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Biometric description of fruits and seeds, germination and imbibition pattern of desert rose [*Adenium obesum* (Forssk.), Roem. & Schult.][1]

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ABSTRACT – This study has aimed to carry out the description of fruits and seeds and germination process of desert rose, from two years of observations. The fruits and seeds were characterized based on length, diameter and number of seeds per fruit. The seeds internal structure and germinating process were also described. Germination test was performed at 25 and 30 °C temperatures, determining the germination percentage and germination speed index. Seeds harvested in 2013 were stored for 12 months and submitted to a new germination test. Parallel to these tests, the seeds imbibition curve was determined in substrates over and between sheets of paper at 15, 20, 25 and 30 °C temperatures. The fruits and seeds had similar lengths and diameters in both years of observations. The seeds can be stored for up to 12 months without loss in viability and temperatures of 25 and 30 °C are suitable for performing the germination test of this species. The water absorption curve of desert rose seeds follows a triphasic pattern of soaking.

Index terms: *Adenium obesum* (Forssk.), Roem. & Schult., Apocynaceae, seeds storage.

Descrição biométrica de frutos e sementes, germinação e padrão de embebição de rosa do deserto [*Adenium obesum* (Forssk.), Roem. & Schult.]

RESUMO – Objetivou-se nesse estudo realizar a descrição de frutos e sementes e do processo de germinação de rosa do deserto, a partir de dois anos de observações. Os frutos e as sementes foram caracterizados com base no comprimento, diâmetro e número de sementes por fruto; também, descreveu-se a estrutura interna das sementes e o processo de germinação. Realizaram-se testes de germinação às temperaturas de 25 e 30 ºC, determinando-se a porcentagem e índice de velocidade de germinação. As sementes colhidas em 2013 foram armazenadas por 12 meses e submetidas a novo teste de germinação. Paralelo a esses testes determinou-se a curva de embebição das sementes em substrato sobre e entre papel às temperaturas de 15, 20, 25 e 30 ºC. Os frutos e as sementes apresentaram comprimentos e diâmetros semelhantes nos dois anos de observações. As sementes podem ser armazenadas por até 12 meses sem perdas na viabilidade e as temperaturas de 25 e 30 ºC são adequadas para realizar o teste de germinação dessa espécie. A curva de absorção de água das sementes de rosa do deserto segue um padrão trifásico de embebição.

Termos para indexação: *Adenium obesum* (Forssk.), Roem. & Schult., Apocynaceae, armazenamento de sementes.

Introduction

The *Adenium* genus belongs to the botanical family Apocynaceae, which includes many tropical ornamental species such as *Catharanthus* spp., *Beaumontia* spp., *Carissa* spp., *Allamanda* spp., *Mandevilla* spp., *Nerium* spp. and *Plumeria* spp.

Adenium obesum [(Forssk.), Roem. & Schult.], commonly known as desert rose, is found in sub-Saharan Africa from Sudan to Kenya and from west of Senegal to the south of Natal and Swaziland. In areas where it is native, dry and cold winters are sufficient to induce a dormant period, including the loss of leaves. The flowers of this species are tubular and the color ranges from deep purple-red through pink and white. However, commercial cultivars have different nuances of color, shape and size, and some feature an attractive fragrance. The flowers are produced in clusters (corymbs) at the apex of the branches during most of the year, although in some cultivars flowering is more restricted (Brown, 2012; Dimmitt et al., 2009; McBride et al., 2014).

In addition to the ornamental value, Adamu et al. (2005)

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report the antimicrobial potential of extracts prepared from the desert rose suberin, so that the species is considered a broad spectrum in controlling the microorganisms tested. There are also scientific studies related to the cytotoxic compounds produced by this plant in the fight against cancer cells (Nakamura et al., 2000) and the influenza virus (Kiyohara et al., 2012).

The propagation of the species occurs mainly by seeds, whereas plants from seeds present more developed caudex and main root when compared to the ones propagated by cuttings. However, there are few studies describing the seeds and their germination process, as well as other elements involved in this process, such as the ideal temperature for germination and the water absorption curve.

