ice-cold glass plate under safe green light[16]. The crude extracts from root tissues were extracted according to the usual procedure[11] with slight modifications[8]. The residue obtained after the freeze-drying step was dissolved in 2 ml deionized water and was further purified by Dowex 50×8-200 columns (0.6×13 cm). To ensure genuine extraction of cAMP, from crude tissue extracts, a known amount of ^{3}H-cAMP (approximately 20,000 cpm; 0.9 pmole with a sp. act. of 5 μCi) was used as internal standard. The active fractions were collected, freeze dried and redissolved in 2 ml of deionized water, and cAMP was quantitatively determined[17].

Fractions containing cAMP, from Dowex 50×8-200 columns, were found between 1 and 6, and the rate of recovery was 45.5%. The amount of cAMP, present after partial purification with a Dowex 50 column, is shown in the table. After correcting for cAMP losses during tissue preparation, the levels of cAMP in maize roots were found to be 309 (for light exposed roots) and 387 (for roots kept in the dark) pmoles/g fresh tissue.

The presence of cAMP in higher plants, particularly in dicotyledonous plants, has been previously reported[7,8,18,19]. However, very few published reports are available on cAMP in monocotyledonous plants. Recently, cAMP was found in *Zea mays* shoots, in the range of 153 pmole/mg of protein[12]. In the present report, the presence of cAMP in maize tissues is thus confirmed, in particular for root cells in which cAMP was found for the first time. The physiological role of cAMP in plants warrants a thorough analysis in the future.

Gilman[17] assays of cAMP from *Zea mays* L. (cv. LG 11) root extracts after partial purification by Dowex 50 column chromatography. Primary roots kept in white light or in darkness

Experiment No.	cAMP content in pmole/g of fresh weight			
	Light	Corrected values	Dark	Corrected values
1	137.40	301.90	158.70	348.79
2	121.50	267.00	143.80	316.04
3	163.80	360.00	227.10	499.12
	140.90 ± 10.08	309.63 ± 22.17	176.53 ± 20.97	387.98 ± 46.08

All values represent the mean ± SE of these replicates from 2 separate experiments. cAMP losses during purification procedure have been corrected as usual[8].

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Inhibiting effects of volatile constituents of plants on pollen growth

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Summary. Volatile constituents of parts of plants inhibited the germination of *Camellia sinensis* pollen grains. Growing pollen tubes were affected by a 1-sec treatment with volatile constituents of *Allium*, and tubes treated for 4–6 sec swelled abnormally at the tips and stopped growing; the reaction resembled that of pollen irradiated with γ-rays at 200 kR.

It has long been known that pollen grains of higher plants have a strong resistance to environmental factors; they did not lose germination ability when they were irradiated with a high dose (more than 100 kR) of X-rays or γ-rays[1,3], or soaked in various organic solvents such as diethyl ether, chloroform and phenol[4–6]. Further, it has been reported that pollen germination was not inhibited by treatment with the usually inhibitory compounds actinomycin D, bromodeoxyuridine, or cycloheximide[7]. The author observed in the experiments described here that the germination of *Camellia sinensis* pollen was completely inhibited by treatment with volatile constituents released from pieces of various plants.

Pollen grains collected from freshly opened flowers of *Camellia sinensis* were used in this experiment. Species used in the experiment, and the parts of the plants from which volatile constituents were released, are as follow: *Allium cepa* (bulb), *Allium sativum* (tuber), *Allium tuberosum* (leaf), *Citrus limon* [Lemon] (pericarp and flesh), *Citrus medica* [Yuzu] (pericarp and flesh), *Citrus unshu* [Orange] (pericarp), *Eutrema wasabi* (rhizome).

These materials were used freshly from fields or trees in this experiment.

A small chamber made of plastic plate (thickness 2 mm) as shown in figure 1 was used for cultivation and treatment with volatile substances. By using the chamber, the growth of the pollen can be observed continuously through the cover glass and agar culture medium under the microscope. The culture medium consisted of distilled water, agar (1%; Wako Co. Ltd), and sucrose (9%), and the pH of the medium was adjusted to 5.5 by titration with HCl solution.

First, pieces of each plant (2.5×2.5×2.5 mm) were placed

on the bottom of the chamber, and the chamber was covered with the cover glass (23 × 18 mm). Next, agar culture medium (16 × 22 × 1.5 mm) was prepared on another cover glass and pollen grains were sown on the medium. 5 min after the placing of the plant pieces in the chamber, the original cover glass was replaced with the cover glass with the culture medium and pollen grains, which was placed agar-side down over the plastic chamber as shown in figure 1, so that the pollen grains were cultured in air containing the volatile constituents released from the plant pieces.

