Musaの生長点の形態形成に及ぼすIAAの効果と貯蔵培地中のIAAの分解

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Effect of IAA on Morphogenesis of *Musa* Apices, and IAA Degradation During Storage of Media

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Summary

IAA (indole-3-acetic acid) added to MS (Murashige and Skoog) medium was quantified by HPLC (high performance liquid chromatography) during storage of the media under various conditions. Illumination (5,000 lx) of the media with fluorescent white lamps degraded IAA significantly, but temperature treatment (25 °C) under the dark condition had no strong effect on IAA degradation. Under the light conditions (5,000 lx) at 25 °C where *in vitro* rooting and shoot growth of *Musa* apices were enhanced well by adding IAA, the amount of IAA decreased papidly and almost no IAA was detected 8 days after treatment. The effect of IAA on morphogenesis of *Musa* apices was clearly observed in the present study. These facts suggest that shoot apices of *Musa* on the media absorb and use IAA at initial times after inoculation.

Introduction

Usually, auxines have a definite influence on rooting of explants cultured *in vitro* (8). In micropropagation of *Musa*, IAA also has an important role in shoot growth and rooting(1). On the other hand, it is known that some plant growth regulators such as IAA, abscisic acid and gibberellins are unstable to high temperature or illumination. In many cases, tissue culture has to be carried out under light and the explants require relatively high temperature. However, the behavior of IAA under tissue culture conditions is not well understood. The present paper reports on IAA degradation in the MS medium, with reference to the effects of IAA on morphogenesis of *Musa*.

Materials and Methods

Plant materials

Banana plants, *Musa acuminata* Colla, var. Sanjaku banana, were kindly provided by Daijuen, Co. Ltd., Toyohashi, Japan.

Preparation of the media and tissue culture

IAA was first dissolved with 0.1 N NaOH and adjusted at pH 5.7 with 0.1 N HCl. The IAA solution was then added to the autoclaved medium (pH 5.7) kept at about 60 °C, through a milliporefilter(0.45 μm, Millipore Corporation Co. Ltd., Massachusetts) under sterile conditions. BA dissolved in 0.1 N NaOH was added to the medium and adjusted to pH 5.7 with 0.1 N HCl before autoclaving. Glass tubes with alminum-foil caps containing about 10 ml of medium were employed in all experiments.

Shoot apices were excised on a clean bench and planted on solid MS(Murashige and Skoog) (7) media containing 30 g/l sucrose and 8 g/l agar supplemented with 3 mg/l BA or 1 mg/l IAA or 3 mg/l BA plus 1 mg/l IAA. They were cultured at 25 °C under continuous light of 5,000 lx supplied with white fluorescent lamps. Shoot length, number of shoots, root length and number of roots were recorded weekly.

Illumination and temperature treatments of the media

The glass tubes containing about 10 ml of MS media with 1 mg/l IAA were placed under following conditons; light (5,000 lx): 25 °C, dark: 25 °C, light: 4 °C, dark: 4 °C and dark: −20 °C. Explants were not inoculated in these tubes.

Extraction and purification of IAA

Five grams of each treated medium were

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sampled and IAA was extracted with 25 m*l* of ethanol overnight. Then, they were filtrated through filtration paper (Toyo No. 2) and the residue washed with 5 ml of ethanol 3 times. The combined filtrate was evaporated *in vacuo* to water phase, adjusted at pH 3.0 with HCl and passed through Sep-pack C18 cartridge (Waters, Co. Ltd., Massachusetts) 2 times. The cartridge was equilibrated previously at pH 3.0 with CH_3COOH. The IAA in the cartridge was eluted with 5 ml of ethyl acetate(EtOAC) 2 times, evaporated *in vacuo* to dryness and redissolved with EtOH. The solution was passed through milliporefilter (Nihon Millipore Kogyo, Co. Ltd., Japan) to remove the precipitates, evaporated again to dryness and dissolved with 100 μl of EtOH. Twenty microliters of the solution were subjected to HPLC analysis. All operations were carried out below 40 °C. Samples were kept from light as far as possible during these operations. The procedures are summarized in Fig. 1. Although the sampling was performed at intervals of 3 weeks in the preliminary experiments, almost no IAA was detected 3 weeks later under light conditions. Therefore, the media were sampled at intervals of 3 days.

