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Differential expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase of *Andrographis paniculata* in andrographolide accumulation

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ABSTRACT

The methyl-D-erythritol 4-phosphate pathway (MEP) and Mevalonate Pathway (MVP) is responsible for the biosynthesis of a substantial number of natural compounds of biological and biotechnological importance. In recent years, this pathway has become an obvious target to develop new herbicides, pharmaceuticals and antimicrobial drugs. The numerous secondary metabolites are produced through this pathway which involved in defense response against biological and non-biological agents. Some of them have broad economical functions like improving the interaction between the plant and environment. Andrographolide is one of the principal secondary metabolite of A. paniculata. It has multiple health promoting effects including anti HIV property. In this study we have exogenously applied Jasmonic acid (JA) and Gibberelic acid (GA_3) as an elicitor, in variable concentration, to access the effect on andrographolide accumulation and chlorophyll content of A. paniculata. The result indicated that the biosynthesis of andrographolide has enhanced by 31.25% and 56.1% by application of GA_3 and JA respectively. Increment in the diterpene content has shown its association with the expression level of hmgr genes in the andrographolide accumulation regulatory cascade.

Key words: *Andrographis paniculata*, andrographolide accumulation, *hmgr*, Differential expression.

INTRODUCTION

Higher plants have a common building blocks for the synthesis of several thousand isoprenoid-derived primary and secondary metabolites and now it is well established that IPP and its isomer DMAPP (Dimethylallyl diphosphate) is synthesized in the plastids through MEP/DXP (2 C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose-5-phosphate) pathway and in the cytosol

through the MVA (Mevalonic acid) pathway (Figure 1) [1, 2]. The MEP/DXP pathway is present in many eubacteria, apicomplexa parasites, and photosynthetic eukaryotes, but is absent from other eukaryotes, including humans [3]. Thus, the genes and enzymes of this pathway are attractive targets for the development of new pharmaceutical drugs, herbicides and pesticides [2, 4, 5].

The primary metabolites derived from the MEP/DXP pathway are the plant hormones GAs (Gibberelic acid) and ABA (Abscisic acid), and photosynthesis actors such as chlorophylls and carotenoids [1, 6, 7]. A number of secondary metabolites compound also produced through these pathways have nutritional or medical value. Andrographolide is one of the most important diterpene lactones, which is abundantly found in *A. paniculata* which possess great pharmaceutical value. It is an herbal drug used in siddha and ayurveda system of medicines in India and China. The biosynthesis of andrographolide in *A. paniculata* seems to accumulate by both MVA and MEP/DXP pathways [8].

The present study targets the signals that regulate the expression of 3-hydroxy-3-methylglutaryl-coenzyme A of *A. paniculata*. The influential role of JA (Jasmonic acid) and GA_3 on the MVP and MEP/DXP pathway was asses by foliar application to know the effect on gene expression, andrographolide accumulation, chlorophyll content and biomass production.

EXPERIMENTAL SECTION

Plant growth condition and treatment

A well characterized genotype of *A. paniculata* (Indira megha) of Indira Gandhi Krishi Vishwavidyalaya, Raipur, C.G. India was grown under greenhouses condition with a 15-h light/9-h dark cycle and a temperature of 26±2°C. Relative humidity was maintained at 65-75% in whole growing periods. Thirty day old plantlet of was transplanted in the plastic tray containing soil: sand: FYM with the ration of 1:1:1. There are no chemical fertilizers and plant protection measures were adopted during the investigation. Three different concentrations of GA_3 (3mM, 4mM, 5mM) and JA (0.1mM, 0.3mM, 0.5mM) were used for treatment after 40 days of transplantation. Sampling for expression analysis was done at 3, 6, 12, and 24 h after foliar application. Andrographolide and chlorophyll estimation was done just before the blooming of plants.

Pathway based gene selection

After intensive searches of literatures and databases, initially 3-hydroxy-3-methylglutaryl-coenzyme A reductase (hmgr1), a pathway regulatory enzymes was selected for estimating the gene expression and association analysis with andrographolide accumulation, chlorophyll content and biomass production. The putative sequence of these enzymes was selected from the database for designing the suitable primer pairs for detecting the expression pattern.

RNA isolation and QPCR analysis

Total RNA was extracted and quantified. A total of 3µg of total RNA was reverse transcribed for each samples. QPCR was carried out in 2 ml of the RT product, using Stratagene (M x 3000p) Real-time PCR system with Brilliant SYBR Green QPCR Master Mix (Stratagene, La Jolla, CA,

USA), according to the manufacturer's instructions. The thermal profile was: 94 °C for 3 min; 45 cycles of 94 °C for 1min, anneal 60 °C for 30 s; extension 72 °C for 30s.

Andrographolide and Chlorophyll estimation

The estimation of total andrographolide accumulation was done by HPLC and the chlorophyll content through the SPAD meter. The estimation was replicated thrice for each treatment of GA_3 and JA.

