J. Plant Biol. 39(4) : 287~293 (1996)

The Effect of Taxol and Ethyl-N-phenylcarbamate (EPC) on Growth and Gravitropism in *Zea mays* L.

Park, Yun-Hee, Yoon Hi Choy, and June Seung Lee*

Department of Biological Science, Natural Science College, Ewha Womans University, Seoul 120-750, Korea

The effect of taxol and ethyl-N-phenylcarbamate (EPC) on the growth and gravitropism of maize roots and coleoptiles was studied. Taxol is known to promote the assembly of microtubules (MTs) and stabilizes MTs by preventing depolymerization. EPC, on the contrary, is an anti-microtubule drug that promotes disassembly of MTs. Taxol, at 1 μM, inhibited gravitropic response of maize roots to about 40%, but did not inhibit growth; at 10 μM, it inhibited the gravitropic response of coleoptile segments of maize by approximately 50%, but did not inhibit growth, while 0.5 mM EPC inhibited both the gravitropic response and growth of maize roots by approximately 50%. Taxol, which inhibited the gravitropic response of maize roots and coleoptile segments, had no effect on either the polar or the bilateral transport of auxin. These results indicated that MT polymerization could not occur normally with taxol or EPC, so that if there was any abnormal rearrangement of MT, the gravitropic response was inhibited, which resulted from the inhibition of neither growth nor auxin transport. This results suggested that gravitropic response was related to the MT arrangement, and that both straight growth and the differential growth in gravitropic response could be regulated by different mechanisms.

Keywords : maize, gravitropism, microtubule, taxol, EPC

The growth of plant tissue is mainly regulated by epithelial cortical cell known to be responsive to auxin. The epithelial cortical cell plays an important role in growth and gravitropic response of general plants. According to Evans (1986), any gravitropic response did not appear in roots or stems from which the epidermis was eliminated. In addition, auxin accumulated in cells of a rapid growth region, while auxin in cells of a slow growth region decreased; the auxin level was regulated by the transport occurring between epidermis and the epithelial cortical cell (McDonald & Hart, 1987). Among a series of experimental results noted the importance of epithelial cortical cells in plant growth, Bergfeld and his followers (1988) had shown that MT rearrangement took place in epithelial cortical cells when elongation of coleoptile was stimulated by auxin. MT played a role in forming the skeleton of the cell and was important functions of various cell activities such as cell movement, the signal transduction concerning cell division, and intracellular transport of metabolites (Dustin, 1980). Nick and his coworkers (1990) observed that in gravitropic response of corn coleoptiles placed horizontally, the MT arrangement of epithelial cortical cells growing fast at the lower part was similar to that in stem growing vigorously with auxin. Braun *et al.* (1994) reported that in *Chara* the MT arrangement caused by gravitropic responses appeared in the same way as that of higher plants. Hauser and his co-workers reported the oppsite results in *Arabidopsis* root. It was suggested that MT might be material likely to accept the pressure of statolith in *Chara* rhizoids (Friedrich and Hertel, 1973), and that MT might perform important roles in perceiving gravity in *Ceratodon* (Schwuchow *et al.*, 1989) In order to prove this, MT arrangements and gravitropic responses were studied by using EPC and propyzamide which inhibits MT polymerization by binding reversibly with tubuline (Nick and Hertel, 1991). Most of the material which has effects on the action of MT either destroys or depolymerizes MTs, but taxol stimulates the assembly of MTs and inhibits their depolymerization (Schiff & Horwitz,

