THE INVOLVEMENT OF GIBBERELLINS
IN PHYTOCHROME-CONTROLLED FLOWERING
OF PHARBITIS NIL

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ABSTRACT
The seedlings of Pharbitis nil, a sesitive short-day plant (SDP), were cultivated under special photoperiodic conditions: 72-h-long darkness, 24-h-long white light with low intensity, 24-h-long inductive night. During 24-h-long inductive darkness the total content of gibberellins in cotyledons underwent fluctuations with a maximum at 0 h and 8 h, and a decrease at the end of the dark period. FR light applied at the end of the 24-h-long white-light period inhibited flowering. R light flash and partially exogenous GA3 added on cotyledons could reverse the effect of FR. The seedling growth was not affected by FR and R light irradiation, but was promoted by exogenous GA3 application. The obtained results suggest that gibberellins are involved in photoperiodic control of SDP P. nil flowering. This involvement has nothing in common with participation of gibberellins in the control of the elongation growth of seedlings.

KEY WORDS: flowering, gibberellins, Pharbitis nil.

INTRODUCTION
Gibberellins have a significant influence on flowering of photoperiodically sensitive plants (Metzger 1990). Pharbitis nil is a sensitive short-day plant (SDP) which flowers when 4-5-day old seedlings undergo 16-h-long inductive night. Seedlings cultivated in continuous light remain vegetative. Exogenous GA3 application on subductive 12-h-long photoperiod can slightly stimulate flowering. However GA3 application during 16-h-long fully inductive night has no influence on flowering (Kulikowska-Gulewska et al. 2000).

Reactions induced by red (R) and far-red (FR) light are mediated by phytochrome. This chromoprotein occurs in two molecular forms, with different spectral properties and biological functions: Pr form with absorption maximum within red light (about 660 nm) and Pfr absorbing far-red light (about 730 nm). Pfr is the active phytochrome form (Furuya 1987; Quail 1994). Pfr occurs in two pools: labile (Phy A, PfrA) and stable (Phy B, Pfr B). Phy B is much more stable in light than Phy A, with a half-life more than 12 hours.

Abbreviations
CCC - 2-chloroethyltrimethylammonium chloride; FR - far-red light; GA3 - gibberelin n; R - red light; SDP - short-day plant; LDP - long-day plant; P. nil - Pharbitis nil

Studies on the mechanism of photoperiodic flower induction should be conducted under conditions enabling the proper functioning of one phytochrome fraction, the stable or labile one (Thomas 1991; Cymeraski and Kopcewicz 1994, 1995). We obtained such an experimental system using different methods of seedling cultivation. Such a system is as follows: 72-h-long dark period, 24-h-long period of low intensity white light and 24-h-long inductive night. Under such conditions the seedlings enter the inductive 24 h night with incomplete processes dependent on stable phytochrome (Cymeraski and Kopcewicz 1994). As a result of this kind induction seedlings produced 4-5 flower buds.

The aim of the present work was to investigate the content of endogenous gibberellins and the influence of FR, R and exogenous GA3 application on P. nil flowering under a specific photoperiod regime.

MATERIAL AND METHODS

Plant Material
Seeds of P. nil Chois, cv Violet (Marutane Seed Co., Kyoto, Japan) were scarified with a scalpel and imbibed for 24 h in distilled water (25°C). The swollen seeds were sown into pots filled with perlite: sterile sand (2:1). There
were 15 seeds in each pot. The pots were transferred to a
growth chamber at 25°C with darkness for 72 hours. After
steeping they were irradiated with low intensity white light
(6 W m⁻²) for 24 h followed by a 24-h-long inductive dark
period.

Plant material was harvested at the 72nd hour of dark-
ness, every 4 hours during 24-h-long low intensity white
light and during 24-h-long inductive night. Plants were cut
into cotyledons, hypocotyls and roots, frozen and stored
until analysis.

