Selection and Characterization of a Gibberellin-Deficient Mutant of \textit{Thlaspi arvense} L.\textsuperscript{1}

James D. Metzger* and Amy T. Hassebrock\textsuperscript{2}

U.S. Department of Agriculture/Agricultural Research Service Biosciences Research Laboratory, State University Station, Fargo, North Dakota 58105

ABSTRACT

Field pennycress (\textit{Thlaspi arvense} L.) is a winter annual weed with a cold requirement for reproductive development. Previous work in this laboratory has demonstrated that the bolting aspect (rapid stem growth) of reproductive development is mediated by gibberellins (GA). The present paper describes the selection and characterization of a mutant lacking the capacity for thermoinduced stem growth. Seeds of an inbred field pennycress line (CR\textsubscript{1}) were treated with the chemical mutagen ethyl methane sulfonate, germinated, and allowed to produce seed. Plants derived from these seeds were screened for reduced stem growth. A mutant line, EMS-141, in which the lack of stem growth can be fully overcome with exogenous GAs, was selected for further analysis. Other phenotypic abnormalities exhibited by the mutant line include reduced petiole growth, slightly delayed floral initiation, and failure of flowers to develop fully. These are also reversed with exogenous GAs. Evidence is presented indicating that all of the alterations in growth and development exhibited by EMS-141 are conferred by a recessive mutation of a single nuclear gene. Through quantitative analysis of endogenous GA and GA precursors and a comparison of the abilities of various compounds to restore normal growth when applied to plants of EMS-141, the physiological basis for the mutant phenotype was determined to be the result of highly reduced endogenous GA levels. Moreover, the affected site in GA biosynthesis appears to be the accumulation of \textit{ant} kaurene, probably at the level of \textit{ant} kaurene synthase. The relative abilities of exogenous GA and GA precursors to restore normal growth of petioles and stems are compared, and the results are used to make inferences on the functions of the two different pathways of GA metabolism that exist in field pennycress.

Work in this laboratory has been focused on the mechanisms by which low temperatures induce stem elongation in field pennycress (\textit{Thlaspi arvense} L.), a widely distributed cruciferous weed of the northern Great Plains of North America. Previous work has shown that GA\textsubscript{3} mediates thermoinduced stem growth in this species, and it was hypothesized that thermoinduction alters GA biosynthesis and metabolism such that a specific GA accumulates above a threshold level resulting in stem growth (11). Recently, evidence from two lines of experimentation has been obtained supporting this hypothesis. These studies also implicated the conversion of the GA precursor KA to GA as a major reaction in GA metabolism under thermoinductive regulation (7, 13).

One important question that remains to be resolved is which endogenous GA is responsible for biological action in thermoinduced stem growth. Field pennycress contains two parallel pathways for GA metabolism branching from GA\textsubscript{12} (15). As in many species, field pennycress contains the early C-13 OH pathway, which includes GA\textsubscript{1} and its C-2\textsubscript{3} hydroxylation deactivation product, GA\textsubscript{4} (Fig. 1). In both maize (16) and peas (8, 9), there is good evidence for GA\textsubscript{1} being the endogenous regulator of stem growth. However, field pennycress also contains an additional parallel pathway for GA metabolism whose members are C-13 and C-3 deoxy analogs of the GA in the early C-13 OH pathway (Fig. 1). Our initial evidence indicates that, unlike maize and peas, stem growth in field pennycress is regulated by GA\textsubscript{3} rather than its C-3,13 dihydroxy analog GA\textsubscript{1} (7, 13).

Although the physiological significance of two separate pathways for GA metabolism in field pennycress is not clear, a reasonable possibility is that they function in the regulation of different processes. Thus, while GA\textsubscript{3} may initiate thermoinduced stem growth, GA\textsubscript{1} is perhaps involved in other GA-mediated processes such as petiole growth in young leaves that occur in noninduced (non-cold-treated) plants (12).

One experimental approach in clarifying the roles of the two pathways is to use mutants with blocks in GA biosynthesis and metabolism to probe the dependency of various processes on the presence of specific GA. Such a strategy has been extremely valuable in delineating the pivotal role of GA\textsubscript{1} in controlling stem growth in maize and peas (8, 9, 16). In the present paper, efforts to obtain mutants of field pennycress with altered GA biosynthesis and metabolism through chemical mutagenesis are described, and a dwarf mutant with reduced endogenous levels of GA and GA precursors is characterized.