*Corresponding author: Fax +82-2-360-2385

1981). Taxol is an alkaloid compound extracted from spindle tree of the Taxaceae family that has the structure of a diterpene and is used as an anti-cancer drug. It also stabilizes MTs because it keeps the polymerizational condition even under the disassembly condition of MTs. Nick and Hertel showed that the gravitropic response in EPC-treated tissues was inhibited (1991). But this phenomenon did not seem to be a result of growth inhibition because EPC did not inhibit the phototropic response even at much higher concentrations. The inhibition of gravitropic response occurred at low concentration of EPC, but did not impair MT of the outer epithelial cortical cell. The effect of EPC on perception of gravity was shown at the lower concentration than that in case of growth. That meant MT related to growth responses could differ in essence from that related to gravitropic responses in roots (Akashi *et al.*, 1988). According to Björkman and Leopold (1987), chlorpromazine which is an inhibitor of calmoduline inhibits gravitropic responses, the bilateral transport of calcium and voltage gradient. But it hardly inhibits growth or the polor transport of auxin. These results indicate that although growth, gravitropism and phototropism of the stem or root are all physiological phenomena regulated by auxin, these phenomena is somewhat different from the preexistent concept of growth. The differences possibly result from the kind of MT in cells and it's arrangement. So, the aim of this study is to examine growth, gravitropic responses and transport of auxin in stems and roots using taxol and EPC to analyze growth and growth response occurring in gravitropic response.

MATERIALS AND METHODS

Plant material and reagents

Maize (*Zea mays* L., Golden cross Bantam) was germinated and grown for 2-4 days in darkness at $26\pm1^{\circ}C$. Straight root (1.5-2 cm long) from 2 days old seedling and coleoptile from 3-4 days old seedling were used for experimental materials.

^{3}H-indol-3-acetic acid (^{3}H-IAA, 27 Ci/mmol) were purchased from Amersham (U.K.), IAA and taxol from Sigma, and EPC was gift of Dr. Peter Nick (Freiburg Univ., Germany).

Gravitropic response of coleoptiles

To elucidate taxol effect on negative gravitropic response, coleoptiles of maize was cut into segments of 1-1.3 cm length at the lower part of 3 mm under the apical tip and were incubated in 5 mM Mes/Tris, pH 6.4 buffer (solution) containing taxol (1-10 μM) at $26\pm1^{\circ}C$ in a dark room for 17 h. And then, we measured the curvature of coleoptile segments that supplied continuously with 3×10^{-6} μM auxin and taxol for 3 h to measure the degree of short term gravitropic response.

Gravitropic response of roots

Vertically fixed maize root was pretreated with 5 mM Mes/Tris, pH 6.4, buffer (solution) containing taxol at every concentration (0.4-10 μM) for 90 min with aeration. Then, the maize root was fixed horizontally in humid chamber at room temperature, and the degree of gravitropic response was measured at 30 min interval. In measuring the gravitropic response, we sketched the features of the changing curvature of the maize root fixed horizontally on transparent cellophane paper and measured it with a graduator.

Measurement of growth

For the measurement of degree of short-term growth, we used a computer system (program title; SECANT) that connected a camera with a VTR monitor so as to magnify the coleoptile and maize root at room temperature. The growth of coleoptile was measured by putting 5 coleoptile segments (5 mm long) vertically on the one side of the transparent acryl chamber containing 5 mM Mes/Tris, pH 6.4, buffer solution with aeration. There was hardly any growth of coleoptile perceived by us in the control contained only Mes/Tris buffer solution. Only after pretreatment with IAA (10^{-5} M) for 2 h, we observed growth of coleoptile segments treated with taxol.

For measurement of root growth, we fixed intact maize root vertically on one side of the transparent acryl chamber and poured 5 mM Mes/Tris, pH 6.4, buffer solution in it. Then we had the images of the root tip in the same manner as above. After the roots were treated with a buffer solution containing taxol, we observed some change in growth.

Auxin transport

The agar (1.5% w/v) block containing 5 mM Mes/Tris, pH 5.0, ^{3}H-IAA (0.5 Ci/mL) was used as

donor block, and the agar block containing only 5 mM Mes/Tris, pH 6.8. as receiver block. That block size was 3×3×1 mm applied to segments of coleoptile or 2×2×1 mm applied to roots. The radioactivity level of donor block (3×3×1 mm) was about 15,000 cpm. As for the polar transport of auxin in coleoptile, 1 cm-long coleptile segments was incubated in 5 mM Mes/Tris buffer containing a various concentration of taxol for 90 min by shaking in a dark room at 26±1°C. And then middle part of the coleoptile segment was cut into 0.5 cm-long segments. Donor block (3×3×1 mm) was put the upper end of 0.5 cm-long segment and receiver block (3×3×1 cm) was applied to the lower end because basipetal transport predominated in coleoptile and vegetative shoots. After 2 h, radioactivity of receiver block at the lower end was measured with a Liquid Scintillation Counter (LSC).

