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Synthesis of organic osmolytes and salt tolerance mechanisms in *Paspalum vaginatum*

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Abstract

Synthesis of organic compounds in response to salinity stress and their contribution to organic osmotic adjustment were investigated in seashore paspalum (*Paspalum vaginatum* Swartz). Nine genotypes exhibiting the widest range of salt tolerance were grown in sea-salt amended nutrient solution in a greenhouse. Salinity ranges were 1.1 (EC_w0, control) to 49.7 dS m^{-1} (EC_w50) based on electrical conductivity of the solution (EC_w). Organic osmolytes most important within seashore paspalum under salinity stress were proline, Gly-betaine, and trigonelline in terms of explaining intraspecific salt tolerance differences and, therefore, should be the focus of biotechnology approaches to enhance these traits. While these osmolytes differed in accumulation with increasing salinity and absolute concentrations among salt tolerant and intolerant genotypes, the magnitude of responses was not sufficiently large to suggest use for salt screening as physiological/biochemical markers. Fructose concentration increased with salinity, especially for salt sensitive ecotypes, and may have potential as a marker. Glucose, sucrose, and *myo*-inositol tended to increase with salinity, but changes did not relate to intraspecific salt tolerance, while mannitol and sorbitol were not affected by salinity. Proline demonstrated a 20.8-fold increase averaged across genotypes from EC_w0 to EC_w50 salinity. Proline was the primary organic osmolyte for osmotic adjustment accounting for an average of 9.3% to total solute potential (Ψ_s) at EC_w50 and 56% of the organic solute contribution to Ψ_s. In the salt tolerant genotype, SI 93-2, proline and Gly-betaine exhibited greater absolute concentration and accumulation rate relative to the least salt tolerant, Adalayd. The intraspecific role of Gly-betaine did not relate to osmotic adjustment differences, suggesting another role perhaps in protection of the thylakoid membrane.

Keywords: Salinity; Seashore paspalum; Compatible osmolytes; Proline

1. Introduction

Salinity stress is a major factor limiting plant growth and productivity in many areas of the world (Epstein et al., 1980; Flowers, 1999). For several halophytic grasses, Hester et al. (2001) demonstrated that intraspecific variation in morphological and physiological traits expressed under salinity stress was as great as interspecific variation. Knowledge of intraspecific salt tolerance mechanisms in the salt tolerant genotypes of a species relative to the least tolerant types can assist in developing physiological-based screening protocols in traditional breeding and focus biotechnological approaches toward important biochemical traits contributing to superior salt tolerance in the species (Bohnert and Jensen, 1996; Duncan and Carrow, 1999; Hester et al., 2001; Ashraf and Harris, 2004).

Under the variation of saline environments, plants have developed different adaptative mechanisms (Rhodes et al., 2002; Borsani et al., 2003; Sairam et al., 2006). One adaptive plant response to salt stress is synthesis and accumulation of low-molecular weight organic compounds in the cytosol and organelles (Ashraf and Harris, 2004; Bartels and Sunkar, 2005; Sairam et al., 2006). These compounds are collectively called compatible osmolytes because they accumulate and function without perturbing intracellular biochemistry, such as enzyme or protein activities in the cytoplasm. A major function of compat-

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ible osmolytes that accumulate is provision of osmotic stress protection by allow osmotic adjustment to counteract higher inorganic salts in the vacuole and in root medium (Bohnert and Shen, 1999; Zhu, 2001; Rhodes et al., 2002). Another function of compatible osmolytes that may occur at lower concentrations is osmoprotection, which includes protection of thylakoid and plasma membrane integrity, stabilizing proteins, a sink for energy or reducing power, a source of carbon and nitrogen for recovery, or scavenging of reactive oxygen species that are by-products of salinity stress (Bartels and Sunkar, 2005; Sairam et al., 2006). Compatible osmolytes reported to be affected by salinity stress include: simple sugars (fructose, glucose); disaccharides (trehalose, sucrose); sugar alcohols or polyols (sorbital, mannitol, galactitol, and cyclic polyols such as *myo*-inositol, ononitol, pinnitol); amino acids (proline); quaternary amino acid derivatives (glycine betaine, proline betaine, trigonelline) and sulfonium compounds (Ashraf and Foolad, 2007; Sairam et al., 2006; Bartels and Sunkar, 2005; Ashraf and Harris, 2004; Borsani et al., 2003; Zhu, 2001).

Seashore paspalum, a halophytic warm season grass, has recently gained attention for use on saline turfgrass sites and for forage production, drainage water reuse schemes, and land reclamation under saline conditions (Duncan and Carrow, 2000; Semple et al., 2003; Grattan et al., 2004; Rogers et al., 2005). Osmotic adjustment through inorganic ion uptake or synthesis of organic compounds has been postulated to have a significant role in salt tolerance in seashore paspalum by Marcum and Murdoch (1994); but, only one genotype was included in their comparative study between grass species and a limited number of synthesized osmolytes were investigated. Accumulation of compatible organic osmolytes is affected by genotypes, salinity levels, and tissue types (Morgan, 1984; Alian et al., 2000). Lee et al. (2005b) reported a wide variation in salt tolerance among seashore paspalum genotypes; thus, elucidation of the intraspecific response of different osmolytes would be useful for physiological selection markers and for directing biotechnological approaches to salt tolerance improvement within the species.

Therefore, the objectives of this study were (a) to assess the influence of increasing salinity on synthesis of organic compounds potentially functioning as organic osmolytes or osmoprotectants in seashore paspalum genotypes; and (b) to evaluate organic compatible compound differences and potential significance between the most and least salt tolerant genotypes.

