水耕におけるトウモロコシ幼植物の種子根の屈曲の品種間差異に及ぼす内的要因

誌名	日本作物學會紀事
ISSN	00111848
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巻/号	50巻2号
掲載ページ	p. 148-156
発行年月	1981年6月

Japan. Jour. Crop Sci. 50(2) : 148-156 (1981)

Endogenous Factors Affecting the Varietal Differences in the Curvature of Seminal Roots of *Zea mays* L. Seedlings in Water Culture

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Received July 9, 1980

Seminal roots of *Zea mays* seedlings grow rotating their tips rhythmically in water cultures and in soil[8]. And they often show curved growth in water cultures. They grow straight if the root caps are removed before growing in nutrient solution[9].

It seems likely that there must be some varietal differences in root curvature in the same underwater culture condition.

With this idea 12 cultivars of *Zea mays* L. were compared to find out the varietal differences in the behavior of root growth in a nutrient solution. Some experiments were also conducted to confirm the mechanism of the root curvature by applying IAA and GA_3 exogenously to the decapped roots and growing the seedlings by applying antiauxins such as *p*-chlorophenoxyisobutylic acid (PCIB) and triiodobenzoic acid (TIBA) to see how much antiauxin we need to adjust the root curvature. Cycloheximide (CHM) was tested to find whether this protein-synthesis inhibitor can control the root curvature of *Zea mays* seedlings.

Materials and Methods

Experiments began in April, 1978 and finished in February, 1979. In most experiments the cultivar used was Great Bell unless otherwise stated. This cultivar showed the most typical curved root growth in a nutrient solution among the 12 cultivars examined. Pioneer 3715 was also used as a control cultivar as its roots grew straight even in the same environment.

Procedures of seed soaking, germination and collecting a uniform root growth of seedlings is described elsewhere[9]. Seedlings were fixed through holes of black plastic shelf fixed in big two-liter beakers. After pouring in 1 liter of Hoagland's solution, seedlings were grown for 48 h under a 12 h illumination scheme in a controlled room of 22±1°C.

The nutrient was aerated throughout the experiments unless otherwise stated. Toshiba Solar Lamps D-400 were used 80 cm above the plant materials so that the light intensity was 8,600 lx at the plant surfaces. In each experimental plot 20 seedlings were grown in each beaker with four replicates. After harvesting, the shoot length, the mesocotyl length, the number of lateral roots, the lateral root length, the seminal root length and the behavior of root curvature were measured. Root curvature was graded into six categories; straight, waving, hook, rolling, spiral clockwise and anticlockwise. Rate of root curvature was expressed as percentage of curved roots to total roots of 20 seedlings according to the categories.

Results and Discussions

Twelve cultivars were compared with reference to the root curvature. The results are shown in Table 1 and Table 2. Table 1 shows that there are wide variations in root curvature when grown in aerated nutrients. Root length fluctuates among the cultivars tested and so does the root depth. The sweet corn cultivars have fewer laterals and their seminal roots curve more, while dent corn cultivars have more laterals and their seminal roots grow straight. The extent of root curvature can be compared with D/L figures among the 12 cultivars. Top growth e.g. mesocotyl length and shoot length are not related to the feasibility of root curvature. Table 2 shows the frequency distribution of the root curvature with respect to 6 types of growth behavior and the D/L figures in Table 1 coincide with the order of straight growth of the seminal roots in Table 2. From these data, two typical cultivars were selected as experimental materials; Pioneer 3715 as straight growth type and Great Bell as curving growth type.

Temperature, aeration and light were examined to see whether these factors can affect the root curvature when using the Great Bell cultivar. The effects of temperature, low (17°C), medium (22°C) and high (27°C) on the Pioneer 3715 and Great Bell seedling growth are shown in Fig. 1. Both cultivars show faster growth in tops and roots in higher temperatures than in lower ones. Root curvature occurs in Great Bell seedlings and seems to be promoted in higher temperatures, indicating that physiological metabolism in the root tissues as well as those of tops may be accelerated in higher temperatures resulting in the root curvature.

