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Physiological potential of creole bean seeds subjected to alternative adhesive agents and sururu shell in Alagoas, Brazil

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Abstract

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Various productive sectors have pursued sustainability, and agriculture is no exception. As a result, studies have been conducted on potential techniques for utilizing waste and enhancing the quality of materials used in plant production. Seed coating is probably the oldest, most economical, and safest measure, offering the best results for improving the physiological aspects of seeds. Lima bean (*Phaseolus lunatus* L.) belongs to the legume family; its seeds are edible and frequently cultivated as food in various parts of the world. Lima bean cultivation is primarily carried out by family farming in the Northeast of Brazil. In the state of Alagoas, the sururu (*Mytella charruana*) is found. The collection of this mollusk annually generates about five tons of shell waste in the Mundaú Lagoon, located on the southern coast of Alagoas. Therefore, the objective of this study was to evaluate the effect of different adhesive agents in the pelleting of creole lima bean seeds with sururu shell powder, analyzing the impact of these treatments on the physiological potential of the seeds. The adhesive materials used for the coating were water, extracts of aloe vera, mandacaru, and prickly pear, as well as cassava gum and polyvinyl acetate-based glue. Immediately after the application of the adhesive agent, the seeds were pelleted with sururu shell powder. The analyzed variables were: Thousand-seed weight, water content, first germination count, germination rate, germination speed index, mean velocity of germination, mean germination time, germination uncertainty, and seedling length and dry mass. The analysis of these variables allowed us to verify that pelleting the Creole lima bean seeds resulted in increased weight of a thousand seeds, mean germination speed, seedling length, and dry mass, as well as decreased mean germination time and germination uncertainty. Concluding, cassava gum is thus the most efficient adhesive agent in pelleting Creole lima bean seeds with sururu shell powder.

Keywords: germination; sustainability; vigor

Introduction

Legumes, also known as *Fabaceae*, are an important source of nutrients, including minerals, carbohydrates, vitamins, and dietary fiber, and are an excellent source of plant-based protein compared to animal products (Anor et al., 2014). Popoola et al. (2019) described legumes as being classified into smaller and larger species, with smaller legumes, such as lima beans (*Phaseolus lunatus* L.), being considered neglected, underutilized, and less explored.

Even though it is highly relevant to agriculture, especially in Brazil, this species is considerably understudied (Moraes et al., 2017). Considering that lima bean seeds are heirloom, it is important to prioritize studies. Primarily, this is because its cultivation is traditionally carried out by family farmers who use heirloom varieties that lack defined growth and characteristics, making their physiological characteristics unknown (Carvalho et al., 2015).

Heirloom seeds are considered the cultural and genetic heritage of traditional peoples and are indispensable for the *in situ* conservation of resources and biodiversity (Santos et al., 2017). Crisóstomo et al. (2018) explain the importance of heirloom seeds, as they are indispensable for genetic improvement due to their high adaptability to specific environmental conditions. Being a source of genetic diversity, they can be used to search for genes with tolerance and/or resistance to biotic and abiotic factors.

The value addition to seeds through production technologies, such as coating, has become a market demand, and the competition is increasingly intense (Melo et al., 2023a). In Brazil, seed pelleting is still considered a relatively new technology, especially in family farming, due to the limited information available about types of adhesives and treatment strategies (Melo et al., 2020). Techniques aimed at optimizing seed utilization, such as coating, have been a technology that has grown and thrived over the years, adding value to seeds and providing a more competitive trade. Furthermore, it helps to solve problems related to the shape and size of seeds, standardizing them and increasing precision in sowing (Santos, 2016).

Commonly known as "sururu," *Mytella charruana* is a mollusk widely produced in the states of the Northeast region of Brazil. The shells are composed of a chemical substance called calcium carbonate ($CaCO_3$), which is widely used in various areas, including the production of primary and slaked lime (Ca (OH) 2), fertilizers and pesticides, animal feed, polyethylene foam, glass production, pharmaceuticals, and construction (Lima and Oliveira, 2016).

However, the improper disposal of sururu shells represents an environmental problem. Due to the environmental impact of synthetic fertilizers during production and their high acquisition costs, the use of waste in agriculture is a good alternative. The agronomic and economic advantages of applying waste in the field add to the issues related to environmental sustainability, recycling, and protection of mineral deposits that supply the ore used in the production of synthetic fertilizers (Nascimento et al., 2014).

