Gibberellins in Relation to Growth and Flowering in *Pharbitis nil* Chois1

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**ABSTRACT**

Flowering can be modified by gibberellins (GAs) in *Pharbitis nil* Chois, in a complex fashion depending on GA type, dosage, and the timing of treatment relative to a single inductive dark period. Promotion of flowering occurs when GAs are applied 11 to 17 hours before a single inductive dark period. When applied 24 hours later the same GA dosage is inhibitory. Thus, depending on their activity and the timing of application there is an optimum dose for promotion of flowering by any GA, with an excessive dose resulting in inhibition. Those GAs highly promotory for flowering at low doses are also most effective for stem elongation (2,2-dimethyl GA4 > GA3 > GA1 > GA2 > GA5 > GA7). However, the effect of GAs on stem elongation contrasts markedly with that on flowering. A 10- to 50-fold greater dose is required for maximum promotion of stem elongation, and the response is not influenced by time of application relative to the inductive dark period. These differing responses of flowering and stem elongation raise questions about the use of relatively stable, highly bioactive GAs such as GA3, to probe the flowering response. It is proposed that the 'ideal' GAs for promoting flowering may be highly bioactive but with only a short lifetime in the plant and, hence, will have little or no effect on stem elongation.

There are many reports of both promotive and inhibitory effects of applied GA1 on flowering of herbaceous higher plants and for woody species (20, 21, 30 and references therein). Even for a single woody species, apple, both inhibition and promotion of flowering have now been reported (9 and references therein). Depending on the species, the site(s) and mode(s) of GA action could differ, and this may account for the contradictory responses observed between and within a species. The complexity of response has been much discussed (7, 26). As a further illustration, Warm (24) concluded that GA3 acted at two sites, in either or both the leaf and the shoot apex, in promoting the flowering of *Hyoscyamus niger*.

Although some of the contrasts noted in the responses to GAs may reflect differences in the site and timing of treatment, the GA used and its uptake and metabolism in the plant could also be important. To examine the various factors on the response of flowering to GAs, we have used the SD plant *Pharbitis nil*. Its flowering can be promoted by GA3 both for the dwarf strain Kidachi (16, 17), which is naturally low in bioactive GAs (1) and for the tall strain, Violet, after its treatment with CCC (26). Flowering of both these strains can be triggered by a single exposure to a dark period of about 12 h in duration (5), and this responsiveness has allowed us to examine with precision the effects of various GAs on early processes of floral induction. Simultaneously, the effectiveness of various GAs on stem elongation has been examined.

**MATERIALS AND METHODS**

Growing Conditions and Plant Material. Seeds of *Pharbitis nil* Chois, the dwarf strain Kidachi (obtained from Y. Ogawa, Mie Univ., Tsu City, Japan), and the tall strain Violet (obtained from Muratane Seed Co. Kyoto, Japan) were treated with concentrated H2SO4 for 35 min and then washed in running water overnight at 30°C. The seeds were sown into a mixture of equal parts of perlite plus vermiculite in 12-cm diameter pots. The seedlings were raised in continuous light (200 μmol m⁻² s⁻¹ PAR) at 30°C for 2 d, then at 25°C for a further 2 d before a dark period of about 12 h at 27°C. After darkness the seedlings were moved to continuous light at 21°C until dissected for flowering response 10 to 14 d later. Seedlings were watered twice daily, once with water and once with Hoagland nutrient solution.

Dwarfing of strain Kidachi reflects its inability to produce high levels of GAs (1). Therefore, GA response was also examined in one experiment using near-isogenic lines derived from a cross between strains Violet and Kidachi (RW King, unpublished data). This use of near-isogenic lines provided a comparison of the tall/dwarf character in an otherwise common genetic background.

The dwarf strain Kidachi was used because of the known history of GA3 promoting its flowering (16, 17). A marginally inductive photoperiod was chosen to provide us with the possibility of seeing both promotive and inhibitory effects of the growth regulators. Initially, we applied less-polar, monohydroxylated GAs. This allowed us to 'pulse' the GA by using the plant's own capability of metabolism to remove excessive levels of the GA (or its bioactive metabolites) which might have a negative effect on flowering (e.g. by promoting stem elongation). We thought that this technique might allow the applied GA per se or one of its several metabolites, to yield a positive morphogenetic effect on flowering without significantly affecting vegetative growth. The use of a broad range of GA doses for the less polar GAs further increased the likelihood of finding a dosage that might be optimal for flowering, with minimal effects on (or from) other growth processes. Finally, the use of varied times of application (relative to the inductive photoperiod) allowed us to

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3 Abbreviations: GA/GAs, gibberellins as a class; CCC, 2-chloroethyltrimethylammonium chloride.
determine whether there were periods of increased sensitivity (with regard to either positive or negative effects) and, when combined with a broad range of dosages, further increased the likelihood of finding an optimal dosage.

