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Analysis of Growth Patterns During Gravitropic Curvature in Roots of *Zea mays* by Use of a Computer-based Video Digitizer

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Abstract. A computer-based video digitizer system is described which allows automated tracking of markers placed on a plant surface. The system uses customized software to calculate relative growth rates at selected positions along the plant surface and to determine rates of gravitropic curvature based on the changing pattern of distribution of the surface markers. The system was used to study the time course of gravitropic curvature and changes in relative growth rate along the upper and lower surface of horizontally-oriented roots of maize (*Zea mays* L.). The growing region of the root was found to extend from about 1 mm behind the tip to approximately 6 mm behind the tip. In vertically-oriented roots the relative growth rate was maximal at about 2.5 mm behind the tip and declined smoothly on either side of the maximum. Curvature was initiated approximately 30 min after horizontal orientation with maximal (50°) curvature being attained in 3 h. Analysis of surface extension patterns during the response indicated that curvature results from a reduction in growth rate along both the upper and lower surfaces with stronger reduction along the lower surface.

In a recent review on root gravitropism, Jackson and Barlow (1981) pointed out that "before any theory that invokes the action of growth-inhibiting or -promoting hormones can be properly formulated, it is necessary to ascertain whether bending results from an *acceleration* of growth on the upper side, or an *inhibition* on the lower, or both, when compared with roots that are not undergoing geocurvature." Unfortunately, attempts to measure relative growth rates (RGR) along the upper and lower surfaces of graviresponding roots have led to variable and sometimes conflicting reports (Pilet and Ney 1981).

Horizontal positioning of roots is reported either to accelerate root growth (Keeble et al. 1931), inhibit root growth (Brain 1935; Bennet-Clark et al. 1959; Konings 1964; Bejaoui and Pilet 1977), or to have no effect (Cholodny 1932; Navez 1933). In most cases the data indicate an overall reduction in growth rate in horizontally-oriented roots.

A number of workers have made comparisons of the growth rate along the upper and lower surfaces of gravistimulated roots to determine the growth pattern responsible for gravicurvature. Results from such experiments have varied depending on the species studied and the conditions employed. Data from studies of primary roots of *Vicia faba* indicated that downward curvature arose mainly via an acceleration of elongation on the upper side (Veen 1964) or via an acceleration on the upper side accompanied by a deceleration on the lower side (data of Sachs described by Jackson and Barlow 1981). Konings (1964) reported that the growth pattern behind gravitropic curvature in submerged pea roots is complex. During the first hour, growth is somewhat enhanced along the top and strongly reduced along the bottom. Later, growth is enhanced along the bottom and reduced along the top, resulting in a temporary partial reversal of curvature. Still later, the pattern returns to one of strong inhibition along the bottom with either a slight promotion or less inhibition along the top, resulting in resumption of positive curvature. The pattern is much simpler in pea roots kept in moist air. Positive curvature occurs as a result of growth inhibition along both the top and bottom of the root with stronger inhibition along the bottom. Audus and Brownbridge (1957) also studied pea roots and noted that the early stages of gravitropic curvature appeared to arise primarily from decelerated growth along the lower side, with only a slight enhancement along the top. Later in the curvature response, the pattern shifted to one in which the growth rate was reduced on both the upper and lower sides, with stronger growth reduction on the lower side.

Darbelley and Perbal (1984) reported that curvature within the main portion of the elongation zone of gravistimulated roots of lentil results primarily from reduced growth on the lower side. Similarly, Pilet et al. (1974, 1981) observed that georeaction in maize roots is initially due to an overall decrease in growth with a greater decrease on the lower side. In the later stages of curvature, however, they noted that growth appeared to be slightly enhanced on the upper part of the elongating zone. Barlow and Rathfelder (1984) used a photographic method to measure localized growth patterns in gravistimulated roots of maize. They found that, in the 20 to 25°C temperature range, gravitropic curvature resulted both from a stimulation of growth along the upper side and a reduction of growth along the lower side. In the temperature range 30 to 35°C, curvature resulted only from a reduction in growth along the lower side without acceleration along the upper side.

