Endogenous Gibberellins and Shoot Growth and Development in *Brassica napus*¹

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**ABSTRACT**

Greenhouse-grown oilseed rape (*Brassica napus*, annual Canola variety 'Westar') plants were harvested at six dates from the vegetative phase until the early pod (silique)-fill/late flowering stage. Endogenous gibberellin (GA)-like substances were extracted from stems, purified, and chromatographed on silica gel partition columns prior to bioassay in serial dilution using the 'Tan-ginbozu' dwarf rice microdrop assay. The concentrations of total endogenous GA-like substances were low during vegetative stages (1 nanogram GA₉ equivalents/gram dry weight), and rose 300-fold by the time of floral initiation. After floral initiation the concentration of GA-like substances fell, then rose again during bolting to maximal levels during the early pod-fill stage (940 nanograms per gram dry weight). The qualitative profiles of GA-like substances varied across harvests, with higher proportions of a GA₉-like substance at the early pod-fill stage. In a second study stems were similarly harvested at eight dates and the concentrations of endogenous GA₉, the principal bioactive native GA of oilseed rape, were determined by gas chromatography-selected ion monitoring using [17,17-²H]GA₀, as a quantitative internal standard. The concentration of GA₉ increased at about the time of floral initiation and then subsequently fell, thus confirming the pattern noted above for total GA-like substances. The exogenous application of paclobutrazol (PP333), a persistent triazole plant growth regulator (PGR) which blocks GA biosynthesis, or another triazole, triafoxenol (RSW0411), prevented flowering as well as bolting; plants remained at the vegetative rosette stage. These results implicate a causal role for endogenous GA in the control of bolting, which normally precedes anthesis. Further, the rise in the concentration of total endogenous GA-like substances, including GA₀, which was associated with floral initiation, and the prevention of visible floral development by the triazole PGRs, also indicates a role for endogenous GAs in the regulation of flowering in *B. napus*.

A hypothesized causal relationship between GAs, bolting, and flowering in *Brassica* predicts that the concentration and distribution of endogenous GAs should be positively correlated with the initiation of each of these physiological processes. In particular, an increase in the concentration of certain endogenous GAs, such as GA₀, might be expected to precede and accompany bolting. Consistent with this, a cold treatment (vernalization) which induces floral initiation and then bolting in biennial *Brassica* genotypes, has been reported to increase the endogenous concentration of GA-like substances (11, 27, 29). It is generally concluded that endogenous GAs probably play a regulatory role in stem elongation in *Brassica* (4, 30). However, a causal role for GAs in the control of flowering in *Brassica*, as with other cold-requiring biennials remains in dispute (1, 9, 14, 26, 30).

There is a wide range of genetic variation in the genus *Brassica*, thus providing a unique experimental system to examine the putative roles and interactions of GAs, flowering and bolting. The principal native GAs of *Brassica* (GA₀, GA₁₉, GA₃₀) have recently been characterized (20) and are members of the early-13-hydroxylation biosynthetic pathway which leads to GA₀, the pivotal GA for shoot elongation in maize, pea, and probably, rice (16). We have thus investigated the developmental changes in concentrations of endogenous GA-like substances and of GA₀, to determine if there are correlations between the concentration of GAs and shoot elongation and flowering in the shoots of an annual variety of oilseed rape, *Brassica napus*. Further, PGRs² which block GA biosynthesis were applied to reduce endogenous GA content, and thereby possibly modify bolting and flowering (2).

**MATERIALS AND METHODS**

**Plant Materials**

Six seeds of oilseed rape (*Brassica napus*, canola cultivar 'Westar') were planted in each 14 × 13 cm pot filled with Metro-mix 200 (W. R. Grace & Co., Ajax, Ontario), a peat and vermiculite medium on January 16, 1986. Pots were watered to saturation daily and fertilized weekly with 0.25 g 28-14-14 with added micronutrients (Plant Prod 28-14-14, Plant Products Co. Ltd., Bramalea, Ont.). Following seedling emergence on January 27, 1986, pots were thinned to two

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² Abbreviations: PGR, plant growth regulator; a.m.u., atomic mass units; EtOAC, ethyl acetate; GC-SIM, gas chromatography-selected ion monitoring; MeOH, methanol; MeTMSI, trimethylsilyl ether of the methyl ester; Rt, retention time; SiO₂, silicic acid.
uniform plants. Plants were grown in a greenhouse at the University of Lethbridge (latitude 49.6°N) at about 22°C (night) and 25°C (day). Supplemental lighting for 14 h daily was provided with cool-white fluorescent tubes (140 μmol s⁻¹ m⁻² PAR, determined with a Li-Cor quantum sensor [Li-Cor Inc., Lincoln, NE]). Shoot heights to the shoot apex were measured and developmental stages (5) were recorded weekly.

