

Seed viability changes during fruit ripening of *Tapirira guianensis* Aubl.: Implications for collection

Alterações na viabilidade de sementes durante o amadurecimento de frutos de *Tapirira guianensis* Aubl.: Implicações para a coleta

Cambios en la viabilidad de las semillas durante la maduración del fruto de *Tapirira guianensis* Aubl.: Implicaciones para la recolección

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Abstract

Environmental changes provoked by man have caused great impacts on nature with irreparable consequences to the environment, life, and biodiversity. Species as *Tapirira guianensis* that have high ecological potential, due to adaptability, fast growth, and abundant fruit production,

can contribute to the restoration of these environments. The objective of this work was to verify the influence of the fruit maturation stage on the physiological potential of the *Tapirira guianensis* seeds from three genitor plants and, thus, to define seed collection patterns based on the fruit external characteristics. The experiment was performed in a completely randomized design, in factorial scheme 3 x 3, three parent trees (1, 2, and 3) and three fruit maturation stages (green, intermediate and mature fruits), containing 4 replicates of 25 seeds per treatment. Seeds from mature fruits in comparison to those of green fruits presented significant reductions in the water content in the parent trees 1, 2 e 3 de 11%, 21%, and 28%, respectively. Otherwise, seeds from mature fruits presented significant increases in the emergence and emergence speed index of 7% and 11% for parent tree 1, 76%, and 108% for parent tree 2, and 40% and 40% for parent tree 3, in comparison to the seeds from green fruits. This research revealed that was possible to define physiological maturation of seeds according to the fruit maturation stages, being the seeds from intermediate and mature fruits that present higher germination power and development of seedlings and, therefore, indicated for collection of *Tapirira guianensis* seeds.

Keywords: Fruit color; Forest seeds; Germination; Maturation; Vigor.

Resumo

As mudanças ambientais provocadas pelo homem têm causado grandes impactos na natureza com consequências irreparáveis ao meio ambiente, à vida e à biodiversidade. Espécies como *Tapirira guianensis* que apresentam alto potencial ecológico, devido à adaptabilidade, rápido crescimento e abundante produção de frutos, podem contribuir para a restauração desses ambientes. Objetivou-se verificar a influência do estágio de maturação do fruto no potencial fisiológico das sementes de três plantas genitoras e, assim, definir padrões de coleta com base nas características externas do fruto. O experimento foi realizado em delineamento inteiramente casualizado, em esquema fatorial 3 x 3, três árvores matrizes (1, 2 e 3) e três estágios de maturação dos frutos (frutos verdes, intermediários e maduros), contendo 4 repetições de 25 sementes por tratamento. Sementes de frutos maduros em comparação com as de frutos verdes apresentaram reduções significativas no teor de água nas árvores matrizes 1, 2 e 3 de 11%, 21% e 28%, respectivamente. Por outro lado, sementes de frutos maduros apresentaram aumentos significativos no índice de emergência e velocidade de emergência de 7% e 11% para a árvore matriz 1, 76% e 108% para a árvore matriz 2, e 40% e 40% para a árvore matriz 3, em comparação com o sementes de frutas verdes. Esta pesquisa revelou que foi possível definir a maturação fisiológica das sementes de acordo com os estágios de maturação dos frutos, sendo as sementes de frutos intermediários e maduros que apresentam maior poder de germinação e

desenvolvimento de mudas e, portanto, indicadas para coleta de sementes de *Tapirira guianensis*.

Palavras-chave: Cor do fruto; Sementes florestais; Germinação; Maturação; Vigor.

