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Effects of Electric Current on Flowering in *Pharbitis* and Floral Stimulus Activity in the Phloem Exudate of Cotyledons

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Direct current (DC) applied to cotyledons during a 16 h inductive dark period inhibited the flowering in the short-day plant, *Pharbitis nil* Choisy cv. Violet. The inhibitory effect of DC was more profound when the current flowed from roots to cotyledons, showing its polarity-dependent action. The second half of the inductive dark period was more sensitive to DC stimulus. The flowering was significantly depressed only when DC stimuli were applied to the translocation path of the floral stimulus from the induced cotyledon to the apex, suggesting that the transport of floral stimulus was damaged by the DC treatment. The vegetative apex culture bioassay system showed that a significant level of the floral stimulus activity existed in the phloem exudate from the cotyledons which would fail to form their own floral buds. These results strongly support the hypothesis that DC partially impede, at least temporarily, the transmission path of the floral stimulus from florally-induced cotyledon to the apex, rather than depressing *in situ* synthesis of the floral stimulus.

Key words: *Pharbitis nil*, flowering, direct current, phloem exudate

Under appropriate light environment photoperiodic plants are induced to produce interorgan signal in their leaves or cotyledons which is transmitted to apex to shift ongoing vegetative growth to reproductive phase. Transmissible signals, nature of which is yet to be identified, have so far been proposed to be a floral promoter in *Nicotiana tabacum* (Chailakhayan *et al.*, 1989), an inhibitor in *Pisum sativum* (Murfet, 1985), a balanced mixture of promoters and inhibitors (Thomas, 1993), or interacting inhibitors (Law *et al.*, 1993).

In the study to characterize the action mode of unknown floral stimulus, Bernier *et al.* (1981) and Vince-Prue and Gressel (1985) introduced localized blockage along the transmission path by girdling the stem above the induced leaves or cutting the supply of floral stimulus by removing induced leaves. Along with these mechanical manipulations to prevent the plants from flowering, Montavon and Greppin (1983, 1986) applied 10 V of direct current (DC) between the petiole of the leaf being induced and the root of long-day *Lactuca* to prevent flowering. Adamec *et al.* (1989) and Macháčková *et al.* (1990) have observed the inhibitory DC action in *Chenopodium*, a short-day plant, by applying the current between cotyledons and the root. They proposed a hypothesis that DC inhibited flowering in *Chenopodium* by interfering with the transport of a floral stimulus from the induced leaf to the apex. However, their results could not completely exclude the possibility that synthesis of the floral stimulus might have been blocked by DC treatment.

To verify the alternative, the present study adopted a bioassay system as described by Ishioka *et al.* (1990, 1991) and intended to confirm the activity of floral stimulus in phloem exudates from DC-stimulated cotyledons of *Pharbitis nil*.

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MATERIALS AND METHODS

Plant material

Seeds of *Pharbitis nil* Choisy cv. Violet, a short-day plant were soaked in concentrated sulfuric acid for 30 min with occasional stirring and were rinsed with running water at least for 20 h. The seeds thus imbibed were sown on mixture of perlite and vermiculite (v/v 1 : 1) in 12 cm diameter pots and grown with Hoagland nutrient solution under continuous light. The irradiance from mixture of fluorescence lamps and incandescent bulbs was adjusted at 15 $W \cdot m^{-2}$ at the plant level and temperature was kept at 26± 2 ℃. The plants 6 days old were used in all experiments.

DC application

DC application to the plants was performed with setup described by Adamec *et al.* (1989) and Macháčková *et al.* (1990) with slight modification. DC was applied through salt bridges made of polyethylene tubing (inner diameter 1.2 mm, outer 1.8 mm) filled with 10 mM KCl solution. A bridge was fixed on either center of abaxial side of a cotyledon or elsewhere designated in the experiments, and connected the plants with a Ag/AgCl-electrode. By a salt bridge, another Ag/AgCl-electrode of opposite polarity was connected with the nutrient solution bathing the roots. From a power supply (± 15 V), the DC of various intensities (3, 6 or 9 μA) was set by means of a rheostat on the microampermeter scale.

