Root-Zone Salinity Alters Raffinose Oligosaccharide Metabolism and Transport in Coleus

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Exposure of variegated coleus (Coleus blumei Benth.) plants to a saline root-zone environment (60 mM NaCl:12 mM CaCl₂) resulted in a significant decline in elongation growth rate over the 30-d experimental period. During the initial 5 to 10 d of exposure, mature source leaves showed strongly diminished rates of photosynthesis, which gradually recovered to close to the control rates by the end of the experiment. In green leaf tissues, starch levels showed the same transient decline and recovery pattern. Low starch levels were accompanied by the appearance of several novel carbohydrates, including high-molecular-weight raffinose family oligosaccharides (RFOs) with a degree of polymerization (DP) of 5 to 8, and an O-methylated inositol (OMI). New enzyme activities, including galactan:galactan galactosyltransferase, for the synthesis of high-DP RFOs and myo-inositol 6-O-methyltransferase for O-methylation of myo-inositol, were induced by salinity stress. Phloem-sap analysis showed that in the stressed condition substantially more sucrose than RFO was exported, as was the OMI. In white sink tissues these phloem sugars were used to synthesize high-DP RFOs but not OMIls. In sink tissues galactan:galactan galactosyltransferase but not myo-inositol 6-O-methyltransferase was induced by salinity stress. Models reflecting the changes in carbohydrate metabolism in source and sink tissues in response to salinity stress are presented.

Salinity is only one of many environmental factors resulting in suboptimal crop growth, yet its impact is perhaps one of the most far-reaching in agronomic terms. One frequently reported effect of salinity stress is a reduction in the shoot/root ratio of the stressed plant (Hanson and Hitz, 1982). This observation has led to the conclusion that carbon allocation is altered by salt stress, a conclusion that is supported by observed changes in photosynthetic rate under stress conditions (Everard et al., 1994). The reductions in photosynthesis may be at least partially due to nonstomatal factors, suggesting that chloroplast metabolism, and therefore carbon partitioning processes, are also adversely affected by salinity stress (Everard et al., 1994). However, despite the clear alterations in growth and photosynthetic metabolism that occur in response to salt stress, the exact nature of the changes in carbon metabolism that are induced by salt stress have not been elucidated (Munns, 1993).

Apart from some recent studies on celery, a mannitol-translocating species (Everard et al., 1994; Stoop and Pharr, 1994), and iceplant (Vernon and Bohnert, 1992), a cyclitol-accumulating species, most research on salinity stress responses has been performed on species that synthesize and export only the disaccharide Suc. In salt-tolerant species such as celery and iceplant, salt stress has been found to induce synthesis of polyols (linear polyhydric sugar alcohols) and cyclitols (cyclic polyhydric sugar alcohols), at the expense of more common storage carbohydrates such as starch and Suc (Loescher and Everard, 1996). These observations have led to the suggestion that naturally occurring changes in carbohydrate metabolism may have an adaptive role in allowing plants to survive under saline conditions (Pharr et al., 1995; Bohnert and Jensen, 1996; Loescher and Everard, 1996). Support for this idea comes from experiments using transgenic plants, in which significant tolerance to salinity stress has been imparted to salt-sensitive plants by expression of a gene coding for the synthesis of novel and specific carbohydrates, for example the polyol mannitol in tobacco (Tarczynski et al., 1993). What these transgenic plant studies suggest is that subtle changes in carbohydrate biochemistry can result in significant increases in salinity tolerance.

Coleus (Coleus blumei Benth.) is a species that is widely planted in arid and semiarid urban regions that frequently experience soil salinity problems. Our interest in coleus lies in the fact that it synthesizes and translocates RFOs (Madore, 1990). Recent reports (Bachmann et al., 1994; Bachmann and Keller, 1995) using Ajuga reptans L., another member of the Lamiaceae, show that the synthesis of high-molecular-weight RFO sugars (DP > 6) can be induced by low-temperature stress. These high-DP RFOs accumulate and are thought to be a factor in the induction of cold tolerance for the overwintering period in this species. At present, it is unknown if this formation of high-molecular-weight RFOs represents a universal stress response of RFO-exporting plants.

Abbreviations: DP, degree of polymerization; GGT, galactan:galactosyltransferase; Gol, galactinol; IMT, myo-inositol 6-O-methyltransferase; OMI, O-methyl-inositol; Raf, raffinose; RFO, raffinose family oligosaccharide(s); Sta, stachyose.

