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Adesmia subterranea Clos germination physiology and presowing treatments

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Abstract

The genus *Adesmia* (Fabaceae, Papilionoideae) is one of the scarce forage resources at high altitude and arid zones of South America. Its germination behavior has not been examined. Seeds of *Adesmia subterranea* "Cuerno de Cabra" were pretreated with sulfuric acid (1, 3, and 5 minutes soaking) and mechanical scarification to determine their impact upon dormancy and percentage and speed of germination. Treatments were evaluated under a range of constant temperatures (5 to 30° C) and 2 day/night cycles resembling the extreme environmental conditions of this species habitat. Water uptake and leachate conductivity were higher in the seeds scarified mechanically or with 5 minutes chemical scarification. These treatments also had the greatest total germination and rate at all temperatures in a petri dish germination test. However, in a cell tray experiment using a commercial substrate, the highest seedling emergence and rate were observed with chemical scarification (5 and 3 minutes). The high amount of leakage caused by the scarifications affected emergence in a non-sterile media. The results indicate that *A. subterranea* seeds have an impermeable seed coat which restricts water uptake, and the efficiency of sulfuric acid scarification to overcome seed coat impermeability and improve germination and emergence.

Key Words: Legumes, scarification, hard seed coat, imbibition, temperature

Resumen

El género *Adesmia* (Fabaceae, Papilionoideae) es uno de los escasos recursos forrajeros de zonas áridas y de altura en Sud América. El comportamiento de la germinación no ha sido evaluado hasta el momento. Semillas de *Adesmia subterranea* "Cuerno de Cabra" fueron pretratadas con ácido sulfúrico (inmersión 1, 3, y 5 minutos) y escarificadas mecánicamente para determinar su impacto sobre la dormancia, el porcentaje de germinación y la velocidad de germinación. Los tratamientos fueron evaluados en un rango de temperaturas constantes (de 5 a 30 °C) y en 2 ciclos de día/ noche similares a las extremas condiciones climáticas del habitat de esta especie. La imbibición y la conductividad eléctrica del sobrenadante fueron superiores en las semillas escarificadas mecánicamente o las escarificadas con ácido 5 minutos. Esos tratamientos también presentaron el mayor porcentaje y velocidad de germinación sobre papel en todas las temperaturas evaluadas. Sin embargo, cuando se utilizó un sustrato comercial el mayor porcentaje y velocidad de emergencia se observó en las semillas escarificadas químicamente durante 5 y 3 minutos. Los resultados indican que las semillas de *A. subterranea* poseen una cobertura impermeable que impide el ingreso del agua afectando la germinación y que la escarificación con ácido sulfúrico es muy eficiente para facilitar el ingreso del agua y consecuentemente mejorar la germinación y la emergencia

The genus *Adesmia* (Fabaceae, Papilionoideae), endemic to South America, includes more than 230 species (Burkart 1967). Widely distributed in the arid regions of Chile, Argentina, Bolivia, Perú, and southern Brazil, *Adesmia* provides forage for guanacos, goats, and small animals and wood for peasants at high altitude (Cajal 1983). In Patagonia (Argentina) different species of *Adesmia* are part of the goat diet (Pelliza-Sbriller et al. 1985). *Adesmia subterranea* "Cuerno de Cabra" a flat small bush, occurs between 3,000 and 4,000 m in the Andes mountains of Chile and Argentina (Kiesling 1994). Information on its propagation and germination behavior is not available.

The dormancy imposed by hard seed coats has been reported as a mechanism to survive extreme harsh environmental conditions, and it is a common phenomenon in leguminous seeds (Bradbeer 1988) and other families and species (Egley 1989). At high altitude, the polyphenols accumulated in thick seed coats also inhibit germination in several species (Gutterman 1993). According to Tran and Cavanagh (1984) there is a specifically located structural weakness in impermeable seeds, which is the focus for breakdown of hard seeds by natural agents. In *Papillionaceae* legumes, the point of weakness is located beneath the lens or strophiole (Probert 1992). Chemical scarification is widely used to overcome the strength of seed covering structures in many species: *Lupinus havardy* (Mackay et al. 1995), *Pistacia mutica* (Caloggero and Parera 2000), *Prosopis cineraria, Leucaena leucocephala, Acacia nilotica* and *Acacia tortilis* (Sacheti and Al-Rawahy 1998) and *Rubus* sp. (Peacock and Hummer 1996).

