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Comparison of *Ent*-Kaurene Synthetase A and B Activities in Cell-Free Extracts from Young Tomato Fruits of Wild-Type and *gib-1*, *gib-2*, and *gib-3* Tomato Plants

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Abstract. The nonallelic *gib-1* and *gib-3* tomato (*Lycopersion esculentum* Mill.) mutants are gibberellin deficient and exhibit a dwarfed growth habit. Previous work has shown that this dwarfed growth pattern can be reversed by the application of a number of gibberellins and their precursors, including *ent*-kaurene (*ent*-kaur-16-ene). This indicates that they are blocked in gibberellin biosynthesis at a step prior to *ent*-kaurene metabolism. The normal accumulation of carotenoids observed in these mutants suggests a functionally normal isoprenoid pathway. *Ent*-kaurene is synthesized from geranylgeranyl pyrophosphate in a two-step process with copalyl pyrophosphate as an intermediate. *In vitro* assays using young fruit extracts from wild-type and *gib-2* plants resulted in the conversion of geranylgeranyl pyrophosphate to copalyl pyrophosphate, and the conversion of copalyl pyrophosphate to *ent*-kaurene. Similar assays using *gib-1* plants indicated a reduced ability for synthesis of copalyl pyrophosphate from geranylgeranyl pyrophosphate, and thus a reduced *ent*-kaurene synthetase A activity. Furthermore, *gib-3* extracts demonstrated a reduced ability to synthesize *ent*-kaurene from copalyl pyrophosphate, and thus a reduced *ent*-kaurene synthetase B activity. These results establish the enzymatic conversion of geranylgeranyl pyrophosphate to copalyl pyrophosphate, and copalyl pyrophosphate to *ent*-kaurene, as the sites of the mutations in *gib-1* and *gib-3* tomatoes, respectively. We also note that tomato fruit extracts contain components which are inhibitory to *ent*-kaurene synthesis.

Gibberellins (GAs), in particular GA_1, are thought to have integral roles in seed germination and stem elongation. Screening for mutants, which depend on applied GAs for germination or have dwarf growth habits that can be reversed by applied GAs, has led to the selection of a number of GA-deficient mutants. No evidence has emerged to suggest that any of these mutants are GA-deficient as a result of an increased catabolism of bioactive GAs. On the other hand, a number of mutants have been shown to be deficient in GA biosynthesis. These include the *d1*, *d2*, *d3*, and *d5* mutants in maize (see Phinney 1984, for review), the *le* and *na* mutants in peas (Ingram et al. 1984, Potts and Reid 1983), *dx* and *dy* mutants in rice (Kobayashi et al. 1989, Murakami 1972), and the *gib-1*, *gib-2*, and *gib-3* mutants in tomato (Zeevaart 1986).

Carotenoids and GAs are synthesized via isoprenoid pathways. While most cellular geranylgeranyl pyrophosphate (GGPP) is converted into carotenoids, a small portion is metabolized into GAs. The first committed step in GA biosynthesis is the conversion of GGPP to copalyl pyrophosphate (CPP), followed by the conversion of CPP to *ent*-kaurene. The enzymes which catalyze these two steps are known as the A and B activities of *ent*-kaurene synthetase, respectively (Fig. 1). *Ent*-kaurene synthetase activity was first described in *Echinocystis macrocarpa* (Graebe et al. 1965). Both GGPP and CPP were shown to be converted to *ent*-kaurene in cell-free preparations of *Gibberella fujikuroi* (Shechter and West 1969) and the wild cucumber *Marah macrocarpus* (Frost and West 1977). Furthermore, the A and B activities of *Marah macrocarpus* proved to be chromatographically distinct, but interacting enzymes (Duncan and West 1981). The A and B activities have also been described in a number of additional species (see Coolbaugh 1983, for review). Quaternary ammonium compounds, such as Amo-1618 [2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenyl

