A Maize Vacuolar Invertase, *IVR2*, Is Induced by Water Stress. Organ/Tissue Specificity and Diurnal Modulation of Expression[1]

Jae-Yean Kim, Aline Mahé, Judy Brangeon, and Jean-Louis Prioul*

Institut de Biotechnologie des Plantes, Centre National de la Recherche Scientifique-Unité Mixte de Recherche 8618, Bât 630, Université de Paris-Sud, 91405, Orsay cedex, France

The expression of invertases was analyzed in vegetative organs of well-watered and water-stressed maize (*Zea mays*) plants. Early changes in sucrose metabolism and in acid soluble invertase expression were observed in vegetative sink and source organs under mild water stress. The organ-specific induction of acid invertase activity was correlated with an increase in the *Ivr2* gene transcripts and in the vacuolar invertase proteins. In addition diurnal changes in activity and *Ivr2* transcripts for vacuolar invertase were noted in shoots. Hexoses (glucose and fructose) accumulated in all organs examined from water-stressed plants. In situ localization studies showed that glucose accumulation, vacuolar invertase activity, invertase protein, and the *Ivr2* transcripts colocalized specifically in bundle sheath and vascular tissue cells of mature stressed leaf; in primary roots the stress-induced increase of *Ivr2* transcripts was detected only in root tips. Based on these results different regulatory roles are proposed in sink and source organs for the stress induced *Ivr2* vacuolar invertase.

Water deficit is one of the most detrimental environmental stresses in plants; it leads to large physiological modifications, such as photosynthesis reduction, transcriptional and post-transcriptional regulation of various genes, protein turnover, and osmolyte biosynthesis (Bohnert et al., 1995). In maize (*Zea mays*) the increase of soluble and insoluble invertase activities during pollination and early kernel development was blocked by water stress conditions (Zinselmeier et al., 1995); this effect was correlated with a low level of reducing sugars, an increase in Suc concentration, starch depletion, and an inhibition of ovary growth leading to abortion. In contrast it was shown recently that a marked accumulation of hexoses was correlated to an induction of vacuolar invertase activity in mature maize leaves under water stress (Pelleschi et al., 1997). These results address the question of an organ- and tissue-specific response of invertase to water stress.

Suc is the major end product of leaf photosynthesis and also is the phloem-transported sugar in most higher plants. Suc cannot be used directly for metabolic processes, but must be cleaved into hexoses by invertase (β-fructosidase, EC 3.2.1.26) or Suc synthase (UDP-D-Glc: D-Fru-2-α-D-glucosyl-transferase, EC 2.4.1.13; Susy) before entering into carbohydrate metabolism.

Invertase is present in most plant organs in multiple forms, which are classified by pH optima (acid and neutral/alkaline), solubility properties (soluble and insoluble), and cellular locations. Cell wall-bound and vacuolar forms are acidic, the first one being insoluble and the second one soluble; the cytosolic is both neutral/alkaline and soluble. The physiological role of invertase isozymes appears to be diverse and recent studies suggest that their function varies depending upon the organ/tissue or cells in which they are expressed. Invertases take part in Suc partitioning between source and sink organs. In young sink organs cell wall-bound invertases can enhance carbohydrate movement by maintaining a Suc concentration gradient between phloem and sink tissues (Sonnewald et al., 1991; Sturm et al., 1995; Cheng et al., 1996); in mature organs they can alter Suc and starch balance (Huber, 1983). More specifically, in source organs, invertase may limit carbohydrate export rate by hydrolyzing Suc, thus hindering Suc loading into phloem. The resulting hexose could also be involved in a feedback regulation of photosynthesis (Foyer, 1987).

To study the roles of invertases expressed in different cellular compartments, transgenic plants expressing yeast invertase have been produced in potato, tomato, tobacco, and Arabidopsis (Sonnewald et al., 1991, 1997; Heineke et al., 1994). Expression of a yeast-derived invertase in the cell wall or in the vacuole in potato plants leads to an accumulation of hexoses, Pro and starch, an inhibition of photosynthesis, and an increase in respiration (Heineke et al., 1994; Büssis et al., 1997). Furthermore in transgenic tobacco plants expressing yeast-derived cell wall or vacuolar invertase the reduction of photosynthesis-related gene transcripts and accumulation of pathogenesis-related gene transcripts were associated with hexose accumulation (Herbers et al., 1996).

[1] J.-Y.K. was financially supported by the Korean government.

* Corresponding author; e-mail prioul@ibp.u-psud.fr; fax 33–1–69–33–64–24.

The effect of invertase on the expression of transcripts may be better understood when considering sugars as physiological signals able to repress expression of photosynthesis-related genes (Sheen, 1990; Jang et al., 1997), or activate gene expression in Suc and starch synthesis and/or degradation (Chen et al., 1994; Koch, 1996), as well as specific stress-response genes in infection and wounding (Herbers et al., 1996; Ehness et al., 1997). Sugars can also regulate cell differentiation, elongation, and growth (Wu et al., 1993; Weber et al., 1996). In this regard some works deal with invertases as potential producers of signaling factors (Glc and Fru) via Suc hydrolysis (Koch et al., 1995; Herbers et al., 1996; Weber et al., 1996).

In maize two genes/cDNAs (*Incw1* and *Incw2*) encoding two cell wall-bound invertases (Shanker et al., 1995; Cheng et al., 1996; Taliercio et al., 1999) and two partial cDNAs (*Ivr1* and *Ivr2*) encoding two vacuolar invertases (Koch et al., 1995; Xu et al., 1995) have been cloned. We have recently cloned two new genes (*Incw3* and *Incw4*) encoding cell wall invertases (Kim et al., 2000).

In the present paper the expression of three types of invertase activities (vacuolar, cell wall, and cytosolic) and of Susy activity was analyzed in well-watered and water-stressed vegetative organs (mature leaf, leaf sheath, young leaf, primary roots, and adventitious roots). The transcript levels for all the genes cloned so far and the invertase protein levels of two types (vacuolar and cell wall-bound) were investigated. It was observed that *Ivr2*, encoding a vacuolar invertase, was the only gene among all the known invertase genes to be specifically induced under water stress and in an organ/tissue specific manner. In addition a diurnal regulation of the induced invertase activity and of the *Ivr2* mRNA content was observed in shoots. The putative role of specific temporal and spatial patterns of *Ivr2* expression during water stress is discussed in relation to photoassimilate source-sink partitioning, osmotic adjustment, and sugar signaling.

RESULTS

Activity of Vacuolar Invertase Is Induced by Water Stress in Specific Organs

The activity of all Suc cleaving enzymes (vacuolar, cell wall, cytosolic invertases, and Susy) in well-watered and water-stressed maize source and sink

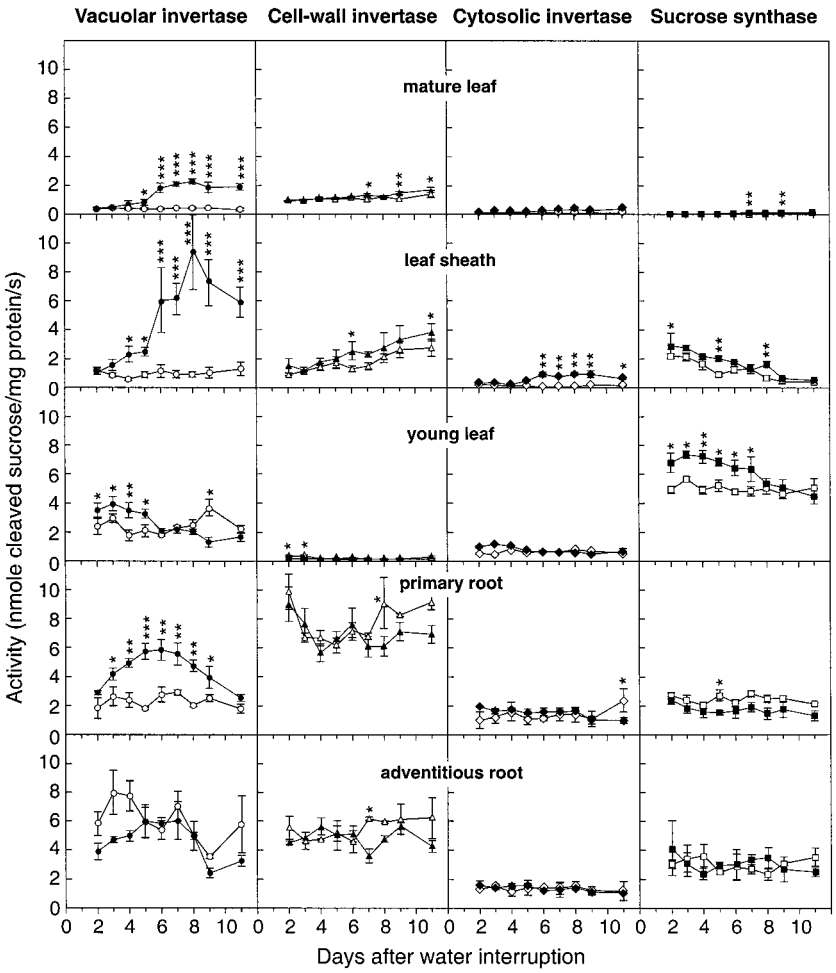

Figure 1. Effect of drought on acid soluble (vacuolar), acid insoluble (cell wall), and neutral (cytosolic) invertase activity, and Susy activity in five vegetative organs from well-watered and water-stressed maize plants. Samplings were carried out at 9 AM (3 h after light on) from d 2 to 11 after water interruption. White symbols, Control plants; black symbols, water-stressed plants. Mean ± SE from four independent determinations. Control/stress Student's comparison: *, $P < 0.05$ significant; **, $P < 0.01$ very significant; ***, $P < 0.001$ highly significant.

