doped hydroxyapatite nanoparticles in agriculture.

Keywords: Agriculture, enhanced growth and yield, hydroxyapatite nanoparticles, maize, seed coatings.

THE quality of agricultural products must be maintained through advanced technological practices to achieve sustainability[1]. A plant needs nutrients even before emergence. Among the many available methods, seed coating is an efficient carrier of nutrients. It is more effective than applying nutrients to the soil since it can act as a protective outer covering for the seeds[2–4]. Healthy growth of a plant and an optimal yield level can be obtained by providing nutrients in appropriate quantity in usable form at the precise time. However, farmers experience difficulty in providing nutrients precisely because of the inherent drawbacks of traditional fertilizers[1]. One such example is the premature decomposition and volatilization of urea, providing a low amount of nitrogen (N) to the plants[5]. This can result in a primary nutrient deficiency affecting photosynthesis, all enzymatic reactions and eventually crop yield[6,7]. Therefore, developing effective fertilizers with precise and delayed-release properties is important.

In this study, we focus on maize seed coating. In 2020, it was projected that there would be a million tonnes of maize produced globally, that has numerous uses[8–10]. Currently, the worldwide maize crop yields show a decrease in quantity as a result of micro-nutrient deficiency, especially Zn, which is involved in water uptake and plant reproduction while also being an enzyme cofactor[11,12]. Although the plant requirement of this micronutrient is minute, its deficiency can cause drastic effects such as depression in plant growth, yield and quality of the crop[13]. These issues underscore the necessity of novel, precise and environment-friendly techniques to deliver plant nutrients in adequate amounts.

Our previous work led to the identification of hydroxyapatite (HA) nanoparticles not only as a plant nutrient delivery vehicle, but also as a competent candidate to minimize urea loss in the soil[5,14–18]. HA nanoparticles are an excellent carrier matrix for urea and zinc along with the impressive ability to act as a phosphorus (P) source, which is a primary nutrient important for early root development.

Importantly, we recently developed a novel seed coating incorporating Zn-doped HA–urea nanoparticles to deliver macro–micro plant nutrients[19,20]. This nano-seed coating demonstrated a significant escalation in the growth, development and germination rate of *Zea mays* L. (maize) at the seedling stage by enhancing seed germination by 69%, highlighting its suitability for effective delivery of Zn, N and P to the plant at the seedling stage. In the present study, pot trials were carried out using the coated maize seeds to determine how their germination success reported earlier translates to higher crop yield. It was concluded that potassium and nitrogen have a remarkable effect on trace element accumulation of maize plants[21]. Moreover, enhanced uptake of Zn upon the addition of a P-containing

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Table 1. Fertilizer recommendation for maize cultivation by the Department of Agriculture (DOA), Sri Lanka

Time of application	Fertilizer recommendation by DOA for irrigated cultivation of corn in Sri Lanka			Calculated amount as per pot		
	Urea (kg/ha)	TSP (kg/ha)	MOP (kg/ha)	Urea (g/pot)	TSP (g/pot)	MOP (g/pot)
Before planting	75	100	50	2	3	1
Four weeks after planting	250			7		
Total	325	100	50	9	3	1

TSP, Triple super phosphate; MOP, Muriate of potash.

compound was demonstrated[22]. Therefore, the literature supports the importance of this triple-nutrient system in enhancing plant growth. The novelty of this study is the application of zinc-doped HA–urea nanohybrid as a plant nutrient delivery system.

Materials and methods

Materials

All analytical-grade reagents and chemicals were purchased from Sigma-Aldrich, USA and were used without further purification. Distilled water was used for solution preparation.

Preparation of seed coatings for maize seeds

Seeds of maize with a germinability of 75% were used to test the effectiveness of the seed coating material on plant growth and reproduction. Seeds were sterilized for 20 min in a 10% sodium hypochlorite solution to eliminate dust and microbial contaminants from the seed surface. The seeds were then rinsed in distilled water.

