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Identification of New Soluble Sugars Accumulated in a Halophytic Seashore Paspalum Ecotype under Salinity Stress

Geungjoo Lee[1], Robert N. Carrow[2*], and Ronny R. Duncan[3]

[1]Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeong-Eup 580-185, Korea
[2]Department of Crop and Soil Sciences, University of Georgia, Griffin, GA 30221, USA
[3]Turf Ecosystem, LLC., 110 Arroyo, Boerne, TX 78006, USA

Abstract. Seashore paspalum (*Paspalum vaginatum* Swartz) is a warm season turfgrass recognized as the most salt tolerant among the turfgrass species. This experiment was designed to analyze carbohydrate components in a salt tolerant ecotype HI 101 of seashore paspalum turfgrass, which was grown in a nutrient solution at EC_w50 (50 dS·m^{-1}) salinized with a sea-salt mixture (i.e., sea water = 54 dS·m^{-1}). Freeze-dried and ground aerial parts were extracted in 80% or 100% methanol, which were further hydrolyzed for a composition analysis by using a GC or GC-MS method. A GC method for detecting carbohydrate types showed that HI101 contained small amounts of arabinose (3.6 mole%), xylose (2.6%), mannose (1.2%), and glucuronic acid (5.1%), while the major components were galactose and glucose at 18 and 70%, respectively. Another alditol acetate method (GC-MS), for detection of neutral sugars, also indicated different kinds of carbohydrate with a higher amount of galactose (17 mole%) and glucose (62%), as well as minor carbohydrates such as xylose (7.8%), arabinose (4.7%), rhamnose (4.4%), and mannose (3.9%). In the previous analysis, some sucrose, fructose, myo-inositol, and glucose had already been accounted for; thus, the major peak might be a hexose galactose which was positively accumulated in the HI 101 with increasing salinity and incorporated into a cell-wall polysaccharide, revealing it as a compatible solute and osmoprotectant. The calculated solute potential by galactose and glucose was -2.5 and -12.4 bar, respectively, which accounted for 10 and 48% of a solute potential decrease at the 50 dS·m^{-1} salinity level.

***Additional key words*:** galactose, osmolyte, osmotic contribution, salinity tolerance, soluble carbohydrate

Introduction

During salt stress, an accumulation of compatible solutes in the cytoplasm of a cell has been recognized as one of the adaptive mechanisms of a plant for the counteraction of higher inorganic salts in a vacuole and in a root medium (Bohnert and Shen, 1999; Zhu, 2001; Rhodes et al., 2002). These compatible osmolytes can be classified into three classes encompassing amino acids and their derivatives: polyols and sugars, and methyamines (Yancey et al., 1982; Anjum et al., 2000; Adams et al., 2005). These compatible solutes were also found to have an osmoprotection role including stabilizing a protein conformation and activity, maintaining the membrane integrity for a plasma or thylakoid membrane transport, and scavenging of reactive oxygen species that are by-products of abiotic stresses such as drought, extreme of temperature, or a salt stress (Saneoka et al., 1995; Bartels and Sunkar, 2005; Sairam et al., 2006).

Among those compatible osmolytes, soluble carbohydrates, reported to be affected by a salinity stress, can be grouped into simple sugars (fructose, glucose), disaccharides (trehalose, sucrose), and sugar alcohols or polyols (sorbital, mannitol, galactitol, and cyclic polyols such as *myo*-inosotol, ononitol, pinnitol) (Zhu, 2001; Bartels and Sunkar, 2005; Sairam et al., 2006). A diasaccharide trehalose was reported to be the most efficient for protecting the structure and activities of yeast cytosolic enzymes by preventing a protein unfolding from a salt stress (Sola-Penna et al., 1997).

