Effects of 1-Aminocyclopropane-1-carboxylic Acid Production on the Changes in the Polyamine Levels in Hiproly Barley Callus after Auxin Withdrawal

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Contents of polyamines and 1-aminocyclopropane-1-carboxylic acid (ACC) in Hiproly barley callus were examined under different culture conditions. After auxin withdrawal, the contents of free polyamines changed conversely to the contents of ACC. In the absence of auxin, incorporation of L-[3,4-¹⁴C]methionine into polyamines and the activity of *S*-adenosylmethionine decarboxylase (SAMDCase) in the callus increased, then remained stable, but incorporation of L-[3,4-¹⁴C]methionine into ACC, precursor of ethylene and ACC synthase activity once declined and increased again.

Aminoxyacetic acid (AOA) affected the increase in the levels of polyamines in the callus. 1-Aminoisobutyric acid (AIB) had a slight effect on the polyamine production. The incorporation of L-[3,4-¹⁴C]methionine into ACC and ACC synthase activity were inhibited by AOA, but not by AIB. AOA stimulated the activity of SAMDCase, and also enhanced the incorporation of L-[3,4-¹⁴C]methionine into polyamines in the callus. Methylglyoxal-bis(guanyldrazone) (MGBG) greatly enhanced the ACC production. The rate of incorporation of L-[3,4-¹⁴C]methionine into ACC and ACC synthase activity in the callus were significantly enhanced by MGBG. MGBG strongly inhibited SAMDCase activity and the incorporation of L-[3,4-¹⁴C]methionine into polyamines. Moreover, the synthesis of polyamines was inhibited by MGBG.

These results suggested that in Hiproly barley callus ACC production has an important effect on changes in the polyamine levels, and that polyamine and ethylene biosynthetic pathways are regulated by competition against each other.

Putrescine, spermidine, and spermine are synthesized from arginine, ornithine, and methionine. In plants, putrescine is first synthesized from both arginine and ornithine through their decarboxylation,^{1,2)} and spermidine and spermine are formed from putrescine by transfer of the aminopropyl moieties from methionine.³⁾

Spermidine and spermine biosynthesis is closely related to ethylene evolution. Apelbaum *et al.*⁴⁾ and Suttle⁵⁾ reported that ethylene production was regulated by exogenously applied polyamines and that ethylene inhibited polyamine formation. Robert *et al.*⁶⁾

also reported that the levels of spermidine and spermine in carnation flowers were elevated by inhibition of the ethylene biosynthetic pathway. Even-Chen *et al.*⁷⁾ noted an increase in incorporation of methionine into polyamines when ethylene production was inhibited by aminoethoxyvinylglycine in aged orange peel.

Ethylene is synthesized through the conversion of methionine to *S*-adenosylmethionine (SAM) then to ACC.^{8~10)} SAM is pivotal in the biosynthesis of both polyamines and ethylene. Thus, polyamines and ethylene have been thought to be related biosynthetically.

Results reported in literatures cited above indicated that ethylene produced might be important in changes in the polyamine levels in plant tissues. We investigated how polyamines and ethylene biosynthetic pathways were regulated in the callus after auxin withdrawal. This paper describes the effects of inhibition of ethylene production on changes in the polyamine levels in Hiproly barley callus after cultivation in the absence of 2,4-D.

MATERIALS AND METHODS

Materials and chemicals. Hiproly barley (*Hordeum disticum* L. var. Hiproly) was used in the experiment. Spermidine, spermine, and methylglyoxal-bis(guanyldihydrazone) (MGBG) were obtained from Aldrich Chemical Co, aminooxyacetic acid (AOA), SAM, and ACC from Sigma Chemicals, 1-aminoisobutyric acid (AIB) from Wako Pure Chemicals, and 2,4-dichlorophenoxyacetic acid (2,4-D) from the Tokyo Kasei Co. Aquazol-2 and L-[^{14}C -carboxyl]-S-adenosylmethionine (59.8 mCi/mmol) were obtained from New England Nuclear, and L-[3,4- ^{14}C]methionine (40~60 mCi/mmol) from Commissariat à l'Energie Atomique. Murashige and Skoog's medium was obtained from Flow Laboratories.