Sodium alginate solution (10% (w/v), 20.00 ml) was mixed with 10.00 ml of HA nanoparticles. Then, the HA–sodium alginate mixture was added to a carboxymethyl cellulose (CMC) solution (1.6%, 75 cm^3) and stirring continued for 2 h to obtain the polymer–HA mixture. Seed coating was applied by dipping sterilized maize seeds (0.30 ± 0.05 g) in the polymer–HA mixture, then immersing them in a 1.0 mol dm^{-3} calcium chloride solution and drying them for 48 h at 35°C in a vacuum oven. Similarly, instead of using the 10.00 ml solution of HA nanoparticles, Zn doped HA, HA–urea, and Zn-doped HA–urea nanoparticle suspensions were prepared[19]. The preparation of Zn-doped HA–urea nanoparticles was carried out by adding phosphoric acid (0.60 mol dm^{-3}, 50.00 cm^3) dropwise into a suspension of 3.81 g of calcium hydroxide and 0.50 g of urea in 50.00 cm^3 distilled water while stirring at 500 rpm over 30 min. The mixture was further stirred for 1 h. A solution of Zn^{2+} (0.50 mol dm^{-3}, 10.00 cm^3) was added to the above mixture and stirring continued for 2 h followed by drying at 50°C for 48 h. Finally, another seed coating was prepared using sodium alginate and CMC solutions without using any nanoparticles, followed by dipping in $CaCl_2$ solution.

Pot experiment

Pot experiments were carried out from December 2020 to March 2021 in the Western Province, Sri Lanka. Maize seeds of the ‘Bhadra’ variety from the Department of Agriculture, Sri Lanka, were used. Evenly coated, undamaged seeds were placed in clean plastic pots (30 cm diameter; 16 cm high) with 5000 g of soil (Typic Plinthudultsin *Kalutara*) in each pot. Identifiable litter, woody debris and roots in the soil were removed by hand, then dried well and sieved using a 2 mm sieve and homogenized before the experiment.

Coated seeds were established in the completely randomized design with six replicates (three seeds for each replicate) for each treatment. After germination (at week 2), two plants were thinned out, leaving the healthy plant in the pot. The fertilizer application was done according to the recommendation of the Department of Agriculture, Sri Lanka (Table 1). Initial soil pH and electrical conductivity were measured as 6.7 and 1.69 mS cm^{-1} respectively. The temperature was recorded as 25°C at 74% humidity. All the pots were treated similarly, including N, P and K fertilizer application and watering. The microelement solution (50 cm^3) was supplied to all the experimental pots after 28 days of seed sowing, as recommended[23].

Vegetative growth measurements

Plant height was measured weekly after planting, from the ground surface to the end of the longest leaf tip of each maize plant. Stem girth was measured 10 cm above the ground level using a Vernier caliper. The number of unrolled leaves was estimated weekly after planting.

Yield measurements

After completing their life cycle at 12 weeks, the plants were carefully removed from the soil and cleaned. Harvested shoots, seeds and roots were evaluated separately. The average dry weights were obtained after the plants had been dried in an oven at 60°C for 48 h. The number of seeds per

