

## Effects of Inverted Incubation on Adventitious Root Formation in Epicotyl Cuttings of *Vigna angularis* Owhi et Ohashi

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### 팔의 유경절편에서 부정근형성에 대한 도립배양의 효과

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Disbudded epicotyl cuttings from light-grown 6-day-old seedlings of *Vigna angularis* Owhi et Ohashi were preincubated in  $2 \times 10^{-4}$ M IAA solution for 48 hr to promote adventitious root formation in upright or inverted direction and then incubated in upright direction for 96 hr. Adventitious root formation occurred only at the morphological base of the cuttings which were preincubated in upright direction, while at the both ends in inverted direction. IAA treatment enhanced the adventitious root formation in all cuttings regardless of their orientation during preincubation. To elucidate localized root development, the activity of enzymes involved in root initiation and development was measured 24 hr, 48 hr, and 148 hr after epicotyl incubation. IAA oxidase, peroxidase and catalase were assayed in the apical, middle and basal segment of the epicotyls, and their fresh weight and length were measured. Elongation occurred the most in the upper segment of the epicotyl while fresh weight gain was the most in the basal segment. At root initiation phase, 24 hr after incubation IAA peroxidase and catalase activities appeared high at rooting zone while IAA oxidase activity was low at both ends, IAA oxidase and peroxidase activities declined at the rooting zone during the adventitious root formation at 48 hr. Inversion of cuttings during preincubation caused a change of enzyme activities along their epicotyl cuttings. Only peroxidase activity showed a high correlation with root initiation. Therefore, the biochemical change is highly correlated with change in IAA level in the rooting zone of the epicotyl, resulting in root formation in unusual rooting zone of epicotyl.

key words : rooting, catalase, IAA oxidase, peroxidase

Adventitious root formation in epicotyl or hypocotyl cuttings occurs only at the morphological basal end of the cuttings (Massei and Valio, 1983; Cho and Soh, 1989) and is enhanced by exogenous application of auxin (Cho and Soh, 1989; Pluss et al., 1989). Since auxin is synthesized at the apical end of the shoot and is transported basipetally, the excision of roots allows the accumulation of auxins at the base of the cuttings where adventitious roots are formed (Liu and Reid, 1992). However, the distribution and metabolism of auxin and the process of root organogenesis in cuttings are little understood well (Cho, 1985).

In the present paper, the dependence of root formation on auxin transport was studied. In order to interrupt the basipetal transport of auxin, epicotyl cuttings were incubated in both upright or inverted position. Morphologically reversed incubation of cuttings is known to result in the interruption of basipetal movement of auxin in epicotyl cuttings (Wright, 1980; Cho and Soh, 1989).

In addition, IAA treatment induces changes in the activities of IAA oxidase and peroxidase as oxidative inactivator of IAA (Mato and Vietez, 1980). These results prompted us to analyse oxidases and catalase in the epicotyls in relation to

rhizogenesis. Therefore, we compared IAA oxidase, peroxidase, and catalase activities in three segments of epicotyls with equal length. It is important that biochemical metabolism in the rooting zone is correlated with the developmental stage of root formation. Thus, microscopical observation was carried out to define the developmental stage.

## MATERIALS AND METHODS

### Plant Material

Seedlings of *Vigna angularis* Owhi et Ohashi (Azukia, Azuki bean) were cultivated for 148 hr in a culture room at  $25 \pm 1^\circ\text{C}$  under 16 hr photoperiod with a light intensity of  $40 \mu\text{mol}/\text{m}^2\text{s}$ . The seedlings were cut 15 mm below the apex and 40 mm epicotyl segments were taken.

### Application of IAA

As a control, cuttings were preincubated in distilled water in morphologically normal and inverted positions for 48 hr. For auxin treatment, morphological basal or apical end of the cuttings was kept in upright direction in  $2 \times 10^{-4}\text{M}$  IAA solution for 48 hr, followed by 96 hr in upright direction in distilled water. The cuttings were then cut into three segments of equal size marked before the incubation (apical, middle and basal) and their fresh weight and length were recorded. In each experiment, observation was recorded at three different phases: adventitious root initiation phase (after 24 hr), adventitious root formation phase (after 48 hr), and adventitious root elongation phase (after 148 hr). The epicotyl segments of each category were immediately frozen in liquid nitrogen and kept at  $-70^\circ\text{C}$  until use.

