

Utilizing Chitosan Nanoparticles To Deliver Natural Growth Promoters To Optimize Seed Germination In Glycine Max

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Abstract:

This project aims to create biodegradable nanoparticles that deliver zinc ions to the soybean plant using a slow-release mechanism, allowing for accelerated seed germination without introducing synthetic materials into the environment and limiting overfertilization of agricultural land.

Nanoparticles were formed using Ionic Gelation through an alginate pre-gel matrix. Once the nanoparticle solution was formed, the solution was then diluted to concentrations of 0.25, 0.5, and 1 mg/mL for further testing. Nanoparticle supplementation promoted shorter, thicker root growth in Glycine Max versus non-supplemented plants, suggesting improved zinc uptake as traditionally applied zinc fertilizers leach through the soil, requiring plants to develop longer roots to access it. Optimal growth was seen at 0.25 mg/mL, where plants doubled root quantity, increased root density by 74%, and shoot length by 25% under normal conditions. Under drought and nutrient deficient conditions, 0.25 mg/mL plants grew similarly to control conditions, suggesting nanoparticles improve water conservation within the plant and uptake of nutrients present in the soil ecosystem.

1. Introduction

Currently, 600 million farms are feeding 8 billion people around the world. [1] By the end of the century, we will have ½ as many farms feeding double the amount of people. The current solution that farmers employ is to provide more fertilizers to plants, yet that results in fertilizers contaminating local water supplies. Therefore I aim to find a medium where farmers can improve crop yield, yet not farm the environment. Nanoparticles, especially in the agriculture industry, have gained prominence over the years as an efficient growth supplement. Therefore this study aims to examine if these same nanoparticles can be utilized as a vessel for nutrient delivery in an eco-friendly manner.

2. Background

Nanoparticles have played a prominent role in drug delivery. In such applications, drugs are attached to the nanoparticle surface and are then guided by an external magnetic field to the disease site. [2] The slow-release mechanism of these nanoparticles ensures the drug's gradual entry into the bloodstream, targeting the specific site of the disease for effective treatment.

While drug delivery research has been extensive, the exploration of nanoparticles in other industries like agriculture hasn't matched the depth seen in the pharmaceutical sector. The bulk of research has concentrated on initial interactions between plants and nanoparticles, ensuring there aren't any adverse toxic effects.[3] However, chitosan-based nanoparticles have been promising due to their biodegradable nature, as microorganisms slowly degrade the nanoparticle matrix, initiating the slow-release mechanism. The loading material, zinc, promotes the formation of various essential enzymes like Carbonic Anhydrase. Not to mention Chitosan itself has positive effects on plant growth by initiating defense responses that protect against harmful pathogens, promoting overall nutrient uptake, and upregulating genes involved in the chlorophyll production pathway, leading to more efficient photosynthesis. [4]

3. Methods

3.1. Pre Gel Matrix

Nanoparticle formation was facilitated through an Ionic Gelation method which consists of the formation of an alginate pre-gel matrix. This was performed through the addition of 134 milligrams of Calcium Chloride and 600 milligrams of Sodium Alginate in 240 mL of Distilled Water. The ionic cross-linking of the alginate solution with the Calcium cation will form a gel that will be used for the formation of nanoparticles. This solution should be stirred in a magnetic stirrer for 30 minutes.

3.2. Fertilizer Loading

After 30 minutes create a 1% solution with 20 milligrams of Zinc Sulphate in 200 mL of Distilled Water. Gently add this into the alginate pre-gel solution and then stir in a magnetic stirrer for another 30 minutes. The fertilizer is now loaded into the pre-gel matrix.

3.3. Chitosan Layering

Create a 80 mL solution by adding 64 mg of Chitosan in 80 mL of Distilled Water. This is performed to have the Chitosan biomolecule coat the alginate gel that was previously made. This should be stirred in a magnetic stirrer for 60 minutes. Once the time is up turn off the stirrer and let the solution equilibrate overnight.

3.4. Nanoparticle Stabilization

Once the solution has finished equilibrating, the gel should settle at the bottom of the solution. Extract this gel and wash it two times with distilled water so excess ions are removed from the solution. Re-disperse the gel into water and in the magnetic stirrer at 2 grams of gum arabic into the solution which will stabilize the nanoparticles in water. Let it equilibrate overnight once again and once it settles to the bottom wash off excess gum arabic and redisperse in water for dilution.

3.4. Dilution & Testing Procedure

Dilute the solution into three separate concentrations of 0.25 mg/mL, 0.5 mg/mL, and 1.0 mg/mL. Create four groups of twelve plants, and in each group, there is a control with no nanoparticle supplementation and each of the three concentrations. Each group is tested for something different, one being to see if nanoparticles can improve water uptake and the other to see if nanoparticles indeed deliver zinc to the plant.