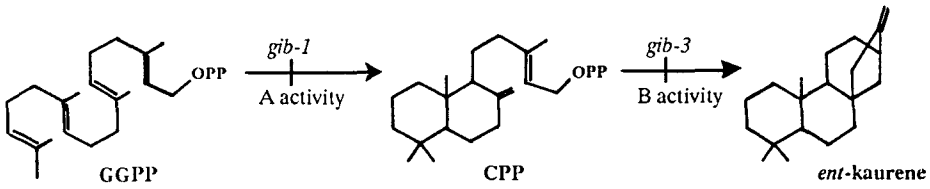

Fig. 1. The two-step conversion of GGPP to *ent*-kaurene via the intermediate CPP. Two distinct activities, A and B, catalyze the conversion of GGPP to CPP and CPP to *ent*-kaurene, respectively. The steps blocked in the *gib-1* and *gib-3* mutants are indicated.

piperidine-1-carboxylate] inhibit A activity, whereas B activity is more sensitive than A to inhibition by sulfhydryl-group-blocking agents, such as *N*-ethylmaleimide (Fall and West 1971, Frost and West 1977).

The dwarf growth habit in the GA-deficient tomato mutants, *gib-1* and *gib-3*, is overcome by the application of *ent*-kaurene (Zeevaart 1986). This suggests that their GA deficiency (Bohner et al. 1988) is the result of a block in the GA biosynthetic pathway prior to *ent*-kaurene metabolism. In addition, since the observed carotenoid accumulation in these mutants is similar to wild type, it would appear that isoprenoid biosynthesis through GGPP is normal. However, the possibility cannot be ruled out that GGPP biosynthesis for utilization in *ent*-kaurene biosynthesis is limiting in the mutants, if separate enzymes and pools of intermediates are involved in carotenoid and *ent*-kaurene biosynthesis (Gray 1987). Thus, the A and B activities, and components which regulate their activity, are excellent candidates for the mutation sites in *gib-1* and *gib-3* tomatoes. In fact, we now report that young fruits of *gib-1* are deficient in the A activity, and young fruits of *gib-3* are deficient in the B activity. We also determined that components are present in tomato fruit cell-free extracts which inhibit *ent*-kaurene synthesis.

Materials and Methods

Plant Growth and Fruit Harvest

Seeds of wild-type tomato (*Lycopersion esculentum* Mill. cv. Moneymaker) and GA-deficient mutants, *gib-1* (W335), *gib-2* (W270), and *gib-3* (W182), in the same genetic background, were obtained from Professor J. H. van der Veen, Department of Genetics, Agricultural University, Wageningen, The Netherlands. Mutant seeds were germinated in the presence of GA_7 (5 mg/L). Following germination, seedlings were grown in a growth chamber at 23°C with 20 h light for approximately 4 weeks, followed by transfer to a greenhouse maintained at 23°C with 20 h of light. In order to obtain normal fruits on the mutants, inflorescences were sprayed weekly with GA_7 (5 mg/L). Fruits were harvested when 1 cm in diameter and immediately frozen in liquid N_2. A comparison of flower, seed, and fruit development of the most extreme dwarf, *gib-1*, following application of GA_{4+7}, and wild type has been reported (Groot et al. 1987). Care was taken to avoid harvesting parthenocarpic or fruits with few seeds, which were recognizable due to their nonspherical shape.

