Determination of physical properties and viability in different colors of Pueraria seeds

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ABSTRACT

The differences observed in the physical properties of the seeds may indicate types of equipment for harvesting and processing, in addition to variability between plants. We studied the characterization of the dimensions of the seeds, linear and geometric and defined viability in categories. The results showed that the studied seeds did not reach a spherical shape, and their mass underwent variation resulting from the sorption and desorption process. The morphological characteristics suggested slight differences in the shape of the seeds. A high coefficient of variation was observed for fresh seed mass (18.34%), volume index (18.07%), seed volume (18.07%), and seed surface area (12.47%). Seed colorations showed differences in germination. The analysis concluded that all categories of seeds are viable. These descriptive data indicate that the combination of seed characteristics and the germination process help in methodologies for planting, harvesting, processing, and storage of seeds.

Keywords: Linear and Geometric Dimensions. Morphology. Pueraria phaseoloides.

Introduction

The knowledge about seed physical and structural characteristics is still relatively incipient, mainly of some forest and/or forage species, as is the case of Pueraria phaseoloides (Roxb.) Benth.). Also known as Pueraria, a forage species that presents seeds with integumentary numbness (PINHEIRO et al., 2019). It is widely used in the Amazon, intercropped with crops and soil cover (PINHEIRO et al., 2017). In addition, germinative processes influenced by quiescence and dormancy also reflect the absence of viable seed germination (PINHEIRO et al., 2021). However, germination requires mechanisms that offer ideal conditions to achieve optimum seedling development.

For this to occur, it is necessary to know the levels of dehydration, physical and structural characteristics, and the factors that affect germination (environment, types of dormancies, ecosystems). So, the aggregated knowledge generated by different studies results in various information on seeds. This information is critical to assist in large-scale analyses and supports decision-making in seed conservation operations.

Studies by Miguel et al. (2015), Fawzi et al. (2016), and Pontes et al. (2018a) affirm that seed morphometric discrimination is an important tool for the intra and interspecific detection of differences in plant populations in taxonomic, phylogenetic, and ecological. Moreover, the quantification of biological parameters by mathematical models and statistical methods obtained from experimental data is a widely established practice in several branches of biology (MONTEIRO; REIS, 1999). Analyzing
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morphometric characteristics is an essential parameter for understanding the dispersion syndrome and successional stages, providing additional data for germination physiology, natural regeneration, programs, or restoration of vegetation cover in altered areas (SANTIAGO; PAOLI, 1999; FENNER; THOMPSON, 2005).

In recent years, morphometric analysis (length, width and thickness, and fresh and dry mass) has been used to detect and interpret differences in the shape and size of seeds (SANTOS et al., 2015; PINHEIRO et al., 2017; ROSO et al., 2019; SANTOS et al., 2020), allowing a better understanding of the relationship between individuals and groups (CHRISTO et al., 2014). The effect of variability in seed size (dimensions) and genetic, physical, and physiological quality has been the subject of several studies with different approaches, considering different performance components in seeds and their resulting seedlings (PELILLISSIER, 2013) as well as their mass. Understanding the dimensional and three-dimensional shapes of the seeds is essential to the design and construction of machines used for processing and pre-processing.

Furthermore, according to Altuntas et al. (2005), it assisted in the decision-making in a project to store bulk materials. However, few studies reported measuring the physical properties of seeds in most species. These data can be organized into dimensions (seed volume index, geometric mean diameter, equivalent average diameter, arithmetic mean diameter, seed sphericity, surface area, aspect ratio, seed volume).

In addition, biotypic characteristics (dimorphism) of the color of the seed coat can also be a factor that interferes with the germination of a seed. This dimorphism can be accompanied by the physiological process that occurs in the heterogeneous maturation of the species. The seed has uneven colors at harvest time, but its physiological maturity is never the same. In this way, the maturation aspects may have been accompanied by cutaneous and/or embryonic numbness and may result in greater longevity.