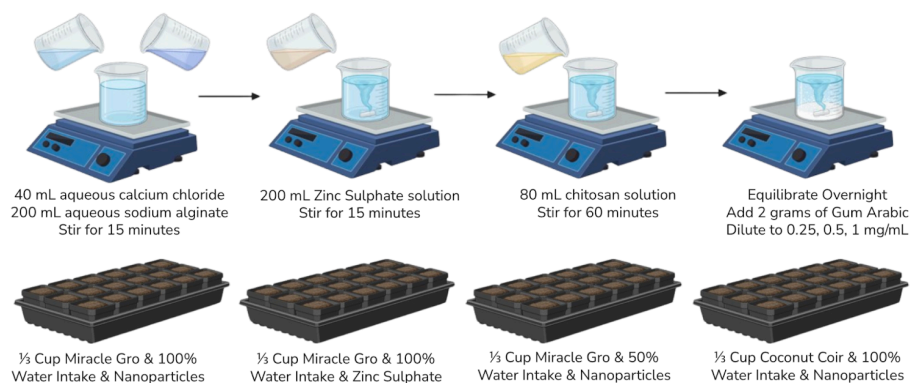


Figure 1: Roadmap of the Production Process and Testing Procedure

4. Results

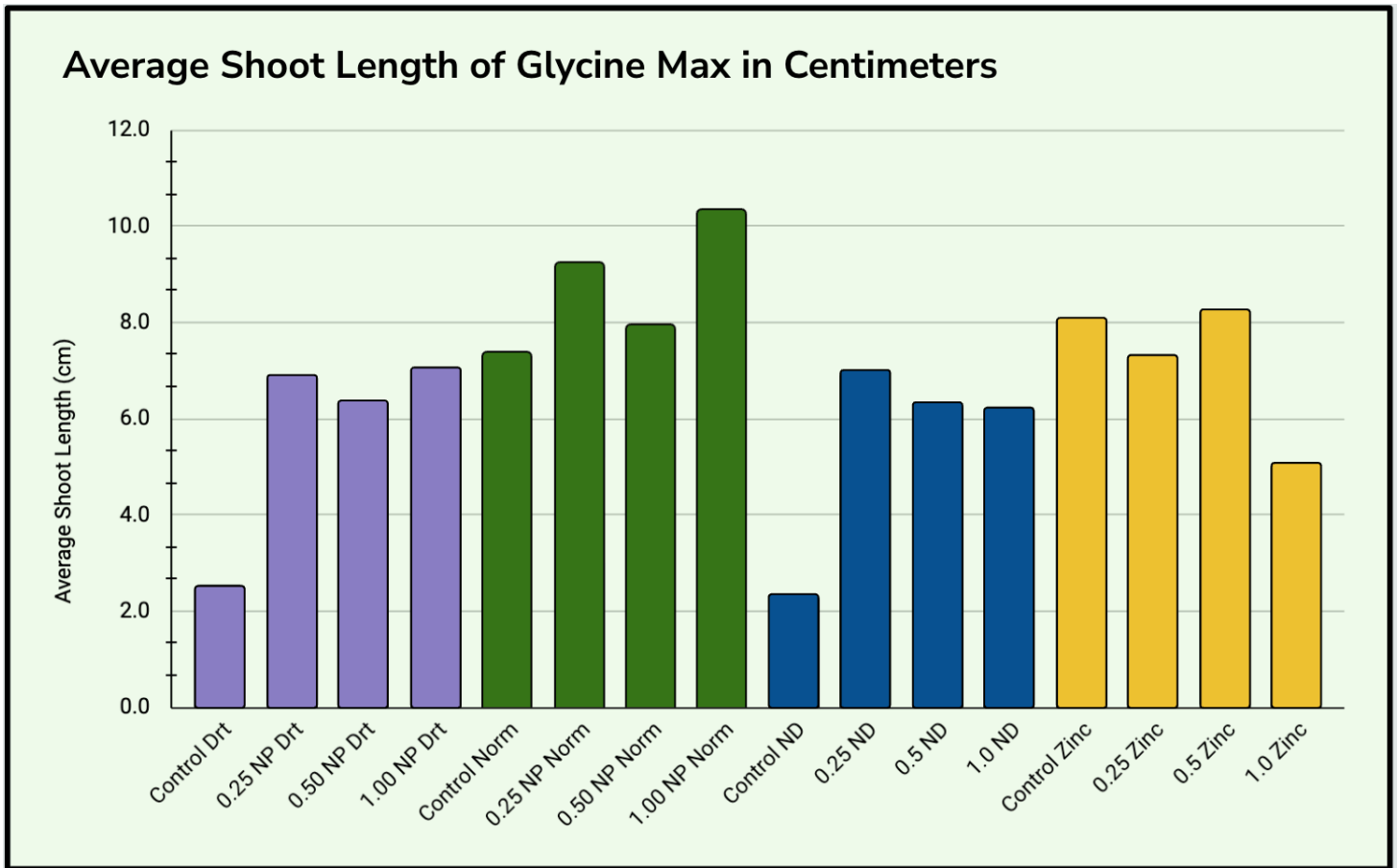


Figure 2: The Average Shoot Length of Glycine Max in Drought Conditions, Normal Conditions, Nutrient