2. Materials and methods

Nine seashore paspalum (*Paspalum vaginatum* Swartz) genotypes (each experimental was collected as a single plant in nature) were selected for this study based on salinity tolerance, which include the most (SI 93-2, HI 101), intermediate (Sea Isle 2000, TCR1, TCR 6, Sea Isle 1, HI 34, SI 90), and least (Adalayd) tolerant genotypes (Lee et al., 2004a, 2005a). All grasses except Adalayd were cultivar releases or genotypes from the breeding program of Dr. Ronny Duncan at the University of Georgia. This study was conducted using a solution/sand culture under controlled climate greenhouse conditions at the Griffin Campus/UGA at Griffin, GA, from May to November 1998. On cloudy days, supplemental light with 400 W metal halide lights was used. The temperature in the greenhouse was $30 \pm 2/27 \pm 2\,^{\circ}C$ (day/night) with a 14-h photoperiod. In each pot (13.0 cm long × 10.0 cm wide × 12.5 cm high) five 2-cm plugs of a genotype was planted with six replications of each grass and salinity combination. Genotypes were maintained under the same irrigation (once a day) and cutting practices at 2.5 cm (once a week) throughout the study. Each pot with nine holes at the bottom was filled with washed sand. The culture solution was prepared using a half-strength of Hoagland and Arnon's (1950) nutrient solution (#2), modified with Fe-EDTA as an iron source to give 5 mg L^{-1} of Fe (Sprint 138, 6% Fe, Becker-Underwood, Ames, IA). Nine pots were held in a wooden frame, which was placed in a 28 L container of nutrient solution (1.2 dS m^{-1} and pH 6.3 ± 0.5) formulated with deionized water. After a 4-week acclimation period, nutrient solution was salinized with sea-salt mixture and gradually increased to 10.3, 20.5, 30.7, 39.5, 49.7 dS m^{-1} by addition of 6.9 g L^{-1} of sea-salt mixture every day (Dudeck et al., 1993). Solutions were renewed each week, aerated constantly, and restored to a constant volume from evapotranspiration losses by adding deionized water every 2 or 3 days. Salinity level was monitored by measuring electrical conductivity of the solution (EC_w) twice a week at 25 °C with an Orion conductivity meter (Model 160, Boston, MA).

Proline content was determined with 0.5 g of fresh leaves by using the acid-ninhydrin method with spectrophotometer analysis (Beckman Model DU-600, Beckman Instrument Inc., CA) at 520 nm (Bates, 1973). Freeze-dried ground leaves (0.4 g) were used for quantification of glycine betaine and trigonelline. The samples were soaked in 10 mL of methanol at 70 °C for 1 h with three times vortex. All upper aqueous extract was transferred, dried on heating block using N_2 gas, and partitioned in equal volumes of water and chloroform (3 mL of water:3 mL of chloroform) by shaking for 5 min. The supernatant collection was deionized by addition of ion exchange resins (2:1 Dowex anion exchanger to Amberlite cation exchangers, about 0.2 g of total weight). After shaking for 5 min, the deionized extract was filtered using a 0.45 μm syringe filter (13 mm diameter) and then used directly to measure glycine betaine and trigonelline with a Gilson high performance liquid chromatography (HPLC) system with a 20 μL injection loop, a pump, an autosampler, and a UV (ultra violet)/visible detector (Gilson Medical Electronics, Middleton, WI). Separations were performed on a 250 mm × 4.6 mm i.d. stainless-steel column packed with Whatman Partisil 10-SCX directly connected with a guard column (Fisher Scientific, Pittsburgh, PA). A 50 mM of KH_2PO_4 with 5% methanol (pH 4.6) as eluent was used at a 1.5 mL min^{-1} of flow rate. Glycine betaine and trigonelline standards were run at 0.1–4.0 g L^{-1} and 0.001–0.04 g L^{-1}, respectively. Recovery tests were carried out using standard addition method and average of 98% and 91% recoverability was obtained for glycine betaine and trigonelline ($n = 5$), respectively.

For sugar analysis, 50 μL of supernatant extracted from the freeze-dried and ground leaves in 80% of methanol was pipetted into gas chromatography (GC) vials and dried under air stream.

Oxime-trimethylsilyl (Oxime-TMS) method of Chapman and Horvat (1989) was adopted for derivatizing organic compounds in GC vials for the GC detection. Twenty-five microliters of hydroxylamine–HCl (25 mg mL^{-1} in pyridine) was pipetted for the oximation, followed by adding the mixture of 70 μL of *N*,*O*-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (1:1). Separations were performed by GC, which consisted of a flame ionization detector (FID), an autosampler, and an integrator (5890 series II, Hewlett-Packard Co., Naperville, IL). A DB-5 capillary GC column with fused silica (30 m × 0.32 mm i.d. × 0.25 μm film) was used to detect the organic compounds (Agilent Technologies, Santa Clara, CA). Helium was used as a carrier gas at a flow rate of 53.1 mL min^{-1}. Flow rates of air, hydrogen, and nitrogen as make-up gases were 370–380, 28, and 30 mL min^{-1}, respectively.

The contribution of each organic osmolyte to total solute potential (Ψ_s) was determined using the van't Hoff equation as reported by Alarcon et al. (1993), where the calculated contribution of individual osmolytes to measured Ψ_s was based on relative dry weight at saturation [dry weight/(saturated weight − dry weight)] and osmolyte concentration on a dry weight basis. Total solute potential (Ψ_s) and leaf water potential (Ψ_w) of leaf tissue sap was determined as reported by Lee et al. (2005b). We assumed that osmolytes behaved as ideal osmotica as noted by Alarcon et al. (1993).

The experimental design was a split-plot design with six replications where salinity level and genotype were the main and subplot, respectively. Six salinity levels (one salt level per container) were arranged randomly within each replication. Contents of organic ions were statistically analyzed using least significant difference (LSD) to separate means of genotypes at each salinity level and among salinity levels for each genotype (SAS Institute, 2001). Multiple regression analysis was used to determine the most significant organic osmolytes that correlated with variation in shoot and root growth (dependent variables) across all salinity levels for linear, quadratic, and cubic relationships. The unit used for regression was each ecotype-salinity replication. Independent variables significant at $P \leq 0.10$ level were included in the forward selection model, and partial R^2 and coefficient values were assessed for the relationship of variables.

