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Influences of Radiation Intensity, Plant Age and Benzyladenine on Photoperiodic Flowering of *Pharbitis nil* Seedlings

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Abstract

Effects of different radiant energies of the fluorescent light (photosynthetic photon flux densities of 10 to 700 μmol $m^{-2}s^{-1}$) and plant ages (3 to 8 day-old after planting) on the flowering response of *Pharbitis nil* seedlings, strains Violet and Kidachi, have been examined.

Flowering response to an inductive dark period declined remarkably with increasing age at any irradiances. The flowering response was strong under the irradiance between 70 and 300 μmol $m^{-2}s^{-1}$, with the maximum response shifting to the higher irradiance with increasing age. High irradiance of 700 μmol $m^{-2}s^{-1}$ always reduced the flowering response regardless of seedling age. The daily alternation of irradiation with sunlight (1000 μmol $m^{-2}s^{-1}$) from 09:00 to 17:00 AST and fluorescent light (200 μmol $m^{-2}s^{-1}$) resulted in stronger flowering than continuous irradiation of fluorescent light. The decline of flowering due to the irradiance as low as 30 μmol $m^{-2}s^{-1}$ or to ageing of the plant was dramatically reversed by benzyladenine (BA) application to the cotyledons, but that due to the high irradiance of 700 μmol $m^{-2}s^{-1}$ was not.

The implications of these findings are discussed in relation to senescence of cotyledons with increasing in age, photosynthate supply, endogenous rhythm and also action of BA in the light period preceding the inductive dark period.

Key words: Benzyladenine-Flowering-*Pharbitis*-Plant age-Radiation intensity.

Introduction

There have been many reports concerning the photoperiodic control of flowering of *Pharbitis* seedlings using either the tall strain, Violet or the dwarf one, Kidachi. They include the influence of light qualities in Violet[20,21], the response to exogenous cytokinins in Violet[8,15] or gibberellins in Kidachi[9,12]. However, growing conditions differed in those studies and especially the light condition and the age of plant before dark induction, both of which influence flowering response.

Pharbitis seedlings raised in darkness may require light period of about 1000 lux for flowering, suggesting that photosynthesis may be one of the factors affecting the photoperiodic sensitivity[7]. However, dark-grown seedlings became photoperiodically sensitive when exposed only one exposure for a few minutes to red light followed by benzyladenine (BA) treatment[14]. This indicates that the light period process involves more factors than photosynthesis, suggesting that there exists a role for phytochrome-active light[6]. Furthermore, the flowering response of 4 day-old *Pharbitis* seedlings raised under 200 μmol $m^{-2}s^{-1}$ photosynthetic photon flux densities can be promoted by the BA applied to the cotyledons[13].

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In the studies presented here *Pharbitis* seedlings of strains, Violet and Kidachi, have been examined for their flowering response to different irradiances and ages of the plant before exposing to an inductive dark period. Effects of BA applied to the cotyledons on the flowering response to different irradiation intensities and plant ages have also been examined for the strain Violet.

Materials and Methods

Seeds of *Pharbitis nil* Choisy, strains Violet and Kidachi, were treated with concentrated H_2SO_4 for 45 min and then washed in running water overnight at 29°C. The seeds were sown in 12 cm diameter pots which contained a mixture of equal parts of perlite plus vermiculite. In one series of experiments, the seedlings were raised for 3 to 8 days (4 to 9 days after seed imbibition) under continuous white fluorescent light of different photosynthetic photon flux densities (10 to 700 μmol $m^{-2}s^{-1}$, 400 to 700 nm) which were set using a LICOR quantum sensor (LICOR Instruments, NB). Each group of plants was exposed to a 15 h inducitve dark period at 27°C from 17:00 AST on various days. In the other series, the seedlings were raised at 29°C under the sunlight (800 to 1000 μmol $m^{-2}s^{-1}$ at midday) from 09:00 to 17:00 and then placed under fluorescent light (200 μmol $m^{-2}s^{-1}$) until 09:00 next morning, and designated here as sunlight alternation. Just before the dark period, the hypocotyl length in cm and the area of cotyledons were measured, the latter being represented in cm^2 by multiplying length and width. For the treatments with BA, aqueous solutions of various concentrations with 0.05% (v/v) Tween-20 was applied to the cotyledons using a paint brush. Control plants were treated with water containing 0.05% (v/v) Tween-20. After the inductive darkness the seedlings were raised under the continuous fluorescent light at 25°C until the dissection for measurement of flowering response. The seedlings were watered twice a day, once with water and once with the Hoagland nutrient solution. There were two or three pots with 14 to 21 plants for each treatment. Values given in the figures are means $\pm$ the standard error of the mean.

