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Regions of Differential Cell Elongation and Mitosis, and Root Meristem Morphology in Different Tissues of Geotropically Stimulated Maize Root Apices[1]

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ABSTRACT

We examined cell length, mitosis, and root meristem "cuticle" in different tissues of geostimulated, red light-exposed primary roots of corn (Zea Mays, Wisconsin hybrid 64A × 22R). The examination was done at 15-minute intervals for a period of 240 minutes. Differences in cell elongation between the upper and lower sides were most prominent between 1.5 and 2.5 mm from the root meristem; the outer cortex had the greatest elongation growth, and the upper cells showed a significant increase in length compared to the lower. A differential mitosis was also found, with the lower tissue being greater. We infer that the mitotic activity is indicative of cell division, and this division occurs strictly in the first 1.5 mm of the root meristem. The combined effect of differential cell elongation and cell division results in the localization of the geotropic curvature in the 1.5- to 2.5-mm region from the root meristem. Mitosis that occurs primarily in the cortex and stele were asynchronous; the peak of cortical division preceded that of the stele. Both peaks occurred before the peak of geotropism. A densely stained layer separates the cap from the root meristem. This layer is thinner at the apex of the root meristem. The area of the thin region increased with time and peaked at 180 minutes after geostimulation, which was coincidental with the peak of the geotropic response.

This study was initiated to locate precisely the region and the cells that are responsible for the geotropic curvature in primary roots of corn. We estimated cell lengths in the first 4 mm of the epidermis, and one row each of the outer, mid, and inner cortex, and counted the mitotic figures in the above tissues plus the entire cortex, the endodermis, pericycle, and vascular tissues. The correlation of the cell length and cell division data enable us to explain why the curvature response begins at a specific location of the roots. Examinations were at 15-min intervals for 240 min following red light irradiation and geotropic stimulation.

MATERIALS AND METHODS

Planting. Corn seeds (*Zea mays*, Wisconsin hybrid 64A × 22R) were soaked in initially warm water (50 C) for 8 hr, then the water was decanted and the seeds held at 4 to 6 C for about 20 hr in complete darkness. The cold treatment induced uniform germination. The seeds were planted on the edges of lucite bars (23 × 2.5 × 5 cm) wrapped with moist filter paper. The bars were placed in deep enamel pans partially filled with water. The pans were covered and kept in complete darkness at 25 ± 1 C. After 40 hr of growth, the primary roots were ready for use at a length of 2 to 3 cm. Most of the roots were straight and grew horizontally; they were under continuous geotropic stimulation during growth and experimentation. A dim green light, 540 to 580 nm with peak transmission at 560 nm (16), was used for planting of the seed.

Irradiation and Gravity Stimulation. The primary roots of corn show no geotropic curvature until they are exposed to light (unpublished data). The horizontal roots were exposed to red light of 661 nm at 1 Jm^{-2} for 60 sec using a Baird Atomic interference filter with a bandwidth at half-maximum of ± 23 nm. Irradiation was from 55 cm above the roots with a 500 w-120 v Sylvania projection lamp in a Sawyer projector. The intensity of the lamp was controlled by varying the voltage, and measured by a Hewlett-Packard radiant flux meter. The roots were not exposed to any light other than the red irradiation mentioned above. All manipulations were done in complete darkness. The red-exposed roots, in horizontal position, were returned to the growth pan and kept moist again in complete darkness until harvest.

Histology. Harvesting was carried out in a dim green light at 15-min intervals for 240 min following the red exposure. The tip 5 mm of six roots was harvested by a slantwise cut to differentiate the upper and lower side, the shorter end being the lower side with respect to gravity. The tips were dropped immediately into a fixative containing 3% glutaraldehyde, 0.05 M Na·cacodylate buffer (pH 6.8), and 0.1% $CaCl_2$. The fixation was at room temperature for 3 hr and then at 31 ± 1 C for 3.5 hr on a slanted horizontal rotor (5 rpm). The tissues were then rinsed repeatedly with the buffer (at room temperature). The tissues were fixed again in 1% OsO_4 in 0.1 M Na-cacodylate buffer (pH 6.8) at room temperature for 4 hr in the dark. They were rinsed twice in the 0.1 M buffer.

Dehydration was done in a graded series of acetone, terminated with 100% dry acetone (dried by molecular sieve) for 30 min. The dehydrated tissues were infiltrated by a Spurr's low viscosity epoxy resin, medium firm (19). Infiltration was done at 31 C on the same rotor used for fixation, with a series of resins within a 24-hr period. The infiltrated tissues were embedded in 100% resin in flat boats and cured at 60 C for at least 24 hr.

