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in

Acar Z. (ed.), López-Francos A. (ed.), Porqueddu C. (ed.).
New approaches for grassland research in a context of climate and socio-economic changes

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 102

2012
pages 161-164

Comparative estimation of crude protein, phenols and tannins concentration of *Lotus corniculatus* growing in different habitats

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Abstract. *Lotus corniculatus* is a temperate perennial legume that contains condensed tannins which increase the animal production and decrease bloating in ruminants. The aim of this study was to investigate differences in crude protein (CP), total phenols (TPH), total tannins (TT) and condensed tannins (CT) concentration of *L. corniculatus* individuals growing in oak forest and in open grasslands. The collection of plant material was conducted in Taxiarchis area, Chalkidiki, Northern Greece from four sites in an oak forest and four sites in adjacent open grasslands. Twenty individual plants of *L. corniculatus* were randomly collected at the flowering stage in summer 2011 from each of the eight sites and were analysed for CP, TPH, TT and CT concentration. According to the results, the CP concentration was significantly higher in individuals from the forest in comparison to individuals from the open grasslands. Inversely, the individuals from the open grasslands had significantly higher concentration of TPH, TT and CT compared to those from the forest.

Keywords. Legumes – Condensed tannins – *Lotus corniculatus.*

Estimation comparative des concentrations de protéines brutes, de phénols et de tannins de* Lotus corniculatus *provenant de différents habitats

Résumé. *Lotus corniculatus est une légumineuse vivace, tempérée, qui contient de tanins condensés qui augmentent la production animale et diminuent le météorisme chez les ruminants. L'objectif de cette recherche était d'étudier les différences de protéines brutes (CP), des phénols totaux (TPH), des tanins totaux (TT) et la concentration des tanins condensés (CT) de génotypes de* L. corniculatus *qui poussent dans la forêt de chênes et dans les prairies ouvertes. La collection de matériel végétal a été réalisée dans la région de Taxiarchis, à Chalkidiki, en Grèce du nord à partir de quatre sites d'une forêt de chênes et de quatre sites des prairies ouvertes adjacentes. Vingt plantes individuelles de* L. corniculatus *ont été prélevées au hasard au stade de la floraison en été 2011, de chacun des huit sites et ont été analysées pour le CP, TPH, TT et la concentration de CT. Selon les résultats, CP était significativement plus élevée chez les individus de la forêt par rapport aux individus des prairies ouvertes. Inversement, les plantes provenant des prairies ouvertes avaient des concentrations de TPH, TT et TC significativement plus élevées par rapport à ceux de la forêt.*

Mots-clés. *Légumineuse – Tanins condensés* – Lotus corniculatus.

I – Introduction

Lotus corniculatus is a widely distributed legume of high nutritive value (Escaray *et al*., 2012) with good adaptability to different soil and climatic conditions, fact that results in its genetic diversity (Steiner *et al*., 2001). It contains phenolic compounds and especially proanthocyanidins, also known as condensed tannins (CT), which prevent bloating and allow the control of internal parasite infections, without using anthelmintic drugs (Aerts *et al*., 1999, Min and Hart, 2003). However,

depending upon its CTs concentration (Marshall *et al*., 2010) it could have a beneficial or detrimental effect on ruminant production. Marshall *et al*. (2010) found significant variation in CT within and between varieties of *Lotus*. It is known, that CT concentration is affected by genetic and environmental variables (McMahon *et al*., 2000). Therefore, the knowledge of germplasm diversity in response to environmental condition is a useful tool for its utilization in breeding efforts.

The objective of this research was to estimate the variation in phenols, tannins, condensed tannins and crude protein (CP) content among *L. corniculatus* individual plants growing under two different environmental conditions, namely in forest and in open grassland.

II – Materials and methods

The study was conducted in the area of Cholomontas, Chalkidiki prefecture, northern Greece (40°23'N, 23°28'E) at 800 m a.s.l. The climate of the area is classified as subhumid Mediterranean, with a mean air temperature of 11.1°C and an annual rainfall of 767 mm. The area is situated in the *Quercion confertae* subzone of the *Quercetalia pubescentis* (sub-Mediterranean) zone (Athanasiadis, 1986). The whole forested area was grazed by goats and sheep.