Results

1. *Violet*

Hypocotyl elongation of Violet is shown in Fig. 1-a. The lower the irradiance, the longer the hypocotyl at every plant ages. The hypocotyl of plants irradiated at 10 μmol $m^{-2}s^{-1}$ was 1.5 times longer than that at 70 μmol $m^{-2}s^{-1}$, while irradiation of 700 μmol $m^{-2}s^{-1}$ reduced hypocotyl to half in length compared with that of 70 μmol $m^{-2}s^{-1}$. The hypocotyl length in the other experimental series, the sunlight alternation, was similar to that irradiated continuously at 300 μmol $m^{-2}s^{-1}$. As seen in Fig. 1-b, the cotyledon gradually expanded with higher irradiance up to 70–200 μmol $m^{-2}s^{-1}$. Cotyledon expansion levelled off at about this irradiant intensity. The flowering response at different irradiances is shown in Fig. 1-c. Three day-old plants flowered strongly reaching a peak response at an irradiance of 70 μmol $m^{-2}s^{-1}$ and their flowering was decreased with higher irradiances. The flowering response at 700 μmol $m^{-2}s^{-1}$ was very weak and almost the same as poor as that at 10 μmol $m^{-2}s^{-1}$. Four day-old plants flowered best at about 200 μmol $m^{-2}s^{-1}$. With older seedlings the flowering response decreased and especially remarkable was the decrease at lower irradiances than 200 μmol $m^{-2}s^{-1}$. Eight day-old plants produced no flower at any irradiances.

Plants raised under the sunlight alternation flowered better than those raised under the continuous