### Observation of adventitious root development

To examine root development with light microscope, the cuttings were fixed in FAA, dehydrated in t-butyl alcohol and embedded in paraffin. Cross sections were stained with 0.1% toluidene blue.

### Measurement of enzyme activities

The segments of each category were ground in a mortar with equal volume of 50 mM potassium phosphate (pH 7.2). The result homogenate was centrifuged at  $4^\circ\text{C}$  for 10 min,

at 12000 xg, and the supernatant was assayed for enzyme activities. The activity of IAA oxidase was measured as described by Mato and Vietez (1986). Peroxidase activity was assayed according to Castillo et al. (1984) and catalase activity was assayed as described by Avei (1971). Assays were carried out in triplicate and averaged.

## RESULTS

Disbudded epicotyl cuttings of *Vigna angularis* seedlings were vertically preincubated in morphologically upright or inverted direction in  $2 \times 10^{-4}\text{M}$  IAA solution for 48 hr to promote adventitious root formation and then incubated in distilled water in the upright direction for 96 hr. As control, cuttings were incubated in water in normal and inverted direction.

IAA solution increased number of adventitious roots by about 167% in normal direction (Table 1). Interestingly, fewer roots emerged on the apical end when the cuttings were preincubated in inverted direction in IAA solution. Root primordia were formed only near the basal end in the normal incubation, but in both ends in inverted incubation. The development of the primordia during root initiation phase was restricted near the cutting edges, named as rooting zone. The root initials were made up of small and densely cytoplasmic cells which appeared in the interfascicular region in the rooting zone. The initials grew toward the cortex layer at the root formation phase and then the root emerged through the epidermal layer at the root elongation phase (Figure 1).

To analyze the effects of IAA along the epicotyl axis, fresh weight and length of the three segments of an epicotyl were recorded (Figures 2, and 3). IAA treatment stimulated an increase in both weight and length of epicotyl segments. Changes appeared inversely in fresh weight to the length of

**Table 1.** Effect of Inverted incubation on adventitious root formation in epicotyl cuttings of *Vigna angularis* Owhi et Ohashi.

Treatment(hr)		Number of root*	
0-48	48-148	morphological base	morphological apex
△	△	$1.08 \pm 0.09$	$0.00 \pm 0.00$
▲	△	$2.88 \pm 0.29$	$0.00 \pm 0.00$
▽	△	$0.22 \pm 0.21$	$0.00 \pm 0.00$
▼	△	$1.16 \pm 0.15$	$0.84 \pm 0.28$

△: normal incubation in distilled water, ▽: inverted incubation in distilled water.

▲: normal incubation in IAA solution, ▼: inverted incubation in IAA solution.

\*Average of 30 cuttings with standard error. IAA was used at  $2 \times 10^{-4}\text{M}$ .

