

Gravity-Induced Polar Transport of Calcium across Root Tips of Maize¹

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ABSTRACT

Calcium movement across primary roots of maize (*Zea mays*, L.) was determined by application of ⁴⁵Ca²⁺ to one side of the root and collection of radioactivity in an agar receiver block on the opposite side. Ca movement across the root tip was found to be at least 20 times greater than movement across the elongation zone. The rapid movement of Ca across the tip was severely inhibited in roots from which the root cap had been removed. Ca movement across the tip was also strongly retarded in roots pretreated with 2,4-dinitrophenol or potassium cyanide. Orientation of roots horizontally had no effect on Ca movement across the elongation zone but caused a strong asymmetry in the pattern of Ca movement across the tip. In gravistimulated roots, the movement of Ca from top to bottom increased while movement from bottom to top decreased. The data indicate that gravistimulation induces polar movement of Ca toward the lower side of the root cap. An earlier report (Lee, Mulkey, Evans 1983 Science 220: 1375–1376) from this laboratory showed that artificial establishment of calcium gradients at the root tip can cause gravitropic-like curvature. Together, the two studies indicate that Ca plays a key role in linking gravistimulation to the gravitropic growth response in roots.

There is increasing evidence that Ca plays an important role in the gravitropic response of plant organs (16). In 1976, Goswami and Audus (10) confirmed earlier reports (1, 3) that Ca moves toward the upper side of horizontally placed shoots, and they showed that this occurs prior to the initiation of gravitropic curvature. More recently, Slocum and Roux (17) have found evidence for gravity-induced accumulation of Ca on the upper side of horizontally placed *Avena* coleoptiles. The interest in Ca as a potential mediator of growth in plant cells has intensified with the discovery of the Ca-activated, enzyme-modulator protein, calmodulin, in plant tissues (6, 7, 13). Calmodulin activates plasma membrane Ca ATPases in plant cells (4, 9) and it has been pointed out that changes in Ca movement across the plasma membrane may play a role in regulating growth rates in plants (912). Indirect evidence for the participation of calmodulin in gravitropic responses is provided by the experiments of Biro *et al.* (2) showing that agents which inhibit calmodulin function can interfere with gravitropism in *Avena* coleoptiles.

During the past year, we have been examining the potential role of Ca in mediating gravitropic curvature in roots of maize. We reported that Ca-chelating agents applied to the tip of maize

roots cause a loss of gravitropic sensitivity and that gravitropism can be restored by replacing the chelating agent with Ca (14). We also reported that unilateral application of Ca near the root tip causes roots to curve toward the Ca source (14). These results indicate that free Ca in the root tip is necessary to gravitropism and that Ca gradients across the root tip may play a role in linking graviperception to gravitropic curvature.

In view of the evidence that imposed gradients of Ca across the root tip can induce gravitropic-like curvature, we have studied the influence of gravity on ⁴⁵Ca²⁺ movement across root tips of maize. We find that gravistimulation induces a strong polar movement of Ca across the root tip toward the lower side of the root.

MATERIALS AND METHODS

Plant Material. Maize seedlings (*Zea mays* L., Bear hybrid WF 9 × 38 MS, Customaize, Momence, IL) were raised as described by Mulkey *et al.* (15). The grains were placed between wet paper towels held between opaque plastic trays. Although the trays were kept under laboratory lighting, the developing seedlings received little light and were etiolated. The seedlings were used when the primary roots were approximately 1.5 cm long (about 3 d after planting). In some of the experiments, the root caps were removed prior to testing for Ca transport. Root cap removal was done using a surgical scalpel with a number 11 blade. Working under a dissecting scope, the edge of the scalpel was inserted at the junction of the root cap and the root apex and a gentle force was applied to pry the cap off. Microscopic examination showed no damage to the root apex by this procedure, and the roots continued to grow.

Application of ⁴⁵Ca²⁺. To measure transport of Ca across the root, ⁴⁵Ca²⁺ (as ⁴⁵CaCl₂, 5.41 mCi/mg Ca) was incorporated into agar (1.5%) donor blocks (1.5 mm cubes, 30,000 dpm ⁴⁵Ca²⁺/block) and the donor blocks were placed on one side of the root either along the first 1.5 mm of the tip or in the elongation zone in the region from 3 to 4.5 mm behind the tip. Since the total Ca content of the donor blocks was low (approximately 2.5 ng/block), application of donor blocks to the surface of the root would not be expected to significantly alter apoplasmic Ca levels in the root. A receiver block the same size as the donor was placed on the opposite side of the root directly across from the donor block. After a transport period of either 45 or 90 min, the donor and receiver blocks were placed in separate scintillation vials for determination of radioactivity.

Pretreatment with Metabolic Inhibitors. In order to test the dependence on metabolism of the movement of Ca, experiments were done using roots which had been pretreated with DNP³ (0.1 mM) or KCN (1 mM). The seedlings were held vertically for 90 min with the roots immersed in a solution containing the inhibitor plus quarter-strength modified Hoagland nutrient solution

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³ Abbreviation: DNP, 2,4-dinitrophenol.

