Effects of Gibberellins and Gibberellin-biosynthesis Inhibitors on Stem Elongation and Flowering of *Raphanus sativus* L.

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Summary

The effect of exogenous applications of several gibberellins (GAs) native to a cold-requiring plant *Raphanus sativus* L. was investigated to uncover the role of the GAs in cold-induced stem elongation and flowering of the plant. GA1 and GA4 promoted the stem elongation and flowering, whereas GA2 and GA20, which are thought to be precursors of GA1 and GA1, respectively, were less active. Prohexadione calcium, which inhibits hydroxylation of GAs including 2β- and 3β-hydroxylation, reduced the effects of GA2 and GA20; in contrast, it enhanced the effects of GA1 and GA4. These results strongly indicate that GA1 and GA4 are the endogenous biologically-active GAs in cold-induced stem elongation and flowering of *R. sativus*. We found no difference between GA1 and GA2 in their relative activity to stem elongation vs. flowering; thus, we suggest that they have the same role in the promotion of stem elongation and flowering. Thus, it will be necessary to reduce GA1 and GA4 concentrations to control undesired stem elongation and flowering.

Key Words: flowering, gibberellin, *Raphanus sativus* L., stem elongation, vernalization.

Introduction

Japanese radish (*Raphanus sativus* L.) is a cold-requiring plant (CRP), both imbibed seeds and green plants of the species are cold inducible (Eguchi and Koide, 1944; Kagawa and Sata, 1957). Control of the bolting, i.e. stem elongation and flowering, is important for taproot production, because unusually cool temperature and low light intensity often induce untimely bolting (Katsura and Kunishige, 1994).

It is generally concluded that endogenous gibberellins (GAs) play a regulatory role in cold-induced stem elongation of CRPs (Zeevaart, 1983; Pharis and King, 1985). However, participation of GAs in the flowering of CRPs is still unclear because exogenous applications of GAs and GA-biosynthesis inhibitors do not affect flowering of many CRPs (Zeevaart, 1983; Pharis and King, 1985).

In our previous experiment (Nishijima et al., 1997), not only cold-induced stem elongation but also flowering of *R. sativus* were significantly retarded by uniconazole (UCZ), a GA-biosynthesis inhibitor. This result suggests involvement of GAs not only in cold-induced stem elongation but also in the flowering. Because the early 13-hydroxylation and the early nonhydroxylation pathways of GA-biosynthesis may operate in shoot of *R. sativus* (Nishijima et al., 1995; Nakayama et al., 1995), it is possible that GA1 and GA4 are the endogenous biologically-active GAs by analogy to several other species (Murakami, 1972; Phinney and Spray, 1982; Zeevaart et al., 1993; Nakayama et al., 1991). Because GA1 and GA4 concentrations in stems increased in long-day (LD) after a cold treatment, the increase probably promoted cold-induced stem elongation and flowering (Nishijima et al., 1995; Nakayama et al., 1995).

In this study, we applied GA1, GA4, and their respective precursors, GA20 and GA2, to test their effects on stem elongation and flowering. The investigation have two major purposes; one, to ascertain whether
GA$_1$ and GA$_4$ are the endogenous biologically-active GAs in cold-induced stem elongation and flowering of *R. sativus*; two, to investigate the functional difference of endogenous GAs in stem elongation vs. flowering, because, in a few other plants, the relative activity of reproductive vs. vegetative growth differs depending on GA species (Evans et al., 1990; Katsura et al., 1991). Thus, we applied proxehadione calcium (DOCHC), an inhibitor of hydroxylation of GAs (Nakayama et al., 1990), together with GAs to retard the conversion of the applied GAs in plant tissue. Based on results of those experiments, the role of endogenous GAs in cold-induced stem elongation and flowering is discussed with respect to the physiological basis of flowering.

