Scientific Paper

Quality and physical dormancy of seeds from *Desmodium incanum* DC. in the Entre Ríos province, Argentina

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Abstract

Objective: To determine the quality and physical dormancy of seeds from *Desmodium incanum* DC. (Fabaceae, Desmodieae) in the Entre Ríos province, Argentina.

Materials and Methods: Fruits from plants of two neighboring natural areas, located in the Entre Ríos province, Argentina, were harvested. The collection was carried out between December and February, 2017-2018, on a surface of 20 m^2 for each area. From the homogeneous set of seeds, the moisture content, seed weight, germination and viability, were determined. The permeability to water and longevity were modeled. The least squares was used as estimation method, and the higher determination coefficient, R^2, was used as criterion for the model selection.

Results: The harvested seeds showed low moisture content (8,2 %) and high quality. The germination essay showed low percentage of normal seedlings (3,5 %) and 95 % of hard seeds. In turn, 89,5 % of the embryos were observed viable. The proposed model proved different depth of physical hardness, and the fraction with high dormancy depth prevailed.

Conclusions: The seeds of the species produced in the region showed high quantity of viable embryos; although most of the seeds showed deep physical dormancy. The hard seeds reached slow breaking of the physical dormancy in time, which originated normal seedlings, even after 22 months of storage.

Keywords: storage, seed quality, permeability

Introduction

The loss of autochthonous species increases remarkably, and one of the main reasons is the lack of knowledge about their benefits and the characteristics that favor their persistence (Oscanoa-Lagunas, 2005). The genus *Desmodium* Desv. belongs to the family Fabaceae (Legumes), subfamily Faboideae, tribe Desmodieae. *Desmodium incanum* DC., (syn. *Desmodium canum* Schinz & Thell.). *Desmodium incanum* DC is distributed throughout America, from Canada to central Argentina and Uruguay (Burkart, 1987). In Argentina it is present in ten provinces (Vanni, 2008).

It is a perennial forage species, of spring-summer cycle, with flowering period in November and fructification in December, and another flowering one in February and fructification in March (Fernández *et al.,* 1988). It is appetizing for the animals, and due to its nutritional properties is a feedstuff of good quality. It is very common in natural pasturelands. Until now no toxicity problems associated to it are known (Scandaliaris *et al*., 2013). Preliminary studies of the digestibility of its leaves, carried out in a population of this species, located in areas around Paraná report 14,8 and 19,5 % of crude protein in the vegetative and reproductive states, and metabolic energy of 2,31 and 2,44 Mcal/kg DM, respectively (Galussi, A. and Gillij, Y., pers. com., 2017; 2018).

To determine the planting density of this species, it is necessary to know the weight and the different categories of seeds and seedlings found in the germination analysis. Although there are methodologies stipulated by the rules of the International Seed Testing Association (ISTA, 2016), no information is available about the seed quality of this species produced in the Entre Ríos region. In this regard, it is known that the moisture content of the seeds and the temperature, are determinant factors in their conservation (Marcos Filho, 2005). In *Medicago sativa* L. seeds, for example, for adequate storage it is necessary to decrease the moisture content from 25-20 % (harvest) to lower values than 12 % (Rivas-Jacobo *et al*., 2007).

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In the seed, water is found in different association forms: water type 1, 2, 3 and 4, with lower content than 7,5 %, between 7,5 and 20 %, between 20 and 33 % and from 33 to 41 %, respectively (Villela *et al*., 2003). The deterioration degree of the seed through time will occur according to its water content (Villela *et al*., 2003; Marcos-Filho, 2005).

In *D. incanum* seeds, harvested in Entre Ríos, the moisture content at the harvest moment is not known. However, this is a quality indicator that is important to determine in order to establish its relation to seed longevity.

