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Hormonal Effect and Cytokinin Autonomy in Callus Culture of *Phaseolus vulgaris* L.

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강낭콩의 Callus 組織培養에 있어서 植物 Hormone의 영향과 Cytokinin Autonomy

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ABSTRACT

The activities of auxins and cytokinins have been examined in the growth of callus tissue derived from *Phaseolus vulgaris* L. cv. Damyang. The synthetic auxin, picloram was the most effective in promoting callus growth and the range of effective concentrations (0.1 μM to 32 μM) was broad. 2,4-D also enhanced callus growth at the optimal concentration of 3.2 μM. NAA promoted callus growth at relatively higher concentrations than other auxins tested. IAA was less effective in supporting callus growth. Cytokinin bearing saturated side chain (N^6-isopentyladenine) was approximately 30 times more active than the corresponding unsaturated compound, N^6-(Δ^2-isopentenyl) adenine. The abilities of cytokinin-autonomous growth were also examined. Callus tissues previously grown on concentrations lower and/or higher than optimal concentrations of cytokinins were better habituated in the subsequent passage. It was suggested that the development of cytokinin autonomy may be related to dosage-concentrations of cytokinin in the previous passage.

INTRODUCTION

Genetic approaches as a model for the study of hormonal metabolism have been accelerated by the development of new strategies using *Phaseolus* tissue cultures (Armstrong *et al.*, 1981). The traits identified to date include the intrinsic genetic variations in the cytokinin metabolism (Mok *et al.*, 1978, 1982) and in the induction of cytokinin autonomy (growth on cytokinin-free medium) in tissue cultures of *Phaseolus* (Kim, 1980; Mok *et al.*, 1980). Thus the pronounced differences on cytokinin metabolism are in

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response to cytokinins bearing saturated and unsaturated isoprenoid side chains on callus cultures between interspecies, *P. vulgaris* and *P. lunatus*. Moreover, the cytokinin-autonomous growth of *Phaseolus* callus tissues is genotype-specific and this trait is heritable and controlled by nuclear genes.

In the work reported here, we have attempted to identify the traits of a geographically established cv. Damyang of *Phaseolus vulgaris* L. with regard to the requirements of auxins and cytokinins and the ability of the development of cytokinin autonomy on callus growth.

MATERIALS AND METHODS

Chemicals. N^6-furfurylaminopurine (kinetin), N^6-(Δ^2-isopentenyl) adenine (i^6Ade), indole-3-acetic acid(IAA), α-naphthalene acetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) were purchased from Sigma. N^6-isopentyladenine (hi^6Ade) was synthesized in Dr. D. J. Armstrong's laboratory, Oregon State University, Corvallis, Oregon, according to the procedures described by Leonard *et al.* (1968). Picloram (4-amino-2,3,5-trichloropicolinic acid) was a gift from Dow Chemical.

Plant Materials. Seeds of *Phaseolus vulgaris* L. cv. Damyang were obtained from Dr. K. E. Yoon, Korea Ginseng and Tobacco Res. Inst.

Tissue Culture Medium. Tissue culture medium used was consisted of the mineral nutrients described by Murashige and Skoog (1962) with the following organic substances added: sucrose(30 g/l), *myo*-inositol (100 mg/l), thiamine-HCl (1 mg/l), nicotinic acid (5 mg/l), and pyridoxine-HCl(0.5 mg/l). Picloram (2.5 μM) was used to supply the auxin requirements of the callus tissue, except as indicated. Kinetin(5 μM)was included in the medium used for callus initiation and for stock cultures. The pH of the medium was adjusted to 5.7 and Difco Bacto-agar(8 g/l)was added. The medium was dispensed into 125-ml Erlenmeyer flasks (50 ml/flask) and autoclaved at 120°C for 15 min. For examining the response to auxins and cytokinins of *P. vulgaris* cv. Damyang callus tissue, the appropriate amounts of auxins and cytokinins were dissolved in dimethylsulfoxide (Schmitz and Skoog, 1970) and added to the autoclaved tissue culture flasks prior to solidification of the medium. The final amount of dimethylsulfoxide in the tissue culture medium was 0.05 ml/flask.

Growth and Harvest of *Phaseolus* Callus Cultures. Seeds of Damyang were surface sterilized with 2.5% sodium hypochlorite and germinated under sterile conditions. The hypocotyl of 7 day-old seedling was cut into slices and three discs (approximately 2 mm thick) were planted in each flask. After a culture period of 21 days, the callus tissue formed on the initial explants was transferred once (the first passage) on the medium containing 2.5 μM picloram and 5 μM kinetin. The duration of the first passage was 21 days. Tests for response to auxins and cytokinins were performed on the second passage

except as indicated. Tests for cytokinin autonomy (growth on cytokinin-free medium) were examined on the subsequent passage. All cultures were grown in the dark at 27° C. Tissues were harvested and weighed after 35 days. The average fresh callus weight of the four replicate flasks was determined. All experiments were repeated at least once by using newly established cultures.

