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Research Article

Innovations in lettuce seed pelleting: sustainable materials and machine learning analysis[1]

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ABSTRACT

The low sowing efficiency of lettuce seeds stemming from their small size, irregular shape, and low mass poses a significant barrier to yield. The present study aimed to evaluate the physiological quality of lettuce seeds pelleted with combinations of sustainable coating materials (MB4, Ekosil, BTgran, and mangrove sururu shell powder) and adhesives (PVA glue, FTA glue, and cassava gum). The experiment followed a completely randomized design, in a 3 × 4 + 1 factorial scheme, with germination and vigor variables assessed. The combination of BTgran and PVA glue provided the best performance for germination, seedling length, and dry mass. Machine learning analysis identified the most influential variables with 75 % of accuracy, providing analytical support for the development of more sustainable and accessible agricultural technologies.

KEYWORDS: *Lactuca sativa* L., *Mytella charruana*, seed germination, rock powder.

RESUMO

Inovações na peletização de sementes de alface: materiais sustentáveis e análise por aprendizado de máquina

A baixa eficiência de semeadura de sementes de alface, devido ao seu pequeno tamanho, formato irregular e reduzida massa, representa uma barreira significativa à produtividade. Objetivou-se avaliar a qualidade fisiológica de sementes de alface peletizadas com combinações de materiais de recobrimento sustentáveis (MB4, Ekosil, BTgran e pó de concha de sururu) e adesivos (cola PVA, cola FTA e goma de mandioca). O experimento foi conduzido em delineamento inteiramente casualizado, em esquema fatorial 3 × 4 + 1, com avaliação de variáveis de germinação e vigor. A combinação de BTgran com cola PVA apresentou os melhores resultados para germinação, comprimento de plântulas e massa seca. A aplicação de aprendizado de máquina permitiu identificar as variáveis mais decisivas com 75 % de acurácia, fornecendo suporte analítico para o desenvolvimento de tecnologias agrícolas mais sustentáveis e acessíveis.

PALAVRAS-CHAVE: *Lactuca sativa* L., *Mytella charruana*, germinação de semente, pó de rocha.

INTRODUCTION

Lactuca sativa L., an annual herbaceous species of the Asteraceae family, is the most widely consumed leafy vegetable worldwide and holds substantial social and economic importance (Shi et al. 2022). Its high nutritional value rich in fibers, vitamins (A, B, B2, and C), and minerals such as calcium and iron contributes to its recognition as a food of considerable dietary relevance (Dylag et al. 2023).

Besides its nutritional value and broad consumer acceptance, lettuce plays a crucial role in food security, particularly in regions with limited access to fresh vegetables. However, challenges related to sowing and seedling establishment may compromise its production and availability, since vegetable seeds often exhibit characteristics that hinder their handling, such as irregular shape, small size, and low mass, all of which impair singulation during sowing (Sprey 2018).

Seed pelleting has emerged as an important technology due to the added value it provides and its growing acceptance in the competitive agricultural market (Melo et al. 2020, Liyakat 2024). To optimize this technique, however, a deeper understanding of the interactions among the used materials, particularly their ideal particle size and proportions, is required. A significant information gap persists regarding the effects of coating materials, application methods, and

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additives such as inoculants on seed performance (Lopes & Nascimento 2012).

Rock dust is a potential coating material for seed pelleting, especially within the context of soil remineralization (Lima et al. 2024). This approach incorporates ground rocks or minerals into the soil, functioning as natural fertilizers that enhance fertility without causing environmental harm (Carpenter et al. 2025). Over time, rock dust undergoes physical, chemical, and biological weathering, promoting a slow and continuous release of essential nutrients, including macro- and micronutrients (Swoboda et al. 2022). This gradual release prevents abrupt nutrient peaks and reduces leaching losses, addressing common drawbacks of chemical fertilizers, which are typically highly soluble and more prone to environmental contamination (Manning 2010). Additionally, controlled nutrient release improves nutrient-use efficiency and contributes to the long-term sustainability of production systems (Carpenter et al. 2025).