In the process of germination, the water regulates the tissues rehydration, with the consequent intensification of respiration, and digestion and translocation metabolic activities, which are essential to supply energy and nutrients for growth resumption of the embryonic axis (Kikuchi et al., 2006; Ataíde et al., 2014).

Determining the water absorption curve is also related to integument impermeability studies, determining the treatments duration with plant growth regulators, osmotic conditioning and pre-hydration (Carvalho and Nakagawa, 2012). Still, it is interesting to understand this process for determining the temperature and humidity conditions when willing to store the seeds for an indeterminate period of time, without, however, affecting their physiological quality.

As in the germination process, humidity and temperature can trigger physiological processes during the storage phase and thereby accelerate the seeds deterioration or promote the growth of pathogens (Pontes et al., 2006; Medina et al., 2009).

Thus, this work aimed to do a descriptive study of fruits and seeds and the *A. obesum* germination process from two years of observations.

Material and Methods

Plant material

Fruits of *A. obesum* were collected between the months of February and March 2013 and March and April 2014 from 20 plants grown in a greenhouse at University State of Londrina when they started the spontaneous opening. The fruits were transferred to the Seed Analysis Laboratory and packaged in plastic trays until completing the natural opening and releasing the seeds.

Characterization of fruits and seeds

The biometrical description of the fruit (2013 and 2014) was done from a sample of 13 fruits for each year. Therefore, the length and equatorial diameter of each fruit at harvest were measured; the number of seeds per fruit was also determined.

For biometrical description of seeds (2013 and 2014) determining the thousand seed weight from eight samples of 100 seeds was held; and water content, determined by the oven method at 105 ºC (Brasil, 2009), using two samples of 20 seeds. The seeds length and diameter were measured in a sample of 200 seeds with a digital caliper.

For internal morphological observations, the seeds were previously soaked in distilled water for three hours to soften and moisturize the tissues. After this period, longitudinal sectioning was done with a metallic blade and then they were observed through a Motic® SMZ-168 magnifying glass.

Germination test and water absorption curve

Freshly harvested seeds in 2013 were submitted to the germination test at 25 ºC. Simultaneously, the seeds water absorption curves were determined at temperatures of 15, 20, 25 and 30 ºC in two substrates: over and between sheets of paper.

The remaining seeds were stored in a glass bottle with a polyethylene cap at a temperature of 25 °C and relative humidity of 65% ± 5 for a period of 12 months and submitted to seed germination test under the same conditions in the year 2014.

In 2014, a fresh lot of seeds was harvested and they were submitted to a germination test at temperatures 25 and 30 °C. Similarly, the seeds water absorption curves were determined at 25 and 30 °C temperatures over and between sheets of paper, based on the results obtained in 2013.

For the germination tests, four replications of 50 seeds were employed, which were placed on towell paper moistened with distilled water in the amount of 2.5 times the dry paper mass (Brasil, 2009), stored in crystal polystyrene boxes (Gerbox®) in a B.O.D. (Biochemical Oxygen Demand) incubator under constant light. The variables evaluated were germination percentage and germination speed index (GSI) (Maguire, 1962), starting the counts from the third (2013) and fourth days (2014) after installing the tests.

The imbibition curve was performed with four replications of ten seeds for each treatment. The seeds were laid out over or between sheets of paper, as described for the germination test. Initially, the seeds were weighed every hour during the first eight hours of imbibition by means of an analytical precision scale (0.0001 g). After this time, the seeds were weighed at intervals of 24 hours, marked from the beginning of the experiment, ending the weighing 24 hours after the primary root protrusion of 50% of seeds. At every weighing the seeds were removed from the Gerbox® boxes and placed over paper to absorb external moisture, weighed and then returned to the

Gerbox® and B.O.D.

Statistical analysis

The measures relating to the length and diameter of fruits and seeds of years 2013 and 2014 are followed by descriptive statistics and grouped in a diagram of frequency distribution. The averages obtained for the germination percentage in both lots were submitted to analysis of variance and compared based on the *p-value*. For the soaking curves in both years, a regression model was adjusted by testing the polynomial regression of first, second and third degrees.

