식물학회지 제32권 제4호
Korean J. Bot. 32(4) : 323-330(1989)

Effect of Endogenous IAA Transport on Adventitious Root Formation in *Phaseolus vulgaris* Hypocotyl Cuttings

Cho, Duck Yee[1] and Woong Young Soh[2]
(1. Department of Biology, Chonju Woo Suk University 2. Department of Biology, College of Natural Science, Chonbuk National University, Chonju)

강낭콩 下胚軸 切片의 不定根形成에 미치는 內在 IAA의 移動

趙 德 以[1]·蘇 雄 永[2]
(1. 全州又石大學 生物學科 2. 全北大學校 自然科學大學 生物學科)

ABSTRACT

This work was carried out to elucidate effects of endogenous and exogenous IAA transport on adventitious root formation in *Phaseolus vulgaris* hypocotyl cuttings. For inverted or normal incubation in distilled water, the adventitious root is always formed at the morphological base but not at the morphological apex. For inverted incubation, in both distilled water and certain chemical solution, the root formation is retarded more at the first stage (0-24 hr) than at the second stage (24-48 hr). When p-chlorophenoxyisobutyric acid (PCIB) was applied to the cuttings at the first stage, the root formation was inhibited more than at the second stage. Treatment of 2,3,5-triiodobenzoic acid (TIBA)markedly inhibited the adventitious root formation in Phaseolus vulgaris hypocotyl cuttings. This inhibition influenced the root according to the applied stage and period. Therefore, the root formation is more related to the stage of root primordium formation than to the stage of root elongation from the primordium. Inhibition of auxin transport or action by TIBA or PCIB could also be reversed when hypocotyl cuttings are incubated in exogenously applied IAA solution.

INTRODUCTION

The formation of adventitious root primordia is regulated by plant hormone such as auxin (Went 1934; Went and Thimann 1937). Auxins accumulated in the rooting zone induced the adventitious root formation (Altman and Wareing 1975; Friedman *et al.* 1979; Jarvis and Booth 1981). Furthermore, the adventitious root formation is related to polar transport of "rooting substances" (Priestly and Swingle 1929). Recently the role of polar transport of exogenous IAA (indole-3-acetic acid) was proved by Greenwood and Goldsmith (1970).

It was reported that IAA treatment accelerated root formation of Azuki-bean more at the second stage (24-48 hr)(Shibaoka 1971). On the other hand, there were reports that IAA treatment to the cuttings of pea at the first stage were more effective (Eriksen and Mohammed

1974; Fabijan *et al.* 1981). These above reports are from the studies on exogenous auxin treatment (Eriksen and Mohammed 1974; Fabijan *et al.* 1981; Shibaoka 1971). However the effect of endogenous auxin transport on adventitious root formation was rarely reported.

The basipetal polar transport of auxin is reduced in the case of the inverted cuttings compared to the normal cuttings. In general, it is thought that the basipetal auxin transport in the normal incubation is far superior to the acropetal transport by the gravity (Naqivi and Gordon 1965; Leopold and Hall 1966; Little and Goldsmith 1967; Goldsmith 1982; Wright 1981). In addition, the content of endogenous auxin accumulated in the root forming zone depends on the direction of incubation. In the present study, we will examine the relation between the adventitious root formation and endogenous auxin in the cuttings incubated both in the normal and inverted direction.

MATERIALS and METHODS

Plant material Seeds of *Phaseolus vulgaris* L., kidney bean were soaked in gauze, moistened with distilled water for 24 hr and then seeds appearing 2 mm-long root were sown in clean fine sand, inside stainless steel trays ($15\times20\times5$ cm^3). Seedlings were raised in a controlled growth chamber at 25 ± 1℃, 16 hr photoperiod supplied by cool white fluorescent light of 1900 lux. Hypocotyl was cut at the point of 40 mm from the cotyledonary node of 6-day-old seedlings (Fig. 1,A).