Induction and proliferation of callus from Hiproly barley. Hiproly barley callus was induced and grown on White's medium as described in a previous paper.¹¹⁾ The basal culture medium tested was Murashige and Skoog's medium. Calli were subcultured every 8 weeks. Calli were kept for at least two passages on White's medium containing 2 mg/l of 2,4-D, before experiments, and calli were transferred to Murashige and Skoog's basal media containing various concentrations of AOA, AIB, or MGBG. Hiproly barley calli at different times during cultivation were placed into Erlenmyer flasks containing a basal medium with L-[3,4- ^{14}C] methionine (1 μCi). After incubation for 24 hr, the calli were removed, rinsed twice with distilled water, and stored at -20°C .

Polyamine extractions. Calli (100 mg) were homogenized in 1 ml of chilled 5% perchloric acid with a glass homogenizer. This homogenate was centrifuged at $20,000 \times g$ for 20 min, and the supernatant, containing the free polyamines, was stored at -20°C .

Benzoylation and high performance liquid chromatographic analysis of polyamines. The polyamine concentration in the extract was measured by a method modified from that of Redmond and Tseng.¹²⁾ Two ml of 2 N sodium hydroxide was added to a 1.0-ml sample followed by 10 μl of benzoyl chloride. The mixture was shaken with

a Vortex mixer for 45 sec, and left for 20 min. Two ml of saturated sodium chloride solution was added and the solution was extracted with 4 ml diethyl ether. After centrifugation at $1,500 \times g$ for 15 min, two ml of the upper ether phase was removed and evaporated under a stream of nitrogen. The residue was dissolved in 100 μl of methanol and 10 μl of the solution was used for high performance liquid chromatography (HPLC).

HPLC of benzoylpolyamines was run by a Hitachi model 655A-11 with an ultraviolet detector on a 8NVC₁₈ column (radial pak, Waters Associates) at 254 nm. A linear gradient of acetonitrile from 36.5% to 100% in 1% acetic acid over a period of 20 min was used as a mobile phase at a flow rate of 3 ml per min.

Labeled polyamines were extracted by 5% perchloric acid, benzoylated by benzoyl chloride, and fractionated by HPLC as described above. One ml of fraction eluted from HPLC was collected in scintillation vials. Ten ml of Aquazol-2 was added to each vial and the radioactivity was measured by a liquid scintillation counter. Specific incorporation of ^{14}C activity into polyamines was defined as dpm per one nmol of polyamines per 24 hr.

Ethylene assay. Hiproly barley calli were placed in vials containing Murashige and Skoog's medium with or without 10 μM 2,4-D, and vials were sealed with rubber septa. After 24 hr in the dark, 1 ml air samples were taken with gas-tight syringes.

Ethylene in the samples was measured by a gas chromatograph (GC) (Hitachi, model 073) with a flame ionization detector. A 2-m column containing Porapak Q of 80 to 100 mesh was used at 80°C with N_2 gas as the carrier at a flow rate of 40 cm^3 per min.

Extraction and gas chromatographic analysis of ACC. ACC was measured by GC after its conversion to ethylene by the method of Lizada and Yang.¹³⁾ Calli (1.0 g) were homogenized in 1 ml of 3% sulfosalicylic acid with a glass homogenizer. The homogenate was centrifuged for 2 min. The precipitate was extracted three times with 1 ml of 3% sulfosalicylic acid. The supernatant was put on a column of Dowex 50 $\times$ 8 (H^+ form) (bed volume, 0.5 ml). After the column was washed with 6 ml of water, the ACC fraction was eluted with 3 ml of 3 N NH_4OH . The eluate was concentrated *in vacuo* at 40°C , and the residue was dissolved in 1.0 ml of water. Seven hundred μl of the sample, 100 μl of 10 mM HgCl_2 and 800 μl of distilled water were placed in glass vials. The vials were sealed with rubber septa and chilled on ice-water for 15 min. One hundred μl of NaOCl reagent (5% NaOCl and saturated $\text{NaOH} = 2:1$, v/v) was introduced into the reaction vials by a syringe. The vials were agitated on a Vortex mixer for 30 sec to liberate ethylene. Liberated ethylene was measured by GC. The contents of ACC were defined as nmol of ethylene produced per gram of callus.

