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Effect of BAP and Osmotic Regulators on the Germination of Synthetic Seeds of Sugarcane (*Saccharum officinarum*)

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Abstract. Sugarcane is the raw material for sugar. Limited availability of planting materials has led to a decline in sugar production. The development of synthetic sugarcane seeds offers an alternative for large-scale propagation with long-term storage. This study aims to evaluate the effect of BAP and osmotic regulators on the germination and storage capacity of synthetic sugarcane seeds. The synthetic seeds were produced using the encapsulation technique, whereby basal shoots of the NXI 1-3 sugarcane variety were inserted into 3% sodium alginate and treated with various concentrations of BAP (0 ppm, 0.5 ppm, 1 ppm, 1.5 ppm, 2 ppm), mannitol (0%, 1%, 2%, 3%), and PEG (0%, 1%, 2%, 3%) and then dripped into a 100 mM $CaCl_2$. The results showed that BAP at a concentration of 1.5 ppm provided the best response, with the fastest germination time (4.6 days) and the highest germination percentage (100%). Among the osmotic regulators, 3% mannitol produced the longest germination time (21.75 days), with seed viability of 85% and germination percentage of 60%, demonstrating its effectiveness in extending seed storage life. BAP increases seed viability and growth performance, while mannitol effectively maintains seed dormancy so that it can be used to maintain sugarcane seed availability. This study also supports the achievement of the SDGs, particularly SDG 2 (Zero Hunger) through increased seed production to ensure sugarcane seed availability, and SDG 9 (Industry, Innovation, and Infrastructure) through the application of encapsulation methods as a biotechnology innovation to increase seed production.

Keywords: BAP; mannitol; PEG; synthetic seed; sugarcane

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INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of Indonesia's important commodities, especially as the main raw material in national sugar production. In 2023, sugar production decreased by 168.41 thousand tons (7.01%) compared to the previous year (BPS, 2023). One of the causes of this decline in production is the shortage of available sugarcane seeds (Lestari et al., 2023; Rizqi et al., 2023). To overcome this, the in vitro production of synthetic sugarcane seeds is one of the efforts that can be made to produce sugarcane seeds on a large scale (Nongdam, 2016). Mass seed production is also carried out to maintain the availability of sugarcane seeds through storage. Synthetic seeds can be used for the propagation of vegetatively propagated plants such as sugarcane, thereby supporting the production, conservation, and preservation of sugarcane genotypes (Maheswari & Garg, 2023; Dewanti et al., 2021). Synthetic seeds that are resistant to damage also have the potential to facilitate seedling distribution (Rihan et al., 2017).

Synthetic seeds are made by wrapping explants (which can be somatic embryos or other meristem tissues) in gel capsules using encapsulation techniques (Abbas et al., 2022). The gel capsule is made of sodium alginate, which functions as artificial endosperm and can protect

the explants from mechanical damage (Dewanti et al., 2021; Nongdam, 2016). There are several factors that can affect the success of synthetic seeds, including explant selection, the addition of growth regulators, and the selection of the appropriate type and concentration of gel matrix (Loganathan et al., 2022; Nicolae et al., 2023; Reshi et al., 2025). Sodium alginate is a type of hydrogel that is suitable for synthetic seed production because it has low toxicity, is not too sticky, and hardens quickly (Devi et al., 2018). However, synthetic seeds have low viability and cannot be stored for a certain period of time, so certain substances such as phytohormones, osmotic regulators, or other growth regulators need to be added.

