

Molecular and biochemical approximation of polyamine roles in tolerance mechanisms to salt stress in *Lotus* spp.

ROSALÍA PAZ, DIEGO H. SANCHEZ, FERNANDO PIECKENSTAIN, SANTIAGO MAIALE, ANALÍA SANNAZZARO, JUAN CRUZ CUEVAS, AMALIA CHIESA, GONZALO BONA and [OSCAR A. RUIZ*](#).

Unidad Biotecnológica 1. IIB-INTECH/UNSAM-CONICET. Ruta Circunvalación Laguna km 6, (7130) Chascomús, Pcia. de Buenos Aires, Argentina.

* *Corresponding author*

Lotus glaber is the most important legume in the saline-alkaline lowlands of the Salado River basin. This region (approximately 9,000,000 ha), located in Buenos Aires Province (Argentina) is devoted to the breeding of beef cattle. In order to increase forage yield and improve the quality of their pastures, regional farmers utilize *L. glaber*, whose adaptability to saline soils is well-known. The economic importance of this legume has led to an increasing number of studies regarding the physiological basis of its salt tolerance. Polyamines are aliphatic amines of low molecular weight charged positively at physiological pH. The distribution of these positive charges permits their interaction with proteins, membrane lipids and DNA. It's well known that the activity of the plant enzymes of polyamine biosynthesis is induced under abiotic stress, including salinization. With this idea in mind, we evaluated the effect of salt stress in polyamine pathway in *Lotus glaber*. As proline is a traditional stress marker in plants, we evaluate their levels under similar stress conditions. To understand the response of *L. glaber* to salt stress, we evaluated 15 day-old seedlings germinated and grown under 0, 25, 50 and 75 mM NaCl. We observed a progressive accumulation of Na⁺ and loosening of K⁺. Simultaneously we observed an increasing in proline levels and accumulation in Spm, coincidently with a diminishing in Spd levels.

In addition, we analyzed a natural population of *L. glaber* collected from lowlands of Salado River Basin. We isolated 103 genotypes of *L. glaber* and cloned them by nodal cutting. Thirty days old genotypes were exposed to 300 mM NaCl (n=5), and death days average was determined. A Gaussian distribution was obtained. The time survival varied from 12 till 30 days. Five genotypes of each extreme were selected and cloned and sub irrigated with 0 and 150 mM NaCl. Based on their differential relative growth rates the genotypes were classified in sensitive and tolerant. We concluded that the preliminary classification based on average days of "survival-time" under strong saline conditions are not representative of their tolerance physiological conditions because some genotypes identified firstly as sensible were tolerant and vice versa.

Analysis in polyamine content demonstrated that spermine accumulates less in tolerant genotypes than in sensitive. Simultaneously, proline presents a progressive accumulation in

both genotypes but was smaller in tolerant ones. Complementary and taking in to account the results obtained, we generate *Lotus corniculatus* transgenic plants that over express under control of the constitutive promoter CaMV35S, a putative gene of spermidine synthase previously cloned from tobacco. The lines obtained presented constitutive high levels of Spd and Spm than controls non-transformed. Moreover, we observed that these transgenic plants under saline stress showed a decrease in the spermidine concentrations and an increment in the spermine levels suggesting activation in the spermine synthase activity. Under similar conditions the proline levels diminished.

Actually, we are working in the generation of *Lotus spp* transgenic plants that potentially over express regulatory enzymes in the polyamine biosynthesis under control of a promoter inducible by stress. This promoter denominated RD29A was cloned from *Arabidopsis thaliana* and is under evaluation in our lab. Binary vectors harboring the sequence of arginine decarboxylase (ADC) under control of this promoter was assayed successfully in hairy roots of *Lotus corniculatus*, suggesting a conservative stress signaling pathway between species.