The radioactivity of ACC was measured by liberated ethylene.¹⁴⁾ Ethylene was absorbed into mercury per-

chlorate. The absorbed radioactivity was measured by a liquid scintillation counter. Specific incorporation of ^{14}C activity into ACC was defined as dpm per nmol of ACC per 24 hr.

Preparation and assay of enzymes. For preparation of SAMDCase, a callus was homogenized in a glass homogenizer with 1 ml of 10 mM sodium phosphate buffer (pH 7.5) containing 0.1 mM dithiothreitol and 1 mM pyridoxal-5'-phosphate at 4°C . The homogenate was centrifuged at $12,000 \times g$ for 20 min at 0°C . The supernatant fraction was used for the SAMDCase assay.

The SAMDCase was measured by a method modified from that of Montague *et al.*¹⁵ The reaction was started by adding 100 μl of crude enzyme to 10 μl of L-[^{14}C -carboxyl]-S-adenosylmethionine and 10 μl of 5 mM pyridoxal-5'-phosphate. The reaction mixture was placed in a Warburg flask. A 7-mm diameter filter paper disc (Whatman No. 1) was impregnated with 100 μl of 2 N potassium hydroxide and then used to trap the $^{14}\text{CO}_2$ liberated. After incubation at 37°C for 60 min, the reaction was stopped with 0.5 ml of 1 N sulfuric acid, and incubated for an additional 60 min, after which the paper discs were removed, dried, and placed in vials containing 1 ml of distilled water and 10 ml of Aquazol-2. ^{14}C activity was measured by a scintillation counter. The enzyme activity is expressed as $^{14}\text{CO}_2$ nmol per mg of protein per hours.

For the preparation of ACC synthase, a callus was homogenized in a glass homogenizer with 1 ml of 100 mM HEPES buffer (pH 8.5) containing 4 mM dithiothreitol, 0.5 mM pyridoxal-5'-phosphate, and 10 mM EDTA at 4°C . The homogenate was centrifuged $12,000 \times g$ for 20 min at 0°C . The supernatant was dialyzed with 2 mM HEPES buffer (pH 8.5) containing 0.5 mM dithiothreitol and 0.5 μM pyridoxal-5'-phosphate for 12 hr at 4°C . The dialyzed supernatant was used for the ACC synthase assay.

The ACC synthase was measured by a method modified from that of Bollner *et al.*¹⁶ The reaction was started by adding 200 μl of 50 mM HEPES buffer (pH 8.5) containing 600 μM SAM to 500 μl of crude enzyme. After incubation at 30°C for 4 hr, the amounts of ACC produced were measured by the method described above.

Enzyme protein was measured by the method of Lowry *et al.*¹⁷

RESULTS

Changes in the incorporation of L-[3,4- ^{14}C]methionine into polyamines and ACC, and the activities of SAMDCase and ACC synthase in Hiproly barley callus after auxin withdrawal

In Hiproly barley callus after auxin withdrawal, the changes in the incorporation of L-[3,4- ^{14}C]methionine into polyamines and the

FIG. 1. Incorporation of L-[3,4- ^{14}C]Methionine into Polyamines and S-Adenosylmethionine Decarboxylase (SAMDCase) Activity in Hiproly Barley Callus after Auxin Withdrawal.

Calli at various stages were incubated for 24 hr in the presence of L-[3,4- ^{14}C]methionine (1 μCi), and ^{14}C activities of polyamines and the activity of SAMDCase were measured.

○, spermidine; ●, spermine; △, SAMDCase.

FIG. 2. Incorporation of L-[3,4- ^{14}C]Methionine into 1-Aminocyclopropane-1-carboxylic Acid (ACC) and ACC Synthase Activity in Hiproly Barley Callus after Auxin Withdrawal.

