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Expression of a 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Gene from *Camptotheca acuminata* Is Differentially Regulated by Wounding and Methyl Jasmonate[1]

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We have isolated a gene, *hmg1*, for 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) from *Camptotheca acuminata*, a Chinese tree that produces the anti-cancer monoterpenoid indole alkaloid camptothecin (CPT). HMGR supplies mevalonate for the synthesis of the terpenoid component of CPT as well as for the formation of many other primary and secondary metabolites. In *Camptotheca*, *hmg1* transcripts were detected only in young seedlings and not in vegetative organs of older plants. Regulation of the *hmg1* promoter was studied in transgenic tobacco using three translational fusions (-1678, -1107, -165) with the β-glucuronidase (GUS) reporter gene. Histochemical analysis of plants containing each of the three promoter fusions showed similar developmental and spatial expression patterns. In vegetative tissues, GUS staining was localized to the epidermis of young leaves and stems, particularly in glandular trichomes. Roots showed intense staining in the cortical tissues in the elongation zone and light staining in the cortex of mature roots. *hmg1*::GUS expression was also observed in sepals, petals, pistils, and stamens of developing flowers, with darkest staining in the ovary wall, ovules, stigmas, and pollen. Leaf discs from plants containing each of the translational fusions showed a 15- to 20-fold wound induction of *hmg1*::GUS expression over 72 h; however, this increase in GUS activity was completely suppressed by treatment with methyl jasmonate. Taken together, these data show that a 165-bp fragment of *Camptotheca hmg1* promoter is sufficient to confer developmental regulation as well as wound induction and methyl jasmonate suppression of GUS expression in transgenic tobacco.

Mevalonate is the primary building block for isoprenoid synthesis in higher plants. Thus, it serves as a precursor for the production of a number of compounds vital to normal plant growth and development, including carotenoids, the phytol tail of Chls, plastoquinone, and ubiquinone, as well as the phytohormones ABA, cytokinins, and GAs. Mevalonate also contributes to the formation of a wide variety of plant secondary metabolites such as phytoalexins, rubber, and terpenoid indole alkaloids.

The final step in mevalonate production is catalyzed by the branch-point enzyme HMGR (EC 1.1.1.34), which shunts HMG-CoA into the isoprenoid pathway. Molecular studies have shown that plant HMGRs are encoded by small gene families whose members exhibit complex developmental and environmental regulation (Caelles et al., 1989; Learned and Fink, 1989; Natrita and Gruissem, 1989; Yang et al., 1991; Choi et al., 1992; Chye et al., 1992; Genschik et al., 1992).

We are interested in the regulation of HMGR because of its role in providing terpenoid intermediates for indole alkaloid formation (Fig. 1). Biosynthesis of these compounds begins with the decarboxylation of Trp to tryptamine, catalyzed by the enzyme TDC (Lückner, 1984). SS then assembles strictosidine, a key intermediate in the indole alkaloid pathway, by coupling tryptamine to the monoterpene glucoside secologanin (Fig. 1; Stöckigt and Zenk, 1977). Over 1000 alkaloids are derived from strictosidine, including strychnine, quinine, and the anticancer compounds vinblastine, vincristine, and camptothecin (Cordell, 1974).

Previous molecular studies have focused on the cloning and expression of SS and TDC genes because of their importance in the synthesis of strictosidine. cDNAs for SS have been isolated from *Rauvolfia serpentina* (Kutchan et al., 1988) and *Catharanthus roseus* (McKnight et al., 1990; Pasquali et al., 1992), and a cDNA for TDC has been cloned from *C. roseus* (De Luca et al., 1989).

Although an HMGR cDNA has recently been isolated from *C. roseus* (Maldonado-Mendoza et al., 1992), little is known about the regulation of HMGR or other genes involved in secologanin biosynthesis in species that accumulate indole alkaloids. Feeding experiments with *C. roseus* tissue cultures suggest that terpenoid synthesis may represent the limiting step in indole alkaloid production (Merillon et al., 1986, 1989); therefore, it is of interest to begin to dissect the regulation of this portion of the pathway.

Toward this goal, we have isolated and characterized an HMGR gene from *Camptotheca acuminata*, a Chinese tree that is the natural source for the anti-tumor indole alkaloid camptothecin. Unlike the bisindole alkaloids of *C. roseus*, which

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Abbreviations: GUS, β-glucuronidase; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; JA, jasmonic acid; MeJA, methyl jasmonate; PCR, polymerase chain reaction; SS, strictosidine synthase; TDC, tryptophan decarboxylase; X-Gluc, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide.

