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Involvement of Spermine in Control of Ethylene-Mediated Growth Response in *Ranunculus sceleratus* Petioles

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Ranunculus sceleratus 엽병의 에틸렌 매개 생장반응조절에 있어서 Spermine의 관여

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ABSTRACT

Cell elongation is known to be promoted by ethylene in petioles of *Ranunculus sceleratus*. Treatment of petiole segments with spermine resulted in an inhibition of cell elongation and of ethylene biosynthesis in the presence of applied auxin. Dose response curve for the spermine inhibition of auxin-induced ethylene production appeared similar to that of ACC-based ethylene production suggesting that the polyamine inhibits ethylene biosynthesis by blocking the conversion of ACC to etylene. Auxin-induced ethylene production was significantly promoted by treatment of the tissue with either DFMA or DFMO, specific inhibitors of polyamine biosynthesis. Increased level of ethylene production by DFMA was found to be completely abolished by application of exogenous spermine at a high concentration. These results indicate that endogenous spermine plays a regulatory role in the growth response of *Ranunculus* petioles to auxin and ethylene.

INTRODUCTION

In petioles of the semi-aquatic plant *Ranunculus sceleratus*, ethylene is known to promote cell elongation (Musgrave and Walters, 1973; Horton, 1987). The ethylene effect in the petiole segments is thought to be manifested by increased sensitivity of this tissue to auxin; auxin dose response curve for ethylene-treated segments is shifted to the left compared with air control (Nam, 1987; Kang *et al.*, 1992).

In view of the well-documented interactions between hormones and polyamines in a variety of plant processes (Smith, 1985; Evans and Malmberg, 1989), the possibility of polyamines playing a regulatory role in the control of growth reponse in *Ranunculus* petioles was explored. In the present work, our experimental results are presented to indicate that polyamines inhibit auxin-induced ethylene production and thus consequently ethylene-mediated cell growth, and that endogenous polyamines may be involved in the growth control of this tissue.

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MATERIALS AND METHODS

Plant material. Locally collected seeds of *Ranunculus sceleratus* L. were germinated and grown in a pot placed in a pan of water in a greenhouse. Young leaf petioles about 3 cm long were excised from 6 to 8 week-old plants, and 1 cm segments isolated from these petioles were used in all experiments.

Incubation of tissue segments. Ten petiole segments were incubated in 1 ml of 50 mM K-phosphate buffer at pH 6.8 containing 2% sucrose and test substances in a 25 ml Erlenmeyer flask for 20 or 23 h at 26°C in total darkness. For measurements of ethylene production, each flask was sealed with a silicone stopper, and 1 ml gas sample was withdrawn from the flask with a hypodermic syringe at the end of the incubation period. Ethylene in the gas sample was determined with a gas chromatograph (Shimadzu, GC-3BF). For growth measurements, increase in length of each segment was measured under a dissecting microscope at the end of the incubation period.

Chemicals. Indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC), aminooxyacetic acid (AOA), spermine, and other fine chemicals were purchased from Sigma (St. Louis, MO, USA). The inhibitors of polyamine biosynthesis, α-difluoromethylarginine (DFMA) and α-difluoromelthylornithine (DFMO) were kindly supplied by Dr. E. H. W. Bohme of Merrell Dow Research Institute, Cincinnati, OH, USA.

RESULTS AND DUSCUSSION

Polyamines are naturally occuring polycationic substances that regulate a variety of cellular processes. In plants, polyamines are mainly known to be associated with developmental processes involving cell division (Walker *et al.*, 1985) and differentiation (Feirer *et al.*, 1984; Jarvis *et al.*, 1985) However, in one instance reported for anaerobic elongation of rice coleoptiles, exogenously applied putrescine increased cell elongation and an increase in the titer of putrescien was closely correlated with coleoptile elongation (Regginani *et al.*, 1989). Growth of rice coleoptiles (Metraux and Kende, 1984; Raskin and Kende, 1984) and that of *Ranunculus* petioles (Horton, 1987) have one common characteristic feature in that cell elongation is rather promoted by ethylene, in contrast with most other actively growing tissues. Data presented in Fig. 1 indicate that externally applied spermine strongly inhibited growth of *Ranunculus* petioles in the presence of auxin. Spermidine and putrescine also had similar effects to inhibit cell elongation in this tissue (data not shown).

