REGULATION OF PLANT ISOPRENOID METABOLISM:

Characterization and Induction of Potato HMGR Genes in Relation to Antimicrobial Isoprenoid Synthesis

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INTRODUCTION

The acetate-mevalonate pathway in plants provides a diverse array isoprenoid compounds that have a vital role in cell structure a function(Bach *et al.*, 1987, 1988; Gray, 1987; Kleinig, 1989) These incl phytosterols, dolichols, ubiquinones, phytols, carotinoids, a phytohormones such as ABA and gibberellins. This pathway also produces variety of stress-induced metabolites such as steroid-glycoalkaloids phytoalexins that may contribute to defense against insects and pathoge The basic isoprene unit also is present in a number of other importa compounds such as the cytokinines. Recent research suggests th isoprenylation of proteins is an important regulatory mechanism in ani cell, and similar mechanisms may occur in plants(Goldstein and Brown, 199 In spite of the significance and vital role of these compounds in plan the organization and regulation of the isoprenoid biosynthetic pathway poorly understood. HMGR catalyzes the conversion of HMG CoA to mevalona HMGR is the rate limiting step in sterol biosynthesis in yeast and mammal

cells and is also considered a key regulatory enzyme in plant isoprenoid biosynthesis. Biochemical and molecular studies with animal and yeast HMGR revealed that regulation of this enzyme is extremely complex and includes controls at the levels of transcription and translation, as well as post-translational modification of the protein(Goldstein and Brown, 1990). Plants have the capacity to synthesize a more complex array of isoprenoid compounds than animals or yeast, suggesting that the regulation of the key enzymes in the pathway may also reflect this diversity and complexity(Gray, 1987). Indeed, earlier biochemical studies of plant HMGR suggest functional compartmentation at the subcellular level and also indicate that multiple forms of HMGR are present(Stermer and Bostock, 1987; Russell and Davidson, 1982). The first plant HMGR gene was cloned from *Arabidopsis* by Learned and Fink(1989). Following this work, plant HMGR genes were cloned from potato(Choi *et al.*, 1992), tomato(Narita and Gruissem, 1989), rubber plant(Chye *et al.*, 1991), and raddish(Ferrer *et al.*, 1990). Using these cloned genes and different plant organs, studies have progressed rapidly on the regulation of HMGR gene expression in different developmental and stress-related contexts. Isoprenoid metabolism is strongly activated in solanaceous plants following mechanical injury or infection(Kuc and Rush, 1985; Tjamos and Kuc, 1982; Vogeli and Chappell, 1988; Threllfall and Whitehead, 1988). Earlier biochemical studies in potato revealed that wounding of the plant induced the rapid accumulation of steroid glycoalkaloids. When the wounded tissues were inoculated with the incompatible race of the fungal pathogen *Phytophthora infestans* or treated with arachidonic acid, an elicitor produced by this fungus, steroid glycoalkaloid biosynthesis was strongly and rapidly inhibited and sesquiterpenoid phytoalexins were accumulated(Tjamos and Kuc, 1982; Threlfall and Whitehead, 1988). Subsequently, Vogeli and Chappell(1988) reported that squalene synthetase and sesquiterpene cyclase, the first

enzymes in the branches leading to sterols and sesquiterpenes, respective responded inversely following elicitor treatment of tobacco cells. T inverse response also was observed in potato tissues following elici treatment or inoculation with incompatible races of *P. infestans* or fu non-pathogenic on potato that elicit a hypersensitive response(HR; Zook and Kuc, 1991, see fig. 1).

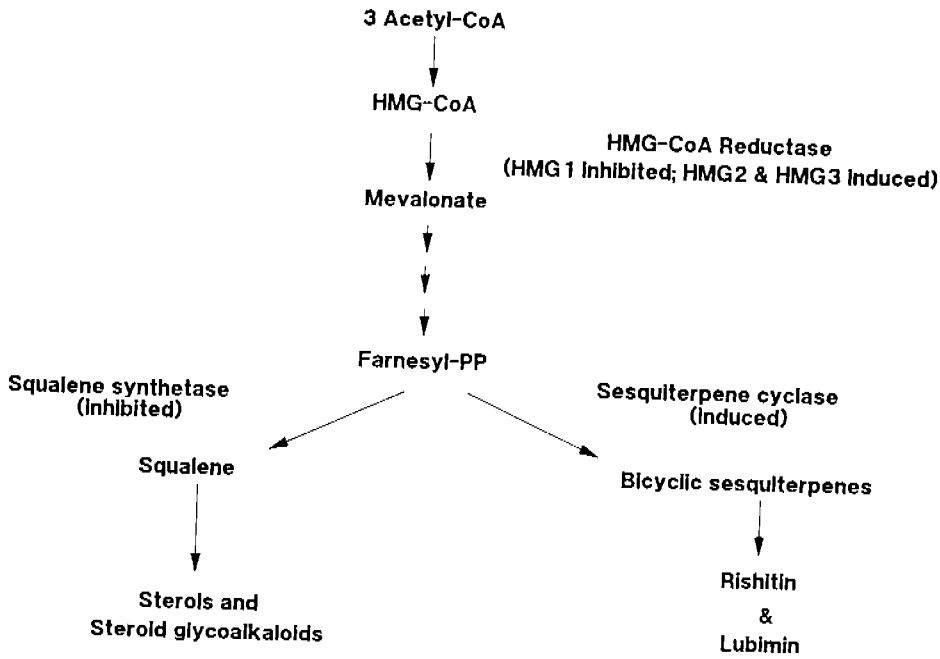

Fig. 1. Outline of stress-responsive isoprenoid biosynthesis in Solanaceous plants. During expression of the hypersensitive response, wound induced sterol/steroid glycoalkaloid synthesis is inhibited in favor of sesquiterpenoid phytoalexin synthesis. HMGR, squalene synthetase, and sesquiterpene cyclase are key enzymatic steps in the pathway affected by elicitor treatment or inoculation with fungi. The subcellular compartmentation of the various steps in the pathway has not been resolved.