Analysis of Gibberellin-like Substances

Shoots were harvested at weekly intervals, leaves and pods (siliques) were removed and the remaining stem tissue was weighed and ground in −20°C 80% aqueous methanol (MeOH) (Table I). Additional stems were harvested to dissect shoot apices and these shoots were dried to determine moisture content (Table I). After vacuum filtration, MeOH was removed in vacuo at 35°C, the pH raised to 9.0 with KOH and the aqueous extract was extracted three times with water-saturated diethyl ether to remove pigments. The pH was then neutralized with HCl and the extract was slurried with polyvinylpolypyrrolidone and filtered. The pH was then reduced to 3.0 with HCl and GAs were extracted three times with water-saturated EtOAc. Water in the EtOAc-soluble extract was removed by freezing and filtration of ice, and the EtOAc was taken dryness in vacuo at 35°C. After freeze-drying, the extract residue was dissolved in MeOH:EtOAc (1:1) and loaded onto glass-fiber filter paper discs prior to stepwise elution SiO₂ partition column chromatography (3, 21). The resulting 26 fractions were bioassayed at 1/200 and 1/400 dilutions using a modified (19) Tan-ginbozu dwarf rice microdrop assay (13).

For each of the six harvests three replicate samples were analyzed. Due to the considerable variation in absolute sensitivity of the GA bioassay, the experimental design involved the simultaneous analysis of all six samples from one complete replicate of the six harvests. The six samples were processed side-by-side and bioassayed in a given trial. Thus, quantitative comparisons across each replicate are valid but differences across the three replicates reflect differences in bioassay sensitivity superimposed on the differences due to ontogeny.

Four regions of GA-like activity were eluted eluting from the SiO₂ columns and the level of GA-like activities were summed for each region. Statistical comparisons of levels of GA-like activity in each of the four regions, as well as for concentration of total GA-like activity, were based on Kruskal-Wallis analyses, the non-parametric equivalent of one-way analyses of variance.

Analysis of Endogenous GA, by GC-SIM

Seeds were planted January 13, 1987, under conditions similar to those described above and harvests of stem tissue were collected at 3 to 7 d intervals around the time of floral initiation. Freeze-dried stem tissue was ground in −20°C 80% aqueous MeOH and 100 ng [17,17-³H]GA₁ (99.2% enrichment) (20) was added as an internal standard. Purification was similar to that previously described, although 16.7 Bq [³H]GA₁ 1.21 TBq per mmol, (Amersham) was added immediately prior to SiO₂ partition column chromatography. The fractions coeluting from SiO₂ partition columns with [³H]GA₁ were subsequently purified on reversed-phase C₁₈ HPLC (8, 19). The HPLC fractions containing the [³H]GA₁ were derivatized and analyzed by GC-SIM as previously described in detail (19). Quantities of endogenous GA₁ were based on ratios from the abundances of the 506 (GA₁) and 508 a.m.u. (²H₂-GA₁) although two other characteristic ion pairs were also monitored (491/493 and 448/450 a.m.u.). Two or three replicate samples were analyzed for the eight GA₁ harvests. Variation across the replicates was always less than 100% and averaged about 30%.

Plant Growth Regulators

Two oilseed rape cultivars, 'Westar' and 'Pivot', were grown under the same conditions and timing as outlined above in "Plant Materials." Thirty-three d after seedling emergence, at which time plants were at growth stage 3 (5), PGRs were applied with a hand-sprayer delivering about 1 mL to each plant at one of three concentrations: paclobutrazol (PP333; 1-(4-chloroethyl) 4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-0) (18) at 70, 64, or 0.6 mg mL⁻¹; triapenthenol (RSWO411; R-(cyclohexal methylene)-γ-(1,1-dimethyl)-1H-1,2,4-triazole-1-ethanol) (18)—100, 91.1, 0.8 mg mL⁻¹, both in 0.05% v/v Tween 20. Heights to the shoot apex and developmental stage were recorded weekly for each of the 10 replicate plants for each treatment.