(11) adjusted to pH 6.4. After the pretreatment period, the roots were blotted and agar donor and receiver blocks were applied as described above.

RESULTS

Movement of Labeled Ca across Vertical Roots. When labeled Ca was applied to one side of the elongation zone of intact roots held vertically, there was little movement of label to a receiver block on the opposite side (Table I). By contrast, the movement of labeled Ca from donor to receiver across the apical 1.5 mm of the tip was large, with 20 to 50 times (depending on duration of the transport period) more label moving across the tip as compared with the elongation zone. The lateral transport of Ca across the root tip appeared to depend strongly upon the presence of the root cap. When the cap was removed, labeled Ca movement across the apical 1.5 mm of the decapped root was inhibited by 80 to 90% (Table I).

Movement of Labeled Ca across Horizontal Roots. Changing the root from a vertical to horizontal orientation had little or no effect on the movement of labeled Ca across the elongation zone (Table I). However, placing the root horizontally caused a large change in the movement of Ca across the root tip. In horizontally placed roots, Ca movement from top to bottom increased to 163% (45-min transport period) or 118% (90-min transport period) of that occurring across tips of vertical roots. Ca movement from bottom to top of horizontal roots was reduced to 53% (45-min transport period) or 62% (90-min transport period) of that occurring across tips of vertical roots (Table I). As with vertical roots, the movement of labeled Ca across the tips of horizontally placed roots was strongly inhibited by removal of the root cap (Table I).

Effect of Metabolic Inhibitors. To test the dependence of calcium movement across the root on metabolic energy, we examined $^{45}\text{Ca}^{2+}$ movement across roots pretreated for 90 min 0.1 mM DNP or 1 mM KCN (Table II). Neither DNP nor KCN inhibited the already slight movement of Ca across the elongation zone in vertical or horizontal roots. In fact, pretreatment with DNP appeared to increase the movement somewhat of $^{45}\text{Ca}^{2+}$ across the elongation zone. By contrast, both inhibitors strongly suppressed Ca movement across the tips of vertical roots. They also inhibited movement of $^{45}\text{Ca}^{2+}$ from top to bottom across the tips of horizontally placed roots but did not inhibit movement from bottom to top. If anything, the inhibitors stimulated the movement of $^{45}\text{Ca}^{2+}$ from bottom to top of horizontally placed roots during the first 45 min of transport (Table II). The small stimulation of $^{45}\text{Ca}^{2+}$ movement from bottom to top may have been a reflection of the inhibition of movement from top to bottom.

DISCUSSION

The results indicate that there is rapid transport of exogenously applied $^{45}\text{Ca}^{2+}$ across the tips of intact primary roots of maize but not across the elongation zone. The movement of Ca does not appear to be simply by diffusion or by movement along the moist surface of the root since: (a) metabolic inhibitors strongly retard the movement; (b) movement across the tip is much greater than movement across the elongation zone; (c) movement across the tip is asymmetric in horizontal roots; and (d) the pattern and magnitude of Ca movement across the root remains unchanged when the two sides of the root (donor and receiver) are isolated by a layer of vacuum grease along the surface on each side of the root (data not shown).

The rapid movement of Ca across the tip appears to occur through, or at least to depend upon, cells of the root cap. Removal of the cap greatly retards movement of Ca across the tip. Gravitostimulation causes asymmetric movement of Ca across the tip toward the lower side. The induction by gravitostimulation of polar Ca movement across the tip appears to result both from suppression of Ca movement from bottom to top and enhancement of Ca movement from top to bottom.

In work on shoot gravitropism, Arslan-Cerim (1) reported that metabolic energy is necessary for lateral transport of Ca toward the top of gravistimulated *Helianthus* hypocotyls. We found that DNP and KCN strongly suppress Ca movement across the tips of vertical roots and retard $^{45}\text{Ca}^{2+}$ movement from top to bottom across tips of horizontally placed roots. This indicates that gravity-induced polar movement of Ca toward the lower side of the root tip may arise from metabolically dependent pumping of Ca or from a coupling of Ca movement to metabolically dependent transport of some other factor. The reduced movement of Ca from bottom to top may be a consequence of the active movement of Ca in the opposite direction. This possibility is supported by the finding that inhibitors which suppress Ca movement from top to bottom, increase Ca movement from bottom to top.

These results, in conjunction with our earlier work (14) showing that tip gradients of Ca can induce gravitropic-like curvature in roots, indicate that gravity-induced polar movement of Ca across the root tip may play a key role in coupling gravitostimulation to gravitropic curvature.

