Varietal Difference in Photoperiodic Behavior of
Pharbitis nil

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In their classical study on photoperiodism, Garner and Allard found that various strains of soybean show different photoperiodic behavior, and later this was studied in detail. Similar results have been reported for many other plants, such as Xanthium, Chenopodium, Oryza, Solidago, Andropogon, Eupatorium, Gossypium and Hordeum. That Pharbitis nil is also one of them has been reported by Muramatsu and Sakamoto. They planted several strains of Pharbitis from December to June in conditioned greenhouses of Kyoto and Misima which locate at about 35°N. Under constant temperature of 30° in the greenhouse, the position of node bearing the first flower bud of a strain from North China was always lower than in the southern strains. This varietal difference was conspicuous especially under long-day condition of the summer.

This paper deals with (1) photoperiodic behavior of six strains of Pharbitis nil and (2) physiological basis of the photoperiodic difference investigated by grafting two strains with different photoperiodic behavior.

Materials and Methods

The following strains were used in the present investigation.

1) Tendan (in abbreviation T), collected by H. Kihara in the suburb or Peking in North China in 1938. Since many of characters of this strain are like those of wild types, it is almost certain that the original population in Peking was a wild one. However, it is not known for certain whether it was indigenous or fairly recently naturalized.

2) Violet (abbr. V), received from H. Kihara in 1938. This strain has been used for the investigation of photoperiodism in Kyoto University. This cultivated form has large violet flowers.

3) Shif ukurin (abbr. S), one of the Japanese garden varieties used by S. Nakayama at Miyazaki University. Since it has large violet flowers with white margin, it was named “Violet Flower with White Margins” in his early report. The new name was recently given by him.

4) Kidachi (abbr. K), a dwarf mutant of a Japanese garden variety closely related to Violet. It has a stunted stem which attains only the length of one meter at the maximum. In late fall, it often develops one meter long or longer side branches.
5) Nepal No. 850 (recorded as Nepal, abbr. N), one of the wild strains collected in the mountainous region of Nepal by S. Nakao, a member of Nepal-Himalayan Expedition of Kyoto University in 1952. This strain has small blue flowers like those of Tendan and Africa.

6) Africa (abbr. A), collected by K. Furusato at Konakry of Guinea in Western Africa in 1955. This strain differs from the five strains mentioned above in many characters, especially in that the plant has abundant hairs and its axillary inflorescence has 6-8 flowers, while the other strains have 1-3 flowers only. The present authors are convinced that this strain can be classified as a variety of Pharbitis nil, because it easily crosses with other strains of Pharbitis nil, and the F1 plants are completely fertile.

Those six strains have been maintained in the Laboratory of Applied Botany, Kyoto University, and were expected to be highly homozygous. No phenotypically recognizable segregation was observed among their self-pollinated progeny.

Before seeding the seeds were treated with conc. H₂SO₄ for 30-40 minutes and washed thoroughly in running water. They were then sown under continuous illumination. The photoperiodic response of various strains was examined when the young seedlings had developed the cotyledons only. For dark treatment small cabinets furnished with daylight fluorescent lamps were used. Light intensity in the cabinets was 4000 lux at the plant level and the temperature was maintained at 22±1°C. Each dark period was terminated by daylight fluorescent light which was switched by an automatical device. Although the photoperiodic behavior of an adult plant may differ slightly from that of a seedling, the varietal difference among strains should remain unchanged.

In grafting experiments, three strains Tendan, Nepal and Africa were used. One experiment was performed at a constant temperature of 30°C, and others were carried out in an ordinary greenhouse where the temperature varied considerably.

### Results

I. Photoperiodic response of various strains.

   a) Flowering response under varying night lengths

Four-day-old seedlings grown in the greenhouse under continuous illumination supplemented at night with incandescent light were placed in the cabinets at 6:00 p.m. and kept in darkness for 7 to 14 hours increasing the time by one hour. After that all plants were returned to the greenhouse at 9:00 a.m. and exposed to sunlight until 6:00 p.m. The treatment was repeated for 7 days, then all plants were removed to continuous illumination in the greenhouse. Flowering responses were observed three weeks after the end of the treatments. The results are expressed by three criteria; 1) percentage of plants with flower buds, 2) number of flower buds per plant, and 3) percentage of plants with a terminal flower bud (Fig. 1).