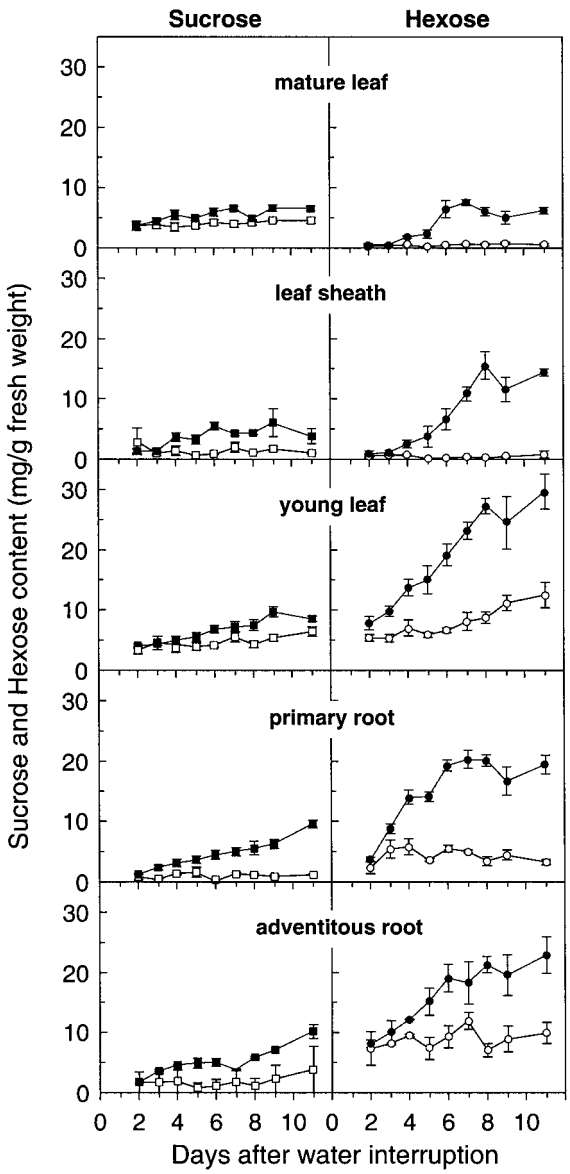

Figure 2. Effect of drought on Suc and hexoses (Glc and Fru) concentrations on a fresh weight basis in five vegetative organs from well-watered and water-stressed maize plants. Same samples as in Figure 1.

organs was investigated for 11 d after deprivation (Fig. 1).

In well-watered plants activities of all invertases were higher in sink organs (young leaf, primary, and adventitious roots) than in the source leaf with the exception of cell wall-bound invertase activity, which was undetectable in young leaf (Fig. 1). Water stress effect was either nil or marginal on cell wall-bound and cytosolic invertases and on Susy activities, whatever the organ tested (Fig. 1). In contrast a vacuolar invertase activity was markedly induced by water stress in mature leaf, leaf sheath, and primary roots (Fig. 1), but not in adventitious roots. Early effects were detectable at 2 d in primary roots and young leaves and a few days later in mature leaf laminae and sheath. Maximum effect was, in general, noted around 5 to 8 d.

Hexoses Accumulate in All Water-Stressed Organs

To determine if stress-induced vacuolar invertase activity alters carbohydrate pools, Suc and hexose (Fru and Glc) contents were measured (Fig. 2). In control plants Suc levels were higher than hexose levels in both mature leaf (source) and sheath (sink), whereas the opposite was observed in other sink organs (young leaf and both root types). Water stress produced a marked hexose accumulation in all organs, whereas Suc increased, to a lesser extent, in leaf sheath and in primary and adventitious roots. The daily hexose accumulation rate was higher in sinks (from 15–30 mg g^{-1} fresh weight d^{-1}, depending the organ) than in source leaf (7 mg g^{-1} fresh weight d^{-1}). This accumulation was not well correlated to invertase activity, but it was related to water loss: relative water content (RWC) decreased earlier and more rapidly in young leaf and primary root than in mature leaf (Fig. 3). This raised the question as to a concentration effect due to water loss. Nonetheless

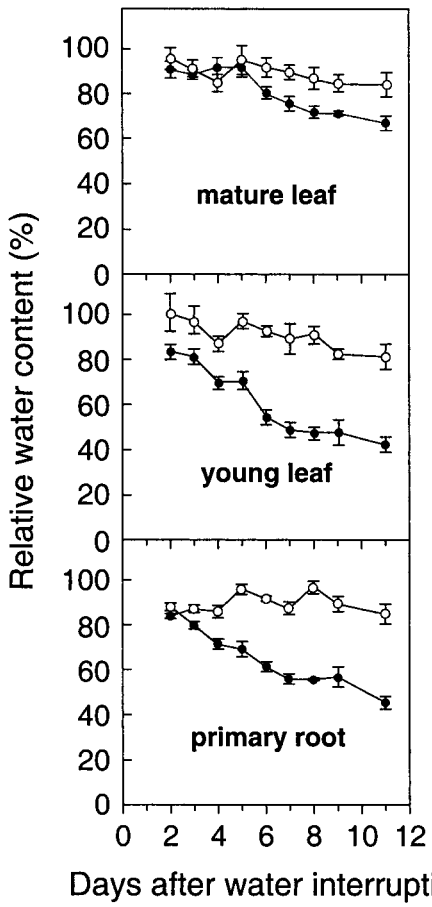

Figure 3. Variation of RWC in mature leaf, young leaf, and root after water deprivation.

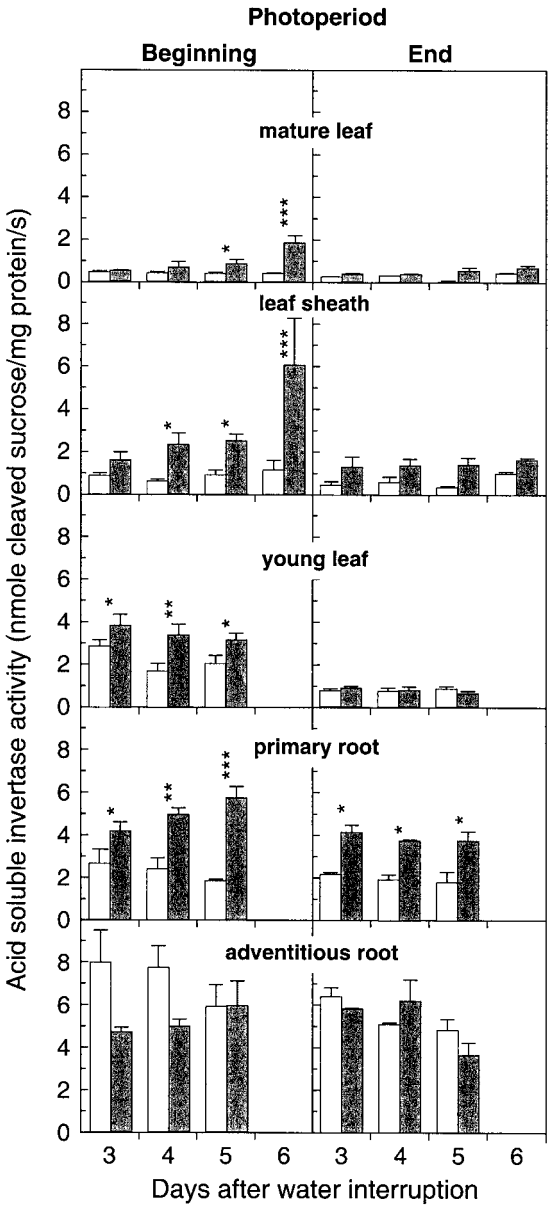

Figure 4. Diurnal variation of acid soluble invertase (vacuolar) in five vegetative organs of watered (white bars) and water-stressed plants (gray bars). Samplings were carried out at the beginning, 9 AM (3 h after light on), and at the end, 9 PM, of the photoperiod (1 h before light off). Symbols for statistical comparisons between stressed and control plants as in Figure 1.

this question may discarded in mature leaf since vacuolar invertase activity and hexose content increased in parallel before any significant changes occurred in RWC (compared values at 5 and 6 d in Figs. 1–3). This retarded RWC response tends to dismiss leaf RWC as being the trigger for a response to water stress.

The Induced Activity Presents Diurnal Changes

It is interesting that the stress-induced vacuolar invertase activity showed diurnal modulations in stressed mature leaf, in sheath, in young leaf, and to a lesser extent in primary roots (Fig. 4, compare beginning and end of photoperiod). This was confirmed by measuring vacuolar invertase activity every 3 to 4 h for 3 d in water-stressed leaves under 16-h light-day cycles (not shown). The invertase activity increased during the dark period, reached a peak before the end of the dark period, and decreased rapidly after 3 h in the light period (Kim, 1998).