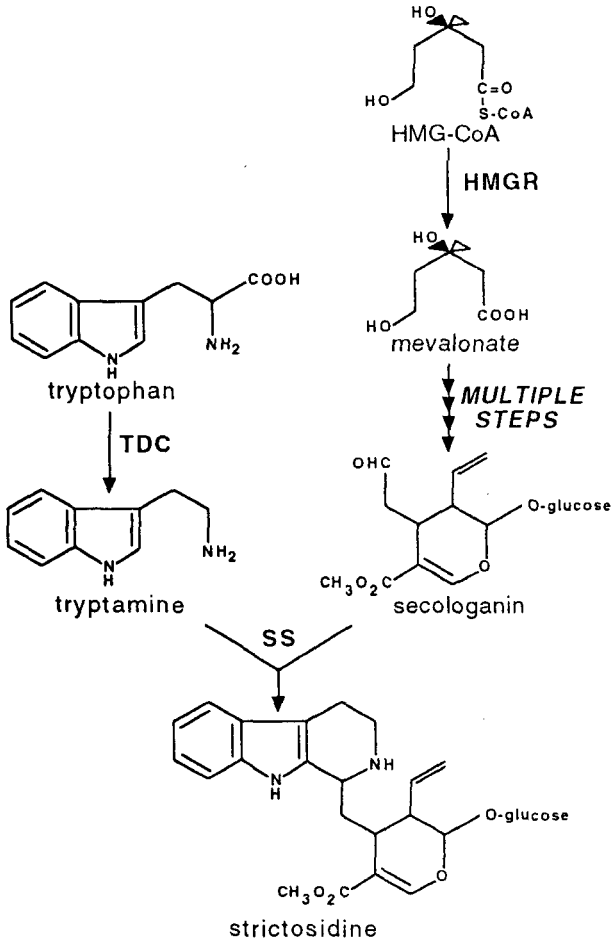

Figure 1. The role of HMGR in strictosidine biosynthesis. Strictosidine, a key intermediate in monoterpenoid indole alkaloid synthesis, is produced from the condensation of tryptamine and secologanin by SS. The indole tryptamine is derived from the decarboxylation of Trp by TDC. Secologanin, the monoterpenoid portion of strictosidine, is synthesized in multiple enzymic steps from mevalonate supplied by HMGR.

disrupt microtubule assembly, the monoindole alkaloid camptothecin is a specific inhibitor of DNA topoisomerase I. Thus, because of their distinct subcellular targets, the *Camptotheca* and *Catharanthus* alkaloids can be used in combination or sequentially for cancer chemotherapy.

In the present report we describe the complex developmental and environmental regulation of the *Camptotheca* HMGR gene in transgenic tobacco using a series of three HMGR promoter-reporter gene constructions. Using these constructs, we also demonstrate that the *Camptotheca* gene is differentially regulated by wounding and the elicitation transducer MeJA. Furthermore, we show that the smallest promoter fragment of 165 bp is sufficient to regulate each of these responses.

MATERIALS AND METHODS

Plant Materials

Camptotheca acuminata trees were grown from seeds under greenhouse conditions. Tobacco plants (*Nicotiana tabacum* cv Xanthi) used for transformations were grown on one-half Murashige-Skoog media (Murashige and Skoog, 1962) in sterile Magenta boxes with 16 h:8 h light:dark cycle.

Nucleic Acid Isolation and Analysis

DNA was isolated from *C. acuminata* leaves using the hexadecyltrimethyl ammonium bromide method (Taylor and Powell, 1982). Total RNA from plant tissues was isolated using the procedure of Jones et al. (1985). Poly(A)$^+$ RNA was selected by oligo-dT cellulose chromatography with the FastTrack mRNA isolation kit (Invitrogen, San Diego, CA).

For DNA gel-blot analysis, DNA (10 μg/lane) was digested overnight with restriction enzymes, separated by electrophoresis on 0.8% agarose gel, and blotted to nylon filter (Micron Separations, Inc., Westboro, MA). For RNA gel-blot analysis, poly(A)$^+$ RNA (2 μg/lane) was denatured, separated on formaldehyde gels, and blotted to nylon (Micron Separations, Inc.). Nucleic acid blots were probed with ^{32}P-labeled HMGR PCR fragment (see below) in 5× SSC, 0.5% SDS, 5× Denhardt's reagent, 100 μg/mL of denatured calf thymus DNA, 50% formamide at 42°C. Final wash conditions were 1× SSC, 0.5% SDS at 68°C for 1 h.

Library Construction and Screening

C. acuminata DNA was digested to completion with *Bam*HI and size selected on a 15 to 60% linear Suc gradient by the protocol of Sambrook et al. (1989). Fractions containing 9- to 15-kb fragments were pooled, ligated into λEMBL 3 (Stratagene, La Jolla, CA), and packaged in vitro.