Application of Gibberellins. The GAs were dissolved in 80, 90, or 95% ethanol/water (v/v) and applied as a 1 μl drop to the underside of the petiole of each cotyledon. Control plants were treated with solvent alone. Sources or methods of synthesis of GAs were: GA₁ and 2,2-dimethyl GA₄ (2, 12 and references therein); GA₃ (Sigma Chemical Co.); GA₄ (>97% A₄) and GA₅ (>95% A₅) (Abbott Laboratories, North Chicago, IL); GA₅ (14); GA₅ was purified from immature fruit of Prunus persica (25).

The choice of GA₄ and GA₅ was based on their known rapid metabolism in several plant systems, including Pharbitis (6) and apple (10), as well as being active in the promotion of flowering (19, 20). The use of GA₃ constituted a 'control' of sorts (e.g. it was used by Ogawa [16, 17]), and it is known to be relatively long-lived (11). Once activity was evident from the use of GA₄, the use of the highly biologically active C-2 dimethyl derivative of GA₄ (see Refs. cited in [12]) seemed logical. Gibberellin A₅ was chosen because of its high efficacy on conebud differentiation in Pinaceae conifers (21), and GA₅ was chosen because of its known role as the 'effector' GA in vegetative stem elongation of both a monocot and a dicot (23). Gibberellin A₅ was chosen because a compound(s) of similar chromatographic polarity appeared immediately following the inductive photoperiod in Lolium (22), and Lona (8) had earlier noted a significant flowering effect on Perilla of extracts of immature peach seeds, which are known to be especially rich in GA₃ (25).

There were three or four pots per treatment with six or seven seedlings per pot. For any one treatment the variance between pots was less than that between plants, so variance between pots within treatments was neglected for statistical analysis. Values given are means, ±1 SE of the mean, except where otherwise noted.

RESULTS AND DISCUSSION

Gibberellin Dosage and Stem Elongation. Stem elongation increased with GA dosage up to at least 20 μg per plant (Fig. 1). In one experiment (not shown) GA₅ at various doses was included along with 2,2-dimethyl GA₄, GA₃, and GA₄. Final stem length depended on the harvest date. However, it is clear that effectiveness of the various GAs was: 2,2-dimethyl GA₄ > GA₅ > GA₃ > GA₅ > GA₇ > GA₄. Thus, greater activity of 2,2-dimethyl GA₄ than for GA₅ (50–100 times) holds not only for monocotyledonous plants as has been noted (12), but also for at least one dicotyledonous plant. This ranking was maintained in the tall and dwarf strains and for GAs dissolved in 80, 90, or 95% ethanol. In an earlier study Ogawa (16) reported a somewhat similar ranking of effectiveness for GAs dissolved in water and applied to strain Kidachi (GA₅ > GA₇ = GA₁ > GA₃ > GA₄).

An approximately 10-fold greater dose of any GA was required to trigger elongation of the dwarf strain, Kidachi, compared with the tall strain, Violet (Fig. 1). A comparable difference for GA₇ is also evident for an isogenic tall/dwarf line (see later). These differences in response could be related to the earlier finding of Barendse and Lang (1) of an approximately 3-fold lower level of bioactive GA in Kidachi than in Violet. Their data relating to responsiveness to exogenous GA₃ are ambiguous but also point to greater doses being required for Kidachi. It is clear that the relative ineffectiveness in promotion of elongation by GA₃ or GA₅ is not an indication of a late biosynthetic block as both these GAs were equally ineffective in the GA-sufficient tall, or the GA-limited dwarf strains. Rather, their low activity in stem elongation probably reflects a reduced uptake or transport (12), enhanced metabolism (catabolism/conjugation), and/or whether or not the GA is active per se or must first be converted to an active form (23). Differences in metabolism are probably the most likely explanation for activity differences (e.g. GA₅ and GA₇ versus GA₃, GA₅, GA₇, or GA₄). The dramatically enhanced effectiveness of 2,2-dimethyl GA₄ for stem elongation (Fig. 1) may result primarily from increased uptake and transport (12) rather than from any hindrance of 2β-hydroxylation (12).

