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A Similar Dichotomy of Sugar Modulation and Developmental Expression Affects Both Paths of Sucrose Metabolism: Evidence from a Maize Invertase Gene Family

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Invertase and sucrose synthase catalyze the two known paths for the first step in carbon use by sucrose-importing plant cells. The hypothesis that sugar-modulated expression of these genes could provide a means of import adjustment was initially suggested based on data from sucrose synthases alone; however, this hypothesis remained largely conjectural without critical evidence for invertases. Toward this end, a family of maize invertases was cloned and characterized. Here, we show that invertases are indeed sugar modulated and, surprisingly, like the sucrose synthase genes, fall into two classes with contrasting sugar responses. In both families, one class of genes is upregulated by increasing carbohydrate supply (*Sucrose synthase1* [*Sus1*] and *Invertase2* [*Ivr2*]), whereas a second class in the same family is repressed by sugars and upregulated by depletion of this resource (*Shrunken1* [*Sh1*] and *Invertase1* [*Ivr1*]). The two classes also display differential expression during development, with sugar-enhanced genes (*Sus1* and *Ivr2*) expressed in many importing organs and sugar-repressed, starvation-tolerant genes (*Sh1* and *Ivr1*) upregulated primarily during reproductive development. Both the *Ivr1* and *Ivr2* invertase mRNAs are abundant in root tips, very young kernels, silk, anthers, and pollen, where a close relationship is evident between changes in message abundance and soluble invertase activity. During development, patterns of expression shift as assimilate partitioning changes from elongating silks to newly fertilized kernels. Together, the data support a model for integrating expression of genes differentially responsive to carbohydrate availability (i.e., feast and famine conditions) with developmental signals. The demonstration that similar regulatory patterns occur in both paths of sucrose metabolism indicates a potential to influence profoundly the adjustment of carbon resource allocation.

INTRODUCTION

Sugar-responsive expression of specific genes provides a means for optimizing metabolism in microorganisms relative to supplies of critical carbohydrate resources (e.g., the *lac* operon and glucose-responsive genes). A similar but expanded function is evident in multicellular organisms. Distinctive features of plants place special demands on their capacity for gene regulation in response to changes in carbohydrate availability. In contrast to animals, plants display far less sugar homeostasis. Large variations in endogenous levels present a greater challenge to long-term, coarse control mechanisms as well as a broader range of signals to carbohydrate-regulated genes. In addition, the sessile nature of vascular plants makes environmental adjustment crucial. In this regard, the plants' flexibility of form can be invaluable, and this versatility in turn can entail sugar-responsive changes in the expression of genes altering development and carbohydrate partitioning.

Sucrose-metabolizing enzymes are particularly important in this regard, because allocation of carbon resources is mediated primarily through long-distance transport of sucrose in the phloem. Shifts in sucrose distribution and import by different organs correlate strongly with capacities for carbon flux through sucrose cleavage reactions (Sung et al., 1989, 1994), catalyzed in some instances by sucrose synthase (operating in the degradative direction) (Sung et al., 1989, 1994; Zrenner et al., 1995) and in others by invertases (Morris and Arthur, 1984a, 1984b; Sung et al., 1994). Although both soluble and insoluble invertases are important in developing grains (Shannon and Dougherty, 1972; Miller and Chourey, 1992; Cheikh and Jones, 1995; Chourey et al., 1995; Zinselmeier et al., 1995), it is the soluble activity that appears critical to the earliest phases of growth (Hanft and Jones, 1986a, 1986b; Cheikh and Jones, 1995; Zinselmeier et al., 1995). A specific role has been proposed for soluble invertase during the development of bean seeds (Weber et al., 1995). In addition, soluble invertase activity also most closely parallels import into many enlarging structures, such as stems (Hatch and Glasziou, 1963; Kaufman et al., 1973; Morris and Arthur, 1984a; Arai et al., 1992), bean pods (Sung et al., 1994), flowers (Hawker et al., 1976), and leaves at specific phases of development (Morris and Arthur, 1984b; Schmalstig and Hitz, 1987).

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Other proposed roles of soluble invertases in plant growth and development include influence on fruit sugar composition (Hubbard et al., 1989; Yelle et al., 1991; Klan et al., 1992; Darnell et al., 1994), osmotic adjustment (Sharp et al., 1990), and tissue expansion (Hatch and Glasziou, 1963), and association with sugar import in a variety of tissues (Morris and Arthur, 1984a, 1984b; Schmalstig and Hitz, 1987; Sung et al., 1994; Roitsch et al., 1995; Sturm et al., 1995; Weber et al., 1995). In addition, soluble invertases can contribute to changes in morphology (Kaufman et al., 1973; Arai et al., 1992; Chausse et al., 1995), early seed development (Hanft and Jones, 1986a, 1986b; Reed and Singletary, 1989; Cheikh and Jones, 1995; Weber et al., 1995; Zinselmeier et al., 1995), silk elongation (Bassetti and Westgate, 1993), flowering (Hawker et al., 1976; Bernier et al., 1993; Miller and Ranwalla, 1995), and hexose effects on the sugar-sensing system (as in yeast [Gancedo, 1992] and possibly plants [Sheen, 1990, 1994; Graham et al., 1994; Jang and Sheen, 1994; Stitt et al., 1995]).

Recent data have shown that sugar effects on plant gene expression are highly selective and span a full range from activation to repression (Jang and Sheen, 1994; Sheen, 1994; Thomas and Rodriguez, 1994; Koch et al., 1995; Thomas et al., 1995). Overall, however, carbohydrate abundance tends to favor expression of genes for storage, biosynthesis, and other processes of utilization, whereas sugar depletion favors genes for photosynthesis and/or remobilization (Thomas and Rodriguez, 1994; Koch, 1996).

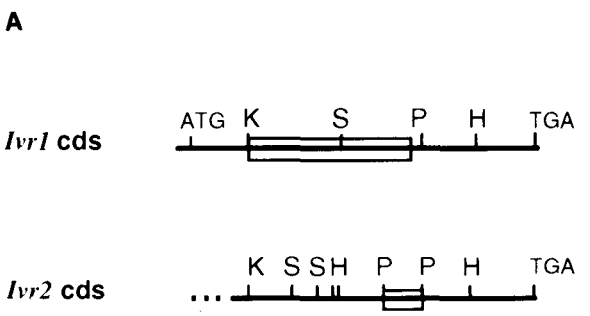
An intriguing exception to this generalization was revealed by the finding that sugars could both induce and repress sucrose synthase genes (Koch et al., 1992b). The importance of these enzymes to the essential first step in sucrose metabolism placed the sugar responsiveness of their respective genes into a particularly interesting context. Carbohydrate effects on the expression of these genes could theoretically facilitate changes in sucrose utilization and import relative to available supplies (Koch et al., 1992b). Such a role for this aspect of gene expression is also relevant to the proposal by Farrar and Williams (1991) that sugars could exert a coarse control over sink metabolism. In addition, contrasts in gene expression associated with sugar supply (enhanced versus repressed) and overall localization (broad versus narrow) have led to the suggestion that the responses could optimize balance in their expression relative to resource availability (Koch et al., 1992b). However, despite the fascinating potential of such a possibility, results obtained thus far have related to only one of the two known enzymatic paths for sucrose metabolism.

