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Effect of water stress on the nutritive value of three *Lotus corniculatus* L. populations at the regrowth stage

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Abstract. *Lotus corniculatus* L. is a promising drought resistant forage legume of high nutritive value. The aim of this study was to estimate the effect of limited irrigation on the nutritive value at the regrowth stage of three natural populations of *L. corniculatus* growing at different elevation in northern Greece. Plants of these populations were tested under two irrigation levels: (1) watered up to soil field capacity and (2) at 40% of field capacity for three months. Plant materials of *L. corniculatus* were randomly collected at the regrowth stage in October 2013 from each population at each treatment and were analysed for chemical components. There was no significant difference among the populations (across irrigation levels) in their CP, NDF, ADF, and ADL, content. On the other hand, limited irrigation had significantly decreased NDF (-43 g/kg DM), ADF (-28 g/kg DM) and ADL (-10 g/kg DM) contents in all tested populations, while it did not affect the CP content. According to the results, the phenol (TPH) and tannins (TT) concentrations were significantly higher in Drama (8 and 6.1g/kg DM) and Taxiarchis population (10.2 and 8 g/kg DM), compared to Kilkis (4.7 and 3.4 g/kg DM), while in condensed tannins (CT) concentration there was no significant difference. Inversely, limited irrigation significantly increased TPH, TT and CT concentrations in all tested populations compared to those received full irrigation. It can be concluded that limited irrigation decreased fiber content of *L. corniculatus*, but increased TPH, TT and CT concentrations. Thus, the next step is to evaluate the water stress on forage yield of *L. corniculatus* and its potential regrowth.

Keywords. Legumes – Drought – Autumn – Forage quality – Tannins.

Effet du stress hydrique sur la valeur nutritive de trois populations de *Lotus corniculatus* L. au stade de repousse

Résumé. *Lotus corniculatus* L. est une légumineuse fourragère prometteuse, résistante à la sécheresse et à forte valeur nutritive. Le but de cette étude était d'estimer l'effet d'une irrigation limitée sur la valeur nutritive au stade de repousse pour trois populations naturelles de *L. corniculatus* poussant à différentes altitudes dans le nord de la Grèce. Des plantes de ces populations ont été testées sous deux niveaux d'irrigation: (1) irriguées jusqu'à atteindre la capacité de rétention du sol et (2) à 40% de capacité de rétention pendant trois mois. Les matériels végétaux de *L. corniculatus* ont été collectés au hasard au stade de repousse en octobre 2013 pour chaque population à chaque traitement et ont été analysés pour les composants chimiques. Il n'y a pas eu de différence significative entre les populations (entre niveaux d'irrigation) pour les teneurs en CP (protéine brute), NDF, ADF, et ADL. Par ailleurs, une irrigation limitée avait baissé significativement les teneurs en NDF (-43 g/kg MS), ADF (-28 g/kg MS) et ADL (-10 g/kg MS) dans toutes les populations testées, tandis que ceci n'avait pas affecté la teneur en CP. Selon ces résultats, les concentrations en phénol (TPH) et tannins (TT) étaient significativement plus élevées chez les populations Drama (8 et 6,1 g/kg MS) et Taxiarchis (10,2 et 8 g/kg MS), comparées à Kilkis (4,7 et 3,4 g/kg MS), tandis que la concentration en tannins condensés (CT) ne montrait pas de différence significative. Inversement, une irrigation limitée augmentait significativement les concentrations en TPH, TT et CT chez toutes les populations testées en comparaison à celles recevant la pleine irrigation. On peut en conclure qu'une irrigation limitée diminuait la teneur en fibre de *L. corniculatus*, mais augmentait les concentrations en TPH, TT et CT. Ainsi, l'étape suivante consiste à évaluer l'effet du stress hydrique sur le rendement fourragère de *L. corniculatus* et sa repousse potentielle.

Mots-clés. Légumineuses – Sécheresse – Automne – Qualité du fourrage – Tannins.

I – Introduction

Forage legumes differ in drought stress sensitivity (Dierschke and Briemle, 2002) and water deficit may have strong effects on their production. However, knowledge about the influence of water stress on the nutritive value of legumes is limited and inconsistent (Kuchenmeister *et al.*, 2013). *Lotus corniculatus* L. is a promising drought resistant forage legume of high nutritive value (Escaray *et al.*, 2012). Its overall forage quality under drought conditions is better than that of *Medicago sativa* due to higher leaf:stem ratio and delayed maturity (Peterson *et al.*, 1992). Additionally, its condensed tannins in leaves protect plant proteins from degradation in the rumen, prevent bloating in ruminants (Li *et al.*, 1996) and allow the control of internal parasite infections, without using anthelmintic drugs (Min and Hart, 2003).

Condensed tannins (CT) have a significant variation according to the genetic and environmental variables (McMahon *et al.*, 2000), particularly in high-condensed tannin accessions of *L. corniculatus*. Marshall *et al.*, (2010). The dietary effects of condensed tannins varies with their concentration, structure, and molecular weight, and are generally considered nutritionally detrimental at levels over 40 g/kg dry matter (Waghorn and Shelton, 1992). The aim of the present study was to investigate the effect of limited irrigation on the nutritive value of three natural populations of *L. corniculatus* at the regrowth stage.

