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Classificação e qualidade de sementes de *Acca sellowiana* (O. Berg) Burret quanto à tolerância à dessecação

Classification and quality of *Acca sellowiana* (O. Berg) Burret seeds as to tolerance to desiccation

Clasificación y calidad de las semillas de *Acca sellowiana* (O. Berg) Burret según la tolerancia a la desecación

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Resumo

Este trabalho teve como objetivo determinar o comportamento fisiológico e a qualidade de sementes de cinco genótipos de *Acca sellowiana* quanto à dessecação. As sementes foram obtidas de cinco plantas de quintais urbanos nos municípios de Dois Vizinhos - PR (A1, A2) e Vacaria - RS (A3, A4, A5). O experimento foi realizado em arranjo bifatorial (5x3), sendo cinco genótipos e três teores de água (28%, 15% e 5%), organizado em blocos inteiramente casualizados, com quatro repetições de 50 sementes cada. As sementes foram germinadas em papel absorvente, embebido com água, e mantidas em câmara BOD a temperatura de 25±3ºC com fotoperíodo de 16 horas. Foram avaliadas a porcentagem de germinação, índice de velocidade de germinação e tempo médio de germinação, pela contagem das sementes a cada dois dias, por 50 dias a partir da emissão da radícula. Não foram observadas diferenças significativas para porcentagem de germinação, ultrapassando 79% de todos os genótipos. A dessecação das sementes teve baixa influência no índice de velocidade de germinação e tempo médio de germinação, apesar de verificada influência do genótipo em função da variabilidade genética. O genótipo A5 possui sementes com qualidade superior as demais. As sementes de A. sellowiana possuem comportamento ortodoxo.

Palavras-chave: Feijoa; Recursos genéticos vegetais; Conservação *ex situ*; Germinação; Banco de germoplasma.

Abstract

The objective of this paper was to determine the physiological behavior and seed quality of five genotypes of *Acca sellowiana* regarding desiccation. Seeds were obtained from five plants of urban backyards in the cities of Dois Vizinhos - PR (A1, A2) and Vacaria - RS (A3, A4, A5). The experiment was conducted in a bifactor arrangement (5x3), with five genotypes and three levels of water content (28%, 15% and 5%), arranged into complete randomized block design, with four replicates of 50 seeds each. Seeds were germinated on absorbent paper soaked with water, being kept in BOD chamber under temperature of 25±3°C with photoperiod of 16 hours. Germination percentage, speed index and average time were assessed by counting seeds every two days for 50 days, starting from the moment of radicle emergence. No significant differences were observed for germination percentage and all genotypes surpassing 79% of germination. Desiccation of seeds had low influence on germination speed index and average time of germination, although genotype influence was verified due to genetic variability. Genotype A5 has seeds with superior quality from the others. Acca sellowiana seeds have orthodox behavior.

Keywords: Feijoa; Plant genetic resources; Ex situ conservation; Germination; Seed bank.

Resumen

Este trabajo tuvo como objetivo determinar el comportamiento fisiológico y la calidad de la semilla de cinco genotipos de *Acca sellowiana* con respecto a la desecación. Las semillas se obtuvieron de cinco plantas de jardín urbano en las ciudades de Dois Vizinhos - PR (A1, A2) y Vacaria - RS (A3, A4, A5). El experimento se realizó en un arreglo bifatorial (5x3), con cinco genotipos y tres contenidos de agua (28%, 15% y 5%), organizados en bloques totalmente aleatorios, con cuatro repeticiones de 50 semillas cada una. Las semillas fueron germinadas en papel absorbente, empapadas con agua y mantenidas en una cámara DBO a una temperatura de 25±3ºC con un fotoperíodo de 16 horas. Se evaluaron el porcentaje de germinación, el índice de velocidad de germinación y el tiempo medio de germinación contando las semillas cada dos días durante 50 días a partir de la emisión de la radícula. No se observaron diferencias significativas en cuanto al porcentaje de germinación, que supera el 79% de todos los genotipos. La desecación de las semillas influyó poco en la tasa de germinación y en el tiempo medio de germinación, aunque el genotipo se vio influido por la variabilidad genética. El genotipo A5 tiene semillas de calidad superior a las otras. Las semillas de A. sellowiana tienen un comportamiento ortodoxo.
Palabras clave: Feijoa; Recursos fitogenéticos; Conservación ex situ; Germinación; Banco de semillas.