Resumen

Los cambios ambientales provocados por el hombre han provocado grandes impactos en la naturaleza con consecuencias irreparables para el medio ambiente, la vida y la biodiversidad. Especies como *Tapirira guianensis* que tienen un alto potencial ecológico, debido a su adaptabilidad, rápido crecimiento y abundante producción de frutos, pueden contribuir a la restauración de estos ambientes. El objetivo fue verificar la influencia de la etapa de maduración del fruto sobre el potencial fisiológico de las semillas de tres plantas parentales y, así, definir patrones de recolección en función de las características externas del fruto. El experimento se realizó en un diseño completamente al azar, en un esquema factorial 3 x 3, tres plantas matrices (1, 2 y 3) y tres etapas de maduración de frutos (frutos verdes, intermedios y maduros), conteniendo 4 repeticiones de 25 semillas por tratamiento. Las semillas de frutos maduros en comparación con las semillas de frutos verdes mostraron reducciones significativas en el contenido de agua en las plantas matrices 1, 2 y 3 de 11%, 21% y 28%, respectivamente. Por otro lado, las semillas de frutos maduros mostraron incrementos significativos en el índice de emergencia y la velocidad de emergencia de 7% y 11% para la planta matriz 1, 76% y 108% para la planta matriz 2 y 40% y 40% para la planta matriz 3. en comparación con las semillas de frutos verdes. Esta investigación reveló que fue posible definir la maduración fisiológica de las semillas de acuerdo a las etapas de maduración de los frutos, con semillas de frutos intermedios y maduros que tienen mayor poder de germinación y desarrollo de plántula y, por tanto, indicadas para la recolección de semilla de *Tapirira guianensis*.

Palabras clave: Color de la fruta; Semilla florestal; Germinación; Maduración; Fuerza.

1. Introduction

Over the last decade, the environmental changes provoked by the man to meet the necessity of the called "modern life" have been causing great impacts to nature with consequences, in many cases irreparable to the environment, life and biodiversity. The loss of biodiversity has been spreading with the pressure of urban occupation, occurring mainly in function to irrational deforestation, directly contributing to the fauna and flora reduction,

consequently, promoting the disappearance of forest species with socio-economic importance (Sodhi, et al., 2010; Norris, 2016; Rosa, et al., 2016).

Among the species with great potentials to be explored, *Tapirira guianensis* Aubl., popularly known as tapirira, belongs to the Anacardiaceae family, is present throughout the Brazilian territory, can be found widely distributed in dry hillsides, but is mainly present in moist terrains, varzea areas (flood forest); can be used in heterogeneous reforestations, control of marginal erosion in rivers and recovery of degraded areas (Santana, et al., 2009; Santos-Moura, et al., 2012). In addition, this species has high ecological potential, due to adaptability in different environments, fast growth, and abundant fruit production.

A key stage in the life cycle of plants is seed stage. The seeds are closely related to the reproduction and dispersion of a plant, is considered the main propagation form of many species. In addition, *ex situ* storage of seeds provides an insurance policy against the extinction of species in nature and provides material for future restoration efforts (Guerrant Junior, et al., 2015). Therefore, obtaining quality seed is important to any seedling production program for commercial plantations, forest rehabilitation and conservation of genetic resources (Vidigal, et al., 2011; Zade, et al., 2018).

In the propagation by seeds of tropical species, one of the difficulties faced is the obtaining of seeds of physiological, physical and sanitary quality in areas of natural occurrence (Buisson, et al., 2017). The production of high-quality seeds depends on the appropriate moment of harvest (Silip, et al., 2010). In general, the seed harvests must be carried out at the physiological maturity point, at the moment of maximum production and higher product quality, where they have maximum dry matter weight (Castro, et al., 2004).

During the harvest process, the coloration present in the fruits can be an important indicator used to determine the physiological maturity of seeds (Rubio, et al., 2013; Schulz, et al., 2014). However, many forest species show unevenness fruit maturation and, therefore, can produce seeds with different levels of physiological quality. In addition, there are species in which the seeds that become viable before the fruits become fully mature (Cruz-Tejada, et al., 2018). In addition, the physiological maturation process of seeds can differ between natural populations of a specie, due the genetic structure of a single plant to influence the germination potential of seeds (Yuan, et al., 2016).

Despite the growing work developed with forest species, the knowledge about the plants propagation these species is limited, especially about basic conditions for seedling production, such as fruits collection, seeds germination, growth and development seedlings (Luna-Nieves et al., 2017). In this context, the objective of this work was to verify the influence of the fruit

maturation stage on the physiological potential of the *Tapirira guianensis* Aubl. seeds from three matrices plants and, thus, to define seed collection patterns based on the fruit external characteristics.

2. Methodology

2.1 Location and Fruits collection

Tapirira guianensis Aubl. fruits were collected from three matrices tree located at the Belém Campus in the Universidade Federal Rural da Amazônia, Belém-PA, Brazil (01°27'29" S, 48°26'06" W). The fruits collection with different maturation stages was performed directly in the tree with the aid of trimmer. Posteriorly, the fruits were separated into three maturation stages: green fruits (green-colored epicarp), intermediate (epicarp with green and dark-brown spots) and mature fruits (dark-brown epicarp) (Figure 1).