Treatment of the petiole segments with various concentrations (0.3 to 30 mM) of spermine likewise resulted

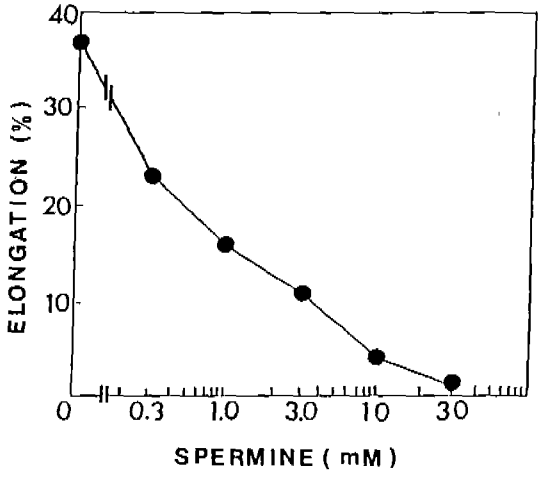

Fig. 1. Effect of spermine on cell elongation in *Ranunculus* petioles. Isolated segments were incubated in a medium containing 0.1 mM IAA and various concentrations of spermine for 20 h. Growth is expressed as percent elongation.

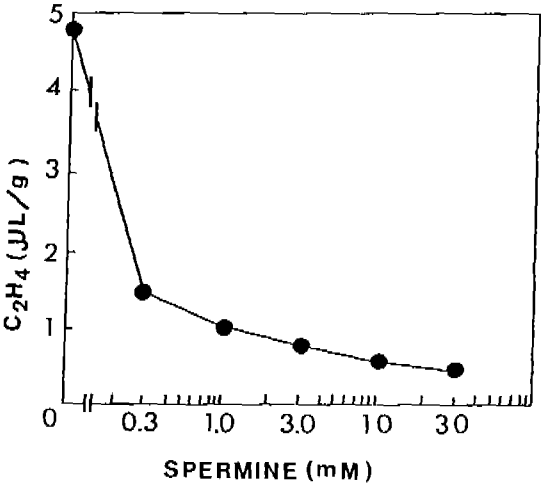

Fig. 2. Effect of spermine on IAA-induced ethylene production from petioles. Isolated segments were incubated in a medium containing 0.1 mM IAA and various concentrations of spermine for 20 h. Ethylene produced from the tissue was determined at the end of the incubation period.

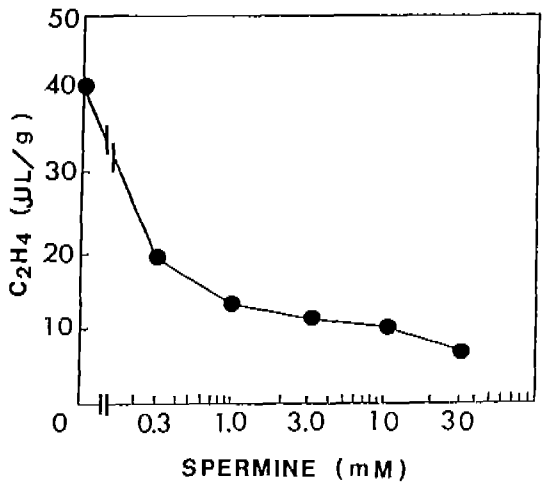

Fig. 3. Effect of spermine on ACC-based ethylene production. Isolated tissue segments were incubated in a medium containing 10 μM ACC, 10 μM AOA and various concentrations of spermine. Ethylene produced from the tissue was determined at the end of the incubation period.

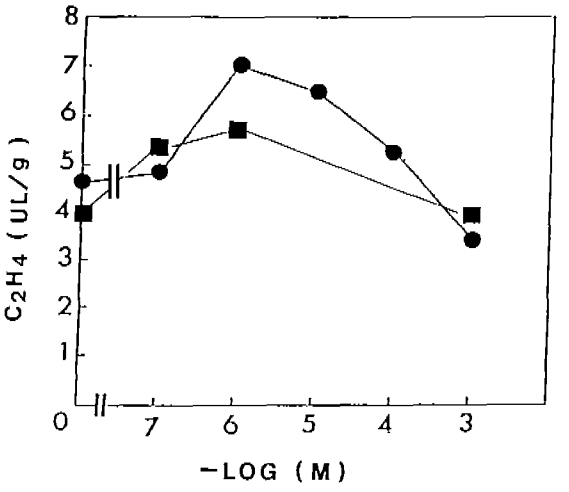

Fig. 4. Effects of DFMA (circles) and DFMO (squares) on auxin-induced ethylene production. Isolated tissue segments were incubated in a medium containing 0.1 mM IAA and various concentrations of DFMA and DFMO, respectively. Ethylene produced from the tissue was determined at the end of a 23 h incubation period.