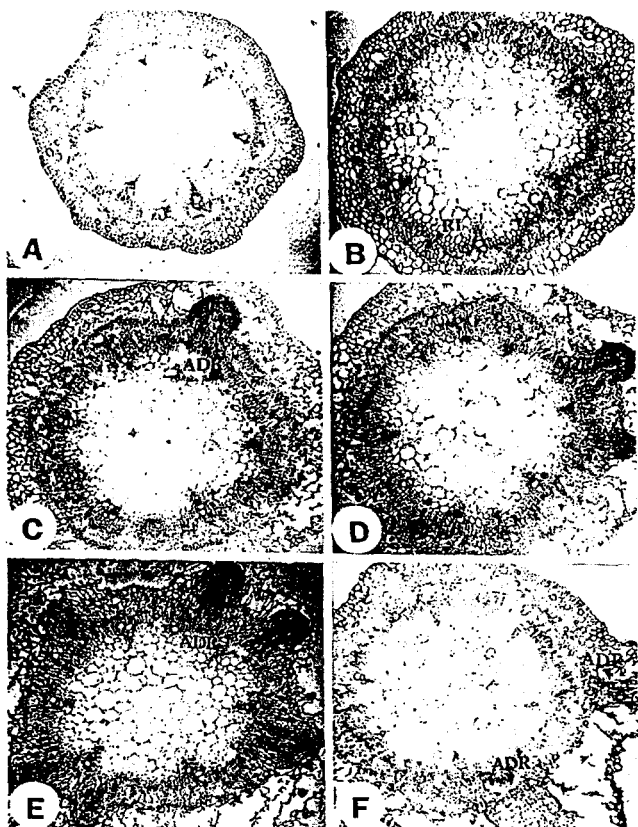


Figure 1. Cross sections of epicotyl cuttings of *Vigna angularis* Owhi et Ohashi incubated in IAA solution. Epicotyl cuttings were sectioned after being incubated in  $2 \times 10^{-4}$ M IAA solution for 0 hr(A), 24 hr(B), 48 hr(C), 60 hr(D), 72 hr(E), and 148 hr(F). AD: adventitious root CA: cambium, CO: cortex, EN: endodermis, RI: root initial.

epicotyl. While the apical segment had vigorously elongated, the fresh weight increase was most extensive in the basal segment which was incubated in water. In the inverted position, fresh weight gain was somewhat inhibited but, elongation was not inhibited (Figures 2, and 3).

Peroxidase activity was the highest in the rooting zone at root initiation phase and markedly declined with root development. However, after 148 hr, peroxidase activity was the highest in the upper part of the segments which were exposed in  $2 \times 10^{-4}$ M IAA solution. In inverted direction peroxidase activity was the highest at the upper part of the cuttings. The pattern of catalase activity along the cuttings was somewhat reverse of the peroxidase pattern (Figures 4, and 5). IAA oxidase showed relatively high activity at both ends of the cuttings and a marked decline in the activity was observed in the lower part of the cuttings after 48 hr (Figure 6). The change of enzyme activities at the rooting zone was the most during the root initiation phase but tended to be

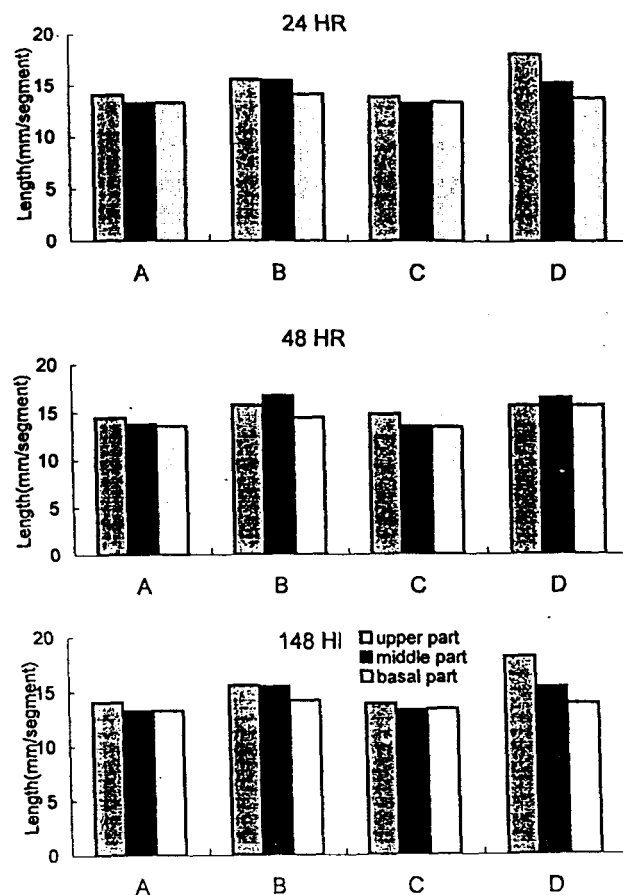


Figure 2. Effect of normal and inverted position on length of segmented epicotyl cuttings in *Vigna angularis* Owhi et Ohashi.

A: normal incubation in DW, B: normal incubation in IAA solution, C: inverted incubation in DW, D: inverted incubation in IAA solution. IAA was used at  $2 \times 10^{-4}$ M.

equalized later.