On the other hand, bicolor tones are part of the egg since its fertilization. In some cases, seed bicoloration is related to the accumulation of flavonoids (including anthocyanins) and carotenoids, and betalains. The first two classes are generalized, while betalains are found exclusively in a group of angiosperms, Caryophyllales (including beets and amaranth), but never in combination with anthocyanins (STAFFORD, 1998). Other factors interfere in the species are the age of the mother plant, the position of the seed in the fruit, the inflorescence, as well as the canopy and can affect the properties of the seeds, often accompanied by a dimorphism, in the seeds themselves, in the fruits and the flowers (ROACH; WULFF, 1987).

In this study, the color of the seeds accompanies the physiological maturation of the fruits at harvest time, as they accompany the seeds, making the color uneven. Thus, the focus of this study was to evaluate linear and geometric dimensions and viability of the seeds as a function of the tegument colors in Pueraria seeds.

Material And methods

The seeds were obtained in Acre, a state that is part of the legal Amazon, where this species was used to recover soil fertility. For the biometric characterization of the Pueraria seeds, a lot of 20,000 units was used. First, random seeds were selected and evaluated for the fresh mass’s length, width, thickness, and weight. To determine the fresh weight of the seeds after harvest, the material selected for weighing was 150 individual units. An analytical balance (Bel Mark 210A) was used precision to four decimal places, and the results were expressed in milligrams. Then, based on an arrangement
of seed hilum, the length from the hilum to the opposite side and width from one end to the other was measured. Moreover, the thinner apparent side determined the thickness (Figure 1).

The evaluations were made with a King Tools 150BL digital caliper (sensitivity of 0.01 mm) and a digital scale (sensitivity of 0.001g). Then, the following properties were calculated according to equations (Eq. 1; Eq. 2; Eq. 3; Eq. 4; Eq. 5; Eq. 6; Eq. 7 and Eq. 8).

\[ SVI = \text{length} \times \text{width} \times \text{thickness} \]  
\[ GMD = SVI^{\frac{1}{3}} \]  
\[ EMD = \left( \frac{\text{length}(\text{width} \times \text{thickness})}{4} \right)^{\frac{1}{2}} \]  
\[ AMD = \frac{\text{length} + \text{width} + \text{thickness}}{3} \]  
\[ SA = \pi D MG^2 \]  
\[ \varnothing = \left[ \frac{GMD}{\text{length}} \right]^{100} \]  
\[ Ar = \left[ \frac{\text{width}}{\text{length}} \right]^{100} \]  
\[ V = \frac{SVI}{6} \]

Seed volume index = SVI – Vieira et al., (2008) eq. 1; Geometric mean diameter = GMD - eq. 2, Equivalent mean diameter = EMD - eq. 3, Arithmetic mean diameter = AMD - eq. 4 – Sahay and Singh, (1994); Surface area = SA eq. 5 – McCabe, Smith and Harriot, (2005); Seed sphericity= \( \varnothing \) eq. 6, Seed volume = V eq. 8 – Mohsenin, (1986); Aspect ratio = Ar eq. 7 – Varnamkhashi et al., (2008).

Before measuring their linear properties, the water content of the seeds was determined according to the Brazilian rules for seed analysis (RAS) (Brasil, 2009). Then, the maximum and minimum values, mean, standard deviation (SD), coefficient of variation (CV), 95% CI, and relative frequency were calculated for all evaluated variables. Next, four classes were determined based on the frequency, and the percentage of their amplitude was determined in each one, using a rule of three. Finally, descriptive statistics and relative frequency were calculated with the statistical programs.

As for the color, the viability of the seeds and the information provided by the producers, who say that the black seeds are not viable (dead), were verified as follows. The seeds were visually separated by color into four categories: black seeds (BS), light red (LRS), dark red (DRS), and mixed (MS) (Figure 2). The germination test was conducted with five subsamples of 50 seeds per repetition, with a constant temperature of 25 °C on substrate blotting paper. According to the RAS, the evaluations were carried out at four and ten days (Brasil, 2009).

Figure 1 - Characteristics of the three-dimensional geometry of the seeds: a-length; b-width; c-thickness. Average seed size: length mm x width mm x thickness mm.
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Three colors, in addition to a mixed sample with all seed colors, four categories, and five repetitions were established. The experimental design was completely randomized in scheme 4 (categories or stains) x 5 (repetitions) and subjected to an analysis of variance. When significant, it was subjected to Duncan’s test (P <0.05). Pearson’s correlation coefficient (r) measures the degree of linear correlation between two quantitative variables and ranges from -1 to +1. The closer the result to these values, the stronger the correlation between the variables examined (Ayres et al., 2007).