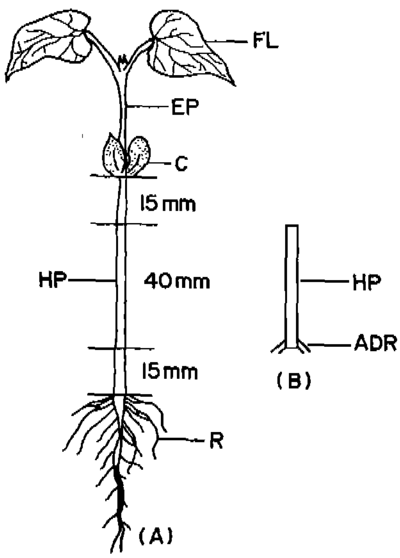

Fig. 1. Diagram of kidney bean seedling used in the experiment(A) and adventitious root formation on hypocotyl cuttings treated with test solution (B). FL : first leaves, EP: epicotyl, C : cotyledon, R: root, HP : hypocotyl, ADR : adventitious root.

Rooting experiment Five freshly prepared hypocotyl cuttings were incubated in glass vials containing 5 ml of test solution with the depth of 25 mm. Test solution(1×10^{-4}M IAA or PCIB) and distilled water were prepared for the incubation of cuttings.

Each vial with five cuttings were placed in a petri-dish (ϕ 90mm) in the incubator under fluorescent lamp (16 hr photoperiod) at 25±1℃ for six days. The hypocotyl cuttings were washed with tap water and rinsed with glass distilled water every 24 hr. The tops of the glass vials were not covered. In order to examine the effect of the endogenous IAA for rooting, the hypocotyl cuttings were incubated in a morphologically inverted or in a normal direction in distilled water. The hypocotyl cuttings were ringed with lanolin paste of 20 mm width. The lanolin paste contained 1%(w/w) TIBA.

Ten mg of TIBA was solved in 1 ml of absolute ethyl alcohol and then mixed with 1 g of lanolin at 50℃ for 24hr. The ethyl alcohol was removed by heating the mixture in a water bath (Iwami and Masuda 1976). After a week of incubation, the adventitious roots were counted with the naked eye (Fig. 1,B).

RESULT

Whether normal or inverted incubation the adventitious root formation was always shown only around the morphological base.

The average number of adventitious roots were 2.30 in the normal incubation as a control. The normal incubation for 168 hr after 12 hr inversion of the cuttings showed 67.39% of root forming rate in comparison with the control. The inverted incubation of 24, 36, 48 hr showed 61.30%, 58.52% and 33.91% of the control respectively in the root formation. The result showed 26.96% of the control in the case of the inverted incubation for the entire 168 hr. So it was shown that root formation ratio decreased in proportion to inverted incubation time. Inverted incubation for 12 hr (24-36 hr) followed by normal incubation for 36-168 hr resulted in root forming rate of 81.62%. Inversion of the cuttings at the first 24 hr resulted in 56.52%, while that at the second 24 hr showed 88.7% (Table 1). When the cuttings were incubated in an inversed direction in IAA solution for the first and second 24 hr. they showed 52.17%, and 43.48% rooting respectively. When the cuttings were invertedly incubated in IAA solution after the first 24 hr under normal conditions, the result was 108.70%. On the other hand, the cuttings that were incubated in distilled water the normal direction for the first 24 hr and subsequently in IAA solution in an inverted direction for the second 24 hr, produced 134.78% rooting. PCIB treatment in the first stage was more inhibitory than the second stage (Table 3).

Application of lanolin paste containing 1% TIBA to the middle portion of the cuttings caused inhibition of adventitious root formation (Table 4). TIBA treatment following distilled water treatment in the first 24 hr and 48 hr, showed 30.00%, and 89.57% rooting respectively. Combination treatment with TIBA and IAA for the second 24 hr after distilled water treatment of the first stage showed 71.30%. Treatment with lanolin paste containing TIBA for 24-168 hr

Table 1. Effects of normal and inverted incubation on adventitious root formation in *Phaseolus vulgaris* hypocotyl cuttings.

