Proc. Natl. Acad. Sci. USA
Vol. 74, No. 6, pp. 2412–2416, June 1977
Botany

Promotion and inhibition of flower formation in a dayneutral plant in grafts with a short-day plant and a long-day plant

(*Nicotiana*/photoperiodism)

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Contributed by A. Lang, March 17, 1977

ABSTRACT **Flower formation in the dayneutral tobacco (*Nicotiana tabacum* L.) cultivar "Trapezond" was accelerated by graft union with the short-day tobacco "Maryland Mammoth" when the grafts were kept on short days and by graft union with the long-day plant *N. silvestris* L. when they were kept on long days. When Maryland Mammoth/Trapezond grafts were kept on long days, flower formation in Trapezond was not, or only slightly, delayed compared to Trapezond/Trapezond controls; but when *N. silvestris*/Trapezond grafts were kept on short days, flower formation in Trapezond was inhibited and its growth changed to dwarf-like habit. The former results indicate transmission of flower-promoting material(s) ("florigen") from photoperiodic plants maintained under flower-promoting daylength conditions to a dayneutral graft partner; the latter indicate the presence in the long-day plant *N. silvestris* under short-day conditions of potent flower-inhibiting and growth-regulating material(s) that can also be transmitted to a dayneutral partner. Analogous flower-inhibitory materials seem not to be present, or to be present to a much lesser extent, in the short-day plant Maryland Mammoth under long-day conditions.**

It is widely held that flower formation in plants involves transmissible, hormone-type messengers or flower hormones. Much of the evidence for this theory derives from grafting experiments with plants in which flower formation is controlled by specific environmental conditions, so that the plants can be either kept in the vegetative condition or "induced" to form flowers, at will. The majority of these experiments have been carried out with plants in which the environmental factor controlling flower formation is length of day or photoperiod. A photoperiodic plant maintained on noninductive photoperiods (i.e., photoperiods in which no flower formation occurs) can be induced to form flowers by graft union with a plant that is capable of flower formation. This grafting partner may be another photoperiodic plant that has been subjected to inductive photoperiods (i.e., photoperiods in which flower formation does take place) and may belong to the same species or to another species or genus and may be of the same or a different photoperiodic response type. Also, it may be a dayneutral plant (i.e., a plant in which flower formation is independent of daylength). The only limiting factor for these combinations is graft compatibility of the partners.

The flower hormone, the existence of which has thus been deduced, has been called "florigen." It appears to be identical in, or at least interchangeable between, different photoperiodic response types and to be present in dayneutral plants, also. It is known that the main source of florigen is the leaves of the plant whereas the target tissue is the growing points (buds). However, efforts at extracting florigen, as the first step toward its chemical identification, have persistently met with limited success at the best, and florigen—and flower-promoting hormones in general—is still a physiological rather than a chemical concept. (For background for these issues see refs. 1 and 2.)

The literature also contains reports on effects in flower formation that have served as a basis for postulating the existence of flower inhibitors. The most common case occurs when some of the leaves of a plant are maintained on an inductive photoperiod and other leaves are maintained on a noninductive photoperiod; flower formation is then often delayed compared with a plant having all leaves on the inductive photoperiod. In most cases, however, this effect can be explained by the source-sink relationship of buds and leaves. The inhibitory effect is apparent mainly when the noninduced leaves are inserted between the induced ones and the bud in which the flowering response is registered. Buds receive assimilates primarily from the nearest mature or nearly mature leaves, and florigen appears to move with the assimilate stream. Thus, noninduced leaves inserted near a bud prevent florigen from induced but more remote leaves from reaching the target bud (see ref. 1). In a careful, quantitative study of this type of inhibition, King and Zeevaart (3) found no necessity for assuming an actual inhibitor.