For measurement of the bilateral transport of auxin in coleoptile, the coleoptile segments (1 cm long) was preincubated in 0.1% DMSO, 5 mM Mes/Tris, pH 6.4, with a various concentration of taxol (4-10 μM) for 90 min in dark room at 26±1°C. The middle region of the pretreated coleoptile was cut into 0.5 cm long, and the donor block was placed on the upper end of middle region for 30 min. After this, we bisected the receiver block (5×5×1 mm) by using a capillary tube and placing the coleoptile on it, so that the coleoptile segments attached the donor block was placed exactly on the middle of receiver block. And the coleoptile was fixed horizontally on the receiver block (1/2 block) attached to the upper part of the coleoptile in the upper region. after 1 h, we measured the radioactivity of receiver block attached to both the upper and lower part of the coleoptile with LSC.

For the experiment on the bilateral transport of auxin in roots, we pretreated a vertically-fixed maize root for 90 min with aeration in solution containing 5 mM Mes/Tris, pH 6.4, 0.1% DMSO and a various concentration of taxol. In order to observe the pattern of auxin transport in both directions, a donor block (2×2×1 mm) was attached to the upper part of the elongation zone of the pretreated root fixed horizontally, a receiver block on the lower part of it and at this time *vice versa* [i. e. the receiver block on the upper side and the donor block on the lower side]. After approximately 2 h, we removed the donor and receiver blocks, then bisected the elongation zone of the root with a sharp needle vertically and cut side attached receiver block into 0.5 cm long pieces to measure the radioactivity with LSC.

RESULTS AND DISCUSSION

The upper region of maize coleoptile placed horizontally has low growth rate, and the MT of epithelial cortical cell is rearranged form transversely to longitudinally under gravitropic stimulus (Nick *et. al.*, 1990), suggesting that MT is involved in the transmission of gravity response. Nick and Hertel (1991) observed that the gravitropic response was inhibited when polymerization of MTs was inhibited in maize coleoptile by using MT assembly inhibitors such as EPC and propyzamide. We, on the contrary, used taxol in this experiments to see the effect on the gravitropic response. As a result, the gravitropic response was inhibited in maize root and coleoptile (Fig. 1, 2 and 3). The results in the treatment of EPC was similar as the experiments with taxol (Fig. 2). As the concentration of taxol increased, the gravitropic response in the taxol-treated root was inhibited, that is, the gravitropic response of the tissues treated with 1 μM and 10 μM taxol was inhibited approximately 40% and 90% in comparison with the control. EPC inhibited the gravitropic response by a stronger degree than taxol: the gravitropic response was inhibited by about 50% in the roots pretreated with 1 mM EPC compared to the control, while root treated with 0.1 mM EPC, the rate was 14%. Parallel to the above, the curvature of the coleoptile segments treated with taxol was measured to observe the vari-