## DISCUSSION

Regulation of IAA content is important in shoot and root differentiation (Steward et al., 1957; Cho, 1985). It is well recognized that auxin plays an important role in the initiation of adventitious root (Jarvis, 1986; Jarvis and Shaheed, 1986; Cho and Soh, 1989; Liu and Reid, 1992). Several studies have shown basipetal transport of IAA in the cuttings. Surgical and IAA transport experiments in epicotyls of sunflower showed that adventitious roots formed only in the zone where auxins accumulated (Liu and Reid, 1992). Even basal supply of IAA enhanced basipetal transport from upper leaves (Jarvis and Shaheed, 1986). Root regeneration is not correlated with total amount of supplied auxin found in the

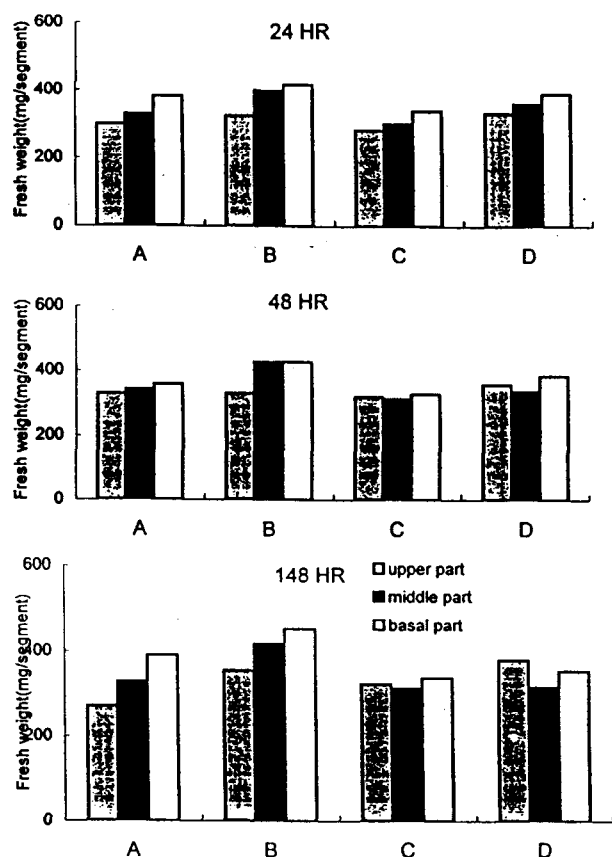


Figure 3. Effect of normal or inverted position on fresh weight of segmented epicotyl cuttings in *Vigna angularis* Owhi et Ohashi. A: normal incubation in DW, B: normal incubation in IAA, C: inverted incubation in DW, D: inverted incubation in IAA. IAA was used at  $2 \times 10^{-4}$ M.

cuttings but is related to auxin moving downward in the hypocotyl.

Planting the cuttings in inverted position resulted in fewer adventitious roots formation on the normal rooting zone due to a decreased rate of basipetal movement of auxin (Baadmand and Andersen, 1984; Cho 1985; Murayama and Ueda, 1973). Inversion of epicotyl cuttings would abolish the predetermined polar axis of IAA quantity. The changes resulted in the adventitious root formation in different direction, and also growth of the epicotyl itself would be affected as shown by our study. However, without exogenous auxins, the amount of the accumulated endogenous auxin in the rooting zone of the cutting may not be enough to initiate root formation as the case of inverted cuttings which did not form roots developed.

In other experiment, variation in peroxidase activity may indicate root development (Berthon et al., 1987). Many authors reported that a sharp increase of peroxidase activity at root initiation phase was followed by a sharp decrease