Root growth and curvature were examined under aeration and non-aeration in both distilled water and nutrient solution. The removal of root caps from the seminal roots was examined in each treatment plot. In the experiment the Great Bell cultivar was used. The results of the experiment are shown in Fig. 2. Decapping and aeration promote root growth irrespective of the kinds of media and the root grows straight if the root cap is removed. Aeration reduces root curvature if the seedling is grown in distilled water, but it promotes if the root is grown in the aerated solution. Under moist air condition non-decapped seminal roots curve to some extent. A similar tendency was found when the Pioneer 3715 and Great Bell seedlings were grown in pots with wet vermiculite.

Maize roots seem to be very sensitive to curve by perceiving light break even when grown under complete darkness depending on cultivars. In this experiment all the procedures including seed imbibition, germination and growth in a nutrient were conducted under complete darkness with double covers of black curtains in dark room. A small electric torch covered with two sheets of red cellophane paper was used to inspect the seedling growth for several seconds periodically. The cultivar examined was Great Bell. The effects of removing shoots and/or root caps from Great Bell and Pioneer 3715 seedlings were also examined with reference to the growth and curvature of the seminal roots under 12 h illumination in water

Table 1. Varietal differences of the growth of seminal roots in underwater culture.

Cultivar	Type	Mesocotyl	Plumule	No. of laterals	Lateral length	Root length(L)	Root depth(D)	D/L
		mm	mm		mm	mm	mm	
Pioneer 3715	D	5.22	31.94	2.94	17.00	94.43	92.19	0.99
Ko No. 8	D	8.37	34.45	3.01	28.34	114.45	109.20	0.95
Hi Dent	D	11.53	46.13	3.71	51.91	106.01	100.89	0.95
Hokuyu	D	9.87	35.36	1.67	24.52	99.54	93.51	0.94
Shiromochikibi	F	9.07	23.60	4.25	*	96.09	89.44	0.93
Honey Bantam	F	8.00	35.85	1.80	15.24	108.39	100.18	0.92
Caldera 535	D	11.10	43.55	2.94	29.45	99.55	88.15	0.89
Pioneer 3853	D	11.63	42.53	3.29	27.81	87.95	76.55	0.87
Heigenwase	D-F	8.08	39.86	2.99	21.90	107.89	93.26	0.86
Early Dominator	F	5.27	28.41	1.59	8.84	78.25	65.43	0.84
Golden Cross Bantam	S	9.60	28.10	0.76	*	71.69	50.99	0.71
Great Bell	S	14.28	44.43	0.11	22.83	84.93	54.45	0.64

Note: D; dent corn, F; flint corn, D-F; Hybrid between dent corn and flint corn, S; sweet corn *; Not measured

Table 2. Varietal differences of the curvature of seminal roots in underwater culture.

Cultivar	Clockwise	Anticlockwise	Rolling	Waving	Hook	Straight
	%	%	%	%	%	%
Pioneer 3715	1.3	0	0	0	0	98.7
Ko No. 8	2.6	1.3	1.3	0	3.8	91.0
Hi Dent	3.8	5.0	1.3	0	6.3	83.6
Hokuyu	0	0	1.3	0	12.5	86.2
Shiromochikibi	7.9	2.6	1.3	2.6	0	95.6
Honey Bantam	4.0	2.5	11.2	0	7.5	74.8
Caldera 535	11.3	6.3	5.0	1.3	17.5	58.6
Pioneer 3853	3.8	12.5	7.5	7.5	18.8	68.7
Heigenwase	8.8	8.8	11.3	5.0	12.5	53.6
Early Dominator	8.8	12.5	25.0	2.5	8.8	42.4
Golden Cross Bantam	11.3	11.3	62.5	1.3	10.0	3.6
Great Bell	12.5	7.5	66.3	1.3	8.8	3.6

culture.

