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Comportamiento y Longevidad de *Aspidosperma polyneuron* Müll. Arg. frente al Ultrasecado

Seed conservation of palo-rosa

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Resumen

Aspidosperma polyneuron es una especie climax del bosque tropical que ha sido intensamente explotada provocando su reducción en las poblaciones de Misiones. Para conservarla en bancos de germoplasma, se estudió el comportamiento y la longevidad de las semillas frente al ultra-secado. Como mostraron comportamiento ortodoxo frente a la deshidratación, se ultra-desecaron hasta 3% de humedad y se almacenaron en envases herméticos a 8 ± 2 °C. En estas condiciones se midieron la viabilidad y el vigor a los 0, 7, 20, 48 y 60 meses mediante las variables: porcentaje de germinación, tiempo medio de germinación e índice de velocidad de germinación. La primera de ellas no mostró diferencias significativas mientras que las otras dos, detectaron cierto deterioro causado por el almacenamiento. Estos resultados indican que es posible prolongar la longevidad de las semillas de *A. polyneuron* durante largos períodos solucionando el faltante de semillas entre temporadas de producción.

Palabras clave: Palo rosa; Almacenamiento de semillas; Ultradesecación; Semillas forestales.

Abstract

Aspidosperma polyneuron is a climax species of the rainforest tropical forest that has been intensively exploited causing its population decline in the province of Misiones. their reduction in populations of Misiones. To preserve conserve it in gene banks, the behavior and longevity of ultra-dried seeds were studied. The seeds were dehydrated to 3% of moisture content and stored in airtight containers at 8 ± 2 °C. Viability and vigor were measured through germination percentage, and vigor, through mean germination time and germination rate index, at 0, 7, 20, 48 and 60 months of age using the variables germination rate, mean germination time and germination speed index.. Seeds of *A. polyneuron* behaved as orthodox, preserving its viability and vigor for 60 months when they were subjected to ultra-dry method. This method could solve the shortage of seeds during the long periods between production seasons. The first germination showed no significant difference while the other two presented some deterioration caused by storage. Results indicate that it is possible to extend the longevity of *A. polyneuron* seeds for long periods solving the shortage of seeds between production seasons.

Keywords: Palo-rosa; Seed storage; Ultra-drying; Forest seeds.

Introduction

The Atlantic Forest of South America, is the second largest rainforest eco-region of America, and is one of the world's most endangered lands. Currently, isolated fragments of vegetation persist in this forest corresponding to about 7% of the original vegetation cover, recognized worldwide for hosting high biological diversity with an exceptional concentration of endemic species [1]. Palo rosa (*Aspidospermapolyneuron*), Apocynaceae, a native species present in Argentina, is restricted to the Iguazú and General Belgrano departments in Misiones province, where is recognized for its large size. Due to the good quality of its timber and its essential oils content, it has been subjected to overexploitation with a consequent reduction in its natural distribution area and its population size. In Brazil and Venezuela, is included in the list of species for *ex situ* and *in situ* conservation [2]. In Misiones it was declared Provincial Natural Monument as well as pinoparanaby Law XVI - Nº 19 (1986) [3] while the IUCN Red List [4] classifies it as an endangered species.

The breeding season of this species startsat 20 years of age producing abundantseeds but at intervals of 2-4 years. It has also been reported low germination rates (35-70%) in nursery production attempts [5]. Moreover, the great height of the individuals along with the anemochory dispersion hinders the harvesting of mature seeds. That is why seeds storage is a very appropriate alternative for conservation of genetic resources for the production of plants for reforestation and it is especially recommended for those species that have no annual fruiting [2].

According to Valentini *et al*. [6] palo rosa seeds are orthodox, while for the Royal Botanic Gardens Kew [7] this behaviour has yet to be confirmed. The word orthodox is applied to those seeds capable of maintaining the viability after being dehydrated and exposed to low temperatures [8]. Among the species studied, *A. quebrachoblanco*is considered recalcitrant for Sandoval [9] but is orthodox for Alzugaray *et al*. [10] and *A. cylindrocarpon* is considered orthodox [2], while *A. pyryfolium* failed to exceed 15 months of storage under natural conditions and remains unclassified as to its tolerance to dehydration [11].