We have previously observed that seashore paspalum (*Paspalum vaginatum* Swartz) ecotypes exhibited fructose, glucose, sucrose, and *myo*-inositol accumulations with an increasing salinity, while some sugar alcohols (mannitol and sorbitol) were not detected (Lee et al., 2007b). During the quantification of some known organic solutes by using a chromatography system, we detected some significant unknown compounds that occupied 45 and 7 to 16% of the total peak area from an HPLC and GC chromatography, respectively, for a salt tolerant HI 101 ecotype at a 50 dS·m^{-1} salinity level. Although these unknown compounds seemed not to be related to an intraspecific difference in an absolute concentration among ecotypes, osmotic adjustment to a salinity stress in the halophytic seashore paspalum was evident in terms of a greater accumulation rate with an increasing salinity.

The objectives of this study, therefore, were to separate

***Corresponding author:** rcarrow@uga.edu
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and identify various soluble carbohydrates accumulated under a high salinity stress, and to estimate potential contribution to a total solute potential (Ψ_s) decrease in the halophytic seashore paspalum ecotype HI 101. When considering the various kinds of soluble carbohydrates during metabolism under salinity stress, certain sugars and polyols may also contribute to an osmotic adjustment. Results from this study will provide new information on soluble carbohydrates potentially functioning as compatible osmolytes, in response to a salinity stress in the halophytic turfgrass.

Materials and Methods

A seashore paspalum ecotype, 'HI 101' which is one of the most salt tolerant among the collections at the University of Georgia and grown at EC_w50 was selected for this study (Hoagland. and Arnon. 1950; Lee et al., 2007a). During the HPLC and GC analysis to quantify the organic compounds including proline, glycine betaine, trigonelline, and soluble sugars (Lee et al., 2007b), some unknown compounds were detected and their peak areas (%) were estimated to track the relative concentration changes with salinity and ecotype treatments.

Some unknown compounds with dramatic changes as salinity increased were analyzed at the Complex Carbohydrate Research Center, Athens, GA. A composition analysis (York et al., 1986) was performed using leaf samples of the ecotype HI 101 that were used to extract carbohydrates with the same methods as for the GC (150 mg of freeze-dried and ground leaves was used to extract by homogenizing for 1 min. in 80% of methanol) or HPLC preparation (0.4 g of the freeze-dried and ground leaf samples were soaked in 10 mL of 100% methanol at 70℃ for 1 hour with three vortexes during the extraction process). The extracts were hydrolyzed in freshly prepared 1 M methanolic-HCl for 16 h at 80℃. The released sugars were derivatized with Tris-Sil and the samples were resolved on a 30 m DB-1 column (0.25 mm × 0.25 mm, i.d.; Supelco, St. Louis, USA) in a Hewlett Packard 5985 GC-MS system. Twenty μg of *myo*-inositol was also added as an internal standard.

The alditol acetate method was adopted to detect the neutral sugars (Sawardeker et al., 1965). The extracts in the method were hydrolyzed in 2 M TFA at 121℃ for 2 hrs and then reduced with sodium borodeuteride at the selected retention time. The product was acetylated using acetic anhydride at 120℃ for 3 hrs. The derivatized samples were analyzed by a GC-MS using a Sp2330 column (Supelco, St. Louis, USA). Internal standard, myo-inositol, was added to the samples prior to the reduction step.

From the composition and alditol acetate methods, the concentration and mole percentage of the detected carbohydrate residues were estimated based on an internal standard, myo-inositol. The contribution of each organic osmolyte to the 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 the measured Ψ_s was based on a relative dry weight at saturation [dry weight/(saturated weight-dry weight)] and an osmolyte concentration on a dry weight basis. Total solute potential (Ψ_s) and leaf water potential (Ψ_w) of the leaf tissue sap was determined as reported by Lee et al. (2005). We assumed that the detected soluble carbohydrates behaved as ideal osmotica as noted by Alarcon et al. (1993).