Drought Induced a Parallel Increase of Both Activity and Protein for Vacuolar Invertase

To understand the induction of vacuolar invertase activity it is necessary to evaluate the variation in invertase protein. Specificity of the anti-IVR2 antiserum was checked on two-dimensional isoelectric focusing (IEF)-SDS-gel blots by comparison with antiserums raised against a carrot vacuolar invertase and an Arabidopsis cell wall invertase peptide. The carrot vacuolar invertase antiserum displayed the same immunospot pattern as the IVR2 antiserum on two-dimensional IEF-SDS gel blots (data not shown). The IVR2 pattern (Fig. 5, A and B, spots a and b) was clearly distinct from that detected by Arabidopsis cell wall invertase antiserum (Fig. 5, C and D, spots e, f, and g) in mature leaf. Thus IVR2 antiserum does not recognize cell wall invertases and appears to be specific to vacuolar invertases. In mature leaf vacuolar invertase IVR2 antiserum showed at least two peptide groups with different M_rs (Fig. 5, A and B): group a (68 kD) composed of an apparent spot cluster showing a unique pI of 5.0, and group b (74 kD) with aligned spots between pI 4.7 and 7.0, but with two main spots between pI 5.8 and 6.0. Main spots in a and b groups from the morning samples (at 9 AM) were larger in water-stressed (Fig. 5B) than in control organs (Fig. 5A). The same samples probed with cell wall antibodies (Fig. 5, C and D) labeled three peptides groups: e (52 kD, pI 4.8–5.1), f (60 kD, pI 4.9–5.6), and g (44 kD, pI 7.8–8.2). The fragment g, being only 44 kD, could be a partial fragment, which was frequently detected by SDS-PAGE during invertase purification. This intrinsic protein lability is reported for many plant invertases (Unger et al., 1992). Comparison of band intensities between stress and control samples showed no significant differences in the cell wall invertase spots, except for the g band. As there is no difference in activity (Fig. 1) this would mean that the g peptide is inactive; this is consistent with a degradation product.

The immunoblot patterns for vacuolar invertase were rather complex in the primary roots. Two additional peptide groups were noted: c (68 kD, pI 5.3) and d (80 kD, with a broad pI range pI 5.5–6.3); both spots were slightly enhanced under stress (Fig. 3F). The b and d groups had the same spot pattern for pI, but the d group was slightly shifted toward a higher mass than b group.

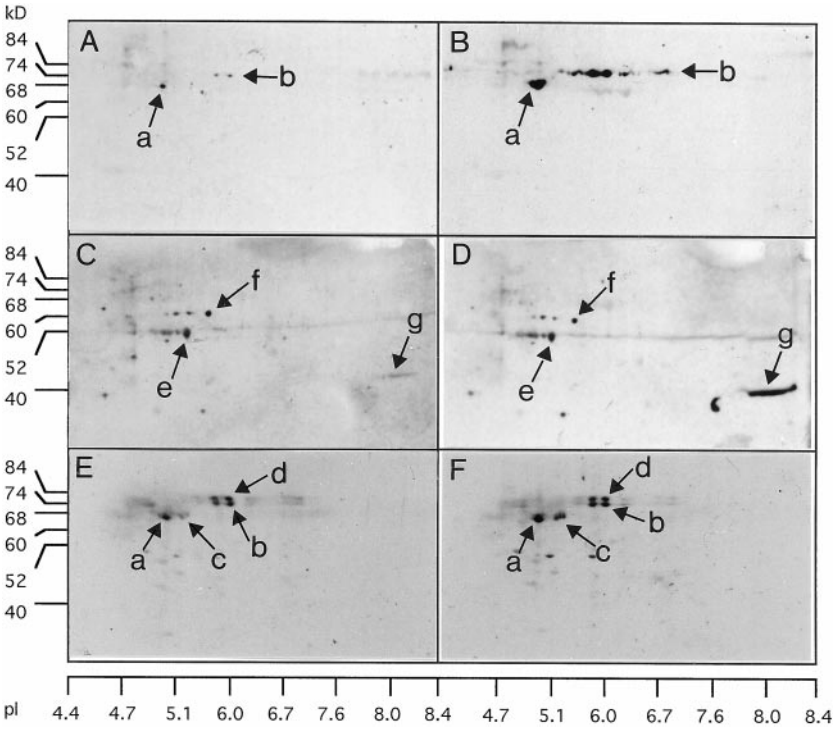

Figure 5. Immunodetection from two-dimensional gels of vacuolar and cell wall invertase in mature leaf (A–D) and primary root (E and F) from well-watered (left column: A, C, and E) or water-stressed plants (right column: B, D, and F) for 7 d . Homologous groups of spots were designated a, b, c, and d for vacuolar invertase antibodies and e, f, and g for cell wall invertase antibodies. Antiserums raised against an IVR2 oligopeptide (A and B for mature leaf; E and F for primary root) and a cell wall invertase peptide (C and D for mature leaf) were used for invertase immunodetection from crude protein extracts (50 μg) in mature leaf and root. All sampling was done at 9 AM. Comparison among loaded protein quantities was carried out from Coomassie Blue gel staining (data not shown). To measure pI, four gels were cut into 15 fragments and four gel fragments were incubated together in 1 mL of distilled water overnight; pH was measured from these eluted solutions.

Ivr2 Transcripts, Encoding a Vacuolar Invertase, Are Specifically Induced by Water Stress and Correlate with Vacuolar Invertase Activity

Since vacuolar invertase activity induction was accompanied by an increase in vacuolar invertase proteins, it is expected that the corresponding transcript levels would also be correlated to vacuolar invertase activity. Membrane blots of total RNAs from five organs of well-watered and water-stressed plants were hybridized with specific gene probes encoding two vacuolar invertases (*Ivr1* and *Ivr2*), four cell wall invertases (*Incw1*, *2*, *3*, and *4*), and two Susy (*Sus1* and *Sh1*).

No significant changes in the expression pattern for genes encoding the cell wall invertases were detected in water-stressed organs, nor for the two genes encoding Susy (Kim, 1998); in contrast, the expression of one vacuolar gene (*Ivr2*) was affected.

Ivr1 transcripts were detected at very low levels in primary and adventitious roots and were undetectable in mature and young leaf, irrespective of the water treatment (data not shown). Only *Ivr2* gene transcripts increased under water stress (figure 6) displays the time-course expression of *Ivr2* gene transcripts in various organs under watered or water-stress conditions. Water stress clearly modified the *Ivr2* gene expression pattern in mature leaf, leaf sheath, and roots. The *Ivr2* expression pattern followed that of vacuolar invertase activity in all organs (Fig. 1), except in adventitious roots in which there was no significant induction of vacuolar invertase activity. Diurnal fluctuations of the *Ivr2* transcripts were also apparent in stressed young leaf, mature leaf, and leaf sheath, but not in roots. Transcript levels were consistently the highest at the beginning and the lowest at the end of the light period. These fluctuations suggest a diurnal regulation of the gene.

Water Stress Induces *Rab17* Expression and Reduces *RbcS* Expression

To examine if hexoses accumulated under water stress may trigger modifications in gene expressions, total RNAs from mature leaf were hybridized with probes of a maize *RbcS* gene encoding the small subunit of ribulose-bisphosphate carboxylase, known to be repressed by Glc (Sheen, 1990), and of a maize *Rab17* gene, called Abscisic acid (ABA)- or water-stress responsive gene (Vilardell et al., 1990). The *RbcS* gene transcripts decreased rapidly after 5 to 6 d in stressed mature leaf compared with controls (Fig. 7). At this time point hexose content reached about 6 mg g^{-1} fresh weight (Fig. 7), which is the reported threshold for hexose regulation of gene expression in tobacco (Herbers et al., 1996). In mature leaf the induction of vacuolar invertase activity and the accumulation of hexoses preceded the reduction of *RbcS* gene transcripts. In contrast the *Rab17* gene expression was induced by water stress from d 5; it is interesting that its temporal expression pattern suggested a diurnal regulation similar to that of *Ivr2* transcripts (Figs. 6 and 7). The *EF-α* gene transcript level used as a constitutive expression standard remained constant and unaffected by stress.