The accumulation of phytoalexins often is associated with the tissue necrosis characteristic of the HR. Although phytoalexins are considered a possible defense response against microbial invasion, definitive proof for a causal role of these compounds in restricting pathogen ingress and colonization is lacking. The identification and cloning of genes for phytoalexin detoxifying enzymes from pathogenic fungi has strengthened the case for phytoalexins as defense compounds (Van Etten *et al.*, 1989; Kuhn and Smith, 1979). However, at this time there is no direct evidence that phytoalexins are crucial for disease resistance in any given interaction. The phytoalexin hypothesis could be tested with phytoalexin-deficient mutants, this approach, however, requires a more detailed understanding of phytoalexin biosynthesis. Accumulation of phytoalexins has been demonstrated in many plant families. Generally, species within a family of plant primarily synthesize phytoalexins of similar structure and biosynthetic origin. For example, in the *Leguminosae* there are more than 100 different phytoalexins that have been characterized and 80% of these are isoflavonoid derivatives. Similarly, the majority of the phytoalexins that have been described in solanaceous plants are terpenoid derivatives(Kuc and Rush, 1985). Examples of the latter are the sesquiterpenoids rishitin in potato and tomato, and capsidiol in tobacco and pepper. The research presented in this proceding represents a contribution towards understanding the organization and regulation of the isoprenoid phytoalexin pathway in plants. It is anticipated that this information will be useful for designing strategies to better define the role of these compounds in plant growth and development and in responses to pathogens and other environmental stresses.

MATERIALS AND METHODS

Plant and Fungal Materials

Certified seed grade potatoes(*Solanum tuberosum* L. cv Kennebec) were obtained from Herman Tim(Department of Vegetable Crop, UC-Davis) and stored at 4°C until 24 hour prior to use when they were placed in darkness at room temperature. To avoid the light effect, manipulation of potato tubers were carried out under a green safelight comprised of two green fluorescent tubes(Sylvania F40-G) wrapped with one sheet each of Rosecolene filter No. 874(medium blue green) and No. 874(medium green). Tubers were washed, surface sterilized in 70% ethanol for 3 minutes, then disks(22x5-7 mm) were cut from the central parenchymous tissue with a sterile cork borer. The disks were washed 3 times with sterile distilled water and five were placed on sterile filter paper in petri dish and excess water was removed using sterile filter papers. All treatments and inoculations were applied to upper surface of disk in 50 μl volume and incubated at 20°C. RNAs from other than tuber were extracted from green house grown plant tissue which is directly froze in liquid N_2. Sporangial inoculums were prepared from *Phytophthora infestans*(Mont.) deBary race 0 and 1, 2, 3, 4 grown on lima bean agar or rye seed media at 20°C. Sporangial suspensions containing 10^5 sporangia were applied to tuber disk aged 20 hours after preparation.

Chemicals

Arachidonic acid(AA)and other chemicals were purchased from Sigma. Immediately before application, AA solution was dried under N_2 gas and resuspended in sterile water by brief sonication. Total 50 μg of AA was applied in 50 μl volume to each tuber disk which show approximately 50% of the maximum response for sesquiterpenoid phytoalexin accumulation(Bostock *et al.*, 1982). Methyl-jasmonate(MJ), Salycilate(SA), Acetylsalicylate(ASA),

Salicylhdroxamate(SHAM), and n-Propylgalate(nPG) were treated similar way as AA.

cDNA Library Screening

670,000 plaques from potato tuber cDNA library(obtained from Okita) in λ gt 11 were transfered to Nylon filters(Ausubel *et. al.*, 1987). About 800 nucleotides long cDNA probe was constructed from the highly conserved region of *Arabidopsis* HMGR cDNA (obtained from Learned and Fink, 1989) by digestion with *Nhe*I. The filters were hybridized with radiolabeled probe under low stringency hybridization condition(5X SSC, 5X Denhardt's, 10% formamide. 100 μg/ml denatured salmon sperm DNA at 42°C) and washed same condition for 2 hours. Forty-seven positive clones were obtained and 11 of them were subcloned on pBluescriptII KS(+)(Strategene) and partially mapped using 8 different restriction endonucleases. Based on the maps, probe were constructed from each end region of cDNA and hybridized with elicitor induced and uninduced potato RNA. As a results, we selected 3 different cDNA clones which are expressed in potato tuber.