Deficient Conditions, and Zinc Supplemented Conditions.

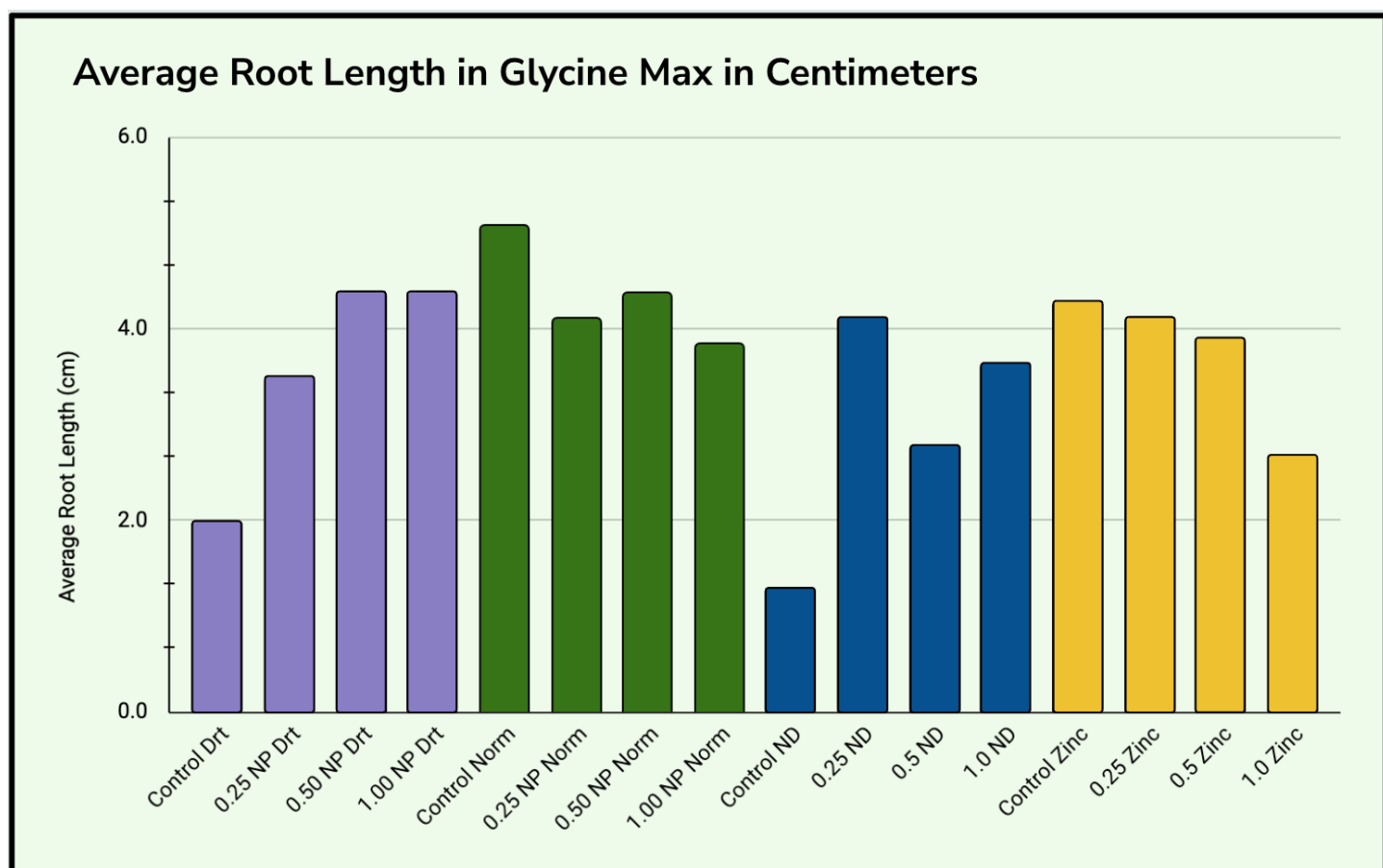


Figure 3: The Average Root Length of Glycine Max in Drought Conditions, Normal Conditions, Nutrient Deficient Conditions, and Zinc Supplemented Conditions.

