Correlations between Gravitropic Curvature and Auxin Movement across Gravistimulated Roots of Zea mays[1]

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ABSTRACT

We compared the kinetics of auxin redistribution across the caps of primary roots of 2-day-old maize (*Zea mays*, cv Merit) seedlings with the time course of gravitropic curvature. [³H] indoleacetic acid was applied to one side of the cap in an agar donor and radioactivity moving across the cap was collected in an agar receiver applied to the opposite side. Upon gravistimulation the roots first curved upward slightly, then returned to the horizontal and began curving downward, reaching a final angle of about 67°. Movement of label across the caps of gravistimulated roots was asymmetric with preferential downward movement (ratio downward/upward = *ca.* 1.6, radioactivity collected during the 90 min following beginning of gravistimulation). There was a close correlation between the development of asymmetric auxin movement across the root cap and the rate of curvature, with both values increasing to a maximum and then declining as the roots approached the final angle of curvature. In roots preadapted to gravity (alternate brief stimulation on opposite flanks over a period of 1 hour) the initial phase of upward curvature was eliminated and downward bending began earlier than for controls. The correlation between asymmetric auxin movement and the kinetics of curvature also held in comparisons between control and preadapted roots. Both downward auxin transport asymmetry and downward curvature occurred earlier in preadapted roots than in controls. These findings are consistent with suggestions that the root cap is not only the site of perception but also the location of the initial redistribution of effectors that ultimately leads to curvature.

The Cholodny-Went model of root gravitropism emphasizes the role of auxin redistribution across the elongation zone in the development of the differential growth inhibition that results in downward curvature (18). Although the differential growth occurs in the elongation zone, the root cap is essential to the response. Removal of the cap results in loss of responsiveness (1, 2). Konings (13, 14) suggested that the columella tissue of the root cap is not only the site of perception (12) but also the site at which transverse redistribution of auxin occurs (see review by Goldsmith [8]). He presented

evidence that, in gravistimulated roots, auxin is asymmetrically transported across the root cap toward the lower side and that auxin can subsequently move back into the elongation zone with asymmetric auxin distribution extending at least 6 mm from the apex. Although Koning's model requires auxin movement from the cap into the elongation zone, much of the evidence from experiments with excised, decapped root segments indicates that auxin transport is preferentially toward the tip (16). However, Davies and co-workers (3, 4) showed that auxin moves basipetally from the cap into the elongation zone in intact roots and they suggested that this occurs through cortical cells. Tsurumi and Ohwaki (17) also found basipetal movement of auxin through segments of *Vicia faba* roots and presented evidence that the predominant pathway for basipetal movement was through the outer part of the root. Hasenstein and Evans (10) compared the asymmetry of auxin transport in intact *versus* decapped roots of maize. They found strong basipetal asymmetry in intact roots and noted that the asymmetry was reversed when the roots were decapped. These findings are consistent with Koning's suggestion that the root cap is the site of auxin redistribution as well as the site of graviperception.

In view of the increasing evidence that the cap may be the site of auxin redistribution during the gravitropic response of roots, we re-examined transverse auxin movement across the caps of gravistimulated roots of maize and compared lateral auxin movement across the cap with the time course of curvature. Our findings support the suggestion that asymmetric auxin movement across the root cap is an important factor in root gravitropism.

MATERIALS AND METHODS

Plant Material

Caryopses of maize (*Zea mays* L. cv Merit) were soaked in distilled water for 10 h prior to planting. The grains were placed between wet paper towels on vertical opaque plastic trays and germinated at room temperature (20–23°C) under fluorescent laboratory lighting (intensity 175 μE · m^{-2} · s^{-1}). After 2 d, seedlings with straight primary roots (approximately 2 cm long) were selected and mounted vertically in Petri dishes lined with moist filter paper. Roots were allowed to equilibrate for 1 h prior to experimentation.