Calli at various stages were incubated for 24 hr in the presence of L-[3,4- ^{14}C]methionine (1 μCi), and the ^{14}C activity of ACC and ACC synthase activity were measured.

○, ACC; ●, ACC synthase.

TABLE I. EFFECTS OF 1-AMINOISOBUTYRIC ACID (AIB), AMINOXYACETIC ACID (AOA), AND METHYLGLYOXAL-BIS(GUANYLHYDRAZONE) (MGBG) ON THE POLYAMINE LEVELS OF HIPROLY BARLEY CALLUS

Chemical	Concentration (mM)	Cultivation time					
		7th day		14th day		30th day	
		Spd	Spm	Spd	Spm	Spd	Spm
		nmol/g callus					
Control	—	52.1	13.1	63.8	18.2	79.3	32.0
AIB	1.0	66.2	16.7	69.4	19.5	100.2	26.5
	5.0	67.7	18.9	71.7	22.5	100.6	23.4
AOA	0.5	82.2	25.6	114.9	28.0	90.9	39.7
	1.0	86.2	26.3	105.1	32.9	97.7	43.0
MGBG	1.0	61.4	16.5	59.7	19.3	59.2	20.0
	10.0	66.1	17.9	54.4	15.5	58.3	8.5

Spd, spermidine; Spm, spermine.

activity of SAMDCase are shown in Fig. 1. When the callus was deprived of 2,4-D, specific incorporation of ^{14}C into polyamines and SAMDCase activity in the callus increased greatly, then remained stable. In the absence of 2,4-D, changes in the specific incorporation of ^{14}C into polyamines paralleled changes in the activity of SAMDCase.

Figure 2 shows changes in the incorporation of L-[3,4- ^{14}C]methionine into ACC and the activity of ACC synthase in the callus after auxin withdrawal. The changes in the activity of ACC synthase paralleled the changes in the specific incorporation of ^{14}C activity into ACC. The incorporation of L-[3,4- ^{14}C]methionine into ACC and ACC synthase activity first decreased, then increased.

The results of Figs. 1 and 2 demonstrated that changes in the polyamine biosynthesis are symmetrically related to changes in the ACC production. Hiproly barley callus placed on Murashige and Skoog's medium without 2,4-D formed adventitious roots by the 30th day of cultivation. The symmetrical relationship between polyamines and ACC continued until complete adventitious roots were formed.

Effects of AIB, AOA, and MGBG on changes in the levels of polyamines and ACC in Hiproly barley callus

This experiment was designed to observe the

TABLE II. EFFECTS OF 1-AMINOISOBUTYRIC ACID (AIB), AMINOXYACETIC ACID (AOA), AND METHYLGLYOXAL-BIS(GUANYLHYDRAZONE) (MGBG) ON THE 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) PRODUCTION OF HIPROLY BARLEY CALLUS

Chemical	Concentration (mM)	Cultivation time		
		7th day	14th day	30th day
		ACC nmol/g callus		
Control	—	48.5	35.6	23.2
AIB	1.0	31.5	32.1	24.7
	5.0	47.4	31.7	27.5
AOA	0.5	47.5	5.1	1.7
	1.0	37.3	3.5	1.5
MGBG	1.0	44.6	55.8	45.2
	10.0	50.1	73.6	68.2

effects of AOA, AIB, and MGBG on changes in the levels of polyamines in Hiproly barley callus that had been deprived of 2,4-D for a period of 30 days.

When MGBG was used to inhibit specific steps in the polyamine biosynthesis, spermidine and spermine synthesis were repressed. Table I indicates a strong inhibition of the biosynthesis of spermidine and spermine at a millimolar concentration of MGBG. Levels of spermidine and spermine were reduced by 10 mM MGBG to about 30% and 70%, respectively, of the controls on the 30th day after cultivation.