The root tips were sectioned longitudinally on an AO-Spencer 820 microtome which was modified to use a glass knife. The tissue sections were stained on a warm (60 C) hot plate in a solution of 1% toluidine blue and 1% borax for 3 min, rinsed with double-distilled H_2O, and counter-stained in 2% basic

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fuchsin for 1.5 to 2 min and then rinsed. Three longitudinal sections, 3 μm thick, taken from sections 50 μm apart, were obtained from the central region of each root. The first tissue section was taken at the appearance of the metaxylem cells.

Cell Length. Three sections from each of three roots were examined at each harvest. For average cell length determination, the number of cells in each 0.5-mm section was counted from 0.5 to 3.5 mm from the cap-to-root juncture (the junction between the root cap and root meristem [Fig. 1)]. To estimate cell lengths, cell numbers per unit length, in both upper and lower sides, were counted for the epidermis, outer cortex (the row of cells next to the epidermis), midcortex (the middle row of the cortex), and inner cortex (the row next to the endodermis).

Mitotic figures were counted in each 0.5-mm segment, at each harvest time, by rows, beginning at the root meristem (cap junction) in the epidermis, the entire cortex, and in the stele (endodermis, pericycle, and all of the vascular cells). No attempts were made to distinguish the different stages of mitosis. Mitosis is absent 1.5 mm behind the cap junction. The mitotic counts were taken at time intervals of 15 min for a total of 240 min.

Root Meristem Morphology. The junction between the root cap and the root proper (the "dome"), or the root meristem region, is separated by a densely stained (purplish blue) layer. The layer is thinner in the center and thicker toward the periphery of the root meristem, and extends beyond the root cap and becomes the outer layer covering the root epidermis (Figs. 1 and 2). The area (or diameter) of the thin region was measured with a micrometer under a microscope at each harvest.

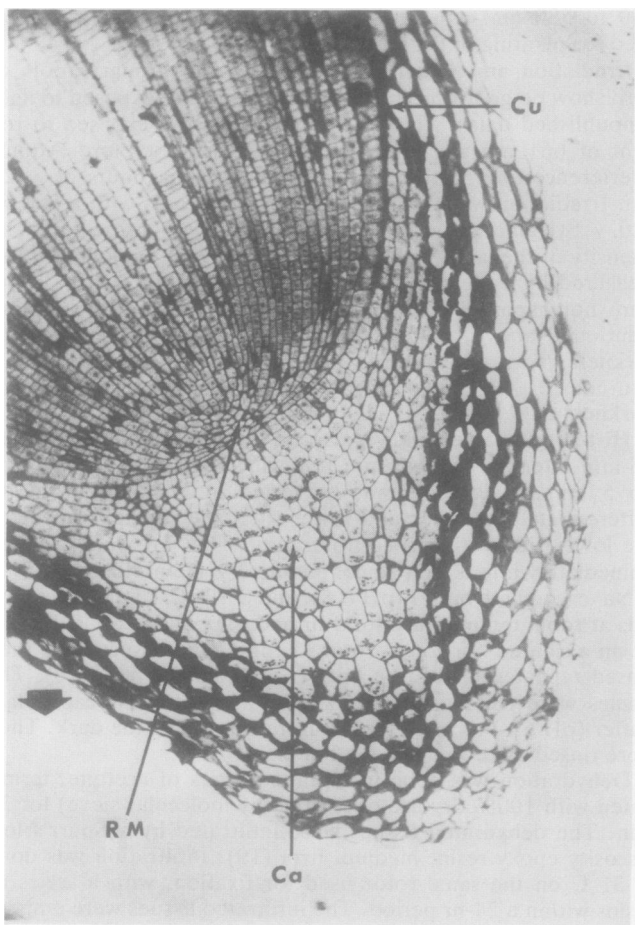

FIG. 1. Corn root apex showing the root cap (Ca) and the root meristem (RM). The cap and the root meristem are separated by a densely stained layer we call the cuticle (Cu); this layer is thinner at the apex of the root meristem and becomes thicker at the arrow indicating Cu. The diameter across the apex encompassing the thin region is measured and presented in Figure 5. The thick arrow indicates the direction of gravity.

RESULTS

Cell Length. Average cell lengths in the different regions of four different tissues are shown in Tables I to IV. Since the geotropic curvature begins 75 min after red light exposure and geostimulation, the statistical comparisons of cell lengths between the upper and lower tissues were done from 75 through 240 min. In the epidermis (Table I), the cells of the upper tissue in the regions of 0.5 to 1.5 and 2.0 to 2.5 mm from the apex of the root meristem are significantly longer than the cells in the lower tissue. Significant differences are seen also in the outer cortex in the region 1.5 to 2.5 mm (Table II) and in the midcortex, 0.5 to 1 mm (Table III). The inner cortical cells show no difference in length between the upper and lower tissues (Table IV). The greater cell length of the outer cortex in the upper tissues can be visualized in Figure 2. The 1.5- to 2.5-mm region (measuring from the root meristem) shows prominent and persistent curvature. This region measures 2 to 3 mm from the root tip, the root cap being 509 ± 3.87 μm long. A comparison of cell length for the first 4 mm of the corn root shows the outer cortical cells to be longer than the cells in any other row of tissue.