Nuclear DNA isolation and genomic blot

Nuclei were isolated from potato leaves by the method of Timberlake(1978) except that the extraction buffer was pH 9.5(Fisher and Goldberg, 1982) and Triton X-100 was added to a final concentration of 0.5% to lyse chloroplasts. Nuclei were lysed in 1% sarkosyl, 0.05 M Tris, 0.02 M EDTA(pH 9.5), centrifuged, the DNA was precipitated with 0.5 volume of cold isopropanol, and further purified by CsCl/ethidium bromide density gradient centrifugation. DNA were digested 5 x recommended units of restriction enzyme and 10 μg/lane were fractionated by electrophoresis in 0.7% agarose gels in TAE buffer and transfered to a Nytran membrane. The DNA blots were hybridized with random-prime labeled probes using standard procedures. Two

classification of the cultivated potato as a tetraploid species(2N=4X=48 chromosome).

Sequencing and Probe Construction

cDNAs subcloned in pBluscriptII KS(+) were digested with proper restriction endonucleases. Nested deletion sets of cDNAs were obtained by Exonuclease III and Mungbean nuclease digestion according to manufacturers guide(Strategene). DNA sequences were determined by chain termination method(Sanger *et al.*, 1977) using T7 and T3 universal primers and the Sequanase enzyme according to the manufacturers instructions(United States Biochemicals). Synthetic primers were also designed and used for sequencing unoverlapped regions. DNA sequences were analyzed using personal computer program Microgenni. From the sequences of potato HMGR 1, 2, 3 cDNA variable regions were selected for gene specific probe(5′ untranslated region and N-terminal coding region for HMGR 1, 3′ untranslated region for HMGR 2 and 3, see Choi *et al.*, 1992 and Fig. 2).

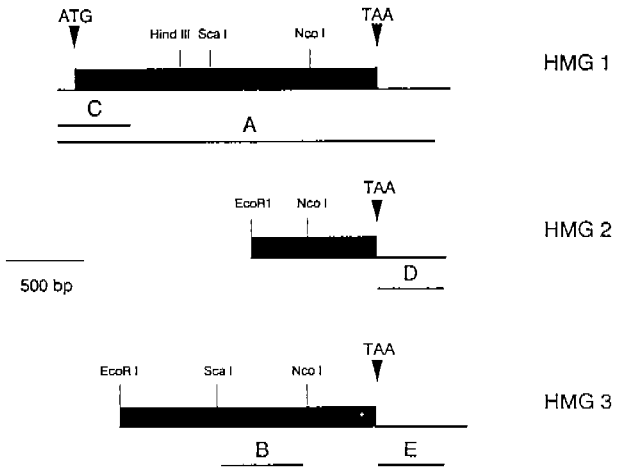

Fig. 2. Gene-specific and conserved region probes used in this study. Regions used for DNA probes in the RNA and DNA gel blots are indicated by corresponding bars with the following designations; A) probe containing entire ORF of hmg1; B) ScaI-NcoI fragment containing a region that is highly conserved among HMGR genes; C) gene-specific probe of hmg1; D) gene-specific probe of hmg2; E) gene-specific probe of hmg3

RNA Isolation and Northern Blot

Total RNAs were prepared from potato tuber and other tissues by a modification of the method of Parish and Kirby(1966) to include precipitation in 2 M LiCl. Total RNA(20 μg/lane) or polyA$^+$ RNA(1 μg/lane) was fractionated by electrophoresis through 1% agarose gels containing formaldehyde, and transfered to Nytran membranes(S & S inc.). Hybridization of cDNA probes to RNA blots routinely carried out at the same stringency using standard procedures. The amount of labeled probe hybridized to each RNA sample was estimated with a 2-D radioimaging system(Ambis system Inc.).

Determination of SGA and phytoalexins

SGA were extracted from potato tuber disks by a modification of the procedure of Allen and Kuc(1967). The amount of SGA was determined by the spectrophotometric assay and calculated based on the standard curve of a-solanine. The sesquiterpenoid phytoalexins were quantified by a semimicro method(Henfling *et al.*, 1980). Concentration of rishtin and lubimin the major sesquiterpenoid phytoalexins in potato, were determined by gas chromatography using standard rishtin and lubimin with methyl-arachidate as an internal standard.

RESULTS

Potato HMGRs are encoded by small gene family and expressed in various organs.

To determine the number of genes encoding potato HMGR, nuclear DNA was hybridized with conserved region and gene specific probes. As shown in figure 3, when nuclear DNA was digested with *Eco*RI and hybridized with conserved region probe, which comes from C-terminal conserved coding region

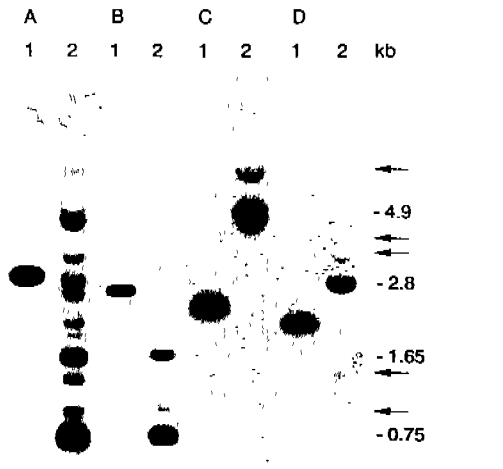

Fig. 3. Southern blot hybridization of potato nuclear DNA with potato HMGR cDNA probes. Nuclear DNA(10 ug/lane) from potato leaves were digested with SacI and HindIII and fractionated by electrophoresis in 0.7 % agarose gel. Blots were hybridized with conserved region and gene specific probes.Panel A; conserved region probe, B;hmg1, C;hmg2, D;hmg3 specific probe respectively. Lane 1 in each panel is two copy reconstruction f HMGR genes.