1. Introduction

Studies aiming to understand behavior of seeds during storage involving native species have enabled the proper management of seed batches (Donazzolo et al., 2015). Considering that not all seeds are tolerant to desiccation, prior knowledge on physiological behavior is necessary for successful conservation (Marques et al., 2019). This is due to the need to conserve seeds for several purposes, and it demands development of research related to their physiological classification. Studies about seeds desiccation, as well as on which conditions are ideal for their storage allow for the extension of their use period, additionally, contributing to the conservation of species germplasm (Da Silva et al., 2017).

Over the initial development of seeds, still inside the mother plant, intense cell division occurs, in addition to tissue formation and differentiation, which are water-dependent processes. Right after cellular expansion happens, with accumulation of reserves, mostly proteins and carbohydrates, leading to a substantial increase in dry matter and, in general, reduced water content, preventing early germination (Borghetti & Ferreira, 2004).

At the end of the ripening stage, seeds can be classified as orthodox, recalcitrant or intermediate. Orthodox seeds tolerate desiccation at low levels of water content, relying on this process to redirect their metabolic path towards germination; whereas recalcitrant seeds present certain desiccation tolerance limits (Barbedo et al., 2002), in addition to suffering damage when stored at low temperature (Ali et al., 2016; Silva et al., 2019). Intermediate seeds acquire desiccation tolerance at the end of their development, after vacuolar volume reduction and decrease in metabolism (Oliver et al., 2020). Seeds considered intermediate have characteristics similar to orthodox seeds, and sometimes characteristics similar to recalcitrant seeds, being able to tolerate desiccation between 10 and 12% humidity (Mayrinck et al., 2019) and be damaged by low temperatures (Hui et al., 2019).

During the ripening of the seed, several events take place, including the accumulation of storage products, the suppression of early germination, the acquisition of desiccation tolerance, and often the induction of dormancy, potentially being affected by biotic and abiotic factors (Hussain et al., 2020). Additionally, seeds ability to tolerate desiccation varies according to species and origin of the batches, demonstrating that their genetic load is also able to exert influence (Oliver et al., 2020).

It is important to analyze seeds behavior based on different genotypes and regions, characterizing levels of desiccation tolerance in seeds according to a particular species, since many species have not had their characterization elucidated or further studies evaluating behavioral issues are required, as is the case of *Acca sellowiana* (O. Berg) Burret (Myrtaceae). *A. sellowiana* is well-known as goiabeira-serrana, goiabeira do mato or feijoa (Ducroquet & Ribeiro, 1991), and is native to the southern Brazilian plateau (Ducroquet, et al., 2000). It is under a domestication process, occurring within natural populations in understory layer of areas with altitudes above 800 m, a fleshy fruit, sweet and acidulous taste with excellent aroma, consumed in natura or processed (Ducroquet et al., 2000; Thorp & Bieleski, 2002), and demonstrating great market potential (Amaral et al., 2019). It also has the peculiarity of presenting edible petals (Mattos, 1986) and important characteristics for ornamental use (Gomes et al., 2017) and is recommended for the recovery of affected ecosystems (Fockink et al., 2020) also in Pharmacology (Santos et al., 2019). In this way, the use of this species tends to increase and the knowledge about the behavior concerning desiccation and longevity of seeds is of great importance, especially for the conservation of germplasm for future use (Donazzolo et al., 2015) and use in genetic improvement (Gomes et al., 2013).

A. sellowiana seeds maintain high viability when stored under refrigeration for up to two years (Donazzolo et al., 2015). However, seeds physiological classification is still controversial, since Gomes et al. (2013) classify the species as intermediate and Sarmento, et al. (2013) as orthodox. The objective of this study was to determine physiological behavior and quality of seeds from five genotypes of *A. sellowiana* regarding desiccation.

2. Material and Methods

This study was conducted from March to June 2014 in the Seeds Laboratory of the Federal University of Technology – Paraná, Campus Dois Vizinhos. This work is framed as an experimental work in the laboratory, with a quantitative approach (Pereira et al., 2018). The seeds were obtained from fruits of five plants (genotypes A1, A2, A3, A4 and A5). The first two were present in backyards in an urban area of the municipality of Dois Vizinhos, Paraná (25º 44' 01" South, 53º 03' 26" West and 509 m altitude), and the remainder from backyards in an urban area of the municipality of Vacaria, Rio Grande do Sul (28°30’39” South, 50°55’47” West and 950 m altitude). After fruits ripened in March 2014, approximately 30 fruits per plant were collected and pulped. The separation of seeds from the pulp were carried out with the aid of the enzyme pectinase at a proportion of 1.6 mL Kg pulp, for 48 hours at room temperature (Kashyap et al., 2001).