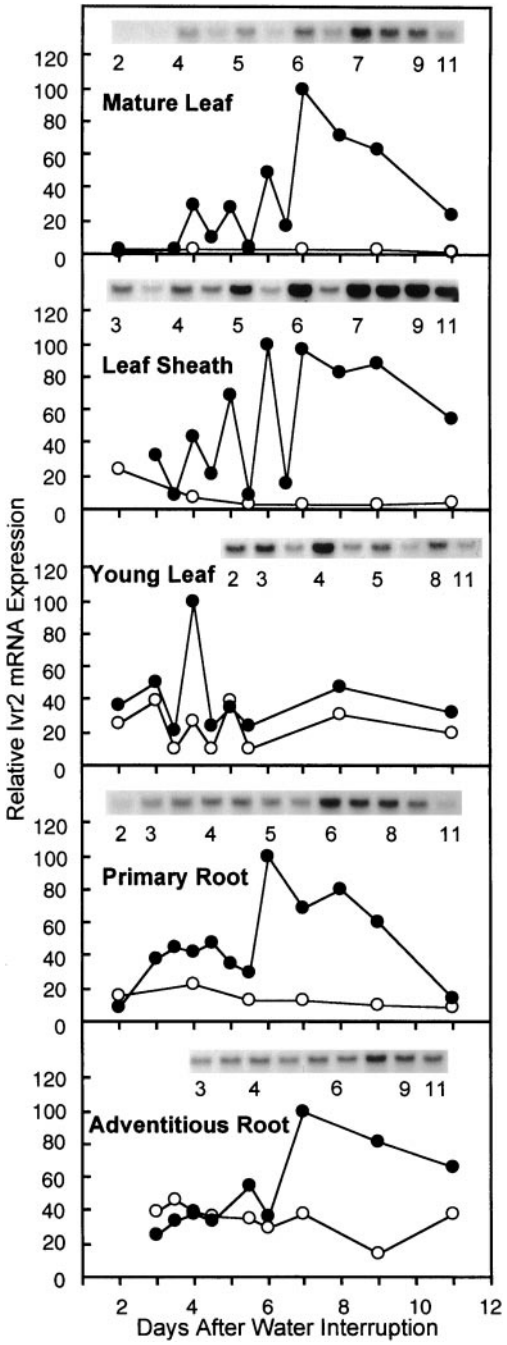

Figure 6. Time course of *Ivr2* transcript accumulation in water-stressed (black symbols) and watered plants (white symbols) in five vegetative organs. Total RNA samples (20 μg/lane) extracted from organs were hybridized with an *Ivr2* cDNA probe, radioactivity was stripped, and the membrane was rehybridized with *EF-α* cDNA. The hybridization signal intensity was measured with an image analyzer and the relative *Ivr2* mRNA expression was normalized from the average between the intensity of *EF-α* and of 18S rRNA staining.

Vacuolar Invertase Protein and *Ivr*2 mRNA Show the Same in Situ Localization as Induced Vacuolar Invertase Activity and Glc Content

In situ localization of Glc and invertase activity was carried out on hand-sliced fresh material by using a Glc oxidase/peroxidase-diaminobenzidine (DAB) staining method. In well-watered mature leaf only traces of Glc staining were detectable (data not shown), whereas under stress a brown DAB precipitate indicating the presence of Glc was seen in bundle sheath cells and vascular cells (Fig. 8A). Invertase activity staining was also localized within vascular and perivascular cells of water-stressed leaves (Fig. 8C), which contrasted with a cell wall localization in the various tissues of well-watered leaf (Fig. 8B). Such cell wall staining is consistent with measured activity from whole leaf extracts (Fig. 1) indicating that cell wall-bound invertase activity, although at a low level, is the only Suc cleaving enzyme in control mature leaf. The colocalization of vacuolar acid invertase activity and Glc strongly suggest that accu-

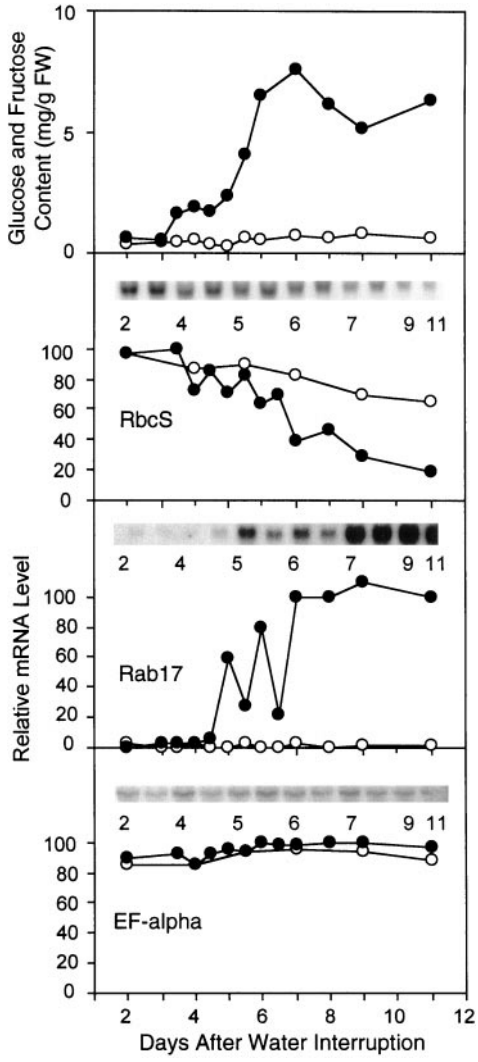

Figure 7. Time course of hexose accumulation in mature leaf in comparison with the expression of a hexose repressed gene (*RbcS*), an ABA-responsive gene (*Rab17*) and a constitutively expressed gene (*EF-α*), under watered and water-stressed conditions. The membrane for mature leaf used in Figure 6 was rehybridized with ^{32}P-labeled *Rab17*, *RbcS*, and *EF-α* cDNA probes.

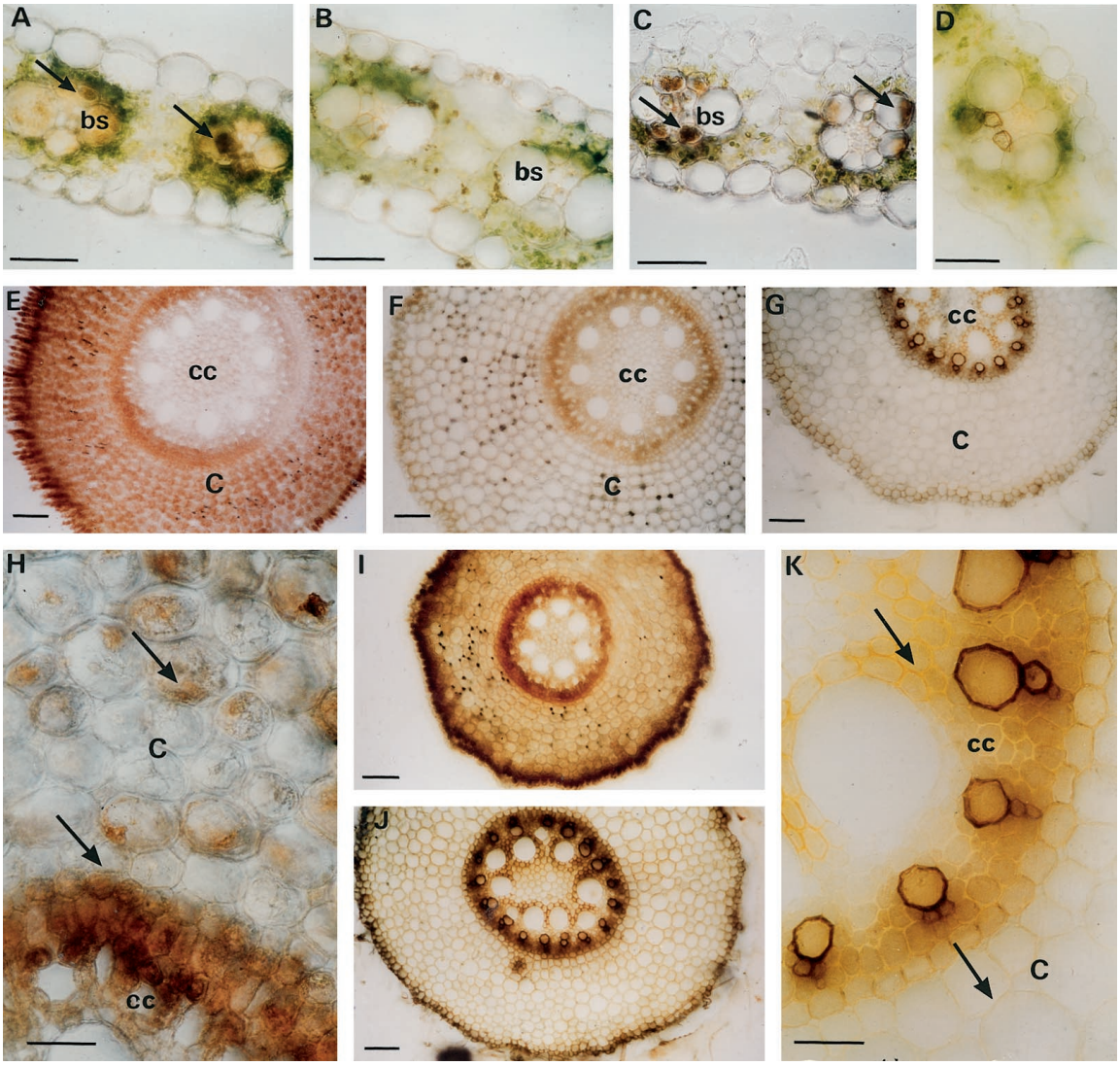

Figure 8. In situ histochemical staining for Glc and invertase activity in hand-sliced fresh mature leaf (A–D) and primary root (E–K) sampled on d 7 from well-watered and water-stressed plants. A, Stressed mature leaf; Glc localized within bundle sheath and vascular bundle cells (arrows). B, Watered mature leaf; acid invertase activity only detected in cell walls. C, Stressed mature leaf; acid invertase activity localized within bundle sheath and vascular bundle cells (arrows). D, Stressed mature leaf; histochemical control (tissue flushed, no sugar addition). E, Stressed root; strong signal for Glc in cytosol of cortex and outer central cylinder. F, Watered root; weak signal for Glc in both cell walls and cytosol of cortex and central cylinder. G, Stressed root; negative histochemical control showing artifactual xylem staining. H and I, Stressed root; strong intracellular acid invertase activity in epidermis and central cylinder (arrow), weaker signal in cortex (arrow). J and K, Watered root; moderate cell wall signal and weak intracellular signal for acid invertase activity in epidermis, cortex and central cylinder (arrow). bs, Bundle sheath; C, cortex; cc, central cylinder. Bars in A through D, 50 μm; E through G and I and J, 100 μm; H and K, 25 μm.

mulated Glc is the product of the invertase activity. The non-substrate histochemical controls indicate that DAB-labeling of xylem elements in mature stressed leaf is likely to be artifactual (Fig. 8D).