Results and Discussion

Characterization of fruits and seeds

The desert rose fruits are classified as follicles, dehiscent, having a break line in the longitudinal direction, and striking when the fruits are in an advanced ripening stage. Their length and diameter can vary depending on factors such as pollination efficiency, environmental conditions and nutritional status of the plants. Table 1 shows desert rose fruits length and diameter values measured in years 2013 and 2014. The fruits average length was between 18.7 and 20.0 cm and the diameter between 12.9 and 13.3 mm.

The number of seeds per fruit is also related to the same conditions affecting the development of the fruit. In 2013 there was an average of 75 seeds per fruit and 84 seeds in 2014 (Table 1).

The seeds have a cylindrical shape, a brown staining integument and a brown-gold pappus (bristles) on both ends, which helps the dispersion by wind (Figure 1A). Desert rose seeds are similar to the the ones in oleander (*Nerium oleander*), but in this species bristles are on only one end thereof.

Table 1. Biometric characterization of *Adenium obesum* fruits harvested in 2013 and 2014.

	Length (cm)		Diameter (mm)		Number of seeds	
	2013	2014	2013	2014	2013	2014
Minimum	15.1	16.7	11.1	11.3	28.0	55.0
Maximum	25.1	23.4	15.2	15.4	117.0	118.0
Average	18.7 ± 2.9	20.0 ± 1.7	13.3 ± 1.2	12.9 ± 1.3	74.7 ± 27.9	84.4 ± 19.6

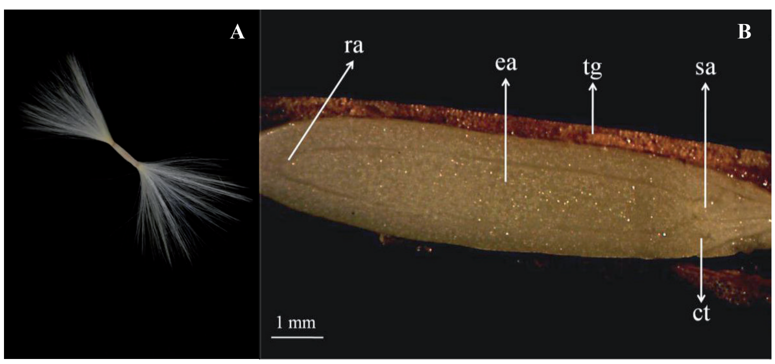

Figure 1. Morphological characterization of *Adenium obesum* seeds, external (A) and internal (B) aspects.
Caption: (B): tg – integument; ea – embryonic axis; ra – root apex; sa – stem apex; ct – cotyledons.

For seeds length and diameter, it can be seen in Table 2 and Figure 2 that in 2013 the predominant classes of length and diameter were 9.1 to 10.0 mm and 1.81 to 2.0 mm, respectively. In 2014, the highest frequency for diameter was observed in the same class of 2013; however, the predominant length was between 11.1-12.0 mm.

For the thousand seed weight were obtained the average values of 15.8 g (2013) and 15.6 g (2014). Water content had an average of 7.1% in 2013 and for this same lot reduction of the water content to 6.5% was found after a 12-month storage. However, for the seeds harvested in 2014 the water content was 5.6%, which explains the reduction in the thousand seed weight in that year.

Table 2. Biometric characterization of *Adenium obesum* seeds harvested in 2013 and 2014.

	Length (mm)		Diameter (mm)	
	2013	2014	2013	2014
Minimum	7.7	7.9	1.5	1.4
Maximum	13.1	14.4	2.7	2.6
Average	9.8 ± 1.0	10.7 ± 1.3	1.9 ± 0.2	2.0 ± 0.2

As for the seeds internal structure (Figure 1B), it was found that when they are hydrated, the embryo is easily observed: it is cylindrical, has white coloring and occupies almost the whole kernel space. The cotyledonary reserve tissue is also white colored and has a firm consistency.