Results

Unknown compounds detected in the HPLC and GC analysis

The GC analytical data identified some of the simple carbohydrate of hexoses (fructose, glucose, inositol) and disaccharide sucrose peaks (Fig. 1). Several unknown compounds were observed from the GC running among which 5 major peaks were detected at retention times of 7.1, 8.5, 10.1, 11.6, and 18.2 min. The relative peak area (%) indicated that some of the unknown peaks were detected at 8.5, 10.1, and 18.2 min. accumulated in the shoot tissue with increasing salinity, especially with the maximum content at EC_w50 (Table 1). Another extraction method for the HPLC analysis identified proline, glycine betaine, and trigonelline (Fig. 2). The HPLC data also showed that 6 unknown peaks were identified at the retention time of 1.8, 2.5, 3.1, 3.4, and 6.5 min. One of the unknown peaks

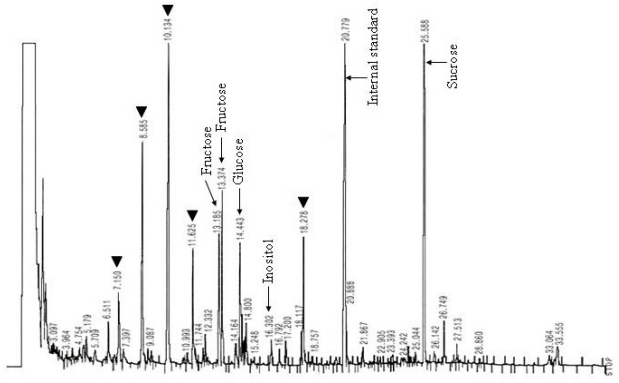

Fig. 1. A GC chromatogram for separating carbohydrate residues in the shoot tissue of HI 101 ecotype of seashore paspalum turfgrass.grown at EC_w50 which was prepared by an 80% methanol extraction followed by the Oxime-trimethylsilyl (Oxime-TMS) derivatizing method. Triangle symbols (▼) indicate the peaks of the unknown compounds.

Table 1. Changes in the relative peak area of some unknown compounds of HI 101 seashore paspalum turfgrass grown at different salinity levels when compared with the peak area of an internal standard.

Chromatography used	Unknown compound at retention time (min)	Relative peak area (%)[z] at					
		EC_w0	EC_w10	EC_w20	EC_w30	EC_w40	EC_w50
GC	8.5	28.0±0.6[y]	24.1±1.8	28.2±2.5	25.8±3.3	28.1±3.7	37.5±0.3
	10.1	5.4±1.1	10.9±0.6	15.2±2.1	31.7±8.3	37.2±12.8	82.3±2.2
	18.2	11.2±2.9	9.8±3.3	7.5±1.2	11.2±0.7	14.3±0.5	21.5±0.6
HPLC	6.5	67.8±6.1	95.6±4.4	161.0±21.0	191.1±19.2	225.5±15.9	279.6±15.7

[z]Reflects the modified area of a unknown peak based on the peak area of the internal standard.
[y]The values represent means±S.E. of three observations.

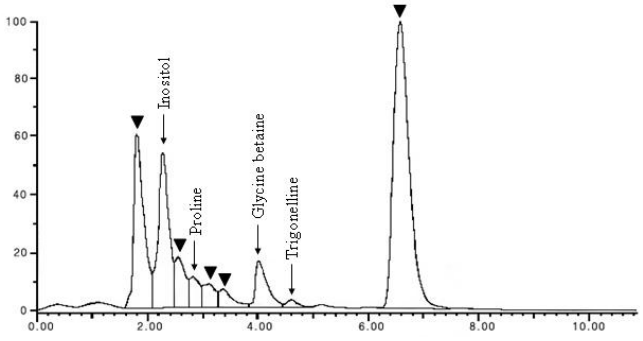

Fig. 2. An HPLC chromatogram for separating carbohydrate residues in the shoot tissue of HI 101 seashore paspalum turfgrass grown at EC_w50 which was prepared by a 100% methanol extraction at 70℃ for 1 hour. Triangle symbols (▼) indicate the peaks of the unknown compounds.

having the highest relative peak area (%) at 6.5 min. was also found to be positively accumulated (4 times) with a salt addition to the culture solution (Table 1).

