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Organ-Specific Invertase Deficiency in the Primary Root of an Inbred Maize Line[1]

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ABSTRACT

An organ-specific invertase deficiency affecting only the primary root system is described in the Oh 43 inbred maize (*Zea mays*). Invertases (acid and neutral/soluble and insoluble) were assayed in various tissues of hybrid (NK 508) and inbred (Oh 43, W 22) maize lines to determine the basis for an early report that Oh 43 root tips were unable to grow on sucrose agar (27). Substantial acid invertase activity (7.3 to 16.1 micromoles of glucose per milligram of protein per hour) was evident in extracts of all tissues tested except the primary root system of Oh 43. This deficiency was also evident in lateral roots arising from the primary root. In contrast, morphologically identical lateral roots from the adventitious root system had normal invertase levels. These results suggest that ontogenetic origin of root tissues is an important determinant of invertase expression in maize. Adventitious roots (including the seminals) arise above the scutellar node and are, therefore, of shoot origin. The Oh 43 deficiency also demonstrated that invertase activity was not essential for maize root growth. Sucrose synthase was active in extracts from all root apices and theoretically provided the only available avenue for sucrose degradation in primary root tips of Oh 43. The deficiency described here will provide a useful avenue of investigation into the expression and significance of root invertase.

Sucrose breakdown is critical to the vast majority of plant species because nonphotosynthetic tissues depend on import of this sugar for their growth and development. Initial cleavage of sucrose can be catalyzed by either invertase (EC 3.2.1.26) or the reversible enzyme sucrose synthase (EC 2.4.1.13). Invertases are especially active in tissues undergoing rapid cell division, such as shoot and root apices (1). Previously, the role of invertases in sucrose transfer was considered particularly important in plants such as sugar cane and maize in which substantial sugar movement occurred through the cell wall space and was accompanied by action of an extracellular invertase (13, 15). Recent evidence, however, indicates that, although much hydrolysis is often observed, invertase activity may not be essential for sucrose uptake into either sugar cane stems (21, 31) or maize kernels (30).

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Sucrose generally is believed to enter root tips without traversing the extracellular space (12); however, growing roots can differ markedly in their capacity to lose (29) and retrieve (27) exogenous sugars. Net losses do occur. The extent of sugar efflux from roots can be affected by irradiance level, nutritional status, moisture availability, and temperature (29). The composition of root sugars exuded is variable but includes both reducing and nonreducing sugars (29). Glucose and fructose are often taken up from the extracellular space more rapidly than is sucrose (17). Retrieval of solutes from the apoplast and the form in which they are available may thus be a potentially important attribute of root carbon balance.

A deficiency in this retrieval process was first indicted by Robbins (27) who reported that roots of an inbred maize, Oh 43, were unable to grow on sucrose agar medium, and yet roots of another line, Hy 2, grew well. Only when roots of both were cultured immediately adjacent to one another were those of Oh 43 able to grow. Growth of excised Oh 43 root tips also occurred when glucose was substituted for the sucrose. Oh 43 was concluded to be "incapable of inverting sucrose" in its root tips. Preliminary investigations by B. Burr (Brookhaven National Laboratory, personal communication) indicated that a lack of invertase may have been the reason for the inability of Oh 43 roots to metabolize sucrose.

The absence of invertase activity could have important implications for sucrose import, not only because of potential effects on the retrieval system but also because sucrose utilization in such an instance could theoretically be initiated only via action of sucrose synthase. In addition, genetic material that lacks activity of a specific enzyme can be useful in investigations of physiological processes normally mediated by these enzymes (18). This report demonstrates that invertase is not essential for primary root growth, despite probable advantages of its presence, and indicates an unusual organ-specific difference in expression between primary and adventitious roots.

MATERIALS AND METHODS

Plant Material

Maize seeds (*Zea mays* L. NK 508, W 22, and Oh 43) were germinated on moist filter paper in Petri dishes. Seeds were imbibed for 24 h, and pericarps were removed, allowing more uniform germination and more effective surface sterilization (20-min soak in 0.525% sodium hypochlorite).