**Materials and Methods**

**Plant material**

Seeds of *Raphanus sativus* cv. Taiybo-sobutori (Takii Seed Co. Ltd., Japan) were sown in 1/5000 a Wagner pots filled with mixture of commercial horticultural medium (Kureha-engei-baido, Kureha Chemical Co., Japan) and sand (1:1). As shown in Fig. 1, two courses of treatments were designed for the present experiments. During the treatments, the temperature was kept at 22 ± 3°C except during a cold treatment (CT). The plants were grown in a short-day condition (SD) for 10 days after sowing, then the plants were thinned, leaving 3 per pot. SD consisted of 8 h daily natural sunlight, followed by a 16 h dark period. Thereafter the plants were kept at 5°C under a 8 h photoperiod (fluorescent lamps at 10 μmol·m$^{-2}$·s$^{-1}$) for 20 days (CT). The plants were then subjected to 80 days of long-day photoperiodic treatment (LD), which consisted of the same 8 h light period as that of SD, but supplemented with the subsequent 8 h of light supplied by fluorescent and incandescent lamps at 5 μmol·m$^{-2}$·s$^{-1}$ in total. Incandescent lamps were used to increase the light intensity ratio of red light against far red light to get sufficient long-day effect. In contrast, the unchilled control plants were transferred to LD immediately after the 10 days of growth in SD after sowing.

**Application of chemicals**

For application of uniconazole [UCZ, (±)-(E)-1-(4-chlorophenyl)-4, 4'-dimethyl-2-(1, 2, 4'-triazol-1-yl)-1-penten-3-ol, Sumitomo Chemical Co., Japan], each pot was soil-drenched with 100 ml of aqueous solution of 4 μmol UCZ 3 days before CT. Authentic gibberellins (GAs, 10-1000 μM) were dissolved and 50 mM of proxehadione calcium (DOCHC, Calcium 3-hydroxy-4-propionyl-5-oxo-3-cyclohexanecarboxylate, Kumiai Chemical Co., Ltd., Japan) were suspended into 10% aqueous acetone containing 0.05% Tween 20. Forty microliters of the solution were applied with a syringe to the apical portion of a plant at 3-day intervals beginning 2 days after the onset of LD treatment until the end of the experiment. When both GA and DOCHC were applied to the same plants, DOCHC were applied first, then GA was applied immediately after the DOCHC solution had dried.

**Growth measurement and statistical analysis**

Stem length and number of main-stem leaf were recorded at flower opening. In contrast, apical portions of the plants which did not bloom were collected at the end of LD, and observed, using a dissecting microscope, to count the leaves including leaf primordia, and to identify the floral stage based on the criteria by Eguchi and Koide (1944). Days to flower opening were counted from beginning of LD (Fig. 1). Floral initiation was the time when floral primordia were formed.

Six plants in 2 pots were used per a treatment, and the experiments were duplicated. Since the duplicated experiments gave nearly the same results in all experiments, the data were pooled before being subjected to an analysis of variance. The averages were compared by Tukey’s LSD at 5% significance.

**Results**

**Effect of GAs on stem elongation and flowering of the cold–nontreated plants**

Without GA application, stem elongation and floral initiation did not occur on unchilled plants (Table 1). However, significant amount of stem elongation and floral initiation were induced by GA application except for GA$_{20}$, although no plant achieved flower opening until the end of LD. GA$_4$ at or more than 10 μM induced stem elongation and floral initiation, whereas more than 100 μM of GA$_1$ and GA$_9$ were required to attain the same results; but the percentage of floral initiation was higher in the GA$_1$ than in the GA$_9$ treatment. Consequently the efficacy of the GAs for stem elongation and flowering was GA$_4$ > GA$_1$ > GA$_9$ >
GA_{20}. The degree of the promotion of flowering was proportional to that of the stem elongation, regardless of GA species and dosage.

**Effects of DOCHC on exogenous GA-induced stem elongation and flowering**

DOCHC alone did not induce stem elongation and flowering (data not shown); but it slightly enhanced stem elongation induced by GA_{1} and GA_{4} (Fig. 2); it did not affect floral initiation induced by those GAs. Contrarily, DOCHC almost completely reversed the stem elongation and floral initiation induced by GA_{0}.

**Effect of GAs on the UCZ-retarded stem elongation and flowering of the cold-treated plants**

Uniconazole strongly retarded cold-induced stem elongation and flowering (Table 2); but four GAs at 10 \( \mu \text{M} \) completely reversed the retardation of flowering. However, except for GA_{4}, concentration higher than 10 \( \mu \text{M} \) was necessary to reverse completely the retardation of stem elongation. The efficiency of reversing UCZ-retardation of stem elongation was GA_{4} \geq GA_{1} \geq GA_{0} \geq GA_{20}.

