Water deficit effect on physiological responses of Lotus corniculatus plants of different origin

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Water deficit effect on physiological responses of *Lotus corniculatus* plants of different origin

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Abstract. *Lotus corniculatus* seedlings of two different origins were used to evaluate the effect of water deficit on physiological parameters. Plants from two natural populations of Northern Greece (Kilkis and Taxiarchis) were selected and transplanted to pots. After a period of plant establishment, two water regimes were used: (a) irrigation up to field capacity and (b) limited irrigation in order to maintain water deficit conditions in the soil. The measurements were taken during the growing season at four different phenological stages. Assimilation rate, transpiration rate, stomatal conductance, chlorophyll fluorescence and water potential were measured, while water use efficiency (WUE) was calculated. The results showed that plant origin significantly affected the assimilation rate, stomatal conductance and transpiration rate, with plants of Taxiarchis origin showing higher values. On the other hand, the water treatment used in the present study did not induce any significant changes of the measured physiological parameters, suggesting some degree of drought tolerance of this species.

Keywords. Birdsfoot trefoil – Water stress – Stomatal conductance – Transpiration – Photosynthesis.

Effet du déficit hydrique sur les réponses physiologiques de plants de* Lotus corniculatus *d'origine différente

Résumé. *Des semis de* Lotus corniculatus *provenant de deux régions différentes ont été utilisés pour évaluer l'effet du déficit hydrique sur les paramètres physiologiques. Des plantes de deux populations naturelles du nord de la Grèce (Kilkis et Taxiarchis, respectivement) ont été sélectionnées et transplantées dans des pots. Après une période d'établissement des plantes, deux régimes d'eau ont été appliqués: (a) l'arrosage jusqu'à saturation et (b) l'arrosage limité afin de maintenir des conditions de déficit hydrique dans le sol. Les mesures ont été prises au cours de la saison de croissance à quatre stades phénologiques différents. Le taux net de photosynthèse, le taux de transpiration, la conductance stomatique, la fluorescence de la chlorophylle et le potentiel hydrique ont été mesurés, tandis que l'efficience d'utilisation de l'eau (WUE) a été calculée. Les résultats montrent que le taux net de photosynthèse, ainsi que le taux de conductance stomatique et de transpiration sont significativement influencés par l'origine des plantes ; ceux de* Taxiarchis *montrant des valeurs plus élevées. D'autre part, le traitement de l'eau utilisée dans la présente étude n'a pas induit de changements significatifs des paramètres physiologiques mesurés, ce qui suggère un certain degré de tolérance à la sécheresse de cette espèce.*

Mots-clés. *Conductance stomatique – Lotier corniculé – Photosynthèse – Stress hydrique – Transpiration.*

I – Introduction

Water deficit is one of the most important abiotic factors affecting plant growth in arid and semi-arid regions (Wood, 2005). Plants have developed different strategies to overcome water deficit conditions in the field (Karatassiou *et al.*, 2009). *Lotus corniculatus* L. (birdsfoot trefoil) is a common herbaceous, perennial species, native to areas experiencing dry summers in Greece, and other Mediterranean countries, as well as, in many temperate areas all over the world. Due to its non-bloating features when grazed directly by livestock it is considered as agronomically important. It has also been used for soil remediation and erosion control (Inostroza *et al.*, 2015). Although it is regarded as drought resistant the mechanisms that cause its resistance have not

yet been fully understood (Inostroza *et al.*, 2015). This study analyzes the effect of water deficit on the physiological responses of *L. corniculatus* seedlings from two middle elevation grasslands of Northern Greece.

II – Materials and methods

The experiment was conducted in the farm of the Aristotle University of Thessaloniki, Northern Greece (longitude: 40°31'91", latitude: 23°59'58"), at an altitude of 6 m a.s.l. The climate of the area could be characterized as Mediterranean semiarid with dry summers. The mean annual precipitation of the area is approximately 400 mm and the mean annual air temperature is 15.5°C. During the experimental period, the temperature at the canopy level (T), photosynthetic photon flux density (PPFD) and vapor pressure deficit (VPD), were also acquired using a microclimatic sensor (Novasima MS1, Novatron Scientific Ltd, Horsham, UK). Temperature ranged between 24.3 and 32.6°C, PPFD between 822 and 1304 μmol $m^{-2}s^{-1}$ and VPD between 2.02 and 4.24 kPa.