**Results and discussion**

The values of the analysis of physical properties, morphometry, and fresh mass of the seeds are shown in Table 1. The result of weighing the fresh mass of the seeds ranged from 1.8 to 17.1 mg (mean = 11.24 mg), demonstrating that they can be classified into the small and light seed categories. The dimensions of the seeds can also confirm this, considering that the length of the seeds presented 1.68 to 2.78 mm (average 2.33 mm), the width 2.06 to 3.86 mm (average 3.04 mm), and the thickness was 1.41 to 2.47 mm (mean 2.05 mm). It should be noted that the dimensions presented demonstrate that the variable width has a greater amplitude than the others, whereas the length and thickness are similar in size. The seed length is always measured from the hilum opposite, even if the width is greater than the length.

Some studies classify seeds according to the mass of a thousand seeds. For example, Cavalcante et al. (2017) consider the weight of moringa seeds, of 202.20 g/1000 seeds, as light. Ramos et al. (2010) evaluated the mass of a thousand seeds of the same species previously mentioned and obtained values ranging from 194.25 to 199.75 g. Bezerra et al. (2004) consider the average weight value of 218.88 g/1000 seeds and consider 177.07 g/1000 seeds. With this, it can be considered that Pueraria seeds are extremely light and small since their average mass is 75 mg/1000 seeds, which only reinforces the data of this study.

Generally, in seeds with two dimensions (diameter and length), called rounds, the length is always greater. On the other hand, those with three dimensions may have an inversion in size, where width can be greater than length, and the thickness is always the less thick (thinner)
part. The least thick part (thinnest) is always considered to measure thickness, and this measure will always be smaller than the others. These dimensions vary from species to species, being more common in some forages and forests (Fabaceae) since they are the ones that have characteristic three-dimensional seeds, as well as some agricultural species such as corn, rice, and beans.

When studying the same species, Pinheiro et al. (2019) found that the fresh mass of the seed was the one with the highest coefficient of variation (CV = 19.51%). These results corroborate our findings since a higher coefficient of variation was also observed in the fresh weight of the seeds (CV = 18.37%). These high CV results for the fresh mass can be related to the hygroscopicity of the seeds, which means they can yield or absorb water from the environment. Thus, if the water vapor pressure in the seed is lower than that of the air, moisture absorption (sorption) occurs, and when the opposite happens, the seed yields water to the air (desorption). Hygroscopic equilibrium is achieved when the partial pressure of water vapor in the product equals the partial pressure of water vapor in the air that surrounds it at the same temperature (PESKE et al., 2019).

Corrêa et al., 2005 affirm that the oscillation in seed sizes within a lot can influence a balance between water content and ambient air conditions. Also, the majority of the seeds are not being collected with equal physiological maturity since seeds with advanced maturity tend to lose mass and viability faster. According to Macedo et al. (2009), the influence of the environment on seed development mainly translates to variations in size, weight, physiological potential, and health.

The physical properties of Pueraria seeds showed a more significant coefficient of variation in the seed volume index and in the seed volume (18.07%), both calculated based on their dimensions, which explains why the relationship between the three-dimensional seeds is uneven. Thus, the volume (V) and the seed volume index (SVI) are the amplitude indicators that help determine the direction of the sizing and regulation of equipment for harvesting.

Table 1 - Biometric and physical properties and fresh weight characterization of Pueraria phaseoloides seeds. Seed volume index (SVI), Geometric mean diameter (GMD), Equivalent mean diameter (EMD), Arithmetic mean diameter (AMD), Surface area (SA), and Seed volume (V).