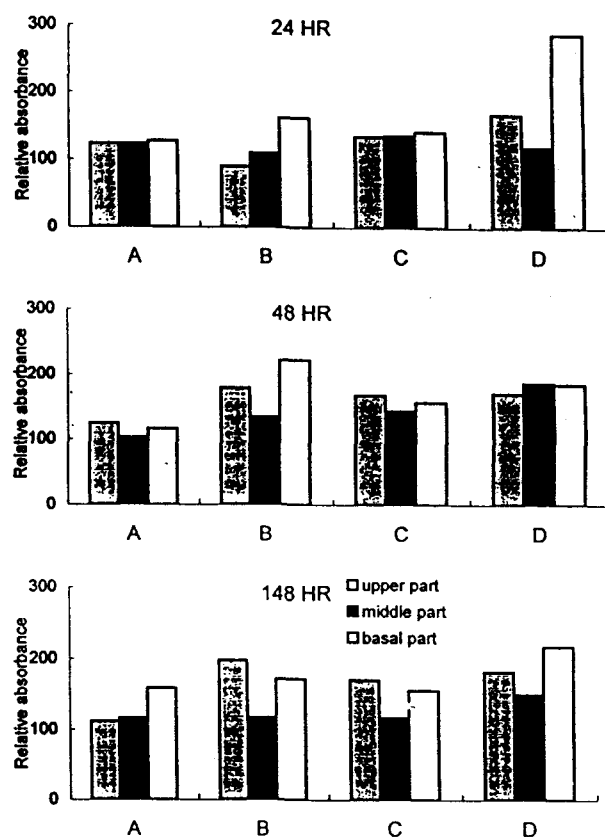


Figure 4. Effect of normal and inverted position on catalase activity of epicotyl cuttings of *Vigna angularis* Owhi et Ohashi. A: normal incubation in DW, B: normal incubation in IAA, C: inverted incubation in DW, D: inverted incubation in IAA. IAA was used at  $2 \times 10^{-4}$ M.

(Castillio et al., 1984; Gaspar et al., 1985). However, the subsequent decrease of enzyme activity was not observed in microcuttings of *Mallus* (Klerk et al., 1990). Our results, the rooting induction phase is characterized by a rise in peroxidase activity. Inversion of cuttings caused an unusual increase of the enzyme activity at the apical end of the cuttings. Our results indicate a high correlation between peroxidase activity and root initiation. On the other hand, IAA inactivation is related to the activity of IAA-oxidase. Peroxidase has also been known to catalyze the oxidation of IAA (Grambow and Langen-Schwich, 1983). However, the relative distribution of peroxidase did not parallel that of IAA oxidase. The efficiency of IAA protection is inversely related to the efficiency of oxidase activities as reflected by the activity of catalase.

The IAA level would be enhanced by both basipetal transport and biochemical events in the rooting zone of the cuttings. Exogenously supplied auxin may regulate IAA oxidase directly by altering the level of auxin protectors

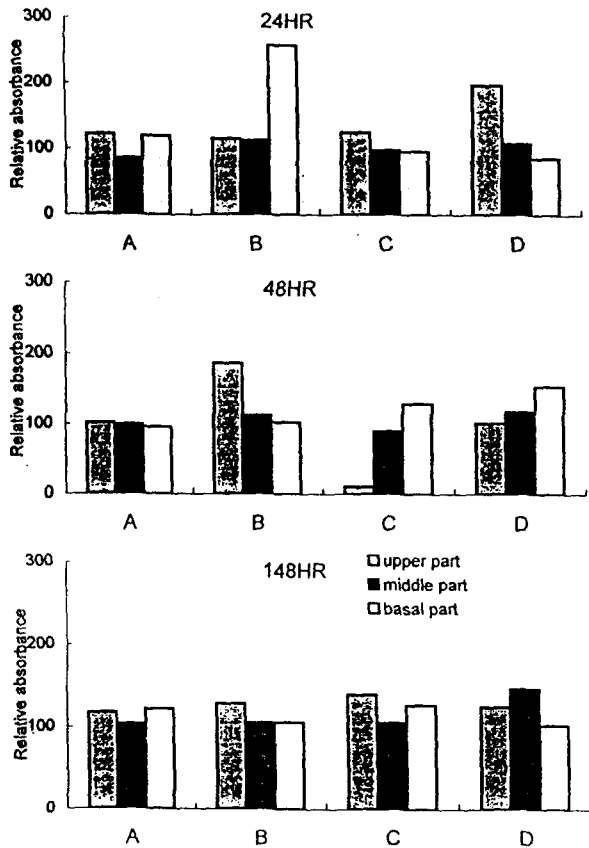


Figure 5. Effect of normal and inverted position on peroxidase activity in epicotyl cuttings of *Vigna angularis* Owhi et Ohashi. A: normal incubation in DW, B: normal incubation in IAA, C: inverted incubation in DW, D: inverted incubation in IAA. IAA was used at  $2 \times 10^{-4}M$ .