Carbohydrate residues from the composition analysis

This method was designed to analyze the carbohydrate composition which occurred in two different extraction samples. Regardless of the extraction methods, the composition analysis on the gas chromatography managed to separate two pentose (arabinose and xylose) and four hexose monosaccharides (glucuronic acid, mannose, galactose, glucose) (Fig. 3 and 4). The result showed that the shoot samples contained small amounts of arabinose, xylose, glucuronic acid, and mannose. The relative percentage of the minor carbohydrate residues was less than 5%, while the major components were galactose at 15 to 18% and glucose at 70 to 73% at the EC_w50 salinity level (Table 2). The average content of the carbohydrates ranged from 41.4 (7.5 $mg \cdot g^{-1}$ DW) to 2406.1 $nmol \cdot mg^{-1}$ (433.5 $mg \cdot g^{-1}$ DW) for mannose and glucose, respectively, with a total of 3463.7 $nmol \cdot mg^{-1}$.

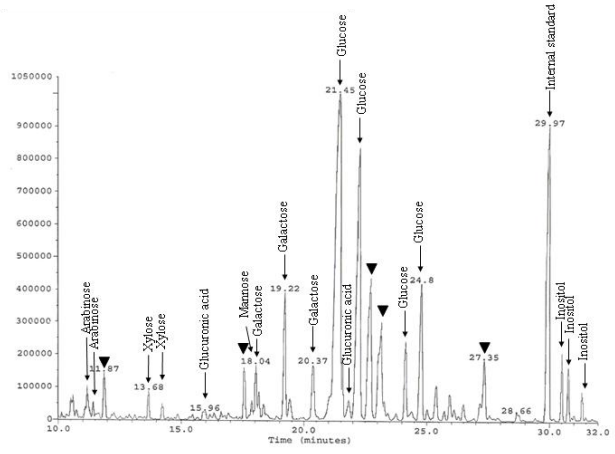

Fig. 3. A composition analysis from a GC that shows carbohydrate compositions in the shoot tissue of HI 101 seashore paspalum turfgrass grown at EC_w50 where the sample was prepared by 100% methanol extraction. Triangle symbols (▼) indicate the peaks of the unknown compounds.

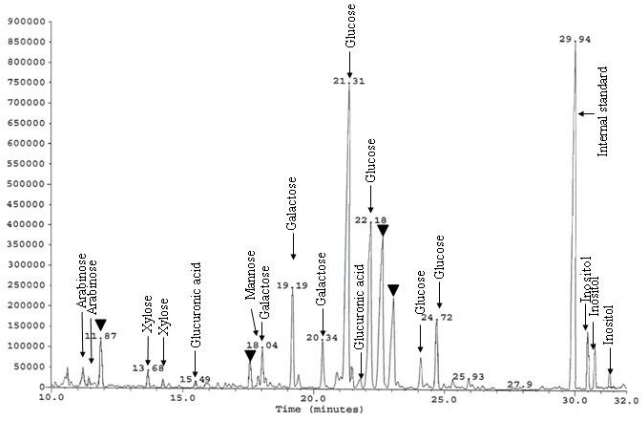

Fig. 4. A composition analysis from a GC that shows carbohydrate compositions in the shoot tissue of HI 101 seashore paspalum turfgrass grown at EC_w50 where the sample was prepared by 80% methanol extraction. Triangle symbols (▼) indicate the peaks of the unknown compounds.

Carbohydrate residues from the alditol acetate method

Another composition analysis, the alditol acetate method for the detection of the neutral sugars managed to separate

Table 2. Carbohydrate residues of HI 101 seashore paspalum turfgrass grown at EC_w50 from a composition analysis for which the samples were prepared by two different extraction method for a GC or HPLC analysis.