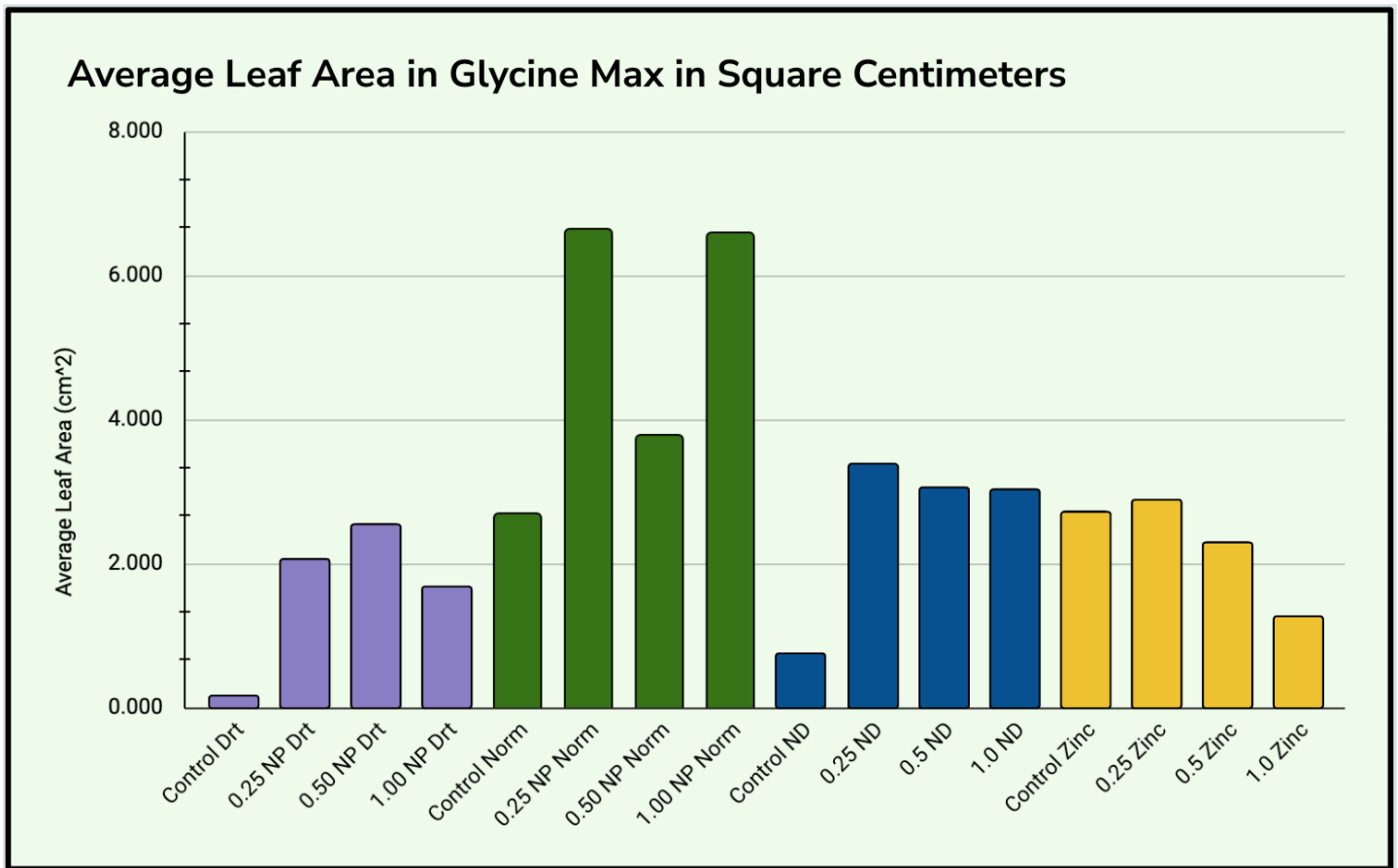


Figure 4: The Average Leaf Area of Glycine Max in Drought Conditions, Normal Conditions, Nutrient Deficient Conditions, and Zinc Supplemented Conditions.