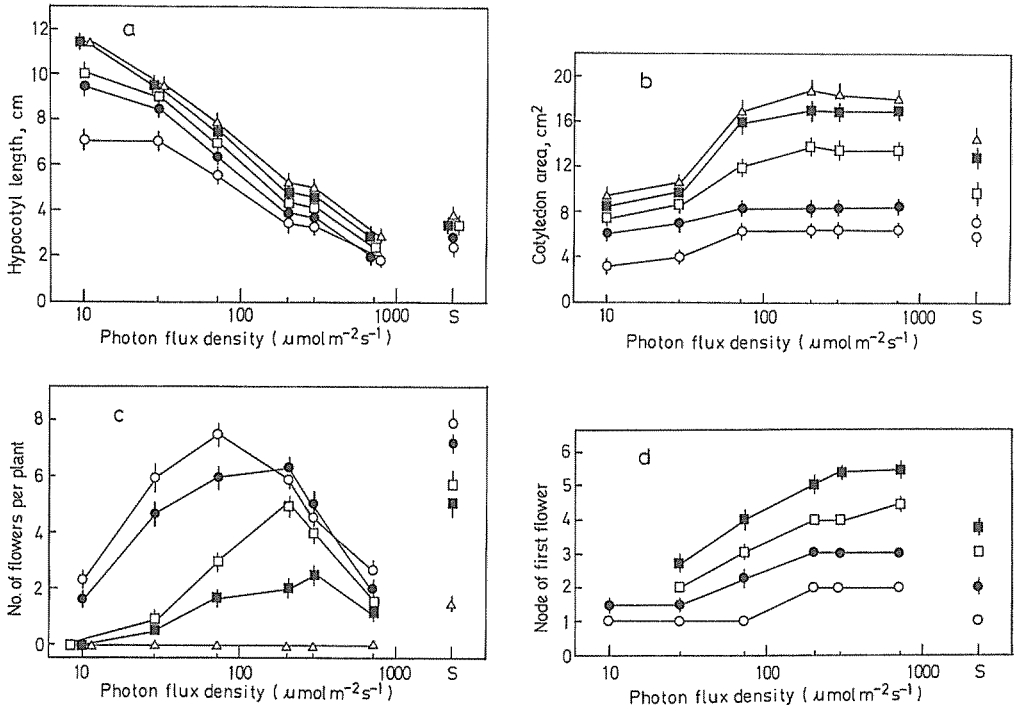

Fig. 1. Hypocotyl length (a), cotyledon area (b), flowering (c) and node bearing first flower (d) of *Pharbitis*, Violet, irradiated with fluorescent light of different photosynthetic photon flux densities (PPFD) and sunlight alternation (S) as function of plant age; 3 (○), 4 (●), 5 (□), 6 (■) and 8 (△) day-old.

fluorescent light of any irradiances. Even 8 day-old plants in the sunlight alternation were capable of flowering (Fig. 1-c). As seen in Fig. 1-d, for 3 day-old plants the first flower was formed at the first node of the main axis at 10 to 70 μmol $m^{-2}s^{-1}$ and at the second node at 200 μmol $m^{-2}s^{-1}$ or more. The node number bearing the first flower increased by about one node per day with the increasing age at 70 to 700 μmol $m^{-2}s^{-1}$ and in the sunlight alternation.

The flowering response to various durations of an inductive dark period was examined with 5 day-old plants raised under different irradiances. As seen in Fig. 2, the plants irradiated at 70 to 300 μmol $m^{-2}s^{-1}$ flowered in

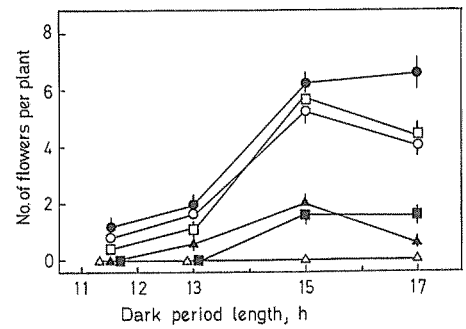

Fig. 2. Flowering response of 5 day-old *Pharbitis* seedlings, Violet irradiated with fluorescent light of different photosynthetic photon flux densities at 10 (△), 30 (▲), 70 (○), 200 (●), 300 (□) and 700 (■) μmol $m^{-2}s^{-1}$ to various durations of the dark period.