RESULTS AND DISCUSSION

Endogenous GA-like Substances

Four regions of GA-like activity eluted from the SiO₂ partition columns loaded with extracts from developing oilseed rape stems (Fig. 1). Region I eluted coincidentally with [³H]GA₂₅₀, region III with [³H]GA₁, and region IV eluted in the MeOH wash in which GA glucosyl conjugates would elute, if present. Since the extracts represented only the acidic

<p>| Table I. Description of Plants Harvested in 1986 for Analysis of GA-Like Substances |
|---------------------------------------------|-----------------|---------------|-----------------|-----------------|---------------|</p>
<table>
<thead>
<tr>
<th>Harvest</th>
<th>Days from Emergence*</th>
<th>Development Stage</th>
<th>No. of Plants/Sample</th>
<th>Moisture Content</th>
<th>Dry Wt/Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>g</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>Vegetative (2.3) ⁷</td>
<td>10</td>
<td>95</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>Vegetative (2.4) ⁷</td>
<td>4</td>
<td>95</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>prior to bolting (3.1)</td>
<td>3</td>
<td>94</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>early bolting (3.2)</td>
<td>3</td>
<td>93</td>
<td>7.6</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>late bolting (4.1)</td>
<td>2</td>
<td>92</td>
<td>17.3</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>early pod-fill (4.2)</td>
<td>2</td>
<td>88</td>
<td>15.1</td>
</tr>
</tbody>
</table>

* Emergence (50%) occurred on January 31, 1986.  
⁷ Stage as per Harper and Berkenkamp (5).
EtOAc-soluble fraction from the extracts, many glucosyl conjugates would have been previously discarded with the acidic aqueous phase. Further, the dwarf-rice microdrop assay is principally responsive to free GAs and GA glycosyl esters and relatively insensitive to GA glucosides (22). Hence, the level of GA-like activity in region IV does not represent the total of GA glucosyl conjugates present in the original extracts.

Different ratios of the four regions of GA-like activity from the SiO2 partition column were apparent in the sequential harvests of stem tissue (Table II). Harvests 1 and 2 contained very low concentrations of GA-like substances which were generally of a less-polar nature, largely coincidental with the Rt of [3H] GA20 (Fig. 1). GA20 is native to oilseed rape and a logical precursor of GA1 (23). At harvests 3 and 6, which contained the highest GA concentrations (Table II), the more polar GA1-like substance predominated. The principal GA in the harvest 6 tissue was confirmed to be GA1, although modest amounts of GA3 and small amounts of iso-GA3 were also found (20). Since GA1 is the effector GA for shoot elongation in maize, pea, and probably rice (16), changes in the concentration of GA1 are particularly relevant in an analysis of the regulation of stem elongation (bolting) in *Brassica*.

Comparisons of total GA-like concentrations (Fig. 2b) of the shoots (stems and apex, no leaves) indicated statistically significant differences (Table III; H = 14.2, P = 0.007). The total concentration of GA-like substances was low during the vegetative phases of growth (harvests 1 and 2). Harvest 3 occurred just prior to bolting (Fig. 2a) and the concentration of total GA-like substances at harvest 3 was 200-fold higher than that of the preceding week (Fig. 2b). The observation that a high concentration of GA-like substances precedes the actual stem elongation supports a regulatory role for GAs in the bolting response in oilseed rape. The total GA concentration then fell from its very high level prior to bolting but still remained reasonably high during the period of rapid stem elongation (harvests 4 and 5, Fig. 2b). The maximal concentration of GA-like substances was detected in the final harvest (harvest 6) which was taken near the end of stem elongation, when anthesis was continuing (upper stem) and pod-filling had commenced (lower stem).

**Analysis of Endogenous GA1 by GC-SIM**

The analysis of endogenous GA1 by GC-SIM using [3H] GA1 as an internal standard (Fig. 2c) confirmed the early peak observed for total GA-like activity (Fig. 2b). The rise in endogenous GA1 preceded bolting and also coincided with the approximate time of floral initiation. Whether this early increase in GA1 serves as a trigger for floral initiation is unknown, but it is noteworthy that Thomas *et al.* (27) observed a similar bimodal pattern of endogenous GA-like substances following vernalization of *Brassica oleracea*. Further, Pocock and Lenton (17) observed a rise in endogenous GAs which immediately followed vernalization in sugar-beet.

