ABSTRACT. Raffinose family oligosaccharides (RFOs) perform several physiological functions in plants. In addition to accumulating during seed formation, raffinose and stachyose are translocated in the phloem and may accumulate in response to low temperatures, drought, or salt stress. Although the synthesis of galactinol, as mediated by galactinol synthase (GAS), is the first committed step in RFO formation, its expression patterns are poorly understood in most species. We have cloned and characterized the expression of two galactinol synthase gene family members in melon (Cucumis melo L. Cantalupensis Group). Both CmGAS1 and CmGAS2 are highly expressed in mature leaves. Galactinol synthase transcription in leaves was not upregulated by either water or low temperature stresses. Transcripts of CmGAS1 were present in developing melon seeds at a time coincident with the formation of raffinose and stachyose. Based on the GAS expression and RFO accumulation patterns, we propose that RFOs in melon function in carbon translocation and seed desiccation tolerance.

The synthesis of raffinose family oligosaccharides (RFOs) has been implicated in many developmental and physiological processes in plants. The enzyme galactinol synthase (GAS; UDP-galactose:inositol galactosyltransferase) catalyzes the formation of galactinol from myo-inositol and UDP-galactose. This galactinol serves as a galactose donor for raffinose and stachyose synthesis.

Members of the Cucurbitaceae, Scrophulariaceae, Labiatae, Celastraceae and Oleaceae, and probably other families, translocate significant quantities of RFOs in the phloem (Turgon et al., 2001; Zimmerman and Ziegler, 1975). These plants have numerous plasmodesmata between the specialized companion cells (intermediary cells, ICs) and surrounding mesophyll layers that are thought to permit the passage of sucrose from the mesophyll cells into the ICs, where it is then converted to galactinol and RFOs before transport (Haritatos et al., 1996).

Raffinose and stachyose are also synthesized in many orthodox (desiccation tolerant) seeds. They may provide protection to membranes that surround cellular compartments, preventing sucrose crystallization in vegetative tissues destined to undergo severe dehydration, for example in Xerosicyos, Ramonda, Haverlea, and Boea (Albini et al., 1999; Caffrey et al., 1988; Corbineau et al., 2000; Muller et al., 1997; Peterbauer and Richter, 2001).

Many species make RFOs in response to low temperature stress conditions. RFOs may serve as carbon storage pools in low temperature tolerant plants since the enzymes that catalyze RFO formation are thought to be less sensitive to low temperatures than those which catalyze the formation of starch (Bachmann et al., 1994). It has been shown that GAS transcripts accumulate in vegetative tissues of Arabidopsis thaliana and in some seeds after exposure to low temperatures (Liu et al., 1998; Peterbauer and Richter, 2001). In addition, GAS enzyme activity is higher in winter-hardy than in summer-hardy species (Liu et al., 1998; Peterbauer and Richter, 2001). Although the presence of RFOs has been documented in a variety of systems, there are few studies demonstrating the expression of the GAS genes. Sprenger and Keller (2000) published a study that described the expression patterns of two galactinol synthase genes, GolS-1 and GolS-2, in Arabidopsis. Ajuga is a frost-hardy evergreen labiate that both accumulates and translocates RFOs. The gene, GolS-1 was primarily expressed in the mesophyll in response to low temperatures while GolS-2 was expressed in intermediary cells and most likely catalyzes the formation of RFOs destined for translocation. The Arabidopsis GAS genes AtGolS1 and AtGolS2 are expressed in response to drought and salt stress while AtGolS3 accumulates in response to low temperature stress (Taji et al., 2002). The expression patterns of other Arabidopsis GAS genes have not been characterized.

We have cloned two GAS genes in melon (Cucumis melo L. Cantalupensis Group). One gene, CmGAS1, is highly homologous to the zucchini CmGAS1 gene identified by Kerr et al. (1993). A CmGAS1 promoter-gusA fusion was made and used to transform Arabidopsis and tobacco plants. In these transgenic plants, GUS expression localized to the smallest veins of mature Arabidopsis and tobacco leaves (Haritatos et al., 2000). Although galactinol is not normally synthesized in wild-type tobacco leaves, stain localized to the companion cells and sieve elements in the minor veins (Haritatos et al., 2000), indicating that the CmGAS1 promoter drives vein-specific gene expression. There are no previous reports documenting the expression pattern of the second melon GAS gene, CmGAS2.

In melon, raffinose and stachyose serve as phloem translocation sugars and also accumulate in maturing seeds. Other functions of RFOs in melon have not been documented. We have used RNA blots and RT-PCR techniques to characterize the mRNA expression patterns of the CmGAS1 and CmGAS2 genes in melon.