Transport of Label Applied as [³H]IAA

Agar (1.5%) sheets (1.0 cm × 1.0 cm × 1.5 mm) were incubated overnight in distilled water (pH adjusted to 6.5 with NaOH). They were then cut into small blocks (1.5 mm cubes). Blocks used as receivers were used directly while blocks used as donors were allowed to equilibrate with a small volume of [³H]IAA (925 GBq/mmol, Amersham) such that equilibrated blocks contained 1.24 kBq [³H]IAA/block (approximately 0.4 μM).

To measure auxin transport across the cap, donor blocks were applied to one side of the caps of vertically oriented roots. After 30 min of label preloading, receiver blocks were applied to opposite sides of the root caps and the seedlings were gravistimulated by rotating the Petri dishes 90°. Half of the seedlings were oriented so that the donor block was on the top of the root and half with the donor on the bottom. Total auxin transported was measured as movement of label across the cap following stimulation periods of 0 to 15, 0 to 30, 0 to 45,. . . , 0 to 180 min. Following the transport period, the donor and receiver blocks were placed into separate scintillation vials for determination of radioactivity. The value for each data point is the mean of at least 45 roots ± standard error of mean.

Auxin transport across the caps of roots was also monitored at 15 min intervals during a 2 h period of continued gravistimulation. Donor and receiver blocks were simultaneously applied to opposite sides of the root caps of vertically oriented seedlings within the dishes. Following 15 min of gravistimulation, the receiver blocks were removed and placed in scintillation vials for determination of radioactivity. Fresh receiver blocks were then applied to the root and this procedure was repeated every 15 min for 120 min. A minimum of 48 roots was used for each data point.

Determination of Rate of Gravicurvature

Approximately 10 seedlings were mounted vertically in a humid chamber. At zero time the chamber was rotated 90° to bring the roots to a horizontal position and the roots were photographed at 5 min intervals for 180 min. The degree of curvature was measured using a protractor and rate of curvature determined by plotting curvature *versus* time. This experiment was repeated six times.

Prestimulation of Roots

In some experiments roots were intermittently prestimulated prior to steady exposure to the stimulus. Roots mounted vertically in Petri dishes were first rotated 90° and stimulated for 2 min. They were then reoriented 180° and again stimulated for 2 min. This alternate stimulation on opposite flanks was repeated every 2 min for 1 h. Roots prestimulated by alternate gravistimulation in this fashion are referred to here as prestimulated roots. To control for effects that may arise from the manipulation of the roots during prestimulation, some roots were prestimulated every 2 min for 1 h as described but then rotated 90° out of the plane of prestimulation to expose a nonadapted surface to the gravity stimulus. These roots are referred to as prestimulated/rotated roots. Auxin

transport across the root cap and the kinetics of curvature during prolonged stimulation were measured using both prestimulated and prestimulated/rotated roots. Ten seedlings were used for each treatment and the treatments were repeated four times.

RESULTS

Time Course of Gravitropic Curvature

Figure 1 shows the time course of development of curvature under the conditions used for the auxin transport experiments. During the first 20 min of stimulation, control roots displayed a mean upward curvature (−2°) but returned to the horizontal position by 30 min. Continued stimulation resulted in normal downward curvature reaching a maximum angle of approximately 67°. The angle of curvature did not increase with gravistimulation beyond 3 h.

Studies of the kinetics of gravicurvature of roots of the Bear hybrid (WF9 × 38) cultivar of maize have shown that they can be divided into two classes on the basis of their response (5). Class I roots begin downward curvature within 15 min of stimulation. They curve past 90° (70–100 min) and eventually return to an angle of curvature less than 90°. Class II roots exhibit a transient upward curvature initially. They then return to a horizontal position. After about 20 min they curve downward reaching a maximum curvature of about 80°. We have observed that seedling roots of the Merit cultivar are largely class II. Hild and Hertel (11) observed similar initial backward (*i.e.* downward) curvature in gravistimulated coleoptiles of maize. They suggested that this response may result from an overstimulation of the gravity-sensing mechanism, and they found that the initial backward curvature could be eliminated by allowing adaptation to the gravity stimulus by prestimulation of the organ. To test the hypothesis that the initial upward curvature of Merit roots results from overstimulation of the gravisensing mechanism, roots were adapted to the gravity stimulus by prestimulation.