Mangrove sururu (*Mytella charruana*) shells represent a viable alternative for nutrient supply in seed pelleting, as their composition, primarily calcium oxide (CaO) and magnesium oxide (MgO), allows a partial substitution for commercial nutrient sources (Silva et al. 2024).

In this context, machine learning tools are used to uncover hidden patterns in the experimental data, enabling a more precise interpretation of the effects of coating materials and adhesives on seed physiological performance. This analytical approach serves as a complementary alternative to the conventional seed testing analysis widely applied worldwide, supporting more assertive decision-making by revealing complex relationships among variables (Silva et al. 2025).

Therefore, this study aimed to evaluate the combination of coating materials and adhesives on the physiological quality of pelleted lettuce seeds.

MATERIAL AND METHODS

The assay was conducted at the Universidade Federal de Alagoas (Rio Largo, Alagoas state, Brazil), in November 2024.

Lettuce seeds of the Moana variety, supplied by ISLA Seeds (Brazil), organically certified and free of chemical agents, were used in the experiment, due to their hardiness and suitability for year-round cultivation.

The 2024 seed lot was previously characterized for germination, showing values above the minimum standard for the species, and the seeds were stored at 10 ± 2 ºC and 40-50 % of relative humidity in a controlled, light-protected environment, until the beginning of the assays. The experimental design was completely randomized, in a 3 × 4 + 1 factorial scheme, comprising 3 adhesive agents and 4 coating agents, along with bare seeds used as control.

For pellet production, the used coating agents were Btgran, Ekosil, MB4, and mangrove sururu shell powder, and the adhesive agents were PVA glue (polyvinyl acetate), FTA glue (based on sugar, water, and wheat flour), and cassava gum (cassava starch). For the preparation of the FTA glue, 100 g of sugar and 7 g of wheat flour were dissolved in 200 mL of water. The binding point was determined by evaluating the viscosity and flow characteristics on a cooled surface. Continuous stirring was applied during heating, to ensure homogeneity and prevent excessive foaming or overflow (Voss & Benvegnú 2008). For the cassava gum, 2 tablespoons of cassava starch were dissolved in 500 mL of water and gently heated over low temperature until a transparent, homogeneous gel formed, with constant agitation to prevent aggregation (Meira et al. 2016).

The pelleting process consisted of selecting seeds with a diameter below 2 mm, using a 2-mm mesh sieve to ensure homogeneity and absence of physical damage, whereas seed diameters were measured in triplicate using a digital calliper and compared with predefined quality control thresholds. The seeds were pre-moistened with distilled water or diluted adhesive solution under gentle agitation to enhance the adhesion of coating materials. The coating agents were sieved to obtain particle sizes between 50 and 150 µm, using standardized sieves (mesh size, material, and manufacturer detailed), with particle size confirmed through optical measurement in triplicate. Adhesive solutions of 5 % w/v cassava starch gum, 3 % w/v PVA glue, and 4 % w/v FTA glue were prepared and maintained under constant agitation to ensure homogeneity. Pelleting was performed in a rotary drum (~30 rpm), alternating fine sprays of adhesive with the application of coating material, until the desired pellet diameter (3-5 mm) was achieved. Pellets were dried for at least 24 hours in a ventilated, shaded environment until moisture content decreased below 12 %, and finally calibrated by sieving to ensure uniformity. All parameters, including rotation speed, processing time, material quantities, and number of repetitions, were recorded to guarantee reproducibility.

Bare and pelleted seeds were placed in Gerbox-type boxes, between sheets of Germitest paper moistened with distilled water at 2.5 times the weight of the seeds. The boxes were then placed in a B.O.D. (Biochemical Oxygen Demand) incubator at 20 ºC (Brasil 2025). For each treatment, 100 seeds were used, distributed in 4 replicates of 50 seeds, totaling 2,400 seeds per treatment.

Germination was recorded upon the formation of normal seedlings. The first germination count was performed on the 4th day after sowing, followed by daily evaluations for 7 days (Brasil 2025).