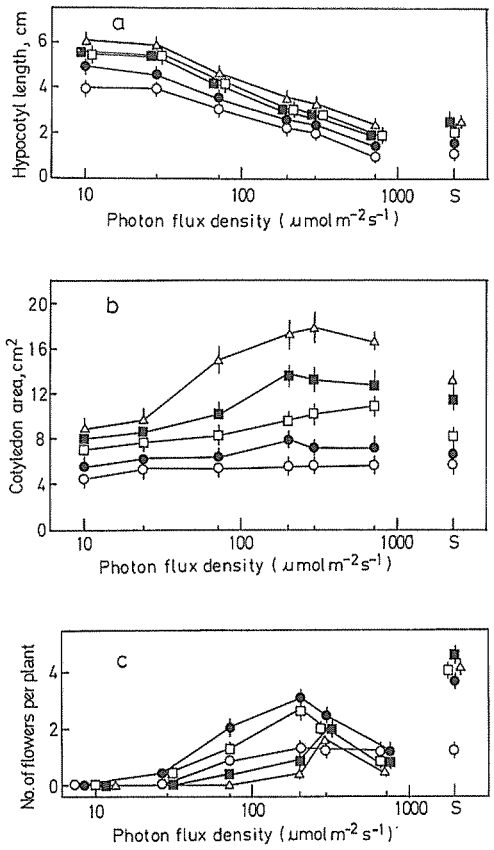

Fig. 3. Hypocotyl length (a), cotyledon area (b), flowering (c) of *Pharbitis*, Kidachi, irradiated with fluorescent light of different photosynthetic photon flux densities and sunlight alternation (S) as function of plant age; 3 (○), 4 (●), 5 (□), 6 (■) and 8 (△) day-old.

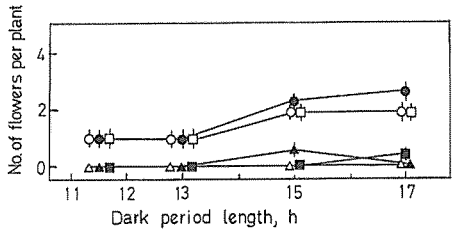

Fig. 4. Flowering response at 5 day-old *Pharbitis* seedlings, Kidachi irradiated with fluorescent light of different photosynthetic photon flux densities at 10 (△), 30 (▲), 70 (○), 200 (●), 300 (□) and 700 (■) μmol $m^{-2}s^{-1}$ to various durations of the dark period.

response to a 11.5 h dark period while those at 30 or 700 μmol $m^{-2}s^{-1}$ flowered only with a longer dark period than 13 h. Plants irradiated at 10 μmol $m^{-2}s^{-1}$ did not flower with any duration of dark period.

2. *Kidachi*

As shown in Fig. 3, compared with the strain Violet, Kidachi was about half in hypocotyl length (a) but was similar in cotyledon area (b). At higher irradiances the hypocotyl became shorter and cotyledon expansion was promoted up to 200 μmol $m^{-2}s^{-1}$. The pattern of flowering (c) depended on the irradiation and the plant age in a similar manner to that of Violet although the flowering of 3 day-old Kidachi levelled off at 200 to 700 μmol $m^{-2}s^{-1}$ and the number of flowers at any age was much less. At low or high irradiances (below 70 μmol $m^{-2}s^{-1}$ or above 300 μmol $m^{-2}s^{-1}$) flowering did not occur or did weakly. On the other hand, in the sunlight alternation the plants older than 4 days flowered much more than those in the continuous fluorescent light. The flowering response of Kidachi in the sunlight alternation was better than that of Violet.

The flowering response to various durations of darkness given to 5 day-old plants raised under different irradiances was examined (Fig. 4). Flowering of the plants irradiated by 70 to 300 μmol $m^{-2}s^{-1}$ occurred at 11.5 h dark period and increased with increase in dark period length up to 15 h. However, there was little or no flowering for plants irradiated either by 10 and 30 μmol $m^{-2}s^{-1}$ or 700 μmol $m^{-2}s^{-1}$.

3. *Benzyladenine treatments*

The effect of applying BA on flowering under different irradiances was examined. An aqueous solution of 0.22 mM BA was applied to the cotyledons of 5 day-old Violet irradiated at 30, 200 or 700 μmol $m^{-2}s^{-1}$ before exposure to various durations of inductive dark periods of 11.5 to 17 h. The results

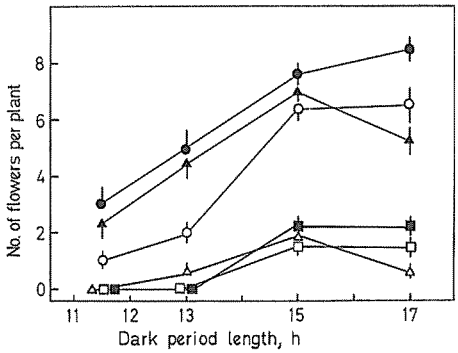

Fig. 5. Flowering response of 5 day-old *Pharbitis*, Violet irradiated by 30 (△, ▲), 200 (○, ●) and 700 (□, ■) μmol $m^{-2}s^{-1}$ (PPFD). The cotyledons were treated with 220 μM BA (▲, ●, ■) or water (△, ○, □) prior to various durations of dark period.