Under complete darkness, shoots of Great Bell seedlings etiolated with the length of 43.18 mm and mesocotyl length 44.19 mm and seminal root length 85.29 mm, but roots curved with the ratio; clockwise spiral growth 3%, anticlockwise spiral growth 4%, rolling 32%, waving 3%, hook 54% and straight growth 4% respectively. These data are comparable with those of the same cultivar in Table 2 and it can be concluded that under complete darkness roots curve less as compared with those grown under illuminated environments. However the expectation that they might grow straight under complete darkness did not occur, implying that roots of this cultivar will curve very easily in a nutrient even under complete darkness.

ABA is produced in the root cap in perceiving light and this chemical is supposed to control root growth, but from this experiment ABA may not largely affect the curvature of seminal roots. Under water culture live and actively growing roots float as small amount of various gasses may be produced within the root tissues because of the metabolic activities. This force of buoyance may promote the root curvature. But the relationship between the decapped roots and the buoyance in relation to root curvature is not known as seen in Fig. 2 and Fig. 3.

Great Bell seedlings with decapped roots were grown in a nutrient solution with various concentrations of IAA and GA_3 to reproduce the root curvature. And IAA was applied in a lanolin paste onto the cut surfaces of shoot axes of seedlings after removing the root caps. In the former treatments both chemicals were dissolved in several drops of 0.1 N of KOH solution before adding it to the nutrient solution and in the latter IAA was dissolved in ethylether before mixing in lanolin paste and stirred until the ether-like smell disappeared.

As shown in Fig. 4 root curvature can be reproduced by applying 10^{-7}M IAA and 10^{-5}M GA_3 in a nutrient and 10^{-4}M IAA in lanolin. Seedling growth of tops and root was retarded more in 10^{-5}M GA_3 than in higher concentrations. GA_3 promotes auxin production within the plant tissues and controls the activity of auxin oxidases, eventually increasing the auxin level in plant tissues. Experimental results prove that bean roots show a two phase type growth response to auxin with promotion at lower concentrations up to 10^{-10} M and inhibition at higher ones[6]. In this experiment the auxin concentrations exogenously applied into the nutrient seem rather high, but the efficiency of auxin intake into the root tissues may be low and the activation of auxin oxidase may take place there.

The transport of labelled IAA from shoot axis to root tip was followed by means of

3-indolyl (1-^{14}C) acetic acid of specific activity 57.6 mCi/m mol obtained from Radiochemical Centre, Amersham, Bucks, U.K. through Japan Isotope Association, Tokyo. One ml of the IAA (in a mixture of toluene 9: acetone 1) was dissolved in 40 ml of ethylether and mixed with 15 g of liquid lanolin heated in the water bath and left until the ether evaporated thorough-