In primary roots marked differences in tissue staining for Glc and invertase activity were visible between stressed and watered conditions. In water-stressed root the cortex and outer cylinder cells were heavily Glc-stained in the intracellular compartment (Fig. 8E) compared with a weak apoplastic and cytoplasmic staining in well-watered root (Fig. 8F). The staining pattern of acid invertase activity was again very similar to that of Glc; in stressed primary roots a strong intracellular staining in cortex and central cylinder cells was detected (Fig. 8, H and I) corresponding to the observed induction of vacuolar invertase activity (Fig. 1). In well-watered primary roots a moderate cell wall-staining and a weaker intracellular signal for invertase activity was detected in the central cylinder and in epidermal cells (Fig. 8, J and K), which is again

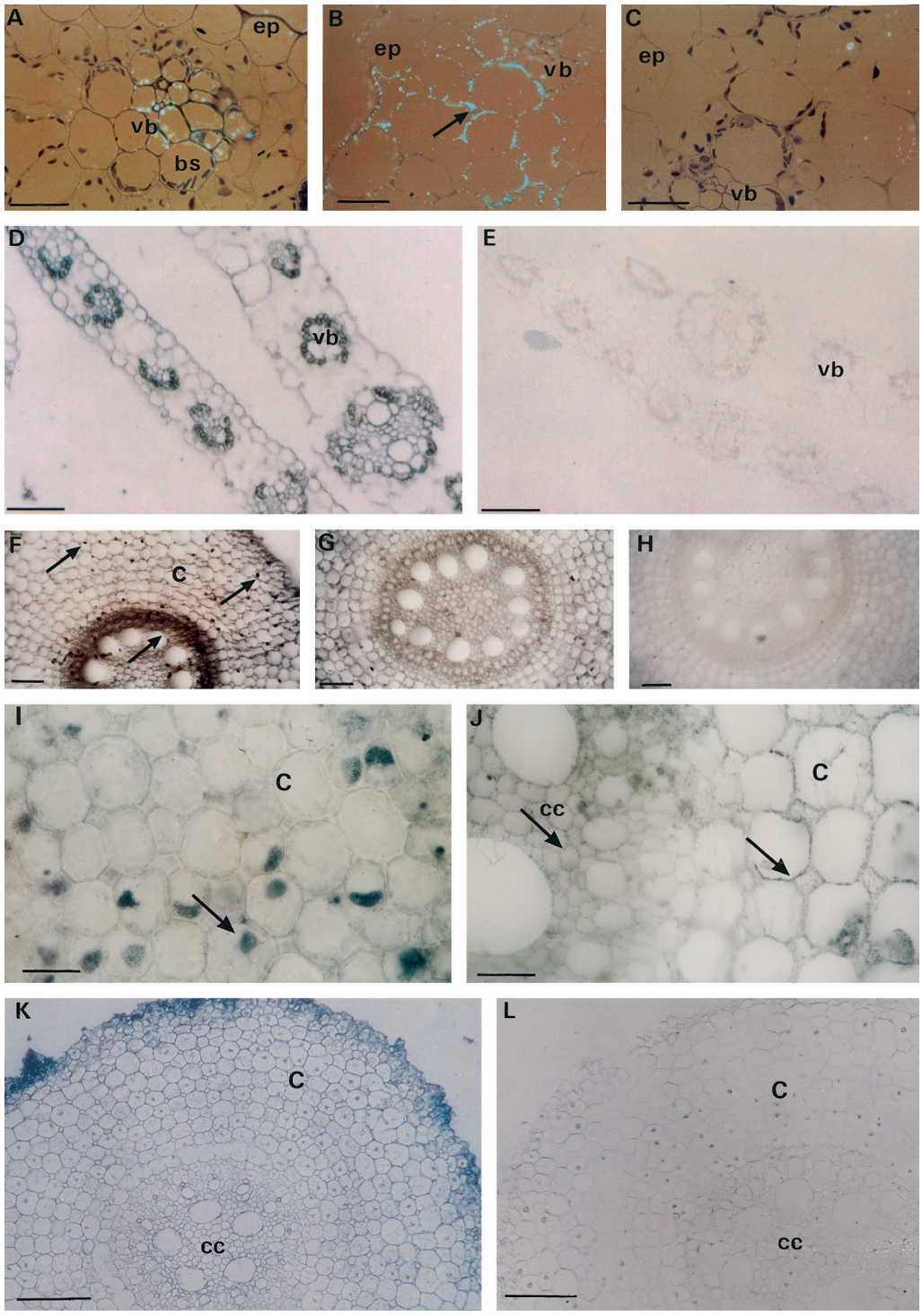

Figure 9. In situ immunolocalization of invertase protein (A–C and F–J) and in situ hybridization of *Ivr2* mRNA transcripts (D–E and K–L) in mature leaves (A–E) and roots (F–L) sampled on d 7 from water-stressed plants. Epipolarization optics of water-stressed mature leaf section exposed to A, Vacuolar invertase antibodies showing intracellular labeling in the vascular bundle; B, cell wall invertase antibodies showing cell wall labeling (arrow); and C, nonimmune serum. D, leaf section of water-stressed mature leaf hybridized to *Ivr2* probe, in antisense orientation; E, leaf hybridized to *Ivr2* probe in sense orientation. F and I, Root section of water-stressed plants incubated with vacuolar invertase antibodies showing intracellular localization in cortex and central cylinder (arrows); G and J, water-stressed roots exposed to anti-cell wall invertase serum showing immunopositive cell walls in all root tissue (arrows in J); H, root section incubated with nonimmune serum yielded no labeling; K, root section of water-stressed plants hybridized to *Ivr2* antisense probe; L, root hybridized to sense probe. c, Cortex cells; cc, central cylinder; ep, epidermis; bs, bundle sheath cells; vb, vascular bundle cells. Bars in A through C, I, and J, 25 μm; D through H, K, and L, 100 μm.

consistent with enzyme assays showing a higher cell wall invertase activity than vacuolar activity (Fig. 1).

In situ immunolocalization of the vacuolar invertase protein showed an intracellular tissue specific localization, namely in bundle sheath and vascular cells of mature leaf (Fig. 9A). In contrast, immunolocalization of cell wall invertases was confined to cell walls in all leaf tissues (epidermal, mesophyll, and vascular; Fig. 9B). The nonimmune serum-treated leaf was immunonegative (Fig. 9C). The cellular distribution of vacuolar invertase protein was similar to that of invertase activity and accumulated Glc in stressed leaf (compare with Fig. 8, A and C). To determine if the location of *Ivr2* gene transcripts was the same as that of vacuolar invertase protein and activity, in situ hybridization using antisense (Fig. 9D) and sense (Fig. 9E) *Ivr2* probes was carried out. Indeed strong signals indicated that under water stress *Ivr2* transcripts were detected in bundle sheath cells and to a lesser extent in vascular cells (Fig. 9D).

Vacuolar invertase proteins were also immunodetected in the epidermis, central cylinder, and cortex cells of primary roots. The signal was intracellular in cortex cells, supporting a vacuolar location (Fig. 9, F and I, arrows). Using anti-cell wall invertase serum, immunopositive cell walls were seen throughout the central cylinder and cortex cells (Fig. 9, G and J). Stressed root sections were hybridized in situ; the antisense probed roots showed a strong staining for *Ivr2* transcripts in epidermal, cortex, and central cylinder cells (Fig. 9K) in comparison with sense-probed roots, which remained unstained (Fig. 9L). Taken together these results suggest that the induction of vacuolar invertase is due to the cell-specific expression of the *Ivr2* gene.

