STUDIES ON A CATENARY PROCESS OF THE LIGHT-INDUCED GEOTROPIC RESPONSE IN *ZEA* ROOTS

Takashi SUZUKI

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CONTENTS

General Introduction

The direction of growth and development of the young growing organs is decided by tropic response or morphogenesis. At an early stage in seed germination it is important that the primary root and shoot become so orientated that the former grows down towards a reliable supply of water and mineral nutrients, and the latter upwards into the light to allow leaf expansion and photosynthesis to take place. The environmental parameter used by plants to achieve the vital orientation of their young roots and shoots as they emerge from the seed is "gravity", and the response of these plant organs to gravity is known as "geotropism".

The guidance systems by which the young growing organs achieve their orientation in a gravitational field have been studied for more than a century, and in this time some appreciation of their complexity and mechanism has been acquired. Geotropism can be regarded as a catenary process, a chain of reactions causally linked in sequence. The first step is the immediate action of gravity on some component mass of the system causing therein a physical change. This has been called susception. The second step is the transformation of that physical information into biochemical information. This has been called reception. The third step is the transmission of the information from the site of perception to the region of response. This step is rea-

sonably certain to be mainly one of differential transport or supply of growth regulator(s). The final step is the response of the reacting zone to this differential concentration of growth factor.

Another type of gravity-sensing orientation system is found on the primary roots of some plant specise where, in addition to gravity, light is indispensable to the induction of geotropic response, so this response is called the light-induced geotropic response. Although little is known on this photo-sensing mechanism, some results have suggested that light makes available a critical component in the geotrpoic response mechanism, and the simplest possibility is that a specific amount of an important growth inhibitor is synthesized or released in the cap following irradiation, and then transported basipetally and ultimately metabolised after having exerted its effect in the growing zone of the root.

In this thesis, thus, I describe experiments conducted to determine the effect of light on the induction of geotropic responsiveness, moreover, to determine the growth regulator which leads to the appearance of downward curvature, and its action to the cell elongation of Zea primary roots.

PART I

SPECTRAL DEPENDENCE OF THE LIGHT-INDUCED GEOTROPIC RESPONSE IN *ZEA* ROOTS

Part I

Spectral Dependence of the Light-induced Geotropic Response in *Zea* Roots

Abstract

The induction by light of geotropic responsiveness in the primary roots of *Zea mays* L. (cv. Golden Cross Bantam 70) was found to be governed by the "all-or-none law". The response was induced by light energies above a threshold value, but the maximal curvature of geo-stimulated roots was constant irrespective of the light energy above that threshold. The action spectrum for this light effect showed a large peak at 650, a small peak at 410, and a shoulder at 663 nm. The effect of red light was not reversed by far-red light. Thus, the geotropic response in *Zea* roots may not be controlled by phytochrome.

Introduction

It is known that light may affect both the presence and the extent of the geotropic response in plant organs (see reviews by Wilkins, 1966; and Juniper, 1976). In shoots, the effect appears to be generally on the sensitivity (extent) of the response, while roots may show a geo-

tropic response only after they have been exposed to light, i.e., their georesponsiveness has to be established by light.

It has been shown that induction of georesponseiveness in the roots in some plant species is dependent on a particular wavelength of light. Thus, Vanilla roots, which are diageotropic (i.e., are oriented horizontally) in the dark and in far-red, red, orange and green light, become positively geotropic after exposure to blue light (Irvine and Freyre, 1961). Georesponsiveness in Convolvulus roots is induced by red light and reversed by far-red light if given immediately after the red (Tepfer and Bonnett, 1972). The primary roots of some cultivars of Zea mays are diageotropic in the dark and in green (510-530 nm) light, but become positively geotropic after exposure to white, red (600-700 nm) and blue (400-500 nm) light, and at a quantum flux of 2.67×10^{-11} E $cm^{-2}s^{-1}$red and blue lights were reported to be equally effective in the induction of the positive geotropic response (Scott and Wilkins, 1969). Shen-Miller (1973, 1978) reported that the geotropic response (degree of curvature) in Zea roots exposed to red light was log-linear in the irradiance range of 10^{-2}-10^{-6}ergs cm^{-2}. When Zea roots were exposed to equal quantum fluxes of 2.24×10^{14} photons cm^{-2} at different wavelengths, three major peaks for the induction of geotropic responsiveness were obtained, at 460, 560 and 660 nm (Shen-Miller, 1978). However, since this action curve

was not obtained by plotting the reciprocal of the numbers of incident quanta necessary to induce a constant geotropic curvature at each wavelength, the curve cannot be considered a genuine action spectrum.

In this Part, I describe experiments conducted to determine the exact light dependence of the induction of geotropic responsiveness in Zea roots, and to establish the actual action spectrum for this light effect.

Material and Methods

Plant Material

Caryopses of Zea mays L., cv. Golden Cross Bantam 70 (Sakata Seed Comp., Yokohama, Japan), were imbibed for ca. 12 h in a Petri dish in the dark in running tap water covering the grains, and were then planted, with the embryo pointing upward, on moist vermiculite in containers covered with a nylon sheet. The primary roots were allowed to elongate horizontally on the vermiculite at 26 ± 2°C in the dark, except that during transfer and other operations they were exposed to very weak green safe light. The safe light was obtained from a 20-W cool-green fluorescent tube (Mitsubishi Electric Co., Tokyo:SL-20SG) through 6 sheets of green cellophane, transmission range 490-580 nm, with peak transmittance at 530 nm. The seedlings were exposed to this light for not more than 5 min, and even 1 h exposure did not

establish georesponsiveness. Caryopses with primary roots 2.0-2.5 cm in length, obtained ca. 35 h after the transfer were used for the experiments.

Geotropic Stimulation and Light Sources

The roots of the seedlings were horizontally oriented by pinning the grains on a polysteyrene plate, exposed to light, and then allowed to bend for 4 h in a moist chamber. Geotropic responsiveness was recorded by making shadow photographs of the roots. The percent of roots exhibiting curvature and the mean curvature of the roots which did curve, were used as parameters. Each result represents the response of at least 30 roots. Standard errors of the mean have been calculated and significant differences assessed by the t-test. The time course of the curvature was recorded with a transducer (Hoshi Electric Works, Kawasaki, Japan : DTD 1B) and an electronic polyrecorder (Toa Electronics, Tokyo : EPR-10A).

In the dose-response experiments, red light at 663 and 649 nm was obtained from a tungstem lamp (300W; Kondo Electrical Industrial Co., Tokyo) through 30 mm of water, interference filters (Toshiba Glass Co., Shizuoka, Japan : KL-66, peak 663 nm; KL-65, peak 649 nm), respectively. Far-red light at 729 nm was also obtained from a tungstem lamp through 30 mm of water, an interference filter (Toshiba

Glass Co. : KL-73, peak 729 nm) and a glass filter (Toshiba Glass Co. : V-R-69). The quantum flux densities were altered by passing the light through neutral density filters (Toshiba Glass Co. :ND-2x, -4x, -8x).

To test the spectral sensitivity of the roots, spectral bands between 380 and 740 nm were isolated from the light of a Xenon lamp (Ushio Electric Works, Tokyo : UXL-500 D, 500W) by passing it through a monochromator (Nippon Kogaku, Tokyo: G-250). The half band width was 10.7 ± 5 nm. The radiant energies at each spectral band were measured with a Kipp-Zonen (Delft, Netherlands) compensated thermopile (CA-1) and a DC Microvoltmeter (Toa Electronics : PM-16A).

Results

Light Fluence and Geotropic Responsiveness in Zea Roots

Zea roots were exposed to three different treatments of red light at 663 nm, kept in the horizontal position, and the time course of curvature of the roots was recorded (Fig. 1). Curvature started about 1 h after the light exposure, continued for the following 1.5 h, and then ceased, irrespective of fluence level. This result indicates that the time course of the actual geotropic curvature of the roots under our conditions does not depend on the fluence. In the following experiments, the curvature therefore was measured only once, 4 h after the light exposure.

Zea roots were exposed to 2.43 x 10^{-10}E $cm^{-2}s^{-1}$ of red light at 663 nm for various periods of time. The results, obtained after 4 h geostimulation, are shown in Figure 2. Curvature is hardly observed at exposure times shorter than 26 s; above this exposure period, the percentage of curved roots increases drastically with increasing exposure time, to reach a plateau at 31 s. However, the degree of curvature of those roots that do undergo curvature is constant, irrespective of the exposure time, with a mean of ca. 35°. Thus, the geotropic responsiveness in our material appears to be induced at light doses exceeding a definite energy value, whereas the actual response (the degree of curvature) does not seem to depend on the light dose above that threshold value. In other words, the induction of the geotropic responsiveness in Zea roots seems to be an all-or-none response.

The Minimum Light for the Induction of Georesponsiveness in Zea Roots

To determine the minimum light energy needed to induce geotropic responsiveness, Zea roots were exposed to different fluences of red light at 663 nm for various times, and then given 4 h geostimulation. The results are shown in Figure 3. At each irradiation time, the induction of the geotropic responsiveness showed the same pattern as in Figure 2. The

minimum fluence was calculated by multiplying the light energy of light was determined by finding the intersection point of a tangent line in the steepest part of each curve and the horizontal line in the lag part of the curve. The calculated values are shown in Table 1. The minimum light fluences were very similar, regardless of light energy, the mean value being 6.51×10^{-9}E cm^{-2}. These results confirm the conclusion that there is a threshold light energy value for the induction of geotropic responsiveness in Zea roots. Moreover, the results show the validity of Bunsen-Roscoe reciprocity law, in that identical responses are produced by identical products of light energy and duration.