The seeds were washed and left resting at ambient temperature for 24 hours to reach hygroscopic balance. For each batch of seeds, the variables weight of a thousand seeds (WTS) and water content in the greenhouse at 105°C for 24 hours were obtained, both in accordance with Rules for Seed Analysis - RAS (Brazil, 2009).

For the tests aiming for physiological classification according to drying, seeds were analyzed based on the methodology proposed by Hong and Ellis (1996), where they can be considered: (1) recalcitrant seeds – absence of germination at 10-12% humidity; (2) orthodox seeds, germination at 5% humidity; (3) intermediate seeds, germination at 10% humidity. For this purpose, germination was obtained for each batch of seeds (genotype) with initial water content of 28% and contents of 15% and 5% after desiccation.

Desiccation was conducted through seed drying in hothouse at 40°C. For each drying treatment, 200 seeds for later usage with the germination test were used, in addition to being subjected to two replicates of one gram each. The replicates with one gram had their weight monitored to acquire the weight loss needed to reach the desired water level by means of mathematical estimation that considers initial humidity content. As soon as this estimated weight was reached, the seeds were put to germinate and both samples of one gram were used for determination of humidity in a hothouse at 105° C for 24h, as established by RAS (Brazil, 2009).

For the germination test, seeds were placed in acrylic boxes with lid of type gerbox, size of 11x11x3.5 cm, with four repetitions of 50 seeds per treatment. The seeds were placed under absorbent paper sterilized in autoclave for 15 min at 221° C, which has been dampened with distilled water at a ratio of two times the weight of the substrate. Samples were placed in BOD, regulated at 25±3° C and a photoperiod of 16 hours. Counting the seeds was performed every two days starting from the moment of first radicle emergence for a 50-day period. To count germinated seeds, the criterion of 5 mm radicle emergence was used (Popinigs, 1985). Germination percentage (G%), germination speed index (GSI) and average germination time (AGT), as suggested by Maguire (1962) were measured.

The experiment was arranged in a randomized complete block design with bifactor arrangement, with five genotypes and three levels of water content, with four replicates. Percentage data were transformed by means of arcsen ((x+1)/100) ½, and GSI and AGT data were transformed to (x+0.5) before variance analisis. For average comparisons, the Tukey test was applied ($p<0.05$). All tests were analyzed using the Assistat software (Silva & de Azevedo, 2009).

3. Results and Discussion

Regarding the weight of a thousand seeds (WTS), differences were observed between the seeds of the evaluated *A. sellowiana* genotypes (Table 1). The A1 genotype was the one with the highest weight (8.58g) and the genotype A5 that presented the smallest WTS (5.12g), in relation to the other genotypes the *A. sellowiana* evaluated. These results demonstrate the great variability for certain characteristics of native species, which is likely to occur due to the existing genetic diversity of these native species, which often do not have genetic improvement studies, having wide bases of genetic variability (Sánchez-Mora et al., 2019; Borsuk et al., 2016). However, with WST averages within the expected for Brazil group (Ducroquet et al., 2000).

According to Saldanha et al. (2020), during development seeds grow in size, caused by cellular multiplication and development that constitutes the embryonic axis and reserve tissue. At the end of the ripening period, the seed undergoes dehydration and a relative decrease in its size depending on the species (Carvalho & Nakagawa, 2000). Thus, there are variations in seed size between individuals of the same species (Saldanha et al., 2020), probably due to the genetic variability between arrays, along with environmental influence on their development (Felippi et al., 2012).

Table 1. Weight of a thousand seeds (WTS) and germinal percentage (GP) (%) of *A. selloiana* seeds based on interaction between factors genotype and level of seed desiccation.

		Germinal Percentage (GP) (%) *			**Average (%)**
Genotype	**WTS**	**Humidity**			
		28%	15%	5%	
A1	8,58 ^{a}	74.50	91.00	79.00	81.50 c*
A2	6,47 ^{b}	88.50	89.50	90.50	89.50 b
A3	5,28 ^{c}	89.42	86.53	86.53	87.50 bc
A4	8,46 ^{a}	83.17	83.65	86.05	84.29 bc
A5	5,12^{c}	98.50	98.50	98.50	98.50 a
Average (%)		86.81^{ns}	89.83	88.1	

*Averages not followed by the same letters differ statistically among themselves by Tukey test at 5% probability.
Source: Authors.