DISCUSSION

Vacuolar Invertase Expression under Water Stress

The most remarkable response to water stress in maize organs is the induction of vacuolar invertase activity. This invertase activity is mainly induced in mature leaf, leaf sheath, and primary root and is accompanied by an increase in vacuolar invertase protein and *Ivr2* transcripts. In situ localization data support the view that *Ivr2* gene expression is linked to the change in vacuolar invertase proteins under water stress. Two genes, *Ivr1* and *Ivr2*, encoding vacuolar invertases show different organ/tissue-specific and developmentally specific expression patterns (Xu et al., 1996). *Ivr2* is expressed in various vegetative and reproductive organs and its expression is related to an increase in soluble sugars, whereas *Ivr1* shows a narrower expression pattern, being essentially limited to roots and reproductive organs, and inhibited by high sugar level (Xu et al., 1996). The accumulation of soluble sugars by water stress could trigger the induction of *Ivr2 gene* expression. However, this is unlikely since the induction of *Ivr2* expression is not well correlated with soluble sugar content, with the exception of young leaf. Moreover, the expression of other sugar regulated genes (*Ivr1*, *Sus1*, and *Sh1*) encoding Suc cleavage enzymes did not change under the same condition (Kim, 1998). Furthermore, *Ivr2* expression showed diurnal variations, whereas the sugar-induced gene regulation tends to override light, tissue-dependent, or developmental regulations (Sheen, 1990). Alternatively the *Ivr2* gene can be induced via a water stress-specific pathway such as ABA-related signal transduction, since exogenous ABA treatment can induce an accumulation of *Ivr2* transcripts (K.E. Koch, personal communication; J. Trouverie and J.L. Prioul, unpublished data). This effect is consistent with the fact that the expression of *Rab17*, an ABA responsive gene, was also induced under water stress, with a diurnal and temporal pattern similar to that of *Ivr2* (Figs. 6 and 7). However, it remains to be elucidated whether or not diurnal fluctuation of *Ivr2* and *Rab17* transcripts in shoots is regulated by ABA fluctuation.

To our knowledge, no reference about immunodetection of invertase on two-dimensional IEF-SDS gel is available to date. The increase in size of immunospots a and b (Fig. 5) under water stress would suggest that at least one of these groups corresponds to the IVR2 protein. The root specific spot c (Fig. 5) may correspond to the IVR1 protein since its pI fits with the theoretical pI of 5.0 for prepropeptide deduced from the *Ivr1* sequence and since both spots and transcripts are absent in mature leaf, but present in roots. However, we cannot exclude the possibility of unknown gene(s) encoding vacuolar invertase(s) considering *Ivr1* transcript levels in roots were constant. In fact we have detected two invertase groups in mature leaf and four groups in primary roots. RFLP map analysis using *Ivr2* gene probe showed two to four gene copies of which two were localized on chromosome 4L and 5S, respectively (Xu et al., 1996). There are also two copies for *Ivr1* on chromosome 2S and 10L. These copies may code for proteins with different molecular masses and pI properties. Post-translational glycosylation could also result in forms with altered M_r and possibly pI. Indeed maize vacuolar invertases are *N*-glycosylated proteins. The maize IVR1 and IVR2 invertase protein sequences, deduced from their cDNAs, show at least five potential glycosylation sites. The d spots could be derived from the glycosylation of the b spots, since they present the same pI pattern with a shift toward higher M_r, possibly due to multiple glycosylation sites.

Ivr2 expression was induced in water-stressed adventitious roots, but the vacuolar invertase activity remained unchanged, implying that there would be some post-transcriptional/translational regulation for vacuolar activity in this organ. Indeed the existence of proteinaceous invertase inhibitor has been reported for cell wall-bound invertase and more

recently for the vacuolar form (Krausgrill et al., 1996).

Invertase as a Putative Factor of Hexose-Signaling System

Under water stress, hexose accumulation was associated with an increase in vacuolar invertase activity in mature leaf and primary roots. Glc accumulation in both organs colocalized in situ with vacuolar invertase activity, invertase protein, and *Ivr2* transcripts. It is interesting that our results showed that *RbcS* expression, a bundle sheath cell specific-photosynthetic gene, was significantly diminished by water stress when leaf hexose content was higher than the threshold level (6 mg g^{-1} fresh weight) reported by Herbers et al. (1996; Fig. 7). This observation would support the hypothesis of a feedback inhibition of *RbcS* expression by excess hexoses. In a maize mesophyll protoplast system hexose treatment resulted in the repression of photosynthetic gene expression via hexokinase-associated signal transduction (Jang et al., 1997).

However, the sugar response is difficult to separate from the ABA one. Pelleschi (1997) reported that an accumulation of Glc and Fru in maize mature leaf is associated to and slightly precedes xylem ABA concentration in water-stressed maize. We also detected that the induction of *Ivr2* transcript and the accumulation of hexose preceded *Rab17* transcript induction in mature leaf under water stress. In addition since exogenous ABA and sugar treatments could stimulate Ivr2 expression, we suggest that ABA- and sugar-signaling pathways could crosstalk under water stress. This hypothesis is supported by Smeekens (1999), who observed that sugar repression of *Cab* gene expression is inhibited in *Abi1* and *Abi2* ABA-insensitive mutants in Arabidopsis.

Vacuolar Invertase Activity Plays a Role in Establishing Sink Organs and in Suc Mobilization under Water Stress

In maize sink organs the association of a high hexose/Suc levels with a high invertase activity supports the role of Suc-cleaving enzyme in Suc partitioning. During the day Suc is mainly synthesized in leaf mesophyll cells from current photosynthates, whereas at night Suc mainly originates from starch hydrolysis in the bundle sheath cells. In well-watered leaf with low vacuolar invertase activity Suc is exported or stored principally in the vacuole of photosynthetic cells (Huber, 1989; Heineke et al., 1994). This vacuolar Suc is remobilized for export in the early hours of the dark period, before starch remobilization. Under water stress the physiological significance of IVR2 diurnal variations may be explained by the following model (Fig. 10). In mature leaf at the beginning of the dark period under water stress, Suc

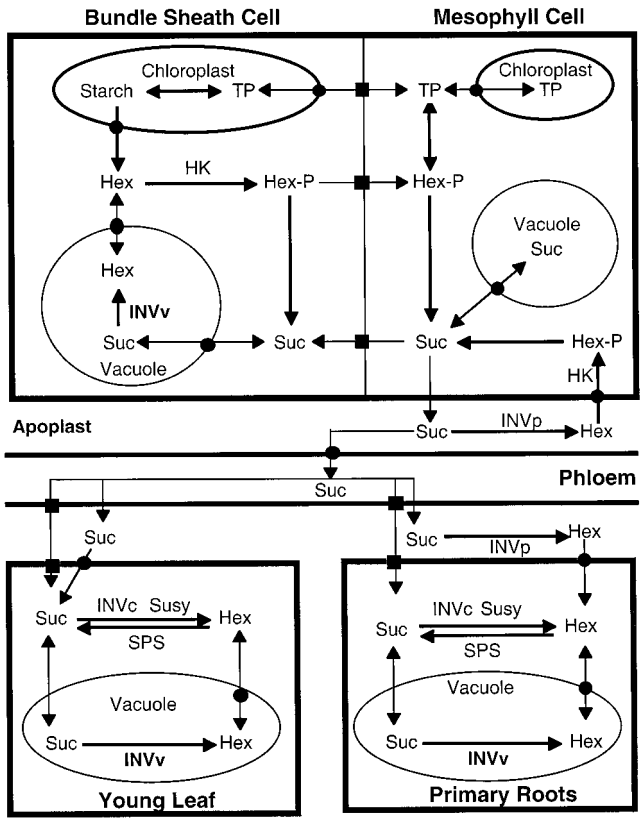

Figure 10. Flowchart of Suc and hexose mobilization among different organs, cells, and compartments in maize. Suc is synthesized in leaf mesophyll cells during the daytime from current photosynthates and in bundle sheath cells at night from starch hydrolysis products. Suc is loaded into phloem or temporally stored in the vacuole and remobilized. Induction of invertase activity in bundle sheath cells during the night in water-stressed maize slows down the Suc export and produces a Suc degradation/synthesis futile cycle. In young leaves Suc is imported without hydrolysis, whereas it is imported with or without hydrolysis in roots. Black squares represent plasmodesmata and black circles represent membrane translocation. Hex, hexoses (Glc or Fru); Hex-P, hexose phosphates (Glc- or Fru-Phosphate); TP, triose-P; INVv, INVc, and INVcw: vacuolar, cytosolic, and cell wall-bound invertases respectively; SPS, Suc-P synthase.

stored in mesophyll vacuoles would be remobilized as in watered plants. However, Suc stored in bundle sheath cells would be hydrolyzed by stress-induced vacuolar invertase activity during the first hours of the dark period. Resulting hexoses could not be exported until resynthesis of Suc via hexokinase and Suc-P synthase, thus forming a futile cycle. These events would result in altered starch remobilization as in transgenic plants expressing yeast-derived vacuolar invertase (Heineke et al., 1994; Büssis et al., 1997). Accordingly, Pelleschi (1997) observed larger starch granules in bundle sheath chloroplasts of water-stressed maize than in watered plants at the beginning of the light period.

Suc from the phloem could be unloaded into sink organs via an apoplastic or symplastic pathway. In young sink leaves with minimal wall invertase activ-

ity Suc is unloaded symplastically or apoplastically without cleavage. Compared with young leaves, roots not only contain Susy, vacuolar, and cytosolic invertase, but also a high level of cell wall invertase activity, which could itself explain the higher hexose to Suc ratio. However, in some sink organs such as maize primary roots and carrot tap roots, vacuolar invertase and/or cytosolic Susy are considered to be more important than cell wall invertase in maintaining Suc gradient (Duke et al., 1991; Strum et al., 1995). Our in situ data showed the induction of *Ivr2* expression in root tips under water stress suggesting that it may play a stimulating role in carbohydrate partitioning in active sink tissues. Activation of Suc hydrolysis via the induced vacuolar invertase activity under water stress would stimulate Suc unloading from phloem, sequestration into vacuoles, and consequently contribute to an osmotic adjustment by vacuolar hexose accumulation. The increase in root to shoot dry weight ratio under water stress (Pelleschi et al., 1997; Kim, 1998) is consistent with an enhanced unloading due to a stronger induction of vacuolar acid invertase activity in primary roots than in young leaves.