Efforts to maintain seed viability can be made by adding phytohormones such as auxin and cytokinin, which can stimulate the growth and development of explants (Mehbub et al., 2022; Sosnowski et al., 2023; Tesse et al., 2025). BAP is one of the cytokinins that can be added to the encapsulation matrix to maintain the viability of synthetic seeds and enhance the development of plant propagules (Ghosh et al., 2025). Meanwhile, to extend the shelf life of seeds, osmotic regulators such as mannitol and PEG can be added. Osmotic regulators can increase the osmotic pressure of the culture medium, thereby inhibiting water and nutrient absorption by plants, which can inhibit seed germination (Plumula et al., 2024). The addition of osmotic regulators in the production of synthetic seeds is done to slow down seed germination so that the seeds can be stored for a certain period of time. This inhibition method is also a sugarcane conservation strategy to maintain the availability of quality sugarcane seeds. Therefore, this study aims to ensure the availability of sugarcane seeds throughout the year through the provision of synthetic seeds and to provide further studies on the germination ability and storage life of synthetic seeds by providing BAP and osmotic regulators.

METHODS

Explants

The explants used in the production of these synthetic seeds were basal shoots of sugarcane originating from 2-month-old NXI 1-3 sugarcane planlets.

Culture Medium

The medium used to make synthetic seeds is basic MS medium + vitamins plus treatment and sodium alginate. MS medium consists of 20 mL/L stock A, 20 mL/L stock B, 5 mL/L stock C, 5 mL/L stock D, 5 mL/L stock E, 5 mL/L stock F, 5 mL/L myo-inositol, 5 mL/L vitamin PY-TY, and 30 g/L sucrose. The sodium alginate solution used was 3%.

Treatments

Two types of treatments were applied in the production of synthetic seeds: (1) the addition of 6-benzylaminopurine (BAP) and (2) the use of osmotic regulators, namely mannitol and polyethylene glycol (PEG).

The BAP treatment consisted of five concentration levels: 0, 0.5, 1.0, 1.5, and 2.0 ppm. Each treatment was repeated five times, resulting in a total of 25 experimental units.

For the osmotic regulator treatment, mannitol was applied at concentrations of 1%, 2%, and 3%, while PEG was tested at the same concentration levels. Each treatment was repeated four times, resulting in a total of 32 experimental units.

Encapsulation Technique

Synthetic seed production was carried out using the encapsulation technique with encapsulation medium and 100 mM $CaCl_2$ (Figure 1). Synthetic seed production was carried out by cutting the basal shoot from the sugarcane plantlet using a scalpel, approximately 0.5-1 mm. The basal shoot is placed in the encapsulation medium that has been treated, then the explants are taken one by one using a plastic pipette and dripped into a 100 mM $CaCl_2$ solution. The seeds are soaked in the $CaCl_2$ solution for 15-20 minutes, and after the seeds harden, they are rinsed using sterile distilled water.

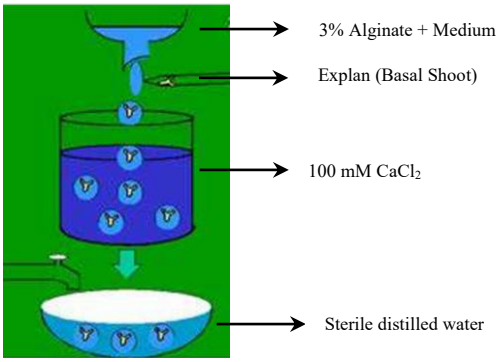


Figure 1. Encapsulation Technique (Chavan & Jayewar, 2021)

Storage Technique

The encapsulated synthetic seeds are stored in sterile distilled water and labeled according to

their respective treatments. The stored granules are observed periodically to monitor their development response based on predetermined observation parameters.

Observation Parameters

The observation parameters included seed germination time, seed germination percentage, shoot height, and number of leaves.

Data Analysis

Experimental data were analyzed using the Standard Error of the Mean (SEM) to evaluate the variation and differences between treatment means in the germination performance of synthetic sugarcane seeds.

