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LEAKAGE OF HEXOSE PHOSPHATES FROM THE MAIZE SCUTELLUM*

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Abstract—Maize seedlings were incubated at room temperature in the dark with their scutella immersed in fructose solutions. Hexose phosphates and sucrose leaked from these scutella when the root-shoot axes were removed and the scutella placed in water at 1°. Considerable quantities of mannose-6-phosphate (M6P) accumulated in the scutella during incubation of seedlings in mannose or mannose plus fructose solutions; M6P leaked from the scutellum only after removal of the root–shoot axis. During a 4-hr period at 1°, the leakage of newly synthesized sucrose was proportionately greater than the leakage of newly synthesized M6P. The leakage of hexose phosphates at 1° only after removal of the root–shoot axis is additional support for the contention that leakage takes place in a mass flow of solution which empties into the bathing solution through the cut ends of the phloem and which originates in the mesophyll parenchyma cells.

INTRODUCTION

FROM previous work with the maize scutellum, both sliced[1] and whole,[2] it was concluded that sucrose leakage takes place in a pressure flow of solution which leaves the tissue through the cut ends of the phloem. Moreover, the solution is thought to originate in the mesophyll parenchyma cells and to flow from these cells into the phloem. A pressure flow of solution moving through non-vascular cells by way of plasmodesmata would be expected to carry with it any substances dissolved in the cytoplasmic solution including phosphorylated metabolites and cofactors.

In this paper it is shown that hexose phosphates do, in fact, leak from the scutellum along with sucrose when the root–shoot axis is removed.

RESULTS AND DISCUSSION

Seedlings (endosperm removed) were treated by placing the scutella in 1·0 M fructose for 4 hr. Small amounts of hexose phosphates and a large amount of sucrose leaked from these scutella into 1° water following removal of the root–shoot axes (Table 1). The amount of hexose phosphate that leaked was less than 10% of the amount in the tissue. Tissue sucrose was not measured in the experiment of Table 1. However, in other experiments, the tissue sucrose following a 4-hr fructose incubation was about 200 μmoles/12 scutella; so the 50·3 μmoles of sucrose that leaked (Table 1) were about 25% of the tissue sucrose at the time the scutella were detached from the root–shoot axes. If the idea that leakage results from a pressure flow of solution is correct, it would be expected that different relative amounts of hexose phosphates and sucrose would leak since those portions of the total tissue levels of these substances occupying cell organelles or vacuoles would be excluded from the

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[1] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* in press (1971).

[2] T. E. HUMPHREYS, *Phytochem.* in press (1971).

flowing solution; and, in addition, the hexose phosphates might be anchored against the flow at fixed, positively charged, sites.

TABLE 1. LEAKAGE OF G6P PLUS F6P AND SUCROSE FROM SCUTELLA TREATED IN 1·0 M FRUCTOSE*

Source	Amount, μmoles/12 scutella	
	G6P + F6P	Sucrose
A Tissue, initial	1·15	—
B Tissue, after leakage	1·02	—
C Leakage into 1° water	0·09	50·3

* The endosperm was removed from 72 seedlings; 48 seedlings were placed individually in tubes with their scutella immersed in 1·0 M fructose whereas the scutella were excised from the remaining 24 seedlings and extracted in ethanol (for A above). After 4 hr, the seedlings were removed from the fructose, and the scutella were excised into flasks (12/flask) containing 10 ml of water at 1°. After 4 hr of leakage at 1° the scutella were killed and extracted in ethanol (for B above). Portions (0·1 ml) of the bathing solution were removed from each flash for sucrose analysis. The bathing solutions from the four flasks were then combined and taken to dryness in vacuo. The residue was dissolved in 2·0 ml of tris (0·1 M, pH 7·5) and the resulting solution used for the analysis of hexose phosphates (for C above).