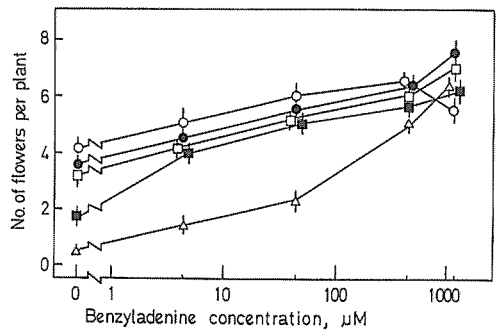

Fig. 6. Flowering response of 3 (○), 4 (●), 5 (□), 7 (■) and 8 (△) day-old *Pharbitis*, Violet irradiated by 200 μmol $m^{-2}s^{-1}$ (PPFD). At 3 day-old the cotyledons were treated with BA at various concentrations.

are shown in Fig. 5. The flowering of plants irradiated by 200 μmol $m^{-2}s^{-1}$ was promoted by BA in any duration of darkness as shown in our previous report[13]. However, the promotion by BA was much more evident in plants irradiated by 30 μmol $m^{-2}s^{-1}$ but not in plants by 700 μmol $m^{-2}s^{-1}$.

In order to examine effects of exogenous BA on the decline of flowering response with the ageing of plants, BA solutions at various concentrations (0 to 1100 μM) were applied to the cotyledons of 3 day-old Violet irradiated by 200 μmol $m^{-2}s^{-1}$. Thereafter one series of the plants was exposed to a 13 h inductive dark period at different days. As shown in Fig. 6, BA application prevented the decline of flowering due to plant ageing even at a low concentration of 4.4 μM. The higher BA concentrations, the greater the promotion of flowering and the blocking of the decrease of flowering with ageing. Thus, even 7 or 8 day-old plants flowered as strongly as 3 day-old plants when treated with 440 or 1100 μM BA.

Discussion

It is clear for both strains, Violet and Kidachi of *Pharbitis nil* that plant age influences flowering response in a manner quite distinct from its effect on hypocotyl elongation and cotyledon expansion (Figs. 1-c vs 1-a and b, and Figs. 3-c vs 3-a and b). The change in ability to flower with age also appears to differ from changes seen in various cellular components in cotyledon. For example, DNA and RNA (Ogawa, unpublished), protein and endogenous auxin[10], gibberellins[11] and cytokinins (Ogawa, unpublished) decrease rapidly or gradually with cotyledon age. At irradiance below photosynthetic compensation point of 15 μmol $m^{-2}s^{-1}$ the absence of and/or weak flowering response probably reflects a photosynthetic limitation although even at the low irradiance phytochrome-mediated light-energy reactions could be set in. Alternatively, exposure to continuous low irradiance could cause damping of endogenous rhythms as described by Bünning[1] especially for older seedlings (Ogawa in preparation). Such rhythms are known to be important for flowering response of *Pharbitis* seedlings[16]. A good flowering response under alternating irradiances with sunlight and fluorescent light could result from daily synchronization of the flowering rhythm to give an inductive phase coinciding with the 17:00 start of any inductive darkness (Ogawa in preparation).