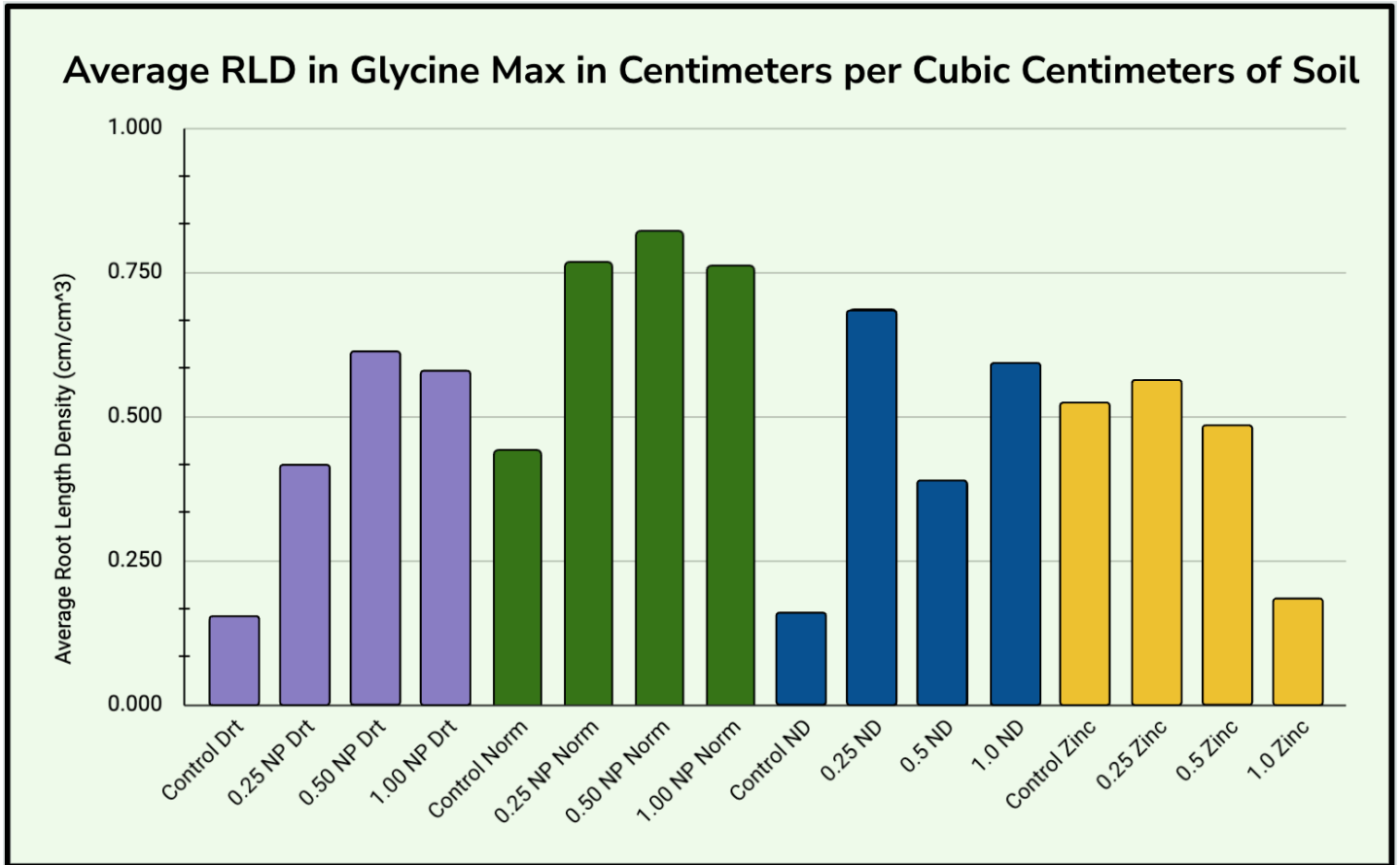


Figure 5: The Average Root Length Density of Glycine Max in Drought Conditions, Normal Conditions, Nutrient Deficient Conditions, and Zinc Supplemented Conditions.