**Effects of DOCHC on exogenous GA-reversal of UCZ-retarded stem elongation and flowering**

Without GA application, DOCHC did not affect the retardation of stem elongation and flowering by UCZ, whereas DOCHC without GA and UCZ application significantly retarded stem elongation without affecting flowering when compared with the untreated control plants (data not shown). DOCHC did not affect GA-reversal of the UCZ-retardation of flowering except that it significantly suppressed the reversal by GA_{0} and GA_{20} in the delay of flower opening (Fig. 3). Furthermore, DOCHC did not reverse UCZ-retardation of stem elongation by GA_{1} and GA_{4}. However, the reversal of UCZ-retardation of stem elongation by GA_{0} and GA_{20} was effectively reduced by DOCHC.

**Table 1.** Effect of exogenous gibberellins (GAs) on stem elongation and flowering of unchilled plants of *Raphanus sativus* cv. Taibyo-sobutori.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (( \mu \text{M} ))</th>
<th>Stem length (cm)</th>
<th>Floral initiation (%)</th>
<th>Leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT(^z)</td>
<td>10</td>
<td>1.7 e</td>
<td>0</td>
<td>55.6 a</td>
</tr>
<tr>
<td>GA_{1}</td>
<td>10</td>
<td>7.4 e</td>
<td>17</td>
<td>54.2 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>47.0 c</td>
<td>100</td>
<td>45.9 d</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>76.2 a</td>
<td>100</td>
<td>47.0 bc</td>
</tr>
<tr>
<td>GA_{4}</td>
<td>10</td>
<td>47.4 c</td>
<td>100</td>
<td>44.9 d</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>57.8 b</td>
<td>100</td>
<td>45.3 d</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>76.8 a</td>
<td>100</td>
<td>43.0 d</td>
</tr>
<tr>
<td>GA_{9}</td>
<td>10</td>
<td>1.6 e</td>
<td>0</td>
<td>54.6 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>24.2 d</td>
<td>58</td>
<td>51.2 abc</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>53.8 bc</td>
<td>100</td>
<td>46.5 cd</td>
</tr>
<tr>
<td>GA_{20}</td>
<td>10</td>
<td>1.7 e</td>
<td>0</td>
<td>54.2 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.1 e</td>
<td>0</td>
<td>54.7 a</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>5.4 e</td>
<td>0</td>
<td>51.4 ab</td>
</tr>
</tbody>
</table>

\(^z\) Non-treated

The plants were grown as shown in Fig. 2. No plant reached flower opening until the end of LD. Means followed by the same letter in the same column were not significantly different by Tukey’s LSD at 5% level.

**Discussion**

In several plant species, 3\( \beta \)-hydroxylated C-19 GAs seem to be the biologically-active GAs (Murakami, 1972; Phinney et al., 1982; Nakayama et al., 1991; Zeevaart et al., 1993). In our experiment with *R. sativus*, the endogenous 3\( \beta \)-hydroxylated C-19 GAs, GA_{1} and GA_{4}, were highly active in the induction of stem elongation and flowering. Furthermore, the activity of GA_{0} and GA_{20}, the precursors of GA_{1} and GA_{4}, respectively, were reversed by DOCHC (Figs. 2, 3 and 4).
Table 2. Effects of exogenous gibberellins (GAs) on cold-induced stem elongation and flowering retarded by uniconazole in *Raphanus sativus* cv. Taiyo-so-butori.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (µM)</th>
<th>Stem length (cm)</th>
<th>Flower opening (%)</th>
<th>Days to flower opening</th>
<th>Leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniconazole</td>
<td>0</td>
<td>NTZ</td>
<td>-</td>
<td>54.3 ab</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>NTZ</td>
<td>-</td>
<td>7.3 f</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA1</td>
<td>10</td>
<td>35.8 d</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>45.8 bc</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>52.6 ab</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA2</td>
<td>10</td>
<td>47.2 bc</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>51.5 ab</td>
<td>100</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>56.5 a</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA9</td>
<td>10</td>
<td>40.0 cd</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>40.8 c</td>
<td>100</td>
</tr>
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<td>1000</td>
<td>58.6 a</td>
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<td></td>
<td>GA20</td>
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<td>25.3 e</td>
<td>100</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>30.8 de</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000</td>
<td>41.9 c</td>
<td>100</td>
</tr>
</tbody>
</table>

The plants were grown as shown in Fig. 2. Means followed by the same letter in the same column were not significantly different by Tukey's LSD at 5% level.

Z Nontreated

4), suggesting that GA0 and GA20 are probably inactive unless they are converted into GA2 and GA4 through 3β-hydroxylation in plant tissue (Fig. 4). In contrast, DOCHC enhanced activities of GA1 and GA4 probably through inhibition of their inactivation by 2β-hydroxylation (Figs. 2 and 4). These results strongly suggest that GA1 and GA4 are the endogenous biologically-active GAs in cold-induced stem elongation and flowering of *R. sativus*.