RESULTS

Response to Auxins in Tissue Culture of *P. vulgaris*. To determine the optimal conditions for establishing callus culture of *P. vulgaris* cv. Damyang, callus growth was tested for response to different auxins on the medium containing 5 μM kinetin as cytokinin source (Fig. 1). The tissues were grown well on the synthetic auxin, picloram at moderate to high concentrations, 0.1 μM to 32 μM. The quality of the tissue was good and the color was light-yellow at these concentrations. The range of optimal concentration of 2,4-D was markedly narrower than that of picloram. Thus, the optimal concentration of 2,4-D for callus growth of Damyang was 3.2 μM. NAA enhanced callus growth when supplied at relatively high concentrations (10 μM and 32 μM). But at all the concentrations tested the callus was poor and dark-brown except it was light-brown at higher concentrations than 10 μM. Thus, the optimal growth might not have been reached within the range of NAA tested. IAA was less effective in supporting callus growth. Although fresh weight of the callus was higher on IAA than on 2,4-D at high concentrations, browning of the tissues developed at the early stages of the culture period and the callus became almost necrotic after 3 weeks.

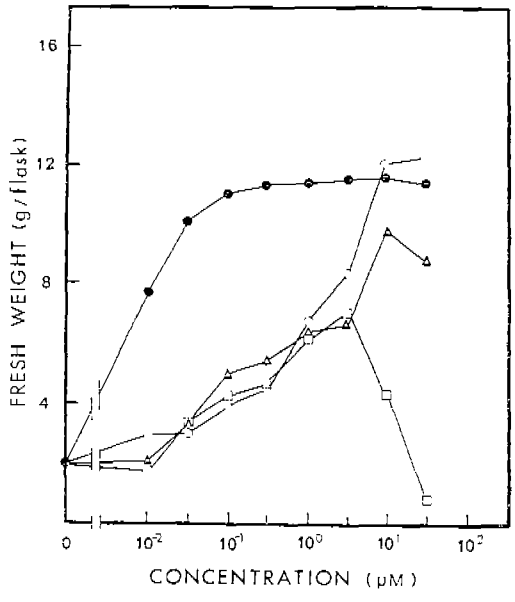

Fig. 1. Auxin activities of IAA(△), 2,4-D(□), NAA(○), and picloram(●) in promoting callus growth of *P. vulgaris* cv. Damyang.

Response of *P. vulgaris* Callus Tissue to Cytokinins. The effects of cytokinins bearing saturated and unsaturated N^6-isoprenoid side chains were tested for their ability to promote the growth of callus tissues derived from *P. vulgaris* cv. Damyang. The growth response to kinetin was also included in comparison. As shown in Fig. 2, the activity of hi^{6}Ade with saturated side chain was approximately 30-fold greater than that of

i^6Ade with unsaturated side chain in promoting the growth of Damyang callus tissue. However, both cytokinins tested promoted vigorous growth and produced the maximal yield of callus tissue up to 10g per flask at appropriate concentrations. Kinetin showed almost the same activity as hi^6Ade in this tissue.

Cytokinin Autonomy in *P. vulgaris* Callus Tissue. To determine whether Damyang callus tissue grown on media containing different auxins had gained the capacity for cytokinin-autonomous growth, callus tissues previously grown on various concentrations of each auxin (Fig. 1) were transferred to the media containing the same concentration but no cytokinin. Fig. 3 shows the best callus tissues which were grown on cytokinin-free medium for seven weeks with 2.5 μM of IAA, 2,4-D, and NAA each as auxin sources. The callus tissue grown

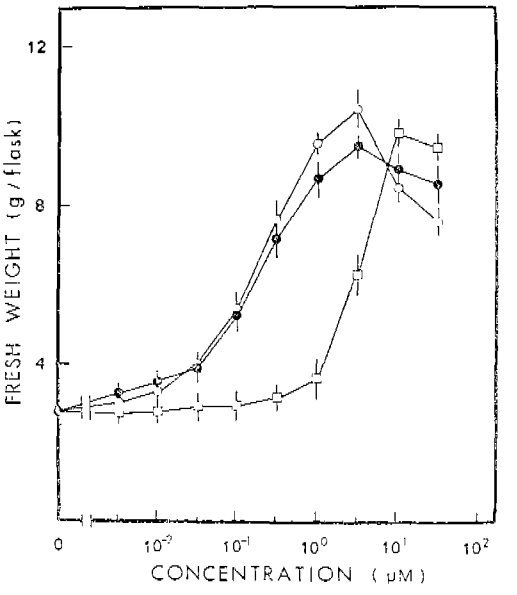

Fig. 2. Cytokinin activities of i^6Ade(□), hi^6Ade (○), and kinetin(●) in promoting callus growth of *P. vulgaris* cv. Damyang. The vertical bars indicate SE.