Regarding the initial water content, all genotypes of *A. sellowiana* had an initial content of 28% (Table 1), value relatively lower than what was found by Gomes et al. (2013) (36%), and higher than the one described by Santos et al. (2004) (21%). The high amount of water in seeds of Myrtaceae family is common and may reach 70% for some species (Amaral et al., 2019). According to the same authors water content was an effective index to assist in determining the physiological maturity of seeds, which is directly connected to the germination process.

Regarding the desiccation process, no significant differences ($p > 0,05$) were observed for seed germination based on the desiccation levels tested (28%, 15% and 5%), nor with the lack of interaction of the genotypes assessed (Table1). These results indicate the existence of a drying tolerance by all the evaluated *A. sellowiana* genotypes (Table 1), a behavior that is characteristic of orthodox seeds according to the classification by Hong & Hellis (1996).

Desiccation tolerance is one of the most important properties of seeds as an adaptation strategy, allowing their survival during storage or under conditions of environmental stress, ensuring the spread of species in time and space (Oliver et al., 2020). In this sense, orthodox seeds can be kept more easily in *ex situ* collections, such as seed banks using reduction of humidity content (Cruz & Souza, 2006). To Oliver et al. (2020), when orthodox seeds reach ripening, they can reach humidity levels at around 15% to 20%. Drying these seeds until a humidity level of 5 to 15% can lead seeds to a quiescence state, which causes reduction in metabolism to minimum levels, helping seeds to stay alive even under adverse conditions, only after hydration these seeds can resume the metabolism directed towards the germination process (Mendes-Lopes et al., 2020).

Gomes et al. (2013) also found that 83% of *A. sellowiana* seeds germinate at 5% of water content. However, after 90 days of storage, germination percentage decreased dramatically to 38%, leading the authors to classify these seeds as intermediate. Based on it, the authors highlight the difficulty in determining a physiological classification for the seeds of this species, most likely due to their sensitivity and tolerance, in addition to the influence of batches, being it for genotypic or phenotypic reasons. On the other hand, Donazzolo et al. (2015) found high germination rates for seeds stored up to two years under refrigeration, indicating there was no damage due to the low temperatures tested.

For Mendes-lopes et al. (2020) during the ripening stage, seeds acquire desiccation tolerance, which is maintained after dispersion. If there are differences in maturation between batches, it is assumed that there are behavioral differences in seed desiccation, with some batches being able to extend their storage period and thus keeping high germination viability. Groot et al. (2003) reported variation in storage capacity of seeds between and within batches from same species. Possibly, amongst the explanations provided, the presence and amount of sugars would be involved in desiccation tolerance, stabilizing the membranes and avoiding structural changes of the proteins (Wang et al., 2019).

There was influence of genotypes on size and seed germination (Table 1). The bigger seeds had the lower germination rates, which reveals a possible genetic variability. Nevertheless, it is possible to affirm that *A. sellowiana* seeds present high germination rates, since the index of germinated seeds exceeded 80%. However, the importance of studies aimed at the storage of different batches is stressed, in order to assess the viability of seeds according to time, considering the temperature below 10°C according to Hong and Ellis (1996), reducing the longevity of intermediate seeds.

By analyzing the germination speed index (GSI) and average germination time (AGT), it was possible to observe that there is interaction between the factors seed humidity and the genotypes of *A. sellowiana* evaluated (Tables 2 and 3).

For GSI, there were genotypes that did not suffer desiccation influence (A2, A3 and A5) while others (A1 and A4) had an increase in GSI under desiccation of 15% or 5%, whereas GSI was by itself lower with 28% humidity. This behavior may indicate that the higher humidity content is related to less vigor in seeds. In the case of GSI, a great influence of genetic variation was observed, as there was no difference for the genotypes and all humidity levels tested (Table 2). As it is a species considered allogamous (Finatto et al., 2011) it is expected that its heterozygosity stays high in populations, differentiating individuals genetically. Comparing genotypes, GSI was higher for A5, a result that corroborates with the GP (Table 1). Considering that there is a direct relation between GSI and seed vigor, (Sohail et al., 2018), genotype A5 has seeds with superior quality than the others.

Table 2. Germination speed index of *A. sellowiana* seeds based on interaction between factors genotype and level of seed desiccation (humidity).

