

Osmolytes in salinity-stressed *Iris hexagona*

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Abstract We characterize the salinity stress response of *Iris hexagona*, a freshwater species, by measuring three putative osmolytes, betaine, proline, and dimethylsulphoniopropionate (DMSP) in plants after short (3 days) and long-term (4–5 months) exposure to NaCl. HPLC analyses show that untreated control leaves contained 2.1, 0.2 and 3.2 mg g⁻¹ DW of betaine, proline, and DMSP, respectively, and establish the presence of these compounds in the Iridaceae. Within 4 days of salinity stress (200 mM NaCl) betaine, proline and DMSP increased significantly. Among vegetative tissues, the highest level of proline occurred in roots; betaine and DMSP were highest in leaves. Analyses of generative tissue (flowers and flower stalks) after long-term exposure showed the highest levels (>10 mg g⁻¹ DW) of all examined compounds in petals but only proline and betaine increased with salinity. All three substances showed a basipetal gradient in flower stalks. Although the examined compounds responded to salinity, the osmometry of the sap indicated that they comprise less than 10% of the osmotically active solutes. The temporal and spatial changes in the distribution of the analyzed compounds indicate complex responses to salinity.

Keywords *Iridaceae* · Betaine · Dimethylsulphoniopropionate (DMSP) · Proline · Salinity response

Introduction

We are interested in mechanisms by which freshwater species survive and adapt to salinity stress. Our model species, *Iris hexagona* Walter (Iridaceae), is native to wetlands of the North American Gulf Coast and is exposed to several stresses such as salinity (Van Zandt and Mopper 2004), herbivory (Mopper et al. 2004; Schile and Mopper 2006), and florivory (Tobler et al. 2006). The large iris rhizomes protect coastal wetlands from erosion but ever increasing salinity negatively affects growth. Previous work has shown that salinity-stressed *I. hexagona* strongly increase production of ABA and JA hormones (Wang et al. 2001) and confirmed other reports of hormonal control of osmoprotective pathways. For instance, abscisic acid (ABA) is required for salt-stress induced proline accumulation (Strizhov et al. 1997), and betaine production (Nakamura et al. 2001).

Nitrogen-containing compounds (NCCs) serve as osmoprotectants but vary with plant species and include amino acids (proline), amides, imino acids, proteins, quaternary ammonium compounds such as betaines and polyamines (Rabe 1990). These compounds attenuate the inhibitory effect of ions on enzyme activity (Solomon et al. 1994), increase the thermal stability of enzymes (Galinski 1993) and prevent the dissociation of the oxygen-evolving complex of photosystem II (Papageorgiou and Murata 1995; Zhu 2002). The wide distribution of proline, betaine and dimethylsulphoniopropionate (DMSP) prompted our quantification of these putative osmoprotectants.

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The amino acid proline has multiple stress-reducing functions in *Spartina anglica* (Mulholland and Otte 2002), sugar beet (Ghoulam et al. 2002), and *Phragmites australis* (Rolletschek and Hartzendorf 2000). Proline reduced levels of free radicals and improved the ability of transgenic seedlings to grow in salt-containing media (Hong et al. 2000). Addition of proline to cultures of salt-sensitive cell lines of peanut (*Arachis hypogaea* L.) reduced salt-induced decrease in fresh weight and peroxidative damage to membranes (Jain et al. 2001). Suppression of proline degradation improved tolerance to freezing and salinity in *Arabidopsis* (Nanjo et al. 1999).

Betaine (glycine betaine) is a quaternary ammonium compound, which can be induced by salt in barley (Wang and Nii 2000), *Ipomoea pescaprae* (Venkatesan and Chellappan, 1998), and in salt-tolerant grasses (*Anneurolepidium chinense*, Ochiai and Matoh, 2001; *S. anglica*, Mulholland and Otte, 2002). Importantly, betaine is induced by salts (KCl, MgCl₂, Na₂SO₄), H₂O₂, and organic compounds such as ABA, salicylic acid and *n*-butanol (Jagendorf and Takabe 2001), as well as drought (Hitz et al. 1982) and cold stress (Kishitani et al. 1994). In addition to its protection of protein complexes and membranes (Sakamoto and Murata 2000), betaine also induced low temperature responsive genes (Allard et al. 1998).