Pérez-Garcia*et al*. [12] explain that low humidity is definitely the key to the conservation of orthodox seeds in the medium and probably in the long term, being this condition much more important for the conservation of seeds that a low temperature. However, it is necessary to determine experimentally the minimum critical moisture value of the seeds of each species and this would be related to the chemical composition of the seed. While the chemical composition of the seeds of *A. polyneuron* is unknown, Bewley and Black [13] suggest that lipids are the major components of the endosperm in the Apocynaceae family. Seeds with high lipid content have lower breathing thresholds and a possible reduction in the optimum moisture content [14].

Considering that the scarce literature existing about conservation of *A. polyneuron* is also contradictory, the objectives of this study were to confirm the performance of the seeds against dehydration and to study the response of seeds to dehydration and storage timeusing the ultra-drying method to obtain viable seeds to accomplish restoration works.

Materials and Methods

Palo rosa fruits were harvested from trees located in a natural growth area in Andresito, at 25° 46' south latitude, 54° 04› west longitude and an altitude of 320 meters above sea level in Misiones province (Argentina). The harvest was carried out when the fruits begun its natural dehiscence process, ensuring thus the maturity of the seeds. They were immediately taken to the Seeds Laboratory, College of Exact, Chemical and Life Sciences, National University of Misiones, where they were kept at room temperature until the opening of all follicles are completed. The seeds were removed manually and stored at room temperature until processed within 24 hours.

Initial characteristics of seeds

The moisture content (MC), the germination percentage (GP) and the vigour by the germination speed index (IVG) and mean germination time (TMG) were evaluated immediately after the seeds removal. The initial moisture content determination (IMC), based on fresh weight was performed by the high-temperature method [15] using two replicates of 2 g each.

To determine the initial germination and vigour and all subsequent periods, 4 replicates of 20 seeds were sown on sand and were brought to a germination chamber at 25 °C and 8 h of light exposure. Seeds were considered as germinated only when they gave rise to normal seedlings [15]. Daily evaluations were made over a period of 28 days, until the time when the number of germinated seeds became stable. The results were expressed in percentage of normal seedlings. The germination speed index [16], was calculated as

$IVG = n_1/t_1 + n_2/t_2 + \ ... + n_i / t_i$, where:

IVG = germination speed index; n_i = number of seedling emerged on first, second and umpteenth day; t_1, t_2 y t_i = time in days.

Performance of seeds against drying

The effect of dehydration on the seeds was determined according to Hong *et al.* [8], by reducing the MC of the seeds in two stages. The first of these (10% of MC) was obviated because it agreed with the MC already found in the freshly harvested seeds. For the second step, two samples of seeds were exposed to the dehydrating action of the silica gel to achieve a 5% MC. The desired moisture content (DMC) was calculated with the equation described by Ellis and Hong [17] which allows the monitoring of the MC, if the initial moisture content (IMC) is known, avoiding the loss of seeds:

Weight of seeds (g) to DMC = (100 - IMC %) / (100 - DMC %) x initial weight of seeds (g)

Once reached the 5% moisture content, the viability was determined by a germination test on one of the samples while the other was brought to -20 °C over a period of 3 months after which a new germination test to check viability was performed.

Performance of seeds with ultra-desiccation

The methodology proposed by Gómez-Campo [18] consisting of reducing MC seeds to values between 1-3% by exposure to silica gel was performed. GP on a sample of these seeds was determined while the rest was stored in flame-sealed glass vials with an equivalent amount of silica gel with cobalt chloride as indicator at a temperature of 8 °C for 60 months. Viability was determined at 7 (T7), 20 (T20), 48 (T48) and 60 (T60) months, by measuring the germination percentage

(GP) and vigour through the IVG and TMG mentioned by Ranal and Santana [15] as: TMG =, where: TMG = mean germination time; *Ti* = number of days from the beginning of germination until day i and *Ni* is the number of seeds germinated on day i.

For each assay, a vial containing 80 seeds was opened and the seeds were exposed to a saturated atmosphere for12 hours to avoid damage by imbibition, prior to sowing [17]. No more measurements of MC of the seeds were made because the colour of the silica gel of the vials was unchanged, indicating that the seed moisture and the environment inside the vials did not change.

Statistical Analysis

The results were analysed using the statistical package provided by the software Statgraphics Centurion XVI [19]. The values in percentage were transformed into arcsin √ (% / 100) prior to analysis of variance (ANOVA) and Tukey's Average comparison test ($P < 0.05$).