Tissue Extraction

Frozen material (45–60 g fresh wt/preparation) was ground with a pestle in a mortar to which liquid N_2 was periodically added. All subsequent operations were conducted at 4°C. After tomato fruits were finely powdered, 0.5 g of polyvinylpolypyrollidone/g fresh wt was added, followed by additional grinding. Then 2.5 ml of chilled extraction buffer A [50 mM Tes-KOH (pH 7.4), 5 mM $MgCl_2$, 5 mM 2-hydroxyethylmercaptan, 5 mM ascorbate, 0.1 mM NaS_2O_5, 10% (vol/vol) glycerol, 1 mM phenylmethylsulfonylfluoride, and 2 μM leupeptin] per gram fresh weight of tissue was added, followed by additional grinding. Extracts were squeezed through six layers of chilled, prewetted cheesecloth, then centrifuged at 25,000 *g* for 30 min. The resulting supernatant was further centrifuged at 100,000 *g* for 90 min. The second centrifugation step was necessary to remove, by pelleting, enzymes in the microsomal fraction. The 100,000 *g* supernatant fraction would not be expected to include those enzymes involved in the conversion of *ent*-kaurene to *ent*-kaurenoic acid (see Graebe 1987, for review). This centrifugation did not result in a measurable loss of *ent*-kaurene synthetase activity. The resulting supernatant was then applied to a QAE-Sephadex A-25 column (Pharmacia, Piscataway, NJ, USA) (Duncan and West 1981), 0.25 ml/mg protein, preequilibrated in extraction buffer B (buffer A without phenylmethylsulfonylfluoride and leupeptin). The column was then washed with three column volumes of buffer B containing 0.1 M KCl, followed by the elution of the A and B activities in three column volumes of buffer B containing 0.25 M KCl. The 0.10–0.25 M KCl eluate was concentrated to a volume of ≤5 ml using a Amicon PM10 filter (Amicon, Danvers, MA, USA), then dialyzed against two changes of 100 volumes of buffer B, frozen, and stored at −80°C until used in activity assays.

Substrate Synthesis

The synthesis of [^{3}H]GGPP and [^{3}H]CPP was performed as previously described (Coates et al. 1978, Upper and West 1967). Briefly, all-*trans*-geranylgeraniol was synthesized from all *trans*-farnesyl acetone (K and K Laboratories, Plainview, NY, USA) by the addition of reduced trimethylphosphono acetate (Aldrich Chemical, Milwaukee, WI, USA), followed by a lithium aluminum hydride (Aldrich) reduction of all-*trans*-geranylgeranyl -methyl ester to all *trans*-geranylgeraniol. Geranylgeraniol and copalol (a gift from Professor R. M. Coates, University of Illinois, Champaign, IL, USA) were separately oxidized with activated manganese dioxide to their respective aldehydes, and then

tritiated by reduction with sodium borotritide (155 mCi/mmol for geranylgeraniol, Amersham Corp., Arlington Heights, IL, USA; 600 mCi/mmol for copalol, E. I. Dupont DeNemours and Co., Inc., Wilmington, DE, USA). Tritiated geranylgeraniol and copalol were reactivated with trimethylchlorosilane (Aldrich) and pyrophosphorylated with tris(tetra-*n*-butylammonium) pyrophosphate (synthesized from tetra-*n*-butylammonium hydroxide and tetrasodium pyrophosphate decahydrate, Aldrich) (Davisson et al. 1985). Final specific activities were 38 mCi/mmol for [^{3}H]GGPP and 75 mCi/mmol for [^{3}H]CPP.

Enzyme Assays and Thin-Layer Chromatography (TLC)

Assays for the conversion of GGPP to CPP (A activity) contained 0.25–1.0 ml of tomato extract (adjusted to 1 mg protein/ml extract, so that 1 ml of extract was derived from 10–15 g fresh wt of tomato fruits) in buffer B, plus 5 mM $MgCl_2$, 2 mM ATP, and approximately 1.5×10^6 dpm [^{3}H]GGPP/0.25 ml final assay volume. Once mixed, assays were incubated 60 min at 30°C, then acidified with 1 M HCl and incubated for 15 min at room temperature. This acid treatment resulted in the conversion of the assay product, CPP, into copalol. The copalol was then partitioned into benzene:acetone (4:1), the organic phase collected, dried under N_2 gas and redissolved into a small volume of hexane. This hexane soluble [^{3}H]copalol was then supplemented with nonradioactive copalol, applied to an $AgNO_3$-[4% (vol/vol) in acetonitrile:ethanol (9:1)] treated Silica Gel 60 F_{254} TLC sheet (5 × 20 cm, 0.2 mm thick, EM Science, Cherry Hill, NJ, USA), and developed sequentially for 15 cm with hexane:EtOAc (3:1) and for 10 cm with benzene:EtOAc (9:1). Migration of geranylgeraniol and copalol standards was determined. Standard lanes were sprayed with 5% (vol/vol) H_2SO_4 in ethanol, heat treated at 115°C for 10 min, and visualized under UV light. Geranylgeraniol typically migrated 2 cm and copalol 6 cm under the described conditions. Sample lanes were scraped in 1-cm wide bands corresponding to the migration of standard copalol. Copalol from the scraped portion of the TLC plate was eluted with EtOAc, then dried under N_2 gas, and redissolved in 100% MeOH, which was made 70% aqueous-MeOH prior to high-performance liquid chromatographic (HPLC) analysis.