of hmg3, 5.3 kb, 4.8 kb, 3.0 kb, and 2.7 kb DNA fragment were detected. In contrast, when same DNA was hybridized with gene specific probe of hmg3, which comes from 3′ untranslated region, only 3.0 kb, and 2.7 kb fragments were detected. When DNA was digested with *Hind*III, conserved region probe detect 16 kb, 6.5 kb, 5.6 kb, 4.8 kb, 2.4 kb, and 2.2 kb but hmg3 specific probe can detect only 16 and 5.6 kb fragments. These results mean that the potato HMGRs are encoded by small gene family and our gene specific probe detect subset of DNA fragments which were hybridized with conserved region probe. Potato cultivars are known as allotetraploid and organization of certain gene in this plant genome is quite complicate. We also obtain nuclear DNA Southern blot results using hmg2 specific probe and replicated same DNA blot. Hmg2 specific probe detect subset of fragments which is not hybridized with hmg3 gene specific probe(data not shown).

Total RNA was isolated from uninduced plant organs including roots, tubers, etiolated sprouts, stems, leaves, flower buds, and blossoms to determine expression of HMGR mRNAs(Fig. 4).

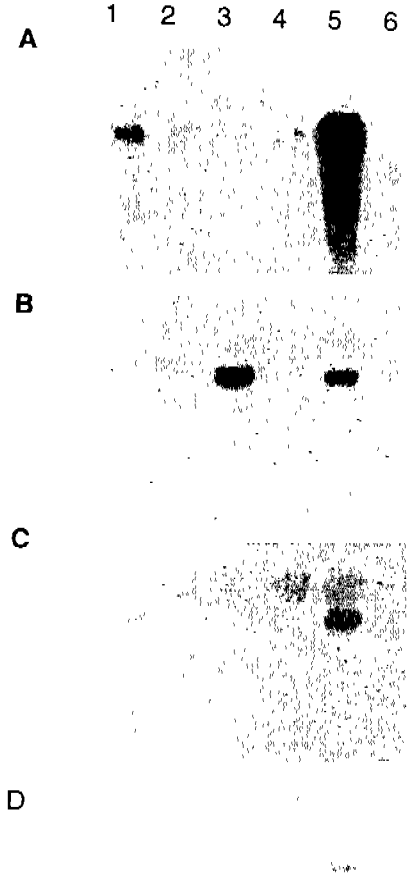

Fig. 4. Costitutive expression of HMGR genes in various organs of the potato plants. Total RNA(20 ug) from roots(lane 1), stems(lane 2), flower primordia(lane 3), petals(lane 4),anthers(lane 5), and pistils(lane 6) were hybridized with A) conserved region probe, B) hmg1 specific probe, C) hmg2 specific probe, D) hmg3 specific probe. These probes detected transcripts of approximately 2.5 kb.

Total HMGR mRNA which is hybridized with conserved region probe show extremely high level expression in blossoms. This can not be explained by adding up of hmg1, 2, and 3 mRNA expression in this organ therefore possibly another gene(s) for HMGR is present and expressed in this organ other than hmg1, 2, or 3. High level HMGR mRNA was also detected in etiolated sprouts, stems. and flower buds. Apparently lower level was expressed in roots, tubers, and leaves. Amount of HMGR mRNA expressed in each organs was correlated with the sum of hmg1,2, and 3 except in blossom and each gene is differentially expressed in different organs. Hmg1 is expressed high level in etiolated sprouts, stems, and flower buds and lower level expression was detected in roots, tuber, leaves, and blossoms. Hmg2 is expressed in roots, etiolated sprouts, flower buds, and blossoms but low level expression were detected in tubers, stems, and leaves. In contrast, hmg3 is only expressed in blossoms and in other organs the expression was below detection level with our condition.

Expression of HMGR genes following wounding and elicitor treatments

To determine the effect of elicitor(arachidonic acid) on the expression of HMGR genes in potato tuber, gene specific probes and conserved region probe were subjected to Northern blot with potato RNA extracted from AA-induced and wounded potato slices. Total HMGR mRNA, which is hybridized with conserved region probe was dramatically increased right after wounding and elicitor treatment(Fig. 5-A). The relative abundance of total HMGR mRNA was about 3 times more in elicitor treatment and this induced level stayed until 96 hour after treatment. The amount and time of transcripts increase is correlated with the increase of HMGR activity after elicitor treatment(Stermer and Bostock, 1987). In contrast to the HMGR enzyme activity is decreased 24 hours after treatment and stay background level after 48 hour, the induced mRNA stayed until 96 hour which is the last data