Figure 1. Green, intermediate and mature fruits of *Tapirira guianensis* Aubl.



Source: Authors (2020).

Figure 1 shows how the green, intermediate and mature fruits of *T. guianensis* can demonstrate different fruit maturation stages by their epicarp color and size difference. It is evident that each coloration is directly related to the size of the fruit (data not shown) and both can be related to the development of *T. guianensis* seeds.

2.2 Experimental design

The experiment was performed in a completely randomized design, in factorial scheme 3 x 3, three parent trees and three fruit maturation stages, containing 4 replicates of 25 seeds

per treatment. The seeds were disinfected in 5% sodium hypochlorite for five minutes, washed with distilled water for one minute and placed on a paper towel to dry for 24 hours, according to Souza et al. (2011) with modifications.

2.3 Water content

Water content (WC) of seeds was determined through four replicates of 25 seeds in a forced ventilation oven at 105 ± 3 °C for 24 hours (Brasil, 2009).

2.4 Germination conditions

Germination test was performed in plastic trays with dimensions of 23 cm x 15 cm x 7 cm (length, width, and depth, respectively) containing commercial substrate (Germina plant®). The substrate was moistened with distilled water at 60% of its water retention capacity (Brasil, 2009). The plastic trays were placed on workbenches and maintained under laboratory conditions with controlled temperature (28 ± 3 °C).

2.5 Germination evaluation

The evaluations were conducted daily during 26 days after sowing. The parameters evaluated were: emergence (E), corresponds to the number of seedlings with 0.5 cm of the hypocotyl above substrate surface; emergence speed index (ESI) (Maguire, 1962); and germination, through the percentage of normal seedlings (NS), abnormal seedlings (AS) and dead seed (DS) (Brasil, 2009).

2.6 Seedling length

After the germination test, where evaluated shoot length (hypocotyl and epicotyl) and root length of the normal seedlings from each treatment, with the aid of a ruler, graduated in centimeter. The mean values obtained were divided by the total number of seeds contained in the replicate (25 seeds), the result was expressed in cm/seedling (Vanzolini, et al., 2007).

2.7 Seedling dry matter

Normal seedlings separated into shoot and root were placed in Kraft paper bags and dried in a regulated oven at 65 ± 3 °C for 24 hours until reaching constant weight. The samples

were weighed in the analytical balance with an accuracy of 0.001 g. The mean values obtained were divided by the total number of seeds contained in the replicate (25 seeds). The dry matter result was expressed in g/seedling.

2.8 Data analysis

The data were subjected to variance analysis (ANOVA) and significant differences between the means were determined by the Scott-Knott test at a probability level of 5%. Standard deviations were calculated for each treatment. Statistical analyses were performed using software Sisvar (Ferreira, 2011). The percentage of data were submitted to arc sin $(x/100)^{1/2}$ transformation.

3. Results and Discussion

3.1 Seeds from green fruits did not complete the maturation process

The water content (WC) of seeds, emergence (E) and emergence speed index (ESI) of *Tapirira guianensis* Aubl. seedlings were influenced by the fruit maturation stage and parent tree by the Scott-Knott test ($p < 0.05$) (Table 1). Seeds from intermediate and mature fruits presented low WC and higher E and ESI than to seeds from green fruits in all the parent trees. WC, E and ESI values of parent trees (1, 2 and 3) varied within the maturation stages. Seeds from mature fruits in compared to those of green fruits presented significant reductions in the WC in the parent trees 1, 2 e 3 de 11%, 21% and 28% (Table 1), respectively. Otherwise, seeds from mature fruits presented significant increases in the E and ESI of 7% and 11% for parent tree 1, respectively, 76% and 108% for parent tree 2, respectively, and 40% and 40% for parent tree 3, respectively, in compared the seeds from green fruits.

Table 1. Quality of *Tapirira guianensis* Aubl. seeds in function of fruit maturation stage and parent tree.