In conclusion, under water stress *Ivr2* gene expression is likely to coordinately regulate changes in vacuolar invertase proteins and activity in spatial and temporal specific manners. The induced vacuolar invertase results in hexose accumulation that in turn may be involved in a signal transduction pathway or in an increase of osmotic pressure leading to water stress resistance. The modulations of vacuolar invertase activity between source and sink and between sink organs, concurrent with the diurnal regulation of IVR2 activity in bundle sheath cells, would alter the competition for assimilates and thus modulate source-sink strength.

MATERIALS AND METHODS

Plant Material and Sampling

Maize (*Zea mays*; F_2) plants were grown in a green house with a 16-h light period supplemented with fluorescent lamps (400 μmol quanta m^{-2} s^{-1}, Philips Sun-T Agro, Paris) as described by Pelleschi et al. (1997). Plants were watered with a nutrient solution (Hydrocani C2 + Hydroplus Fer H23, HURELARC) eight times a day by an automatic system. Nutrition solution was interrupted in the water-stressed group when the ligule of the fourth leaf was visible, about 15 d after germination. In the water-stressed and control groups, five vegetative organs (fourth mature leaf, fourth leaf sheath, young leaf, primary, and adventitious roots) of four plants were sampled once or twice a day (3 h after the beginning of the photoperiod and/or 1 h before the dark period) until the 11th d after water interruption. At this time leaves were severely wilted. For young leaf, the basal yellow part of the youngest visible leaf was sampled.

Biochemical Measurements

Susy and invertase activities, and Suc and hexose contents were measured as described by Pelleschi et al. (1997) except for the following modifications. HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]-NaOH buffer was replaced by 50 mM sodium phosphate buffer (pH 7) for neutral/alkaline invertase assays because the HEPES buffer inhibits this activity. Insoluble cell wall-bound invertase activity was assayed from well-mixed cell debris suspension. Protein concentrations were determined using a Bio-Rad (Hercules, CA) protein assay kit with bovine serum albumin (BSA; Sigma, St. Louis) as standard.

Northern Blots

Samples were harvested, quick frozen in liquid nitrogen, and stored at −80°C. Total RNA was isolated by the phenol-chloroform method as described by Mahé et al. (1992). Total RNA samples (20 μg/lane) were separated in denaturing formaldehyde 1.2% (w/v) agarose gel (Sambrook et al., 1989) and transferred to a nylon membrane in 2× SSC as a transfer buffer. Membranes were hybridized to ^{32}P-labeled DNA probes generated using an oligo-labeling kit (Pharmacia Biotech, Piscataway, NJ). Probes, encoding two vacuolar invertases (*Ivr1*, accession no. U16123: 1-kb *Nco*I/*Eco*RI cDNA fragment; and *Ivr2*, accession no. U31451: 0.8-kb *Eco*RI/*Pst*I 3′-cDNA fragment, including a 0.45-kb 3′-coding and a 0.34-kb 3′-untranslated region), four cell wall invertases (*Incw1*, accession no. U17695: 0.55-kb *Eco*RI/*Sal*I cDNA fragment; *Incw2,* accession no. AF050631: 0.8-kb *Eco*RI/*Pst*I 3′-cDNA fragment; *Incw3*, accession no. AF043346: 0.75-kb *Eco*RI/*Hin*dIII fragment consisting of 3′ end of exon 3 to intron 3, exon 4, and to the 5′ part of exon 5; and *Incw4,* accession no. AF043347: 0.9-kb *Eco*RI/*Hin*dIII fragment consisting of 0.1-kb intron 2 and 0.8-kb exon 3), two Susy (*Sh1*, Pww110/1 from Werr et al. [1985]: 1.2-kb *Pvu*II:*Pvu*II fragment; and *Sus1,* PshD13 from Gupta et al. [1988]: 1.4-kb *Eco:Pst*I fragment), a small subunit of Rubisco (*RbcS*, pC1 from Broglie et al. [1984]: *Pst*I/*Pst*I 0.5-kb cDNA fragment), an ABA-responsive gene (*Rab17*, from Vilardell et al. [1990]: .8-kb-*Eco*RI/*Xho*I cDNA fragment), and a translation elongation factor (*EF-α*, 0.8-kb *Eco*RI/*Bam*HI cDNA fragment) were used in succession. The loading differences were corrected with respect to the average between the *EF-α* probe signal and 18S rRNA ethidium bromide staining intensity. These probes were specific under the hybridization conditions used: i.e. 50% (v/v) formamide, 42°C with either 2× SSC for *Ivr1*, *Incw2*, and *Incw3*; 3× SSC for *Ivr2*; 4× SSC for *Sh1*, *Sus1*, *Rab17*, *RbcS*, and *EF-α*; or 5× SSC for *Incw1*.

In Situ Glc and Invertase Activity Staining

Tissue slices (200 μL) were hand-cut from fresh roots and leaves previously embedded in 4% (w/v) agarose for 5 to 10 min. Slices were washed extensively to remove soluble sugars for in situ detection of acid invertase activity

or left unwashed for in situ Glc detection. Activity was visualized by an incubation in the reaction medium consisting of 200 mM citrate/tris [tris(hydroxymethyl)aminomethane], pH 6.0, 100 mM Suc, 20 μg mL^{-1} Glc oxidase (Sigma), 250 μg mL^{-1} horseradish peroxidase (Sigma), and 300 μg mL^{-1} DAB (Sigma). Suc was omitted for Glc detection. The controls for activity and for Glc detection were performed with flushed and denatured (3 min at 65°C) preparations, respectively, using a reaction medium without Suc. Under our conditions only acid invertase staining was detected because 200 mM Tris totally inhibits neutral invertase activity (Kim, 1998).

Immunoblots

A rabbit antiserum raised against a synthetic oligopeptide (KRVVGSAAVPVLEDEA) of IVR2 invertase deduced from *Ivr2* gene (GenBank accession no. U31451) was used the for vacuolar invertase detection. The oligopeptide is vacuolar invertase-specific (IVR1 or IVR2), but has no homology with corresponding parts of cell wall invertase. Proteins were separated on a 10% (w/v) SDS-polyacrylamide gel. For two-dimensional IEF-SDS polyacrylamide gel proteins were separated on IEF gel using pH 3.0- to 10-range ampholites (Pharmacia Biotech). Proteins on polyacrylamide gel were transferred to nitrocellulose membranes by using an electrotransfer system (Bio-Rad). The resulting membranes were blocked in 5% (w/v) non-fat dry milk in Tris-buffered saline (TBS)-T (100 mM Tris-HCl, pH 7.5, 300 mM NaCl, and 1% [w/v] Tween 20), incubated with the primary antibody and horseradish peroxidase-conjugated secondary antibody, and detected with enhanced chemiluminescence reagents as recommended by the manufacturer (Amersham, Buckinghamshire, UK).

Immunolocalization

Immunolocalization of vacuolar and cell wall-bound invertases was carried out either on tissue slices of fresh material prepared as described above and/or on sections of chemically fixed and resin-embedded material. Leaf and root tissues were harvested on the morning of the 7th d after water interruption and were fixed by immersion in a solution of 0.1 M phosphate buffer (pH 7.2), 4% (w/v) paraformaldehyde, and 0.5% (w/v) glutaradehyde. Each sample was cut into small pieces with a razor blade and fixed overnight at 4°C. The samples were washed four times for 20 min with 50 mM phosphate buffer, pH 7.2, dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, and 100%), embedded in LR white resin (Electron Microscopy Science, Fort Washington, PA), and polymerized at 55°C for 48 h. Semi-thin sections (5 μm) were cut with a rotary microtome and affixed to Superfrost plus glass slides. Antiserums for carrot vacuolar and Arabidopsis wall-bound invertases (gifts from Dr. A. Sturm and Dr. A. Kingston-Smith) were used for immunodetection as follows: pre-incubation was carried out for 2 h with TBS-T containing 0.1% (w/v) gelatin, 2% (w/v) BSA, and 5% (w/v) non-fat powdered milk. Incubation with each primary antiserum (cell wall-bound or vacuolar invertase) 1/1,000 and 1/500 diluted in TBS-T containing 0.1% (w/v) BSA and 0.1% (w/v) gelatin was carried out overnight at 4°C; sections were then washed in TBS-T and incubated with 1 nm of gold-conjugated antirabbit immunoglobulin G diluted 1/100 for 2 h at room temperature. Control preparations were treated in the same manner, except that rabbit nonimmune immunoglobulin G was used as the primary serum. The sections were washed in TBS-T, in distilled water and amplified for 20 min using a silver enhancement kit according to the manufacturer's (British Biocell International, Cardiff, UK) instructions.