MATERIALS AND METHODS

Plant Culture and Growing Conditions

The specific details of plant culture, photoperiodic conditions, and thermoinductive cold treatments have been described before (11). Briefly, young seedlings were transplanted...
Figure 1. Probable metabolic relationships between endogenous GA and GA precursors found in field pennycress shoots (14, 15). *Compounds have not been detected in field pennycress but are presumed to be intermediates (5).
to 10-cm plastic pots containing vermiculite and grown for 6 weeks in a greenhouse at approximately 21°C. The plants were then subjected to a 4-week thermoinductive cold treatment at 6°C and then transferred to a growth chamber at 21°C. Depending on the specific experiment, a SD or LD photoperiod was used during this final phase of growth. SD photoperiods consisted of 8 h of light from fluorescent and incandescent lamps. LD conditions consisted of the same 8-h photoperiod as SD followed by 16 h of low-intensity light from incandescent lamps (11, 12). Plants were continuously subirrigated with one-quarter strength Hoagland nutrient solution during all phases of growth (3).

Selection of Mutants

Seeds of the inbred line CR₁ were soaked in a 0.35% (v/v) aqueous solution of ethyl methane sulfonate for 24 h, rinsed under running tap water for 30 min, and then germinated in 9-cm Petri dishes lined with blotter papers that were soaked with 7 mL of water. Approximately 1000 seedlings (M₁ generation) were transplanted to 54 × 27 × 6-cm plastic flats divided into 48 equal compartments (Compaks; T. O. Plastics, Inc., Minneapolis, MN) containing vermiculite. These plants were raised in a greenhouse at approximately 21°C with daily watering with one-quarter strength Hoagland nutrient solution as described before (14). Six-week-old M₁ plants were thermoinduced at 6°C for 4 weeks, returned to the greenhouse, allowed to flower and self. The resulting seed (M₂ generation) were collected from each plant and placed in separate manila envelopes to dry and afterripen on a laboratory bench for at least 4 weeks before germination.

The M₂ seeds were germinated in 9-cm plastic Petri dishes containing a blotter paper moistened with 7 mL of an aqueous solution of GA₃ (1 mg mL⁻¹). After 1 week, 48 M₂ seedlings, originally derived from each M₁ plant, were transferred to the compartmented plastic flats as described above. The M₂ plants were then grown for 4 to 5 weeks in the greenhouse, thermoinduced at 6°C for 4 weeks, and then returned to the greenhouse. The thermoinduced M₂ plants were observed for the ability to bolt; plants exhibiting little or no stem growth 3 weeks after the end of the cold treatment were selected and treated with 10 μg of GA₃ to the shoot tip in order to promote flower development and seed production. Seeds produced by these plants (M₃ generation) were collected, dried, and then assessed for the stability of phenotypic characteristics. Stable dwarf lines were propagated from M₃ seed. One dwarf line, designed as EMS-141, was then subjected to further analysis.

Inheritance Pattern of the Dwarf Phenotype

Plants of EMS-141 were crossed with wild type (CR₁) plants using a method similar to that described by McIntyre and Best (10). Seeds of both EMS-141 and CR₁ were germinated simultaneously in Petri dishes containing blotter papers soaked with a solution containing 1 mg mL⁻¹ GA₃ or deionized water, respectively. The seedlings from both lines were raised in the greenhouse at approximately 21°C for 6 weeks and thermoinduced at 6°C for 4 weeks as described previously. When the plants were returned to a growth chamber at 21°C, the EMS-141 plants were treated with 10 μL of a 50% (v/v) aqueous acetone solution containing 1 μg μL⁻¹ GA₃.