DMSP is a tertiary sulfur compound and a precursor of dimethyl sulfide (DMS), which is involved in the global cycling of sulfur (Colmer et al. 2000). Salinity, light, temperature and nitrogen availability affect DMSP biosynthesis (Stefels 2000). Many marine algae accumulate this compound but some vascular plants also contain DMSP (Rhodes and Hanson 1993) but high levels occur only in *Spartina*, *Wollastonia* and *Saccharum* (Otte and Morris, 1994). Although DMSP increased in response to salinity in *S. anglica* (Mulholland and Otte, 2000, 2002), *S. alterniflora* showed no changes that correlated with salinity or soil sulfide concentrations (Otte and Morris 1994). The increase of DMSP at low water temperature (0 vs 10°C) in some macroalgae suggests that DMSP also functions as a cryoprotectant (Karsten et al. 1992).

We hypothesize that the documented hormonal responses of *I. hexagona* to salinity stress stimulate accumulation of secondary compounds that contribute to salt tolerance. Here we report that the concentrations of the putative osmolytes vary with salinity but constitute only a minor portion of iris osmolytes.

Materials and methods

Plant materials and salinity treatment

Responses to short-term salinity stress were examined in about 8-month old, pre-reproductive plants that were

exposed to 0, 200, 400 mM NaCl for 1, 2, 3, and 4 d in 4 l plastic containers. Fifteen plants were assigned to each treatment and grown under fluorescent light (12 h day/night cycle, 20 μmol m⁻² s⁻¹) at 23–25°C. The experiments were performed in triplicate with five plants harvested after one to five days of salinity exposure. Leaves, rhizomes and roots were either extracted immediately, stored in a freezer (–70°C), or lyophilized and stored at room temperature. To assess variation in metabolite concentrations, we analyzed untreated controls for each measurement. A second set of experiments examined long-term salinity exposure of plants and focused on the osmolyte content of flowers and flower stalks. These structures were collected from plants grown in a common garden at 100 mM NaCl for 4–5 months at the Center for Ecology and Environmental Technology of the University of Louisiana at Lafayette. Controls were grown in soil that contained <3 mM NaCl. Flowers were separated into petals and ovaries, stalks were separated into apical, central and basal segments, and all material was stored in a freezer until analysis.

Extraction

About 2 g of tissue was weighed and ground into powder under liquid N₂. From fresh or frozen samples a similar amount as the analyzed sample was weighed and used to determine dry weight. The samples were extracted in 5 ml of 5% cold perchloric acid at 4°C overnight (Colmer et al. 2000) and centrifuged (4,000g, 10 min). The volume of supernatant was measured and centrifuged (12,000g, 5 min) before HPLC analysis.

HPLC analysis

A DionexTM GC40 pump and GP40 UV detector were connected to a strong cation exchange column (AlltechTM 5 SCX, 250 × 4.6 mm). The column was primed with 50 mM KH₂PO₃, pH 2.25 plus 5% acetonitrile (Colmer et al. 2000) for 55 min (2 ml/min). Ten minutes before sample injection (50–150 μl) the flow rate was adjusted to 1 ml/min. Sample separation was performed isocratically for 45–60 min and absorption (190 nm) was recorded (PeakNet ver. 5.2). After each separation the column was washed with 400 mM NaCl plus 50 mM KH₂PO₃ pH 2.25 for 5 min (2 ml/min). Under these conditions, the retention times for betaine (Sigma # B3501), proline (Sigma # P0380) and DMSP (Research Plus, Manasquan, NJ) were 13, 17, and 31 min, respectively.

Betaine was confirmed by examining the HPLC eluate according to Barak and Tuma (1979). Proline was authenticated by ninhydrin (Trotel et al. 1996). The identity of DMSP was verified by head-space gas chromatography of the separated fraction. The sample was transferred into a

sealed vial, hydrolyzed with 1 mL 5N NaOH and the resulting dimethyl sulfate was separated on a Super Q 80/100 column (Alltech #9781) at 90°C, retention time = 10.8 min). We determined the sample quantity from the injected aliquot, volume of headspace and calibrated peak area.

Osmometry

To assess the osmotic potential we squeezed sap out of ca. 0.5 g frozen material in microcentrifuge tubes with a perforation at the bottom. The liquid was collected and centrifuged (10,000g × 10 min) and 10 µl of the supernatant was measured using a 5500 WESCOR vapor pressure osmometer.

Statistical analysis

The data were analyzed by a full-factorial and repeated-measure analysis of variance to assess the effects of salinity, time and tissue type on the proline, betaine and DSMP (SAS PROC GLM, SAS Version 8). Data were log-transformed when necessary to meet normality and homogeneity of variance. Tukey-adjusted, paired comparisons determined differences between individual groups.

