RESEARCH ARTICLE

Ethylene treatment improves diosgenin accumulation in *in vitro* cultures of *Dioscorea zingiberensis* via up-regulation of CAS and HMGR gene expression

Sabake T. Diarra[1] · Ji He[1] · Jianbo Wang[1] · Jiaru Li[1] ✉

1 Wuhan University, College of Life Sciences, State Key Laboratory of Hybrid Rice, Wuhan, PR China

✉ Corresponding author: jrli@whu.edu.cn

Abstract

Background: The perennial medicinal herb *Dioscorea zingiberensis* is a very important plant used for steroid drug manufacturing for its high level of diosgenin in rhizome. Although the stimulation of diosgenin accumulation by ethylene has been reported in a few of plant species, its regulation is not yet characterized at the molecular level, the underlying molecular mechanism remains elusive.
Results: In this study, the effects of ethylene on diosgenin biosynthesis in *in vitro* cultures of *D. zingiberensis* were described. The results showed that, in samples treated with ethylene at concentration E3 (10^4 dilution of 40% ethephon), the diosgenin biosynthesis was significantly promoted in comparison with the control samples. Treatment with high concentrations of ethylene had inhibitory effect, whereas with low concentration of the gas elicitor brought about no detectable deleterious effect on the growth rate and diosgenin content of the cultures. The considerable increase of diosgenin level in *in vitro* cultured *Dioscorea zingiberensis* by ethylene application is accompanied by the concomitant increase of soluble proteins and chlorophyll content. The gene expressions of cycloartenol synthase and 3-hydroxy-3-methylglutaryl-CoA reductase but not of squalene synthase or farnesyl pyrophosphate synthase were up-regulated by applied ethylene.
Conclusions: Our results suggest that ethylene treatment enhanced diosgenin accumulation via up-regulation of the gene expressions of cycloartenol synthase and 3-hydroxy-3-methylglutaryl-CoA reductase.

Keywords: diosgenin, *Dioscorea zingiberensis,* ethylene, gene expression.

INTRODUCTION

Diosgenin, (25R)-Spirost-5-en-3β-ol (Figure 1), is a steroidal sapogenin isolated from the plants (Marker et al. 1940). It is one of the most important raw materials for steroid drugs manufacturing. Diosgenin represents the main starting material for commercial synthesis of sex drugs and corticosteroids such as cortisone, testosterone, and progesterone. Estrogenic, progesterogenic and anti-inflammatory effects have been hypothesized for diosgenin due to its structural similarity to estrogen and progesterone precursors (Dewick, 2002). Anticancer activity, controlling hyperlipidemia, inhibiting melanogenesis, delaying skin aging at the time of climacteric have also been demonstrated (Lee et al. 2007; Tada et al. 2009; Yan et al. 2009; Gong et al. 2010). The perennial medicinal herb *Dioscorea zingiberensis* is a very important plant used for steroid drug manufacturing for its high level of diosgenin in rhizome (Huai et al. 1989; Ding et al. 1991; Cheng et al. 2008).
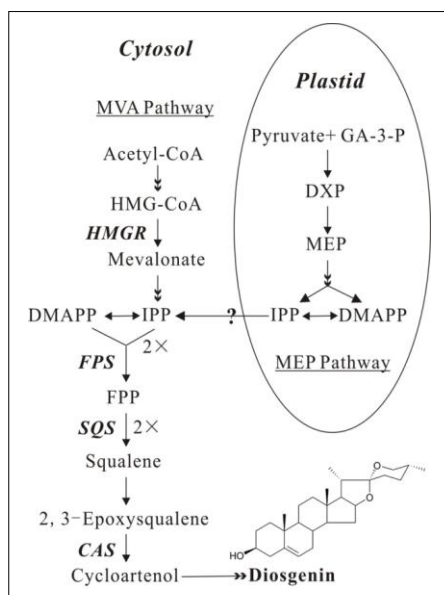
Fig. 1 The biosynthesis pathway and structure of diosgenin. MVA: mevalonic acid; GA-3-P: glyceraldehyde-3-phosphate; DXP: 1-deoxy-D-xylulose-5-phosphate; MEP: 2-C-methyl-D-erythritol-4-phosphate; HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA; HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase; HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase; IPP: isopentenyl diphosphate; DMAPP: dimethylallyl diphosphate; FPP: farnesyl diphosphate; FPPS: FPP synthase; SQS: Squalene synthase; CAS: Cycloartenol synthase.