The time course of curvature development in prestimulated roots differed from that displayed by control roots. Gravi-
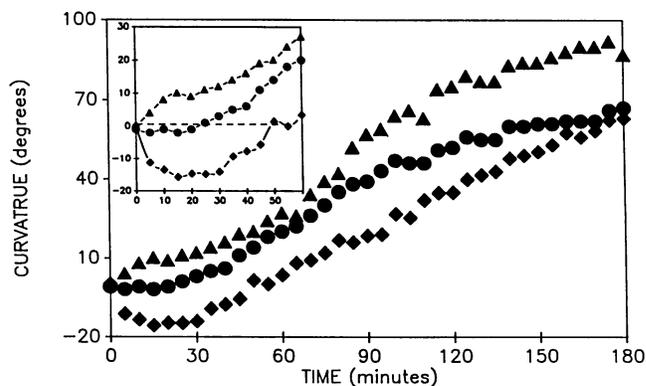

Figure 1. Gravitropic response of control (circles), prestimulated (triangles), and prestimulated/rotated (diamonds) roots. Each data point represents the mean value of 60 roots. Standard error bars not included as values were small (maximum standard error = ± 4°). Inset shows early time course of gravitropic response.

stimulation of prestimulated roots resulted in immediate downward curvature (Fig. 1) with a mean maximum curvature of approximately 87°. Prestimulated/rotated roots showed initial negative curvature (Fig. 1, inset) similar to control roots except that their backward curvature was exaggerated during the first hour of stimulation. The maximum curvature of prestimulated/rotated roots (63°) was similar to that of the controls (67°).

Asymmetric Movement of Label from [³H]IAA Across the Caps of Gravistimulated Roots

Movement of label from [³H]IAA from top to bottom was 28.7 Bq ± 0.5 and from bottom to top was 17.8 Bq ± 0.3 across the caps of gravistimulated roots. Radioactivity was allowed to accumulate in the receivers during the 90 min period immediately following stimulation. A consistent asymmetric movement (downward movement/upward movement) of 1.6 was observed. No asymmetric movement was observed when this experiment was performed using roots from which the caps had been removed (19).

Time Course of Development of Asymmetric Movement of Label from [³H]IAA Across the Caps of Gravistimulated Roots

Figure 2 shows results from experiments testing the time course of the development of asymmetric movement of label from [³H]IAA as a function of time after gravistimulation. Each data point is based on total activity accumulated in the receivers during a transport period from zero time (initial horizontal orientation) to the time indicated by the particular data point. No asymmetric movement of label was detected for the measurements taken 15 to 30 min after stimulation. However, preferential upward movement (upward movement = 833 ± 58 cpm, downward movement = 472 ± 23 cpm, asymmetry = 0.6, n = 14) was consistently observed during the first 5 min. After 45 min preferential downward movement of label (asymmetry = 1.3) was detected. The magnitude of this preferential downward movement increased with time reaching a maximum (1.6) following 60 min of gravistimula-
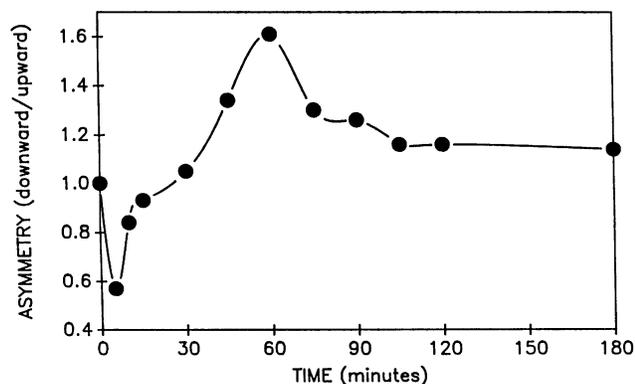
tion. Further stimulation resulted in a decrease in preferential downward movement, with the downward/upward ratio approaching 1.2 after 105 min.

