Cold tolerance of forage legumes growing in controlled continental Mediterranean conditions

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Cold tolerance of forage legumes growing in controlled continental Mediterranean conditions

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Summary - The growth of forage crops in Mediterranean areas is seriously limited by the dry summer but also by the cold winter. Potential forage species should be tested to improve the forage availability in these periods in which herbage production is limited. The objectives of this study were to compare seedling survival and viability in response to cold conditions and to identify ecophysiological mechanisms of cold response in annual legumes cultivated in Mediterranean zones. Experiments were carried out in several annual forage legumes (*Trifolium brachycalycinum, T. michelianum, Medicago polymorpha, M. truncatula and M. scutellata)* that were compared with some annual grain legumes *(Vicia ervilia, V. narbonensis, V. articulata* and *Pisum sativum*) and with some perennial forage legumes (*Medicago sativa, Hedysarum coronarium* and *Onobrychis viciaefolia*). A set of seeds was planted in pots containing a mixture of peat: sand (1:1, v/v) and grown in a growth chamber at 10/5°C, 70/80% RH (d/n), a photoperiod of 11h and a photosynthetic photon flux density (PPFD) of 400 µmol m^{-2} s^{-1} during 20 days. Another set of seeds was planted in the same conditions during 40 days. Visual foliar damages, low temperature survival, leaf water status (RWC and leaf water potential), dry matter production, relative growth rate, content of substances implicated in cold tolerance (starch, total soluble sugar, soluble proteins and proline) and leaf pigments were measured. Results will be discussed in relation to differences in cold tolerance.

Key-words: Cold tolerance, forage legumes, growth, low temperature survival, solute content

Résumé - *La croissance des plantes fourragères cultivées dans la région méditerranéenne est profondement limitée durant les chaleurs de l'été ainsi que pendant le froid d'hiver. L'utilisation des espèces fourragères pendant ces périodes requiert, entre autres, une bonne connaissance de leur sensibilité au froid. Dans cette étude nous avons cherché à comparer la survie et la viabilité des plantes au froid, ainsi que identifier les mécanismes écophysiologiques de plusieurs espèces de plantes légumineuses cultivées dans le pourtour méditerranéen. On a comparé des espèces fourragères annuelles (*Trifolium brachycalycinum, T. michelianum, Medicago polymorpha, M. truncatula *et* M. scutellata*), , avec des espèces de graines (*Vicia ervilia, V. narbonensis, V. articulata *et* Pisum sativum*) et avec des espèces fourragères vivaces (*Medicago sativa, Hedysarum coronarium *et* Onobrychis viciaefolia*). Les plantes sont placées durant 20 et 40 jours en chambres de croissance dont les conditions sont contrôlées: température 10°C le jour, 5°C la nuit; humidité relative 70% le jour, 80% la nuit, photopériode 11h et l'intensité lumineuse (PAR) de 400 µmol m^{-2} s^{-1}. Suite à ces expositions au froid, on a determiné différents paramétres: les pourcentages de germination, les lésions visuelles des feuilles, les paramétres hydriques des feuilles, la production de matière sèche, le taux de croissance relatif, la teneur en substances qui interviennent dans la résistance au froid (amidon, sucres solubles totaux, protéines solubles et proline) et la composition pigmentaire des feuilles. Les auteurs discutent la signification des résultats mis en rapport avec des différences en leur tolérance au froid.*

***Mots-clés**: tolérance au froid, plantes fourágéres légumineuses, croissance, survie au basses températures, la teneur en solutes*

Introduction

The growth of forage crops in Mediterranean areas is seriously limited by the dry summer but also by the cold winter. Potential forage species should be tested to improve the forage availability in these periods in which herbage production is limited. Therefore, this work

aimed to compare seedling survival and viability in response to cold conditions and to identify ecophysiological mechanisms of cold response in annual legumes cultivated in Mediterranean zones.