In general, the amount of diosgenin naturally accumulated in plant tissues is small, but can be increased by various elicitors (Radman et al. 2003; Namdeo, 2007). The gaseous phytohormone ethylene has wide ranging effects on plant growth and developmental processes, including senescence abscission, floral transition, fruit ripening, germination and morphogenic response in seedlings (Kieber, 1997). Several line of evidence indicated the stimulating effect of ethylene on diosgenin content and enzymes activities in *Trigonella foenum-graecum* (Oncina et al. 2002; De and De, 2003; Gómez et al. 2004) and in *D. floribunda* (De and De, 2005).

Four enzymes, *i.e.*, cycloartenol synthase (CAS), squalene synthase (SQS), farnesyl pyrophosphate synthase (FPPS), and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), are well recognized as key enzymes responsible for the biosynthesis of triterpene diosgenin in plants (Figure 1). The synthesis of steroidal sapogenins and alkaloids involves cyclization of squalene 2, 3-epoxide, mediated by cycloartenol synthase, with the formation of tetracyclic, C_{30}-compound- cycloartenol (Brown, 1998). It has been well documented that CAS plays essential roles in the plant cell viability, and in the regulation of triterpenoid biosynthesis. SQS is a bifunctional enzyme that catalyzes the condensation of two molecules of farnesyl diphosphate (FPP) to give presqualene diphosphate (PSPP) and the subsequent rearrangement of PSPP to squalene, a key precursor for the sterol and triterpene biosynthesis (Huang et al. 2007; Lee and Poulter, 2008). FPPS is essential for the organ development in plants although it has not previously been identified as a key regulatory enzyme in triterpene biosynthesis (Kim et al. 2010a). The enzyme HMGR catalyzes the first committed step of isoprenoids biosynthesis in MVA pathway (Wang et al. 2007). Accumulating research results suggested that plant HMGRs are encoded by a gene family members and regulated by light, growth regulators, wounding and treatment with pathogen or elicitors (Park et al. 1992; Maldonado-Mendoza et al. 1997).

Several studies have been done to assess the stimulating effects on diosgenin production by ethylene or other elicitors, but the possible genes responsible for the stimulation are largely unknown. The aim of this present work is to understand the molecular mechanism of ethylene induced diosgenin accumulation and the gene expression patterns of CAS, SQS, FPPS, and HMGR in *in vitro* cultures of *D. zingiberensis*.

MATERIALS AND METHODS

Plant materials

The *in vitro* cultures were established from *Dioscorea zingiberensis* C. H. Wright which was originally collected from Wudang Mountain, Hubei Province, China. The cultures were maintained on Murashige and Skoog (1962) salt medium (MS medium) containing 1 mg/L 6-BA, 25 g/L sucrose and 8 g/L agar. The pH of the medium was adjusted to 5.8. The medium was sterilized at 121ºC for 20 min. The tissue cultures were grown and maintained in a culture chamber at 25ºC with a 12 hrs light photoperiod, provided by fluorescent tubes (Philips, 3000Lx); and were subcultured at 4 week intervals.

Diosgenin and BSA were from the Sigma Company. Methanol and ethephon (40% ethylene) were from Fisher Scientific and Sichuan Guo Guang Company, respectively. All other chemicals and reagents used were of the highest purity commercially available. Agilent 1100 workstation was used for HPLC analysis.

