Trehalose metabolism in root nodules of the model legume *Lotus japonicus* in response to salt stress

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The effect of NaCl stress (50 mM) and validamycin A treatment (30 μM) on growth and nitrogen fixation of *Lotus japonicus* was investigated in plants cultured under symbiotic and hydroponics conditions for ten weeks (flowering stage). Validamycin A was used as a potent trehalase inhibitor, and was able to produce a five-fold increase in the level of trehalose during salt treatment, concomitant with an enhance in biomass (20%) in salinized plants. Alterations of nodule metabolism related to some carbohydrates and some enzyme activities were also examined. The shoot and total plant dry weight were severely affected by saline conditions decreasing by 40% and only 15–20% in plant treated without or with validamycin A, respectively. Nitrogenase activity (E.C. 1.7.9.92) was inhibited almost 40% by salt stress and no effect of validamycin was observed. Based on these results, *L. japonicus* might be considered as a salt-sensitive legume. In addition, the saline conditions also inhibited the enzyme activities of sucrose synthase (E.C. 2.4.1.13), alkaline invertase (E.C. 3.2.1.26) and trehalose-phosphate synthetase (E.C. 2.4.1.15). The validamycin A treatment mainly decreased enzyme activities: sucrose synthase, trehalose-phosphate phosphatase (E.C. 3.1.3.12) and trehalase (E.C. 3.2.1.28). On the other hand, a high concentration of the carbohydrates, starch, sucrose and glucose, seems not to be the mechanism induced in *L. japonicus* to protect nodules exposed to NaCl because all these sugars decreased in such conditions. Results of the present study support the possible role of trehalose as an osmoprotectant under salt stress.

Introduction

*Lotus japonicus* is a perennial temperate pasture species that is closely related to birdsfoot trefoil (*L. corniculatus* L.) and exhibits characteristics that are useful for genomics, such as a short life cycle, self-fertility, diploidy, relatively small genome size and a short generation time. Phylogenetically, *L. japonicus* is included in the so-called “temperate” or “galegoid” legume group and forms determinate nodules as do “tropical” or “phaseoloid” legumes such as soybean (*Glycine max* L.) and common bean (*Phaseolus vulgaris* L.). It has therefore been proposed to be a model legume for classical and molecular genetic studies on symbiotic plant-microbe interactions (Handberg and Stougaard 1992), and has been demonstrated suitable as a platform for legume crop improvement (Sato and Tabata 2005).

Abbreviations – AI, alkaline invertase; ANA, apparent nitrogenase activity (H₂ production in air, units of μmol H₂ g⁻¹ FW h⁻¹); ANOVA, analysis of variance; DW, dry weight; EDTA, ethylenediaminetetraacetic acid; FW, fresh weight; HK, hexokinase; LSD, least significant difference; NAD⁺, nicotinamide adenine dinucleotide; NDW, nodule dry weight; NFR, nitrogen-fixation rate; PDW, plant dry weight; RDW, root dry weight; SDW, shoot dry weight; SS, sucrose synthase; TPS, trehalose-phosphate synthase; TSS, total soluble sugars; TRE, trehalase.