Lotus corniculatus seedlings were sampled from two semi-arid areas of central Macedonia, Greece, namely Kilkis (K, temperature 12.2ºC; precipitation 585 mm; altitude 570 m a.s.l.) and Taxiarchis (Tax, temperature 11.1ºC; precipitation 767 mm; altitude 645 m a.s.l.) in September and October of 2012 and transplanted in small pots. At the beginning of March 2013, 32 seedlings from each origin were transplanted –one plant per pot– in larger pots (16 cm diameter and 45 cm height), filled with medium texture soil collected from the farm. After a two-month establishment period, a transparent shelter was placed upon plants in order to prevent rainfall intervention. Two drip irrigation regimes were applied: a) irrigation up to field capacity (Full irrigation –FI) and (b) limited irrigation (Limited irrigation –LI) (40% water of that received by FI) in order to maintain water deficit conditions in the soil. The pots were placed in a completely randomized design with four replicates. Measurements were taken during spring 2013 on four different dates corresponding to four phenological stages: early vegetative, vegetative, flowering and start of fruit formation.

In each phenological stage four plants were randomly selected for physiological measurements. The water potential (Ψ) was measured in stems using a pressure chamber (SKPM 1400, Skye Instruments Ltd, Llandrindod Wells, UK). Assimilation rate (A), stomatal conductance (g_s) and transpiration rate (E) were measured with a portable photosynthesis system (LCpro-SD, ADC Bioscientific Ltd, Hoddesdon, UK) on the abaxial leaf surface. Water use efficiency (WUE) was estimated as the ratio of assimilation to transpiration rate. The ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) was measured at 20min dark-adapted leaves using a chlorophyll fluorometer (OS 30p+, OptiSciences Inc, Hudson, USA). All measurements were carried out between 9.30 and 12 a.m. on sunny days.

Analysis of variance (ANOVA) was used to determine the effects of origin, irrigation treatment and plant phenological stage ($P<0.05$) for all measured parameters (Steel and Torrie, 1980). Statistical analysis was performed using the statistical package SPSS (SPSS for Windows, release 22.0; SPSS, Inc., Chicago, USA).

III – Results and discussion

According to the statistical analysis of the data, transpiration rate, stomatal conductance and assimilation rate significantly differed between origins (Table 1), with plants from Taxiarchis presenting higher values of these parameters compared to plants from Kilkis (Table 1). On the other hand, no significant effect of water treatment on *L. corniculatus* physiological responses was found, suggesting some degree of drought tolerance of this species. Water potential, F_v/F_m, E, g_s and A significantly differed among the four phenological stages (Table 1). At the first two phenological stages, Ψ remained at relatively high values, while after the flowering stage it

rapidly decreased reaching values about -19 bar. Stomatal conductance and A were higher at the flowering stage, while transpiration rate remained relatively low, leading to high levels of WUE at that particular stage. Even under very low Ψ values, however, plants from both origins continued to photosynthesize at a lower rate. Interestingly, *L. corniculatus* plants showed similar values of F_v/F_m at all phenological stages, which were close to the values reported for non-stressed terrestrial leaves (Bjorkman and Demmig, 1987). Some plants are adapted to photosynthesize even under low water potential, using mechanisms that tend to maintain turgor, in order to protect tissues from dehydration (Jones, 2004). Sanchez *et al.* (2012) and Inostroza *et al.* (2015) working with *L. corniculatus*, found that this species uses osmotic adjustment as a means to overcome water deficit.

