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Kinetic Analyses of Spermine Effects on Petiole Elongation in *Ranunculus sceleratus*

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Possible roles of polyamines in the inhibition of cell elongation in *Ranunculus* petioles were investigated. Exogenously applied polyamines greatly inhibited the auxin-induced petiole growth, while treatment of the tissue with α-difluoromethylarginine, the inhibitor of putrescine biosynthesis, further enhanced the growth in the presence of IAA. Inhibitory effect of spermine can also be apparent for fusicoccin-induced elongation, but not for growth induced by a low pH. Spermine also suppressed the ethylene-enhanced growth in the presence of auxin. Using computer-based video digitizer system, the inhibitory effects of spermine on petiole growth were kinetically analyzed. Auxin-induced growth was characterized by an initial and transient growth with a highly elevated rate followed by a steady growth with a slightly reduced rate. Spermine treatment was found to shorten the duration of the initial phase of growth, and to reduce the rates of both the initial and steady growth as well. The latent period for auxin induction was not affected by spermine.

Keywords: petiole elongation, spermine, ethylene, auxin induction, *Ranunculus sceleratus*

Polyamines are important modulators of many biological processes such as cell division, cellular differentiation, and stress responses in plants (Smith, 1985; Altman, 1989; Galston and Kaur-Sawhney, 1990). Polyamines also have been suggested to be involved in the control of cell elongation. Polyamine contents and activities of polyamine biosynthetic enzymes were positively correlated to the growth rates in corn coleoptiles (Dumortier *et al.*, 1983), pea epicotyls (Dai and Galston, 1981; Goren *et al.*, 1982) and deep-water rice internodes (Cohen and Kende, 1986).

In petioles of the semi-aquatic plant *Ranunculus sceleratus*, ethylene was known to promote cell elongation (Musgrave and Walters, 1973). Ethylene-enhanced growth was responsible for the response of rice plant to survive under oxygen-restricted environments (Raskin and Kende, 1983). Accumulation of putrescine was suggested to be involved in the regulation of ethylene-induced elongation of internodes (Cohen and Kende, 1986) and coleoptiles (Reggiani *et al.*, 1989; Lee and Chu, 1992) of rice. However, effects of exogenously applied polyamines were controversial. Putrescine had no effect of aerobic elongation of rice coleoptiles (Lee and Chu, 1992), while it stimulated their elongation under anaerobic conditions (Reggiani *et al.*, 1989).

We previously reported that spermine inhibited the elongation of *Ranunculus* petiole segments in relation to its effect on ethylene biosynthesis (Jeong *et al.*, 1992). The inhibitory effects of spermine on the petiole elongation were further characterized in view of the possibility of polyamines as an important regulator of growth in *Ranunculus* petioles.

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

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plants, and 1 cm or 1.5 cm segments isolated from these petioles were used for all experiments as indicated.

Chemicals

Indole-3-acetic acid (IAA), aminoethoxyvinylglycine (AVG), fusicoccin and other fine chemicals were purchased from Sigma (St. Louis, MO, USA). Three major polyamines (putrescine, spermidine, spermine) and methylglyoxal bis-guanyhyldrazone (MGBG) were also obtained from Sigma. The inhibitors of polyamine biosynthesis, α-difluoromethylarginine (DFMA) and α-difluoromethylornithine (DFMO) were kindly supplied by Dr. E. H. W. Bohme of Merrel Dow Research Institute, Cincinnati, OH, USA.

Long-term growth measurements

Ten petiole segments were preincubated for 4 h in distilled water followed by incubation in 10 mL of medium [2% sucrose buffered with 10 mM 2-[N-morpholino]ethanesulfonic acid (Mes) at pH 6.8] with test chemicals where indicated. After 18 h incubation at 28℃, increase in length of each segment was measured under a dissecting microscope.

Short-term growth kinetics

Growth kinetics of single segment was measured with a computer-based video digitizer system (Video Van Gogh, Tecmar, Inc., Cleveland, OH, USA) as described by Nelson and Evans (1986). A 15-mm petiole segment positioned vertically in a lucite chamber was incubated in a medium (10 mM Mes, pH 6.8) at 28℃ with constant aeration. After preincubation of the segment for 60~90 min where the endogenous growth rate reached a steady state, test chemicals were added to the medium and growth changes were monitored with video camera and recorded into an IBM computer.