Group	Treatment (hr)					Number of roots*
	0–12	12–24	24–36	36–48	48–168	Mean±S.E.(%)
1.	△	△	△	△	△	2.30±0.12(100.00)
2.	▼	△	△	△	△	1.55±0.14(67.39)
3.	▼	▼	△	△	△	1.30±0.10(56.52)
4.	▼	▼	▼	△	△	1.41±0.12(61.30)
5.	▼	▼	▼	▼	△	0.78±0.08(33.91)
6.	▼	▼	▼	▼	▼	0.62±0.12(26.96)
7.	△	▼	△	△	△	1.36±0.08(59.13)
8.	△	△	▼	△	△	1.90±0.18(82.61)
9.	△	△	△	▼	△	1.87±0.10(81.30)
10.	△	△	▼	▼	△	2.04±0.12(88.70)
11.	△	▼	△	▼	△	1.30±0.14(56.52)

* Average of 50 cuttings with standard error.
▼ : incubated in inverted direction.
△ : Incubated in normal direction.

Table 2. Effect of IAA on adventitious root formation during the inverted or normal incubation in *Phaseolus vulgaris* hypocotyl cuttings.

Group	Treatment (hr)			Number of roots*
	0–24	24–48	48–168	Mean±S.E. (%)
1.	△	△	△	2.30±0.12(100.00)
2.	◬	▼	▼	1.20±0.06(52.17)
3.	▼	◬	▼	1.00±0.08(43.48)
4.	◬	△	△	2.50±0.12(108.70)
5.	△	◬	△	3.10±0.20(134.78)

*Average of 50 cuttings with standard error.
IAA was used at 1×10^{-4}M.
△ : Incubation in morphologically normal direction.
▼ : Incubation in morphologically inverted direction.
⧨ : Inccuubation in IAA solution in morphologically inverted direction.

Table 3. Effect of p-chlorophenoxyisobutyric aid (PCIB) on adventitious root formation in *Phaseolus vulgaris* hypocotyl cuttings.

Treatment (hr)			Number of roots*
0–24	24–48	48–168	Mean±S.E. (%)
H_2O	H_2O	H_2O	2.30±0.12(100.00)
PCIB	H_2O	H_2O	1.20±0.08(52.17)
H_2O	PCIB	H_2O	1.40±0.12(60.87)
PCIB	PCIB	H_2O	0,70±0.08(30.43)
PCIB+IAA	H_2O	H_2O	1.53±0.14(66.52)
H_2O	PCIB+IAA	H_2O	1.67±0.12(72.61)
PCIB+IAA	PCIB+IAA	H_2O	2.00±0.14(86.96)

* Average of 20 cuttings with standard error.
PCIB and IAA were used at 1×10^{-4}M.

Table 4. Effect of 2,3,5-triiodobenzoic acid (TIBA) on adventitious root formation in *Phaseolus vulgaris* hypocotyl cuttings.

Group	Treatment (hr)			Number of roots*
	0–24	24–48	48–168	Mean±S.E. (%)
1.	○	○	○	2.30±0.12(100.00)
2.	▲	▲	▲	0.37±0.06(16.09)
3.	△	▲	▲	0.69±0.03(30.00)
4.	△	△	▲	2.06±0.12(89.57)
5.	△	◬	▲	1.64±0.14(71.30)
6.	◬	▲	▲	1.55±0.10(67.39)

* Average of 60 cuttings with standard error.
○ : Treated with plain lanolin paste.
△ : Treated with glass distilled water.
▲ : Treated with 1% TIBA in lanolin paste.
◬ : Treated TIBA and IAA in lanolin paste.

after combination treatment with TIBA and IAA for the first 24 hr period showed 67.39%.

DISCUSSION

From the inhibition of adventitious root formation in the inverted incubation, we can deduce that the endogenous auxin in the cuttings were accumulated in the basal part through basipetal polar transport where it can induce the root primordium initiation.

Eriksen and Mohammed (1974) also reported that IAA promotes adventitious root formation at the first atage. In sunflower the incubation of hypocotyl cuttings during the first 24 hr period increases the adventitious root primordia (Fabijan *et al.* 1981) and root primordia formation was promoted by an accumulation of endogenous IAA in hypocotyl during the first stage (Wample and Reid 1979). Cho(1985) reported that endogenous and exogenous IAA accumulation increased during the early stage in epicotyl cuttings of Azuki-bean through a quantitative analysis with high performance liquid chromatograhy (HPLC). It resembles the present results in that basipetal auxin transport in iverted incubation of the segment decreased by 50-70% for *Avena sativa* and 50% for *Zea mays*(Little and Goldsmith 1967).