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In RFO-synthesizing plants the cytokol myo-inositol acts as a carrier of Gal residues for the synthesis of the RFOs (Kandler, 1967; Dey, 1985). Cytokolts such as myo-inositol, and, more importantly, its methylated derivatives, pinitol and ononitol, are now known to play some role in salinity and drought tolerance in species such as iceplant (Paul and Cockburn, 1989; Vernon and Bohnert, 1992; Ishitani et al., 1996). Therefore, in RFO-synthesizing species the potential for cytokol accumulation as a stress-tolerance mechanism also exists, but whether this occurs is unknown at this time.

Previous studies on salinity tolerance in coleus have been limited to simple cultivar-screening studies (Ibrahim et al., 1991). Although substantial cultivar tolerance has been demonstrated in this species, the physiological basis behind this tolerance has not been addressed. The present study was designed to characterize the effects of salinity stress on RFO synthesis, export, and metabolism in source and sink leaf tissues of coleus to determine whether alterations in carbohydrate metabolism could also account for salinity-stress tolerance in this species.

MATERIALS AND METHODS

Clonal coleus (Coleus blumei Benth. cv Fairway White) plants were propagated from a single plant purchased at a local nursery and rooted cuttings from these plants were used for all experiments. Six cuttings were transplanted to each of six sand tanks in a greenhouse under natural lighting and a day/night temperature regime of approximately 78°C/65°C (Wilson et al., 1992). Each tank was flood-irrigated four times daily with a solution containing the following (in mol m⁻³): Ca(NO₃)₂, 2.5; KNO₃, 3.0; MgSO₄, 1.5; KH₂PO₄, 0.17; Fe (as sodium ferric diethylenetriamine pentaacetate), 0.05; H₃BO₃, 0.023; MnSO₄, 0.005; ZnSO₄, 0.0005; CuSO₄, 0.0002; and H₂MoO₄, 0.0001 (Wilson et al., 1992). After equilibration for 2 weeks under these growth conditions, three tanks of coleus plants were salinized by the addition of a 5:1 ratio of NaCl to CaCl₂ in three equal increments on consecutive days until a salinity level of 60 mM NaCl and 12 mM CaCl₂ was reached. This salinity level was maintained over a 30-d period. The remaining three tanks were maintained as unsalinized controls.

Photosynthesis Measurements

Instantaneous measurements of net photosynthesis were made using an open-system portable CO₂ analyzer (Analytical Development Co., Hoddesdon, UK) on nine different leaves from randomly chosen control and stressed plants. Measurements were made at midday following 0, 5, 15, and 30 d of salinization. Leaves were allowed to equilibrate in the leaf chamber for at least 2 min before the CO₂ assimilation was measured. Air from outside the greenhouse was passed through the portable system to maintain a stable CO₂ concentration during the measurements. The area of leaves placed in the leaf chamber was determined with an area meter (model 3000, Li-Cor, Lincoln, NE) and was corrected for leaf area corresponding to nonphotosynthetic white tissues.

Carbohydrate Analysis

Leaf tissue samples of approximately 0.2 g fresh weight were excised from green (source) or white (sink) areas of mature leaves every 3 d from each tank of control and stressed plants (n = 9 for each treatment and tissue type at each sampling time). Samples were collected at midday, immediately frozen in dry ice, and maintained at ~20°C until the tissue was analyzed. Soluble carbohydrates were extracted, isolated, and analyzed by HPLC using a Sugar-Pak 1 column (Waters), as described previously (Madore, 1990). High-DP RFOs in the deionized extracts were analyzed by HPLC using an oligosaccharide column (Rezex RSO, Phenomenex, Torrance, CA) at 85°C using water as an eluant at a flow rate of 0.3 mL min⁻¹. Sugars were detected by refractive index monitoring and quantified against known standards.

Starch remaining in the extracted tissues was digested with amyloglucosidase and released Glc was quantified spectrophotometrically using a commercially available Glc detection kit (HK 20, Sigma) following the manufacturer’s recommendations (Madore, 1990).

¹⁴CO₂ Pulse-Chase Experiments

These experiments were performed on C. blumei plants exposed to 8 d of salinization. Branches of C. blumei (containing 10–20 leaves) were excised from the plant using a razor blade. The cut ends were immediately immersed in water, and the stems were recut at the base under water to eliminate any trapped air in the xylem. After a 1-h incubation period under a water-filtered, 150-W light source, the branch was enclosed in a plastic bag. The branches were then pulsed with 3.7 MBq ¹⁴CO₂ for 1 min, as previously described (Flora and Madore, 1993, 1996). At specific time intervals throughout the 90-min chase, three leaves were randomly removed from the branches, separated into green and white regions, and immediately frozen in aluminum-foil envelopes below dry ice. The leaves were stored at ~20°C until extracted and processed as described above for carbohydrates. The radioactivity in each ion-exchange fraction and in the insoluble residue was determined by scintillation counting. The neutral fraction was further partitioned into individual sugars by HPLC and radiolabel in each sugar was determined by postcolumn fraction collection of the sugar peaks and scintillation counting (Flora and Madore, 1993, 1996).