The objectives of this experiment were to determine the factors that influence seed germination in *A. subterranea* and to evaluate the effects of chemical and mechanical scarification on germination and emergence.

Materials and Methods

Seed material

The seeds of *A. subterranea* were collected in 3 different areas (Los Morrillos, Agua Negra, and San Guillermo) 32° 17'S 69°

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42'W of the Andean Cordillera in San Juan, Argentina, in March 1999. The altitude of the collection sites varies between 2,500 to 3,500 m. The seeds from the 3 collection areas were mixed, shelled and placed in hermetically sealed containers at room temperature (25° C) until the seed scarifications treatments were applied. The average weight of 1,000 seeds was 2.62 g and the moisture content was 12% (ISTA 1985).

Seed treatments.

Chemical scarification.

The seeds (5,000) were soaked in 50 ml of concentrated sulfuric acid (36 N) for 1, 2, or 3 minutes. After treatment, seeds were rinsed several times with tap water and dried at room temperature (25° C).

Mechanical scarification.

The seed coat on one side of the seed was perforated with a needle avoiding cotyledon damage.

Measurements

Water uptake and leakage conductivity. Four replications of 26 mg of seed (~100 seeds) were soaked in 30 ml of distilled water at 20° C. The weight of imbibed seed was recorded at 10, 20, 40, 60, or 90 minutes. Water uptake was expressed as the percentage increase over initial fresh weight. The conductivity of the leachate was measured with a conductivity meter (Oacton Instruments, Vernon Hills, Ill) at the same temperature and intervals and referred as $\mu S\ cm^{-1}\ g^{-1}$ of seed.

Germination test.

Four replicates of 25 seeds of each treatment were sowed in a petri dish with germination paper (Munktell filter 1700, Sweden) moistened with distilled water and incubated at 5, 10, 15, 20, 25, or 30° C. Additional seeds, sowed in a Petri dish, were placed in growth chambers providing 2 day/night temperature 16 hours/8 hours cycles (14° C / –2° C) and (19° C/ –0.5° C) to simulate the average maximum and minimum temperatures in January at the collection sites. Light was coincident with the high temperature period of each cycle and supplied by both fluorescent and incandescent lamps at a photosynthetic photon flux density of 420 $\mu mol\ m^{-2}\ sec^{-1}$. Seed was considered germinated when the radicle length exceeded 2 mm. Germinated seeds were counted every 24 hours and removed. The experiment was concluded after 12 days.

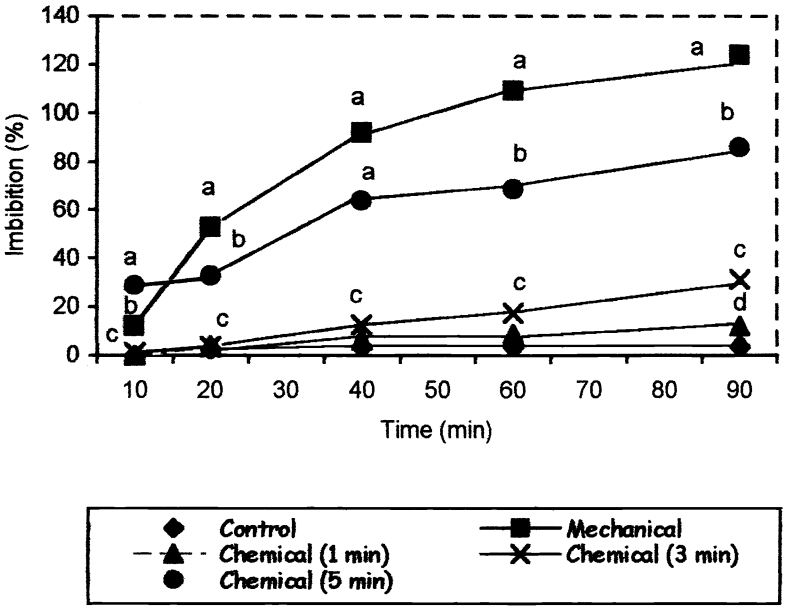

Fig. 1. Seed imbibition (percentage increase over initial fresh weight) of control and scarified seeds incubated at 20° C. Means followed by the same letter are not significantly different at P = 0.05 using least significant difference test (LSD).