The validity of this "feast-and-famine" model for adjustment of carbon resource allocation will depend in many instances on the sugar-response characteristics of invertase genes. Here, we test the hypothesis that the expression of invertases, which are widespread effectors of carbon import and utilization, is sugar regulated and subject to contrasting patterns of induction and repression. Further, we present evidence for differential expression of these genes during development and for temporal changes that correspond to shifts in carbon allocation during key phases of reproduction. Together, the data support the conclusion that a similar dichotomy of sugar modulation and developmental expression regulates both paths of sucrose metabolism and therefore can have a profound effect on the adjustment of the use of sucrose among plant parts.

RESULTS

Expression of Maize Invertases Is Differentially Sugar Responsive

Identification of a soluble invertase gene family in maize enabled analysis of the carbohydrate responsiveness and developmental control of these genes. Two subfamilies were distinguished through selective hybridization with *Invertase1* (*Ivr1*) or *Invertase2* (*Ivr2*) cDNAs for soluble invertase, as shown



B

Probe:	Copy number:	Map location:
Ivr1	2	2S, 10L
Ivr2	2-4	4L, 5S, +

Figure 1. *Ivr1* and *Ivr2* Maize Invertases.

(A) Restriction maps of the coding sequence (cds) for *Ivr1*, cDNAs for *Ivr1*-1 and *Ivr2*-1, and probes (boxed regions) used for each. Restriction sites are indicated for KpnI (K), SmaI (S), HincII (H), and PstI (P). The *Ivr1* and *Ivr2* GenBank accession numbers are U16123 and U31451, respectively, with further description in Xu et al. (1995) and Koch et al. (1995). The *Ivr1* and *Ivr2* specific probes (boxed regions) were tested for cross-hybridization with *Ivr1* and *Ivr2* cDNA clones at high stringency. The dashed line indicates unavailable sequence.
(B) Organization of the *Ivr1* and *Ivr2* invertase gene subfamilies. Gene number and map locations were determined by restriction fragment length polymorphism analysis of recombinant inbred populations courtesy of B. Burr and E. Matz (Brookhaven National Laboratory), S. Wright (Linkage Genetics), and G. Davis, T. Musket, and S. Chao (Maize Genome Center).

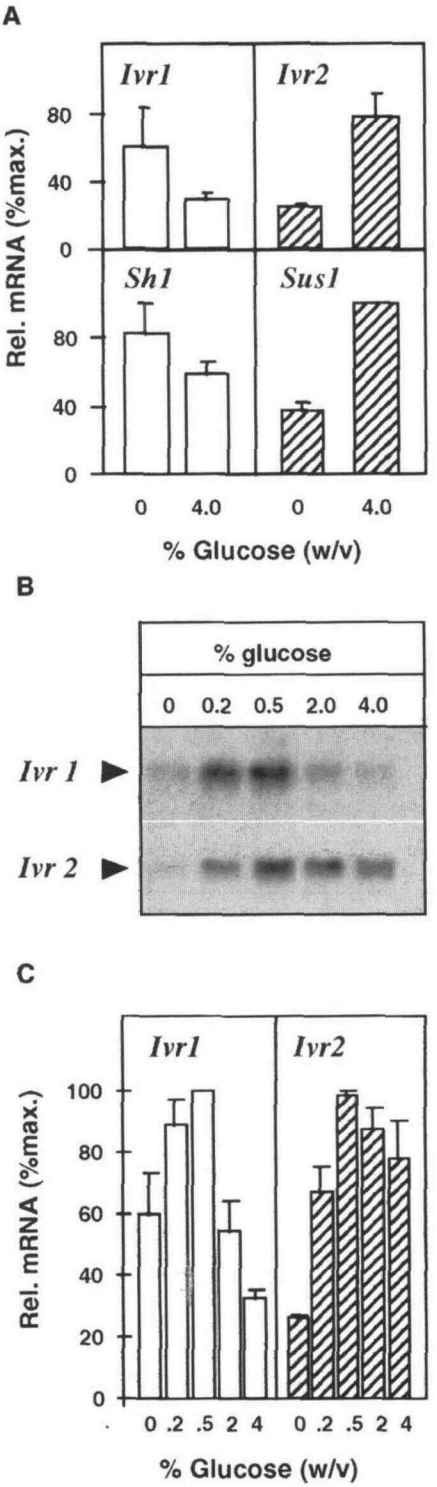


Figure 2. Sugar-Modulated Expression of the *Ivr1* and *Ivr2* Invertases: Evidence for Similar Response Classes among Genes for Sucrose Metabolism.

in Figure 1. At least four different genes and chromosomes are involved. The 2S site is near that of the *miniature1* kernel mutant described by Miller and Chourey (1992) but appears to be several map units distant (Coe, 1995). Organization of the soluble invertase gene family also indicates that the origin of the *Ivr1* and *Ivr2* subfamilies predates an evolutionarily recent duplication affecting much of the maize genome (Helentjaris, 1993).

Figure 2A shows a marked contrast between sugar-modulated expression of the *Ivr1* and *Ivr2* invertases, which are, respectively, repressed and enhanced by carbohydrate availability. Responses to glucose, sucrose, and other metabolizable sugars were similar and independent of their osmotic influence on media (data not shown). Glucose was used in preference to sucrose because in root tips, cells lack sucrose carriers and the latter sugar is typically hydrolyzed before entry (Duke et al., 1991). Hexoses are also believed to be more directly involved in sugar signaling in yeast (Gancedo, 1992) and plants (Sheen, 1991, 1994; Graham, 1994; Jang and Sheen, 1994; Stitt et al., 1995).

A comparison of differential expression of invertase and sucrose synthase genes in the same set of material revealed some important similarities (Figure 2A). Contrasting regulation was apparent in both families and provides evidence for similar response classes among genes for sucrose metabolism. A sugar-repressed, depletion-tolerant class can be discerned in the expression of both the *Ivr1* and *Shrunken1* (*Sh1*) genes. In contrast, a sugar-enhanced, depletion-sensitive class is evident in responses of the *Ivr2* and *Sucrose synthase1* (*Sus1*) genes. Differences in expression are less apparent near the midrange of the typically broad variation in sugar levels available to plant tissues (Figures 2B and 2C). However, contrasts become increasingly evident toward the extremes of the physiological range of sugar levels.

