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

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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 ECw50 (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 (sorbitol, mannitol, galactitol, and cyclic polyols such as myo-inositol, ononitol, pinnitol) (Zhu, 2001; Bartels and Sunkar, 2005; Sairam et al., 2006). A disaccharide 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 abiotic stress 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
and identify various soluble carbohydrates accumulated under
a high salinity stress, and to estimate potential contribution
to a total solute potential (Ψₚ) 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 Uni-
versity of Georgia and grown at ECₜ₅₀ 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 Carbo-
hydrate 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 homo-
genizing for 1 min. in 80% of methanol) or HPLC pre-
paration (0.4 g of the freeze-dried and ground leaf samples
were soaked in 10 mL of 100% methanol at 70°C 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°C. 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°C for 2 hrs
and then reduced with sodium borodeuteride at the selected
retention time. The product was acetylated using acetic
anhydride at 120°C 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 carbo-
hydrate residues were estimated based on an internal stan-
dard, myo-inositol. The contribution of each organic osmolyte
to the total solute potential (Ψₚ) 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 Ψₚ, 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 (Ψₛ) and leaf water potential (Ψₖ) 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 com-
ounds 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 EC₅₀ (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 EC₅₀ 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.](image-url)
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 ECw50 salinity level (Table 2). The average content of the carbohydrates ranged from 41.4 (7.5 mg·g⁻¹ DW) to 2406.1 nmol·mg⁻¹ (433.5 mg·g⁻¹ DW) for mannose and glucose, respectively, with a total of 3463.7 nmol·mg⁻¹.

### 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.

<table>
<thead>
<tr>
<th>Chromatography used</th>
<th>Unknown compound at retention time (min)</th>
<th>ECw0</th>
<th>ECw10</th>
<th>ECw20</th>
<th>ECw30</th>
<th>ECw40</th>
<th>ECw50</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC</td>
<td>8.5</td>
<td>28±0.6</td>
<td>24.1±1.8</td>
<td>28.2±2.5</td>
<td>25.8±3.3</td>
<td>28.1±3.7</td>
<td>37.5±0.3</td>
</tr>
<tr>
<td></td>
<td>10.1</td>
<td>5.4±1.1</td>
<td>10.9±0.6</td>
<td>15.2±2.1</td>
<td>31.7±8.3</td>
<td>37.2±12.8</td>
<td>82.3±2.2</td>
</tr>
<tr>
<td></td>
<td>18.2</td>
<td>11.2±2.9</td>
<td>9.8±3.3</td>
<td>7.5±1.2</td>
<td>11.2±0.7</td>
<td>14.3±0.5</td>
<td>21.5±0.6</td>
</tr>
<tr>
<td>HPLC</td>
<td>6.5</td>
<td>67.8±6.1</td>
<td>95.6±4.4</td>
<td>161.0±21.0</td>
<td>191.1±19.2</td>
<td>225.5±15.9</td>
<td>279.6±15.7</td>
</tr>
</tbody>
</table>

*Reflects the modified area of a unknown peak based on the peak area of the internal standard. The values represent means±S.E. of three observations.
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 ECw50 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 ECw50 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 2.** Carbohydrate residues of HI 101 seashore paspalum turfgrass grown at ECw50 from a composition analysis for which the samples were prepared by two different extraction method for a GC or HPLC analysis.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>GC extract</th>
<th>HPLC extract</th>
<th>Mean²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol·mg⁻¹</td>
<td>Mole%</td>
<td>nmol·mg⁻¹</td>
</tr>
<tr>
<td>Arabinose</td>
<td>64.8</td>
<td>3.3</td>
<td>182.6</td>
</tr>
<tr>
<td>Xylose</td>
<td>50.9</td>
<td>2.6</td>
<td>130.9</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>104.2</td>
<td>5.3</td>
<td>253.9</td>
</tr>
<tr>
<td>Mannose</td>
<td>18.7</td>
<td>1.0</td>
<td>64.0</td>
</tr>
<tr>
<td>Galactose</td>
<td>293.6</td>
<td>14.8</td>
<td>951.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>1444.5</td>
<td>73.1</td>
<td>3367.8</td>
</tr>
<tr>
<td>Total</td>
<td>1976.7</td>
<td>100</td>
<td>4950.6</td>
</tr>
</tbody>
</table>