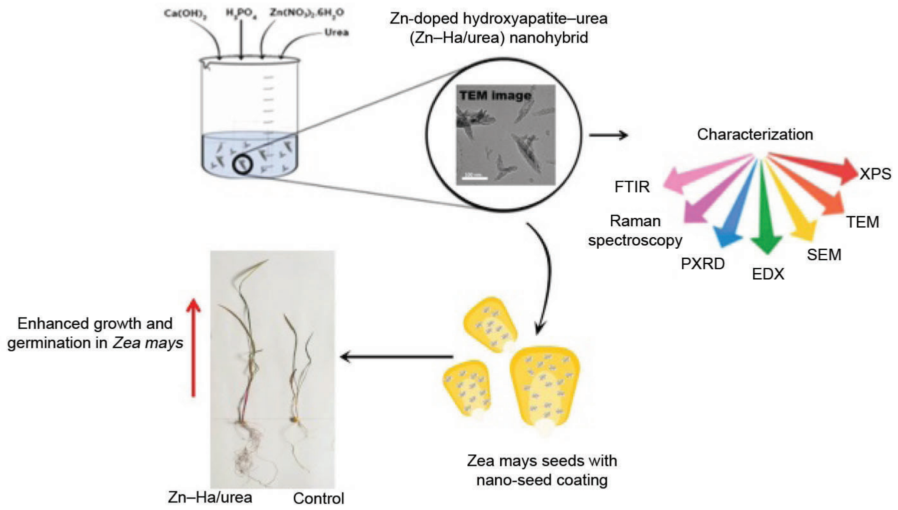

Figure 1. Graphical representation of synthesis and characterization of Zn-doped HA–urea nanohybrid and its use in enhanced germination and seedling growth.

cob, cob diameter, cob length, total dry weight and biomass of each plant were estimated for maize. Harvested seeds were sun-dried up to 10% of moisture by thoroughly mixing once for two days. Then the thousand-grain weight was measured by weighing 100 seeds and multiplying by 10 to obtain the mass of 1000 grains[24].

Root–shoot nutrient analysis

Plant root and shoot samples were oven-dried separately at 72°C to a constant weight. Then, the N and C contents of the finely powdered root and shoot samples were analysed in triplicates (using Perkin-Elmer 2400 Series II CHNS/O Analyzer).

Statistical analysis

The collected data were statistically analysed as a completely randomized block design. Data were subjected to analysis of variance (ANOVA) using the statistical analysis system (SAS) software program. Mean separation was done using Duncan's multiple range test (MRT), and *P*-values <0.05 were considered statistically significant.

Results and discussion

The effectiveness of the modified HA nanoparticles as a plant nutrient was comprehensively studied structurally and morphologically; the structural features have been discussed in our previous publications[19,20]. Figure 1 illustrates the synthesis and detailed characterization of the nanohybrid, its utilization as a seed coating on maize seeds, and the resulting enhanced germination and seedling growth. It is anticipated that when nutrients are coated on the seed, they will completely enter it and become available for the growth functions of the plant instead of draining into the soil. A soil leaching experiment was conducted to test this, and the results have been emphasized in our previous study[19]. The leachate of Zn-doped HA–urea coating contained a negligible amount of nitrogen, and there was no indication of Zn discharging into the soil at the parts per billion level from any of the seed covers. The results specify that all of the N and Zn in the coatings were efficiently utilized for the growth and development of the seed rather than penetrating the soil. This study provides evidence for the efficacy of seed coating on the growth, development and crop production in *Z. mays*. Initial soil pH and electrical conductivity were measured as 6.7 and 1.69 mS cm^{-1} respectively. Figure 2 shows the weekly growth of maize plants. At week 3, just after germination, there was a noticeable increase in the plant height (70 cm) of Zn-doped HA–urea-treated seeds compared to the control (27 cm). Similarly, a 50% rise in the plant girth was observed at week 3 with Zn-doped HA–urea seed covering.

The results of the pot experiment with maize seeds showed a significant increase of 60% in plant height ($P < 0.0001$) in Zn-doped HA–urea-coated seeds (139.3 cm) compared to the control at the twelfth week (Figure 3 *a*). Notably, there was no significant difference between the control (87.2 cm) and seeds coated with just the alginate and CMC biopolymeric mixture without nanoparticles (87.1 cm).

