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Seasonal Variation of Glycine Betaine in Plants from a Littoral Salt - Marsh in SE Spain

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SUMMARY

A general response of plants to salt stress, observed in salt-sensitive as well as tolerant species, relies on the accumulation of compatible osmolytes in the cytoplasm of the plant cells; osmolytes help to maintain osmotic balance, act as direct 'osmoprotectans' of macromolecular structures under stress conditions, and prevent oxidative stress as scavengers of ROS. However, it is not clear the relevance of this conserved response for the mechanisms of salt tolerance of halophytes – defined as plants able to tolerate and complete their life cycle in conditions of high soil salinity. The aim of the present study was to determine the levels of glycine betaine (GB) – one of the most common plant osmolytes – in several species from a littoral salt marsh in SE Spain, and to correlate changes in GB contents with the environmental conditions of the plants. Plant material was collected three times per year – in spring, summer and autumn – in 2009 and 2010, from three 100 m^2 (10 x 10 m) experimental plots with different soil salinity. The species included in the study were the following:

- ***i)*** from plot 1, located at the edge of the salt marsh and with low salinity: *Rosmarinus officinalis*, *Helianthemum syriacum*, *Schoenus nigricans*, *Plantago crassifolia* and *Juncus acutus*;
- ***ii)*** from plot 2, with intermediate soil salinity level: *P. crassifolia*, *J. acutus*, *J. maritimus*, and *Sarcocornia fruticosa*;
- ***iii)*** from plot 3, located in the centre of the marsh and with the highest salinity: *J. maritimus*, *S. fruticosa* and *Inula crithmoides*.

Plant material (green leaves or culms) were dried in an oven at 65 ºC until constant weight, ground in a coffee grinder and stored at room temperature. Glycine beatine was quantified using standard spectrophotometric assays. Among the taxa under study, *Sarcocornia fruticosa* was by far the species which accumulated the highest GB content (437 μmol g^{-1} DW, as average), followed by *Inula crithmoides* (188), both succulent plants; all other taxa showed similar low values, below 15 μmol g^{-1} DW. In most investigated species, including those with relatively low levels of the osmolyte, we could detect a significant seasonal variation of GB contents, which were particularly higher in the very dry summer of 2009 – except for the non-tolerant *R. officinalis* and *H. syriacum*. Regarding those species present in two different plots – *P. crassifolia*, *J. acutus*, *J. maritimus*, and *S. fruticosa* – a positive correlation between GB accumulation and soil salinity was observed in the samples collected in summer 2009; in the other seasons, differences between plots were not

significant, or no clear patterns of variation were detected. Therefore, the main trigger of GB biosynthesis appears to be water stress, specifically the summer drought characteristic of Mediterranean climates, with at least two consecutive months of high temperatures and no rain, conditions which also increase the salinity of the soil in the salt marsh. Taken together, these data support the notion that glycine betaine biosynthesis is indeed involved in the mechanisms of salt tolerance of halophytes in their natural habitats, even in those species which do not accumulate GB to high levels and use other osmolyte(s) – such as proline or some sugars – for maintaining osmotic balance.

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