When slices of the scutellum are incubated in mannose solutions, considerable quantities of mannose-6-phosphate (M6P) accumulate in the tissue[3]. M6P also accumulated when seedlings were incubated with their scutella in mannose or mannose plus fructose solutions (Table 2). Appreciable amounts of this M6P leaked from the scutellum into 1° water when the root–shoot axis was removed (Table 2). The much greater leakage of M6P following treatment of the scutella in 0·1 M mannose plus 0·9 M fructose than following treatment in 0·1 M mannose alone was probably the result of the higher turgor driving the flow of solution through the fructose-treated cells.

TABLE 2. ACCUMULATION AND LEAKAGE OF HEXOSE PHOSPHATES*

Treatment	Amount, μmoles/12 scutella		
	Tissue G6P + F6P	Tissue M6P	Leakage M6P in 1° water
1 Mannose (0·1 M), 4 hr	0·40	8·45	1·11
2 Mannose (0·1 M) + fructose (0·9 M), 4 hr	0·81	10·03	2·80

* Seedlings were placed individually in tubes with their scutella in the above solutions. At the end of the 4-hr treatment period, the scutella (12/treatment) were detached and extracted in ethanol to obtain the tissue hexose phosphate. A separate set of 12 seedlings was used in each of the first two treatments; at the end of the 4-hr treatment, the scutella from these seedlings were detached from the root–shoot axes and placed in 10 ml of 1° water. After 4 hr at 1° the bathing solutions were collected for determination of M6P.

M6P did not leak from the scutellum until the root–shoot axis was removed (Fig. 1). Sucrose also does not leak until the root–shoot axis has been removed.[1,2] It is concluded

[3] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **6**, 647 (1964).

that both sucrose and M6P leave the scutellum through the cut end of the main vascular strand; presumably the phloem is the conduit.[4,5] The amount of M6P in the scutellum declined during the leakage period (Fig. 2), indicating that the M6P that leaked was formed before the root–shoot axis was detached from the scutellum. Moreover, the low temperature (1°) appears to preclude extracellular synthesis (at the cut surface of the scutellum) of M6P from mannose, sieve tube ATP,[6] and a cell wall or membrane-bound hexokinase.

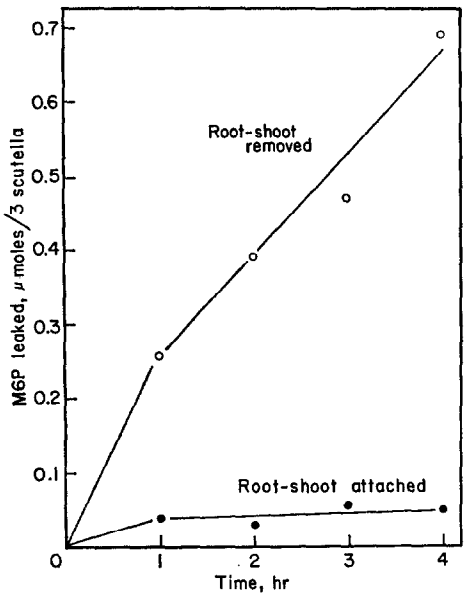

FIG. 1. THE EFFECT OF REMOVAL OF THE ROOT–SHOOT AXIS ON M6P LEAKAGE FROM THE SCUTELLUM. Seedlings (24) were treated with their scutella immersed in fructose (0·9 M) + mannose (0·1 M) for 4 hr. At the end of the treatment, one set of 12 seedlings was washed and the seedlings were placed individually in tubes with their scutella immersed in 1·0 ml of water at 1°. The scutella were detached from the other set of 12 seedlings; each scutellum was placed in a tube with 1·0 ml water at 1°. At the end of each hr during the 4-hr leakage period, 3 tubes from each set were removed from the ice, the seedlings or excised scutella were discarded, and the bathing solutions were pooled and analyzed for M6P.