Hormonal treatment

After four weeks of subculture, the *in vitro* cultures were moved into new MS medium which was 6-BA free. Ethephon were diluted 10^2, 10^3, 10^4, 10^5 times with sterilized distilled water, and marked as E1 to E4 respectively. The ethephon solution was sterilized by filtration through a sterile 0.22 µm membrane filter. The control samples (CK) were treated with distilled water only. For ethylene addition, 1 cm-wide strips of sterilized filter paper were soaked in elicitor solution and placed on the wall of the culture boxes. Three replicates of 20 boxes (60 plantlets) were done for each treatment. In order to record the initial weight of the materials, the culture bottles were weighed before and after the tissues were moved into the medium. These cultures were incubated under conditions as described above for 40 days before used in further experimental investigations.

Growth measurement

The treated and control plantlets were harvested after 40 days culturing, the fresh weight (FW) was determined, and then dried to constant weight at 60ºC for several days and used to measure dry weight (DW). The dried materials were used to assess the diosgenin content.

Measurement of diosgenin content and yields

Preparation of samples and the indirect competitive ELISA assay method was performed as previously described (Li et al. 2010). Diosgenin yields in 10 boxes' culture were measured using the following formula, Diosgenin yields = diosgenin content (% DW) x DW of 10 boxes culture (mg).

Measurement of soluble protein

Total soluble proteins were extracted with 100 mM sodium phosphate buffer (pH 7.5) containing 2 mM ascorbate and 0.5 mM ethylene diamine tetraacetic acid (EDTA), and were quantified according to Bradford (1976), using bovine serum albumin as a standard.

Measurement of chlorophyll content

The chlorophyll content was measured spectrophotometrically in 80% acetone extracts as described by Arnon (1949). Total chlorophyll (Ct) were calculated using the equation $Ct = 1000* A652/34.5$.

RNA isolation and semi-quantitative RT-PCR analysis

Cultures treated with ethephon at concentration E3 were used to examine the expression level of genes involved in diosgenin biosynthesis in comparison with the control samples.

Total RNA from the treated and control samples, was isolated using TRI plant Total RNA Fast Extraction Kit (Spin-column) (BioTeke Corporation, Beijing) according to manufacturer's instructions. Fragments containing coding zones of different genes were amplified by RT-PCR. Semi-quantitative PCR reactions were performed using 31 cycles for CAS (GenBank accession no. AM697885), 33 cycles for SQS (GenBank accession no. JN693497), 31 cycles for FPPS (GenBank accession no. JN693498), 28 cycles for HMGR (GenBank accession no. DQ017377) and 27 cycles for actin (GenBank accession no. JN693499), the quantitative primers were designed according to known sequence listed in the Table 1. PCR reactions were carried out in an ABI thermocycler using Taq DNA polymerase (Fermentas) under the following conditions: an initial 5 min denaturing at 94ºC, followed by cycles of 94ºC 20 sec, 58ºC 20 sec, and 72ºC 30 sec. PCR products were sampled at the aforementioned specified cycles and analyzed by 1.5% agarose gel electrophoresis.

Table 1. Primer sets used for the semi-quantitative RT-PCR.

Gene name	Accession number	Primer name	Primer sequence (5′ to 3′)
HMGR	DQ017377	HMGR-qF	GTTTCCAAGGGTGTCCAAAA
		HMGR-qR	GCCCCACAGACAACTGATTT
FPPS	JN693498	FPS-qF	TAAGGTGGGCCTTATTGCTG
		FPS-qR	TCGCAAGGTCTTGTTCTCCT
SQS	JN693497	SQS-qF	ATTCCGGCTGATGTCAAAGT
		SQS-qR	ATAACCTTTGCCAAGCTCCA
CAS	AM697885	CAS-qF	CATCCGGAAAGCTTGTGATT
		CAS-qR	AAGCGCTAACATGACCCAAC
Actin	JN693499	Actin-qF	ATGCCATTCTTCGTTTGGAC
		Actin-qR	CTACTCTTGGCGGTTTCCAG

Statistical analysis

Differences between the treatments and the controls of the data were analyzed by an analysis of variance, and the post hoc Duncan's multiple range test was used when appropriate. Pearson's correlation test was carried out between diosgenin and other parameters. All statistical analyses were performed using the SPSS package (SPSS Inc., Chicago, IL) and were considered significant at $P <$ 0.05.