Results

Short-term response in leaves, rhizomes and roots

The various tissues of control plants contained between 0.1 and 0.2 mg g⁻¹ DW proline but responded differently to salinity. Leaves, rhizomes, and roots contained different quantities of proline [$F_{(2,17)} = 17.2$, $P < 0.0001$, Fig. 1a–c] and showed a significant interaction between salinity and tissue [$F_{(4,17)} = 6.81$, $P = 0.002$]. Proline increased in leaves after 3 and 4 days exposure to 200 mM NaCl ($P = 0.0421$) but 400 mM NaCl did not cause additional effects (Fig. 1a). Compared with controls, proline in rhizomes of salinity-treated plants increased 5.6-fold ($P = 0.0003$) after 1 day and 3.7-fold ($P < 0.0001$) after 2 days (Fig. 1b). However, proline in untreated plants also steadily increased such that after 4 days of treatment all plants contained similar levels. In roots (Fig. 1c), controls maintained constant levels of proline (<0.3 mg per g DW) while both salinity levels increased proline during the first 3 days, but increased further on the fourth day only in 200 mM NaCl (Fig. 1c).

Betaine levels in vegetative tissue were about tenfold higher than those of proline. Salinity affected betaine in leaves, rhizomes and roots [$F_{(2,17)} = 13.36$, $P = 0.0003$]. Betaine levels increased in leaves (Fig. 1d) after 1 day of

400 mM NaCl exposure ($P = 0.0184$) and after 2 days in 200 mM NaCl treatment ($P = 0.0421$). In rhizomes, betaine did not respond to salinity (Fig. 1e). Betaine concentrations fluctuated strongly in roots (Fig. 1f) but, overall, were higher after salinity exposure ($P = 0.0004$).

The DMSP concentrations in leaves (Fig. 1g) were higher than proline or betaine and responded to both salinity treatments. It accumulated transiently after 200 and 400 mM NaCl treatment ($P = 0.037$) but, by day 4, identical levels of DMSP were observed. DMSP did not show a salinity dependent response in rhizomes (Fig. 1h) or roots (Fig. 1i).

Distribution of proline, betaine and DMSP in generative structures after long-term (4–5 months) exposure to salinity

Flowers (petals and ovaries) contained higher concentration of proline, betaine or DMSP than leaves, roots or rhizomes. Proline and betaine increased significantly in petals of salt-treated plants [Fig. 2a, b; $F_{(1,29)} = 6.78$, $P = 0.017$ for proline and $F_{(1,29)} = 13.13$, $P = 0.002$ for betaine]; ovaries showed no difference. Salinity treatments did not affect DMSP concentrations. The stalks of all salinity-treated plants contained more of the examined compounds than controls (Fig. 2a–c) with concentration decreasing from apex to base. The basal segments contained quantities comparable to leaves (compare Fig. 2, basal stalk segments with Fig. 1a, d, g). A strong interaction [$F_{(4,29)} = 5.65$, $P = 0.003$] between salinity, flowers and stalks suggest a differential salinity response for these tissues.

Proline, betaine and DMSP showed a basipetal gradient in stalks (Fig. 2). Proline (Fig. 2a) and DMSP (Fig. 2c) were equally distributed in petals and ovaries, but amounts of betaine were much higher in petals than ovaries (Fig. 2b). DMSP concentrations were highest in floral tissues but there was no strong salinity effect [$F_{(4,18)} = 2.56$, $P = 0.07$, Fig. 1c]. The lack of interaction between salinity and floral tissue [$F_{(4,29)} = 1.26$, $P = 0.319$] indicates that DMSP does not accumulate in flowers as a result of salinity. However, DMSP was elevated by salinity in stalks.

Osmolality

To examine the extent to which proline, betaine and DMSP functioned as osmolytes, the relative amount of the measured compounds was compared to the total osmolality of the sap. Proline, betaine and DMSP comprised only a small proportion (1–4%) of the total osmolytes in vegetative tissue but made up about twice this amount in flowers (see Tables 1, 2). Although we did not adjust for apoplastic

