Flowering Response of *Pharbitis nil* to Agents Affecting Cytoplasmic pH

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**ABSTRACT**

Permeant weak acids and auxins have been shown to reduce the cytoplasmic pH in several systems. Lactic, citric, formic, butyric, salicylic, parahydroxybenzoic, propionic acid, and sodium propionate inhibited the flowering response of *Pharbitis nil* seedlings when applied immediately before an inductive dark period. The acidic auxins IAA, indolebutyric, and α-naphthaleneacetic acid, as well as the nonacidic auxin α-naphthaleneacetic acid, also inhibited the flowering response. Inhibition was generally more pronounced with a 12-hour than with a 16-hour dark period. Salicylic acid and sodium propionate shifted the response curve of the dark period by about 2 hours. Salicylic acid, sodium propionate, and indolebutyric acid were inhibitory when applied during the first few hours of the dark period. The permeant weak bases NH₄Cl, procaine, and trisodium citrate enhanced the flowering response. NH₄Cl reduced the length of the critical dark period. The inhibition of flowering by acids and auxins as well as the promotion of flowering by bases was obtained even when only the cotyledons had been treated. The inhibition of floral induction by auxins may not be dependent on their effect on the cytoplasmic pH.

*Pharbitis nil* is a short-day plant that can be induced to flower by a single photoinductive dark period (21). Notwithstanding extensive research efforts during the past decades, the cellular events leading to floral induction are still largely unknown (2, 21). The metabolic activities of cellular and organellar compartments are known to be strongly influenced by pH (13, 16, 20). The intracellular pH is believed to be under tight control (13, 16, 20). Even transient changes in intracellular pH may have regulatory significance (1, 3). We, therefore, examined the possibility that changes in cytosolic pH during the inductive dark period may affect the flowering response.

Reliable measurement of cytosolic pH in plant cells is still difficult, particularly in mature cells in which most of the cellular volume is occupied by the vacuole. Modification of cytosolic pH has been achieved, however, by the use of weak acids. Such acids permeate into cells in their undissociated form and dissociate subsequently, releasing protons and acidifying the cytosol (1, 10, 16–18). Weak bases such as ammonium salts and procaine, on the other hand, raise the cytosolic pH (7, 13, 16, 19).

Auxins have been known for many years to inhibitphoto-periodic floral induction in SD plants (2, 11). The mode of action of this inhibition is unknown (11). Application of exogenous auxins has been shown to decrease the cytosolic pH in several plant systems (3, 5–8). There is a possibility, therefore, that auxins influence floral induction through their effect on the pH of the cytoplasm. This hypothesis has also been tested in the present study.

**MATERIALS AND METHODS**

Seeds of *Pharbitis nil* Chois cv ‘Violet’ (Marutane Seed Co. Kyoto, Japan) were stirred with concentrated H₂SO₄ for 45 min, rinsed well and imibed for 24 h in running tap water. The seeds were sown in vermiculite at noon and transferred to a growth chamber at 24 ± 2°C with continuous light (17 W m⁻², cool-white fluorescent; Tadiran, Israel). Plants were induced to flower on the 6th or the 7th d by a single dark period of various lengths as indicated in each experiment. Thereafter the plants were returned to continuous light.

Plants were sprayed (about 1 mL per plant) immediately before the inductive dark period, or as otherwise indicated in each experiment. In some experiments treatment solutions were applied to cotyledons alone (about 400 μL per plant), to the shoot tip alone (about 10 μL per plant) as well as to the whole plant. All chemicals were dissolved in distilled water except salicylic acid which was solubilized by the addition of dilute NaOH, raising the pH of the solution to 5.0. Control plants were sprayed with distilled water. No wetting agents were used.

The number of flowers per plant and the percentage of plants exhibiting terminal flowering were recorded 14 d after induction. At least 10 replicate plants were used per treatment, and each experiment was conducted 3 times or more with similar results.

**RESULTS**

Organic acids, auxins, and bases were applied to plants immediately before the start of the inductive dark period. The effect of the various agents was examined at 2 lengths of the dark period, 12 and 16 h.

Lactic and citric acid were inhibitory at 20 mM in plants receiving a 12 h dark period, while there was no effect with a 16-h dark period (Fig. 1, A and B). With formic acid, sodium propionate (Fig. 1, C and D) and propionic acid (Table I) inhibition was obtained at higher concentrations. An inhibitory trend could be seen with formic acid and sodium propionate even with a 16-h dark period. A much stronger inhibition and at a lower concentration (5 mM) was obtained with salicylic acids (Fig. 2) and butyric acid (data not shown).
which were much more effective at a 12-h than at a 16-h dark period. Parahydroxybenzoic acid, an analog of salicylic acid, was also strongly inhibitory at 5 mM in plants receiving a 12-h dark period (data not shown).

