한국식물병리학회지 12(3) : 285-290(1996)
Korean J. Plant Pathol. 12(3) : 285-290(1996)

Increase in 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase mRNA Level in Tomato by Fungal Elicitors and Mechanical Wounding

Hee Sung Park* and Yong Se Lee[1]
Department of Plant Breeding and Genetics, Catholic University of Taegu-Hyosung, Kyungbuk 713-702, Korea
[1]Department of Agronomy, Taegu University, Kyungbuk 713-702, Korea

Fungal Elicitor와 기계적 상해에 의한 토마토 HMGR mRNA의 증가

박희성* · 이용세[1]
대구효성가톨릭대학교 식물육종학과, [1]대구대학교 농학과

ATRACT : 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), as a key enzyme, regulates biosynthesis of numerous isoprenoid compounds including phytoalexins. In tomato, host defense responses are associated with accumulation of sesquiterpenoid phytoalexins. Tomato HMGR DNA (HMG2) cross-hybridizes to mRNA of about 2.7 kb in size which is greatly induced in tomato cells treated with elicitors isolated from *Verticillium albo-atrum* or *Fusarium oxysporum* in a dose-response manner. Wounding to tissues such as stems, leaves, or roots induced HMGR mRNA synthesis in a biphasic mode. In both of experiments, HMGR mRNA levels are transient with maximum levels 9 to 12 hr after treatment.

Key words : **HMG2, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), mRNA expression, fungal elicitors, wounding.**

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) catalyzes the conversion of HMG-CoA to mevalonic acid, a key step in the biosynthesis of isopentenyl pyrophosphate as a building block of isoprenoid compounds. In animals, HMGR mediates the production of a discrete number of isoprenoids: sterols, ubiquinone, dolichol, isopentenylated adenosine, and isoprenoid moieties involved in post-translation modification of a number of cellular proteins (15, 27, 30). In contrast, plants synthesize a significantly greater array of unique isoprenoid compounds including phytosterols, plant growth regulators, phytoalexins, carotenoid pigments, components of chlorophyll and plastoquinone, rubber, and a variety of specialized isoprenoids associated with insect attraction, fragrance, flavor, feeding deterrents, and allelopathy. HMGR is also considered to be a major control point in plant isoprenoid biosynthesis and thus has been studied in a number of plant species (2, 3, 7). Consistent with the increased complexity of the isoprenoid pathway in plants, multiple isozymes of HMGR appear to differ in both kinetic properties and regulation by light, growth regulators, or pathway end-products (4, 35). Increases in HMGR enzyme activity in response to pathogens, wounding, or elicitors are associated with accumulation of diterpene (13) or sesquiterpene phytoalexins (18, 31, 32) suggesting its active participation to host defense-related system. Rishitin, the major sesquiterpene phytoalexin in tomato, is elevated in response of inoculation with the wilt-inducing fungi, *Verticillium albo-atrum* and *Fusarium oxysporum*, and the leaf spot pathogen, *Cladosporium fulvum* (11, 23, 34). To initiate the molecular study of HMGR in relation to defense responses in tomato, a cloned gene (HMG2) encoding tomato HMGR (26) has been used as a probe to monitor its mRNA synthesis either in tomato suspension cell cultures during challenges with elicitors isolated from tomato pathogenic fungal cell wall or in tomato plant tissues subjected to mechanical wounding The results from northern gel and slot blot hy-

*Corresponding author.

bridization are presented in this report.