Not only was plumule/stem elongation enhanced by GAs, but the petiole and hypocotyl elongated and the area of the cotyledons almost doubled. These latter changes were first evident 2 d after treatment and had gone to completion within 9 d (results not shown).

Effect on Stem Elongation of Time of Gibberellin Treatment. For both Kidachi and Violet sensitivity of stem elongation to GA₃ or GA₅ did not change dramatically with time (Fig. 2). Effects of GAs on elongation were no different whether seedlings

![Fig. 1. Effect on stem length of varying amounts of different GAs applied once to the petiole of Pharbitis, at 16 h (a) or 5 h (b, c) before induction for the dwarf strain, Kidachi (a, b) or the tall strain, Violet (c). Flowering data for Kidachi are given in Figure 3. All GAs were applied in 95% ethanol unless otherwise indicated. The inductive dark period of 13.25 h began about 130 h after seed sowing. Means are for at least 16 replicate seedlings. Bars are 2 × the SE of the mean.](https://www.plantphysiol.org/doi/abs/10.1104/pp.1987.76.2.1127)
had been kept in continuous light or induced to flower by exposure to a single dark period. The reduced stem elongation following later applications possibly reflects the fact that stem length was measured at a fixed real time and not at a fixed time from treatment. In addition, older seedlings may not have been as responsive. Over a 24-h period Ogawa (17) also found no effect of time of application on shoot elongation, although in his data the response to GA3 was limited (e.g., stem lengths of 20 mm without GA3 versus 50 mm after GA3 application).

**GA Dosage and Flowering.** Flowering of strain Kidachi (but not Violet) could readily be promoted by a single application of GA prior to an inductive dark period (Fig. 3). However, there was a clear optimum, and both the minimum effective dose and the optimum dose were higher the less the activity of the GA in promoting stem elongation (see above). GA3 was equally effective in these two experiments when applied in 95 or 80% ethanol. The highly water-soluble GA12, however, appeared to be less effective in 95% than in 80% ethanol (data not shown). Ogawa (17) found only stimulation of flowering up to a dose of 0.1 μg GA3 per plant and clearly from our data this was at or below the optimum. His applications were to the plumule and the GA3 was dissolved in water. However, these differences in technique are unimportant. As shown in Table I, flowering was inhibited by high doses of GA3 (10 μg/plant) whether application was in ethanol or water, or to the plumule or petiole. Clearly, had Ogawa (17) examined higher dosages of GA3, then inhibitory effects of GA3 would also have been evident.

**Flowering and the Time of Gibberellin Application.** The inhibition of flowering at high GA dosage correlated with considerable stimulation of stem elongation (Fig. 1 versus Fig. 3), and its action was consistent at all times of GA application before and after the inductive dark period (Fig. 2). However, for flowering, both promotive and inhibitory responses were evident depending on the timing of treatment. Low GA dosages prior to the inductive dark period were generally promotory as illustrated for GA3 and GA4 (Fig. 4). The two separate experiments with GA3, and the one with GA4 (Fig. 4) highlight the phase of inhibition that occurs after the inductive dark period. Promotion before darkness was, however, not always evident. This we attribute to difficulties in applying an optimal GA dose at just the right time before darkness, yet one which is not so high that it remains active in the plant until the inhibitory phase occurs, some 24 h later.

**Genotype and Gibberellin Response.** Strain Violet has a high content of bioactive GAs (1), and its stem elongation in response to GAs is more sensitive than for the dwarf strain Kidachi (see above). Any effect of GAs on flowering of strain Violet could, however, be demonstrated only with great difficulty. Only in one
Table 1. Inhibition of Flowering by GA3

<table>
<thead>
<tr>
<th>Site of GA3 Application (20 μg/plant)</th>
<th>Control</th>
<th>Ethanol as Solvent</th>
<th>Water as Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petiole</td>
<td>2.85 ± 0.10</td>
<td>1.55 ± 0.10</td>
<td>2.09 ± 0.23</td>
</tr>
<tr>
<td>Plumule</td>
<td>2.85 ± 0.10</td>
<td>1.81 ± 0.10</td>
<td>1.62 ± 0.15</td>
</tr>
</tbody>
</table>

mean flowering response ± 1 se of the mean

FIG. 4. Effect of time (relative to the start of the inductive dark period) of a single petiole application of GA3 (20 μg/plant) on flowering of Pharbitis strain Kidachi. Controls (dosage 0) treated once only with 95% ethanol. Three separate experiments are shown (a, b, c).