The amount of material wasted in the production process has become a global problem and has been worsening over time. Efforts to minimize these impacts continue to extend to various sources of pollution, including agriculture, mining, fishing, and industry. Most of the time, these pollutants dump their waste into the environment criminally, causing irreparable damage. (Monaco et al., 2015). Therefore, the objective of this study was to evaluate the effect of different adhesive agents on the pelleting of heirloom lima bean seeds with powdered sururu shells, analyzing the impact of these treatments on the physiological potential of the seeds.

Materials and Methods

Location

The study was conducted at the Fitotecnia Laboratory of the Campus of Engineering and Agricultural Sciences (CECA) at the Federal University of Alagoas (UFAL), Rio Largo, AL, Brazil. The seeds used were heirloom lima bean seeds (donated by family farmers – 2023 harvest).

Adhesive agents

The adhesive agents used were: 1 – water (control) (50 mL distilled water / 100 g seeds), 2 – mandacaru extract (200 g pulp + 50 mL distilled water / 100g seeds), 3 – polyvinyl acetate glue (50 mL glue / 100 g seeds), 4 – cassava gum (200 g gum / 1 L-1 distilled water / 100g seeds), 5 – palm extract (200 g pulp + 50 mL distilled water / 100 g seeds), and 6 – aloe vera extract (200 g pulp + 50 mL distilled water / 100 g seeds).

Procedures

For the preparation of the adhesive agents mandacaru, palm, and aloe vera, raw materials for each extract were collected. Next, the collected materials were washed with running water and mild soap. Then, using a sterilized knife, the thorns were removed, and the leaves were opened. The gel from the aloe vera leaves was used, while for the mandacaru and palm, their leaves were peeled. After extracting the pulp, each pulp was processed in a blender with the mentioned amount of distilled water until a homogeneous solution was obtained. For the preparation of the PVA glue and cassava gum, the raw materials were already arranged in disposable cups (150 mL). Distilled water was then added in the specified amount until the mixture became homogeneous.

The workbenches, instruments used, and seeds were sanitized with 70% alcohol before pelleting. The seeds were immersed in alcohol for 1 minute, then washed with running water (Rios et al., 2016). After the initial preparations of the experiment, the seeds were added to a plastic bottle. Then, the adhesive agent was added, and the seeds were gently shaken to ensure a uniform coating. With the seeds already coated with the adhesive agent, they were placed in a plastic tray (0.40 x 0.40 x 0.11m) containing powdered sururu shell. The tray was gently shaken until the seeds were perfectly covered.

Subsequently, with the treated seeds, five rows of 5 seeds each were arranged on paper towels in roll format. The papers had already been moistened with distilled water in an amount equivalent to 2.5 times their weight. Then, they were placed in a B.O.D. (Biochemical Oxygen Demand) type germinator at a temperature of 20–30°C (Brasil, 2009). Seeds that produced normal seedlings, meaning they exhibited all essential structures, were considered germinated. This suggests the potential for completing their development and producing healthy plants when subjected to favorable conditions. (Brasil, 2009). With the experiment set up, daily counts were conducted to monitor the quantity of germinated seeds. These counts were performed for nine days, at the same time each day. The experimental design employed was a completely randomized design (CRD), with four replications of 25 seeds per replication.

Variables analyzed

Water content: To determine the water content of the seeds, the oven method at 105 ± 3°C for 24 hours was adopted, following the Seed Analysis Rules. (Brasil, 2009).

Thousand seed-weight: The thousand seed weight was determined by weighing eight replicates of 100 units, according to the Seed Analysis Rules (Brasil, 2009): TSW = SA × 100/N, where TSW = Thousand Seed Weight (g); SA = Sample Weight (g); N = Total Number of Seeds.

First germination count: Along with the germination test, a first germination count was performed, recording the percentage of normal seedlings obtained on the fifth day after the test was set up.

Germination: $gi = (\Sigma ki = 1ni/N) \times 100$, where ni is the number of germinated seeds/seedlings emerged at time *i* and N is the total number of seeds placed for germination (Carvalho et al., 2005).

Germination Speed Index: G1/N1 + G2/N2 + ... + Gn/Nn, where IVG = G1, G2, and Gn = number of germinated seeds counted in the first, second, and last count, and N1, N2 and Nn = number of days from sowing to the first, second, and last count (Maguire, 1962).

Average germination time: $t = \Sigma ki = 1(niti)/\Sigma ki = 1ni$, where *ti* is the time from the start of the experiment to the i^{th} observation (days or hours); *ni* is the number of germinated seeds at time *i* (corresponding to the i^{th} observation); *k* is the last day of germination (Czabator, 1962).