Phloem Exudation Experiments

The experimental protocols for these procedures were essentially as previously described (Flora and Madore, 1993, 1996) and were performed on coleus plants exposed to 8 d of salinization. Prior to labeling, branches containing at least four mature leaves were excised from each plant and immediately immersed in 20 mM NaEDTA (pH 7.0) to prevent callose formation. The plant was enclosed in a plastic bag and exposed to ¹⁴CO₂ (produced by the acidification of 1.0–1.5 MBq of NaH¹⁴CO₃), as previously described (Flora and Madore, 1996). After a 30-min labeling
period, the plastic bag was removed, and the plant was left under the light for a 30-min chase period in ambient air. Branches were then immersed in water, and individual leaves were removed with a razor blade at the base of the petiole. The petiole was then recut under water and placed in a microfuge tube containing 1.5 mL of 20 mM EDTA (pH 7.0).

The amount of 14C exuded by each labeled leaf was monitored at 30-min intervals by scintillation counting until the total label exuded from each leaf had reached approximately 50 Bq. The exudate was separated into neutral, acidic, and basic fractions as described above, and the amount of radioactivity in each fraction was determined by scintillation counting. The neutral fraction was further analyzed by HPLC and radiolabel in each sugar was determined by postcolumn fraction collection of the sugar peaks and scintillation counting (Flora and Madore, 1993). For each experiment the exudate was collected from eight leaves (from four different plants) from both stressed and control plants 8 d after salinization began. This procedure was repeated twice.

Sugar Metabolism Studies

These experiments were performed on coleus plants exposed to 8 d of salinization. Leaf discs (8 mm in diameter) were excised from the interveinal areas of the green and white tissues of salinity-stressed and control leaves. Nine discs from each tissue type were vacuum-infiltrated with a 10 mm solution of radioactively labeled sugars ([14C]Sta, specific activity = 1 mCi mmol⁻¹), prepared as described in Madore [1990]; or [2-3H]myo-inositol, specific activity = 15 mCi mmol⁻¹, ICN), and then placed on damp filter paper under a 150-W light source for 20 min. The radioactivity in neutral, basic, acidic, and ethanol-insoluble fractions was determined by scintillation counting. The neutral fractions were further partitioned into individual sugars by HPLC, and radiolabel in each sugar was measured by postcolumn fraction collecting and scintillation counting (Flora and Madore, 1993). This procedure was repeated three times.

Enzyme Extraction and Assay

Leaf tissue (1 g) from green and white areas of mature leaves was obtained from several different control or stressed plants after 8 d of salinization treatment. The leaf material was ground on ice using a mortar and pestle in 3 mL of grinding buffer (for GGT: 50 mM Hepes-KOH, 5 mM DTT, 5 mM MgCl₂, and 20 mM ascorbic acid [pH 7.5]; for IMT: 150 mM Tris-HCl, 100 mM NaCl, 20 mM EDTA, 10 mM 2-mercaptoethanol, and 5 mM DTT [pH 8.0]). The extracts were filtered through cheesecloth, transferred to microfuge tubes, and centrifuged for 2 min at 10,000g in a microcentrifuge. Portions (0.5 mL) of the supernatant were desalted on Sephadex G25 columns equilibrated with grinding buffer (IMT) or Mcllvaine buffer, pH 5.0 (GGT). Protein contents were then determined by the Bradford method (Bradford, 1976).

GGT activity was assayed as previously described (Bachmann et al., 1994) using 50 μL of crude, desalted enzyme and 40 mM Sta at 30°C for 3 h in a reaction buffer at pH 5.0. The reaction was stopped by boiling for 1 min, and the assay mixture was deionized on coupled Dowex-1 and Dowex-50 ion-exchange columns. High-DP oligosaccharides were eluted with 10 mL of water and taken to dryness in a vacuum centrifuge (Speed-Vac, Savant Instruments, Farmington, IL). Dried samples were resuspended in 100 μL of water, and 20 μL was injected onto an RS0 oligosaccharide HPLC column (Phenomenex) held at 85°C. The high-DP oligosaccharides were separated using water as an eluant at a flow rate of 0.3 mL min⁻¹ and quantified by refractive index monitoring against known standards.