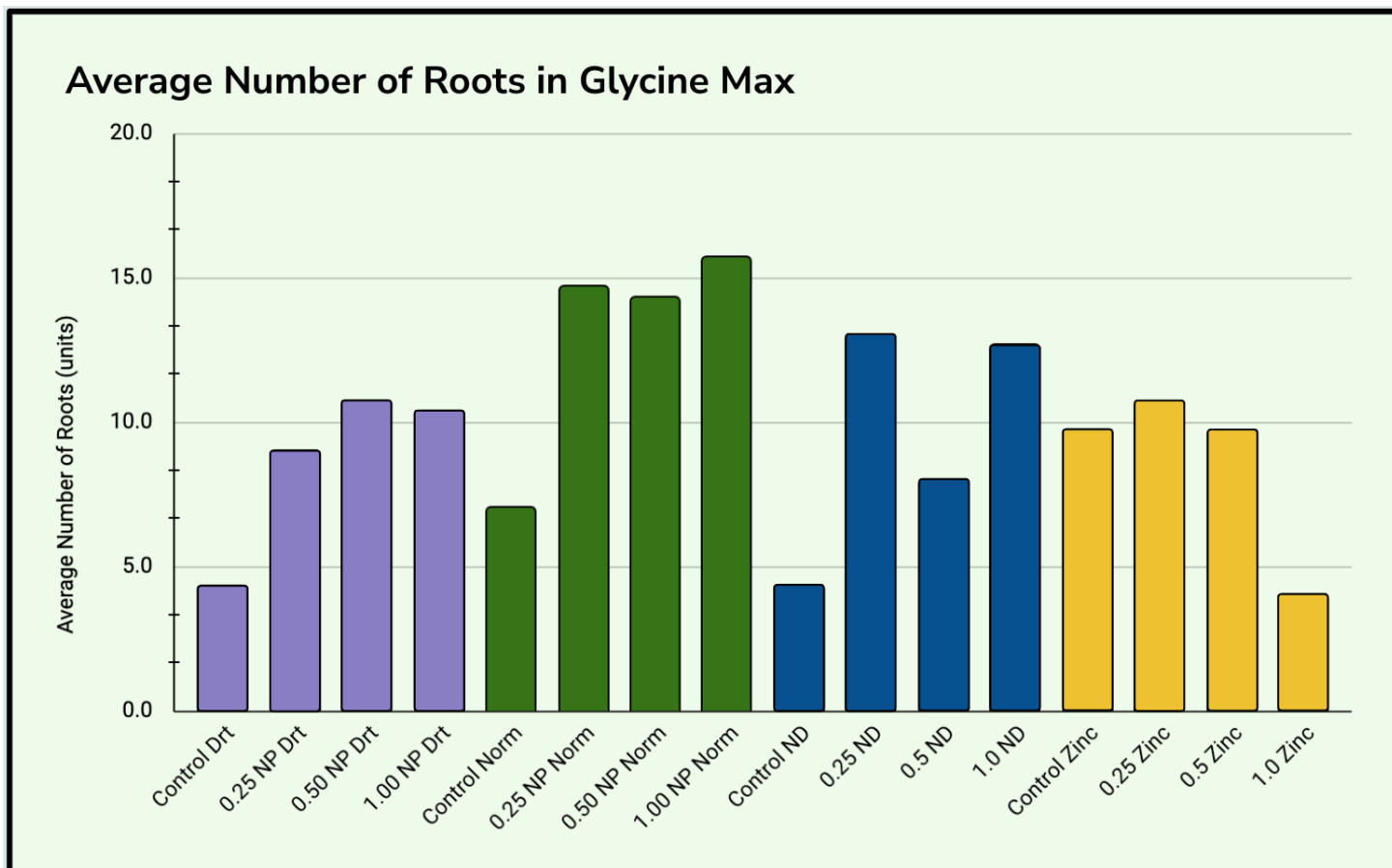


Figure 6: The Average Number of Roots in Glycine Max in Drought Conditions, Normal Conditions, Nutrient Deficient Conditions, and Zinc Supplemented Conditions.

Zeta Potential (mV)	Mobility (umcm/Vs)	Conductivity (mS/cm)
-17.1	-1.337	1.53
-16.8	-1.313	1.59
-17.4	-1.363	1.73

Table 1: Unloaded Chitosan/Alginate Nanoparticles without fertilizer

Zeta Potential (mV)	Mobility (umcm/Vs)	Conductivity (mS/cm)
0.505	0.03958	1.89
-0.556	-0.04355	2.26
-0.374	-0.02930	2.28

Table 2: Loaded Chitosan/Alginate Nanoparticles with Zinc Sulphate

Zeta Potential (mV)	Mobility (umcm/Vs)	Conductivity (mS/cm)
-20.4	-1.597	1.45
-20.9	-1.641	1.53
-20.9	-1.639	1.57

Table 3: Modified Nanoparticles with Gum Arabic for Stability

4. Discussion

4.1. Analysis of Shoot Length

Under drought conditions, the average shoot lengths for control plants were significantly lower (ranging from 2.5 to 5.0 cm) compared to those treated with nanoparticles. The 0.5 mg/mL concentration showed the highest shoot length, averaging 7.0 to 7.6 cm across trials. Under normal conditions, nanoparticle treatments significantly enhanced shoot length, with the 1.0 mg/mL concentration achieving the highest average of 8.8 to 12.4 cm. In nutrient-deficient conditions, the control plants showed reduced shoot length (0.5 to 5.6 cm), whereas nanoparticle treatments improved shoot length significantly, with 1.0 mg/mL achieving up to 8.2 cm. Zinc sulfate supplementation without nanoparticles resulted in lower shoot lengths (7.1 to 8.6 cm) compared to nanoparticle treatments under similar conditions.