Considering Figure 3 *b* at $P = 0.0003$, it was noted that the plant girth of Zn-doped HA–urea coating was higher (4.3 cm) compared to the control and other treatments. Moreover, the number of average unrolled leaves indicated that Zn-doped HA–urea-coated seeds showed the highest value (19 leaves), while all the other treatments and control had no significant difference among them ($P < 0.0001$) (Figure 3 *c*). Figure 3 *d* compares average root length measurements of maize plants. The plant received all three

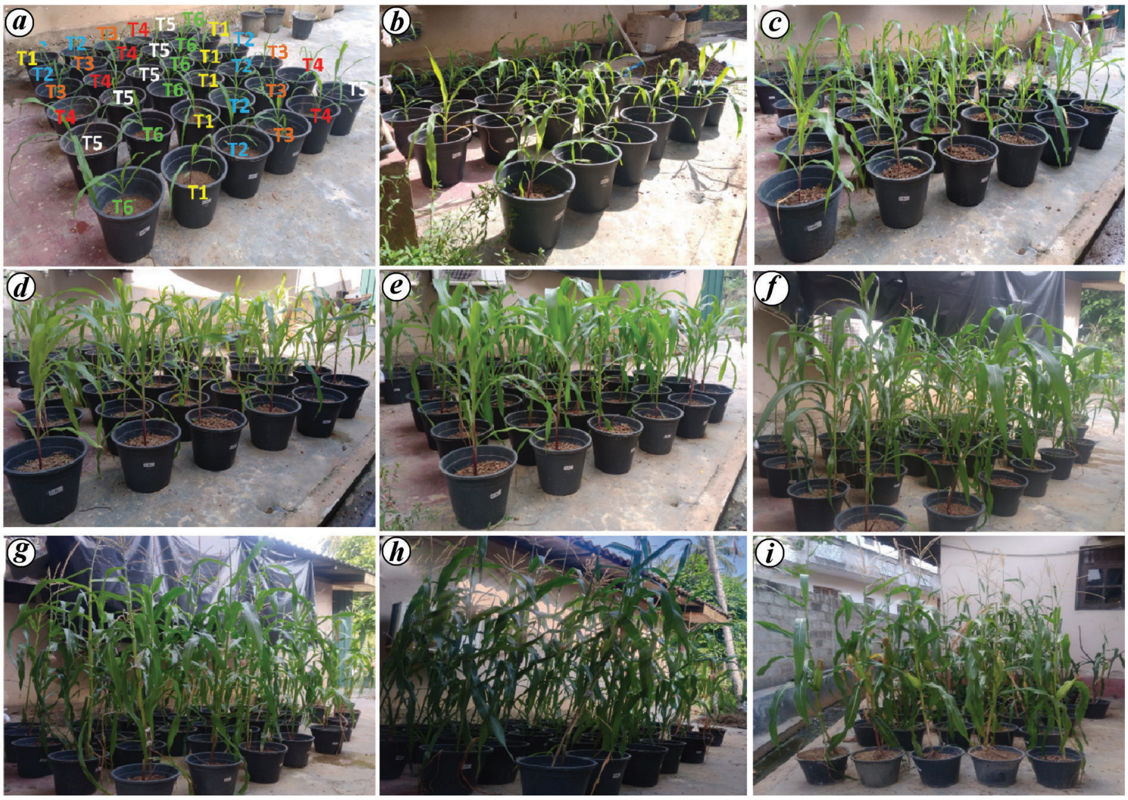

Figure 2. Images of plants at week (***a***) 3, (***b***) 4, (***c***) 5, (***d***) 6, (***e***) 7, (***f***) 8, (***g***) 9, (***h***) 10 and (***i***) 11 after planting.