FIG. 5. Effect of time (relative to the start of the inductive dark period) of application of GA3 (20 μg/plant) on flowering of Pharbitis strain Violet. Data is for three separate experiments with inductive dark periods of: 12.0 (♀); 12.5 (♂); 13.25 h (♀). Bars are 2 × the se of the mean. Values are significantly different from control (P ≤ 0.05) for promotion by GA3 (12 h dark) and for inhibition by GA3 (12.5 h dark).

King, unpublished data. As for the parent strains, a lower GA dose was required in the tall than in the dwarf line for stimulation of stem elongation (Fig. 6). Again, as for dwarf Kidachi (Fig. 3), in the isogenic dwarf, for applications prior to the inductive treatment, promotion of flowering was found at low doses, and at higher doses inhibition was observed. Flowering of the tall isogenic line, however, was not influenced by any dose of GA3.

In a further experiment even lower GA3 doses (<2 ng/plant) were tested but, again, there was no response. Possibly for this tall isogenic line endogenous levels of GAs are saturating for flowering, and in strain Violet the level may be close to saturating.

CONCLUSIONS

Promotion of flowering of dwarf lines of Pharbitis nil is possible but only with careful choice of GA dosage, type, and the timing of treatment relative to an inductive dark period.

Although GAs appear to be nonlimiting for flowering in normal height, high-GA lines of Pharbitis (e.g. Figs. 5 and 6), they were limiting in dwarf lines (e.g. Fig. 3) and in tall seedlings treated with CCC (26). Use of B-955 (N,N-dimethylamino-succinamic acid) not only retarded stem growth of P. nil (cv Violet), it also inhibited flowering, implying a GA requirement for both processes (27). In noninductive conditions GAs have no effects on flowering of Pharbitis (18), but this lack of activity could equally well reflect the inhibition of flowering resulting from too much GA or from a GA that may have been more readily taken up and/or transported to a site of action (e.g. 2,2-dimethyl GA4 versus GA4, Figs. 1 and 3), or, after a SD exposure, from applications made after the inductive treatment. In the present study, this inhibition of flowering was correlated with excessive stem elongation and, as summarized in Figure 7, an ‘effective’ GA for flowering may be relatively ineffective for stem elongation, as is also evident for GA4 on apple (9) and GA14 on Lolium temulentum (22). Distinctions between GA type and vegetative versus flowering responses may also explain the effectiveness for flowering of GA4 on Chrysanthemum (19) and of GA14 on another SD plant, Impatiens (15).

One explanation for our finding of an optimum in the response
of flowering to GAs is that they play a dual role. A single site of response appears probable, and there is compelling evidence that action is at the shoot apex in *Pharbitis* (17, 18, 26; Table I). Perhaps this action involves a change in the plastochron at the shoot apex. Such a change, for example, is known to be associated with the GA$_3$-promoted flowering in *Xanthium* (3). The switch from promotion to inhibition could relate to enhancement of stem elongation in too close an association with the progression of events of flower initiation and early differentiation. At higher doses of GA (prior to induction), however, a potentially massive promotion of flowering may be counteracted by too long a retention of the GA, which then leads to inhibition of flowering.

An implication of these findings is that the role of GAs in flowering may need reexamination. The reports with other species (20) of GAs either inhibiting or promoting flowering might reflect extremes of a dual response that would only be evident from applications of the appropriate GA at the right dose and timing. One such case is seen in apple in which inhibition of flowering has generally been reported for treatments with GA$_3$, a GA$_3$/7 mixture, or GA$_7$, whereas promotion results with GA$_4$ or 3-epi-GA$_4$ applied at the appropriate time (9). Attention to timing and GA type has also allowed, routinely, the promotion of flowering by GA$_{4/7}$ and other less-polar GAs applied to Pinaceae family conifers (see literature cited in Refs. 20 and 21).

If the more effective GAs for flowering are essentially "pulsed," that is, remain active for only a short period before metabolism renders them ineffective, then it is intriguing that change in the photoperiod causes a brief switch in availability of endogenous GAs. Work by Zeevaart and colleagues (4, 13, 28, 29) indicates that conversion of the C$_9$ GA$_{19}$ to the C$_9$ GA$_{20}$ is under photoperiodic control. Our further studies of GA changes at the shoot apex of the LD plant *Lolium temulentum* (22) also support such a scenario. The availability of GA precursors to deliver such a pulse of GA at the apex on receipt of a flowering stimulus also fits with our findings herein of a brief "time window" and narrow 'optimum dose range' for GAs to promote flowering.

**LITERATURE CITED**