To identify the most relevant physiological attributes for treatment classification, a Random Forest machine learning model was applied. The variables included germination percentage, first germination count, germination speed index, mean germination time, mean germination rate, synchrony, uncertainty, seedling length, and seedling dry mass. Observations were labeled as either "High" or "Low" physiological performance, based on principal component analysis (PCA) and distribution of scores. The model was trained with a stratified data sample and validated using k-fold cross-validation with $k = 5$, ensuring that all observations were used for both training and testing. Data processing and model development were conducted in a Python 3.10 environment, using the Scikit-learn (Pedregosa et al. 2011), Pandas (McKinney 2010), Seaborn (Waskom 2021), Matplotlib (Hunter 2007), and NumPy (Harris et al. 2020) libraries.

The physiological quality of seeds was assessed through standard tests, including moisture content (oven method at 105 ± 3 ºC, for 24 h; Brasil 2025), first germination count (on the fourth day after sowing), and total germination (normal seedlings with radicles ≥ 2 mm, counted daily for 7 days). The germination speed index was calculated following Maguire (1962), whereas the mean germination time and mean germination rate were determined using the formulas of Czabator (1962) and Santana & Ranal (2004), respectively. Germination synchrony and uncertainty were calculated as proposed by Labouriau (1983) and Santana & Ranal (2004), using the GerminaQuant 1.0 software. Seedling length was measured at the end of the germination test, and dry mass was obtained after drying at 80 ºC for 24 h, and weighing on an analytical scale (Krzyzanowski et al. 2020).

The resulting data were subjected to analysis of variance (Anova), using the PAST 4.03 software (Hammer et al. 2001). When the F-test indicated significance, the means were compared using the Tukey test at 1 % of probability, and, when necessary, the Dunnett's test was also applied at 1 % of probability. Additionally, a principal component analysis (PCA) was conducted to identify patterns and relationships among the variables, complementing the overall analysis.

RESULTS AND DISCUSSION

The initial moisture content of non-pelleted lettuce seeds (bare seeds) was 11.5 %, whereas pelleted seeds coated with various adhesive and coating agents exhibited moisture contents ranging from 11.95 to 12.3 %. The statistical analysis indicated that these differences were significant ($p < 0.05$), likely reflecting the hygroscopic nature of the pelleting materials, which can retain slightly higher amounts of water, when compared to bare seeds. Maintaining optimal moisture levels, typically between 8 and 12 % for lettuce seeds during storage, is crucial for preserving physiological stability and ensuring uniform germination, as deviations beyond this range may impair early metabolic processes and seed vigor (Lobo et al. 2020).

The germination results for the pelleted lettuce seeds subjected to adhesive and coating agents are presented in Table 1. The treatments using BTgran in combination with all adhesive agents (PVA glue, cassava gum, and FTA glue), as well as Ekosil + PVA glue, MB4 + gum, and sururu shell powder + gum, showed no statistical differences among themselves or in relation to the bare seeds. The efficiency of these treatments and the absence of negative effects on germination can be attributed to the ability of coating and adhesive agents to create more stable conditions for initial seed imbibition (Coraspe et al. 2015). Uniformity in water absorption is crucial for activating hydrolytic enzymes, such as amylase, which is essential for mobilizing energy reserves stored in seed tissues (Weits et al. 2020). The incorporation of organic and inorganic components with bioactive potential into seed coatings expands the use of natural elements defined here as substances derived from plants, minerals, or other non-synthetic

Table 1. Germination of lettuce seeds subjected to coating agents and adhesives.