Action Spectrum for Induction of Georesponsiveness in Zea Roots

Zea roots were exposed to equal quantum fluxes of 1.43×10^{-9}E $cm^{-2}s^{-1}$ at different wavelengths and the minimum exposure time for inducing a response to 4 h geostimulation was determined for each wave band. Then the minimum quantum doses were calculated and the reciprocals of these values were plotted against wavelength. The action spectrum thus obtained (Fig. 4) shows a large peak at 650, a small peak at 410, and a shoulder at 663 nm.

Does Phytochrome Control the Induction of Georesponsiveness in Zea Roots ?

Zea roots were exposed to red light (663 nm), far-red light (729 nm), or red followed by far-red, and then subjected to 4 h geostimulation. The results are shown in Table 2. Although the energy of a 6-s exposure to the red light at 1.52×10^{-9}E $cm^{-2}s^{-1}$ was only slightly above the threshold value for inducing the response, both the percentages of curved roots and the magnitude of the curvatures were not affected by far-red light.

In another experiment, Zea roots were exposed for various times to red light (649 or 663 nm, 2.04×10^{-10}E $cm^{-2}s^{-1}$) or to red light mixed with far-red (729 nm, 1.12×10^{-9}E $cm^{-2}s^{-1}$) light. As can be seen from Figure 5, no significant difference was observed in the geotropic responsiveness. With red light of 649 nm quite similar results were obtained as with red light of 663 nm. These results imply, together with the results in Figure 4, that the induction of geotropic responsiveness in Zea roots is not controlled by phytochrome.

Discussion

The results (Fig. 2) indicate that the induction of geotropic responsiveness in the roots of Zea mays (cv. Golden Cross Bantam 70) is an all-or-none response, and that all roots that do curve upon a constant geotropic stimulation always show a constant curvature, irrespective of the light dose, once the latter exceeds a threshold value. These

results disagree with those of Shen-Miller (1973, 1978) which were obtained with roots of Zea mays cv. Wisconsin hybrid 64A x 22R. Even if the data in Figure 2 were recalculated as the mean curvatures of all roots, the curvature was not dependent on the light energy (Fig. 6). In other words, light regulates only the induction of geotropic responsiveness in Zea roots, but not the extent of the actual geotropic response. Responsiveness was fully induced by quantum fluxes exceeding 6.5×10^{-9}E cm^{-2} (Fig. 3, Table 1), although the precise threshold value cannot be obtained because of the high variability of roots (compare Mohr, 1972). Furthermore, the induction of this response by light satisfies the Bunsen-Roscoe reciprocity law. This fact indicates that the response is initiated by the photochemical transformation of a photoreceptor.

The spectral response curve obtained in my experiments has a large peak at 650, a small one at 410 and a shoulder at 663 nm (Fig. 4). Scott and Wilkins (1969) have reported that red (600-700 nm) and blue (400-500 nm) light has an equal effect on the induction of geotropic response in roots of Zea (cv. Giant Horse Tooth). In my experiments the minimum quantum energies at 663 and 650 nm for inducing the response were one-sixth and one-thirteenth of that at 410 nm, respectively (see Fig. 4). The light-doses used by Scott and Wilkins (1969) were probably above the threshold value for

inducing the response, although differences in behavior and sensitivity between the roots of different maize cultivars cannot be ruled out.

Shropshire (1972) has pointed out some of the screening errors that may be involved in the determination of an action spectrum, and may cause shifts in the action peaks. The presence of other pigments could give rise to screening effects. If this argument is applicable to the determination of the action spectrum in Zea roots, the main peak at 650 nm in my results could be derived from phytochrome. However, I could not observe red, far-red reversibility in the light induction of georesponsiveness in Zea roots (Table 2). Moreover, even if Zea roots were exposed simultaneously to red and far-red light, when the apparent percent of P_{fr} would be expected to be very low (Hillman, 1965), the response was not affected, and the threshold value was the same as obtained in red light only (Fig. 5). These results indicate that the geotropic responsiveness in Zea roots may not be controlled by phytochrome.

Table 1. Minimum light doses for inducing geotropic responsiveness at various intensities of red (663 nm) light. Minimum light doses were recalculated from Figure 3.

Light intensity (ratio)	Minimum dose (x 10^{-9}E cm^{-2})
1[a]	6.08
0.8	7.30
0.4	5.78
0.32	5.83
0.2	6.38
0.16	6.57
0.08	7.66

[a] 1.52 x 10^{-9}E $cm^{-2}s^{-1}$

Table 2. The geotropic response in Zea roots exposed to the sequence of red and far-red light.

The time interval between red and far-red light was within 0.1 s. Quantum fluxes of both red (663 nm) and far-red (729 nm) light were 1.52×10^{-9}E $cm^{-2}s^{-1}$.
At least 30 roots were used in each treatment.

Light treatment	Curved roots (%)	Curvature[a]
R (10s)	92.5	34.5 ± 5.10
R (6s)	88.9	36.4 ± 4.21
FR (5 min)	6.7	17.5
R (10 s)→FR (5 min)	91.7	34.7 ± 3.39
R (6 s)→FR (5 min)	91.2	32.6 ± 3.49

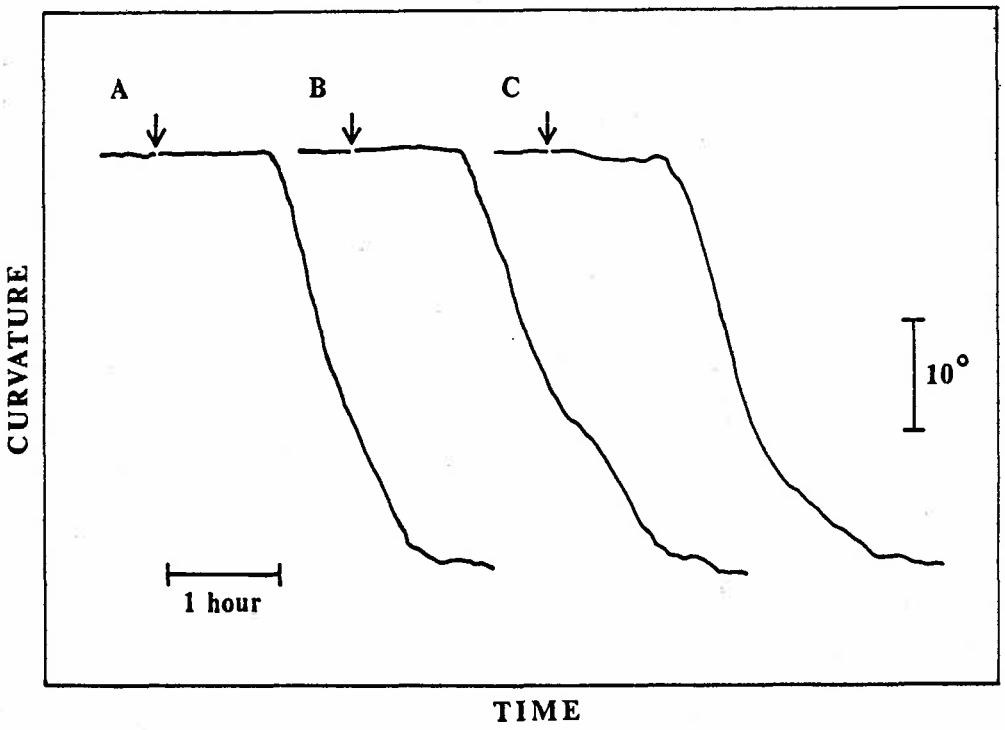

Fig. 1. Time course of development of geotropic curvature in Zea roots. The roots were exposed to 1.52 x 10^{-9}E $cm^{-2}s^{-1}$ of red light (663 nm) for 3 h (curve A) and 20 s (curve B), and to 3.03 x 10^{-10}E $cm^{-2}s^{-1}$ for 20 s (curve C), and observed in the horizontal position for 4 h. Arrows indicates the start of light treatment.

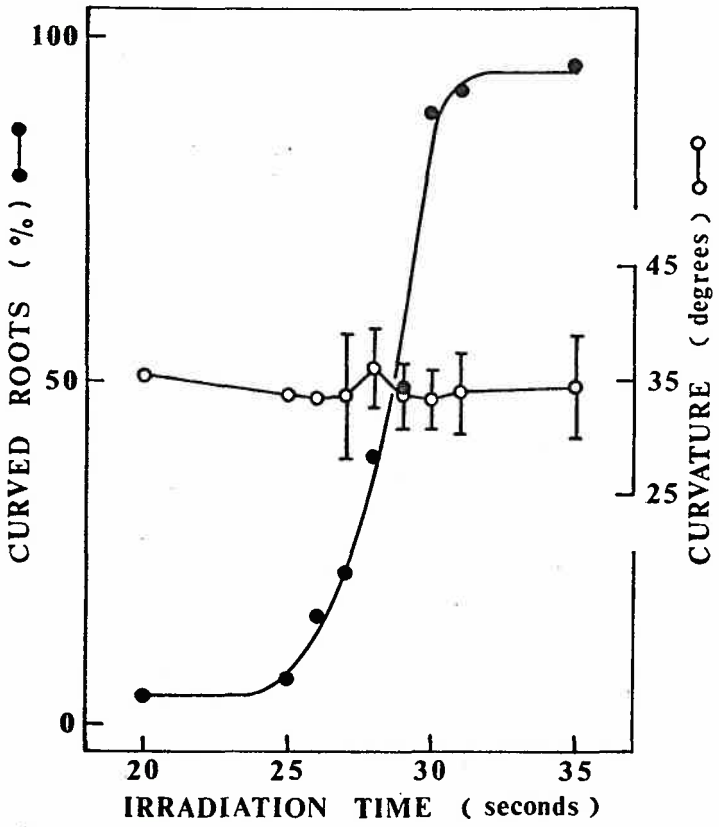

Fig. 2. Effects of light on the percent of curved roots and the geotropic curvature of curved roots in Zea. The roots were exposed to 2.43 x 10^{-10}E $cm^{-2}s^{-1}$ of red light (663 nm). Results were determined after keeping them for 4 h the horizontal position. Each datum represents the response of at least 30 roots.