IMT activity was assayed using a modified radioisotope assay (Vernon et al., 1993; Wanek and Richter, 1995). Fifty microliters of enzyme was assayed in a microfuge tube containing 100 μL of a reaction mixture consisting of 50 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 10 mM myo-inositol, and 0.5 mM S-adenosyl-Met containing 20,000 dpm (methyl-[14C]-S-adenosyl-Met (specific activity = 56 mCi mmol⁻¹; ICN). Blank reactions were run by adding water in place of the myo-inositol. The reactions were run at 30°C for 2 h, and stopped by boiling for 1 min. Unreacted S-adenosyl-Met was then removed by adding 0.1 g of Dowex-1 (formate) ion-exchange resin and shaking the reaction tubes on an orbital shaker for 30 min. One milliliter of water was then added, and the tubes were shaken vigorously and centrifuged for 1 min in a microfuge to pellet the resin. The radioactivity in 0.5 mL of the supernatant was then determined by scintillation counting, and the amount of methylinositol formed was determined from the specific activity of the S-adenosyl-Met in the reaction mix.

RESULTS

Plant elongation growth, as determined by plant height, was severely curtailed in response to the salinized root-zone environment (Fig. 1). Growth reduction was noted beginning about 8 d after the onset of the salinity treatment and continued throughout the experimental period. Despite their smaller stature, the stressed plants tolerated the salt stress for a period of over 2 months, and then flowered and set seed at the same time as the control plants (data not shown).

Root-zone salinity also greatly reduced the photosynthetic capability of the plants (Fig. 2A), with maximum effect seen about 5 to 10 d following exposure of the plants to salt. By the end of the experimental period (30 d) photosynthetic rates had recovered to close to the control values (Fig. 2A). Starch levels in the green (source) leaf tissues reflected the photosynthetic activity patterns (Fig. 2B). White (sink) leaf tissues showed no significant starch accumulation in either the control or stressed condition (Fig. 2B).

During the period when photosynthesis was most affected by salt, significant changes were noted in carbohydrate content of the source tissues (Fig. 3). In particular, this period was characterized by the appearance of high-DP (6–8; Fig. 3A; Verbascose: DP = 5, Fig. 3B) RFOs, as well as a methylated inositol (Fig. 3D). The exact nature of the OMI was not determined, but from its chromatographic behavior on the HPLC column, it appeared to be
either ononitol or its epimer pinitol. It was not determined in these studies whether the methylated inositol derivative is further metabolized in coleus, as occurs in *Mesembryanthemum crystallanum* (Vernon et al., 1993) via the epimerization enzyme ononitol epimerase. Elevated levels of Raf (Fig. 3D) and, to a lesser extent, Suc (Fig. 3E) were also observed, whereas levels of Sta (Fig. 3C) and Gol (Fig. 3F) remained essentially unaltered by the salt stress. Levels of hexose sugars, particularly Glc (Fig. 3G) and, to a lesser extent, Fru (Fig. 3H), were reduced in response to salt stress.

During the same period the white nonphotosynthetic sink tissues also showed altered carbohydrate patterns (Fig. 4). Again, as seen in the source-leaf tissues, high-DP RFOs (Fig. 4A) and verbascose (Fig. 4B) accumulated, as did Raf (Fig. 4D) and Suc (Fig. 4E), whereas no changes were observed for Sta (Fig. 4C) or Gol (Fig. 4F). However, in contrast to the observed changes in source tissues, levels of the hexose sugars Glc (Fig. 4G) and Fru (Fig. 4H) were elevated in the stressed condition in sink tissues. Also, sink tissues did not accumulate OMI during salt stress (Fig. 41).

The altered carbohydrate patterns seen during this initial phase of salt exposure were also reflected in the radiolabeling patterns in carbohydrates recovered in phloem sap exudates from stressed source leaves. In general, salt stress did not significantly alter the overall distribution of 14C in neutral, basic, and acidic phloem sap constituents (Fig. 5A), but did alter significantly the pattern of radiolabeling of specific phloem-mobile carbohydrates (Fig. 5B). In particular, a substantial shift occurred such that most of the label exiting the leaf was in the form of Suc under the stressed condition (Fig. 5B). Also, salt stress resulted in the appearance of OMI in the phloem sap (Fig. 5B).

Pulse-chase studies of the source tissues during the same initial stress period revealed that more recently fixed carbon was diverted to the high-DP oligosaccharides (Fig. 6A) and to the OMI (Fig. 6C) and myo-inositol (Fig. 6H) during the initial phases of salt stress, and that salt-stressed leaves contained less labeled Sta (Fig. 6B). No significant changes in labeling patterns in Raf (Fig. 6C), Suc (Fig. 6D), Gol (Fig. 6E), or Glc (Fig. 6F) were induced by the salinity stress. When the pulse of carbon was followed into the sink tissues, radiolabeling patterns showed that high-DP oligosaccharides (Fig. 7A) had higher levels of label under salt stress, as did Suc (Fig. 7D), Gol (Fig. 7E), Glc (Fig. 7F), and to a slight extent the OMI (Fig. 7G). No change in labeling was noted for Raf (Fig. 7C) but radiolabel in Sta (Fig. 7B) and Fru-myoinositol (Fig. 7H) were reduced in response to the salinity treatment.

