

# Seeds technologies: performance of sodium alginate and calcium chloride as seed coating

Tecnologias de sementes: desempenho do alginato de sódio e cloreto de cálcio como revestimento de sementes

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#### ABSTRACT

Direct seeding is a restoration method that can benefit from the use of seed coating techniques to increase efficiency in the field. This study aimed to evaluate the effectiveness of sodium alginate as a seed-encapsulating agent and calcium chloride as a crosslinking agent for a model species. Three concentrations of alginate (1.5%, 2%, and 3%) and two concentrations of calcium chloride (5% and 10%) were used. Encapsulation occurred through a drip process. The treatments consisted of ten replications of ten *Solanum lycopersicum* L. (Solanaceae) seeds, totaling 600 seeds. We counted the number of seeds emitting radicle (SER), the mean germination time (MTG), the germination speed index (GSI), and the synchronization index (Z). Encapsulation using an alginate concentration of 2% and the lowest concentration of calcium chloride (5%) showed the best results for SER ( $66\pm18.5$ ), MGT (10.5), GSI (0.63), and Z (-0.3562). The combination of a 2% alginate concentration with the lowest concentration of calcium chloride (5%) shows potential for applications in seed encapsulation.

Keywords: direct seeding; encapsulation; germination; seed treatment; vigor.

#### **RESUMO**

A semeadura direta é um método de restauração que pode se beneficiar do uso de técnicas de revestimento de sementes para aumentar sua eficiência no campo. Este estudo teve como objetivo avaliar a eficácia do alginato de sódio como agente encapsulante de sementes e do cloreto de cálcio como agente de reticulação para uma espécie modelo. Foram utilizadas três concentrações de alginato (1,5%, 2%) e 3%) e duas concentrações de cloreto de cálcio (5% e 10%). O encapsulamento ocorreu por gotejamento. Os tratamentos consistiram em dez repetições de dez sementes de *Solanum lycopersicum* L. (Solanaceae), totalizando 600 sementes. Contaram-se o número de sementes que emitem radícula (SER), o tempo médio de germinação (MTG), o índice de velocidade de germinação (GSI) e o índice de sincronização (Z). O encapsulamento usando uma concentração de alginato de 2% e a menor concentração de cloreto de cálcio (5%) apresentou os melhores resultados para SER ( $66\pm18,5$ ), MGT (10,5), GSI (0,63) e Z (-0,3562). A combinação de uma concentração de 2% de alginato com a menor concentração de cloreto de cálcio (5%) mostra potencial para aplicações em encapsulamento de sementes.

**Palavras-chave:** encapsulamento; germinação; semeadura direta; tratamento de sementes; vigor.

Recebido em: 26 jul. 2024 Aceito em: 12 nov. 2024

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## INTRODUCTION

The search for high-quality seeds has always been a priority for farmers, as it directly impacts the success of culture establishment in the field (ZINDMEISTER *et al.*, 2020). Seeds are subject to various biotic and abiotic stresses, which can significantly affect their production, thus, the adoption of seed treatment technologies is crucial, and chemical, biochemical, and biological treatments can be used to protect seeds and improve their establishment, growth and potential productivity in the field (AFZAL *et al.*, 2020).

Seed improvement technologies, such as seed priming and seed coating, have been increasingly used to optimize the performance of seeds exposed to specific conditions and environments (HALMER, 2008; MA, 2019; ZHANG *et al.*, 2022). Seed coating involves the application of external substances to the seed coat (PEDRINI *et al.*, 2020). This technique is used to change the physical characteristics of the seed, improving its handling through the standardization of weight, shape, and size (KAUFMAN, 1991; AVELAR *et al.*, 2012). The implementation of seed coating in large-scale direct seeding of agricultural and horticultural crops helps achieve seed size uniformity (MADSEN *et al.*, 2016). These, in turn, facilitate mechanized planting and accelerate and synchronize germination in the field, increasing the use of seeds for global restoration (PEDRINI *et al.*, 2017).

Alginate has stood out as the most commonly used substance d for seed coating due to its favorable characteristics, such as a filling and binding material (HEO *et al.*, 2008; KHAN *et al.*, 2011; ANIS *et al.*, 2012; LALLY *et al.*, 2017). It exhibits solubility at room temperature when used in low concentrations, has permeable gel-forming and swelling properties, offers cost-benefit and ease of application, and is non-toxic (GUERRA *et al.*, 1999). Alginate has demonstrated its capacity in the development of artificial seeds, serving as a solid matrix that provides resistance and mechanical stability (GANTAIT *et al.*, 2014), in addition to being used in seed coatings combined with beneficial compounds and microorganisms to increase germination efficiency and in early plant growth (BASHAN *et al.*, 2002; YOUNG *et al.*, 2006; BERNINGER *et al.*, 2016; ZHOU *et al.*, 2017). Sodium alginate has demonstrated effectiveness in coating native forest seeds intended for use in the direct seeding technique employed in ecological restoration efforts (DUTRA *et al.*, 2023). However, there are few studies on the seed encapsulation method with alginate and its ideal concentration.

