Physiological Response of 14 *Lotus tenuis* Genotypes Subjected to Drought Conditions

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Introduction

Lotus tenuis L. is a perennial forage species of European origin. It is found in Chile in a wide range of environments between the Valparaíso (32°S) and Bíobío Regions (38°S), with a strong presence in areas with clay and volcanic soils of medium texture with problems of moisture retention. The Instituto de Investigaciones Agropecuarias (INIA) perennial forage legume breeding program has focused on developing drought-tolerant cultivars of the species. A population of 100 individuals was selected in previous studies, and all individuals were agronomically and physiologically characterized under field and greenhouse conditions. Results led to the selection of a sub-population of 14 individuals composed of drought-sensitive (7) and drought-tolerant (7) genotypes. The objective of this study was to interpret drought tolerance/sensitivity of the sub-population by analyzing plant physiological response under field drought conditions.

Methods

One hundred *L. tenuis* genotypes were multiplied vegetatively in a greenhouse to characterize their agronomic and physiological behavior under field conditions. Two experiments were established at the INIA Santa Rosa Experimental Station in Chillán, Chile (36º03'S, 72º07'W) and were maintained under contrasting water regimes (irrigation and dryland). Each experiment used an alpha lattice design with 5 replicates.

Plants were established in a soil derived from volcanic ash of silt loam texture (medial, thermic, Humic Haploxerands, Andisol) with a shallow profile (30-40 cm) and low fertility levels. The 100 genotypes were planted with 90-cm row spacing on 3 January 2011. Each row had 10 individuals with a 70-cm row length. The assay under irrigated conditions was irrigated daily for 1.5 h with micro-sprinklers and an application rate of 4.4 mm/h.

Soil moisture was monitored with capacitance sensors (5TE, Decagon) in both experiments (irrigation and dryland) and during the whole experimental period. Plant water status was evaluated by determining xylem (Ψx), osmotic ($\Psi\pi$), and pressure (Ψp) potential, relative water content (RWC), normalized difference vegetation index (NDVI), isotope discrimination of ^{13}C (Δ^{13}C), and leaf temperature. The latter was used to calculate the crop water stress index (CWSI) according to Inostroza and Acuña (2010); growth analysis was performed by estimating specific leaf area (SLA), stem diameter, relative stem elongation rate (RSER), leaf/stem relationship, and shoot dry matter (DM) production. Data were analyzed through analysis of variance by the MIXED procedure in SAS.

Results

The combined analysis of variance of both environments (irrigation and dryland) showed a significant effect of the genotypes (G) and water environments (E) on all evaluated physiological traits. The G×E interaction was only significant in NDVI, RSER, and CWSI ($P < 0.05$). Water status under drought conditions was better for the group of 'tolerant' genotypes than the 'sensitive' genotypes, and this was reflected in Ψx values that were 17% higher (Table 1). Calculated with plant temperature, CWSI estimates stomatal conductance and thus water status. In this respect, CWSI values confirm the better water status of the 'tolerant' genotypes because their CWSI was 7% lower than the 'sensitive' genotypes (Table 1).

Plant drought tolerance is associated with physiological mechanisms that maintain an adequate water status under drought conditions and/or maintain metabolic functions under normal conditions with a depressed water status. Based on this definition, we infer that 'tolerant' genotypes exhibit both strategies, that is, water status and metabolic functions associated with the photosynthetic capacity are higher than those of 'sensitive' genotypes. The latter becomes clear when observing NDVI and SLA values, two physiological traits associated with plant photosynthetic capacity (Inostroza and Acuña 2010). 'Tolerant' genotypes under drought conditions showed NDVI and SLA values 5% and 11% higher than 'sensitive' genotypes. The consequence of these physiological adjustments was greater growth (RSER, Table 1) and DM production by approximately 22% and 70%, respectively, in the 'tolerant' genotypes.