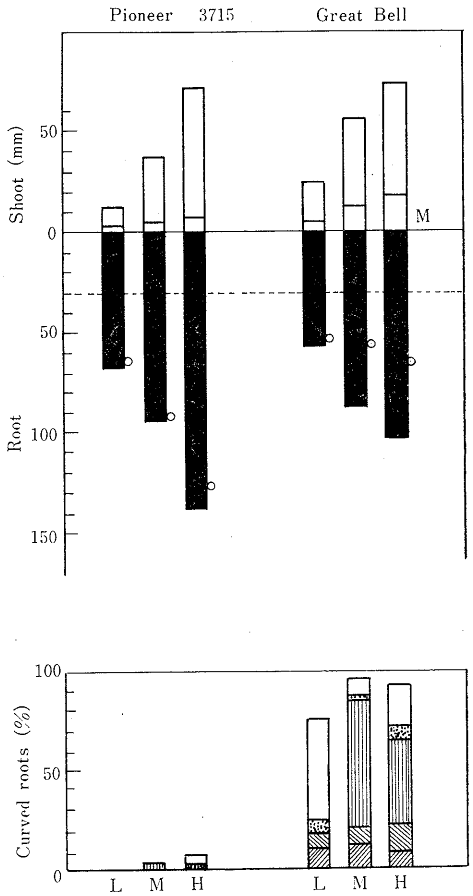


Fig. 1. Effects of temperature on the growth and curvature of seminal roots of seedlings of *Zea mays* L. var. Great Bell.
L ; 17°C M ; 22°C H ; 27°C
M; mesocotyl length
∘ ; depth of root growth in nutrient solution
… ; root length at the beginning of experiment
Curvature ; □ waving ▨ clockwise ▧ anti-clockwise ▦ hook |||| rolling
M : mesocotyl

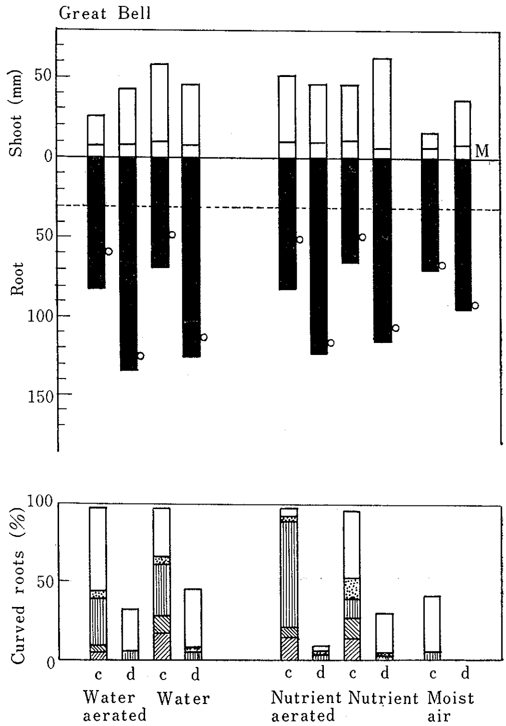


Fig. 2. Effects of nutrient, aeration and decapping on the growth and curvature of seminal roots of Great Bell seedlings.
d ; decapped root
c ; control for romoval of root cap

ly. The IAA concentration was 6×10^{-6} M. The IAA was supplied onto the cut surfaces of shoot axes of Great Bell and Pioneer 3715 seedlings and plants were grown in a nutrient for 48 h under a 12 h illumination scheme in 22±1°C. The lanolin was applied to 600 seedlings altogether. After harvesting, two consecutive segments 3 mm in length both from the root tips and root bases of 20 seedlings with four replicates were sampled together as 1 sample of each treatment plot. Forty segments in a group were placed in scintillation vial and the radioactivity was determined with LSC 900 scintillation spectrometer (Aloka Ltd.). Samples were counted for 5 min and expressed in dpm and the data were presented per 40 segments.

As shown in Table 3, labelled IAA moves to all parts of the seedlings when grown in a nutrient for 48 h and it moves more in

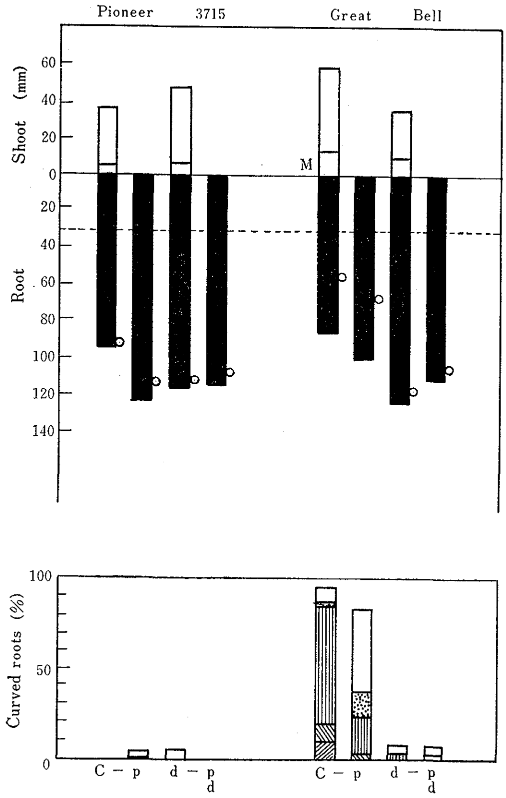


Fig. 3. Effects of removing shoots and/or root caps from seedlings on the growth and curvature of Great Bell seminal roots.
C ; control -p ; removing shoot
d ; removing root cap