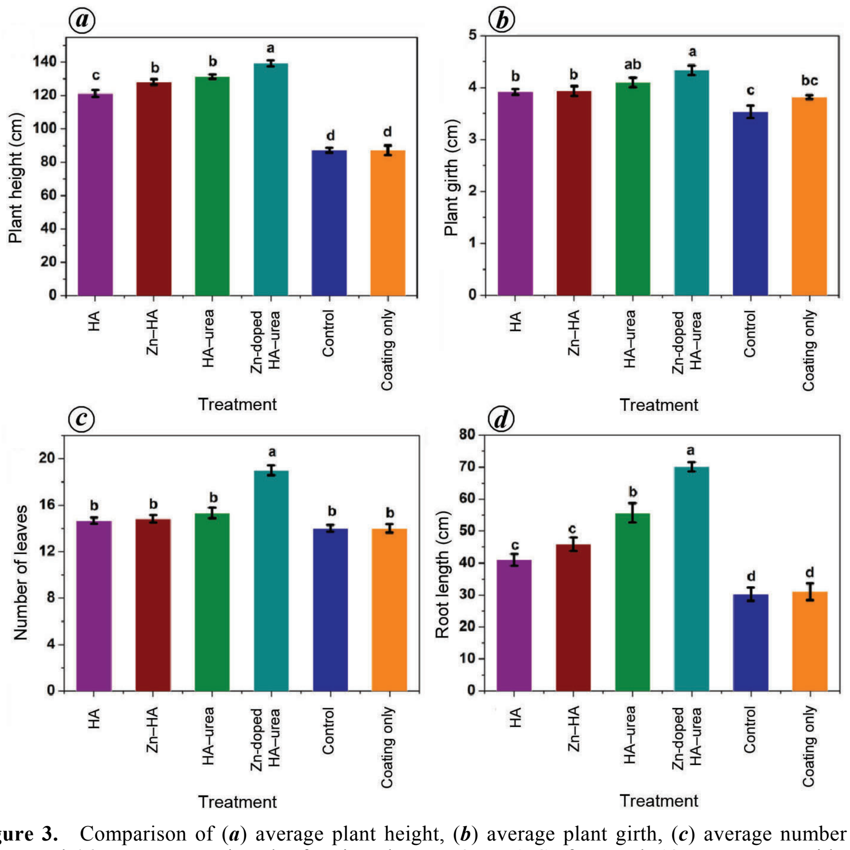

Figure 3. Comparison of (***a***) average plant height, (***b***) average plant girth, (***c***) average number of leaves and (***d***) average root length of maize plants at 25° ± 1°C after week 12. Treatments with the same letter above the bars are not significantly different in their means at $P > 0.05$.

