Effect of Salinity on Accumulation of *Chiro*-inositol and Other Non-structural Carbohydrates in *Limonium*

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Salt stress is a worldwide environmental factor seriously limiting crop productivity. Reducing salinity impact on crop production and increasing crop salt tolerance are important global issues, which rely greatly on the understanding of plant physiological and biochemical bases for salt tolerance.

Salt present in the root zone decreases the osmotic potential and causes water stress in the plant. One almost universal response by plants is the synthesis of small osmotically active metabolites in the cell, which lowers cellular osmotic potential and increases the plants ability to retain or take up water (Hare *et al.*, 1998). These metabolites are usually derived from soluble nitrogenous compounds and carbohydrates. Most of these metabolites are considered as compatible (noninhibitory to cell normal metabolism) solutes and, in addition to their osmolytic function, are able to assume various protective functions for alleviating further salt stress impacts such as overproduction of toxic oxidative reactive agents, disruptions of membrane and protein structures and functions (Bohnert and Shen, 1999). Their synthesis and accumulation are likely correlated with salt tolerance (Popp and Smirnoff, 1995). Compatible solute accumulation during stress is thought to be one of the most effective and fundamental adaptation mechanism for enhancing plant salt tolerance.

Floral crops exhibit a great diversity in their salt tolerance. *Limonium* (*L.*) is a genus containing several popular floral crops which are salt-tolerant. Recently, pinitol, a cyclic sugar alcohol (cyclic polyol, or cyclitol), was identified to occur in *L. gmelini* subsp. *hungarica,* in response to salt stress. However, for *L. perezii* and *L. sinuatum,* two members of USA floral industry, little is known about their sugar composition and their response to salt stress. In this study, we characterized sugar accumulation in the two *L.* species grown under different salinities. Starch, the other main form of non-structural carbohydrate, was also examined for a further understanding of carbohydrate storage in the salt-stressed plants.

MATERIALS AND METHODS

L. perezii cv. 'Blue Seas' and *L. sinuatum,* cv. 'American Beauty', were grown in sand tanks (1.2 × 0.6 × 0.5m deep) in greenhouse (Riverside, California) (day/night temperature: 12-36/11-32 °C; humidity: 42-48%) with a randomized block design for 7 salinities (three sand tanks/salinity). Fifteen seedlings were grown in each tank. Salinities were constructed based on the projected salt composition of drainage of San Joaquin Valley of California (Table 1) (Suarez and Simunek, 1997). In the second year experiment (2001), Colorado River water salt composition (relatively lower SO_4^{2-} and higher Cl^- constituents) was also used for salt treatments. A modified Hoagland's base nutrient solution (BNS) for irrigation served as the control (electrical conductivity, 2.5 dS m^{-1}). The salt treatments were achieved by the addition of the salts to the BNS. Each irrigation was of 15 min duration (three times daily). Overall, the solution pH ranged from 7.7 to 8.2.

Two to five plants were taken between 11:30 and 12:30 from one tank and the samples were immediately processed, deep frozen under -80 °C and then freeze-dried at - 50 °C for 72 h in a Freeze Dry System. The dried samples were ground using a Wiley mill to pass through 0.635 mm screen and 0.1 g ground tissue was used for carbohydrate determination. Soluble sugars were extracted and quantified using HPLC (Liu *et al.*, 1999) with a Dionex CarboPac

Table 1. Composition of salinizing salts in irrigation solutions constructed to simulate increasing salinities typically in San Joaquin Valley drainage waters.

Salinity level (dS m^{-1})	Ca^{2+}	Mg^{2+}	Na$^+$	SO$_4{}^{2-}$	Cl$^-$
			(mol m^{-3})		
2.5	2.5	1.5	13.8	7.0	7.0
6.0	6.3	4.9	43.7	21.0	21.1
7.0	7.8	5.5	50.9	29.5	24.7
8.0	8.3	6.6	58.2	29.5	28.2
10.0	10.4	8.3	73.3	37.2	35.5
11.0	11.8	9.3	82.0	42.0	38.5
12.0	12.6	10.0	88.5	44.9	42.8
15.0	13.0	13.9	123.0	58.2	59.6
16.0	13.4	15.5	137.0	63.9	66.4
20.0	13.5	20.1	178.0	79.0	86.3
25.0	13.8	27.9	247.0	104.0	111.0
30.0	14.0	33.6	298.0	124.0	144.0

PA1 column connected to an ESA Coulochem II Electrochemical Detector. *Chiro*-inositol and *myo*-inositol were separated and identified using paper chromatography (Liu *et al*., 2002), and confirmed by NMR 500 MHz (^{1}H) spectra. Starch was enzymatically hydrolyzed to glucose and quantified by comparison to a known glucose standard using a Microplate Spectrophotometer at 340 nm.

Significance of salt effect on sugar and starch concentrations and of their differences between species or between salt compositions were analyzed at $P \leq 0.05$ using GLM and TTEST procedures, respectively, in SAS-2001 statistical package.