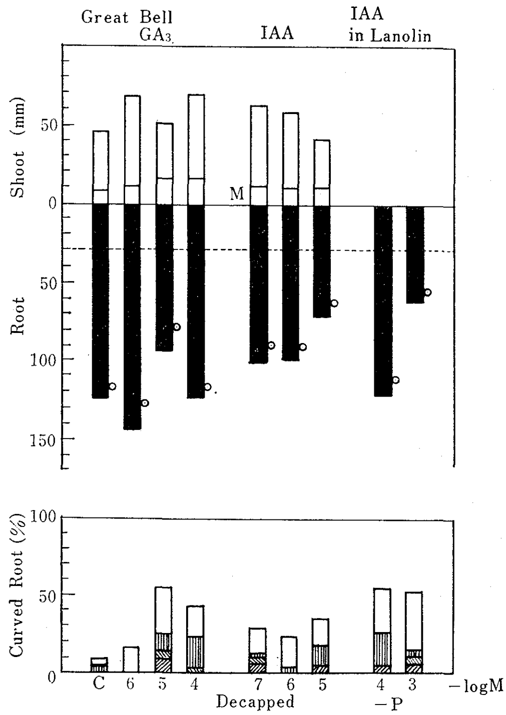


Fig. 4. Effects of applied IAA and GA_3 on the growth and curvature of decapped Great Bell. C ; control

Great Bell than in Pioneer 3715 to each organ; the embryo receives most followed by endosperm and mesocotyl. IAA transport to the root is low when compared to that of the top and it is interesting to notice that the IAA transport to the root tip is comparatively low as compared to that of the root base. Here again in the Great Bell root tip, showing a typical curvature, more IAA is accumulated. It was shown in Fig. 3 that the decapped Great Bell roots grew longer than those of Pioneer 3715, while the non-decapped roots of the former grew shorter than those of the latter. And in Table 4, IAA is accumulated more in the decapped Great Bell roots than those in the other cultivar. These data imply that IAA transport is enhanced in the decapped Great Bell roots and presumably promote straight root growth.

From the above-mentioned results it was suspected that the main cause of the root curvature in a nutrient might be the auxin effect and in next experiment antiauxins were used with various concentrations to confirm this mechanism with Great Bell seedlings. PCIB which inhibits auxin activity and TIBA which hinders basipetal auxin transport were used in a nutrient.

As shown in Fig. 5, root curvature is controlled by concentrations higher than 10^{-5} M PCIB and 10^{-7} M TIBA. Root growth is strongly enhanced with the former concentration of PCIB. This result supports the data reported by Burström[4].

The prominent candidate for a precursor of ethylene in plant is methionine[11] and this hormone is supposed to regulate protein synthesis and membrane metabolism in

Table 3. Differences in labelled IAA transport to each organ of seedling in *Zea mays* L. (dpm)

Cultivar	Mesocotyl	Embryo	Endosperm	Root base	Root tip	Lateral
Great Bell	409.7	987.9	717.5	121.6	14.9	—
Pioneer 3715	398.3	604.6	385.9	31.5	3.5	3.4

plants. Ethylene production is promoted by auxin and it induces a certain enzyme to link auxin with amino acids and let the auxin inactive[12]. Ethylene effects on growth involve alteration in polarity of growth, swelling response and the inhibition of tropistic responses. In this experiment CHM as an inhibitor of RNA protein synthesis was used to test its effect on root curvature with various concentrations in a nutrient. Great Bell seminal roots could grow straight with 10^{-6}M CHM.

