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BRIEF COMMUNICATION

Sucrose metabolism in *Lupinus albus* L. under salt stress

F.M. FERNANDES*[1], M.C. ARRABAÇA** and L.M.M. CARVALHO***

*Escola Superior de Educação de Beja, Rua Pedro Soares s/n, 7800 Beja, Portugal**
*Faculdade de Ciências da Universidade de Lisboa, Departamento de Biologia Vegetal, Campo Grande, 1749 Lisboa, Portugal***
*Escola Superior Agrária de Beja, Rua Pedro Soares s/n, 7800 Beja, Portugal****

Abstract

Salt stress (50 and 150 mM NaCl) effects on sucrose metabolism was determined in *Lupinus albus* L. Sucrose synthase (SS) activity increased under salt stress and sucrose phosphate synthase activity decreased. Acid invertase activity was higher at 50 mM NaCl and decreased to control levels at 150 mM NaCl. Alkaline invertase activity increased with the salt stress. Glucose content decreased with salt stress, sucrose content was almost three times higher in plants treated with 150 mM NaCl and fructose content did not change significantly. The most significant response of lupin plants to NaCl excess is the increase of sucrose content in leaves, which is partially due to SS activity increase under salinity.

Additional key words: invertases, sucrose phosphate synthase, sucrose synthase, sugars.

Salinity limits plants distribution throughout natural habitats and is a severe agricultural problem in many regions on the Earth. Salt stress involves osmotic and ionic components as a consequence of soil osmotic pressure increase and a higher concentration of potentially toxic ions. Generally, plant responses to salinity are evaluated by their growth, ion balance, compatible organic solutes synthesis and osmotic adjustment (Sánchez-Blanco *et al*. 1991). Salinity may cause disturbances in plant water balance, including reduction in pressure potential, growth inhibition, stomatal closure and photosynthesis reduction (Poljakoff-Mayber 1982). Among the other significant salt stress consequences are changes in contents of sucrose and reducing sugars as well as in sucrose synthase (SS; EC 2.4.1.13), sucrose phosphate synthase (SPS; EC 2.4.1.14), acid invertase (EC 3.2.1.26), and alkaline invertase (EC 3.2.1.26) activities (Elavumoottil *et al*. 2003, Kaur *et al*. 2003).

In *Lupinus albus* L. salt stress has been reported to reduce growth, transpiration rate, photosynthetic rate and pigments content (Fernandes and Arrabaça 1999). Therefore, a study on the salt stress effect on enzymes of sugar metabolism can provide relevant information about biochemical responses of lupin plants to excess of NaCl.

Plants of *Lupinus albus* L. were grown according to Shaddad *et al*. (1990) in controlled chambers at 25/18 ºC (day/night), 80 % relative humidity, a 16-h photoperiod with irradiance of 250 μmol $m^{-2}s^{-1}$ provided by *Sylvania Gro-Lux* (F 30 W / Gro-T8) lamps. Seeds germinated 4 d and then seedlings were supplied twice a week with Hewitt solution. After 16 d, plants were treated either with 50 or with 150 mM NaCl for the next 12 d. The youngest fully expanded leaf of 28-d-old plants was used for assays.

Leaves were harvested after 4 h illumination, frozen in liquid nitrogen and maintained at -70 ºC. A leaf tissue (0.25 - 0.5 g) was grounded in a mortar with 4 cm^3 extraction medium, according to Weiner *et al*. (1992). After centrifugation the extract for 10 min at 30 000 *g* and 4 ºC, the supernatant was desalted and concentrated in a stirred cell (*A 8400*, *Amicon*, Beverly, USA), with an ultra filtration membrane of cut off 10 000, under nitrogen atmosphere and 50 Pa pressure.

Sucrose phosphate synthase (SPS) activity was determined in the synthesis direction. Enzyme was

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Abbreviations: Glc-6-P - glucose- 6-phosphate; Fru-6-P - fructose-6 - phosphate; SPS - sucrose phosphate synthase; SS - sucrose synthase; UDP-Glc - uridine diphosphate glucose; V_{lim} - limiting velocity; V_{max} - maximal velocity.
[1] Corresponding author; fax: (+35) 1284326824, e-mail: ffernandes@eseb.ipbeja.pt

assayed either with Pi and limited substrates concentration or assayed with a saturating substrate concentration and in the presence of the activator glucose-6-phosphate (Glc-6-P). Both assays were performed according to Johnson *et al*. (1988) with exception of uridine diphosphate glucose (UDP-Glc) and fructose-6-phosphate (Fru-6-P) concentrations, which were 20 mM. Sucrose synthase (SS) assay was performed in the synthesis direction according to Fiew and Willenbrink (1990) except for fructose and UDP-Glc concentrations, which were 30 mM and 10 mM, respectively. The reactions were stopped by addition of 0.2 cm^3 1 M NaOH and boiling for 10 min. The sucrose formed by SS and sucrose-P formed by SPS was determined colorimetrically at 520 nm according to Roe (1934). Invertases were assayed according to Johnson *et al*. (1988) except for sucrose concentration, which were 150 mM in acid invertase assays and 50 mM in alkaline invertase assays. Reactions were stopped according to Irving and Hurst (1993). Glucose produced by invertase activity was determined by a modification of *Boehringer-Mannheim* UV test (Hatterscheid and Willenbrink 1991).