	Germination speed index (GSI) (%)			**Average (%)**
Genotype	**Humidity**			
	28%	15%	5%	
A1	1,08 dB*	1,46 cA	1,26 cAB	1,26 d
A2	1,56 cA	1,65 cA	1,69 bA	1,63 c
A3	2,46 aA	2,23 bA	2,46 aA	2,38 b
A4	2,02 bB	2,69 aA	2,67 aA	2,46 b
A5	2,78 aA	2,75 aA	2,75 aA	2,76 a
Average (%)	1,98 B	2,15 A	2,17 A	

*Averages not followed by the same capitalized letters by row and lowercase letters by column differ statistically among themselves by Tukey test at 5% probability.
Source: Authors

The behavior for AGT followed the same pattern of GSI, presenting interaction and differentiation with the genotypes and humidity tested (Table 3).

In the case of the factor humidity, the highest AGT average occurred with 28% humidity occurred in the seeds, indicating that with less amounts of water, the seeds germinate faster, reinforcing previous results. Genotype A5 also presented the lowest AGT, result that corroborates with the other variables analyzed, since it also presented higher GP and GSI, results that indicate this genotype has the highest physiological quality of the seeds analyzed regard to germination parameters. Therefore, from these results, it is possible to say that higher humidity levels in seeds of *A. sellowiana* significantly reduce germination quality parameters.

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Table 3. Average germination time of *A. sellowiana* seeds based on interaction between factors genotype and level of seed desiccation (humidity).

Genotype	Average germination time (AGT) (%)			Average (%)
	Humidity			
	28%	15%	5%	
A1	35,49 aA*	32,20 aB	32,22 aB	33,3 a
A2	29,81 bA	28,35 bA	27,80 bA	28,6 b
A3	20,75 dAB	22,56 cA	19,95 cB	21,1 c
A4	23,97 cA	18,64 dB	18,69 cB	20,4 c
A5	18,81 dA	19,17 dA	18,74 cA	18,9 d
Average (%)	25,77 A	24,18 B	23,48 B	

*Averages not followed by the same capitalized letters by row and lowercase letters by column differ statistically among themselves by Tukey test at 5% probability
Source: Authors.

The results of our work show that the genotypes of *A. sellowiana* had low influence when undergone to a reduction in water content, showing higher values of GSI and AGT under these conditions, indicating an orthodox behavior. These results differ from those found for seeds of other species from the family Myrtaceae, which are sensitive to low levels of water, such as *Eugenia involucratae*, *Eugenia pyriformis* (Delgado & Barbedo, 2007), *Eugenia pleurantha* and *Myrcia venulosa* (Mayrinck & Davide, 2016).

Today *A. sellowiana* is grown commercially in New Zealand, California, the Caucasian Republics of Georgia and Azerbaijan, Colombia and Israel (Moretto et al., 2014). In Brazil, where the species is native, there is little commercial exploitation in small orchards located in Santa Catarina, Rio Grande do Sul, in Mountain of Mantiqueira, and between the states of São Paulo and Minas Gerais (Moretto et al., 2014). According to Saifert et al. (2020) the available germplasm banks of *A. sellowiana* outside Brazil have a restricted genetic base due to the use of few plants used with progenitors. Unlike Brazil where the genetic basis is broad related to phenotypic diversity among the existing materials, this diversity of materials is related to several geographical origins (Sánchez-Mora et al., 2019).

This shows the importance of conservation of these important genetic resources in the preservation of the species itself, as its ecosystem is increasingly threatened by the expansion of agriculture (Lopes et al., 2010). In our work we found that the seeds of *A. sellowiana* showed orthodox behavior, which allows the seeds to be submitted to the desiccation process without compromising their germination process. Thus, the formation of active twin banks from the seeds of different materials is possible, since plants of *A. sellowiana* are susceptible to the fungus *Colletotrichum gloeosporioides*, which in many cases makes it impossible to maintain collections of plants used as a source of genetic resources of the species (Fantinel et al., 2017). The orthodox behavior presented by *A. sellowiana* seeds will play an important tool in the formation of germplasm banks to assist in the preservation and conservation of the diversity of materials existing in Brazil.

4. Conclusion

With the results achieved, the proposed objectives were achieved and we can say that: the *A. sellowiana* seeds can be physiologically classified as orthodox; there is influence of the genotype on percentage, speed and average time of germination; reduction in water content improves speed and average time of germination.

This orthodox behavior of *A. sellowiana* seeds will allow the formation of germplasm banks and helping the process of use and conservation of the species.

We believe it is necessary to expand the research focusing on the influence of the maturation point, origin of the genotypes and on the conservation time when the seeds are stored.

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