4.2. Analysis of Root Length

Under drought conditions, root length in control plants was minimal (0.2 to 3.3 cm), whereas nanoparticle treatments significantly increased root length, with 0.5 mg/mL showing the highest values (3.4 to 5.1 cm). In normal conditions, the 1.0 mg/mL nanoparticle treatment showed the highest root length (3.6 to 5.5 cm). Nutrient-deficient conditions saw an increase in root length with nanoparticle treatments, where the 0.5 mg/mL concentration was particularly effective (4.2 to 6.3 cm). Zinc sulfate supplementation alone showed root lengths between 3.8 and 5.8 cm, which were lower than those observed with nanoparticle treatments.

4.3. Analysis of Root Length Density (RLD)

Under drought conditions, the control plants exhibited low RLD (0.003 to 0.293 cm/cm³), while nanoparticle treatments significantly increased RLD, with the 0.5 mg/mL concentration showing the highest improvement (0.426 to 0.970 cm/cm³). Under normal conditions, RLD was highest with 1.0 mg/mL nanoparticle treatment (0.832 to 1.132 cm/cm³). In nutrient-deficient conditions, nanoparticle treatments also improved RLD significantly, particularly at 0.5 mg/mL concentration (0.457 to 0.923 cm/cm³). Zinc sulfate alone had lower RLD values (0.385 to 0.809 cm/cm³) compared to nanoparticle treatments.

4.4. Analysis of Leaf Area

Drought conditions resulted in minimal leaf area for control plants (0.0 to 2.286 cm²), whereas nanoparticle treatments improved leaf area, with the 0.5 mg/mL concentration showing the highest values (2.014 to 2.927 cm²). Under normal conditions, the 0.5 and 1.0 mg/mL nanoparticle treatments nearly doubled the leaf area compared to control plants, reaching up to 7.008 cm². Nutrient-deficient conditions saw significant improvements in leaf area with nanoparticle treatments, particularly at 0.5 mg/mL (4.449 to 4.669 cm²). Zinc sulfate alone had lower leaf areas (1.839 to 2.892 cm²) compared to nanoparticle treatments.

4.5. Number of Roots & Leaves

The number of roots under drought conditions was significantly higher with nanoparticle treatments (6.0 to 15.0) compared to control plants (1.0 to 7.0). Under normal conditions, nanoparticle treatments resulted in the highest number of roots (13.0 to 18.0), particularly at 1.0 mg/mL. Nutrient-deficient conditions also saw improvements in the number of roots with nanoparticle treatments (10.0 to 14.0), while zinc sulfate alone resulted in lower numbers (3.0 to 11.0). The number of leaves followed a similar trend, with nanoparticle treatments resulting in higher leaf counts under all conditions compared to controls and zinc sulfate alone.

4.6. Analysis of Zeta Potential, Mobility, and Conductivity

The Zeta potential tests indicated successful zinc loading on the nanoparticles. Unloaded nanoparticles had zeta potential values of -16.8 to -17.4 mV, whereas loaded nanoparticles exhibited a significant increase in surface charge. Conductivity values were higher for loaded nanoparticles (1.89 to 2.28 mS/cm) compared to unloaded ones, indicating better interaction with the medium. The modification of nanoparticles with Gum Arabic further improved colloidal stability, as indicated by more negative zeta potential values (-20.4 to -20.9 mV) and adequate conductivity (1.45 to 1.57 mS/cm).

4.7. Zinc Supplementation

Zinc is an essential micronutrient that serves as a cofactor for numerous enzymes and proteins involved in plant growth and development. [5] One critical enzyme is Carbonic Anhydrase, encoded by the CA5A gene. This enzyme catalyzes the reversible hydration of carbon dioxide, a reaction pivotal for photosynthesis. [6]

Carbonic Anhydrase Activity:

- **Zinc Binding:** Zinc ions (Zn^{2+}) bind to the active site of Carbonic Anhydrase, facilitating the conversion of CO_2 and H_2O to HCO_3^- (bicarbonate ion) and H^+ (proton).
- **Bicarbonate Production:** The bicarbonate ion produced is a substrate for Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo), the primary enzyme in the Calvin cycle responsible for carbon fixation.
- **Photosynthesis Enhancement:** Increased availability of bicarbonate enhances RuBisCo's carboxylation efficiency, leading to higher rates of photosynthesis. This results in increased production of carbohydrates, which are essential for plant growth and biomass accumulation. [7]

The enhanced photosynthetic activity due to increased Carbonic Anhydrase activity and subsequent bicarbonate production leads to improved growth metrics, including shoot length, root length, and leaf area. [8] Zinc supplementation via nanoparticles ensures a sustained release of zinc ions, maintaining optimal enzyme activity and promoting consistent growth.