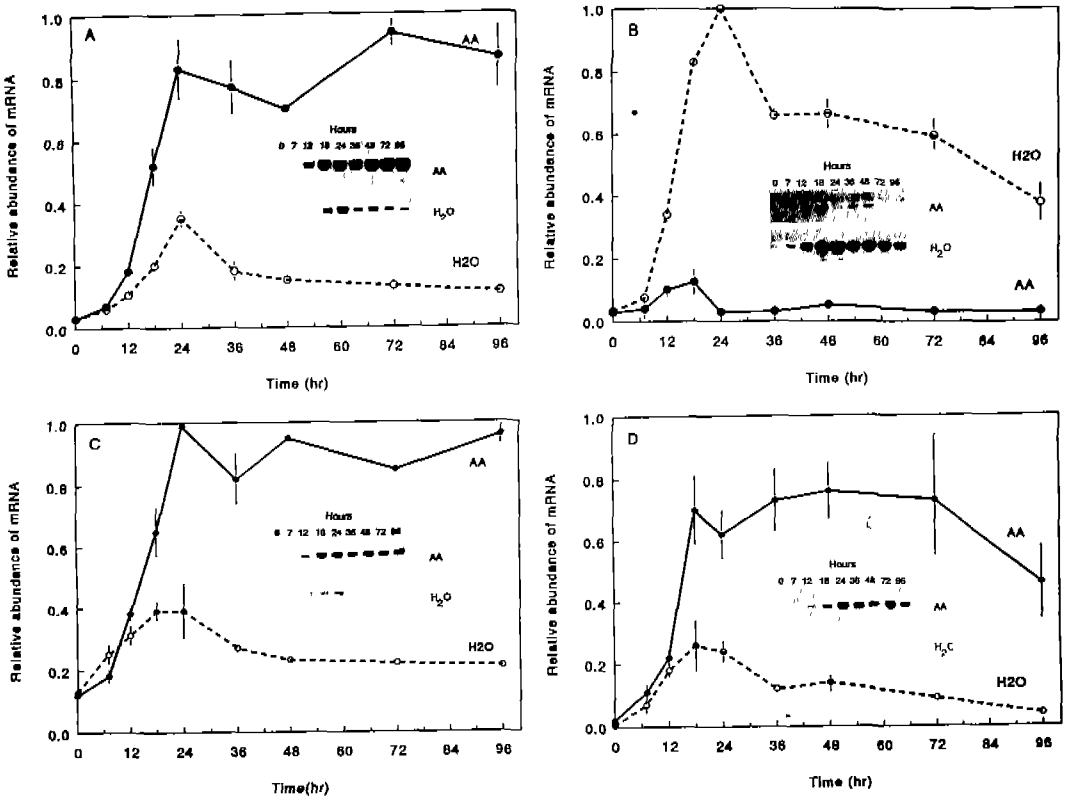

Fig. 5. Differential expression of potato HMGR genes following wounding and arachidonic acid treatment. A;expression of total HMGR mRNA which is hybridized with conserved region probe following wounding and elicitor treatment. B; expression of HMG1, C; expression of HMG2, C;expression of HMG3. RNA samples were extracted following AA application(50 ug in 50 ul H_2O per potato disk) and wound control(50 ul H_2O).

6-B) and stay at this level until 48 hours. Usually disease symptom caused by *P. infestans* is appear 48 to 72 hour after inoculation and the suppression of hmg1 mRNA during this period is surprising. Hmg2 mRNA is induced by both pathogen races and peaked at 12 hours after inoculation(Fig. 6-C). Water control stay 20% of maximum mRNA level. Hmg3 mRNA is also induced by both pathogen(Fig. 6-D) but the induction was rather rapid and earlier in incompatible pathogen than compatible pathogen inoculation. In water control, hmg3 mRNA is only expressed at about detection level with our condition(0-5% of maximum).

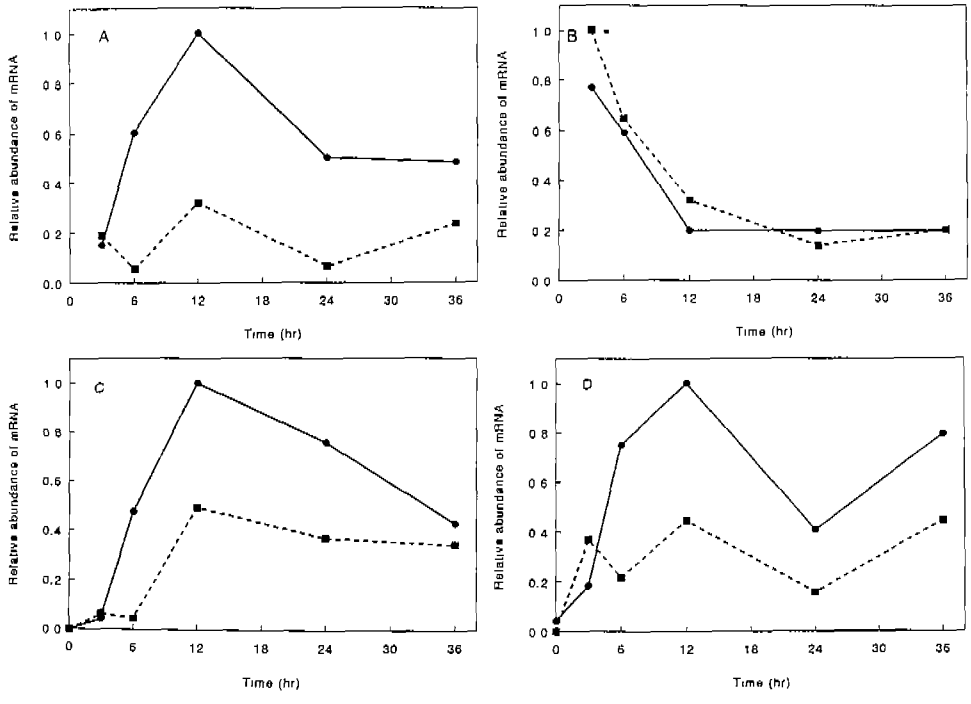

Fig. 6. Differential expression of potato HMGR genes following inoculation with Phytophthora infestans. A:, B:, C:, and D: same as fig.5. Phytophthora infestans race 0 is incompatible to the potato cultivar kennebec which we used in this experiment and race 1,2,3,4 is compatible to this potato cultivar. Pathogens were inoculated with 10^6 sporangia per disk in 50 ul water 20 hours after preparation of potato disks. As a control 50 ul water was applied.