Under these conditions, open flowers appear on plants of both EMS-141 and CR₁ in about 4 weeks. However, in field pennycress, the stamens mature and shed pollen before flower opening, thereby ensuring self-pollination (10). Thus, for the female parent (CR₁), about 10 flower buds, 2 to 3 d before opening were emasculated with the aid of a low-powered microscope. The stigmas were carefully examined for the presence of any pollen, and all of the remaining flowers, and unopened flower buds were removed. Pollination was achieved by grasping the stamens from the male parent (EMS-141) and gently rubbing the anthers against the stigma of the female parent. The stigmas were then checked with a low-powered microscope for the presence of pollen. The plants were placed in a small transparent chamber with 100% RH for 2 d before returning the plants to a growth chamber at 21°C. The progeny from the crosses were collected and germinated. The phenotypes of the F₁ plants were determined 2 weeks after the end of a 4-week cold treatment as described before. The plants were allowed to flower, self, and produce seed. The phenotypes of the F₂ progeny were assessed as described for the F₁ generation.

Growth Measurements

Stem heights were measured from the subtending node of the first visibly elongating internode to the last true leaf (11). For petiole growth, young leaves with petioles 3- to 5-mm long were selected and marked on the blade with a small spot of indelible black ink. After 2 weeks the length of the petiole from its base to the bottom of the blade was measured (12).

Application of Compounds

The effects of various compounds on the growth of petioles and stems of EMS-141 and CR₁ plants were compared. Leaves of noninduced plants (4–6 weeks old) were treated by applying 10 μL of a solution containing 1 μg of the test compound, 50% (v/v) acetone, and 0.05% Tween 20 to the blades on alternate days for a total of six applications (12). At the start of an experiment, petioles of treated leaves were between 2 and 5 mm long. Petiole lengths were measured as described before, 2 weeks after the first application. SD photoperiods were used when comparing the effects of compounds on petiole growth.

The effects of the test compounds on stem growth in thermo- and noninduced plants were determined by applying 1 μg dissolved in 10 μL of a solution containing 50 and 0.05% (v/v) acetone and Tween-20, respectively, as described previously (11, 13). Applications were made three times a week for 4 weeks. Stem heights were measured 5 weeks after the first application. LD photoperiods were used when comparing the effects of compounds on stem growth.
Quantitative Analysis of Endogenous GA and GA Precursors

The endogenous levels of GA and GA precursors in the shoots of EMS-141 and CR₁ plants were compared by GC-MS. In addition, comparisons were made for thermo- and noninduced material from both lines as well. The thermo- and noninduced plants used for quantitative analysis were harvested 10 d after the end of the thermoinductive cold treatment. Separate extraction procedures were used for GA and the kaurened precursors of GA. GA were extracted from lyophilized shoots of 96 CR₁ and 96 EMS-141 plants, by homogenization with 250 mL of ice-cold 80% (v/v) aqueous acetone in a Waring Blender. The homogenate was filtered and the residue extracted once more in an identical fashion. The two filtrates were combined and 1 µg each of the following $^{2}$H-labeled internal standards added: GA₁, GA₇, GA₉, GA₂₀, and GA₃₅. Activated charcoal and celite were added (1 g each/ g dry weight) with stirring. After 30 min, the extract was filtered, and the acetone was removed under reduced pressure at 35°C. Following adjustment of pH to 2.5 with 6 N HCl, the remaining aqueous solution was partitioned four times with equal volumes of ethyl acetate. The organic phases were combined, dried over anhydrous Na₂SO₄, reduced in volume, and subjected to silicic acid adsorption chromatography (15).

The eluates from silicic acid chromatography were then fractionated by gradient-eluted reverse-phase HPLC as described previously (14). Fractions were collected every minute from 5 to 27 min after injection. Each fraction was dried, methylated with ethereal diazomethane, and redissolved in 50 µL of N-methyl-N-trimethylylilfluoracetamide. Two microliters of this solution were analyzed by GC-MS using a Finnegan ion trap mass spectrometer interfaced with a Hewlett-Packard gas chromatograph as described before (14). Absolute levels of endogenous GA for which there were $^{2}$H-labeled internal standards were estimated using isotope dilution (1). Full-scan mass spectra were obtained and the ratio of the intensities of the base peaks from the $^{2}$H-labeled GA and their unlabeled endogenous counterparts were used in the calculations of GA levels. Appropriate adjustments in ion intensities were made to account for the natural isotope abundances as well as the small contamination by the unlabeled GA in each $^{2}$H-labeled internal standard. Relative levels of endogenous GA for which internal standards were unavailable were also compared in the extracts of shoots from CR₁ and EMS-141. Full-scan mass spectra were obtained and the intensities of the base peaks were used as a measure of GA amount. Because only 4% of each derivatized sample was analyzed, the maximum amount of labeled internal standard contained in any injection was 40 ng, if no losses occurred during the work-up procedures. Therefore, the linearity of the instrument response was examined by co-injecting 25 ng of $^{3}$H-GA₁ with varying amounts (1–100 ng) of unlabeled GA₁. The curve resulting from a plot of the ratio of the ion current responses for m/z 506 and 508 (the base peaks for GA₁ and $^{3}$HGA₁, respectively) as a function of the response for m/z 506 was a straight line for the entire concentration range. It was assumed that a similar linear relationship existed for the other compounds examined.