Measurement of Asymmetric Movement of Label from [³H]IAA during Discrete Intervals of the Gravitropic Response

When movement of label was measured at intervals by replacement of receiver blocks every 15 min, we detected a period of constant, preferential downward movement (ratio about 1.4) beginning 45 min after gravistimulation and lasting about 60 min (Fig 3). Thereafter, the downward asymmetry decreased rapidly reaching a value of about 1.2 after 120 min.

Comparison of Curvature and Asymmetric Movement of Label during Gravitropism of Roots of Prestimulated versus Control Seedlings

To test further the correlation between movement of label and curvature we measured curvature and asymmetric movement of label after 30 min of stimulation in both control roots and prestimulated roots. This time period was selected since prestimulated roots were well into positive curvature by that time while control roots showed approximately zero net curvature after going through the phase of negative curvature followed by return to the horizontal (Fig. 1). After 30 min of stimulation strong preferential downward movement of label had developed in prestimulated roots. In fact the magnitude of preferential downward movement of label (1.7) in prestimulated roots measured at 30 min exceeded the maximum asymmetry (1.6, see Fig. 2) obtained for control roots (60 min of stimulation). By contrast, there was little or no asymmetric movement of label across the caps of control roots or prestimulated/rotated roots measured at 30 min.

DISCUSSION

The data obtained in this study are consistent with Konings's (13, 14) suggestion that the cap is the site of auxin
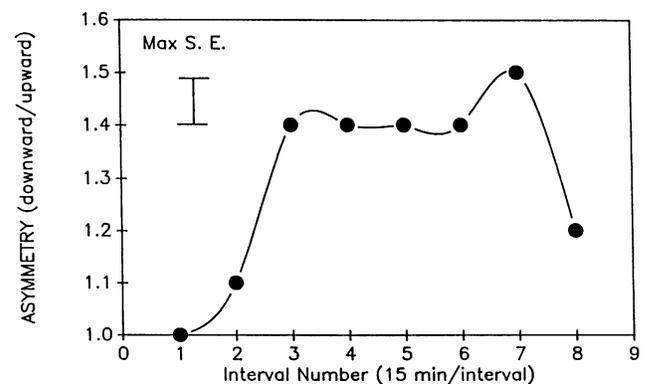
Figure 2. Time course of the development of asymmetric movement of label from [³H]IAA across caps of gravistimulated roots. Each data point represents the mean value of at least 28 roots. The maximum standard error was 0.1 asymmetry unit.

Figure 3. Measurement of asymmetric movement of label from [³H] IAA during discrete intervals of the gravitropic response. Asymmetric movement of label across the root tip was determined at 15 min intervals by regularly replacing the receivers. Each data point represents the mean value of at least 48 roots. Standard error was 0.1 asymmetry unit.

redistribution in gravistimulated roots. They are also consistent with models for root gravitropism suggesting that auxin redistributed in the cap moves back toward the elongation zone through the cortical cells (6, 8). According to this model, auxin moves toward the root cap through the stele and then moves back from the cap toward the elongation zone through cortical cells. In vertically oriented roots, this flow is proposed to be radially symmetrical while in gravistimulated roots it is proposed that auxin entering the cap moves preferentially toward the lower side of the cap. It then enters the transport stream moving auxin back into the elongation zone where the resulting asymmetry in auxin concentration causes the differential growth inhibition leading to curvature. In a study of auxin transport through intact (not decapped) apical sections of the maize cultivar Giant Horse Tooth, Scott and Wilkins (16) reported that very little label moved into the root cap when label was applied either basally or directly to the cap. Although this observation is inconsistent with the model described above, Hasenstein and Evans (10) have since shown that label from applied IAA moves freely through the cap of the maize cultivar B73 × Missouri 17, and Hasenstein (9) has recently found that partial drying of the cap severely retards auxin movement into the cap. That auxin can be transported from the cap to the elongation zone has been demonstrated by Davies et al. (3, 4), Tsurumi and Ohwaki (17), and Hasenstein and Evans (10).