MATERIALS AND METHODS

Plant and fungal materials. Tomato (*Lycopersicon esculentum* cv. EP7) plants were grown under greenhouse conditions. Tomato suspension cultures were maintained in the dark in a modified MS medium (16). *Verticillium albo-atrum* (race 1) and *Fusarium oxysporum* (race 1), both in compatible relations with tomato plants were maintained on 2.4% potato dextrose (PD) agar and grown in 2.4% PD liquid medium for cell wall isolation. To obtain fungal elicitor, the method by Ayers *et al.* (1) was modified. Mycelia grown in PD liquid media were collected by suction filtration through sintered-glass funnel and washed with distilled water, 100 mM potassium phosphate, pH 7.2 and then 500 mM potassium phosphate pH 7.2. The washed mycelia were homogenized in a Waring Blender (50 g of the mycelia in 200 ml of the 500 mM buffer), filtered through Whatman filter paper supported on a sintered-glass funnel and washed with the same buffer four times. The remaining material was autoclaved (121°C for 15 min) and the heat-released material was passed through Whatman filter paper. The filterable was dialyzed against distilled water for 48 hours and its glucose equivalents were determined by anthrone assay (14).

DNA probe. Total RNA (30 μg) isolated from tomato cells harvested 9 hr after treatment with *V. albo-atrum* elicitor (50 g glucose equivalents/ml) was used for the first strand cDNA synthesis utilizing oligo(dT)-18mer and AMV reverse transcriptase according to Promega manual. Based on sequence of tomato HMG2 genomic DNA (26), primer1 (CGCAAGCTTGGTGATGCAATGGGAATGAACATGGT) containing 26 bases of coding strand plus a 5' *Hin*dIII site and primer 2 (TGAGATGCAAGCTGAGTTCCACCTCC) complementary to sequence beginning 202 bases upstream from the stop codon were designed. Polymerase chain reaction (PCR) was performed for 35 cycles (95°C, 1 min; 55°C, 2 min; 72°C, 3 min) followed by post reaction for 5 min. A 0.49 kb DNA fragment from PCR was identified on agarose gel electrophoresis and its *Hin*dIII digested product was inserted into pBluescript SK- (pCD1). The insert of pCD1 was confirmed to represent the carboxy terminal region of HMG2 cDNA by partial nucleotide sequencing (the data not shown). Tomato HMG2 inserted in pSK- (pTH295; see ref. 26) was digested with *Ava*I and *Eco*RI to produce 0.7 kb fragment which may represent divergent region among HMGR gene families (21, 26) and, therefore, specific for tomato HMG2.

Northern Hybridization. Total RNA was isolated from 1 to 3 g fresh weight of tissues or cells from suspension cultures which was ground in the presence of liquid nitrogen and homogenized directly in 0.1 M Tris (pH 9.0) saturated phenol as described previously (17). For northern analyses, total RNA (5 to 20 μg/lane) was denatured by treatment with glyoxal prior to electrophoresis in 1.2% agarose for gel analyses or application directly to nytran filters utilizing a slot blotting apparatus. pCD1 HMGR probe DNA was [α-^{32}P] dCTP-labeled by the random priming method (Prime-a-Gene labelling system, Promega). Membranes were prehybridized overnight in a solution containing 50% formamide, 6× SSC, 5× Denhardt's solution, 5 mM EDTA, 0.1% SDS, 100 μg/ml denatured salmon sperm DNA and then the labeled probe DNA was added for hybridization for 24 hr at 42°C. For monitoring sequence specific hybridization only for the tomato HMG2 gene, the 0.7 kb *Ava*I/*Eco*RI fragment encoding 5-untranslated regions and the 5' end of the gene was utilized. The hybridization results were obtained by autoradiography (29).

RESULTS

HMGR mRNA synthesis by fungal elicitors. Suspension cells of tomato cultivar EP7 was maintained in growth regulator-supplemented MS liquid media in the dark condition. Suspension cells at different growth phase (from early log to stationary stage, Fig. 1.A.) were treated with various concentrations of elicitors (0~100 μg glucose equivalents/ml) isolated from cell wall fractions of *Verticillium albo-atrum* or *Fusarium oxysporum*. Total RNA isolated after elicitor treatment for a certain period of time, was used for RNA blot hybridization analyses using the 0.49 kb fragment of pCD1 as a probe for conserved HMGR cDNA region. A transcript of about 2.7 kb was induced by treatment of the elicitors for 9 hrs in a dose-dependent manner (lanes 3~5 and 6~8 in Fig. 1.B.). Elicitor from *V. albo-atrum* was a more potent inducer of HMGR mRNA synthesis than *F. oxysporum* elicitor (compare lane 3 to 6 or 4 to 7 or 5 to 8). In contrast, no emergence of HMGR mRNA was observed from the control experiments (lane 1 and lane 2 for 0 hr and 9 hr

mock treatment, respectively) at all.