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**Table II. Qualitative Groupings of GA-Like Substances from Oilseed Rape Stems**

<table>
<thead>
<tr>
<th>Harvest</th>
<th>I (tr. 1-7)</th>
<th>II + III (8-21)</th>
<th>IV (22-25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA0*</td>
<td>GA1, GA0, GA0*</td>
<td>GA-conjugates</td>
<td></td>
</tr>
<tr>
<td>ng GA equiv/g dry wt (percentage of total GA-like substances)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.8 (45)</td>
<td>0.9 (55)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>135 (31)</td>
<td>155 (63)</td>
<td>12 (6)</td>
</tr>
<tr>
<td>4</td>
<td>4.8 (58)</td>
<td>2.9 (25)</td>
<td>1.8 (16)</td>
</tr>
<tr>
<td>5</td>
<td>1.1 (6)</td>
<td>6.2 (34)</td>
<td>19.6 (59)</td>
</tr>
<tr>
<td>6</td>
<td>3 (6)</td>
<td>917 (83)</td>
<td>47 (11)</td>
</tr>
</tbody>
</table>

* Bioactive native GAs which would elute in these regions (Rood *et al.* [20]).
  a Only traces of GA-like substances were detected in these extracts. Concentrations were thus below 5 ng/g dry wt.
  b Percentages from the three replicates were calculated separately and then averaged. Hence, the mean levels and percentages do not produce identical ratios.

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**Figure 1.** Profiles of GA-like substances (I–IV) from step-eluted SiO2 partition columns loaded with extracts from oilseed rape plant shoots (stems and apex, no leaves) harvested on d 30 (top) and d 76 (bottom). In each plot the lower dashed line represents the leaf sheath length of control seedlings while the upper dashed line represents the response to 100 pg GA2 per rice seeding.
butrazol, which blocks GA biosynthesis, or triapenthenol, reduced shoot elongation at lower dosages, or entirely prevented it at high doses (Fig. 3). Additionally, flowering was delayed or completely inhibited by high dosages of either PP333 or RSW0411 (e.g. plants remained at the rosette stage of development for three months [Fig. 4]).

and concluded that GAs were involved in the transition from the vegetative to the floral stage.

The Application of Plant Growth Retardants

The application of the persistent triazol PGRs (18), paclobutrazol, which blocks GA biosynthesis, or triapenthenol, reduced shoot elongation at lower dosages, or entirely prevented it at high doses (Fig. 3). Additionally, flowering was delayed or completely inhibited by high dosages of either PP333 or RSW0411 (e.g. plants remained at the rosette stage of development for three months [Fig. 4]).

Viewed within the context of previous reports, our results support the involvement of endogenous GAs not only in the regulation of bolting in *Brassica*, but also in the regulation of its flowering. Exogenous GA3 promotes flowering in many *Brassica* genotypes, and under marginally noninductive temperature conditions GA3 will induce flowering without a complete vernalization treatment in some biennial genotypes (9, 28). The application of plant growth retardants which block GA biosynthesis, delay (at lower concentrations), or even prevent (at higher concentrations) flowering. The finding in the present study that an increase in endogenous GA concentration accompanies floral initiation is consistent with a promotive role for GAs in the regulation of flowering in *B. napus*. However, we analyzed GAs of the entire shoot (stems and apex, no leaves or pods) and not only the apex. Hence, the specific relationship between GA type and concentration in the shoot apex and floral initiation is unknown.

The involvement of GAs in the regulation of bolting and flowering has been implicated in photoperiodic plants as well as cold-requiring plants, thereby providing a precedence for the relationship between GAs and reproductive development (14, 30). It has also been concluded that GAs are involved in the regulation of stem elongation in *Thlaspi arvense*, a winter annual weed, which is also a member of the Cruciferae (12). Although GA3 may be the primary stem-elongation hormone...
its role in the regulation of flowering is still unclear (14, 30). Additional research may result in the further separation of GA effects on shoot elongation versus flowering in *Brassica* (24, 26) as has been achieved for certain other plants (7, 15, 25, 30). Finally, future research with *Brassica* may allow the separation of different classes or concentrations of GAs with regard to effects on stem elongation versus floral initiation (7, 15).

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