Five successive 2-mm segments were sampled from the tips of primary roots 4 to 5 days after germination. Intact roots of

Oh 43 seedlings grew more slowly than did those of NK 508 or W 22, but all roots had reached 2 cm before excision. Tissue samples were weighed, frozen in liquid N_2, and stored at −80°C until assayed for invertase activity. In subsequent experiments, 5-mm root tips were excised from primary and adventitious roots for invertase and sucrose synthase activity measurements. Plants and tissues were as above.

Tissue Extraction

Frozen tissue samples were ground to a fine powder in liquid N_2 using a mortar and pestle. Frozen powder was transferred to a second mortar containing ice-cold 200 mM Hepes buffer (pH 7.5) with 1 mM DTT, 5 mM MgCl, 1 mM EGTA, 20 mM sodium ascorbate, and 10% (w/w) polyvinylpolypyrrolidone. One milliliter of extraction buffer was used for every 100 mg of tissue fresh weight. Buffered extract was centrifuged at 14,000*g* for 1 min to sediment particulate matter. Supernatant was dialyzed (27,000 mol wt cutoff) at 4°C for 24 h against extraction buffer diluted 1:40. Buffer was changed after 1 h and thereafter every 4 h. Soluble dialyzed extract was assayed for invertase as described below. Previously separated particulate matter was rinsed with 1 volume of extraction buffer and assayed for insoluble, cell wall-bound invertase (soluble acid invertase includes both vacuolar and loosely bound extracellular enzyme [1]).

To test the possibility that the soluble enzyme was present in primary roots of Oh 43 but was being bound or inactivated during the extraction procedure, two additional extraction/assay methods were used. First, adventitious root extracts, previously shown to contain active invertase activity, were added to those of primary apices. The resulting mixture was dialyzed and assayed for enzyme activity. Second, 3-cm apices of both primary and adventitious roots Oh 43 roots were excised. Apices of these roots (0.5 cm) were suspended in extraction buffer for 3 h at 27°C. Buffer alone was subsequently dialyzed as described above. The portion of each root that had been immersed in the extraction buffer was excised for fresh weight measurement. After dialysis, the buffer-enzyme solution was analyzed for enzyme activity.

Enzyme Assays

Soluble and insoluble forms of acid invertase were assayed as described by Lowell *et al.* (23). Reaction media contained 50 mM sucrose, and pH of 4.5 was adjusted with a sodium acetate buffer. Neutral invertase was assayed using the same reaction medium adjusted to pH 7.5 with potassium phosphate buffer. Initial assays were also performed at pH ranges of 4.0 to 5.5 for acid invertase and 7.0 to 8.0 for neutral invertase. After a 15-min incubation at 30°C, glucose production was quantified by the glucose oxidase method (Sigma). Sucrose synthase was assayed in the degradative direction using a radiometric assay quantifying the production of [^{14}C] uridine diphosphoglucose (7).

Histochemical Staining

Free-hand cross-sections from apices of both primary and adventitious roots were fixed in 4% formalin (pH 7.0) for 30 min and rinsed in water at least 10 times throughout a period of 3 h to remove endogenous sugars (5). Sections were then incubated in a sodium phosphate buffer (0.38 M, pH 6.0) containing 0.24 mg mL^{-1} nitroblue tetrazolium, 0.14 mg mL^{-1} phenazine methosulfate, 25 units mL^{-1} glucose oxidase, and 5 mg mL^{-1} sucrose (5). Control sections were incubated in the same mixture without sucrose. After the sections were rinsed in water, they were postfixed in 4% formalin (pH 7.0) and photographed under a microscope.

RESULTS

Primary roots of Oh 43 showed little or no acid invertase activity (Table I). In contrast, acid invertase was active in extracts from apical areas of roots from other maize lines examined (NK 508 and W 22). Activity, per unit fresh weight, was greatest in root apices, decreasing with distance from the tip until no longer detectable farther than 8 mm from the apex. Soluble enzyme accounted for 88 to 92% of the total acid invertase activity, and the remainder was due to action of the insoluble enzyme. Neutral invertase activity was insignificant or absent from both lines (data not shown). Although invertase action was essentially undetectable in the primary

Table I. *Soluble and Insoluble Acid Invertase Activity in Sequential 2-mm Segments of Primary Roots of 5- to 6-d-old Seedlings from One Hybrid and Two Inbred Lines of Zea mays*

Each value represents the mean of three separate samples ± SEM.