RESULTS AND DISCUSSION

In addition to *myo*-inositol, another cyclitol, *chiro*-inositol, was identified for the first time to occur in the two *L.* species. Murakeözy *et al*. (2002) reported that *L. gmelini* accumulated significant amount of pinitol, a prevalent stress-inducible cyclitol. However, it was not detected in the shoots of both *L. perezii and L. sinuatum* under either salt- or nonsalt-stress condition. Apparently different species within the same genus may accumulate different cyclitols. Three common sugars, fructose, glucose and sucrose, were also detected in the two *L.* species.

Before salt treatment, *myo*-inositol and *chiro*-inositol concentrations were relatively low in both leaves and roots even though the three common sugar concentrations were quite high (Table 2). Starch level was high in the leaves but low in the roots (Table 2). Salt treatments altered soluble sugar accumulation. Most noteworthy is the finding that leaf *chiro*-inositol concentration increased dramatically from 6.4 to 52.8 (*L. perezii*) and from 2.6 to 72.9 (*L. sinuatum*) µmol g^{-1} dry weight as salinity increased from 2.5 to 30 dS m^{-1} (Figure 1A). Such large increases indicates that *chiro*-inositol can contribute significantly to osmotic adjustment in stressed plants. Because the seedlings contained little *chiro*-inositol prior to salt treatment, the increase in *chiro*-inositol level with increasing salinity did not come from its pre-accumulation but was a salt-induced response of more *chiro*-inositol synthesis per unit of biomass formation. Meanwhile, leaf *myo*-inositol concentration remained low and showed no significant response to salinity (Figure 1B).

A significant ($P \leq 0.05$) salt effect on leaf glucose and fructose concentrations for both *L.* species was also found. However, the changes in their levels with increasing salinity were apparently much less remarkable than *chiro*-inositol (Figure 1C-D). Sucrose concentration

Table 2. Distributions of soluble sugars (μmol g^{-1} $_{dwt}$) and starch (mg g^{-1} $_{dwt}$) in leaves and roots of *L.* seedlings of 29 days old grown under nonsalt-stress condition. Values are means ±1 SE, n=3 for leaves and n=2 for roots (a pooled sample of 12-40 plants was for one replication.). Total sugar: sum of the individual sugars. dwt: dry weight.

	*Chiro-*inositol	*Myo-*inositol	Fructose	Glucose	Sucrose	Total sugar	Starch
L. perezii							
Leaf	2.7±0.7	15.7±0.5	44.9±0.9	42.2±1.9	82.7±5.4	188±7.8	166±3.9
Root	1.5±0.1	6.8±0.1	51.9±1.0	32.7±0.5	120±1.9	213±0.2	26.5±2.0
L. sinuatum							
Leaf	2.3±0.2	15.4±1.2	42.1±8.1	37.9±5.8	95.6±11.3	193±26.0	204±16.7
Root	1.8±0.1	7.7±0.8	49.6±4.2	37.5±3.5	159±18.3	256±26.9	25.8±1.7

decreased significantly ($P \leq 0.05$) in *L. perezii* but showed no significant ($P>0.05$) change in *L. sinuatum* with increasing salinity (Figure. 1E). Probably as a result, *L. sinuatum* leaves had higher sucrose but lower glucose and fructose concentrations than *L. perezii* leaves under salt stress. This may be related to the difference of their growth vigor under salt stress (Grieve et al., 2004) although further evidence is needed to support this contention.

No significant ($P>0.05$) salt effect was found on the sum of the three common sugar concentrations for both species. Thus, the *chiro*-inositol response to salinity mainly accounted for the increase trend in total soluble sugar concentration (Figure 1F), and also accounted for a carbon partitioning change between soluble sugars, observed in the increase in the ratio of *chiro*-inositol over the sum of fructose, glucose and sucrose (rcofgs), from 0.034 to 0.29 (*L. perezii*) and from 0.012 to 0.32 (*L. sinuatum*) as salinity increased from 2.5 to 30 dS m^{-1} (Figure 1H). This preference of more *chiro*-inositol accumulation over the common sugars under the salt stress did not affect starch accumulation in the both *L.* species ($P>0.05$) (Figure 1G), and did not appear to cause a carbohydrate limitation.

Cells might need to accumulate more *chiro*-inositol for dealing with increasing salinity because *chiro*-inositol is nonreactive (Popp and Smirnoff, 1995), and as a polyol, more compatible than the common sugars (Gorham *et al*., 1981). Polyols are shown to be effective in scavenging radical oxygen species and have protective functions for proteins and membrane (Williamson *et al*., 2002). Both *L.* species survived of all salt treatments and finished their life cycle showing no any visual stress injury (Grieve, *et al*., 2004). The increased *chiro*-inositol accumulation might be inevitably involved in protecting plants from salt-stress damages via those polyol protective functions and played adaptive role for *L.* species salt tolerance.

The results from the second year experiment with *L. perezii* showed the similar responses of all leaf soluble sugar and starch concentrations as well as rcofgs to salinities of either San Joaquin Valley drainage water or Colorado River water salt composition, compared with that from the first year experiment (Figure 1). Overall, the two salt compositions with the same series of salinities resulted in no significant ($P>0.05$) difference in leaf non-structural carbohydrate accumulation in *L.* species.