Table 1. Main factor effects on *Lotus corniculatus* physiological parameters. Data represent means±S.E

Effect	Ψ (bar)	F_v/F_m	E (mmol $m^{-2}s^{-1}$)	g_s (mol $m^{-2}s^{-1}$)	A (μmol $m^{-2}s^{-1}$)	WUE (μmol $m^{-2}s^{-1}$/ mmol $m^{-2}s^{-1}$)
Origin	NS	NS	*	*	*	NS
Taxiarhis	-6.1±1.8	0.81±0.01	2.3±0.3	0.10±0.01	5.8±0.5	2.89±0.22
Kilkis	-7.0±1.6	0.82±0.01	1.7±0.2	0.06±0.01	4.4±0.6	2.75±0.24
Water treatment	NS	NS	NS	NS	NS	NS
Full	-6.4±1.9	0.82±0.01	1.9±0.2	0.08±0.01	5.2±0.6	2.98±0.25
Limited	-6.7±1.5	0.81±0.01	2.1±0.3	0.08±0.01	5.0±0.6	2.66±0.21
Phenological stage	***	***	***	***	***	***
Early vegetative	-2.4±0.2	0.86±0.01	2.7±0.4	0.09±0.02	5.7±0.9	2.04±0.17
Vegetative	-1.2±0.2	0.79±0.01	2.7±0.4	0.08±0.02	5.5±0.8	2.10±0.18
Flowering	-3.8±1.0	0.82±0.00	1.6±0.2	0.11±0.02	6.8±0.5	4.51±0.19
Fruit formation	-18.9±2.2	0.79±0.01	1.0±0.1	0.03±0.01	2.4±0.3	2.63±0.28

*P<0.05, **P<0.01, ***P<0.001, NS: non significant

Under full irrigation, for the same values of Ψ plants from Taxiarchis had higher values of g_s compared to Kilkis (Fig. 1A). Under limited irrigation and for Ψ>-5 bar, plants from Taxiarchis showed higher g_s values. However, for Ψ<-5 MPa, g_s of plants from Kilkis remained relatively stable and higher than the Taxiarchis one. In addition, in Kilkis origin under both water treatments g_s was less strongly correlated to Ψ (r^2= 0.32 for FI and 0.22 for LI), compared to the Taxiarchis origin (r^2=0.84 for FI and 0.88 for LI), indicating that other factors apart from water potential control the opening of the stomatal apparatus. The changes of g_s in relation to Ψ are expected to also affect the assimilation rate of the species. Indeed, under full irrigation for the same values of Ψ, plants from Taxiarchis showed higher values of A (Fig. 1B). However, A decreased rapidly when Ψ reached very low values. Plants from Kilkis, on the other hand, decreased A more slowly with the decrease in Ψ. Under limited irrigation, for Ψ <-5 bar, plants from Kilkis showed higher assimilation rate compared to Taxiarchis. These differences in the physiological responses indicate that plants of different origin may have developed varying strategies to overcome water deficit conditions. *L. corniculatus* populations obtained from origins of Northern Greece also exhibited different growth parameters in response to water stress (Karatassiou *et al.*, 2015).

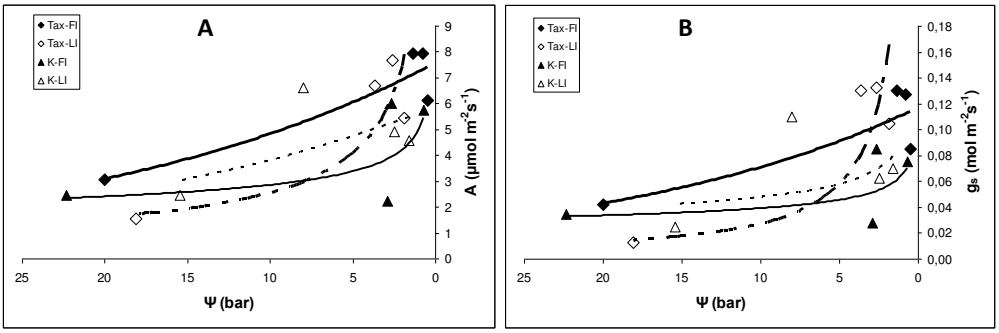

Fig. 1. Changes of stomatal conductance (gs) and assimilation rate (A) in relation to water potential (Ψ). Tax-Taxiarchis, K – Kilkis, FI – Full irrigation, LI – Limited irrigation.

IV – Conclusions

Our results showed that water treatment did not affect *L. corniculatus* physiological responses, indicating some degree of drought tolerance of this species, through the development of strategies that allow photosynthesis even under very low water potential. However, the differences in the physiological responses of the two origins found in the present study may indicate that plants could have employed other plastic and/or adaptive mechanisms to persist and thrive in the environmental conditions of each area.

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