RESULTS AND DISCUSSION

Germination Time

Seed germination is indicated by the explant's ability to penetrate the encapsulation capsule. This ability indicates that the cells in the explant are capable of growing and developing within the capsule (Muslihatin et al., 2018). The addition of BAP at various concentrations showed differences in the time it took for the explant to penetrate the capsule. Seeds added with BAP had a faster germination time than seeds without BAP. Table 1 shows that the 1.5 ppm BAP treatment produced the fastest germination time of 4.6 days. This treatment produced a germination time that was twice as fast as synthetic seeds without BAP addition. Plant growth regulators in synthetic seeds showed a seed survival rate and explant regeneration of 100% (Sharma et al., 2021). Seed germination speed is influenced by the ability of the explants to grow in the encapsulation matrix. The addition of BAP can prolong sugarcane shoots, so that seeds with BAP addition have a faster germination time than seeds without BAP addition. In the 0 ppm BAP treatment, the longest germination time was obtained, namely 10.4 days. BAP in synthetic seeds not only encourages seed germination but also acts as an artificial endosperm to prevent explants inside the capsule from dying or failing to germinate. In research conducted by (Prasayu & Ratnasari, 2021), artificial endosperm is essential for increasing the percentage of viable seeds and the number of seeds that penetrate the capsule.

The addition of mannitol and PEG causes germination time to become longer as the concentration increases. The higher the concentration of osmotic regulator added, the longer the seed germination time. As shown in Table 1, the addition of 3% mannitol resulted in a germination time that was 3.6 times longer than without the addition of mannitol, and the addition of 3% PEG resulted in a germination time that was 3.2 times longer than without the addition of PEG. The addition of mannitol as an osmotic regulator can reduce internal osmotic potential (dehydration), which can interfere with the germination metabolism of synthetic seeds (Darko et al., 2019). Similarly, in the PEG treatment, higher PEG concentrations increased the osmotic pressure in the medium, which reduced water potential, thereby inhibiting the germination of synthetic seeds due to the inhibition of water absorption by the seeds (Mehmandar et al., 2023). Based on these results, the application of 3% mannitol resulted in a longer germination time compared to PEG at the same concentration. Therefore, the 3% mannitol treatment is the best treatment for extending the shelf life of synthetic sugarcane seeds, with the longest germination time of 21.75 days.

Shoot Height

Successful germination and seed elongation are characterized by shoot penetration through the encapsulation matrix, indicating a relationship between germination ability and shoot growth. The ability of explants to elongate shoots is influenced by the cytokinin hormone. In (Saleem et al., 2022) research, the addition of cytokinin accelerated the elongation of sugarcane shoots compared to without the addition of cytokinin. BAP, which acts as a cytokinin in synthetic seeds, accelerated the rate of shoot growth so that in synthetic seeds with 2 ppm BAP, the highest average shoot were obtained with a height of 9.4 mm, while the lowest (6.16 mm) occurred in the 0 ppm BAP treatment (Table 1). These results are consistent with the germination data, where seeds treated with 2 ppm BAP showed the highest germination percentage, while seeds without BAP showed the lowest percentage. BAP, a cytokinin-type plant hormone, stimulates cell division by activating genes involved in the cell cycle (Klaocheed et al., 2020). Therefore, the addition of BAP at the optimal concentration stimulated rapid shoot elongation (Dewi et al., 2022; Rahayu & Banowati, 2022). This finding is in line with Amente & Feyissa's (2022) study, which showed that BAP concentrations between 1.5 and 2 ppm optimally stimulated shoot growth in sugarcane.

The effect of osmotic regulators on shoot height varied between treatments. In the mannitol

treatment, shoot height tended to increase with increasing concentration, although the control (0% mannitol) produced slightly taller shoots than the mannitol-treated seeds. Mannitol, a sugar alcohol similar to sucrose, can provide energy to the explants and stabilize the osmolality of the medium (Singh, 2013). Although the initial germination rate was lower under osmotic stress, the explants that germinated later showed better adaptability and growth performance. In contrast, the 2% PEG treatment produced the highest shoots (6.23 mm) compared to other PEG concentrations and the control. These findings are supported by the results of research by Sahat et al. (2022), which reported that higher PEG concentrations (30 g/L) produced higher shoots than the control treatment (0 g/L PEG) for several potato genotypes. After germination, PEG-exposed explants became more tolerant to osmotic stress, allowing for normal subsequent development. Although the differences between treatments were not statistically significant, the 2% PEG treatment showed better shoot growth performance than the other treatments.