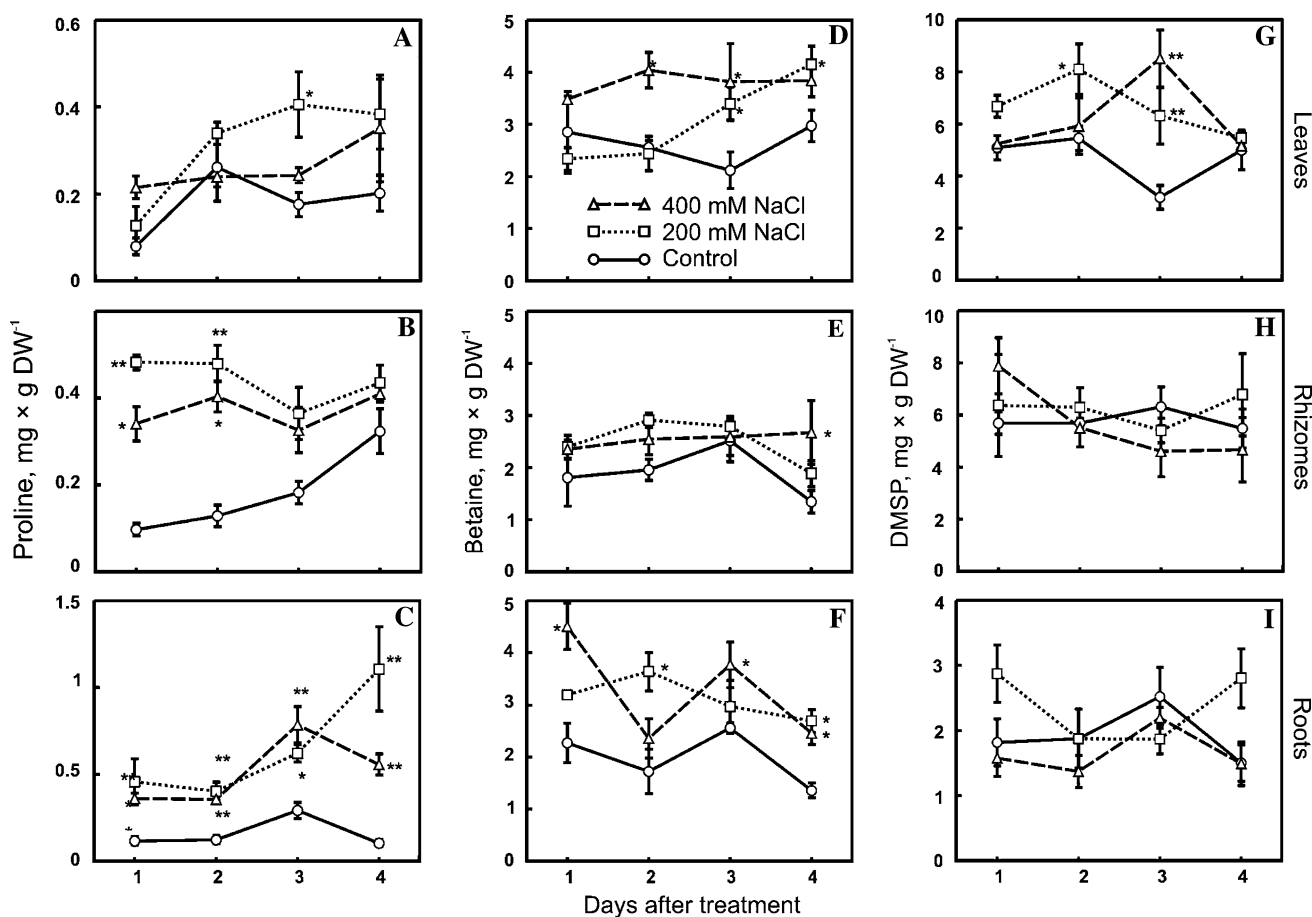


Fig. 1 Distribution of proline (a–c), glycine betaine (d–f), and DMSP (g–i) in *Iris hexagona*. Short-term effects (1–4 days) were tested in leaves (a, d, g), rhizomes (b, e, h), and roots (c, f, i) after exposure to <math><3</math> (solid line controls), 200 (stippled line), or 400

(dashed line) mM NaCl, respectively. Significance between treatment and control within the same panel is indicated by * $P < 0.05$ and ** $P < 0.001$

water, the osmolality of the cell sap after 4 days of 400 mM NaCl treatment increased substantially in all examined parts. Roots but not leaves or rhizomes also responded to 200 mM NaCl (Table 1, last column) with increased sap osmolality. Control plants contained comparable osmolalities in short-term and the long-term experiments (average of 397 and 409 mOsm, respectively). In short-term experiments, the osmolality increased with salinity and was 397, 448, and 504 mOsm for controls, 200 and 400 mM salinity treated plants, respectively.

