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EFFECTS OF CHITOSAN NANOPARTICLES AND MS MEDIUM ON POTATO (*Solanum tuberosum*) SYNTHETIC SEED REGENERATION

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ABSTRACT

This study was conducted to determine the in vitro regeneration response of synthetic potato (*Solanum tuberosum* L.) plantlet seeds to different concentrations of chitosan nanoparticles and MS medium. The study was conducted at the Plant Physiology and Biotechnology Laboratory of Sultan Ageng Tirtayasa University from December 2025 to April 2026. This study used a factorial randomized block design (RAK) consisting of two factors. The first factor was chitosan nanoparticle (C) with levels of concentration were 0 ppm, 50 ppm, 100 ppm, 150 ppm. The second factor was MS medium (T) with levels of ½ MS, ¼ MS, 0 MS. The results showed that the number of germinated seeds in the 50 ppm + ½ MS medium treatment combination yielded a higher average value of 0.6 seeds. Plant height showed that the 150 ppm + ½ MS medium treatment had the best effect, at 1.05 cm. The success rate of synthetic seed regeneration reached 26.7% in the 50 ppm + ½ MS medium treatments. The study showed that neither treatment had a significant effect on any of the observed parameters.

Key words: Chitosan, Nanoparticles, Synthetic seed

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the major horticultural commodities in Indonesia and plays a strategic role in supporting food security and dietary diversification. The Granola variety is widely preferred by local farmers due to its high productivity and favorable tuber characteristics, making it one of the most consumed agricultural products in Indonesia after wheat, rice, and maize (Pratama *et al*., 2020). In 2021, global potato production reached 376 million tons, compared to 510 million tons for rice, 780 million tons for wheat, and 1.15 billion tons for maize (Devaux *et al*., 2020; Steglińska *et al*., 2024). In Indonesia, potato production grew at an average annual rate of 8.27% during 2020-2022. However, production declined by 8.19% annually during 2022–2024. The low productivity of potato cultivation in Indonesia is influenced by several factors, one of which is the use of planting materials with suboptimal quality (BPS, 2025; Rosdiana *et al*., 2023).

In vitro propagation of potato (*Solanum tuberosum* L.) has become an effective alternative for overcoming the limited availability and poor quality of planting materials produced through conventional methods. Nevertheless, tissue culture-derived products still face several challenges, including the risk of contamination and damage to culture media and plant materials during distribution. Culture bottles containing explants are susceptible to physical damage caused by transportation shocks. In addition, explants produced through tissue culture are vulnerable to bacterial and fungal contamination due to non-sterile conditions during culture maintenance and distribution processes (Herman *et al*., 2017).

The development of synthetic seed technology through explant encapsulation offers a strategic solution to overcome distribution constraints and contamination risks. Synthetic seeds are an advancement of tissue culture technology, produced from somatic embryos or vegetative propagules derived from tissue-cultured plants and encapsulated in a hydrogel matrix, enabling them to function similarly to true seeds. The success of regeneration depends heavily on the type of explant, the nutrients used, and the growth-promoting hormones for that plant.

Chitosan is a natural biopolymer derived from the deacetylation of chitin found in crustacean shells, such as crabs and shrimp. It possesses antimicrobial properties and is considered environmentally friendly due to its biodegradable nature. Chitosan nanoparticles are made by small-sized particles designed to improve the solubility and stability of bioactive compounds, thereby enhancing the effectiveness of secondary metabolites. The application of chitosan at a concentration of 100 ppm has been shown to increase leaf production in tomato plants and provide effectiveness comparable to fungicide treatments (Islam *et al*., 2016; Hersanti *et al*., 2023).

This research investigating the combined effects of chitosan nanoparticles and MS medium on the regeneration of synthetic seed-derived potato plantlets under in vitro conditions. This knowledge gap is expected to provide valuable information that contributes to the advancement of plant biotechnology and synthetic seed technology.

MATERIALS AND METHODS

The research was conducted from December 2025 to April 2026 at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Sultan Ageng Tirtayasa University. The equipment used included an autoclave, laminar air flow cabinet, magnetic hotplate stirrer, dropper pipette, micropipette, tweezers, an analytical balance, scalpel and blade, Erlenmeyer flask, measuring cylinder, pH indicator, spatula, Bunsen burner, refrigerated cabinet, and sprayer. The basic materials used in seed encapsulation were 3% sodium alginate, calcium chloride 100 mM, Murashige & Skoog (MS) basal medium, nano-chitosan, 1.5 mg/L BAP, and 0.5 mg/L NAA.