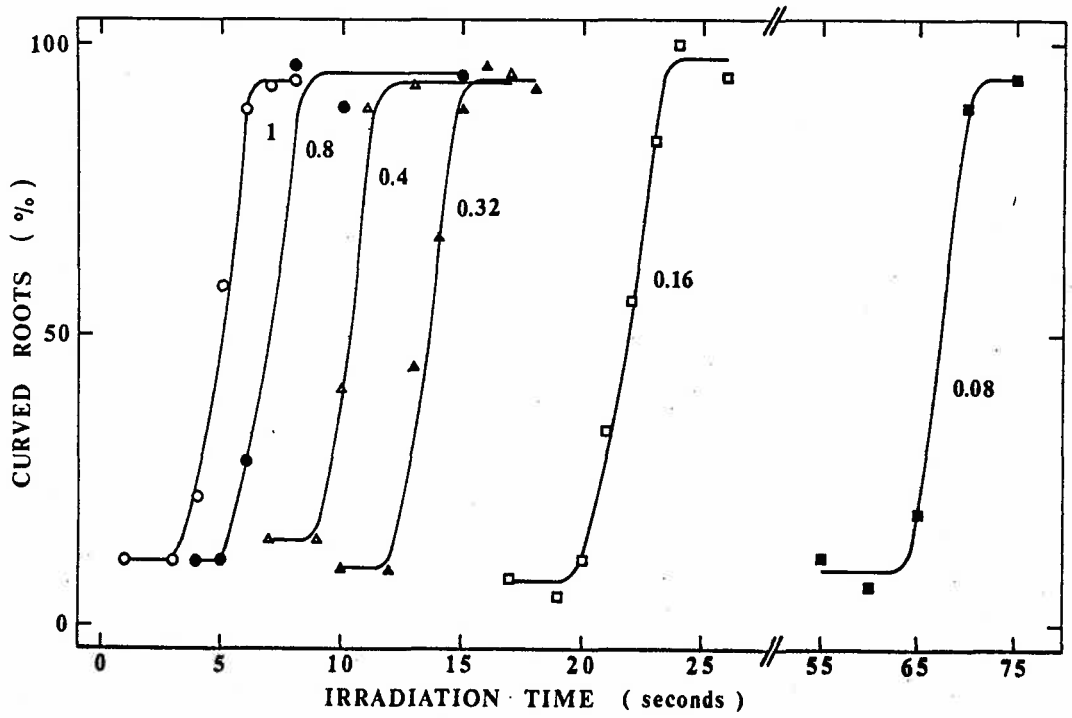

Fig. 3. Relationship between light energy and exposure time in the induction of georesponsiveness in Zea roots. The roots were exposed to different energies of red light (663 nm) for various lengths of time. Results were determined after keeping them for 4 h the horizontal position. The numbers to the right of each curve is the relative light energy, 1 being 1.52×10^{-9} E $cm^{-2}s^{-1}$. Each datum represents the response of at least 30 roots.

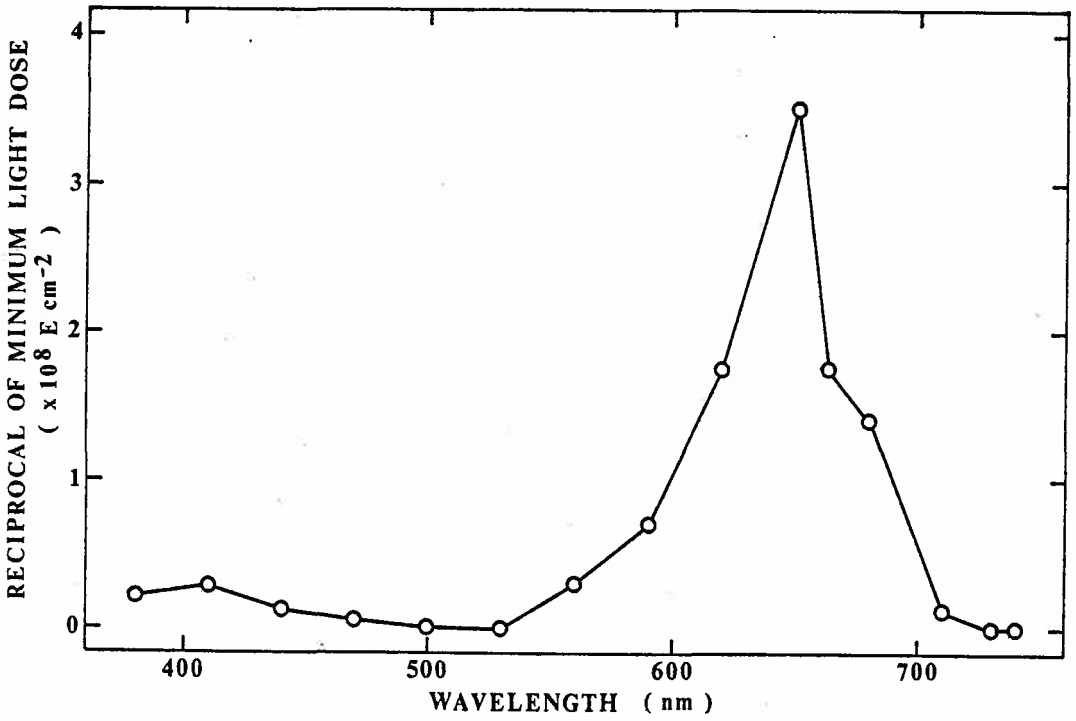

Fig. 4. The spectral dependence of geotropic responsiveness. The roots were exposed to an equal quantum energy of 1.43 x 10^{-9}E $cm^{-2}s^{-1}$, and the reciprocal of minimum quantum energies at each wavelength for inducing the response was plotted.

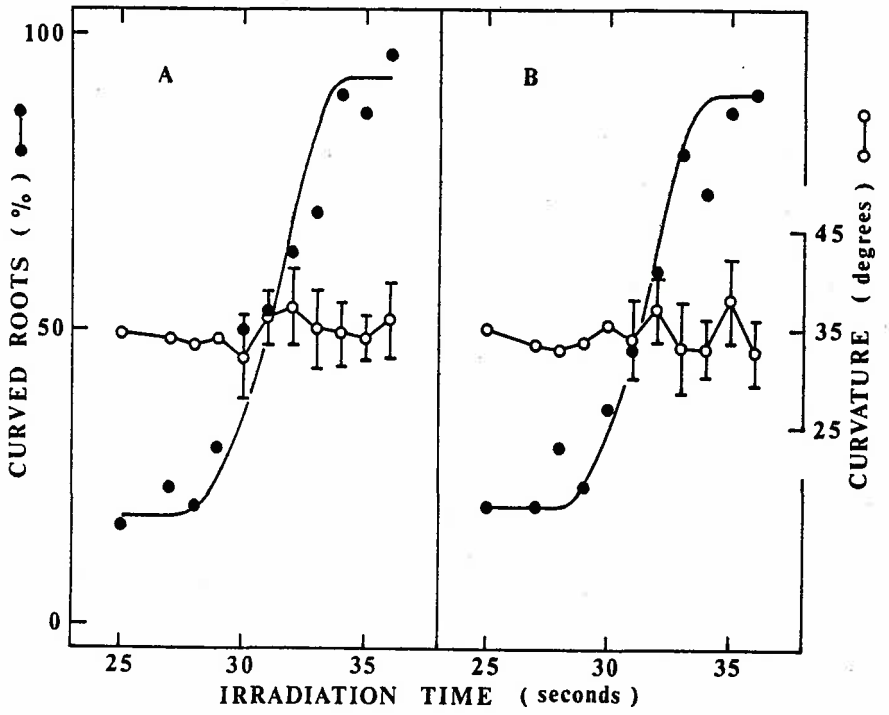

Fig. 5A and B. The effect of far-red light on red-light-induced geotropic responsiveness in Zea roots. The roots were exposed to 2.04 x 10^{-10}E $cm^{-2}s^{-1}$ of red light at 663 nm only A and to red light mixed with 1.12 x 10^{-9}E $cm^{-2}s^{-1}$ of far-red light at 729 nm B for various lengths of time. Results were determined after keeping them for 4 h the horizontal position. Each datum represents the response of at least 30 roots.