Carhohydrate	GC extract		HPLC extract		Mean[z]	
	nmol·mg^{-1}	Mole%	nmol·mg^{-1}	Mole%	nmol/mg·mg^{-1}	Mole%
Arabinose	64.8	3.3	182.6	3.7	123.7	3.6
Xylose	50.9	2.6	130.9	2.6	90.9	2.6
Glucuronic acid	104.2	5.3	253.9	5.1	179.1	5.1
Mannose	18.7	1.0	64.0	1.3	41.4	1.2
Galactose	293.6	14.8	951.4	19.2	622.5	17.9
Glucose	1444.5	73.1	3367.8	68.0	2406.1	69.5
Total	1976.7	100	4950.6	100	3463.7	100

[z] Average value from GC and HPLC extracts.

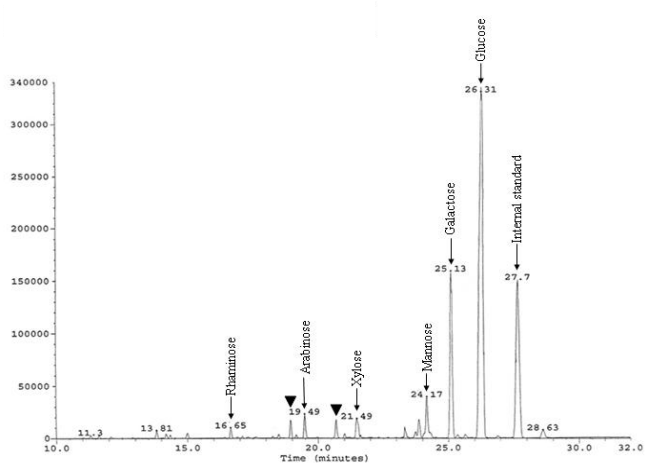

Fig. 5. An alditol acetate analysis from a GC-MS showing carbohydrate compositions in the shoot tissue of HI 101 seashore paspalum turfgrass grown at EC_w50 where the sample was prepared by 100% methanol extraction. Triangle symbols (▼) indicate the peaks of the unknown compounds.

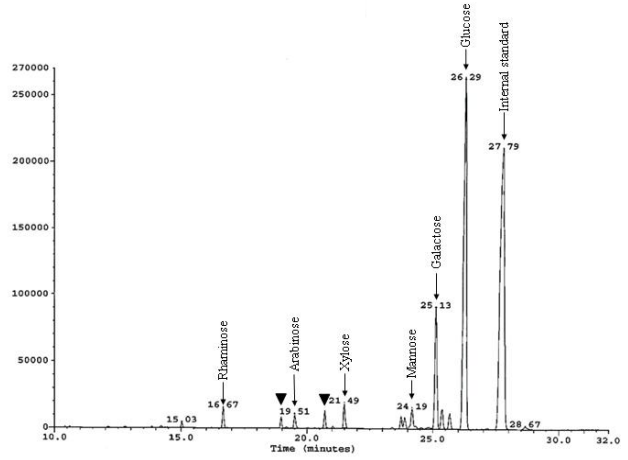

Fig. 6. An alditol acetate analysis appeared on a GC-MS showing carbohydrate compositions in the shoot tissue of HI 101 seashore paspalum turfgrass grown at EC_w50 where the sample was prepared by 80% methanol extraction. Triangle symbols (▼) indicate the peaks of the unknown compounds.

small amounts of arabinose, xylose, mannose, and a new component rhaminose, while the chromatogram again showed a major presence of hexoses galactose and glucose (Fig. 5 and 6). Shoot carbohydrate content at EC_w50 was higher from the alditol acetate method on the GC-MS than the GC composition analysis with an average range of 76.8 (12.6 mg·g^{-1} DW) to 1339.0 nmol·mg^{-1} (241.2 mg·g^{-1} DW) (Table 3). The minor components contributed relatively less percentage than 8%, while the hexoses galactose and glucose occupied 16 to 19% with an average of 69.8 mg·g^{-1} DW and 62% with an average of 241.2 mg·g^{-1} DW, respectively.