Floral induction and growth analysis

When grown under continuous light for 6 days, *Pharbitis* developed a pair of fully opened cotyledons at the cotyledonary node approximately 2 mm below the apex. The plants were transferred to the dark room where the flowering was induced by a single 16 h dark period. One of the cotyledons, if necessary, was excised just before the dark treatment and lanolin was pasted on the cut area to block the phloem exudation. At the end of the dark period, the plants were grown under continuous light for 2 weeks and the flowering was evaluated in terms of numbers of floral buds per plant. Five plants were treated in each experiment repeated three times and means with standard errors were calculated from the results.

Collection of phloem exudate

Phloem exudate was obtained by the procedure as described by Ishioka *et al.* (1990, 1991). Cotyledons with petioles excised from the plants immediately after the end of the inductive dark treatment were placed in a vial containing 20 mM EDTA solution such that the ends of the petioles were submerged in the chelating solution for 1 h. The cotyledons then were transferred to deionized distilled water (10 mL/10 cotyledons) and incubated for 6 h, keeping the ends of the petioles well submerged in the bathing solution. The solution containing the diluted phloem exudate was used for bioassay for floral stimulus activity. The incubation was performed at 26 ± 2℃ in the light.

Bioassay for floral stimulus

The bioassay was performed according to Ishioka *et al.* (1990, 1991) with slight modification. After treated with sulfuric acid, the imbibed seeds of *Pharbitis nil* were surface sterilized with 1-1.5% NaOCl solution for 20 min and rinsed three times with sterilized deionized distilled water. The seeds were sown aseptically on autoclaved Murashige and Skoog (MS) medium supplemented with 3% sucrose and 0.8% bactoagar (Difco, U.S.A.) in 125 mL Erlenmeyer flasks. The cultures were kept in a growth chamber under continuous light for 7 days. The apices of the seedlings thus grown were excised from seedlings and used for bioassay. Each apex, 5 mm long, was placed on 10 mL of autoclaved MS medium with 5% sucrose, 0.8% bactoagar and the phloem exudate in a test tube. The exudate had been added to the medium before autoclaving. The apex cultures were maintained under continuous light in a growth chamber. At the end of 6 week culture period, the activity of floral stimulus in the phloem exudate was analyzed by measuring percentages of plantlets with floral buds. Each bioassay was repeated three times with ten replicates. The bioassay system is shown in Fig. 1 diagrammatically.

Table 1. Floral responses of *Pharbitis nil* affected by 16 h DC (6 μA) stimulus with various application schedules and polarities. Anode and cathode connected to roots or a cotyledon, with untreated cotyledon cut off before DC treatment. The plants were induced to flower by a 16 h dark period (IDP). Data are shown with SE

Application period (16 h)	Polarity		Flowering (floral buds/plant)
	Anode	Cathode	
None	None	None	2.88± 0.25
Light before IDP	Root	Cotyledon	0
	Cotyledon	Root	0.97± 0.15
IDP	Root	Cotyledon	0
	Cotyledon	Root	0.65± 0.10
Light after IDP	Root	Cotyledon	0.42± 0.13
	Cotyledon	Root	0.48± 0.06

period (Fig. 3). DC of 3 μA depressed flowering linearly as the stimulus lengthened from 4 to 16 h. The maximal depression was only 47.4% of the control (Fig. 3A). Complete inhibition of flowering was brought by even 8 h DC stimulus when the intensity of the current doubled (Fig. 3B). When stimulated by DC of 9 μA during the first 4 h of a 16 h inductive dark period, the plants remained vegetative (Fig. 3C).

Flowering was inhibited not only by DC stimulus during the inductive dark period but also by that imposed either before or after the dark (Table 1). When DC of 6 μA flowed from roots to a cotyledon for 16 h either before the beginning or after the end of a 16 h dark period, the flowering was inhibited completely or by 85.4% respectively. The current of opposite polarity depressed the flowering either by 66.3% or 83.3%. The plants were found to be sensitive to DC treatments given during the noninductive light periods.