Differential expression following MJ treatment

Because the maximum level transcripts were detected 24 hr after wounding and AA treatment, HMGR mRNAs were monitored at this time point in the experiments described here. RNA samples that were hybridized with conserved region probe yielded a single size transcript of approximately 2.5 kb that were strongly induced following treatment of potato disks with high concentration of MJ(2 or 4 μmole per disk, figure not shown). The abundance of total HMGR mRNA transcripts did not correspond with SGA accumulation(Fig. 7). Wound induced transcript levels of hmg1 were strongly enhanced by treatment of the tissues with low concentration of MJ(400 pmole per disk). The increase in hmg1 mRNA levels following MJ treatments were concentration dependent, and high concentration of MJ(2 μmole per disk or more) completely abolished hmg1 mRNA accumulation(Fig. 7). The maximum level of hmg1 mRNA accumulation was detected following treatment with 0.4 nmole MJ per disk, and enhancement was detected as low as 40 pmole per disk. The maximum level of SGA accumulation was detected when 4 nmole of MJ was applied on a potato disk and a significant increase in SGA accumulation was detected even when 0.4 pmole MJ was applied on a disk(Fig. 7).

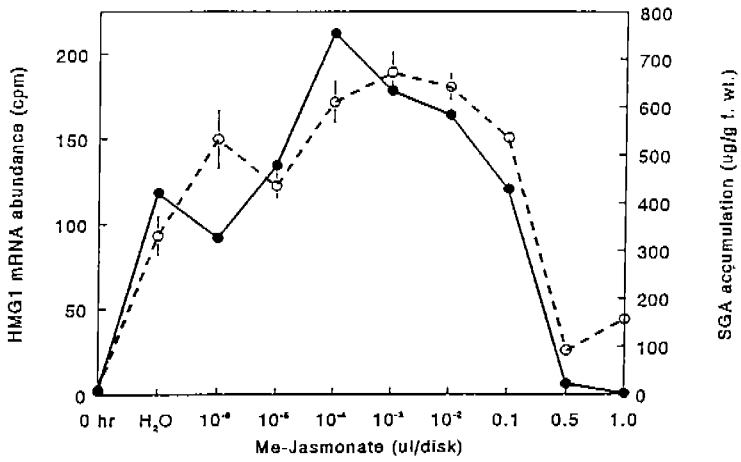

Fig. 7. Effects of different MJ concentrations on hmg1 mRNA and SGA accumulation. An RNA blot was hybridized with hmg1 specific probe and counted(solid line) with an Ambis 2-D radioisotope imaging system. Steroid-glycoalkaloid accumulation(broken line) was determined 96 hrs after treatment are indicated. MJ 0.1 ul is correspond to 0.4 umole.

The abundance of hmg1 transcripts corresponded closely with SGA accumulation. In contrast to the expression pattern for hmg1, hmg2 and hmg3 mRNA levels appears to be unrelated to SGA accumulation following treatment of the disks with MJ(data not shown). Hmg3 mRNA abundance was strongly enhanced following treatment with high concentrations of MJ which suppressed SGA accumulation.

Effects of lipoxygenase inhibitors on HMGR mRNA and SGA

Because of the effect of exogeneous MJ on hmg1 mRNA and SGA accumulation, we tested the hypothesis that endogeneous jasmonate is responsible for wound induction of hmg1 and SGA accumulation. SHAM and nPG were used to inhibit lipoxygenase activity which is required for the biosynthesis of jasmonate in plants(Vick and Zimmerman, 1984). The wound induction of total HMGR mRNA levels were not significantly affected by SHAM or nPG treatment(data not shown). However, wound induced hmg1 mRNA levels were reduced to 38% and 15% of the control levels by SHAM and nPG treatment respectively(Fig. 8).

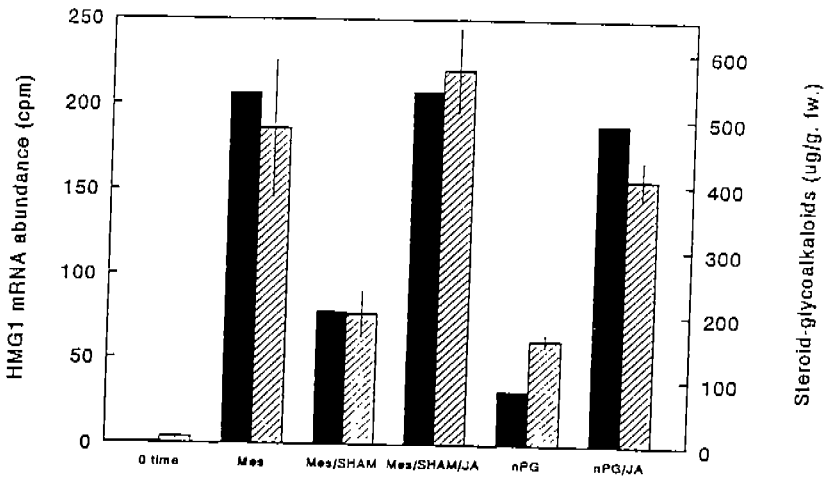

Fig. 8. Effect of lipoxygenase inhibitors, MJ, and AA on hmg1 mRNA and SGA accumulation. Hmg1 mRNA level(solid bar) and steroid-glycoalkaloid accumulation(dashed bars) are indicated. Five mMsolution(SHAM and nPG) and fifty nmole MJ was treated.