To extract the kaurened precursors of GA, lyophilized shoots from 96 thermo- and noninduced plants of both lines were homogenized in 250 mL of ice-cold methanol with a Waring Blender. The homogenate was filtered and the residue extracted once more with methanol. The two filtrates were combined and 1 µg each of $^{3}$H-labeled kaurene, kaurenol, and kaurenoic acid was added as an internal standard. In addition, 50,000 dpm of $[^{14}C]$kaurene (specific activity = 97 µCi µmol⁻¹) was also included. The volume of the filtrates was then reduced to about 100 mL under reduced pressure. The composition of each solution was adjusted with deionized water to a final composition of 90% methanol, 10% water. The solution was stirred for 30 min with 75 g of preparative C₁₈ reverse-phase packing material (55–105 µm; Waters Associates, Milford, MA) and then filtered. This process was repeated once more with fresh C₁₈. This filtrate contained both kaurenol and kaurenoic acid and was further fractionated by gradient-eluted reverse-phase HPLC as described before (14). Fractions where both of these compounds elute were collected, derivatized, and analyzed by GC-MS.

Under these conditions, kaurene, Chl, and other nonpolar pigments remained absorbed to the C₁₈. These compounds were eluted from the C₁₈ with 250 mL of acetone followed by 250 mL of tetrahydrofurane. The two solutions were combined, and the kaurene separated from the bulk of the pigments by size exclusion chromatography (14) using a 2 × 100 cm column of Biobeads SX-8 (Bio-Rad) eluted with ethyl acetate at 5 mL min⁻¹. Five-minute fractions were collected and a 10% aliquot assayed for radioactivity following combustion (14). The fractions containing radioactivity were then combined and subjected to adsorption chromatography on a 15 × 120-mm column of silica. Kaurene was eluted with 2 bed volumes of hexane. The solvent was removed, and kaurene was derivatized as described for the GA-containing fractions and then subjected to GC-MS. Procedures for determining the levels of the three kaurenoids by isotope dilution were identical to those described previously for GA.

Sources of Chemicals

Unlabeled GA and GA precursors were obtained as described before (13). $^{3}$H-Labeled kaurene, kaurenol, kaurenoic acid, and GA₆ were synthesized from the C-16 noroketone via the Wittig reaction (4, 7). $[^{3}H]$GA₃ was obtained by feeding $[^{3}H]$steviol, synthesized from steviol noroketone via the Wittig reaction, to cultures of Gibberella fujikuroi (4). Steviol was obtained by treating an aqueous extract of dried Stevia rebaudiana leaves (Sigma) with a pectinase (Pectinol AC, Rohm and Haas) as described before (14). $^{3}$H-Labeled GA₁, GA₁₀, and GA₂₀ were synthesized by L. Mander (Australian National University) and obtained from R. P. Pharis (University of Calgary). $[^{14}C]$Kaurene was obtained by incubating $[^{14}C]$mevalonic acid with pumpkin endosperm preparations as described before (7) except that NADPH₂ was omitted.

RESULTS

Selection and Characterization of a GA-Sensitive Dwarf Mutant

The progeny of nearly 900 selfed M₁ field pennycress plants following chemical mutagenesis of CR, seed were screened
for the ability to initiate stem growth following a 4-week thermoinductive cold treatment. Although there were a number of M₁ plants with reduced stem growth, exogenous GA₃ applied to the shoot tips was effective in restoring the normal phenotype in only three lines. One of these, designated as EMS-141, was selected for further analysis. Figure 2 shows a comparison of a wild type (CR₁) and an EMS-141 plant 1 month after the end of a 4-week thermoinductive cold treatment. The three most obvious alterations in growth and development of thermoinduced EMS-141 plants are the lack of stem growth, reduced size of leaves, particularly evident in the lengths of the petioles, and retarded flower development (Fig. 2). However, application of 1 μg of GA₃ to the shoot tips of thermoinduced EMS-141 plants three times a week for 4 weeks completely restored the normal phenotypic appearance exhibited by thermoinduced CR₁ plants (Fig. 2).