Regarding germination, even when the seed is viable, slow and poor germination under natural and appropriate conditions (temperature, light, water and oxygen availability) is explained as product of the physiological phenomenon known as seed dormancy (Quinlivan, 1971). According to Bewley and Black (1986), seed dormancy is an important factor in the dynamics of natural populations, and is related to plant adaptation to environmental heterogeneity. In preliminary germination essays with *D. incanum* seeds, obtained in Entre Ríos, during the period from 2016 to 2018 (Galussi, A., pers. com., 2016; 2018), low values of normal seedlings and high quantity of non-germinated seeds, categorized as hard seeds, were observed.

In the germination analysis in Fabaceae and other botanical families the presence of hard seeds is mentioned as manifestation of physical dormancy, trait that has been referred by different authors (Galussi *et al*., 2013a; Orsenigo *et al*., 2019; Paul *et al*., 2019).

The term hard seed is applied to the seed that shows a water-impermeable coat, and is not able to germinate because its embryo cannot imbibe (ISTA, 2016). Physical dormancy is related to diverse characteristics of the seeds, such as anatomy and composition of chemical substances in the seed tegument, which cause different degrees in the dormancy depth (Galussi *et al*., 2019). The depth degrees of physical dormancy originate different levels of permeability in the seeds, which can be analyzed through mathematical models. These models allow an adequate description of the imbibition process (Chapra and Canale, 2004). For breaking physical dormancy several methodologies are cited (ISTA, 2016; Zapata *et al*., 2017; Castillejo-Jiménez, 2019).

Taking into consideration the scarce information about the quality attributes of *D. incanum* seeds, produced in the Entre Ríos region, in Argentina, the objective of this study was to determine the quality and physical dormancy of such seeds.

Materials and Methods

Location. Fruits (loments) were harvested from plants of two neighboring natural areas: Ensayo Colony, Diamante (31°51'14.0''S and 60º 33'02.0''W) and Paraná, La loma Sanctuary (31°45'44.0"S and 60°31'57.0"W), located in the Entre Ríos province, Argentina.

The collection was carried out between December and February, 2017-2018 on a 20-m^2 surface for each area, when the endocarps with seeds were found mature with brown color. The color of the pericarp and seed coat was determined through comparison with the color chart proposed by Munsell (2000). The fruits were stored in the laboratory, in plastic containers, at room temperature (20 ± 1 °C) until the beginning of the essays. From the homogeneous set of harvested endocarps (650 g) the seeds were manually extracted to conduct the quality evaluation essays.

Moisture content. This analysis was carried out according to the method of high constant temperature, established according to the rules indicated by ISTA (2016), with slight modifications in seed weight. For such purpose, once the fruit seeds were extracted, two repetitions of 1,5 g of seeds per essay and per harvest were placed in metallic containers, at 130-133 ºC, during one hour. Then they were left to cool down in the desiccator for one hour. The tolerance between repetitions was within the accepted one (≤ 0,2%). The moisture content was calculated, as percentage in weight, with three decimal figures for each repetition and the result was reported rounded off to the closer 0,1 %.

Weight of 1 000 seeds. It was determined from the homogenized sample, with eight repetitions of 100 seeds (ISTA, 2016).

Germination and viability. The evaluations were carried out with four repetitions of 100 seeds. The seeds were placed within moisturized paper for germination (in roll) and were put in polyethylene bags to prevent evaporation. They were taken to chamber at 25 ºC, with a photoperiod of 8/16 hours, during 21 days. The seedlings were evaluated according to ISTA (2016) rules. In the non-germinated seeds the viability was evaluated through topographic tetrazolium test. For such purpose, a cut was performed on the seed coat on the distal end of the cotyledons (antiraphe zone). Afterwards the seeds were placed in water until completing imbibition. Then, they were submerged in an aqueous solution at 0,5 % of 2, 3, 5-triphenil tetrazolium chloride at 20 ºC for 12 hours. The staining topography on the embryos was evaluated and viability was determined (ISTA, 2016).