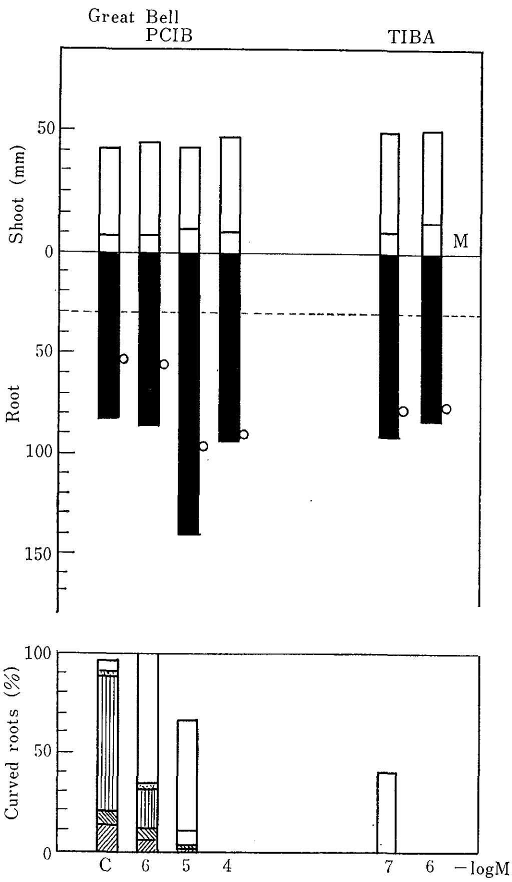


Fig. 5· Effects of various concentrations of PCIB and TIBA on the growth and curvature of Great Bell seminal roots grown in liquid culture.

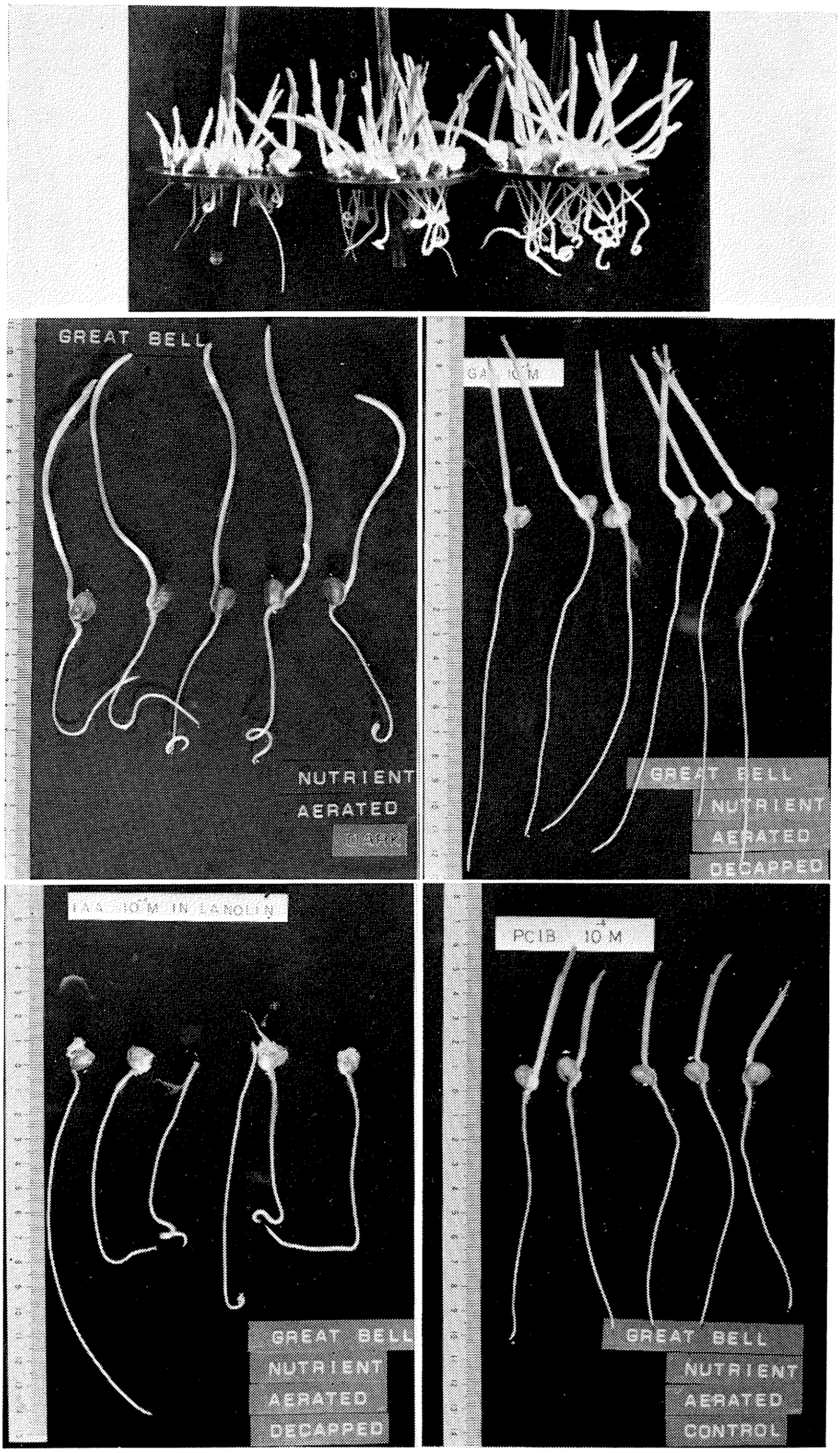
Table 4. Effects of decapping of seminal root on labelled IAA transport from plumular axis to root tip. (dpm)