A 444-bp fragment of the *C. acuminata* HMGR coding region was obtained by PCR using the conserved HMGR primers as described by Maldonado-Mendoza et al. (1992) and a *C. acuminata* seedling cDNA library for template. A 14-kb HMGR clone, designated λ*hmg1*, was isolated from the λEMBL3 genomic library by plaque hybridization (Sambrook et al., 1989) using the *C. acuminata* HMGR PCR fragment as a probe. A 4.5-kb *Eco*RI fragment containing the HMGR gene and flanking sequences was subcloned into Bluescript KS+ (Stratagene). This plasmid, designated pHMG1, was used for sequencing template and promoter isolations.

Plasmid Construction and Plant Transformation

An *hmg1*::GUS translational fusion was made by ligating a 1790-bp *Eco*RI-*Nco*I fragment from the *hmg1* promoter to a GUS reporter gene (pRAJ275; Jefferson, 1987) containing the nopaline synthase polyadenylation signal at its 3′ end. This construct was designated pCAH-1678 to reflect the size of the region 5′ to the transcription start site. Promoter deletions were made by removal of sequences 5′ to internal *Sph*I (pCAH-1107) and *Sal*I (pCAH-165) restriction sites (see Fig. 3). HMGR1::GUS constructs were assembled in pUC18 and then transferred to the binary vector Bin19 (Bevan, 1984). Constructs were electroporated into *Agrobacterium tumefaciens* LBA 4404 and used to transform tobacco leaf discs (Horsch et al., 1985).

Nucleotide Sequencing and Analysis

Nucleotide sequencing was performed by the staff of the Advanced DNA Technologies Lab at Texas A&M University using the dideoxy chain termination method with double-stranded plasmid templates (Sanger et al., 1977). The 5′ end of the *hmg1* transcript was mapped by primer extension (Sambrook et al., 1989) using 2-week-old *Camptotheca* seedling RNA. Sequences for other HMGR genes were retrieved using the National Center for Biotechnology Information BLAST network service. Computation for sequence comparison was performed with the GeneWorks programs (version 2.2.1; Intelligenetics, Mountain View, CA).

GUS Assays and Histochemistry

Quantitative GUS assays were performed as described by Jefferson et al. (1987). Fluorescence of methylumbelliferone cleaved from methylumbelliferyl-β-D-glucuronide was measured in a fluorometer (Hoeffer Scientific, San Francisco, CA) and expressed as nmol of MUG per min per mg of protein as measured by the method of Bradford (1976).

GUS expression was histochemically localized by incubating tissues in the chromogenic substrate X-Gluc for 3 to 12 h at 37°C as described by Jefferson et al. (1987). Stained tissues were bleached for 30 to 60 min in 10% Clorox at 37°C and hand sectioned for photomicrography.

Wounding and MeJA Treatment

A total of 20 primary transformants were used for histochemical and quantitative analyses: nine pCAH-1678 plants, nine pCAH-1107 plants, and two pCAH-165 plants. For wound induction, 7-mm leaf discs were punched from 10- to 15-cm leaves with a cork borer and incubated in one-half Murashige-Skoog medium in constant light at 25°C. Samples were removed at 0, 3, 6, 12, 24, 48, and 72 h and frozen in liquid nitrogen for later enzyme assay. Interactions between the wound response and MeJA treatment were assayed in leaf discs incubated in one-half Murashige-Skoog medium containing 10^{-4} M MeJA for the same time intervals. MeJA used in this study was a gift from Dr. John Mullet (Department of Biochemistry, Texas A&M University).

RESULTS

Isolation and Analysis of a *Camptotheca* HMGR Gene

A PCR probe encoding the conserved active site of HMGR was generated from a *Camptotheca* seedling cDNA library and used to probe a genomic DNA gel blot (Fig. 2). Based on this information, a *Bam*HI size-selected genomic library was constructed in EMBL3 and screened with the PCR probe, and a 14-kb *Camptotheca* HMGR clone (λ*hmg1*) was isolated. The λ*hmg1* insert was mapped with restriction enzymes (data not shown) and has a banding pattern identical to the darkest bands seen in Figure 2. The presence of additional, lighter bands under high-stringency wash conditions (Fig. 2) suggests that the *Camptotheca* genome may also contain additional, divergent HMGR genes. As noted in the introduction, HMGRs are encoded by small gene families in all of the plant species examined to date.