However, there are some reports that are difficult to explain on this basis. For example, Lang and Melchers (4) found that if *Hyoscyamus niger*, a long-day plant, was completely defoliated, flower buds were formed irrespective of the daylength regimen but, if a single leaf was left on (A. Lang, unpublished data) or grafted back onto the plant (4), flowers were formed only on long days, as in intact *Hyoscyamus* plants. They (4) concluded that an inhibitory effect of short days, localized in the leaves, was an essential element of the photoperiodic response of long-day plants. Evans (5), working with the long-day plant *Lolium temulentum*, found that short-day leaves inhibited flower formation even if not inserted between a long-day leaf and the growing point. Raghavan and Jacobs (6) showed that shoot apices of the short-day plant *Perilla*, when excised and cultured *in vitro*, alone or with the first pair of unfolded leaves attached initiated flowers (inflorescences) on short days as well as on long days but, if the second pair of unfolded leaves was also left attached, flower formation occurred only on short days. Jacobs and Suthers (personal communication) observed marked flower inhibition in a dayneutral *Coleus* species under the influence of a short-day graft partner (another *Coleus* species) maintained on long days.

We conducted experiments designed to obtain direct evidence for the existence of flower-*inhibitory*, graft-transmissible materials particularly in such plants in which the existence of flower-*inducing*, graft-transmissible material(s) (i.e., florigen) had been unequivocally demonstrated. We chose as materials the dayneutral tobacco (*Nicotiana tabacum* L.) cultivar "Trapezond," the short-day tobacco cultivar "Maryland Mammoth," and the long-day plant *Nicotiana silvestris* L. Flower formation in Maryland Mammoth on long days has been induced by graft union with Trapezond and other dayneutral tobaccos and with *N. silvestris* (7, 8); flower formation in *N. silvestris* on short days has been obtained in graft union with

Trapezond, another dayneutral tobacco, and Maryland Mammoth (8, 9). The experimental design was simple: prior to grafting, Maryland Mammoth and *N. silvestris* were maintained on their noninductive photoperiods (i.e., long and short days, respectively); after grafting, part of each graft combination was placed under long days and part, under short days, and the flowering response of Trapezond was measured.

MATERIALS AND METHODS

The plant material came from our own stocks which have been maintained by self-pollination for many years and represent a genetically highly uniform material. The plants were grown in a greenhouse in a standard soil mixture and, after being well-established, received supplementary mineral nutrition, plus water when needed. The supplementary feeding was discontinued when the grafts were made but was resumed, although at a reduced rate, when they were well-established.

Short-day conditions (8 hr of light daily) were provided by covering the plants with a black cloth curtain from 4 p.m. (1600) to 8 a.m. (0800); long-day conditions (16–20 hr of light) were provided by extending the natural daylight with light from incandescent lamps. Temperature was maintained at a minimum of about 20°; excessively high temperatures were reduced by regular greenhouse ventilation. When grafts had to be maintained into the fall and winter seasons, supplementary light was also provided during the daylight period.

Grafts were simple cleft-grafts. Trapezond was used as stock throughout. All leaves were removed, and the plants were decapitated in the younger, not yet fully grown, part of the stem. The scion was cut wedge-shape at its base (2.5–3.5 cm) and was inserted into a vertical cut made into the uppermost part of the stem stump; the graft was tied firmly with raffia or, in some cases, with a viscose rayon ribbon (Swistraw, white, from Artis Inc., Temple City, CA). One or, in a few cases, two of the axillary buds in the upper portion of the Trapezond shoot were allowed to grow out as "indicator shoots" for the flowering response; all other buds on the stock were removed together with the leaves.

As scions, either shoots or single leaves were used. Shoot scions of Trapezond and Maryland Mammoth consisted of the apical portions of the main shoots of the plants, 10–12 cm in length and with the four or five youngest, unfolded but still expanding, leaves; shoot scions of *N. silvestris* consisted of about the same number of the youngest leaves, the stem, and the upper part of the root (*N. silvestris*, having a pronounced rosette habit of growth when kept on short days, has a very short shoot axis which is insufficient for grafting purposes). For leaf grafts, young but fully expanded leaves were used.