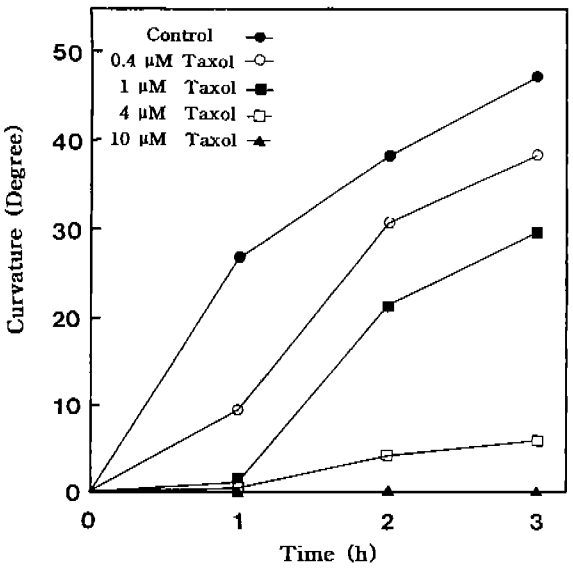

Fig. 1. Effect of taxol on maize root gravitropic curvature. Vertically placed roots were preincubated in buffer with or without taxol for 2 h and the root were placed in horizontally at time zero in humid chamber for 3 h.

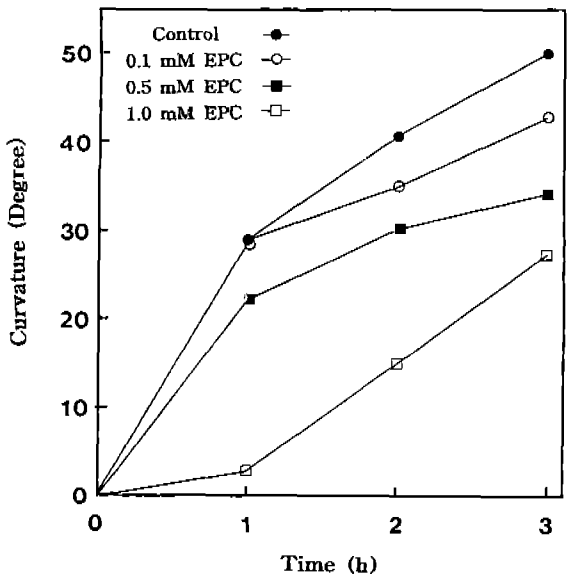

Fig. 2. Effect of EPC on maize root gravitropic curvature. Vertically placed roots were preincubated in buffer with or without EPC for 2 h and the root were placed in horizontally at time zero in humid chamber for 3 h.

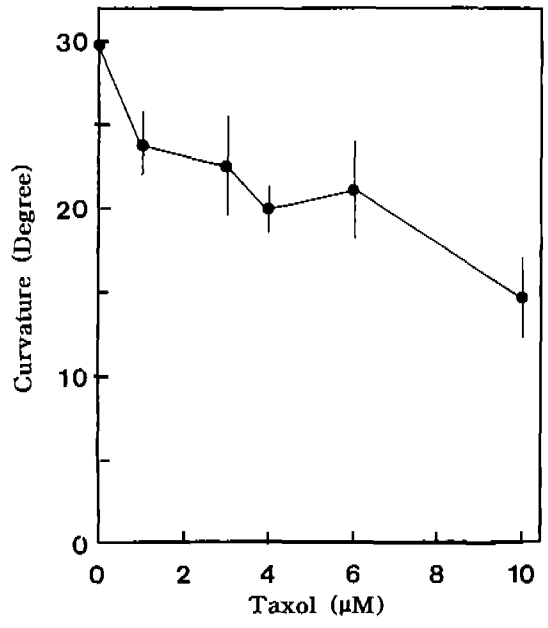

Fig. 3. Long term effect of taxol on gravitropic curvature of maize coleoptile segments. Maize coleoptile segments were incubated for 24 h at indicated concentrations of taxol. Each point is an average of measurements from ≥20 coleoptiles; bars indicate the standard deviation.

ation in gravitropic responses by taxol. As Fig. 3 shows, coleoptile segments treated with 10 μM taxol for 17 h curved 12 degree, indicating that the inhibition by about 50% on average compared to the