The present experiment also agrees with the report that basipetal polar transport of the endogenous auxin was retarded in inverted stem segments (Hertel and Leopold 1962; Wareing and Phillips 1981). Thus the basipetal polar transport of the endogenous auxin is influenced by gravity (Goldsmith 1982).

From the inhibitory effect of adventitious root formation in inverted incubation at the first stage, it can be interpreted that the endogenous auxin accumulation is more effective in the stage of root primordia initiation. Altman and Wareing (1975) also reported that auxin accumulation followed by an accumulation of sugar at the rooting zone induced root formation.

. In the experiments with PCIB and TIBA, it was found that the adventitious root formation was influenced by auxin more at the first stage than the later stage. However, Shibaoka (1972) reported that the late stage was more sensitive to auxin in root formation.

The TIBA treatment inhibits the basipetal transport of auxin (Hoad *et al.* 1971; Sievers and Ladage 1973; Haissig 1974). When the internode of willow was, therefore, treated with TIBA, cell division during adventitious root initiation was inhibitd by about 75% (Haissig 1970). And when IAA level was low the root development was inhibited (Wample and Reid 1979). Fabijan *et al.*(1981) also asserted that the ratio of the adventitious root formation decreased by 24% when treatment was done with 0.1% TIBA. Similar results were reported in corn coleoptiles and tobacco stem segments by Niedergang and Skoog (1956). The present results in kidney bean hypocotyl cuttings on adventitious root formation by TIBA were close to those in many other plants. Adventitious root formation always occurred on the morphological base whether the cuttings were incubated under normal or inverted conditions (Table 1,2). In the experiment with the epicotyl cuttings of Azuki-bean, similar phenomena has occurred (unpublished data, Cho). These resembles the results in willow stem cuttings and dendlion root segments (Pfeffer 1903; Warmke and Warmke 1950). On the other hand, Hoad *et al.*(1971) reported that, in willow segment, adventitious root formation occurred both on a morphological base and apex.

적 요

강남콩 하배축 절편에 있어서 부정근형성과 내재및 외래 IAA 이동에 관해서 실험하였다. 도립 및 정상 배양에 있어서 언제나 형태적 기부에서 부정근형성이 일어났다. 도립배양의 결과 초기 24 시간(0—24hr)에 더욱 억제적 효과를 보였고 PCIB의 처리에서도 같은 경향을 보였다.

TIBA 처리에서는 현저하게 부정근형성을 억제하였고 이 억제적 효과는 처리시간에 비례하며 근 신장기 보다는 근 원기에 더욱 억제효과가 컸다. PCIB와 TIBA의 오옥신 이동억제효과는 외래 IAA 용액 처리에 의해서 회복 되었다.

REFERENCE

Altman, A. and P.F. Wareing. 1975. The effect IAA on sugar accumulation and basipetal transport of ^{14}C-labelled assimilated in relation to root formation in *Phaseolus vulgaris* cuttings. *Physiol, Plant.* **33** : 32-38.

Cho, D.Y. 1985. Distribution and quantitative determination of IAA by HPLC concerning adventitious root formation in Azukia epicotyl cuttings. *Korean J.Plant Tissue Cult.* **12**(2) : 79-87.

Eriksen, E.N. and S. Mohammed. 1974. Root formation in pea cuttings Ⅱ. The influence of indole-3-acetic acid at differental stages. *Physiol. Plant.* **30** : 158-162.

Fabijan, D., E. Yeung. L. Mukherjee and D.M.Reid 1981. Adventitious rooting in hypocotyls of sunflower(*Helianthus annus*) seedlings. Ⅰ. Correlative influences and developmental sequence. *Physiol. Plant.* **53** : 589-597.

Friedman, R.,A. Altman and E. Zamski. 1979. Adventitious root formation in bean hypocotyl cuttings in relation to IAA translocation and hypocotyl anatomy. *J. Exp. Bot.* **30**(117) : 769-777.