Discussion

We present the first report of the quantitative relationship between proline, betaine, and DMSP in the Iridaceae. Concentrations of proline, betaine and DMSP in vegetative tissues of *I. hexagona* (leaves, rhizomes, roots) were similar to those found in the cold-stressed wheat seedlings (Naidu et al. 1991; 1–47 $\mu\text{mol g}^{-1}$ DW). The proline

levels in stalks, petals, and ovaries were comparable to levels in salinity-stressed *S. anglica* (Mulholland and Otte, 2002; 10–160 $\mu\text{mol g}^{-1}$ DW). However, the relatively low concentration in leaves (Fig. 1a) and the small proportion of the total osmolality of leaf, root and rhizome sap suggest that proline is not a major osmoprotective factor in vegetative organs of *I. hexagona*. The relatively large amount of proline in flowers and stalks suggests some function related to generative tissue such as storage for reduced C and N (Stefels 2000). However, the lack of induction by salinity (Figs. 1a–c, 2a; Tables 1, 2) argues against a role as osmoprotectant. In support of this conclusion, the correlation between salinity and proline in leaves, rhizomes, and roots was weaker than detected in radish (Muthukumarasamy et al. 2000) sugar beet (Ghoulam et al. 2002) or *I. pes-caprae* (Venkatesan and Chellappan, 1998).

Although betaine has been detected in a wide range of plant species, large accumulations have been reported only in Chenopodiaceae (Storey and Wyn Jones 1977). To the best of our knowledge, this is the first report that salinity

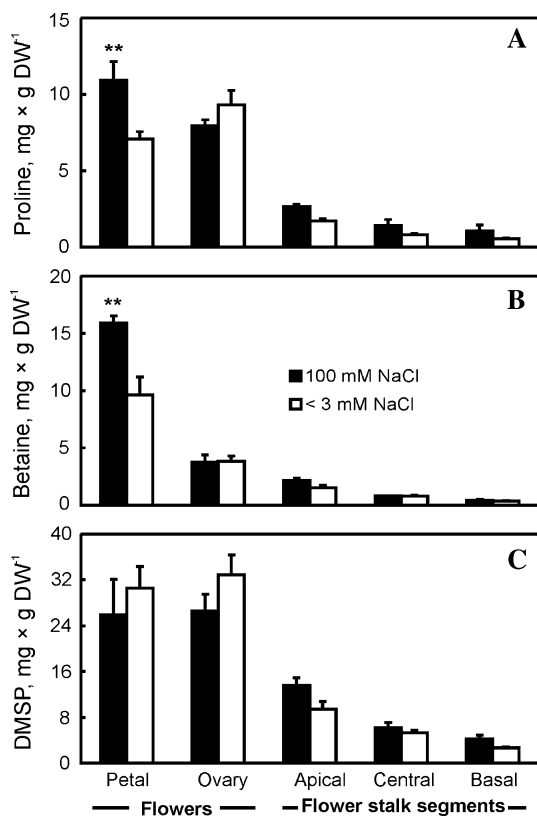


Fig. 2 Distribution of proline, glycine betaine, and DMSP in flowers and stalks of *Iris hexagona* after long-term exposure to salinity. Iris were grown in soil supplemented either with NaCl to establish 100 mM NaCl or left untreated (<3 mM NaCl). **Indicates $P < 0.001$ difference between treatments

leads to betaine accumulation in the Iridaceae. However, the accumulation is much less than that reported for salt-tolerant *S. anglica* (Subbarao et al. 2001; 25–150 $\mu\text{mol g}^{-1}$ DW, about 3–18 mg g^{-1} DW), except for salinity-stressed

petals (17 mg g^{-1} DW), which contained similar amounts as leaves of salinity-stressed *S. anglica* (Subbarao et al. 2001; Mulholland and Otte, 2002). The betaine levels in vegetative parts of iris (2–4 mg g^{-1} DW) were about 3–10 times larger than proline levels. Only flowers contained approximately equal levels of betaine and proline. The high content of betaine in flowers (Fig. 2b) may contribute to the higher seed yield and performance observed in salinity-stressed iris (Van Zandt and Mopper 2002).

In addition to osmotic adjustment, betaine-like compounds protect cellular macromolecules, provide nitrogen storage, buffering of cellular pH, detoxification, and scavenging of free radicals. Accumulation of nitrogen compounds is usually correlated with plant salt tolerance (Mansour 2000), but the exact mechanism is unknown. If osmolytes affect metabolism, hexose content or redox state, the observed gradient in the stalks (Fig. 2b) may also constitute a long-distance signal (Hare et al. 1998).