Materials and Methods

PLANT MATERIAL. Melon plants (Cucumis melo cv. Hale’s Best Jumbo, Burpee/Ball Seed Co., West Chicago Ill.) were grown in 15-cm-diameter clay pots with potting soil in a growth chamber (CEL 37-14, Sherer-Gillette/Revco/Lindberg, Asheville, N.C.) with 425 mmol·m⁻²·s⁻¹ illumination provided by 160-W VHO cool white fluorescent bulbs. A 16-h/8-h light/dark photoperiod and 27°C/22°C light/dark temperatures were provided.
Replicate pots of 3-week-old melon plants were treated to 8°C dark periods for 1, 2, or 3 consecutive days. Entire plants were harvested and individual organs were frozen in liquid nitrogen either 2 or 8 h after the low temperature treatment. Controls were not subjected to 8°C night temperatures.

In water stress studies, water was withheld from 3-week-old melon plants for 4, 5, 6, 7, or 8 d. Two replicate plants were harvested midday after the water-stress period. Control plants were well-watered throughout the experiment.

Field melon plants were started in growth chambers, hardened in cold frames, and transplanted to black plastic mulch covered rows approximately three weeks after germination. Flowering occurred two weeks later, at which time female flowers were hand-pollinated and tagged. Fruit were thinned to two per plant. Five melons were harvested at 3- to 6-d intervals (15 times during fruit development). The largest and smallest melons were discarded and sugars were extracted from 50-seed subsamples of the remaining three melons at each time point as described by Turgeon et al. (1993). After ion exchange, the extract was dried, dissolved in water, and filtered before separation by high-performance liquid chromatography.

| Primer          | Sequence                              |
|-----------------|---------------------------------------|
| 5’RACE CmGAS1   | 5’-GTAGCGCTGCACAAATAGTGGAC-3’          |
| 3’RACE CmGAS1   | 5’-CTTGAGACTTGGCAAGGCCCACCT-3’        |

Table 1. Sequences of gene-specific primers used for PCR.

| Gene     | Common name | DNA GenBank No. | Protein GenBank No. | Expression      |
|----------|-------------|-----------------|---------------------|-----------------|
| AtGolS1  | Arabidopsis | AC002537        | AAB63818            | Drought, salt   |
|          |             |                  | NP_182240.1         |                 |
|          |             | NM_130286.1     | AAL07218.1          |                 |
|          |             | AY056139        | AAM14365.1          |                 |
|          |             | AY091426        | BAB78530.1          |                 |
|          |             | AB062848        | AAM15468.1          |                 |
|          |             | AC007236        | AAB63818.1          |                 |
|          |             | AY085006        | AAM161564.1         |                 |
| AtGolS2  | Arabidopsis | NM_104537       | NP_176653.1         | Drought, salt   |
|          |             | AY058238        | AAL15412.1          |                 |
|          |             | AY050410        | AAK91426.1          |                 |
|          |             | AB062849        | BAB78531.1          |                 |
|          |             | AC009323        | AAG9103.1           |                 |
|          |             | AF412094        | AAL06547.1          |                 |
| AtGolS3  | Arabidopsis | AC003970        | AAC33195.1          | Cold            |
|          |             | AF370546        | AAK49793.1          |                 |
|          |             | NM_100805       | NP_172406.1         |                 |
|          |             | AY0581452       | AAM10014.1          |                 |
|          |             | AF370546        | AAK49793.1          |                 |
|          |             | AB062850        | BAB78532.1          |                 |
|          |             | AC003970        | AAC33195.1          |                 |
| AtGolS4  | Arabidopsis | NM_104734       | NP_176250.1         |                 |
|          |             | AC002292        | AAB71970.1          |                 |
| AtGolS5  | Arabidopsis | AB005244.2      | BAB10052.1          | Siliques        |
|          |             | ABO00015        | ABO00015            |                 |
| AtGolS6  | Arabidopsis | NM_122284       | NP_197768.1         |                 |
|          |             | AL049171        | CAB38954.1          |                 |
|          |             | AL161564        | CAB79480.1          |                 |
| AtGolS7  | Arabidopsis | NC_003075.1     | NP_194355           |                 |
|          |             | AC004473        | AAC24075.1          |                 |
|          |             | NM_104732       | NP_176248.1         |                 |
| Brassica | Brassica    | AF109654        | AAD26116.1          |                 |
| Pisum    | Pea         | AJ243815        | CAB51130.1          | Embryo          |
| ArGolS1  | Ajuga reptans | AJ237693       | CAB51331.1          | Source lvs, cold|
| ArGolS2  | Ajuga reptans | AJ237694       | CAB51343.1          | Sink leaves     |
| Vitis    | Grape       | AF178569        | AAD55726.1          |                 |
| Oryza    | Rice        | D26537          | BAA05538.1          | Water stress    |
| CpGAS1   | Zucchini     | AY077642        | AAL78687.1          | Posts           |
| Glycine  | Soybean     | AY077641        | AAL78686.1          | Leaves          |
| CmGAS1   | Melon       | AY077642        | AAL78687.1          | Leaves          |
| CmGAS2   | Melon       | AY077641        | AAL78686.1          |                 |
| Thellungiella | Thellungiella | AF499723       | AAM19710.1          |                 |
| At NM_122802 | Arabidopsis | NM_122802       | NP_56816.1          |                 |
| Lycopersicom | Tomato     | AF311943        | AAL26804.1          |                 |
Poly(A)+ RNA was separated from total RNA using an oligo(dT)-
procedure (Goldsbrough et al., 1986; Kirby and Cook, 1967).