Hydration rate. Essays of seed permeability to water were carried out, according to Galussi *et al.* (2013a; 2013b). Three repetitions were taken from the sample, of 400 seeds each, previously observed under magnifying glass (x 10). Those seeds that were cracked, with fissures, or lacking part of the testa, were discarded. The seeds were submerged in water and were considered completely hydrated when an increase of maximum size was observed (80 % of their fresh weight). Thus, their permeability time was determined. During the period of immersion in water, they were observed every day for one week; afterwards, twice a week during one month, and once a week until 269 days of being in water and finishing the trial. In turn, the number of permeable (imbibed seeds) and the time in days until observing increase size and rupture of the seed tegument, were recorded. Then, to evaluate germination, they were placed within moisturized paper, according to the above-explained methodology.

Longevity test. The evaluation of longevity (germination through time) was conducted by germination essays (GT), according to the above-explained methodology. For such purpose, three moments were established: a) planting immediately after carrying out harvest, b) at 12 months and C) at 22 months. From each fraction four repetitions were sown, of 100 pure seeds, placed in moist paper for germination, arranged in a stove at 25 ºC, with a photoperiod of 8/16 h light and darkness.

Statistical analysis. The results of accumulated imbibed seeds percentage (y) were modeled with the time (days) as only independent variable (x) and the model that best represented the release from physical dormancy was selected. The utilized estimation method was least squares, and the criterion for the selection of the model was the highest determination coefficient, R^2. The program InfoStat (Di Rienzo *et al.*, 2019) was used.

Results and Discussion

Seed quality. Table 1 shows the mean characteristics of the *D. incanum* seeds, corresponding to weight, moisture, germination and viability (hard seeds).

Regarding seed weight, it could be established that the recorded value was 4,1 g, which is close to the weight of *Medicago polymorpha* L., which was 4,5 g (Ovalle *et al*., 2005), and is higher than that of *M. sativa* (2,21-2,49 g). According to Galussi *et al.* (2013a), the *D. incanum* seed is similar in shape to the seed from these species, but it is larger. The weight could also be conditioned by the dormancy mechanism, specifically if it is started during the period of dry matter accumulation or early dehydration (Bewley and Black, 1986).

The moisture content of the seeds (8,2 %) was slightly higher than the one cited for *Desmodium ovalifolium* (7,6 %) and without presence of hard seeds (Muñoz *et al*., 2009). Nevertheless, it was lower than the one reported by Rivas-Jacobo *et al.* (2007) and Galussi (2013a) in samples of *M. sativa* seeds stored in the region (9-12 %).

This can be explained because the hard seeds of *M. sativa* and *Trifolium repens* L. showed low water content (6-7 %), according to Galussi *et al.* (2013a; 2013b). probably, the high number of hard seeds present in the *D. incanum* sample (95 %) would explain the low water content found.

Moisture indicated the presence of type 2 water (quantity very close to type 1), which shows very low metabolic activity in the seed, and favors the conservation of viability of orthodox seeds (Marcos-Filho, 2005).

If it is related to the rule proposed by Harrington (1972), valid for moisture contents in the range from 4 to 14 %, which sustains that for each point by which the moisture content of the seed is reduced its storage potential is doubled, hard seeds would have high conservation potential.

Meyer *et al.* (2007) determined that in hard soybean seeds water conductivity in the teguments is lower than in non-hard seeds, in correspondence

Table 1. Quality of *D. incanum* seeds.

Weight of 1000 seeds, g	Percentage, %					
	Moisture	Seedlings		Non-germinated seeds		
		Normal	Abnormal	Dead	Hard	
					Viable	Non-viable
4,12 ± 0,04	8,2	3,5 ± 1,0	1,0 ± 0,96	0,5 ± 0,5	89,5 ± 1,3	5,5± 2,0

with this, hard seeds are not exposed to a fast exchange with the ambient temperature and, consequently, they would be less affected by the sorption mechanism (adsorption-desorption), maintaining its minimum respiratory activity, which causes deterioration to be lower.

The germination test showed low percentage of normal seedlings (3,5 %) and high quantity of hard seeds (95 %). Nevertheless, according to the topographic tetrazolium essay, 89,5 % of the embryos were observed viable (table 1).