Number of Leaves

The highest average number of leaves was obtained from the 1.5 ppm BAP treatment (3.4 leaves), while the lowest was observed in the 0.5 ppm BAP treatment (1.76 leaves). The appropriate BAP concentration promotes leaf differentiation in the explants, while excessive cytokinin levels can inhibit leaf expansion and reduce bud formation (Prayoga et al., 2024). This pattern is consistent with the findings of Tarigan et al. (2024), who noted that increasing BAP concentration increased leaf production to an optimal point, beyond which growth was inhibited. Accordingly, seeds treated with 2 ppm BAP showed fewer leaves than those treated with 1.5 ppm BAP. Tesfa et al.'s (2016), research on sugarcane varieties N52 and N53 showed that the BAP concentration that produced the highest number of leaves was 1 ppm for sugarcane variety N52 and 0.5 ppm BAP for sugarcane variety N53. Increasing BAP concentration after that showed a decrease in the number of leaves. The decrease in leaf number with increasing BAP concentration is due to cytokinins stimulating proteins and participating in cell cycle control during cell division. This cell division will encourage shoot multiplication, thus reducing the number of leaves produced (Hailu, 2017).

Regarding osmotic regulators, the 3% mannitol treatment produced the highest number of leaves (2.44 leaves), exceeding the control (0% mannitol). Similarly, the 3% PEG treatment produced 2.39 leaves, exceeding the 0% PEG control. The increase in the number of leaves in both osmotic regulator treatments indicates that the explants developed better physiological tolerance after germination, resulting in faster growth compared to the control without osmotic regulation.

Percentage of Seed Germination

The effects of BAP and osmotic regulators on seed germination are shown in the following graph.

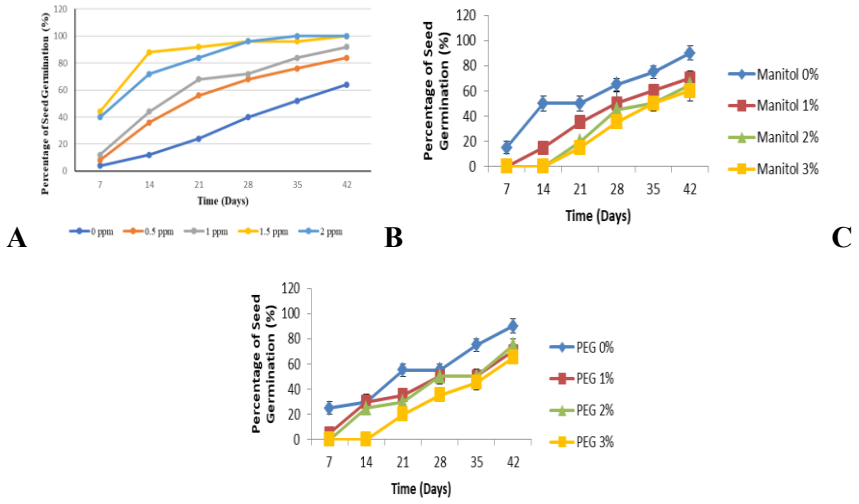


Figure 2. The Effect of BAP and Osmotic Regulators on the Germination Percentage of Synthetic Seeds. Treatments: BAP (A), Mannitol (B), and PEG (C)

Table 1. The Effect of BAP and Osmotic Regulators on Germination Time, Shoot Height, and Number of Leaves

Treatments	Germination Time	Shoot Height (mm)	Number of Leaves
0 ppm BAP	10.4±3.55	6.16±1.04	1.96±0.56
0,5 ppm BAP	9±1.87	7.2±0.93	1.76±0.50
1 ppm BAP	8±1.14	7.28±0.44	2.2±0.34
1,5 ppm BAP	4.6±0.4	9.16±0.38	3.4±0.32
2 ppm BAP	5.2±0.96	9.4±0.48	3.2±0.28
0% Mannitol	6±0.71	6.15±0.22	1.54±0.08
1% Mannitol	14.5±0.87	5.05±0.33	1.85±0.22
2% Mannitol	19.25±0.75	5.50±0.53	1.81±0.21
3% Mannitol	21.75±0.75	5.76±0.18	2.44±0.26
0% PEG	6.25±0.48	5.88±0.27	1.54±0.08
1% PEG	8.5±0.87	5.47±0.47	1.54±0.25
2% PEG	13±0.58	6.23±0.57	1.89±0.16
3% PEG	20±0.58	5.46±0.41	2.39±0.23