The sensitivity of the plants to 4 h application of DC during each quarter of a 16 h inductive dark period was examined (Fig. 4). The flowering was depressed by more than 50% of the control in all cases. The plants were most sensitive to DC stimulus applied in the third and the last quarters of the dark showing 25.7% and 27.6% of the control respectively. While short period of DC application before the dark period could not block flowering significantly, DC given to the plants after the inductive dark pe-

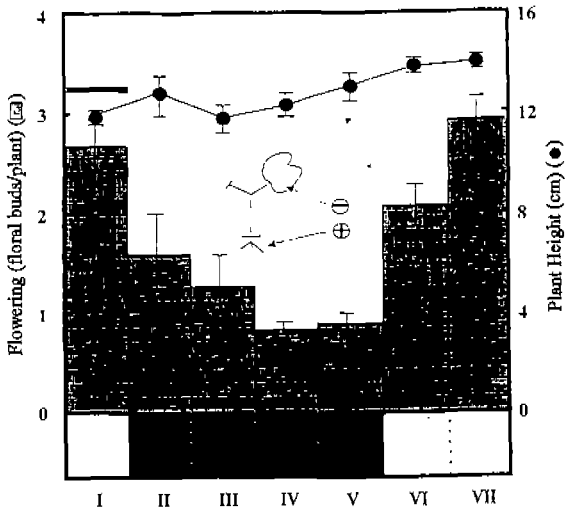

Fig. 4. Flowering in *Pharbitis nil* inhibited by 4-h-DC stimulus (6 μA) at various application timings (I-VII). The polarity of the current is shown in a diagram inserted in the figure. DC was applied to plants; for 4 h before the start of 16 h-dark (I); for the first quarter of the dark (II), for the second quarter of the dark (III), for the third quarter of the dark (IV); for the last quarter of the dark (V), for 4 h after the end of the dark (VI), or for the second 4 h after the end of the dark (VII). A horizontal bar in the figure indicates control flowering level. Vertical bars represent SE.

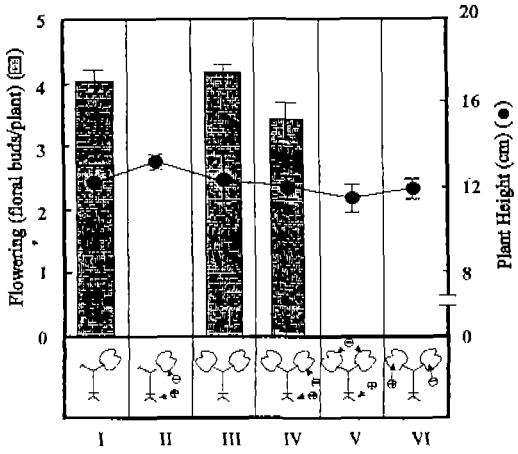

Fig. 5. Effect of DC stimulus (6 μA) on the flowering in *Pharbitis nil* in the presence or absence of untreated cotyledon. DC was applied to plants for 16 h of inductive dark period. Plants with a single cotyledon received no DC stimulus (I); plants with one cotyledon received DC stimulus (II); plants with both cotyledons received no DC stimulus (III); plants with both cotyledons received DC stimulus (IV); plants received DC stimulus with cathode connected to both cotyledons (V); plants received DC stimulus with anode or cathode attached to each of the cotyledons (VI).

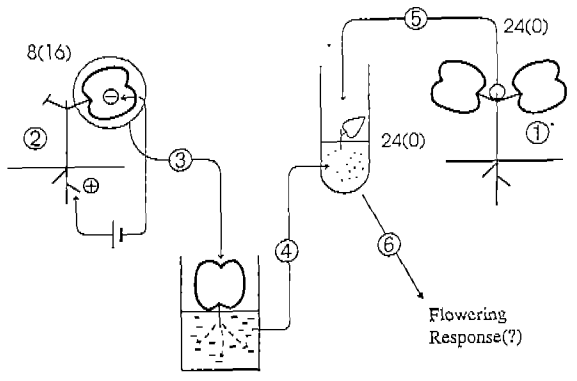

Fig. 1. Bioassay system for flower-inducing activity in phloem exudate; ① vegetative seedling grown under continuous light; ② florally-induced cotyledon with or without DC stimulus; ③ excised cotyledon dipped into distilled water to get phloem exudate; ④ phloem exudate added to the culture medium; ⑤ apex region excised and cultured in the medium prepared as ④; ⑥ flowering response of the apex culture analyzed to confirm the floral stimulus activity in the exudate.