Root Segment	Soluble[a]			Insoluble		
	Oh 43	NK 508	W 22	Oh 43	NK 508	W 22
mm	*μmol glucose g^{-1} fresh wt h^{-1}*					
0–2	0.2 ± 0.2[b]	43.8 ± 5.2	40.2 ± 3.7	—[c]	3.8 ± 0.5	3.6 ± 0.8
2–4	—	32.8 ± 2.2	28.6 ± 3.1	—	3.1 ± 1.9	2.7 ± 1.5
4–6	—	20.6 ± 5.0	15.4 ± 4.6	—	—	—
6–8	—	1.4 ± 0.9	0.9 ± 0.5	—	—	—
8–10	—	—	—	—	—	—

[a] Soluble activity is expressed per unit fresh weight to allow comparison with insoluble activity. [b] Activity in root tips from axenic culture was 0.0 ± 0.0 μmol g^{-1} fresh weight h^{-1}; thus, microorganisms are a likely source of the residual activity in approximately one of three assays. [c] —, not detectable.

roots of Oh 43, adventitious root extracts showed levels of activity at least as great as those from the primary roots of other lines tested (Table II).

Active acid invertase was released into buffer in the extracellular space around adventitious root tips of Oh 43 during 3 h of emersion (13.4 ± 3.5 μmol glucose g^{-1} fresh weight h^{-1}—approximately 25% of the activity on a fresh weight basis of N_2 and buffer extracted roots). The same was not observed during emersion of primary roots from this line. Also, from 83 to 94% (13.3 to 15.1 μmol glucose mg^{-1} protein h^{-1}) of the invertase activity in adventitious root extracts remained after addition of extracts from primary roots of Oh 43.

Unlike invertase, sucrose synthase was active in extracts of both primary and adventitious roots from Oh 43 seedlings. Activity of this enzyme was approximately similar in both root types, as observed for the other two lines tested (Table II).

Extracts of lateral roots originating from primary roots exhibited no detectable acid invertase activity (Table III), in contrast to counterparts derived from adventitious roots. Shoots of 5-d-old Oh 43 seedlings and endosperm or scutellum tissue from developing kernels (23 days after pollination) also exhibited activity of both soluble and insoluble acid invertase (Table III).

Histochemical staining indicated that no invertase activity was detectable in the primary roots of Oh 43 (Fig. 1). Cross-sections of primary and adventitious roots of NK 508 and adventitious roots of Oh 43 all stained positively for invertase activity. Invertase activity was primarily in the cortex and was localized intercellularly.

Table III. *Soluble Acid Invertase Activity in Various Tissues of Oh 43, an Inbred Line of Zea mays*

Shoot, primary roots, and adventitious roots were sampled from 6-d-old seedlings. Endosperm and scutellum were from developing kernels 23 d after pollination. Lateral roots developed after 2 to 3 weeks of growth and were then excised. Each value represents the mean of three separate samples ± SEM.

Plant Segment	Enzyme Activity	
	μmol glucose mg^{-1} protein h^{-1}	*μmol glucose g^{-1} fresh wt h^{-1}*
Shoot	10.8 ± 0.6	25.8 ± 1.5
Endosperm	7.3 ± 1.2	12.6 ± 2.1
Scutellum	9.0 ± 0.8	19.4 ± 1.7
Primary roots	0.1 ± 0.1	0.2 ± 0.2
Lateral roots from 1° roots	0.1 ± 0.1[a]	0.2 ± 0.1[a]
Adventitious roots	16.1 ± 3.2	60.8 ± 12.1
Lateral roots from adventitious roots	10.4 ± 0.9	38.7 ± 3.2

[a] Activity in root tips from axenic culture was 0.0 ± 0.0 μmol g^{-1} fresh weight h^{-1}; thus, microorganisms are a likely source of the residual activity in approximately one of three assays.