Sugar-feeding experiments showed that both source (Fig. 8A) and sink (Fig. 8B) tissues were capable of forming high-DP oligosaccharides from exogenously supplied Sta, and that the amount of carbon diverted to these sugars was elevated by salt stress (Fig. 8). In contrast, source tissues (Fig. 8C) were capable of synthesizing OMI from exogenously supplied myo-inositol, whereas sink tissues (Fig. 8D) were not. Enzyme studies (Fig. 9) showed that the GGT
enzyme required for synthesis of high-DP oligosaccharides was present in both source and sink tissues (Fig. 9A) and that its activity was amplified in both tissues in response to salt stress (Fig. 9A). In contrast, the OMT enzyme responsible for synthesis of OMI was present only in source tissues and was not detected in sink tissues (Fig. 9B). Salinity stress resulted in an almost 10-fold greater activity of this enzyme in source tissues, but no induction of the enzyme in sink tissues (Fig. 9B).

**DISCUSSION**

The production of low-molecular-weight metabolites seems to be a virtually universal response to stress, and commonly occurs in both prokaryotes and eukaryotes (for review, see Bohnert et al., 1995; Bohnert and Jensen, 1996). The most common hypothesis to explain the role of these molecules in stress tolerance is that they serve as osmolytes, helping cells to osmotically adjust when faced with low water potentials. However, it is becoming clear that these compounds frequently do not accumulate at high enough levels to be acting as true osmolytes, and it is now envisioned that some may be serving alternate roles in stress protection, such as radical scavenging, protection from photoinhibition, or detoxification (for review, see Rabe, 1990; Bohnert et al., 1995; Pharr et al., 1995; Popp and Smirnoff, 1995; Bohnert and Jensen, 1996; Loescher and Everard, 1996). Recently, molecular techniques have been utilized to try to enhance stress tolerance in crop plants, and it has been conclusively shown that the production of carbohydrates such as mannitol (Tarczynski et al., 1993; Thomas et al., 1995), fructans (Pilon-Smits et al., 1995), or trehalose (Holmstrom et al., 1996) in plants that do not normally produce these compounds can induce substantial tolerance. Even quite low levels (5–100 mM) of these osmoprotectants can have a substantial impact on the ability of plants to survive dehydration and/or salinity stress.

Coleus produces novel carbohydrates, namely high-DP RFOs and OMI, in response to salinity stress. The enzymes responsible for the synthesis of these carbohydrates are

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**Figure 3.** Effects of root-zone salinity on midday soluble carbohydrate content of green photosynthetic source tissues of mature variegated coleus leaves. Data represent the means of nine measurements per data point (± se). High-DP oligosaccharides, RFOs with DPs of 6 to 8; O, control; ●, salt-stressed. f.wt., Fresh weight.
Figure 4. Effects of root-zone salinity on midday soluble carbohydrate content of white nonphotosynthetic sink tissues of mature variegated coleus leaves. Data represent the means of nine measurements per data point (± SE). High-DP oligosaccharides, RFOs with DPs of 6 to 8; ○, control; ●, salt-stressed. f.wt., Fresh weight.

### SINK TISSUES (WHITE)

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<thead>
<tr>
<th>Sugar Type</th>
<th>DAYS OF SALT TREATMENT</th>
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<tr>
<td><strong>A</strong> High DP Oligosaccharides</td>
<td>0 5 10 15 20 25 30</td>
</tr>
<tr>
<td><strong>B</strong> Verbascose</td>
<td>0 5 10 15 20 25 30</td>
</tr>
<tr>
<td><strong>C</strong> Stachyose</td>
<td>0 5 10 15 20 25 30</td>
</tr>
<tr>
<td><strong>D</strong> Raffinose</td>
<td>0 5 10 15 20 25 30</td>
</tr>
<tr>
<td><strong>E</strong> Sucrose</td>
<td>0 5 10 15 20 25 30</td>
</tr>
<tr>
<td><strong>F</strong> Galactinol</td>
<td>0 5 10 15 20 25 30</td>
</tr>
<tr>
<td><strong>G</strong> Glucose</td>
<td>0 5 10 15 20 25 30</td>
</tr>
<tr>
<td><strong>H</strong> Myo-inositol + Fructose</td>
<td>0 5 10 15 20 25 30</td>
</tr>
<tr>
<td><strong>I</strong> O-methyl-inositol</td>
<td>0 5 10 15 20 25 30</td>
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Undetectable in the absence of stress, which suggests, in agreement with other studies (Pharr et al., 1995; Loescher and Everard, 1996), that their production is a response to stress and is not simply a consequence of a slowdown of carbohydrate utilization. These carbohydrates are also not produced in large quantities (Figs. 3 and 4), which suggests that they are probably not acting as osmolytes, but rather are playing some alternate, as yet unknown, role.