Assays for the conversion of CPP to *ent*-kaurene (B activity) were performed as for the A activity, except for the following: ATP was omitted from the assay; 1.0×10^6 dpm [^{3}H]CPP/0.25 ml final assay volume replaced GGPP; and no HCl treatment or TLC separation followed incubation. Following an organic partition into benzene:EtOAc (4:1) and drying under N_2 gas, samples were redissolved in 100% MeOH, which was made 70% aqueous-MeOH prior to HPLC analysis.

Assays testing tomato extracts for components which inhibit *ent*-kaurene synthesis were performed as described above for A activity, except that 5×10^5 dpm of [^{14}C]isopentenyl pyrophosphate (IPP) (56 mCi/mmol, Amersham) was substituted for [^{3}H]GGPP. Following incubation, an organic partition, and drying under N_2 gas, as described above, samples were solubilized in a small volume of hexane and applied to a silica gel TLC plate (as described above, without $AgNO_3$ treatment). The TLC plate was developed 15 cm in hexane. *Ent*-kaurene standards ([^{14}C]*ent*-kaurene, 3.65 mCi/mmol, Amersham) migrated with an R_f of 0.66. Sample lanes were scraped in 1-cm wide bands corresponding to migration of the *ent*-kaurene standard, and radioactivity was measured using a Beckman LS 5000TD counter.

HPLC and Radiocounting

Quantitation of both radioactive copalol and *ent*-kaurene was performed on a Waters M-6000A HPLC using a Waters 660 Solvent Programmer and a Nova Pak C_{18} column (15 × 0.4 cm i.d.) attached to a C_{18} Guard-PAK precolumn module (Waters, Milford, MA, USA). Radioactivity was detected using a Flo-One HP radioactive flow detector (Radiomatic Instruments and Chemical Company, Tampa, FL, USA). A ratio of 1:2.3, column effluent: scintillation fluid (Flo-Scint II, Radiomatic), was used. Counting efficiency was 26%. Column flow rate was 1 ml/min with a 2-min delay between sample loading and gradient initiation. The gradient for copalol quantitation was 70–85% aqueous-MeOH, curve no. 3 (i.e., initially rapid, then very gradual between 80 and 85%) over 20 min. Elution time for the copalol standard was 12.2 min and for the geranylgeraniol standard 13.0 min. The gradient for *ent*-kaurene quantitation was linear at 70–100% aqueous-MeOH over 10 min, followed by 10 min at 100% MeOH. Elution time for the copalol standard was 13.2 min and for the *ent*-kaurene standard 16.6 min.

Results

Preliminary Results

Since it was difficult to obtain substantial amounts of either A or B activity, a number of buffering agents at a range of pHs, buffer components, columns, and column elution schemes were tested. Also, a variety of tomato tissues were tested, including green leaves, axillary buds, and 5-day-old whole dark-grown seedlings, none of which yielded measurable amounts of *ent*-kaurene synthetase activity. Even in young fruits, the ability to obtain substantial amounts of either A or B activity was limited. Two factors were identified as problematic. One was the presence of phosphatases in the extracts, which in the enzyme assays resulted in the dephosphorylation of the substrates GGPP or CPP. The inclusion of specific phosphatase inhibitors, such as 50 mM KF, in the assay inhibited both these contaminating phosphatases and the B activity, thus proving counterproductive. Phosphatase contamination was minimized by eluting the A and B activities from a QAE-Sephadex A-25 column at a 0.10–0.25 M KCl step, which was lower than the KCl concentration at which the majority of the phosphatase activity eluted. Nevertheless, 5–10% of the substrate was dephosphorylated during the assay incubation period using the 0.10–0.25 M KCl fraction. A second factor limiting successful quantitation of A and B activity was the presence of inhibitory components. Extracts from the liquid endosperm of *Cucurbita maxima* contain high levels of A and B activity (Turnbull et al. 1985). *Ent*-kaurene synthesis from [^{3}H]IPP in *Cucurbita maxima* extracts was inhibited by adding tomato extracts (Table 1). Fractionated tomato extracts,