(A) Sugar response classes (sugar repressed versus sugar enhanced) among genes for sucrose metabolism. Abundance of mRNA was quantified by PhosphorImager analysis of gel blots from replicate experiments. RNA was extracted from maize root tips incubated for 24 hr in White's basal medium with or without supplemental glucose (4.0% [w/v], 222 mM). Gel blots of total RNA (10 μg per lane) were probed with ^{32}P-labeled *Ivr1* or *Ivr2* subfamily-specific invertase probes or with *Sh1* or *Sus1* sucrose synthases. Error bars denote SEM. Rel., relative; max., maximum.

(B) and **(C)** Glucose gradient responses. Comparative abundance of mRNA was visualized on x-ray film **(B)** or quantified by PhosphorImager analysis of gel blots from replicate experiments **(C)**. RNA was isolated from maize root tips incubated for 24 hr in White's basal medium supplemented with glucose (0, 0.2, 0.5, 2.0, or 4.0% [w/v], corresponding to 0, 11, 28, 111, or 222 mM, respectively). Data from sucrose (and other metabolizable sugars) were similar, but glucose is shown due to typical hydrolysis of exogenous sucrose before entry into root cells (see text). Error bars denote SEM.

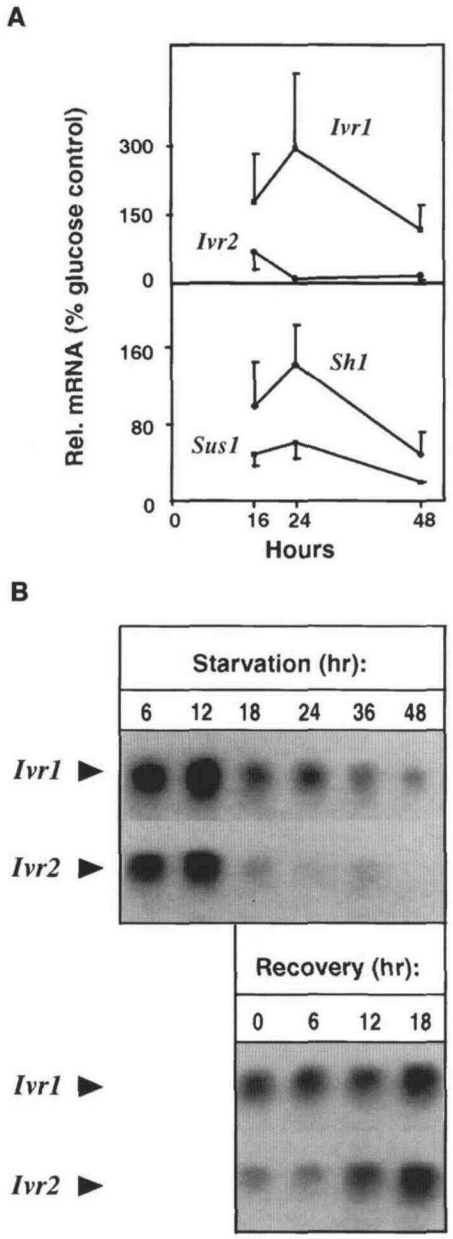


Figure 3. Starvation Time Course for Expression of *Ivr1* and *Ivr2* Invertases: Tolerance to Sugar Depletion Differs with Response Classes among Genes for Sucrose Metabolism.

(A) Time course of starvation response. Abundance of mRNA was quantified by PhosphorImager analysis of gel blots from replicate experiments. RNA (10 μg per lane) was extracted from maize root tips depleted of carbohydrates for 16, 24, or 48 hr in sugar-free White's basal medium and analyzed as in Figure 2. Error bars denote SEM. Rel., relative.

(B) Expanded time course of starvation and recovery. Gel blot analysis of RNA was conducted on extracts from maize root tips depleted of carbohydrates as given for **(A)** except that at 18 hr, root tips were transferred to fresh, glucose-free media (top gel) or media supplemented with 2.0% (w/v) glucose (111 mM) to initiate recovery (bottom gel).

Maintenance of Expression during Starvation Stress Differs among Maize Invertases

A contrast in starvation tolerance was evident when the persistence of *Ivr1* and *Sh1* mRNAs was compared with that of *Ivr2* and *Sus1* (Figure 3A). Transcripts of both *Ivr1* and *Sh1* could be detected for at least 48 hr in sugar-depleted root tips, and those of *Ivr1* were maintained at levels equal to or surpassing those of glucose-fed controls. In contrast, abundance of *Ivr2* and *Sus1* mRNAs decreased during the same time period to the limits of detection or below. In excised root tips, soluble sugars drop within 10 hr to levels suboptimal for expression of *Ivr1* as well as *Ivr2* if no supplemental sugars are added (Brouquisse et al., 1991). Other factors in addition to sugars can affect regulation of *Ivr1* and *Ivr2* in intact and newly cultured root tips (0 to 12 hr); however, the starvation and recovery experiment shown in Figure 3B indicates longer term differences in their expression due to carbohydrate availability. The depletion of *Ivr2* mRNAs under starvation is reversed by glucose, whereas *Ivr1* transcript abundance remains relatively constant.

Maize Invertases Show Differential Organ and Developmental Expression

Figure 4 shows that the *Ivr1* and *Ivr2* gene classes have distinctly different profiles of organ and developmental expression. *Ivr1* message levels were markedly higher in reproductive structures than in vegetative tissues, whereas *Ivr2* transcripts were prevalent in each of the sucrose-importing structures examined. Both *Ivr1* and *Ivr2* mRNAs were lacking in mature photosynthetic leaves and present in structures in which elongation and expansion could be linked to import. The latter structures included root tips, very young kernels, silk, and anthers. Pollen, too, had abundant levels of these transcripts and a capacity for rapid germ tube elongation. In expanding kernels at 8 days after pollination (DAP), both *Ivr1* and *Ivr2* invertases were expressed. *Ivr2* mRNAs were abundant in the lower kernel (pedicel region) and barely detectable in the upper portion (pericarp and nucellus), whereas the reverse was observed for *Ivr1*. In both instances, individual cross-reactive genes could be more locally expressed; however, the collective limits of each class are shown here to differ.

Temporal Changes in Invertase Expression and Activity Occur during Crucial Stages of Reproductive Development

Changes during kernel development shown in Figure 5 indicate that although *Ivr1* and *Ivr2* mRNA levels increased in young kernels until 8 to 10 DAP, they dropped rapidly thereafter. Total soluble acid invertase activity followed a compatible pattern given a somewhat greater protein longevity, accumulation over time, and limited persistence (Figure 5C). In contrast, insoluble activity, assayed for comparison, peaked consider-

ably later (~16 DAP) and persisted until well past maturity under local growing conditions (32 DAP) (Figure 5D).