RESULTS AND DISCUSSION

In *Ranunculus sceleratus*, ethylene has been known to promote petiole growth (Horton, 1987) in contrast with other plant tissues. Kang *et al.* (1992) suggested that such characteristics may be attributable to an increased sensitivity of the tissue to auxin by ethylene. However, ethylene-induced polyamine accumulation was parallel to the ethylene-induced growth in rice (Lee and Chu, 1992), implicating that endogenous polyamines could mediate the ethylene-enhanced cell elongation. To the contrary, we found that exogenously applied spermine rather inhibited growth of *Ranunculus* petioles. This may, in part, be due to the suppressed ethylene biosynthesis by spermine treatment (Jeong *et al.*, 1992). To clarify this confusion, we investigated the growth inhibitory effects of polyamines in the presence of AVG, a strong inhibitor of ethylene biosynthesis. Data presented in Fig. 1 indicate that exogenously applied polyamines strongly inhibited the auxin-induced petiole elongation. We could detect the inhibitory effect of polyamines at 0.3 mM. Among three major polyamines, spermine showed the strongest inhibition of petiole growth. In Fig. 2, the spermine effect was characterized in relation to IAA dose-response curve. Spermine could not shift the dose-response curve to high auxin concentrations, but reduce the magnitude of the maximum response to auxin. It has been reported that exogenous polyamines, espe-

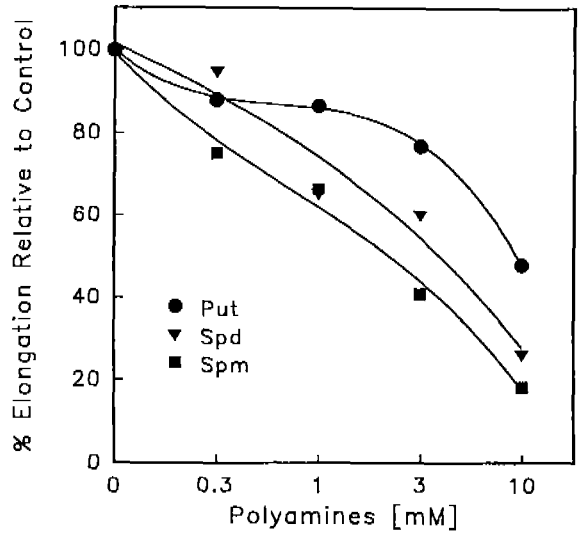

Fig. 1. Effect of polyamines on cell elongation in *Ranunculus* petioles. Isolated segments were incubated in a medium containing 10^{-4} M IAA and various concentrations of polyamines for 18 h following 4 h preincubation in distilled water. To prevent ethylene evolution, 3×10^{-6} M AVG was added to the medium. Growth is expressed as percent of the control.

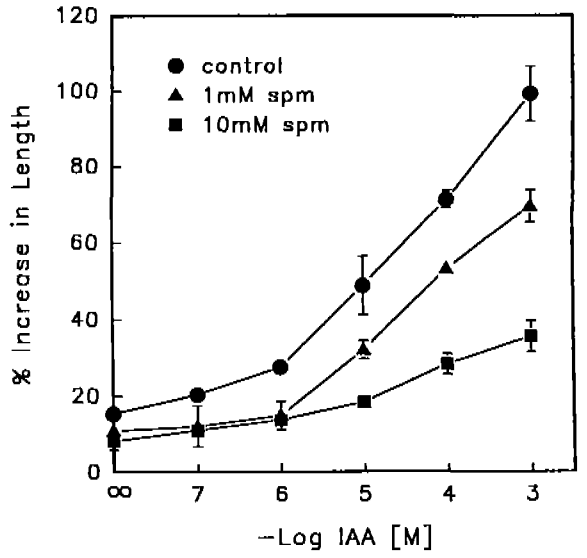

Fig. 2. Effect of spermine on IAA-induced cell elongation in *Ranunculus* petioles. Isolated segments were incubated in a medium containing 1 and 10 mM spermine and various concentrations of IAA for 18 h following 4 h preincubation in distilled water. To prevent ethylene evolution, 3×10^{-6} M AVG was added to the medium. Growth is expressed as percent elongation and vertical bars represent SE of the mean values from 4 separate experiments.