RESULTS AND DISCUSSION

GA_3 and JA (elicitor molecules) Selection

GA_3 was changes the amounts of metabolites by regulating the activity of the key enzymes like DXS and HMGR [9]. The strategy behind selection of GA_3 is that, it increases HMGR activity and expected increase the accumulation of andrographolide and chlorophyll. JA was selected because of its property of activating transcription of downstream target genes and its effect in increasing the phytoalexins, a diterpenoid.

HMGR expression under GA_3 and JA induction

The relative expression levels of the HMGR gene in leaves showed different patterns of mRNA accumulation over the course of treatments. The expression of HMGR increased gradually within 24 hour reached the highest level at 6 hours when treated with JA, 12 hours with the treatment of GA_3. The transcripts for both GA_3 and JA-treated plant had increased rapidly by the first time point measured and maintained levels well above those found in non-treated control plant (Figure 2). The 5µM concentration GA_3 induced HMGR expression at the maximum level after 12 h. In the other hand JA has also influenced the expression level of HMGR. The maximum expression of HMGR was recorded by treating 0.1mM JA at 6 hours after treatment. Treatment with JA produced greater effects than GA_3 and the timing of HMGR transcript changes also appeared to be somewhat different when comparing GA_3 treatment with JA. The study indicated that the GA_3 and JA has substantially influenced the HMGR activity and shows that GA_3 and JA has induced the HMGR activity and regulates the production of Andrographolide accumulation in *A. paniculata*.

Elicitor treatment stimulates accumulation of andrographolide and chlorophyll

After treatment, significant increase in andrographolide accumulation and chlorophyll content was recorded. The highest level of andrographolide accumulation in GA_3 treated plants was recorded at concentration of 5µM. It was 2.94% as compared to control (2.24%) and the chlorophyll content was increased by 23.15% as compared to untreated plants. The andrographolide concentration was 31.25% higher than the control plants. The accumulation was more profound in case of JA treatment. 0.1mM JA treated plants has enhanced the andrographolide by 56.25% when compared to control. The plant contains 3.5% of andrographolide, whereas in control, the content is 2.24%. The chlorophyll content was increased by 27.76% in 0.1mM JA treated plants as compared to control. When the correlation analysis was done between the chlorophyll content and andrographolide accumulation, both were found to be highly correlated and significant at 1% level (R=0.843** for JA treatment and R=0.822** GA_3 treatment). Highly significant increase in biomass content was observed after the application of JA & GA_3 (data not given). The results of expression profiling,

andrographolide accumulation and chlorophyll content revealed that the application GA_3 and JA alters the expression of the HMGR gene and it reveals that HMGR is actively participating as a key enzyme in the accumulation of andrographolide and chlorophyll content. This was the first report showing the participation and differential expression of genes involved in andrographolide biosynthetic pathway. Numerous studies in different plant systems based on terpene production using various elicitors are well reported [10, 11, 12]. Therefore, it is worthwhile studying expression patterns of genes in andrographolide biosynthesis under various treatments including GA_3 and JA, which will be helpful to uncover molecular induction mechanism for improving andrographolide biosynthesis in *A. paniculata*.