Among all strains, the minimum dark length required to initiate flower buds was shortest for Kidachi followed by the other four strains in the order: K<T<V<S<N (Fig. 1). Africa did not initiate flower buds after 7 short days consisting of 10-hour light and 14-hour dark periods. This order for flowering response among the strains remained firm when the length of the dark period was fixed so that it would bring about 50% flowering, 100% flowering or three flower buds per plant. All plants of Tendan and Violet initiated terminal flower buds after 12 hours and
Shifukurin after 13 hours of darkness, whereas only 20% of Nepal plants produced terminal flower buds after 14 hours of darkness. The strain Kidachi had a peculiar behavior. It has the shortest critical dark period to produce axillary flower buds, but produced no terminal flowers even if the dark period was prolonged up to 14 hours (Fig. 1).

b) Number of short-day cycles required for flowering.

In this experiment the dark period was kept constant, namely after an 8-hour light a 16-hour dark period followed, and the number of short days was taken as a variable. The results are given in Fig. 2. To induce 100% flowering one short day was enough for Tendan, Violet, Shifukurin and Kidachi, but two short days were necessary for Nepal and 10 short days for Africa. One short day was enough to induce terminal flowers in all plants for Violet and Tendan, and two short days were required for Shifukurin. In Nepal 39% of the plants produced a terminal flower after 7 short day cycles. In Kidachi and Africa no terminal flower appeared even if they were subjected to 10 short days.

An interesting contrast in the flowering response was found between Kidachi and Nepal. With one short day, all plants of Kidachi initiated flower buds, but only 33% of Nepal. After 1-5 short day treatments the number of flower buds produced was larger in Kidachi than in Nepal. Nevertheless Kidachi did not initiate any terminal flower with 10 short days, while some Nepal plants produced terminal flowers with 7 short days.

With the exception of Kidachi, the strains with high photoperiodic sensitivity tended to have a shorter critical dark period, and, under a certain definite photoperiod, to initiate more flower buds and to produce terminal flower buds more easily than the strains with low sensitivity (cf. a)).

II. Grafting experiments between strains with low and high photoperiodic sensitivity.

Since the flowering stimulus is produced in leaves and induces flower buds at
the growing apex, the difference could be attributable, at least, to two factors. One could be the reactability of the shoot apex to the flowering stimulus, and the other could be the productivity of the flowering stimulus in the leaves. Should the latter be the deciding factor, the leaves of the sensitive strain would produce a larger amount of flowering stimulus during a definite dark period than those of the less sensitive strain. On the other hand, if the former holds true the shoot apex of the sensitive strain should respond stronger to a definite amount of floral stimulus than that of the less sensitive strain. We thought that grafting experiments between strains with different sensitivity may provide some critical information. Two strains, one with high and the other with low sensitivity, were chosen and grafted by approach at the first node. The grafted plants were divided into two groups. In one group, one just fully expanded leaf on a highly sensitive plant and one bud on a less sensitive plant were left intact and all other leaves and buds were removed. In the other group, on the contrary, a single leaf on a less sensitive plant and a bud on a highly sensitive plant were left intact. As controls, two other groups of grafts were made. They were between two plants from the same strain, and all leaves and buds were removed leaving one leaf on the donor plant and one bud on the receptor. The four groups of grafted plants were subjected to short days. Adequate treatment, i.e. duration of dark period and number of short day cycles to be given, was determined according to the results obtained from preliminary experiments. The results are presented in Table 1.

Experiment 1: Grafting between Tendan and Nepal.