A 4.5-kb *Eco*RI fragment from λ*hmg1* was subcloned (*hmg1*), mapped (Fig. 3), and sequenced. The *Camptotheca hmg1* gene encodes a predicted protein of 593 amino acids with a calculated M_r of 63,277. The *Camptotheca hmg1* coding region is interrupted by three introns in the same relative positions as those reported for *hmg1* and *hmg3* from *Hevea brasiliensis* (Chye et al., 1992). The predicted amino acid sequence of *Camptotheca hmg1* shares 75% amino acid identity with the *hmg1* genes of *Hevea* (Chye et al., 1991) and *Nicotiana sylvestris* (Genschik et al., 1992), and 74% identity with *hmg1* from *Solanum tuberosum* (Choi, 1992). All of these proteins are extremely conserved in the C-terminal region, which contains the active site.

As has been seen in other plant HMGRs, the deduced protein for *Camptotheca hmg1* has two hydrophobic regions toward the N terminus, which may represent transmembrane domains (Caelles et al., 1989). The *Camptotheca hmg1* gene product also has a putative "PEST" proteolytic cleavage sequence (Rogers et al., 1986) at residues 164 to 184, which potentially targets the enzyme for rapid degradation.

The *hmg1* transcription start site was localized 111 bp upstream of the translational start by primer extension (data not shown). Although the *hmg1* gene has a TATAAA sequence at −57, its distant position from the transcription start makes it an unlikely candidate for a typical TATA box. HMGR genes from humans (Reynolds et al., 1984) and *Hevea*

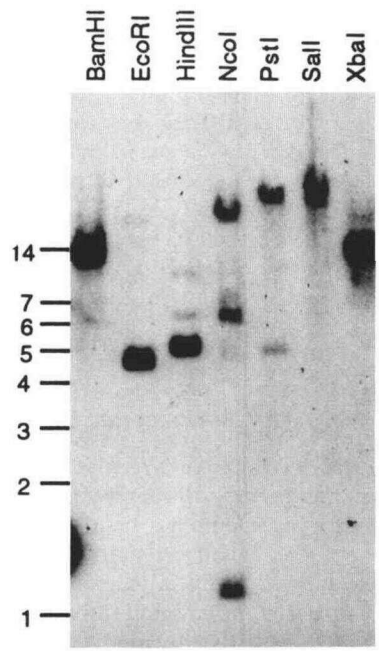

Figure 2. DNA gel blot of *Camptotheca* genomic DNA probed with an HMGR PCR fragment. Ten micrograms of DNA per lane were digested with *Bam*HI, *Eco*RI, *Hin*dIII, *Nco*I, *Pst*I, *Sal*I, or *Xba*I, separated on a 0.8% agarose gel, blotted, and hybridized with a labeled HMGR probe. The 444-bp probe was produced by the PCR using *Camptotheca* seedling cDNA as template and primers from conserved sequences in the HMGR active site (Maldonado-Mendoza et al., 1992). The blot was washed in 1× SSC, 0.1% SDS at 68°C and exposed to x-ray film. Sizes are marked in kb.

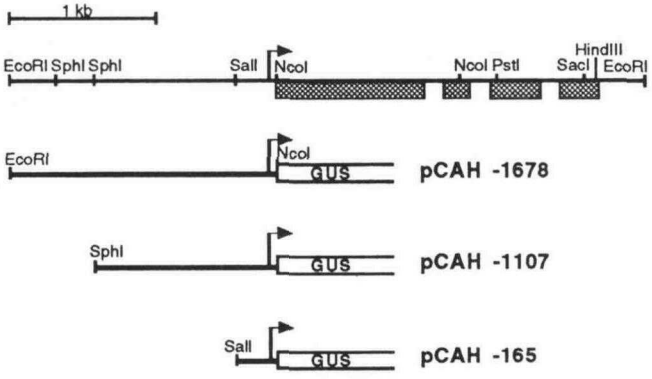

Figure 3. Physical map of *Camptotheca hmg1* gene and chimeric *hmg1*::GUS constructs. The 5′ ends of the hmg1 promoter are −1678 (pCAH-1678), −1107 (pCAH-1107), and −165 (pCAH-165) relative to the transcription start site.

(Chye et al., 1992), as well as a number of other "housekeeping" genes related to general metabolism, also appear to have TATA-less promoters (Dynan, 1986).

Expression of *hmg1* in *Camptotheca*

Camptotheca RNA gel blots showed an abundant 2.4-kb *hmg1* transcript in mRNA extracted from 2-week-old seedlings that was not detectable in roots, stem, or leaves of older plants (Fig. 4). This suggests that at least a portion of the increased HMGR enzyme needed for isoprenoid production during the early development of *Camptotheca* seedlings may be supplied from *hmg1* transcripts.

These data are consistent with the results of Choi et al. (1992), who report that the *Solanum hmg1* and *hmg3* transcripts are not detectable in vegetative tissues, whereas the *hmg2* message is detectable only at low levels in roots. The absence of detectable *hmg1* mRNA in adult vegetative organs of *Camptotheca* may also reflect a rapid turnover of the HMGR message, because the *hmg1*::GUS fusions are expressed, at least at low levels, in all tissues of transgenic tobacco.