3. Results and discussion

Genotypes are listed in the tables in descending order of salinity tolerance as reported by Lee et al. (2004a). The need for elucidation of the role of organic osmolytes in salt tolerance mechanisms of seashore paspalum and development of superior cultivars are highlighted by the recent interest in genotypes of this species for forage and land reclamation schemes on saline sites (Semple et al., 2003; Rogers et al., 2005), drainage water reuse (Grattan et al., 2004), and turfgrass sites (Duncan and Carrow, 2000) coupled with the ability to develop seeded types (Turfseed, 2006). Accumulation of many organic compounds under saline conditions is a well-documented metabolic feature exhibited by many salt tolerant plants (Bohnert and Shen, 1999; McNeil et al., 1999). Within seashore paspalum genotypes varying in salt tolerance, the current study deals with endogenous levels of proline, quaternary amino acid derivatives (Gly-betaine, trigonelline), sugars (fructose, glucose, sucrose), and sugar alcohols or polyols (*myo*-inositol, sorbitol, mannitol) in response to salinity stress and their role in salinity tolerance.

3.1. Proline

Content of the amino acid proline was strongly influenced by salt level with content of 0.36–0.72 at EC_w0 dS m^{-1}, while at EC_w50 the range was 9.12–13.17 mg g^{-1} FW (Table 1). The greatest increase in proline was between EC_w30 and EC_w50. Differences among grasses occurred at the higher salinity levels of EC_w30 and EC_w50. The salt tolerant genotypes SI 93-2 and HI 101 demonstrated a somewhat higher rate of proline accumulation at EC_w50 compared to the EC_w0 control (29- and 21-fold, respectively) than Adalayd (17-fold). As salinity increased, there was a trend (but not significant) for higher abso-

Table 1
Difference in shoot proline content in seashore paspalum genotypes under different salinity levels

Entry	Proline content (mg g^{-1} FW)				*F*-test[a]	LSD (0.05)[a]
	EC_w0	EC_w10	EC_w30	EC_w50		
SI 93-2	0.36	0.54	2.03 bc	10.41 ab	***	2.79
HI 101	0.49	0.82	1.94 bc	10.23 ab	***	1.62
Sea Isle 2000	0.44	0.48	2.59 bc	10.01 ab	***	3.62
TCR 1	0.56	0.89	5.48 a	11.38 ab	***	4.66
TCR 6	0.72	0.85	3.58 ab	13.17 a	***	2.51
Sea Isle 1	0.40	0.93	2.11 bc	9.29 bc	***	2.76
HI 34	0.52	0.71	2.09 bc	11.67 ab	***	3.70
SI 90	0.54	0.99	2.92 bc	10.19 ab	***	3.13
Adalayd	0.54	0.68	1.47 c	9.12 bc	***	2.54
F-test[a]	0.09	0.13	*	*		
LSD (0.05)[a]	0.22	0.40	2.03	3.24		

Means within a column followed by the same letter are not significantly different based at the $P = 0.05$ level for the given salinity.

[a] *F*-test and LSD test (0.05) are to compare mean performances among entries or salinity levels where the denoted symbols indicate significant difference at the 0.001 (***), 0.01 (**), and 0.05 (*) levels.

Table 2
Calculated organic solute potential contributed by individual organic compounds and their contribution to shoot total solute potential (Ψ_s) measured at the highest salinity level (EC_w50 dS m^{-1}) by genotype

Entry	Calculated organic solute potential (Ψ_s) (bar) by compound[a]							Sum of organic solute, Ψ_s (bar)	Total Ψ_s measured (bar)[b]	Organic contribution (%)[c]
	Gly-betaine	Trigonelline	Proline	Fructose	Glucose	*Myo*-inositol	Sucrose			
SI 93-2	−1.44	−0.003	−2.82	−0.18	−0.07	−0.02	−0.17	−4.71	−25.0	19
HI 101	−1.87	−0.005	−2.92	−0.22	−0.10	−0.02	−0.21	−5.34	−25.9	21
Sea Isle 2000	−1.74	−0.004	−2.78	−0.23	−0.09	−0.03	−0.20	−5.07	−24.3	21
TCR 1	−1.96	−0.005	−3.24	−0.35	−0.09	−0.03	−0.27	−5.95	−27.0	22
TCR 6	−1.45	−0.004	−3.56	−0.25	−0.08	−0.02	−0.26	−5.62	−25.1	22
Sea Isle 1	−2.52	−0.005	−2.84	−0.25	−0.11	−0.03	−0.22	−5.98	−24.9	24
HI 34	−1.73	−0.004	−3.28	−0.21	−0.07	−0.02	−0.18	−5.50	−24.9	22
SI 90	−1.46	−0.004	−2.79	−0.23	−0.09	−0.03	−0.21	−4.81	−25.0	19
Adalayd	−1.79	−0.004	−2.79	−0.4	−0.17	−0.04	−0.24	−5.42	−24.8	22

[a] van't Hoff equation; Ψ_s (MPa) = $-c_s RT$, where $R = 0.0083143$ L MPa mol^{-1} K^{-1} and $T = 293$ K were considered; 1 bar = 0.10 MPa.
[b] Total Ψ_s measured is total solute potential (Lee et al., 2005b).
[c] Organic contribution = (calculated sum of organic solutes listed in this table Ψ_s/measured total Ψ_s) × 100.

lute proline content in the more salt tolerant grasses relative to Adalayd.

Proline accounted for 51% of the organic contribution to total organic solute potential for Adalayd [i.e. (−2.49 bar calculated potential from proline/−5.42 bar of total solute potential from organic osmolytes) × 100)], but 55% and 60% for the two salt tolerant genotypes SI 93-2 and HI 101, respectively (Table 2). As with proline content, these results suggest proline may be involved in salt tolerance differences at the intraspecific level.

In contrast, multiple regression analysis to determine content of compatible osmolytes related to shoot growth across salinity levels and genotypes support a negative role for proline accumulation under salt stress for this species (Table 3). For shoot growth, the partial contribution by proline was 76% [shoot growth = 1.760 − 0.041 (proline) + 0.003 (sucrose^2) + 0.0002 (proline^2), $R^2 = 0.88$]. A similar regression for root growth did not show proline as important, where [root = 0.535 − 0.033 (fructose), $R^2 = 0.34$].