In photosynthetic coleus source tissues, the primary effects of salt stress on carbohydrate metabolism appear to be on myo-inositol metabolism via the induction of a methylation reaction. This methylation has major consequences on subsequent RFO metabolism, for a methylated myo-inositol is effectively removed from the RFO biosynthetic pathway and cannot recycle as a Gal acceptor to form Gol. For RFO biosynthesis to continue during salinity stress, a de novo synthesis of myo-inositol must be induced. Pulse-chase studies (Fig. 6) show that more photosynthetic carbon is indeed diverted to myo-inositol (Fig. 6H) under salinity stress conditions. A complete reaction scheme is depicted in Figure 10.

Increased de novo synthesis of myo-inositol via myo-inositol-1-P synthetase (reaction 1, Fig. 10) and myo-inositol-1-P phosphatase (reaction 2, Fig. 10) (Loewus and Loewus, 1983) would require increased utilization of photosynthetically generated reducing power in the form of NAD(P)H. The de novo pathway therefore parallels that leading to polyol synthesis and may serve as a mechanism for bleeding off excess photochemical energy under conditions of limited carbon dioxide fixation, as is currently postulated for mannitol biosynthesis in other species (Pharr et al., 1995; Loescher and Everard, 1996).

The synthesis of high-DP RFOs most likely operates as a mechanism allowing for a buildup of temporary carbon reserves to replace the normal starch storage reserves, which are greatly reduced during the initial phases of salinity stress (Fig. 2B). The advantage of storing vacuolar carbon in this form, as opposed to the more common Suc.
Carbohydrate Metabolism in Salt-Stressed Coleus

Figure 5. Effects of root-zone salinity on distribution of radiolabel in basic, acidic, and neutral compounds (A) and individual carbohydrates obtained from phloem exudates from mature, variegated coleus leaves radioalabeled with \(^{14}\)C\(_2\). Plants were exposed to root-zone salinity for 8 d prior to the experiments. White bars, Control; shaded bars, salt-stressed. VER, Verbasose; STA, Sta; RAF, Raf; O-MI, 6-OMI. Data represent the means ± se of 16 measurements per data point.

Figure 6. Effects of root-zone salinity on distribution of radiolabel in carbohydrates in green photosynthetic leaf tissues of mature variegated coleus leaves following a 1-min pulse of \(^{14}\)CO\(_2\) to intact leaves. Plants were exposed to root-zone salinity for 8 d prior to the experiments. High-DP RFOs, RFOs with DPs of 6 to 8. Data represent the means ± se of nine measurements per data point. ⚫, Control; ○, salt-stressed.

The results presented here suggest that the mesophyll cells are the most likely location for the synthesis of the high-DP RFOs, since no high-DP oligosaccharides were found in the phloem sap (Fig. 5B). Current phloem-loading models hold that RFOs destined for export are synthesized from Gol and Suc within the phloem tissues themselves. RFOs are then released into the sieve elements (Turgeon, 1991; Turgeon and Beebe, 1991). If high-DP RFOs are synthesized in the phloem, these sugars should also be exported. That they are not is a good indication that these carbohydrates originate in the mesophyll cells, and are prevented from entering the phloem by the dimensions of the symplastic connections that connect the mesophyll with the phloem cells. Mesophyll pools of RFOs do occur in Xerosicyos danguyi H. Humb. (Madore, 1992) and Ajuga reptans (Bachmann and Keller, 1995), and these pools become greatly enlarged in response to chilling (Bachmann and Keller, 1995) or desiccation (Madore and Gadus, 1993).

The high-DP pathway depicted in Figure 10 would result in the removal of a significant proportion of photosynthetically produced Sta from the phloem-transport pathway by vacuolar transfer. The end result would be a reduction in the amounts of Sta available for export, which is what is observed (Fig. 5B). In addition, however, the synthesis of high-DP oligosaccharides also effectively removes the Suc biosynthetic pathway itself from the RFO-reaction equations. Once Raf is made, it recycles as a galactosyl acceptor (reaction 5, Fig. 10) and no longer requires de novo synthesis from Suc. This would leave more of the photosynthetically produced Suc available for phloem transport under stress conditions and, again, this is what is observed...
This may represent a way of preventing early senescence of the source tissues, as the production of methylated compounds utilizes S-adenosyl-Met, an important precursor of both ethylene and polyamines (Bohnert and Jensen, 1996). If methyl groups are diverted from S-adenosyl-Met and exported from the leaf, this may reduce the amount of ethylene produced by the source tissues during stress conditions. The same function may be served by enhanced export of photosynthetic-produced Ser (Madore and Grodzinski, 1984). OMI transport and metabolism may therefore represent a means for "detoxifying" excess methyl groups produced by photosynthetic tissues under stressful conditions.