In some series, the following modification was introduced. The Trapezond stocks were defoliated except for the uppermost leaves but were not immediately decapitated, and the scions were grafted sideways into the stems of the stocks. The stocks were decapitated only after the scions had resumed active growth (between 11 and 19 days after grafting), somewhat above the graft region, and the uppermost axillary (lateral) bud was allowed to develop into the indicator shoot. Thus, the Trapezond indicator shoot in these grafts was situated *above* the scion. The objective of this modified procedure was twofold. First, the indicator shoot was less subject to inhibition (apical dominance) by the scion than when it was inserted below the scion; in grafts with this latter arrangement growth of the indicator shoots was sometimes strongly decreased, rendering the results ambiguous. Second, because the indicator shoot did not start growth until after decapitation of the stock, it was under the influence of the scion from the very beginning of its development.

In order to prevent wilting of the scion, the upper portion of the grafts was enclosed with a clear plastic bag of appropriate size (so-called food storage bags) directly after grafting; the bag was tied around the stem of the stock below the graft region. After tissue union was well established, usually after about 10 days, the bag first was untied and then 3–5 days later was removed. During the same period, the grafts were protected from direct sunlight by means of cheesecloth.

Dayneutral tobaccos exhibit a marked gradient in the flowering tendency of their lateral shoots: laterals in the lower portions of the main shoot, if released from apical dominance, form flowers only after having made a substantially larger amount of vegetative growth than laterals nearer the inflorescence region (10, 11). To minimize any effect of this gradient on our results, we made grafts only in a relatively limited region of the Trapezond shoot, mostly between the 15th and 20th nodes of the elongate portion of the shoot, and were careful to randomize the stocks, in this respect, across the various graft combinations.

In order to maintain the indicator shoot in as great a dependency on the scion as possible, leaves developing on the indicator shoots were regularly removed when they reached a length of 12–15 cm. In the control grafts (Trapezond/Trapezond), the scions were decapitated after appearance of visible flower buds, and side shoots were also removed before they formed flowers because preliminary observations had shown that these measures were favorable for a rapid and relatively uniform flower response of the stocks. In the grafts with Maryland Mammoth and *N. silvestris*, flower buds on the Trapezond stocks appeared in almost all cases before those of the scions and their development did not seem to be affected if the flowers of the scions were allowed to develop, too.

The flowering response of the Trapezond stocks was measured by the following parameters: (*i*) number of flowering and vegetative grafts; (*ii*) number of days from decapitation of the stock (= start of growth of the indicator shoot) to the appearance of the first visible flower bud on the indicator shoot; (*iii*) days from decapitation of the stock to the opening of the first flower (first anthesis) on the indicator shoot (not reported in this paper); (*iv*) length of the indicator shoot, on the latter date, to the base of the first (terminal) flower; and (*v*) number of nodes (leaves) formed on the indicator shoot before the lowermost inflorescence branch. When a graft did not form visible flower buds after a certain period of time, the apex of the indicator shoot was dissected under a low-power microscope, and its condition (flowering or vegetative) and the number of nodes (leaves and leaf primordia) were determined.

RESULTS

Only the results of one large experiment—in which the indicator shoot was inserted above the graft region and was not allowed to grow before the union between the partners had been well established (see *Materials and Methods*)—are reported here (Table 1); the entire experimental material will be presented more fully elsewhere.