Expression of *hmg1*::GUS in Transgenic Tobacco

To examine the developmental regulation of the *Camptotheca hmg1* promoter, three different-length 5′ fragments from the gene were placed into translational fusions with the GUS reporter gene (Fig. 3) and transformed into tobacco. The three constructs showed identical temporal and spatial patterns of GUS expression in transgenic tobacco; however, the relative levels of GUS activity varied as much as 24-fold between individual transformants containing the same construct (data not shown). The typical pattern of X-Gluc staining in these *hmg1*::GUS constructs is presented in Figure 5.

In young leaves, 1- to 5-cm long, the majority of GUS staining was localized in the epidermis and developing trichomes (Fig. 5A). Both the adaxial and abaxial epidermal cells were stained, as were the bases of glandular and nonglandular hairs. In the mid-rib of young leaves, the darkest staining was confined to the epidermis and the bundle sheath surrounding the vein (Fig. 5C). As the leaves matured, GUS staining became more restricted to the heads of glandular trichomes (Fig. 5B).

The epidermis was also uniformly stained in sections of young stems taken from internodes within the first 5 cm of the shoot apex (Fig. 5D). Localized patches of GUS staining were also frequently observed in the internal phloem parenchyma associated with bundles of developing phloem fibers (Fig. 5E).

Roots of R_0 *hmg1*::GUS plants generally exhibited widespread, although variable, patterns of GUS staining. In contrast to the shoot system, roots showed the most intense staining in cortical cells and minimal staining in the epidermis and root hairs. Staining was also typically absent from both the root cap and apical meristem of young roots, whereas adjacent tissues, corresponding to the zone of elongation, were often deeply stained (Fig. 5F). GUS staining was progressively less intense in the maturation zone as identified by its developing root hairs (Fig. 5F), although localized patches of GUS staining were also commonly seen along older branched roots (Fig. 5G) throughout development.

Flower buds showed at least some *hmg1*::GUS expression in all four whorls of the flower during development. In young buds (stages 1–6 of Koltunow et al., 1990), faint blue staining was detected in sepals and throughout the corolla, in both the tube and limb (data not shown). In stamens, the most pronounced staining was localized to the developing pollen grains (Fig. 5, H and K), although at the time of anther dehiscence, most grains stained lightly, if at all.

Both the receptacle and ovary wall of the pistil (Fig. 5I) were deeply stained throughout flower development. Although ripening ovules also exhibited considerable GUS activity, very little staining was observed in placental tissues and its associated vasculature (Fig. 5L). Stigmas stained darkly at all stages of flower development, even after anthesis (Fig. 5J).

R_1 seedlings that had just emerged from the seed coat showed intense GUS staining at the base of the cotyledons, along the hypocotyl, and in the region of radicle elongation (Fig. 5M). After the cotyledons had fully expanded, at about 6 d postimbibition, faint staining could still be observed at the hypocotyl/root junction (Fig. 5M, arrow) and in the

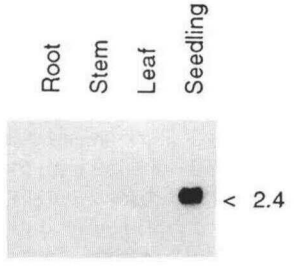

Figure 4. RNA gel blot of *hmg1* mRNA from *Camptotheca* vegetative tissues. Two micrograms per lane of poly(A)$^+$ RNA from root, stem, leaf, and 2-week-old seedlings were denatured with formamide, separated on 1.2% agarose, blotted, and hybridized with the *Camptotheca hmg1* PCR probe. Sizes of bands are indicated in kb.