Contribution of the carbohydrate residues to the total solute potential

The calculated solute potential of the minor carbohydrate components (arabinose, xylose, glucuronic acid, mannose) ranged from -0.04 to -0.15 MPa from a composition analysis on a GC. Similarly solute potentials of the minor components (arabinose, rhaminose, xylose, mannose) from an alditol acetate method on a GC-MS ranged from -0.07 to -0.14 MPa. The calculated solute potential of the major carbohydrates was -0.33 to -0.53 MPa for galactose, and -1.15 to -2.06 MPa for glucose. The measured solute potential of HI 101 at EC_w50 was -2.59 MPa which was used for an estimation of the osmotic contribution of the detected carbohydrates (Table 4). Osmotic contribution to the shoot solute potential decrease of each carbohydrate residue from two different analyses was less than 6% for the minor components, while galactose and glucose accounted for 13 to 21% and 44 to 80%, respectively. If all the detected components of the carbohydrate were totally used up for the solute potential decrease, they accounted for 71 to 115% of an osmoregulation.

Table 3. Carbohydrate residues of HI 101 seashore paspalum turfgrass grown at EC_w50 from an Alditol acetate analysis on a GC-MS system for which the samples were prepared by two different extraction methods for a GC or HPLC analysis.

Carhohydrate	GC extract		HPLC extract		Mean[z]	
	nmol·mg^{-1}	Mole%	nmol·mg^{-1}	Mole%	nmol·mg^{-1}	Mole%
Arabinose	49.6	4.1	161.2	5.2	105.4	4.7
Rhamnose	75.6	6.3	78.0	2.5	76.8	4.4
Xylose	96.4	8.1	230.6	7.4	163.5	7.8
Mannose	35.1	2.9	148.8	4.8	92.0	3.9
Galactose	194.3	16.2	580.5	18.5	387.4	17.4
Glucose	747.0	62.4	1930.9	61.7	1339.0	62.1
Total	1198.0	100	3130.0	100	2164.1	100

[z] Average value from GC and HPLC extracts.

Table 4. Contribution of the carbohydrate compositions to the shoot solute potential of HI 101 seashore paspalum turfgrass grown at EC_w50 (=49.9 dS·m^{-1}).

Method	Carbohydrate	Content (mg·g^{-1} DW)	Osmolality (mol·L^{-1})	Ψ_s calculated (MPa)[z]	Contribution (%)[y]
Composition analysis	Arabinose	18.6	0.044	-0.11	4
	Xylose	13.7	0.032	-0.08	3
	Glucuronic acid	34.8	0.063	-0.15	6
	Mannose	7.5	0.015	-0.04	1
	Galactose	112.1	0.219	-0.53	21
	Glucose	433.48	0.845	-2.06	80
	Sub total				115
Alditol acetate analysis	Arabinose	15.8	0.037	-0.09	3
	Rhamnose	12.6	0.027	-0.07	3
	Xylose	24.6	0.057	-0.14	5
	Mannose	16.6	0.032	-0.08	3
	Galactose	69.8	0.136	-0.33	13
	Glucose	241.2	0.471	-1.15	44
	Sub total				71

[z] van't Hoff equation; Ψ_s (MPa) = $-c_s$RT, where R=0.0083143 L MPa mol^{-1}K^{-1} and T = 293°K, was considered.
[y] Contribution = (Ψ_s calculated / Ψ_s measured) x100 where the denominator value was -2.59 MPa (Lee et al., 2005).

Discussion

Some carbon compounds such as carbohydrates, sugar alcohols, and organic acids play important roles as food reserves, constitutive substances, cell signalling, and a cell protection from abiotic stresses (Loewus and Murthy, 1999; Hasegawa et al., 2000; Williamson, et al., 2002). Williamson et al. (2002) noted that the polyols present in plants varies with species and usually a single type of the polyol is present in a species. Our previous measurements of the principal organic compounds of the halophytic seashore paspalum detected some mono- and disaccharides (fructose, glucose, *myo*-inositol and sucrose) and no polyols such as mannitol and sorbitol under salinity stress conditions (Lee et al., 2007b). Some unknown compounds were also highly accumulated at EC_w50 when compared with those measured in a non-salinized control (Table 1, Fig. 1 and 2). Composition analyses indicated that seashore paspalum ecotype HI 101 synthesized some minor (arabinose, rhamnose, xylose, glucuronic acid, and mannose) and major (galactose and glucose) carbohydrates (Table 2 and 3).