Goldsmith, M.H.M. 1982. A saturable site responsible for polar transport of indole-3-acetic acid in sections of maize coleoptiles. *Planta.* **55 :** 68-75.

Greenwood, W.S. and M.H.M. Goldsmith. 1970. Polar transport and accumulation of indole-3-acetic acid during root regeneration by *Pinus lambertiana* embryos. *Planta.* **95 :** 297-313.

Haissig, B.E. 1970 Influence of indole-3acetic acid on adventitious root primordia of *Brittle willow*. *Planta.* **95 :** 27-35.

Hertel, R., and A.C. Leopold. 1962. Versuche zur analyyse des Auxin transports in der koleoptile von *Zea mays* L. *Planta(Berl.).* **59 :** 535-562.

Hoad, G.V., S.K. Hillman and P.F. Wareing. 1971. Studies on the movement of indole auxins in willow (*Salix vimimalis* L.) *Planta(Berl.).* **99 :** 73-88.

Iwami. S., and Y. Masuda. 1976. Distribution of labeled auxin in geotropically stimulated stems of cucumber and pea. *Plant & Cell Physiol.* **17 :** 227-237.

Jarvis, B.C. and A. Booth. 1981. Influence of indole-butyric acid, boron, myoinositol, vitamine D_2 and seedling age on adventitious roots. *Physiol. Plant.* **53 :** 213-218.

Jarvis, B.C., A. Booth. and P.R.M. Shanon. 1983. Involvement of polyamines with adventitious root development in stem cuttings of mung bean. *ibd.* **24 :** 677-683.

Leopold, A.C. and O.F. Hall. 1966. A mathematical model of polar auxin transport. *Plant Physiol.* **41 :** 1476-1480.

Little, C.H.A., and M.H.M. Goldsmith. 1967. Effect of inversion on growth and movement of indole-3-acetic acid in coleoptiles. *Plant Physiol.* **42 :** 1239-1245.

Naqivi, S,M. and S.A. Gordon. 1966. Auxin transport in *Zea mays* L. Coleoptiles influence of gravity on the transport of IAA-2-C^{14}. *Plant Physiol.* **42 :** 831-839.

Niedergang-Kaminen and F. Skoog. 1956. Studies on polarity and auxin transport by triiodobenzoic acid. *Physiol Plant.* **9 :** 60-73.

Pfeffer, W. 1903. Pfeffers physiology of plants. 2nd ed., Clarendon press oxford.

Priestly, J.H. and C.F. Swingle. 1929. Vegetative propagation from the standpoint of plant anatomy Techn. Bull. U.S. Dept. Agriculture 151.

Shibaoka, H. 1971. Effeccts of indoleacetic, *p*-chlorophenoxy-isobutyric and 2,4,6-trichlorophenoxyacetic acids on three phase of rooting in Azukia cuttings. *Plant & Cell Physiol.* **12 :** 193-200.

Siebers, A.M., and C.A. Ladage. 1973. Factors controlling cambial developmet in the hypocotyl of *Ricinu communis* L. *Act. Bot. Neerl.* **22 :** 416-432.

Thomson, K.,R. Hertel and S. Muller. 1973. 1-N-Naphthylphthalamic and 2,3,5-triiodobenzoic acids. invitro binding and auxin transport. *Planta.* **109 :** 337-352.

Wample, R.L., and D.M. Reid. 1979. The role of endogenous and ethylene in the formation o adventitious roots and hypocotyl hypertrophy in flooded sunflower plants (*Helianthus annus*). *Physiol Plant.* **45 :** 219-226.

Wareing, P.F., and I.D.J. Phillips. 1981. The control of growth and differentiation in plants. 3rd Edition Pergamon.

Warmke, H.E. and G.L. Warmke. 1950. *Amer. J. Bot.* **37 :** 272.

Went, F.W. 1938. Specific factors other than auxin affecting growth and root formation. *Plant Physiol*

13 : 55-80.

Went. F.W. and K.V. Thimann. 1937. Phytohormones. Thc MacMillan Company. New York.

Wright. M. 1981. Rcversal of the polarity of IAA transport in the leaf sheath base of *Echinochloa colenum*. *J. Exp. Bot.* **32** : 159-169.

(Receioed Sept. 26, 1989)