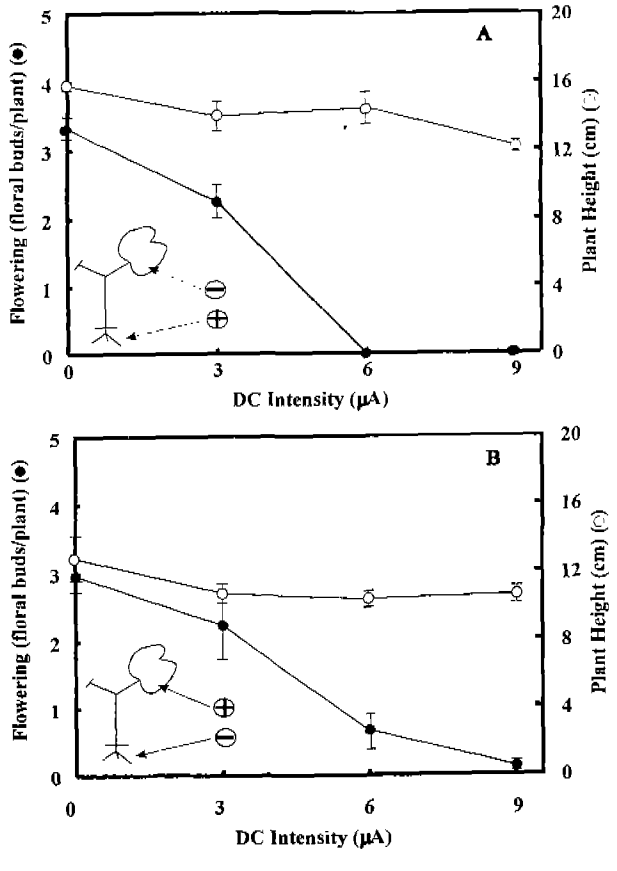

Fig. 2. Effect of DC stimulus for 16 h-dark period with various intensities and polarities on flowering in *Pharbitis nil*. Vertical bars represent SE.

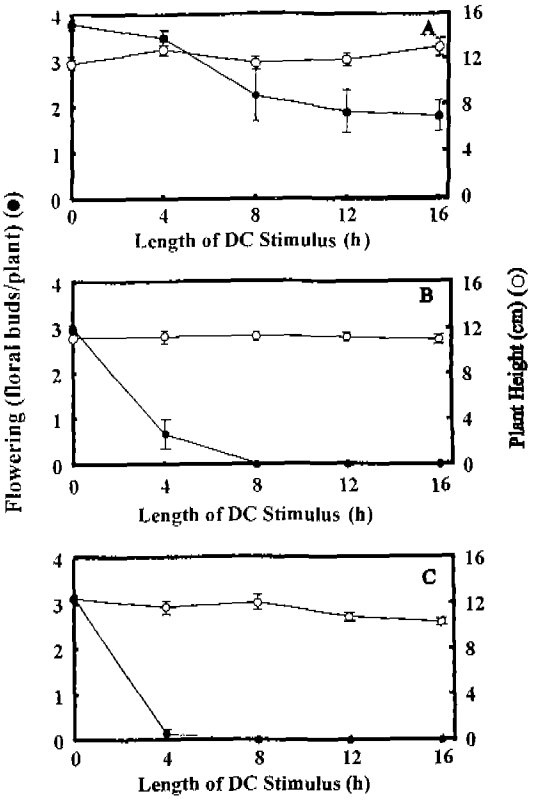

Fig. 3. Effect of DC stimulus of various lengths on flowering in *Pharbitis nil*. The current began to stimulate the plants at the start of a 16 h-dark period. DC intensities are; 3 μA (A), 6 μA (B), 9 μA (C). Anode was connected to roots and cathode to a cotyledon. Untreated cotyledon was cut off. Vertical bars represent SE.