Accumulation of glucose, galactose, arabinose, and rhamnose was found in halophytic forages growing in marshy habitats (Joshi et al., 2005). In the previous GC analysis, we had detected some sucrose, fructose, *myo*-inositol, and glucose in the sample where galactose was not identified (Fig. 1 and Lee et al., 2007). Accordingly, galactose is a new residue contained in the sample and the unknown peak might be galactose. In addition to an osmotic adjustment, galactose has been reported to be increased in tonoplast lipids, thus enhancing the activities of the transporting enzymes such as H^+-ATPase and H^+-PPase of barley under a salt stress (Gong et al., 1999). A UDP-glucose which is generated from sucrose is proposed to be

converted to UDP-galactose with a uridine -galactose-4-epimerase (UDP-Gal epimerase) (Dormann and Benning, 1998; Reiter and Vanzin, 2001; Liu et al., 2007). A *PvUGE1* gene which encodes UDP-galactose epimerase was isolated in a salt tolerant seashore paspalum (Endo et al., 2005). Endo et al. (2005) reported that the root growth and survival rate of transgenic rice with the *PvUGE1* gene from seashore paspalum were significantly increased. In this respect, it is speculated that by the *PvUGE1* gene, the elevated galactose level of HI 101 under a salinity stress can be converted to UDP-galactose which was further incorporated into the cell-wall polysaccharides allowing a cell-wall integration or used for a biosynthesis of numerous carbohydrates such as a raffinose (Liu et al., 2007).

Because the mole percentage of some minor carbohydrates was less than 8% (Table 2 and 3), their contribution to the decrease in the solute potential due to those carbohydrates did not exceed a total of 14%, while the osmotic contribution of galactose and glucose reached the maximum values of 21 and 80%, respectively. Also, some portion of osmotic adjustment by organic compounds could be from the unidentified compounds which were revealed in the GC and HPLC chromatograms. Since inorganic or organic osmolytes are accumulated in more tolerant ecotypes as salinity increases, it seemed likely that excessive contents beyond an osmoregulation against a higher osmotic stress by salt addition could be partitioned to an enhanced plant biomass production (Lee et al., 2007b). Results indicated that the major sugar components detected in the HI 101 shoots under salinity stress may serve both as a compatible solute and an osmoprotectant.

Further investigation needs to be conducted to identify some of the organic compounds detected from the two composition analyses and to clarify the relationship of a shift in the carbohydrate partitioning between soluble sugars or between nonstructural and structural sources in seashore paspalum under salinity stress. Also, it remains to be determined whether the carbon sources are from a photosynthetic assimilation or from a conversion of the stored carbohydrates. This information as a whole should be useful for seashore paspalum managers and breeders for enhancing the performance of seashore paspalum in response to salinity stress.

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Literature Cited

Adams, M.A., A. Richter, A.K. Hil, and T.D. Dolmer. 2005. Salt tolerance in Eucalyptus spp.: identity and response of putative osmolytes. Plant Cell Env. 28:772-787.

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

Anjum, F., V. Rishi, and F. Ahmad. 2000. Compatibility of osmolytes with Gibbs energy of stabilization of protein. Biochimica Biophysica Acta 1476:75-84.

Bartels, D. and R. Sunkar. 2005. Drought and salt tolerance in plants. Critical Reviews in Plant Sciences 24:23-58.

Bohnert, H.J. and B. Shen. 1999. Transformation and compatible solutes. Scientia Hort. 78:237-260.

Dormann, P. and C. Benning. 1998. The role of UDP-glucose epimerase in carbohydrate metabolism of Arabidopsis. Plant J. 13:641-652.