Mitotic Figures. Light microscopy of corn roots showed that cells with mitotic figures are concentrated in the first 1-mm region from the apex of the root meristem (Table V). Mitotic figures are absent in the root cap cells, except in the cap

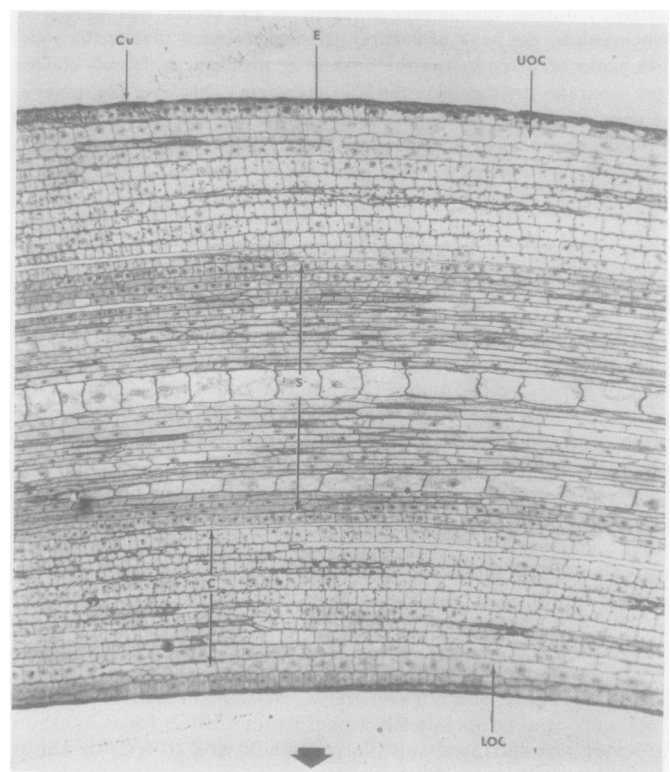

FIG. 2. Geotropic curving region of the corn root, 1.5 to 2.5 mm from the root meristem. The thick arrow indicates the direction of gravity. The cells in the upper outer cortex (UOC) are longer than those in the lower (LOC). The epidermis is covered by a densely stained cuticular layer (Cu). This layer covers the root meristem and extends basally to the root epidermis. E: epidermis; C: cortex (about 11 rows of cell on the lower side), S: stele.

Table I. Average Cell Length of Upper (U) and Lower (L) Epidermal Cells of Primary Roots of Corn.

Stimulation (min)	Distance from Root Meristem (mm) 0.5-1.0 U	0.5-1.0 L	1.0-1.5 U	1.0-1.5 L	1.5-2.0 U	1.5-2.0 L	2.0-2.5 U	2.0-2.5 L	2.5-3.0 U	2.5-3.0 L	3.0-3.5 U	3.0-3.5 L
	(μm)											
0	10.4	10.3	10.9	10.7	15.3	15.2	24.6	24.0	34.9	35.0	49.0	(50.0)
15	10.4	9.98	11.2	11.4	16.8	16.8	27.5	27.5	38.0	40.0	52.6	-
30	11.0	10.1	11.3	10.8	16.7	16.0	27.3	28.6	38.5	41.7	50.0	56.6
45	10.7	11.5	10.6	11.2	16.0	14.9	23.6	22.9	36.1	35.3	50.8	-
60	11.0	10.6	11.2	11.7	18.2	18.2	30.9	33.3	47.6	50.0	69.8	55.6
75	9.96	9.49	11.2	11.7	16.8	16.4	29.9	26.3	40.6	39.1	54.4	-
90	10.8	11.0	11.0	11.4	14.9	15.4	24.4	22.9	32.3	35.7	46.7	47.6
105	11.0	11.1	10.5	10.5	14.5	16.0	28.2	27.0	43.5	40.5	51.5	(58.5)
120	10.4	10.0	11.5	10.9	16.5	16.6	27.0	26.6	36.5	39.0	51.5	(50.0)
135	10.5	9.50	11.7	10.8	16.4	15.5	25.0	25.0	35.7	35.7	47.6	-
150	11.2	10.1	11.3	10.4	17.3	17.1	27.8	28.2	39.4	40.0	51.5	-
165	10.6	10.0	10.6	10.2	18.2	15.1	27.8	25.2	40.0	38.0	49.2	51.0
180	10.5	10.2	11.1	9.90	16.8	15.2	27.3	25.3	37.9	38.5	45.5	51.0
195	10.6	10.2	10.6	10.2	15.4	14.8	25.0	24.0	36.1	36.6	50.8	45.4
210	10.9	11.1	12.2	11.9	19.4	20.4	30.9	31.6	46.2	42.7	55.6	(66.7)
225	10.3	10.2	11.1	10.9	16.8	17.3	26.3	26.3	35.3	33.0	44.1	(44.1)
240	-	-	-	-	18.1	16.8	26.8	25.0	-	-	-	-
$F^{\dagger}$	7.02		5.15		1.07		8.43		0.40		-	
$P^{\dagger}$	< 0.025*		< 0.05*		> 0.2		< 0.025*		> 0.2		-	