Although stem growth was completely arrested in thermoinduced EMS-141 plants, flower initiation was only delayed in comparison to CR₁ (Table I). Microscopic examination of apices from both lines periodically after the end of a 4-week-old cold treatment revealed that flower primordia were first visible after 4 and 10 d in CR₁ and EMS-141, respectively. Further floral development in the mutant line was also retarded. Flower buds appeared 7 to 10 d later than in wild type plants. However, these buds failed to open and never produced any seed (Table I). These effects on floral development were completely reversible with exogenous GA₃ (Fig. 2).

Leaf growth in EMS-141 and CR₁ was also compared (Table II). Under SD conditions, petiole growth in both lines was complete 14 d after the first measurement. However, the mean final length of petioles from EMS-141 was about two-thirds of that attained by those from CR₁. This growth inhibition in EMS-141 was fully reversed following application of 1 μg of GA₃ on alternate days (Table II). Petioles from both lines also responded to a 16-h extension of the 8-h photoperiod with low-intensity light from incandescent lamps. As reported previously (12), petioles from CR₁ plants grew more than twice as long under LD than SD. Likewise, petiole growth was markedly enhanced in EMS-141 by LD treatment as well, although both petiole final length and the proportion of growth increase over SD was less than in CR₁ (Table II).

In contrast to petiole growth, there were no differences between EMS-141 and CR₁ in leaf shape before and after thermoinduction (data not shown). Thus, the typical foliar dimorphism displayed by this species (11) is preserved in the mutant.

Evidence that the alterations in the growth and development of EMS-141 are conferred by a recessive mutation of a single nuclear gene is presented in Table III. All of the F₁ progeny resulting from crossing EMS-141 and CR₁ had the wild type phenotype following thermoinduction. However, the dwarf phenotype appeared in the F₂ generation following selfing F₁ plants. The traits for growth habit segregated nearly three normals to one dwarf. Selfing dwarfs from the F₂ resulted in progeny with only the dwarf growth habit (Table III).

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**Table I. Time Course of Reproductive Development in the EMS-141 and CR₁ Lines of Field Pennycress**

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Days after the End of the Cold Treatment*</th>
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<tr>
<td></td>
<td>EMS-141</td>
</tr>
<tr>
<td>Flower primordia</td>
<td>10–12</td>
</tr>
<tr>
<td>Botting</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Flower buds</td>
<td>24–29</td>
</tr>
<tr>
<td>Open flowers</td>
<td>&gt;60</td>
</tr>
</tbody>
</table>

* Results show the range of five replicates for each line.
Comparison of Endogenous GA and GA-Precursor Levels in Normal and Dwarf Plants

In a preliminary study, the levels of GA-like substances in thermo- and noninduced shoots of CR₁ and EMS-141 were compared using the d5-maize bioassay. No GA-like substances were detected in extracts of shoots from 96 plants of EMS-141 from either treatment (data not shown), indicating that the dwarf has severely reduced endogenous GA levels. This conclusion was further substantiated following quantitative analysis of GA and GA-precursor levels by GC-MS. Table IV shows that none of the GA native to CR₁ field pennycress (Fig. 1) was detected by GC-MS in the shoot extracts from either thermo- or noninduced EMS-141 plants. Likewise, 7β, 13-dihydroxykaurenoic acid, a side branch product originating from kaurenoic acid (5), was detected only in the extracts of CR₁ shoots. Trace amounts of the GA precursors kaurene and kaurenoic acid were measured in EMS-141 shoots, the levels of which ranged from 15- to 35-fold less than in corresponding extracts from CR₁ (Table IV). Comparison of individual GA levels between thermo- and noninduced wild-type (CR₁) plants also revealed that GA from the early C-13 hydroxylation pathway (Fig. 1) were about the same or higher in noninduced than in thermoinduced plants. In contrast, the C-13 desoxy GA were detected only in thermoinduced plants (Table IV).