DISCUSSION

These data confirm that Oh 43 (an inbred line of maize) lacks invertase activity in its primary root tips. Surprisingly, however, a deficiency was not evident in structurally and functionally similar adventitious roots (Table II), other tissues of the same Oh 43 plants (Table III), or in the developing kernels of this line (6). The genetic potential for invertase expression is therefore present. The lack of activity may result from altered regulation of gene expression (transcription or translation), the loss of a tissue-specific invertase isozyme, or the existence of an unidentified effector of enzyme function. The significance of results described here is twofold. First, evidence is presented for differential expression of invertase in morphologically identical organs that differ primarily in point of origin. This is most strikingly illustrated in the apparent distinction between lateral roots arising from primary and adventitious root systems. Differential expression of genes in morphologically identical structures are unusual; however, organ- or tissue-specific differences have been well documented (9, 32). Xie and Wu (32), for example, found that genes for alcohol dehydrogenase were differentially expressed in root and shoot tissues of rice plants. One isozyme predominated in shoot-derived organs (leaves, sheaths, nodes, and pollen), and the other isozyme showed highest activity in the roots. It is interesting in this respect that the adventitious roots of maize arise above the scutellar node of the developing

Table II. *Soluble Acid Invertase and Sucrose Synthase Activity in 0.5-cm Apices of Primary and Adventitious Roots of 5- to 6-d-old Seedlings from One Hybrid and Two Inbred Lines of Zea Mays*

Each value represents the mean of three separate samples ± SEM

Enzyme Measured	Oh 43		NK 508		W 22	
	Primary	Adventitious	Primary	Adventitious	Primary	Adventitious
	μmol glucose mg^{-1} protein h^{-1}					
Invertase	0.1 ± 0.1[a]	16.1 ± 3.2	16.0 ± 2.5	14.0 ± 2.5	12.6 ± 2.2	11.9 ± 1.6
Sucrose synthase	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	0.9 ± 0.1
	μmol glucose g^{-1} fresh wt h^{-1}					
Invertase	0.2 ± 0.2[a]	60.8 ± 12.1	56.5 ± 8.7	51.6 ± 9.2	53.0 ± 9.3	48.9 ± 6.7
Sucrose synthase	4.7 ± 0.4	4.2 ± 0.1	3.5 ± 0.5	4.2 ± 0.6	4.0 ± 0.4	3.7 ± 0.4

[a] Activity in root tips from axenic culture was 0.0 ± 0.0 μmol g^{-1} fresh weight h^{-1}; thus, microorganisms are a likely source of the residual activity in approximately one of three assays.