†2-way analysis of variance between 75 and 240 min of gravity stimulation and red-light irradiation

*Significantly different between U & L Cell Lengths

( ) Only 1 to 2 root sections tabulated

Table II. Cell Lengths of Upper (U) and Lower (L) Outer Cortical Cells of Primary Roots of Corn.

Stimulation (min)	Distance from Root Meristem (mm) 0.5-1.0 U	0.5-1.0 L	1.0-1.5 U	1.0-1.5 L	1.5-2.0 U	1.5-2.0 L	2.0-2.5 U	2.0-2.5 L	2.5-3.0 U	2.5-3.0 L	3.0-3.5 U	3.0-3.5 L
	(μm)											
0	10.2	10.2	14.9	14.9	26.7	26.0	41.3	42.0	76.9	60.1	81.9	(71.4)
15	10.4	10.2	17.6	17.5	30.0	33.3	46.9	52.6	69.8	71.4	93.8	-
30	11.8	11.3	17.0	19.1	30.3	33.3	53.6	50.8	69.8	75.0	90.9	107
45	10.6	11.2	16.0	16.4	28.8	30.0	48.4	45.4	81.1	63.8	88.2	-
60	11.4	10.9	17.3	19.4	33.0	30.0	50.0	55.6	75.0	83.3	120	100
75	9.84	9.77	15.6	13.4	28.9	26.3	54.4	49.0	83.3	73.5	132	-
90	10.5	10.6	14.7	14.7	29.1	25.0	41.7	39.4	66.7	60.0	74.6	71.4
105	11.7	10.6	16.1	16.1	31.6	30.9	55.6	50.8	80.6	76.9	111	(100)
120	11.7	10.4	16.8	17.5	32.7	35.2	64.9	49.0	74.6	83.3	100	(100)
135	10.9	10.2	16.7	17.4	32.7	30.3	48.5	49.0	76.9	69.0	94.3	-
150	10.9	11.4	18.5	18.7	32.7	31.6	53.8	50.0	73.5	69.4	94.3	-
165	10.7	10.6	18.0	15.9	37.5	30.6	56.6	57.7	81.1	85.7	115	109
180	10.7	10.0	17.2	16.2	30.3	29.9	43.5	43.5	64.1	66.7	79.4	116
195	10.4	9.55	15.3	15.5	30.3	27.5	51.7	50.0	73.2	73.2	100	83.3
210	11.8	12.4	20.3	20.7	35.3	33.0	53.6	47.6	68.2	71.4	88.2	(143)
225	10.3	11.1	18.0	17.6	31.2	26.6	46.9	46.9	60.0	68.2	88.2	(93.8)
240	-	-	-	-	31.2	32.3	50.0	49.2	-	-	-	-
$F^{\dagger}$	1.40		1.06		7.59		5.88		0.05		-	
P	> 0.2		> 0.2		< 0.025*		< 0.05*		> 0.2		-	

†2-Way analysis of variance between 75 and 240 min of stimulation

* Significantly different between U & L Cell lengths

( )Only 1 to 2 root sections tabulated

meristem, and also absent in cells 1.5 mm from the root meristem. Similar results were found also in an inbred line of corn (7). In this study, we counted mitotic figures (inclusive of all stages of mitosis) in five different tissues of corn roots at different times of geostimulation. We infer that the nuclear

Table III. Average Cell Lengths of Upper (U) and Lower (L) Mid-Cortical Cells of Primary Roots of Corn.