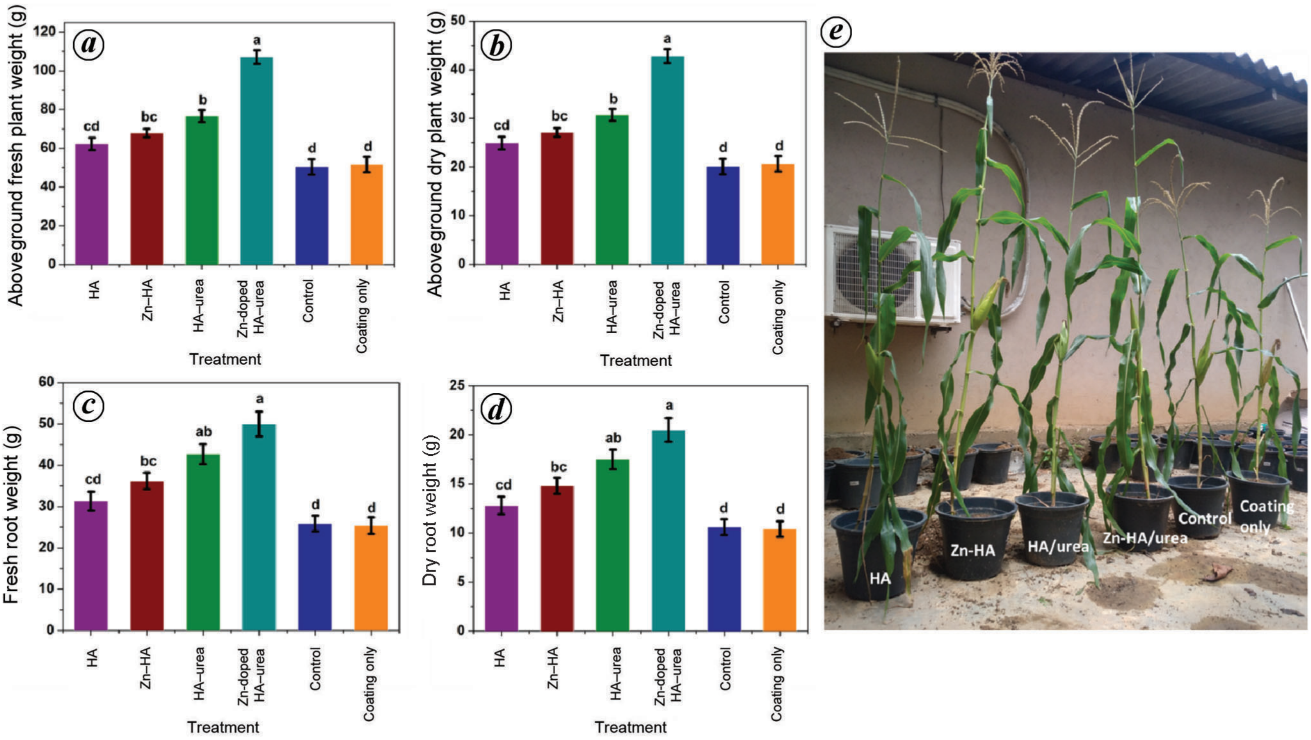

Figure 4. Comparison of (***a***) aboveground fresh plant weight, (***b***) aboveground dry plant weight, (***c***) fresh root weight and (***d***) dry root weight of maize plants. (***e***) Images of maize plants at 25° ± 1°C after week 12. Treatments with the same letter above the bars are not significantly different in their means at $P > 0.05$.

supplements at its early growth stage (Zn-doped HA–urea) and displayed the highest root length (70.1 cm) at $P < 0.0001$.

The average aboveground fresh plant (shoot) weight showed the highest value with an increase of 112.5% for Zn-doped HA–urea-coated seeds compared to the control at $P < 0.0001$ (Figure 4 *a*). After drying the plants, Zn-doped HA–urea treatment showed the highest dry shoot weight of 42.8 g compared to other treatments, while the control exhibited the lowest dry shoot weight (20.1 g) at $P < 0.0001$ (Figure 4 *b*).

Importantly, the cob length of the Zn-doped HA–urea seed treatment showed the highest significant value of 15.0 cm with a 56% increment analogous to control at $P < 0.0001$ (Figure 5 *a* and as confirmed by Figure 5 *f*). However, no significant difference in cob diameter was observed between the treatments and control (Figure 5 *b*). Promisingly, the pot experiment further revealed that the Zn-doped HA–urea coated seeds exhibited the highest total seed weight per pot of 87 g (Figure 5 *c*) and thousand-grain weight of 183 g (Figure 5 *d*), highlighting the effectiveness of the Zn-doped HA–urea treatment compared to the control at $P = 0.002$. All growth parameters and yield parameters revealed no significant difference between the control and seeds that had just been covered with the polymeric coating mixture without nanoparticles. This confirms that there is no considerable effect of the coating substance on seed growth.

Since the grain production of maize is dependent on the availability of carbon and nitrogen, we analysed root–shoot C and N contents[25]. As indicated in Figure 6, the highest root–shoot N and C contents were observed in the Zn-doped HA–urea treatment, while the control showed the lowest at a 95% confidence interval.

One of the most crucial steps in the plant life cycle is seed germination, suggesting the importance of making nutrients available at the early growth stage. In this study, Zn-doped HA–urea seed coating exhibited the highest growth and yield among all treatments and the control. According to the literature, many important parameters affect the rate of leaf emergence, such as soil strength, depth of sowing and nutrition availability[26,27]. Among them, expanding the accessibility of supplements, especially N, plays an important role in the appearance of leaves. Considering earlier observations, it can be assumed that the plant received N along with P and Zn, resulting in an elevated number of leaves compared to the other treatments, which did not contain the above three in the same mixture. Moreover, the highest root length was represented by the plant receiving triple nutrients, indicating a synergistic effect of incorporating urea (N) and Zn into the HA matrix that originally accommodated P, a vital element for the root development of a plant, as mentioned in the literature[28,29]. The extent of the root system can corroborate the shoot weight observations since plants tend to equilibrate the