CONCLUSIONS

Chiro-inositol occurs in the two *L.* species and its enhanced accumulation by salt stress contributes to cell stable osmotic pressure increase and appears an important physiological process for *L.* plants to adapt to salt stress. This work also provides new information for gene target search in transformation for enhanced crop salt tolerance.
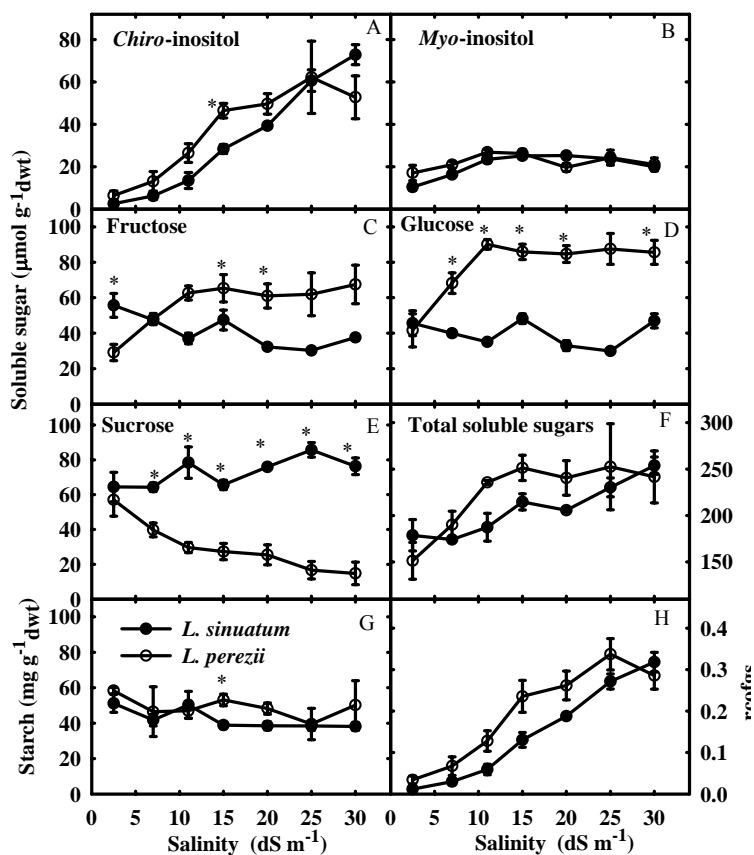
Figure 1. Leaf soluble sugar (A-F) and starch (G) concentrations, and ratio of *chiro*-inositol over the sum of fructose+glucose+sucrose (rcofgs) (H) in response to salinity (San Joaquin Valley drainage water salt composition, 67 and 31 days of salinization during vegetative growth in corresponding to the long (180-200 days) and short (110-120 days) time-to maturity for *L. perezii* and *L. sinuatum*, respectively). Total soluble sugars are the sum of all the detected sugars. Vertical bars represent ±1 SE, n=3 (tanks). Significant difference (*P*#0.05) between the two species is marked with *. No this mark means no significant difference (*P*>0.05).

REFERENCES

Bohnert, H.J. & Shen, B. 1999. Transformation and compatible solutes. Sci. Hort. 8:237-260.

Gorham, J. Hughes, L.I. & Wyn Jones, R.G. 1981. Low-molecular-weight carbohydrates in some salt-stressed plants. Physiol. Plant 53: 27-33.

Grieve, C.M. Poss, J.A. Draper, J.H. Grattan, S.R. Shouse, P.J. Lieth, J.H. & Zeng, L. 2004. Productivity and mineral nutrition of *Limonium* species irrigated with saline waters. Submitted to J. Amer. Soc. Hort. Sci.

Hare, P.D. Cress, W.A. & Van Staden, J. 1998. Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ. 21:535-553.

Liu, X. Robinson, P.W. Madore, M.A. Witney, G.W. & Arpaia, M.L. 1999. 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology. J. Amer. Soc. Hort. Sci. 122:671-675.

Liu, X. Sievert, J. Arpaia, M.L. & Madore, M.A. 2002. Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. J. Amer. Soc. Hort. Sci. 127:108-114.

Murakeözy, É. P. Smirnoff, N. Nagy, Z. & Tuba, Z. 2002. Seasonal accumulation pattern of pinitol and other carbohydrates in Limonium *gmelini* subsp. *hungarica*. J. Plant Physiol. 159:485-490.

Popp, M. & Smirnoff, N. 1995. p.199-215. Polyol accumulation and metabolism during water deficit. In Smirnoff N. (ed.), Environment and plant metabolism Springer-Verlag, Berlin.

Suarez, D.L. & Simunek, J. 1997. UNSATCHEM: Unsaturated water and solute transport model with equilibrium and kinetic chemistry. Soil. Sci. Soc. Am. J. 61:1633-1646.

Williamson, J.D. Jennings, D. B. Guo, W.W. Pharr, D. M & Ehrenshaft, M. 2002. Sugar alcohols, salt stress, and fungal resistance: polyols-multifunctional plant protection? J. Amer. Soc. Hort. Sci. 127:467-473.