Cytokinins such as BA retard leaf senescence of various plant species for intact[2,3,4] as well as excised leaves[19]. For intact leaves of *Phaseolus* Naito et al[5] reported that BA treatment increased DNA, RNA, protein and chlorophyll content, keeping the leaves young. Perhaps such changes occurred also in *Pharbitis* cotyledons after BA treatment and this may explain its ability to retard the age-dependent decline in flowering (Fig. 6). It is not clear why BA enhancement of flowering was greatest at low irradiance (30 μmol $m^{-2}s^{-1}$) but it was little at a high irradiance (700 μmol $m^{-2}s^{-1}$) (Fig. 5). The role of BA for flowering at the low irradiance is supposed to enhance assimilate supply to the apex as shown earlier by Ogawa and King[13,14]. This action might be great at the low irradiances but could be saturated at high irradiances. It is difficult, nonetheless, to distinguish the indirect action of BA on cotyledon senescence that may influence flowering from direct actions on floral processes or on cotyledon photoresponse. However, flowering was depressed by high irradiance even in very young seedlings with still expanding cotyledons (Figs. 1-a and b vs 1-c, and Figs. 3-a and b vs 3-c) and the seedlings exposed to shorter or longer dark period (Figs. 2 and 4). This suggests a further action of high irradiance than senescence. A further interaction between irradiance and flowering involves induction of flowering of *Pharbitis* irradiated by continuous fluorescent light at high intensity of 16,000 lux[18], probably equal to about 300 μmol $m^{-2}s^{-1}$. Such high irradiance strongly inhibited shoot elongation as was observed by Shinozaki[18], but reduction of flowering was resulted here. The discrepancy between these two sets of data may indicate difference in the mechanism of photoperiodically induced flowering as here and continuous light-induced flowering as studied by Shinozaki[18]. The common effect of inhibition of shoot elongation may be quite unrelated to short day-flowering and/or continuous light-induced flowering. Further studies are required from the viewpoint of photobiology as well as plant physiology.

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References

1) BÜNNING, E. *In* Physiological Clock. pp. 21, 56 and 74. Springer-Verlag (1964).
2) ENGELBRECHT, V. L. Über Keinetinwirkungen bei intakten Blättern von *Nicotiana rustica*. Flora, 154: 57–69 (1964).
3) FLETCHER, R. A. Retardation of leaf senescence by benzyladenine in the intact bean plants. Planta, 89: 1–8 (1969).
4) JACOBY, B. and J. DAGON. Effects of ^{6}N-benzyladenine on primary leaves of intact bean plants and on their sodium absorption capacity. Physiol. Plant. 23: 397–403 (1970).
5) NAITO, J., H. TSUJI and I. HATAKEYAMA. Effect of benzyladenine on DNA, RNA, protein and chlorophyll contents in intact bean leaves: Differential responses to benzyladenine according to leaf age. Physiol. Plant. 43: 367–371 (1978).
6) NAKAYAMA, S., H. A. BORTHWICK and S. B. HENDRICKS. Failure of photoreversible control of flowering in *Pharbitis nil*. Bot. Gaz. 121: 237–243 (1960).
7) MARUSHIGE, K. and Y. MARUSHIGE. Effect of light on the appearance of photoperiodic sensitivity of etiolated *Pharbitis nil* seedlings. Bot. Mag. Tokyo, 79: 397–403 (1966).
8) OGAWA, Y. Über die Wirkung von Kinetin aut die Blütenbildung von *Pharbitis nil* Chois. Plant Cell Physiol. 2: 311–329 (1961).
9) OGAWA, Y. Weitere Untersuchungen über die Wirkung von gibberellin-ähnlichen Substanzen auf die Blütenbildung von

Pharbitis nil. Plant Cell Physiol. 3: 5–21 (1962).