Carbon isotope discrimination (Δ^{13}C) has been proposed as a trait highly associated with water use efficiency of crops and their productivity under water deficit conditions (Condon *et al.* 2004). However, these relationships are not observed in perennial forage

Table 1. Specific leaf area (SLA, cm^2/g), xylem water potential (Ψx, MPa), normalized difference vegetation index (NDVI), relative stem elongation rate (RSER, cm/cm/d), crop water stress index (CWSI) and isotope discrimination of ^{13}C (Δ^{13}C) evaluated in 14 *Lotus tenuis* genotypes in two water environments (irrigation (Irr) and dryland (Dry)).

Sensitive	SLA		Ψx		NDVI		RSER		WSI		Δ^{13}C	
	Irr	Dry	Irr	Dry	Irr	Dry	Irr	Dry	Irr	Dry	Irr	Dry
LT11-06	155.0	117.4	-0.63	-1.21	0.59	0.52	0.036	0.031	0.37	0.57	-27.5	-27.9
LT11-51	144.5	121.8	-0.29	-1.30	0.72	0.62	0.044	0.028	0.41	0.52	-28.1	-28.8
LT14-05	155.5	129.3	-0.24	-0.96	0.63	0.62	0.028	0.036	0.40	0.63	-28.2	-27.9
LT14-17	166.6	122.1	-0.17	-0.65	0.69	0.48	0.031	0.022	0.34	0.50	-28.0	-28.8
LT14-30	162.3	130.5	-0.13	-1.13	0.50	0.62	0.036	0.035	0.33	0.57	-27.0	-27.4
LT14-34	151.4	139.4	-0.62	-1.16	0.58	0.53	0.038	0.020	0.42	0.49	-28.7	-28.1
LT4-84	112.7	117.4	-0.38	-0.99	0.47	0.57	0.046	0.019	0.42	0.57	-27.9	-29.1
$\bar{x}_{sensitive}$	179.7	125.4	-0.35	-1.06	0.60	0.57	0.037	0.027	0.38	0.55	-27.9	-28.3
Tolerant												
LT14-36	167.7	135.4	-0.17	-1.05	0.47	0.48	0.055	0.033	0.35	0.51	-27.7	-28.2
LT14-37	173.3	138.1	-0.25	-1.10	0.66	0.73	0.051	0.027	0.33	0.47	-28.5	-28.5
LT14-80	168.6	130.4	-0.28	-0.78	0.58	0.71	0.058	0.039	0.36	0.54	-28.7	-28.4
LT4-21	136.2	102.9	-0.43	-0.79	0.72	0.54	0.049	0.043	0.37	0.59	-26.7	-27.3
LT4-43	146.9	150.3	-0.52	-0.74	0.78	0.67	0.061	0.034	0.31	0.53	-27.6	-27.7
LT4-60	220.5	130.5	-0.50	-0.81	0.80	0.65	0.052	0.029	0.37	0.46	-28.5	-29.0
LT4-78	176.1	138.1	-0.22	-1.07	0.67	0.69	0.055	0.040	0.29	0.50	-26.9	-27.9
$\bar{x}_{tolerant}$	169.9	132.2	-0.34	-0.90	0.67	0.64	0.054	0.035	0.34	0.51	-27.8	-28.1
$LSD_{population}$	40.2	31.7	0.35	0.61	0.09	0.10	0.016	0.015	0.10	0.09	1.60	1.60
$\bar{x}_{Population}$	156.2	130.4	-0.33	-0.91	0.63	0.59	0.047	0.032	0.37	0.52	-27.8	-28.2

LSD, least significant difference for paired comparisons between genotypes (P=0.05). Population=100 genotypes.

legumes. Similar results have been reported by Inostroza and Acuña (2010) in white clover (*Trifolium repens* L.) and by Lucero et al. (2001) in perennial ryegrass (*Lolium perenne* L.) and white clover. Despite significant differences in Δ^{13}C values found among genotypes, means of 'sensitive' and 'tolerant' groups are equal. Finally, the 'tolerant' genotypes under irrigation conditions exhibited a higher-value phenotype for the physiological traits being studied.

Conclusion

'Tolerant' genotypes are more productive under drought conditions because they exhibit a more favorable water status than the 'sensitive' genotypes. This allows them to grow and produce more DM than the 'sensitive' genotypes.

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