The explants used in this study were derived from sterilized potato (*Solanum tuberosum* L.) shoots of the Granola variety, 12 MST old. This study employed an experimental design using a factorial Randomized Block Design involving 12 treatments across to the two factors. The first factor was the concentration of chitosan nanoparticles, consisting of four levels 0 ppm (C_1), 50 ppm (C_2), 100 ppm (C_3), 150 ppm (C_4), and the second factor was the concentration of Murashige & Skoog growth medium, consisting of three levels ½ MS (T_1), ¼ MS (T_2), 0 MS (T_3). Each treatment was replicated three times and then analyzed using ANOVA with the DSAASTAT test.

The study began with the sterilization of equipment and the work area, followed by the preparation of MS medium according to the treatments and the preparation of stock solutions of chitosan nanoparticles, BAP, and NAA. Synthetic seeds were planted using Lembang Soil under sterile conditions in a Laminar Air Flow (LAF). The planted synthetic seeds were then sprayed and stored under in vitro conditions. Observations were conducted for 10 weeks after planting. The observation indicators in this study included seed germination time, regeneration success rate, number and color of leaves, total number of germinated seeds, seedling height, and the degree of browning of seeds/plantlets.

Sterilization was performed using an autoclave for 15 minutes at 121° C and 15 psi. Preparation of the encapsulation medium began by preparing an MS medium solution according to the treatment protocol with added sterile BAP 1.5 mg L^{-1} and NAA 0.5 mg L^{-1} into MS medium solution. The medium solution divided into Erlenmeyer flasks containing 3% sodium alginate, and chitosan nanoparticles. Chitosan nanoparticles were treated according to the type of treatment, after which the medium were heated until reached the boiling point using a magnetic hotplate stirrer. Potato explants measuring ±0.5 cm were placed in a sodium alginate solution, which was then dropped into 100 mM $CaCl_2 \cdot 2H_2O$. The treatment combination of the first group used shoot explants, the second group used the first potato explant nodes, and the third group used the second potato explant nodes. This study using chitosan nanoparticles produced by the ionotropic gelation method.

RESULTS AND DISCUSSION

Number of germinated seeds

The number of germinated seeds was determined by counting those that grew and successfully formed living plants, characterized by bright green leaves, the emergence of shoots, and an increase in the height of the explants. Observations were made by counting the sprouted seeds and comparing them to the number of synthetic seeds planted in each observation unit. In this research parameter, the total sample used was 5 samples for each treatment combination. If we consider the average result of one treatment combination, and there are three treatment groups/replicates, then the total number of observations in one treatment combination is 15 observations.

As shown in Figure 1, the regeneration of synthetic potato (*Solanum tuberosum* L.) seeds was successful under several treatment conditions. In each observation unit, only a few seeds germinated out of the total of 5 seeds planted. The results of the data analysis showed that there were 8 treatments that successfully germinated. The highest average results were found in treatments C2T1 and C3T2, with an average of 0.27 germinated seeds. These were followed by treatments C1T1, C2T1, and C3T3, with an average of 0.20 germinated seeds. Furthermore, an average value of 0.1333 was obtained for the treatment combinations C1T2, C4T2, and C4T3. Non-viable or non-germinating seeds were found in the germination treatments C1T3, C2T2, and C4T1. Furthermore, an average value of 0.1333 was obtained for the treatment combinations C1T2, C4T2, and C4T3. The data on seed germination are shown in Figure 2.

The success of synthetic seed regeneration in *in vitro* culture depends heavily on the availability of mineral nutrients and growth regulators present in the medium at specific concentrations. Chitosan has positively charged amine groups (NH^{3+}) that are active under acidic conditions, while sodium alginate is negatively charged (anionic) and active in water due to its carboxyl groups (COO^-). When these two solutions are mixed, the carboxylate groups (COO^-) and the amine groups (NH^{3+}) form ionic bonds with each other, creating a solid membrane or gel structure (Conzatti *et al*., 2017). Consequently, in this case, sodium alginate mixed with chitosan will affect plant growth and make it difficult for the seeds to germinate.

This is essentially because chitosan exhibits very high viscosity when dissolved. Chitosan is a natural, long-chain biopolymer with a high molecular weight. The addition of growth regulators such as naphthaleneacetic acid (NAA), which is acidic, affects the gelation of chitosan nanoparticles. When these long chains interact in an acidic solution, their movement becomes restricted, resulting in a viscous solution. This is consistent with the findings of Alves *et al*. (2025), who reported that in chitosan applications, higher pH values (approaching neutral) result in lower hydrogel viscosity. Conversely, under more acidic conditions, the hydrogel's viscosity increases because the polymer chains are more susceptible to cross-linking. This is due to high protonation, which increases the electrostatic repulsion between chitosan polymer chains.

Synthetic seed plant height

Observations were made using a ruler or similar measuring instrument, and expressed in centimeters (cm). This parameter describes the vegetative growth rate of plantlets derived from synthetic seeds. Plant height was measured on the last day of observation (10 weeks after planting) by measuring from the base of the leaf to the tip of the stem of the planted explant.