Coating agents	Adhesives		
	Glue	Gum	FTA
Ekosil	78 ABaz	73 Baby	61 Abby
MB4	51 Cby	90 Aaz	46 Bby
BTgran	86 Aaz	80 Aabz	75 Aaz
Sururu	69 Bay	82 Aabz	47 Bby
Germination (%) = 85 z			
F for coating agents (CA)	11.13**		
F for adhesive agent (AA)	34.44**		
F for interaction (CA x AA)	8.47**		
CV (%)	11.68		

Means followed by the same lowercase letter in the row and the same uppercase letter in the column within the factorial do not differ significantly at 1 % of probability by the Tukey test. Means followed by the same letter (z, y) indicate comparison with the control (without coating agents and adhesives) and do not differ significantly at 1 % of probability by the Dunnett's test. ** Significant at 1 % of probability.

sources in sustainable agricultural practices. These natural elements can provide nutritional, protective, or growth-promoting benefits, enhancing seed performance while minimizing environmental impact (Amirkhani et al. 2019).

Moreover, the materials used in the treatments may have mitigated physical stresses such as fluctuations in temperature or moisture, thus providing a more favorable environment for metabolic processes leading to germination. This stability supports ATP synthesis and the production of metabolic compounds essential for embryonic axis growth, thereby promoting a vigorous seedling development (Zhang et al. 2020).

These findings are consistent with the observations of Sprey & Ferreira (2018), who reported no significant differences in the physiological quality between pelleted and non-pelleted tomato seeds. In this study, pelleting was performed using dolomitic limestone and cassava starch as coating materials, applied at 4 % w/v in the adhesive solution with particle sizes of 50-150 μm. Among the evaluated coating materials, dolomitic limestone demonstrated a slightly superior performance, when compared to cassava starch, in promoting seed uniformity and handling. Similar results were observed by Melo et al. (2023) and Melo et al. (2020), who used aloe vera, cactus extract, and sugar syrup as binding agents, and rock powders as coating materials for pelleting maize and bean seeds. In both studies, germination and vigor parameters were not negatively affected by pelleting, regardless of the employed materials. These findings reinforce the potential of alternative and sustainable pelleting techniques as effective strategies to maintain seed viability and physiological performance.

The principal component analysis (PCA) was conducted to evaluate the influence of BTgran rock powder in combination with PVA glue, cassava gum, and FTA glue. The results revealed that the first two principal components (PC1 and PC2) explained 41.75 and 20.52 % of the total variance, respectively, accounting for 62.27 % of the overall variability (Figure 1). The PCA enabled the identification of correlations among the analyzed variables and treatments.

The BTgran + gum treatment exhibited the highest values for mean germination rate, germination synchrony, germination percentage, and germination speed index, all concentrated in the PC1. These results suggest that the cassava gum contributed to an efficient coating by forming a film that reduced water loss and promoted uniform seed imbibition (Assis 2022), which is essential for metabolic activation and germination (Liyakat 2024). Similar findings were reported by Pirola et al. (2016) for *Poncirus trifoliata* seeds coated with cassava starch, where germination was not impaired. In contrast, the BTgran + FTA treatment led to increased germination uncertainty in the PC2, indicating a reduced germination synchrony, likely due to the high water solubility of FTA, which compromised coating stability. Conversely, the BTgran + PVA glue treatment performed favorably in both principal components (PC1 and PC2), especially for first germination count, seedling length, and seedling dry mass. These results demonstrate that the PVA glue facilitated a uniform coating application, promoting improved early seedling development. The correlation between seedling length and seedling dry mass further supports the understanding that seedlings with greater length and dry mass exhibit superior physiological performance (Vanzolini et al. 2007).

Overall, the performance of the treatments was influenced by both the used materials and the pelleting process itself. As reported by Lobo et al. (2020), pelleting enhances early seedling development by improving growth- and vigor-related variables. These findings highlight the potential of cassava gum and PVA glue, when combined with BTgran, as promising materials for lettuce seed pelleting and as viable technological alternatives for improving field establishment.

Figure 2 presents the effects of MB4 rock powder combined with binding agents. The first two principal components explained 93.23 % of the total variance, with PC1 representing 85.94 % and PC2 7.29 %. Mean germination time, seedling length, and synchrony were strongly positively associated with PC1 and correlated with the MB4 + gum treatment. Due to its composition, cassava gum forms a uniform coating layer that enhances water retention, a key factor for activating hydrolytic enzymes (Melo et al. 2024). This structure also facilitates gas exchange, supporting respiratory metabolism (Weits et al. 2020) and contributing to vigorous seedling development (Marini et al. 2020).

