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Cold response of annual Mediterranean pasture legumes

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SUMMARY – In southern Europe, the trend towards low input agriculture gives new incentives to sow forages, legumes in particular, with the aim of improving natural pastures, increase the quality of animal feeding and improve soil fertility. However, growth of forage crops in these areas is seriously limited by the ability of each species to grow during cold winters. Therefore, the objective of this work was to evaluate several annual medics cultivated in Mediterranean zones for cold response. Annual species tested were *Medicago polymorpha* L.*, M. truncatula* Gaertn*, M. rugosa* Desr. and *M. scutellata* (L.) Mill. Twenty day old plants were subjected to two temperature regimes: 20/15ºC or 10/5ºC (d/n) for 40 d. Leaf area, dry matter production, growth parameters and solute content (proline, starch and total soluble sugars) were determined. Results will be discussed in relation to differential response of annual medics to cold conditions.

Key words: Annual medics, cold tolerance, growth, solute content.

RESUME – *"Réponse au froid de légumineuses de prairie annuelles". L'utilisation de légumineuses annuelles, en particulier les médics, pour l'amélioration des prairies, le contrôle de l'érosion ou la restauration des sols prend actuellement de l'importance dans le sud d'Europe. Cependant, un niveau suffisant de tolérance au froid pourrait être nécessaire pour étendre la surface d'utilisation des médics dans des milieux à températures hivernales basses. Dans cette étude nous avons étudié le comportement au froid de plusieurs espèces de légumineuses annuelles cultivées dans le pourtour méditerranéen. Les espèces employées ont été* Medicago polymorpha *L.,* M. truncatula *Gaertn,* M. rugosa *Desr. et* M. scutellata *(L.) Mill. Des plantes âgées de 20 jours ont été exposées aux températures 20/15ºC ou 10/5ºC (j/n) pendant 40 jours. Suite à ces expositions à différentes températures, on a déterminé la production de matière sèche, les paramètres de croissance et la teneur en solutés (proline, amidon et sucres solubles totaux). Les auteurs discutent la signification des résultats par rapport aux différences de réponse au froid de ces espèces.*

Mots-clés : *Légumineuses annuelles, tolérance au froid, croissance, teneur en solutés.*

Introduction

The legume-based pasture systems provide a source of forage for livestock and confers benefits such as symbiotically fixed nitrogen, which becomes available to subsequent crops. Annual pasture legumes are an important component of the extensive systems used to improve low quality native pastures and to seed new pastures. Subterranean clover and annual medics are the main species utilized particularly due to their self-reseeding ability and grazing tolerance.

In the Mediterranean region, annual legumes tend to grow and reproduce during the cool and wet season because growth is seriously limited by the dry summer months. The adaptation and long-term persistence of annual legumes depends largely on the capacity of plants to complete their reproductive cycle, in order to ensure seed production and on the survival of seeds in the soil over several seasons (Lloyd *et al.,* 1997). In this sense, some aspects of ecophysiology of annual legumes have been recently studied. For example, flowering responses of annual legumes showed great variability and a high degree of plasticity in flowering time (Ehrman and Cocks, 1996; Del Pozo *et al.,* 2000), similar variability was also found in seed germination (Norman *et al.,* 1998) and seed dormancy components (Porqueddu *et al.,* 1996). However, since most vegetative growth of annual legumes occurs under cold winter conditions more detailed studies of specific responses to these conditions are required. Therefore, the objective of this research was to evaluate several annual medics cultivated in Mediterranean zones for cold response and to identify their ecophysiological mechanisms for cold tolerance.

Material and methods

Experiments were carried out using five annual medic cultivars: *Medicago polymorpha* L. cv. Santiago, *M. polymorpha* L. cv. Anglona, *M. truncatula* Gaertn. cv. Paraggio, *M. rugosa* Desr. cv. Paraponto and *M. scutellata* (L.) Mill. cv. Kelson. Seedlings were grown in 14 9 cm pots containing a mixture of peat: sand (1:1, v/v) (4 plants/ pot) under controlled environmental conditions with a temperature regime of 20/15ºC (day/night), photosynthetic photon flux density (PPFD) of 400 mol/m^2/s, 70/80% RH (day/night) and 11 h photoperiod. After 20 days, half of the plants were shifted to growth conditions of 10/5ºC with all other conditions kept constant. Control plants remained at the 20/15ºC growth regime. Measurements were made after 40 days of temperature treatment. Solute concentrations (free proline, total soluble sugars and starch) were estimated as described previously (Sánchez-Díaz *et al.*, 2000). Leaf area was measured by using an automatic leaf area meter and specific leaf area (SLA) was calculated as the leaf DM per unit leaf area. Finally, plant dry matter (DM) production was determined after drying at 70ºC for two days. Means ± SE (n = 16) were calculated, and when the F ratio was significant, least significant differences were evaluated by the Tukey's *t*-test.

