原　　著

Effects of short chain carboxylic acids on pollen growth

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花粉管の伸長に対する短鎖脂肪酸の影響

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Introduction

It has been reported that some carboxylic acids having 8-10 carbon atoms and a carboxyl group, such as enanthic acid, pelargonic acid, capric acid and 2-decenoic acid strongly inhibited pollen tube germination, pollen tube elongation, and pollen tube mitosis.[1-7] These compounds were regarded as a new constitution of inhibitors "Myrmic acids" by Iwanami and Iwadare.[3] Our latest study revealed that introduction of additional carboxyl group to the extreme end of carbon chain of monocarboxylic acids caused loss of the activity. For example, sebacic acid, one of C_{10}-carboxylic acids did not inhibit pollen germination.[3]

In 1980, Iwanami[7] found that short and middle chain dicarboxylic acids not only did not inhibit pollen growth, but also stimulated pollen tube elongation.The stimulation was stronger than that of indoleacetic acid, a typical plant growth hormone. In addition, he pointed that traumatic acid (Traumatin)[8] found in 1936 as wound hormone, was one of these dicarboxylic acids. Later, present authors noticed that short monocarboxylic acids (C_2-C_6) as well as dicarboxylic acids have stimulating activity when they were added into culture media. This paper deals with the subject, and the experimental results and discussion are stated hereunder.

Material and methods

Pollen grains of *Camellia japonica* were collected from freshly opened flowers and stored over silica gel

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in a plastic box They were used in the experiment within three months. Names and chemical structures of compounds used in this study are shown in Fig. 1. Number of carbon atoms of seven monocarboxylic acids are 1, 2, 3, 4, 5, 6, and 7. Two myrmic acids (caprylic acid, capric acid) and two dicarboxylic acids (suberic acid, sebacic acid) were used as reference compounds.

These compounds were disolved in distilled water and pHs of the solutions were adjusted to 5.0 by titration with sodium hydroxide solution or hydrochloric acid. Culture media were prepared with solutions containing the carboxylic acids (0-100ppm), sucrose(8%) and sugar(1%). *Camellia japonica* pollen grains were sown on the media and cultured in a moist chamber at 25°C. Number of germinated pollen grains were counted by means of a microscope after one hour cultivation. Length of the pollen tubes was measured after 20 hour cultivation according to Iwanami's method[9] (1959) using a small projector (Olympus SP-150). All the experiments were repeated more than three times, and maximum and mean values are presentad in the table.

Results and discussion

Table 1 shows germination percentage and pollen tube length of *Camellia japonica* pollen grains when they were cultured in the presence of or without carboxylic acids (control). As shown in the table, monocarboxylic acids having three through seven carbon atoms showed stimulating activity, especially, acetic acid (C_2) and propionic acid (C_3) stimulated pollen tube elongation at all examined concentrations. However, 100 ppm of valeric acid (C_5) 100 ppm caproic acid (C_6), and 50-75 ppm of enanthic acid (C_7) inhibited pollen germination and pollen tube elongation. The stimulating activity of monocarboxylic acid has been perceived in treatment with 100 ppm of acetic acid (Iwanami and Iwadare[3] 1979).

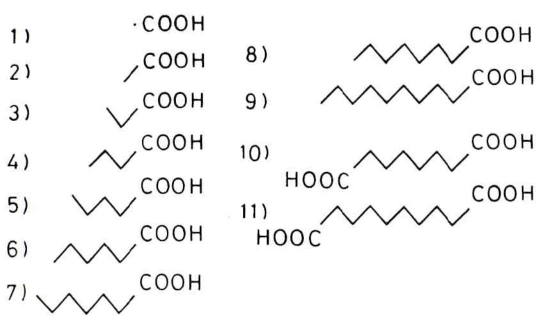

Fig. 1. Chemical structure of the compounds used in this study. 1) formic acid, 2) acetic acid, 3) propionic acid, 4) butylic acid, 5) valeric acid, 6) caproic acid, 7) enanthic acid, 8) caprylic acid, 9) capric acid 10) suberic acid, 11) sebacic acid

It has been known that middle chain monocarboxylic acids strongly inhibit pollen germination and pollen tube elongation and that short and middle chain dicarboxylic acids stimulate pollen tube elongation. Fig.2 shows the comparison of pollen tube growth of *Camellia japonica* pollens cultured on sucrose-agar media containing various carboxylic acids. The figure shows that dicarboxylic acids (C_8-D and C_4-D) and short chain monocarboxylic acids (C_2-C_7) stimulate pollen tube elongation and that middle chain monocarboxylic acids (C_8 and C_{10}) strongly inhibit pollen tube elongation. In the case of enanthic acid (C_7-monocarboxylic acid), it stimulates the pollen tube growth at low concentration (5 and 12.5 ppm) and inhibits at high concentration (25, 50 and 75 ppm).

