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Condensed tannins in the Genus *Lotus*

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Proanthocyanidin (PA), also called condensed tannins (CT), are polymeric flavanols formed by condensation of monomeric flavanols such as flavan-3-ols (catechins, as initiating unit) and flavan-3-4-diols (leucoanthocyanidins, as extension units). PA generally accounts for the majority of flavanols present in plants, and they are also more effective protein precipitators than monomeric flavanols avoiding bloating. Bloat is a serious digestive disorder of beef cattle and dairy cows, grazing of forage legumes such as alfalfa and white clover. Bloat is caused by the formation of stable proteinaceous forms in the rumen, preventing gas escape and resulting in death or morbidity. Forage diets containing medium level of PA (i.e. 1-5 mg CT/g DM) are desired, but excess of CT levels in plants may reduce voluntary intake and lower the nutritional value of forage diets. Others authors refer that tannins also prevent intestine parasitic infection (personal communication INTA Balcarce) and modulate the mechanism related to abiotic stress tolerance. The *Lotus* species has significant differences between them and within deferent's populations of the same species (especially alogamous ones i.e.: *Lotus corniculatus* and *L. tenuis* = *L. glaber* Mill.).The differences are observed on leaves, stems and roots. In our group analyze with special interest the *L. tenuis*, because, it is a keystone specie in the Salado River basin.

The objectives of our group include the evaluation of the possible relationships between the saline stress and the tannins levels, and if such modifications regulate the efficiency of the nodulation. To analyze this, we work with species with importance on the forage production (*L.tenuis* and *L corniculatus*) and for sustainability of Mediterranean farming or systems (*L. creticus*). Simultaneously we evaluated the saline stress in *Lotus* model species used in the genomic sequencing Project (*L. burttii; L. japonicus* MG20*; L. japonicus* Gifu and *L. filicaulis*). Salinization was imposed by gradually increasing NaCl from 0 to 150 mM during a 20 day-period, after which plants were irrigated with 150 mM sodium chloride for an additional 19 day-period. After harvesting, the following growth parameters were determined: shoot and leaf fresh (FW) and dry weight (DW) and leaf area and tannin levels were quantified for each fraction. The values were expressed in Catequine equivalents (CE) Sometimes, dry leaf and shoot material was used for Na and K determination in order to check the saline stress status.

L corniculatus and *L creticus* present higher levels than *L. tenuis* and opposite tendency under saline stress; while *L. corniculatus* diminished, *L. creticus* increase their CT

concentration. Similar results were obtained in the *Lotus* model species, presenting differences in stem, leaves and roots.

For the second objective, that is to evaluate if CT concentrations affect the nodulation process, we utilize *L. corniculatus* transgenic lines harboring the transcriptional factor denominate Sn1 isolate from wheat and differences in the CT levels in roots and leaves. Genotypes cloned vegetative propagations were inoculated with *Mesorhyzobium* (NZP 2213 and NZP 2037) and *Bradyrhyzobium* (NZP 2309). The alterations observed in the nodulation patterns will be discussed.