Nucleic acid extraction. Genomic DNA was extracted from
dark-grown melon seedlings as described by Haritatos et al. (2000).

Ten-microgram samples of melon genomic DNA were digested
with BamHI, EcoRI, EcoRV, HindIII, KpnI, SstI, or XhoI restriction
enzymes (Gibco BRL, Life Technologies, Gaithersburg, Md.).

Library construction. A partial melon genomic library was
generated by partially digesting genomic melon DNA (100 μg) with
MboI, fractionated on a sodium chloride gradient, and separated
by agarose gel electrophoresis. DNA from the 9 to 23 kB bands on the
gel was ethanol precipitated, ligated to BamHI restricted arms of the
bacteriophage λ GEM11, and packaged with Packagene Lambda
DNA Packaging System (Promega, Madison, Wis.). The library
(400,000 phage) was screened by hybridization to a radiolabeled
probe using a 784 bp HindIII open reading frame of the CpGASI
cDNA [CpGASI probe was provided by DuPont (Kerr et al., 1993)].

Phage were plaque-purified, subcloned using SstI restriction
sites into pBluescript II KS+(Stratagene), and maintained in DH5α
bacteria. A melon subgenomic library was constructed to clone a
specific band that the CpGASI probe binds to on a Southern blot as
described by Haritatos et al. (2000). Radioabeled probes and hybridization. The CpGASI probe
was made from a 784 bp HindIII fragment of the CpGASI cDNA.

The CpGASI gene specific probes were made from a cloned 186 bp
fragment between two adjacent HincII restriction sites in the 3'
untranslated region of the melon CmGAS1 gene. The SspI site in the
3’ untranslated region and an XbaI site within the multiple cloning
site of a subcloned CmGAS2 gene fragment were used to generate
a 302 bp fragment from which CmGAS2 gene specific probes were
made.

Fragments of DNA were labeled with α32P-dCTP (DuPont-New
England Nuclear) using a randomly primed DNA-labeling kit
(Boehringer Mannheim, Indianapolis, Ind., USA) and unincorporated
nucleotides were removed by passage over a sephadex G50
column (Sigma, St. Louis) before standard hybridization protocols
(Sambrook et al., 1989).

PCR and cloning. To confirm expression results obtained by
northern blotting, poly(A)+ RNA was reverse-transcribed and
amplified using CmGAS1 gene-specific primers following a RACE
protocol (Clontech, Palo Alto, Calif.) (Table 1). The RACE kit AP1
primers were ligated to cDNA made from 1 μg of poly(A)+ RNA
extracted from melon seeds 39 d after polination (DAP). Polymerase
chain reaction (PCR) solutions of 50 μL included 5 μL 10x
PCR buffer (Perkin-Elmer, Foster City, Calif.), 1 μL 10 mM dNTPs
(Clontech), 1 μL each of 10 μM AP1 primer and gene specific
primers (Table 1), and 1 U AmpliTaq DNA polymerase (Perkin-
Elmer). Either 0.05 or 0.01 μg of cDNA template was included in
PCR reactions. Hot-start PCR amplification was performed in an
Amplitron II thermocycler (Barnstead-Thermolyne Corp., Dubuque,
Iowa) for 30 cycles with 30 s at 94°C and 4 min at 68°C or for 30
cycles with 94°C for 30 s, 60°C for 30 s, and 68°C for 4 min.