According to the data on Table 2, it was found that the treatments involving chitosan nanoparticle concentration, MS medium concentration, and a combination of the two did not have a significant effect on the height of synthetic potato seedlings. The lowest average plant height was observed in the control group 0 ppm, at 1.82 cm, while the highest average height was achieved at a concentration of 150 ppm, at 2.58 cm. Among the MS medium concentration treatments, the lowest average height was 1.99 cm (½ MS treatment variables), while the highest average MS medium concentration was 0.17 (0 MS treatment variables). The average overall growth in plant height across all treatments was 2.45 cm. This suggests that the application of chitosan nanoparticles up to a concentration of 150 ppm, as well as variations in MS medium concentration, did not significantly increase potato plantlet height at 10 weeks of age.

Better result according to MS medium concentration was reduced to 0 MS in certain treatments, plant height did not differ significantly. This indicate that the nutrient reserves stored in the synthetic seeds or the plantlets' ability to utilize available nutrients were still sufficient to support height growth during the observation period. The physiological state of the explants plays an important role in determining their growth response during in vitro culture. When explants exhibit relatively similar genetic and physiological characteristics, their growth performance can also become relatively uniform, resulting in non-significant differences in plant height despite variations in culture treatments (Kalsoom *et al*., 2023).

Regeneration success rate (%)

The regeneration success rate is the percentage of synthetic seeds that successfully develop into living plants in each observation unit. This parameter is used to evaluate the ability of synthetic seeds to maintain viability and continue the growth process after encapsulation. This parameter is used to assess the effectiveness of treatments involving different concentrations of chitosan nanoparticles and MS nutrient medium. In this research parameter, the total sample used was 5 samples for each treatment combination. Therefore, the total percentage obtained when all seeds regenerate is calculated as 100%.

Figure 2. shows the regeneration success graph shows that the regeneration percentage of synthetic potato plantlet seeds ranged from 0–26.7%. The highest regeneration percentage was obtained in the treatments with 50 ppm chitosan nanoparticles + ½ MS and 100 ppm chitosan nanoparticles + ¼ MS, at 26.70%, whereas several treatments, such as 0 ppm chitosan nanoparticles + 0 MS, 50 ppm chitosan nanoparticles + ¼ MS, 50 ppm chitosan nanoparticles + 0 MS, and 150 ppm chitosan nanoparticles + ½ MS, showed no regeneration success. Although there were differences in the percentage values among the treatments, statistical analysis results showed that the chitosan nanoparticle and MS nutrient treatments did not have a significant effect on regeneration success. This indicates that the variation in regeneration success was more influenced by internal factors of the explants, the viability of the synthetic seeds, and the adaptability of the propagules during the regeneration process than by the treatments applied.

The regeneration ability of synthetic potato seeds can be affected by the presence or absence of nutrients in the growing medium. Many of the samples that failed in this parameter were those treated without MS (0 MS). This is because the synthetic seeds failed to regenerate properly at 0 MS (without additional nutrients and vitamins basal) because the somatic embryo lacks internal food reserves (endosperm). Encapsulation media without any additional nutrients cannot meet the immediate energy and micro-macronutrient needs that trigger germination and initial growth of regenerated synthetic potato seeds.

The highest yield medium in this study, 150 ppm chitosan nanoparticles + ½ MS, produced the lowest value, at 0% of 100%. This may be due to an imbalance in the composition of sodium alginate, chitosan, and the very high concentration of calcium chloride. Sodium alginate is a negatively charged (anionic) polymer. When exposed to calcium chloride, the positively charged calcium ions (Ca^{2+}) bind to the carboxylate groups (COO^-) in the alginate chains. At the same time, adding chitosan at high concentrations can trigger coagulation of sodium alginate and increase viscosity. This can lead to failure of synthetic seeds to regenerate, potentially leading to plant death.

The low growth rate in the nano-chitosan treatment is thought to be due to the formation of an encapsulation matrix that is too dense, thereby inhibiting imbibition and gas exchange. Chitosan possesses excellent gel-forming ability due to its polymeric structure, allowing the formation of stable hydrogel matrices through intermolecular interactions between polymer chains. This characteristic makes chitosan a suitable encapsulating material for synthetic seed technology, as the formed gel matrix can provide physical protection for encapsulated propagules while maintaining moisture and supporting controlled nutrient release during regeneration (Bidabadi & Mohan, 2020).