In some experiments the seedlings were irradiated with
a 15-min-long pulse of far-red (FR) light (λ_max=730 nm,
T,177=7 nm, intensity 4.3 μW/cm²) at the end of the 24-h-
long white light period. The effect of FR photoreversibili-
ity by red (R) light was also studied. Exogenous GA₃
(1 mM) was applied to the cotyledons (100 μl per plant)
7 times using a soft brush during 24-h-long inductive dark-
ness (at 0, 4, 8, 12, 16, 20 and 24 h).

After the completion of treatments the plants were grown
in a growth chamber under continuous light at 25°C for
14 days. The number of flower buds per plant was then
determined using a dissection microscope. Also at that time,
the elongation growth of treated plants was measured. Fifteen
plants were used in each experiment, which was repeated
at least 3 times.

Determination of endogenous GAs

GAs were extracted from frozen plant material (2 g of
cotyledons, hypocotyls or roots per sample) by 80% methanol (2 parts of 25 ml each), fractionated and purified
according to the method described by Hedden (1987).
After filtration the extract was passed through a Baker –
Bondapak octadecyl C₁₈ column (J.T. Baker, S. Wilko –
Eurocolor, Łódź, Poland) loaded as follows; 5 ml of 80% methanol, 5 ml of 5% acetic acid, 50 ml of plant extract, 5
ml of 5% acetic acid, 5 ml of distilled water and 1.2 ml of
100% methanol. The last fraction (1.2 ml of methanol
eluates) was used in thin layer chromatography (TLC) on
aluminum oxide (Kavalier, Czech Republic) covered with silica
gel. The solvent system was acetic acid – chlorophorm –
ethyl acetate (5:90:10 v/v). Gibberellins showed activity in
four Rₗ zones; 0.0-0.2, 0.3-0.5, 0.6-0.8 and 0.9-1.0. P. nil
extracts were analysed for GA biological activity by the
lettuce hypocotyl test (Frankland and Wareing 1960).
Extracts were also analysed by Shimadzu GC – 14 B
equipped with a capillary column according to the method
of Jolliffe et al. (1979) and Kulikowska-Gulewska et al.
(2000). The results were statistically evaluated and LSD
was calculated at a significance level of 0.01 and 0.05.

RESULTS

Endogenous GA level in cotyledon, hypocotyl and root

During 24-h-long inductive night the content of gib-
berellins in cotyledons underwent fluctuations with a maxi-
mum at 0 h and 8 h of darkness and a decrease during the
second half of inductive night (Fig. 1). In hypocotyls maxi-
mal content of these substances was observed at the 0 h
and 12 h of darkness, and in roots there was successive in-
crease of GAs which could suggest transport of gibberellins
from cotyledons to hypocotyls and roots, as the cotyledons
did not show any gibberellin – like activity at 12 h of dark-
ness. During the second half of inductive night gibberellin
content in hypocotyls was rather small. In roots increase at
the 20th hour of darkness and then decrease in these com-
ponents was observed. Generally, a P. nil seedling ends the
inductive night with a low endogenous gibberellin level,
amounting to about 0.5 μg g f.w.⁻¹ (Fig. 1).

Effects of photoperiod, GA and FR light on flowering and
e elongation growth

Fig. 1. Gibberellin (GA₁, GA₃, GA₄, GA₅, GA₉) content in cotyledons,
hypocotyls and roots of Pharbitis nil during 24-h-long inductive

Far-red light applied at the end of the 24-h-long period of
white light with low intensity inhibited flowering of P. nil
seedlings. Control plants formed about 5 floral buds and FR
treated ones 0.6 (Fig. 2A). Terminal flowering was reduced by
FR treatment from 100% in control plants to less than
20% in the treated ones (Fig. 2B). Red light applied imme-
diately after FR light pulse could reverse the effect of FR
irradiation (Fig. 2A and B). Seedling shoot length was not
influenced by FR and R irradiations (Fig. 3).