Materials and methods

Experiments were carried out using 8 annual forage legume cultivars: *Trifolium brachycalycinum* Clare (Cl.), *T. brachycalycinum* Rosedale (Ros.), *T. michelianum* Giorgia (Gio.), *Medicago polymorpha* Santiago (Sant.), *M. polymorpha* Anglona (Angl.), *M. truncatula* Paraggio (M.t.), *M. rugosa* Paraponto (M.r.), *M. scutellata* Kelson (M.s.); 3 perennial forage legume cultivars: *Hedysarum coronarium* Grimaldi (H.c.), *Onobrychis viciaefolia* (O.v.) and *M. sativa* Aragon (Arag.) and 4 grain legumes cultivars: *Vicia ervilia* (V.e.), *V. narbonensis* (V.n.), *V. articulata* (V.a.) and *Pisum sativum* Esla (Esla). Seeds were planted in 14 x 9 cm pots containing a mixture of peat: sand (1:1, v/v) (2 plants/pot). Plants were grown in a controlled growth chamber with a day/night regime of 10/5°C and 70/80% RH. A photosynthetic photon flux density of 400 µmol m^{-2} s^{-1} was provided for an 11h photoperiod. A set of plants grew in these conditions during 20 days and a second set of plants was maintained during 40 days.

The degree of injury was visually assessed using a scale of 0 to 3 (0, dead; 1, some development but dying; 2 some damage but surviving; 3, no damage) (Sandli *et al.*, 1993) and the percentage of plant survival was calculated. The leaf relative water content (RWC) was calculated as described by Wheatherley (1970). Free proline was estimated by the ninhydrin reaction (Paquin and Lechasseur, 1979), total soluble sugars (TSS) with the anthrone reagent (Yemm and Willis, 1954), soluble proteins using protein dye-binding (Bradford, 1976) and starch content with the iodine reagent (Jarvis and Walker, 1993). Leaf pigments (chlorophylls and total carotenoids) were determined according to Lichtenthaler (1987) in ethanol (95% v/v) extracts of fresh leaves. Finally, plants were separated into leaves, stems and roots and dry matter (DM) production was determined after drying at 70°C for two days. Relative growth rates (RGR) were also calculated.

Means ± S.E. (n=8) were calculated, and when F ratio was significant, least significant differences (LSD) were evaluated by the Student's t-test.

Results and discussion

Giorgia, Santiago, Paraponto, *O. viciaefolia* and Aragon had lower percentage of plant survival in both moments of harvest whereas Anglona did not survive until 40 days (Fig. 1). Anglona and Paraponto presented a negligible growth and for this reason both species were discarded (Fig. 2).

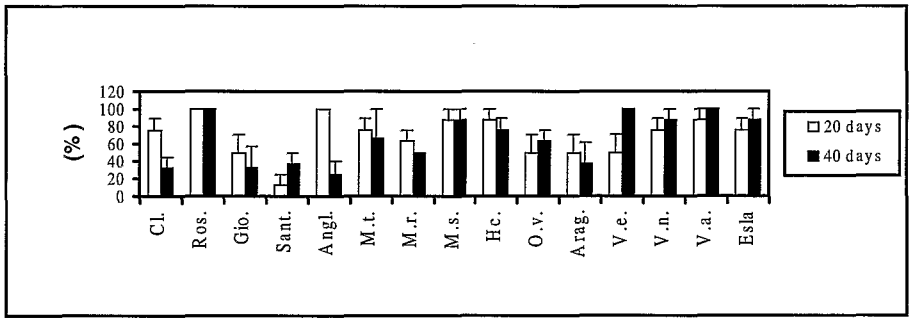

Fig. 1. Survival percentage.