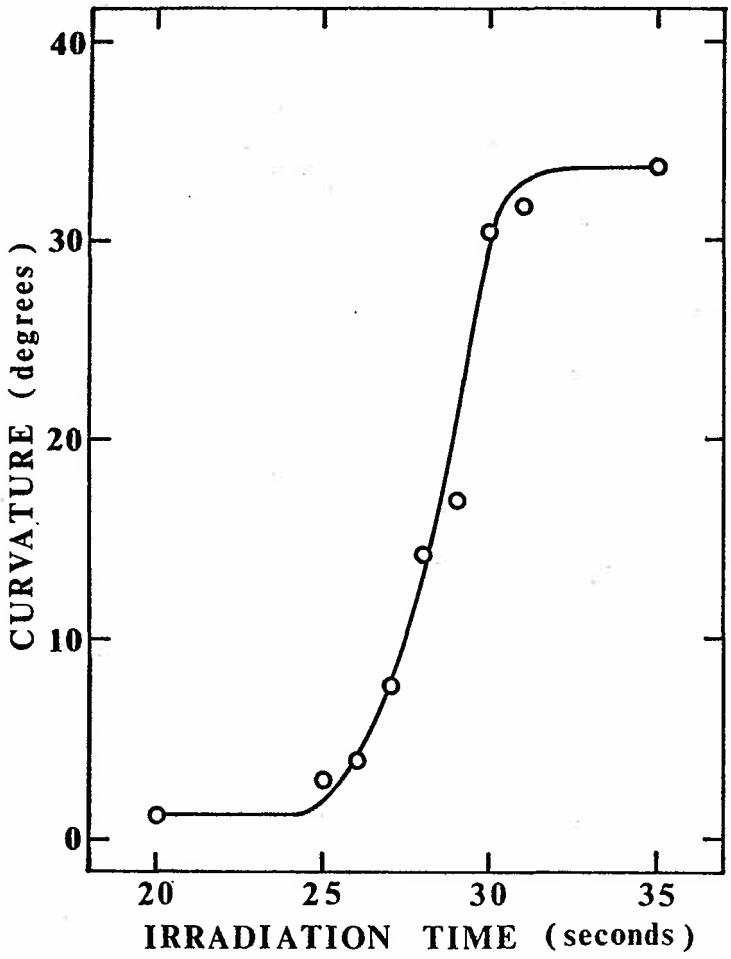

Fig. 6. Effects of light on geotropic curvature in Zea roots. The data were recalculated from Figure 2 and show the mean curvature of all roots.

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PART II

DISTRIBUTION OF GROWTH REGULATORS IN RELATION TO THE LIGHT-INDUCED GEOTROPIC RESPONSIVENESS IN *ZEA* ROOTS

Part II

Distribution of Growth Regulators in Relation to the Light-induced Geotropic Responsiveness in *Zea* Roots

Abstract

Growth regulators were measured in extracts from the upper and lower halves of 7-mm apical segments of horizontally oriented, red-light-irradiated and non-irradiated roots of *Zea mays* L., cv. Golden Cross Bantam 70, which exhibit a geo-response only after an exposure to light. Abscisic acid (ABA) was measured by gas-liquid chromatography, auxin (indole-3-acetic acid, IAA) by the *Avena* straight-growth assay, and an unidentified growth inhibitor by a *Zea* root-growth assay. The ratio of ABA in the upper and lower halves was 1.6 in the irradiated roots and 1.0 in the non-irradiated ones. The total amount of ABA after irradiation was in-

creased by a factor of ca. 1.8. The ratio of IAA in the upper and lower halves of irradiated and non-irradiated roots was 1 : 3.4 and 1 : 2.9, respectively. The content (or activity) of an unidentified growth inhibitor was highest in the lower halves of horizontally oriented roots which had been irradiated with red light. The unidentified growth inhibitor, rather than IAA or ABA, may be the major factor in the light-induced geotropic responsiveness in Zea roots.

Introdnction

The primary roots of some Zea mays cultivars are diageotropic in the dark and become positively geotropic after exposure to light (Scott and Wilkins, 1969; Pilet, 1971; Shen-Miller, 1973; H. Wilkins and Wain, 1975a). I have recently demonstrated that the action spectrum for this light effect shows a peak at 650 nm and that there is a threshold energy value for inducing the geotropic responsiveness (Suzuki and Fujii, 1978). The simplest explanation is that at least one growth-inhibiting substance is produced or released under the influence of light in the root cap of maize, moves basipetally from the tip to the extending

zone, is laterally displaced in geo-stimulated (horizontally oriented) roots, and thus gives rise to curvature even if the root is subsequently kept in darkness (Pilet, 1975a; H. Wilkins and Wain, 1975a; see also reviews by Juniper, 1976. and M. B. Wilkins, 1978). Light does not seem to have an effect on the transport of the inhibitor(s) from the cap to the elongating part of the roots or on their action in this part (Pilet, 1976a).

That growth inhibitors actually occur in Zea roots was first reported, and their possible relation to root geotropism was discussed by Gibbons and Wilkins (1970). Shaw and Wilkins (1973) found evidence that in horizontally-oriented roots the inhibitor was transported laterally in the root cap whereas Pilet (1976b) reported that this transport took place in the apex rather than the cap. Indole-3-acetic acid (IAA) has been shown to occur in Zea roots by some chemically rigorous methods (Bridges et al., 1973; Elliott and Greenwood, 1974; Rivier and Pilet, 1974) and is known to inhibit root growth. However, it has been strongly suggested that the growth inhibitor produced in the cap of Zea roots and responsible for geotropic curvature is abscisic acid (ABA) and not IAA (Audus, 1975; Pilet, 1975b; H. Wilkins and Wain, 1975b), although the possibility of other inhibitors has been recognized. In geostimulated roots of Ribes nigrum (El-Antably, 1975) and Vicia faba

El-Antably and Larsen, 1974)--which show a positive geotropic response in complete darkness--the amount of ABA was higher in the lower than in the upper halves. If ABA is also the inhibitior mediating the light-induced geotropic response of Zea roots, the ABA level in the lower halves would be expected to increase only when the horizontally oriented roots are exposed to light. I have accordingly investigated the levels of some endogenous growth regulators in geotropically stimulated Zea roots exposed to red light or kept in darkness.

Material and Methods

Plant Material and Geotropic Stimulation

Caryopses of Zea mays L., cv. Golden Cross Bantam 70 (Sakata Seed Co., Yokohama, Japan), were imbibed for 24 h in a Petri dish in the dark in running tap water which covered the grains, and were then planted, with the embryo upward, on 0.4% solidified agar in containers covered with a nylon sheet. The primary roots were allowed to elongate horizontally on the agar at 26°C in the dark, except that during transfer and other operations they were handled in very weak green light, transmittance 490-580 nm, with a peak at 530 nm, obtained from a 20-W cool green fluorescent tube (Mitsubishi Electric Co., Tokyo, Japan : SL-20 SG) filtered through 6 sheets of green cellophane.

The procedures for gravity and light treatment were the same as described in Suzuki and Fujii (1978). Seedlings with primary roots 2.0-2.5 cm in length, obtained ca. 30 h after transfer to the agar, were exposed to 2.04×10^{-10}E cm^{-2} s^{-1} of red light for 5 min, and then kept for 1 h in the dark. Curvature starts about 1 h after the light exposure and continues during the following 1.5 h (Suzuki and Fujii, 1978). Red light was obtained from a tungsten lamp (300 W; Kondo Electrical Industrial Co., Tokyo) through 30 mm of water, an interference filter (Toshiba Glass Co., Shizuoka, Japan: KL-65, peak 649 nm, half-band width 11.0 nm), and a glass filter (Toshiba Glass Co.: V-R-63) for cutting stray lights. The radiant energies were measured with a Kipp-Zonen (Delft, Netherlands) compensated thermopile CA-1 and a direct-current Microvoltmeter (Toa Electronics, Tokyo, Japan: PM-16A). One h after the light treatment, the apical segments, 7 mm long and including the elongating zone, were cut from the roots and were carefully bisected longitudinally with a steel razor blade into the upper and lower halves.

Extraction Procedures for Growth Regulators

Immediately after cutting, the upper and lower halves of 1,500 segments were frozen with liquid N_2 and crushed to powder in a chilled mortar. The powder was suspended

in 50 ml of 80% cold methanol, homogenized at 4000 rpm for 10 min with a Teflon-glass homogenizer (Takashima Shoten Co., Tokyo), and allowed to stand overnight with stirring, at 4°C. The homogenate was then centrifuged for 10 min at 8000x*g* at 4°C. The extraction was repeated 3 times, the methanolic extracts were combined and were concentrated with a vacuum evaporator (Tokyo Rikakikai Co., Tokyo: N-1) at 37°C to remove the methanol. The resulting aqueous solution of *ca*. 3 ml was diluted with distilled water up to 20 ml, and was first partitioned 3 times against equal volumes of *n*-hexane at pH 2.5, and thereafter against equal volumes of dicholromethane, once at pH 9.0 and then once at pH 2.5. The acidic dichloromethane extract was evaporated to dryness and analyzed by thin-layer chromatography (TLC).

Thin-layer Chromatography of Acidic Growth Regulators

The dried samples were dissolved in small amount of ethyl acetate and loaded on 20 x 20 cm, 0.2 mm thick plates of Silica gel 60 F_{254} (Merck, Darmstadt, Germany; fluorescent). Authentic IAA or ABA were placed on either side of the streak and the plates were developed with toluene-ethyl acetate-acetic acid (40:5:2, v/v) or, in certain cases, with *iso*-propanol-28% ammonia-water (10:1:1, v/v). After developing, IAA, ABA and certain other compounds were

localized under ultraviolet (UV) radiation (Fl-3S UV lamp; Irie Factory, Tokyo) and the corresponding zones marked. The individual zones were scraped off the plates, the Silica gel was eluted with water-saturated ethyl acetate, and the eluates were dried in a vacuum evaporator at 37°C.

Gas-liquid Chromatography of Some Growth Regulators

The eluates from zones corresponding to ABA on a thin-layer plate were redissolved in 3 ml of ether and methylated with diazomethane (Schlenk and Gellerman, 1960). The methylated eluates were evaporated again to dryness, dissolved in 10 µl of ethyl acetate, and injected onto the gas-liquid-chromatography (GLC) column. The GLC analyses were carried out with a gas chromatograph (Hitachi 163; Hitachi city, Japan) equipped with a ^{63}Ni electron-capture detector. The glass column 0.3 cm diameter, 2 m long was packed with 1% XE 60 on AW-DMCS Chromosorb W (Shimadzu Seisaku-sho, Kyoto, Japan; 60-80 mesh). The carrier gas was N_2, used at a flow rate of 60 ml/min. Samples were analysed at 205°C. Injection and detection temperature was 250°C. Quantitative estimation was achieved by using standard (±)-ABA (Sigma Chemical Co., St. Louis, USA) during gas chromatographic analyses of TLC eluates. The ABA was quantified by measuring the area under the peak.