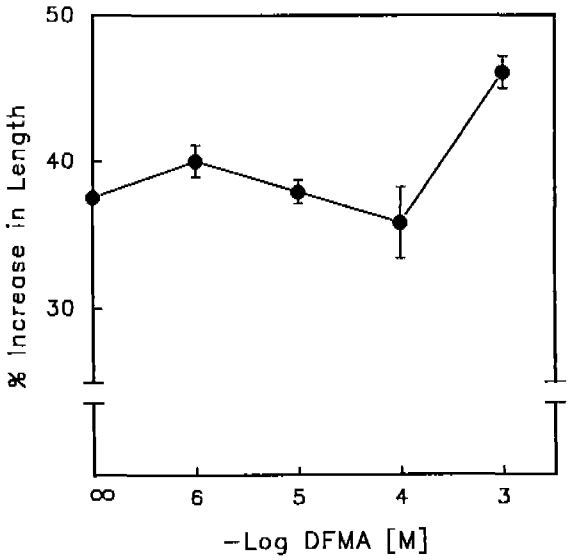

Fig. 3. Effect of DFMA on petiole elongation. Isolated segments were incubated in a mediun containing 10^{-5} M IAA and various concentrations of DFMA for 18 h following 4 h preincubation in distilled water. Growth is expressed as percent elongation and vertical bars represent SE of the mean values from 4 separate experiments.

cially spermine and spermidine, can be bound to cell wall compartment in mungbean hypocotyls (Goldberg and Pedrizet, 1984) and carrot cell culture (Pistocchi *et al.*, 1988). However, it is not likely that the inhibitory effects of exogenously applied polyamines at rather high concentrations are simply due to a restraint of the cell wall extensibility. When the antagonist of arginine decarboxylase, DFMA, was added to incubation medium to inhibit polyamine biosynthesis, the auxin-induced petiole elongation was further stimulated at 1 mM DFMA (Fig. 3). This implicates that endogenous polyamine may be involved in the control of petiole growth. Unlike DFMA, DFMO and MGBG which act on ornithine decarboxylase and S-adenosylmethionine decarboxylase, respectively, had no effect at all (data not shown). In *Ranunculus* petioles, DFMA treatment reduced putrescine content while DFMO and MGBG did not change the putrescine level (Chang and Kang, in preparation). This may explain the lack of effects of these compounds on the auxin-induced petiole elongation. Putrescine biosynthesis was suggested to be involved in the elongation of rice (Lee and Chu, 1992).

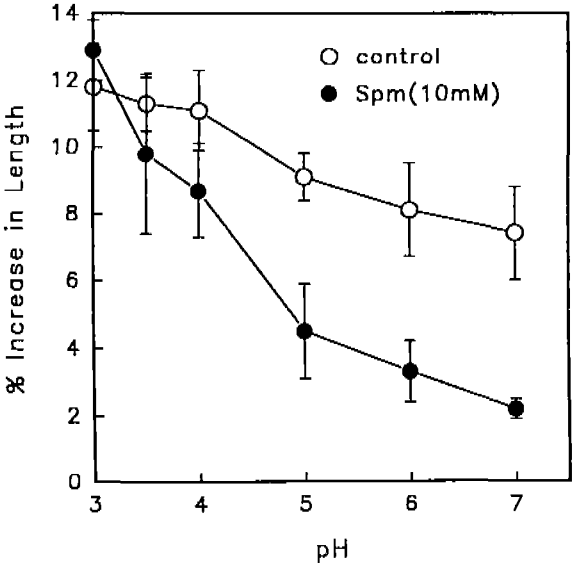

Fig. 4. Effect of spermine on cell elongation at various pH in the presence or absence of 10 mM spermine. Isolated segments were incubated in buffers for 8 h. Growth is expressed as percent elongation and vertical bars represent SE of the mean values from 4 separate experiments.

With regard to the growth-inhibitory effect of polyamines, we found that spermine did not inhibit the acid-induced growth (Fig. 4). With decreasing pH, where acid-mediated wall loosening becomes domi-

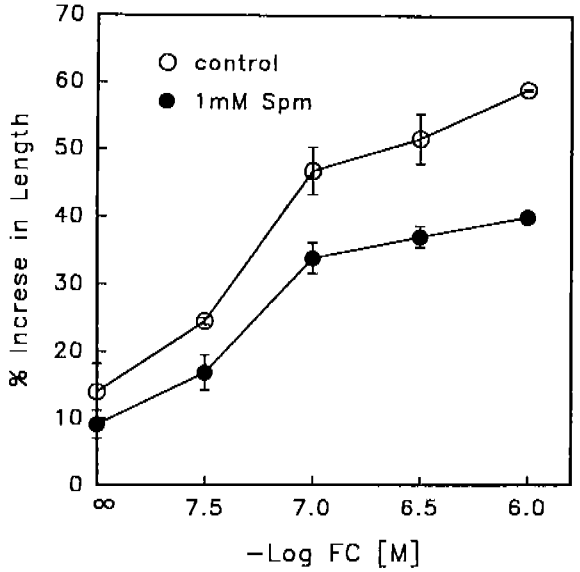

Fig. 5. Effect of spermine on fusicoccin-induced *Ranunculus* petiole elongation. Preincubation and incubation were carried out as described for Fig. 1, except that medium contained various concentrations of fusicoccin in the presence or absence of 1 mM spermine. Growth is expressed as percent elongation and vertical bars represent of the SE of mean values from 3 separate experiments.