In terms of the role of proline in osmotic adjustment, proline was the primary organic osmolyte contributing to total solute potential (Ψ_s) by accounting for an average of 11.9% of Ψ_s at EC_w50, based on an average proline solute contribution of −3.00 bar to the average genotype Ψ_s of −25.2 bar (Table 2). Relative to the total organic osmolyte contribution (−5.38 bar average) to Ψ_s, proline accounted for 41–63% of contribution by organic compounds. No apparent differences among salt tolerant and intolerant genotypes were noted for either proline contribution to Ψ_s or contribution of proline to total organic osmolyte component of Ψ_s. Thus, proline was the primary organic osmolyte contributing to osmotic adjustment of Ψ_s and the contribution of 11.9% indicates proline, while not a primary osmolyte for osmotic adjustment in *P. vaginatum*, does contribute at high salinity (Table 2). Marcum (2002) reported that proline often accumulates in grasses under salinity stress, but that proline content was insufficient for osmotic adjustment in grasses.

Ashraf and Foolad (2007) noted that accumulation of proline, primarily in the cytosol, often occurs in plants under salinity stress with strong correlation between stress tolerance and proline accumulation, but the relationship is not universal and may be species dependent. Bartels and Sunkar (2005) and Ashraf and Foolad (2007) reported that other roles proposed for proline besides osmotic adjustment in stressed plants include acting as hydroxyl scavenger, stabilization of membrane and protein structure, as a sink for carbon and nitrogen for stress recovery, and buffering cellular redox potential under stress. Since salt tolerance seashore paspalum genotypes did accumulate more proline on a relative basis (i.e. compared to the control) and there was a trend for higher absolute proline contents, perhaps a significant role for proline in this species is as a carbon and nitrogen sink for recovery as well as being the primary compatible osmolyte for osmotic adjustment.

Table 3
Regression analysis of variables of organic osmolyte attributing to shoot and root growth under salinity stress

Shoot growth				Root growth			
Variable[a]	Coefficient	Partial R^2	*P*-value	Variable[a]	Coefficient	Partial R^2	*P*-value
Intercept	1.76		0.0001	Intercept	0.535		0.0001
Proline	−0.041	0.76	0.0001	Fructose	−0.033	0.34	0.0001
Proline^2	0.0002	0.09	0.0001				
Sucrose^2	0.003	0.03	0.0101				
Overall		0.88***		Overall		0.34***	

*** Significant differences at the 0.001 probability level.
[a] Included all variables to meet 0.10 significance level for entry into the model.

Table 4
Difference in shoot Gly-betaine content of seashore paspalum genotypes under different salinity levels[a]

Entry	Glycine betaine content (mg g^{-1} DW)						*F*-test[a]	LSD (0.05)[a]
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	14.54 b	25.62 a	27.07 b–d	28.11 a–e	35.65	35.96 cd	***	2.58
HI 101	18.87 a	21.37 c	25.61 de	27.95 a–e	33.04	37.30 b–d	***	2.91
Sea Isle 2000	10.05 c	21.47 c	28.98 ab	30.00 a	35.76	38.64 bc	***	2.4
TCR 1	18.10 a	21.24 c	26.03 c–e	27.65 c–e	35.70	39.24 ab	***	4.1
TCR 6	10.12 c	25.30 ab	27.01 b–d	29.61 a–c	34.70	36.03 cd	***	2.01
Sea Isle 1	19.39 a	24.60 ab	30.10 a	26.49 e	35.18	39.33 ab	***	2.92
HI 34	14.86 b	23.12 bc	28.20 a–c	26.88 de	33.98	36.44 b–d	***	3.22
SI 90	19.79 a	23.49 a–c	25.57 de	28.71 a–d	31.55	34.25 d	**	5.75
Adalayd	20.26 a	22.08 c	24.27 e	27.81 b–e	32.65	27.97 e	***	3.55
F-test[a]					0.07			
LSD (0.05)[a]	2.85	2.38	2.3	2.07	3.61	3.08		

[a] See Table 1 for explanations.

3.2. Quaternary amino acid derivatives

As salinity level increased, leaf Gly-betaine content in all genotypes was enhanced by 1.38–3.8-fold from EC_w0 to EC_w50 with the salt sensitive Adalayd (1.38-fold) and SI 90 (1.73-fold) exhibiting the least increase relative to the control (Table 4). Except at EC_w40, significant grass differences were evident at each salinity level. At EC_w50, the most salt tolerant genotypes (SI 93-2 and HI 101) synthesized Gly-betaine more (35.96 and 37.30 mg g^{-1}, respectively) than the least tolerant Adalayd (27.97 mg g^{-1} DW). Gly-betaine level decreased at EC_w50 compared to EC_w40 for Adalayd, while it increased for the other genotypes. When comparing accumulation level of Gly-betaine between the EC_w0 control and other salinity levels, salt tolerant SI 93-2 exhibited a greater increase in content on an absolute or percent increase basis compared to Adalayd [21.4 (2.5-fold) vs. 12.1 mg g^{-1} DW (1.6-fold), respectively]. Gly-betaine accumulation appears to be involved as a salt tolerance mechanism for the species with greater expression for the more salt tolerant genotypes.

Gly-betaine was the second most important organic compound for osmotic adjustment contributing an average of 7.0% to Ψ_s; and 33.9% (average) of the organic contribution to total organic solute potential adjustment at EC_w50 salinity (Table 2). However, there was no apparent difference between salt intolerant and tolerant genotypes in terms of the osmotic adjustment role by Gly-betaine at the EC_w50 salinity level (Table 2). Therefore, Gly-betaine contributed to osmotic adjustment for all seashore paspalums, but this function of Gly-betaine did not account for salinity tolerance differences among genotypes. Lee et al. (2004b) demonstrated that more salt tolerant genotypes of *P. vaginatum* exhibited enhanced photochemical efficiency of PSII. Since Gly-betaine is most abundant in the chloroplast, it may function in osmotic adjustment and protection of the thylakoid membrane in salt-stressed seashore paspalum, thereby maintaining photosynthetic efficiency (Ashraf and Foolad, 2007).