Stimulation (min)	Distance from Root Meristem (mm) 0.5-1.0 U	0.5-1.0 L	1.0-1.5 U	1.0-1.5 L	1.5-2.0 U	1.5-2.0 L	2.0-2.5 U	2.0-2.5 L	2.5-3.0 U	2.5-3.0 L	3.0-3.5 U	3.0-3.5 L
	(μm)											
0	9.90	10.2	11.9	11.9	17.4	18.2	28.2	27.6	40.0	43.5	64.1	(62.5)
15	10.5	10.5	13.8	13.7	22.1	21.0	29.1	28.6	39.5	48.4	57.7	-
30	11.2	10.6	13.3	13.4	21.1	22.4	29.4	33.7	46.9	51.7	61.2	66.7
45	10.8	11.0	12.6	12.3	19.0	18.3	29.1	29.7	36.1	42.9	57.7	-
60	10.6	10.5	13.2	14.0	22.6	22.9	30.3	39.0	60.0	50.0	69.8	62.5
75	10.4	10.0	12.6	12.3	20.4	19.6	35.7	31.6	51.0	50.0	66.7	-
90	11.0	10.5	12.9	12.0	18.7	18.7	25.4	27.5	37.0	41.7	55.6	55.6
105	10.6	10.2	12.9	11.7	19.5	17.3	34.5	30.9	55.6	50.0	66.7	(62.5)
120	11.1	9.80	12.0	12.7	20.2	21.6	32.9	33.3	44.2	46.7	60.2	71.4
135	11.3	10.6	12.7	12.1	19.5	18.4	26.3	28.9	41.7	47.6	55.6	-
150	10.8	10.7	14.0	12.1	20.7	18.1	34.0	31.6	45.5	47.6	54.3	-
165	10.1	10.4	12.0	12.2	21.0	20.8	35.7	34.1	47.6	51.7	57.7	65.8
180	10.2	10.1	12.3	12.7	18.5	21.1	29.4	32.7	44.2	46.3	62.5	67.6
195	10.3	9.80	11.2	11.6	17.6	19.5	28.3	30.0	42.9	48.4	54.6	74.1
210	11.3	11.0	15.5	15.2	22.7	22.4	31.9	33.7	45.4	46.2	62.5	(76.9)
225	10.7	10.6	12.4	12.8	19.7	21.4	31.2	33.7	41.7	43.5	53.6	(55.6)
240	-	-	-	-	20.6	21.0	30.3	30.9	-	-	-	-
$F^{\dagger}$	9.09		1.34		0.02		0.15		4.43		-	
$P^{\dagger}$	< 0.025*		> 0.2		> 0.2		> 0.2		< 0.1		-	

†2-Way analysis of variance between 75 and 240 min of stimulation

* Significantly different between U & L cell lengths

( ) Only 1 to 2 root sections tabulated

Table IV. Average Cell Lengths of Upper (U) and Lower (L) Inner Cortical Cells of Primary Roots of Corn.

Stimulation (min)	Distance from Root Meristem (mm) 0.5-1.0 U	0.5-1.0 L	1.0-1.5 U	1.0-1.5 L	1.5-2.0 U	1.5-2.0 L	2.0-2.5 U	2.0-2.5 L	2.5-3.0 U	2.5-3.0 L	3.0-3.5 U	3.0-3.5 L
	(μm)											
0	8.90	8.60	9.90	10.4	15.9	14.5	22.9	21.8	32.5	30.5	40.3	(35.7)
15	8.70	8.60	10.3	10.1	15.6	16.7	23.4	25.9	34.1	34.9	46.2	-
30	8.50	8.40	9.74	9.84	16.7	17.9	27.3	24.8	35.3	36.6	44.1	46.9
45	8.98	9.40	9.15	9.40	15.3	14.6	22.6	20.8	33.0	33.7	44.1	-
60	8.88	8.40	10.1	10.2	18.3	17.3	27.3	26.8	41.0	37.0	56.6	52.6
75	7.96	8.70	9.80	9.70	16.8	16.0	28.6	25.4	48.5	37.9	56.8	-
90	8.80	8.85	9.52	9.65	14.9	15.3	22.7	22.4	29.1	30.9	36.5	(37.0)
105	8.40	8.22	9.32	9.01	15.7	14.6	25.9	25.0	38.0	40.0	51.7	(50.0)
120	8.60	8.20	9.40	9.30	16.1	15.7	24.1	22.5	34.5	37.0	42.4	(50.0)
135	8.70	8.30	9.60	10.1	14.2	14.5	20.2	22.7	27.0	32.3	37.7	-
150	9.00	8.60	9.90	13.0	16.3	16.2	25.4	24.6	31.6	35.7	39.0	-
165	7.96	8.11	9.01	9.04	15.5	16.0	31.2	28.6	36.1	41.7	48.4	49.0
180	8.50	8.10	9.30	9.40	14.9	15.2	22.0	24.8	31.6	33.8	38.5	43.1
195	8.17	7.98	8.65	8.31	13.8	13.4	21.9	21.0	30.9	33.3	41.7	40.0
210	9.20	9.01	10.6	10.8	16.4	16.8	23.6	27.8	35.3	42.9	46.9	55.6
225	8.13	8.50	9.65	10.2	15.6	16.8	24.0	25.6	32.3	35.7	36.6	44.4
240	-	-	-	-	14.2	15.5	27.2	25.0	-	-	-	-
$F^{\dagger}$	0.47		1.40		0.40		0.03		2.87		-	
$P^{\dagger}$	> 0.2		> 0.2		> 0.2		> 0.2		< 0.2		-	