A Hitachi 163 gas chromatograph equipped with a flame

ionization detector was also used to check for contamination by ABA of the growth regulator(s) located between cis-trans- and trans-trans-ABA on the thin-layer chromatograms described above. In this case, a glass-capillary column (Chemicolumn OV-101, Hitachi; 0.25 mm diameter, 20 m long), precoated with silicon oil, was used. The column oven temperature was maintained at 190°C during the first 2 min, raised linearly from 190 to 200°C at a rate of 2°C/min during next 5 min, and then maintained at 200°C. The carrier gas was helium; its flow rate was adjusted to 1 ml/min.

Gas Chromatography-Mass Spectrometry

A Hitachi Model RMU-6L MS equipped with a Hitachi 063 gas chromatograph was used for gas chromatography-mass spectrometry (GC-MS). Separations of the methylesters were performed on a silanized glass column, 3 mm diameter and 1 m length, packed with 25 OV-1 on Chromosorb W (Gasukuro-kogyo Co., Tokyo; 60-80 mesh). Helium at 0.1 kg/cm was used as the carrier gas. Injection temperature was 230°C. The column oven temperature was maintained at 165°C. The ionizing potential of the source was 40 eV. The temperature of the single jet separator and the ion source was 250 and 200°C, respectively.

Avena Straight-Growth Assay

For the quantitative estimation of IAA, the Avena straight-growth test was carried out as described in Fujii (1974). Seeds of Avena sativa L. cv. Victory No. 1, obtained from the Canwest Seed Company, Edmonton, Alberta, Canada, were dehusked, and imbibed in Petri dishes in the dark in deionized water not covering the seeds. About 24 h after the start of imbibition, the grains were transferred to 0.5% solidified agar in glass tubes (2 cm diameter, 5 cm height), the basal end of the grain being inserted into the agar. The plants were grown at 26°C in the dark, except when they were exposed to very weak green safe light when transplanted or operated upon. Three-mm-sections were cut from 65-h-old, 6-8-mm long coleoptiles, starting 3 mm below the tip, and were floated at 26°C on 0.02 M potassium-phosphate buffer (pH 5.8) containing various concentrations of IAA or eluates from zones corresponding to IAA on the thin-layer plate. After 2 h, the length of the sections was measured under a microscope to the nearest 0.01 mm.

Zea Root-Growth Assay

Five-mm apical segments were cut from 54-h-old, 2.0-2.5 cm primary roots of Zea mays L. cv. Golden Cross Bantam 70 seedlings grown in the same conditions as described above, and were floated at 26°C on 0.02 M potassium-phosphate

buffer (pH 5.8) containing the test chemicals. After 2 h, the length of the segments was measured as above.

Results

Estimation of ABA

Table 1 shows the Rf values of growth regulators found in the acidic dichloromethane-soluble fraction of Zea roots after TLC with toluene-ethyl acetate-acetic acid (40:5:2, v/v). Zones corresponding to ABA on the thin-layer plates were scraped off the plates and eluted twice with 3 ml of water-saturated ethyl acetate. After methylation with diazomethane, 1 µl of the methylated sample was chromatographed on the GLC column. A GC-MS examination of the methylated eluate yielded a mass spectrum identical with that of authentic ABA as shown by Rivier et al. (1977). The ABA content in each root half is shown in Tabel 2. The upper halves of horizontally oriented and irradiated roots contained 1.6 times as much ABA as the lower ones, while no marked difference in the content of ABA was observed in both the upper and lower halves of non-irradiated ones. The total amount of endogenous ABA in the irradiated roots was greater than in the non-irradiated ones, by a factor of ca. 1.8.

Estimation of IAA-like Activity

The zones corresponding to IAA on thin-layer plates developed with toluene-ethyl acetate-acetic acid (Table 1) were scraped off the plates and eluted twice with 3 ml of water-saturated ethyl acetate. The eluate was rechromatographed by TLC using iso-propanol-28% ammonia-water (10:1:1) as solvent. The chromatogram was divided into 10 equal zones, and each zone was scraped off and eluted twice with 3 ml of water-saturated ethyl acetate. After drying, each extract was dissolved in 1 ml of 0.02 M phosphate buffer (pH 5.8), and the IAA-like activity in each extract was determined with the Avena straight growth test and the Zea root-growth assay. Promotion of Avena coleoptile growth and inhibition of Zea root growth were observed only with the extracts from zones corresponding to IAA (Fig. 1).

In another test, the zones corresponding to IAA on the thin-layer plates were scraped off, eluted with water-saturated ethyl acetate, and after drying, re-dissolved in 3 ml of ether and then methylated with diazomethane. The methylated eluates were evaporated again to dryness, dissolved in 10 µl of acetone, and subjected to GC-MS. The GC-MS examination of the methylated eluate yielded a mass spectrum identical to that of authentic methyl indolyl-acetate, having the molecular ion at m/e 189 and major fragmentation peaks at m/e 130 (base peak), 103, 102 and

77 (Fig. 2).

Finally, zones corresponding to IAA on thin-layer plates developed with toluene-ethyl acetate-acetic acid (Table 1) were scraped off, eluted twice with 3 ml of water-saturated ethyl acetate, and dried in an evaporator. Each extract was then dissolved in 1 ml of 0.02 M phosphate buffer (pH 5.8), and the level of IAA-like activity in each extract was determined by means of the Avena straight-growth test (Table 3). The ratio of these activities in the lower and upper halves of irradiated and non-irradiated roots was 3.4 and 2.9, respectively. No major difference in the total amount of IAA-like activity was observed after irradiation.

Implication of Another Growth Inhibitor

In a preliminary experiment it was found that a substance having an Rf value of 0.16 upon TLC with toluene-ethyl acetate-acetic acid (40:5:2) (Table 1) strongly inhibited the growth of Zea roots. The position of this substance on the thin-layer plates was easily detected by UV radiation. Fluorescence was quenched in this zone and this served as a marker for the inhibitor. Zones corresponding to this substance were scraped off the plates and eluted twice with 3 ml of water-saturated ethyl acetate. After drying in an evaporator, each extract was dissolved

in a volume of 0.02 M phosphate buffer (pH 5.8) equivalent to the fresh weight of the root halves, and the growth-inhibiting activity was assayed with Zea roots. The results are shown in Table 4. The growth of Zea roots was strongly inhibited by the estimated amount of this substance in the lower halves and slightly in the upper ones of irradiated roots, and slightly both in the upper and lower halves of roots kept horizontally in complete darkness. Gas-chromatographic analysis (Fig. 3) showed that this eluate contained no contamination by ABA, although several other peaks were present.

Discussion

As explained in the Introduction, it appears that light makes available a critical component in the geotropic response mechanism of roots of some maize cultivars, and the simplest possibility is that a growth inhibitor is synthesized or released in the cap following irradiation, and then transported basipetally and asymmetrically to the growing zone of a geo-stimulated root. As also explained, occurence of IAA in maize root tips has been demonstrated by chemically rigorous methods. However, auxin transport in roots was found to be strongly polarised in the direction base→apex (Bowen et al., 1972; Shaw and Wilkins, 1974) and no evidence of an active, downward lateral transport of

IAA could be found in geotropically stimulated roots of some maize cultivars. The quantitative estimation of auxin in our material, on the one hand, showed that the ratio of IAA-like activity between the lower and upper halves of horizontally oriented, red-light treated and dark-grown Zea roots was 3.4 and 2.9, respectively, in favour of the lower halves (Table 3); on the other hand, however, the slight increase in IAA-like activity in the lower halves of roots exposed to light was not reflected in the biological activity as determined by the Zea root-growth test (see Table 5). These results imply that asymmetrical redistribution of IAA does occur but is not an essential element for geotropic curvature, occurring also in geo-stimulated but non-curved roots which have not been exposed to light. The fact that asymmetric application of IAA did not induce curvature of decapitated roots of Zea (Pilet, 1975b) would also indicate that IAA is not the "cap inhibitor" necessary for the geotropic response of Zea roots.

On the other hand, it has been suggested that the growth inhibitor produced in the root cap of Zea is ABA (Pilet, 1975b; H. Wilkins and Wain, 1975b; Rivier et al., 1977). ABA inhibited root elongation when applied to decapitated roots of some maize cultivars, and when applied asymmetrically caused a substantial curvature (Pilet, 1975b).

H. Wilkins and Wain (1974) have reported that ABA is not present in Zea root caps in darkness, appearing only after the whole roots are exposed to light. If this is so, asymmetrical redistribution of ABA should be observed only in Zea roots which had been horizontally oriented and exposed to light. However, my experiments (Table 2) with roots of the Golden Cross Bantam cultivar--in which geosensitivity appears only following irradiation--showed that the ABA content in the upper halves of horizontally oriented roots exposed to light was 1.6 times as great as in the lower ones, whilst showing no differences in the halves of non-irradiated roots, and whereas the total ABA content of the roots increased after irradiation of the roots with 5 min of red light, non-irradiated roots did contain considerable amounts of ABA prior to irradiation. On the other hand, ABA scarcely inhibited root growth in this cultivar (Table 5). These results strongly indicate that the growth inhibitor necessary for the geotropic curvature at least in Golden Cross Bantam maize is not ABA.