The competency of tomato cells to respond to elicitors was highly dependent on their growth phase; elicitor induction was most effective in cells during the early-log phase (Fig. 1.C.(a)) implying more demands of HMGR in cells in active division or possibly young

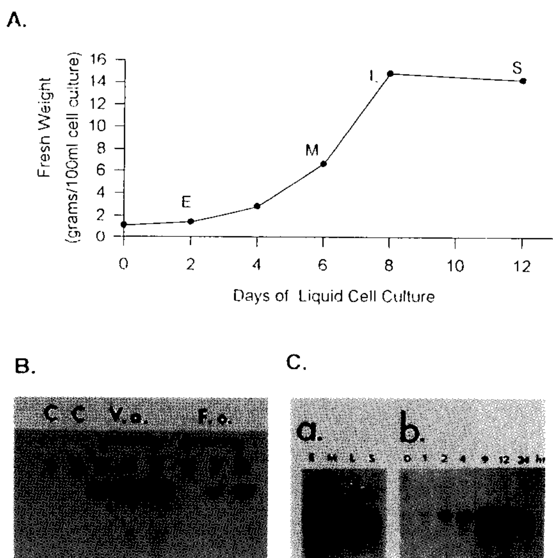

Fig. 1. Induction of HMGR mRNA levels in fungal elicitor-treated tomato cells. Northern hybridization analysis were performed on total RNA isolated from suspension-cultured cells of tomato cultivar EP7 following treatment with water (control) or fungal elicitors. The 0.49-kb fragment from pCD1 containing highly conserved region was used as probe. A. Growth curve of tomato cells in suspension culture. E, M, L and S are denoting early log-, middle log-, late log- and stationary-phase, respectively. B. Elicitor dose response: lane 1, 0 hr control; lane 2, 9 hr mock; elicitor-treated cells in early log stage for 9 hours after addition of 20 (lane 3), 50 (lane 4) or 100 (lane 5) μg of glucose equivalents/ml elicitor from *V. albo-atrum* (V.a.) and of 20 (lane 6), 50 (lane 7) or 100 (lane 8) from *F. oxysporum* (F.o.). C. Growth phase-dependence of HMGR mRNA induction by elicitor treatment. (a) Cells at various stages of growth (E, M, L, S; see A.) were treated with *V. albo-atrum* elicitor (50 μg glucose equivalents/ml) for 9 hr. Total RNA (15 μg) from each growth phase was assayed by northern blot hybridization. (b) Time course of elicitor induction. Total RNA (15 μg/lane) was isolated from early exponentially growing tomato cell treated with *V. albo-atrum* elicitor (50 μg glucose equivalents/ml) at 0 hr and harvested at the times as indicated.

tomato plants than in cells of old ages (note s of Fig. 1. C.(a)). A more detailed time course of elicitor-induced responses indicates that HMG2 mRNA induction is transient with maximal mRNA levels at 9 to 12 hr (Fig. 1.C(b)). The kinetics of HMG2 mRNA induction is similar for both *F. oxysporum* and *V. albo-atrum* elicitors but is distinct from that of another defense-related gene, phenylalanine ammonia-lyase (12) which shows maximal mRNA levels at 3 to 4 hr (data not shown).