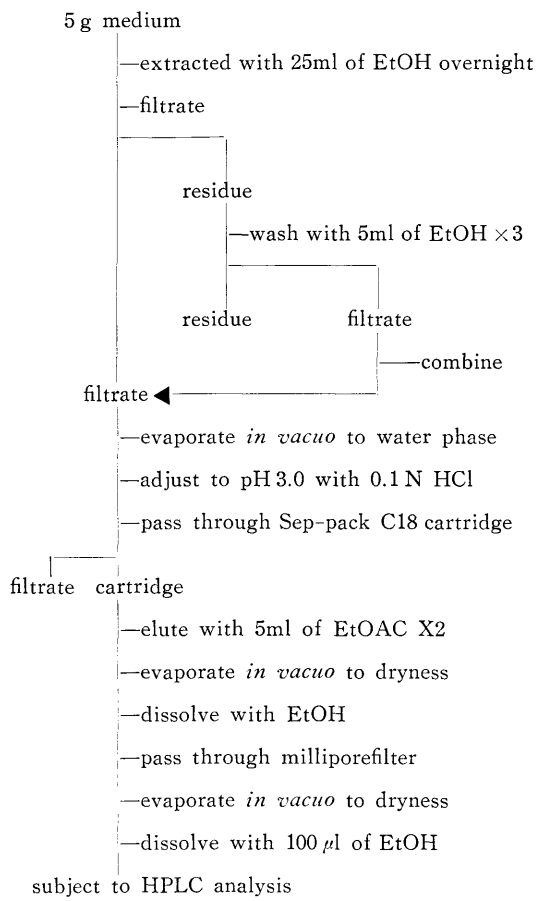

Fig. 1. Extraction and purification procedure of IAA from MS medium.

HPLC analysis of IAA

HPLC (Hitachi, 655A-12) equipped with ODS column (Hitachi YMC pack, A-302, 150 mm in length × 4.6 mm in width) and a UV detector was used. An ethanol gradient system, from 50 to 100%, was employed and flow rate was 0.2 ml/min. Under these conditions, the retention time of IAA was about 21 min (Fig. 2). Peak heights rather than peak areas in the elution profiles of IAA compared well with the amounts of standard IAA injected. Therefore, the amounts of IAA were calculated by their peak heights. The calculation formula was: $y=0.0559x+9.4095$, y: the amount of IAA (ng), x: peak height. Experiments were duplicated and mean values are shown.

Results and Discussion

Fig. 3 shows the growth of shoots generated from *Musa* apices with IAA and/or BA. Shoot growth was promoted by adding IAA to the

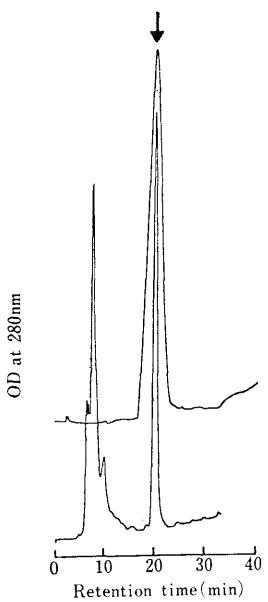

Fig. 2 Elution profiles of IAA. Upper: standard IAA (1 μg), Lower: IAA extracted from MS medium. Arrow indicates IAA.

medium. Shoot length was 3 times that with BA only, 4 weeks after inoculation. In the medium with IAA+BA shoot growth was intermediate between that of the medium with IAA and that with BA. On the other hand, BA stimulated adventitious bud formation of the explants. At 4 weeks after inoculation, the number of shoots per explant was 1.6 with BA, whereas it was 1.1 with IAA. The number of shoots 3 months after inoculation decreased compared with that 2 months later, in both media with BA and BA+IAA (Table 1). Several shoots seemed to brown at this time in these media.