In Situ Hybridization

Root and leaf tissues were cut into small pieces and immediately placed in a solution of formaldehyde-acetic acid (50% [v/v] ethanol, 5% [v/v] acetic acid, and 10% [v/v] formaldehyde). The fixative solution was changed twice and samples were stored at 4°C for several days. Samples were dehydrated in a series of increasing concentration of ethanol-tert-butanol washes and embedded in Paraplast Plus (Monoject Scientific, Fort Washington, PA) according to the manufacturer's instructions. Samples were cut in 8-μm sections and affixed to Superfrost plus glass slides. Paraffin was removed by soaking in Histoclear (National Diagnostics, Albania, GA) for 10 min followed by 5-min washings in Histoclear:ethanol (1:1) and 100% (v/v) ethanol. For in situ hybridization sections were prehybridized for 2 h at 42°C with 4× SSC, 50% (w/v) formamide, 5× Denhart, and 5% (w/v) dextran sulfate containing 1 mg/mL salmon sperm DNA. Hybridization with single-stranded digoxygenated antisense or sense DNA probes (produced by using single primer-specific PCR amplification with digoxigenin-UTP) was carried out overnight at 42°C in the prehybridization buffer. Hybridized RNA was revealed indirectly by incubation with an antidigoxigenin-alkaline phosphatase conjugate (Boehringer Mannheim, Basel) and nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate color substrates as described in the manufacturer's instructions.

ACKNOWLEDGMENTS

We thank O. Roche and Dr. R. Rémy for helping with in situ hybridization and two-dimensional SDS-IEP electrophoresis experiments, respectively. We are grateful to Drs. K.E. Koch, P.S. Chourey, and M. Pagès for their kind gifts of *Ivr1/Ivr2*, *Incw1/Incw2*, and *Rab17* cDNA, and to Drs. A. Sturm and A. Kingston-Smith for the carrot vacuolar invertase and Arabidopsis cell wall invertase antiserums, respectively. The authors wish to thank R. Boyer for photography work.

Received February 9, 2000; accepted May 2, 2000.

LITERATURE CITED

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. Plant Cell **7:** 1099–1111

Broglie R, Corruzi G, Keith B, Chua N (1984) Molecular biology of C_4 photosynthesis in *Zea mays*: differential localization of proteins and mRNAs in two cell types. Plant Mol Biol **3:** 431–444

Büssis D, Heineke D, Sonnewald U, Willmitzer L, Raschke K, Heldt HW (1997) Solute accumulation and decreased photosynthesis in leaves of potato plants expressing yeast-derived invertase either in the apoplast, vacuole or cytosol. Planta **202:** 126–136

Chen MH, Liu LF, Chen YR, Wu HK, Yu SM (1994) Expression of a-amylases, carbohydrate metabolism, and autophagy in cultured rice cells is coordinately regulated by sugar nutrient. Plant J **6:** 625–636

Cheng WH, Taliercio EW, Chourey PS (1996) The *Miniature 1* seed locus of maize encodes a cell-wall invertase required for normal development of endosperm and maternal cells in the pedicel. Plant Cell **8:** 971–973

Duke ER, McCarty DR, Koch KE (1991) Organ-specific invertase deficiency in the primary root of an inbred maize line. Plant Physiol **97:** 523–527

Ehness R, Ecker M, Godt DE, Roitsch T (1997) Glucose and stress independently regulate source and sink metabolism and defense mechanisms *via* signal transduction pathway involving protein phosphorylation. Plant Cell **9:** 1825–1841

Foyer CH (1987) The basis for source-sink interaction in leaves. Plant Physiol Biochem **25:** 649–657

Gupta M, Chourey PS, Burr B, Still PE (1988) cDNAs of two on-allelic sucrose synthase genes in maize: cloning, expression, characterization and molecular mapping of the sucrose synthase-2 gene. Plant Mol Biol **10:** 215–224

Heineke D, Wildenberger K, Sonnewald U, Willmitzer L, Heldt HW (1994) Accumulation of hexose in leaf vacuole: studies with transgenic tobacco plants expressing yeast-derived invertase in the cytosol, vacuole or apoplasm. Planta **194:** 29–33

Herbers K, Meuwly P, Frommer WB, Métraux JP, Sonnewald U (1996) Systemic acquired resistance mediated by the ectopic expression of invertase: possible hexose sensing in the secretory pathway. Plant Cell **8:** 793–803

Huber SC (1983) Relation between photosynthetic starch formation and dry weight partitioning between the shoot and root. Can J Bot **61:** 2709–2716

Huber SC (1989) Biochemical mechanism for regulation of sucrose accumulation in leaves during photosynthesis. Plant Physiol **91:** 656–662

Jang JC, Leon P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell **9:** 5–19

Kim JY (1998) Les invertases du maïs: isolement, caraterisation de 4 gènes d'invertases pariétales et analyze de l'expression des invertases vacuolaires et pariétales en réponse au stress hydrique. PhD thesis. Université Paris-Sud, Orsay, France

Kim JY, Mahé A, Guy S, Brangeon J, Roche O, Chourey PS, Prioul JL (2000) Characterization of two members of the maize gene family, Incw3 and Incw4, encoding cell wall invertase. Gene **245:** 89–102

Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 509–540

Koch KE, Xu J, Duke ER, McCarty DR, Yuan CX, Tan BC, Avigne WT (1995) Sucrose provides a long distance signal for coarse control of genes affecting its metabolism. *In* HG Pontis, GL Salerno, EJ Echeverria, eds, Sucrose Metabolism, Biochemistry, Physiology and Molecular Biology. American Society of Plant Physiologists, Rockville, MD, pp 266–277

Krausgrill S, Sander A, Greiner S, Weil M, Rausch T (1996) Regulation of cell wall invertase by a proteinaceous inhibitor. J Exp Bot **47:** 1193–1198

Mahé A, Grisvard J, Dron M (1992) Fungal- and plant-specific gene markers to follow the bean Anthracnose infection process and normalize a bean chitinase mRNA induction. Mol Plant-Microbe Interact **5:** 242–248

Pelleschi P, Rocher JP, Prioul JL (1997) Effect of water restriction on carbohydrate metabolism and photosynthesis in mature maize leaves. Plant Cell Environ **20:** 493–503

Pelleschi S (1997) Recherche de locus a effects quantitatifs liés au métabolisme glucidique au cours d'une contrainte hydrique chez le maïs (*Zea mays* L). PhD thesis. Université de Paris-Sud (XI), Orsay, France

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Shanker S, Salazar RW, Taliercio EW, Chourey PS (1995) Cloning and characterization of full-length cDNA encoding cell-wall invertase from maize. Plant Physiol **108:** 873–874

Sheen J (1990) Metabolic repression of transcription in higher plants. Plant Cell **2:** 1027–1038

Smeekens S (1999) Genetic analysis of sugar sensing mechanisms in *Arabidopsis*. Genetics Society Meeting on *Arabidopsis*. Oxford, UK

Sonnewald U, Hajirezaei MR, Kossman J, Heyer A, Tretheway RN, Willmitzer L (1997) Increased potato tuber size resulting from apoplastic expression of a yeast invertase. Nat Biotech **15:** 794–797

Sonnewald U, Stitt M, Schaewen AV, Brauer M, Willmitzer L (1991) Transgenic tobacco plants expressing yeast-derived invertase in either the cytosol, vacuole or apoplast: a powerful tool for studying sucrose metabolism and sink/source interaction. Plant J **1:** 95–106

Sturm A, Sebkova V, Lorenz K, Hardegger M, Lienhard S, Unger C (1995) Development- and organ-specific expression of the genes for sucrose synthase and three isoenzymes of acid β-fructofuranosidase in carrot. Planta **195:** 601–610

Taliercio EW, Kim JY, Mahé A, Shanker S, Choi J, Cheng WH, Prioul JL, Chourey PS (1999) Isolation, characterization and expression analyses of two cell wall invertase genes in maize. J Plant Physiol **155:** 197–204

Unger C, Hofsteenge J, Strum A (1992) Purification and characterization of a soluble β-fructo-furanosidase from *Daucus carota*. Eur J Biochem **204:** 915–921

Vilardell J, Goday A, Freire MA, Margarita T, Martinez MC, Torne JM, Pagès M (1990) Gene sequence, developmental expression, and protein phosphorylation of RAB-17 in maize. Plant Mol Biol **14:** 423–432

Weber H, Borijuk L, Wobus U (1996) Controlling seed development and seed size in *Vicia faba*: a role for seed coat-associated invertases and carbohydrate state. Planta **10:** 823–834

Werr W, Frommer WB, Maas C, Starlinger P (1985) Stucture of the sucrose synthase gene on chromosome 9 of *Zea mays* L. EMBO J **4:** 1373–1380

Wu LL, Song I, Kim D, Kaufman PB (1993) Molecular basis of the increase in invertase activity elicited by gravistimulation of oat-shoot pulvini. J Plant Physiol **142:** 179–183

Xu J, Avigne WT, McCarty DR, Koch KE (1996) A similar dichotomy of sugar modulation and developmental expression affects both paths of sucrose metabolism: evidence from a maize invertase gene family. Plant Cell **8:** 1209–1220

Xu J, Pemberton GH, Almira EC, Mc Carty DR, Koch KE (1995) The *Ivr 1* gene for invertase in maize. Plant Physiol **108:** 1293–1294

Zinselmeier C, Schussler JR, Jone RJ, Westgate ME (1995) Low water potential disrupts carbohydrate metabolism (*Zea may* L.) ovaries. Plant Physiol **107:** 385–391