During the 4-hr leakage period 28% of the initial M6P leaked from the scutellum into 1° water (Fig. 3). During the same period and from the same scutella sucrose leaked in an amount equal to about 80% of the newly synthesized tissue sucrose. In this experiment the seedlings were treated first in fructose (1·0 M) and then in fructose (0·9 M) plus mannose (0·1 M) because mannose strongly inhibits sucrose synthesis.[7] From the start of the fructose treatment until the end of the mannose treatment, at which time the root–shoot axes were removed and leakage started, the scutellar sucrose increased by 55 μmoles; 46 μmoles leaked into 1° water during the next 4 hr. The sucrose curve of Fig. 3 was calculated assuming that the initial tissue sucrose (107 μmoles) was stored (in the vacuole?) and, therefore, was out of the pressure-flow stream whereas the sucrose synthesized during the treatment period

[4] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **10,** 243 (1971).
[5] T. E. HUMPHREYS and L. A. GARRARD, *Phytochem.* **10,** 981 (1971).
[6] D. C. J. GARDNER and A. J. PEEL, *Nature, Lond.* **222,** 774 (1969).
[7] L. A. GARRARD and T. E. HUMPHREYS, *Phytochem.* **8,** 1065 (1969).

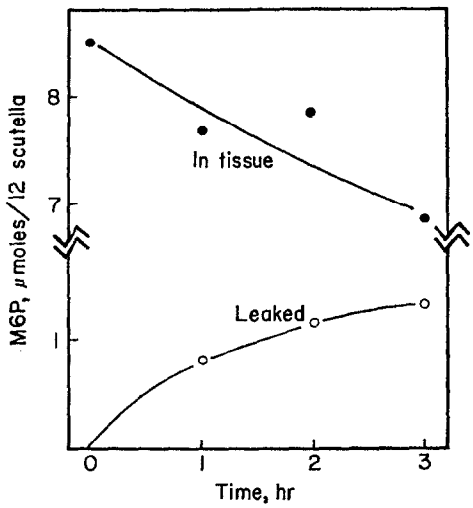

FIG. 2. LEAKAGE OF M6P AND TISSUE LEVELS OF M6P WITH TIME FOLLOWING REMOVAL OF THE ROOT–SHOOT AXIS.

Seedlings (48) were treated with their scutella immersed in fructose (0·9 M) + mannose (0·1 M) for 4 hr. At the end of treatment the scutella were detached. One group of 12 was extracted in ethanol immediately; the other scutella were placed in flasks (12/flask) containing 10 ml of water at 1°. At the end of each hr thereafter, the scutella from one flask were extracted and the bathing solution collected for the determination of M6P.

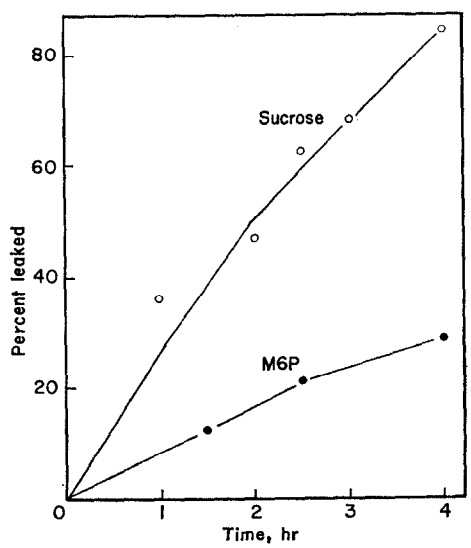

FIG. 3. THE RELATIVE AMOUNTS OF NEWLY SYNTHESIZED SUCROSE AND M6P THAT LEAKED FROM THE SCUTELLA FOLLOWING REMOVAL OF THE ROOT–SHOOT AXES.

Seedlings (48) were treated with their scutella immersed in fructose (1·0 M) for 2 hr and then in fructose (0·9) + mannose (0·1 M) for 2 hr. At the end of the 4-hr treatment period the scutella were detached. One group of 12 scutella were extracted in ethanol; the other 3 groups were placed in flasks (12 scutella/flask) containing 10 ml of water at 1°. The amounts of M6P and sucrose that leaked were determined at the times shown. An additional two groups of scutella were extracted in ethanol at the beginning of the treatment period to determine the initial tissue sucrose level (107 μmoles/12 scutella). At the end of the initial 4-hr treatment, 12 scutella contained 163 μmoles sucrose and 6·00 μmoles M6P.