Amplification products were separated on a 1% agarose gel, blotted
to membrane, and hybridized with the 784 bp HindIII fragment of
the CpGASI probe. Bands that hybridized with the probe were
removed from a low-melt agarose gel and purified using Clontech’s
GENO-BIND matrix. Purified products were reamplified by PCR
using the same conditions. PCR products were cloned using the TA
Cloning Kit (Invitrogen, San Diego).

Results

Galactinol synthase genes. A conserved GAS cDNA probe
(CpGASI probe) from zucchini was used to identify homologous
sequences in melon. Southern blots were made from digested
genomic melon DNA and hybridized with the CpGASI probe (Kerr
et al., 1993). For most restriction enzymes, one band hybridized
strongly with the conserved GAS probe and several minor bands
were apparent (Fig. 1). The band that hybridized most strongly (∼6.6 kb) on the EcoRI digest is highly homologous to the \textit{CpGAS1} probe. Two of the DNA fragments that hybridized to the conserved GAS probe on EcoRI-restricted DNA were cloned (6.6 and 9 kb).

The melon \textit{GAS1} gene (\textit{CmGAS1}) and promoter were subcloned from a subgenomic library made from a 6-kb EcoRI restriction fragment of DNA, as described by Haritatos et al. (2000). Gene specific probes (from the 3' untranslated region) from this clone hybridize to the 6.6 kb band on Southern blots made from EcoRI-digested genomic melon DNA (Fig. 2) and did not cross-hybridize with \textit{CmGAS2} plasmid DNA (data not shown). Sequencing revealed that three introns, of lengths 155 bp, 482 bp, and 132 bp are present in the \textit{CmGAS1} gene. Four exons of lengths 315 bp, 324 bp, 135 bp, and 219 bp probably encode a putative protein 331 amino acids long (Fig. 3A).

The melon \textit{GAS2} gene (\textit{CmGAS2}) was subcloned from a \lambda GEM11 bacteriophage library made from fractionated genomic DNA partially digested with MboI. The 302 bp \textit{CmGAS2} gene specific probe hybridized with the 9 kb band on Southern blots made from EcoRI-digested genomic melon DNA (Fig. 2) and did not cross-hybridize with \textit{CmGAS1} plasmid DNA (data not shown). It appears that the two introns in \textit{CmGAS2} are at the same locations as the first and third introns of \textit{CmGAS1}. The intron of \textit{CmGAS2} corresponding to the 482 bp intron of \textit{CmGAS1} is missing. The three exons of \textit{CmGAS2} are 312 bp, 459 bp, and 228 bp, and are thought to encode a putative protein 333 amino acids long (Fig. 3B).

**GALACTINOL SYNTHASE EXPRESSION IN MELON.** Since the zucchini conserved GAS probe recognized both \textit{CmGAS1} and \textit{CmGAS2} genes as well as a soybean seed GAS gene (data not shown), it was used as a probe to determine which melon tissues expressed galactinol synthase.

Using the \textit{CpGAS1} probe, we did not detect GAS transcript in total RNA extracted from 10- or 21-d seeds, immature (sink) leaves, roots, or mature fruit flesh (Fig. 4). The conserved \textit{CpGAS1} probe as well as \textit{CmGAS1} and \textit{CmGAS2} gene specific probes hybridized strongly to total RNA extracted from mature leaves, and bound to mesophyll/epidermal total RNA to a lesser degree (Fig. 5). Since these mesophyll/epidermal samples were collected by gently scraping buffer-soaked leaves, some vein contamination may be present in the mature mesophyll/epidermal tissue samples.

RNA gel blots made from total RNA extracted from whole mature leaf tissue at 3-h intervals throughout the 16-h light and 6-h dark daily cycle exhibited no dramatic flux in diurnal expression patterns of galactinol synthase transcripts (data not shown).

Early in melon seed development, glucose and sucrose levels were high (Fig. 6A and B). Field grown melons began to accumulate significant levels of raffinose and stachyose 24 DAP, just before the time of maximum dry matter accumulation (Fig. 6A and C). Galactinol synthase transcript was detected in poly(A)^+ RNA extracted from seeds 39 DAP when probed with the conserved GAS probe as well with the \textit{CmGAS1} gene specific probe (Fig. 7). The results indicate that \textit{CmGAS1} is expressed in seeds.