In the control grafts (Trapezond/Trapezond) (Fig. 1) the time of flower formation (flower bud appearance) was the same under both long- and short-day regimens but the number of nodes on the indicator shoots formed prior to the first flower-bearing one was significantly less under the former. In another experiment, flower bud appearance in Trapezond/Trapezond grafts was also significantly accelerated on long days. Thus, flower formation in these grafts may be promoted by long days, and nongrafted Trapezond plants may also flower sooner on long than on short days. However, this difference, most noticeable in suboptimal light conditions (e.g., in the greenhouse

FIG. 1. Control grafts, Trapezond (dayneutral)/Trapezond. (*Left*) On long-day regimen. (*Right*) On short-day regimen. Pairs have been selected to show early and late responses; the right-hand graft of each pair does have flower buds. In these grafts, flowers developing on the scion have been removed. In this and the other figures, the indicator shoot of the stock is on the left, arising above the graft region, the scion is on the right, and some leaves on the latter have been removed for photography to show the graft region (arrows). Grafts were made between August 5 and 10 and photographed on October 12, 1976.

FIG. 2. Grafts of Maryland Mammoth (short-day plant) on Trapezond (dayneutral). Otherwise as in Fig. 1, except flowers on scion not removed.

in winter), is probably not based on a photoperiodic response but is a consequence of the greater quantity of light the plants receive under long days. Grafts of Maryland Mammoth and *N. silvestris* on Trapezond were only compared with Trapezond/Trapezond control grafts kept under the same photoperiod.

When grafts between Maryland Mammoth and Trapezond (Fig. 2) were kept on short days (i.e., with the Maryland

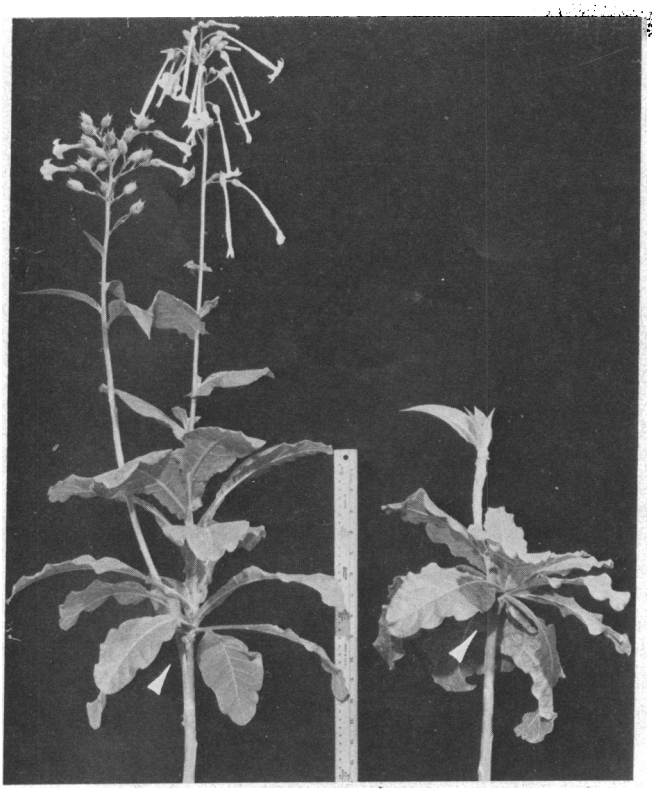

FIG. 3. Grafts of *Nicotiana silvestris* (long-day plant) on Trapezond (dayneutral). Because the response was very uniform, only one graft is shown for each regimen: left side, long-day; right side, short-day. Otherwise as in Fig. 2.

Mammoth partner proceeding to flower), the flowering response in Trapezond was accelerated, both the time to flower bud appearance and the number of nodes produced by the indicator shoot before flower formation being significantly less than in short-day control grafts. When identical grafts were kept on long days, with Maryland Mammoth staying vegetative, the Trapezond partner of 1 of 16 grafts did not initiate flowers, and 3 did not produce visible flower buds before termination of the experiment. However, in all these cases, the growth of the indicator shoots was quite slow, presumably because of correlative inhibition by the vigorously growing scions. The number of nodes on these shoots was not significantly different from that in Trapezond/Trapezond controls on long days—i.e., there was no indication of genuine inhibitory influence from the vegetative Maryland Mammoth partner on flower formation in Trapezond. In another experiment with Maryland Mammoth and Trapezond, the node number in the indicator shoots of the long-day grafts was increased, compared with Trapezond/Trapezond long-day controls, but the difference, although statistically significant, was relatively small (24 versus 34 nodes).