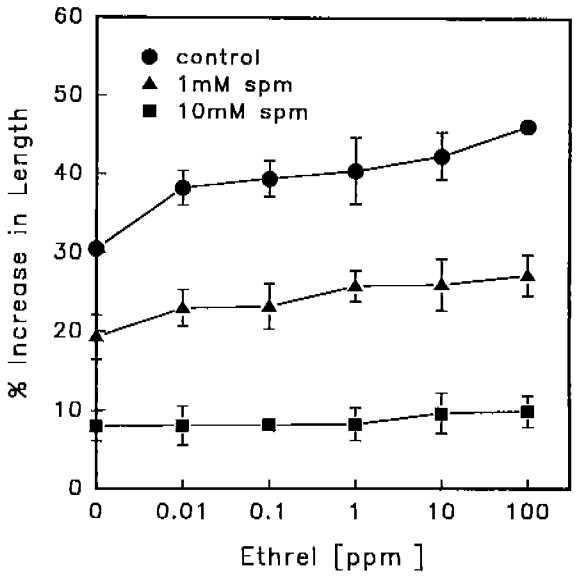

Fig. 6. Effect of spermine on IAA-dependent and ethylene-induced petiole elongation in *Ranunculus* petioles. Ethrel (0.01 to 100 ppm) was added to the medium containing 3×10^{-6} M IAA. Preincubation and incubation were carried out as described for Fig. 1. Growth is expressed as percent elongation and vertical bars represent SE of the mean values from 4 separate experiments.

nating, the spermine effect was also decreased. This result might support the notion that the polyamine effects were not simply due to their cationic property, or merely resulted from reduced cellular uptake of the positively charged compound having the amine groups. However, inhibitory effect of spermine was apparent for fusicoccin-induced growth (Fig. 5). Since fusicoccin was known to cause H^+ extrusion to the greater extent than IAA at the same concentration (Cleland, 1976), it is possible that the spermine effect was decreased due to medium acidification.

Ethylene-enhaced growth of *Ranuncunlus* petioles was known to be dependent on the presence of auxin (Samarakoon and Horton, 1984). In the presence of IAA and AVG, 2-chloroethylphosphoric acid (ethrel) was added to the medium. This compound is known to generate ethylene by decomposition after being taken up to the plant tissue (Warrer and Leopold, 1969). With increasing ethrel concentrations, the auxin-induced growth was further stimulated (Fig. 6). In the presence of 10 mM spermine, the stimulatory effect of ethrel was completely abolished (Fig. 6). Therefore, we can discriminate the inhibitory effect of spermine on auxin-induced growth and that on ethylene-induced growth of *Ranunculus* petioles. However, the possibility that spermine acts on a common site in both cases cannot be ruled

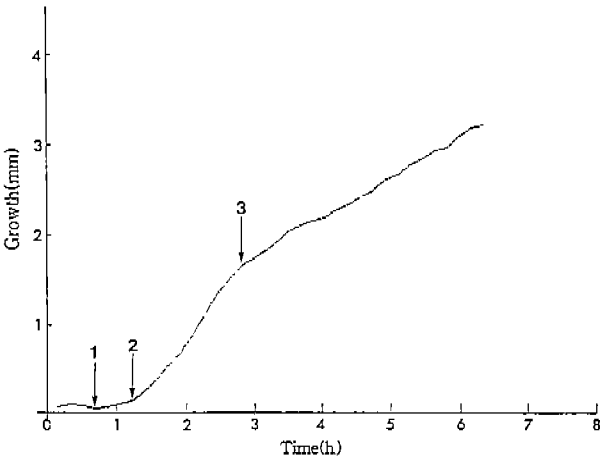

Fig. 7. Typical time course curve of growth response to IAA (10^{-4} M). The arrow 1 indicates the time of auxin application. The period between the arrows 1 and 2 represents the latent period for auxin induction, and that between the arrows 2 and 3 characterizes the initial growth rate. The period following the arrow 3 depicts the steady growth rate.