Figure 6 shows that at the earliest stages of kernel development, rapid, pronounced increases occurred in levels of *Ivr1* and *Ivr2* mRNAs and in activity of total soluble invertase. Within the first 2 DAP and 24 hr of fertilization, soluble invertase activity doubled, rising twofold over that in unpollinated ovules (Figure 6B) and twice as high as insoluble activity from the same kernels (Figure 6C).

During the male reproductive development shown in Figure 7, *Ivr1* and *Ivr2* mRNA levels increased in anthers and were also abundant in pollen alone. Relative levels of these messages were greater in shedding anthers than in pollen per se, indicating a probable presence in both the male gametophyte and its nutritive tissue. The data on enzyme activity are consistent with this interpretation and also show that the invertase message is expressed in pollen rather than being stored with other inactive pollen mRNAs (Mascarenhas, 1994).

In silks, high levels of *Ivr1* and *Ivr2* mRNAs were observed before pollination, and a descending tip-to-base gradient was evident distal to the ovary (Figures 8 and 9). Pollination induced a rapid drop in the levels of both soluble invertase mRNAs and initiated collapse of the gradient. At the enzyme level, temporal and spatial changes in total soluble activity were consistent with those of the *Ivr1* and *Ivr2* message levels, whereas little change was observed in the insoluble invertase activity. Overall activity levels were compatible with soluble acid invertase identity for the *Ivr1* and *Ivr2* clones.

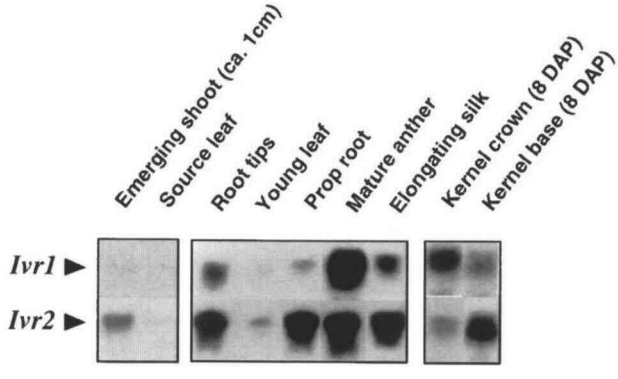


Figure 4. Profiles of Differential Organ and Developmental Expression for *Ivr1* and *Ivr2* Invertases in Maize.

Gel blot analysis of RNA extracted from different plant tissues. Samples included emerging shoots (excised from 5-day-old seedlings when shoots were ~1.0 cm long and not yet green), source leaf (blade of a fully expanded, photosynthetic leaf from a nearly mature plant), root tips (terminal 1.0 cm of roots from 5-day-old seedlings), young leaf (yellow-green blade from the central whorl of a nearly mature plant), prop roots (elongating aerial roots from a nearly mature plant), mature anthers (at anthesis), elongating silks, kernel crowns (upper portion of kernels at 8 DAP, primarily nucellus and pericarp), and kernel bases (basal portion of kernel at 8 DAP, primarily pedicel). Materials compared within the same blot are grouped by panel. Total RNA (10 μg per lane) was analyzed as given in Figure 2.

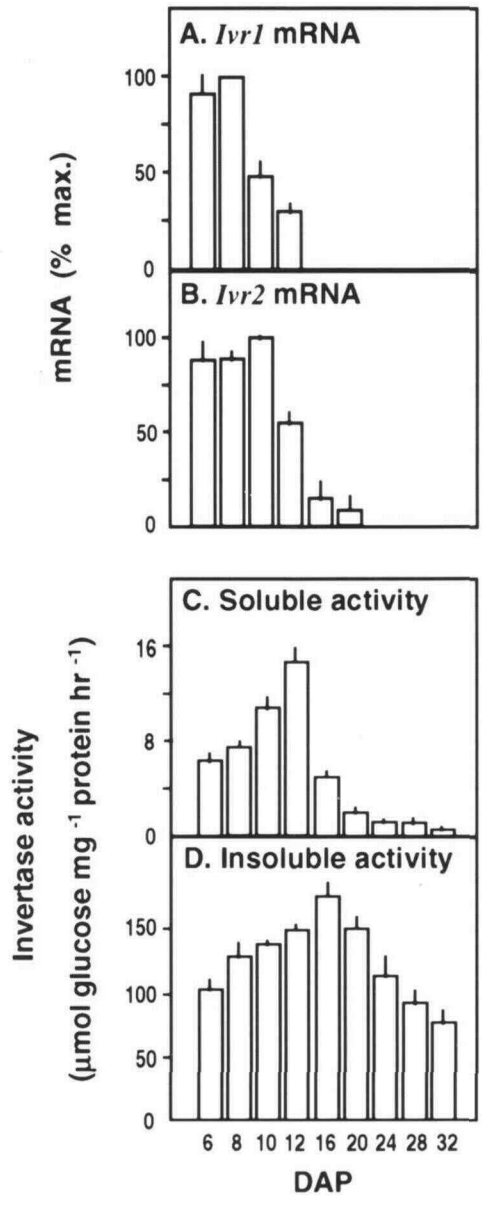


Figure 5. Kernel Growth: Developmental Analysis of *Ivr1* and *Ivr2* mRNAs and Total Invertase Activity.

(A) and **(B)** Gel blot analysis of mRNA abundance for *Ivr1* **(A)** and *Ivr2* **(B)**. Total RNA (10 μg per lane) was analyzed, and mRNA was quantified as given in Figure 2. Developing kernels were sampled during the period from 6 to 32 DAP. Full maturity was reached at ~30 DAP under local conditions. The ground kernel samples were subdivided for RNA and enzyme analyses. max., maximum.

(C) and **(D)** Analysis of invertase enzyme activity in soluble **(C)** and insoluble **(D)** fractions from extracts of the above-mentioned tissues.

