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Leaf growth and osmotic adjustment of subterranean clover under drought

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Summary - The drought effect on osmotic adjustment and leaf expansion was analysed in four subterranean clover cultivars. Both parameters showed wide genetic variability. The two genotypes with highest osmotic adjustment in expanding leaves also showed the lowest reduction in leaf expansion rate.

Key-words: osmotic adjustment, leaf expansion, *Trifolium subterraneum* L., genetic variability

Résumé - *L'effet de la sécheresse sur l'ajustement osmotique et l'expansion foliaire a été analysé chez quatre variétés de trèfle souterrain. Ces deux paramètres montrent une grande variabilité génétique. Le deux génotypes avec la plus grande ajustement osmotique montrent la plus faible réduction de la vitesse d'expansion foliaire.*

Mots-clés: *l'ajustement osmotique, l'expansion foliaire,* Trifolium subterraneum L.*, variabilité génétique*

Introduction

Leaf expansion reduction is one of the first plant responses to water stress (Hsiao *et al.*, 1976). Few days after withholding watering leaf expansion stops almost completely in many species. Under such conditions it is widely accepted the involvement of Abscisic Acid (ABA) in leaf expansion control (McDonald and Davies, 1996). However, it is also generally accepted that cell turgor plays an important role in leaf expansion (Morgan, 1984). The lowering of osmotic potential ($\Psi\pi$) by net solute accumulation, (osmotic adjustment, $\Delta\Psi\pi$), is considered to be a trait that can contribute to maintain turgor under drought thus helping to maintain leaf expansion at low Ψ. The $\Delta\Psi\pi$ in response to drought has been observed in several species and also in *Trifolium subterraneum* (Socías and Medrano, 1994). Provided the existence of $\Delta\Psi\pi$ in response to drought in subterranean clover, the objectives of this work were to evaluate the variability of $\Delta\Psi\pi$ and leaf expansion in different genotypes of this species, as well as to determine the relationship between the presence of $\Delta\Psi\pi$ and the maintenance of leaf expansion under drought.

Material and Methods

The experiment was conducted under field conditions in March 1997 at the University of Balearic Islands, Palma de Mallorca, Spain. Plants of three genotypes of *Trifolium subterraneum* L. ssp. *brachycalycinum* Katznelson et Morley (Clare, a commercial variety; 1022 and Funtanabona, accessions from Greece and Italy respectively), and Aubarca, a genotype of *Trifolium subterraneum* L. ssp. *subterraneum* Katznelson et Morley (collected in Mallorca) were grown in 6 plots of 0.75 m^2 each with 12 plants per plot. The clay-calcareous soil was fertilised with 15g N m^{-2}, 6.1g P m^{-2} and 5.8g K m^{-2} before sowing. A plastic shelter to prevent occasional rains was used. Control treatment was maintained near field capacity by drip irrigation every 3 days. Drought was induced to half of the plots when plants were well developed by withholding watering.

Soil water content (SWC), leaf water potential (Ψ) and leaf osmotic potential (Ψπ) were measured weekly, 0, 7, 14, 21 and 28 days after withholding watering. SWC was determined at midday once a week with a Time-Domain-Reflectometry equipment (Trime® System). Ψ was measured at predawn and midday with a Schölander pressure chamber (Soil Moisture Equipment Corp. CA). Ψπ was determined in folded and unfolded leaves collected at predawn and immediately frozen in liquid nitrogen (dew was previously removed). Ψπ was measured with a vapour pressure osmometer (Wescor 505). Possible dilution by apoplastic water was not considered. Osmotic adjustment was calculated as the difference between Ψπ of irrigated and stressed plants. Leaf expansion rate (LER) was estimated weekly from consecutive measurements of midvein length in expanding leaves, the measurements were done in the linear growth phase (maximum growth rate). LER reduction (ΔLER) was calculated as the difference between irrigation and drought values.

Results and Discussion

Environmental conditions corresponded to that typical for a Mediterranean spring. In irrigated plots, SWC was around 20%, near field capacity (25%), all along the experiment. In drought plots SWC started to fall after 7 days without watering to values down to 16%, being gradually reduced to wilting point (8%) at the end of the experiment. Under irrigation all the genotypes kept their Ψ_{pd} between –0.04 Mpa and –0.08 Mpa until the end of the experiment. Under water stress, Clare, 1022 and Aubarca, decreased slowly to -0.17 MPa and –0.21 Mpa at the end of the experiment while Funtanabona did not experiment any change (Table 1). For Ψ_{md} treatment effects were more evident with a sharp decline to –1.95 in Funtanabona and –1.93 in Aubarca (Table 1).

Table 1. Predawn Water Potential (Ψ_{pd}), Midday Water Potential (Ψ_{md}), Osmotic adjustment (ΔΨπ) and leaf expansion reduction (ΔLER). Mean ± s.e. values of the last week of the treatment.