IAA also had a strong effect on rooting of the *Musa* explants. The mean number of roots per explant was 4.57 and mean length was 1.15 cm at 4 weeks after inoculation, whereas no rooting was observed at this time in the medium with BA only (Fig. 4). The effects of IAA on the morphogenesis of *Musa* apices, especially on root formation, were already observed at 2 weeks after inoculation (Fig. 5-A). Several roots were obseved 2 months later in the media with BA and gradually developed thereafter (Table 1, Fig. 5-B).

In the present study, BA seemed to suppress the effect of IAA on growth of shoots and roots of *Musa*. Antagonistic effects between auxins and cytokinins on root elongation were also found in *Lens*. Cytokinin seems to induce auxin oxidase such as peroxidase in the root, resulting in inactivation of auxin(4). On the con-

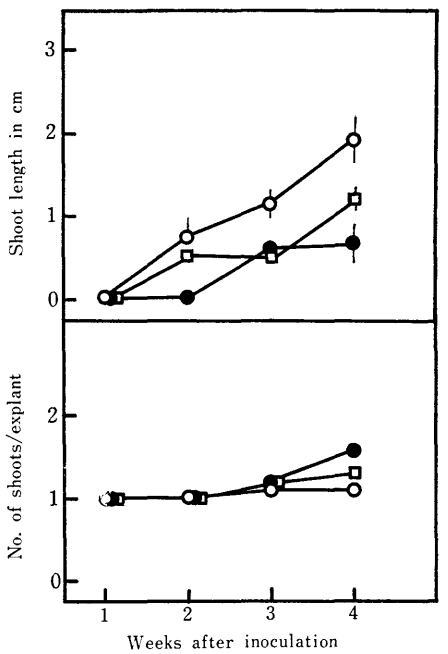

Fig. 3. Effect of IAA and BA on shoot growth (upper)and shoot formation(lower). ●: 3 ppm BA, ○: 1 ppm IAA, □: 3 ppm BA+1 ppm IAA. Vertical bars indicate SE.

Table 1. Effect of IAA and BA on shoot and root growth.

	2 months after inoculation		
	IAA	BA	IAA+BA
Shoot length (mm)	52.3±7.6	16.6±1.4	18.9±3.1
No. of shoots/explant	1.2	4.3	2.7
Root length (mm)	26.9±3.1	14.6±1.7	17.0±1.1
No. of roots/explant	9.0±1.0	2.4±0.4	4.2±1.5
	3 months after inoculation		
	IAA	BA	IAA+BA
Shoot length (mm)	67.4±12.3	31.7±7.1	45.6±5.4
No. of shoots/explant	1.3	4.0	1.7
Root length (mm)	30.2±2.1	25.3±1.5	21.9±1.9
No. of roots/explant	16.8±3.4	16.8±4.0	12.0±3.5

Each value is average of length ± SE or number ± SE.

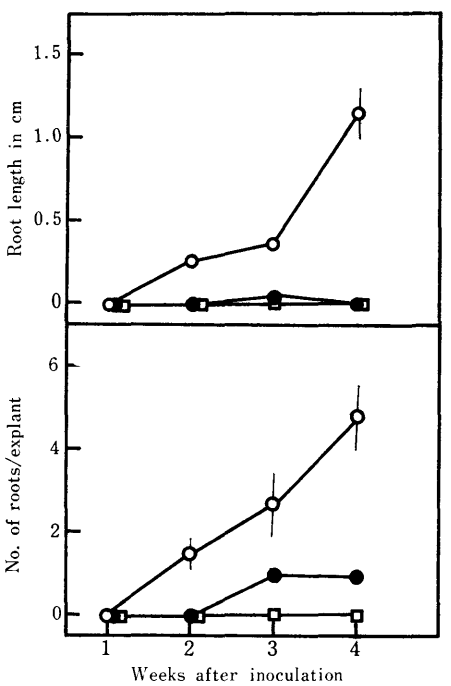

Fig. 4. Effect of IAA and BA on root growth (upper)and root formation (lower). ●: 3 ppm BA, ○: 1 ppm IAA, □: 3 ppm BA+1 ppm IAA. Vertical bars indicate SE.