Since neither auxin (IAA) nor ABA seems to be involved in the light-dependent georesponse of Zea roots, another inhibitor which I extracted from Golden Cross Bantam roots assumes considerable interest. This growth inhibitor has not yet been identified but its Rf value is different from

those of both cis-trans- and trans-trans-ABA, and its growth inhibiting activity in the lower halves of roots which have been kept horizontal in the light is greater than in the upper ones while no such difference is found in horizontally oriented roots kept in complete darkness (Tables 4, 5). Moreover, the amount of (or activity attributable to) this substance increased in roots following exposure to red light. These results indicate that this new growth inhibitor resembles the "cap inhibitor" extracted by Kundu and Audus (1974) and H. Wilkins and Wain (1974), and may be the inhibitor controlling the light-dependent geotropic response in Zea roots. Before this can be firmly established, however, the inhibitor must be chemically identified, and I am currently engaged with this task.

Table 1. Rf values of some acidic, dichloromethane-soluble growth regulators occurring in *Zea* roots upon TLC with toluene-ethyl acetate-acetic acid (40:5:2, v/v)

Regulator	Rf value
cis-*trans* ABA	0.13
Unidentified compound from *Zea* roots	0.16
trans-*trans* ABA*	0.18
IAA	0.29

* Authentic trans-trans ABA was used for comparative purposes.

Table 2. ABA content in the upper and lower halves of roots of Zea placed horizontally and either kept in continuous darkness or treated with light.

Roots of ca. 54-h-old, dark-grown seedlings of Golden Cross Bantam were grown horizontally either in continued darkness or in the darkness after 5 min of red light, and after 1 h extracted in methanol followed by partitioning against n-hexane at pH 2.5 and against dichloromethane at pH 9.0 and 2.5, and TLC with toluene-ethyl acetate-acetic acid (40:5:2,v/v). The Rf zone 0.13 was extracted with water-saturated ethyl acetate, and ABA was determined by gas-liquid chromatography.

Condition	Root halves	ABA content (ng/g fr.wt)			Ratio
		Exp. 1	Exp. 2	Mean	upper/lower
Dark	Upper	38.1	33.2	35.7	1.0
	Lower	39.1	32.0	35.6	
	Total	77.2	65.2	71.3	
Light	Upper	78.9	76.7	77.8	1.6
	Lower	49.5	47.3	48.4	
	Total	128.4	124.0	126.2	

Table 3. IAA-like activity in the upper and lower halves of roots of Zea placed horizontally and either kept in continuous darkness or treated with light.

Extraction procedures were the same as described in Table 2, except Rf zone 0.29. IAA activity was estimated by the Avena straight-growth test.

Condition	Root halves	IAA equivalents (ng/g fr.wt.)			Ratio
		Exp. 1	Exp. 2	Mean	lower/upper
Dark	Upper	12.9	14.9	13.9	·····2.9
	Lower	42.0	38.0	40.0	
	Total	54.9	52.9	53.9	
Light	Upper	13.4	15.6	14.5	·····3.4
	Lower	48.9	49.1	49.0	
	Total	62.3	64.7	63.5	

Table 4. Inhibition of Zea root growth by an unidentified compound extracted from roots of Zea mays cv. Golden Cross Bantam 70 and located at Rf 0.16 on thin-layer plate developed with toluene-ethyl acetate-acetic acid (40:5:2, v/v). For the root-growth inhibition assay, 20 5-mm apical segments were cut from 54-h-old Zea primary roots of same cultivar, and floated for 2 h on a volume of 0.02 M phosphate buffer (pH 5.8) equivalent to the fresh weight of the tissue and containing the unidentified compound. The control length after 2 h was 5.53 ± 0.031 mm. Extraction etc. are described in Table 2.

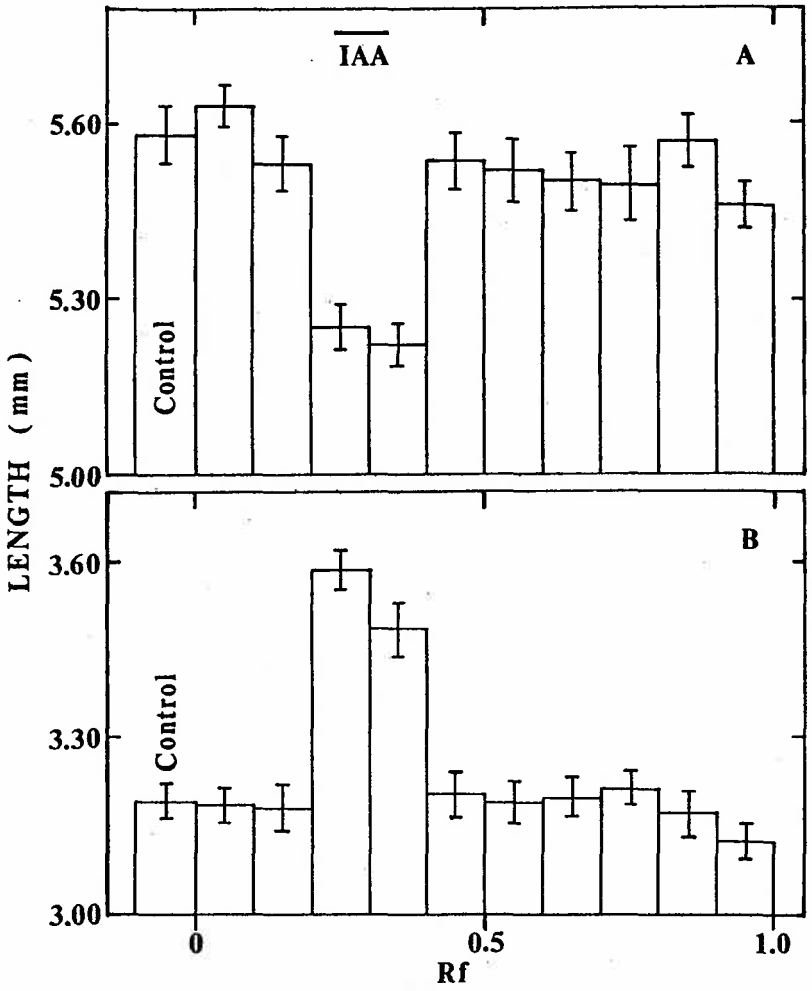
Condition	Root halves	Exp. 1		Exp. 2		Mean Inhibition (%)
		Length of sections (mm)	Inhibition (%)	Length of sections (mm)	Inhibition (%)	
Dark	Upper	5.40±0.032	25.4	5.38±0.040	29.1	27.3
	Lower	5.44±0.027	16.9	5.42±0.025	20.9	18.9
Light	Upper	5.42±0.025	20.8	5.43±0.031	18.3	19.6
	Lower	5.30±0.030	43.1	5.32±0.029	40.0	41.6

Table 5. Inhibition of Zea root growth by ABA, IAA and the unidentified compound (UC) extracted from the upper and lower halves of dark-grown and light-treated, geo-stimulated Zea roots. Extraction procedures are described in Table 2; Rf zones tested=0.13, 0.29 and 0.16 for ABA, IAA and UC, respectively. Root-growth assay as in Table 4. Length of control segments=5.54 ± 0.036 mm. The significant differences between the upper and lower halves were observed only in the inhibition by UC extracted from the light-treated halves.

Condition	Root halves	Inhibition (%)		
		ABA	IAA	UC[a]
Dark	Upper	7.5	47.3	27.3
	Lower	6.9	52.7	18.9
Light	Upper	9.7	47.7	19.6
	Lower	7.9	54.0	41.6

[a] Data from Table 4

Fig. 1. A and B. *Zea* root-growth (A) and *Avena* straight-growth assays (B) of IAA-like activity eluted from TLC plates. Fifteen hundred, 7-mm-apical segments of primary roots of ca. 54-h-old, dark-grown seedlings of cv. Golden Cross Bantam 70 were used as a material. Extraction procedures are described in Table 2. The zones corresponding to IAA on thin-layer plates developed with toluene-ethyl acetate-acetic acid (40:5:2) were scraped off the plates and eluted, and the eluate was re-chromato graphed by TLC using *iso*-propanol-28% ammonia-water (10:1:1) as solvent. Ten equal zones were scraped off and eluted, and each extract was dissolved in 1 ml of 0.02 M phosphate buffer (pH 5.8). The IAA-like activity in each extract was determined with *Avena* straight-growth and the *Zea* root-growth assays. The position of authentic IAA is indicated by horizontal bar. The initial length of the *Zea* roots and *Avena* coleoptiles were 2.95 ± 0.02 and 4.99 ± 0.03 mm, respectively. Twenty segments were used for each experiment. Vertical bars = Standard errors