<table>
<thead>
<tr>
<th>Properties</th>
<th>Minimum</th>
<th>Mean ± SD</th>
<th>Maximum</th>
<th>CV (%)</th>
<th>± 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>L (mm)</td>
<td>1.68</td>
<td>2.33± 0.18</td>
<td>2.78</td>
<td>7.75</td>
<td>2.30 – 2.35</td>
</tr>
<tr>
<td>W (mm)</td>
<td>2.06</td>
<td>3.04± 0.32</td>
<td>3.86</td>
<td>10.43</td>
<td>2.9 – 3.09</td>
</tr>
<tr>
<td>T (mm)</td>
<td>1.41</td>
<td>2.05± 0.17</td>
<td>2.47</td>
<td>8.41</td>
<td>2.02 – 2.08</td>
</tr>
<tr>
<td>M (mg)</td>
<td>1.80</td>
<td>11.24± 2.06</td>
<td>13.60</td>
<td>18.34</td>
<td>10.91 – 11.57</td>
</tr>
<tr>
<td>SVI</td>
<td>4.88</td>
<td>14.65± 2.65</td>
<td>21.46</td>
<td>18.07</td>
<td>14.23 – 15.07</td>
</tr>
<tr>
<td>GMD</td>
<td>1.70</td>
<td>2.44± 0.16</td>
<td>2.78</td>
<td>6.51</td>
<td>2.41 – 2.46</td>
</tr>
<tr>
<td>EMD</td>
<td>1.13</td>
<td>1.44± 0.06</td>
<td>1.60</td>
<td>4.46</td>
<td>1.42 – 1.44</td>
</tr>
<tr>
<td>AMD</td>
<td>1.72</td>
<td>2.48± 0.17</td>
<td>2.88</td>
<td>6.69</td>
<td>2.44 – 2.50</td>
</tr>
<tr>
<td>SA (mm²)</td>
<td>9.04</td>
<td>18.74± 2.34</td>
<td>24.26</td>
<td>12.47</td>
<td>18.36 – 19.11</td>
</tr>
<tr>
<td>V (mm³)</td>
<td>2.56</td>
<td>7.67± 1.39</td>
<td>11.24</td>
<td>18.07</td>
<td>7.45 – 7.89</td>
</tr>
<tr>
<td>Ø (%)</td>
<td>92.29</td>
<td>104.82± 5.28</td>
<td>116.70</td>
<td>5.03</td>
<td>103.97 – 105.6</td>
</tr>
<tr>
<td>Ar (%)</td>
<td>93.52</td>
<td>131.07± 14.25</td>
<td>163.59</td>
<td>10.87</td>
<td>128.8 – 133.34</td>
</tr>
</tbody>
</table>

Where: In columns, C = length; W = width; T = thickness; M = mass; Ø = sphericity; Ar = aspect ratio; CV = coefficient of variation; CI = confidence interval; SD = standard deviation.
sowing, and processing seeds. In addition, this information can be used to identify trends for both dimensions. For example, the sphericity of the seed indicates how close the shape is to a sphere, and our study found that the seeds presented a high average value (104.82%), meaning that the seeds did not follow a spherical trend since the width offered the most remarkable breadth of size.

The geometric and arithmetic mean diameter showed similar results for the variation coefficient (6.51 and 6.69%), and the equivalent mean diameter was the least varied among all variables (4.46%). These actual variations of the mean diameters demonstrate how frequently unevenness occurs in the morphological format and in what is related to the relative aspect of the seed mass. Pontes et al. (2018b) state that these diameters provide information on the intensity of oscillations in seed dimensions.

The surface area of the seeds, which is affected by the drying process and storage, presented a variation coefficient of 12.47%, being a good aspect for the activation of the water maintenance mechanisms in the seeds. According to Zareiforoush et al. (2011), the effects of the conditioning of the seeds depend on the effects of volume and surface, which consequently influence the water uptake during the seed hydrolysis, directly impacting seed germination. Furthermore, the relationship between the surface area and the seed volume is directly related to the seed drying time, soaking, and energy requirements during the germination process. The effects of the surface area on the absorption and water loss rates of particulate materials can also be characterized by the use of the surface concerning its volume (MIR et al., 2013).

It is essential to report that some authors relate the seed format to some survival mechanisms of the species when in adverse situations. For example, the shape of the seeds is consistently linked to the longevity of the seeds in soil seed banks (PINHEIRO et al., 2021), explaining the tolerance to fire or heat of seeds recovered after fires (RUPRECHT et al., 2015). Furthermore, directional selection results from the choice of dispersers when selecting fruits and seeds (HEELWRIGHT, 1993; SOBRAL et al., 2014; RIBEIRO et al., 2016).