4.8. Calcium Alginate

Calcium alginate, derived from alginic acid found in brown algae, is known to enhance plant growth by upregulating genes associated with auxin (Indole-3-acetic acid, IAA) biosynthesis. Auxins are key plant hormones that regulate various aspects of plant development, including cell elongation, division, and differentiation. [9]

Auxin Synthesis and Signaling:

- **Calcium Ion Release:** Calcium ions (Ca^{2+}) released from calcium alginate act as secondary messengers in various signal transduction pathways.
- **IAA Biosynthesis:** These ions stimulate the expression of genes involved in the biosynthesis of IAA, a primary auxin in plants.
- **Cell Elongation and Division:** Higher auxin levels promote cell elongation and division, leading to increased root and shoot growth. Auxins also play a role in root initiation and branching, enhancing the plant's ability to absorb water and nutrients. [10]

The increased auxin production resulting from calcium alginate supplementation enhances root

and shoot development. Improved root architecture allows for better nutrient and water uptake, contributing to overall plant health and growth. [11] The nanoparticles provide a controlled release of calcium ions, ensuring continuous auxin stimulation and sustained growth.

4.9. Chitosan

Chitosan, a biopolymer derived from chitin, has multiple roles in promoting plant growth. It is known to upregulate the synthesis of chlorophyll and osmolytes, crucial for photosynthesis and stress tolerance, respectively. The presence of chitosan stimulates the production of Glutamyl-tRNA, leading to the biosynthesis of 5-aminolevulinic acid (ALA), a precursor for chlorophyll. [12]

Chlorophyll Synthesis:

- **Glutamyl-tRNA Synthetase Activity:** Chitosan enhances the activity of Glutamyl-tRNA synthetase, increasing the production of ALA.
- **Protoporphyrin IX Formation:** ALA is converted to Protoporphyrin IX, which then binds magnesium ions via Magnesium Chelatase to form Chlorophyllide.
- **Chlorophyll Formation:** Chlorophyllide undergoes esterification to produce chlorophyll, the primary pigment for photosynthesis.
- **Photosynthetic Efficiency:** Enhanced chlorophyll content improves light absorption and energy conversion efficiency, boosting photosynthetic capacity and plant growth. [13]

Osmolyte Production:

- **Abscisic Acid (ABA) Synthesis:** Chitosan triggers the synthesis of ABA, a hormone involved in stress responses.
- **P5CS Expression:** ABA stimulates the expression of the gene P5CS, which encodes Pyrroline-5-carboxylate synthetase.
- **Proline Synthesis:** P5CS converts Glutamate to Glutamic- γ -semialdehyde, which is subsequently converted to Pyrroline-5-carboxylate (P5C). P5C is then reduced to Proline by Pyrroline-5-carboxylate reductase (P5CR).
- **Osmoprotection:** Proline acts as an osmolyte, protecting plant cells under stress conditions by maintaining cellular osmotic balance and stabilizing proteins and membranes. [14]

Chitosan's role in enhancing chlorophyll synthesis boosts the plant's photosynthetic capability, leading to increased biomass and improved growth metrics. Its ability to induce osmolyte production, such as proline, helps plants cope with stress conditions like drought and nutrient deficiency. The nanoparticles ensure a sustained release of chitosan, providing continuous stimulation of these beneficial processes.

4.10. Summary

The combined application of zinc sulfate, calcium alginate, and chitosan-loaded nanoparticles results in an improved effect on plant growth. The nanoparticles provide a sustained and controlled release of nutrients, ensuring their availability over an extended period. This prolonged exposure leads to continuous genetic and biochemical stimulation, maximizing growth and stress resilience. The observed improvements in growth metrics under both optimal and stress conditions can be attributed to the enhanced photosynthetic efficiency, improved nutrient uptake, and increased stress tolerance facilitated by the nanoparticles. [15]

5. Conclusion

The initial hypothesis of chitosan nanoparticles effectively delivering growth-promoting nutrients to Glycine Max was successful as seen through the improved growth data, and how nanoparticles are a trigger for gene upregulation for plant cells. Further research includes trying to identify how the nanoparticles interact with plant health on a more genetic level and analyzing nutrient concentrations using more advanced instrumentation to get accurate results before implementation.

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