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## The accumulation of condensed tannins in local populations of sulla

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**Summary** - Aim of the study was the evaluation of the accumulation and content of condensed tannins (CT) during the life cycle of sulla (*Hedysarum coronarium* L.). The colorimetric method «Butanol-HCl-Fe<sup>3+</sup>» has been utilized and adapted to the species. Different phenological phases have been examined separately for both components leaves and stems. A comparison was initially made between the Southern Sardinian natural population «Villanovafranca» and the commercial variety «Grimaldi»; other four Sardinian natural populations of sulla were also analyzed. Leaves showed a considerable higher CT content than stems. Some natural populations showed higher CT concentration than «Grimaldi».

**Key-words:** sulla, condensed tannins

**Résumé** - Dans le but d'évaluer la quantité et le tas d'accumulation de tannins condensés (TC) durant le cycle biologique de la sulla on a employé la méthode colorimétrique «Butanole-HCl-Fe<sup>3+</sup>». On a analysé différents stades phénologiques en séparant les deux composants feuilles et tiges. Initialement on a conduit le travail sur la population naturelle de Sardaigne «Villanovafranca» et sur la variété commerciale de sulla «Grimaldi» et sur des autres populations naturelles de sulla de Sardaigne. Les feuilles ont présenté une quantité de TC considérablement plus élevée. Quelques populations naturelles ont présenté une teneur en TC supérieur à la variété commerciale.

**Mots-clés:** sulla, tannins condensés

### Introduction

Sulla (*Hedysarum coronarium* L.) is a forage legume currently under renewed interest because of its suitability to cropping in semiarid environments, also outside the Mediterranean basin. Condensed tannins (CT), are polyphenolic secondary compounds that can react by hydrogen bonding with plant proteins in the near neutral pH range to form CT-protein complexes, which are stable and insoluble at pH 3.5-7.0, but dissociates and release protein at pH<3.5 (Jones and Mangan, 1977). Thus CT contained in forage plants can protect dietary proteins against degradation in the rumen and increase amino acid supply for absorption in the small intestine of ruminants (Wang *et al.*, 1996). CT only occur in leaves and stems of several forage plants, other species do not contain them. CT-containing species do not cause bloat, while moderate CT concentrations (2-4% of dry matter) enhance forage nutritive value in grazing ruminants by reducing protein degradation from rumen bacteria and increasing protein degradation from the intestine, without depressing rumen fibre digestion or voluntary intake (Douglas *et al.* 1993). CT have also been reported as having nematocidal properties against free living nematodes and are also considered to offer plants protection against bacterial, fungal and insect predation (Niezen *et al.*, 1995). Sulla is among the CT-containing species; in this preliminary study we attempt to set the best method to check the

CT accumulation during the life cycle of *sulla*, with the aim to study the CT content of some *sulla* natural populations from South Sardinia.

### Materials and methods

The plant materials initially compared were the Sardinian natural population of *sulla* «Villanovafranca» and the commercial variety «Grimaldi»; four additional Sardinian natural populations, «Furtei, Suelli, Mandas, Sardara», were also used. Plants were grown in open field in rows spaced 25 cm, the distance between plants was 10 cm. Experimental design was a randomized block with three replicates, 30 plants per plot. Leaves and stems were sampled at the following stages: stage 2 (vegetative), stage 3 (flower bud appearance), stage 6 (flowering), stage R (regrowth after cut). Stages were named according to Borreani *et al.* (1999). Fresh tissues were collected from each plant, samples were kept refrigerated during and after collection until the tissues were freeze-dried and ground to a fine powder. Extraction of free condensed tannins was done according to Terrill *et al.* (1992) with some modifications, by adding to the sample a mixture of 7:3 (v/v) acetone/water containing 1% ascorbic acid, shaking for 60 minutes, then the mixture was centrifuged for 10 minutes at 3100 rpm. Extractable CT determination was performed on a Perkin Elmer spectrophotometer following the butanol-HCl-Fe<sup>3+</sup> assay (Porter *et al.*, 1986). A mixture of butanol-HCl was added to the extracts and Iron Reagent (2% Ferric Ammonium Sulphate in 2N HCl) was included as catalyzing agent, the mixture was incubated at 100°C in a water bath for 40 minutes, then cooled before reading the absorbance at 550 nm. A standard calibration curve was developed by means of purified CT from *Mimosa* sp. leaves.

### Results and discussion

As shown in fig. 1, a different condensed tannin accumulation pattern was found in leaves of the commercial variety «Grimaldi» compared to the local population «Villanovafranca».