In the *N. silvestris*/Trapezond long-day grafts (Fig. 3), the indicator shoots formed flower buds more rapidly and after a lesser number of nodes than in the Trapezond/Trapezond long-day controls. Quite in contrast, in the analogous short-day grafts the indicator shoots remained vegetative for the duration of the experiment (90–94 days); under long days they had by this time produced mature fruits and seeds. The mean node number on the short-day indicator shoots, as determined by microscopic examination of some grafts, at this time was 36 and thus exceeded considerably that on the indicator shoots of the

Table 1. Flowering and growth responses in grafts between the dayneutral tobacco cultivar Trapezond (TR) and the short-day cultivar Maryland Mammoth (MM) or the long-day plant *Nicotiana silvestris* (NS)

Combination	Photoperiod*	Grafts, no.			Mean time to first visible flower bud, days	Mean length of indicator shoot, cm	Mean nodes on indicator shoot, no.
		Total	Forming flowers	Remaining vegetative			
TR/TR (control)	LD	15	15	0	49	49	18
	SD	15	15	0	49	52	22
MM/TR	LD	16	15	1†	>48‡	>46‡	20
	SD	16	16	0	32	41	15
NS/TR	LD	15	15	0	23	45	14
	SD	15	0	15	—	16	36§

In this experiment, the scions (TR, MM, NS) were grafted sideways into the stem of the stock (TR); the indicator shoot of the latter developed from the first axillary bud above the graft union.

* LD = long days, SD = short days.

† Indicator shoot growing poorly (100 days after stock decapitation: only 6 cm long, with 15 nodes).

‡ Indicator shoots of three grafts produced no visible flower buds but contained microscopic ones; growth of shoots was slow.

§ Indicator shoots of five grafts dissected; all were strictly vegetative.

Statistical analysis of Table 1¶

	TR/TR, SD	TR/MM, LD	TR/MM, SD	NS/TR, LD	NS/TR, SD
TR/TR, LD	0 *	0 0	—	* *	—
TR/TR, SD	—	—	* *	—	*

* Difference statistically significant ($P < 0.05$); 0, difference not statistically significant ($P > 0.05$); first symbol, days to first visible flower buds; second symbol, node number.

¶ Most of the confidence intervals were constructed by assuming a normal distribution and using the confidence interval for the difference between two means. In comparing series in which data were censored (e.g., MM/TR,SD with MM/TR,LD) where part of the indicator shoots had not formed visible buds by the termination of the experiment) a nonparametric technique based on the Mann-Whitney test was used [see Conover, W. J. (1971) *Practical Non-Parametric Statistics* (John Wiley & Sons, New York-London-Sydney-Toronto), p. 238].

N. silvestris/Trapezond long-day grafts and of any other flowering grafts. Thus, flower formation in Trapezond had been significantly delayed, if not altogether suppressed, under the influence of *N. silvestris* scions maintained under short-day conditions. In addition to this effect, the growth habit of the indicator shoots was altered in a striking manner. First, the shoots grew more slowly, as measured by the rate of node production. In long days, this rate was about 0.6 node per day (14 nodes in 23 days, the average time to the appearance of the first floral bud); in short days it was about 0.4 (36 nodes in 90 days). Second, the shoots were considerably thicker than under long days. Third, their internodes were considerably shorter: about 20 mature internodes in about 90 days on 16-cm shoots or less than 1 cm per internode under short days versus 14 internodes per 45 cm or more than 3 cm per internode on long days.