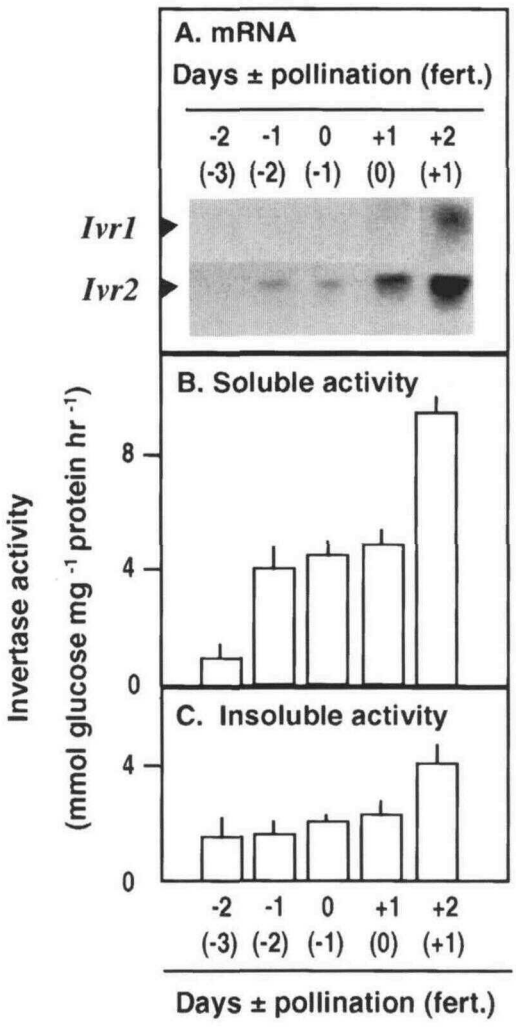


Figure 6. Ovule Ferilization: Developmental Analysis of *Ivr1* and *Ivr2* mRNAs and Total Invertase Activity.

(A) Gel blot analysis of *Ivr1* and *Ivr2* invertase mRNA abundance in ovules before and after pollination/fertilization (fert.). Pollination precedes fertilization (values within parentheses) by ~24 hr in maize. The phase of development was determined by using pretagged individuals and groups of synchronously developing plants (see Methods). Nonpollinated ovules remained similar for several days (data not shown). Total RNA (10 μg per lane) was analyzed as given in Figure 2.
(B) and **(C)** Analysis of invertase enzyme activity in soluble **(B)** and insoluble **(C)** fractions from extracts of the above-mentioned tissues.

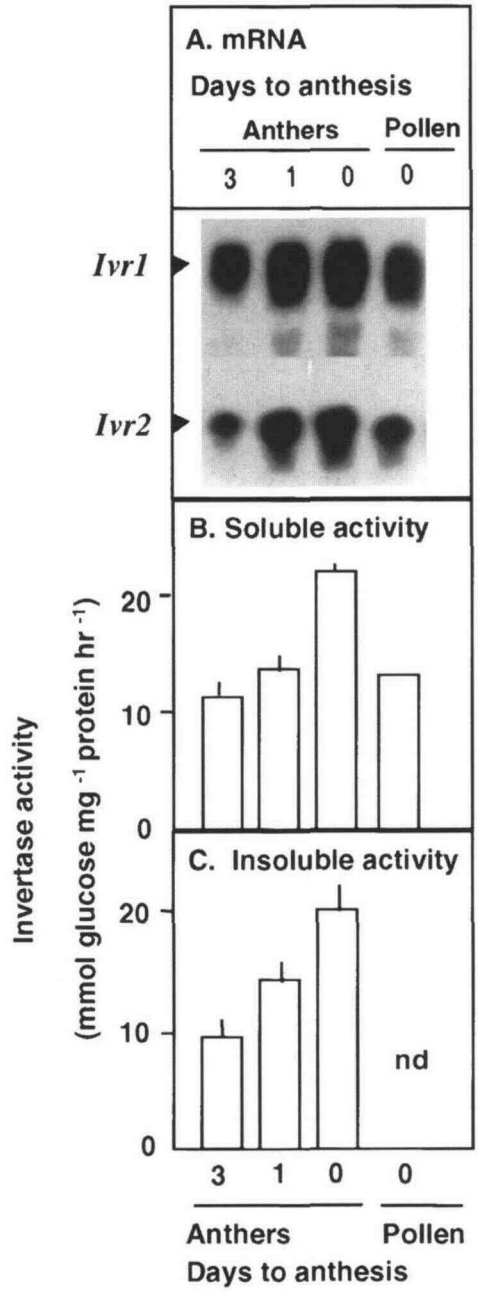


Figure 7. Male Reproductive Development: Analysis of *Ivr1* and *Ivr2* mRNAs and Total Invertase Activity.

(A) Gel blot analysis of *Ivr1* and *Ivr2* invertase mRNAs in developing anthers and mature pollen. The phase of development was determined by using pretagged individuals and groups of synchronously developing plants (see Methods). Total RNA (10 μg per lane) was analyzed as given in Figure 2. Ground samples were subdivided for RNA and enzyme analyses.
(B) and **(C)** Analysis of invertase enzyme activity in soluble **(B)** and insoluble **(C)** fractions from extracts of the above-mentioned tissues. Activity was not determined (nd) for insoluble fractions from pollen.

DISCUSSION

Two levels of significance are proposed for the differential carbohydrate and developmental regulation revealed here for a maize invertase gene family. First, we cloned maize invertase

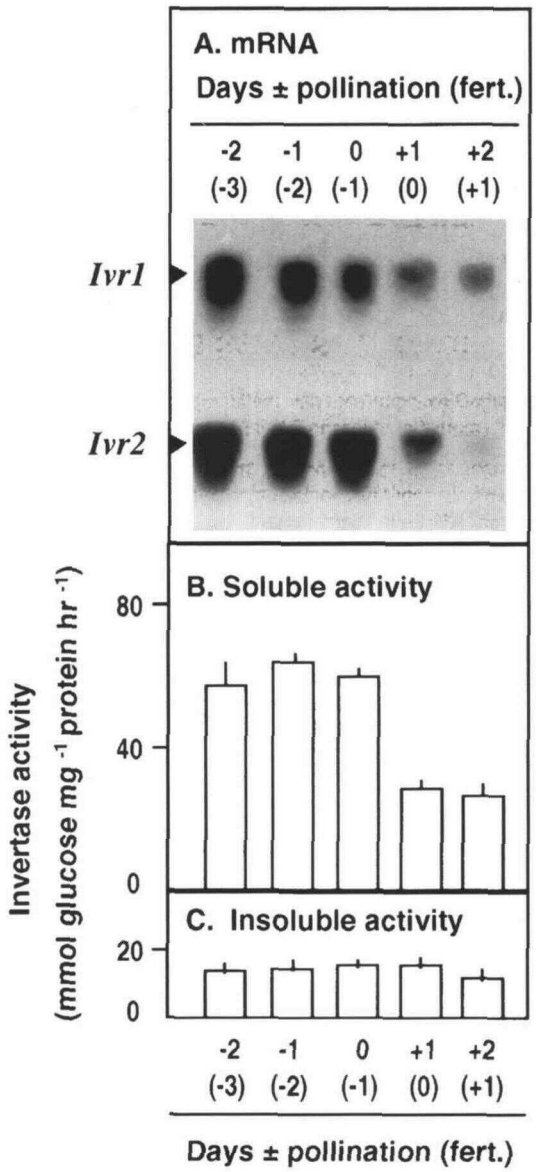


Figure 8. Elongating Silk and Pollination: Responses of *Ivr1* and *Ivr2* mRNAs and Total Invertase Activity.