Some of the conflicting reports on growth patterns during root gravitropism may arise from variations in growing conditions and seedling age, or from real differences in response patterns from species to species. However, it is also likely that some of the variability can be attributed to insufficient precision in measurement of localized growth rates. To overcome this difficulty, we have developed an automated computer-based system for analyzing surface extension patterns in gravistimulated roots. In a preliminary report (Lee et al. 1983), we described our video digitizing method using an Apple II+ computer for analysis of growth patterns in graviresponding roots. Our system is similar to one developed by Telewski et al. (1983), which was used for measurement of changes in cellular dimensions in photomicrographs and for analysis of growth in maize seedlings (Wakefield et al. 1983; Jaffe et al. 1985). Our system has the advantage of allowing measurement of RGR at localized regions along an extending surface.

We have recently enhanced the capabilities of our system by switching to a higher resolution digitizing board in an IBM personal computer, increasing the magnification of the video camera, and developing an automatic search routine for tracking of markers on the surface of the plant organ. Although our system does not measure instantaneous surface extension rates as was done for straight root growth with the streak photography method developed by Erickson and Goddard (1951) and Erickson and Sax (1956), it yields good approximations of relative elemental growth rate (REGR) with sufficiently small time intervals and distances between markers. In addition, it offers a sophisticated means of assessing multi-dimensional growth patterns where continuous growth measurements by streak photography are inapplicable. An alternative method was developed by Silk and Erickson (1978) and Erickson and Silk (1980), employing time lapse photography for analysis of growth patterns during curvature of lettuce hypocotyls. Photographs were taken at 2 h intervals. Our computer system facilitates more frequent data collection, therefore providing a better estimate of REGR. In this paper, we describe the system and discuss its utilization for analysis of localized surface extension patterns in graviresponding roots of maize.

Materials and Methods

Plant Material

Maize grains (*Zea mays* L. B73 × Missouri 17, Mike Brayton Seeds, Ames, Iowa) were soaked in distilled water overnight and then placed between wet paper towels on plastic trays (30 × 40 × 1.5 cm; width, length, height). The embryos of the grains were placed against the back surface of the trays and aligned along the vertical axis. The trays were stacked together vertically in a plastic container, with about 5 cm of water on the bottom. Although the trays were kept under laboratory lighting at 28°C, the developing seedlings received little light and were etiolated. The seedlings were used 2 days after planting when the primary roots were approximately 2.5 cm long.

Preparation for Growth Measurement

To facilitate extension measurement, glass beads (ca. 0.25 mm, Arthur H. Thomas Co., Philadelphia, PA), which were stained black, were placed at regular intervals (ca. 0.5 mm) along one side of a root. The seedling was then mounted on the inside of a vertically-oriented Petri dish using putty to attach the grain to the dish. The back of the dish was covered with white felt to provide maximum contrast with the black glass beads.

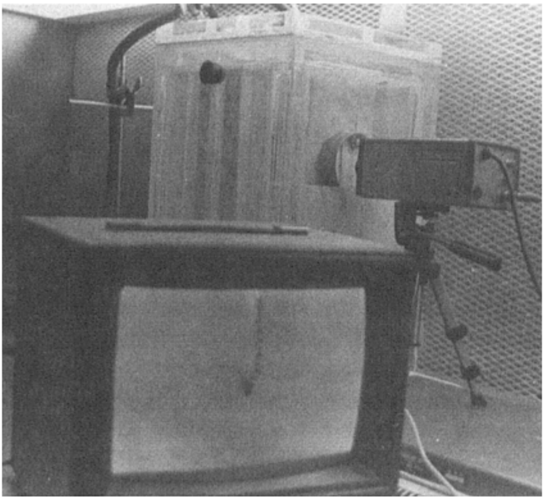

Fig. 1. Video digitizer system showing the TV camera, the high humidity Plexiglas chamber containing the seedling, and the CRT monitor. The IBM PC, with the digitizer board, is located just out of the picture.

The Petri dish containing the seedling was mounted vertically inside a reinforced Plexiglas chamber (30 × 30 × 30 cm) maintained at 28°C and illuminated with Sylvania Lifeline cool white lights (60 μE m^{-2} s^{-1} at seedling level). The relative humidity inside the chamber was maintained at 100%, as measured with a hygrometer (G. Lufft GmbH, Inc., Stuttgart, W. Germany), by lining each wall of the chamber (except the portion of the front wall used as a viewing port) with wet paper towels in contact with 5 cm of standing water at the bottom of the chamber. Air was bubbled into the water through an air stone at a flow rate of 5000 ml min^{-1}.