Tendan and Nepal were sown on February 8, 1956, under continuous illumination at 30° and were grafted on March 14-17. In this experiment, with some exceptions, the receptor bud and the donor leaf were located at the second node. The grafts were planted in randomized order in four wooden boxes. Since the growth was fast under this condition, the connection of tissues was completed within several days. After confirming that all graftings were successful, the plants were subjected
to four consecutive short days consisting of ca. 14-hour light and ca. 10-hour dark periods at 20° from March 21. After the treatments the plants were grown under continuous illumination at 20°. Flowering responses were examined on June 29. By this time, most of the plants have developed more than ten leaves and have restored vegetative growth after having initiated some axillary flower buds.

As shown in Table 1, the leaf of Tendan gave rise to more flower buds on the partner plant than that of Nepal, regardless of the kind of the receptor bud. The bud of Tendan initiated more flower primordia than that of Nepal, regardless of the

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<th>Table 1. Grafting experiment</th>
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<td><strong>Response</strong></td>
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<td>Flowers* quotient</td>
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<td>% Flowering</td>
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<td>No. of flower buds</td>
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<th><strong>Response</strong></th>
<th><strong>Experiment 3</strong></th>
<th><strong>Experiment 4</strong></th>
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<td><strong>Bud</strong></td>
<td><strong>Leaf</strong></td>
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<tr>
<td>Flowers* quotient</td>
<td>Tendan</td>
<td>16/16</td>
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<td>% Flowering</td>
<td>100</td>
<td>73</td>
</tr>
<tr>
<td>No. of flower buds</td>
<td>$4.2 \pm 0.45$</td>
<td>$1.6 \pm 0.43$</td>
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| Flowers* quotient | Africa | 10/10 | 2/9 | | Africa | 15/15 | 1/9 |
| % Flowering | 100 | 22 | | Africa | 100 | 11 |
| No. of flower buds | $2.2 \pm 0.05$ | $0.2 \pm 0.09$ | | $3.9 \pm 0.25$ | 0.1 | 0.12 |

* Number of receptors with flower buds divided by number of grafts observed.
** Two plants initiated terminal flowers.
*** Thirteen plants initiated terminal flowers.

Experiment 1: Sown on Feb. 8, grafted on March 14-17, treated 4 days with dark periods of 10-10 1/4 hours, March 21-25, observed on June 29, 1956.
Experiment 3: Sown on Sept. 3, grafted on Sept. 15, treated by 3 cycles consisting of 8-hour light and 16-hour dark periods from Sept. 26, observed on Oct. 25, 1963.
Experiment 4: Sown on Sept. 7, grafted on Sept. 19, treated by 4 cycles consisting of 8-hour light and 16-hour dark periods from Sept. 30, observed on Oct. 31, 1963.
kind of donor leaf which supplied the floral stimulus. However, no significant difference in flowering response was obtained between the grafts which had a leaf of Nepal and a bud of Tendan and those which had a leaf of Tendan and a bud of Nepal.

This result indicates that the stimulus produced in the leaf of Nepal was strong enough to induce flowering in Tendan, but not in Nepal itself. On the other hand, the bud of Nepal could react to the stimulus produced in the leaf of Tendan, but not to the stimulus produced in its own leaf under the same condition. Thus, the stimulus produced in the leaf of Nepal under this experimental condition was considered to be weaker than that of Tendan, and the bud of Nepal to be less sensitive than that of Tendan.

Experiments 2, 3 and 4: Grafting between Tendan and Africa.

Some experiments similar to that mentioned above using Africa instead of Nepal were performed in an ordinary greenhouse at varying temperature in 1963. Dark treatments were given by enclosing the donor leaf in a small bag of light-proof paper to avoid the obscuration of the developing receptor bud. As shown in the 2nd, 3rd and 4th experiments of Table 1, when two different strains were grafted, the flowering responses were intermediate between those obtained from the two groups of grafts within the same strain. Stronger flowering responses were observed when Tendan contributed the donor leaf than when it contributed the receptor bud. This tendency was particularly clear in experiment 2 where Africa as donor leaf could not induce flower buds in Tendan.

These results indicate that the difference in flowering response of these strains is certainly attributable to a difference both in leaf and bud, and that the physiological difference in the leaves seems to have more pronounced effect upon the varietal behavior.