Emergence test

Four replications of 45 seeds were sowed into 250 cell tray (16 cm^3 cell) filled with a commercial substrate (Fitotec, San Juan, Argentina) composed of sphagnum peat, horticultural vermiculite and perlite, and incubated at the same 2 day-night cycles used for the germination tests. Treatments were also tested simultaneously using perlite as the substrate for a day night temperature 16 hours /8 hours (19° C/ –0.5° C) cycle. Emerged seeds were counted every 24 hours. The experiment was concluded after 12 days when no further seedlings had emerged.

Experimental design and statistical analysis.

All the experiments were conducted as a randomized complete block design with 4 replications. All variables were subjected to analyses of variance using PROC

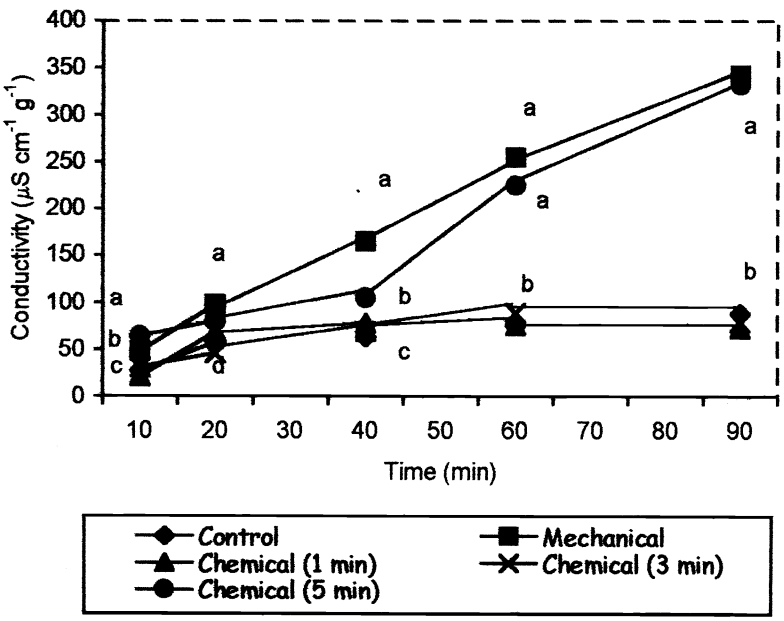

Fig. 2. Seed leakage conductivity ($\mu S\ cm^{-1}\ g^{-1}$) of control and scarified seeds incubated at 20° C. Means followed by the same letter are not significantly different at P = 0.05 using least significant difference test (LSD).

Table 1. Effect of temperature and treatments on petri dish germination, emergence rate index (ERI), Maguire´s equation (MG), and mean time germination (MTG) of *A. subterranea* seeds.

	Germination	ERI	MG	MTG
	%			
Treatment (Tr)	**	**	**	**
Temperature (T)	**	**	**	**
Tr x T	ns	ns	ns	ns
Treatments				
Control	37.0 d	1.73 d	1.78 d	6.06 a
Mechanical scarification	93.6 a	7.33 a	12.08 a	3.19 c
Chemical scarification (1 min)	76.1 c	4.73 c	6.38 c	4.99 b
Chemical scarification (3 min)	85.2 b	6.64 b	11.13 a	3.19 c
Chemical scarification (5 min)	90.0 ab	6.21 b	8.10 b	3.85 c
Temperature (°C)				
5	63.5 b	3.11 c	2.98 d	5.95 a
10	79.0 a	6.09 ab	9.02 b	3.84 cb
15	75.6 a	5.47 cb	8.90 bc	4.05 c
20	83.1 a	6.56 a	12.00 a	3.05 c
25	78.6 a	5.23 c	7.18 c	4.59 b
30	78.6 a	5.50 cb	7.08 c	4.05 b
Linear	**	**	**	**
Quadratic	**	**	**	**

ns, *,** Non significant or significant at the 0.05 and 0.01 probability levels respectively.
Means followed by the same letter are not significantly different at P = 0.05 according LSD test.