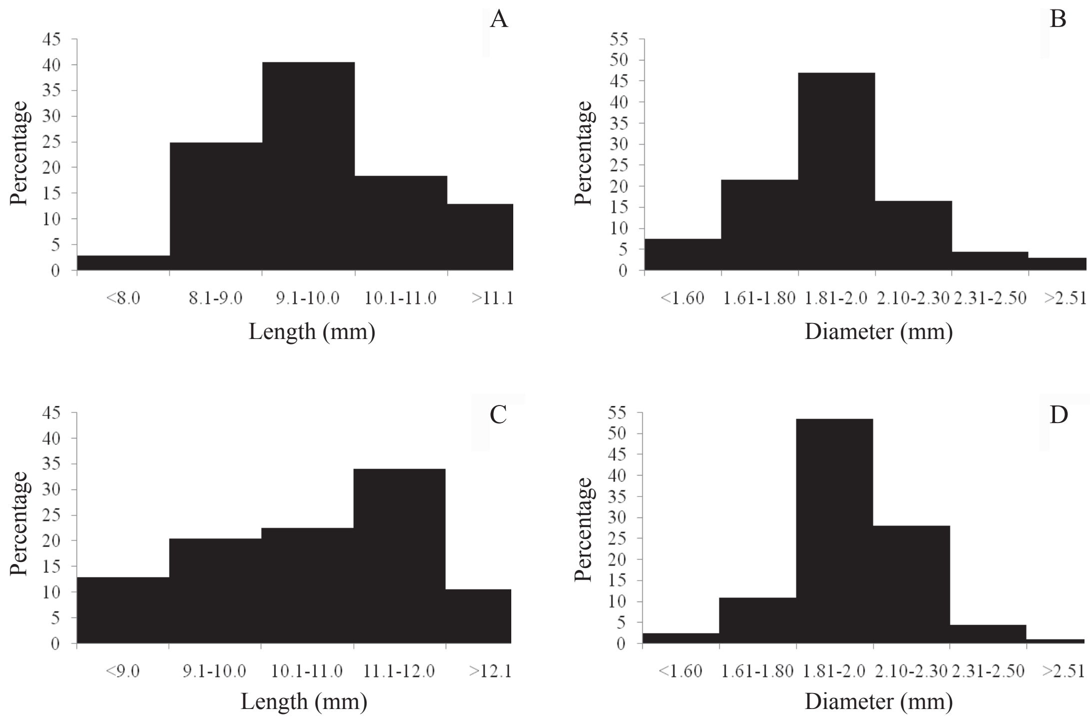

Figure 2. Frequency distribution for seed length and diameter of *Adenium obesum* seeds collected in years 2013 (A and B) and 2014 (C and D).

Germination test and water absorption curve

Parallel to the germination test, the description of the desert rose germination process was carried out at 25 °C temperature, over paper. 96 hours after seeding, seed integument disruption and primary root protrusion were observed. After 120 hours, there was greening of the base stem and an average increase of 3 mm of the radicle. After 168 hours, there was a total disruption of the integument and exposure of the cotyledonary leaves (Figure 3) due to water absorption and increasing volume of seed cotyledons, causing integument disruption and emergence of the root hypocotyl axis and other internal structures of the seed (Borges et al., 2009).

As for the germination tests, it was observed at harvest and 12 months after it that the average values for this variable were 90 and 91%, respectively (Table 3), with little influence of storage at 25 °C on germination percentage. Thus, it turns out that storing seeds of this species at 25 °C for up to 12 months does not alter its germinability ($p > 0.7$). Regarding the germination speed index (GSI), the value observed (7.4) remained constant in the two periods in which the germination test was conducted.

Figure 3. Germination process characterization of *Adenium obesum*. (A) Primary root protrusion, (B) primary root elongation, (C) issuance of root hairs and (D) exposure of cotyledonary leaves.

For the lot of seeds harvested in 2014, the germination test was conducted at temperatures of 25 and 30 ºC. This year, however, there were higher germination percentages compared to 2013. At the temperature of 25 °C, the average germination percentage was 96%, and at 30 °C it was 98%; however, there was no statistically significant difference between them (Table 3). Carpenter and Boucher (1992), in a study of Madagascar periwinkle (or rosy periwinkle) (*Catharanthus roseus*) 'Grape cooler' seeds, found that maximum germination occurs at temperatures between 25 and 35 °C, and temperatures below 25 °C are unfavorable to this process.

Table 3. Germination (%) and germination speed index (GSI) of *Adenium obesum* seeds harvested in 2013 and 2014.