Uncertainty index: $U = -\Sigma ki = 1Filog_2Fi \asymp Fi = ni/\Sigma ki = 1ni$, where *Fi* is the relative frequency of germination; *ni* is the number of germinated seeds at time *i* (corresponding to the i^{th} observation); *k* is the last day of germination (Labouriau, 1983).

Mean velocity germination: $v = 1/t$, where *t* is the mean germination time (Bortolini and Fortes, 2005).

Root and shoot length of seedlings: At the end of the germination test, the seedlings from each adhesive agent were measured from the tip of the radicle to the apex of the seedling. A ruler was used for this measurement, and the results were expressed in centimeters per seedling (Melo, 2011).

Dry mass of seedling roots and shoots: After the germination test, the normal seedlings from each replication were separated into shoots and roots and placed in paper bags. These were then placed in a forced ventilation oven at 80°C for 24 hours. After this period, the samples were placed in desiccators with activated silica gel and weighed on an analytical balance with a precision of 0.0001 g, with the result expressed in g/seedling (Krzyzanowski et al., 2020).

Statistical analysis

The data collected from the experiment were subjected to analysis of variance (ANOVA). A comparison of means was performed using the Tukey test, and when necessary, Dunnett's test was used at a 5% significance level. The statistical analyses were conducted using SISVAR 5.6 software (Ferreira, 2014).

Results and Discussion

Analyzing the results in Table 1, the thousand-seed weight (TSW) variable increased for all agents except those treated with water. The similarity in mass gains among the pellets may be associated with the adherence of the materials used. The benefit of uniformity in the size of the propagation unit lies in the ease of sowing, whether manual or mechanized (Gadotti and Puchala, 2010). For water content (WC), there was no statistical difference between the adhesive agents, as all performed well. Melo et al. (2023a) and Melo et al. (2020) working with pelleted corn seeds, and Melo et al. (2023b) in his study on the pelleting of heirloom bean seeds, concluded that all the adhesive agents chosen to coat the seeds do not retain moisture and that the ambient laboratory temperature (32ºC during the research) is sufficient for drying during the pelleting process. It is emphasized that the Thousand-seed weight is an important datum that indicates the quality of seeds to be used per hectare, as well as generating information to calculate the seeding density. Furthermore, determining the water content of a seed lot is of fundamental importance for evaluating its quality and ensuring proper storage (Sarmento et al., 2015).

Table 1. Thousand Seed-Weight (TSW) and Water Content (WC) of heirloom lima bean seeds subjected to adhesive agents for pelleting with powdered sururu shells

Treatments	TSW (g)	WC (%)
Water	699.0 bz	10.0 az
Aloe Vera	744.1 ay	10.5 az
Glue (PVA)	735.1 ay	11.0 az
Mandacaru	748.2 ay	11.5 az
Palm	730.2 ay	11.2 az
Cassava Gum	750.1 ay	12.0 az
	PMS = 698 z	TA = 12 z
CV (%)	13.10	9.50

Source: Authors' own elaboration, *2024*.
Note: Means followed by the same lowercase letter in the column do not differ at the 5% probability level according to the Tukey test. Means followed by the same letter (z, y), between TSW and WC (control – without adhesive agents + rock powder), do not differ significantly at a 5% probability level according to the Dunnett test

In Table 2, the parameters of first germination count (FGC), germination (GER), and germination speed index (GSI) evaluated did not differ statistically from each other. The treatments showed excellent performance in adhering to the seed coating, meaning that water, aloe vera, glue (PVA), mandacaru, palm, and cassava gum were able to attach the powdered sururu shell effectively and did not affect the germination process of the heirloom lima bean seeds. Melo et al. (2023a), Melo et al. (2023b), and Melo et al. (2020) concluded in their respective studies (with corn and beans) that the germination percentage of the seeds is not reduced by pelleting, regardless of the material used. Therefore, it is a good parameter in the execution of the process. When analyzing the coating of rice seeds, Tavares et al. (2012) found that pelleting resulted in a germination rate of over 85%. In a related study, Nascimento et al. (2009) found that the application of cassava gum to pelleted carrot seeds resulted in reduced germination levels. Despite being different species, the results found are a good parameter to be analyzed and differ from those of this study.