Table 1. Description of collection sites

Site Nr.	Altitude	Aspect	Dominant herbaceous species	Description
Forest				
1	852 m	East	*Vicia* sp*, Platango* sp.	Beech forest
2	867 m	North-west	*L. corniculatus, Cynodon* sp.	Oak forest
3	780 m	West	*Brachypodium* sp.*, Trifolium sp.*	Oak Forest
4	815 m	South	*Trifolium sp., Brachypodium* sp.	Oak forest
Grassland				
1	792 m	North-west	*Cynodon* sp.*, Cinosurus* sp.	Not grazed grassland
2	799 m	South-east	*Agrostis* sp.*, Cynodon* sp.	Grazed grassland
3	765 m	North-east	*Agrostis* sp.*, Chrysopogon* sp.	Grazed grassland
4	812 m	West	*Cynodon* sp.*, Hieracium* sp.	Heavily grazed grassland

Twenty individual plants were collected at the flowering stage in August 2011 from each of four different sites in the forested area and in the open grasslands (Table 1), i.e. total of 160 plants and oven-dried at 50ºC for 48h. All the samples were ground through a 1 mm sieve and analysed for N using the Kjeldahl procedure (AOAC, 1990). Crude Protein (CP) concentration was then calculated by multiplying the N content by 6.25. Samples were analysed for total phenols (TPH), total tannins (TT) and CT assays according to Makkar (2003). Three replicates of 200 mg samples were extracted in 10 ml aqueous acetone (acetone: water, 7:3) twice in an ultrasonic water bath for 20 min. The extracted samples were centrifuged at 3,000 g at 4°C for 10 min and the supernatants were used for tannin analysis on the same day Makkar (2003). Total phenols (TPH) and total tannins (TT) in the extract were determined by a modification of the Folin-Ciocalteu method using polyvinylpolypyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols (Makkar *et al*., 1993). Concentration of TT (mg/g DM) was calculated as follows: TT conc. = (conc. of TPH) - (conc. of TPH remaining after PVP treatment). 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.

One-way ANOVA of the data was performed using SPSS® statistical software v. 18.0 (SPSS Inc., Chicago, IL, USA), in order to determine differences among the habitats and collection sites in each habitats. The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

III – Results and discussion

The individuals originated from the forest had significant lower TPH, TT and CT and higher CP concentration compared to those from grassland (Table 2). Iason and Hester (1993) have also reported that shading reduced TPH. According to Jantineke *et al*. (2009) shading reduced the TPH, TT and CT respectively. It is well known that shade improves the nutritive value of the herbage vegetation as the maturity stage is delayed compared to open field conditions (Burner and MacKown, 2006, Parissi and Koukoura, 2009). The increase of crude protein content in legumes species under shade has also been reported by Kyriazopoulos *et al*. (2012).

Table 2. Means and Coefficient of Variation (CV) of TPH (mg/g DM TAE), TT (mg/g DM TAE), CT(mg/g DM QE) and CP(%) concentration of *L. corniculatus* individual plants from forest and grassland

	TPH		TT		CT		CP	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Forest	5.5	25.1	2.4	35.3	20.3	36.3	19.0	19.0
Grassland	6.6	22.3	3.0	34.8	25.5	24.6	13.7	6.7
F-value/Sign.	5.848	**	4.322	**	5.939	**	40.446	**

** ($P \leq 0.05$).

Table 3. TPH (mg/g DM TAE), TT (mg/g DM TAE), CT (mg/g DM QE) and CP(%) concentration of L. corniculatus individual plants from the collecting sites in forest and grassland

	Forest				Grassland			
	1	2	3	4	1	2	3	4
TPH	4.3b	6.8a	4.7b	6.1a	5.1b	6.4ab	7.7a	7.0a
TT	1.7b	3.3a	1.9ab	2.6a	1.6c	2.8b	4.0a	3.6a
CT	15.2b	26.0a	14.2b	25.5a	24.9	24.7	28.2	24.3
CP	21.0a	21.0a	19.4a	14.7b	13.3	14.3	13.6	13.7

* Means in the same row followed by the same letter are not significantly different ($P \leq 0.05$).

Significant differences were detected for TPH, TT, CT and CP in forest and for TPH in grassland among the collecting sites (Table 3). Significantly higher TPH, TT, and CT concentration was observed in forest habitat at site 2 where *L. corniculatus* was one of the dominant species (Table 1). On the other hand, the ungrazed grassland (site 1 of grasslands) (Table 1) had significant lower TPH and TT compared to the others (Table 3). It is well documented that plants-herbivores interactions led to the development of defence mechanisms of plants against herbivores such as tannins (Barroso *et al.,* 2001). Thus, ungrazed plants had lower content of tannins compared to the grazed ones).

The higher variability in terms of CV estimation was observed for TT and CT among the individuals from both forest and grassland. The CP concentration revealed the lowest variability among the individual from both forest and grassland.

IV – Conclusions

The habitat ecology and management seems that affect the concentration of crude protein, phenols and tannins in individual plants of *L. corniculatus*. The arising question is whether this variation is a result of genetic or environmental effect, in order to use in future breeding efforts.

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