Our northern blot assessment of GAS gene expression in seeds was confirmed with RACE-PCR. This technique is more sensitive than northern blotting and can detect low copy numbers of RNAs. Polymerase chain reaction primers specific to \textit{CmGAS1} were synthesized (Table 1) and used in conjunction with an adapted ligated primer (from SMART RACE kit, Clontech) to retrieve cDNAs made from poly(A)^+ RNA extracted from 39 DAP melon seeds. Only the \textit{CmGAS1} 3’ and 5’ gene specific primers amplified fragments of melon seed cDNA that hybridized to the conserved GAS probe. Amplified PCR fragments were cloned, sequenced, and determined to be identical to the \textit{CmGAS1} gene over the one third of the coding region that was cloned. Thus, \textit{CmGAS1} is expressed in developing melon seeds. These
results confirmed those obtained by RNA gel blot analysis using poly(A)+ RNA isolated from melon seeds 39 DAP.

**Galactinol Synthase Expression in Response to Stress.**

There was no change in mature leaf GAS gene expression either 3 or 9 h after plants were chilled during the dark period. Galactinol synthase gene expression in the leaves of water-stressed melon plants also did not change in response to 4 (nonwilted) or 8 d (wilted) of water stress. No hybridization was evident in RNA gel blots made from total RNA extracted from roots and immature leaves of these stressed plants (data not shown).

The _CmGAS1_ promoter analysis reveals the presence of a sequence that may encode a G box (CACGTG) 2885 bases before the putative _CmGAS1_ translational start site. The _CmGAS1_ promoter also contains motifs that are present in some phloem specific promoters (ANNNGATA, CCA/TGG). The promoter of _CmGAS2_ has not been sequenced.

**Galactinol Synthase Phylogenetic Analyses.** While the phylogenetic analyses suggest similar groupings of GAS gene family members based on known expression patterns of genes, strong correlations between sequence similarity and gene function cannot be determined since the expression patterns of GAS gene family members are not fully characterized (Fig. 8). As would be expected, _CpGAS1_ cDNA and _CmGAS1_ are very similar. It is also interesting to note that the orthologs _AtGolS2_, _Thellungiella_, and _AtGolS3_ may all be expressed in response to stress conditions.

Discussion

**Galactinol Synthase Genes in Melon.** We identified two GAS genes in melon. No additional GAS gene family members in either genomic or subgenomic libraries were found. Although future work may reveal the presence of additional gene family members, the _CmGAS1_ and _CmGAS2_ genes do exhibit expression patterns consistent with our knowledge of RFO formation in melon.

In cucurbits, raffinose and stachyose serve as translocation sugars and also accumulate in developing seeds. Since the location and function of RFOs are diverse, one might expect each member of the GAS gene family to be expressed for a unique physiological purpose. However, there is some overlap in _CmGAS1_ and _CmGAS2_ expression patterns. The roles of the GAS genes are not as specific as the _GolS-1_ and _GolS-2_ _Ajuga_ genes: _GolS-1_ is expressed at a low level in veins, and its expression increases significantly in response to chilling stress; _GolS-2_ is expressed in veins and is thought to catalyze the formation galactinol to be used in the synthesis of higher order RFOs for translocation (Sprunger and Keller, 2000).

Both _CmGAS1_ and _CmGAS2_ RNAs are expressed in mature leaf tissue. The presence of transcript for the GAS gene is not surprising since galactinol is a key substrate for the formation of the raffinose and stachyose that are translocated in the phloem. We detected a low level of _CmGAS1_ and _CmGAS2_ transcript in our mesophyll/epidermal tissue RNA preparations. Since the transcript level was much higher in RNA from total leaf tissue than in mesophyll/epidermal samples, even through veins constitute only a small fraction (4.5%) of total cell volume, most of the _CmGAS1_ and _CmGAS2_ gene expression clearly occurs within the veins of the mature leaf. Including the veins in RNA samples significantly enhances GAS expression. Phloem specific promoter elements are present within the _CmGAS1_ promoter and may affect its expression.

Our mesophyll/epidermal cell sampling technique was not ideal. Leaves were immersed in extraction buffer, and the adaxial surface was lightly scraped with sandpaper. It is plausible that some intermediary cells were ruptured in the process of sampling; however, it is doubtful that gene expression patterns were altered as a result of our scraping technique.
nique since tissues were immersed in extraction buffer during this process. Relative to the entire leaf sample, the mesophyll/epidermal scraping sample was enriched with regard to mesophyll cells, but may also contain some vein contamination. If in fact the observed expression of \textit{CmGAS1} and \textit{CmGAS2} gene expression in the epidermal/mesophyll tissues is real, it is most likely even lower than that detected in our samples.