It should be particularly noted, however, that these salt-induced changes in carbohydrates in both source and sink tissues are transient responses. Although the plants in this study were exposed for a continuous 30 d to the root-zone salinity treatment, the carbohydrate changes disappeared sometime within the second week of exposure. The transient nature of the carbohydrate responses suggest that, in coleus, the novel carbohydrates are only important during the initial phases of stress, when photosynthesis is most profoundly affected. As the plant acclimates to the stress, (Fig. 5B). Salinity stress also results in the appearance of a novel sugar, OMI, in the phloem-transport system.

Incoming OMI is apparently rapidly demethylated and metabolized, as evidenced by the appearance of labeled myo-inositol following pulse-chase labeling (Fig. 7H). Incoming Suc and RFOs appear to be used to form pools of high-DP RFOs. These reactions are depicted schematically in Figure 11. The white sink tissues are apparently not induced to synthesize OMI de novo, as the IMT was not induced (Fig. 9B) in response to salt stress. OMI instead appears to be translocated to the sinks via the phloem transport system, where it is rapidly metabolized to as-yet-unknown compounds. The low levels of OMI recovered in sink tissues may have represented OMI not yet unloaded from the phloem tissues.

The presence of OMI in the phloem sap during stress suggests that one role of OMI formation may be to allow the transport of methyl groups out of the source leaves.
Carbohydrate Metabolism in Salt-Stressed Coleus

Effects of root-zone salinity on activities of Ga1actan:galactan transferase (A) or 6-O-methyltransferase (B) in source or sink tissues from mature variegated coleus leaves. Plants were exposed to root-zone salinity for 8 d prior to the experiments. Data represent the means of three experiments.

and photosynthetic activity and the ability to store starch recovers, the need for the novel carbohydrates apparently diminishes for reasons that are not yet clear. However, during these initial phases, it is also apparent that the changes in source-tissue carbohydrate status are accompanied by substantial changes in the carbohydrates that are exported via the phloem-transport system to the sink tissues. This raises the possibility that alteration in the type of carbohydrates transported in the phloem may act as a signaling system for activation of the acclimation responses in the sink tissues.

It has long been recognized that sugar levels in plant tissues play an important role in source-sink relationships. However, it is now clear that sugar levels also impact on carbohydrate-metabolizing enzymes, changing both gene expression and enzyme activity. In source tissues high carbohydrate levels have been shown to negatively impact on key photosynthetic enzymes (Sheen, 1994), whereas in sink tissues genetic expression of catabolic enzymes such as Suc synthase appear to be regulated by levels of imported carbohydrates (Koch et al., 1992). The data presented here show that stress can alter, at least transiently, not only carbohydrate levels, but also the types of carbohydrates that are synthesized and exported by the plant. Future research should therefore be aimed at the investigation of the genetic effects of these transients, for it may prove that even subtle changes of specific carbohydrate types in source and sink tissues may serve as signals for new gene expression, allowing a redirection of growth responses for the long-term survival of the plant.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Jodie S. Holt and Cheryl Wilen for their help with the photosynthesis measurements, as well as Michelle V. Gadush for her excellent technical assistance.

Received May 9, 1997; accepted August 22, 1997.
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LITERATURE CITED