Fruit maturation	Parent tree	WC (%)	E (%)	ESI
Green	1	37 ± 0.60Ac	83 ± 3.83Aa	1.92 ± 0.11Aa
Green	2	46 ± 2.55Aa	55 ± 5.39Bb	1.07 ± 0.23Bc
Green	3	43 ± 0.27Ab	65 ± 5.40Bb	1.46 ± 0.09Bb
Intermediate	1	29 ± 0.16Cc	93 ± 6.00Aa	2.03 ± 0.34Aa
Intermediate	2	35 ± 0.81Ba	94 ± 5.16Aa	2.24 ± 0.19Aa
Intermediate	3	32 ± 0.83Bb	94 ± 5.16Aa	2.24 ± 0.18Aa
Mature	1	33 ± 0.10Ba	89 ± 8.87Aa	2.14 ± 0.38Aa
Mature	2	31 ± 0.68Cb	97 ± 3.83Aa	2.23 ± 0.25Aa
Mature	3	31 ± 0.94Bab	91 ± 6.63Aa	2.04 ± 0.29Aa
CV (%)		1.75	7.67	9.48

WC = Water content, E = Emergence, ESI = Emergence speed index. Columns with different uppercase letters between fruit maturation stage (green, intermediate and mature under equal parent tree) and lowercase letters between parent trees (1, 2 and 3 under equal fruit maturation stage) indicate significant differences from the Scott-Knott test ($p < 0.05$). Values described corresponding to means from four repetitions and standard deviations. Source: Authors (2020).

Table 1 shows that the E and ESI values of *T. guianensis* seedlings from seeds from green fruits can vary between the parent trees. Otherwise, the values of E and ESI of seedlings from seeds from intermediate and mature fruits are higher than seeds from green fruits and do not show statistical difference between parent trees. Therefore, the quality of seeds from intermediate and mature fruits is superior to that of seeds from green fruit, regardless of the parent tree.

In general, the WC of seeds varied from 29% to 46%. This variable has great importance, contributing to a better understanding of seed behavior. According to Santos-Moura et al. (2012) *Tapirira guianensis* Aubl. seeds behave as recalcitrant maintaining high viability in 24% of the WC. Recalcitrant seeds are discarded by parent plants with relatively high water contents and remain metabolically active and sensitive to desiccation during their development and post-spillage (Subbiah, et al., 2019).

Seeds from intermediate and mature fruits presented low WC and high E and ESI when compared to the seeds of green fruits according to Table 1. During the maturation process, after the cell division, the seeds begin an intermediate phase, where the water content of the cells is replaced by dry matter (carbohydrate, protein, and lipids) (Castro, et al., 2004). Similar results,

in relation to the physiological maturity point of the seeds, where seeds from fruits with darker coloring presented lower water content and higher germination than seeds from green fruits, also were verified in *Phoenix canariensis* hort. ex Chabaud seeds (Pimenta, et al., 2010), *Amburana cearensis* (Allem.) A.C. Smith seeds (Lopes, et al., 2014) *Jatropha curcas* L. seeds (Silva, et al., 2017).

3.2 Seeds of mature fruits present higher germination potential

Normal seedlings (NS) and dead seeds (DS) were influenced by the fruit maturation stage and parent tree by the Scott-Knott test ($p < 0.05$) (Table 2). Seeds from intermediate and mature fruits presented higher NS and low DS than to seeds from green fruits in all the parent trees. NS and DS values of parent trees (1, 2 and 3) varied within the maturation stages. Seeds from mature fruits in compared to those of green fruits presented significant increases in the NS of 13%, 73% and 43% for parent trees 1, 2 and 3, respectively, and significant decreases 38%, 88% and 74% for parent trees 1, 2 and 3, respectively.

Table 2. Germination of *Tapirira guianensis* Aubl. seeds in function of fruit maturation stage and parent tree.