	2013 A*	2013 B	2014	
	25 ºC	25 ºC	25 ºC	30 ºC
Germination (%)	90	91	96	98
F	0.09 ns		1.04 ns	
p-value	0.7		0.3	
CV (%)	4.7		2.9	
GSI	7.4	7.4	8.2	10.7

*2013 A: freshly harvested seeds; 2013 B: seeds stored for 12 months. ns: non significant F-value.

Thus, to evaluate the quality of a given seed lot in a laboratory, there is a need for a germination pattern for each species because each one presents seeds with different characteristics regarding their germinative and physiological behaviors (Wielewicki et al., 2006).

On the other hand, GSI increased due to the germination temperature increase, from 8.2 to 25 °C to 10.7 to 30 °C, so that the temperature of 30 °C accelerated the seeds germination process (Table 3). Similar results are described by Gordin et al. (2012), wherein the *Guizotia abyssinica* seeds GSI increased as the germination temperature increased from 25 to 30 ºC.

As the temperature and kinetic energy are increased, water viscosity is reduced, thus favoring imbibition and the seeds metabolism components reactions rate (Marcos-Filho, 2015), such as changing the structure of proteins and nucleic acids, besides modifying the cell membranes fluidity (Rodrigues et al., 2010; Zinn et al., 2010).

For the seeds water absorption in 2013 (Figure 4), an increase in mass was observed due to imbibition, from 7.24% after the first hour at 15 °C over paper up to 25.46% at 25 °C between sheets of paper. For this same seeds lot after 12 months of storage there is, in the first hour of imbibition, an increase from 7.33% to 10.40% in mass of seeds kept over paper (25 °C) and between sheets of paper (30 °C), respectively (Figures 5A and F). Similar values are observed for seeds harvested in 2014 and submitted to the same conditions (Figures 5C and H).

This rapid water absorption by the seeds occurs due to the large difference in water potential between the seeds and the substrate, which characterizes phase I of the germination process (Bewley and Black, 1994). After five hours from the start of imbibition, seeds harvested in 2013 and kept between sheets of paper at temperatures of 20, 25 and 30 °C reached water contents between 35 and 50% (Figures 4 D, F and H). For imbibition curves reached in 2014, it has also been found, after the fifth hour, that the seeds had already absorbed 35 to 42% of water in all conditions tested, except for those submitted to 25 °C over paper (Figure 5).

For Carvalho and Nakagawa (2012), cotyledonary seeds finish phase I of the germination process once they reach water contents between 35 and 40%, and from these levels phase II would be started. However, the duration of each phase depends on properties inherent to seeds and environmental conditions present (Bewley and Black, 1994).

In the experiments conducted in 2013 and 2014, the primary root protrusion occurred between 48 and 72 hours in seeds kept at a temperature of 30 °C in both substrates (over paper and between sheets of paper); with that, the end of phase II of the germination process is characterized. However, in 2013, the seeds maintained at temperatures of 15 and 20 °C in both substrates began the process of deterioration after 120 hours of incubation under these conditions, and only some seeds issued the primary root. Thus, the importance of temperature to trigger the germination process is evident.

With respect to substrates over paper and between sheets of paper, there was no direct influence of these on the pattern of seeds water absorption. Although not being entirely surrounded by moisture (as it occurs between sheets of paper), seeds absorb water in a similar manner when exposed over paper; it is likely that this is due to the high permeability of the integument. Gordin et al. (2012) have found no differences either in the pattern of *Guizotia abyssinica* seeds water absorption in the substrates over paper and between sheets of paper.