In the minor veins of melon leaves, stachyose and raffinose accumulate to levels of 330 and 70 mM, respectively; however, the mesophyll concentration of these sugars is less than 1 mM (Haritatos et al., 1996) and polyclonal antibodies that recognize galactinol synthase and stachyose synthase have been localized to minor vein intermediary cells in cucurbits (Beebe and Turgeon, 1992; Holthaus and Schmitz, 1991). Since galactinol synthase and stachyose synthase are both present in intermediary cells, it is likely that galactinol is formed and subsequently converted to raffinose and stachyose within intermediary cells before its entry into the sieve elements. Sucrose, the precursor to galactinol, could diffuse into intermediary cells via the numerous plasmodesmata at the intermediary cell-bundle sheath cell interface. Since stachyose and raffinose are both larger than sucrose, they may not be able to diffuse into the mesophyll through plasmodesmata. Galactinol, however, is approximately the same size as sucrose. According to this previously described polymer-trap model, it would be expected that galactinol would diffuse out of the intermediary cells via plasmodesmata; this may explain in part why the concentration of galactinol (assuming compartmentation) is the same in the cytoplasm of both intermediary cells and mesophyll cells (Haritatos et al., 1996).

Although mesophyll protoplasts isolated from \textit{Cucumis melo} had no stachyose or stachyose synthase activity in one study (Schmitz and Holthaus 1986), other labs have published evidence that stachyose is synthesized in the mesophyll (Hendrix, 1982; Madore et al., 1988; Madore and Webb, 1982; Madore and Webb, 1981). In immunolocalization studies using a polyclonal GAS antibody, there was no detectable signal above background in the mesophyll (Beebe and Turgeon, 1992). Since gene-specific antibodies to \textit{CmGAS1} and \textit{CmGAS2} have not been generated, we are unable to determine the specific localization patterns of the GAS genes within melon leaves. Haritatos et al. (2000) succeeded in localizing GUS expression to the veins of tobacco and \textit{Arabidopsis} when expression was directed by the \textit{CmGAS1} promoter, thus providing further evidence that GAS gene expression is highly vein specific.

The significance or the extent of galactinol formation in melon mesophyll cells is not known; however, RFOs do accumulate in the mesophyll cells of low temperature tolerant \textit{Ajuga reptans} and drought tolerant \textit{Xerosicyos danguyi}; both translocate RFOs (Bachmann and Keller, 1995; Madore et al., 1988). In the CAM cucurbit, \textit{Xerosicyos}, galactinol synthase activity was detected in leaf disks made from mesophyll cells (Madore et al., 1988). The RFO biosynthetic pathways may be present in both the intermediary cells of the phloem and in the mesophyll cells in some species (such as \textit{Ajuga} and \textit{Xerosicyos}); the purpose of mesophyll RFO metabolism in other crops (such as melon) may be very limited.
In mature melon leaves, there is no diurnal fluctuation in GAS transcript level. This is in agreement with our data that show that the relative concentrations of stachyose, raffinose, sucrose and hexoses in mature melon leaves do not fluctuate during the 16-h light period (data not shown). Melon accumulates starch within mesophyll chloroplasts, and may convert this stored carbohydrate to translocatable sugars as needed during the diurnal cycle.

**Galactinol synthase expression in seeds.** We documented the formation of sugars in field-grown melons to ensure that the seeds we collected were actively accumulating RFOs. In these plants, translocated RFOs are hydrolyzed in the fruit and are resynthesized in the seeds. The *CmGAS1* transcript is present in melon seeds, where raffinose and stachyose accumulate during the maturation stage of seed development.

The RFOs also accumulate in soybean seeds; a system characterized to a much greater extent than in melon. Raffinose and stachyose begin to accumulate as seeds achieve maximum dry weight (Obendorf et al., 1998; Lowell and Kuo, 1989; Blackman et al., 1992). Sucrose, stachyose, and raffinose in a ratio of 8:4:1 compose 99% of the soluble sugars in mature soybean seeds (Saravitz et al., 1987) and GAS enzyme activity is high during the time in which RFOs accumulate (Kuo et al., 1997). Saravitz et al. (1987) compared four soybean genotypes which differed in RFO content at maturity. A linear relationship was found between the RFO concentration and galactinol synthase activity. Based on their results, it was hypothesized that galactinol synthesis may be a rate limiting step in the formation of soybean seed RFOs. When most developing orthodox seeds achieve physiological maturity, they undergo a programmed desiccation phase and even low levels of RFOs could alleviate some of the cellular damage induced by extremely low water conditions. If seeds are removed from the plant after RFOs have accumulated to an adequate degree, they remain viable after rapid desiccation (Blackman et al., 1992).