TABLE III. EFFECTS OF 1-AMINOISOBUTYRIC ACID (AIB), AMINOXYACETIC ACID (AOA), AND METHYLGLYOXAL-BIS(GUANYLHYDRAZONE) (MGBG) ON ^{14}C ACTIVITIES OF SPERMIDINE AND SPERMINE

Chemical	Concentration (mM)	¹⁴ C Activity					
		14th day			30th day		
		Spd	Spm	Total*	Spd	Spm	Total*
dpm/nmol polyamine/24 hr							
Control	—	58.7	30.0	51.5	79.3	32.1	72.4
AIB	1.0	58.7	64.3	54.8	99.5	103.0	65.3
	5.0	72.6	64.7	70.6	124.4	84.4	83.5
AOA	0.5	98.7	95.5	98.1	201.8	167.0	99.7
	1.0	191.0	177.5	188.3	244.4	219.2	201.6
MGBG	5.0	153.3	213.2	165.3	141.2	256.6	88.4
	1.0	79.2	19.3	69.2	68.9	21.3	31.1
	10.0	25.2	27.6	25.5	28.4	1.4	9.0

Calli were incubated for 24 hr in the presence of the indicated concentration of AIB, AOA, or MGBG, and L-[3,4- ^{14}C]methionine (1 μCi), and ^{14}C activities of spermidine (Spd) and spermine (Spm) were measured.

* Contents of spermidine plus spermine.

To find whether polyamine biosynthesis was stimulated by the inhibition of ethylene production, AOA and AIB were added to the culture medium. The effects of AOA and AIB on polyamine levels in the callus are shown in Table I. Compared with those of the control, the levels of spermidine and spermine in the callus rapidly increased after cultivation in the medium containing 1 mM or 0.5 mM AOA. AIB had no significant effect on changes in the levels of spermidine or spermine in the callus.

The effects of AOA, AIB, and MGBG on changes in the levels of ACC in the callus are shown in Table II. The ACC production in the callus was prevented by AOA, but not by AIB. On the other hand, ACC production was greatly enhanced by MGBG. The contents of ACC in the callus increased about 3-fold after treatment with 10 mM MGBG.

Effects of AIB, AOA, and MGBG on the incorporation of L-[3,4- ^{14}C]methionine into polyamines and ACC in Hiproly barley callus

This experiment was designed to observe the changes in the incorporation of methionine into polyamines and ACC in Hiproly barley callus.

TABLE IV. EFFECTS OF 1-AMINOISOBUTYRIC ACID (AIB), AMINOXYACETIC ACID (AOA), AND METHYLGLYOXAL-BIS(GUANYLHYDRAZONE) (MGBG) ON ^{14}C ACTIVITY OF 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC)

Chemical	Concentration (mM)	¹⁴ C Activity	
		14th day	30th day
dpm/nmol ACC/24 hr			
Control	—	19.1	16.4
AIB	1.0	15.6	10.0
	5.0	16.7	9.1
AOA	0.5	13.5	7.0
	1.0	12.0	6.9
MGBG	5.0	7.3	6.6
	1.0	17.8	23.7
	10.0	35.8	35.0

Calli were incubated for 24 hr in the presence of the indicated concentration of AIB, AOA, or MGBG, and L-[3,4- ^{14}C]methionine (1 μCi), and then ^{14}C activity of ACC was measured.

Table III shows the effects of AIB, AOA, and MGBG on the incorporation of L-[3,4- ^{14}C]methionine into polyamines in the callus. The rates of the incorporation of L-[3,4- ^{14}C]methionine into polyamines increased greatly when the callus was transferred to the medium containing AOA. In the presence of

1 mM AOA, specific incorporation of ^{14}C activity into spermidine and spermine increased about 4 and 3-fold respectively, over the control. MGBG strongly inhibited the incorporation of L-[3,4- ^{14}C]methionine into polyamines. On the 30th day after cultivation, specific incorporation of ^{14}C activity into polyamines in the callus treated with 10 mM MGBG had decreased by about 90% of the control.