Leaf trigonelline content was ~1000 times lower than Gly-betaine, but exhibited appreciable increase as salinity increased for all genotypes (Table 5). A significant difference in trigonelline content among seashore paspalum genotypes was exhibited at all salinity levels. The minimum content was 13.41 μg g^{-1} DW for TCR 6 at EC_w0 and the maximum was 101.93 μg g^{-1} DW for TCR 1 at EC_w50. Interestingly, leaf trigonelline content increased up to EC_w20 and then declined at EC_w30 and re-accumulated substantially at EC_w40 except for

Table 5
Difference in shoot trigonelline content in seashore paspalum genotypes under different salinity levels

Entry	Trigonelline content (μg g^{-1} DW)						*F*-test[a]	LSD (0.05)[a]
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	19.32 de	59.90 a–c	62.44 b–d	33.28 cd	78.23 b–d	86.98 ab	***	20.5
HI 101	51.82 a *vs*	63.54 ab	56.77 b–d	29.02 cd	79.72 a–d	97.55 ab	***	8.06
Sea Isle 2000	14.28 e *vs*	24.80 d	60.45 b–d	23.96 d	60.97 e	87.27 ab	***	7.74
TCR 1	33.37 b–d	45.68 c	56.13 cd	29.57 cd	93.24 a	101.93 a	***	24.8
TCR 6	13.41 e *vs*	60.14 a–c	61.20 b–d	68.08 a	68.41 de	88.36 ab	***	9.42
Sea Isle 1	43.33 ab	72.77 a	86.99 a	36.48 c	88.10 ab	85.27 b	***	13.4
HI 34	22.60 c–e	60.12 a–c	70.02 b	36.23 c	83.18 a–c	89.26 ab	***	12.5
SI 90	44.41 ab	59.41 a–c	66.73 bc	50.71 b	80.07 a–d	84.58 b	*	29.7
Adalayd	46.67 ab	51.43 bc	53.41 d	32.13 cd	72.48 c–e	67.73 b	**	14.8
F-test[a]								
LSD (0.05)[a]	16	15.62	13.31	11.75	13.53	15.41		

[a] See Table 1 for explanations.

Table 6
Difference in shoot fructose content in seashore paspalum genotypes under different salinity levels

Entry	Fructose content (mg g^{-1} DW)						*F*-test[a]	LSD (0.05)[a]
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	3.24 ab	2.83	3.79	3.31 cd	4.73 c	4.76 c–e	*	1.21
HI 101	3.19 ab	2.89	3.62	3.93 a–c	6.41 ab	4.68 c–e	**	1.51
Sea Isle 2000	2.22 c	2.49	3.83	3.19 d	5.80 a–c	5.48 b–d	***	0.94
TCR 1	2.73 bc	2.40	3.58	4.47 a	7.07 a	7.30 a	***	1.62
TCR 6	2.66 bc	2.81	3.75	3.76 a–d	5.69 bc	6.48 ab	***	0.94
Sea Isle 1	2.51 bc	2.53	3.66	3.51 b–d	4.84 c	4.05 de	*	1.24
HI 34	2.62 bc	2.37	3.77	3.63 b–d	5.71 bc	4.58 c–e	**	1.45
SI 90	3.78 a	3.85	3.66	4.10 ab	6.57 ab	5.72 bc	*	2.06
Adalayd	2.44 bc	2.72	3.38	3.74 b–d	5.55 bc	6.51 ab	***	1.39
F-test[a]	*	0.07	0.16	*	*	**		
LSD (0.05)[a]	0.82	0.85	0.59	0.71	1.31	1.45		

[a] See Table 1 for explanations.

TCR 6. When comparing the salt tolerant SI 93-2 and HI 101 types to salt sensitive SI 90 and Adalayd in terms of trigonelline response between EC_w30 and EC_w50, the salt tolerant genotypes exhibited a greater increase in both percent and absolute terms. Trigonelline content increased from 53.7 to 68.5 μg g^{-1} DW and 2.61- to 3.36-fold for the salt tolerant types; and for the salt sensitive genotypes 33.9 to 35.6 μg g^{-1} DW and 1.67- to 2.11-fold.

The low cellular concentrations relative to proline and Gly-betaine suggest that trigonelline would not play a significant role as a compatible osmolyte for osmotic adjustment and this is supported by calculated solute potential data (Table 2). Marcum and Murdoch (1992) reported similar results for *Sporobolus virginus*. Trigonelline may act as an osmoprotectant via enhancing salt stability of pyruvate kinase, inducing defensive metabolic plant responses, prevention of water loss under salt stress, or serving in other cell cycle regulatory roles (Suzuki-Yamamoto et al., 2006; Minorsky, 2002).

3.3. Non-structural sugars

Results for non-structural, water-soluble sugars (fructose, glucose, and sucrose) are shown in Tables 6–8. Leaf fructose content ranged from 2.22 to 7.30 mg g^{-1} DW and accumulation was gradual with increasing salinity with the highest fructose content at EC_w40 or EC_w50 (Table 6). Comparison among genotypes at each salinity level revealed differences in fructose content. No significant difference was found up to EC_w40 between the salt tolerant (SI 93-2 and HI 101) and least tolerant Adalayd, but significantly higher fructose was accumulated for Adalayd at EC_w50 in the shoot tissue.

Interestingly, multiple regression analysis to determine content of compatible osmolytes related to root growth across salinity levels and genotypes showed a negative trend for fructose accumulation in shoots under salt stress for this species where [root growth = 0.535 − 0.033 (fructose), $R^2 = 0.34$] (Table 3). Kerepesi et al. (1998) reported that fructose, glucose, and sucrose rates increased sharply in all plant parts in salt sensitive *Triticum aestivum* L. genotypes, while remaining unchanged (leaves and stems) or decreased (roots) in salt tolerant genotypes. They noted that lower salt tolerance may be related to greater energy cost for organic osmolyte adjustment, including sugars, for salt sensitive plants in contrast to greater reliance on inorganic ions for osmotic adjustment in salt tolerant genotypes. Kerepesi et al. (1998) suggested the potential for using changes in concentration of specific sugar components as a

Table 7
Difference in shoot glucose content in seashore paspalum genotypes under different salinity levels