(A) Gel blot analysis of *Ivr1* and *Ivr2* invertase mRNA abundance in elongating silk before and after pollination/fertilization (fert.). Pollination precedes fertilization (values within parentheses) by ~24 hr in maize, the approximate time required for pollen tube growth through the length of silk (12 to 25 cm). The phase of development was determined by using pretagged individuals and groups of synchronously developing plants (see Methods). Nonpollinated silks remained similar for several days (data not shown). Total RNA (10 μg per lane) was analyzed as in Figure 2. Samples were subdivided for RNA and enzyme analyses. **(B)** and **(C)** Analysis of invertase enzyme activity in soluble **(B)** and insoluble **(C)** fractions from extracts of the above-mentioned tissues.

genes specifically to test the hypothesis that import adjustment could be affected by sugar-modulated expression of these genes for sucrose metabolism. Our data show that invertase expression is indeed sugar modulated and moreover includes contrasting patterns of sugar induction and repression. The correspondence between these responses and those of sucrose synthase genes (Koch et al., 1992b) indicates a dichotomy of sugar-modulated expression that affects both paths of the essential first step in sucrose use by importing cells. Second, a potential significance lies in the relationship between these

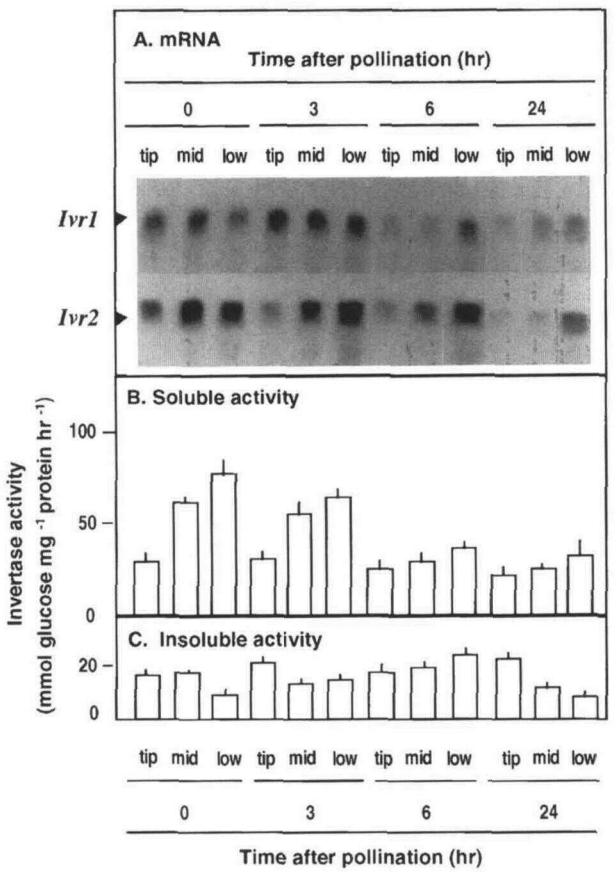


Figure 9. Spatial Gradient in Silk: Time Course of Change in *Ivr1* and *Ivr2* mRNAs and Total Invertase Activity after Pollination.

(A) RNA gel blot analysis of changes in *Ivr1* and *Ivr2* invertase mRNA abundance along a spatial gradient from tip to base of silk after pollination. Fertilization follows pollination by ~24 hr in maize, the approximate time required for pollen tube growth through the length of silk (here ~12 cm). Each silk fraction represents strands dissected into their uppermost third (tip), middle third (mid), or basal third (low). Nonpollinated silks remained similar for several days (data not shown). Total RNA (10 μg per lane) was analyzed as given in Figure 2. **(B)** and **(C)** Analysis of invertase enzyme activity in soluble **(B)** and insoluble **(C)** fractions from extracts of the above-mentioned tissues.

contrasting classes of sugar-responsive genes (sugar enhanced versus starvation tolerant) and differences in developmental expression. In both the sucrose synthase and invertase gene families, the starvation-tolerant genes have more narrowly distributed patterns of developmental regulation, suggesting specialized functions for their response classes. Results also imply that the integration of sugar and developmental signals could have additional functional significance, theoretically affecting the roles of invertases in stress adjustment, morphological change, development, and even the sugar-sensing system itself.

Both Known Paths of Sucrose Metabolism Are Subject to a Similar Dichotomy in Sugar-Modulated Expression

Three features of the differential sugar-regulated gene expression reported here may have particular biological relevance. First, the similarity between patterns of carbohydrate modulation for invertase and sucrose synthase genes (Figure 2A; Koch et al., 1992b) indicates that this shared regulatory feature may have special importance. Because both alternative modes of sucrose cleavage in plants can be thus affected, carbohydrate modulation is likely to exert an important and widespread influence on sucrose import and partitioning. Marked changes in expression of sugar-enhanced versus sugar-repressed, starvation-tolerant genes occur above and below the physiological midrange of sugar concentrations (Figures 2A and 2B). At low sugar levels, a critical capacity for sucrose cleavage could be preserved by expression of the *Ivr1* and *Sh1* genes. Effects of carbohydrate abundance on the *Ivr2* and *Sus1* genes, however, could maintain expression over a broader range of high sugar levels. Carbohydrate modulation of these mRNA levels may more closely parallel overall metabolic activity, including that of mitochondrial respiration (Brouquisse et al., 1991; Farrar and Williams, 1991; Lambers and Atkin, 1995).

The second functionally significant attribute of the sugar response classes was the degree of starvation tolerance exhibited by *Ivr1* and *Sh1* over time (Figure 3A), especially when compared with the markedly greater sensitivity of *Ivr2* and *Sus1* expression to sugar depletion. Responses of the latter (sugar-enhanced genes) during an episode of starvation and recovery are consistent with their proposed role in coarse adjustment of sucrose use relative to supply (Farrar and Williams, 1991; Koch et al., 1992b, 1995; Pollock and Farrar, 1996). In contrast, an alternate function is suggested by the continued presence of the *Ivr1* and *Sh1* mRNAs after as much as 48 hr of severe carbohydrate deprivation. A possible value of this response may lie in its potential to maintain or aid import and metabolism for areas of critical function in the plant (Koch et al., 1992b; Koch and Nolte, 1995) or during important phases of development.