This is the first report of DMSP in the Iridaceae. Levels in *I. hexagona* were higher than in many species listed by Paquet et al. (1995) and comparable to those in *S. anglica* (Mulholland and Otte, 2002). DMSP was more concentrated than proline or betaine in leaves, rhizomes, and roots. The high concentration of DMSP, especially in flowers and stalks, implies that iris responses to salinity include DMSP. However, lower concentrations than in other species, the weak response to increased salinity (Fig. 1g–i) and the decrease in flowers after long-term salinity exposure reduce its significance as an osmolyte. Because DMSP is synthesized in chloroplasts (Trossat et al. 1998), roots and stems are important sinks for DMSP (Mulholland and Otte 2000). Therefore, DMSP could serve as a constitutive solute (Stefels 2000) that responds only transiently to salinity (see Fig. 1g).

Table 1 Concentration of Betaine, DMSP and Proline in leaves, rhizomes and roots of *Iris hexagona*

Treatment (NaCl)	Tissue	Betaine (mOsm)	DMSP (mOsm)	Proline (mOsm)	Water% FW	Sum B&D&P (mOsm)	Sum B&D&P% sap	Sap (mOsm)
Control	Leaf	4.2	6.2	0.3	70.0	10.7	2.3	476 ± 23
	Rhizome	5.0	12.4	0.4	69.8	17.7	4.2	427 ± 22
	Root	2.1	2.0	0.2	87.6	4.3	1.5	288 ± 15
200 mM	Leaf	5.3	9.8	0.6	76.3	15.7	3.4	462 ± 10
	Rhizome	5.7	11.1	0.7	68.6	17.6	3.6	489 ± 6
	Root	2.4	1.5	0.5	87.9	4.3	1.1	393 ± 13
400 mM	Leaf	4.5	10.1	0.3	81.8	14.9	2.6	569 ± 42
	Rhizome	5.3	9.5	0.7	68.4	15.5	3.0	520 ± 5
	Root	3.0	1.8	0.6	87.7	5.4	1.3	423 ± 11

The analytes were determined by HPLC from plants exposed to 200 and 400 mM NaCl for 4 days. The combined values are compared with the osmolality of the cell sap. Although the relative water content (FW%) shows a gradient between leaves and roots, the increase of the analytes (Sum B&D&P mOsm) in salinity-stressed plants did not significantly contribute to the increase of sap osmolality (Sum B&D&P % sap). The mOsm quantities of betaine, DMSP, and proline were calculated from the averages of measured values (see Fig. 1)

Table 2 Concentrations of osmolytes in flowers and stalks of *Iris hexagona*

Treatment (NaCl)	Tissue	Betaine (mOsm)	DMSP (mOsm)	Proline (mOsm)	Water% FW	Sum B&D&P (mOsm)	Sum B&D&P% sap	Sap (mOsm)
<3 mM	Stalk							
	Petal	7.4	5.4	23.4	88.3	36.2	7.3	497 ± 24
	Ovary	2.8	6.7	23.7	89.0	33.2	7.9	419 ± 27
	Apical	1.5	1.6	9.1	85.2	12.2	3.1	400 ± 18
	Central	0.8	0.9	5.5	84.1	7.2	1.9	381 ± 24
	Basal	0.4	0.6	2.8	84.3	3.7	1.1	349 ± 13
100 mM	Stalk							
	Petal	11.8	8.1	19.2	88.6	39.2	6.7	582 ± 52
	Ovary	3.3	7.0	23.3	86.6	33.6	5.7	593 ± 15
	Apical	2.4	3.0	15.2	82.9	20.6	4.0	512 ± 19
	Central	0.9	1.5	6.5	83.7	8.9	1.6	543 ± 21
	Basal	0.4	1.1	4.2	84.7	5.7	1.1	504 ± 6

Tissue of plants grown in 100 mM NaCl for 4–5 months were analyzed for betaine, DMSP, and proline by HPLC. The summed values are compared with the osmolality of the cell sap. The narrow range of water content (FW%) indicates that the sum of betaine, DMSP and proline (Sum B&D&P) comprises only a small percentage (Sum B&D&P% sap) of the total solutes in the sap (sap mOsm). The mOsm quantities of betaine, DMSP, and proline were calculated from the averages of the measured values (see Fig. 2)

The lower quantities of DMSP in flowers at higher salinity (Fig. 2c) may have important ecological consequences. At elevated pH, DMSP is easily cleaved into pungent acrylic acid and dimethyl sulfate. The reduced DMSP content in flowers of salinity-stressed iris may indirectly contribute to rampant deer florivory of salt marsh irises (Tobler et al. 2006) because the lower quantities of DMSP in salinity-stressed plants could make the flowers more palatable for vertebrate browsers.