GA20 was much less active in inducing stem elongation and flowering than was GA9 (Tables 1 and 2). These results may suggest that the conversion of GA20 to GA3 occurs to a lesser degree than does that of GA9 to GA4.

In a few plant species, relative activities of GAs to flowering vs. growth of vegetative organ differ depending on GA species. In *Colocasia esculenta*, 13-hydroxylated GAs promoted both flowering and cornel elongation, while 13-nonhydroxylated GAs promoted flowering without significant increase to cornel elongation (Katsura et al., 1991). In *Lolium temulentum*, the presence of 13-hydroxyl group in GAs enhanced the promotive effect on inflorescence initiation, whereas the 3β-hydroxylation had a similar effect on stem elongation (Evans et al., 1990). The chemical structures of GA1 and GA4 are different by the existence of a 13-hydroxyl group. However there was no difference in activity between GA1 and GA4 relative to stem elongation vs. flowering of *R. sativus* (Table 1, Fig. 2). Thus, endogenous GA1 and GA4 probably have the same role in the promotion of stem elongation and flowering of *R. sativus*.

Small quantities of exogenous GAs completely canceled the UCZ-retardation of cold-induced flowering, while much larger quantities of GAs were necessary to overcome that of cold-induced stem elongation (Table 2). This is contrary to the results with unchilled plants in which both stem elongation and flowering required a large quantity of GA. Consequently, CT may lower the threshold concentration of stem endogenous GAs necessary for flowering greater than that necessary for stem elongation. This decrease in the "threshold GA concentration" in stem and the increase in stem GA1 and GA4 concentrations by cold induction (Nishijima et al., 1995; Nakayama et al., 1995) may interactively promote flowering of *R. sativus*. 

![Fig. 3. Effects of exogenous gibberellins and prohexadione calcium on the cold-induced stem elongation and flowering retarded by uniconazole. The plants of *Raphanus sativus* cv. Taiyo-so-butori were grown as shown in Fig. 1. Flower opening was 100% except that the plants treated with uniconazole alone was 92%. NT: nontreated with chemicals. U: uniconazole (4 µmol/pot). D: prohexadione calcium (50 mM). Gibberellins at 100 µM were applied. Vertical bars indicate standard deviation. The columns with the same letter are not significantly different by Tukey's LSD at 5% level.](image-url)
To control flowering of *R. sativus*, endogenous GA concentration should be reduced before floral evocation (Nishijima et al., 1997). Our results suggest that GA\(_1\) and GA\(_4\), the endogenous biologically-active GAs, are not functionally different in promotion of stem elongation and flowering. Thus, it will be necessary to reduce both GA\(_1\) and GA\(_4\) concentrations before floral evocation to prevent undesired stem elongation and flowering of *R. sativus*.

**Acknowledgment**

We thank Kyowa-Hakko Industry Co. Ltd. for supplying us authentic GAs. We also thank Mrs. T. Araki for her assistance.

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低温処理されたダイコンの花成と茎の伸長に対するジペレリンおよびジペレリン生合成阻害剤の影響

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摘 要

内生ジペレリン（GAs）が、低温処理されたダイコン（Raphanus sativus）の抽出（花成を伴う茎伸長）に果たす役割を明らかにするため、GAの投与が花成および茎の伸長に及ぼす影響を検討した。他にいくつかの植物で内生活性型GAであることが示唆されているGA1およびGA4は、ダイコンの花成と茎の伸長に著しい促進効果を示した。一方、GA3の前駆体であるGA20およびGA4の前駆体であるGA9は、GA1およびGA4と比べて効果が低かった。3βおよび2β水酸化を含むGAの水酸化を阻害するプロヘキサジェオンカルシウムは、GA1およびGA4の効果を高めた反面、GA20およびGA9の効果を低下させた。これらの結果から、ダイコンの花成と茎の伸長に対する内生活性型GAは、GA1およびGA4であることが強く示唆された。なお、GA1およびGA4とも、花成または茎の伸長のどちらを選択的に促進することはなかった。従って、これらのGAは花成および茎の伸長の促進に対して同じ機能をもつと考えられた。以上の場合、低温処理による花成と茎の伸長を抑制するためには、内生GA1およびGA4の両者の濃度を低下させことが必要であると示唆された。

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