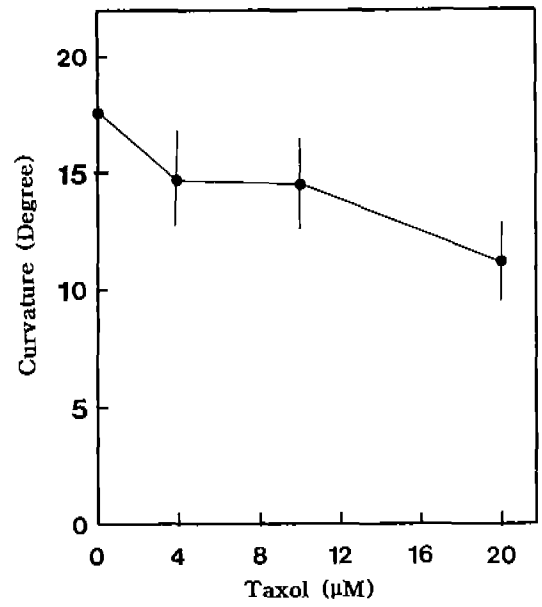

Fig. 4. Short term effect of taxol on gravitropic curvature of maize coleoptile segments. Maize coleoptile segments were incubated with IAA (3×10^{-6} M) for 3 h at indicated concentrations of taxol. Each point is an average of measurements from ≥30 coleoptiles; bars indicate the standard deviation.

control which curved 25 degree. When the coleoptile segments was treated with taxol at a lower concentration (1-6 μM), its curvature was inhibited by 30% on average. And when we observed the degree of short-term gravitropic response of those supplied with auxin continuously (Fig. 4), the segments treated with 20 μM taxol curved about 11 degree in 3 h compared to the control which curved 18 degree, so that the gravitropic response was inhibited approximately 40%. Two factors, both inhibition of polymerization of MT with EPC or propyzamide and the stimulative effect of polymerization with taxol, resulted in the inhibition of the normal arrangement of MT. The fact that treatment of these reagents inhibited gravitropic response meant that the arrangement of MT was related to gravitropic responses. To see whether this effect resulted from (1) inhibition of growth or (2) auxin transport or (3) the loss of perceiving gravity, growth of maize roots and coleoptile was measured in the presence of taxol with a video computer system for 4 h (Fig. 5). It turned out that the roots treated with 1 μM taxol (hardly grew compared to those before treatment. This indicated that 1 μM taxol did not inhibit the growth of roots. When the EPC effect on growth of root was studied in the same manner as Fig. 5, elongation began to decrease

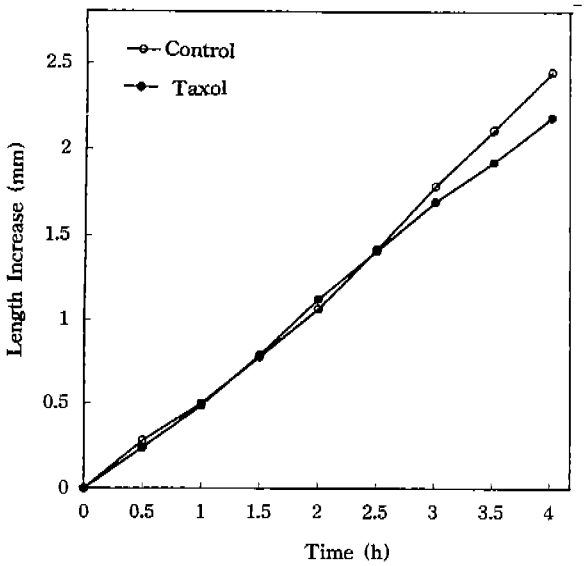

Fig. 5. Effect of taxol on the mazie root elongation. Taxol (1 μM) was applied in 5 mM Mes/Tris buffer, pH 6.8.

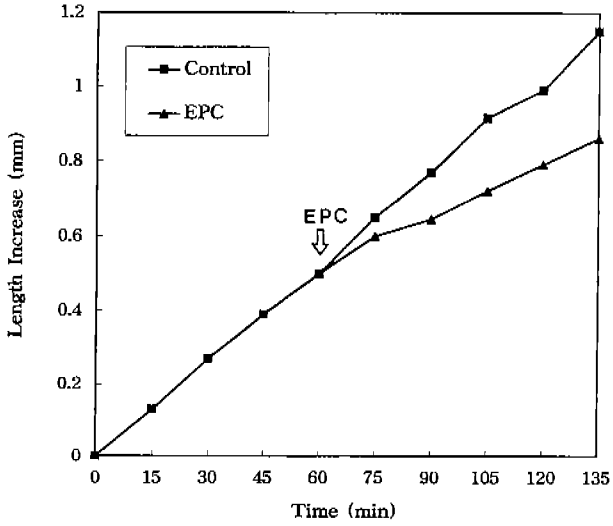

Fig. 6. Effect of EPC on the maize root elongation. EPC (0.5 mM) was applied to the incubation medium at time indicated by the arrow.