Table1. Effects of seven monocarboxylic acids on pollen germination and pollen tube elongation of *Camellia japonica*. (pH of each medium was adjusted to 5.0)

Compound	Concentration (ppm)	Germination(%) maximum	mean	Pollen tube length(mm) maximum	mean
Control	0	96.9	95.1	6.9	5.6
Formic acid (C_1)	12.5	96.8	95.4	6.7	5.8
	25	95.2	95.0	6.6	5.9
	50	97.3	95.2	6.0	5.8
	75	98.6	96.1	6.9	5.7
	100	96.3	91.7	5.8	5.4
Acetic acid (C_2)	12.5	97.6	94.5	8.1	**6.7**
	25	98.0	95.1	10.3	**8.7**
	50	97.7	94.5	10.3	**8.6**
	75	97.2	96.3	9.6	**8.4**
	100	98.1	95.9	8.5	**7.3**
Propionic acid (C_3)	5	95.9	97.0	6.0	5.8
	12.5	97.2	94.6	8.0	**7.5**
	25	95.8	94.3	7.9	**7.6**
	50	97.5	94.5	8.1	**7.5**
	75	96.1	95.0	7.5	**7.0**
	100	96.4	96.0	6.2	5.0
Butylic acid (C_4)	5	98.4	95.5	8.6	**7.5**
	12.5	98.0	94.7	8.0	**7.3**
	25	97.0	95.0	8.8	**7.2**
	50	95.3	94.3	7.0	**6.6**
	75	94.5	93.1	5.9	5.3
	100	94.4	79.7	5.6	5.1
Valeric acid (C_5)	5	97.7	96.1	8.7	**8.0**
	12.5	98.2	96.7	8.1	**7.7**
	25	97.5	94.7	8.6	**7.3**
	50	98.1	95.0	5.6	5.1
	100	88.6	82.5	2.7	1.9
Caproic acid (C_6)	5	97.0	93.1	7.8	**7.5**
	12.5	96.4	92.3	7.7	**7.2**
	25	95.3	89.8	7.8	**6.7**
	50	97.6	91.6	6.6	6.1
	100	46.9	50.3	2.4	1.1
Enanthic acid (C_7)	5	95.3	93.1	7.8	**7.5**
	12.5	96.4	92.3	7.7	**7.2**
	25	97.1	95.2	5.0	4.5
	50	95.8	89.1	2.1	1.4
	75	57.3	48.3	0.6	0.3

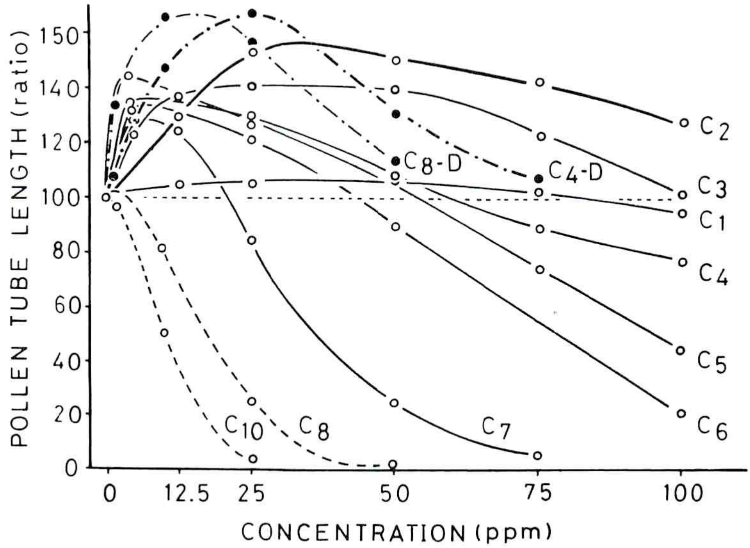

Fig. 2. Comparision of the effects of various carboxylic acids on pollen tube length of *Camellia japonica* pollen. C_1-formic acid, C_2-acetic acid, C_3-propionic acid, C_4-butylic acid, C_5-valeric acid, C_6-caproic acid, C_7-enanthic acid, C_8-caprylic acid, C_{10}-capric acid (monocarboxylic acids) C_4-D -succinic acid, C_8-D-suberic acid (dicarboxylic acids)