(55 μmoles) was in the cytoplasmic solution. The M6P curve was calculated assuming all the M6P was in the cytoplasmic solution. However, these assumptions are open to question since, when the percentages of sucrose leaked are calculated from the total tissue sucrose as was done with M6P, the sucrose curve closely approximates the M6P curve of Fig. 3. Apparently, this means that the cytoplasmic solution contains proportionally the same amounts of sucrose and M6P; either both substances are restricted to the cytoplasmic solution or they have similar distributions within the cell. Previous results with scutellum slices[1,8] indicate that there are two cellular compartments for sucrose, cytoplasmic and storage. However, treatment with mannose has been shown to cause a drastic reduction in the scutellum ATP level,[7] and under these conditions the natural compartmentation of the cell may not be maintained.

It was argued before[1,2] from sucrose leakage data that the pressure flow of solution includes all the cells of the scutellum, not just the phloem. The results of the M6P leakage experiments are in accord with this idea. Since leakage occurred only after removing the root–shoot axis, since leakage occurred at 1°, and since nearly one-third of the total tissue M6P leaked during a 4-hr leakage period, it is concluded that leakage of M6P is a physical process (probably a pressure flow of solution[2]) which extends through all the living cells of the scutellum and empties into the bathing solution through the cut ends of the phloem. The fact that M6P and sucrose readily leak from scutella after mannose treatment (which reduces the ATP level to 10% or less of its initial value[7]) is a further indication that an active vein-loading process is not occurring here. These arguments imply that movement of M6P from cell to cell occurs through plasmodesmata, and that the plasmodesmata function as simple connecting tubes allowing free flow of solution. Similar conclusions were reached in studies of sucrose leakage.[1,2]

EXPERIMENTAL

Plant material. Maize grains (*Zea mays* L., cv. Funks G-76) were soaked in running tap water for 24 hr and then placed on moist filter paper in the dark at 24–25° for 72 hr. The endosperms were carefully removed and the seedlings were placed in ice water as they were prepared, and they were rinsed in ice water 3 times before use.

Experimental procedure. Detailed procedures are given in the tables and figures. Each seedling was placed in a plastic, round bottom, 50 ml, centrifuge tube which had been cut down to about one-third its original height. The seedling was placed so that the scutellum (abaxial surface down) rested on the bottom and the primary root and shoot were bent upward along the wall of the tube. Then enough treatment solution was added to reach but not cover the scutellar node. The tubes were placed in a specimen jar which was lined with moist paper and covered on the outside with aluminium foil. The jar was kept at room temperature (*ca.* 23°) during the treatment period. At the end of treatment, the seedlings were quickly washed and the root–shoot axis was removed by making a single cut at the scutellar node. The scutella were killed in boiling 80% EtOH either immediately after excision or after a period in 1° H_2O during which leakage of hexose phosphates and sucrose occurred. Procedures for the determination of sucrose leakage and for the preparation of EtOH extracts of the scutella have been described.[9] The sucrose contents of the bathing solution and the tissue extracts were determined by analyzing these solutions for glucose before and after invertase treatment. Glucose was determined by the glucose oxidase method. The procedures for the analysis of hexose phosphates were described previously[3] except that a purified phosphomannose isomerase (Sigma Chemical Co.) instead of a crude muscle powder was used in the M6P determination.

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[8] T. E. Humphreys and L. A. Garrard, *Phytochem.* **5**, 653 (1966).
[9] L. A. Garrard and T. E. Humphreys, *Phytochem.* **6**, 1985 (1967).

Key Word Index—Zea mays; gramineae; maize; hexose phosphates; leakage of sugar; mass flow.