RESULTS

Plants grown under continuous light for vegetative growth were induced to flower by a single 16 h dark period during which DC stimulus with various intensities and polarities was applied, and the floral response was analyzed (Fig. 2). The flowering was decreased as the DC intensity increased. Direct electric current passing through the plant with the anode connected to roots and the cathode to a cotyledon was found to be more inhibitory compared to the current of opposite polarity. Six μA DC completely depressed flowering. The apex growth was not significantly affected by the DC stimulus with its intensity up to 9 μA.

Sensitivity of the plants to DC stimulus with various intensities and duration which was imposed to the plants at the start of a 16 h inductive dark

Table 2. Effect of DC stimulus (6 μA) with various attachment loci of cathodes on the flowering in *Pharbitis nil* with a single cotyledon. Anodes were connected to the root. Plants were stimulated by DC throughout a 16 h inductive dark period. Data are shown with SE

Cathode connected to	Flowering (floral buds/plant)
Control (no stimulus)	3.67± 0.12
Cotyledon	0
Distal end of cotyledonary petiole	0
Proximal end of cotyledonary petiole	0.20± 0.10
Hypocotyl	3.25± 0.22
Apical bud	0

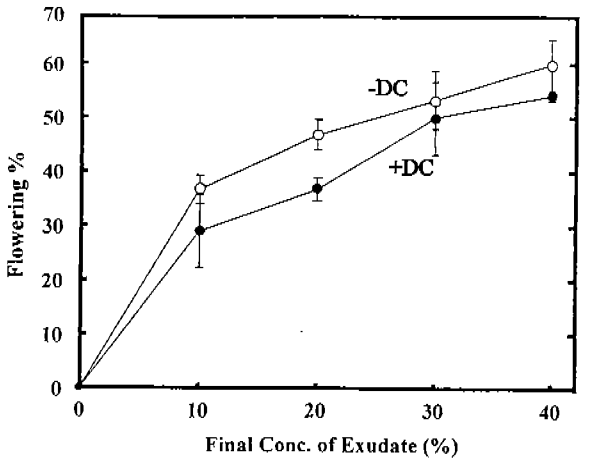

Fig. 6. Flowering responses of vegetative apex cultures of *Pharbitis nil* to the phloem exudate collected from cotyledons of *Pharbitis nil* exposed to a single 16 h dark period with or without DC (6 μA) stimulus. Cathodes were connected to the cotyledons and anodes to the root. Data are shown with SE.

riod still exerted its inhibitory effect bringing floral response down to 64.1% of the control. The DC stimulus applied 4 h after the end of the inductive dark period showed no effect on the flowering.

In the experiment so far performed, DC stimulus was set between roots and one of the cotyledons with untreated one cut off just before the DC application, so that the depressive effect of DC on the flowering might not be masked by floral stimulus activity possibly produced in the untreated cotyledon. It was shown that floral response in *Pharbitis* became saturated by a 16 h dark period regardless of the presence of an untreated cotyledon on the plant (Fig. 5, I and III). Once one of the cotyledons was excised and DC was applied to the remaining cotyledon which was the only possible source of the floral stimulus, the flowering was completely inhibited (Fig. 5, II). The production of the floral stimulus in the untreated cotyledon, however, was not much hampered by DC treatment on the opposite cotyledon, i.e. the flowering was depressed only by 17.7% by DC (Fig. 5, III and IV). When DC flowed either from roots to both cotyledons or from one cotyledon to another throughout the inductive dark period, the flowering was completely blocked (Fig. 5, V and VI).