If the cap is the site of auxin redistribution, one would predict a close correlation between the kinetics of curvature and the kinetics of the development of asymmetric auxin movement across the cap. We found this to be the case.

When auxin movement across the tip was monitored as redistribution of label applied as [³H]IAA we saw significant asymmetric (preferentially downward) movement of label across the caps of stimulated roots. The magnitude of preferential downward movement increased with the duration of stimulation reaching a peak asymmetry of about 1.6 (Fig. 2). The time course of the development of asymmetric auxin movement paralleled the time course for curvature (Fig. 4).

Although the general correlation between curvature and movement was strong, we could not detect development of

Figure 4. Correlation between asymmetric movement of label from [³H]IAA (circles) and the rate of curvature (triangles). Standard error (transport asymmetry) ± 0.1 asymmetry unit. The rate of curvature was determined by plotting curvature *versus* time and taking tangents to the curve at the indicated times.

asymmetric auxin movement prior to the initiation of curvature. In the context of the Cholodny-Went theory of root gravitropism it is surprising that curvature develops so quickly relative to the development of asymmetric auxin redistribution. This indicates that there may be very rapid signal transmission from the cap to the elongation zone, more rapid than can be accounted for by normal velocities of auxin transport. Alternatively, the slow development of apparent asymmetric movement of label may be attributable to the method of measurement. Movement was determined by collecting label in agar blocks applied to the surface of the root cap. Using this method, one would expect internal auxin asymmetry to develop prior to asymmetric accumulation in applied agar blocks. Also, according to the model for auxin redistribution during gravistimulation (6), much of the label taken up by the root cap would enter the basipetally moving auxin transport stream, thus delaying the development of auxin asymmetry within the cap.

Prolonged stimulation led to a decline in the rate of curvature as well as a drop in asymmetric movement of label. It seems likely that these changes reflect a reduction in the strength of the gravity stimulus as curvature brings the root apex back toward the vertical orientation. Decreased asymmetry of auxin movement across the cap would result in decreased auxin delivery to the elongation zone on the lower side. This may account for the shape of the curvature rate *versus* time curve.

Although the initial upward curvature in roots of Merit appears to correlate with initial upward auxin movement, we cannot explain why this temporary reverse in preferred direction of auxin movement occurs. Hild and Hertel (11) reported a similar phenomenon in coleoptiles of maize. Initially the coleoptiles responded to gravity with slight downward curvature. The coleoptiles then returned to the horizontal and began normal upward curvature. Hild and Hertel noted that the initial downward curvature correlated with auxin transport to the upper side. When similar experiments were performed using amylomaize, a cultivar with small, slowly sedimenting amyloplasts, the initial phase of downward curvature did not occur. Hild and Hertel interpreted these results as an indication that the initial wrong way curvature of the wild type results from an overstimulation of the gravity-sensing mechanism. They proposed that prestimulation may allow adaptation of the sensory mechanism and permit a response to subsequent stimulation without an initial period of wrong-way curvature.

Based on the work reported here, it appears that the initial upward curvature of Merit roots may also result from overstimulation of the gravity-sensing mechanism. Prestimulated roots curved downward with no initial wrong-way curvature (Fig. 1) and showed greater overall curvature. Surprisingly, prestimulated/rotated roots showed even stronger wrong-way curvature than controls. Although we cannot explain this observation, it merits investigation in more detail. The prestimulation appears somehow to increase sensitivity to gravity on the non-prestimulated sides of the detector cells. Prestimulated roots also exhibited earlier and stronger preferential downward movement of label than control roots.