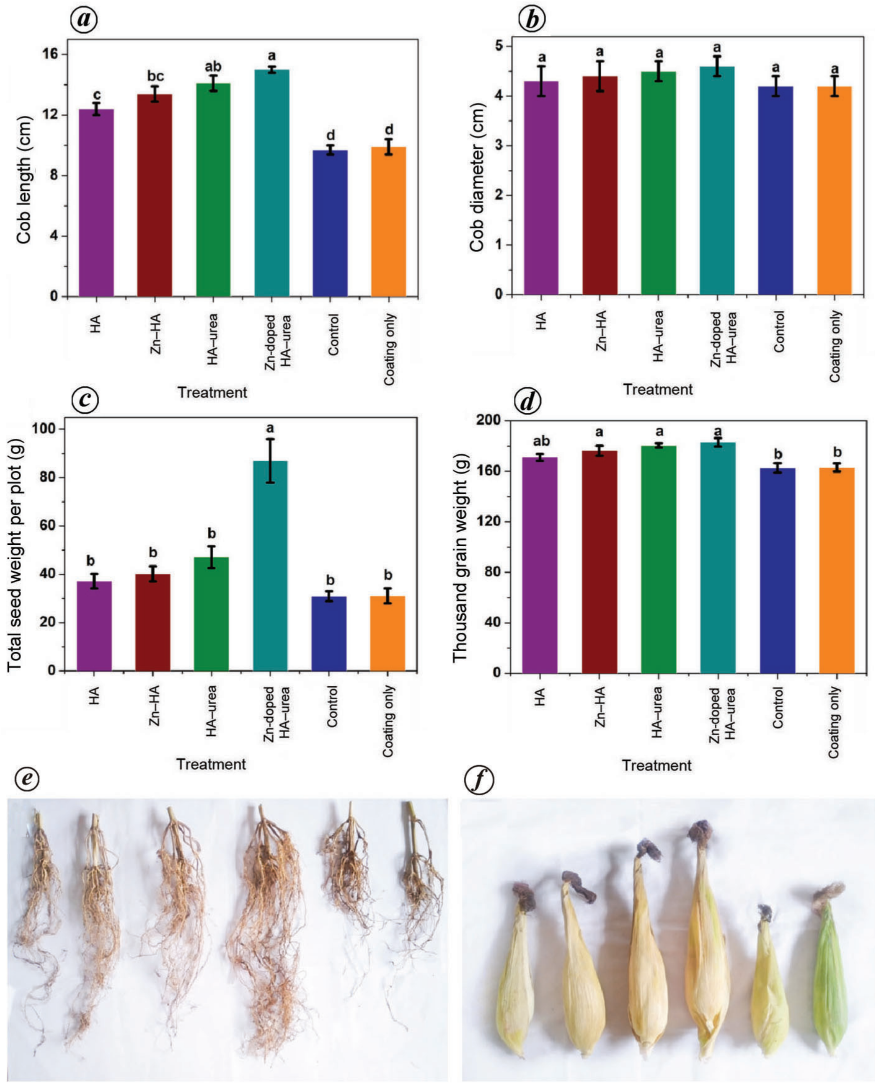

Figure 5. Comparison of (***a***) cob length, (***b***) cob diameter, (***c***) total seed weight per pot and (***d***) thousand-grain weight. (***e***, ***f***) Images of (***e***) roots and (***f***) cobs at 25° ± 1°C after week 12. Treatments with the same letter above the bars are not significantly different in their means at $P > 0.05$.

growth of shoots and roots[30]. These outcomes suggest that the seed coating comprising Zn-doped HA–urea nanoparticles resulted in the highest plant growth (Figure 4 *e*) and efficient development of the root system (Figure 5 *e*), which discharged all three vitamins concurrently compared to the other treatments.

Further, the experimental results captured at week 3, just after germination, indicated an increase in the growth parameters demonstrating the necessity of supplementing plants early in their growth. In the present study, the plant that received all three nutrients at the germination stage exhibited the highest root–shoot nutrient content.