II – Material and methods

Plants of three populations of *L. corniculatus* L. from northern Greece growing in different elevation were collected in September 2012. These populations were named after the location as follows: Drama (D) (100-500 m asl), Kilkis (K) (100-300 m asl) and Taxiarchis (T) (>500 m asl). Thereafter, 32 plants of each population (total 96 uniform plants) were selected and transplanted in pots (diameter 16 cm, height 45 cm), filled with medium texture soil and placed under a transparent shelter in spring 2013 at the farm of the Aristotle University of Thessaloniki, northern Greece at an altitude of 10 m asl. The climate of the area is characterized as Mediterranean semiarid, with mean annual precipitation of 443 mm and mean annual temperature of 15.5°C. Drip irrigation was applied in two levels: (1) up to soil field capacity (W) and (2) 40% of field capacity (WL). The pots were placed in completely randomized design. The plants were harvested four times in different phenological stages. After that all the plants were harvested in the same level and remained uncut for two months and their regrowth biomass was harvested in early October.

Aboveground biomass from every individual plant was oven-dried at 60°C for 48 h, ground through a 1 mm screen and analyzed for Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) (Van Soest *et al.*, 1991) using the ANKOM fibre analyzer. Nitrogen was determined using the Kjeldahl procedure (AOAC, 1990), and crude protein was calculated as N content $\times 6.25$. Samples were also analysed for total phenols (TPH), total tannins (TT) and CT assays according to Makkar (2003). Total phenols (TPH) and total tannins (TT) in the extract were determined by a modification of the Folin-Ciocalteu method using polyvinylpyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols (Makkar *et al.*, 1993). Both total phenols and total tannins were expressed as tannic acid equivalent (mg/g TAE). Condensed tannins (CT) were determined according to the method of Porter *et al.*, (1986), using purified quebracho CT as the reference standard. The CT contents are therefore expressed as quebracho equivalent.

General linear models procedure (SPSS® 18 for Windows) was used for ANOVA. The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

III – Results and discussion

There were no significant differences for CP, NDF, ADF, and ADL contents among the populations (data not shown). On the other hand, all the above components except the CP content (overall populations) of (WL) plants were significantly reduced (Table 1). According to Kuchenmeister *et al.* (2013) moderate water stress reduced NDF and ADF, while it had no severe effects on CP content of *L. corniculatus*.

Table 1. Chemical composition (g/kg DM) of the three populations (D, K,T) of *L. corniculatus* in the two irrigation levels (W, WL)

	W				WL			
	D	K	T	Mean	D	K	T	Mean
CP	178 ^{ab}	185 ^a	170 ^{ab}	178 ^A	166 ^{ab}	149 ^b	171 ^{ab}	162 ^A
NDF	402 ^a	418 ^a	391 ^{ab}	404 ^A	366 ^{bc}	338 ^c	379 ^b	361 ^B
ADF	263 ^{ab}	274 ^a	256 ^{ab}	264 ^A	242 ^b	209 ^c	256 ^{ab}	236 ^B
ADL	97 ^a	102 ^a	92 ^a	97 ^A	92 ^a	79 ^b	92 ^a	87 ^B

* Means followed by the same small letter in the same row within irrigation level are not significantly different ($P \geq 0.05$). Means of each parameter followed by the same capital letter in the same row are not significantly different ($P \geq 0.05$).

However, there was a significant interaction between the irrigation and populations (Table 1). Population of Kilkis had significantly decreased CP, ADF and ADL in the limited watering samples compared with the well irrigated, while no significant differences were detected for the other two populations. Similarly, Drama's population has the same trend as Kilkis although there were no significant differences. The NDF content of Drama and Kilkis populations was significantly reduced under limited irrigation, but not in Taxiarchis.

Table 2. TPH (mg/g DM TAE), TT (mg/g DM TAE), CT (mg/g DM QE) concentration of *L. corniculatus* in the two water levels and the three populations

	TPH	TT	CT
Water levels			
W	6.35 ^b	4.35 ^b	13.33 ^b
WL	8.95 ^a	7.31 ^a	16.76 ^a
Populations			
Drama	8.03 ^b	6.09 ^b	15.14 ^{a*}
Kilkis	4.69 ^c	3.39 ^c	13.80 ^a
Taxiarchis	10.22 ^a	8.01 ^a	14.83 ^a

* Different letters for the same parameter in each column indicate significant differences ($P \leq 0.05$).

Overall water stress, the population of Taxiarchis has significant higher concentration of TPH and TT compared to the other two populations (Table 2). Kilkis population had the lowest concentrations in TPH and TT. There is a significant variation among the TPH and TT concentrations of the population probably due to the habitat characteristics (Giagourta *et al.*, 2015). On the other hand, there was no significant difference in CT concentration among the three populations.

Concerning the effect of water stress (Table 2), there was a significant increase of TPH, TT and CT concentration in the WL treatment. According to Anuraga *et al.* (1993) CT concentrations in *Lotus* are affected by changes in growth temperature and drought stress.

IV – Conclusions

Generally, limited irrigation stress led to decrease in fiber and CP contents of *Lotus corniculatus*. However, it increased the TPH and TT and CT concentrations. The three populations have significant different concentrations of TPH and TT, but not in condensed tannins. The results indicate that populations of *L. corniculatus* of different origins vary in their response to water stress as evidenced by their different nutritive values. The next step will be the evaluation of water stress on forage yield of *L. corniculatus* and its potential regrowth.

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