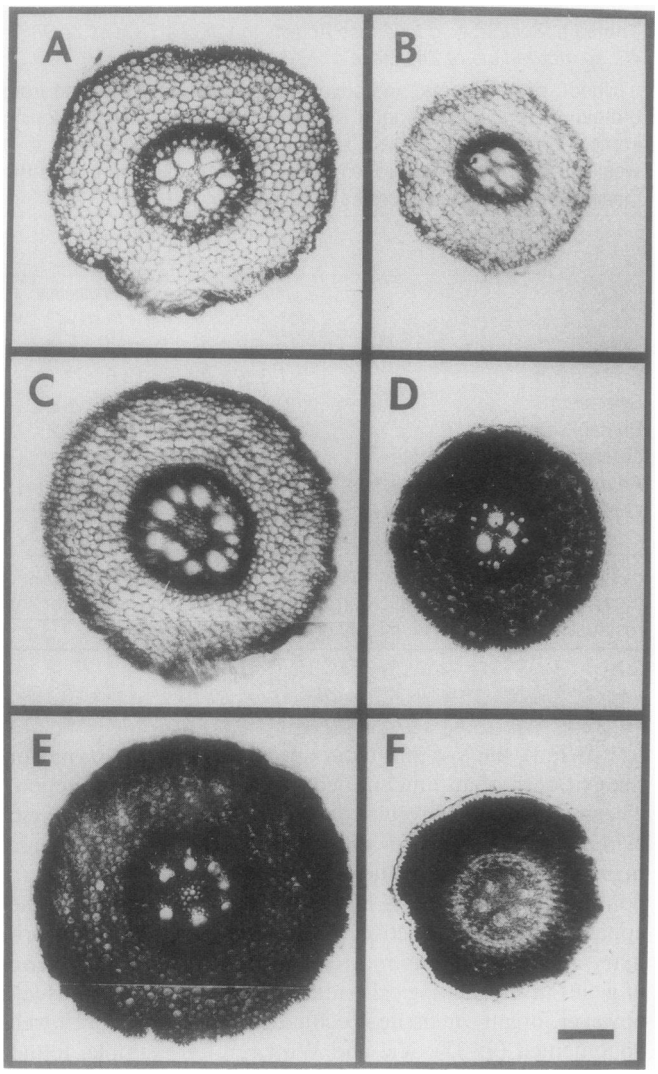

Figure 1. Histochemical localization of invertase activity in free-hand, fresh cross-sections of root apices of 6-d-old maize seedlings. Sections are approximately 50 μm in thickness. Primary (A) and adventitious (B) root cross-sections from NK 508 incubated in reaction medium without sucrose (controls). Primary (C) and adventitious (D) root cross-sections from Oh 43 incubated in reaction medium (note only adventitious root section exhibits blue formazan reaction product [dark areas]). Primary (E) and adventitious (F) root cross-sections from NK 508 incubated in reaction medium (note both sections exhibit reaction product [dark areas]). Bar = 50 μm.

seedling and are, therefore, derived from shoot tissue (14). The difference in invertase expression in primary and adventitious root systems may reflect a similar root/shoot dichotomy. Therefore, the tissue of origin and cell lineage may be more important than organ identity and function in regulating invertase expression. Other variants in invertase expression have been described. Echeverria and Humphreys (8) reported a maize line that exhibited no soluble invertase activity in contrast to previously tested lines. Other tissues of this maize line exhibited normal invertase activity.

Second, data demonstrate that invertase is not essential for primary root growth. The primary roots of Oh 43 exhibited no signs of premature senescence and, if left intact, continued apparently normal growth for many days (data not shown). The role of invertase in sucrose import into roots has been questioned by Chapleo and Hall (3) who concluded that, although present, apoplastic root invertase did not have a direct role in sugar transport in *Ricinus*. However, substantial activity of invertase has been widely documented in roots of plants such as pea (24), bean (28), tomato (4), *Ricinus* (3), oat (25), and maize (2, 16). In maize, the Oh 43 invertase deficiency apparently prevents utilization of exogenous sucrose (27). Specific tissue localization also has been described. Peak activity for root invertase is generally 2 to 3 mm behind the apex and corresponds to the region of expansion and elongation in pea (28) and maize (16). In *Ricinus*, this activity predominates in the cortex (3).

Although invertase may not have a direct role in sucrose import in roots, it still may be important to two major aspects of root biology. First, invertase has been implicated in formation of mycorrhizal associations (26). Maize (10, 19) and 90% of other agriculturally important species form these beneficial symbioses under field conditions (11). Invertase activity typically increases and hexose levels increase at infection sites of biotrophic fungi (22). Elevated hexose content in roots upon infection by mycorrhizal fungi has been attributed to an increase in invertase levels (26). It is not known whether this is host or fungal invertase; however, Oh 43 does not appear to provide the former in its primary root systems.

Second, the purported lack of a sucrose carrier in the plasma membrane of maize root cells (20) would indicate that, if sucrose were released into the apoplast, retrieval might proceed more effectively in the presence of extracellular invertase. Such retrieval could be particularly important during stress or periods when sugar losses from the symplast were elevated. Any physiological consequence of this deficiency would most likely be evident early in seedling development because the root system would consist solely of a primary root at this time. Later in seedling development, adventitious or seminal roots rapidly take over a dominant role, leaving the primary root with little or no essential function (14). In the field, lines having Oh 43 as a progenitor have been observed to suffer from poor emergence rates in damp soils (B. Martin, Pioneer Seed, personal communication). However, conclusive evidence of a physiological effect will require generation of and analysis of isogeneic lines.

In conclusion, the deficiency described here will be useful for investigation into the regulation and physiological function of root invertase. Because the primary root system in maize is nonessential, invertase-deficient roots can be studied without deleterious effects on the overall physiology of the plant. In addition, this deficiency is significant because it reveals an unexpected distinction between primary and adventitious root development in maize. At some level, the mechanisms that regulate invertase in these root systems must differ.

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