Endo, N., K. Yoshida, M. Akiyoshi, Y. Yoshida, and N. Hayashi. 2005. Putative UDP-galactose epimerase and metallothioneine of *Paspalum vaginatum* enhanced the salt tolerance of rice, *Oryza sativa* L. from transplanting to harvest stages. Breeding Sci. 55:163-173.

Gong, H.M., B.J. Yu, and Y.L Liu. 1999. Effects of fatty acids on lipid composition and function of tonoplast vesicles in barley seedlings under salt stress. Acta Bot. Sin. 41, pp. 414-419.

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

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. California Agric. Exp. Stn. Circular 347.

Joshi, A.J., B.S. Mali, and H. Hinglajia. 2005. Salt tolerance at germination and early growth of two forage grasses growing in marshy habitats. Env. Exp. Bot. 54:267-274.

Lee, G.J., R.N. Carrow, and R.R. Duncan. 2005. Growth and water relation responses to salinity stress in halophytic seashore paspalums genotypes. Scientia Hort. 104:221-236.

Lee, G.J., R.N. Carrow, R.R. Duncan, M.A. Eiteman, and M.W. Rieger. 2007b. Synthesis of organic osmolytes and salt tolerance mechanisms in *Paspalum vaginatum*. Env. Exp. Bot (*In press*).

Lee, G.J., R.R. Duncan, and R.N. Carrow. 2007a. Nutrient uptake responses and inorganic ion contribution to solute potential under salinity stress in halophytic seashore paspalum. Crop Sci. 47: 2504-2512.

Liu, H.L., X.Y. Dae, Y.Y. Xu, and K. Chong. 2007. Over-expression of *OsUGE-1* altered raffinose level and tolerance to abiotic stress but not morphology in *Arabidopsis*. J. Plant Physiol. 164:1384-1390.

Loewus, F.A. and P.P.N. Murthy. 1999. *myo*-inositol metabolism in plants. Plant Sci. 150:1-19.

Reiter W.D. and G.F. Vanzin. 2001. Molecular genetics of nucleotide sugar interconversion pathways in plants. Plant Mol. Biol. 47:95-113.

Rhodes, D. and A.D. Hanson. 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 44:357-384.

Sairam, R.K., A.Tyagi, and V. Chinnusamy. 2006. Salinity tolerance: cellular mechanisms and gene regulation. P121-176. In: B. Huang (Ed.). Plant-environment interactions. 3rd Edition. CRC Press, Boca Raton, FL.

Saneoka, H., C. Nagasaka, D.T. Hahn, W. Yang, G.S. Premachandra, Joly R.J., and D. Rhodes. 1995. Salt tolerance of glycinebetaine-deficient and -containing maize lines. Plant Physiol. 107:631-638.

Sawardeker, J.S., J.H. Sloneker, and A. Jeanes. 1965. Quantitative determination of mono-saccharides as their alditol acetates by gas liquid chromatography. Anal. Chem.37:1602-1604.

Sola-Penna, M., A. Ferreira-Pereira, A.P. Lemos, and J.R. Meyer-Fernandes. 1997. Carbohydrate protection of enzyme structure and function against guanidinium chloride treatment depends on the nature of carbohydrate and enzyme. Eur. J. Biochem. 248: 24-29.

Williamson, J.D., D.B. Jennings, W. Guo, and D.M. Pharr. 2002. Sugar alcohols, salt stress, and fungal resistance: polyols-multifunctional plant protection? J. Amer. Soc. Hort. Sci. 127(4): 467-473.

Yancey, P.H., M.E. Clark, S.C. Hand, R.D. Boulus, and G.N. Somero. 1982. Living with water stress: evolution of osmolyte systems. Science 217:1214-1222.

York, W.S., A.G. Darvill, M McNeill, T.T. Stevenson, and P. Albersheim. 1986. Isolation and characterization of cell walls and cell wall components. Methods Enzymol. 118:3-40.

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