50% after 30 min treatment with 0.5 mM EPC, i.e., EPC inhibited the growth of roots more obviously than taxol (Fig. 6).

The growth of coleoptile segment treated with taxol was measured at 10 min intervals for about 6 h in order to examine the effects of taxol on growth in maize coleoptiles (Fig. 7). Because coleoptile segments had hardly grown in buffer solution, they were pretreated with 10^{-5} M auxin for 2 h, and after the effect of auxin on growth had been stabilized, 10 μM taxol was added. But any difference in growth

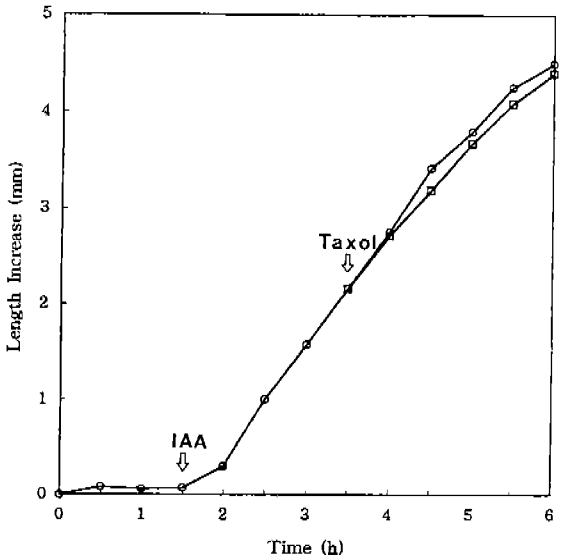

Fig. 7. Effect of taxol on IAA-induced elongation of coleoptile segments in maize. IAA (10^{-5} M) and taxol (10 μM) were added to the medium at time indicated by the arrows. Each point is an average of measurements for 5 segments in above 5 times.

was not observed continuously, that is, taxol did not have any effect on the growth of roots. Meaning that the inhibitory effect of gravitropic response was not caused by inhibition of growth, which again supports Nick and Hertel (1991)'s hypothesis that MT concerning growth and gravitropic response are not the same. This hypothesis explains MTs participating in growth and gravitropic response are of different kinds and that they have differential growth aspects towards each other. According to Nick and his coworker's report (1990), if epithelial cortical MT concerns transmission of gravity stimulus, gravitropism would never be inhibited without destructing MT when is treated with EPC and thus causing destruction of MT by inhibiting MT polymerization. When EPC is treated in maize coleoptile, however, EPC solution at low concentrations inhibits gravitropic response obviously and does not impair the outer epithelial cortical MT which plays a role of growth regulator and has no direct relation with gravitropic response. This means that the perception of gravity has different regulation modes than that of growth, which is supported by the experiment using chlorpromazine, calmodulin inhibitors (Bjoerkman & Leopold, 1987). According to Bjoerkman & Leopold's report, when chlorpromazine is treated in maize root, the gravitrpic response of root is inhibited, but growth or polar transport of auxin remain unaffected. With several reports mentioned above and the re-

