

Drought stress conditions during seed development of narrowleaf birdsfoot trefoil (*Lotus glaber*) influences seed production and subsequent dormancy and germination

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Introduction

The drought stress is considered the main factor that imposes crops yield limitations. The effects of water deficiencies depend of several factors such as its intensity, duration, phenological phase of growth and genetic resistance capacity of plants. The water limitation affects plant growth and productivity. The most typical symptom of water deficiencies in higher plants is a retarded growth due to inhibition of cell elongation by water limitation (Nieman, 1965).

In the pampas lowlands, water limitation is usually an environmental restriction and in spite of natural *Lotus glaber* drought tolerance, this factor affects forage production and its natural persistence in grassland communities through seeds production.

Like other legume species, *Lotus glaber* has hard seeds with a coat-imposed dormancy which prevents water uptake and gaseous exchange (Mujica and Rumi, 1993). Factors involved in the onset of the impermeable seed coat condition of *Lotus glaber* during seed development, and in the development of the endogenous dormancy before seed coat impermeability is reached, are not known.

Seed coat-imposed dormancy in several other species is believed to be controlled by genetic and/or environmental factors present during seed development. These include soil moisture content (Barton, 1965), relative humidity (Harrington, 1949; Quinlivan, 1971), temperature alternation (Wurzbürger and Koller, 1976), daily average temperature or thermo-period (Von Abrams and Hand, 1956) and photoperiodic regime (Koller, 1962; Evenari *et al.*, 1966; Karssen, 1970; Gutterman and Evenari, 1972; Gutterman and Heydecker, 1973; Kigel *et al.* 1977; Kigel *et al.*, 1979).

The objective of this study was to test the hypothesis that water availability during seed development and maturation affects seeds production and degree of hard-seededness in *L.*

glaber by changing seed coat properties, conditioning water uptake through seed coat, and subsequently affecting dormancy, germination and speed of germination.

Materials and methods

Seeds of *Lotus glaber* obtained from a naturalized population of Saladillo, Buenos Aires Province, were disinfected with Ca hypochlorite, washed thoroughly and sown in 250 cc pots filled with culture soil. Pots were placed under natural conditions in the field and watered daily. One plant per pot was selected 30 days after sowing and plants were transplanted to 4 kg pots filled with same substrate 60 days after sowing.

Likewise soil, moisture retention curve was calculated through increase of soil water potential and soil moisture percent decrease. Weight of each pot until constant soil water potential was calculated with these values.

Twenty plants were used for each treatment in a complete randomized experimental design. The trial was initiated at the beginning of flowering and treatments were: (a) -0.4 Mpa soil water potential (E -0.4 Mpa); (b) -1.5 Mpa soil water potential (E -1.5 Mpa) and (c) control (soil to field capacity). These soil water potential levels were maintained constant during all trial through weight pots determination every 2 days intervals. The pots were maintained in light and temperature natural conditions during all trial. Pods were manually collected at complete ripening and before the spontaneous dehiscence of them during all flowering period. The number of total pods, number of pods per plant, number of total seeds, number of seeds per pod and 1000 seeds weight was determined for each treatment.

Immediately after harvest, seeds were stored in the dark simulating natural conditions of pressure, temperature and relative humidity. Each month for 12 months each treatment was evaluated for germination at 21° C in darkness in batches of 200 seeds in each Petri dish, starting from 1 May 2005, to determine changes in dormancy through time. The number of imbibed, non-imbibed and germinated seeds was recorded every 24 hours for 15 days and the accumulated rate was calculated. Final germination rate, estimated according to Pollock and Roos (1972), was evaluated after 150 days. Speed of germination was expressed as the time elapsed (h) since the beginning of the test until half of the final germination rate was reached (T_{50}). The imbibed seeds were recognized by their greater volume and bright teguments, and seed was considered germinated when radicle length reached 2 mm. The design was a complete randomized block experiment with 4 replicates.

Data were analyzed statistically by ANOVA and differences between means were tested for significance using least significance difference test according to Snedecor and Cochran (1980).

Results

Both drought stress treatments, E -0.4 MPa and E -1.5 Mpa, showed a significant reduction in number of total pods, compared with control treatment, representing 29 % and 12.7 % of

control values, respectively (Table 1). Likewise control treatment showed a significantly greater number of pods per plant compared with both drought stress treatments, which differs between them, with a significantly greater number of pods per plant for E -0.4 MPa compared with E -1.5 Mpa treatments.