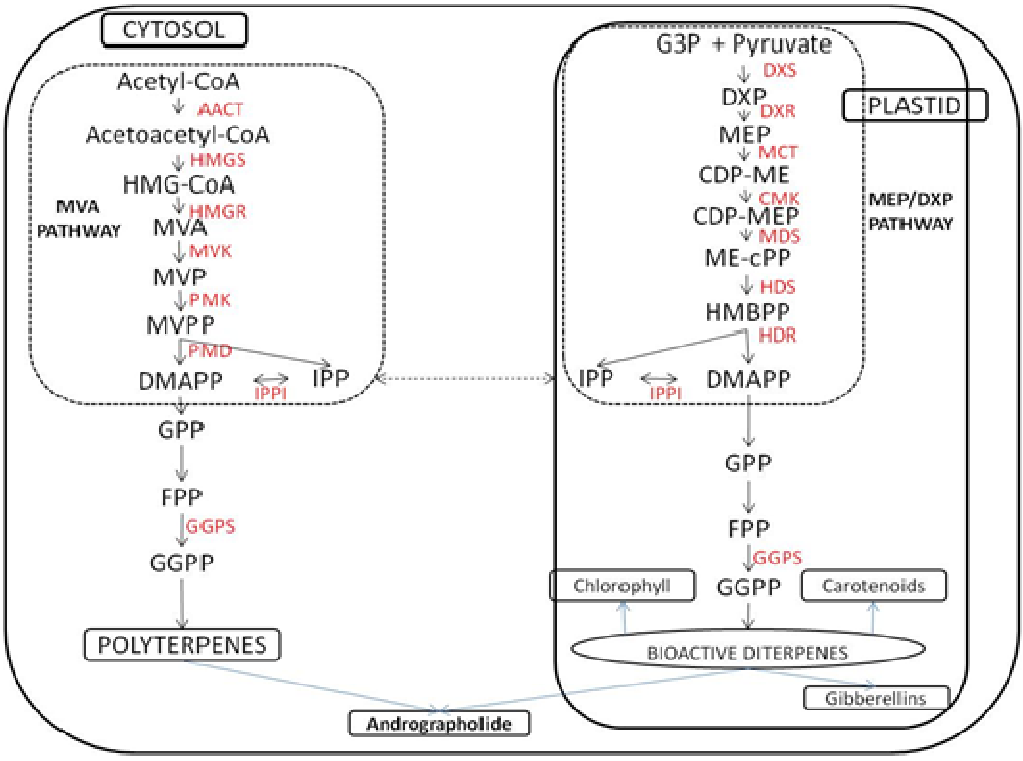
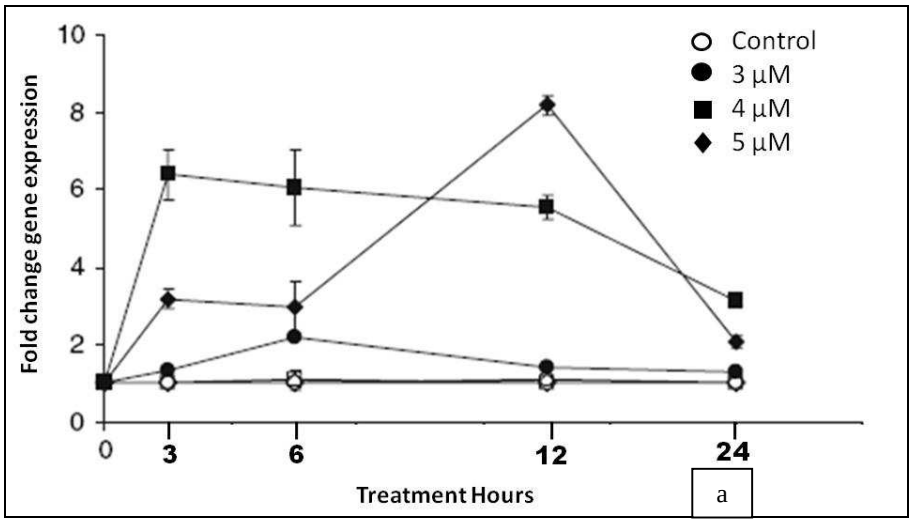


Fig. 1. Biosynthetic routes of terpene biosynthesis pathway show the tentative production of the andrographolide red colour text shows the enzymes of respective product.

Understanding the regulation of the genes described here might aid ultimately in manipulation of plant response in the biosynthesis of valuable andrographolide. The role of HMGR gene in andrographolide production suggests that elicitation causes strong and rapid induction of respective transcripts. Unrevealing the exact function of the enzymes encoded by the pathway related genes and their promoter sequences that control their regulation, may provide valuable tools in production of secondary metabolite. This work clearly demonstrates that HMGR is one of the key players in andrographolide accumulation.



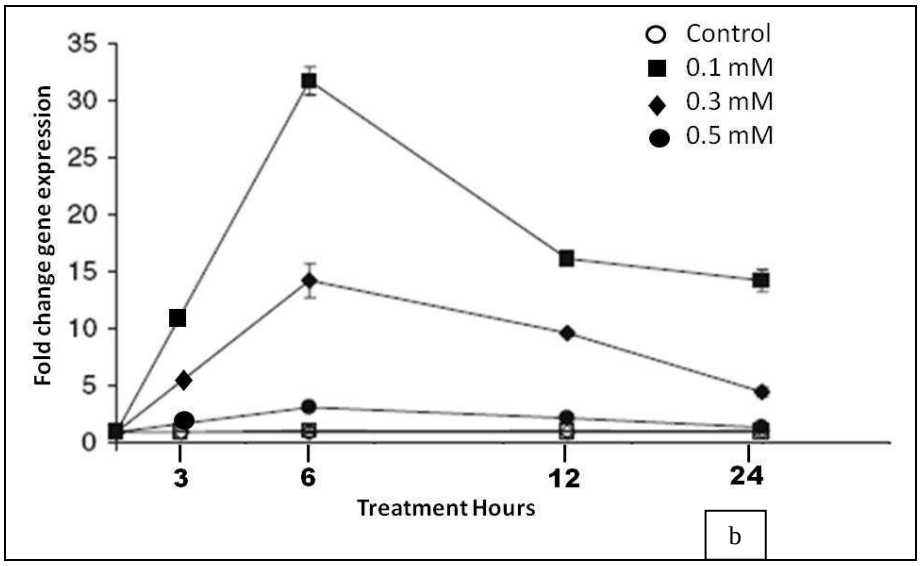


Fig. 2. a. Changes in steady-state transcript levels of HMGR genes in *A. paniculata* following GA_3 treatments b. Changes in steady-state transcript levels of HMGR genes in *A. paniculata* following JA treatments. A minimum of three saplings per replicate were harvested and pooled, used in RNA extractions, and analyzed by qRT-PCR in triplicate.

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