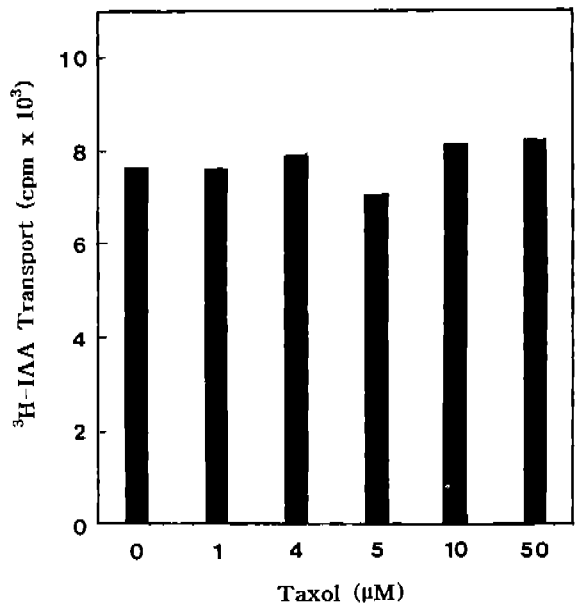

Fig. 8. Effect of taxol on polar transport of auxin in maize coleoptile segments. Maize coleoptile segments were preincubated with various concentrations of taxol for 90 min. Transport represents the radioactivity (cpm) collected in the receiver blocks at the end of 2 h transport period.

sults of Fig. 5 or 7. it seems to be that the outer epithelial cortical MT concerning growth regulation does not have any direct impact on the transmission of gravitropic response. To examine whether taxol's selective inhibition of gravitropic response had anything to do with transport of auxin, we observed the polar and bilateral transport of auxin in coleoptile tissue segments pretreated with taxol (Fig. 8). The level of auxin which transported endogenously in coleoptile segments pretreated with taxol (1-50 μM) for 90 min, was 8000 cpm. The result was very similar to the auxin level in the control pretreated with a buffer solution; taxol inhibited only gravitropic responses without affecting the polar transport of auxin. So, we examined what effects of taxol had on bilateral transport of auxin in roots or coleoptiles that were placed horizontally to confirm that the gravitropic was regulated by bilateral transport of auxin rather than polar transport. There was a similarity between tissues treated with taxol and those in the control as in ratio of bilateral transport of auxin (^{3}H-IAA) in the "elongation zone" of root (Table 1). 1 μM taxol-treated tissue showed a slighty higher rate of bilateral transport of auxin toward the lower part than the control did; while 4 μM taxol-treated ones showed a slightly lower transport rate. We have also examined the effects of taxol on bilateral

Table 1. Effect of taxol on lateral transport of auxin in maize roots

Pretreatment[1]	Radioactivity in tissue (cpm)[3]		
	Bottom→Top	Top→Bottom	Ratio[2]
None	314.2	443.2	1.41±0.34
1 μM Taxol	326.3	507.4	1.56±0.37
4 μM Taxol	355.9	484.7	1.36±0.35

[1] 90 min preincubation
[2] Ratio of cpm (Top→Bottom/Bottom→Top)±SD
[3] ^{3}H-labelled auxin was transported laterally to each side (upper and lower) of the tissue. In the "Bottom→Top", a donor (^{3}H-IAA) block was placed on the lower side of horizontally placed roots and the radioactivity in the upper half of the tissue (0.5 mm) was measured, and *vice versa* for the "Top→Bottom". Transport time was 45 min. Data represented are an average from five experiments with ≥4 roots each.

Table 2. Effect of taxol on lateral transport of auxin in maize coleoptile segments

Pretreatment[1]	Radioactivity in recciver block (cpm)[3]		
	Battom→Top	Top→Bottom	Ratio[2]
None	1880.0	2449.7	1.30±0.20
4 μM Taxol	2235.6	2765.5	1.24±0.19
10 μM Taxol	2483.4	3067.1	1.24±0.21

[1] 90 min preincubation
[2] Ratio of cpm (Top→Bottom/Bottom→Top)±SD
[3] Vertically oriented coleoptile segments were preloaded with donor (^{3}H-IAA) block for 1 h. after then, the segments with donor and receiver block were horizontally placed for another 1 h. In the "Bottom→Top", radioactivity in the upper half of the receiver block was measured and *vice versa* for the "Top→Bottom". Data represented are an average from the experiments with ≥4 coleoptile segments each.