If it can be shown that Ca redistribution links gravitostimulation to gravitropic curvature, two major questions arise. (a) How does gravitostimulation lead to polar Ca movement across the root tip? (b) How does asymmetric distribution of Ca at the root tip lead to asymmetric growth in the elongation zone? Chandra *et al.* (5) reported that the amyloplasts of root cap cells contain Ca, and it seems possible that Ca movement and release by settling amyloplasts initiates or contributes to asymmetric Ca distribution in

Table I. Influence of Gravity on $^{45}\text{Ca}^{2+}$ Movement across Roots of Maize

Agar donor blocks containing 30,000 dpm $^{45}\text{Ca}^{2+}$ were applied to the tip (0–1.5 mm region) or the elongation zone (3–4.5 mm region) with a blank agar receiver block applied to the opposite side. Each number represents the average cpm (with SD) in receiver blocks after 45 min of transport. Numbers in parentheses represent average cpm in receiver blocks after 90 min. Averages determined from five experiments with five roots in each experiment.

	Elongation Zone		Tip	
	cpm in receiver block			
Vertical roots	← ^a	→	←	→
+ cap	28 ± 1.4 (141 ± 15)	20 ± 1.6 (106 ± 8)	1492 ± 311 (2757 ± 426)	1111 ± 277 (3367 ± 411)
– cap	11 ± 2.8 (52 ± 4)	6 ± 1 (32 ± 3)	165 ± 17 (439 ± 51)	62 ± 12 (576 ± 66)
Horizontal roots	↓	↑	↓	↑
+ cap	20 ± 3.8 (190 ± 16)	14 ± 1.6 (128 ± 17)	2123 ± 416 (3635 ± 670)	612 ± 88 (1165 ± 137)
– cap	8 ± 1.4 (51 ± 3)	9 ± 2.3 (59 ± 7)	241 ± 33 (683 ± 134)	192 ± 30 (508 ± 100)

^a The arrows represent the direction of transport, *i.e.* in vertical roots from left to right or right to left across the root and in horizontal roots from top to bottom or bottom to top. In vertical roots, direction is defined from the standpoint of a viewer facing a seedling mounted vertically with the primary root toward the viewer and the grain away from the viewer.

Table II. Effect of Metabolic Inhibitors on $^{45}\text{Ca}^{2+}$ Movement across Roots of Maize

Roots were pretreated 90 min with DNP (0.1 mM) or KCN (1 mM) and $^{45}\text{Ca}^{2+}$ movement across the root was determined by applying agar donor blocks containing 30,000 dpm $^{45}\text{Ca}^{2+}$ to the tip (0–1.5 mm region) or the elongation zone (3–4.5 mm region) with a blank agar receiver block applied to the opposite side. Each number represents the average cpm in receiver blocks after 45 min of transport. Numbers in parentheses represent average cpm (with SD) in receiver blocks after 90 min. Averages determined from five experiments with five roots in each experiment.

	Elongation Zone		Tip	
	cpm in receiver block			
Vertical roots				
Control		24 ± 3.2 (123 ± 38)		1301 ± 236 (3062 ± 320)
DNP		58 ± 12 (359 ± 45)		664 ± 74 (1443 ± 264)
KCN		24 ± 9 (120 ± 33)		397 ± 80 (1895 ± 638)
Horizontal roots				
Control	↓ ^a	↑	↓	↑
Control	20 ± 3 (190 ± 34)	14 ± 2.1 (128 ± 18)	1915 ± 157 (5030 ± 240)	697 ± 169 (2389 ± 310)
DNP	51 ± 20 (175 ± 59)	45 ± 21 (231 ± 57)	689 ± 221 (1927 ± 394)	843 ± 291 (1945 ± 737)
KCN	17 ± 4 (104 ± 18)	9 ± 0.9 (148 ± 40)	973 ± 119 (2456 ± 478)	952 ± 244 (2132 ± 418)

^a The arrows represent direction of transport in horizontal roots, i.e. top to bottom or bottom to top. In vertical roots, transport was measured in only one direction since there is no preferential direction of calcium transport across vertical roots (Table I).

tips of gravistimulated roots.

The relationship of asymmetric Ca distribution at the tip to asymmetric growth in the elongation zone is unknown. Calmodulin-stimulated ATPase activity has been detected in crude microsomal fractions from maize tissue (9), while a microsomal Ca^{2+} , Mg^{2+} -ATPase has been identified in barley roots and shown to be stimulated by barley root calmodulin. These findings, plus reports (2, 16) that inhibitors of calmodulin interfere with gravitropism, suggest that calmodulin might play a role in gravity-induced Ca redistribution or in the potential coupling of asymmetric Ca distribution at the tip to asymmetric growth in the elongation zone.

According to the Cholodny-Went hypothesis of gravitropism (18), gravitropic curvature in roots results from a gravity-induced accumulation of auxin to inhibitory levels on the lower side of the root. Since Ca is essential to the polar transport of auxin (8, 16), Ca asymmetry at the tip may play a role in the establishment of the hypothetical auxin asymmetry in the elongation zone. We are testing these possibilities by examining the effects of calmodulin inhibitors and Ca antagonists both on auxin movement and on the initiation of asymmetric growth in gravistimulated roots.

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