**Galactinol synthase expression in response to stress.** GAS transcript levels were not altered in total RNA extracted from roots, immature leaves, or mature leaves from melon plants after exposure to our low temperature or water stress conditions. This confirms the observation of Mitchell et al. (1992) in which there was no increase in stachyose or other galactosyl oligosaccharides in *Cucumis melo* after 72 h of 10 °C treatment.

Other species do exhibit a cold-acclimation response with regard to GAS gene expression. Kidney bean (*Phaseolus vulgaris*) leaves, which have no galactinol synthase expression when grown at 27 °C, exhibit galactinol synthase enzyme activity after one to four hours of 4 °C exposure (Casillo et al., 1990). Bachmann and Keller (1995) determined that 10 d at a temperature regime of 10/3 °C causes a 10- to 20-fold increase in *Ajuga* leaf RFO content. *Arabidopsis* vegetative tissues (and siliques to a lesser extent) also exhibit a transient increase in galactinol synthase expression during and immediately af-
plants to tolerate desiccation, we did not observe an increase in GAS transcription as a result of drought stress in melon. Similarly, Pattanagul and Madore (1999) reported that in coleus (Coleus blumie Benth.), galactinol synthase activity and RFO metabolism were actually depressed by drought stress. Since both of these species translocate significant quantities of RFOs under nonstress conditions, additional synthesis may not be required for protection.

Conclusion

We have identified two members of the GAS gene family in melon. We were only able to identify GAS gene expression in mature leaves and seeds. The CmGAS1 gene was expressed in seeds as well as in mature leaves whereas CmGAS2 was expressed in mature leaf tissue. Phloem loading is most likely the primary function of RFOs in melon leaves while GAS expression in seeds is probably beneficial for survival after desiccation.

Literature Cited

Albini, F.M, C. Murelli, P.V. Finzi, M. Ferrarotti, B. Cantoni, S. Puliga, and C. Vazzana. 1999. Galactinol in the leaves of the resurrection plant Boea hygroscopica. Phytochemistry 51:499–505.

Bachmann, M. and F. Keller. 1995. Metabolism of the raffinose family oligosaccharides in leaves of Ajuga reptans L. Inter- and intracellular compartmentation. Plant Physiol. 109:991–998.

Bachmann, M., P. Matile, and F. Keller. 1994. Metabolism of the raffinose family oligosaccharides in leaves of Ajuga reptans L. Plant Physiol. 105:1335–1345.

Beebe, D.U. and R. Turgeon. 1992. Localization of galactinol, raffinose and stachyose synthesis in Cucurbita pepo leaves. Planta 188:354–361.

Blackman, S.A., R.L. Obendorf, and A.C. Leopold. 1992. Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. Plant Physiol. 100:225–230.

Caffrey, M., V. Fonseca, and A.C. Leopold. 1988. Lipid–sugar interactions relevance to anhydrous biology. Plant Physiol. 86:754–758.

Castillo, E.M., B.O. de Lumen, B.O. P.S. Reyes, and H.Z. de Lumen. 1990. Raffinose synthase and galactinol synthase in developing seeds and leaves of legumes. J. Agric. Food Chem. 38:351–355.

Castonguay, Y. and P. Nadeau. 1998. Enzymatic control of soluble carbohydrate accumulation in cold-acclimated crowns of alfalfa. Crop Sci. 38:1183–1189.

Corbineau, F., M.A. Picard, J.-A. Fougeroux, F. Ladonne, and D. Comte. 2000. Effects of dehydration conditions on desiccation tolerance of developing pea seeds as related to oligosaccharide content and cell membrane properties. Seed Sci. Res. 10:329–339.

Goldsborough, P.B., S.B. Gelvin, and B.A. Larkin. 1986. Expression of maize zein genes in transformed sunflower cells. Mol. Gen. Genet. 202:374–381.

Haritatos, E., B.G. Ayre, and R. Turgeon. 2000. Identification of phloem involved in assimilate loading in leaves by the activity of the galactinol synthase promoter. Plant Physiol. 123:920–937.

Haritatos, E., F. Keller, and R. Turgeon. 1996. Raffinose oligosaccharide concentrations measured in individual cell and tissue types in Cucumis melo L. leaves: Implications for phloem loading. Planta 198:614–622.

Hendrix, J.E. 1982. Sugar translocation in two members of the Cucurbitaceae. Plant Sci. Lett. 25:1–7.