Comparative Abilities of Exogenous GA and Precursors to Elicit Stem Growth in EMS-141

Table V shows that the relative biological activities associated with the compounds tested for the ability to elicit stem growth in thermoinduced EMS-141 were similar to those previously observed for thermoinduced CR₁ (13). GA₃ was the most active, followed by GA₁. The three endogenous precursors and GA₂₀ had intermediate biological activity, whereas GA₃₃ and steviol (C-13 OH kaurenoic acid) were relatively inactive. The three kaurenoid precursors of GA, kaurene, kaurenol, and kaurenoic acid, were about as active as GA₂₀ (Table V).

In contrast to the effects on thermoinduced plants, neither kaurene, kaurenol, nor kaurenoic acid elicited stem growth in noninduced EMS-141 plants. Both steviol and GA₃₃ were likewise inactive (Table V). The relatively large increases in the biological activities of the three GA precursors following thermoinduction compared with GA₃₃ agrees well with previous observations (13) made for these compounds on CR₁.

Comparative Abilities of Exogenous GA and Precursors to Elicit Petiole Growth

A comparison of the effect of eight GA and GA precursors on petiole growth in noninduced EMS-141 plants is shown in Table V. There were indeed notable differences in the relative biological activities of the test compounds in their abilities to elicit petiole or stem growth. (a) GA₁ and GA₃ were equally active and were the only test compounds that completely restored petiole growth of the mutant to that of the wild type. (b) In contrast to their lack of an effect on stem elongation in noninduced plants, exogenous kaurene, kaurenol, and kaurenoic acid caused a significant growth response in petioles. (c) GA₃₃ and steviol, although less active than the three kaurenoid GA precursors for stem growth in thermoinduced plants, stimulated petiole growth to a similar extent (Table V).

Table III. Inheritance Patterns of the Dwarf Trait following Crosses between the EMS-141 and CR₁ (Wild Type) Lines of Field Pennycress

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of Plants with Each Phenotype</th>
<th>χ²</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Dwarf</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>CR₁ (♂) x EMS-141 (♀)</td>
<td>0</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>F₁ self (F₂)</td>
<td>202</td>
<td>705</td>
<td></td>
</tr>
<tr>
<td>F₂ self (F₃)</td>
<td>250</td>
<td>0</td>
<td></td>
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</table>

* Phenotype (stem growth) was determined 2 weeks after the end of a 4-week thermoinductive cold treatment. *b χ² values are for goodness of fit to a 3:1 ratio. *c F₂ dwarfs were selfed for the F₃.
conversion of geranylgeranyl pyrophosphate to kaurene, catalyzed by the enzyme complex kaurene synthase (5). This reaction is a major branch in terpenoid metabolism. Many major plant secondary products such as carotenoids, the phyto chain of Chl, and sterols are also terpenes and therefore share much or all of the same basic biosynthetic reaction sequence required for the formation of kaurene (5). Inhibition of a reaction before the one catalyzed by kaurene synthase would probably result in a multitude of effects, some perhaps lethal, on the plant. Moreover, in such a situation, exogenous GA should not be able to reverse all of the phenotypic alterations caused by the mutation. However, the only apparent change in the physical appearance of EMS-141 from wild-type plants is the dwarf growth habit and exogenous GA fully restored normal growth (Fig. 2). Mutations at the level of kaurene synthase are common in other species including d5 maize (16), gib-1 tomato (2), and ga-1 Arabidopsis thaliana, respectively (6). The extreme reduction in endogenous GA and GA precursor levels, coupled with a total lack of thermoinduced stem growth (Tables I and IV), also indicates that the mutant allele may be less "leaky" than some GA biosynthesis mutations that have been described in other species (8, 9, 17).