Table 1. Inhibitory effect of tomato extracts on *Cucurbita maxima ent*-kaurene synthesis.

KCl-step eluate	*Ent*-kaurene (dpm)
Cucurbita maxima control	20,167
0.00 M (flow through)	714
0.05 M	3517
0.10 M	1429
0.15 M	9857
0.20 M	9571
0.30 M	4571
0.15 + 0.20 M	5714
0.15 + 0.20 M (100 µl)	43
0.15 + 0.20 M (100 µl heated)	857

The S_{110} supernatant of crude, wild-type tomato extracts was fractionated on a QAE-Sephadex A-25 column using a 0.00–0.30 M KCl-step gradient. Following dialysis, 10 µl (or 100 µl where indicated) of each step eluate was added to a 250 µl *Cucurbita maxima* assay. In the last three assays, 0.15 and 0.20 M fractions were combined. The conversion of [^{3}H]IPP to [^{3}H]*ent*-kaurene was determined as described in Materials and Methods.

eluted from a QAE-Sephadex A25 column using a KCl gradient (from 0.00–0.30 KCl, in 0.05 M increments) and dialyzed against buffer B, all inhibited *Cucurbita maxima* activity. Increasing the amount of tomato extract added to the assay further reduced *Cucurbita maxima* activity, eventually eliminating it. The least inhibitory fractions were found between 0.15 and 0.25 M KCl, which approximately corresponded to the tomato fractions which contained A and B activity. Heating tomato fractions for 10 min in boiling water resulted in their being slightly less inhibitory toward *Cucurbita maxima ent*-kaurene synthesis (Table 1). Similarly, the B activity of *Cucurbita maxima ent*-kaurene synthetase measured with [^{3}H]CPP as the substrate was inhibited by mixing with tomato extracts (data not shown). These results suggested that both heat stable, as well as heat labile inhibitory factors, which were not dialyzable, are present in extracts from immature tomato fruits. These factors are thought to have contributed to the difficulty in obtaining substantial amounts of A and B activity in extracts from tomato fruits.

Ent-*Kaurene Synthetase Activity in Young Tomato Fruits*

The results of *in vitro* enzyme assays optimized for the synthesis of CPP from GGPP and *ent*-kaurene from CPP are shown in Table 2. The numbers given are from a single assay performed on extracts prepared and assayed simultaneously. Replicate assays of these preparations, as well as other preparations, yielded similar relative values between the four genotypes. A comparison of the amount of CPP synthesized in the three GA-deficient mutants and the wild-type fruit extracts clearly demonstrates a deficiency of A activity in *gib-1* fruits. In the experimental results shown, as well as in other preparations, the *gib-1* extract consistently produced less than 10% as much CPP as the wild-type extract. We were unable to detect, by HPLC, any *ent*-kaurene synthesis in assays using GGPP as a substrate (data not shown). In contrast, CPP synthesis in *gib-2* and *gib-3* extracts did not significantly differ from that in wild type. The A activity in wild-type extracts was reduced by 76% when 1 mM Amo-1618 was included in the assay (data not shown).

The *gib-3* extracts were clearly deficient in the conversion of CPP to *ent*-kaurene (B activity). They consistently produced less than 10% as much *ent*-kaurene as the wild-type extract. The results shown also suggest that *gib-2* may have a reduced capacity to synthesize *ent*-kaurene. This reduced capacity, approximately 50% of the wild-type, was reproducible. A previous report demonstrated *gib-2* to be blocked in the conversion of *ent*-7α-hydroxykaurenoic acid to GA_{12}-aldehyde (Zeevaart 1986). We believe this earlier report to be correct and are unable to explain the apparent reduced B activity observed here.