The MB4 + FTA treatment showed moderate but less pronounced effects, positioned near the center

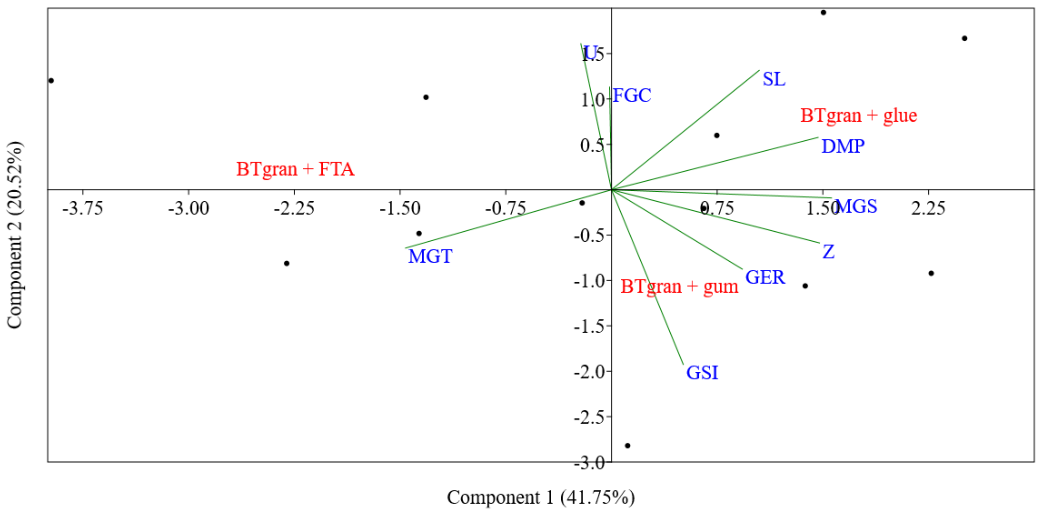

Figure 1. Principal component analysis (PCA) of lettuce seed germination and seedling growth variables influenced by BTgran and binding agents (PVA glue, gum, FTA). GER: germination percentage; FGC: first germination count; GSI: germination speed index; MGT: mean germination time; MGS: mean germination speed; Z: synchrony; U: uncertainty; SL: seedling length; SDM: seedling dry mass.