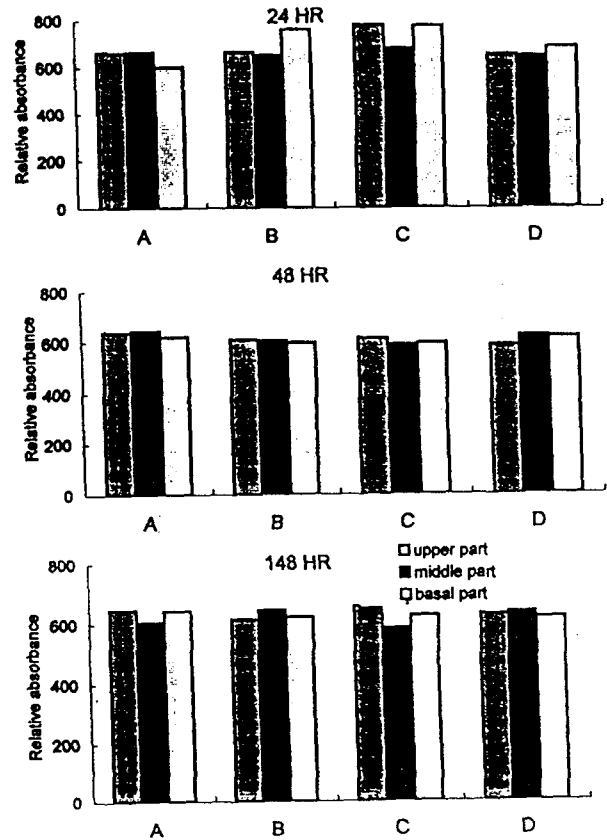


Figure 6. Effect of normal and inverted position on IAA oxidase activity in epicotyl cuttings of *Vigna angularis* Owhi et Ohashi. A: normal incubation in DW, B: normal incubation in IAA, C: inverted incubation in DW, D: inverted incubation in IAA. IAA was used at  $2 \times 10^{-4}M$ .

and/or inducing new ones and as a result may control the level of endogenous auxin. Considering that oxidase activities are responsible for the in vivo auxin catabolism, it suggests that rooting is mainly controlled by the auxin level in the inductive as well as in the root developmental phase.

### 적 요

부정근발생에 관한 도립 및 직립배양의 영향을 구명하기 위하여 6일간 자란 팥의 유경 절편을 사용하였다.  $2 \times 10^{-4}M$  IAA 용액을 48시간 처리할 때에 절편의 위치를 직립 또는 도립으로 배양후 96시간 직립으로 배양하였다. 48시간 처리 시 직립배양하면 부정근의 형성은 형태학적 기부에서만 발생하지만, 도립 전처리 배양을 하면 유경절편의 양끝에서 부정근이 발생하였다. IAA 전처리는 절편의 방향과 관계없이 부정근 형성을 촉진시켰다. 부정근 발생시기에 유경절편 내의 생화학적 변화를 구명하기 위하여 직립배양 및 도립 배양을 한 후 부정근 발생 단계별로 24시간, 48시간, 148시

간 후 절편을 상부, 중부, 하부로 등분하여 부정근 발생에 수반되는 효소활성, 절편의 길이 신장 및 생증량을 측정하였다. 절편의 길이 신장은 절편의 상부에서 높게 나타났고 생증량은 발근부위에서 높게 나타났다. peroxidase와 catalase 활성은 부정근원기의 형성단계인 24시간에 절편의 발근부위에서 높게 나타났고 이에 비해 IAA oxidase는 양쪽 기부에서 낮았다. 그리고 IAA oxidase와 peroxidase 활성은 48시간 배양인 부정근의 형성동안 발근 부위에서 감소하였다. 도립 전처리 유경 절편에 따라서 효소활성의 변화가 일어났으며 peroxidase의 활성은 부정근원기형성과 밀접한 관계를 나타내었다. 따라서 생화학적인 변화는 유경의 발근영역에서 IAA의 수준의 변화와 밀접하게 관련되어 특정부위에서의 부정근 형성을 유도하는 것으로 판단된 것이다.

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(1997년 11월 1일 접수)