Discussion

Despite the number of reports of *ent*-kaurene synthetase A and B activities, their purification has proved to be difficult. Two patterns have emerged from these studies. First, inhibitors of activity have been reported in a number of species, including castor bean (Gafni and Shechter 1981), *Helianthus annuus* (Shen-Miller and West 1982), and tomato fruits (present study). However, whether or not these inhibitors are specific or general, or if they are significant *in vivo*, has not yet been demonstrated. For example, factors inhibiting *ent*-kaurene synthetase activity *in vitro* may be compartmentalized *in vivo* such that they do not have access to *ent*-kaurene synthetase. Second, for reasons hypothesized to be related to GA function, A activity appears to be most abundant in immature tissues and in tissues with high endogenous GA contents. This may be the result of A activity being developmentally regulated, or may result from the accumulation of compounds, in mature tissues, to which A is sensitive.

The GA biosynthetic pathway, from mevalonate to bioactive GAs, has been elucidated (see Graebe 1987, for a review). Since the conversion of

Table 2. The conversion of [^{3}H]GGPP to [^{3}H]CPP (A activity) and [^{3}H]CPP to [^{3}H]kaurene (B activity) in cell-free extracts of young tomato fruits from wild-type, *gib-1*, *gib-2*, and *gib-3* plants.

	[^{3}H]GGPP substrate (A activity)		[^{3}H]CPP substrate (B activity)	
	[^{3}H]CPP	Specific activity	[^{3}H]*Ent*-kaurene	Specific activity
Wild type	2198	102	1204	29
gib-1	166	6	1637	40
gib-2	2058	96	628	15
gib-3	1587	78	74	2

[^{3}H] product given in dpm. Specific activities are in pmoles product/mg protein/h.

GGPP to CPP is the first committed step in the biosynthesis of GAs, it is an excellent point at which to study the regulation of GA biosynthesis. Although there has been little success in understanding the regulation of the A and B activities, the use of mutants presents a potentially rich source of information. The *d5* mutant in maize has been shown by TLC to accumulate *ent*-isokaurene instead of *ent*-kaurene when CPP was used as a substrate in cell-free extracts (Hedden and Phinney 1979). This accumulation was postulated to be the result of an altered B activity which converted CPP to *ent*-isokaurene instead of *ent*-kaurene. Using HPLC analysis of assay products from wild-type and *gib-3* extracts, we observed no difference in the accumulation of products eluting with polarities between those of copalol and *ent*-kaurene (i.e., where *ent*-isokaurene would be expected to elute, although we ran no standards of it). However, this lack of evidence for *ent*-isokaurene accumulation may be due to the already low amount of B activity in wild-type tomato extracts. Therefore, a conversion, similar to that proposed by Hedden and Phinney (1979), would be difficult to detect and cannot be ruled out for the *gib-3* mutant.

In addition to the B activity deficiency reported in *gib-3*, we have observed *gib-1* to be deficient in the A activity. Thus, *gib-1* represents the first mutant characterized as blocked in the A activity of *ent*-kaurene synthetase. Our results do not, however, preclude the possibility that the mutations may not be in the enzymes, but rather in components which regulate the enzymes.

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References

Bohner J, Hedden P, Bora-Haber E, Bangerth F (1988) Identification and quantitation of gibberellins in fruits of *Lycopersicon esculentum*, and their relationship to fruit size in *L. esculentum* and *L. pimpinellifolium*. Physiol Plant 73:348–353

Coates RM, Ley DA, Cavendar PL (1978) Synthesis and carbon-13 nuclear magnetic resonance spectra of *all-trans*-geranylgeraniol and its nor analogues. J Organ Chem 43:4915–4922

Coolbaugh RC (1983) Early steps of gibberellin biosynthesis. In: Crozier A (ed) The biochemistry and physiology of gibberellins, 1983. Praeger Publishers, New York, pp 53–98