The non-viable embryos showed damaged tissue in the radicle and in the hypocotyl-radicle axis. The occurrence of abnormal seedlings in the germination test was low. It corresponded to seedlings with the primary root trapped in the seed coat and seedlings with dead primary root. These results are in agreement with the report by Rojas and Herrera (1988) for *D. ovalifolium*, who stated that low germination is a common problem in the seed lots of this species and responds to physical dormancy.

In the *D. incanum* sample analyzed in this essay high percentage of hard seeds (95 %) was found. That is why, in the studied sample, the weight, moisture and germination/viability showed, mostly, results that respond to the attributes of the category hard seeds. The low germination coincides with the report by Guerrero and Alfandy (2019) for *Desmodium heterocarpon (L.) DC.* (20 % germination). The hard percentage of hard seeds can express the adaptation of the species to the environment in its sexual reproduction, according to the results indicated by Bewley and Black (1986).

Hydration rate. Regarding the trait of water permeability of the seeds, the mathematical analysis of the cumulative percentage of permeable seeds (response variable) regarding the immersion time, expressed as days until imbibition (explicative variable), showed different depths of physical dormancy. The curve of the physical dormancy of the seeds from the sample was of sigmoid type (fig. 1), which showed that the depth of physical dormancy was gradual. The curve responded to a third-degree polynomic model, which is expressed as:

The variable *y* represents the percentage of fully imbibed seeds. The *x* corresponds to the immersion days until being completely imbibed.

This model showed an adjustment coefficient of 97,1 %. Regarding the residue analysis, 1,08 % of imbibed seeds (13 individuals from 1 200) in the first four days expressed fast permeability to water. Three physical dormancy categories could be established: a) moderate, seeds that required up to 42 days of immersion; b) deep, seeds of very slow imbibition, with more than 42 days of immersion and up to 250 days; c) very deep, seeds that were hydrated after 250 days of remaining in water.

In the sample, categories a and b showed 5 and 10 % of permeable seeds, respectively. The seeds that when put to germinate broke physical dormancy, developed normal seedlings. The rest, 90 % of the hard seeds (category c), remained with physical dormancy until the end of the essay, at 269 days.

The results confirmed the presence of a high quantity of hard seeds with different categories of physical dormancy. The performance found in the sample was different from the report for *M. sativa* and white clover (Galussi *et al*., 2013a; 2013b). The different degrees of physical dormancy in *D. incanum* can be related to the chemical components found in the hard seeds (Galussi *et al*., 2019).

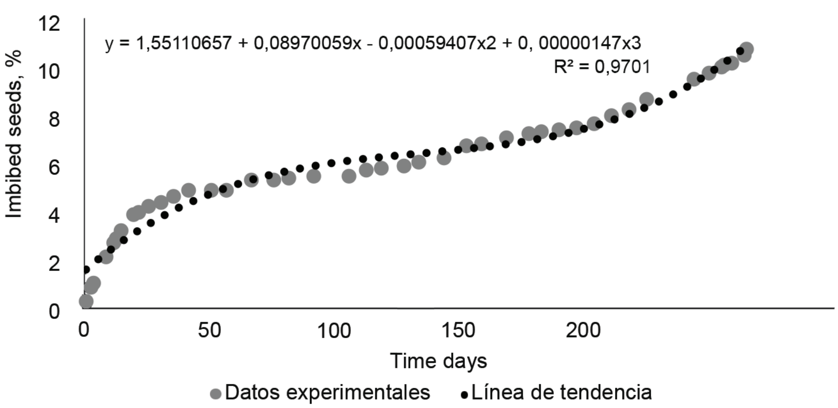

Figure 1. Imbibition rate of *D. incanum* seeds.

Observation: All the imbibed seeds originated normal seedlings

Longevity test. The cumulative values of normal seedlings responded to the seeds with different storage period under dry conditions, which remained in the essay until their germination. During the storage time until the moment of the germination test (0 month, 12 months and 22 months), the seeds showed a natural breaking of dormancy, which was manifested in the first seedling count.

