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Stress-induced Accumulation of Osmoprotectants in four *Plantago* Species

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Abstract. We have studied whether two common osmolytes – proline (Pro) and glycine betaine (GB) – accumulate in plants of different *Plantago* species in response to salt and water stress, which would suggest that these compatible solutes and 'osmoprotectants' fulfil a functional role of in the plants' stress tolerance mechanisms. Plants of *P. coronopus*, *P. major*, *P. crassifolia* and *P. lagopus* were treated with increasing NaCl concentrations or subjected to water stress, under controlled conditions in the greenhouse. GB and Pro contents in control, non-stressed plants were relatively low and, in most cases, did not change, or increased only moderately when the plants were maintained without water or were grown in the presence of up to 400 mM NaCl, for four weeks. However, higher salt concentrations (450 – 600 mM NaCl), which these taxa never encounter in their natural habitats, induced a significant accumulation of Pro in all species but *P. major*, the most salt-sensitive one. These data indicate that GB and Pro do not act as functional osmolytes in *Plantago* species, and do not contribute to osmotic adjustment under natural conditions; however, these plants have the potential to activate Pro-mediated mechanisms of salt tolerance at very high, artificial salinity levels.

Keywords: *Plantago*, osmolytes, sorbitol, glycine-betaine, proline

Introduction. Sorbitol is well established as the major, functional osmolyte in the genus *Plantago*, responsible for osmotic adjustment under abiotic stress conditions (Flowers et al., 2010). However, the contribution of minor osmolytes to stress tolerance – based on their roles as 'osmoprotectants' (low-molecular-weight chaperons and/or ROS scavengers) – remains largely unknown.

Aims and objectives. We have determined the levels of two of the most common osmolytes in plants, proline (Pro) and Glycine betaine (GB), in plants of different *Plantago* species subjected to salt and water stress treatments, to investigate the possible role of these osmoprotectants in the plants' stress tolerance mechanisms.

Materials and methods. Seeds of *P. coronopus*, *P. major*, *P. crassifolia* and *P. lagopus*, 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 four weeks, under controlled conditions in the greenhouse. Pro and GB contents in plant leaves were determined using standard spectrophotometric assays (Bates et al., 1973; Grieve and Grattan, 1983).

Results and Discussion. Pro levels in control, non-stressed plants were very low in *P. coronopus* (ca. 0.5 μmol/g DW), *P. major* and *P. crassifolia* (ca. 1.5 μmol/g DW) and increased only slightly (< 2-fold) upon water stress treatments or when grown in the presence of NaCl up to 400 mM. In the case of *P. lagopus*, control Pro contents were somewhat higher (about 5.0 μmol/g DW), but did not change in stressed plants (Tab. 1). Higher salt concentrations (450 – 600 mM NaCl), however, resulted in a significant accumulation of Pro (20 to > 50-fold as compared to the controls) (Fig. 1, and data not shown) in all species except *P. major*, which is the most stress-sensitive taxon and did not survive such a high salinity

level. Pro levels also increased significantly in *P. coronopus* (~ 100-fold) after 4 weeks of water stress (Table 1). Regarding GB contents, control values reached ca. 6.5 µmol/g DW in *P. coronopus* and about double in the other three species. In all cases, salt and water stress treatments led to a slight increase in GB levels, about 1.5-fold in *P. major*, *P. crassifolia* and *P. lagopus*, and 2 to 2.5-fold in *P. coronopus* (data not shown).

Tab. 1

Proline contents in plants of different *Plantago* species after 4 weeks of water and salt stress treatments, as indicated

Proline (µmol/gr DW)	Stress treatments (4 weeks)				
	Water Stress	control	100 mM NaCl	200 mM NaCl	400 mM NaCl
P. crassifolia	0.75	1.70	2.36	1.37	1.41
P. lagopus	2.39	2.20	3.44	2.15	1.56
P. coronopus	57.22	0.48	0.44	0.59	0.68
P. major	11.2	1.98	1.98	2.35	2.03

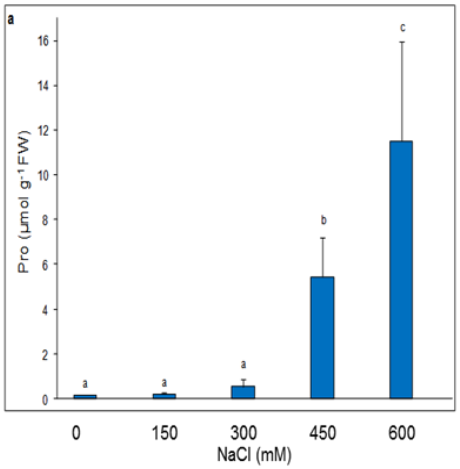

Fig. 1. Pro accumulation in *Plantago crasifolia* plants treated for four weeks with NaCl at the indicated concentrations

Conclusion

Pro and GB levels in the investigated *Plantago* species were too low to have any significant effect on osmotic adjustment under stress conditions. However, salt treatments over 400 mM NaCl induced the biosynthesis and accumulation of Pro at relatively high concentrations, at least in *P. coronopus*, *P. crassifolia* and *P. lagopus*. Therefore, these plants have the potential to activate Pro-mediated stress tolerance mechanisms, probably unrelated to maintenance of osmotic balance, but based on Pro osmoprotectant functions. Presumably, they do not need to do it in their natural habitats, where they are normally not exposed to such salinity levels and sorbitol accumulation is sufficient to confer tolerance.

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