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Organ Correlation in Long-Day Flowering of *Pharbitis nil*

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Abstract. *Pharbitis nil*, strain Kidachi and Violet, were grown at 20 °C under continuous light in various cases of different sizes, containing the nutrient solution or tap water. Kidachi initiated flowers when seedlings were cultured in small cases containing nutrient solution but did not flower in large cases. The application of NAA, kinetin, ABA or 5-chlorobenzoic acid *via* roots in large cases caused flowering, Floral initiation was always accompanied by the suppression of root elongation. The promotion of root elongation by aeration inhibited flowering. Root cutting inhibited flowering. On the other hand, Kidachi did not flower in tap water irrespective of vessel size. Violet did not flower in the nutrient solution even in small vessels, but initiated floral buds in tap water irrespective of vessel size, though the plants grown in large cases developed very long roots. In both types of flowering, cotyledons are necessary to induce flowering and the removal of cotyledons before the 16th day after the start of culture in Kidachi and before the 12th day in Violet completely inhibited flowering. GA_3 promoted stem elongation without preventing flowering of Kidachi.

Floral initiation itself is the event occurring in the shoot apex but it is greatly influenced by external and internal factors. It is well known that day-length acts through leaves and plays decisive roles in the induction of flowering. In some short-day plants, leaves have inhibitory effects on flowering under unfavorable day-length, and removal of leaves caused flowering under long-day condition (RAGHAVAN and JACOBS 1961, SHINOZAKI 1974). Roots have also inhibitory effect on flowering of *Scrofularia* and *Chenopodium*, and removal of roots promoted flowering under unfavorable day-length. The addition of cytokinin inhibits flowering caused by removal of roots (MIGINIAC 1978). *Pharbitis nil*, a short-day plant, can be induced to flower under continuous light in various ways (WADA 1973, SHINOZAKI 1974), though the responses to various treatments differ from strain to strain. In this paper the effects of the presence of cotyledons, root elongation and stem elongation on long-day flowering of Kidachi and Violet caused by different factors are described.

MATERIALS AND METHODS

Pharbitis nil, strain Kidachi (dwarf strain) and Violet, were used. The general methods and procedures used were the same as described previously (SHINOZAKI and TAKIMOTO 1982 a, b, 1983). Culture vessels used in the present

experiments were test tubes (1.8 × 18 cm), glass tubes (3 × 12 cm), small plastic cases (13 × 11 × 6.5 cm) and large plastic cases (33 × 26 × 11.5 cm). Seedlings with just unfolded cotyledons were planted singly in each test tube and glass tube, 12 plants in each small box and 24 plants in each large box. In tap water experiment, seedlings with cut-off roots were grown in tap water for 25 days and then cultured in the nutrient solution for 10 days.

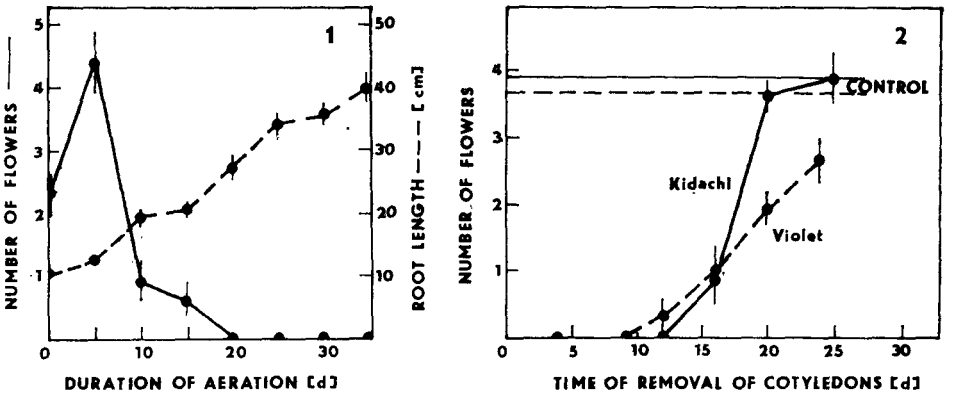

Fig. 1. Effect of root aeration on flowering and root elongation of *Pharbitis nil*, strain Kidachi, cultured in small plastic cases at 20 °C under continuous light. — Solid line: flowering; dashed line: root length.

Fig. 2. Effect of removal of cotyledons on flowering of *Pharbitis nil*, strain Kidachi, cultured in glass tubes containing the nutrient solution, and Violet cultured in glass tubes containing tap water at 20 °C under continuous light. — Solid line: Kidachi; dashed line: Violet.

Growth substances were added to the nutrient solution at various concentrations (table shows only representative data) or applied *via* the apical part of hypocotyl through cotton thread (SHINOZAKI and TAKIMOTO 1983). Chemical treatment was carried out for the first 15 days and then the plants were transferred to the basic nutrient solution. All experiments were carried out at 20 °C under continuous light of 5000 lx provided by day-light fluorescent lamps. The plants were dissected to examine flowering responses and vegetative growth on the 35th day.