Genotype	Treatment	Ψ_{pd}(Mpa)	Ψ_{md}(Mpa)	ΔΨπ Leaf 0 (Mpa)	ΔΨπ Leaf 3 (Mpa)	ΔLER (mmd^{-1})
Clare	I	-0,08±0,008	-0,18±0,023	0,23	0.35	0,90
	D	-0,17±0,008	-1,67±0,058			
1022	I	-0,04±0,008	-0,13±0,026	0,62	0,26	0,60
	D	-0,17±0,043	-1,34±0,348			
Aubarca	I	-0,06±0,007	-0,16±0,023	0,62	0,6	0,67
	D	-0,21±0,051	-1,93±0,082			
Funtana bona	I	-0,08±0,007	-0,15±0,062	0,45	0,35	1,05
	D	-0,07±0,011	-1,95±0,080			

For unfolded, leaf (leaf 3), Ψπ showed some decrease in irrigated plants only in genotype Clare. In water stressed plants Ψπ underwent some decrease after 7 days without watering (a 4% decrease in SWC) in Clare and Aubarca, but around day 14th (a 6% decrease in SWC) in 1022 and Funtanabona. All genotypes showed values around –1.50 MPa at the end of the experiment (data not shown). Osmotic adjustment (ΔΨπ), in mature leaves, was present in all tested genotypes, showing an important genetic variation in its extent, from 0.26 MPa for 1022 to 0.60 for Aubarca (table 1). For folded leaves (leaf 0), under irrigation, Ψπ was constant along the experiment but for water stressed plants started to fall down after 14 days

Figure 1: Predawn leaf Osmotic Potential (Ψπ) in folded leaves in irrigated (closed symbols) and water stressed (open symbols) plants

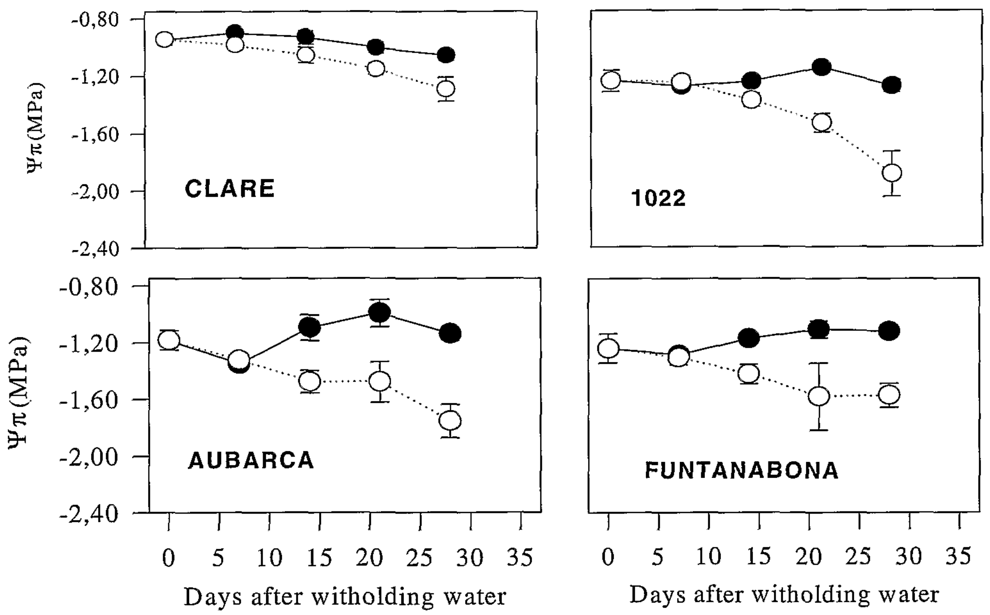
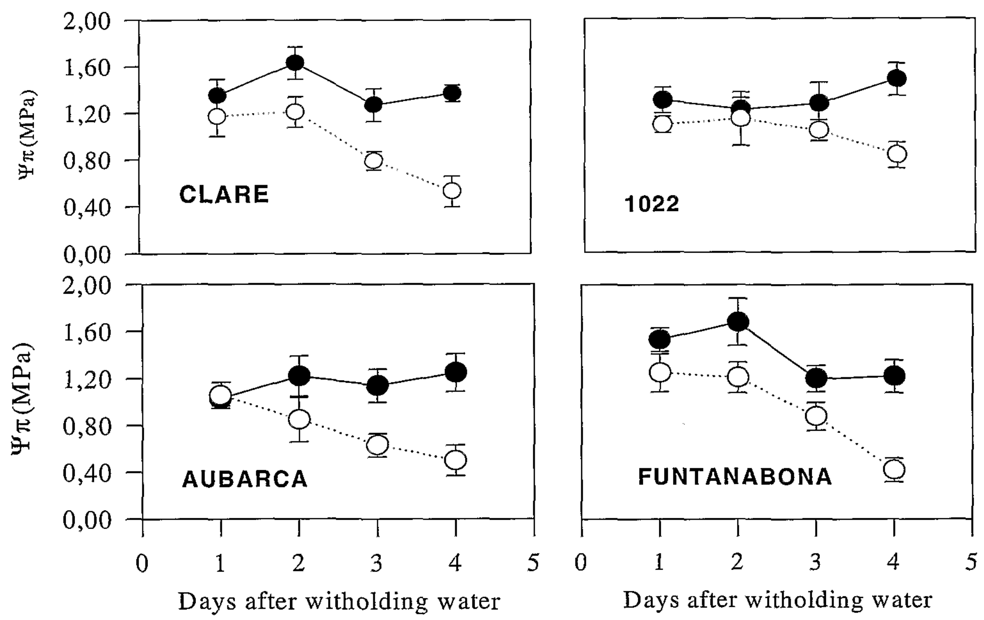