Entry	Glucose content (mg g^{-1} DW)						*F*-test[a]	LSD (0.05)[a]
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	2.14 ab	1.86	2.49 a–c	1.45	1.54 e	1.78	0.22	0.94
HI 101	2.14 ab	1.89	2.03 de	1.71	2.12 a–c	2.12	0.94	1.10
Sea Isle 2000	1.48 c	1.59	2.65 a	1.78	2.22 a	2.07	**	0.58
TCR 1	1.83 a–c	1.40	2.05 c–e	1.91	2.05 c–e	1.96	0.63	0.90
TCR 6	1.57 bc	1.76	2.36 a–d	1.77	1.88 a–e	2.02	0.34	0.76
Sea Isle 1	1.87 a–c	1.49	2.55 ab	1.84	1.78 c–e	1.87	0.39	1.03
HI 34	1.65 bc	1.31	2.59 a	1.61	1.82 b–e	1.62	*	0.67
SI 90	2.38 a	2.55	2.09 c–e	1.71	2.16 ab	2.24	0.79	1.32
Adalayd	1.44 c	1.77	2.13 b–e	1.86	1.89 a–d	2.77	*	0.67
F-test[a]	*	0.11	*	0.78	*	0.06		
LSD (0.05)[a]	0.63	0.75	0.46	0.53	0.35	0.63		

[a] See Table 1 for explanations.

Table 8
Difference in shoot sucrose content in seashore paspalum genotypes under different salinity levels

Entry	Sucrose content (mg g^{-1} DW)						*F*-test[a]	LSD (0.05)[a]
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	5.60 c	8.69	9.63 ab	10.14 b–d	8.82 d–f	8.61 b–d	*	2.65
HI 101	7.82 bc	9.81	9.76 ab	9.38 c–e	13.02 a	8.22 b–d	0.07	3.35
Sea Isle 2000	8.55 b	10.35	9.44 ab	10.26 b–d	9.61 c–e	9.00 b–d	0.82	3.40
TCR 1	8.77 b	11.24	11.78 a	12.62 a	11.75 a–c	10.90 ab	0.23	3.20
TCR 6	12.40 a	11.29	12.05 a	11.95 ab	11.43 a–c	13.02 a	0.97	5.06
Sea Isle 1	7.61 bc	10.08	7.72 b	6.94 f	8.18 ef	7.02 cd	0.18	2.63
HI 34	8.75 b	10.59	8.14 b	9.51 c–e	12.45 ab	7.72 cd	*	2.89
SI 90	7.69 bc	10.82	9.19 ab	10.63 a–c	10.56 b–d	9.78 bc	0.68	4.75
Adalayd	9.68 ab	10.37	9.99 ab	8.59 d–f	8.39 d–f	7.48 cd	0.19	2.57
F-test[a]	**	0.25	*	***	***	**		
LSD (0.05)[a]	2.73	3.04	2.91	2.04	2.34	3.13		

[a] See Table 1 for explanations.

physiological or biochemical marker for salinity screening. Our results suggest that this may be possible in seashore paspalum with respect to fructose.

Leaf glucose content was lower than fructose with a range of 1.44–2.77 mg g^{-1} DW for all grasses across salinity levels used in the study (Table 7). Most genotypes had the highest glucose content at EC_w20, with decreased glucose content at EC_w30, followed by an increase at >EC_w30. Differences in glucose content among grass entries were demonstrated only at EC_w0, EC_w20, and EC_w40 and were small in magnitude with no apparent trend between tolerant and less tolerant genotypes.

Sucrose content in leaf tissues was the highest among the non-structural sugars with a maximum accumulation of 10.08–13.02 mg g^{-1} DW for the genotypes used in this study (Table 8). Hester et al. (2001) reported sucrose to be the most abundant sugar for several halophytic grasses. Increasing salinity significantly affected leaf sucrose accumulation in only two grasses, SI 93-2 and HI 34. Grass differences within each salinity level were evident at all levels except for EC_w10. Leaf sucrose content did not seem to relate to salt tolerance between the most and the least salt tolerant genotypes.

Each of the soluble sugars contributed to Ψ_s adjustment with fructose and sucrose exhibiting somewhat greater contribution than glucose at EC_w50 salinity (Table 2). Adalayd showed somewhat higher reliance on sugars for osmotic adjustment relative to SI 93-2 and HI 101, but the magnitude was not great at 14.9 versus 8.9, and 9.9%, respectively, for the total sugar contribution. These results indicate that fructose, glucose, and sucrose functioned in osmotic adjustment for all genotypes with only minor differences between tolerant and intolerant types. The osmotic adjustment function of sugars in plants under salt stress is consistent with the observations of others (Hasegawa et al., 2000). Only fructose exhibited differences between salt tolerant and salt sensitive grasses as well as being inversely related to root growth. The lack of marked differences between genotypes and relative modest response of sucrose and glucose content to increasing salinity suggest that for this species these sugars were not functioning as signalling molecules to regulate source and sink metabolism (Roitch, 1999; Hasegawa et al., 2000).

3.4. Sugar alcohols

There was no significant change in content of the polyols sorbitol and mannitol for all genotypes responding to salinity stress so no data are shown. Williamson et al. (2002) note that the specific polyols present in plants varies with

Table 9
Difference in shoot *myo*-inositol content in seashore paspalum genotypes under different salinity levels

Entry	*Myo*-inositol content (mg g^{-1} DW)						*F*-test[a]	LSD (0.05)[a]
	EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50		
SI 93-2	0.59	0.39	0.54	0.33	0.43	0.57	0.42	0.37
HI 101	0.41	0.34	0.38	0.36	0.55	0.44	0.56	0.26
Sea Isle 2000	0.38	0.36	0.33	0.40	0.53	0.60	*	0.18
TCR 1	0.32	0.31	0.33	0.37	0.55	0.64	**	0.15
TCR 6	0.37	0.43	0.35	0.34	0.48	0.65	***	0.11
Sea Isle 1	0.39	0.39	0.35	0.41	0.52	0.51	0.29	0.19
HI 34	0.29	0.32	0.27	0.36	0.53	0.48	0.20	0.25
SI 90	0.46	0.42	0.35	0.35	0.55	0.65	0.15	0.25
Adalayd	0.71	0.41	0.39	0.42	0.60	0.68	0.48	0.46
F-test[a]	0.44	0.35	0.51	0.77	0.35	0.39		
LSD (0.05)[a]	0.37	0.12	0.30	0.11	0.18	0.23		

[a] See Table 1 for explanations.