ANOVA and PROC GLM of SAS (SAS Institute, Inc., Cary, N.C.). The main effect of temperature was partitioned into linear and quadratic orthogonal contrasts. Means were compared with a least significant difference test (LSD) at P = 0.05. In each experiment germination percentage, emergence rate index (Shmueli and Goldberg 1971) (ERI = ΣX_n (c-n), where X_n = number of germinated seeds counted in day n; c = number of days from sowing until germination ended; n = day on which counts are made, expressed as the number of days after sowing), mean time to germination (Bewley and Black 1986) (MTG= Σ(t.n)/Σn, where t = time in days, starting from day 0, the day of sowing and n= number of seeds completing germination on day t) and Maguire´s equation (Maguire 1962) (MG= n1/t1 + n2/t2 +....+ n12/t12; where n1,n2...,n12= number of germinated seeds at times t1, t2,..., t12 in days) were calculated. All percentage data were arc sine transformed previous to the analysis.

Results and Discussion

The water uptake and electrical conductivity of the leachate were significantly modified by the treatments. Higher leakage conductivity and imbibition were observed with mechanical scarification and the longer chemical scarification time (Fig. 1 and 2). As soaking time increased the difference between these 2 treatments and the others increased. Conductivity and water uptake were always lowest for the control seeds. Weight of control seeds and those chemically scarified for 1 and 3 minutes increased 20% or less after 90 minutes incubation, whereas weight of mechanically scarified seeds and those chemically scarified for 5 minutes increased 120% and 90%. The data indicate that seeds of *A. subterranea* have an impermeable seed coat which restricts water uptake and also shows the efficiency of the scarification treatments to overcome seed coat impermeability.

Scarification of the seed significantly improved the germination percentage and germination rate expressed as Emergence Rate Index, Maguire´s equation or Mean Time Germination compared to control, at all incubation temperatures (Table 1). The results confirm the efficacy of sulfuric acid to increase germination in hard leguminous seeds reported by other authors (Masamba 1994, Mackay et al. 1995, Teketay 1996). Increasing the time of chemical scarification improved germination and its velocity, however there were no significant differences between mechanical scarification and the 5 minutes chemical scarification.

Scarified seeds germinated over a wide range of constant temperatures (Table 1). There was a significant linear and quadratic response of the percentage and velocity of germination to temperature, where the higher germination, emergence rate index and Maguire´s equation were reached at 20° C.

When treatments were evaluated at 2 different temperature cycles in a petri dish test, percentage and rate of germination were significantly greater in a warmer cycle (Table 2). The 5 and 3 minutes chemical scarification and mechanical scarification resulted in significantly higher germination percentage compared to the control and 1 minute chemical scarification (Table 2).

Rate and amount of emergence changed dramatically when the seeds were sowed in a commercial substrate at the same temperature cycles. The temperature significantly modified the Emergence Rate Index, Maguire´s equation and Mean Time Germination (Table 3). The final germination percentage was significantly higher with 5 and 3 minutes acid scarification compared to the mechanical scarification, con-

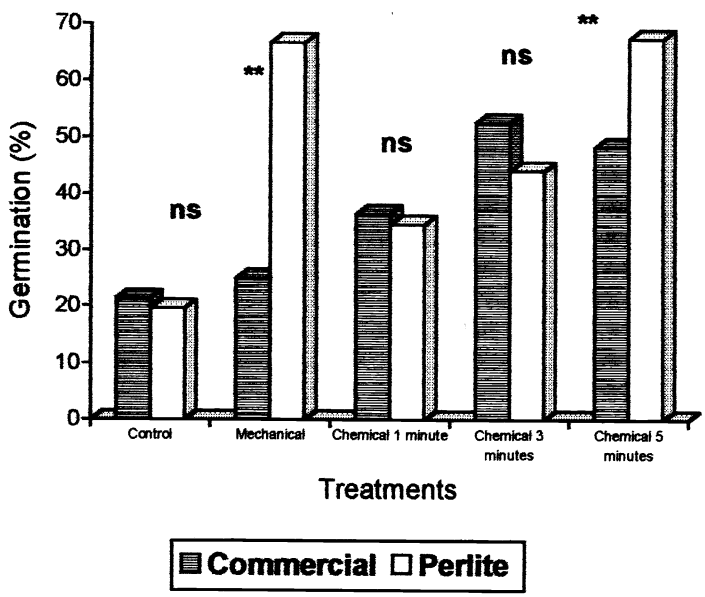

Fig. 3. Effects of the substrate on emergence of *A. subterranea* scarified seeds and control, incubated at a day night temperature 16 hours/8hours (19°C/–0.5° C) cycle. ns, *, Non significant or significant at the 0.05 and 0.01 probability levels respectively.**