10) OGAWA, Y. Über die photoperiodische Empfindlichkeit der Keimpflanzen von *Pharbitis nil* Chois. mit besonderer Berücksichtigung auf dem Wuchsstoffgehalt der Kotyledonen. Bot. Mag. Tokyo 75: 92–101 (1962).
11) OGAWA, Y. Changes in the amounts of gibberellin-like substances in the seedlings of *Pharbitis nil* with special reference to expansion of cotyledon. Plant Cell Physiol. 5: 11–20 (1964).
12) OGAWA, Y. Stimulation of the flowering of *Pharbitis nil* Chois. by gibberellin A_3: Time dependent action at the apex. Plant Cell Physiol. 22: 675–681. (1981).
13) OGAWA, Y. and R. W. KING. Indirect action of benzyladenine and other chemicals on flowering of *Pharbitis nil* Chois. Plant Physiol. 63: 643–649 (1979).
14) OGAWA, Y. and R. W. KING. Establishment of photoperiodic sensitivity by benzyladenine and a brief red irradiation in dark grown seedlings of *Pharbitis nil* Chois. Plant Cell Physiol. 20: 115–122 (1979).
15) OGAWA, Y. and R. W. KING. Flowering in seedlings of *Pharbitis nil* induced by benzyladenine applied under a non-inductive daylength. Plant Cell Physiol. 21: 1109–1116 (1980).
16) PARASAKA, J. R. and C. SPECTOR. The initiation of an endogenous rhythm affecting flower bud formation in *Pharbitis nil*. Physiol. Plant. 32: 62–65 (1974).
17) SHAW, M., P. K. BHATTACHARYA and W. A. QUICK. Chlorophyll, protein and nucleic acid levels in detached, senescing wheat leaves. Can. J. Bot. 43: 739–746 (1965).
18) SHINOZAKI, M. Floral initiation of *Pharbitis nil*, a short day plant, under continuous high-intensity light. Plant Cell Physiol. 13: 391–393 (1972).
19) SUGIURA, M., K. UMEMURA and Y. OOTA. The effect of kinetin on protein level of tobacco leaf disks. Physiol. Plant. 15: 457–464 (1962).
20) TAKIMOTO, A. and K. IKEDA. Studies on the light controlling flower initiation of *Pharbitis nil*. I. Intensity and quality of the light preceding the inductive dark period. Bot. Mag. Tokyo, 72: 137–145 (1959).
21) TAKIMOTO, A. and K. IKEDA. Studies on the light controlling flower initiation of *Pharbitis nil*. VIII. Light-sensitivity of the inductive dark processes. Bot. Mag. Tokyo, 73: 468–473 (1960).

アサガオの光周性花成に及ぼす照射光の強度，草令とベンジルアデニンの作用

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アサガオの光周性花成（短日性）に及ぼす照射光の強度（光量子束密度，10から700 μmol $m^{-2}s^{-1}$）および草令（播種後，3から8日）の作用を，系統，ムラサキとキダチの幼植物を用いて調べた。

光の強度に関係なく，草令が進むにつれて花成は減少した。30 μmol $m^{-2}s^{-1}$ 以下で，3日草令のムラサキの花成は弱く，また，キダチの花成はいずれの草令にでも起らなかった。両系統の花成は，70あるいは100から300 μmol $m^{-2}s^{-1}$ の範囲で強く起り，その最適強度は草令の進行にともない高くなった。しかし，700 μmol $m^{-2}s^{-1}$ では，草令に関係なく花成は著しく減少した。昼間（午前9時から午後5時）は太陽光（最大値，1000 μmol $m^{-2}s^{-1}$），夜間は200 μmol $m^{-2}s^{-1}$ の日周変化の照射による花成は，いずれの強度の連続照射の場合より強かった。

30 μmol $m^{-2}s^{-1}$ の弱い花成はベンジルアデニンを子葉に処理すると著しく促進されたが，700 μmol $m^{-2}s^{-1}$ の弱い花成にはベンジルアデニンの促進は認められなかった。また3日草令の子葉にベンジルアデニンを処理すると，草令の進行による花成の減少は著しく抑制された。

上記の実験結果は，アサガオの子葉の短日性花成反応に，草令，子葉の光合成及び内生リズムの消長が著しく影響していることを示唆している。ベンジルアデニンの花成促進の機構の一つは，草令進行の抑制によると推察される。また，アサガオの短日性花成の機構は，高い強度の連続照射により誘導される花成[18]と異なることは明らかである。高い強度の照射による短日性花成の抑制機構はさらに研究しなければならない。