Table 10
Contribution of inorganic and organic osmolytes to shoot solute potential Ψ_s at EC_w50 dS m^{-1}

	Ψ_s (bar) measured	Ψ_s (bar) calculated[a]			Contribution (%)[c]		
		Inorganic	Organic[b]	Total	Inorganic[d]	Organic	Total
SI 93-2	−25.0	−14.1	−4.7	−18.8	56	19	75
HI 101	−25.9	−18.3	−5.3	−23.6	71	21	92
Sea Isle 2000	−24.3	−16.0	−5.1	−21.1	66	21	87
TCR 1	−27.0	−18.9	−5.9	−24.8	70	22	92
TCR 6	−25.1	−14.1	−5.6	−19.7	56	22	78
Sea Isle 1	−24.9	−24.2	−6.0	−30.2	97	24	121
HI 34	−24.9	−16.8	−5.5	−22.3	68	22	90
SI 90	−24.9	−15.6	−4.8	−20.4	63	19	82
Adalayd	−24.8	−20.3	−5.4	−25.7	82	22	104

[a] van't Hoff equation; Ψ_s (MPa) = $-c_s RT$, where $R = 0.0083143$ L MPa mol^{-1} K^{-1} and $T = 293$ K were considered. 1 bar = 0.10 MPa.
[b] Includes Gly-betaine, trigonelline, proline, fructose, glucose, *myo*-inositol and sucrose.
[c] Contribution = (Ψ_s calculated/Ψ_s measured) × 100.
[d] Includes K, Na, Cl, Mg, Ca, P, Fe, Mn, Zn, Cu, and B. See Lee et al. (2007).

species and usually a single type of polyol is present in a species.

The cyclic polyol, *myo*-inositol, was influenced by salinity (Table 9). The range of leaf *myo*-inositol content was lower than the soluble sugars with a range of 0.29–0.71 mg g^{-1} DW across grasses and salinity levels. As salinity level increased, leaf *myo*-inositol increased significantly for Sea Isle 2000, TCR 1 and TCR 6 compared to control. At each salinity level, no significant difference in *myo*-inositol content among genotypes was evident. The osmotic adjustment contribution of *myo*-inositol was limited and did not vary with genotype. *Myo*-inositol has been reported to function in some plants under salt stress as a compatible solute and in a cell signalling role (Hasegawa et al., 2000; Williamson et al., 2002); however, the minimal changes with salinity stress would suggest that neither role is important for seashore paspalum.

3.5. Organic osmolyte contribution to total solute potential

Organic osmolytes reported in this paper accounted for 19–24% of the Ψ_s at EC_w50 in seashore paspalum genotypes, while inorganic ions were responsible for 56–97% (Lee et al., 2007) (Table 10). Since synthesis of organic compounds is a high energy-requiring process, halophytes (ion includers) may rely on inorganic ion preferentially for osmoregulation (Rains, 1987; Glenn et al., 1992). Interestingly, the salt tolerant SI 93-2 genotype compared to the less tolerant Adalayd exhibited lower contributions of both inorganic and organic osmolytes to solute potential (SI 93-2 at 56 and 19%; and Adalyad at 82 and 22% for inorganic and organic osmolytes, respectively). Additionally, Lee et al. (2005b) reported that relative water content increased from 75% to 78% from EC_w40 and EC_w50 for SI 93-2, but decreased from 75% to 69% for Adalayd. Thus, the relative low contribution of osmolytes to Ψ_s in more salt tolerant genotypes could be at least partially explained by succulence effect, where higher water content can dilute solute contents and lead to less negative values of calculated Ψ_s. Because osmolality (mol L^{-1}) is an expression of moles of total dissolved solutes in water, Ψ_s is less negative when tissue water content is high (Alarcon et al., 1993). Also, salt tolerant SI 93-2 apparently has strong membrane integrity to maintain osmolytes against leaking out, which requires less organic osmolytes for osmotic adjustment. Since inorganic or organic osmolytes are accumulated in more tolerant SI 93-2 as salinity increased, excessive contents beyond osmoregulation might be partitioned to production of plant biomass.

In summary, this research indicates that the organic osmolytes most important within the salt tolerant seashore paspalum genotypes under salinity stress are proline, Gly-betaine, and trigonelline. Therefore, these organic osmolytes should be the focus of biotechnology approaches to enhance these traits. While these organic osmolytes differed in rate of accumulation or absolute concentrations between salt tolerant and intolerant genotypes, the magnitudes of response were not sufficiently large to allow for accurate use of any of these for physiological/biochemical marker assisted salt screening. Fructose concentration exhibited an inverse relationship to salinity tolerance and may have potential as a marker.

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References

Alarcon, J.J., Sanchez-Blanco, M.J., Bolarin, M.C., Torrecillas, A., 1993. Water relations and osmotic adjustment in *Lycopersicon esculentum* and *L. pennellii* during short-term salt exposure and recovery. Physiol. Plant 89, 441–447.

Alian, A., Altman, A., Heuer, B., 2000. Genotypic difference in salinity and water stress tolerance of fresh market tomato cultivars. Plant Sci. 152, 59–65.

Ashraf, M., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59, 207–216.

Ashraf, M., Harris, P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166, 3–16.

Bartels, D., Sunkar, R., 2005. Drought and salt tolerance in plants. Crit. Rev. Plant Sci. 24, 23–58.

Bates, L.S., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207.

Bohnert, H.J., Jensen, R.G., 1996. Metabolic engineering for increased salt tolerance—the next step. Aust. J. Plant Physiol. 23, 661–667.

Bohnert, H.J., Shen, B., 1999. Transformation and compatible solutes. Sci. Hortic. 78, 237–260.

Borsani, O., Valpuesta, V., Botella, M.A., 2003. Developing salt tolerant plants in a new century: a molecular biology approach. Plant Cell Tissue Organ Cult. 73, 101–115.

Chapman, G.W., Horvat, R.J., 1989. Determinat of nonvolatile acids and sugars from fruits and sweet potato extracts by capillary GLC and GLC/MS. J. Agric. Food Chem. 37, 947–950.