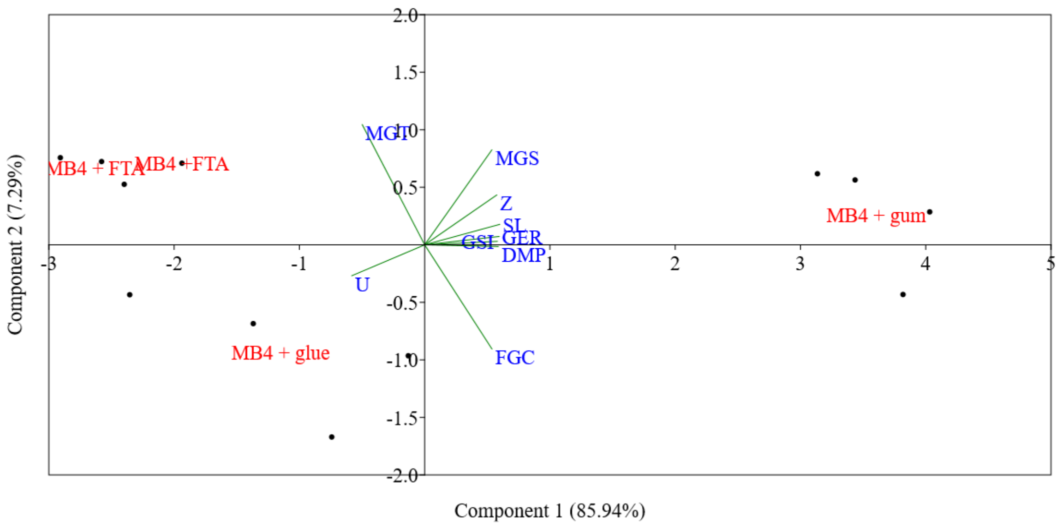

Figure 2. Principal component analysis (PCA) of lettuce seed performance variables influenced by the coating agent MB4 and binding agents (PVA glue, gum, FTA). GER: germination percentage; FGC: first germination count; GSI: germination speed index; MGT: mean germination time; MGS: mean germination speed; Z: synchrony; U: uncertainty; SL: seedling length; SDM: seedling dry mass.

of the plot, whereas MB4 + PVA glue exhibited weak correlations with the evaluated variables, located in the lower left quadrant. These results indicate that the PVA and FTA glues did not achieve the same effectiveness in interacting with MB4, possibly due to differences in solubility and adhesion properties required for efficient pelleting. This limitation may be related to the requirement that binding and coating agents be water-soluble to ensure consistent adhesive performance rather than simply losing consistency upon hydration (Sprey 2018).

Through multivariate interpretation, the PCA demonstrated that cassava gum, when used as a binding agent in combination with MB4, contributes positively to seed physiological performance and early seedling development.

The influence of the EKOSIL coating agent combined with the adhesive agents (PVA glue, gum, and FTA) is presented in Figure 3, where the principal components explained 80.97 % of the total variance, with PC1 and PC2 contributing with 70.54 and 10.43 %, respectively. The variables germination, seedling dry mass, seedling length, and germination synchrony showed a strong association with the EKOSIL + PVA glue treatment, concentrated in the first principal component (PC1). In turn, the treatment containing gum positively influenced the variables first germination count, germination speed index, and mean germination speed, as indicated by the proximity of the points.

The performance of EKOSIL + PVA glue can be attributed to the solubility of the coating in water, which facilitates absorption and gas exchange, promoting the activation of metabolic processes essential for germination and early growth (Melo et al. 2020). In contrast, the EKOSIL + FTA treatment was positioned in the upper left quadrant, associated with PC2, showing a longer mean germination time. These results suggest that the combination formed a barrier, hindering water uptake and delaying germination. This limitation may lead to a longer time interval for the reactivation of seed metabolic processes (Mendonça et al. 2007). EKOSIL demonstrated effectiveness when combined with PVA glue and cassava gum, promoting a higher seed vigor and more uniform germination. Specifically, the treatments enhanced the germination speed index, first germination count, and mean germination speed, indicating a faster and more synchronized seedling emergence. In contrast, the association with FTA glue reduced these vigor-related variables, likely due to the formation of a water-impermeable barrier that delayed water uptake and the reactivation of metabolic processes, resulting in slower and less uniform germination.

Figure 4 shows the sururu shell powder combined with the binding agents PVA glue, cassava gum, and FTA. The principal components explained 84 % of the total variance, with 70 % attributed to PC1 and 14 % to PC2. The results indicated that