Soluble protein content was measured according to Bradford (1976) using BSA as a standard. Soluble sugars present in desalted and concentrated extract were determined by a modification of *Boehringer-Mannheim* UV test at 490 nm in a microplate reader (*Du Pont MPR-A4*, Tessenderlo, Belgium).

Data were analysed by factorial analysis of variance (*ANOVA*) followed by Fisher's PLSD and Scheffé F tests, for the separation of averages in significant groups. *Microsoft Excel* and *StatView 512* were the computer programs used.

Both SPS and SS activities varied during the light period, in lupin leaves, reaching a peak after 4 h of illumination. Thus, enzymes activities were determined in leaves illuminated for 4 h. Salt stress led to an increase in SS activity (Table 2). SPS reaction limiting velocity (V_{lim}) and SPS reaction maximum velocity (V_{max}) decreased significantly under 150 mM NaCl (Table 2). Acid invertase activity was higher in leaves of plants treated with 50 mM NaCl but a similar value to control was achieved under 150 mM treatment. On the contrary, alkaline invertase activity increased significantly in treated plants, although no significant difference was obtained between treatments (Table 2). Fructose content did not change significantly with salt stress whereas glucose content decreased (Table 1). Sucrose content did not change at 50 mM NaCl treatment but it was almost three times higher in plants treated with 150 mM NaCl.

We obtained, under salt stress, significantly lower values of SPS maximal velocity (V_{max}) and SPS limiting velocity (V_{lim}) (Table 2), which were probably due to a lower photosynthetic rate in lupin plants treated with NaCl (Fernandes and Arrabaça 1999). Sucrose content increased almost three times with salt treatment (Table 1). Simultaneously, SS activity increased with salinity (Table 2) and sucrolytic SS activity decrease under salt stress, which has already been reported (Balibrea *et al*. 2001). Therefore SS is, at least, partially responsible for high sucrose content.

High invertases activities induced by salt (Table 2) were presumably due to enzymes synthesis *de novo* (Davies *et al*. 1991), but ion accumulation in leaves of *Phaseolus vulgaris*, under salt stress, cause acid invertase synthesis inhibition without interferes with alkaline invertase (Hawker 1980). So, ion accumulation in leaves of *Lupinus albus* L., submitted to salt stress, could explain acid invertase activity reduced to control levels without affecting alkaline invertase.

Fructose content (Table 1) in lupin leaves did not change significantly under salt stress, as also happened in *Lycopersicon esculentum* L. leaves submitted to NaCl excess (Pérez-Alfocea and Larher 1995). On the contrary,

Table 1. Effect of salt stress on glucose, fructose and sucrose contents of lupin leaves [mg g^{-1}(f.m.)]. Means ± SD of seven replicates. *ANOVA* followed by Fisher's PLSD test was applied. Means with the same letters are not significantly different at $P \leq 0.1$.

Treatment	Glucose	Fructose	Sucrose
Control	0.65 ± 0.18a	0.05 ± 0.01a	0.68 ± 0.13a
50 mM NaCl	0.52 ± 0.12b	0.05 ± 0.01a	0.58 ± 0.04a
150 mM NaCl	0.48 ± 0.11b	0.06 ± 0.01a	1.58 ± 0.12b

Table 2. Effect of salt stress on SS activity [nmol (sucrose synthesized) mg^{-1}(prot.) s^{-1}], SPS reaction limiting velocity, SPS reaction maximal velocity [nmol (sucrose synthesized) mg^{-1}(prot.) s^{-1}], acid invertase activity and alkaline invertase activity [nmol (sucrose hydrolysed) mg^{-1}(prot.) s^{-1}] in lupin leaves. Means ± SD of seven replicates. Means with the same letters are not significantly different at $P \leq 0.1$.

Treatment	SPS V_{lim}	SPS V_{max}	SS	Acid invertase	Alkaline invertase
Control	1.12 ± 0.18a	2.13 ± 0.22a	0.97 ± 0.23a	0.74 ± 0.11a	0.04 ± 0.00a
50 mM NaCl	0.85 ± 0.22ab	2.67 ± 0.12a	1.32 ± 0.26b	2.43 ± 0.63b	0.35 ± 0.01b
150 mM NaCl	0.47 ± 0.14b	1.10 ± 0.08b	2.75 ± 0.49c	0.73 ± 0.35a	0.50 ± 0.10b

salt stress led to glucose content decrease (Table 1), which can reflect an increased glucose demand for cell respiration (Guerrier 1988) in order to assure continuous ATP and NAD(P)H supply, required to avoid or repair salt damages (Krishnaraj and Thorpe 1996). High sucrose content in *Lupinus albus* L. leaves (Table 1) might be a physiological adaptation mechanism to NaCl excess as Munns *et al*. (1982) and Elavumoottil *et al*. (2003) had reported.

We concluded that the most significant response, of lupin plants to NaCl excess, is the increase of sucrose content in leaves, which is, partially, due to SS activity increase under salinity.

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