DMSP may accumulate as a result of high levels of chemically reduced compounds and may serve as a buffer when influx of sulfur-containing compounds exceeds the plant's capacity to convert them into amino acids or proteins (Stefels 2000). The high load of reduced sulfur in marsh soil is likely to provide a suitable and metabolically inexpensive precursor for DMSP synthesis.

The much higher osmolality of the sap (Tables 1, 2) than the sum of the three analytes supports perhaps a minor role as osmolytes and suggests that other, non-identified solutes account for the bulk of osmotically active compounds. Based on the osmolality of the cell sap (about 400 mOsm, Tables 1, 2), non-challenged controls appear capable of sustaining ca. 200 mM NaCl (approximate osmolality 320 mOsm). This value is derived from three observations: sap from control plants contains higher osmolality than 200 mM NaCl (despite some dilution from apoplastic water), earlier investigations found strong ABA and JA induction at that concentration (Wang et al. 2001), and in laboratory experiments, *I. hexagona* grew for more than 4 weeks without major adverse responses at that concentration.

The lack of additional accumulation of proline, betaine, or DMSP at higher salinity (>200 mM, see Fig. 1) suggests that either the metabolic cost exceeds the capacity of the plants, or other metabolites compensate for salinity stress, or salinity inhibits the production of necessary precursors. Although the sum of proline, betaine, and DMSP represents only a fraction of the total osmolality, their response to salinity and variable distributions indicate roles in the adaptive metabolism of salt-stressed iris. Future work will identify additional osmolytes, quantify ion concentration and distribution, and assess their effects on secondary metabolism.

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References

- Allard F, Houde M, Krol M, Ivanov A, Huner NPA, Sarhan F (1998) Betaine improves freezing tolerance in wheat. *Plant Cell Physiol* 39:1194–1202
- Barak AJ, Tuma DJ (1979) Simplified procedure for the determination of betaine in liver. *Lipids* 14:860–863
- Colmer TD, Corradin F, Cawthray GR, Otte ML (2000) Analysis of dimethylsulphoniopropionate (DMSP), betaines and other organic solutes in plant tissue extracts using HPLC. *Phytochem Anal* 11:163–168
- Galinski EA (1993) Compatible solutes of halophilic eubacteria—molecular principles, water-solute interaction, stress protection. *Experientia* 49:487–496