Holmstrom KO, Mantyla E, Welin B, Manda1 A, Palva ET, Tun-
Koch KE, Nolte KD, Duke ER, McCarty DR, Avigne WT
Kandler
Ishitani M, Majumder AL, Bornhouser A, Michalowski CB,
Ibrahim KM, Collins JC, Collins HA
Flora LL, Madore MA
Loescher WH, Everard
Everard JD, Gucci R, Kann SC, Flore JA, Loescher WH
Madore MA
Dey PM
Bradford MM
Bachmann M, Matile P, Keller F (1994) Metabolism of the raffinose
Bonoert HJ, Jensen RG (1995) Strategies for engineering water
stress tolerance in plants. Trends Biotechnol 14: 89–97
Bonoert HJ, Jensen D, Jensen RG (1995) Adaptations to environ-
mental stresses. Plant Cell 7: 1099–1111
Bradford MM (1976) A rapid and sensitive method for the quan-
titation of microgram quantities of protein utilizing the principle
Dey PM (1985) D-Galactose-containing oligosaccharides. In PM
Dey, RA Dixon, eds, Biochemistry of Storage Carbohydrates in
exchange and carbon partitioning in the leaves of celery (Aptum
groenianum L.) at various levels of root zone salinity. Plant Physiol
106: 281–292
Flora LL, Madore MA (1993) Stachyose and mannitol transport in
olive (Olea europea L.). Planta 189a: 484–490
Flora LL, Madore MA (1996) Significance of minor-vein anatomy
to carbohydrate transport. Planta 198: 171–178
Hanson A, Hitz W (1982) Metabolic responses of mesophytes to
Holstrom KO, Mantyla E, Welin B, Manda1 A, Palva ET, Tun-
Nature 379: 683–684
Ishitani M, Majumder AL, Bornhouser A, Michalowski CB,
Jenson RG, Bonoert HJ (1996) Coordinate transcriptional induc-
tion of myo-inositol metabolism during environmental stress.
Plant Physiol 106: 537–548
Kandler O (1967) Biosynthesis of poly- and oligosaccharides dur-
ing photosynthesis in green plants. In A Pietro, FA Greer, TJ
Army, eds, Harvesting the Sun: Photosynthesis and Plant Life.
Academic Press, New York, pp 131–152
Sugar levels modulate differential expression of maize sucrose
synthase genes. Plant Cell 4: 59–69
Loescher WH, Everard JD (1996) Sugar alcohol metabolism in
sinks and sources. In E Zamanski, AA Schaffer, eds, Photosyn-
thesis and Carbon Partitioning in Plants and Crops. Marcel Dekker, New York,
pp 185–207
Loewus FA, Loewus MW (1983) Myo-inositol: its biosynthesis and
Madore MA (1990) Carbohydrate metabolism in photosynthetic and
nonphotosynthetic tissues of variegated leaves of Coleus blumei
Madore MA (1992) Nocturnal stachyose metabolism in leaf tissues
synthase from succulent plants. Plant Physiol 102S: 43
on 14C-photosynthetic carbon fixation in leaves of Salvia splendens L.
Plant Physiol 76: 782–786
Munns R (1993) Physiological processes limiting plant growth in
saline soils: some dogmas and hypotheses. Plant Cell Environ
16: 15–24
Pharr DM, StooP JMH, Williamson JD, Studer Feusi ME, Masel
MO, Konking MA (1995) The dual role of mannitol as osmo-
protectant and photosynthetic assimilate in celery. Hort Sci 30: 1182–1188
Flion-Smits EA, Eksbamp MJM, Paul MJ, Jeukan MJK, Weis-
beek PJ, Smeekens SC (1995) Improved performance of trans-
genic fructan accumulator tobacco under drought stress. Plant
Physiol 107: 125–130
during water deficit. In N Smirnoff, ed, Environment and Plant
Metabolism: Flexibility and Acclimation. Bios Scientific Publishers,
Oxford, UK, pp 199–215
Rabe E (1990) Stress physiology: the functional significance of the
accumulation of nitrogen containing compounds. J Hort Sci 65:
231–243
Res 39: 427–438
Stoop JHM, Pharr DM (1994) Mannitol metabolism in celery
Tarczynski MC, Jenson RG, Bonoert HJ (1995) Stress protection of
transgenic tobacco by production of the osmolyte mannitol.
Science 259: 508–510
Thomas JC, Sepanski M, Arendall B, Bonoert HJ (1995) Enhance-
ment of seed germination in high salinity by engineering man-
itol metabolism in Arabidopsis thaliana. Plant Cell Environ 18:
801–806
Turgeon R (1991) Symplastic phloem loading and the sink-source
transition in leaves: a model. In JL Bonnemain, S Delrot, WJ
Lucas, J Dainty, eds, Recent Advances in Phloem Transport and
Assimilate Compartmentation. Ouest Editions, Nantes, France,
18–22
Turgeon R, Beebe DU (1991) The evidence for symplastic phloem
Vernon D, Tarczynski MC, Jenson RG, Bonoert HJ (1993) Cycli-
tol production in transgenic tobacco. Plant J 4: 199–205
Vernon DM, Bonoert HJ (1992) A novel methyltransferase in-
duced by osmotic stress in the facultative halophyte Mesembry-
themum crystallinum. EMBO J 11: 2077–2085
Wanek W, Richter A (1995) Purification and characterization of
myo-inositol 6-O-methyltransferase from Vigna umbellata Owhi
cycle and age on nucleotide pools of bean leaves. J Exp Bot 43:
1009–1014