Table 1. Effect of spermine on IAA-induced petiole elongation

Treatment		Kinetic parameters			
IAA	Sperminc	Latent period	Duration of initial growth rate	Initial growth rate	Steady growth rate
		(min)	(min)	(μm/min)	
3×10^{-6} M	−	46.1 ± 15.9	84.5 ± 26.8	10.3 ± 2.8	6.0± 1.4
	+	35.2 ± 5.5	60.4 ± 24.6	4.2 ± 0.9	2.8± 1.8
1×10^{-4} M	−	20.4 ± 4.7	151.2 ± 18.2	11.6 ± 2.0	9.4± 1.3
	+	18.4 ± 4.2	104.8 ± 10.3	9.2 ± 2.4	3.4± 1.5

Various concentrations of IAA and spermine (10 mM) were applied to *Ranunculus* petiole segments (1.5 cm) at time zero. AVG (10^{-6} M) was added to the medium to block ethylene biosynthesis. Data are presented as average values from at least 6 to 14 separate experiments with SD.

out since the ethylene effect in *Ranunculus* petioles is auxin-dependent as previously mentioned.

Using computer-based video digitizer system, kinetic studies of spermine effect on auxin-induced growth was carried out. Fig. 7 illustrates a typical growth kinetics of single petiole segment in response to IAA. Auxin-induced growth was characterized by an initial and transient growth with an elevated rate followed by a steady growth with a somewhat reduced rate. Spermine treatment was found to shorten the duration of the initial phase of growth and to reduce the rates of both initial and steady growth as well (Table 1). The latent period for auxin induction was not significantly affected by spermine (Table 1).

It is difficult to interpretate the *in vivo* effect of exogenously applied polyamines. The uptake of polyamines is slow and has many obstacles such as cell wall and cuticles (Goldberg and Pedrizet, 1984), whereas their concentrations in the cell are highly regulated (Heby and Persson, 1990). The general inhibitory effect of spermine at high concentrations (Figs. 1, 2, 5, 6) on petiole growth and the lack of effects of DFMO and MGBG may reflect these problems. The growth inhibition by 10 mM spermine may, in part, be due to nonspecific effect on cell wall extensibility. However, we could detect the inhibitory effects of polyamines at 0.3 mM (Fig. 1), and DFMA increased the petiole growth (Fig. 3). This stomgly suggests that endogenous polyamine negatively control the petiole growth in *Ranunculus sceleratus*, in contrast to rice coleoptiles. In spite of the slow and limited uptake of spermine, it greatly reduced the initial growth rate induced by auxin (Table 1), the implications being that spermine may act at the early stage in the process of growth response. Auxin-induced growth requires RNA and protein synthesis (Edelmann and Schopfer, 1989). Auxin-mediated cell elongation is associated with rapid changes in the expression of primary genes. One of the early auxin-responsive genes, PS-IAA is recently identified to encode a short-lived nuclear protein which contains a putative DNA binding domain of prokaryotic repressive polypeptides (Abel *et al.*, 1994). We identified a 30 kD nuclear protein which could be phosphorylated by spermine and dephosphorylated with IAA and ethylene in *Ranunculus sceleratus* (Chang and Kang, in preparation). In line with our results, it is interesting to note that spermine specifically releases some chromosomal protein homologous to maize HMG 1 protein (Van den Broek *et al.*, 1994).

ACKNOWLEDGEMENTS

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개구리자리 葉柄生長에 미치는 Spermine의 抑制 키네틱스 分析

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적 요

개구리자리 엽병 세포 신장에 대한 폴리아민의 효과를 조사하였다. 외부에서 폴리아민을 처리하면 옥신에 의한 세포신장이 크게 억제되는 반면, putrescine의 생합성 억제제인 α-difluoromethylarginine을 처리하면 옥신에 의한 신장이 증가한다. Spermine은 fusicoccin에 의한 신장도 역시 뚜렷이 억제하였으나 낮은 pH에 의하여 유도되는 생장에는 효과가 없었다. 또한 spermine을 처리하면 옥신이 있는 조건에서 에틸렌에 의한 신장촉진 효과가 사라진다는 결과를 얻었다. 컴퓨터에 연결된 video digitizer system을 이용하여 IAA에 의하여 유도되는 엽병 생장에 미치는 spermine 억제 키네틱스를 분석한 결과, spermine은 초기 신장률과 안정 신장률을 감소시켰으나 잠복기의 길이에는 효과를 미치지 않았다.

주요어: 엽병 세포 신장, 옥신 유도, spermine, 에틸렌, 개구리자리

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