Fruit maturation	Parent tree	NS (%)	AS (%)	DS (%)
Green	1	79 ± 6.83Ba	8 ± 7.30Aa	13 ± 2.03Ab
Green	2	55 ± 7.39Bb	4 ± 8.00Aa	41 ± 6.00Aa
Green	3	63 ± 3.83Bb	2 ± 2.31Aa	35 ± 2.00Aa
Intermediate	1	91 ± 3.83Aa	6 ± 2.31Aa	3 ± 2.00Bb
Intermediate	2	94 ± 5.16Aa	0 ± 0.00Aa	6 ± 5.16Bab
Intermediate	3	85 ± 3.94Aa	0 ± 0.00Aa	15 ± 3.93Ba
Mature	1	89 ± 7.87Aa	3 ± 3.83Aa	8 ± 2.66Ba
Mature	2	95 ± 2.00Aa	0 ± 0.00Aa	5 ± 2.00Ba
Mature	3	90 ± 7.12Aa	1 ± 2.00Aa	9 ± 4.50Ba
CV (%)		8.15	17.38	19.42

NS = Normal seedlings, AS = Abnormal seedlings, DS = Dead seeds. Columns with different uppercase letters between fruit maturation stage (green, intermediate and mature under equal parent tree) and lowercase letters between parent trees (1, 2 and 3 under equal fruit maturation stage) indicate significant differences from the Scott-Knott test ($p < 0.05$). Values described corresponding to means from four repetitions and standard deviations. Source: Authors (2020).

Table 2 shows that the NS and DS values of *T. guianensis* seeds from green fruits can vary between the parents trees. Otherwise, the NS and DS values of intermediate and mature fruits is higher than that of green fruits and can present statistical difference in the DS values between parent trees. Therefore, the germinative potential of seeds from intermediate and mature fruit is greater than that seeds from green fruit, regardless of the parent tree.

Seeds from intermediate and mature fruits presented high NS values and low DS values when compared to the seeds of green fruits according to Table 2. Seeds of mature fruits can present higher germination potential when the maturity of the seed coincided with the maturation of its corresponding fruit (Schulz, et al., 2014). This behavior can be explained by increases in E and ESI values observed this work, providing vigorous seedlings with all the necessary structures to develop a new plant (higher NS). Anastácio et al. (2010) studying the influence of the fruit maturation stage on the physical characteristics and germination of *Schefflera morototoni* pyrenees submitted to pre-germination treatments, observed that the pyrenees from green-purplish fruits obtained 22% of germination higher to the registered in green fruits.

3.3 Vigorous seeds yield seedlings with higher growth rates

Shoot length (SL) and root length (RL) were influenced by the fruit maturation stage and parent tree by the Scott-Knott test ($p < 0.05$) (Table 3). Seedlings from intermediate and mature fruits presented higher SL and RL than to seedlings from green fruits in all the parent trees. SL and RL values of parent trees (1, 2 and 3) varied within the maturation stages. Seedlings from mature fruits in compared to those of green fruits presented significant increases in the SL of 31%, 118% and 68% for parent trees 1, 2 and 3, respectively, and significant increases in the RL values of 38%, 88% and 74% for parent trees 1, 2 and 3, respectively.

Table 3. Length of *Tapirira guianensis* Aubl. seedlings in function of fruit maturation stage and parent tree.

Fruit maturation	Parent tree	SL (cm seedling ⁻¹)	RL (cm seedling ⁻¹)
Green	1	9.53 ± 1.03Ba	6.48 ± 0.42Aa
Green	2	6.55 ± 1.38Cb	5.14 ± 0.76Bb
Green	3	7.65 ± 0.89Bb	5.47 ± 0.59Bab
Intermediate	1	11.84 ± 1.16Aa	7.36 ± 0.38Aa
Intermediate	2	11.40 ± 0.60Ba	7.51 ± 0.74Aa
Intermediate	3	10.99 ± 0.44Aa	7.91 ± 0.73Aa
Mature	1	12.54 ± 1.65Aa	7.43 ± 0.62Aa
Mature	2	14.27 ± 1.52Aa	8.45 ± 0.39Aa
Mature	3	12.87 ± 1.87Aa	8.23 ± 0.78Aa
CV (%)		10.40	9.11

SL = Shoot length, RL = Root length. Columns with different uppercase letters between fruit maturation stage (green, intermediate and mature under equal parent tree) and lowercase letters between parent trees (1, 2 and 3 under equal fruit maturation stage) indicate significant differences from the Scott-Knott test ($p < 0.05$). Values described corresponding to means from four repetitions and standard deviations. Source: Authors (2020).

Table 3 shows that the SL and SR values of *T. guianensis* seedlings from seeds from green fruit can vary between the parent trees. Otherwise, the SL and SR values of seedlings from seeds from intermediate and mature fruits is higher than seeds from green fruits and have no statistical difference between parent trees. Therefore, growth in seedling from seeds from intermediate and mature fruits is greater to that seeds from green fruit, regardless of the parent tree.