Biphasic mode of HMGR mRNA expression by mechanical wounding. HMGR enzyme activity is elevated in potato tubers or sweet potato following wounding (36). To test if wounding triggers an increase in HMGR mRNA levels in tomato as a presumptive mechanism of host defense, total RNA isolated from

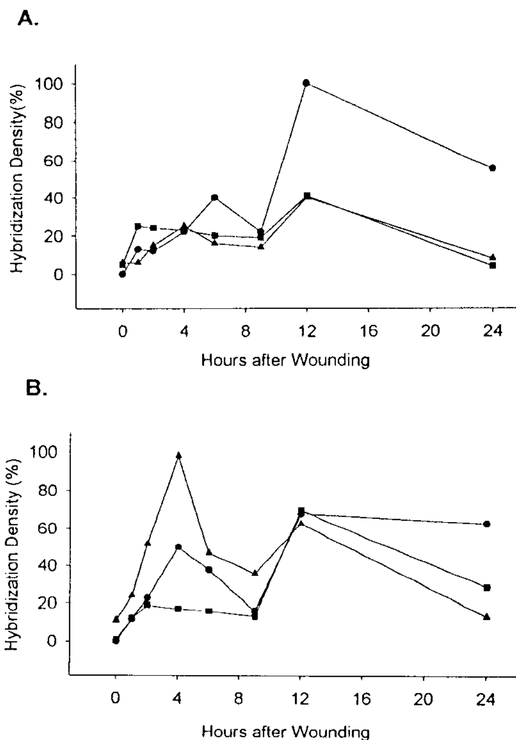

Fig. 2. HMGR mRNA levels in wounded tissue. Total RNAs isolated from wounded tissues (stems, leaves, roots), were analyzed by northern slot blot hybridization using 0.49 kb (A) or 0.7 kb (B). DNA fragment of HMG2 (see DNA probe in Materials and Methods) as DNA probes. Hybridization density was numerically converted by densitometer and the highest point was expressed as 100%. Curved lines with symbols ■, ●, and ▲ represent roots, stems and leaves, respectively.

wounded roots, stems, or leaves of tomato was blotted on the filter membrane using dot blotter under vacuum and fixed by UV-crosslinking. The degree of cross-hybridization after northern slot blot experiments was numerically measured in densitometer and graphically represented as seen in Fig. 2. Technically in our wounding experiments for HMGR mRNA induction, severe chopping with a razor blade was much more effective than cutting the samples into tiny pieces (2 to 5 mm in diameter). The level of HMGR mRNA expression was elevated in all tissues in response to mechanical wounding. In all tissues, HMGR mRNA synthesis was transiently increased in biphasic mode, with first peak at 3~4 hr followed with maximal level at around 12 hr as a second peak. Wound-induced HMGR mRNA cross-hybridized to the pCD1 conserved probe for the HMG2 gene (Fig. 2.A.) as well as to the 0.7 kb *AvaI*/*Eco*RI fragment as a gene-specific probe (see Fig. 2.B.) This result may suggest that HMG2 gene expression is actively related to host defense-response under mechanical damage.