There was a corresponding reduction in the accumulation of SGA(Fig. 8). The reduction of hmg1 mRNA and SGA accumulation by these inhibitors was completely overcome by treatment of the tissue with 40 nmole MJ per disk(Fig. 8). SHAM at the levels that were effective in suppressing the hmg1 and SGA response did not inhibit the expression of hmg2, hmg3, or total HMGR mRNAs(data not shown). In contrast, and unlike earlier studies wherein SHAM and nPG partially suppressed AA phytoalexin elicitor activity, these inhibitors did not suppress the AA effect on the levels of hmg2 and hmg3 mRNAs(data not shown). These results show that levels of hmg1 mRNA are closely corelated with SGA accumulation, and that the levels of hmg2 and hmg3 mRNAs are not related to SGA accumulation in potato tuber.

Specificity of AA and MJ effects on HMGR gene expression

To further examine the specificity of the response of HMGR genes, several compounds known to induce disease resistance and to activate defense related genes in plants were tested for their effects on potato hmg1 gene expression. SA and ASA induce pathogenesis related proteins in plants and SA may function as a signal for systemic acquired resistance in some species of plants. In potato disk, both SA and ASA inhibited wound induction of hmg1 gene expression and suppressed SGA accumulation(Fig. 9). The concentration of SA and ASA used in this experiment did not affect the expression of hmg2 and hmg3(data not shown). The high concentration of MJ(2 μmole per disk) used in this set of experiments induce hmg3 gene expression but inhibited hmg1 gene expression and did not elicit SGA or sesquiterpenoid phytoalexins.

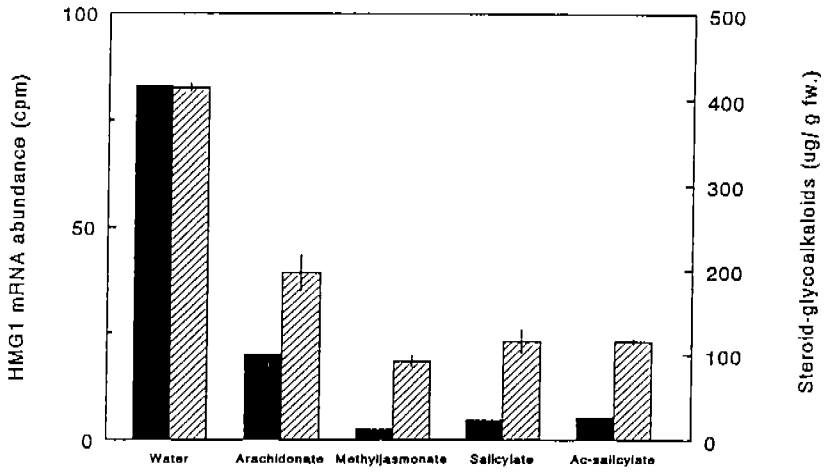

Fig. 9. Effects of chemicals which inhibit wound-inducible hmg1 mRNA levels on SGA accumulation. RNA samples were extracted 24 hrs after treatment of the disks and hybridized with hmg1 specific probe(solid bars). Accumulation of SGA(dashed bars) were analyzed 96 hrs after treatment. Values for SGA are the means and standard deviations from 3 replicated samples. Results for hmg1 mRNA are representative of at least two different experiments. AA(0.16 umole/disk), MJ(2 umole/disk), SA(5 mM solution), and ASA(5 mM solution) were tested.

DISCUSSION

In this paper we demonstrated cloning of three genes for potato HMGR and expression of these genes following wounding, elicitor treatment, and pathogen inoculation. At least three genes are expressed in potato tuber, one is wound inducible(HMG1) but the wound induction of this gene expression is suppressed by elicitor or pathogen treatment and the others(hmg2, and 3) are elicitor and pathogen inducible which are also slightly induced by wounding. This is the first report that a members of same gene family in plant is undergoing reverse regulation by same environmental signal.

Special Feature of Potato HMGR genes

Sequences of potato HMGR genes have high level homology both in amino acids and nucleotides with other cloned plant HMGR genes(Learned and Fink, 1989; Narita and Gruissem 1989; Chye *et. al.*, 1991). Potato HMGR cDNA show highly conserved amino acid sequences in catalytic domain. Furthermore, each potato HMGR gene has higher homology with corresponding tomato HMGR genes(Narita and Gruissem personal communication) than homology among potato HMGR genes. Extremely high nucleotide homology was also extended to 3'-untranslated region of each pairs of gene in potato and tomato which is much higher than homology within same plant. It is not known that why these genes from different plants have more homology even in 3'-untranslated region is not clear but we assume that each pair of corresponding HMGR genes in potato and tomato may have specialized role in plant isoprenoids biosynthesis. It is known that yeast and mammalian HMGR have 7 membrane spanning domain in amino terminal region of HMGR protein(Gil *et al.*, 1985) which is involved in sterol mediated degradation of HMGR protein(Goldstein and Brown, 1990). In plant, only 2 membrane spanning domains are known in *Arabidopsis*(Learned and Fink, 1989) and tomato(Narita and Gruissem, 1989, unpublished results). Potato hmg1 has identical pattern of hydropathy with other known plant HMGR protein but the role of this domain in protein degradation or posttranslational regulation is unknown. N-terminal amino acid sequences of plant HMGR is highly conserved among plant(MDxRRRPxKP) but the role of this conserved amino acids is not clear.