The variables analyzed in Table 2 are of utmost importance, as they assess the germination speed. This indicates that the higher the germination of seeds in the first count, the greater their vigor might be (Krzyzanowski et al., 2020). In Table 3, regarding the mean time (MT), it was possible to observe that cassava gum stands out compared to the other treatments, differing statistically from each other. Concluded that some materials allow better diffusion of gases and water between the seed and the external environment, reducing the mean germination time. Melo (2017) explains that the best MT is associated with the lowest value found for this variable. In terms of uncertainty (I), cassava gum also performed better than the other adhesive agents.

Table 2. First germination count (FGC), germination (GER), and germination speed index (GSI) of heirloom lima bean seeds subjected to adhesive agents for pelleting with powdered sururu shells

Tratamentos	FGC (%)	GER (%)	GSI
Water	99 a	100 a	5,183 a
Aloe Vera	100 a	100 a	5,201 a
Glue (PVA)	100 a	100 a	5,133 a
Mandacaru	100 a	100 a	5,111 a
Palm	98 a	100 a	5,105 a
Cassava Gum	98 a	100 a	5,150 a
CV (%)	4.44	8.13	10.87

Source: Authors' own elaboration, *2024*.
Note: Means followed by the same lowercase letter in the column do not differ at a 5% probability level according to the Tukey test

The uncertainty is related to the distribution of the relative frequency of germination, meaning that a low value for uncertainty indicates germination is more concentrated at a specific time (Carvalho et al., 2015). The glue, mandacaru, and palm did not differ statistically from each other, while the aloe vera was the least synchronized treatment compared to the others. According to Santana and Ranal (2004), when interpreting germination uncertainty (U), the lower the value, the more synchronized the germination, regardless of the total number of germinated seeds. In the mean velocity germination (MVG) of the studied seeds, cassava gum and water treatments stood out, not differing statistically from each other. Aloe vera and glue showed a greater delay in the mean velocity of germination. Seed coating can cause problems such as delaying the germination rate (Mendonça et al., 2007; Conceição and Vieira, 2008).

For the variable seedling length (Table 4), cassava gum stands out, differing statistically from the others. Mandacaru had the second longest length, followed by palm, aloe vera, water, and glue. Applying substances with nutritional support directly to the seed coating provides a significant increase in seedling growth, similar to what occurred in this research (Sampaio and Sampaio, 2009). Caldeira

Table 3. Mean time (MT), uncertainty (U), and mean velocity germination (MVG) of heirloom lima bean seeds subjected to adhesive agents for pelleting with powdered sururu shells

Tratamentos	MT (days)	U (bit)	MVG
Water	5.30 b	0.152 c	0.302 a
Aloe Vera	5.21 b	0.705 a	0.135 d
Glue (PVA)	6.11 a	0.313 b	0.145 c
Mandacaru	6.00 a	0.268 b	0.182 b
Palm	5.13 b	0.284 b	0.149 c
Cassava Gum	4.50 c	0.014 d	0.251 a
CV (%)	11.30	10.11	12.58

Source: Authors' own elaboration, *2024*.

Note: Means followed by the same lowercase letter in the column do not differ at a 5% probability level according to the Tukey test

Table 4. Length (L) and dry mass (DM) of seedlings from heirloom lima bean seeds subjected to adhesive agents for pelleting with powdered sururu shells

Tratamentos	L (cm)	DM (g)
Water	19.60 e	1.37 d
Aloe Vera	20.67 d	1.80 b
Glue (PVA)	18.75 e	1.30 d
Mandacaru	24.40 b	1.05 e
Palm	21.85 c	1.62 c
Cassava Gum	27.00 a	1.85 a
CV (%)	17.10	15.02

Source: Authors' own elaboration, *2024*.

Note: Means followed by the same lowercase letter in the column do not differ at a 5% probability level according to the Tukey test

et al. (2016) stated that the substrate used during the pelleting process can act as a physical barrier, impacting the emergence of the primary root and resulting in seedlings of shorter lengths, a different outcome from what was found in this study. For dry mass (DM), cassava gum also stood out among the adhesive agents. Oliveira et al. (2014) emphasize the importance of assessing dry mass to understand seedling development, contributing to their healthy establishment in the field.

Conclusions

The pelleting process of heirloom lima bean seeds resulted in an increase in thousand seed weight, mean velocity germination, length, and dry mass of seedlings. It also decreased the mean germination time and uncertainty.

Cassava gum is the most efficient adhesive agent in pelleting heirloom lima bean seeds with powdered sururu shells.

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