The authors have observed in other experiment that pollen grains burst in the medium containing high concentration of dicarboxylic acids or high concentration of monocarboxylic acids, for example, plasmoptysis percentage of *Camellia japonica* pollens was almost 100 when they were sown on the medium containing 100 ppm of suberic acid. Further, it is also observed that myrmic acids (capric acid, pelargonic acid, 3-hydroxydecanoic acid) inhibited the propagation of HeLa cells and membrane internalization of human erythrocyted (unpublished data).

On the basis of the above results, it seems reasonable to assume the function of the carboxylic acids in inhibition and stimulation as described below. When monocarboxylic acids interact with cell membrane, the acids would approach to the membrane from the lipophilic hydrocarbon side, which is most distant from the hydrophilic carboxyl or hydroxly (in the case of myrmicacin) group, of the molecules and det in the membrane as shown in Fig.3. In some acids such as MYAs, the length of hydrocarbon chains struck in the membrane would exeed a certain length. Normal actions of the cell membrane would become hard in such cases and inhibition of physiological reactions of the cell would be caused. On the contrary, the inhibition does not take place in the cases of the acids such as formic acid (C_1) through caproic acid (C_6) acids, lengths of which should be smaller than the length required for the inhibition.

Dicarboxylic acids have two carboxyl groups at both extreme ends of carbon chains such as suberic and sebacic acids. They are not able to get in the membrane in normal (thermodynamically stable) conformation. However, it would be possible to approach to membrane from the part of lipophilic hydrocarbon chain

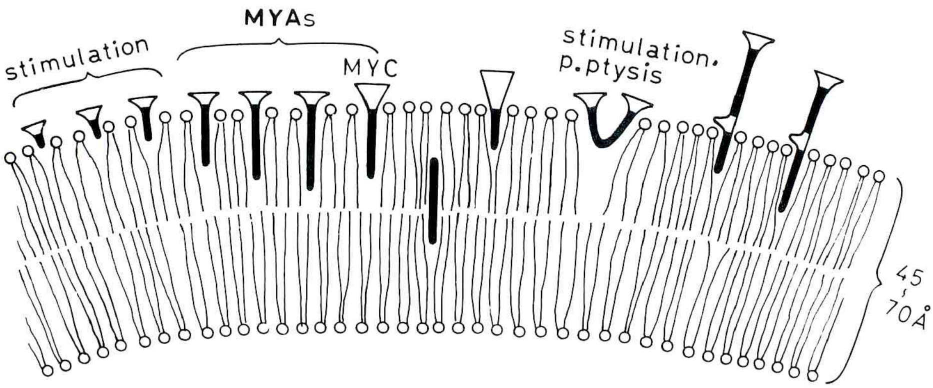

Fig. 3. Explanatory diagram of the structure of cell membrane and action of various carboxylic acids to the lipid molecules of cell membrane. Black part of each compound has lipophilic property and white part has hydrophilic property. (MYAs–myrmic acids, MYC–myrmicacin)

between the carboxyl groups and thinly get in the membrane extending spacing among lipid molecules of the membrane as shown in the diagram. The extention would teaken tention of the cell membrane and therefore plasmoptysis would take place. However, when the concentration of the compound is low, the weak extending force induces stimulation of cell growth. The stimulation with short chain monocarboxylic acids may be explained by almost same way as this speculation. The strength of the stimulation of pollen growth with C_8-carboxylic acid is half of that with C_8-monocarboxylic acid as shown in Fig.1. The fact may support this hypothesis.

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要　　約

カルボキシル基を一つもつ C_8 ～ C_{10} の脂肪酸は、花粉の発芽や花粉管伸長を強く阻害するが、カルボキシル基を両側に二つもつ物は阻害作用を示さない(Fig. 2)。短鎖の脂肪酸でカルボキシル基を一つもつもの（酢酸、プロピオン酸、酪酸、吉草酸など）は低濃度で花粉管の伸長を促進する（Tablel, Fig. 2）。これらの実験結果をもとにして脂肪酸の促進・阻害機構について考察し、脂肪酸は親脂質性をもつ方から細胞膜の脂質分子の間に入り込むことによって花粉の生長を阻害したり促進したりすると考えた（Fig. 3）。