The absence of significant differences among chitosan nanoparticle treatments may indicate that the applied concentrations were not sufficient to induce a measurable physiological response during synthetic seed regeneration. Although chitosan has been reported to possess growth-promoting and antimicrobial properties, its effectiveness is highly dependent on concentration, application method, and plant species. The polymeric nature of chitosan may affect its interaction with the culture environment, limiting the availability of active compounds required to stimulate regeneration

CONCLUSION

The conclusion of this study is that the addition of 100 ppm nanoparticles chitosan had a best tendency in terms of the number of germinated seeds and regeneration success rate. In ½ MS medium addition had a best tendency in terms of the number of germinated seeds and regeneration success rate. The interaction between chitosan nanoparticles and MS medium had no significant effect on the observed parameters of the number of germinated seeds, plant height, and regeneration success rate. The most favorable results were observed in the treatment involving 50 ppm of chitosan nanoparticles and ½ MS medium.

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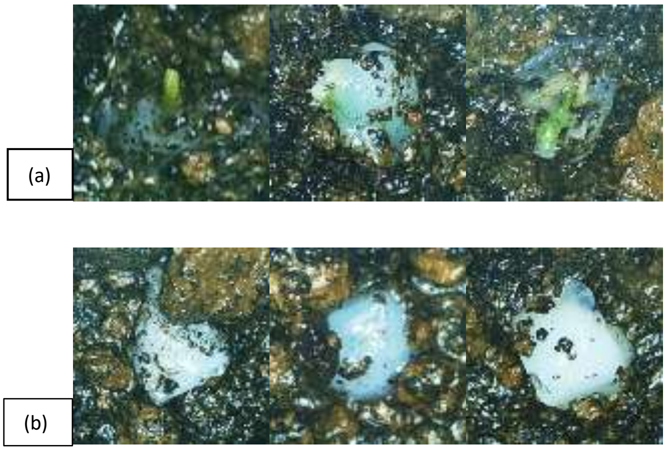
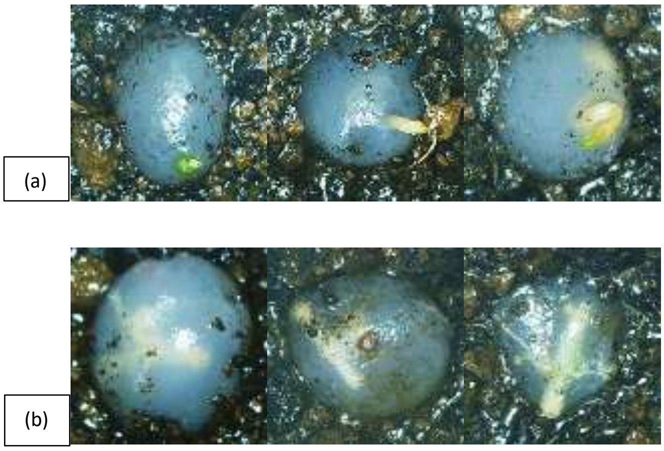
Treatment with 50 ppm chitosan nanoparticles + ½ MS medium



Treatment with 100 ppm chitosan nanoparticles + ¼ MS medium



Note: a). Synthetic seeds that successfully germinated
b). Synthetic seeds that failed to germinate

Figure 1. Seed Germination of *Solanum tuberosum* L. Under Various Treatments at 10 Weeks

Table 1. Effect of chitosan nanoparticle concentration and MS medium on the plant height of germinated synthetic potato seeds at 10 weeks.

Nanoparticles chitosan concentrations (C)	MS medium (T)			**Average**
	½ MS	¼ MS	0 MS	
	……………………………… (cm) ………………………………			
0 ppm	1.15	2.2	2.1	1.82
50 ppm	1.7	1.7	2.35	1.92
100 ppm	1.95	2.85	1.85	2.22
150 ppm	3.15	1.6	3.0	2.58
Average	1.99	2.09	2.33	**2.45**

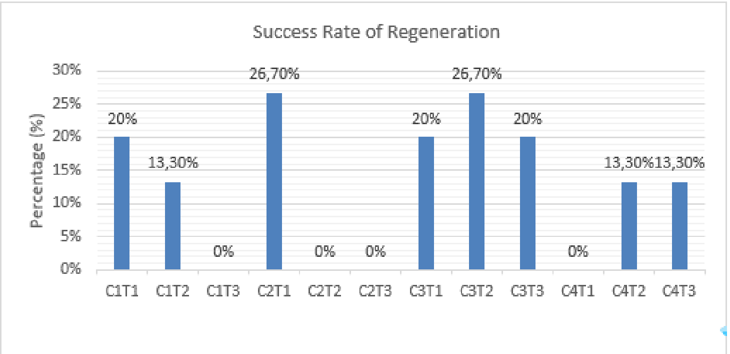


Figure 2. Average percentage of potato synthetic seed regeneration success rates for several combinations of chitosan nanoparticle treatments and MS medium at 10 weeks.