² Average value from GC and HPLC extracts.
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 ECw50 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

Table 3. Carbohydrate residues of HI 101 seashore paspalum turfgrass grown at ECw50 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.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>GC extract</th>
<th>HPLC extract</th>
<th>Mean†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol·mg⁻¹</td>
<td>Mole%</td>
<td>nmol·mg⁻¹</td>
</tr>
<tr>
<td>Arabinose</td>
<td>49.6</td>
<td>4.1</td>
<td>161.2</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>75.6</td>
<td>6.3</td>
<td>78.0</td>
</tr>
<tr>
<td>Xylose</td>
<td>96.4</td>
<td>8.1</td>
<td>230.6</td>
</tr>
<tr>
<td>Mannose</td>
<td>35.1</td>
<td>2.9</td>
<td>148.8</td>
</tr>
<tr>
<td>Galactose</td>
<td>194.3</td>
<td>16.2</td>
<td>580.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>747.0</td>
<td>62.4</td>
<td>1930.9</td>
</tr>
<tr>
<td>Total</td>
<td>1198.0</td>
<td>100</td>
<td>3130.0</td>
</tr>
</tbody>
</table>

† 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 ECw50 (=49.9 dS·m⁻¹).

<table>
<thead>
<tr>
<th>Method</th>
<th>Carbohydrate</th>
<th>Content (mg·g⁻¹ DW)</th>
<th>Osmolality (mol·L⁻¹)</th>
<th>Ψₛ calculated (MPa)‡</th>
<th>Contribution (%)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>Arabinose</td>
<td>18.6</td>
<td>0.044</td>
<td>-0.11</td>
<td>4</td>
</tr>
<tr>
<td>analysis</td>
<td>Xylose</td>
<td>13.7</td>
<td>0.032</td>
<td>-0.08</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Glucuronic acid</td>
<td>34.8</td>
<td>0.063</td>
<td>-0.15</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Mannose</td>
<td>7.5</td>
<td>0.015</td>
<td>-0.04</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>112.1</td>
<td>0.219</td>
<td>-0.53</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>433.48</td>
<td>0.845</td>
<td>-2.06</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Sub total</td>
<td></td>
<td></td>
<td></td>
<td>115</td>
</tr>
<tr>
<td>Alditol acetate</td>
<td>Arabinose</td>
<td>15.8</td>
<td>0.037</td>
<td>-0.09</td>
<td>3</td>
</tr>
<tr>
<td>analysis</td>
<td>Rhamnose</td>
<td>12.6</td>
<td>0.027</td>
<td>-0.07</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Xylose</td>
<td>24.6</td>
<td>0.057</td>
<td>-0.14</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Mannose</td>
<td>16.6</td>
<td>0.032</td>
<td>-0.08</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>69.8</td>
<td>0.136</td>
<td>-0.33</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>241.2</td>
<td>0.471</td>
<td>-1.15</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Sub total</td>
<td></td>
<td></td>
<td></td>
<td>71</td>
</tr>
</tbody>
</table>

‡ van’t Hoff equation: Ψₛ (MPa) = cₛRT, where R=0.0083143 L MPa mol⁻¹ K⁻¹ and T = 293°K, was considered.
§ Contribution = (Ψₛ calculated / Ψₛ measured) x100 where the denominator value was 2.59 MPa (Lee et al., 2005).
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 paspalam (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 paspalam 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 paspalam 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 paspalam managers and breeders for enhancing the performance of seashore paspalam in response to salinity stress.

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**Literature Cited**