Auxins were inhibitory with a 12-h dark period at a still lower range of concentrations—IAA at 1 mM, IBA at 0.05 mM (Fig. 3), and α-NAA at 0.05 mM (Table II). The weak antiauxin, β-NAA (15), was not inhibitory and was, in fact, slightly promotive (Table II). The nonacidic auxin α-NAAm was inhibitory at both 12-h and 16-h dark periods, producing 50% inhibition at 0.5 mM and 100% inhibition at 1 mM.

The effects of sodium propionate and salicylic acid were further investigated by exposing control and treated seedlings to dark periods of various lengths. Sodium propionate extended the length of the critical dark period (Fig. 4) and salicylic acid shifted the response curve by about 2 h (data not shown). Whereas in control plants 50% of the maximum flowering response was obtained with a 11.5-h dark period, with sodium propionate and salicylic acid this could be achieved only with a 13-h dark period.

The time of application was also important. When 40 mM sodium propionate were applied at various times during a 15-h dark period, inhibition was strongest during the first 4 h and gradually diminished thereafter until 8 h (Fig. 5). A second period of inhibition was evident from 8.5 to 12 h. No inhibition was obtained when sodium propionate was applied after 12 h.

**Table 1. Effect of Organic Acids Applied to Whole Seedlings, Cotyledons, or Shoot-Tip Immediately Before a 12-h Dark Period on the Flowering Response, as Compared with Nontreated Control Seedlings**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Whole seedling</th>
<th>Cotyledons</th>
<th>Shoot-tip</th>
<th>Nontreated control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic acid, 5 mM</td>
<td>1.2 ± 0.2 (3.2)*</td>
<td>1.2 ± 0.2 (6.6)</td>
<td>2.1 ± 0.3 (16.6)</td>
<td>4.3 ± 0.3 (70.0)</td>
</tr>
<tr>
<td>Butyric acid, 10 mM</td>
<td>1.6 ± 0.2 (3.0)</td>
<td>0.8 ± 0.2 (0)</td>
<td>2.5 ± 0.5 (30.0)</td>
<td>2.1 ± 0.5 (11.7)</td>
</tr>
<tr>
<td>Propionic acid, 50 mM</td>
<td>1.0 ± 0.3 (4.1)</td>
<td>0.6 ± 0.2 (0)</td>
<td>3.6 ± 0.5 (44.4)</td>
<td>3.1 ± 0.6 (40.0)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are percent terminal flowering.
supplied at the end of a 16-h dark period. The effect of salicylic acid was examined at 2 night lengths (Fig. 6). Strong inhibition was obtained from 0 (11.45 h dark) or 2 h (13 h dark) to 5 h, and a second period of slight inhibition was observed at 10 h. No inhibition was obtained with either material when applied at 6 to 8 h.

Application of IBA (1 mM) at various times during a 12-h dark period showed a strong inhibition during the first 5 h, with a diminishing trend thereafter (data not shown).

The alkalizing agents NH₄Cl, procaine and trisodium citrate, were also examined in our system. NH₄Cl enhanced the flowering response over a broad range of concentrations and almost doubled it at the optimum 5 mM concentration (Fig. 7). When applied to plants exposed to various lengths of dark period, NH₄Cl (10 mM) promoted some flowering, including terminal flowering, even at 10 and 10.5-h dark periods, while control plants remained vegetative (Fig. 8). At longer induc-

**Table II. Effect of Auxin Compounds Applied to Whole Seedlings, Cotyledons, or Shoot-Tip Immediately Before a 12-h Dark Period on the Flowering Response, as Compared with Nontreated Controls**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Whole Seedling</th>
<th>Cotyledons</th>
<th>Shoot-tip</th>
<th>Nontreated control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBA, 0.5 mM</td>
<td>1.2 ± 0.3 (5.0)*</td>
<td>1.0 ± 0.4 (10.0)</td>
<td>4.2 ± 0.4 (61.0)</td>
<td>5.2 ± 0.2 (94.0)</td>
</tr>
<tr>
<td>α-NAA, 0.5 mM</td>
<td>1.3 ± 0.3 (0)</td>
<td>1.0 ± 0.2 (0)</td>
<td>5.2 ± 0.2 (94.0)</td>
<td>5.2 ± 0.2 (94.0)</td>
</tr>
<tr>
<td>β-NAA, 1.0 mM</td>
<td>3.7 ± 0.4 (27.7)</td>
<td>4.1 ± 0.4 (50.0)</td>
<td>4.4 ± 0.4 (50.0)</td>
<td>2.9 ± 0.3 (9.1)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are percent terminal flowering.
tive dark periods NH₄Cl had little effect (Fig. 8). Procaine (at 5 mM) and trisodium citrate (at 50 mM) also enhanced the flowering response when applied immediately before a 12-h dark period (Table III).

