

Lotus RILs analyses under salt stress: establishing discriminative growth conditions

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The aim of this work was to study some functional markers associated to saline stress tolerance in Lotus recombinant inbred lines (RILs) as an activity inside the LOTASSA project (EU). Tolerance responses to NaCl based on growth parameters were characterized in RILs obtained by *Lotus filicaulis* x *Lotus japonicus* ecotype Gifu (NS- RILs) and *Lotus japonicus* ecotype Gifu x *Lotus burtii* (GxLb-RILs) crossings.

Differential tolerance to 0, 75, 100, 125, 150 mM NaCl in each diploid parents was previously assayed using 7 days old plantlets. Salt treatments were applied in a stepped-increase fashion. There were defined length of salts stress treatment, salt levels, and growth parameters in which genotypes differences in growth were statistically significant.

On control conditions, shoot length and growth velocity were higher in *L. filicaulis*. Salt stress diminished shoot length in all genotypes. Lj Gifu was the less affected genotype. Salt effects were notably evident at 13 d after the beginning of 125 mM NaCl treatment (9 days under final salt concentration). Both total and shoot length, allowed discriminating Lj Gifu from the other genotypes; being a useful data given that Lj Gifu is a common parent in both kinds of RILs.

RILs seed were scarified and cultured during 5 days on agar (0.8%). Before salt treatments, percentage of germination and development of NS-RILs (*Lotus filicaulis* x *Lotus japonicus* ecotype Gifu) and GxLb-RILs (*Lotus japonicus* ecotipo Gifu x *Lotus burtii*) untreated plantlets were determined. Seventy five percent of NS-RILs showed a range of germination between 50-100%, however 4% of these RILs showed germination lower than 9%. In GxLb-RILs, 61% showed a range of germination between 50-100% but in 37% of this RILs germination percentage was between 10-49%. Normal plantlets development was also analyzed by ranges. In NS-RILs 34% developed more than 50% of normal plantlets whereas in 54% of these RILs viable plantlets were between 10-49% of germinated seeds. Sixty two percent of GxLb-RILs showed between 50-100% of normal plantlets.

NS-RILs and GxLb-RILs were acclimated at 125 mM NaCl in hydroponic trays and growth parameters were evaluated 9 d after reaching final salt level.

Total length showed two different groups in NS-RILs, one of them included 46 RILs with smaller total length and without differences respect to parental Lj Gifu. The second group,

with higher total length, included 10 NS-RILs and did not show statistically significant differences respect to *L. filicaulis*. GxLb-RILs also showed two statistically different groups: one of them comprised 13 lines with smaller total length, and the other one included 8 RILs and both parents Lj Gifu and *L burttii* without differences among them.

The protocol designed to analyzed salt tolerance in Lotus parents and NS and GxLb-RILs allowed differentiating responses against salt stress. These results will contribute to correlate physiological responses with the molecular markers developed for *Lotus*. Under the defined conditions to discriminate salt stress responses in both groups of RILs, none of them showed any improved responses in growth on respect to their parents, for this reason analyzing the responses in RILs with a higher susceptibility and correlating functional markers associated with NaCl susceptibility, could be of interest.