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BRIEF COMMUNICATION

The effect of various lipids on flowering of *Pharbitis nil* in *in vitro* culture

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Abstract

The effect of applied arachidonic acid, prostaglandin (PGE_1) and various sterols and combinations of arachidonic acid + sterols, on flowering of *Pharbitis nil* were ascertained by using a tissue culture technique. It was found that arachidonic acid, PGE_1 stigmasterol, testosterone, cholesterol, stigmasterol + arachidonic acid, β-sitosterol + arachidonic acid and cholesterol + arachidonic acid all caused earlier flowering. Four inhibitors of prostaglandin biosynthesis (gentisic acid, acetylsalicylic acid, salicylic acid and oleic acid), inhibited flowering completely. The results confirm that the compounds tested could possibly play a role in the flowering of *P. nil.*

Additional key words: arachidonic acid, inhibitors of prostaglandin biosynthesis, prostaglandin, short-day plant, sterols.

Pharbitis nil Chois cv. Violet is a sensitive short-day (SD) plant. It can be induced to flower by a single inductive photoperiod. In the past it has been extensively used as a model plant for flowering studies (Evans 1975, Lang 1984, Aukerman and Amasino 1998). Flowering in photoperiodic sensitive plants is considered to be brought about by transmissible signals which are produced in the leaves and which are either promotive or inhibitory, during inductive or non-inductive photoperiods, respectively. The hypothetical flowering stimulus has been named florigen (Chailakhyan 1937). However the chemical nature of the elusive flowering stimulus has not been identified up to the present (Aukerman and Amasino 1998, Groenewald and Van der Westhuizen 2001).

In this regard it has long been thought that prostaglandins (PGs) play a role in flowering of *P. nil* since certain inhibitors of PG-biosynthesis inhibited flowering of intact plants to a greater or lesser extent (Groenewald and Visser 1974). Moreover it has been found that induced to flower *P. nil* plants contained a 20-times higher concentration of $PGF_{2\alpha}$ than vegetative plants (Groenewald *et al.* 1983). Furthermore it has been discovered that certain inhibitors of steroid biosynthesis, when applied to leaves of intact plants, inhibited flower initiation in the SD plants *Xanthium* and *Pharbitis* (Bonner *et al.* 1963). It therefore seemed to be worthwhile to test prostaglandins and certain steroids and combinations of steroids and a precursor of PGs, on their effect on flowering, using a tissue culture technique.

In the last two decades this type of research, to identify the chemical nature of the flowering signal, has made way for genetic and molecular approaches to the florigen problem. Although at an early stage, the genetic networks that interact to control flowering are being elucidated by analysing flowering-time genes from *Arabidopsis*, maize, pea, *Impatiens* and other species (Aukerman and Amasino 1998, Colasanti and Sundaresan 2000, Hempel *et al.* 2000). However, even by these methods, the chemical nature of the flowering signal(s) has not yet been elucidated. It thus still seems worthwhile to study the effect of chemically defined substances on its ability to induce flowering, since it could provide valuable clues as to the nature of the signal(s) involved, even in the 'molecular age'.

The test system developed by us for *P. nil* consisted of excised shoot apices grown in a nutrient medium, and which allowed the formation of flowers *in vitro* under inductive conditions (Fig. 1). The test compounds were applied in the agar medium in which the apices were placed. Seeds of *P. nil* were obtained from *Muratane Co. Ltd.*, Kyoto, Japan, sterilized (calcium hypochlorite) and aseptically transferred to 250 cm^3 Erlenmeyer flasks

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Abbreviations: LD - long day; PGs - prostaglandins; SD - short day.
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containing sterilized moistened cotton wool. They were allowed to germinate under continuous light (345 μmol $m^{-2}s^{-1}$) and constant temperature of 27 °C. After 3 d the stems of the seedlings were about 4 to 5 cm long and they were then aseptically transferred to sterile Petri dishes. The cotyledons were removed with a sterile scalpel and the hypocotyl was severed 5 - 10 mm below the stem apex. The stem apices were placed with the cut surface pressed onto a suitable agar nutrient medium contained in a test tube (5 cm^3 medium in a 23 × 195 mm tube). The nutrient medium used was the mineral salt mixture of Linsmaier and Skoog (1965) to which 3 % sucrose, 0.8 % agar, 1 g dm^{-3} casein hydrolysate, 0.4 mg dm^{-3} thiamine-HCl, 100 mg dm^{-3} myo-inositol, and 1 mg dm^{-3} kinetin was added. The pH was adjusted to 5.8 prior to autoclaving. The plantlets which developed from the apices produced five leaves and a single terminal flower. Relatively large flowers (*ca*. 3 cm long) were obtained under inductive photoperiods using this medium (Fig. 1.).