† 2-way analysis of variance between 75 and 240 min of stimulation

( ) Only 1 to 2 root sections tabulated

division is indicative of cell division. The entire cortex in the first 1.5-mm region behind the cap consists of 19.1 ± 0.30 columns of cells, and the stele, 22.6 ± 0.66 columns; the cells in the cortex are larger. In spite of these differences, the cortex and the stele (inclusive of endodermis and pericycle) have a similar number of cells in mitosis (Fig. 3 and Table V). Although the peaks of mitosis in cortex and stele occur at different times, both peaks precede the peak of geotropism (Fig. 4). Mitosis is greatest in the first 0.5 mm of the cortex; and in the stele it is in

the first 1 mm from the root meristem (Table V). In the epidermis, mitosis is very low (Tables V and VI), and distributes equally among the first three 0.5-mm sections from the root meristem (Table V). Of the cortical cells, the middle row has the highest mitotic number, and the endodermal cells have the highest mitotic figure of all of the tissues (Table VI).

When the root is taken as a whole (sum of all tissues), two peaks of mitosis occur during the 240 min (Fig. 4B). These peaks reflect those of the cortex and stele (see Fig. 3). A comparison between the upper and lower tissues (Fig. 4B) shows that the cells in the lower tissues have a significantly greater number of mitoses ($P < 0.001$). This difference is noted also in the cortex ($P < 0.005$) and the stele ($P < 0.025$), when these tissues are tabulated separately.

Stained Layer Separating Root Meristem and Root Cap. The area measured as diameter of the thin region of the stained layer located at the tip of the root meristem was examined at successive 15-min intervals for 240 min after red exposure and geostimulation. The measurements are shown in Figure 5. The greatest increase in diameter of the thin area is coincidental with the peak of the curvature; this diameter at 180 min after irradiation is 1.68 times greater than at zero time. This increased area in the thin region is not due to an increase in the dimension of the root. The diameter of the root, measured at the base of the root cap, is 964 ± 24.5 μm (mean of three segments from

Table V. Number of Cell Divisions per Root Section, between 0 and 1.5 mm from Root Meristem, and at Different Times After Geostimulation and Red-Light Exposure. Each Datum is the Average of 3 Roots and 3 Sections per Root.

Stimulation (min)	Epidermis			Cortex			Stele		
	mm from Root Meristem								
	0-0.5	0.5-1.0	1.0-1.5	0-0.5	0.5-1.0	1.0-1.5	0-0.5	0.5-1.0	1.0-1.5
0	0.22	0.11	0.67	4.67	2.78	0	0.89	1.00	0.22
15	0	0	0	7.22	2.56	0.44	0.22	0.22	0.11
30	0	0	0	7.33	4.00	0.33	2.44	2.56	0.56
45	0	0	0	9.56	3.11	0.33	1.78	3.78	0.56
60	0	0	0	5.44	2.44	0.11	1.78	2.33	1.89
75	0	0	0	2.33	1.44	0.44	3.33	5.56	0.89
90	0	0	0	5.56	2.33	0.44	3.33	6.22	1.67
105	0.11	0	0	4.44	3.22	0.33	5.56	3.67	1.33
120	0.11	0	0	3.67	2.89	0.33	5.67	5.22	1.89
135	0.11	0.22	0.22	4.22	1.89	1.00	6.89	6.56	1.78
150	0	0	0	2.89	2.33	0.33	4.67	3.78	0.67
165	0	0	0	3.00	1.00	0.11	1.67	2.00	0.44
180	0.22	0	0	1.78	1.78	0.33	1.89	2.11	0.67
195	0	0	0	1.33	1.22	0.22	0.56	0.67	0.11
210	0.11	0	0	2.67	1.11	0	1.11	1.00	0.22
225	0	0.22	0	2.00	0.67	0.33	0.33	1.44	0
240	0	0	0	1.67	0.89	0	0.89	0.78	0.44
Total	0.88	0.55	0.89	69.8	35.7	5.07	43.0	48.9	13.4
Mean	0.05	0.03	0.05	4.10	2.10	0.30	2.50	2.88	0.79

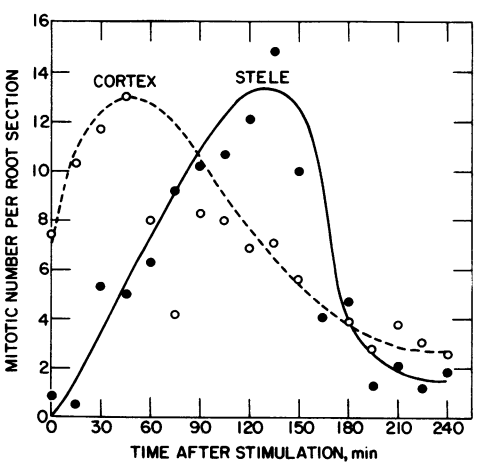

FIG. 3. Mitotic numbers (number of cells in mitosis) per root section (3-μm thickness) in the cortex (all cells) and stele (endodermis, pericycle, and vascular tissues) of primary roots of corn at different times after stimulation by gravity and red light. Each datum is the average of three roots, three sections/root.