Table IV shows the effects of AOA, AIB, and MGBG on the incorporation of L-[3,4- ^{14}C]methionine into ACC in the callus. Strong inhibition of the incorporation of L-[3,4- ^{14}C]methionine into ACC was observed at millimolar levels of AOA. Specific incorporation of ^{14}C activity into ACC decreased by about 40% of the control. MGBG stimulated the incorporation of L-[3,4- ^{14}C]methionine into ACC. On the 14th and 30th days after cultivation, specific incorporation of ^{14}C activity into ACC was increased about 2 and 3-fold respectively by 10 mM MGBG. On the other hand, AIB had only a slight effect on the incorporation of methionine into either ACC or into polyamines (Tables III and IV).

Effects of AIB, AOA, and MGBG on the activities of SAMDCase and ACC synthase in Hiproly barley callus

Table V shows the effects of AOA, AIB, and MGBG on the activity of SAMDCase, which is the rate-limiting enzyme in the pathway of polyamine biosynthesis. When AOA was used to inhibit the conversion of SAM to ACC, the activity of SAMDCase in the callus was greatly elevated. On the 14th and 30th days after cultivation, the activity of SAMDCase was increased about 3 and 2-fold respectively by 1 mM AOA, as compared with the control. AIB, which inhibited the conversion of ACC to ethylene, had no significant effect on the activity of SAMDCase. When MGBG was used to inhibit the decarboxylation of SAM, the activity of SAMDCase in the callus was inhibited about 40% by 10 mM MGBG, as compared with the control.

Table VI shows the effects of AOA, AIB,

TABLE V. EFFECTS OF 1-AMINOISOBUTYRIC ACID (AIB), AMINOXYACETIC ACID (AOA), AND METHYLGlyoxal-BIS(GUANYLHYDRAZONE) (MGBG) ON THE ACTIVITY OF S-ADENOSYLMETHIONINE DECARBOXYLASE (SAMDCase) OF HIPROLY BARLEY CALLUS

Chemical	Concentration (mM)	SAMDCase activity	
		14th day	30th day
$^{14}\text{CO}_2$ nmol/mg protein/hr			
Control	—	64.2	89.7
AIB	1.0	73.9	92.2
	5.0	67.4	112.9
AOA	0.5	106.6	130.7
	1.0	162.0	169.6
MGBG	1.0	69.8	61.4
	10.0	58.0	52.6

TABLE VI. EFFECTS OF 1-AMINOISOBUTYRIC ACID (AIB), AMINOXYACETIC ACID (AOA), AND METHYLGlyoxal-BIS(GUANYLHYDRAZONE) (MGBG) ON THE ACTIVITY OF 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE OF HIPROLY BARLEY CALLUS

Chemical	Concentration (mM)	ACC synthase activity	
		14th day	30th day
ACC nmol/mg protein/hr			
Control	—	11.7	7.6
AIB	1.0	14.3	6.7
	5.0	13.2	6.7
AOA	0.5	9.2	5.7
	1.0	7.8	5.2
MGBG	1.0	15.6	8.5
	10.0	26.0	18.8

and MGBG on the activity of ACC synthase, which is the rate-limiting enzyme in the pathway of ethylene production. The activity of ACC synthase was inhibited by AOA, but not by AIB. On the other hand, when the callus was transferred to the medium with MGBG, ACC synthase activity in the callus increased greatly. On the 14th and 30th days after cultivation, elevated activity of ACC synthase by 10 mM MGBG was about 2-fold, as compared with the control.

DISCUSSION

Giovanelli *et al.*¹⁸⁾ have reported that in

Lemna paucicostata Hegelm, the biosynthesis of SAM is the major pathway for methionine metabolism. Adams and Yang⁸⁾ and Boller *et al.*¹⁶⁾ demonstrated that ACC was produced from SAM, and was an immediate precursor of ethylene. Moreover, SAM serves as a source of aminopropyl moieties for the biosynthesis of spermidine and spermine, as well as a substrate for the production of ACC.³⁾

In many plant tissues,^{8~10,16,18)} ethylene biosynthesis is thought to be processed by the conversion of methionine to SAM and then to ACC. Moreover, polyamine biosynthesis is processed by the decarboxylation of SAM, and incorporation of aminopropyl moieties into polyamines.