Video Digitizer System

The displacement of the marker beads during the growth and gravitropic curvature of the roots was monitored using a Panasonic model WV-1500 TV camera fitted with a 16 mm lens (Fig. 1). Video output went to a CRT monitor (Hitachi model VM-129U), and a video digitizer (Video Van Gogh, Tecmar, Inc., Cleveland, OH) in an IBM personal computer (256K). The digitizer reproduced the video image with an array of 256 × 256 pixels (65,536 total), each with a light level ranging from 0 (black) to 255 (white). With the 16 mm lens, the resolution of the digitized image was 62 μm/pixel.

A program was written for automatic tracking of the glass bead markers during displacement by growth. The program operated by first requiring manual cursor location of all of the markers. It then continually checked their locations based on light levels (markers = 0, background and roots > 0). When a given marker moved between trackings, as a result of extension of the surface on which the marker was situated, the cursor automatically went into a search routine to determine its new location. In this manner, the location of all of the markers was known almost continuously. Experiment duration and the

Table 1. Sample of X,Y-coordinate versus time data.

X-coordinate	Y-coordinate	[a]Time (min)
163	103	0
163	92	0
163	103	2
163	92	2
163	104	4
164	92	4
163	104	6
163	93	6
163	105	8
163	93	8
163	105	10
163	93	10
163	105	12
164	93	12
163	106	14
164	93	14
163	106	16
163	94	16
163	107	18
165	93	18
163	107	20[b]
164	94	20
163	110	30
164	96	30
164	114	40
164	97	40
164	116	50
163	99	50
163	118	60
163	100	60

[a] Individual 2 min readings are shown for the first 20 min and for[b] 10 min intervals thereafter. The data represent the positions of two adjacent markers in the region of maximum elongation, collected at 2 min intervals. The root was mounted in the vertical position (extension along Y axis). One unit on Y axis = 0.062 mm. Initial spacing of the two markers approximately 0.68 mm.

time interval for recording of marker locations were specified by keyboard input at the beginning of each experiment. The collected data (marker locations and elapsed time) were stored in an array (Table 1). Upon completion of each experiment, the stored data were transferred from the array to a floppy or hard disk (IBM) where they were stored as a sequential file.

Analysis of Position versus Time Measurements

As growth occurred, the distances between marker beads increased. The displacement of the beads was used to monitor growth patterns on the surfaces of vertically- and horizontally-oriented roots. The data files, stored as described previously, contained the X and Y coordinates of the marker locations and the elapsed time. These values were used to calculate relative growth rates as a function of position along the root. By analogy with bacterial colony growth, extension of small surface regions over short periods of time can be assumed to be exponential and hence approximated by the formula:

$$r = (\ln L_2 - \ln L_1)/(t_2 - t_1)$$

where r = relative growth rate (% h^{-1}), L_1 = length at time 1 (t_1), and L_2 = length at time 2 (t_2) (Green 1976).

The change in length of a segment of root surface over time was used to calculate the RGR for that segment. The location of that rate was taken to be in the middle of the segment, and the time corresponding to that rate in the middle of the time interval used for the rate calculation. These RGR's are conceptually identical to REGR's. Theoretically, discrepancies between calculated rates and REGR's can be eliminated by reducing the distance between markers and the time interval between measurements. To determine whether the length of the time interval chosen is sufficiently short to allow accurate estimates of instantaneous rates, the time interval between measurements can be repeatedly halved until no significant shift in the growth curve accompanies further shortening of the measurement interval (Silk 1984).

The locations of calculated RGR's were expressed as distance from the root tip (mm), and were continually adjusted to account for the elongation occurring in more apical zones. These values along with their respective RGR's were stored in a new sequential file.

The glass bead marker locations were also used to calculate curvature in horizontally-oriented roots. First, slopes were calculated for lines passing through the first two markers at the basal (non-growing) portion of the root (slope m_1) and for two markers near the tip (slope m_2) by the equation:

$$\tan \theta = (m_2 - m_1)/(1 + m_2 m_1) \quad 0 \leq \theta < 180$$

Although this method of curvature description is somewhat less sophisticated than methods which describe curvature at various points along the curving surface or which evaluate the radius of curvature (Silk and Erickson 1978), it provides an accurate and convenient estimation of the kinetics of curvature development. The degree of curvature was determined at each sample time, and stored along with the elapsed time in a separate sequential file.