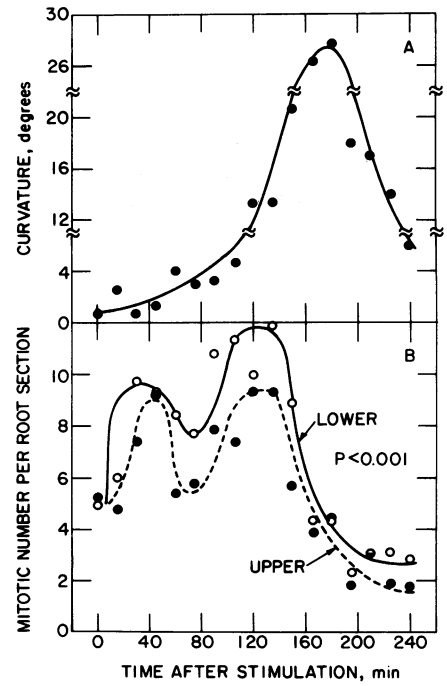

FIG. 4. A: Geotropic curvature of the tip 4 mm of primary corn roots at different times after geostimulation and red irradiation. These same roots were used for the cell length and mitosis studies presented in this paper. B: Comparison of mitotic numbers per root section between the upper and lower cells (sum of epidermis, cortex, and stele) of geotropically stimulated and red light-exposed primary roots of corn. The numbers are significantly different between the upper and lower cells at $P < 0.001$; datum points are means of three roots, three sections/root.

Table VI. Comparison of Mitotic Number Per Root Section in Single Layers of Cells in the 1st 1.5-mm Region Behind the Cap. Each datum is the average of 3 roots and 3 sections per root.

Stimulation (min)	E	OC	MC	IC	End	Peri
	(No.)					
0	1.00	0.11	0.67	0.44	0.56	0.33
15	0	0	0.33	1.11	0	0
30	0	0	0.56	2.22	0.44	0.33
45	0	0	2.11	1.00	2.22	0.89
60	0	0	0.78	0.33	1.22	0.22
75	0	0	0.89	0.22	1.56	1.89
90	0	0.11	1.33	1.22	2.00	2.22
105	0.11	0.33	0.89	0.89	2.56	1.44
120	0.11	0.44	1.78	1.00	4.33	1.67
135	0.56	0	3.11	1.33	2.11	1.67
150	0	0.33	2.83	0.83	2.67	3.33
165	0	0.11	0.89	0.56	0.22	0.56
180	0.22	0.33	0.44	0	0	0.89
195	0	0	0	0.11	0	0.33
210	0.11	0.22	0.22	0.11	0.22	0.67
225	0.22	0	0.11	0	0.44	0
240	0	0	0.33	0.22	0.22	0.33
Mean	0.14	0.12	1.02	0.68	1.22	0.99

E, epidermis; OC, outer cortex; MC, mid cortex; IC, inner cortex; End, endodermis; Peri, pericycle

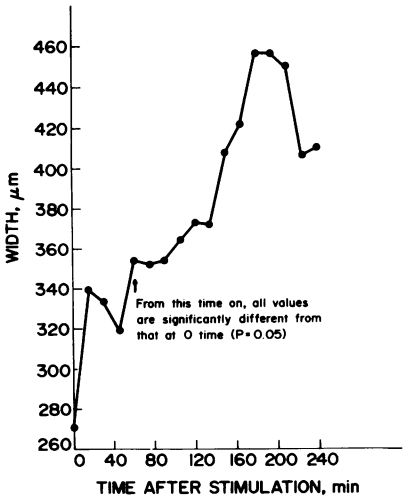

FIG. 5. Diameter (μm) across the thinner stained region at the apex of the root meristem (Fig. 1) of primary roots of corn after geostimulation and red light irradiation. Each datum is the average of three roots, three sections per root. Data analyzed by a one-way analysis of variance.

each of three roots ± $\sigma_{\bar{x}}$) at zero time, and 948 ± 24.1 μm 180 min later (Table VII). The root apex changes very little in diameter during the 240 min of experimentation.