Results and Discussion

The IMC of 10% was lower than those reported by other authors for the same species: 15% registered by Valentini *et al*. [6] and 42.7% registered by Carvalho *et al*. [2].

Moreover, the initial 87% GP was markedly superior to those obtained by the previously mentioned authors suggesting that the seeds were at the point of physiological maturity at the harvesting time. The initial IVG was 1,44 seedlings/day being this value the first one reported for the species.

The seeds dried at 5% MC showed 84% PG, similar to the undried seeds demonstrating that dehydration until this moisture content does not affect the viability of the seeds. Similar results were obtained by Valentini *et al*. [6] after drying in an oven up to 5% of MC. The storage for 3 months at -20 °C did not affect the germination, no significant differences ($p \leq 0.05$) were found in the GP and the IVG compared to initial values (Table 1). According to Hong *et al.* [8] this response is typical of seeds that show orthodox seed storage behaviour; and coincides with those reported by Carvalho *et al*. [2] for the same species.

Table 1: Germination percentage (GP, %) and Germination Speed Index(IVG, seedlings/day) of *A. polyneuron* seeds before (10 %) and after drying (5%).

MoistureContent (%)	Storage Temperature (°C)	Storage Period (months)	GP	IVG
10	8	0	87 a	1,44 a
5	8	0	84 a	1,20 a
5	-20	3	82 a	1,26 a
CV (%)			6,42	22,23

Means followed by the same letter in the columns do not differ by Tukey test at 5% probability.

Moreover, no significant differences(P>0,05)in GP between different storage periods for ultra-dry seeds were detected showing that this severe decrease in moisture content did not affect the viability of the seeds at the beginning or during storage(Table 2). However, the vigour parameters (TMG and IVG) were more sensitive to show some deterioration caused by ultra-desiccation. Differences (P<0,05)were found only in the seeds stored for 7 and 20 months, while those which remained longer (48 and 60 months) did not show this decline(P>0,05).It is possible that this result is due to heterogeneity in the physiological quality of the lot, characteristic of a species that has not been subject to domestication, and that can`t be avoided even if the precaution of using seeds from the same stand is taken. This was the only sign of deterioration found, while Valentini *et al*. [6] reported also the presence of high percentages of abnormal seedlings linked to drying temperatures. These eddrying by exposure to silica gel, although it is recommended for small samples, is much more appropriate than the heat, since the risk of producing alterations in cellular structure is lower.

The seeds with greater lipid content, such as species of Apocynaceae family, have lower optimal moisture content for storage and a stronger desiccation tolerance [20]. This is because the dry matter in starchy seeds attracts and holds water molecules but the oil in oily seeds does not [21].

Furthermore, the use of silica gel in the flame-sealed glass vials provides a relative humidity close to 10-12%, necessary to keep the seeds at the desired moisture content while cobalt chloride indicator allows verifying about possible anomalies in storage conditions throughout the duration of it. In addition, the silica gel used in the vials absorbs toxic gases generated during aging.

Table 2: Viability (GP, %) and vigor measured by mean germination time (TMG, days) and the germination speed index (IVG, seedlings/day) of ultra-dry seeds stored for different periods of time (months)at 8 °C and compared with fresh seeds.

Storage period	GP	TMG	IVG	
Fresh seeds	87 a	20,2 a	1,44	a
0	80 a	20,9 b	1,18	ab
7	78 a	22,0 c	0,95	b
20	71 a	22,6 d	0,80	b
48	75 a	20,2 a	1,22	ab
60	70 a	20,2 a	1,15	ab
CV(%)	16,6	5,04	21,19	

Means followed by the same letter in the columns do not differ by Tukey test at 5% probability.

Both the percentage of germination and the time in which the seeds remained viable during storage were higher than those found by Valentini *et al.* [6] and Carvalho *et al.* [2] showing that the deterioration can be delayed by the ultra-drying if the seeds have physiological maturity at the beginning of storage.

Conclusions

It is confirmed that the *A. polyneuron* seeds exhibit orthodox storage behaviour, as seeds could be dried to 5% MC without affecting viability.

The ultra-dry method used in this work maintains seed viability for a greater period than the interval between two periods of seed production time. The addition of silica gel at the airtight container ensures the stability of the storage conditions, while maintaining the seeds viability per at least 5 years.These results are of considerable practical relevance to seed´s storage in general and for the *ex situ* conservation of plant genetic resources through long-term storage.

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