GA₃ applied to the cotyledons of FR treated plants could
partially reverse the inhibitory effect of FR light on flow-
ering. Control plants formed 4.2 floral buds per plant. A
pulse of FR light resulted in 0.35 floral buds per plant only
(Fig. 4A). Terminal flowering was reduced from 75% for
the control to less than 10% for FR treated plants (Fig 4B).
Exogenous GA₃ application reversed partially this inhibi-
tory effect of FR, especially at the end of a dark period (Fig.
4A and B). However restoring flowering to the control
level was impossible.

The ability of exogenous GA₃ to reverse inhibitory FR
influence at 0 h and 8 h was coincident with an increased
endogenous GAs level in cotyledons of P. nil seedlings
(Fig. 1). At the end of inductive night exogenous GA₃ was
still effective even though the endogenous GAs level was
low. Exogenous GA$_4$ applied during the 24-h-long dark period could stimulate seedling growth above the control level (Fig. 5).

**DISCUSSION**

Endogenous gibberellin content was studied in cotyledons, hypocotyls and roots of *Pharbitis nil* seedlings during 24-h-long inductive night. Plant material was harvested every 4 hours. *P. nil* cotyledons contained a relatively high level of gibberellins at 0 h and 8 h of inductive night. The second half of darkness was accompanied by a decrease in the content of these substances. Similar results were reported earlier for seedlings which underwent 16-h-long inductive night (Kulikowska-Gulewska et al. 2000). The low level of gibberellins after 24-h-long inductive night can be a prerequisite of entering reproductive growth stage. Similar results were obtained by Zeevaart (1964) who induced *P. nil* seedling flowering under continuous light (noninductive conditions) using CCC, gibberellin biosynthesis inhibitor. Oden and Heide (1989) reported a rapid decrease in gibberellin content in *Begonia* when the plants were transferred from noninductive flowering conditions to inductive ones.

Endogenous gibberellin content was high in hypocotyls and roots of *P. nil* seedlings at 12 h of 24-h-long inductive night. One can assume transport of these hormones from cotyledons to hypocotyls and roots because at the same time no gibberellin activity in cotyledons was observed. Yang and coworkers (1996) also suggested endogenous gibberellin translocation from cotyledons to hypocotyls and roots.
On the other hand, endogenous gibberellin content in phloem exudates and cotyledons of P. nil seedlings under inductive and noninductive conditions indicated that the level of these substances was higher under inductive conditions (Wijayanti et al. 1995). Differences in the GAs content in different plant parts can reflect differential biosynthetic regulation, transformation or translocation of GAs (Olsen et al. 1995).

Previously, we have shown that studies on the mechanism of photoperiodic flower induction should be conducted under the conditions enabling the functioning of only one phytochrome system — stable or labile (Cymerski, Kopcewicz 1994, 1995). We have found that far-red light applied at the end of the light phase inhibits the flowering of P. nil. It should be noticed, however, that far-red light does not exert any influence, or the effect is only very slight, if the light phase of the photoperiod is too long, and the light is of high intensity. The most pronounced effect of far-red light on flowering was obtained when P. nil seedlings were grown 72 hrs in darkness, 24 hrs in white light with lowered intensity (day) and then were treated with 24-h-long inductive night. Under such conditions the seedlings enter the inductive 24-h-long night with unfinished processes dependent on stable phytochrome (Cymerski, Kopcewicz 1995). The irradiation at the end of the day with the impulse of far-red light causes the blockade of stable phytochrome — controlled reactions and the inhibition of flowering. In such experimental conditions it has been stated that red light and partially GA3 could reverse the inhibition of flowering caused by far-red light. Because it seems very probable that red light could promote the endogenous content of gibberellins (Black, Vito 1972) it means that gibberellins are taking part in the phytochrome — regulated mechanism of flower induction in P. nil.