IAA
A
5.60
5.30
5.00
Control
LENGTH (mm)
B
3.60
3.30
3.00
Control
0
0.5
1.0
Rf

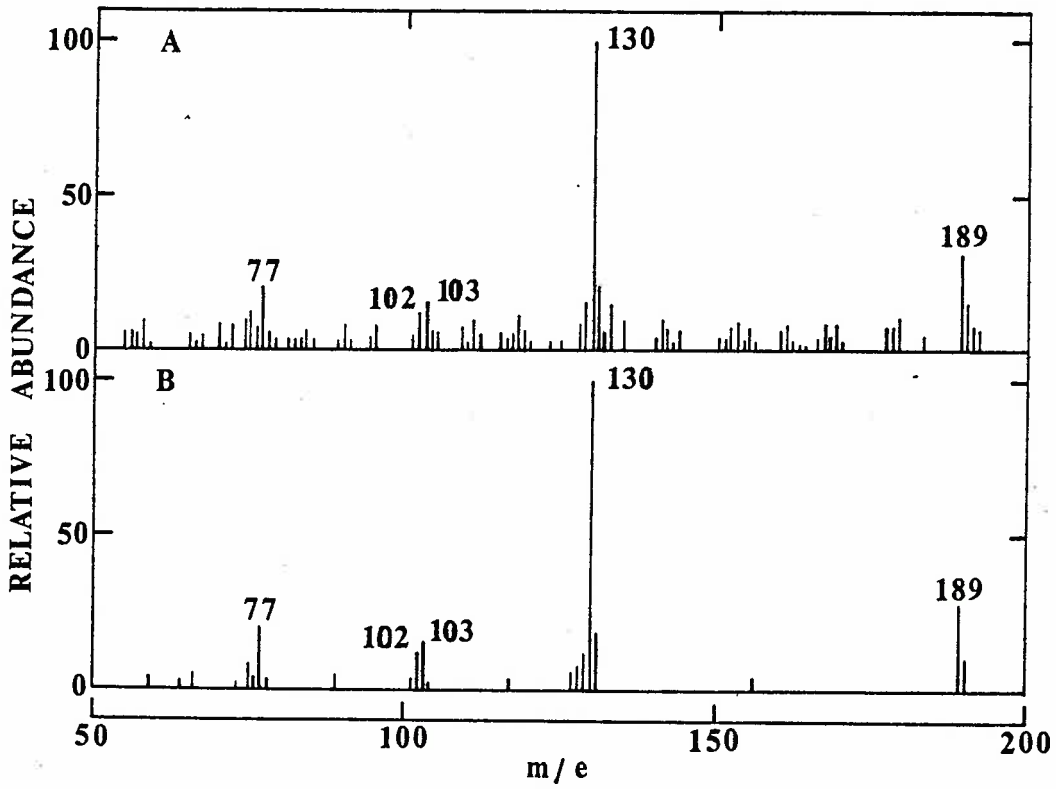


Fig. 2. A and B. Mass spectra for the methylated eluate of zones corresponding to IAA on TLC developed with iso-propanol-28% ammonia-water (A), and methylated authentic IAA (B).

Extraction and TLC procedures are described in Table 2 and Fig. 1. The zones corresponding to IAA on the thin-layer plates were eluted and methylated with diazomethane. After drying, the methylated eluates were dissolved in 10 µl of acetone, 1 µl of which were subjected to GC-MS.

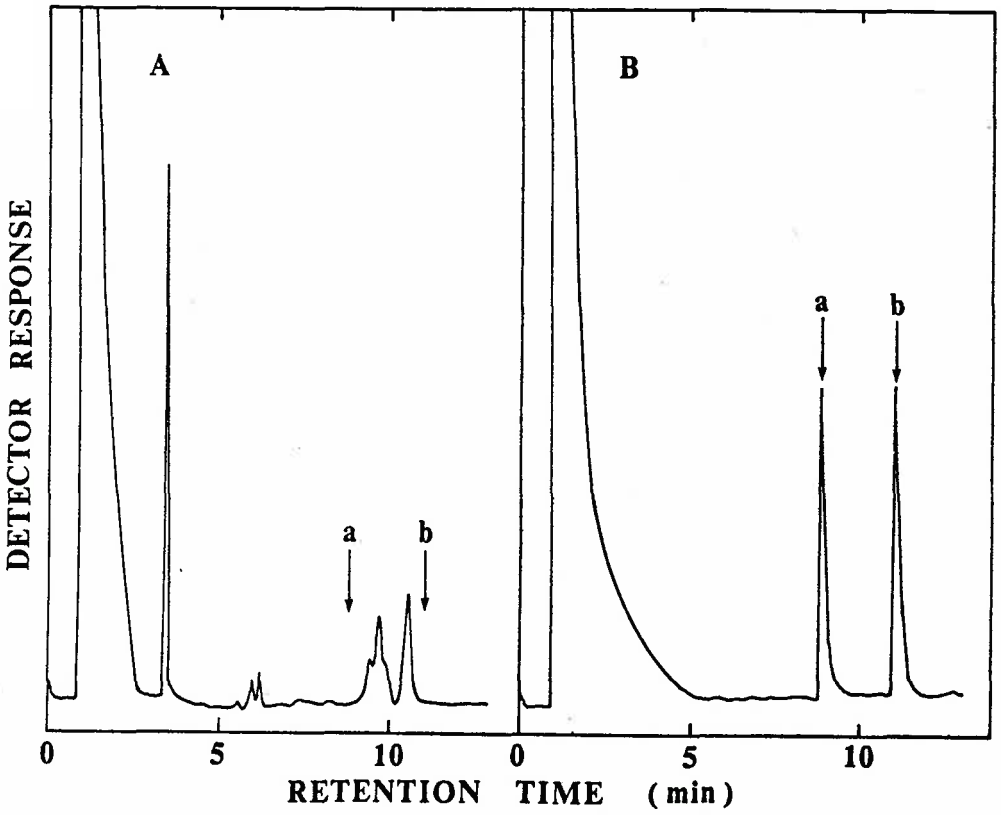


Fig. 3. A and B. GLC traces of methylated eluates (A) from Rf 0.16 zones (Table 1) on TLC plates developed with toluene-ethyl acetate-acetic acid and methylated authentic ABA(B). Arrows (a) and (b) indicate the position of cis-trans and trans-trans ABA, respectively.

Fifteen hundred, 7-mm-apical segments of primary roots of 54-h-old, dark-grown seedlings of cv. Golden Cross Bantam were used as a material. Extraction etc. are described in Table 2. The eluates from Rf 0.16 zones on TLC plates were methylated with diazomethane, and after drying the methylated eluates were dissolved in 10 µl of ethyl acetate, 1 µl of which were injected onto GLC column.

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PART III

A POSSIBLE ROLE OF WALL HYDROXYPROLINE IN GEOTROPIC RESPONSE OF *ZEA* ROOTS

Part III

A Possible Role of Wall Hydroxyproline in Geotropic Response of *Zea* Roots

Abstract

Results of this study provided evidence to support the hypothesis that there is an inverse relationship between hydroxyproline-protein level in the cell wall and the ability of a cell to undergo rapid cell elongation. The unidentified growth inhibitor extracted from *Zea* primary roots accelerated the incorporation of radioactivity derived from ^{14}C-proline into the SLS-insoluble cell wall fraction. However, this inhibitor showed no effect on the ratio of hydroxyproline to proline incorporated into the SLS-insoluble fraction.

Based on these results, I discussed the implication of this unidentified growth inhibitor in the geotropic curvature of *Zea* primary roots.

Introduction

It has been demonstrated that proteins, characterized by a high level in hydroxyproline, are firmly associated with cellulose microfibrils in plant cell walls (Lamport, 1965). The nature of these proteins is still unknown but a few papers have demonstrated a correlation between the increase in the hydroxyproline content of the wall and the decrease of cell elongation (Cleland and Karlsnes, 1967; Ridge and Osborne, 1970; Sadava and Chrispeels, 1973; Vaughan, 1973; Fujii and Shimokoriyama, 1976; Jotterand-Bolivo and Pilet, 1976; Fujii, 1978). Jotterand-Bolivo and Pilet (1976) have shown that for the growing maize roots the increase of the content of wall hydroxyproline was related to a decrease of the root elongation and vice-versa, and that for the geotropic roots the wall hydroxyproline level was lower in the upper part which elongates more than the lower part containing more wall hydroxyproline.

The downward curvature of geostimulated roots arose from the extension rate of the lower part being depressed much more than that of the upper part (Pilet, 1972; Konings, 1965). Such asymmetrical growth has to be related to the production, by the root cap, of some growth inhibitors (see Part II) which move basipetally from the tip to the extending zone of root (Shaw and Wilkins, 1973; Pilet, 1975) and also laterally inside the horizontal root apex (Pilet,

1976). If the increase in wall hydroxyproline is one of the factors which cause cessation of cell elongation in roots, the asymmetrical redistribution of growth inhibitors in geostimulated roots should regulate the wall hydroxyproline content in the upper and lower part of horizontally oriented roots, it being lower in the former than the latter.

The author extracted a growth inhibitor (see Part II) from Zea root, although it has not yet been identified, and demonstrated that its growth inhibiting activity is more in the lower halves than the upper ones of roots which have been kept horizontal in the light. Thus, experiments were attempted in this Part to demonstrate the action of this growth inhibitor on the hydroxyproline level in the cell wall of Zea roots.

Material and Methods

Plant Material

Caryopses of Zea mays L., cv. Golden Cross Bantam 70 (Sakata Seed Co., Yokohama, Japan), were imbibed for 24 h in a Petri dish in the dark in running tap water which covered the grains, and were then planted, with the embryo upward, on 0.4% solidified agar in containers covered with a nylon sheet. The primary roots were allowed to elongate horizontally on the agar at 26°C in the dark, except that during transfer and other operations they were handled in

very weak green light.

Extraction Procedures for Growth Inhibitor

The apical segments, 7 mm long and including the elongating zone, were cut from the roots of 54-h-old, dark-grown seedlings of Golden Cross Bantam, with a steel razor blade. Immediately after cutting, 1500 segments were frozen with liquid N_2 and crushed to powder in a chilled mortar. The acidic dichloromethan-soluble fraction (see Part II) was evaporated to dryness and analyzed by thin-layer chromatography (TLC).

The dried samples were dissolved in small amount of ethyl acetate and loaded on 20 x 20 cm, 0.2mm thick plates of Silica gel 60 F_{254} (Merck, Barmstadt, Germany; fluorescent). The plates were first developed with toluene-ethyl acetate-acetic acid (40:5:2, v/v). After developing, the growth inhibitor was localized under ultraviolet (UV) radiation (Fl-3S UV lamp; Irie Factory, Tokyo) and the corresponding zone marked (see Part II). The Rf 0.16 zone was scraped off the plates, the Silica gel was eluted with water-saturated ethyl acetate, and the eluates were dried with a vacuum evaporator at 37°C. The dried samples were redissolved in a small amount of water-saturated ethyl acetate, loaded again on TLC plates, and developed with n-propanol-n-butanol-28% ammonia-water (6:2:1:2, v/v). The Rf 0.68

zones were scraped off and eluted twice with 3 ml of water-saturated ethyl acetate. After drying with a vacuum evaporator, the extract was dissolved in 0.02 M phosphate buffer (pH 5.8), and used as the growth-inhibitor solution. The position of this substance on the thin-layer plates was easily detected by UV radiation. Fluorescence was quenched in this zone and this served as a marker for the inhibitor.