DISCUSSION

Gene encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase has been identified as a gene family (7, 26). This is not surprising based on protein level studies which indicate multiple isozymes and distinct subcellular localizations (25, 28, 32). However, initial reports on cloned plant HMGR sequences from *A. thaliana* and tomato concluded that the reductase was probably encoded by a single gene (21, 24). Caelles *et al.* (5) independently cloned an *A. thaliana* HMGR cDNA and used this sequence to identify genomic sequences revealing two reductase genes, HMG1 and HMG2, in *A. thaliana*. These two genes, however, did not cross-hybridize with each other on genomic Southern blot experiment of *A. thaliana* DNA. Thus it appears that distinct HMGR isogenes have diverged significantly. It will be of interest to determine whether this divergence has been associated with regulatory and functional specialization of specific isogenes and isogene products. In responses to pathogenic infection, plants synthesize phytoalexins and defense-related proteins. This defense response is also observed in plant cell suspension cultures challenged with fungal elicitors. Increases in HMGR activity leading to terpenoid phytoalexin accumulation have been associated with expression of disease resistance and wound responses in a number of plants including tomato and potato (25, 32, 33, 34). Fungal elicitors have been shown to induce HMGR enzyme activity and sesquiterpene phytoalexin accumulation in suspension-cultured cells of tobacco (6). We, therefore, tested if wounding or fungal elicitor derived from tomato pathogenic fungi induces mRNA levels of HMG2 gene (26). Elicitor-treated tomato cells gave the most dramatic increase in mRNA levels. This increase was transient with maximal level occurring about 9 hr after elicitor treatment. The kinetics of induction are similar to other defense-related genes (8, 9, 10, 19) but were slower than the induction pattern for PAL mRNA (maximum at 4 hr), another defense-related gene product, in the same cells. While, tomato cell suspension cultures respond to fungal elicitors by increasing the level of HMG2 mRNA, further experiments are required to correlate the induction of HMG2 mRNA levels with host response associated with expression of disease resistance in intact plants. HMGR mRNA levels were also elevated in response to wounding in leaves, roots, and stems. Both the elicitor- and wound-induced transcripts cross-hybridized strongly to the gene-specific region of HMG2 indicating that this isogene is involved in these stress responses. Interestingly, wounded tomato tissues did not display the very rapid wound response (mRNA maximum at 30 min after wounding) seen in potato tubers using the same probe (36). The 0.7 kb *AvaI*/*Eco*RI fragment also serves as a gene-specific probe in potato and these studies indicated that the HMG2 mRNA rapidly induced by wounding is distinct from the potato gene analogous to HMG2 in tomato. However, the HMG2-like gene is induced in potato tubers inoculated by the soft rot bacterium, *Erwinia carotovora* subsp. *carotovora* (36). Further studies are required to determine if the rapid wound response is limited to potato or is a tuber-specific response. Phytoalexin accumulation is one of a number of host responses induced during defense responses against pathogens, wounding, or elicitor (8, 12, 19). Increases in stress-related compounds or enzymes involve *de novo* protein synthesis resulting ultimately from activation of the respective defense-related genes (19, 20). So, it is likely that elicitor-and wound-induced increases in HMGR mRNA are due to activation at the gene level. Several of the defense-related genes that have been characterized are encoded by gene families, specific members of which are differentially induced in response to environmental and developmental cues (7, 9, 22). For ex-

ample, PAL from bean is encoded as a gene family, one of which is involved primarily in developmental expression of anthocyanin pigments, another in responses to pathogens associated with phytoalexin production. Our data suggest that an analogous situation exists in the HMGR gene family. Utilizing DNA probes specific for sequences of the HMG2 gene, we determined that wound- and elicitor-induced transcripts were derived from this gene suggesting its difference from HMG1 gene which was expressed in high mRNA level in young fruit (7, 24). This expression is presumably associated with sterol synthesis during this rapid period of growth and not with carotenogenesis which occurs later during ripening.

요 약

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR)는 phytoalexin을 포함하는 수많은 isoprenoid 화합물의 생합성을 조절하는 효소이다. 토마토의 경우 sesquiterpenoid phytoalexin류가 식물방어를 위한 반응산물로서 축적되는 것이 알려져 있다. *Verticillium albo-atrum*이나 *Fusarium oxysporum*으로부터 추출한 elicitor를 토마토의 배양세포에 처리하는 경우 처리량의 증가에 따른 2.7 kb 크기의 HMGR mRNA의 상당한 유도증가가 토마토의 HMG2 DNA를 이용한 northern hybridization에 의해 관찰되었다. 토마토의 잎, 뿌리, 줄기 등에 기계적 상해를 가하는 경우에서도 HMGR mRNA는 2단계를 결쳐 증가함이 관찰되었다. HMGR mRNA는 양 실험의 경우 모두 9시간에서 12시간 사이에서 최대 발현됨이 관찰되었다.

ACKNOWLEDGEMENT

This work was supported by a fund from Catholic University of Taequ-Hyosung, 1996.