Pří B is stable in darkness and can act in the control of flowering for several hours. Furuya and Schäfer (1996) suggested involvement of Phy B and some Phy X in the photoperiodic control of flowering.

Red and far — red light irradiation did not affect P. nil seedling shoot length (Fig. 3). Similar results were obtained for Chenopodium album (Kopcewicz et al. 1992).

Exogenous GA3, applied to P. nil cotyledons, treated with FR light, could partly reverse the inhibitory FR effect on flowering. GA3 ability to reverse the inhibitory effect of FR on P. nil flowering is coincident with an increased endogenous gibberellin level at 0 h and 8 h of inductive night. However during the second half of 24-h-long inductive night GA3 was still effective, although the endogenous GAs level was low. Endogenous gibberellin content in P. nil tissue after R and FR light treatment are under the investigations in our laboratory. Several reports stated that exogenous GA3 applied immediately before inductive night could stimulate flowering (Ingram and Browning 1979; King et al. 1987; Simmons and Coulter 1979).

Far-red light irradiation had no significant influence on P. nil seedling shoot length. End-of-day FR light irradiation usually affects hypocotyl elongation. On the other hand phyB deficient mutants show reduced sensitivity to end-of-day FR irradiation (McNelis and Deng 1995). Under our experimental conditions (72-h-long darkness, 24-h-long white light with lowered intensity, 24-h-long darkness) stable phytochrome-dependent processes were also incomplete.

GAs applied to cotyledons every 4 h during 24-h-long inductive night, following FR treatment, clearly stimulated shoot growth. Ogawa et al. (1990) also reported stem growth stimulation by GAs application. Similar results were obtained for Lolium temulentum (Evans 1990). High seedlings of P. nil var. Tenden contained higher amounts of endogenous GAs than the dwarf variety Kidachi (Ogawa 1965). Olsen et al. (1995) found that gibberellins affected Salix pentandra seedling growth, but photoperiod had no influence on gibberellin metabolism.

Generally it seems that the obtained results show that gibberellins are specifically involved in phytochrome — controlled P. nil transition to flowering. This is an independent phenomenon from the widely known effect of gibberellins on plant elongation growth.

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**LITERATURE CITED**


GIBERELINY
W FITOCROMOWEJ KONTROLI KWITNIENIA PHARBITIS NIL

STRESZCZENIE

Siewki Pharbitis nil uprawiano w specjalnych warunkach fotoperiodycznych: 72-godzinnym okres ciemności, 24-godzinnym okres świtu białego o niskiej intensywności, 24-godzinna indukcyjna noc. W powyższych warunkach siewki rozpoczynają 24 h noc indukcyczną z niezakończonymi procesami zależnymi od fotocromu stabilnego.

W liściach, hipokotyłach i korzeniach siewek Pharbitis nil badano zawartość endogennych giberelin. Do pośrednictwa chromatografii gazowej i bietestu hipokotyla sałaty. Podczas 24-godzinnej indukcyjnej nocy, całkowita zawartość giberelin w liściach uległa wzrostowi, z maksimum w 0 godz. i 8 godz. nocy i maleńko pod koniec okresu ciemnego. Światło dalekiej czerwieni (FR) zaśpikowano na koniec 24-godzinnego okresu światła białego o obniżonej intensywności hamowało kwitnienie. Światło czerwone (R) i częściowo egzogenna GΑ, odwracał harmonijny wpływ. Światło R i FR nie wpływało na wzrost siewek, natomiast wzrost ich był pobudzany przez dodanie egzogennej GΑ.

Otrzymane wyniki sugerują udział giberelin w fotoperiodycznej kontroli kwitnienia SDP Pharbitis nil. Przypuszcza się, że udział giberelin w kontroli wzrostu elonacyjnego siewek odbywa się poprzez inny szlak metaboliczny niż kontrola kwitnienia.

SŁOWA KLUCZOWE: kwitnienie, gibereliny, Pharbitis nil.