These files, containing either relative growth rate or degree of curvature data, were compiled from several experiments using different seedlings. The data were sorted, averaged, and smoothed, and then sent to a Line Chart Program (Energraphics, Enertronics Research, Inc., St. Louis, MO), capable of fitting a variety of line types to the data. The high-order regression line type fits a curve with a polynomial equation of the form:

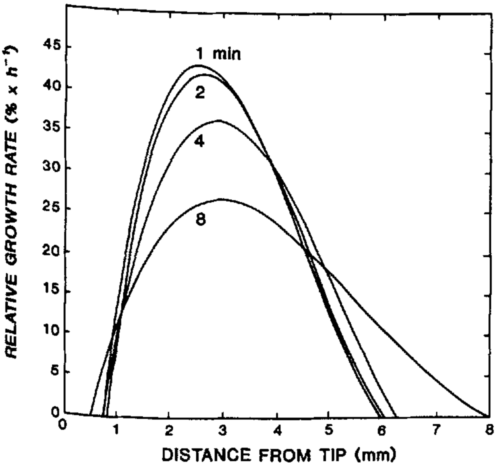

Fig. 2. Dependence of relative growth rate distribution on time interval between recording of marker positions. The four curves represent rates calculated from position data taken from the same 5 roots at different frequencies. Time (min) between data sampling is indicated by number adjacent to each curve.

$$Y = A + (B \times X) + (C \times X^2) \ldots$$

Use of a fourth degree polynomial provided a reasonable balance between good fit and smoothness, avoiding both the strong fine structure oscillations characteristic of higher degree polynomials and the reduced accuracy of lower degree polynomials. All polynomial fitted curves were printed as graphs and used to compare growth and curvature patterns under various conditions.

Results

Dependence of Relative Growth Rate Values on Time Between Measurements

To determine the measurement interval that provides an accurate approximation of instantaneous growth rates and total length of the extending region, data on RGR versus position were collected using measurement intervals varying from 1 to 8 min (Fig. 2). Increasing the frequency of measurements resulted in a shortening of the apparent zone of elongation at the basipetal end, and an increase in the apparent maximal rate of elongation. The maximum RGR appeared to increase from 25% h^{-1} for 8 min intervals to 43% h^{-1} for 2 min intervals, and the basipetal end of the elongation zone appeared to shift from 8 mm to 6 mm from the tip. The apparent shift in the basal limit of the elongation zone away from the apex with decreasing frequency of data collection most likely reflects the additional total root extension that occurs during long measurement intervals. This would be expected to cause an apparent shift in the basal boundary of the elongation zone away from the apex since the position assigned to the calculated rate for each measurement interval is based on the distance between the tip and the center of that measurement interval. The apparent decrease in relative growth rate with decreasing frequency of data collection is most likely indicative of a narrow zone of peak RGR. For short-term measurements, the data would be collected primarily or exclusively from

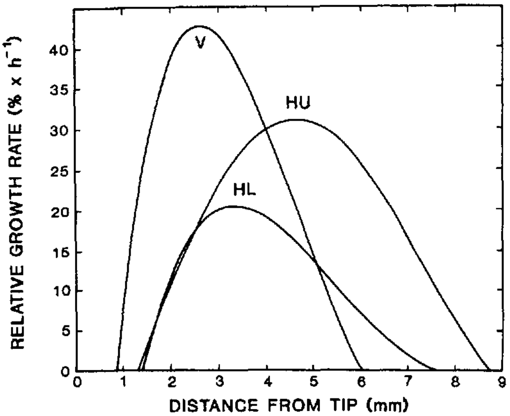

Fig. 3. Relative growth rate (% h^{-1}) as a function of distance from the root tip in vertically- and horizontally-oriented roots. Each curve is the best fit through relative growth rate versus position data from 5 roots, with marker bead positions recorded every 2 min for a 2 h period. Data for vertical and horizontal orientation are from the same roots. V = vertical; HU = horizontal, upper side; HL = horizontal, lower side.

that zone. For longer measurement intervals, the markers may be displaced to adjacent regions of reduced RGR resulting in a lower average rate for that measurement interval. No further change in the growth curve occurred when the measurement interval was reduced from 2 min to 1 min. Therefore, growth rate data obtained using 2 min measurement intervals were used to approximate instantaneous growth rates along the root surface.