in an inhibition of auxin-induced ethylene production as shown in Fig. 2. In view of the potential metabolic connection between polyamine and ethylene biosynthesis through S-adenosylmethionine (SAM) as a common intermediate, possible physiological interactions between the two classes of endogenous regulators have been investigated, and also biosynthesis of one of the two classes as affected by the other have been studied extensively by many investigators. For instance senescence which is typically promoted by ethylene (Yang, 1985) is known to be retarded by polyamines (Galston and K-Sawhney, 1987). Moreover, polyamines inhibit ethylene formation in a number of plant tissues (Appelbaum *et al.*, 1981; Suttle 1981), and conversely ethylene inhibits polyamine biosynthesis (Icekson *et al.*, 1985; Icekson *et al.*, 1986). In the *Ranunculus* petiole, it is apparent that polyamines and ethylene have opposing effects on cell elongation, and that polyamines interfere with ethylene biosynthesis.

Spermine was also found to inhibit ACC-based ethylene production in this tissue, the concentration dependency being almost identical to that for auxin-induced ethylene production (Fig. 3). In these experiments, to avoid the possible interference of endogenous ACC, AOA was added to the incubation medium to suppress cellular activity of ACC synthase, and thus the ethylene produced from these segments could reflect *in vivo* activity of "ethylene forming enzyme (EFE)" (Lizada and Yang, 1979; Hoffman *et al.*, 1982). In view of the well-established pathway of ethylene biosynthesis from methionine via SAM and ACC (Adams and Yang, 1979), it can be concluded from the data presented in Figs. 2 and 3 that the polylamine inhibits ethylene production by blocking the terminal step in the sequence of reactions leading to the formation of ethylene i.e., the conversion of ACC to ethylene.

There are two major approaches that can be deployed to investigate whether or not endogenous polyamines play any role in the control of a particular response where external application of polyamines exerts an effect. One plausible approach would be to determine changes in the cellular polyamine level associated with the particular physiological progress. Another approach, rather indirect, would be to study cellular changes in response to agents which specifically block biosynthesis of the physiologically active substances.

For the second approach mentioned above, two specific inhibitors of polyamine biosynthesis, namely DFMA and DFMO, specific enzyme-activated irreversible inhibitors of arginine decarboxylase and ornithine decarboxylase, respectivey, (Birecka *et al.*, 1988) were applied to see if auxin-induced ethylene production would be affected. Fig. 4 shows data which clearly indicate that auxin-indu-

Table 1. Effects of DFMA on IAA-induced cell elongation and ethylene production and their reversal by exogenous spermine in *Ranunculus* petiole segments

Treatments		Cell elongation	Ethylene production
DFMA	Spermine	(% elongation)	(μl/g fr.wt 20 h)
0	0	28	3.37
1 μM	0	34	7.81
0	30 mM	2.5	1.25
1 μM	30 mM	3.0	1.23

ced ethylene production was significantly increased by DFMA at concentrations upto 0.1 mM. Likewise, treatment of the tissue with DFMO also resulted in increases in auxin-induced ethylene production. These results indicate that reduced polyamine levels in the tissue lead to increased ethylene productionin, and strongly implicate the endogenous polyamine level as an important parameter to control the rate of cellular ethylene production.

One important criterion in evaluating data obtained from this kind of inhibitor studies concerns with a substantiation that the inhibitor effect would be abolished by exogenous application of the substance concerned at a saturating concentration. Data presented in Table 1 indeed confirm this substantiation; both auxin-induced ethylene production and cell elongation were promoted by DFMA treatment, and the promotion in both parameters was completely nullified by external application of spermine at 30 mM. These results strongly imply involvement of endogenous polyamines as an important regulatory agent in the control of ethylene biosynthesis and thus consequently growth manifestation of this aquatic tissue.

적 요

Ranunculs sceleratus 엽병의 세포 신장은 에틸렌에 의하여 촉진되는 것으로 알려져 있다. 오옥신을 처리한 엽병조직 절편에서 spermine은 세포 신장과 에틸렌의 생성을 비슷한 양상으로 억제하였다. Spermine 농도에 대한 오옥신 유도 에틸렌 생성 억제반응은 ACC에 의한 에틸렌 생성의 경우도 유사한 양상을 나타내었으며 이는 폴리아민이 ACC가 에틸렌으로 전환되는 과정을 억제한다는 것을 시사한다. 오옥신 유도 에틸렌 생성은 폴리아민 생합성 억제제인 DFMA와 DFMO에 의하여 각각 현저하게 촉진되었으며 DFMA에 의한 에틸렌 생성의 증가는 spermine을 고농도로 처리하므로써 완전히 소멸되는 결과를 얻었다. 이러한 결과들은 오옥신과 에틸렌에 대한 *Ranunculus*의 세포성장 반응에서 내생 폴리아민이 조절 역할을 수행한다는 가능성을 입증하는 것이다.