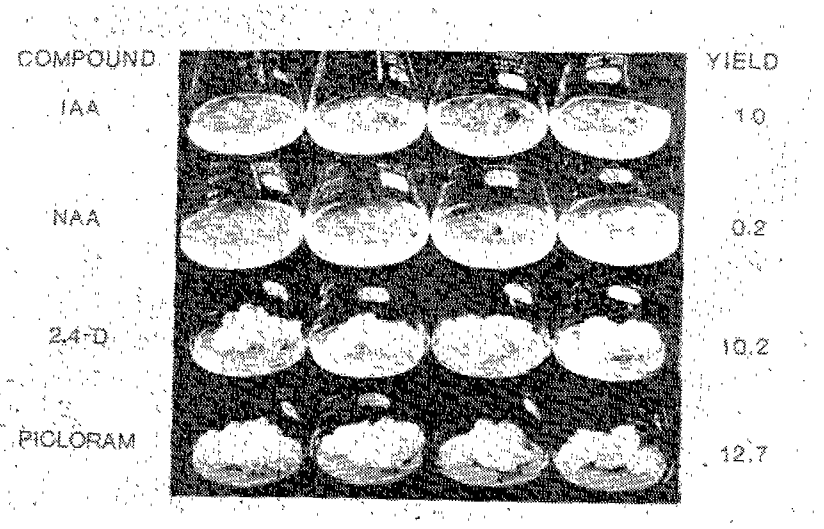

Fig. 3. Representative callus cultures of *P. vulgaris* cv. Damyang after a culture period of seven weeks on cytokinin-free medium. Callus tissues(the second passage) shown in Fig. 1 were used. Average fresh weights(g/flask) are given at the right of the figure.

on 2.5 μM picloram was also shown in comparison. The callus tissues exhibited a poor response to IAA and NAA in the cytokininin-autonomous growth, thus only a slight and irregular increase in background growth was observed. All callus tissues exposed to 2, 4-D and picloram in the previous passage were able to grow on the cytokinin-free medium.

To establish a further characterization of the callus tissue, the growth response of Damyang callus tissue was examined in detail. Growth curves established for tissues grown on medium with and without cytokinin are shown in Fig. 4. In both, tissues grew rapidly after three weeks. The increases in fresh weight were linear from the third to the sixth week of the cuture period and were followed by a slight decrease.

The effects of cytokinins on the development of cytokinin autonomy were also examined. As shown in Table 1, the cultures exposed to concentrations lower and/or

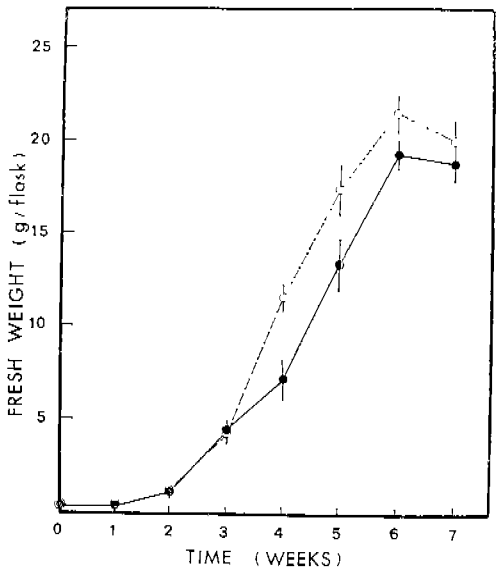

Fig. 4. Growth curves of *P. vulgaris* cv. Damyang callus tissues on medium without cytokinin (●) and medium containing 5 μM kinetin (○). Data were obtained in the fifth passage of callus cultures and cytokinin-autonomous callus was originally derived from the tissue grown on medium containing 2.5 μM picloram and 5 μM kinetin in the second passage. The vertical bars indicate SE.

higher than the optimal concentration in the previous passage showed a better habituation. Thus the optimal concentration of cytokinins was required to suppress the autonomous growth habit. However, suppression of the autonomous growth at the optimal concentration of cytokinins was gradually recovered and callus tissue exhibited maximal growth over 20 g per flask at fresh weight after two or three passages on the cytokinin-free medium (Fig. 4).