Discussion

Usually photoperiodic sensitivity of short-day plants is evaluated by the minimum number of short day cycles required to initiate flower buds. In general the higher is the photoperiodic sensitivity the shorter is the critical dark period. Such may be also the case with Pharbitis nil. The results obtained in the present experiments show that the photoperiodic sensitivity of the strains tested, with the exception of Kidachi, stands in the following order: Tendan > Violet > Shifukurin > Nepal > Africa. Kidachi has a peculiar behavior. It has the shortest critical dark period; 100% flowering is induced by a single dark period of 16 hours and the number of flower buds increases gradually with increasing number of short days, but seldom a terminal flower bud is produced. Thus, this strain differs from the other strains in its manifestation of the photoperiodic response. Such a difference may in some way be related physiologically to the sluggishness of the activity of the shoot apex. Though this is very probable, it still requires further experiments.

It is well known in many plants that the photoperiodic behavior is heritable. I. Ito crossed Tendan with Violet, and found that the photoperiodic response of the F₁ was like that of Tendan. The F₂ segregation ratio was complicated; I. Ito thought that the number of plants used was not large enough for the analysis of the genes responsible for the difference in flowering habit. M. Muramatsu and S. Sakamoto also made some preliminary crosses using several strains without obtaining any conclusive
Varietal differences in the flowering habit of soybean were investigated by Kiyosawa and Kiyosawa by the grafting method. They concluded that a late variety might produce a flowering inhibitor under the conditions under which a midseason variety produces flowers. An early variety seemed to produce a flowering hormone which suppressed the flower-inhibiting action of the late variety. The midseason variety produced equal or smaller amounts of flowering hormone as compared with the late variety.

In the present grafting experiments the intensity of flowering stimulus or amount of flowering hormone produced in the leaf of a sensitive strain during the dark period seems to be higher than that produced in the leaf of a less sensitive strain. Furthermore in sensitive strain flower buds can be induced more easily than in a less sensitive strain in response to the same amount of the stimulus. In many cases, however, the varietal difference in the productivity of the floral stimulus in leaves seemed to be more important than that in the reactability of the bud. It is quite possible that an inhibitory factors and/or a weakening of the stimulus, when it is transmitted, played some significant role in the grafting experiments presented here. To investigate these possibilities further experiment should be carried out.

Grateful acknowledgment is given to Dr. F. Lilienfeld for her kind correction on English.

Summary

1) The photoperiodic sensitivity of Pharbitis nil can be evaluated by the minimum number of short day cycles and minimum duration of the dark period required for flower initiation, and by the readiness to initiate a terminal flower bud. The sensitivity of six strains tested stands in the following order: Tendan > violet > Shifu-kurin > Nepal > Africa. Kidachi, a dwarf mutant, is an exception. It has the shortest critical dark period but seldom produces a terminal flower bud.

2) Grafting experiments indicated that the sensitive strain produces in leaves larger amounts of flowering hormone during a definite dark period, and is induced more easily to flowering in response to the same amount of floral stimulus than a less sensitive strain.

References

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摘　要

今村駿一郎*・村松幹夫**・北条慎一***・淵本 敦*： アサガオ日長習性の系統間差異

種々の長さの暗期を7回与えた場合の限界暗期は木立、テンデン、紫、紫覆輪、ネパールの順に長くなり、アフリカでは14時間の暗期を7回与えても花芽をつけていない。始め挙げた4系統は暗期16時間の短日1回で100%花芽をつけるが、ネパールでは2回アフリカでは10回を要する。

反応性を異にする2系統を呼び掛けして、1. テンデンの葉—テンデンの芽、2. テンデンの葉—ネパールの芽、3. ネパールの葉—テンデンの芽、4. ネパールの葉—ネパールの芽のうつの組合せを作り、短日処理すると1は沢山の花芽をつけ、4は花芽をつけず、2および3は少数の花芽をつける。この事から暗期に対する反応性の弱い系統では一定の長さの暗期中に葉の中に成立する刺激も弱く、同一刺激に対する芽の反応も弱いことが判る。（*京都大学農学部応用植物学教室、**木原生物学研究所、***岐阜県立坂下女子高等学校）