Alginates are natural polysaccharides extracted from algae of the phylum Phaeophyceae, made up of guluronate and mannuronate units, connected by 1,4-glycosidic bonds. These compounds have several attractive properties that make them highly desirable in various industries. They exhibit biocompatibility, accessibility, low toxicity, and easy gelation (LEE & MOONEY, 2012). The presence of carboxyl groups in their molecular structure gives alginates a remarkable ability to undergo the solgel transition when exposed to multivalent ions such as Ca<sup>+2</sup> and Al<sup>+3</sup>, which function as crosslinking agents (ROGER *et al.*, 2006). This process changes the three-dimensional structure of the alginates. Encapsulating seeds with biodegradable and hydrophilic polymers and a biostimulant improves seed quality. This advancement facilitates the use of unmanned vehicles for direct aerial seeding in difficultto-access forest areas, thus reducing environmental impact (VOVCHENKO *et al.*, 2020).

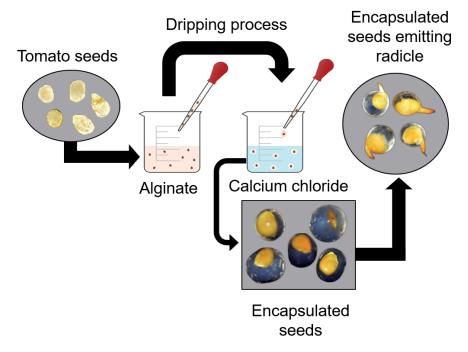
Considering that in direct sowing for ecological restoration purposes small seeds have low establishment (PALMA & LAURANCE, 2015; CECCON *et al.*, 2016) and the use of coating techniques can uniformize seed size to enable the mechanization of planting and accelerate and synchronize germination in the field (PEDRINI *et al.*, 2020), our goal is evaluate the effectiveness of alginate capsules for potential application in native forest seeds. To test the method, it was selected *Solanum lycopersicum* L. (Solanaceae) as a model species for this study, as it is a well-known domesticated species (GERSZBERG *et al.*, 2015) and has small size and shape, which are similar to those used in direct seeding practices for ecological restoration.

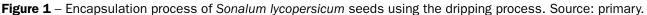
Previous research has often used well-known species with predictable germination behavior, such as lettuce (VALDES & BRADFORD, 1987), onion (TAYLOR *et al.*, 2001), and soybean (JARECKI & WIETECHA, 2021) seeds, to assess the impact of seed coats on quality and yield. Employing agricultural species makes it possible to comprehensively evaluate the coating products. We hope that testing different concentrations of alginate as an encapsulating agent and calcium chloride as a binding agent will help to confirm the efficiency of the method to improve seed germination and vigor and guide its application in native forest seeds.

## **MATERIAL AND METHODS**

#### ENCAPSULATION PROCESS

For the encapsulation of S. *lycopersicum* seeds, six treatments were applied at the following concentrations: T1 (Alginate: 3%, CaCl<sub>2</sub>: 10%), T2 (Alginate: 3%, CaCl<sub>2</sub>: 5%), T3 (Alginate: 2%, CaCl<sub>2</sub>: 10%), T4 (Alginate: 2%, CaCl<sub>2</sub>: 5%), T5 (Alginate: 1.5%, CaCl<sub>2</sub>: 10%) and T6 (Alginate: 1.5%, CaCl<sub>2</sub>: 5%). To assess the effectiveness of alginate capsules, we prepared a sodium alginate dispersion using distilled water as the solvent. The proportions used were 3% (high), 2% (intermediate), and 1.5% (low) w/v (=weight in volume). The S. *lycopersicum* seeds were placed in a beaker containing alginate (figure 1). Subsequently, the seeds were sucked into the Pasteur pipette along with alginate. For encapsulation, the drip method was implemented (PEREIRA *et al.*, 2008), which consisted of adding the covered seeds to a calcium chloride (CaCl<sub>2</sub>) solution, a cross-linking agent, at concentrations of 10% and 5% w/v for capsule formation. After 90 minutes of cross-linking, the capsules were removed from the CaCl<sub>2</sub> solutions and placed on filter paper to remove excess solution.





#### **GERMINATION TESTS**

For every treatment, ten replications of ten S. *lycopersicum* seeds per treatment were used, totaling 600 seeds. The germination test was conducted under international standards established by International Seed Testing Association (ISTA, 2017). The seeds were placed on a paper substrate in Petri dishes moistened with distilled water at a rate of 2.5 times their weight. The germination chambers used were of the Biochemical Oxygen Demand (BOD) type and maintained at a constant temperature of 25°C for 20 days. The experiment was evaluated every 48 hours by adding 0.5 ml of distilled water to the Petri dishes and noting the number of seeds emitting radicle (SER) bigger than two millimeters following the guidelines of the Rules for Seed Analysis (BRASIL, 2009).