Holhaus, U. and K. Schmitz. 1991. Distribution and immunolocalization of galactinol synthase in Cucumis melo L. Planta 185:479–486.

Kerr, P.S., R.W. Pearstein, M.F. Becker-Manley and J.W. Pierce. 1993. Nucleotide sequences of galactinol synthase from zucchini and soybean, p. 1–78. International Patent Publ. No. WO 93/02196, 4 Feb 1993, PCT/US92/06057.

Kirby, K.S. and E.S. Cook. 1967. Isolation of deoxyribonucleic acid from mammalian tissues. Biochem. J. 104:254–257.

Kuo, T.M., C.A. Lowell, and T.C. Nelsen. 1997. Occurrence of pinitol in developing soybean seed tissues. Phytochemistry 45:29–35.

Liu, J.J., D.C. Krenz, A.F. Galvez, and B.O. de Lumen. 1998. Galactinol synthase (GS): Increased enzyme activity and levels of mRNA due to cold and desiccation. Plant Sci. 134:11–20.

Lowell, C.A. and T.M. Kuo. 1989. Oligosaccharide metabolism and accumulation in developing soybean seeds. Crop Sci. 29:459–465.

Mitchell, D.E., M.V. Gadus, and M.A. Madore. 1992. Patterns of assimilate production and translocation in muskmelon Cucumis melo L. I. Diurnal patterns. Plant Physiol. 99:959–965.

Muller, J., N. Springer, K. Bortlik, T. Boller, and A. Wiemken. 1997. Desiccation increases sucrose levels in Rannonda and Hableria, two genera of resurrection plants in the Gesneriaceae. Physiol. Plant. 100:153–158.

Obendorf, R.L., M. Horbowicz, A.M. Dickerman, P. Bruneck, and M.E. Smith. 1998. Soluble oligosaccharides and galactosyl cyclitols in maturing soybean seeds in planta and in vitro. Crop Sci. 38:78–84.

Pattanagul, W. and M.A. Madore. 1999. Water deficit effects on raffinose family oligosaccharide metabolism in Coleus. Plant Physiol. 121:987–993.

Peterbauer, T. and A. Richter. 2001. Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Sci. Res. 11:185–197.

Sambrook, D.M., E.F. Fritsch, and T. Maniatis. 1989, Molecular cloning. A laboratory manual. Cold spring Laboratory Press, Cold Spring Harbor, N.Y.

Saravitz, D.M., D.M. Pharr, and T.E.J. Carter. 1987. Galactinol synthase activity and soluble sugars in developing seeds of four soybean genotypes. Plant Physiol. 83:185–189.

Schmitz, K. and U. Holhaus. 1986. Are sucrosyl-oligosaccharides synthesized in mesophyll protoplasts of mature leaves of Cucumis melo? Planta. 169:529–535.

Sibiri, Y., P. Doireau, and P. Gantet. 2001. Plant bZIP G-box binding factors—Modular structure and activation mechanisms. Euro. J. Biochem. 268:5655–5666.

Spronger, N. and F. Keller. 2000. Allocation of raffinose family oligosaccharides to transport and storage pools in Ajuga reptans: The roles of two distinct galactinol synthases. Plant J. 21:249–258.

Swofford, D. 2002 PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Mass.

Taji, T., C. Ohsumi, S. Iuchi, M. Seki, M. Kasuga, M. Kobayashi, K. Yamaguchi-Shinozaki, and K. Shinozaki. 2002. Important roles of drought- and cold-inducible genes for galactinol synthase stress tolerance in Arabidopsis thaliana. Plant J. 29:417–426.

Turgeon, R. 1991. Symplastic phloem loading and the sink–source transition in leaves: A model, p. 18–22. In: J.L. Bonnemain, S. Delrot, J. Dainty, and W.J. Lucas (eds.). Proc. 1990 Intl. Conf. Phloem Transport and Assimilate Compartmentation. Ouest Editions, Nantes, France.

Turgeon, R., D.U. Beebe, and E. Gowan. 1993. The intermediary cell: Minor vein anatomy and raffinose oligosaccharide synthesis in the scrophulariaceae. Planta 191:456–465.

Turgeon, R., R. Medville, and K.C. Nixon. 2001. The evolution of minor vein phloem and phloem loading. Amer. J. Bot. 88:1331–1339.

Zimmerman, M.H and H. Ziegler. 1975. List of sugars and sugar alcohols in sieve-tube exudates, p. 480–503. In: M.H. Zimmerman and J.A. Milburn (eds.). Transport in plants: Phloem transport. Springer, New York.

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