DISCUSSION

The differences in responses of callus tissue of *Phaseolus vulgaris* L. cv. Damyang to different auxins and cytokinins are evident. The synthetic auxin, picloram is highly ef-

Table 1. Effect of cytokinins in the development of cytokinin autonomy in callus cultures of *P. vulgaris* cv. Damyang

Treatment in previous passage (μM)	Fresh weight (g/flask) on cytokinin-free medium*		
	Kinetin	i^6Ade	hi^6Ade
0.003	10.7	11.0	9.7
0.01	14.2	10.0	13.5
0.03	8.8	9.7	10.6
0.1	9.6	8.5	8.1
0.3	8.1	7.3	6.1
1	4.7	7.4	6.2
3	4.3	6.8	6.0
10	8.1	5.1	4.8
32	6.1	4.2	6.0
0	8.5	8.5	8.5

*Callus tissues (the seconed passage) shown in Fig.2 were used. Average fresh weights of four flasks were determined after a culture period of five weeks.

fective on the callus growth. In comparison to 2, 4-D, it is active at lower concentrations and has a wider range of effective concentrations. Moreover, growth rate and final yield of callus tissue was higher on medium containing the optimal concentration of picloram than that of 2, 4-D. NAA enhanced callus growth only at relatively high concentrations. IAA was less effective in supporting callus growth of Damyang. Thus, the observed difference among auxins tested suggests that it is generally acceptable, as previously reported (Mok and Mok, 1977), that picloram is more effective than the other auxins in promoting callus growth of *Phaseolus*, even though genotypes of *Phaseolus* were differently originated in geography.

The most interesting difference in cytokinin activities in promoting the callus growth of Damyang is seen with cytokinins bearing saturated and unsaturated N^6-isoprenoid side chains. Thus, hi^6Ade is more active than the corresponding unsaturated i^6Ade in promoting the growth of Damyang callus tissue. The similar structure-activity relationships in *Phaseolus* callus tissues were observed and it was suggested that the differences might be related to cytokinin metabolism in these species(Mok *et al.*, 1978; Armstrong *et al.*, 1981; Mok *et al.*, 1982). This suggestion was based on the investigation of Whitty and Hall (1974) that *trans*-zeatin, i^6Ade, and their ribosides were rapidly degraded by a cytokinin oxidase isolated from maize. However, cytokinins with saturated side chains were relatively resistant to attack by this enzyme. Therefore, the low activity of i^6Ade in promoting callus growth of Damyang may be related to rapid conversion of this cytokinin to inactive metabolites.

The abilities of cytokinin-autonomous growth in Damyang callus tissue were also ex-

amined. Callus tissues grown on suboptimal concentrations of cytokinin or at concentrations higher than that required to support optimal growth showed better habituation in subsequent passage of cytokinin-free medium and at optimal concentration this characteristic was a little suppressed. This result suggests that the development of cytokinin autonomy was somewhat related to a dosage of cytokinin in the previous passage. But this result in itself does not establish whether cytokinin autonomy is directly influenced by the exogenous supply of cytokinin. We have previously suggested that cytokinin -dependence or -independence in *Phaseolus* callus cultures might be genotype-specific and heritable (Mok *et al.*, 1980). On the basis of the results obtained here the callus tissue of Damyang appears to be a heritable genotype of cytokinin-autonomous growth and it should provide a useful tool for biochemical and genetic investigations for the regulation of cytokinin metabolism.

摘 要

강낭콩(*Phaseolus vulgaris* L. cv. Damyang)의 callus 組織 生長에 미치는 auxin과 cytokinin의 効果를 본 結果, 處理한 auxin 중에서 picloram이 넓은 범위의 濃度(0.1 μM to 32 μM)에서 組織 生長을 促進시켰으며, 2,4-D 또한 効果的이었으나 picloram보다 活性度가 낮았다. NAA는 相對的으로 高濃度(10 μM 및 32 μM)에서 組織 生長을 促進시켰으며, IAA 處理時에는 組織의 老衰化가 빨랐다.

한편 cytokinin, N^6-isopentyladenine은 N^6-(Δ^2-isopentenyl)adenine보다 callus 組織 培養에 높은 活性을 보여 最適濃度에서 약 30배의 促進効果를 나타냈다. Callus 組織의 cytokinin 非要求性 生長(cytokinin-autonomous growth)實驗에서는 cytokinin의 低濃度와 高濃度에서 자란 組織이 最適 濃度에서 자란 것보다 cytokinin 非要求性 生長 組織으로 더 잘 바뀌었다. 따라서 cytokinin 非要求性 組織으로의 發達에 있어서 前處理時의 cytokinin 濃度가 하나의 重要한 要因으로 思料된다.

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