Cultivar	Control	Decapped
Great Bell	42.4±22.3	79.0±25.1
Pioneer 3715	44.3± 6.2	34.3±11.8

From this result it was considered that root curvature might be induced by ethylene which biochemically produced from methionine and enhanced with the overproduction of auxin within the plant tissues.

Summary

Seminal roots of *Zea mays* L. seedlings show curved growth in an aerated nutrient and this curvature differs with cultivars when grown in 22±1°C under a 12 h illumination scheme for 48 h. Experiments were conducted to find the mechanism of this root curvature under the following headings and the results are also summarized after each study. **1. Cultivar.** Among the 12 cultivars examined there is a wide range of root curvatures, e.g. seminal roots of sweet corn curve more and those of dent corn cultivars grow straight. **2. Environmental factors.** When both Pioneer 3715 and Great Bell seedlings are used, plant roots show faster growth in higher temperatures than in lower ones. Removing root caps and aeration enhance the root growth. When Great Bell seedlings are tested, their seminal roots curve even under complete darkness implying that roots are so prone to curve. **3. IAA and GA_3.** IAA and GA_3 were exogenously applied in the nutrient to reproduce the root curvature with decapped root and IAA was applied in a lanolin paste onto the cut surfaces of the shoot axes of seedlings after removing


GREAT BELL
NUTRIENT
AERATED
GA 10 M
GREAT BELL
NUTRIENT
AERATED
DECAPPED
IAA 10 M IN LANOLIN
GREAT BELL
NUTRIENT
AERATED
DECAPPED
PCIB 10 M
GREAT BELL
NUTRIENT
AERATED
CONTROL

the root caps. Root curvatures can be reproduced by applying 10^{-7}M IAA and 10^{-5}M GA_3 in nutrient and 10^{-4}M IAA in lanolin. **4. Transport of labelled IAA from shoot axis to root tip.** IAA transport was enhanced in the decapped Great Bell roots as compared with Pioneer 3715 and more IAA was accumulated in the root tip of the former than in the latter. **5. Effects of antiauxins.** PCIB and TIBA were applied with increasing concentrations in the nutrient to regulate the root curvature with Great Bell seedlings. Root curvature can be regulated with higher concentrations than 10^{-5}M PCIB and 10^{-7}M TIBA and root growth is enhanced with the former. **6. Effect of CHM on root curvature.** CHM as an inhibitor of RNA protein synthesis was used to test the effects on root curvature. Seminal roots of Great Bell seedlings grow straight with 10^{-6}M of this agent.