Davisson VJ, Woodside AB, Poulter CD (1985) Synthesis of allylic and homoallylic isoprenoid pyrophosphates. Methods Enzymol 110:130–144

Duncan JD, West CA (1981) Properties of kaurene synthetase from *Marah macrocarpus* endosperm: Evidence for the participation of separate but interacting enzymes. Plant Physiol 68:1128–1134

Fall R, West CA (1971) Purification and properties of kaurene synthetase from *Fusarium moniliforme*. J Biol Chem 246:6913–6928

Frost RG, West CA (1977) Properties of kaurene synthetase from *Marah macrocarpus*. Plant Physiol 59:22–29

Gafni Y, Shechter I (1981) Isolation of a kaurene synthetase inhibitor from castor bean seedlings and suspension cultures. Plant Physiol 67:1169–1173

Graebe JE (1987) Gibberellin biosynthesis and control. Annu Rev Plant Physiol 38:419–465

Graebe JE, Dennis DT, Upper CD, West CA (1965) Biosynthesis of gibberellins: I. The biosynthesis of (−)-kaurene, (−)-kauren-19-ol, and *trans*-geranylgeraniol in endosperm nucellus of *Echinocystis macrocarpa* Greene. J Biol Chem 240:1847–1854

Gray JC (1987) Control of isoprenoid biosynthesis in higher plants. Adv Bot Res 14:25–91

Groot SPC, Bruinsma J, Karssen CM (1987) The role of endogenous gibberellin in seed and fruit development of tomato: Studies with a gibberellin-deficient mutant. Physiol Plant 71:184–190

Hedden P, Phinney BO (1979) Comparison of *ent*-kaurene and *ent*-isokaurene synthesis in cell-free systems from etiolated shoots of normal and *dwarf-5* maize seedlings. Phytochemistry 18:1475–1479

Ingram TJ, Reid JB, Murfet IC, Gaskin P, Willis CL, MacMillian J (1984) Internode length in *Pisum*. The *Le* gene controls the 3β-hydroxylation of gibberellin A_{20} to gibberellin A_1. Planta 160:455–463

Kobayashi M, Sakurai A, Saka H, Takahashi N (1989) Quanti-

tative analysis of endogenous gibberellins in normal and dwarf cultivars of rice. Plant Cell Physiol 30:963–969

Murakami Y (1972) Dwarfing genes in rice and their relation to gibberellin biosynthesis. In: Carr DJ (ed) Plant growth substances, 1970. Springer-Verlag, Berlin, Heidelberg, New York, pp 166–174

Phinney BO (1984) Gibberellin A_1, dwarfism and the control of shoot elongation in higher plants. In: Crozier A, Hillman JR (eds) The biosynthesis and metabolism of plant hormones. Cambridge Society of Experimental Biology Seminar Series 23:17–41

Potts WC, Reid JB (1983) Internode length in *Pisum*. III. The effect and interaction of the *Na/na* and *Le/le* gene differences on endogenous gibberellin-like substances. Physiol Plant 57:448–454

Shechter I, West CA (1969) Biosynthesis of gibberellins. IV. Biosynthesis of cyclic diterpenoids from *trans*-geranylgeranyl pyrophosphate. J Biol Chem 244:3200–3209

Shen-Miller J, West CA (1982) *Ent*-kaurene biosynthesis in extracts of *Helianthus annuus* L. seedlings. Plant Physiol 69:637–641

Turnbull CGN, Crozier A, Schwenen L, Graebe JE (1985) Conversion of [^{14}C]gibberellin A_{12}-aldehyde to C_{19}- and C_{20}-gibberellins in a cell-free system from immature seed of *Phaseolus coccineus* L. Planta 165:108–113

Upper CD, West CA (1967) Biosynthesis of gibberellins II. Enzymatic cyclization of geranylgeranyl pyrophosphate to kaurene. J Biol Chem 242:3285–3292

Zeevaart JAD (1986) Characterization of three single gene dwarf mutants of tomato. J Cell Biochem S10B:33