Determination of Proline and Hydroxyproline in Cell Wall

One hundred and fifty, 5-mm-long apical segments cut from 54-h-old Zea primary roots were used. The sections were floated for 2 h on 1.5 ml of 0.02 M potassium phosohate buffer (pH 5.8) containing 6 μCi of [U-^{14}C]-proline (specific activity: 285 mCi/mmole), throughly rinsed with the buffer, and then transplanted on 2 ml of same buffer containing the test chemicals. After 2 h of treatment, the segments were rinsed and frozen with liquid N_2 in the dark and crushed into powder in a chilled mortar. The powder was suspended in 10 ml of 0.02 M phosphate buffer (pH 5.8), homogenized at 4,000 r.p.m. for 5 min with a Teflon-glass homogenizer (Takashima Shoten Co., Tokyo), and allowed to stand for 3 h with stirring, at 4°C. The homogenate was then centrifuged for 10 min at 1,000xg at 4°C. The extraction was repeated 3 times. The water-soluble fractions were combined and designated as "water-soluble fraction". The precipi-

tate (wall fraction) was suspended for 1 h in 8 ml of 1% sodium lauryl sulfate (SLS) at room temperature and the homogenate was centrifuged for 15 min at 10,000x*g*. The extraction was repeated twice, and then the precipitate was extracted with 1% SLS at 65°C, and the supernatant was combined with the above (SLS-soluble fraction). The precipitate was thoroughly rinsed with distilled water to remove the SLS. and was designated as the SLS-insoluble fraction.

To 1 ml of SLS- and water-soluble fractions, we added 10 ml of toluene-Triton X-100 scintillator (2:1) containing 0.4% PPO and 0.01% POPOP and counted the radioactivity with Beckman liquid scintillation spectrometer (Model : LS 8100). The SLS-insoluble fractions were hydrolyzed with 6 N HCl for 6 h at 110°C. After removal of the HCl, proline and hydroxyproline were separated by paper chromatography using *iso*-propanol-formic acid-water (15:2:2, v/v). The chromatogram was cut crosswise into 20 equal pieces, and radioactivity in each division was measured.

Results

In order to test whether the growth inhibition by the inhibitor extracted from *Zea* primary roots (see Part II, Tables 4 and 5) might be the results of the accumulation of hydroxyproline-rich glycoproteins in the cell

wall, ^{14}C-proline labeling of various fractions was recorded. Root segments were floated for 2 h on 1.5 ml of 0.02 M potassium phosphate buffer (pH 5.8) containing ^{14}C-proline, then were thoroughly rinsed with the buffer mentioned above and transplanted on 2 ml of the same buffer with or without the inhibitor. After 2 h treatment, radioactivity in various fractions was measured (Table 1), and its increase in wall fractions during the treatments was calculated (Table 2). Radioactivity incorporated into the SLS-insoluble fraction in segments treated with the inhibitor greatly increased, and that into the SLS-soluble fraction slightly increased (Table 2).

Radioactivity in proline and hydroxyproline in the SLS-insoluble fraction was measured, and its increase during the treatments was calculated (Table 3). The ratio of hydroxyproline to proline incorporated into this fraction during the treatments were also calculated (Table 3); they were ca. 0.25 in both segments treated with and without the inhibitor. This means that the inhibitor does not affect the ratio of hydroxyproline to proline in the SLS-insoluble fraction. Thus, the amount of ^{14}C-labeled hydroxyproline and proline in SLS-insoluble fraction greatly increased in segments treated with the inhibitor, since ^{14}C-proline incorporation into this fraction increased in these segments.

Discussion

Much evidence indicates that there is an inverse relationship between the hydroxyproline-protein level and the ability of a cell to undergo rapid cell elongation (Barnett, 1970; Cleland, 1967; Fujii and Shimokoriyama, 1976; Fujii, 1978; Sadava et al., 1973; Sadava and Chrispeels, 1973). It has been demonstrated that indole-3-acetic acid (IAA), a well known inhibitor of root elongation, increased the level of hydroxyproline rigidly bound to cell wall of Zea primary roots (Fujii and Hori, 1978). Quantitative estimation of IAA in our maize cultivar showed that IAA level was lower in the upper part which elongates more than the lower part containing more IAA (see Part II, Table 3). These results suggested the possibility that IAA may reduce the elongation of the lower halves of horizontally oriented Zea roots by increasing the level of hydroxyproline proteins rigidly bound to the cell wall, resulting in the downward curvature.

However, the author showed that asymmetrical redistribution of IAA occurs, but is not an essential element for geotropic curvature, because it occurs even in geo-stimulated but non-curved roots which have not been exposed to red light (see Part II, Table 3). A slight increase in IAA content in the lower halves seems to be

meaningless for increasing in the hydroxyproline level in the cell wall. Nevertheless, for the georeactive roots the wall hydroxyproline level was higher in the lower halves than the upper one (Jotterand-Dolivo and Pilet, 1976).

If the downward curvature, differential growth between the upper and lower halves, is induced through the differential concentration of hydroxyproline in the wall, the asymmetrical distribution of the inhibitor, extracted from Zea primary roots (see Part II), which strongly inhibits the root elongation should be expected to increase the hydroxyproline level in the lower halves of geostimulated roots which were exposed to red light (see Part I). Indeed, the inhibitor drastically increased the hydroxyproline content in the cell wall.

These results suggest that this inhibitor is one of the factors causing the differential growth between the upper and lower halves, the downward curvature, by increasing the level of hydroxyproline proteins rigidly bound to the cell wall in the lower halves of geostimulated, red-light treated Zea primary roots. How the increased binding of hydroxyproline protein(s) to cell wall polysaccharides is brought about by the inhibitor treatments requires further investigation.

Table 1. [^{14}C]-proline labeling of various fractions of the segments treated with or without the inhibitor extracted from Zea primary roots. The apical segments cut from the primary roots of cv. Golden Cross Bantam were floated for 2 h on [^{14}C]-proline solution (initial) and then treated with or without the inhibitor for 2 h.

	Radioactivity (dpm)		
	Initial	Buffer	Inhibitor
Medium	--------	108025	115339
Water-soluble fraction	883110	821767	767438
Wall fraction	60662	74333	77130
SLS-soluble fraction	59067	72114	73138
SLS-insoluble fraction	1595	2219	3992
Total	943772	1004125	959907

Table 2. Radioactivity (dpm) incorporated into the wall fractions during the treatment.

Apical segments floated for 2 h on [^{14}C]-proline solution (initial) were treated with or without the inhibitor for 2 h. Differences between radioactivities of initial and treated segments were calculated from Table 1.

	Radioactivity (dpm)	
	Buffer	Inhibitor
SLS-soluble fraction	13047	14071
SLS-insoluble fraction	624	1397
Total	13671	15468

Table 3. Radioactivity (dpm) incorporated into proline and hydroxyproline of the SLS-insoluble fraction during inhibitor treatments. Apical segments floated for 2 h on [^{14}C]-proline solution (initial) were treated with or without the inhibitor for 2 h. Differences between radioactivities of initial and treated segments were calculated. Radioactivity in the initial stage was 1273 dpm in proline, 227 in hydroxyproline and 95 in the other group.

	Radioactivity (dpm)	
	Buffer	Inhibitor
Proline	477	1756
Hydroxyproline	122	428
Other	35	113
Hypro/Pro	0.26	0.24

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Concluding Remarks

The light-induced geotropic responsiveness in the primary roots of Zea mays L., cv. Golden Cross Bantam 70, can be regarded as a catenary process, a chain of reactions causally linked in sequence. In this thesis, I have received much information about the mechanism of each process in geotropic responsiveness.

In Part I, I clarified the type of response of geostimulated roots to light stimulus. The light induction of the geotropic responsiveness in Zea roots was an all or none response, satisfying the Bunsen-Roscoe reciprocity law. There was a threshold light energy value for the induction of geotropic response. This fact indicates that the response is initiated by the photochemical transformation of a photoreceptor. Furthermore, the action spectrum for this light effect showed a large peak at 650, a small peak at 410, and a shoulder at 663 nm. The effect of red light was not reversed by far-red light. This suggested that the geotropic responsiveness in Zea roots may not be controlled by phytochrome.

In Part II, I dealt with the transmission of the stimulus from the site of perception to the region of response (i.e., from root apex to extending zone). This process is reasonably certain to be caused by one of differential transport or supply of growth inhibitor(s). According to the Cholodny-Went hy-

pothesis the inhibitor involved in the geotropic response is indole-3-acetic acid (IAA), but recent evidences make this very unlikeky. It has been strongly suggested that the growth inhibitor produced in the cap of Zea roots and responsible for geotropic curvature is abscisic acid (ABA) and not IAA, although the possibility of other inhibitors has been recognized. The results in Part II showed that the growth regulator which underwent lateral transport into the lower half of the horizontally oriented, light-treated Zea roots is neither ABA nor IAA, but rather another growth inhibitor, although it has not yet been identified.

In part III, I studied the role of the inhibitor extracted from Zea roots on the geotropic curvature, and suggested that this inhibitor is one of the factors causing the differential growth between the upper and lower halves, the downward curvature, by increasing the level of hydroxyproline proteins rigidly bound to the cell wall in the lower half of geostimulated, red-light treated Zea primary roots.

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