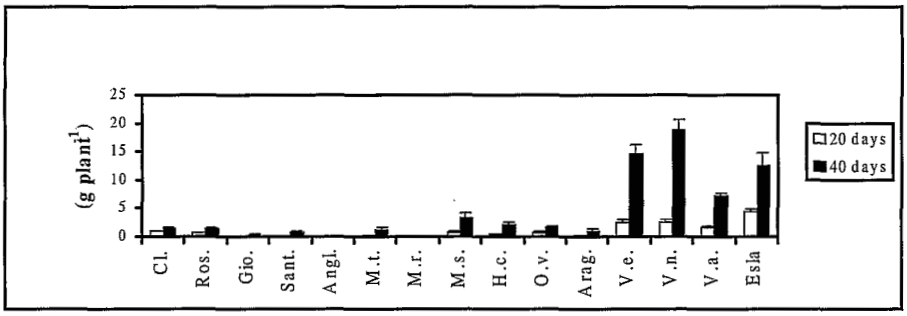

Fig. 2. Total dry matter.

V. articulata was the grain legume which showed the lowest DM production. The survival and mostly DM production in the grain species was in general better than in forage legumes group. In the first harvest (20 days), *V. ervilia* had a low survival percentage rate but in the second harvest (40 days) it had the same rate than the other grain species. For the next measured parameters, the values which are shown are those of the second harvest, because they represent the final status of plants. The RGR of Giorgia and Santiago was significantly higher compared to the rest of forage legumes (Fig. 4). Similarly, in a field study the DM accumulation of Santiago was higher than other annual medics (*M. rugosa*, *M. scutellata* and *M. truncatula*) (Zhu *et al.*, 1998). The lowest were Clare, *O. viciaefolia* and Aragon. Among the grain species, Esla was the one which presented the lower RGR.

Among the annual forage species, Clare, Rosedale and Santiago showed significantly higher RWC (Fig. 3). This could be due to the fact that the roots of the first group were more developed and efficient than those of the second one. *V. articulata* had the highest RWC and into the perennial ones, *O. viciaefolia* had a better water status than Grimaldi and Aragon, that were quite dehydrated.

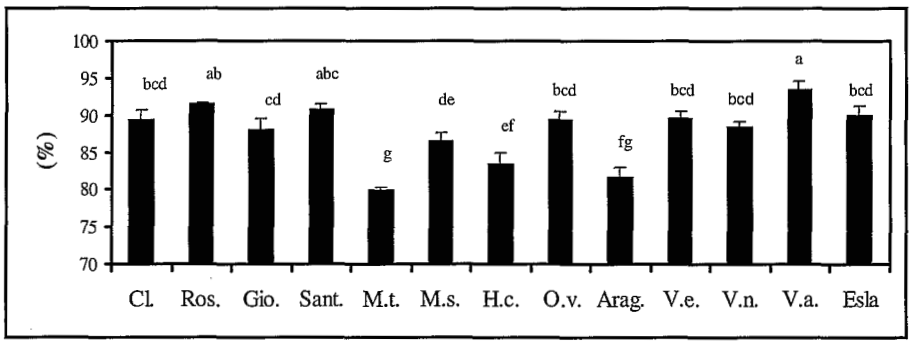

Fig. 3. Relative water content (RWC).

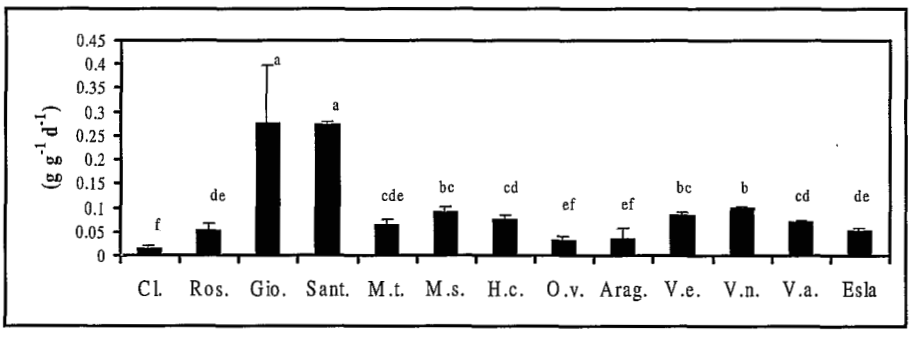

Fig. 4. Relative growth rate.