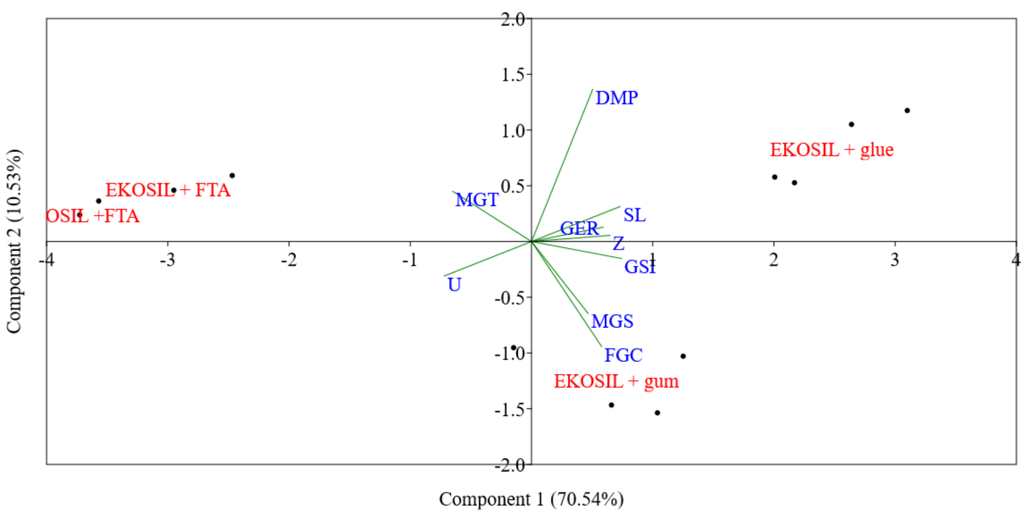

Figure 3. Principal component analysis (PCA) of lettuce seed performance variables influenced by the coating agent EKOSIL and binding agents (PVA glue, gum, FTA). GER: germination percentage; FGC: first germination count; GSI: germination speed index; MGT: mean germination time; MGS: mean germination speed; Z: synchrony; U: uncertainty; SL: seedling length; SDM: seedling dry mass.

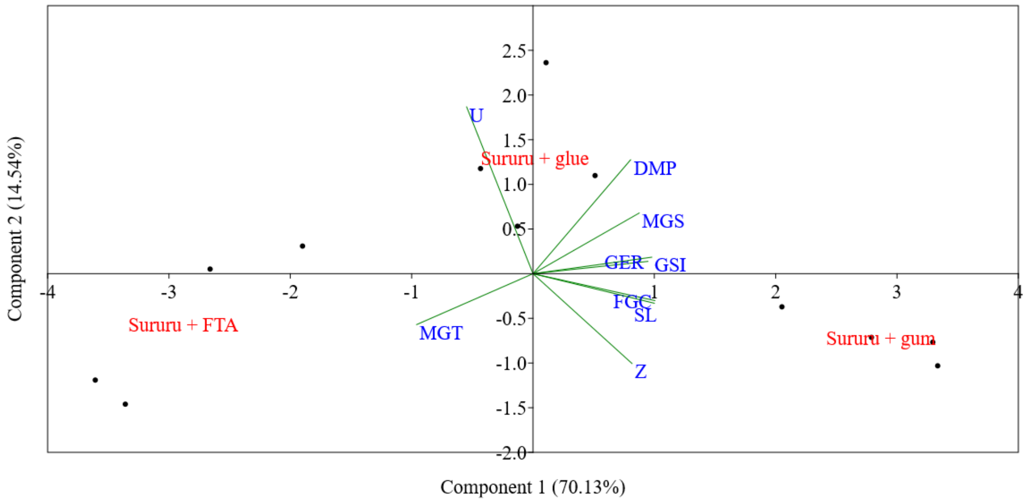

Figure 4. Principal component analysis (PCA) of lettuce seed performance variables influenced by sururu shell powder and binding agents (PVA glue, gum, FTA). GER: germination percentage; FGC: first germination count; GSI: germination speed index; MGT: mean germination time; MGS: mean germination speed; Z: synchrony; U: uncertainty; SL: seedling length; SDM: seedling dry mass.

the sururu + FTA treatment had the lowest mean germination time, associated with PC1, suggesting that FTA enhanced seedling uniformity and vigor. This behavior may be related to the agent's ability to facilitate water absorption and gas exchange, promoting a more efficient development (Nascimento et al. 2009).

The sururu + PVA glue and sururu + gum treatments showed similar results for germination variables and germination speed index, positioning themselves at the center of the plot. These materials likely provide favorable conditions for the germination process. On the other hand, the sururu + PVA glue treatment recorded the highest uncertainty index, indicating a greater variation in germination time, which may compromise batch uniformity. Variables such as germination synchrony were more associated with the sururu + gum treatment, highlighting its efficiency in promoting a more concentrated germination within a shorter time frame. Silva et al. (2024) reported that the use of sururu shell increases the activity of enzymes involved in respiratory metabolism and the citric acid cycle, through the proper availability of cations such as calcium and magnesium. These elements are directly related to membrane stability and mitochondrial integrity, contributing to more efficient metabolism and increased ATP production in plant cells.