With regard to Hiproly barley callus after auxin withdrawal, our results indicated that L-[3,4-¹⁴C]methionine was incorporated into both ACC and polyamines, and that the changes in the polyamine levels were symmetrically related to the changes in the contents of ACC. Furthermore, the contents of ACC decreased when the levels of polyamines in the callus increased, while elevated ACC production induced to decrease the levels of polyamines. Thus, these two compounds are produced by the conversion of methionine to SAM as in other plant tissues, and the biosynthesis of polyamines can be thought to be closely related to the regulation of ACC production.

In Hiproly barley callus, the addition of AOA induced a marked increase in the levels of spermidine and spermine. The incorporation of L-[3,4-¹⁴C]methionine into polyamines increased greatly in the callus treated with AOA. Moreover, the activity of SAMDCase, deciding the fate of which SAM is committed to the polyamine pathway, was strongly stimulated *in vivo* by AOA. On the other hand, MGBG, which is an effective inhibitor of SAMDCase, had a significant effect on the biosynthesis of ACC. The incorporation of L-[3,4-¹⁴C]methionine into ACC and the activity of ACC synthase were

greatly elevated by MGBG. These results suggest that the restriction of ACC production is pivotal in the mechanism leading to elevated polyamine levels in the callus. This hypothesis is supported by the results whereby the addition of AIB, which inhibits ethylene evolution by blocking the conversion of ACC to ethylene, had no significant effect on the biosynthesis of polyamines and ACC. Therefore, in the case of Hiproly barley callus, the biosynthesis of polyamines is thought to be regulated by ethylene, and to inhibit ethylene formation.

REFERENCES

- 1) T. A. Smith, "The Biochemistry of Plants, Secondary Plant Products," Vol. 7, ed. by E. E. Conn, Academic Press, New York, 1980, pp. 249~268.
- 2) Y. M. Heimer, Y. Misraki and U. Bachrach, *FEBS Lett.*, **104**, 146 (1979).
- 3) S. S. Cohen, R. Balint and R. K. Sindhu, *Plant Physiol.*, **68**, 1150 (1981).
- 4) A. Apelbaum, A.-C. Burgoon, J. D. Anderson, M. Lieberman, R. Ben-Arie and A. K. Mattoo, *Plant Physiol.*, **68**, 453 (1981).
- 5) J. C. Suttle, *Phytochemistry*, **20**, 1477 (1981).
- 6) D. R. Roberts, M. A. Walker, J. E. Thompson and E. B. Dubroff, *Plant Cell Physiol.*, **25**, 315 (1984).
- 7) Z. Even-Chen, A. K. Mattoo and R. Goren, *Plant Physiol.*, **69**, 385 (1982).
- 8) D. O. Adams and S. F. Yang, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 170 (1979).
- 9) L. Lürssen, K. Nauman and R. Schröden, *Z. Pflanzenphysiol.*, **92**, 285 (1975).
- 10) N. E. Hoffman and S. F. Yang, *J. Am. Soc. Hortic. Sci.*, **105**, 492 (1980).
- 11) Y. Katoh, T. Hasegawa, T. Suzuki and T. Fujii, *Agric. Biol. Chem.*, **48**, 1405 (1984).
- 12) J. W. Redmond and A. Tseng, *J. Chromatogr.*, **170**, 479 (1979).
- 13) C. C. Lizada and S. F. Yang, *Ann. Biochem.*, **100**, 140 (1979).
- 14) R. E. Young, H. K. Pratt and J. B. Biale, *Anal. Chem.*, **24**, 551 (1952).
- 15) M. J. Montague, J. W. Koppenbrink and E. G. Jaworski, *Plant Physiol.*, **62**, 430 (1978).
- 16) T. Boller, R. C. Herner and H. Kende, *Planta*, **145**, 293 (1979).
- 17) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
- 18) J. Giovanelli, S. H. Mudd and A. H. Datko, *Plant Physiol.*, **78**, 555 (1985).