The higher TSS content in the annual forage group had been noted in stems (Fig. 5). In the grain species the TSS concentration was similarly distributed into the different organs. The perennial forage legumes accumulated more TSS in leaves.

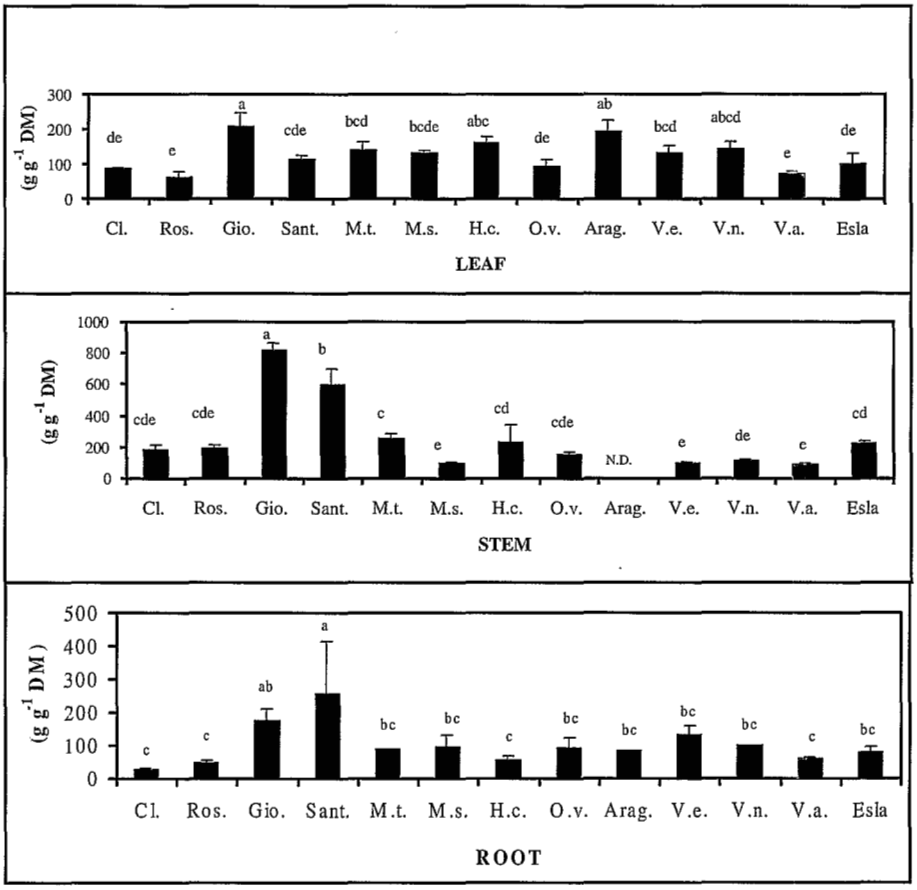

Fig. 5. Total soluble sugar.

A significant correlation between RGR and TSS concentration in stems and roots (R^2=0.771, P<0.001 and R^2=0.794, P<0.001 respectively) has been found. This may be related with a higher cold tolerance as reported by Svenning *et al.* (1997) and Frankow-Lindberg and Von Fricks (1998) in contrasting white clover cultivars. The RWC had also been negatively correlated with the leaf TSS concentration (R^2=0.470, P<0.01). It could be due to the influence of the soluble solutes concentration in plant water status.

There were few differences in protein and proline contents between species (data not shown). Starch content of Giorgia was high in all the organs (Fig. 6); the other species accumulated more starch in stems with the exception of Aragon that had a significant starch accumulation in leaves. Starch is the main form of carbohydrate accumulated in reserve tissues of perennial forage legumes. Field data have demonstrated a clear pattern of seasonal changes in the concentration of starch in stolons of white clover, levels increasing in summer and early fall and decreasing during winter (Bouchart *et al.*, 1998). Proline is also known to confer cryoprotective effects in some species including white clover (Sandli *et al.*, 1993). In agreement with our data, it was also reported that changes in proline levels were not correlated with cold response as strongly as those of TSS (Svenning *et al.*, 1997).