Table 1. Number of total pods, number of pods per plant, number of total seeds, number of seeds per pod and 1000 seeds weight (g) of *Lotus glaber* plants subjected to drought stress of -0.4 Mpa (E -0.4 MPa) and -1.5 Mpa (E -1.5 MPa).

| Treatment | N° total pods | N° pods/ Plant | N° total seeds | N° seeds/pod | 1000 seeds weight |
|------------|---------------|-------------------|----------------|--------------|----------------------|
| Control | 1121 a | 56.0 a | 11990 a | 10.7 a | 1.0614 a |
| E -0.4 MPa | 327 b | 16.3 b | 2527 b | 7.7 b | 1.0710 a |
| E -1.5 MPa | 142 c | 7.1 c | 943 c | 6.6 b | 1.0780 a |

(*) values with different letters within the same column are significantly different (P>0.05)

As a consequence of preceding results, there were differences for total number of total seeds and number of seeds per pod between treatments. Drought stress treatments showed a significant reduction in number of total seeds compared with control treatment, representing 21 % and 8 % of control values, for E -0.4 MPa and E -1.5 Mpa treatments, respectively. Respect to number of seeds per pod, control treatment showed significantly higher values compared with drought stress treatments and without differences between them. In 1000 seeds weight there was not differences between treatments (Table1).

Control treatment seeds had a significantly greater germination rate compared with E -0.4 MPa and E -1.5 Mpa treatments, with a faster germination than E -0.4 MPa and E -1.5 Mpa treatments (Table 2).

Table 2. Germination rate (%) and average speed of germination (T_{50} , hours for a germination rate of 0.50) for *Lotus glaber* seeds harvested from plants subjected to drought stress of -0.4 Mpa (E -0.4 MPa) and -1.5 Mpa (E -1.5 MPa).

| Treatment | Germination rate (%) | Average speed of germination |
|------------|----------------------|------------------------------|
| Control | 35 a | 67.6 b |
| E -0.4 MPa | 14 b | 78.0 a |
| E -1.5 Mpa | 12 b | 80.1 a |

(*) values with different letters within the same column are significantly different (P>0.05)

Discussion

The water deficit in soil can reduce water potential of tissues, provoking different physiological effects as cellular expansion and growth delayed and/or reduces phloem translocation capacity. The latter effect is due to a phloem fluid reduction that can reduce photoassimilates translocation of source to sink organs.

The reduction of soil water and its consequences, depending of its severity, duration and phenological plant stage, is critical for anthesis and fructification periods for a great number of plants species, modifying number of pods and seeds and its filling dynamics. In this work *Lotus glaber* showed a response in accordance with other species.

However, critical water potential for cell expansion is variable between plants and different organs of them. *Lotus glaber* showed large differences in reproductive parameters, such as total number of pods and seeds, with relative low soil water potential variations as $-0,4$ Mpa. The reduction in number of sinks, observed in this work, is associated to aerial biomass growth, according to drought stress treatments imposed by salinity, where was observed 50 % aerial biomass reduction under 200 mM ClNa treatments (Barragán, pers.com). This aerial biomass reduction involves less number of branches and consequently a reduction on flowers and fruits production compared with soil field capacity treatments. These results are according to leaf expansion rate reduction observed for sunflower by -0.2 to -0.4 MPa soil water potential, meanwhile leaf expansion rate reduction was observed in order to -1.2 MPa water potential soil in soybean (Pessarakli, 1999).

The drought stress treatments did not modify number of seeds per pod or 1000 seeds weight. These characters are probably associated to *Lotus glaber* genomical basis and were not affected by environmental conditions.

On the other hand, low water availability in drought stress treatments could have enhanced the number of dormant seeds. The properties of the coat seed could explain the differences found in the rate and average speed of germination between treatments. These results agree with the results obtained by Clúa y Gimenez (2003) for *Lotus glaber* seeds.

The results showed drought stress incidence in *Lotus glaber* persistence in permanent pastures through reduction of seed production. This event can affects the natural reseeding, compromising future plant stands. Therefore, *Lotus glaber* breeding is very important for drought stress tolerance identification. It was determined for this character a great variability between individuals of natural populations and tolerant drought stress clones were identified that showed a high survival capacity in drought stress conditions imposed by high salinity levels (Barragán, pers com).

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