Seedlings from intermediate and mature fruits presented high SL and RL when compared to the seedlings of green fruits according to Table 3. Vigorous seeds yield seedlings with higher growth rates, due to the capacity of the transformation of tissue reserves supply and greater incorporation in the embryonic axis (Taiz, et al., 2017). Carvalho and Nakagawa (2000) affirm that seeds with incomplete maturation can germinate, but do not result in vigorous seedlings when compared to those harvested at the point of physiological maturity.

3.4 Seedlings dry matter in the indication of seed maturity

Shoot dry matter (SDM) and root dry matter (RDM) were influenced by the fruit maturation stage and parent tree by the Scott-Knott test ($p < 0.05$) (Table 4). Seedlings from

intermediate and mature fruits presented higher SDM and RDM than to seedlings from green fruits in all the parent trees. SDM and RDM values of parent trees (1, 2 and 3) varied within the maturation stages. Seedlings from mature fruits in compared to those of green fruits presented significant increases in the SDM of 23%, 68% and 56% for parent trees 1, 2 and 3, respectively, and significant increases in the RDM values of 3%, 51% and 25% for parent trees 1, 2 and 3, respectively.

Table 4. Biomass of *Tapirira guianensis* Aubl. seedlings in function of fruit maturation stage and parent tree.

Fruit maturation	Parent tree	SDM (mg seedling ⁻¹)	RDM (mg seedling ⁻¹)
Green	1	27.50 ± 2.30Ba	6.19 ± 0.59Aa
Green	2	26.64 ± 1.77Ba	5.50 ± 0.54Ca
Green	3	27.07 ± 3.19Ba	5.62 ± 0.80Ba
Intermediate	1	33.11 ± 2.25Ac	7.22 ± 0.90Aa
Intermediate	2	44.11 ± 1.58Aa	7.64 ± 0.42Ba
Intermediate	3	38.61 ± 3.20Ab	8.13 ± 0.35Aa
Mature	1	33.92 ± 2.05Ab	6.37 ± 0.28Ab
Mature	2	44.63 ± 3.99Aa	9.05 ± 0.48Aa
Mature	3	42.27 ± 1.17Aa	7.01 ± 0.42Aab
CV (%)		7.18	9.45

SDM = Shoot dry matter, RDM = Root dry matter. Columns with different uppercase letters between fruit maturation stage (green, intermediate and mature under equal parent tree) and lowercase letters between parent trees (1, 2 and 3 under equal fruit maturation stage) indicate significant differences from the Scott-Knott test ($p < 0.05$). Values described corresponding to means from four repetitions and standard deviations. Source: Authors (2020).

Table 4 shows that the SDM and RDM values of *T. guianensis* seedlings from seeds from intermediate and mature fruits are higher than from seeds from green fruits and show statistical difference between parent trees. Therefore, biomass in seedling from seeds from intermediate and mature fruits is greater to that seeds from green fruit, regardless of the parent tree.

Seedlings from intermediate and mature fruits presented high SDM and RDM when compared to the seedlings of green fruits according to Table 4. Seedlings with higher dry matter content increase the probability of survival and establishment of seedlings in the environment under adverse conditions. Berti et al. (2007) evaluating the effect of the physiological maturity

of *Cuphea* seed on moisture, weight, oil content, fatty acid content, germination and vigor of seedlings, observed that seeds from flower harvested starting 28 days after anthesis produced seedlings with higher dry weight than those collected in less time. This result demonstrates the importance of seedlings dry matter in the indication of seed maturity.

Parameters of Germination and seedling development provide greater safety to confirm seed quality. Mata et al. (2013) searching determines the most adequate indexes for the evaluation of maturity and ideal harvest point of *Inga striata* Benth. seeds, verified that the size, water content, seed dry mass, germination capacity and shoot dry mass of seedling, were the parameters that best determined physiological maturation of these seeds.

4. Conclusions

This research revealed that the seeds from intermediate and mature fruits present higher germination power and development of seedlings and, therefore, indicated for collection of *Tapirira guianensis* Aubl. seeds.

New research about methods of drying and storing seeds and how these factors that influence the physiological quality of seeds can contribute to the species conservation process.

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