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Genetic variation in seed softening of the annual pasture *Medicago minima*

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RESUME – *"Variabilité génétique dans le ramollissement des graines dures de* Medicago minima*". Le modèle de ramollissement des graines dures a été étudié sur 12 populations de* Medicago minima*, originaires d'Australie de l'Ouest, du Bassin Méditérranéen et de l'Argentine. Des différences génétiques ont été trouvées entre les populations étudiées. Deux modèles intra-annuels identifiés dans les populations australiennes suggèrent qu'une différenciation écotypique s'est produite. La plupart des populations méditérranénnes ont révélé différents modèles de ramollissement. Ce ramollissement se fait avant celui des populations australiennes. Les résultats sont interprétés par rapport à l'étendue géographique de l'espèce.*

Mots-clés : *Variabilité génétique, dormance des graines,* Medicago minima.

Introduction

Medicago minima (L.) Bart. is one of the most widely distributed annual medics in mediterranean and semiarid temperate ecosystems. Since the survival of the species is wholly dependent on regeneration from seed, its ability to adapt to highly variable environments suggests that seed dormancy plays an important adaptive role.

In *M. minima*, as in other annual legumes, seed dormancy is imposed by hardseededness, which makes the seed coat impermeable to water. Hence, it is expected that the adaptation of the species to a particular environment will greatly depend on seed softening patterns (increase in seed coat permeability) developed in response to the local conditions. Therefore, the aim of this study was to investigate genetic diversity in softening patterns of *M. minima* populations adapted to a wide range of environmental conditions (125 to 1000 mm annual rainfall).

Origin of the data

The 12 populations of *M. minima* (Table 1) included in this study were obtained from different geographical origins taking annual rainfall as an indicator of environmental variability. The Mediterranean populations were obtained from the Australian Medicago Genetic Resources Centre at Adelaide, South Australia and the first author collected the Western Australian and Argentinean populations.

Ten plants per strain, each obtained from 10 different pods, were grown under uniform conditions at the Shenton Park Field Station of The University of Western Australia (31°56’55”S, 115°47‘34”E and 20 m elevation) in 1995. Pods were harvested and sorted to obtain 50 genetic replicates of 40 pods each per population. Each replicate was placed in a pocket of 10 x 10 cm made of a fine fabric mesh. All pockets were attached to the bare soil surface.

On each sampling date seeds of two replicates per population were manually recovered and tested for softening (germinated seeds + imbibed seeds / total seeds x 100). This process was repeated 21 times over two years. Regression and variance analyses were used to test hypotheses. The most relevant results are presented and discussed in the following sections.

Table 1. Origin, annual rainfall of the area of origin, days to flowering and seed size of populations of *Medicago minima* grown in Western Australia. Parenthesis indicate 1 s.e.m.

Population name	Origin	Annual rainfall (mm)	Flowering time (days)	Seed size[1] (mg)
Naturalized				
Broad Arrow	40 km N Kalgoorlie, WA	240	88 (0.48)	1.20 (0.009)
Kalgoorlie	14 km SE Coolgardie, WA	270	89 (0.38)	1.26 (0.008)
N Norseman	16 km N Norseman, WA	275	88 (2.03)	1.19 (0.006)
S Norseman	11 km S Norseman, WA	300	87 (0.48)	1.19 (0.008)
Salmon Gums	8 km N Salmon Gums, WA	325	88 (0.64)	1.22 (0.009)
Mullewa	15 km NW Mullewa, WA	330	92 (0.53)	1.22 (0.015)
Caldenal	20 km N Anzoategui, Argentina	344	114 (0.53)	1.36 (0.010)
Mediterranean				
Libya	21 km W Ajedabia	125	89 (1.24)	1.18 (0.009)
Tunisia (L)	Dj Bargou	500	112 (0.41)	1.52 (0.012)
Sardinia	5 km N Lotzorai	600	111 (0.34)	1.02 (0.012)
Syria	5 km SW Jisr ash-Shughur	700	116 (0.23)	1.69 (0.021)
Tunisia (H)	Sidi Mechrig beach terrace	1000	108 (0.37)	1.42 (0.014)

[1] Standard errors are for the mean seed weight of total seeds in 40 pods (n = 8).

Genetic variation in seed softening patterns

Our data show strong evidence of genetic variation in seed softening patterns between populations of *M. minima* (Fig. 1). The patterns of all Mediterranean populations were significantly different. These populations (except for the Libyan) softened significantly faster than the Western Australian. The populations from Libya and Argentina were similar to those from Western Australia, while the Syrian was least like the Western Australian strains. Both Tunisian populations also differed significantly in their softening patterns. The Sardinian population softened significantly faster than the rest. These results are consistent with the literature since most studies on hard seed content or seed softening patterns report genetic differences between populations (Ehrman and Cocks, 1996).

A significant ($P \leq 0.01$) relationship ($r = 0.7$) between flowering time and the percentage of soft seeds at the beginning of the winter was observed twice in this study. As a general rule late flowering strains are likely to have softer seeds because there is less time for the seed coat to develop fully. An example of this is found when comparing the Argentinean and Mediterranean populations (except Libya), which softened more rapidly in the autumn, with the Western Australian and the Libyan (Fig. 1, A-B, Table 1). The late-flowering populations of this study, which softened completely at the beginning of the autumn, are likely to have very little success in Western Australia. This is because they are unlikely to survive ‘false breaks’ that take place before or at the beginning of April. Clearly, the pattern of the naturalized Western Australian populations, which soften more slowly, is more appropriate for local conditions.