Table VII. Width of Corn Roots Measured at the Base of the Root Cap During 240 min of Experimentation.

Time after Stimulation (min)	Width ± $\sigma_{\bar{x}}$ (μm)
0	964 ± 24.5
60	925 ± 38.2
120	988 ± 25.4
180	948 ± 24.1
240	1012 ± 21.7

DISCUSSION

Cell Length and Mitosis. We have demonstrated a difference in cell length between the upper and lower tissues following geotropic curvature of corn roots. The difference on the individual cell level is seemingly minute, but it becomes amplified when the entire segment of the root is considered. Further, in shoot phototropism, a difference of 1 mm in length between the light and dark side of the tissue gave rise to a 30° curvature (17). The difference in length (this study) is greatest in the outer cortical cells 1.5 to 2.5 mm from the cap-to-root juncture. It is not certain whether the greater length in the upper cells is due to an increased growth, or to a reduction in the shorter cells in the lower tissue, or both. A study (11), containing a minimal description of methods and presentation of data, purported to show that the differential growth in geotropism of corn root was attributable to an increased elongation in the upper cortical cells, with no change in length in the lower. Obviously more studies are needed, particularly in view of the inhibition of corn root elongation by light (21), and the light requirement of corn roots for geotropic response (14, 15).

Cell division (mitosis), on the other hand, occurs more frequently in the lower tissue. However, this division happens exclusively in the first 1.5-mm region of the root meristem. In this region, the greater elongation in the upper cells (Tables I and III) is offset by the greater number of cell division in the lower tissue (Fig. 4B), hence no curvature is observed. Since cell division is totally absent 1.5 mm behind the meristem, and differential cell elongation persists, a geotropic curvature occurs behind the 1.5-mm region from the root meristem. This region of curvature will be harvested for a subsequent EM[2] study on the association of organelle distribution to the geotropic response (data accepted for publication).

Cell division in corn roots is asynchronous between the stele and the cortex. During the 240 min of geostimulation, peak division occurs 90 min earlier in the cortex than in the stele (Fig. 3). This sequence was noted also in roots of *Vicia faba* (8). Further, the cell cycle of the cortex appears to be longer than the stele. This could be due to a longer G_1 phase (pre-DNA synthetic phase) in the cortical cells (1, 2). A similar trend was observed also in root cells of *Convolvulus* (10). The present study was carried out for only 4 hr (on the entire cortex and stele of the 2-mm region of root tissues behind the root cap). Our observations, although over a shorter period than those reported elsewhere (1, 2, 10), were made at 15-min intervals in comparison to the longer than hourly intervals used by others.

The roots we used in this study were exposed to a red light (660 nm) at 1 Jm^{-2} intensity, which is the most effective wavelength for root geotropism (unpublished data). Red light, at intensities 3 to 4 orders of magnitude greater than we used, had been shown to reduce cell length in roots of rice and wheat, but had no effect on cell division (9). In a preliminary study, we found that red light at about 100 Jm^{-2} intensity inhibited the IAA-promoted (10^{-15} to 10^{-13} M) elongation of corn roots (15). In the light-requiring geotropic response reported here, we find a differential cell elongation and cell division in corn roots; however, the increased or decreased cell length and cell division occur in opposite tissues (Tables I–IV, and Fig. 4B) and in different regions of the tissue. This could indicate that interaction of red irradiation and gravity stimulation triggers a multicellular event involving the interaction of several hormones. Further, one paper reported an asymmetrical differentiation of apical tissues in geotropism of roots (7). The concave side (the lower side) showed an advanced differentiation of xylem and phloem.

Stained Layer Separating Root Meristem and Root Cap. In this study, we noted a layer of densely stained (purplish blue) substance covering the region where the root meristem meets the cap. We are not certain what this layer is. The presence of a cuticle layer on the apical meristem of roots of angiosperms (12) and of root hair of *Allium cepa* (13) had been described. Since this densely stained layer extends from the apex of the root meristem (cap junction) to cover the epidermis of the root, it is likely that this layer is of cuticular nature. In a majority of plants, cuticle layers are associated with epidermal cells (4). Hence, on the whole, the lessening of a barrier between the cap and the root proper by the red light exposure and geostimulation would facilitate the transfer of messages. The root cap is the site of perception for the geostimulus (5) which is then transported to the root for expression of the tropic response. It would be of interest to study the effect of phytochrome on cuticle formation and resorption.

The alterations of cell elongation, cell division, and the increase in the thin area of the cuticle corn roots after red light exposure and gravity stimulation show a complexity of happenings in the geotropic response. These changes could be a result of multihormonal interactions, involving IAA (14), cytokinin (18), ABA-like substance (6, 21) and gibberellic acid (3, 20).

[2] Abbreviation: EM: electron microscopy.

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