The relative biological activities of the various test compounds in eliciting stem growth in EMS-141 were identical to those found previously for CR1 (13). In both thermo- and noninduced plants, both GA5 and GA1 were highly biologically active, with GA5 the more active of the two. GA20 also caused significant stem growth when applied to EMS-141 plants from both treatments, but its activity was substantially less than either GA1 or GA5 (Table V). The other compounds tested had no activity in eliciting stem growth in noninduced EMS-141 plants. However, in thermoinduced plants, the three GA precursors, kaurene, kaurenol, and kaurenoic acid, were as active as GA20. This is in sharp contrast to GA3 and steviol (C-13 OH kaurenoic acid), whose ability to cause stem growth was only slightly enhanced in thermoinduced plants (Table V). Similar results on the comparative biological activities of these compounds in thermo- and noninduced CR1 plants led to the hypothesis that the conversion of kaurenoic acid to GA is under thermoinductive control and that GA5 is the GA responsible for regulating stem growth in field pennycress (13). Additional evidence for the latter aspect of the hypothesis was obtained in the present work through the quantitative analysis of endogenous GA in CR1 plants: the C-13 deoxy GA, GA9, GA12, GA24, and GA31, were detected only in thermoinduced CR1 plants. In contrast, the GA that are members of the early C-13 OH pathway (GA1, GA19, GA20, GA29, GA44, and GA3) occurred in both thermo- and noninduced plants, and in fact, all the levels of these GA were the same or even higher in noninduced CR1 plants (Table V).

The reduced petiole growth in EMS-141 plants (Table II) was probably also the result of lowered endogenous GA content. Petiole growth in the mutant under both SD and LD conditions was very similar to that previously observed for CR1 plants treated with a growth retardant to reduce endogenous GA levels (12). In that work, it was concluded that the increase in petiole growth resulting from extending the photoperiod with low-intensity far-red enriched light was not mediated by GA (12). The present work with a GA-deficient mutant supports that conclusion; under LD, petiole growth in EMS-141 was nearly double that in SD (Table III).

Dramatic differences were observed in the biological activi-
ties of the test compounds in causing petiole growth compared with stem elongation. For stem growth in both thermo- and noninduced plants, GA₉ was the most active compound tested. However, petioles of EMS-141 responded equally well to exogenous GA₁ or GA₆ (Table V). In addition, kaurene, kaurenoic, and kaurenoic acid, all of which were incapable of causing stem elongation in noninduced plants, were less biologically active as GA₂₀ for increasing the growth of petioles in noninduced plants. Thus, in addition to the developmentally related changes in sensitivity to the GA precursors observed in the shoot tips following thermoinduction, there are tissue-related differences as well. Assuming that these compounds must be converted to some GA to elicit a biological response, the results indicate tissue specificity in the regulation of GA biosynthesis and metabolism. This is consistent with a previous study showing that the thermoinductive regulation of the conversion of exogenous [³H]kaurenoic acid to GA was restricted to applications to the shoot tips. When this compound was applied to the leaves, labeled GA₂₀ was formed equally well in thermo- and noninduced plants (7).

Another striking difference in sensitivity to the exogenous test compounds was the ability of GA₃₃ and steviol to elicit increased petiole growth equal to GA₂₀ and the three kaurenoic acid GA precursors. These two compounds had far less activity than any of the other compounds for causing stem growth in thermoinduced EMS-141 plants (Table V). The physiological significance of these results is unclear. One possibility may be that the early C-13 OH pathway leading to GA₁ (Fig. 1) may function to regulate petiole growth. This seems likely in view of the fact that members of the C-13 desoxy pathway (Fig. 1) were not detected in extracts of CR₁, shoots (Table IV). If so, one would expect that expression of biological activity following application of the test compounds is by virtue of their metabolism to GA. Thus, in contrast to shoot tips, the three GA precursors may be preferentially metabolized by the leaves via the early C-13 OH pathway in which the formation of GA₃₃ represents the first committed step (Fig. 1). This may also explain why steviol, which is apparently not endogenous to field pennycress (14), has far less biological activity than kaurenoic acid in stem elongation, but equal activity in petiole growth. The implication from this line of reasoning is that the two pathways of GA metabolism in field pennycress control separate processes: the C-13 desoxy pathway with GA₆ as its bioactive member responsible for thermoinduced stem growth, and the early C-13 OH pathway leading to GA₁ involved in the control of petiole growth and perhaps other aspects of leaf development. We are currently investigating this possibility through quantitative analysis of endogenous GA in specific organs, in vivo metabolism studies, and attempts to isolate other mutants in which one of the two pathways of GA metabolism is selectively affected.

LITERATURE CITED