Ecotypic variation in flowering time is reported extensively in the literature, but there is very little evidence of ecotypic variation in seed softening. Two distinct softening patterns observed in the Western Australian populations suggest that ecotypic differentiation has occurred. One softening pattern was associated with the eastern region (all populations except Mullewa) and the other one with the northern region (Mullewa) (Table 1). The nature of these patterns relates to differences in the environmental features of each region. For example, the growing season in the east, on average, is shorter (10 compared with 16 weeks), drier (239 compared with 332 mm annual rainfall) and starts one week later than at Mullewa. Therefore, it is not surprising that the eastern populations have a longer lag phase and a shorter and steeper log phase than the Mullewa strain (Fig. 1 A). Considering Taylor’s 2-stage model of seed softening (Taylor, 1981), the longer lag phase of the eastern compared with the northern populations suggests that the requirement for appropriate alternating temperatures is met later in the autumn. This, it is suggested, is a response to the late beginning of the growing season.

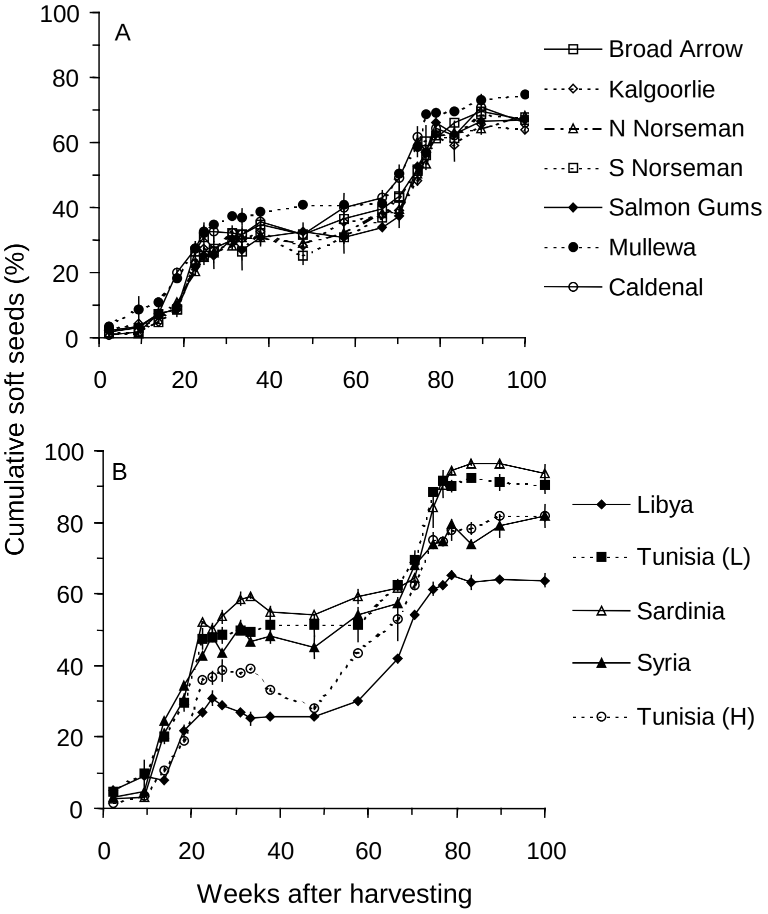

Fig. 1. Genetic variation in seed softening patterns of naturalized (A) and Mediterranean (B) populations of *Medicago minima*). Harvesting date: 26 –10- 1995. Vertical bars represent 1 s.e.m.

Relationship between seed softening patterns and rainfall

A significant ($P \leq 0.01$) relationship between the amount of seeds that softened and the annual rainfall at the various sites at which the strains were collected is shown in Fig. 2. Populations adapted to 400 to 800 mm softened more rapidly than did those from sites drier or wetter sites than that rainfall range.

Although the population from Tunisia (1000 mm) was relatively hardseeded, it is likely that other populations from high rainfall areas will be softer-seeded, as in fact was the case with the strain from Syria, while strains from low rainfall areas will invariably be hardseeded. Hardseededness, as time to flowering, will also evolve as result of uncertainty in the environment. It is likely that in low rainfall areas hardseededness protects the seed from erratic rainfall events during the summer and autumn until the growing season is established. Similarly, in some high rainfall areas the same strategy stops seeds from germinating in response to summer rainfall, so that the reproductive phase occurs concomitantly with higher temperatures in the spring and summer, which are necessary for ripening. Figure 2 shows that there seems to be an ecological limit imposed by the amount and, probably, the distribution of annual rainfall.

The most important outcomes of this study therefore are firstly that the variation found in seed softening patterns within populations of *M. minima* is likely to be of real ecological significance, and secondly, that within species variation is likely to be the result of ecotypic differentiation.

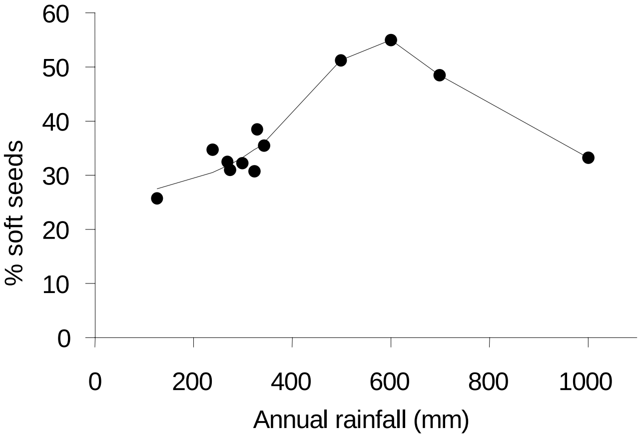

Fig. 2. Relationship between annual rainfall of the area of origin of the population of *Medicago minima* and the percentage of soft seeds at the end of the first autumn.

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