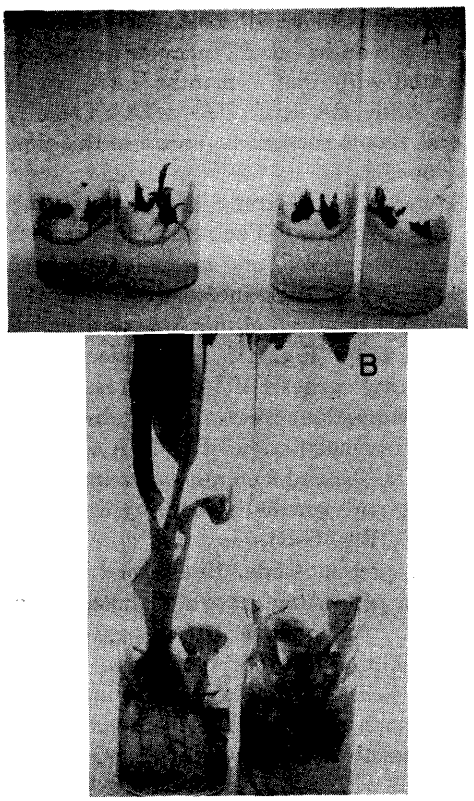

Fig. 5. Shoot apices cultured on MS media. A; Two weeks after inoculation. Right: 3 ppm BA, Left: 1 ppm IAA. Note the formation of adventitious roots. B; Two months after inoculation. Right: 3 ppm BA, Left: 1 ppm IAA.

rary, auxins alter the metabolism of endogenous cytokinins in bark explants (3) and oat stem segments (5).

Although IAA in the media was rather stable in the dark even at 25 °C for several weeks, illumination treatments of 5,000 lx degraded IAA significantly (Table 2). Under light, degradation of IAA was more rapid at 25 °C than at 4 °C. At 8 days after illumination, the amount of IAA reducd by 65% of the initial content at 4 °C, while almost all IAA was disappeared at 25 °C. These results indicate that storage of the media without degradation of IAA is possible for a few weeks when they are kept in the dark below 25 °C. By storing at −20 °C, the solid MS media changed to semisolid liquid when they were transferred to room temperature. However, the IAA in the media was fairly stable at −20 °C.

Table 2. Degradation of IAA in MS media subjected to various treatments.

Treatment	Treatment period (days)			
	0	4	8	12
25 °C Light	1055(ng)	356	108	83
25 °C Dark		975	1147	1182
4 °C Light		876	699	444
4 °C Dark		1280	1279	944
−20 °C Dark		830	1025	1208

Each value is mean of 2 independent analyses.

The degradation process of IAA is not well understood. It is considered that IAA is transformed by IAA oxidase such as peroxidase into 3-methylene oxyindole which is physiologically inactive *in situ*(6). In the present study, IAA in the MS medium was mainly degraded by light oxidation. The derivatives from IAA degradation following light treatment were not identified.

In the tissue culture of pine trees, the pulse method is often employed in order to supply plant growth regulators to the explants; explants were immersed in the solution containing plant growth regulators for several hours immediately before inoculation and cultured on the hormone-free medium(2,9). Present results suggest that the shoot apices of *Musa* planted on MS medium absorb IAA at early stages after inoculation, at most within 8 days, and use it for their morphogenesis and subsequent growth. This may support the usefulness of the pulse method for suppling IAA to *Musa* explants.

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Musa の生長点の形態形成に及ぼすIAAの効果と貯蔵培地中のIAAの分解

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摘　要

MS 培地上で培養した‘三尺バナナ’(*Musa acuminata* Colla) の生長点の形態形成に及ぼす IAA の影響と MS 培養地中の IAA の分解について調査した.

IAA を単独添加した場合，シュートの生長，発根および根の生長は著しく促進された．しかし，そこに BA を添加すると IAA の効果は減少し，不定芽の形成が促進された．一方，BA の単独添加区では不定芽形成の促進効果のみが観察された.

MS 培地中の IAA は，暗黒下ではかなり安定であったが，バナナを培養した 25 °C, 5,000 lx の連続照明下では処理後 8 日めにほとんど分解されていた.

上記のバナナ生長点の形態形成に対する IAA の効果は，生長点置床後 2 週間めには観察された．このことより，培地上に置床された生長点は置床後すみやかに IAA を吸収し，その後の分化，生長に利用しているものと考えられた.