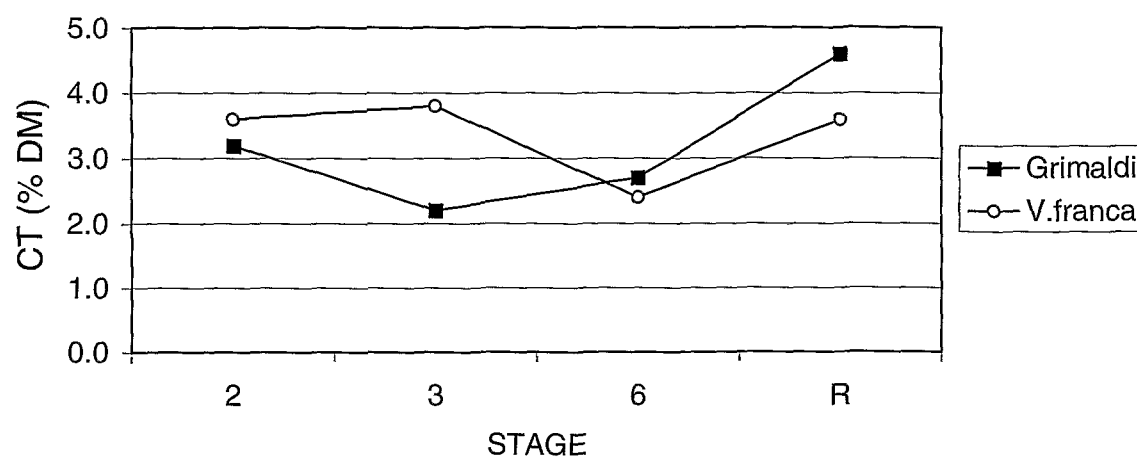


Fig. 1: CT content in leaves of Grimaldi and Villanovafranca at different phenological stages.

The former showed a marked increase in CT content of leaves beginning from the stage 3 (flower bud appearance), while the latter showed a marked decrease from the flower bud appearance (stage 3) until flowering (stage 6). Free CT concentration of leaves was higher in «Villanovafranca» at the first stages while at flowering and regrowth was higher in «Grimaldi».

Regarding the stems (fig. 2), a constant increase in CT was registered for «Grimaldi», while a decrease until flowering was shown by «Villanovafranca». CT content in stems was always substantially lower than in leaves.

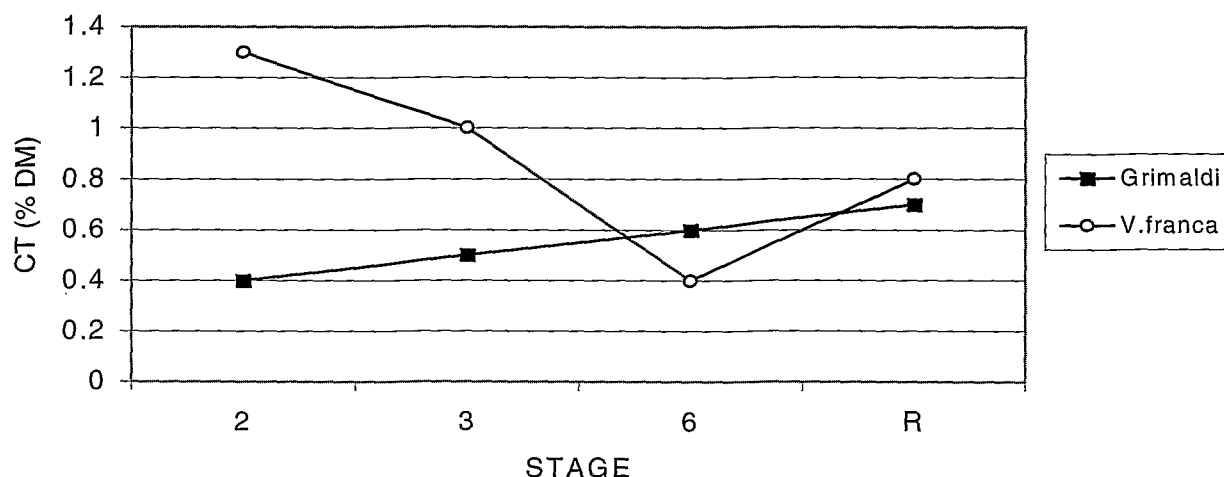


Fig. 2: CT content in stems of «Grimaldi» and «Villanovafranca» at different phenological stages.

The CT content as  $\text{g Kg}^{-1}$  of dry matter (DM) at stage 2 (vegetative) and stage 3 (flower bud appearance) of the five local populations of sulla in comparison with Grimaldi is shown in table 1. Almost always, the local populations showed a CT content significantly higher than Grimaldi for both leaves and stems.

Table 1: Extractable CT of freeze dried sulla leaves and stems at stage 2 (vegetative) and stage 3 (flower bud appearance)

ACCESSIONS	LEAVES $\text{g Kg}^{-1}$ DM		STEMS $\text{g Kg}^{-1}$ DM	
	stage 2	stage 3	stage 2	stage 3
GRIMALDI	32	22	4	5
VILLANOVAFRANCA	36	38	13	10
FURTEI	39	27	11	9
SUELLI	28	28	6	10
MANDAS	25	27	13	5
SARDARA	40	33	14	8
LSD 0.05	3.2	0.7	0.7	1.4

We also tested the CT content by applying (data not presented) the «Vanillin-HCl» assay (Terrill *et al.*, 1992): it required a higher plant sample while the «Butanol-HCl-Fe<sup>3+</sup>» was more sensitive and less tissue consuming. It must be also pointed out that addition of the Iron Reagent was of fundamental importance for the «Butanol-HCl» assay for making a stable reaction with sulla samples, otherwise the assay can be unreliable and the reaction incomplete.

## Conclusions

The free CT content varying from 25 to 40 g kg<sup>-1</sup> DM, as found in the local populations of sulla tested, was within the range of 20-40 g kg<sup>-1</sup> DM reported by Barry *et al.* (1986) as beneficial in ruminant diets: this encourages the breeding programme in progress for the valorization of the Sardinian sulla germplasm. The accumulation pattern needs more investigations specially at flowering, while a decrease in CT accumulation seems almost always a common trend from the vegetative stage to the flower bud appearance stage.

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