Results and discussion

DM production decreased in cold-treated plants of Paraponto, and especially in Anglona and Kelson (Table 1). The root/shoot ratio changed in cold-treated plants of all the species, except for Anglona. *Medicago polymorpha* cv. Anglona is a new cultivar recently selected in Italy (Porqueddu *et al.*, 1998).

Table 1. Dry matter production and growth parameters. Means followed by the same letter are not significantly different (P > 0.05)

Cultivar	Temperature (ºC)	Total DM (g)	Root/shoot (g/g)	Leaf area (cm^2)	SLA (dm^2/g)	LAR (dm^2/g)
Santiago	20	0.49 d	0.30 d	121.5 c	3.37 a	2.35 ab
	10	0.73 d	0.76 b	98.4 c	2.54 ab	1.35 c
Anglona	20	1.73 b	0.49 c	251.3 b	2.67 b	1.44 c
	10	0.49 d	0.50 c	148.2 bc	3.06 ab	2.58 a
Paraggio	20	0.81 cd	0.31 d	143.2 bc	3.15 ab	1.77 bc
	10	0.55 d	0.52 c	110.9 c	2.30 b	2.12 b
Paraponto	20	0.93 c	0.42 cd	236.3 bc	2.36 c	2.58 a
	10	0.48 d	0.20 e	123.4 c	1.80 c	2.73 a
Kelson	20	2.17 a	0.94 a	372.6 a	2.59 bc	1.36 c
	10	0.61 d	0.51 c	102.5 c	1.74 c	1.67 c

When compared the two more affected species (Anglona and Kelson) it was shown that leaf area only decreased in Kelson whereas Anglona increased LAR because it maintained similar leaf area to controls. These results agree with those others that have shown that *M. scutellata* cv. Kelson had quite poor frost resistance (Brandsæter *et al.,* 2000). Cold-treated plants of Anglona also showed higher SLA than Paraponto and Kelson. These changes in SLA and LAR might suggest that Anglona could have some capacity for acclimation to cold temperatures.

There were not differences in leaf total soluble sugars (TSS) in cold-treated plants, but in stems, TSS content generally decreased (Fig. 1). In roots, Anglona and Paraponto increased TSS while Kelson did not change. Leaf proline concentration only increased in the two cultivars of *M. polymorpha,* Santiago and Anglona (Fig. 2). In stems, proline increased in the two cultivars with higher cold tolerance (Santiago and Paraggio), and also in Anglona. This latter cultivar and Paraponto accumulated proline in roots. When the two cultivars more affected by cold conditions (Anglona and Kelson) were compared, Anglona accumulated proline in all vegetative organs, while Kelson was not able to do so.

Leaf starch content only decreased in the two cultivars of *M. polymorpha* (Santiago and Anglona) (Fig. 3). By contrast, Anglona accumulated starch in stems, and especially in roots. Similarly, increased starch content in roots has been related with high capacity for winter acclimation in biennial and perennial legumes (Li *et al.*, 1996).