To get an indication as to the site of action of the pH-modifying agents, the chemicals were applied either to the cotyledons alone, to the shoot tip alone, or to the whole seedling. The inhibitory action of acids and auxins and the promotive effect of the bases was clearly obtained even when the cotyledons alone had been treated (Tables I, II, and III). Effects of treatments of the shoot tip alone were far less pronounced: butyric and propionic acid had no effect and salicylic acid was somewhat inhibitory (Table I). IBA and α-NAA were equally inhibitory when applied to the whole seedlings or only the cotyledons, whereas treatments of the shoot tip had no effect (Table II). The weak antiauxin, β-NAA was slightly promotive in all cases (Table II). The bases, procaine and trisodium citrate, were promotive when applied to the cotyledons alone but had no effect when applied to the shoot tip (Table III). On the other hand, NH₄Cl was equally promotive when applied to the shoot tip or the cotyledons. Treatments of the whole plant (Tables I, II, and III) had, in most cases, similar effects to treatment of the cotyledons alone.

**DISCUSSION**

Treatment of *Pharbitis* seedlings with weak acids at the beginning of the photoinductive dark period inhibited the flowering response. Treatment with weak acids/bases is one of the means for short-term alteration of the cytoplasmic pH (1, 10, 13, 16–19). There is a reason to assume, therefore, that the acid treatments interfered with the flowering response by temporarily reducing the pH of the cytoplasm. Correspondingly, the enhanced induction brought about by weak bases may be attributed to a transient elevation of the cytoplasmic pH.
The inhibitory response to weak acids had four major characteristics:

1. Inhibition was much stronger at the relatively short 12-h dark inductive period than at 16 h (Figs. 1 and 2).

2. Acid-treated seedlings required a longer inductive dark period (Fig. 4), while NHCl treated seedlings responded to shorter dark periods than controls (Fig. 8).

3. The inhibitory effect of the acid treatments was greatly dependent upon the time of application, being particularly inhibitory when applied during the first 4 h of the inductive dark period (Figs. 5 and 6).

4. The inhibitory effects of the acids were more pronounced when applied to the cotyledons than when applied to the shoot tip (Table I).

One explanation that seems to account for the first two characteristics is that treatments that interfere with the first few hours of induction leave the plant with a dark period that is too short and does not suffice for induction. However, when a 16-h dark period is applied, even if the initial few hours are ineffective because of the weak acids treatments, the plant still has a sufficiently long inductive dark period. The fact that acid treatments at the later hours of the dark period were ineffective in reducing the flowering response indicates that the first few hours of the inductive dark period are particularly sensitive to the pH changes. This may suggest that processes occurring during the initial phase of the dark period are dependent upon a relatively high cytoplasmic pH.

It should be pointed out in this context that NHCl treatments were effective mainly at marginal dark periods, under which control seedlings barely flowered (Figs. 7 and 8). This seems to indicate that the elevated cytoplasmic pH itself plays a positive role in the floral induction and compensates, to some extent, for the reduced length of the inductive dark period.

Salicylic acid and a few other phenolic acids promoted flowering of several species of the Lemnaceae (4, 12), while the analog of salicylic acid, parahydroxybenzoic acid, was completely inactive (4). We therefore included both salicylic and parahydroxybenzoic acid in our studies and both were found to be equally inhibitory.

At the onset of the present study we hypothesized that the well-known inhibitory effect of auxins on photoperiodic induction of short-day plants (2, 21) was due to their weak acid properties. The picture which emerged during the course of this study is more complex, however. (a) While the acidic auxins IAA, IBA, and α-NAA were indeed inhibitory, their activity was obtained at a much lower range of concentrations (0.1–1.0 mM) than other weak acids (5–50 mM). (b) The nonacidic auxin NAA was just as inhibitory to photoperiodic floral induction as the acid auxins. (c) The weak auxin, β-NAA (15), slightly promoted the flowering response in spite of its being an acid (Table II).

On the other hand, the much stronger inhibition by auxins at a 12-h as compared to 16-h dark period (Fig. 3) and the sensitivity to auxins during the early hours of the dark period are reminiscent of the effects of weak acids. Also, as with the acids and even more so, the effects of auxins were obtained upon treatment of the cotyledons alone, whereas treatments of the shoot tip were ineffective (Table II). This is in variance with work in Chenopodium (14) which demonstrated the inhibitory effect of auxins mainly upon application to the shoot tip.

We may conclude, therefore, that while the inhibitory effect of auxin may involve acidification of the cytoplasm, this cannot be the sole mechanism through which auxin interferes with the flowering process.

In a previous study (9), we demonstrated a requirement for 

Ca\(^{2+}\)

during the early hours of the inductive dark period. The present study hints at another cellular property—a requirement for elevated cytoplasmic pH during the same hours of the dark period. A positive correlation between acidification and high cytoplasmic Ca\(^{2+}\) levels was found in other systems (5, 6, 13). The results of our studies indicate a reverse trend—the photoperiodic flower induction is inhibited by acidification as well as by transient lowering of cytoplasmic Ca\(^{2+}\) levels. Further studies are required to elucidate these cellular events.

**LITERATURE CITED**


Bandurski, J Krekule eds, Physiology and Biochemistry of Auxins in Plants. Academia Praha, Prague, pp 159–164