#### DATA ANALYSIS

The radicle emission data were used to calculate the number of germinated seeds (SER). The effect of using inputs was evaluated on seed vigor and was estimated by the germination speed index



(GSI), mean germination time (MGT), and synchronization index (Z) (RANAL *et al.*, 2009). Data normality was tested using the Shapiro-Wilk test and residual plots. The Kruskal-Wallis test, complemented by Dunn's post-test, was used to detect differences between treatments at a 5% significance level. We performed exploratory data analysis, including boxplots and average graphs for the analyzed parameters (SER, MGT, GSI, and Z). All analyses were performed with the R software (version 4.0.5) (R Core Team, 2023).

## **RESULTS AND DISCUSSION**

The high concentration of sodium alginate promoted a reduction in the number of seeds emitting radicles, with a percentage of less than 40% (table 1). Between T1 and T2, both with a high concentration (3% sodium alginate concentration), only T1 differed significantly ( $X^2$ = 25.737; *p*= 0.0001003) from the other alginate concentrations (figure 2a). The 2% alginate concentration obtained the best result for all analyzed parameters when associated with low concentrations of calcium chloride (5%) (table 1). Even so, when compared with low alginate concentrations (1.5%), there was no significant difference (figure 2a).

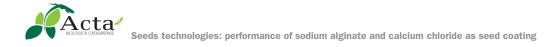
**Table 1** – Mean number of seeds emitting radicle (SER) with standard deviation, mean germination time in days(MGT), germination speed index (GSI) and synchronization index (Z) of the model species Solanum lycopersicumin the different treatments tested.

Treatments	Concentration of Alg		Concentration of CaCl <sub>2</sub>	SER	MGT	GSI	Z
T1	High	3%	10%	29±15.8	12.1	0.26	-0.4571
T2		3%	5%	37±14.2	11.7	0.32	-0.4906
ТЗ	Medium	2%	10%	60±10.0	11.2	0.55	-0,4296
T4		2%	5%	66±18.5	10.5	0.63	-0,3562
Τ5	Low	1,50%	10%	48±9.8	10.9	0.45	-0,4934
Т6		1,50%	5%	57±16.2	11.0	0.53	-0.4277

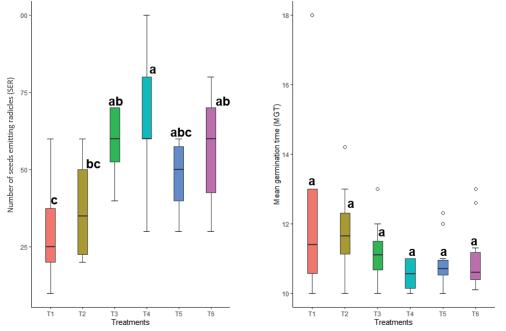
T1 = 3% alginate concentration and 10% calcium chloride concentration; T2 = 3% alginate concentration and 5% calcium concentration; T3 = 2% alginate concentration and 10% calcium chloride concentration; T4 = 2% alginate concentration and 5% calcium concentration; T5 = 1.5% alginate concentration and 10% calcium chloride concentration; T6 = 1.5% alginate concentration and 10% calcium chloride concentration; T6 = 1.5% alginate concentration.

Alginate concentration can influence seed germination responses, limiting water absorption (SILVA *et al.*, 2018; DUTRA *et al.*, 2023). Rehydration of calcium alginate is a slow process that depends on several conditions, mainly the presence of electrolytes in the water (MATSUSHITA *et al.*, 2005). It may be one of the causes of the high variation in the number of seeds emitting radicle, even in treatments with medium (2%) and low (1.5%) alginate concentrations. Classified as a hydrogel, the alginate used as a coating can help prolong the water availability of seeds by acting as a reservoir (MANGOLD & SHELEY, 2007; SERENA *et al.*, 2012). The best performance of alginate was found in relation to cellulose gel based on *Vitis vinifera* L., enabling the synchronization of the growth of seedlings of the forest species *Bixa orellana* L. (DUTRA *et al.*, 2023).