Third, the differential sugar responsiveness of invertase genes puts them in a special position as both effectors and targets of plant sugar signals. In addition to being sugar responsive, enzymes encoded by these genes could have a direct role in the production of hexose input into a sugar-sensing system (theoretically altered by at least half in the sucrose synthase reaction). Despite the well-known effects of regulatory metabolites on controlling metabolism (Stitt et al., 1995; Quick and Schaffer, 1996), these compounds (such as F, 2, 6-BP) do not appear to have similar effects at the gene expression level. Instead, data from yeast indicate that hexose interaction with hexokinase per se provides a primary signal for sugar-regulated genes (Gancedo, 1992). Although details of the mechanism remain an area of active research, the dual role for this enzyme as a catalytic protein in metabolism and a regulatory protein in sugar sensing is widely supported by studies of microbial systems (Gancedo, 1992). Recent evidence indicates that a similar mechanism may operate in plants (Graham et al., 1994; Jang and Sheen, 1994; Stitt et al., 1995), leaving invertase in a key position to produce hexose substrates for the sugar-sensing system.

Some Common Elements Are Evident in the Differential Profiles of Organ and Developmental Expression among Sucrose Synthase and Invertase Genes

In addition to reciprocal responses to carbohydrate availability, *Ivr1* and *Ivr2* invertases show differential tissue and developmental expression (Figure 4). The *Ivr1* mRNAs tend to be associated preferentially with reproductive structures, whereas *Ivr2* mRNAs are more broadly distributed in vegetative sink organs as well as in reproductive structures. The probes used would not have resolved potential differences within families; however, general patterns were consistent with the previously suggested role of soluble invertases in import and metabolism of sucrose during the growth of expanding structures (Hatch and Glasziou, 1963; Morris and Arthur, 1984b; Sung et al., 1994). These patterns included the extent to which invertase mRNA abundance decreased concomitant with sink–source transitions in photosynthetic tissue (shoots and leaves) and cessation of expansion (Schmalstig and Hitz, 1987). The broad distribution of *Ivr2* mRNAs among plant parts (Figure 4) and the sensitivity of these message levels to carbohydrate availability (Figures 2 and 3) indicate that under favorable growing conditions, sugar enhancement of *Ivr2* mRNA has the potential to affect a number of rapidly expanding tissues. Because carbohydrate-induced changes in cell enlargement can result from altered gene expression as well as from substrate availability, both could contribute to changes in organ size and long-term partitioning of carbon resources.

In contrast, the more limited distribution of starvation-tolerant *Ivr1* mRNAs could enhance their potential to aid local expansion and import in critical organs such as root tips and reproductive structures. A similar role for starvation-tolerant, sugar-repressed genes in adjusting to multiple other stresses (osmotic and pathogenic) has recently been proposed for a mannitol dehydrogenase gene (Williamson et al., 1996), and

the sIl invertase of carrot is upregulated by both defoliation and drought (Sturm et al., 1995).

The implication of these results extends beyond the roles of invertases alone. A fascinating similarity is shared between the tissue expression profiles of invertase genes and those encoding sucrose synthases (Koch et al., 1992b). Both the starvation-tolerant *Ivr1* and *Sh1* are expressed primarily in root tips and reproductive organs, whereas the sugar-enhanced *Sus1* and members of the *Ivr2* class are broadly distributed among importing organs ([*Sh1*] Chourey, 1981; [*Sus1*] McCarty et al., 1986). Expression of the two potato sucrose synthases (*Sus3* and *Sus4*) is also compatible, with a broader distribution evident for the sugar-enhanced *Sus4* gene (Fu and Park, 1995; Fu et al., 1995). In addition, Martin et al. (1993) point out that such differences in patterns of tissue expression for sucrose synthases correspond to their sugar and stress responsiveness. Collectively, this information supports the hypothesis that respective broad and narrow patterns of expression may be a characteristic feature of sugar-enhanced and depletion-tolerant genes for sucrose metabolism and may facilitate their respective roles in the plant.

Invertase Expression and Activity Change in Concert with Key Phases of Reproductive Development

Kernel Development

Data in Figures 4 to 6 indicate that soluble as well as insoluble invertases may function in the very early stages of maize kernel growth. Previous analyses have centered largely on insoluble (cell wall) invertases in view of Shannon's original hypothesis for their role in kernel development (Shannon and Dougherty, 1972). However, the very early phases of kernel growth may differ somewhat. A critical early role has been proposed for soluble invertases in the initiation and establishment of normal seed development (Hanft and Jones, 1986a, 1986b; Reed and Singletary, 1989; Cheikh and Jones, 1995; Zinselmeier et al., 1995). During the first week after pollination, embryo and endosperm are minute, whereas growth and import by nucellar tissues predominate in kernels (Randolph, 1936). The very early expression of soluble invertase genes described here may well be associated with such a step in seed set.

Pollination: From Anthers to Silk and Fertilization

Levels of activity and mRNAs in both pollen and anthers are consistent with a twofold role for soluble invertases in these structures (Figure 5). Sugar delivery to the terminal end of the maternal transport path in anthers could be enhanced by cleavage reactions not only supplying elevated rates of respiration, biosynthesis, and secretion but also steepening the sucrose gradient between phloem and the secretory surface. Later, mature pollen must be able to utilize its own stored sucrose as well as external sugar supplies during germination, and sucrose is the primary soluble carbohydrate in most angiosperm pollen grains, including maize (Nakamura et al., 1980). Both sucrose synthase and invertase are likely involved in sucrose use by germinating pollen, although soluble invertases could be especially valuable to expansion of the germ tube. In pollen of *Camellia japonica*, sucrose decreases rapidly during germination, and the activity of soluble invertase, but not sucrose synthase, increases (Nakamura et al., 1980).

The changes in invertase gene expression observed in developing ovules during pollination and fertilization (Figures 8 and 9) correlate with the marked shift in resource allocation from silk elongation to increased expansion of the nucellar constituents in the ovule during the first days of kernel development. Consistent with the rapid cessation of silk elongation, pollination induces a sharp drop in *Ivr1* and *Ivr2* mRNA levels in a gradient from the tip to the base of the silk. In the same time frame, invertase genes are upregulated in the ovule at or before the time of fertilization. In addition to limiting elongation, the reduction in invertase expression in silks at the time of pollination would facilitate reallocation of carbon from the expanding silk to the elongating pollen tube and finally to the enlarging, newly fertilized ovule.

Overall, the data presented here define the presence of sugar-enhanced and sugar-repressed/starvation-tolerant classes among genes encoding enzymes of sucrose metabolism and suggest differences in physiological roles. Localized distribution and starvation-tolerant features of *Sh1* and *Ivr1* genes would favor special-mission functions in priority sites during stress and/or reproduction. The broader distribution and starvation-sensitive expression of sugar-enhanced genes (*Sus1* and *Ivr2*) would facilitate their role in coarse adjustment to photosynthate availability by vegetative tissues and possibly in the sugar-sensitive abortion of reproductive organs. This functional dichotomy has the potential to link sugar responsiveness not only to import and use but also to an array of additional roles ascribed to these genes in plant cells.