Growth Rate versus Position in Vertical and Gravistimulated Roots

Figure 3 indicates the dependence of RGR on distance from the tip in roots of maize oriented vertically and horizontally. The figure is redrawn from a computer print-out obtained from the Energraphics software, after compilation of growth rates measured in 5 roots, at intervals of 2 min for a total of 2 h.

The region of maximal elongation occurred approximately 2.5 mm behind the tip in vertically-oriented roots, with the rate decreasing on either side of the maximum to yield a bell-shaped curve of RGR versus distance from the tip. In horizontally-oriented roots the region of maximal elongation was displaced basipetally, as was the entire extending zone. Maximal surface extension occurred approximately 4.5 mm from the tip on the upper side and 3.5 mm from the tip on the lower side. Preliminary experiments of greater overall duration (4 h), indicate that this basipetal shift in the elongation zone may be transient, and the zone may return to a more acropetal region after several hours (data not shown).

In vertically-oriented roots, the maximal relative rate of elongation was about 43% h^{-1}. The maximal rate observed for horizontally-oriented roots was about 32% h^{-1} along the upper surface, and 20% h^{-1} along the lower surface.

Time Course of Gravitropic Curvature

Curvature began after about 0.5 h in horizontally oriented roots, with maximal (ca. 50°) curvature reached in about 3 h (Fig. 4). The type of line chart which

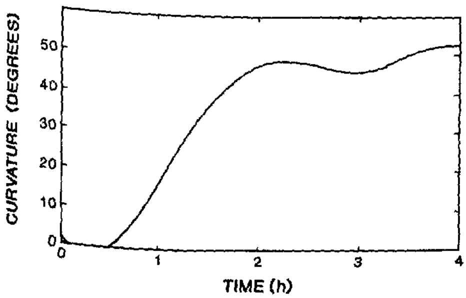

Fig. 4. Time course of curvature development as determined by digitizer measurement of marker bead positions. Curvature represents the angle between the slope of a tangent to the pair of marker beads nearest the basal portion of the root and the slope of a tangent to the pair of beads nearest the tip.

best estimated the relationship of degree of curvature to time in the horizontal position was a 6th degree polynomial regression.

Discussion

The results indicate that gravitropic curvature in roots of this cultivar of maize occurs by an inhibition of growth on both the upper and lower surfaces, with greater inhibition on the lower surface. That is, gravitropic curvature occurs by a differential inhibition of growth. This is consistent with the Cholodny-Went hypothesis for tropisms, and it is in agreement with earlier studies showing that deceleration of growth along the lower side is a major factor in establishing positive gravitropic curvature in roots. However, it should be pointed out that the pattern of differential inhibition observed for root gravitropism in this cultivar of maize may not apply to seedlings of other cultivars or species. The computer-assisted video digitizer method described here would be useful for making comparative measurements of the growth pattern behind gravitropic curvature in roots of various species.

The data indicate that the gravitropic response may involve a shift in the region of maximal elongation and in the overall extent of the elongation zone on both the upper and lower sides. More detailed characterization of this apparent alteration in the elongation zone in gravistimulated roots will require similar experimentation using higher resolution video equipment (see below). The transient nature of this shift could be verified by comparison of growth rate distribution at various times after gravistimulation.

As the resolution is about 62 μm with the video equipment used in these experiments, a tracking marker must be displaced at least 0.06 mm to be detected as a growth increment by the computer. Using a higher resolution lens (50 mm), and silicon carbide particles (Buehler Ltd., Evanston, IL) as markers, the resolution can be increased to about 10 μm. At this magnification, however, only about 1 to 2 mm of the root surface can be analyzed at a time. This higher resolution system should be useful in studying possible changes in the boundaries of the elongation zone upon gravistimulation.

The combination of this higher resolution system (allowing closer spacing of markers) and shorter time intervals for measurement should help to ensure that calculated growth rates closely estimate instantaneous elemental rates. An even more accurate determination of RGR might be obtainable by smoothing and differentiation of position and velocity data. This method would have the advantage of not depending on the validity of the assumption that root growth is truly exponential.

In addition to its use in evaluating multidimensional growth patterns, the surface extension analysis system described here should also prove useful for remote sensing of elongation patterns in plants placed under conditions (e.g., hypobaric treatment) not amenable to study by growth measuring devices requiring direct contact with the plant.

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