These observations suggest that the Zn-doped HA–urea seed treatment is an effective method to supply plant nutrients in the early development phase, which eventually helps boost the yield. The Zn-doped HA–urea-coated seeds showed the highest cob length, cob diameter, total seed weight per pot, thousand-grain weight and root–shoot nutrient availability, validating the effectiveness of seed amendment.

Conclusion

Zinc-doped HA–urea nanohybrid can be considered a competitive solution to overcome common deprivation problems associated with urea and the dwindling of natural phosphorus stocks while supplying Zn to the plant. Furthermore, the biodegradability and biocompatibility of HA make it much less harmful to the environment than routine P fertilizers, while retaining its multifunctional properties. The versatility of HA nanoparticles effectively addresses the solubility issues leading to phosphorus and urea deprivation in the soil associated with traditional fertilizers, along with the high cost of zinc fertilizers. Therefore, structurally

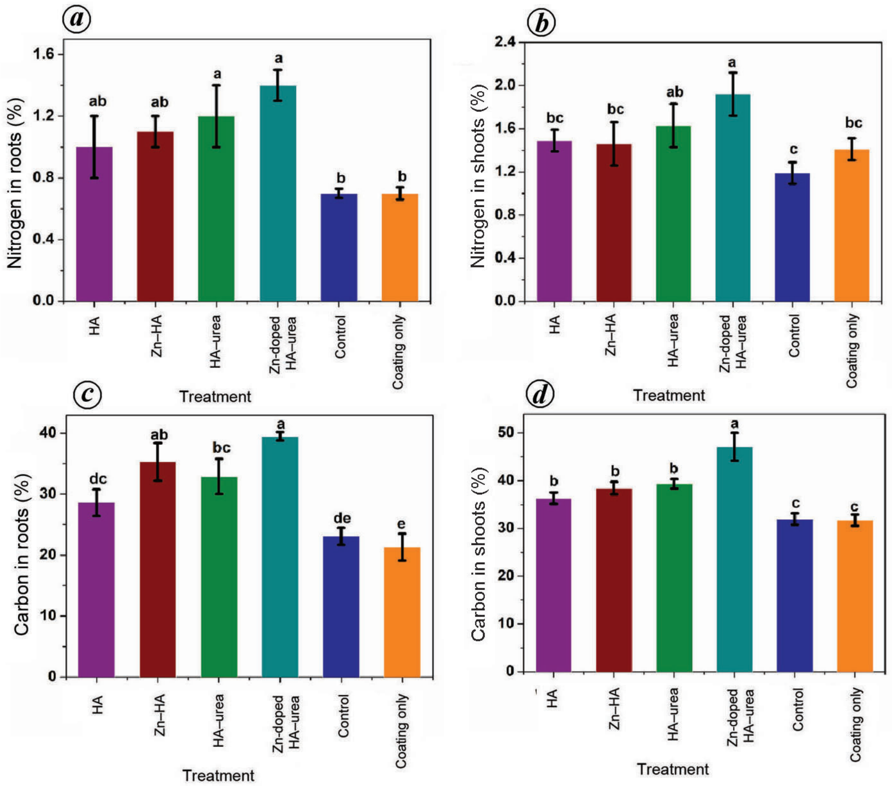

Figure 6. Comparison of nitrogen content in the roots (***a***) and shoots (***b***), and carbon content in the roots (***c***) and shoots (***d***) of maize plants at 25° ± 1°C after week 12. Treatments with the same letter above the bars are not significantly different in their means at $P > 0.05$.

reshaped HA could be the perfect carrier molecule to dispatch fundamental fertilizer supplements during the early phases of plant development. The seed amendment is noteworthy execution in maize by significantly enhancing its growth, development and yield root–shoot nutrient distribution. The outcomes of this study highlight the effectiveness of this nanocoating in the successful supply of N, P and Zn to the maize plant.

Conflict of interest: The authors declare no competing financial interest.

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