Table 2. Morphology of synthetic seeds after 7 and 21 days of storage with BAP treatment

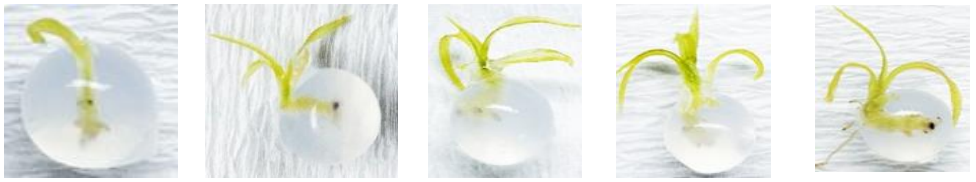
Day	Treatments				
	Control	0.5 ppm	1 ppm	1.5 ppm	2 ppm
7 Day					
21 Day					

Table 3. Morphology of synthetic seeds after 7 and 21 days of storage with Mannitol treatment

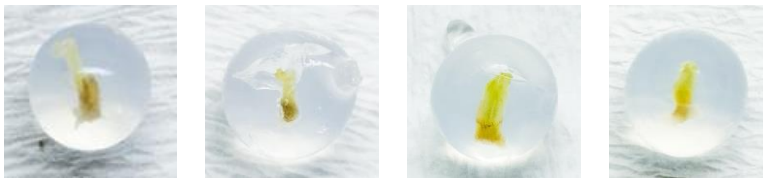
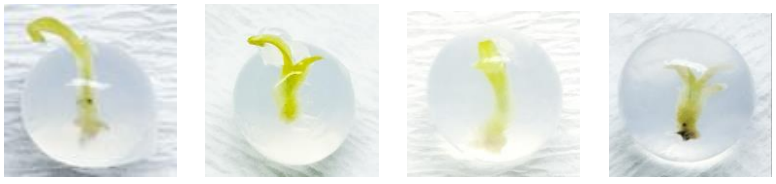
Day	Treatments			
	Control	1%	2%	3%
7 Day				
21 Day				

Table 4. Morphology of synthetic seeds after 7 and 21 days of storage with PEG treatment

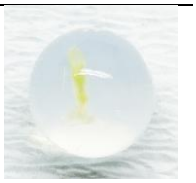
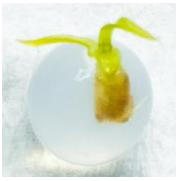
Day	Treatments			
	Control	1%	2%	3%
7 Day				
21 Day				

Figure 2A shows that on the 14th day of observation, synthetic seeds showed the highest germination rate compared to observations on other days. On the 14th day, the percentage of seeds that germinated in synthetic seeds with the addition of BAP reached 34%. This shows that exogenous BAP hormones work most effectively in the early stages of sprout growth. The addition of BAP to the encapsulation matrix serves to accelerate sprout growth because BAP, as a cytokinin hormone, can enhance the performance of endogenous hormones in the explants so that cell division and cell differentiation can be stimulated (Islamia et al., 2022). Then, on day 35, seeds treated with 1.5 ppm BAP reached a germination rate of 100%, which was faster than the 2 ppm treatment, which only reached a germination rate of 100% on day 42. BAP with the right concentration will encourage plant growth. On the contrary, a BAP concentration that is too high can inhibit plant growth (Misbahuljannah et al., 2025). The addition of BAP to the encapsulation matrix increased the percentage of germinated seeds by 56.25% compared to synthetic seeds without BAP addition. This aligns with the research by Nicolae et al. (2024), which states that the percentage of synthetic seeds that germinate and regenerate in alginate with the addition of growth regulators can reach 91.67% in *Solanum tuberosum*, higher than without the addition of growth regulators.

