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Experimental design optimization for the study of saline, alkaline and mixed stress in *Lotus* sp.

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Mixed saline-alkaline stress severely restricts primary production of forages in the Flooding Pampa, the most important area for cattle breeding in Argentina. About 68 % of the soils in this region contain alkaline salts like $NaHCO_3$ and Na_2CO_3 (Mendoza, 1981; Vazquez *et al*, 2001; Costa and García, 1998). In soil solution, alkalinity produced by CO_3^{-2} and HCO_3^- anions of the above-mentioned salts causes a reduction of nutrient availability to the plants, and also produces root damage (Lucena *et al.*, 2007; Cartmill *et al.*, 2008) and leads to chlorosis, growth inhibition and adaptation to nutrients uptake (Alhendawi *et al.*, 1997).

Lotus tenuis is a perennial legume naturalized in saline-alkaline lowlands of the Salado River Basin (Vignolio *et al.*, 1999), highly valuable because of its contribution to forage offer in the region and its influence on growth of associated species (Papadopulous and Kelman, 1999). For this reason, *L. tenuis* is considered as a Keystone specie (Quinos *et al.*, 1998; Paz *et al.*, 2007). These characteristics are common to other species of *Lotus* genus, and for this reason, many efforts were destined to evaluate the mechanisms of salt stress responses in this genus. However, adaptive characteristics of *L. tenuis* and other *Lotus* species indicate that these species are also able to tolerate soil alkalinity, thus being important to discriminate between responsive mechanisms to saline, alkaline and mixed saline-alkaline stresses (Mokhtar *et al.*, 2006).

Preliminary results indicate that mixed saline-alkaline stress is more severe than either the saline or the alkaline stresses, and that there are different degrees of tolerance among species of the genus. In addition, anatomical and morphological were found to be induced by these stresses, with an increase in leaf and stem succulence and plastic differential responses in roots.

In this way, tolerance mechanisms might be acting at different levels (genetic, biochemical, physiological and/or anatomical) and these responses might be different among diverse *Lotus* species. On the other hand, the symbiotic interaction with highly efficient and competitive rhizobacteria able to fix atmospheric nitrogen and well adapted to restrictive

soils might contribute to adaptive responses of *L. tenuis* (Parniske and Downie, 2003; Paz *et al.*, 2008; Bergottini *et al.*, 2008; Estrella *et al.*, 2007).

Finally, it is worth to point out that, in spite of the lack of information about molecular genetics of *L. tenuis*, genomic sequencing of the model species *L. japonicus* is well advanced (>67%) (Udvardi *et al.*, 2005; Sato *et al.*, 2008). As a consequence, the existence of a high level of macro and micro-sinteny between different *Lotus* species was found, which could be relevant for the identification of *L. tenuis* genes induced by stress conditions.

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