The seed aspect ratio, which relates to the distribution of the three linear dimensions of the seeds (Figure 3), classifies and visualizes the degree of size outside standards, separating it into small, medium, and large within a lot. The projected oscillation peaks show that for the 150 seed units analyzed, 79 presented a marked peak of lesser relation, tending to the minimum, characterizing smaller seeds. The highest range of oscillation was number 139, which reached high values of maximum oscillation—bearing in mind that the seeds are already small in size. When analyzed geometrically, a size classification can be established to interpret the aspect ratio of the seeds.

Figure 4 shows seed fresh mass ranges of 10.7 to 13.6 mg (70.7%) for length, from 2.24 to 2.50 mm (56.7%); for width, from 2.97 to 3.41 mm (52.7%); and for thickness, from 1.95 to 2.20 mm (56.7%). The values found show that the seed lot does not present great variations in morphological forms, which the variation coefficient can observe. It is important to report that the greater the variation found in forest seeds, the better the genetic condition of the seed, as there was no loss of genetic variability by drift, which is a change of natural order that affects genotypic characteristics.

It can also involve endogamy, where there is a cross between a certain degree of kinship. In this case, parental plants pollinated between the same species, which can happen in situations of small and dense populations. Some studies report these conditions. Seoane et al. (2000) observed that in small forest fragments,
subpopulations of “guarantã” (Esenbeckia leiocarpa Engl.) are subject to loss of genetic variability due to drift. Pinheiro and Ferreira (2018) affirm that a low variability in fruits and seeds of nearby parental plants can cause a loss in genotypic characteristics. In his study on fruit and seed morphometry of Geonoma maxima subsp. chelidonura (Spruce), A. J. Henderson found that plants with a close degree of kinship affect heterogeneity in primary forests, and it is the same with native plants that are very close to each other, which favors inbreeding (MAUÉS; OLIVEIRA, 2010).

So, endogamy results from a natural condition of the species, which expresses genotypic characteristics to guarantee its survival for a long time and without competition between individuals in that space (PINHEIRO; FERREIRA, 2018). A disperser is needed to carry seeds away, and when this does not happen, parents’ crossings occur, which can be a strategy for survival and longevity by the parental plant.

The classes of established seeds show the variations of amplitudes of the morphological aspects in a linear way, where the three penultimate classes of smaller size for the fresh mass of the seed represent 29.3% (class 1: 1.3%; 2: 6%; 3: 22%), featuring a small number of seeds with extremely light mass. For length in classes 1 and 2, they were 2.7% and 23.3%, respectively, integrating the lowest values and 17.3% corresponding to class 4 with values from 2.51 to 2.78 mm, considered greater length. The classes demonstrate fluctuations in the aspects of the seeds, which is why they are not uniform (CV = 10.87%).

For width and thickness, the behavior between the intervals follows a heterogeneous pattern where the classes oscillate according to the structural morphology of the seeds (class 1: 5.3; 2: 32.7%; 4: 9.3% and class 1: 3.3; 2: 18.7%; 4: 21.3%, both). Both linear and geometric variations demonstrate the decorating effects of several factors, whether environmental, nutritional, physiological, or genetic. According
to Silva et al. (2011), deviations in the symmetry of plant structures with bilateral structures, such as leaves and seeds, occur due to the inability of individuals to contain environmental or genetic variations during their development. In seeds, the results of these genetic and environmental factors, depending on the stage of fruit development, can be biometric oscillations in width, length, and thickness (PONTES et al., 2018a).

The estimates of the correlation coefficients between the biometric and physical characteristics of seeds of *Pueraria phaseoloides*, are shown in Table 3. Such estimates can be used when a specific characteristic can associate its degree of dependence on each other with the element of interest. For example, Farias-Neto et al. (2004) suggest that a more simplified selection can be carried out if this characteristic has a high positive correlation with another dependent element. Since the value proportion between the characteristics has increased, there is undoubtedly a dependency factor that explains their greater amplitudes. Thus, there is no need to adopt restrictions in the selection to obtain gains in the desired evaluation (GONÇALVES et al., 2013).