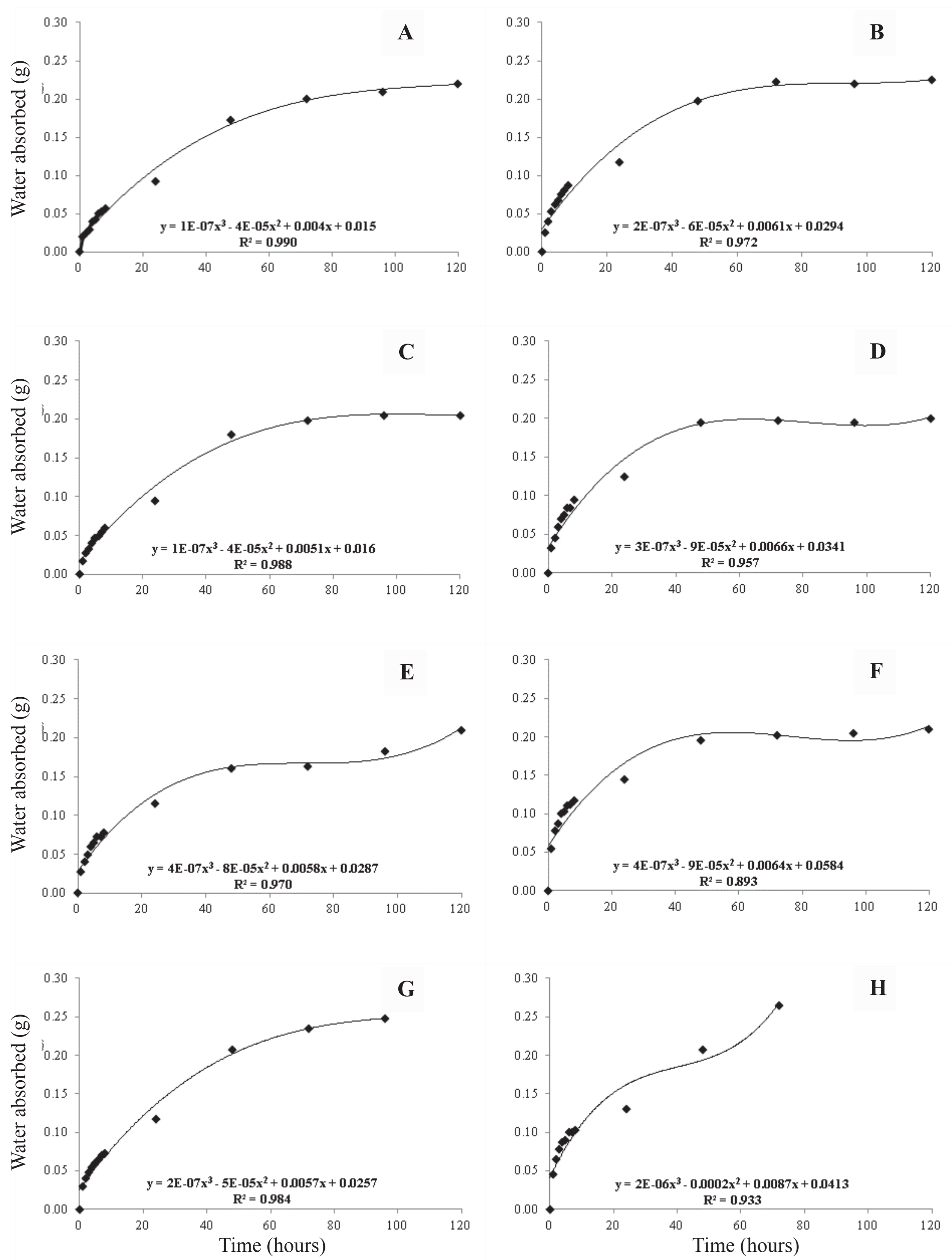

Figure 4. Water absorption curves in *Adenium obesum* seeds harvested in 2013 in a substrate over paper and between sheets of paper, at temperatures of 15, 20, 25 and 30 ºC.

Substrates: over paper (A, C, E and G); between sheets of paper (B, D, F and H). Temperatures: 15 ºC (A and B), 20 ºC (C and D), 25 ºC (E and F) and 30 ºC (G and H).