RESULTS AND DISCUSSION

Root Elongation and Long-Day Flowering

Seedlings of Kidachi and Violet were grown in test tubes, glass tubes and large boxes containing the nutrient solution with or without growth substances added, or tap water for 35 days. Kidachi initiated floral buds when seedlings were cultured in test tubes and glass tubes containing the nutrient solution or in large boxes with growth substances (Table 1). Floral initiation was always accompanied by the suppression of root elongation. Aeration of the seedling roots for more than 15 days completely inhibited flowering and greatly promoted root elongation (Fig. 1). However, the seedlings planted in test tube containing tap water did not flower, though they had very short roots (Table 1). NAA and kinetin applied *via* the apical part of hypocotyl did not cause flowering and the application of them to the plants

TABLE 1

Effects of size of culture vessels, tap water and some growth regulators on flowering responses and root elongation of *Pharbitis nil*, strains Kidachi and Violet, at 20 °C under continuous illuminance 5000 lx

Strain	Culture vessel	Solution chemicals added	No. of flowers per plant	Root length [cm]
Kidachi	test tube	N	2.3 ± 0.4	7.3 ± 0.4
	glass tube	N	2.4 ± 0.3	12.5 ± 0.4
	plastic case	N	0.0	31.7 ± 0.6
		N(NAA, 0.05 μM)	3.6 ± 0.2	10.3 ± 0.2
		N (kinetin, 0.5 μM)	4.4 ± 0.3	7.5 ± 0.2
		N (ABA, 4 μM)	2.0 ± 0.4	6.3 ± 0.4
		N (5-Cl-BA, 20 μM)	2.5 ± 0.5	15.5 ± 0.4
	test tube	T	0.0	7.8 ± 0.6
	plastic case	T	0.0	32.3 ± 0.6
Violet	test tube	N	0.0	2.9 ± 0.2
	glass tube	N	0.0	5.1 ± 0.3
	plastic case	N	0.0	15.3 ± 1.0
	test tube	T	3.4 ± 0.2	7.6 ± 0.6
	glass tube	T	3.0 ± 0.4	10.7 ± 1.2
	plastic case	T	2.6 ± 0.2	34.5 ± 1.7

N: Slightly modified Nakayama's solution, T: Tap water
Vessel size: test tube 1.8 × 18 cm, glass tube — 3 × 12 cm, plastic case — 33 × 26 × 11.5 cm

whose roots were cut off on the 10th day did not induce flowering, that is, not substituted for roots (SHINOZAKI and TAKIMOTO 1983). On the contrary, Violet initiated floral buds irrespective of vessel size when seedlings were cultured in tap water. The plants grown in tap water in a large box had very long roots (Table 1). Thus, the roles of roots in inducing long-day flowering are different from strain to strain.

Effect of Removal of Cotyledons on Flowering

Seedlings of Kidachi were planted in glass tubes containing the nutrient solution, and those of Violet were cultured in glass tubes containing tap water.

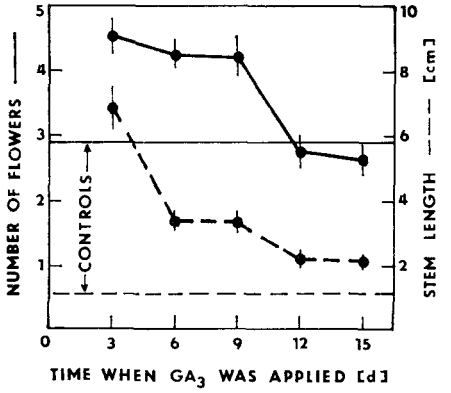

Fig. 3. Effect of GA_3 on flowering and stem elongation of *Pharbitis nil*, strain Kidachi, cultured in glass tubes containing the nutrient solution at 20 °C under continuous light. GA_3 was applied 2 μg/plant *via* the apical part of hypocotyl through cotton thread. — Solid line: flowering; dashed line: stem length.

The cotyledons were cut off at intervals. In both strains, removal of cotyledons earlier than the 15th day strongly inhibited flowering (Fig. 2). It is unlikely that the removal of cotyledons inhibited the development of floral bud, because the first foliage leaf had already unfolded at this stage. Therefore, this result suggests the production of some flower-inducing substance(s) in the cotyledons.

Stem Elongation and Long-Day Flowering

Long-day flowering of *Pharbitis nil* was always accompanied by the inhibition of stem elongation (SHINOZAKI 1974). This is also the case in the present experiment. GA_3 (2μg/plant) was applied to Kidachi seedlings at various times in order to elucidate the relationship between stem elongation and flowering. GA_3 promoted both flowering and stem elongation when applied at early stage (Fig. 3), indicating that this flowering was not caused by the inhibition of stem elongation.

Interaction of organs such as cotyledons, roots and stem in inducing long-day flowering of *Pharbitis nil* differs with strains and experimental conditions.

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