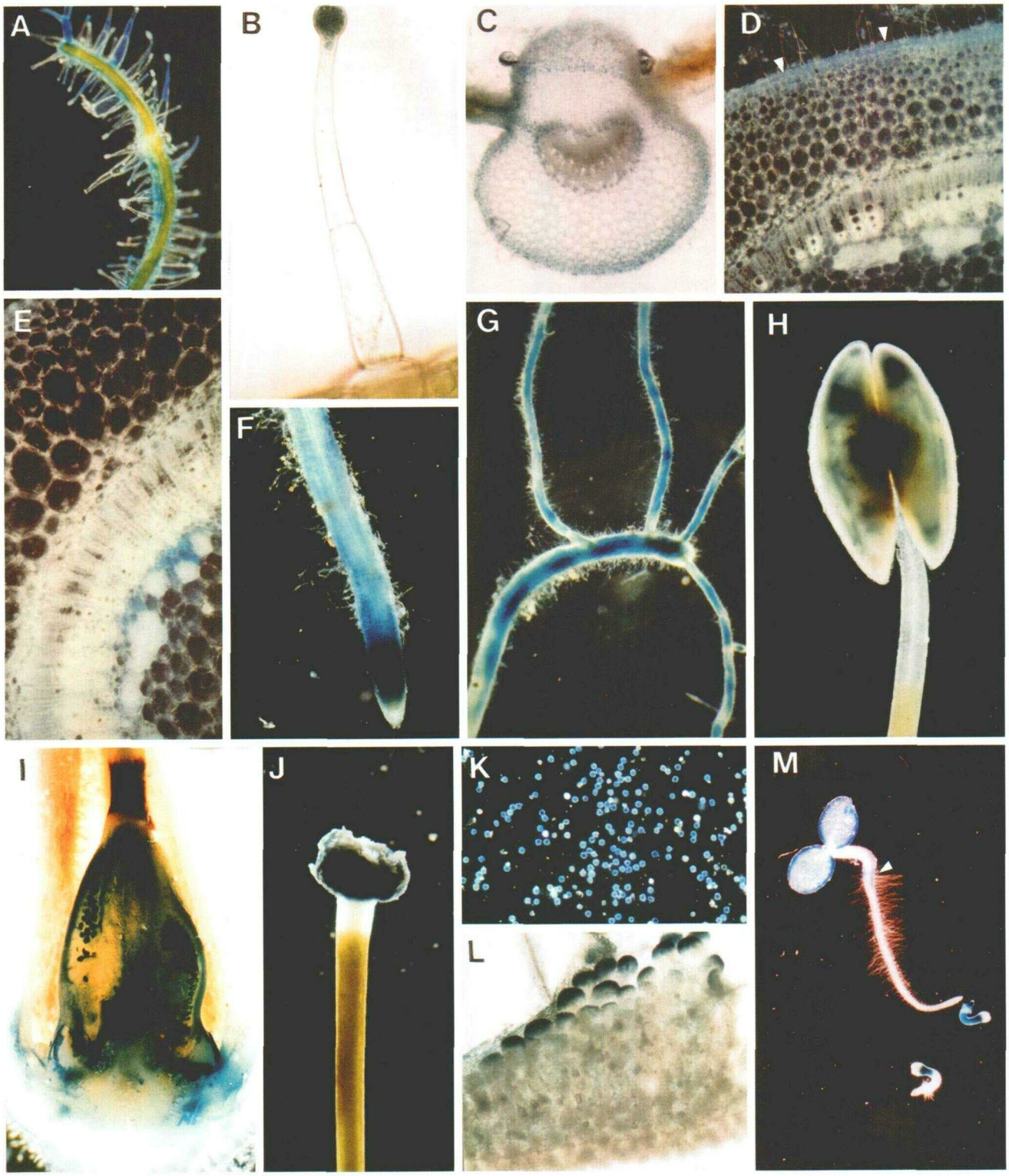

Figure 5. Transgenic tobacco plants expressing *hmg1*::GUS after staining with X-Gluc. Tissues were taken from a pCAH-1678 R_0 plants or R_1 seedlings; however, identical staining was seen in pCAH-1107 and pCAH-165 plants. A, Cross-section from the middle of a 2-cm long leaf. B, Glandular trichome on a 4-cm long leaf. C, Cross-section through the midrib of a 2-cm long leaf. D, Stem cross-section from the internode 4 cm from the apex. Arrowheads point to stained epidermis. E, Stem cross-section from the internode 5 cm from the apex. F, Root from soil-grown plant. G, Branched roots from soil-grown plant. H, Pre-dehiscent anther. I, Longitudinal section through receptacle and ovary of a young flower. J, Stigma and style. K, Immature pollen. L, Cross-section through developing ovules, placental tissue, and ovary wall. M, Germinating seedlings 6 d postimbibition.

cotyledons, with the most intense staining localized along the cotyledon margins. Although the primary root of most young seedlings lost detectable GUS activity soon after germination (Fig. 5M), newly initiated lateral roots of R_1 plants showed GUS staining in their zone of elongation, similar to that shown in Figure 5F. Thus, the general pattern of GUS staining observed in the roots of R_0 plants grown in soil (Fig. 5, F and G) was similar to the pattern seen in roots of R_1 plants grown in agar, regardless of their age.

Response of *hmg1*::GUS to Wounding and MeJA

Response of the *Camptotheca hmg1* promoter to wounding and MeJA treatment was examined in transgenic tobacco plants containing each of the promoter GUS constructs shown in Figure 3. For these experiments 7-mm discs were punched from fully expanded leaves and maintained in liquid media with or without added MeJA for 72 h.