Dudeck, A.E., Peacock, C.H., Wildmon, J.C., 1993. Physiological and growth responses of St. Augustinegrass cultivars to salinity. HortScience 28, 46–48.

Duncan, R.R., Carrow, R.N., 1999. Turfgrass molecular genetic improvement for abiotic/edaphic stress resistance. Adv. Agron. 67, 233–305.

Duncan, R.R., Carrow, R.N., 2000. Seashore Paspalum—The Environmental Turfgrass. J. Wiley & Sons, Hoboken, NJ.

Epstein, E., Norlyn, J.D., Rush, D.W., Kingsbury, R.W., Kelley, D.B., Cunningham, G.A., Wrona, A.F., 1980. Saline culture of crops: a genetic approach. Science 210, 399–404.

Flowers, T.J., 1999. Salinization and horticultural production. Sci. Hortic. 78, 1–4.

Glenn, E.P., Watson, M.C., O'Leary, J.W., Axelson, R.D., 1992. Comparison of salt tolerance and osmotic adjustment of low-sodium and high-sodium subspecies of the C4 halophytes, *Atriplex canescens*. Plant Cell Environ. 15, 711–718.

Grattan, S.R., Grieve, C.M., Poss, J.A., Robison, P.H., Suarez, D.L., Benes, S.E., 2004. Evaluation of salt-tolerant forages for sequential water reuse systems. Agric. Water Manage. 70, 109–120.

Hasegawa, P.M., Bressan, R.A., Zhu, J., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Mol. Biol. 51, 463–499.

Hester, M.W., Mendelssohn, I.A., McKee, K.L., 2001. Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physiological constraints. Environ. Exp. Bot. 46, 277–297.

Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stat. Circ., 347.

Kerepesi, I., Galiba, G., Banyai, E., 1998. Osmotic and salt stress induced differential alterations in water-soluble carbohydrate content in wheat seedlings. J. Agric. Food Chem. 24, 5347–5354.

Lee, G.J., Carrow, R.N., Duncan, R.R., 2004a. Salinity tolerance of seashore paspalums and bermudagrasses: root and verdure responses and criteria. HortScience 39, 1143–1147.

Lee, G.J., Carrow, R.N., Duncan, R.R., 2004b. Photosynthetic responses to salinity stress in halophytic seashore paspalum genotypes. Plant Sci. 166, 1417–1425.

Lee, G.J., Carrow, R.N., Duncan, R.R., 2005a. Criteria for assessing salinity tolerance of the halophytic turfgrass seashore paspalum. Crop Sci. 45, 251–258.

Lee, G.J., Carrow, R.N., Duncan, R.R., 2005b. Growth and water relation responses to salinity stress in halophytic seashore paspalums genotypes. Sci. Hortic. 104, 221–236.

Lee, G.J., Duncan, R.R., Carrow, R.N., 2007. Nutrient uptake responses and inorganic ion contribution to solute potential under salinity stress in halophytic seashore paspalums. Crop Sci. 47, 2410–2419.

Marcum, K.B., 2002. Growth and physiological adaptations of grasses to salinity stress. In: Pessarakli, M. (Ed.), Handbook of Plant and Crop Physiology, 2nd ed. Marcel Dekker, Inc., New York, NY.

Marcum, K.B., Murdoch, C.L., 1992. Salt tolerance of the coastal salt marsh grass, *Sporobolus virginicus* (L.) kunth. New Phytol. 120, 281–288.

Marcum, K.B., Murdoch, C.L., 1994. Salinity tolerance mechanisms of six C4 turfgrasses. J. Am. Soc. Hortic. Sci. 119 (4), 779–784.

McNeil, S.D., Nuccio, M.L., Hanson, A.D., 1999. Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol. 120, 945–949.

Minorsky, P.V., 2002. Trigonelline: a diverse regulator in plants. Plant Physiol. 128, 7–8.

Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. Annu. Rev. Plant Physiol. 35, 299–350.

Rains, D.W., 1987. Metabolic energy cost for plant cells exposed to salinity. In: Newman, D.W., Willson, K.G. (Eds.), Models in Plant Physiology and Biochemistry. CRC Press, Boca Raton, FL, pp. 91–93.

Rhodes, D., Nadolska-Orczyk, A., Rich, P.J., 2002. Salinity, osmolytes and compatible solutes. In: Lauchli, A., Luttge, U. (Eds.), Salinity: Environment-Plants-Molecules. Kluwer Academic Publ., Boston, MA.

Rogers, M.E., et al., 2005. The potential for developing fodder plants for the salt-affected areas of southern and eastern Australia: an overview. Aust. J. Exp. Agric. 45, 301–329.

Roitch, T., 1999. Source-sink regulation of sugar and stress. Curr. Opin. Plant Biol. 2, 198–206.

Sairam, R.K., Tyagi, A., Chinnusamy, V., 2006. Salinity tolerance: cellular mechanisms and gene regulation. In: Huang, B. (Ed.), Plant–Environment Interactions, 3rd ed. CRC Press, Boca Raton, FL.

SAS Institute, 2001. Version 8, SAS Institute, Inc., Cary, NC.

Semple, W.S., Cole, I.A., Koen, T.B., 2003. Performance of some perennial grasses on severely salinized sites on the inland slopes of New South Wales. Aust. J. Exp. Agric. 43, 357–371.

Suzuki-Yamamoto, M., Mimura, T., Ashihara, H., 2006. Effect of short-term salt stress on the metabolic profiles of pyrimidine, purine and pyridine nucleotides in cultured cells of the mangrove tree, *Bruguiera sexangula*. Physiol. Plantarum 128, 405–414.

Turfseed, Inc., 2006. Seaspray Seeded Seashore Paspalum. Turfseed, Inc., Hubbard, OR. http://www.seasprayinfo.com/.

Williamson, J.D., Jennings, D.B., Guo, W., Pharr, D.M., 2002. Sugar alcohols, salt stress, and fungal resistance: polyol—multifunctional plant protection? J. Am. Soc. Hortic. Sci. 127 (4), 467–473.

Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.