Regardless of the concentration of  $CaCl_2$  when used in association with high concentrations of alginate, T1 and T2 presented the lowest numbers of germinated seeds (table 1), but with a significant difference only in relation to the association between 2% alginate with 5% calcium chloride



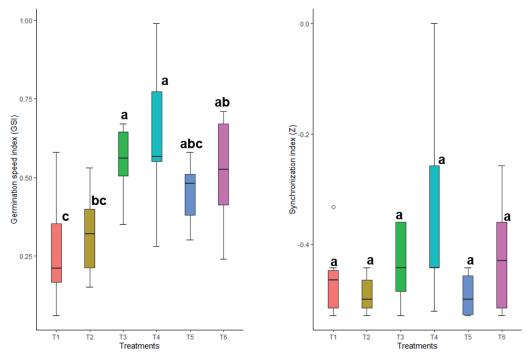
(figure 2a). High alginate concentrations result in improved mechanical properties by increasing intermolecular forces, which makes water absorption difficult in high concentrations (GLADUKH & PODOROZHNA, 2021). On the other hand, the number of seeds emitting radicles in treatments with low concentrations of alginate (1.5%) can be explained by the smaller thickness of the capsule membrane, which promotes the diffusion of Ca<sup>+2</sup> cations, being more effective in smaller than in higher concentrations of sodium alginate (BLANDINO *et al.*, 1999; SILVA *et al.*, 2018).

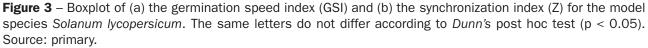


**Figure 2** – Boxplot of (a) the number of seeds emitting radicle (SER) and (b) the number of mean germination time (MGT) for the model species Solanum lycopersicum. The same letters do not differ according to Dunn's post hoc test (p < 0.05). Source: primary.

Although there was no significant difference ( $X^2 = 7.8699$ ; p = 0.1636) between the mean germination time (MGT) (figure 2b), treatments with average concentration (2%) of alginate showed the highest germination values (table 1). The high concentration of alginate also significantly reduced the germination speed ( $X^2 = 27.265$ ;  $p = 5.064^{e-05}$ ), with higher GSI values for the intermediate concentration, associated with 5% calcium chloride, but with no difference between treatments of medium and low concentration (figure 3a). The highest concentrations of alginate (T1 and T2) increased the mean germination time of *S. lycopersicum* seeds (table 1), in addition to presenting the lowest germination speed indices (GSI = 0.26 and 0.32, respectively).

The concentrations of alginate and calcium chloride did not differ in the synchronization index (Z) ( $X^2 = 11.398$ ; p = 0.04404) (figure 3b), but synchronization was better for the treatment with a medium concentration of alginate (2%) and calcium chloride at 5%. Despite showing no differences between synchronization in germination between treatments, at the average concentration [2%] of alginate, T4 showed a lower synchronization index compared to T3 (table 1).





Reducing the mean germination time (MGT) and increasing GSI is very useful in direct sowing, favoring prompt emergence in the field and the establishment of seedlings after sowing. The lower the synchrony index (Z), the more synchronized the germination with time (RANAL & SANTANA, 2006). In all the results, apparently, there was an interaction between the alginate concentration and  $CaCl_2$ . Although the alginate concentration of 2% associated with 5%  $CaCl_2$  promoted a significant increase in germination and its speed, we found that the limiting factor for germination was the combination of high alginate concentrations and  $CaCl_2$ . Regardless of the alginate concentrations or  $CaCl_2$  used, there were no significant changes in the average germination time or its synchrony. However, a tendency towards uniformity in the average germination time between repetitions for medium and low alginate concentrations is evident.

Other studies have demonstrated the potential of using alginate coatings on seeds, with the possible addition of nutrients and biostimulants that contribute to the initial growth of plants (BERNINGER *et al.*, 2016; JARECKI & Wietecha, 2021; SKRZYPCZAK *et al.*, 2021) in addition to inputs to protect seeds against diseases (FU *et al.*, 2022). The use of biodegradable materials as seed coatings is considered a more sustainable practice than other products in the agribusiness market (SOHAIL *et al.*, 2022). Furthermore, these materials can help correct the soil by improving water retention, fertilizers, and soil aeration, reducing evapotranspiration, contributing to the efficiency of seedling emergence, and prolonging the availability of water for plant use (DEMITRI *et al.*, 2013; GUILHERME *et al.*, 2015).

### CONCLUSION

Encapsulation by sodium alginate with calcium chloride was a viable technique for seed coating. However, treatment with 2% sodium alginate concentration and 5% concentration of calcium chloride  $(CaCl_2)$  promoted an increase in germination associated with standardization of the average germination time, relevant factors for the success of direct sowing. Therefore, we recommend this solution for the production of capsules for small seeds, such as the model species *Solanum lycopersicum* L.



#### ACKNOWLEDGMENTS

The authors would like to thank Coordination for the Improvement of Higher Education Personnel [Coordenação de Aperfeiçoamento de Pessoal de Nível Superior] (Capes) for granting research grants for the authors and the Laboratory of Seeds and Forest Seedlings (Lasem) at UFSCar (Federal University of São Carlos) for providing all human and material resources for the field experiment.

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