The Role of HMGR genes in Plant Isoprenoid Biosynthesis

HMGR is known as the rate-limiting step enzyme in sterol biosynthesis in mammalian system but the essential role of HMGR in plant isoprenoid biosynthesis is still controversial(Bach, T.J., 1987). Recent studies in

tobacco cell suspention culture(Chappell *et al.*, 1991) and potato tuber(Stermer and Bostock, 1987) show that the elicitor inducible HMGR activity is required for the accumulation of sesquiterpenoid phytoalexins in Solanaceous plants. Biochemical studies of isoprenoid metabolism also show that sesquiterpene cyclase, a branching point enzyme of sesquiterpene biosynthesis from farnesyl-PP is induced by either elicitor treatment or pathogen inoculation(Vogeli *et al.*, 1988; Zook and Kuc, 1991). In contrast, Squalene synthetase a branching point enzyme from farnesyl-PP to sterols was dramatically suppressed by elicitor or pathogen(Vogeli *et al.*, 1990; Zook and Kuc, 1991). These data are closely correlated with the accumulation of end-products following wounding and elicitor treatment(Kuc and Lisker, 1978; Threlfall and Whitehead, 1988). As a overall feature, accumulation of sterols by wounding is rapidly shot down and carbon flow is redirected toward sesquiterpene biosynthesis by elicitor treatment or pathogen inoculation. Our results, suppression of wound inducible hmg1 mRNA and induction of hmg2 and 3 mRNA after elicitor treatment, have close correlationship with the branching step regulation of sterols and sesquiterpenes biosynthesis. Coordinate regulation of hmg1 mRNA expression and SS activity by wounding and elicitor treatment suggest us hmg1 may have role in sterol biosynthesis. Hmg2 and 3 mRNA is also coordinately regulated with SC activity which lead to accumulation of sesquiterpene phytoalexins. These results may imply that there may be organizational channels for biosynthesis of certain species of isoprenoids and those enzymes which involve in same channels are coordinately regulated in plant.

Lipid-derived signals discriminate wound- and elicitor-responsive pathways in potato.

Jasmonic acid and MJ are potent inducers of proteinase inhibitors in potato and tomato(Farmer and Ryan, 1990, 1992). Because proteinase

inhibitors are also induced by several other types of compounds, such as oligogalacturonides, peptide systemin, and the fungal elicitor chitosan, a model was suggested that jasmonic acid is an intermediate involved in the activation of defense responses to insects and pathogens(Farmer and Ryan, 1992). The notion that jasmonate is involved in plant defense against pathogen attack is also extended by Gundlach *et al.*(1992), who demonstrated that MJ and jasmonic acid accumulate in plant cell suspension cultures following treatment with a fungal elicitor. Furthermore, MJ alone functioned as an elicitor and induced phenylalanine ammonia lyase and phenolic alkaloid accumulation. At present, however, there is no evidence that either proteinase inhibitors or alkaloid accumulation are defense mechanism against pathogens. It is unlikely that proteinase inhibitors contribute significantly to disease resistance since wounding, a treatment that induces high levels of the inhibitors in potato and tomato does not induce resistance. The proposed role of jasmonate as an inducer of disease resistance is premature, at best, and an oversimplification. For the potato responses examined in the present study, MJ affected only wound-inducible hmg1 gene expression and SGA accumulation. No phytoalexin accumulation or induced resistance to *P. infestans* was detected after treatment of the disks with any of the MJ concentrations tested. An earlier study(Doherty *et. al.* 1988) demonstrated that the ASA, SA and related hydroxybenzoic acids inhibit wound responses, including induction of proteinase inhibitors in tomato plants. Subsequently, it was reported that SA is induced systemically following inoculation of tobacco and cucumber plants with various necrotic pathogens, and induces systemic acquired resistance, perhaps, inpart, its elicitation of pathogenesis-related proteins(Ward *et al.*, 1991; Yalpani *et al.* 1991). Wounding does not induce most of the pathogenesis-related proteins in tobacco and does not induce systemic resistance. In the present study, SA and ASA inhibited wound inducible hmg1 gene expression and SGA

accumulation. Hence, there are similarities in the effects of the hydroxybenzoates on tobacco and potato with the effects of AA on potato; specially, SA and its analogs suppress certain wound responses. However, unlike AA, SA does not induce phytoalexin accumulation or resistance to *P. infestans* in potato. This result indicate that the signal transduction pathways connecting AA or SA treatment and the plant response are different. In conclusion, the results of the study show that two stress-responsive isoprenoid pathways are regulated by different lipid-derived signals present during the plant-pathogen interaction. Thus far, these studies are consistent with the hypothesis that MJ is an endogenous signal that participate in the regulation of wound-healing programs. These experiments clearly demonstrate that the consequences of wounding and elicitor treatment are different at the level of gene expression and associated secondary metabolism. Collectively, these results and those of other studies reveal that plants discriminate wounding and pathogen attack, and indicate that the responses observed during hypersensitivity expression are not simply an enhancement of wound-responses.

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