Results from a typical wound-induction experiment are presented in Figure 6. A 2- to 3-fold increase in GUS activity was observed in most transformants 6 h after wounding, the first time point assayed. By the end of the experiment at 72 h, GUS activity continued to increase to a level 15 to 20 times greater than unwounded controls.

The induction of the GUS expression by wounding was completely suppressed by the addition of 100 μM MeJA to leaf discs from each of the three *hmg1*::GUS constructs (Fig. 6). When the wounding and MeJA experiments were repeated with root segments, similar results were obtained (data not shown), suggesting that the wound induction/MeJA suppression of the *hmg1* promoter is not tissue specific.

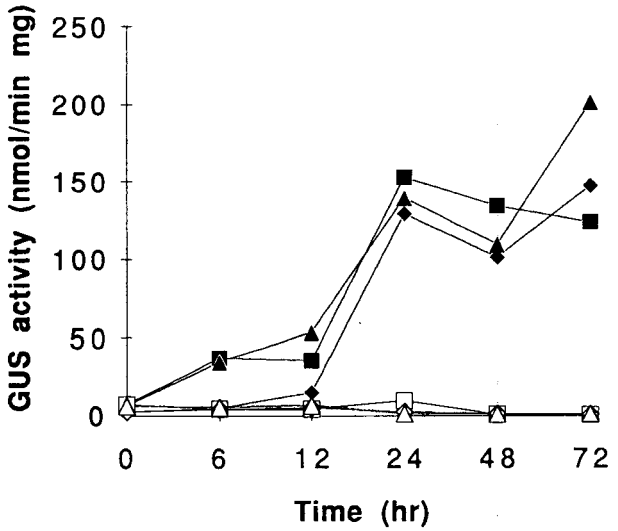

Figure 6. Effect of wounding and MeJA on the expression of *hmg1*::GUS constructs in transgenic tobacco. The effect of wounding (solid symbols) on *hmg1*::GUS expression was assayed by floating 7-mm leaf discs on one-half Murashige-Skoog medium in constant light at 25°C. The effect of MeJA on wound-induced *hmg1*::GUS expression (open symbols) was tested as above, with the addition of 100 μm of MeJA to the medium. Squares represent pCAH-1678, diamonds represent pCAH-1107, and triangles represent pCAH-165.

The influence of MeJA concentration on *hmg1*::GUS expression was examined in pCAH-1678, pCAH-1107, and pCAH-165 plants (Fig. 3) by incubating leaf discs in 0, 10^{-4}, and 10^{-6} M MeJA solutions for 24 h. In all three constructs, the wound induction of *hmg1*::GUS expression was suppressed by MeJA in a dose-dependent manner (data not shown).

DISCUSSION

Structure and Expression of a *C. acuminata* HMGR Gene

We have isolated and characterized a *C. acuminata* HMGR gene using a homologous HMGR probe obtained by PCR from a *Camptotheca* seedling cDNA library. The restriction map of this clone, *hmg1*, is identical to the pattern seen in genomic DNA gel blots; however, one or two additional cross-hybridizing bands are also seen on the blot under stringent hybridization and wash conditions (Fig. 2).

At least two HMGR genes are known from *Arabidopsis thaliana* (Caelles et al., 1989; Learned and Fink, 1989) and tobacco (Genschik et al., 1992), whereas three functional HMGR genes have been reported in potato (Choi et al., 1992) and the rubber tree, *H. brasiliensis* (Chye et al., 1992). As many as four genes may be present in tomato (Park et al., 1992). Based on the appearance of cross-reacting bands in our DNA gel blots (Fig. 2), it is likely that the *hmg1* gene of *Camptotheca* also belongs to a small family of HMGR genes with divergent members.

We did not detect *hmg1* mRNA in *Camptotheca* stems, roots, or leaves; however, abundant transcripts were seen in seedlings. Similar results have been reported for potato (Choi et al., 1992), *N. sylvestris* (Genschik et al., 1992), and rubber tree (Chye et al., 1992), where very little to no HMGR message was seen in mRNA prepared from unstressed vegetative tissues. Because the *Camptotheca* trees used in the present investigation were not of reproductive age (>5 years), we were unable to assess the pattern of *hmg1* expression in the floral organs of this species. Nevertheless, histochemical analysis of *hmg1*::GUS translational fusions in tobacco suggests that this gene may also be highly expressed in developing *Camptotheca* flowers.

Developmental and Tissue-Specific Regulation

Although transformation of *Camptotheca* cell cultures is possible using *Agrobacterium*-based vectors (I.E. Maldonado-Mendoza, unpublished observation), a regeneration protocol has not been developed for this woody species. Therefore, we chose tobacco as a heterologous system in which to study *hmg1*::GUS expression; not only because it is easy to transform and regenerate, but also because it has served as a useful model system for studying terpenoid biosynthesis (Gondet et al., 1992).