The floral stimulus once produced is believed to be translocated through the phloem tissue from the cotyledon to the apex. When somewhere along the translocation path is impaired, the floral stimulus cannot successfully reaches the apex, so that significant level of flowering cannot be expected. With the anode fixed on the roots, the cathode was set to various loci on the plants during the inductive dark period (Table 2). The flowering was completely inhibited when DC was applied to either distal or proximal side of the cotyledonary petiole which was the path of floral stimulus translocation. The current which flowed from roots only up to the hypocotyl so that the DC could not directly reach the translocation path was shown to be ineffective.

Two methods were adopted in the present study for analysis of floral response in *Pharbitis*. The first method was to measure the frequency of floral bud development in the plants which had previously been received DC stimulus during the inductive dark period. And the second one was, as outlined in Fig. 1, to confirm and quantify the flower inducing activity in the phloem exudate to convert vegetative apices to floral ones. Using the bioassay system, it was found that the phloem exudate collected from DC-stimulated cotyledons which would have never evoked floral buds at their own apices contained significant level of flower inducing activity (Fig. 6). Within the range of 10 to 40% final concentrations, in the bioassay medium, the phloem exudates collected from DC-stimulated cotyledons could still evoke flowering which was 78.6% to 93.8% levels of the corresponding controls.

The application of DC stimulus to a cotyledon either in the first or in the second half of a 16 h dark period could reduce the floral stimulus activity in the bioassay by 5.5% and 22.5% respectively, show-

Table 3. Effects of DC (6 μA) on the activity of floral stimulus in phloem exudate (10%) collected from florally-induced cotyledons of *Pharbitis nil* exposed to a 16 h dark period. Cathode was connected to a cotyledon or to cotyledonary petiole, with non-stimulated cotyledon cut off before DC treatment. The activity was measured in vegetative apex culture bioassay system as described in MATERIALS AND METHODS. Anodes were connected to roots of the seedlings. Data are shown with SE

DC treatment period	Cathode on	Flowering percentage
None	None	40.0± 2.4
First half of IDP[a]	Cotyledon	37.8± 8.0
Second half of IDP	Cotyledon	31.0± 8.9
IDP	Cotyledon	29.0± 6.7
IDP	Cotyledonary petiole	36.0± 3.4

[a]IDP, a 16 h inductive dark period.

ing the second half of the dark period was more sensitive to DC treatment with respect to the potency of floral stimulus present in the phloem exudate (Table 3). The activity in the phloem exudate was lowered by 27.5% by DC flowed from roots to blade of a cotyledon, while DC from roots to a cotyledonary petiole could lower the floral stimulus activity in the phloem exudate only by 10%. It was shown that the activity in the phloem exudate was not significantly reduced unless DC directly passed through cotyledons which are the site of the floral stimulus production upon receiving photoperiodic signal.

DISCUSSION

Is the floral inhibitory action of DC applied to leaves or cotyledons due to deteriorating the synthesis of floral stimulus or, to interfering with the transport of the stimulus from leaves or cotyledons being induced to the apex? In reports on *Chenopodium* (Adamec *et al.*, 1989; Macháčková *et al.* 1990) and *Lactuca* (Montavon and Greppin, 1983, 1986) where DC action was limited during the inductive dark period and polarity-specific, it was discussed that DC might probably interfere with the transport to exert its action. In the present study, polarity-dependency of DC action on the flowering in *Pharbitis* was found to be very low, and the inhibitory action of DC was not only limited within the inductive dark period but also extended in the light periods sandwitching the dark (Fig. 2 and Table 1). The flowering was completely arrested although DC application was so limited in the light period before the start of the inductive dark that the flower induction processes in cotyledons could occur without DC stimulus. Severe inhibition of flowering was also observed when cotyledons had been stimulated by DC even right after the end of the inductive dark period. The fact that the flowering was depressed by the pre- and post-treatments by DC let to postulation that the inhibition might probably be caused by; deterioration of physiological and histological environment for the initiation of flower induction processes in cotyledons, degradative biochemical changes leading to improper supply of precursors and enzymes involved in the production of floral stimulus, decreasing potency of floral stimulus having been produced, interfering with the transmission of the stimulus toward the apex, or damage in the apex leading to the failure of responding to the floral stimulus. The last probable cause was ruled out by the result that apex was still responsive to the floral stimulus transmitted from an untreated cotyledon (Fig. 5, IV).