Coating materials that promote greater synchrony and reduce variability enhance seed physiological performance, optimizing germination and early seedling development (Melo et al. 2023). However, materials that form inadequate physical barriers may delay the onset of germination, as observed in some cases in the present study. These findings reinforce the importance of selecting the right coating agents and adhesives in the pelleting process, especially when using organic waste such as sururu shell powder, which has the potential to improve the quality and performance of lettuce seeds. Therefore, the processing of sururu shells into powder can be directly applied to lettuce seeds, helping to reduce the amount of waste from this mollusk in the environment. However, further studies are needed to evaluate the effects of this waste on other agricultural seeds.

The application of the Random Forest model in this study allowed the identification of the physiological variables with the greatest discriminative power among the treatments applied to lettuce seeds. The model's accuracy, at 75 %, was considered satisfactory, given the data complexity and the number of observations per treatment. The variable importance analysis (Figure 5) revealed that germination was the most relevant variable for classifying the treatments, with a relative contribution of 24 %, followed by seedling length with 18 %, and seedling dry mass with 16 %. These three variables, therefore, account for more than half of the model's predictive capacity.

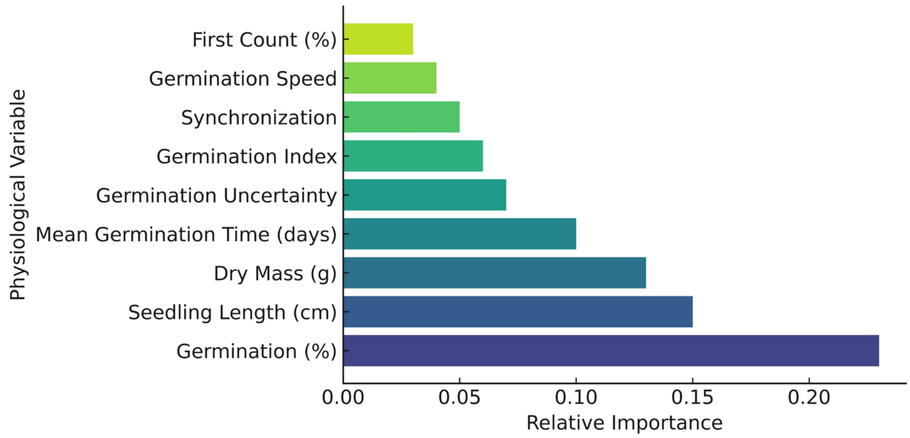

Figure 5. Relative importance of physiological variables in predicting seed germination and early seedling growth using Random Forest.

This result is consistent with the intrinsic nature of the evaluated variables: germination is the primary indicator of seed viability, whereas seedling length and dry mass represent key vigor attributes and reflect the potential for a successful establishment in the field. These variables also stood out in the conventional statistical analyses, particularly in the treatments involving BTgran + PVA glue, confirming the agreement between traditional statistical methods and the machine learning approach.

Other variables such as mean germination time, uncertainty, and germination speed index also contributed substantially to the model, with importance values ranging from 8 to 12 %. This reinforces that the temporal dynamics of germination has a significant influence on overall physiological performance. Therefore, the incorporation of machine learning not only complements multivariate analyses, but also strengthens the decision-making process in developing efficient and sustainable pelleting treatments for lettuce seeds.

CONCLUSIONS

1. The combination of BTgran + PVA glue was the most effective treatment, promoting higher germination, vigor, and seedling development;
2. The pelleting technique using natural materials proved to be a viable and sustainable alternative for lettuce seeds;
3. The Random Forest model identified germination, seedling length, and dry mass as the most relevant variables, reinforcing the experimental findings;
4. The multivariate approach combined with machine learning provided a more precise interpretation of the results and supports the use of agro-industrial residues as a low-cost, high-potential strategy in sustainable agriculture.

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