Fig. 1 Depicts a flowering plantlet which developed from an excised apex of *P. nil* which was subjected to inductive photoperiods.

The compounds tested (concentration 10^{-4}M) were the following: arachidonic acid (precursor of PGs), PGE_1, stigmasterol (steroid), testosterone (steroid), cholesterol (steroid), stigmasterol + arachidonic acid, cholesterol + arachidonic acid, β-sitosterol (steroid), β-estradiol (steroid), testosterone + arachidonic acid, β-estradiol + arachidonic acid, gentisic acid, acetylsalicylic acid, salicylic acid and oleic acid. The last four compounds are inhibitors of PG-biosynthesis. The experiment was performed under inductive conditions (SD) in a temperature controlled room (25 °C) for 45 d. Control plantlets flowered after 45 d.

It was found (Table 1) that arachidonic acid, PGE_1, stigmasterol, testosterone, cholesterol, stigmasterol + arachidonic acid, β-sitosterol + arachidonic acid and cholesterol + arachidonic acid hastened flower formation by 29, 28, 23, 12, 12, 28, 25 and 25 d respectively. It was found that β-sitosterol, β-estradiol, testosterone + arachidonic acid, β-estradiol + arachidonic acid, gentisic acid, acetylsalicilic acid, salicylic acid and oleic acid had no effect on flowering.

Arachidonic acid and eight different PGs, at three different concentrations (10^{-4}, 10^{-5} and 10^{-6}M) were applied to excised shoot apices under non-inductive

Table 1. Effect of various compounds (10^{-4} M) on the flowering of excised shoot apices of *Pharbitis nil* kept under inductive photoperiods (8 h light, 16 h darkness). Each treatment had 5 replicates when the experiment was set up.

Compounds applied	Number of explants		Number of days before first flower
	flowered within 45 d	failed to flower	
None (control)	5	0	45 (± 2)
Arachidonic acid	5	0	16 (± 2)
PGE_1	3	2	17 (± 2)
Stigmasterol	5	0	22 (± 4)
Testosterone	1	3	33
Cholesterol	1	2	33
Stigmasterol + arachidonic acid	4	0	17 (± 2)
β-sitosterol + arachidonic acid	1	1	20
Cholesterol + arachidonic acid	1	1	20
β-Sitosterol	0	2	-
β-Estradiol	0	4	-
Testosterone + arachidonic acid	0	1	-
β-Estradiol + arachidonic acid	0	3	-
Gentisic acid	0	5	-
Acetylsalicylic acid	0	5	-
Salicylic acid	0	5	-
Oleic acid	0	5	-

(long-day, LD) photoperiods. However it was found that none of the treatments induced flower formation. The experiment lasted 60 d. This negative result may be explained by the fact that the plantlets which developed from the apices possessed leaves and produced inhibitors (possibly of low molecular mass phenolic acids), which inhibited flowering.

The most effective treatments were arachidonic acid, PGE_1 and stigmasterol + arachidonic acid. Stigmasterol alone was slightly less effective. Arachidonic acid is a precursor of PGE_2 and $PGF_{2\alpha}$ in mammalian systems (Hinman 1972) and it has been found that applied arachidonic acid can be converted to PGE_2 and $PGF_{2\alpha}$ in plant homogenates (Forster *et al.* 1984, Ali *et al.* 1990, Afzal *et al.* 1991).

Aseptic shoot apices have been used since about 1961 (Raghavan and Jacobs 1961) for the study of various aspects of flowering and a number of researchers have succeeded to induce flowering in shoot apices of SD plants and LD plants, but the results of applied substances were mostly disappointing (De Fossard 1974). Harada (1967) claimed to have obtained plantlets from excised apices of *P. nil* and a *Chrysanthemum* sp. which flowered, but in our hands his nutrient medium failed to induce flowering in *P. nil* and we developed our own successful medium.

De Fossard (1974) is of the opinion that lipids could be involved in flowering. He has tested various lipids and lipoidal extracts on excised apices of *Chenopodium rubrum* (SD plant) which among others included lanosterol, β-sitosterol, stigmasterol, cholesterol and ergosterol. However none of the substances induced flowering under inductive or non-inductive conditions. However, in our system with *P. nil*, we have obtained promising results with various lipids, *e.g.* arachidonic acid, PGE_1 and certain sterols under inductive conditions. It thus seems likely that these compounds play a role in the flowering of *P. nil*.

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