Quantity of plant pieces inhibiting pollen germination of *Camellia sinensis* completely (germination percentage = 0) in a small plastic chamber (30 × 22 × 7 mm)

Plant species	Parts	Quantity of pieces (g)
Citrus limon (Lemon)	Pericarp	0.03–0.08
Citrus limon	Flesh	0.8 –1.0
Citrus medica (Yuzu)	Pericarp	0.05–0.09
Citrus medica	Flesh	0.6 –0.8
Citrus unshu (Orange)	Pericarp	0.04–0.09
Allium cepa	Bulb	0.3
Allium sativum	Tuber	0.4
Allium tuberosum	Leaf	0.2 –0.4
Eutrema wasabi	Rhizome	0.2 –0.5

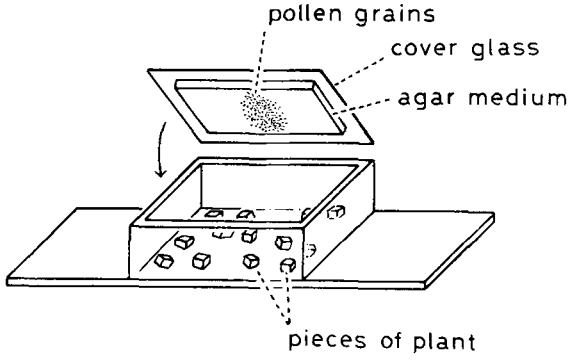

Figure 1. Small plastic chamber used in this experiment. Cultured pollen can be observed continuously through the cover glass and agar medium under the microscope.

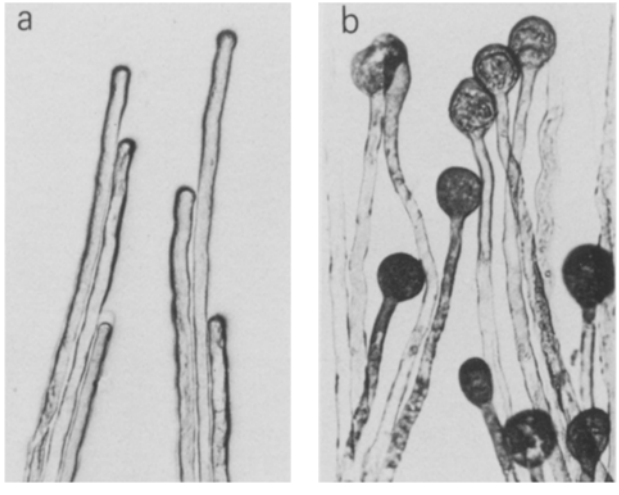

Figure 2. Inhibitory effect of volatile constituents released from *Allium cepa* pieces on pollen tube growth of *Camellia sinensis*. *a* Normal pollen tubes (2 h after sowing). *b* Abnormally swelled pollen tubes which were cultured for 1 h, and then treated with volatile constituents of *Allium cepa* for 5 sec (2 h after sowing).

After 1-h cultivation, the number of germinated pollen grains was counted. The germination percentage of pollen in the control chamber without any plant pieces was 98.5. Pollen treated with volatile constituents of plants, however, showed poor germination, and the germination percentage of the pollen was reduced to zero when more than a certain quantity of plant pieces were placed in the chamber. The table shows the quantity of plant pieces required for complete inhibition of the germination of *Camellia sinensis* pollen.

The inhibitory activity of the volatile constituents of the pericarp of *Citrus limon* was the strongest of all the materials tested; germination of pollen grains was inhibited completely when 0.03–0.08 g of the plant pieces was placed in the chamber. The inhibitory activity of the flesh of *Citrus* fruits was considerably weaker than that of pericarp. In the case of *Allium*, pollen germination was completely inhibited when 0.3–0.4 g of plant pieces was used. It is noteworthy that while pollen grains which were inhibited with *Allium* or *Eutrema* retained their normal water-absorbed shape, all the pollen grains treated with *Citrus* pieces burst. It was confirmed that inhibition was not observed when 0.2 g charcoal (activated carbon, Waco Co. Ltd) was placed in the chamber with the plant pieces. These results show that volatile constituents caused inhibition of pollen growth; however, the mechanism of inhibition is different with different plant species.

Next, the effects of the volatile constituents on growing pollen tubes were studied. Pollen grains of *Camellia sinensis* were cultivated in control agar medium in a small chamber without plant pieces for 2 h, and the pollen grains, which had about 1.2 mm pollen tubes, were then exposed to air containing volatile constituents of plant pieces. The pollen grains, after treatment with volatile constituents for 1, 2, 3, 4, 5, 6, 8, 10, and 20 sec, were brought back to the original chamber, and the effects of the treatment was checked using a microscope.

Pollen tubes which were treated with volatile constituents from 0.5 g of pieces of *Allium cepa* for 1–3 sec stopped their elongation about 1 min after the treatment and the tips of the tubes began to swell. However, after 10–20 min, new pollen tubes started elongating from the corners of the small swellings. When pollen tubes were treated for 4–6 sec, the tips of the pollen tubes swelled abnormally as shown in figure 2; they never burst and never grew new pollen tubes from the large swellings.

It is noteworthy that the inhibitory effects of volatile constituents released from plant pieces are similar to those of irradiation. The author has observed similar abnormal swellings of pollen tubes when *Lilium auratum* pollen tubes were retarded by treatment with γ-rays at 200 kR[3]. It is known that various substances produced from higher plants affect bacteria, plants, and animals. The phenomenon has been called allelopathy[8] or Phytocide[9]. The volatile constituents used in this experiment may be such substances. The author would like to suggest that volatile constituents from plants might be used not only for sterilization but also as specific inhibitors for biological research.

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