Figure 2: Leaf expansion rate (LER) in irrigated (closed symbols) and water stressed (open symbols) plants

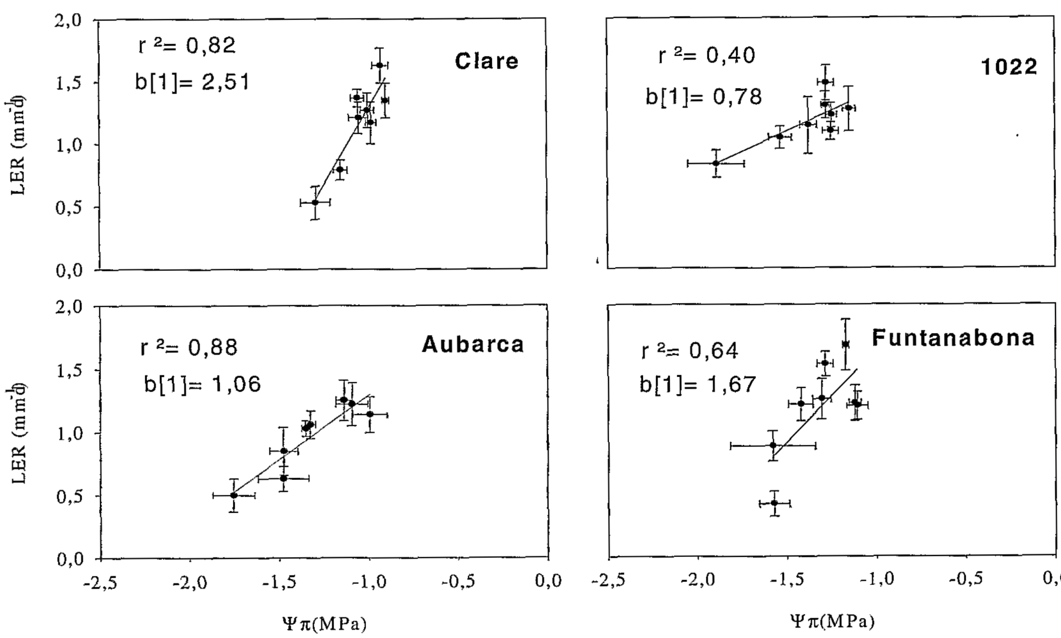


without watering. At the end of the experiment, 1022 presented the lowest Ψπ (-1.89 MPa), and Clare the highest one (-1.29 MPa) (figure 1). As in leaf 3, all tested genotypes showed some reduction of Ψπ in leaf 0 but in a very different extent. 1022 and Aubarca were the genotypes with the highest ΔΨπ, 0.62 MPa, while Clare presented the lowest ΔΨπ, 0.23 MPa (table 1). 1022, Aubarca and Funtanabona showed a similar or higher ΔΨπ in leaf 0 than in leaf 3, while Clare presented the opposite pattern.

The leaf expansion rate was almost constant along the experiment for irrigated plants. Clare, 1022 and Funtanabona presented similar LER, around 1.4 mm d^{-1}, while Aubarca remained around 1.2 mm d^{-1}(Figure 2). All tested genotypes experimented a reduction in LER under water stress, which was early showed by Aubarca under only a 4% reduction in SWC. Clare and Funtanabona maintained their LER until a 6% reduction in SWC and 1022 did not reduce its LER until the last week of treatment. At the end of the experiment the lowest LER was recorded for Funtanabona (0.42 mm d^{-1}). Clare and Aubarca had similar values, around 0.50 mm d^{-1}, and 1022 presented the highest value, 0.82 mm d^{-1}. The highest reduction in LER was present in Funtanabona and Clare while 1022 and Aubarca showed the lowest (Table 1).

LER values were linearly dependent from osmotic potential in leaf 0 because regression coefficients between these two parameters were among 0,40 and 0,88 (fig 3). These results show that the highest leaf elongation rates are achieved for the higher osmotic potential values which corresponds with lower water stress. At the end of experiment, the LER reductions in response to drought were higher for the genotypes with lower osmotic adjustment in leaf 0 and this correspondence was not followed for leaf 3 values (Table 1). These results support the importance of osmotic adjustment in expanding leaves to maintain growth under drought, as well as an important genetic variability for these characteristics which could be exploited to achieve more stable yield in semi-arid pasture crops.

Figure 3: Relationship between Ψπ (Leaf 0) and LER.



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