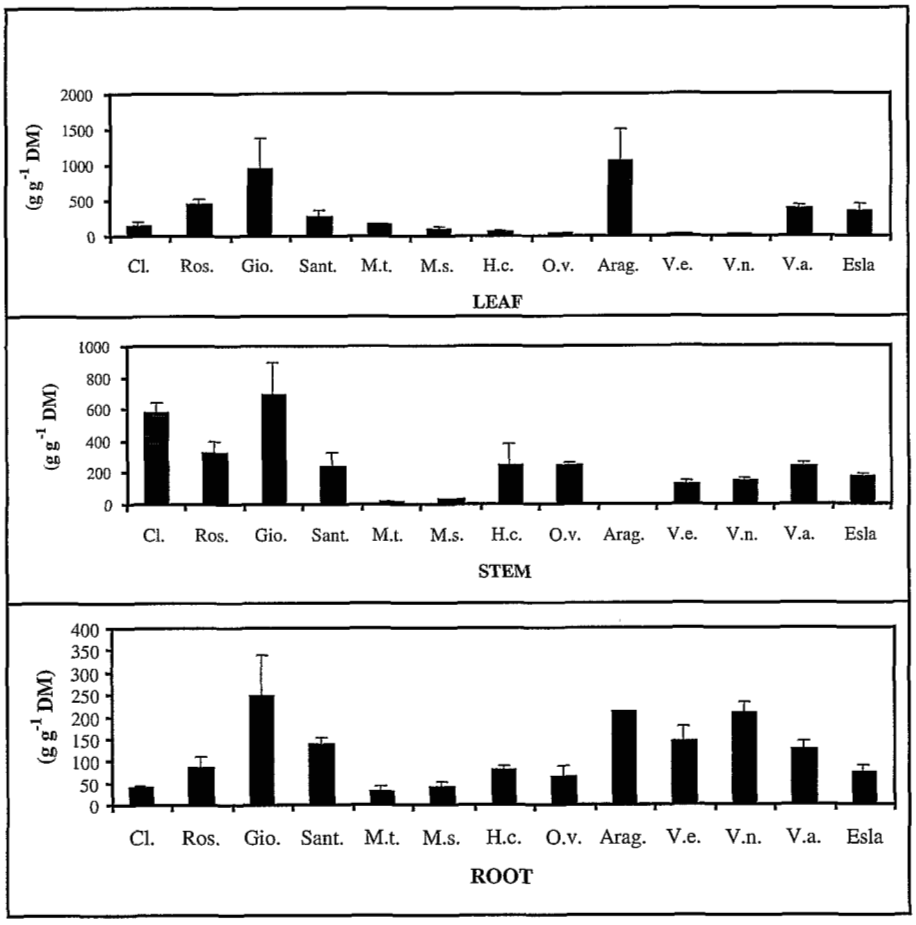

Fig. 6. Starch.

The pigment composition was different in the three groups of plants (Table 1). There were not significant differences in total chlorophyls, but perennial group presented proportionally higher level of chlorophyl b (lower a/b ratio); the grain legumes had significantly greater content of carotens (higher carot/chls ratio).

Table 1. Pigment composition.

Species	Chls (mg g $^{-1}$ DM)	Carot. (mg g $^{-1}$ DM)	a/b	Carot/Chls
Annual forage legumes	6.13±0.23 a	0.61±0.06 b	2.32±0.02 a	0.10±0.01 b
Perennial forage legumes	9.25±1.18 a	2.09±0.27 a	2.67±0.14 a	0.22±0.01 a
Grain legumes	10.20±4.63 a	0.82±0.24 b	1.57±0.36 b	0.11±0.01 b

Conclusions

Cold response in annual forage legumes would appear to be associated with higher content of TSS and starch in stems and roots. Results also showed a strong relationship between TSS reserves and the relative growth rate.

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