transport of auxin in maize coleoptile segments (Table 2). As in the case of root (Table 1), the auxin transport rate was almost the same without any decrease as in coleoptile segment, even with 4 μM or 10 μM taxol treatment compared to the control. Though taxol was considered to be related with the inhibition of gravitropism (see Fig. 1 and 3), taxol hardly had any effects on auxin transport i. e. polar or bilateral transport; it only inhibited the gravitropic response. And even though auxin is transported bilaterally so as to be distributed unevenly, as the rearrangement of MT occurs the gravitropic response and growth are very likely to be physiological phenomena regulated through other processes.

Acknowledgements

This work was support by grants from KOSEF

(grant No. 941-0500-003-2) and from Hormone Research Center 9523 to June Seung Lee.

LITERATURE CITED

Akashi, T., Izumi, K., Nagano, E., Enomoto, M., Mixuno, K. and Shibaoka, H. 1988. Effects of propyzamide on tobacco cell microtubules *in vivo* and *in vitro*. *Plant Cell Physiol.* **29**: 1053-1062.

Bergfeld, R., Speth, V. and Schopfer, P. 1988. Reorientation of microfibrils and microtubules at the outer epidermal wall of maize coleoptiles during auxin-mediated growth. *Bot. Acta* **101**: 57-67.

Björkman, T. and Leopold, A.C. 1987. Effect of inhibitors of auxin transport and of calmodulin on a gravisensing-dependent current in maize roots. *Plant Phsiol.* **84**: 847-850.

Braun, M. and A. Sievers. 1994. Role of the microtubule cytoskeleton in gravisensing *Chara* rhizoids. *European J. Cell Biol.* **63**: 289-298.

Dustin, P. 1980. Microtubules. *Sci. Am.* **243**: 66-76.

Eum, H.K. and Lee, J.S. 1990. Interaction of auxin and Ca^{2+} on corn coleoptile segment elongation. *Korean J. Bot.* **33**: 315-320.

Evans, M.L. 1990. Gravitropism Interaction of sensitivity modulation and effector redistribution. *Plant Physiol.* **95**: 1-5.

Friedrich, U. and Hertel, R. 1973. Abhängigkeit der geotropischen Krümmung der Chara-Rhizode von der Zentrifugalbeschleunigung. *Z. Pflanzenphys.* **70**: 173-184.

Gunning, B.E.S. and Hardham, A.R. 1982. Microtubules. *Annu. Rev. Plant Physiol.* **33**: 651-698.

Hauser, M.T., A. Morikami and P.N. Benfey. 1995. Conditional root expansion mutants of *Arabidopsis*. *Development* **121**: 1237-1252.

McDonald, J.R. and Hart, J.W. 1987. New light on the Cholodny-Went theory. *Plant physiol.* **84**: 568-570.

Nick, P., Bergfeld, R., Schäfer, E. and Schopfer, P. 1990. Unilateral reorientation of microtubules at the outer epidermal wall during photo- and gravitropic curvature of maize coleoptiles and sunflower hypocotyls. *Planta* **181**: 162-168.

Nick, P., Schäfer, E., Hertel, R. and Furuya, M. 1991. On the putative role of microtubules in gravitropism of maize coleoptile. *Plant Cell Physiol.* **32**: 873-880.

Rowinsky, E.K., Cazenave, L.A. and Donehower, R.C. 1990. Taxol-a novel investigational antimicrotubule agent. *J. Natl. Cancer Inst.* **82**: 1247-1259.

Schiff, P.B. and Horwitz, S.B. 1981. Taxol assembles tubulin in the absence of exogenous guanosine 5'-triphosphate or microtubule associated proteins. *Biochemistry* **20**: 3247-3252.

Schwuchow, J., Sack, F.D. and Hartmann, E. 1989. Gravity affects the distribution of microtubules in gravitropic protonema of *Ceratodon* moss. *Plant Physiol.* **89(Suppl.)**: 93.

(Received December 6, 1996)