From the above results it was assumed that root curvatures are induced by ethylene which is biochemically produced from methionine and that such ethylene production may be enhanced by the overproduction of auxin within the plant tissues.

Acknowledgements

Thanks are due to Dr. T. OKABE, National Hokkaido Agricultural Experiment Station and staff of Nagano Agricultural Experiment Station and Hokuren Agricultural Cooporation Ltd. for supplying the seeds of various cultivars tested. Thanks are also due to Dr. T. ISHIBASHI, Faculty of Agriculture, Niigata University for radiochemical analysis of the treated materials.

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Fig. 6. Effects of removal of root caps, auxins and antiauxin on root curvature.

Top; Typical root curvature of seminal roots of Great Bell seedlings grown in aerated nutrient for 48 h.

Middle Left; Etiolated seedlings of Great Bell grown in aerated nutrient under dark for 48 h. Notice that the roots curve even in darkness.

Middle Right; Straight root growth of decapped seminal roots of Great Bell seedlings grown in aerated nutrient when 10^{-4} M GA_3 was exogenously applied to the nutrient.

Bottom Left; Curved growth of decapped seminal roots of Great Bell seedlings grown in an aerated nutrient when 10^{-4} M IAA was applied onto the cut surfaces of shoot axes in lanolin paste.

Bottom Right; Straight root growth of seminal roots in aerated nutrient when 10^{-4} M PCIB was exogenously applied to the nutrient.

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〔和 文 摘 要〕

水耕におけるトウモロコシ幼植物の種子根の屈曲の品種間差異に及ぼす内的要因

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トウモロコシの幼植物を 12 時間照明, 22±°C の条件で通気しながら水耕すると種子根が屈曲を示すことがあるが, その程度は品種によって異なる. 根の屈曲の機作を探るために一連の実験を行い, 次のような結果を得た. 1) 品種: 12 品種を供試した結果, 品種によって根の屈曲が異なり, スィートコーン類 (SC) は曲がりやすく, デントコーン類 (DC) は直線的に伸長した. 2) 環境要因: パイオニア 3715 (DC) とグレートベル (SC) を供試した結果. 高温で根の伸長が促進された. 根冠の除去と通気は根の伸長を促した. グレートベル (GB) を用いた実験では暗黒下でも根が屈曲した. 3) IAA と GA_3: 水耕液に IAA または GA_3 を加えると (GB)の根冠を切除した根でも屈曲し, その濃度はそれぞれ 10^{-7}M と 10^{-5}M であった. また, 地上部を切除し, その切口に IAA を含んだラノリンを塗布すると 10^{-4}M で根の屈曲が再現された. 4) ラベルした IAA の地上部から根端への移動: 根冠を切除したパイオニア 3715 (P) と GB 2品種について前実験と同様ラノリンを用いて比較してみると, IAA の移動量は (P)より (GB) において大であった. 5) 抗オーキシンの影響: (GB) の幼植物を用いて水耕液に抗オーキシンを与えた結果, PCIB M^{-5}M および TIBA^{-7}M で根の屈曲を抑えることができた. 6) 根の屈曲と CHM: GB の幼植物を用いて水耕液にたんぱく質合成阻害物質 CHM を与えた結果, 10^{-6}M で根が直線的に伸長した.

以上の結果から, 根の屈曲は根の組織内でメチオニンから生化学的に生成されたエチレンによって起こり, このエチレンは組織内のオーキシンが過剰になったときに多く生産されるものと考えた.