REFERENCES

Adams, D.O. and S.F. Yang. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the coversion of methionine to ethylene. *Proc. Natl. Acad. Sci. USA* **76**: 170-174.

Apelbaum, A., A.C. Burgoon, J.D. Anderson, M. Lieberman, R. Ben-Arrie, and A.K. Mattoo. 1981. Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplasts. *Plant Physiol.* **68**: 453-456.

Birecka, H., M. Birecki, E.J. Cohen, A.J. Bitonti, and P.P. McCann. 1988. Ornithine decarboxylase, polyamines, and pyrrolizidine alkaloids in *Senecio* and *Crotalaria*. *Plant Physiol.* **86**: 224-230.

Evans, P.T. and R.L. Malmberg. 1989. Do polyamines have roles in plant development? *Annu. Rev. Plant Physiol.* **40**: 235-269.

Feirer, R., G. Mignon, J. Litvay. 1984. Arginine decarboxylase and polyamines required for embryogenesis in the wild carrot. *Science* **223**: 1433-1435.

Galston, A.W. and R. K. Sawhney. 1987. Polyamines and senescence in plants. *In*, Plant Senescence: Its Biochemistry and Physiology. pp. 167-181. Thomson, W.W., Nothnagel, E.A., Huffaker, R.C. eds. The American Society of Plant Physiologists.

Hoffman, N.E., S.F. Yang, A. Ichihara, and S. Sakamura. 1982. Stereospecific conversion of 1-aminocyclopropanecarboxylic acid to ethylene by plant tissues. *Plant Physiol.* **70**: 195-199.

Horton, R. 1987. Ethylene-induced growth in amphibious plants. *In*, Plant Hormone Receptprs. pp. 249-256. Klaembt, D. ed. Springer-Verlag, Berlin.

Icekson, I., M. Bakhanashvili, and A. Apelbaum. 1986. Inhibition by ethylene of polyamine biosynthetic enzymes enhanced lysine decarboxylase activity and cadaverine accumulation in pea seedlings. *Plant Physiol.* **82**: 607-609.

Icekson, I., A. Goldlust, and A. Apelbaum. 1985. Influence of ethylene on s-adenosylmethionine decarboxylase activity in etiolated pea seedlings. *J. Plant Physiol.* **119**: 335-346.

Jarvis, B.C., S. Yasmin, M. T. Coleman. 1985. RNA and protein metabolism during adventitious root formation in stem cuttings of *Phaseolus aureus* cultivar berkin. *Physiol. Plant.* **64**: 53-59.

Kang, B.G., W.J. Park, M.H. Nam, and R. Hertel. 1992. Ethylene-induced increase of sensitivity to auxin in *Ranun-*

culus petioles and its implications regarding ethylene action on adaptation. *In*, Progress in Plant Growth Regulation, eds. Karssen, C., L. van Loon, and D. Vreudenhil. pp. 248-253. Kluwer Acad. Pub. Netherlands.

Lizada, M.C.C. and S.F. Yang. 1979. A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. *Anal. Biochem.* **100**: 140-145.

Metraux, J.-P. and H. Kende. 1984. The cellular basis of the elongation response in submerged deep-water rice. *Planta* **160**: 73-77.

Musgrave, A. and J. Walters. 1973. Ethylene-stimulated growth and auxin transport in *Ranunculus sceleratus* petioles. *New Phytol.* **72**: 783-789.

Nam, M.H. 1989. Relationship between ethylene promoted petiole elongation and auxin transport. Thesis, Yonsei University.

Raskin, I. and H. Kende. 1984. Regulation of growth in stem sections of deep-water rice. *Planta* **160**: 66-72.

Reggiani, R., A. Hochkoeppler, and A. Bertani. 1989. Polyamines and anaerobic elongation of rice coeloptiles. *Plant Cell Physiol.* **30**: 893-898.

Smith, T.A. 1985. Polyamines. *Annu. Rev. Plant Physiol.* **36**: 117-143.

Suttle, J.C. 1981. Effect of polyamines on ethylene production. *Phytochemistry* **29**: 1477-1478.

Walker, M.A., D.R. Roberts, C.Y. Shih, and E.B. Dumbroff. 1985. A requirement for polyamines during the cell division phase of radicle emergence in seeds of *Acer sacchrum*. *Plant Cell Physiol.* **26**: 967-972.

Yang, S.F. 1985. Biosynthesis and action of ethylene. *Hort Science* **20**: 41-45.

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