HIGHLY SIGNIFICANT CORRELATIONS ($p \leq 0.001$) were observed between the evaluated characteristics of the physical variables. The surface area (SA) was highly correlated with volume (V), a positive value close to one ($r = 0.99$), which reinforces the interaction between both on the effects on the influence of water absorption during the breaking of molecules in the process of decomposition of the seeds that will affect the physiological quality. Biometric variables showed a weak correlation between them. Length and thickness were the highest value ($r = 0.48$), demonstrating the species’ natural condition in expressing its characteristics in size variations. According to Zurffo et al. (2014), climatic conditions can promote the expression of biometric characteristics.

The *Pueraria* presents pod-type fruit with several seeds inside, which are dispersed by autochory, technically called ballistic dispersion, in the day’s hottest hours in full sun (ideally between 11:00 am and 1:30 pm). This dispersion occurs when the exocarp reaches dehiscence, from a green to brownish color, indicating that dehiscence is close. When it reaches a black color, dispersion occurs. When the fruit ripens

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**Figure 4** - Setting the percentage between the amplitudes of the seeds distributed in each class. Mass frequency (A), length (B), width (C), and thickness of Pueraria.
at the time of plant reproduction, it breaks, and the seeds are thrown away because of the action of raindrops or mechanical pressure on the fruit. When it reaches the flowering period, the climbing plant emits inflorescence with several flowers, but not all are fertilized at the same time. This situation causes unevenness in the formation of pods and makes harvesting difficult, affecting the physiological quality of seeds. It is essential to highlight that harvesting will not be necessary when all pods reach full maturity because they open easily when collected (black color). Thus, pods are collected when they present brownish to black coloring, and consequently, the seeds follow different maturity colors.

Three different colors were found for Pueraria seeds in this seed lot: light red, dark red, and black seeds. However, the big problem affecting the excellent germination of the seeds is the tegumentary dormancy regardless of the color. Moreover, when mixing all seeds of different colors, run the risk of obtaining more dormant seeds (Figure 5).

When analyzing the mixed-seed category, it was noticed that the average representativeness of normal seedlings was 20%, for abnormal 12% (32% germination). The rest was 68% of hard seeds, which can be considered dormant, with a high percentage that did not germinate, and none died. Normal seedlings were 32% and abnormal 10% (42% germination) for light-dark red seeds, with 50% of hard seeds and 8% dead seeds. The light red seeds had 38% of normal seedlings and 8% abnormal (46% of germination), with 46% of hard seeds and 8% dead seeds. The highest percentage of germination was obtained in black seeds in black seeds, which reached 64%. However, it was where the highest number of dead seeds was obtained, 24% and 12% hard seeds. Normal seedlings corresponded to 52% and abnormal to 12% (Figure 5).

These results imply that black seeds have a high possibility of being in a more significant state of deterioration, causing a loss of physiological quality. Furthermore, when the seeds are mixed, there is an excellent chance of obtaining hard seeds or not. If more dark and light red seeds are selected, the germination will not be uniform. To solve the problem of hard seeds it is recommended to overcome dormancy. Pinheiro et al. (2019) did not perform color selection and obtained a
germination percentage of around 32% in a study on the viability and overcoming of Pueraria seeds; when they overcame dormancy, they germinated to 96% with the abrasive scarification method. The number of hard seeds and the dead seeds dropped to 4%. As for the number of abnormal seedlings, it remained similar, at around 8%.

In some sub-regions in the Amazon where this forage is planted, they believe that black seeds are not viable. According to farmers, these seeds are already dead. However, when analyzing this hypothesis, it was found that these are the ones that germinate in the highest percentage without a process of overcoming integumentary numbness. These seeds have a film that prevents water from entering the integument and the endosperm, a thin membrane surrounding the embryo and the endosperm, called perisperm. So, it is necessary to break two barriers in the seeds, the perisperm, and the tegument.

Conclusions

The results indicated low variability between the biometric and physical property variables, except for the seed mass, which varies according to hygroscopicity. The seeds have an almost rectangular shape with a closer tendency to sphericity. Their variations tend to be homogeneous in their morphological characteristics, resulting in similarly sized lots.

The colorations of the seeds showed a high value of hard or dormant seeds, except for the black ones. Thus, it was concluded that all seed categories are viable and need to overcome dormancy before sowing. Descriptive data indicate that combining three-dimensional seed characteristics and germination process help in seeding, harvesting, processing, and seed storage methodologies.
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