- Ghoulam C, Foursy A, Fares K (2002) Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environ Exp Bot* 47:39–50
- Hare PD, Cress WA, Van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ* 21:535–553
- Hitz WD, Ladyman JAR, Hanson AD (1982) Betaine synthesis and accumulation in barley during field water-stress. *Crop Sci* 22:47–54
- Hong ZL, Lakkineni K, Zhang ZM, Verma DPS (2000) Removal of feedback inhibition of delta1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiol* 122:1129–1136
- Jagendorf AT, Takabe T (2001) Inducers of glycine synthesis in barley. *Plant Physiol* 127:1827–1835
- Jain M, Mathur G, Koul S, Sarin NB (2001) Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). *Plant Cell Rep* 20:463–468
- Karsten U, Wiencke C, Kirst GO (1992) Dimethylsulphoniopropionate (DMSP) accumulation in green macroalgae from polar to temperate regions—interactive effects of light versus salinity and light versus temperature. *Polar Biol* 12:603–607
- Kishitani S, Watanabe K, Yasuda S, Arakawa K, Takabe T (1994) Accumulation of glycinebetaine during cold-acclimation and freezing tolerance in leaves of winter and spring barley plants. *Plant Cell Environ* 17:89–95
- Mansour MMF (2000) Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol Plant* 43:491–500
- Mopper S, Wang YY, Criner C, Hasenstein KH (2004) *Iris hexagona* hormonal responses to salinity stress, leafminer herbivory, and phenology. *Ecology* 85:38–47
- Mulholland MM, Otte ML (2000) Effects of varying sulphate and nitrogen supply on DMSP and glycine betaine levels in *Spartina anglica*. *J Sea Res* 43:199–207
- Mulholland MM, Otte ML (2002) The effects of nitrogen supply and salinity on DMSP, glycine betaine- and proline concentrations in leaves of *Spartina anglica*. *Aquat Bot* 72:193–200
- Muthukumarasamy M, Gupta SD, Panneerselvam R (2000) Influence of triadimefon on the metabolism of NaCl stressed radish. *Biol Plant* 43:67–72
- Naidu BP, Paleg LG, Aspinall D, Jennings AC, Jones GP (1991) Amino-acid and glycine betaine accumulation in cold-stressed wheat seedlings. *Phytochem* 30:407–409
- Nakamura T, Nomura M, Mori H, Jagendorf AT, Ueda A, Takabe T (2001) An isozyme of betaine aldehyde dehydrogenase in barley. *Plant Cell Physiol* 42:1088–1092
- Nanjo T, Kobayashi M, Yoshida Y, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (1999) Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Lett* 461:205–210
- Ochiai K, Matoh T (2001) Mechanism of salt tolerance in the grass species, *Anneurolepidium chinense*—I. Growth response to salinity and osmotic adjustment. *Soil Sci Plant Nutr* 47:579–585
- Otte ML, Morris JT (1994) Dimethylsulphoniopropionate (DMSP) in *Spartina alterniflora* Loisel. *Aquat Bot* 48:239–259
- Papageorgiou G, Murata N (1995) The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving photosystem II complex. *Photosynth Res* 44:234–252
- Paquet L, Lafontaine PJ, Saini HS, James F, Hanson AD (1995) Evidence en faveur de la présence du 3-dimethylsulphoniopropionate chez une large gamme d'Angiospermes. *Can J Bot* 73:1889–1896
- Rabe E (1990) Stress physiology: the functional significance of the accumulation of nitrogen-containing compounds. *J Hort Sci* 65:231–243
- Rhodes D, Hanson AD (1993) Quaternary ammonium and tertiary sulphonium compounds in higher plants. *Ann Rev Plant Physiol Plant Mol Biol* 44:357–384
- Rolletschek H, Hartzendorf T (2000) Effects of salinity and convective rhizome ventilation on amino acid and carbohydrate patterns of *Phragmites australis* populations in the Neusiedler See region of Austria and Hungary. *New Phytol* 146:95–105
- Sakamoto A, Murata N (2000) Genetic engineering of glycinebetaine synthesis in plants: current status and implications for enhancement of stress tolerance. *J Exp Bot* 51:81–88
- Schile L, Mopper S (2006) Negative effects of salinity stress on a leaf-mining insect and its host plant. *Ecol Entomol* 31:1–7
- Solomon A, Beer S, Waisel Y, Jones GGP, Paleg LG (1994) Effects of NaCl, on the carboxylating activity of Rubisco from *Tamarix jordanis* in the presence and absence of proline-related compatible solutes. *Plant Physiol* 90:198–204
- Stefels J (2000) Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. *J Sea Res* 43:183–197
- Storey R, Wyn Jones RG (1977) Quaternary ammonium-compounds in plants in relation to salt resistance. *Phytochemistry* 16:447–453
- Strizhov N, Abraham E, Okresz L, Blickling S, Zilberstein A, Schell J, Koncz C, Szabados C (1997) Differential expression of two P5CS genes controlling proline accumulation during salt-stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in *Arabidopsis*. *Plant J* 12:557–569
- Subbarao GV, Wheeler RM, Levine LH, Stutte GW (2001) Glycine betaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. *J Plant Physiol* 158:767–776
- Tobler M, Van Zandt P, Hasenstein KH, Mopper S (2006) Growth and reproduction of a clonal plant in response to salinity and florivory. *Wetlands* 26:803–812
- Trossat C, Rathinasabapathi B, Weretilnyk EA, Shen TL, Huang ZH, Gage DA, Hanson AD (1998) Salinity promotes accumulation of 3-dimethylsulphoniopropionate and its precursor S-methylmethionine in chloroplasts. *Plant Physiol* 116:165–171
- Trotel P, Bouchereau A, Niogret MF, Larher F (1996) The fate of osmo-accumulated proline in leaf discs of rape (*Brassica napus* L.) incubated in a medium of low osmolarity. *Plant Science* 118:31–45
- Van Zandt PA, Mopper S (2002) Delayed and carry-over effects of salinity on flowering in *Iris hexagona* (Iridaceae). *Am J Bot* 89:1847–1851
- Van Zandt PA, Mopper S (2004) The effects of maternal salinity and seed environment on germination and growth in *Iris hexagona*. *Evol Ecol Res* 6:813–832
- Venkatesan A, Chellappan KP (1998) Accumulation of proline and glycine betaine in *Ipomoea pescaprae* induced by NaCl. *Biol Plant* 41:271–276
- Wang Y, Nii N (2000) Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. *J Hort Sci Biotechnol* 75:623–627
- Wang YY, Mopper S, Hasenstein KH (2001) Effects of salinity on the levels of endogenous ABA, IAA, JA and SA in *Iris hexagona*. *J Chem Ecol* 27:327–342
- Zhu JK (2002) Salt and drought stress signal transduction in plants. *Ann Rev Plant Biol* 53:247–273