DISCUSSION

When the short-day plant Maryland Mammoth tobacco or the long-day plant *Nicotiana silvestris* was grafted onto Trapezond tobacco and the graft maintained on photoperiods inductive for the respective scion, flowering in the dayneutral partner was accelerated. This result indicates transmission of flower-promoting substance(s) (florigen) from photoperiodic plants to a dayneutral plant—analogous to transmission in the opposite direction, from Trapezond or other dayneutral tobaccos to noninduced Maryland Mammoth and *N. silvestris* (7–9)—and thus supports the idea that the florigens of photoperiodic and dayneutral plants are identical or at least fully interchangeable. Promotion of flower formation in dayneutral tobacco by *N. silvestris* graft partners in long-day conditions has already been reported by Zeevaart (9) and Chailakhyan *et al.* (8).

When grafts of *N. silvestris* on Trapezond were subjected to short days (i.e., photoperiods noninductive for *N. silvestris*), flower formation in the dayneutral partner was inhibited in a marked manner while vegetative growth continued; however, the growth habit became dwarf-like, thus somewhat tending toward the rosette-type growth that is characteristic of *N. silvestris*, and most other long-day plants, when on short days. These results strongly indicate that lack of flowering and the growth habit of vegetative *N. silvestris* are determined by flower-inhibitory and growth-regulating material(s) formed in the plant under short-day conditions. They support the conclusion (4) that short days have an inhibitory action in the photoperiodic response of long-day plants. Thus, two types of transmissible substances involved in flower regulation exist in *N. silvestris*—florigen, and the flower-inhibitory and growth-modifying material(s), demonstrable under the inductive and the noninductive photoperiodic conditions, respectively.

In grafts of vegetative Maryland Mammoth on Trapezond there was no or only a small delay of flower formation in Trapezond, as compared to Trapezond/Trapezond controls. Thus, the short-day plant Maryland Mammoth, when maintained under noninductive photoperiods, contains no flower-inhibitory materials or, if they are present, either their quantity or their effectiveness, at least as measured in grafts with Trapezond, is much smaller than that of the flower-inhibitory substance(s) in noninduced *N. silvestris*.

The flower-inhibitory "principle" of noninduced *N. silvestris* is not unique for this long-day plant, because similar results were also obtained in grafting experiments (not reported here) between *Hyoscyamus niger*, another long-day plant, and Trapezond. On the other hand, the lack of an analogous principle in noninduced Maryland Mammoth does not seem to be typical for all short-day plants because Jacobs and associates (ref. 6 and personal communication) found evidence indicating that at least some short-day plants, when maintained on long days, do possess flower-inhibiting, transmissible material(s). These and other questions, including the nature of the inhibitory substance(s) and their relationship to the flower-promoting one(s), require further study. The reports on other flower-inhibitory effects, particularly of leaves maintained on noninductive photoperiods, may also require some reexamination and reevaluation. Generally, the unequivocal demonstration of flower-inhibitory, growth-regulating substances in plants that are also capable of producing florigen makes it necessary, in further grafting and other kinds of experiments on the regulation of flower formation, to consider both flower-promoting and flower-inhibiting transmissible substances.

We are greatly indebted to Kristine Scarbro (East Lansing) and Kh. K. Khazhakyan, L. P. Khlopenkova, and L. I. Yanina (Moscow) for assistance with the grafts; and E. N. Light, H. Wieferich, and S. Ortiz (East Lansing) for care of the grafts in the greenhouses; Professor J. H. Stapleton (Department of Statistics, Michigan State University) for advice on statistical procedures; and Fred Clark (Lansing) for the statistical analysis of the data. This work was started in the summer of 1975 at the Timiryazev Institute when A.L. was at this institute under the Exchange Program between the National Academy of Sciences of the U.S.A. and the Academy of Sciences of the U.S.S.R. and was continued both at the Timiryazev Institute and the MSU-ERDA Plant Research Laboratory. Work at the latter location was supported by the U.S. Energy Research and Development Administration under Contract EY-76-C-02-1338.

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