The harvested and sown seeds (0 month) expressed 0,25 %, at 12 months 0,5 %. Meanwhile, at 22 months 8,5 % of permeable seeds produced normal seedlings. The 0-month seeds, when they reached 269 days of permanence in the germination test, originated 11 % normal seedlings (fig. 1). The ones of 12 and 22 months of age, 300 days after being sown, achieved 65 and 76 % germination, respectively (figure 2).

In the longevity essay it was observed that the humid environment of the germination substrate favored faster the natural breaking of dormancy in the older seeds than the dry storage environment (ambient temperature in laboratory). The seeds that broke physical dormancy originated normal seedlings, for which it can be stated that physical dormancy protects the embryo viability while such characteristic lasts, coinciding with the performance of other forage legumes (Zimmermann *et al*., 1998; Galussi *et al*., 2013a; 2013b).

To ensure a successful implantation of the crop, with produced seeds in the Entre Ríos province, the breaking of physical dormancy is required, which should be in agreement with the dormancy categories that appear. Nevertheless, the diverse treatments for the rupture of physical dormancy (ISTA, 2016; Zapata *et al*., 2017; Castillejo-Jiménez, 2019) had in common one characteristic: when overcoming dormancy, they eliminate partially or totally the mechanism or mechanisms of seed protection against the environmental adversity. That is why it is convenient that the seeds are utilized immediately after receiving the treatment (Marcos-Filho, 2005).

From the results it is advisable to continue with research in seeds from a second harvest, and to conduct anatomical studies of the seed tegument and the chemical components, associated to the physical dormancy categories in *D. incanum* seeds.

Conclusions

The seeds of the species produced in the region showed high quantity of viable embryos, although most had deep physical dormancy and predominance of the fraction with high dormancy depth.

The moisture content at the harvest moment allows the safe storage of the seeds, without the need of previous aeration or drying. The hard seeds, evaluated in time, showed slow breaking of physical dormancy, for which they originated normal seedlings, even after 22 months of storage.

The proposed method allowed to observe the performance of the sample individuals, which showed different depth of physical hardness in the seed coat. Storage in humid environment favored dormancy breaking, mainly in the older seed.

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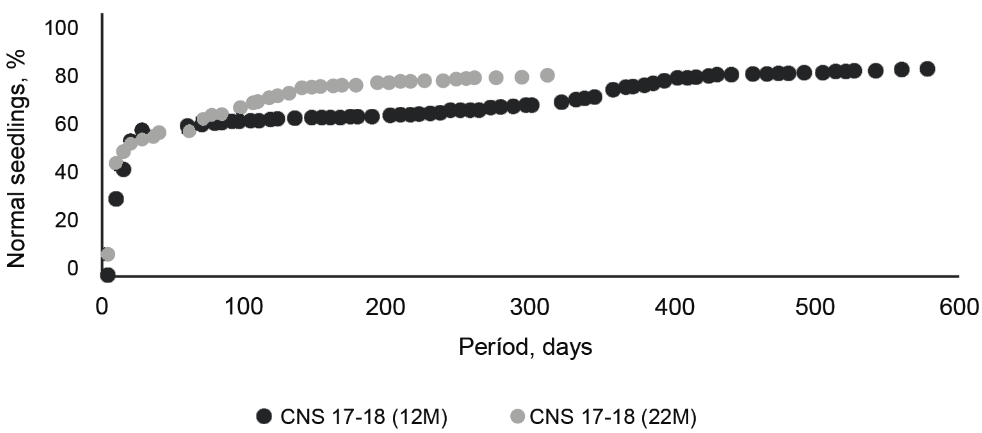

Figure 2. Normal seedlings of *D. incanum* in seeds of different storage age.
CNS: cumulative normal seedlings; ● 12 months of storage; ● 22 months of storage.

Authors' contribution

- Yanina Gimena-Gillij. Data collection, analysis and interpretation, writing and critical revision.
- Alberto Aníbal-Galussi. Conception, design of the study, data collection, analysis and interpretation, writing and critical revision.
- Marcelo Fabián-Prand. Statistical analysis and interpretation of the results and manuscript revision.
- Patricia Perissé. Analysis, writing and critical revision.

Conflict of interests

The authors declare that there is no conflict of interests among them.

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