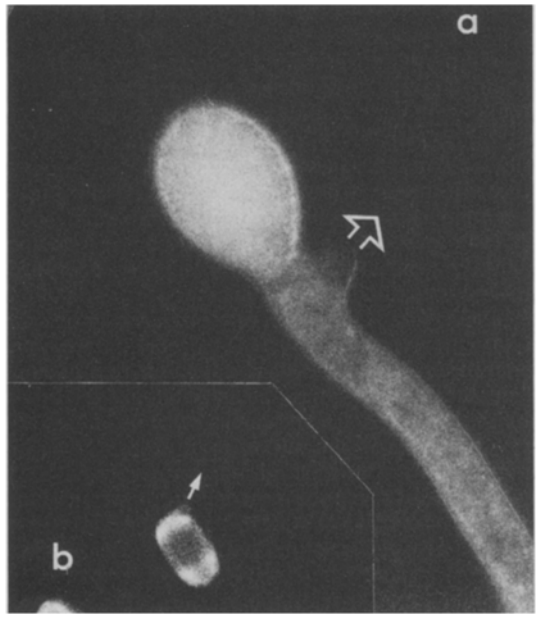
yeast cells were studied. Such cells were obtained by subcultivating a wine strain of *Saccharomyces cerevisiae* for 3 h at 20 °C on slants of malt (2%)-peptone (2%) agar medium (pH 6.2). A small loopful of the actively budding population of yeast cells was dispersed into an aqueous drop of the reagent used. The greenish-blue staining with bromocresol green (10^{-4}, w/v), dark red with chlorophenol red (10^{-4}, w/v) and pinkish violet with alizarin yellow S (10^{-4}, w/v) observed in the peripheral cytoplasm of non-budding cells and budding mother cells are all in agreement with the overall intracellular pH value of 5.8, previously determined by physico-chemical methods in resting baker's yeast[6]. However, the yellow staining of young buds with all indicators indicates an internal pH not higher than 5.0 in such emerging structures.



Quenched fluorescence of the pH probe 4-methylesculetin in the bud initials of: *a* hypha of *Allomyces arbuscula* outgrowing laterally (directional arrow) below an apically differentiating zoosporangium; *b* vegetative cell of *Saccharomyces cerevisiae* at the emergence stage (directional arrow on polarized outgrowth). × 1000.

When budding yeast cells were plunged into a drop of saturated 4-methylesculetin in distilled water, mother cells exhibited a vivid greenish-grey fluorescence in their cytoplasmic 'shoulders' while the bud initials remained fully extinct (fig. b). Only larger buds, just penetrated by mitochondria[7], showed significant fluorescence.

That the lack of observable fluorescence in the bud initials is essentially due to their low pH and not to insufficient concentration of the probe in such small structures can reasonably be assumed based on the observation, facilitated by rolling cells, of some fluorescence in the narrow collar transition between the vividly fluorescing 'shoulders' of mother cells and their extinct buds. As for the possible selective segregation into young buds of quenching compounds absorbing light at the excitation or emission wave length, its effects should be minimized by the overwhelming concentration of fluorescent probe available to the cells in the saturated drops. Assuming therefore that the lack of observable fluorescence in the bud initials is essentially a pH effect, the internal pH of the buds can be estimated at no more than 5.

An understanding of the origin of the increased acidity found in outgrowing buds might involve our recently proposed concept of a randomly occurring event of positioning of mitochondria initiating vectorial dissipation of their extruded protons toward facing plasmalemma[8]. At this consequently polarized site (functionally, while electrically depolarized?), a proton sink would thus be created and self-entrained by further drainage of the protons through the plasmalemma which finally bulges with its acid-plasticized wall, into an outgrowing amitochondrial bud.

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Adenosine 3′,5′-cyclic monophosphate levels in maize roots[1]

S. Sen[2] and P.E. Pilet

Institut de Biologie et Physiologie Végétales de l'Université, 6 place de la Riponne, CH-1005 Lausanne (Switzerland), 9 April 1981

Summary. Endogenous levels of adenosine 3′,5′-cyclic monophosphate (cAMP) in maize (cv. LG 11) root cells, grown in light and dark conditions, were found to be 309 and 387 pmoles/g of fresh tissue respectively.

The occurrence and physiological role of adenosine 3′,5′-cyclic monophosphate (cAMP) in bacteria[3] and mammals[4] have been well established. Despite numerous studies, the presence of cAMP in plants remains uncertain and controversial[5,6]. However, it has been found recently that cAMP does exist in wide varieties of plant species, such as *Phaseolus vulgaris*[7,8], *Lolium multiflorum*[9], *Funaria hygrometrica*[10], *Ochromonas malhamensis*[11] and *Zea mays*[12].

The aim of the present work is to examine the presence of cAMP in the growing roots of *Zea mays*, the elongation of which being clearly regulated by a hormone balance[13].

Caryopses of *Zea mays* L. (cv. LG 11) were grown in darkness at 22 °C[14]. After germination, seedlings were kept in the dark or in white light (Sylvania 220 V/40 W; 1.84 ± 0.12 Wm^{-2} at the root level[15]). When the primary roots reached 12 ± 1 mm length, they were excised on an

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

Y. Iwanami

Biological Institute, Yokohama City University, Kanazawa-ku, Yokohama 236 (Japan), 21 April 1981

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