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Comparative Analysis of Osmolyte Accumulation in three *Juncus* Species, in Response to Abiotic Stress

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Abstract. Accumulation of compatible solutes or 'osmolytes' is a general response of plants to abiotic stress conditions, but we still have very limited knowledge on the relevance of this response for stress tolerance mechanisms of a given tolerant species. We have determined the levels of common osmolytes (proline, glycine betaine and total soluble sugars) in three *Juncus* species, two salt tolerant (*J. acutus* and *J. maritimus*) and one salt-sensitive (*J. articulatus*), subjected to salt or water stress treatments. Correlation of the patterns of osmolyte accumulation with salt tolerance of the three taxa, indicated that biosynthesis of Pro (but not the increase of glycine betaine or sugar contents) plays a relevant role in the stress tolerance of the analysed *Juncus* species.

Keywords: *Juncus*, osmolytes, sugars, proline, glycine betaine

Introduction. The biosynthesis of osmolytes is a general response of all plants to environmental stress conditions causing cellular dehydration, including drought and high soil salinity. These 'compatible solutes' help the plants to maintain cellular osmotic balance and have additional 'osmoprotective' functions, as low-molecular-weight chaperons and/or ROS scavengers. It is generally assumed that this response is important for stress tolerance, but the relative contribution of different osmolytes to tolerance mechanisms of specific taxa remains largely unknown. Comparative analyses of osmolyte accumulation in plant species closely related taxonomically, but with different degrees of stress tolerance, may help to answer this question.

Aims and objectives. This work's aim was to determine changes in the levels of the most common osmolytes – proline (Pro), glycine betaine (GB) and total soluble sugars – upon water and salt stress treatments, in plants of three *Juncus* species, two salt-tolerant (*J. acutus* and *J. maritimus*), and one salt-sensitive (*J. articulatus*).

Materials and methods. Seeds of the three investigated species, collected in the field, were germinated in pots, in a mixture of peat and vermiculite (3:1). Two-month-old plants were treated with increasing NaCl concentrations or subjected to water stress for up to eight weeks, under controlled conditions in the greenhouse. Pro, GB and total sugars contents in plant leaves were determined using standard spectrophotometric assays, as described by Bates et al. (1973), Grieve and Grattan (1983) and Dubois et al. (1956), respectively.

Results and Discussion. Proline contents increased in salt-treated plants in a time- and concentration-dependent manner, but with clear quantitative differences between species. Under the most stressful conditions tested (400 mM NaCl, 8 weeks) Pro levels reached >100 µmol/g DW in the salt-tolerant species, which represents a ~45-fold increase as compared to non-stressed controls. The same pattern was observed in water-stressed plants, although absolute Pro contents in *J. acutus* and *J. maritimus*, after 8 weeks without watering, were even higher than in the 400 mM NaCl-treated plants (e.g., 70-fold increase over the control in *J. maritimus*) (Table 1). In the salt-sensitive *J. articulatus*, background Pro contents

were slightly lower than in the tolerant taxa and only doubled under salt or water stress conditions (Tab. 1). Regarding glycine betaine (Tab. 2) or total sugars (Tab. 3), their levels in the control plants were similar for the three analysed taxa, and the stress-induced accumulation of these osmolytes was much lower than that of Pro (only 1.5 to 3.2-fold increase); most importantly, contrary to what was observed for Pro patterns, there were no relevant differences between the salt-tolerant and sensitive *Juncus* species.

Tab. 1

Proline contents in plants of different *Juncus* species after 8 weeks of water and salt stress treatments, as indicated

Proline (µmole/gr DW)	Stress treatments (8 weeks)				
	Water Stress	control	100 mM NaCl	200 mM NaCl	400 mM NaCl
J. articulatus	3.78	1.90	2.31	2.56	4.31
J. acutus	121.84	2.40	28.68	52.99	110.68
J. maritimus	199.54	2.82	21.77	28.76	128.58

Tab. 2

Glycine betaine contents in plants of different *Juncus* species after 8 weeks of water and salt stress treatments, as indicated

Glycine betaine (µmol/gr DW)	Stress treatments (8 weeks)				
	Water Stress	control	100 mM NaCl	200 mM NaCl	400 mM NaCl
J. articulatus	16.85	9.26	11.37	12.17	18.69
J. acutus	28.93	13.59	21.00	27.16	36.04
J. maritimus	22.24	10.95	15.97	19.34	23.20

Tab. 3

Total sugar contents (expressed as ‘mg equivalent of glucose per gr dry weight’) in plants of different *Juncus* species after 8 weeks of water and salt stress treatments, as indicated

Total sugars (mg eq glucose/g DW)	Stress treatments (8 weeks)				
	Water Stress	control	100 mM NaCl	200 mM NaCl	400 mM NaCl
J. articulatus	73.69	50.71	54.70	64.30	72.63
J. acutus	63.40	31.09	45.95	55.23	77.20
J. maritimus	89.57	30.77	36.18	49.57	98.64

Conclusion

Proline, glycine betaine and sugars may all contribute to cellular osmotic adjustment under salt and water stress conditions in the investigated *Juncus* species, but our results strongly suggest that accumulation of Pro (but not of the other osmolytes) plays a relevant functional role in the mechanisms of stress tolerance in the analysed *Juncus* species.

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