The second half of the inductive dark period was almost twice as much sensitive to DC stimulus as the first half (Fig. 4). Considering that the critical dark period for flower induction in *Pharbitis* was 11.5 h (Vince-Prue, 1985) and the translocation velocity of *Pharbitis* floral stimulus was approximately 0.3 $m \cdot h^{-1}$ (King *et al.*, 1968), the differential sensitivity between the first and second halves of the inductive dark period reflected a probability that impediment of the transmission rather than the formation of the floral stimulus might be more likely to have occured. This suggestion was further supported by the results that the flowering was not affected by DC stimulus when it was applied between roots and hypocotyl so that the transmission path for the floral stimulus toward the apex via cotyledonary petiole was not directly stimulated by DC (Table 2).

Flower inducing activity was confirmed in phloem exudates of *Perilla* (Purse, 1984) and of *Pharbitis* (Ishioka *et al.*, 1990, 1991). The bioassay system for the detection of flower inducing activity in phloem exudate, previously proposed by Ishioka *et al.*, was adopted in the present study. It should be accepted that there might be possible degradation or loss of

the floral stimulus in the process of collecting and handling the phloem exudate for bioassay, so that the floral evocation activity thus measured in the bioassay will not always quantitatively reflect the potency of the flower stimulus working at its apex. Although there was no information on how fast and how much the floral stimulus diluted in bioassay medium could possibly be absorbed into vegetative apices, it was confirmed that within the range of 10 to 40% final concentrations of the phloem exudate in the bioassay medium the floral evocation activity was highly correlated and proportional to the final concentrations of the exudate (Fig. 6). In the vegetative apex culture bioassay, a significant level of floral evocation activity was still exist in the phloem exudate collected from DC-stimulated cotyledons on the plants which otherwise could never be expected to flower (Fig. 6). The result confirmed a hypothesis that the floral stimulus had been synthesized in the DC-stimulated cotyledons of *Pharbitis* but could not successfully reach the apex due to temporary and partial blockage on its transmission path. The idea was further supported by the result shown in Fig. 6 that the floral stimulus could bypass the inhibitory electric current to evoke the vegetative apices to floral buds in the bioassay.

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나팔꽃의 開花와 子葉篩管排出液內 開花刺戟 活性에 대한 電流의 影響

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적 요

단일식물인 나팔꽃(*Pharbitis nil* Choisy cv. Violet)의 개화는 16시간의 개화유도 암기 중 자엽에 가한 직류자극에 의하여 억제되었는데 개화유도 암기중 후반부가 전반부에 비하여 전기자극에 더 민감하였고, 직류의 극성이 뿌리에서 자엽 방향일 때 그 반대 방향의 경우에 비하여 훨씬 억제 영향이 큰 극성의존성 효과가 관찰되었다. 개화는 자엽으로부터 줄기 정단부에 이르는 개화자극의 이동경로에 가해진 전기자극에 의해서만 민감히 억제된 것으로 보아 개화억제가 개화자극 이동과정의 저해 때문인 것으로 추측되었다. 개화유도 암기중 자엽에 전기자극을 받은 식물의 개화과정이 완전히 억제된 경우에도, 이 자엽을 분리하여 얻은 자엽사관배출액내에는 여전히 상당한 수준의 개화자극 활성이 존재하고 있음이 영양형 정단 배양, bioassay를 통하여 확인되었다. 따라서 본 연구의 결과는 직류자극에 의한 나팔꽃의 개화억제 현상은 자엽내의 개화자극 생성 자체의 저해보다는 정단부로 이동하는 개화자극의 이동과정의 일시적 장해에 기인할 것이라는 가설을 강력히 뒷받침한다.

주요어 : 나팔꽃, 개화, 직류전류, 사관배출액, 개화자극

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