In young leaves and stems of transgenic tobacco, most *hmg1*::GUS expression was localized to cells of the epidermis, especially in the heads of glandular trichomes. Diterpenes, particularly duvatrienediol, are particularly abundant in glandular trichomes of tobacco and can be synthesized directly from CO_2 in detached heads (Kandra and Wagner, 1988).

High levels of HMGR activity would therefore be expected in this cell type to provide precursor for diterpene synthesis.

Expression of *hmg1*::GUS in transgenic tobacco roots was higher than expected based on the lack of detectable *hmg1* message in *Camptotheca* roots. Although low levels of HMGR message have been observed in the roots of potato (*hmg2*; Choi et al., 1992) and *N. sylvestris* (6P2; Genschik et al., 1992), it is difficult to ascertain how much of that expression might have been due to "wound induction" caused by the mechanical stress of growing through a solid matrix. Although it is clear from our data that the *Camptotheca hmg1* promoter is highly expressed in the elongation zone of developing roots, the signal(s) responsible for the localized patches of GUS staining often observed in older branched roots remains to be determined.

In young tobacco flower buds, GUS staining was detected in sepals, petals, stamens, and pistils. As the flowers matured, staining became more restricted to developing pollen, stigmas, and ovules, where there is a continued high demand for terpenoids. Similar results have been obtained in potato, where low levels of HMGR transcript were detected in floral primordia, petals, and pistils, and much higher levels of message were seen in anthers (Choi et al., 1992).

Regulation of HMGR Expression by Wounding and MeJA

The existence of multiple HMGR genes in plants offers a molecular explanation for the differential HMGR activities associated with environmental stresses such as wounding and pathogen challenge (Bostock and Stermer, 1989). The HMGR isogenes of potato are differentially activated by wounding, pathogen challenge, and arachidonic acid elicitation (Yang et al., 1991; Choi et al., 1992). Although wounding induces the expression of all three isogenes, only two members of the HMGR gene family (*hmg2* and *hmg3*) are further stimulated when wounded tissues are treated with elicitor or inoculated with the fungal pathogen *Phytophthora infestans* (Choi et al., 1992). For the remaining isogene (*hmg1*), wound-induced expression of HMGR transcription is suppressed rather than enhanced by these treatments (Yang et al., 1991; Choi et al., 1992).

Our data suggest that the *Camptotheca hmg1* gene is regulated in the same manner as, and is therefore functionally homologous to, the potato *hmg1* isogene described by Choi et al. (1992). Not only are both genes wound inducible, but the wound induction of both genes is also suppressed by plant defense signaling compounds. Choi et al. (1992) have reported that treatment with fungal elicitor or arachidonic acid suppresses wound induction of the potato *hmg1* gene. In this report we show a similar suppression of the wound response of the *Camptotheca hmg1* gene using the elicitation signal transducer MeJA.

The role of JA in elicitor-induced signal transduction was first described by Gundlach et al. (1992). Using several well-characterized tissue-culture systems, they showed that fungal elicitation causes a transient increase in JA levels that is well correlated with the onset of secondary product synthesis. Furthermore, they also showed that exogenous MeJA could substitute for fungal wall preparations in these cultures to elicit secondary metabolite production and the de novo transcription of defense genes such as Phe ammonia lyase.

Recently, arachidonic acid treatment has been shown to cause a rapid increase in lipoxygenase activity in potato (Bostock et al., 1992). Lipoxygenase is a key enzyme in the in vivo synthesis of JA (Vick and Zimmerman, 1984). Thus, the suppression of wound inducibility in the potato *hmg1* gene by arachidonic acid and fungal elicitors observed by Choi et al. (1992) is likely to have resulted from a transient increase in endogenous JA.

Because *Camptotheca hmg1* expression is inhibited by MeJA, it is unlikely that this isogene is involved in supplying mevalonate to cells responding to pathogen attack. Isolation and characterization of additional HMGR family members in *Camptotheca* may identify other isogenes that are coordinately regulated to function with other indole alkaloid pathway enzymes during normal development as well as during periods of environmental stress.

Regulation of Expression by Truncated HMGR Promoters

Each of the three *Camptotheca hmg1* promoter fragments (−1678, −1107, −165) used in this investigation showed a similar pattern of developmental regulation in transgenic tobacco. Each promoter fragment also displayed the same quantitative wound induction and suppression of wound inducibility by MeJA.

Thus, it appears that all of the *cis*-acting elements needed for the complex developmental and environmental regulation of this gene are present within 165 bp of the HMGR transcription start site. The relatively small size of this fragment should facilitate the fine mapping of this promoter and identification of specific elements responsible for its regulation.

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