Figure 2B shows that the slowest germination percentage was found in the 2% and 3% mannitol treatments, which only germinated on the 21st day of storage. Similarly, the PEG treatment in Figure 2C shows that PEG 3% only germinated on the 21st day of storage, while other PEG treatments had already started to germinate on the 7th and 14th days. The application of high concentrations of osmotic regulators causes osmotic pressure, which can slow down water absorption by seeds, thereby inhibiting seed germination (Mayang et al., 2021). This inhibition of germination occurs early on when the seeds undergo imbibition to break dormancy; the decrease in water potential caused by the osmotic regulator disrupts seed germination. The results indicate a low germination percentage, suggesting that the addition of osmotic regulators can maintain seed dormancy and inhibit seed germination. Thus, 3% mannitol is the best treatment for extending the shelf life of synthetic seeds, with the lowest germination percentage.

This study aims to ensure the availability of sugarcane seeds throughout the year through the provision of synthetic seeds, as well as to conduct further research on the germination capacity and shelf life of synthetic seeds by providing BAP and osmotic regulators. This study supports Sustainable Development Goal (SDG) 2 (Zero Hunger) because sugar is an important product in food production, and this study aims to ensure the availability of sugarcane seeds throughout the year so that there is no decline in sugar production due to a shortage of quality sugarcane seeds, as well as SDG 9 (Industry, Innovation, and Infrastructure) through the application of seed encapsulation methods as an innovation in the mass production of synthetic sugarcane seeds. Research on synthetic sugarcane seeds is still rare in Indonesia, so this study is expected to be an innovation to increase the large-scale production of synthetic sugarcane seeds efficiently.

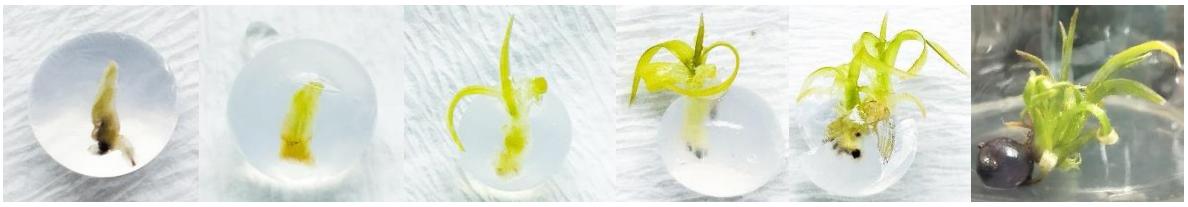


Figure 3. Seed germination process. Browning seed (a), viable seed (b), 7-day-old seed (c), 21-day-old seed (d), 42-day-old seed (e), and seed regeneration (f).

CONCLUSION

Based on the results of the study, it can be concluded that the addition of BAP at a concentration of 1.5 mg/L gave the best results with the fastest germination time of 4.6 days and the highest germination percentage of 100%. BAP did not affect the percentage of viable seeds, as evidenced by the percentage of viable seeds remaining above 90% in both treatments with and without the addition of BAP. Meanwhile, the best osmotic regulator was found at a concentration of 3% mannitol, with a seed viability percentage of 85% and a germination percentage of 60%, as well as the longest seed germination time of 21.75 days. Thus, BAP can increase seed viability and growth performance, while mannitol effectively maintains seed dormancy, making it suitable for maintaining seed availability.

Further research is needed on better treatment combinations to extend the shelf life of synthetic seeds with good viability.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed significantly to the work reported in this manuscript. Author PD conceived and designed the study, conducted the data analysis. Author TH provided feedback on the results of the data analysis. Author N assisted with the literature review and supported data visualization. Authors FFS and FNK were responsible for data collection. Authors HAM and KMK provided substantial feedback during the manuscript drafting process. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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