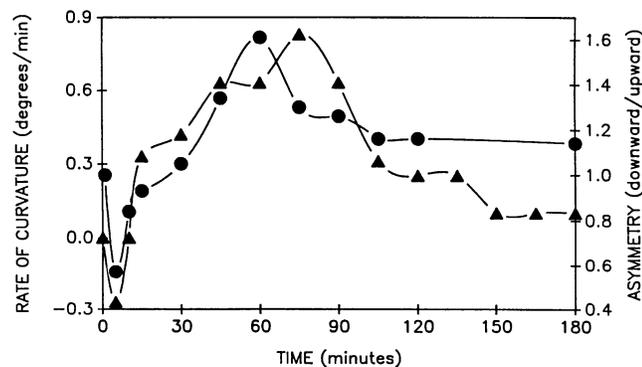These data support the hypothesis that auxin moves into

caps of gravistimulated roots and is then redistributed resulting in curvature (6, 10, 14). It seems unlikely that the auxin controlling curvature is synthesized in the cap since Feldman (7) reported that auxin biosynthesis in roots of this cultivar of maize is strongest in the proximal meristem with no synthesis detectable in the cap. Together, these findings imply that the cap is required for the initial redistribution of auxin during gravitropism.

LITERATURE CITED

1. **Ciesielski T** (1872) Untersuchung über die Abwärtskrummung der Wurzel. Beitr Biol **1**: 1–30
2. **Darwin C** (1880) The Power of Movement in Plants. Appleton and Company, New York
3. **Davies PJ, Mitchell EK** (1972) Transport of indoleacetic acid in intact roots of *Phaseolus coccineus*. Planta **105**: 139–154
4. **Davies PJ, Doro JA, Tarbox AW** (1976) The movement and physiological effect of indoleacetic acid following point applications to root tips of *Zea mays*. Physiol Plant **36**: 333–337
5. **Evans ML** (1985) The action of auxin on plant cell elongation. Crit Rev Plant Sci **2**: 317–365
6. **Evans ML, Moore R, Hasenstein K-H** (1986) How roots respond to gravity. Sci Am **255**: 112–119
7. **Feldman LJ** (1981) Effect of auxin on acropetal auxin transport in roots of corn. Plant Physiol **67**: 278–281
8. **Goldsmith MHM** (1977) The polar transport of auxin. Annu Rev Plant Physiol **28**: 439–478
9. **Hasenstein K-H** (1989) IAA transport in corn roots includes the root cap (abstract No. 555). Plant Physiol **89**: S–93
10. **Hasenstein K-H, Evans ML** (1988) Effects of cations on hormone transport in primary roots of *Zea mays*. Plant Physiol **86**: 890–894
11. **Hild V, Hertel R** (1972) Initial phases of gravity-induced lateral auxin transport and geotropic curvature in corn coleoptiles. Planta **108**: 245–258
12. **Juniper BE, Groves SG, Landau-Schachar A, Audus LJ** (1966) Root cap and the perception of gravity. Nature **209**: 93–94
13. **Konings H** (1967) On the mechanism of the transverse distribution of auxin in geotropically exposed pea roots. Acta Bot Neerl **16**: 161–176
14. **Konings H** (1968) The significance of the root cap for geotropism. Acta Bot Neerl **17**: 203–211
15. Deleted in proof
16. **Scott TK, Wilkins MB** (1968) Auxin transport in roots. II. Asymmetric flux of IAA in *Zea* roots. Planta **83**: 323–334
17. **Tsurumi S, Ohwaki Y** (1978) Transport of ^{14}C-labeled indoleacetic acid in *Vicia* root segments. Plant Cell Physiol **19**: 1195–1206
18. **Went FW, Thimann KV** (1937) Phytohormones. Macmillan, New York
19. **Young LM** (1988) The Roles of Arymmetric Auxin and Calcium Transport in the Gravitropic Response of Maize Roots. Ph.D. thesis, Ohio State University, Columbus, OH