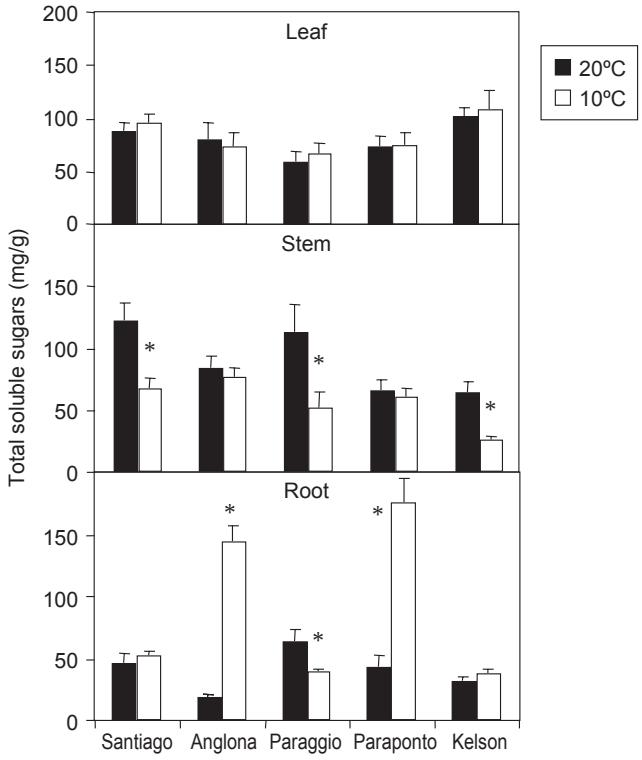

Fig. 1. Total soluble sugar concentration.

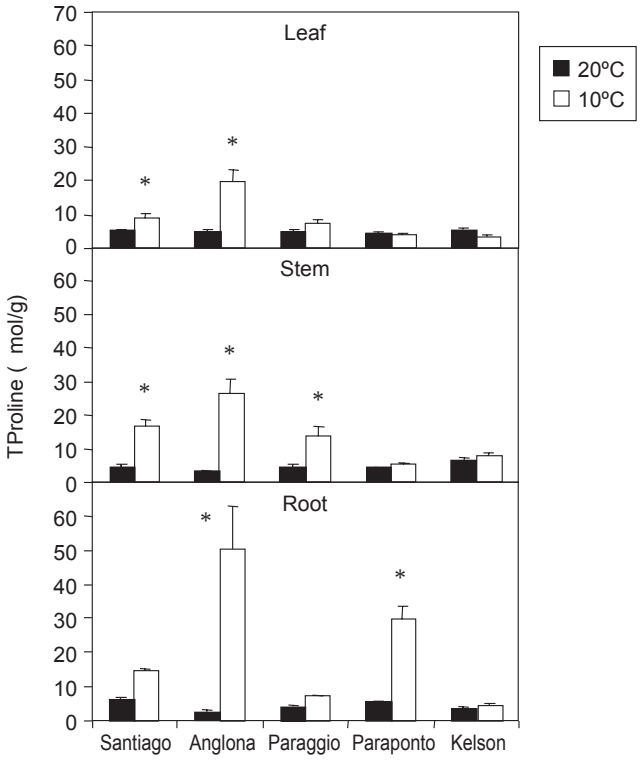

Fig. 2. Proline concentration.

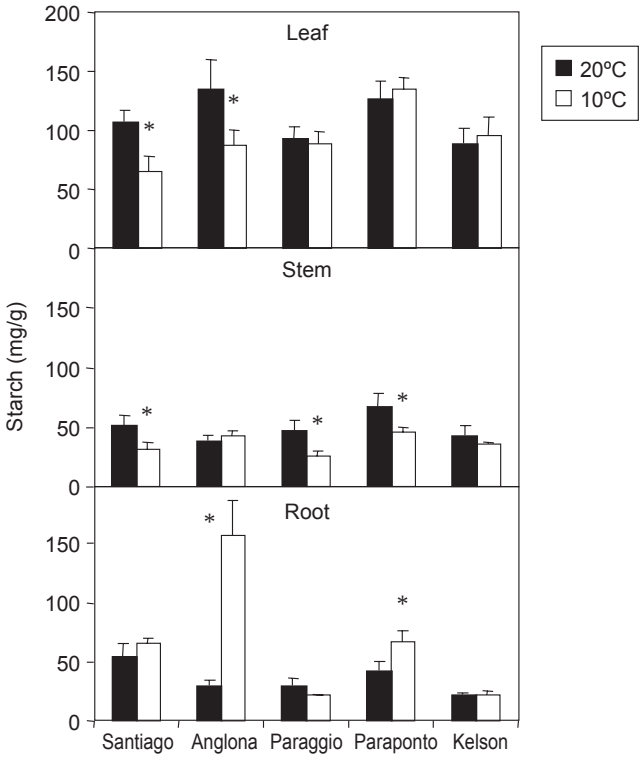

Fig. 3. Starch concentration.

Conclusions

Santiago and Paraggio seemed to be the most tolerant cultivars to cold. Although DM production of Anglona and Kelson was much affected, the high LAR, SLA and solute accumulation in roots showed by Anglona under low temperature, may suggest a high plasticity of this cultivar which will improve acclimation to cold.

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