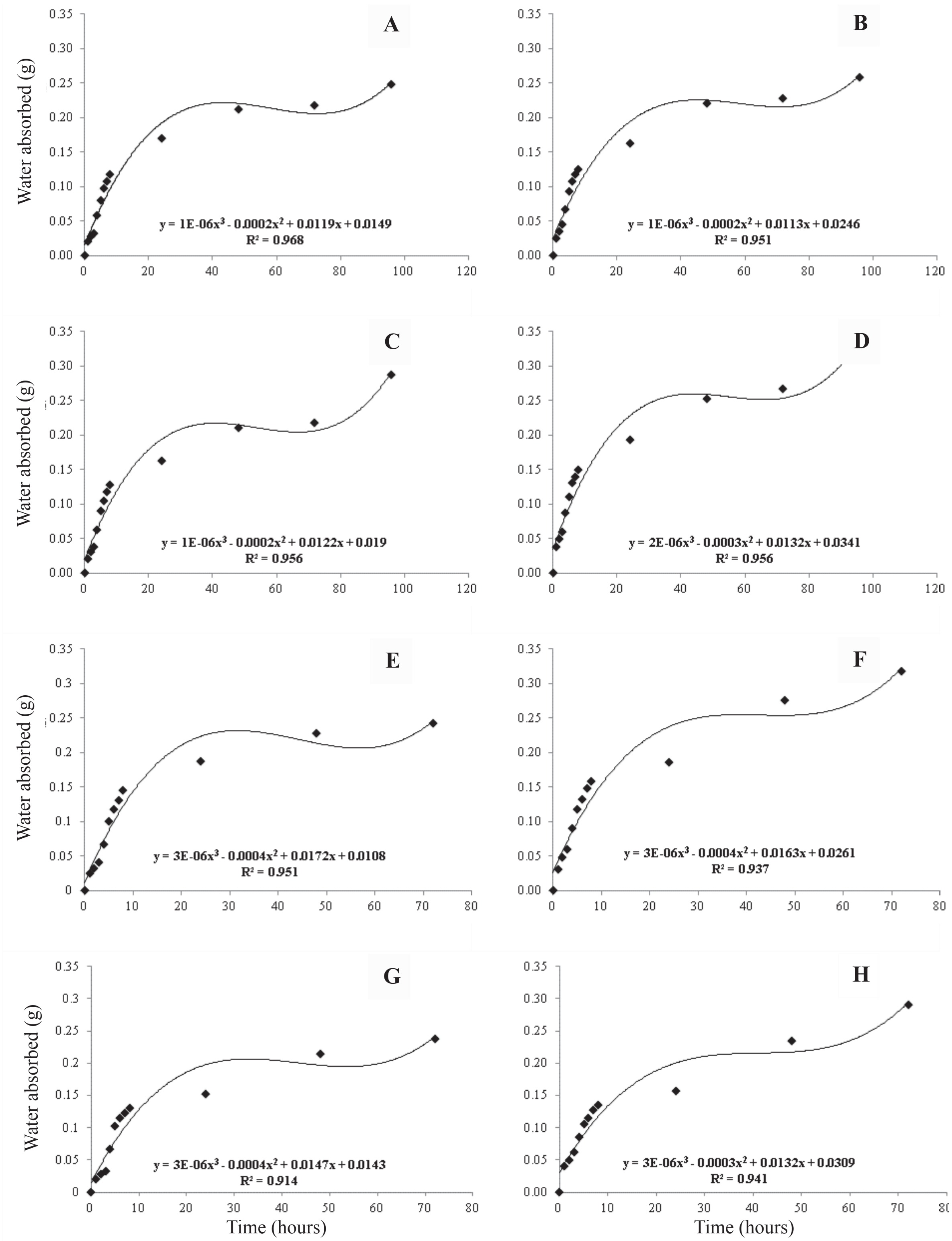

Figure 5. Water absorption curves in *Adenium obesum* seeds harvested in 2013 and stored for 12 months and harvested in 2014 in a substrate over paper and between sheets of paper, at temperatures of 25 and 30 ºC.

Seeds stored for 12 months: (A, B, E and F); seeds harvested in 2014: (C, D, G and H); 25 ºC, over paper: (A and C); 25 ºC, between sheets of paper: (B and D); 30 ºC, over paper: (E and G); 30 ºC, between sheets of paper: (F and H).

Conclusions

Desert rose seeds can be stored at 25 ºC for up to 12 months without loss of viability.

Temperatures of 25 and 30 °C are effective for the desert rose germination test.

The water absorption curve of desert rose seeds follows the triphasic pattern of imbibition, and the seeds reach germination phase I after five hours of imbibiton.

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