REFERENCES

1. Ayers, A. R., Ebel, J., Valent, B. and Albersheim, P. 1976. Host-pathogen interactions X. Fractionation and biological activity of an elicitor isolated from the mycelial walls of *Phytophthora megasperma* var. *sojae*. *Plant Physiol.* 57 : 760-765.
2. Bach, T. J. and Lichtenthaler, H. K. 1983. Inhibition of mevinolin of plant growth, sterol formation and pigment accumulation. *Physiol. Plant* 59 : 50-60.
3. Bach, T. J. 1987. Synthesis and metabolism of mevalonic acid in plants. *Plant Physiol. Biochem.* 25 : 263-278.
4. Brooker, J. D. and Russell, D.W. 1979. Regulation of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase from pea seedlings: Rapid post-translational phytochrome-mediated decrease in activity and *in vivo* regulation by isoprenoid products. *Arch. Biochem. Biophys.* 198 : 323-334.
5. Caelles, C., Ferrer, A., Balcells, L., Hegardt, F. G. and Boronat, A. 1989. Isolation and structural characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Plant Mol. Biol.* 13 : 627-638.
6. Chappell, J. and Nable, R. 1987. Induction of sesquiterpenoid biosynthesis in tobacco cell suspension cultures by fungal elicitor. *Plant Physiol.* 85 : 469-473.
7. Choi, D., Ward, B. L. and Bostock, R. M. 1992. Differential induction and suppression of potato 3-hydroxy-3-methylglutaryl coenzyme A reductase genes in response to *Phytophthora infestans* and to its elicitor arachidonic acid. *Plant Cell* 4 : 1333-1344.
8. Collinge, D. B. and Slusarenko, A. J. 1987. Plant gene expression in response to pathogens. *Plant Mol. Biol.* 9 : 389-410.
9. Corbin, D. R., Sauer, N. and Lamb, C. J. 1987. Differential regulation of a hydroxyproline-rich glycoprotein gene family in wounded and infected plants. *Mol. Cell. Biol.* 7 : 4337-4344.
10. Cramer, C. L. and Radin, D. N. 1990. Molecular biology of plants. In : *Biotechnology of Plant-Microbe Interactions*, ed. by J. P. Nakas and C. Hagedorn, pp. 1-49. McGaw-Hill, New York.
11. deWit, P. J. G. M. and Flach, W. 1979. Differential accumulation of phytoalexins in tomato leaves but not in fruits after inoculation with virulent and avirulent races of Cladosporium fulvum. *Physiol. Plant. Path.* 15 : 257-267.
12. Dixon, R. A., Bailey, J. A., Bell, J. N., Bolwell, G. P., Cramer, C. L., Edwards, K., Hamdan, M. A. M. S., Lamb, C. J., Robbins, M. P., Ryder, T. B. and Schuch, W. 1986. Rapid changes in gene expression in response to microbial elicitation. *Phil. Trans. R. Soc. Lond.* B 314 : 411-426.
13. Dueber, M. T., Adolf, W. and West, C. A. 1978. Biosynthesis of the diterpene phytoalexin casbene. *Plant Physiol.* 62 : 598-603.
14. Dische, Z. 1962. General color reactions. *Meth. Carbohydrate Chem.* Vol. 1, pp. 478-512.
15. Farnsworth, C. C., Gelb, M. H. and Glomset, J. A. 1990. Identification of geranylgeranyl-modified protein in HeLa cells. *Science* 247 : 320-322.
16. Fosket, D. E. and Radin, D. N. 1983. Induction of

carotenogenesis in cultured cells of *Lycopersicon esculentum. Plant Sci. Lett.* 30 : 165-175.