METHODS

Plant Material

Material from the *Zea mays* hybrid NK 508 was used for all experiments. Developmental analyses were conducted using plants grown under greenhouse or field conditions. Samples harvested from nearly mature plants included the blade of a fully expanded leaf, a young yellow-green leaf from the central furl, and prop roots undergoing aerial elongation. During reproductive development, anthers, pollen, silk, ovules, and kernels were sampled at designated phases of development. Timing of the samplings was determined by pretagging individuals in staggered groups of synchronously growing plants. Comparison between groups planted at 2-day intervals allowed initial estimates of developmental progression, with confirmation provided by subsequent maturation of intact individuals in the same tagged,

synchronous group. For experiments involving pollination, silks were trimmed the previous day to a similar distance from the cob tip as a standard procedure for improving uniformity of pollination (~12 cm total length). Individual silks were excised from ovules and pooled for whole-silk samples or dissected into collective fractions of the lower third from each (length nearest the ovule), the middle third, or the third nearest the tip. Kernels were also dissected into upper and lower portions at 8 days after pollination (DAP) when *Ivr1* and *Ivr2* invertase mRNA levels were near their peak. At this stage of development, kernels are primarily maternal tissues throughout, with pericarp and nucellus predominating in the upper portion and pedicel at the base (bracts were removed). Emerging shoots (not yet green) were excised at ~1.0 cm from seedlings grown as described below.

Root tips for experiments were obtained from young seedlings grown under controlled conditions. Seeds were immersed in 20% Clorox for 30 min, rinsed continuously with water for 30 min, and germinated in the dark at 18°C on two layers of moist 3 MM paper (Whatman, Inc., Clifton, NJ) in 17 × 26 cm glass pans. Air flowed continuously at 1 L per min through each pan for the 5-day period, with 40% O_2 supplied during the final 24 hr before root tip excision. Moisture was replenished daily by applying mist and draining excess water. The terminal 1 cm was excised under sterile conditions from roots having reached a 3- to 6-cm total length.

Experimental Conditions

Approximately 100 root tips (~500 mg) were used for each experimental treatment. Each group was incubated in the dark at 18°C for 6 to 48 hr in White's basal medium, with or without an array of supplemental sugars, as described by Koch et al. (1992b). Each group of root tips was agitated at 120 cycles per min in a 125-mL side-arm Erlenmeyer flask with 50 mL of sterile medium. Airflow (40% O_2) through air stones in each flask was maintained at 250 mL per min throughout the incubations. Recutting root tips to 1 cm after incubations did not alter results in these experiments.

Invertase Probes and Restriction Fragment Length Polymorphism Mapping

Molecular materials described in Figure 1 were utilized as probes. Identification, organization, and mapping of the gene family were determined by using DNA gel blot restriction fragment length polymorphism comparisons of recombinant inbred populations. These were analyzed by B. Burr and E. Matz (Brookhaven National Laboratory, Cold Spring Harbor, NY), S. Wright (Linkage Genetics, Salt Lake City, UT), and G. Davis, T. Musket, and S. Chao (Maize Genome Center, University of Missouri, Columbia, MO). The *Ivr1* and *Ivr2* clones (GenBank accession numbers U16123 and U31451, respectively) are described in Xu et al. (1995) and Koch et al. (1995). Each probe hybridized with a specific set of genes at high stringency. The identity of *Ivr1* and *Ivr2* as soluble acid invertases is based on several factors, including extensive sequence similarity to other soluble invertases, similar putative target sequences, locale of key glycosylation sites, and diagnostic domains of sequence conserved among functionally defined soluble invertases (Koch and Nolte, 1995; Xu et al., 1995).

RNA Isolation and Gel Blot Analysis

Samples for RNA analysis were weighed and frozen immediately in liquid N_2. Root tips were first rinsed twice in sterile water and blotted dry. Materials were ground into fine powder in liquid N_2 and subdivided for analysis of RNA and enzyme activity (as described below). Total RNA was extracted, as described previously (Koch et al., 1992b), and quantified spectrophotometrically (Sambrook et al., 1989).

RNA (10 μg per lane) was separated by electrophoresis in 1% agarose gels containing formaldehyde, blotted to nylon membranes, and fixed by UV treatment (Sambrook et al., 1989). Filters were hybridized at 65°C in a solution with 7% SDS, 250 mM Na_2HPO_4, pH 7.2, and 1% BSA, using standard methods described by Koch et al. (1992b). Maize *Ivr1* and *Ivr2* invertase cDNA clones were radiolabeled by the random primer method. No cross-reactivity was observed between the *Ivr1* and *Ivr2* gene probes, their respective cDNAs, or related genomic DNA when hybridizations were conducted at high stringency (data not shown). Blots were washed as described in previous work (Koch et al., 1992b) and either exposed against x-ray film with intensifying screens at −80°C or quantified by PhosphorImager (Molecular Dynamics, Sunnyvale, CA) analysis.

Invertase Extraction and Assay

Soluble invertase was extracted as described by Duke et al. (1991). Frozen samples were ground into fine powder in liquid N_2 and partitioned as above for analysis of RNA and enzyme activity. A designated subsample was extracted in 10 volumes (per unit tissue fresh weight) of ice-cold 200 mM Hepes buffer, pH 7.5, with 1 mM DTT, 5 mM $MgCl_2$, 1 mM EGTA, and 10% (w/w) polyvinylpyrrolidone. Buffered extract was centrifuged at 15,000*g* for 10 min, and pellets were reserved for salt extraction of insoluble, cell wall invertase. Supernatant was dialyzed (50,000 molecular weight cutoff) at 4°C for 24 hr against extraction buffer diluted 1:40. Dialyzed extract was recentrifuged at 15,000*g* for 10 min, and the supernatant was assayed for soluble invertase activity as described below.

Insoluble cell wall invertase was extracted as described by Doehlert and Felker (1987). Particulate material (pelleted as given above) was rinsed and centrifuged three times, each with 5 to 10 mL of extraction buffer, for 10 min at 15,000*g*. The final pellet was resuspended in extraction buffer containing 1 M NaCl and centrifuged at 15,000*g* for 10 min. Insoluble invertase was recovered in the supernatant. Pooled supernatant fractions were assayed for insoluble invertase as described below.

Both soluble and insoluble (salt-extracted) invertase activities were assayed for 15 to 30 min at 37°C in an assay medium with 100 mM sodium acetate, pH 4.5, and 100 mM sucrose in a final volume of 500 μL. Activity was expressed as the glucose portion of total reducing sugar production quantified, as described by Duke et al. (1991).

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