Table 2. Effects of presowing treatments on germination percentage, emergence rate index (ERI), Maguire's equation (MG), and mean time germination (MTG) of *A. subterranea* seeds incubated in a petri dish at 2 different temperature regimes.

	Germination	ERI	MG	MTG
	%			
Treatment (Tr)	**	**	**	**
Temperature (T)	**	**	**	**
Tr x T	*	ns	ns	ns
Treatments				
Control	35.0 c	1.42 d	1.35 d	8.08 a
Mechanical scarification	91.0 a	7.95 a	9.83 a	3.26 cd
Chemical scarification (1 min)	65.5 b	3.80 c	3.58 c	6.17 b
Chemical scarification (3 min)	82.0 a	6.37 b	6.79 b	4.21 c
Chemical scarification (5 min)	85.5 a	7.71 a	10.52 a	2.97 d
Temperature (°C)				
14/-2	80.0	6.07	7.35	4.78
19/-0.5	63.0	4.83	5.48	5.10

ns, *,** Non significant or significant at the 0.05 and 0.01 probability levels respectively.
Means followed by the same letter are not significantly different at P = 0.05 according LSD test.

trol and 1 minute chemical scarification. Similar responses were observed in the calculated germination rate indexes. Evidently, the substrate has a strong effect on the germination of mechanically scarified seeds.

To determine the effect of the substrate on the emergence percentage, all the treatments were evaluated using the cycle 2 temperature with perlite as substrate. The results showed that the emergence percentage of mechanical scarification and chemical scarification for 5 minutes were significantly higher with perlite as the substrate (Fig. 3).

Woodstock (1988) reported that microbial attack stimulated by enhanced leaching from damaged cotyledons may act to further reduce field emergence. In our experiment the greatest seed leaching was observed following mechanical scarification and the most severe chemical scarification treatment. These treatments were severely affected by the use of non sterile germination conditions.

Conclusions

We concluded that the dormancy observed in *Adesmia subterranea* can be attributed to restricted water uptake by the seed coat. The mechanical barrier to radicle emergence was not the reason for the low germination observed in *A. subterranea* because a high germination and emergence were observed in perforated seeds. The hard seed is probably a survival mechanism for the extreme stressful conditions where this species grows. The optimum germination temperature closely resembles what typically occurs in January in the area where this species was collected. Scarification using sulfuric acid provided a practical and easy presowing treatment for increasing germination and rate of *A.subterranea* seeds.

Table 3. Effects of presowing treatments on emergence percentage, emergence rate index (ERI), Maguire's equation (MG) and mean time germination (MTG) of *A. subterranea* seeds incubated at 2 different temperature regimes using commercial substrate.

	Emergence	ERI	MG	MTG
	%			
Treatment (Tr)	**	**	**	**
Temperature (T)	ns	**	**	**
Tr x T	ns	ns	ns	ns
Treatments				
Control	16.9 c	2.03 c	1.29 c	9.33 a
Mechanical scarification	23.2 c	3.46 bc	2.59 bc	5.38 c
Chemical scarification (1 min)	37.5 b	4.65 b	3.21 b	7.79 ab
Chemical scarification (3 min)	51.5 a	6.79 a	5.22 a	7.20 bc
Chemical scarification (5 min)	54.2 a	7.71 a	5.27 a	6.51 bc
Temperature (°C)				
14/-2	36.7	6.52	4.46	4.54
19/-0.5	36.7	3.34	2.58	9.94

ns, *,** Non significant or significant at the 0.05 and 0.01 probability levels respectively.
Means followed by the same letter are not significantly different at P = 0.05 according LSD test.

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