17. Haffner, M. H., Chin, M. B. and Lane, B. G. 1978. Wheat embryo ribonucleates. XII. Formal characterization of terminal and penultimate nucleoside residues at the 5'-ends of capped RNA from imbibing wheat embryos. *Can. J. Biochem.* 56 : 7229-7233.
18. Kuc, J. and Rush, R. J. 1985. Phytoalexins. *Arch. Biochem. Biophys.* 236 : 455-472.
19. Lamb, C. J., Lawton, M. A., Dron, M. and Dixon, R. A. 1989. Signals and transduction mechanisms for activation of plant defenses against microbial attack. *Cell* 56 : 215-224.
20. Lawton, M. A. and Lamb, C. J. 1987. Transcriptional activation of plant defense genes by fungal elicitor, wounding and infection. *Mol. Cell. Biol.* 7 : 335-341.
21. Learned, R. M. and Fink, G. R. 1989. 3-Hydroxy-3-methylglutaryl coenzyme A reductase from Arabidopsis thaliana is structurally distinct from the yeast and animal enzymes. *Proc. Natl. Acad. Sci. USA.* 86 : 2779-2783.
22. Liang, X., Dron, M., Cramer, C. L., Dixon, R. A. and Lamb, C. J. 1989. Differential regulation of phenylalanine ammonia-lyase genes during plant development and by environmental cues. *J. Biol. Chem.* 264 : 14486-14492.
23. McCance, D. J. and Drysdale, R. B. 1975. Production of tomatine and rishitin in tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici. Physiol. Plant Pathol.* 7 : 221-230.
24. Narita, J. and Gruissem, W. 1989. Tomato hydroxymethylglutaryl-CoA reductase is required early in fruit development but not during ripening. *Plant Cell* 1 : 181-190.
25. Oba, K., Kondo, K., Doke, N. and Uritani, I. 1985. Induction of 3-hydroxy-3-methylglutaryl CoA reductase in potato tubers after slicing, fungal infection or chemical treatment, and some properties of the enzyme. *Plant Cell Physiol.* 26 : 873-880.
26. Park, H. Denbow, C. J. and Cramer, C. J. 1992. Structure and nucleotide sequence of HMG2 encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Plant Mol. Biol.* 20 : 327-331
27. Rilling, H. C., Breunger, E., Epstein, W. W. and Crain, P. F. 1990. Prenylated proteins: The structure of isoprenoid group. *Science* 247 : 318-320.
28. Russell, D. W. 1985. 3-Hydroxy-3-methylglutaryl-CoA reductases from pea seedlings. *Meth. Enzymol.* Vol. 110, pp. 26-40.
29. Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, New York.
30. Schindler, S., Bach, T. J. and Lichtenthaler, H. K. 1984. Differential inhibition by mevinolin of prenylipid accumulation in radish seedlings. *Z. Naturforsch.* 40 : 208-214.
31. Shih, M. and Ku'c, J. 1973. Incorporation of ^{14}C from acetate and mevalonate into rishitin and steroid glycoalkaloids by potato tuber slices inoculated with *Phytophthora infestans. Phytopathology* 63 : 826-829.
32. Stermer, B. A. and Bostock, R. M. 1987. Involvement of 3-hydroxy-3-methyl- glutaryl coenzyme A reductase in the regulation of sesquiterpenoid phytoalexin synthesis in potato. *Plant Physiol.* 84 : 404-408.
33. Templeton, M. D. and Lamb, C. J. 1988. Elicitors and defence gene activation. *Plant Cell Environ.* 11 : 395-401.
34. Tjamos, E. C. and I. M. Smith. 1974. The role of phytoalexins in the resistance of tomato to *Verticillium* wilt. *Physiol. Plant Path.* 4 : 24-25.
35. Wong, R. J., McCormack, D. K. and Russell, D. W. 1982. Plastid 3-hydroxy-3-methylglutary coenzyme A reductase has distinctive kinetic and regulatory features: Properties of the enzyme and positive phytochrome control of activity in pea seedlings. *Arch. Biochem. Biophys.* 216 : 631-638.
36. Yang, Z., Park, H., Lacy, G. H. and Cramer, C. L. 1991. Differential activation of potato 3-hydroxy-3-methylglutary coenzyme A reductase genes by wounding the pathogen challenge. *Plant Cell* 3 : 397-405.