RESULTS

Effects of ethylene on the growth of *in vitro* cultures of *D. zingiberensis*

Higher concentrations of ethephon (E1, E2, and E3) had inhibitory effect on the growth of cultures (Figure 2), whereas the lower (E4) concentration didn't induce any evident changes in the treated samples compared with the control samples. The FW decreased in ethephon-treated plants with decrement of 26%, 24%, 14%, and 18% in E1, E2, E3 and E4 compared to CK. Ethephon treatment also significantly lowered dry mass with the highest decrement in E1 (25% compared to CK) and then E3 (23% compared to CK) (Figure 3).
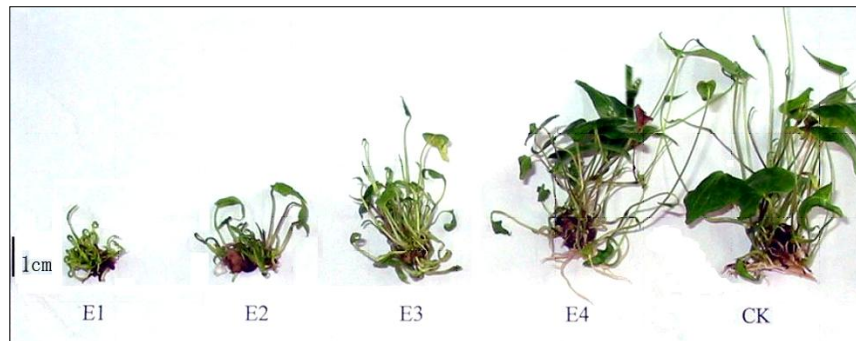
Fig. 2 Effect of ethylene on the phenotypic shape of *in vitro* cultures of *D. zingiberensis*.
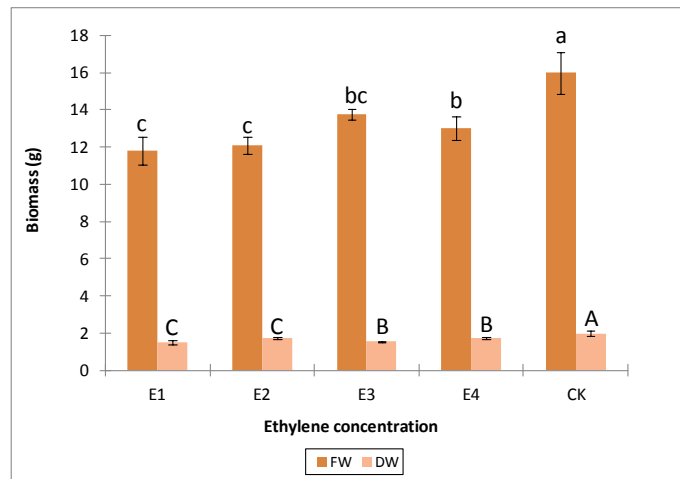

Fig. 3 Effect of ethylene on biomass *in vitro* cultures of *D. zingiberensis*. The fresh mass (FW) and dry mass (DW) were measured in 10 boxes' culture plants. Values are the means ± SD (n = 3). Values with different small letters (FW) or capital letters (DW) are significantly different at the $p < 0.05$ level, according to Duncan's multiple range test.

Effects of ethylene on diosgenin content in cultures

The results (Figure 4a) show that with the decrease of ethephon concentration, the diosgenin level in treated samples temporarily increased and then decreased. The highest level of diosgenin (increase of 149.8% over that of the control samples) was achieved with concentration E3. When expressed with yield, the E3 and E2 showed significant increase (93% and 21% compared to CK) in 10 boxes' culture plants (Figure 4b).

Effects of ethylene on soluble protein and chlorophyll content

The accumulation of soluble protein had a similar trend of diosgenin accumulation (Figure 5a). Their levels increased together with diosgenin, which suggests that a determined level of protein accumulation is required for diosgenin biosynthesis in plant tissue. High ethylene concentration inhibited chlorophyll accumulation, whereas plants with E_3 treatment increased chlorophyll content by 33% (Figure 5b).
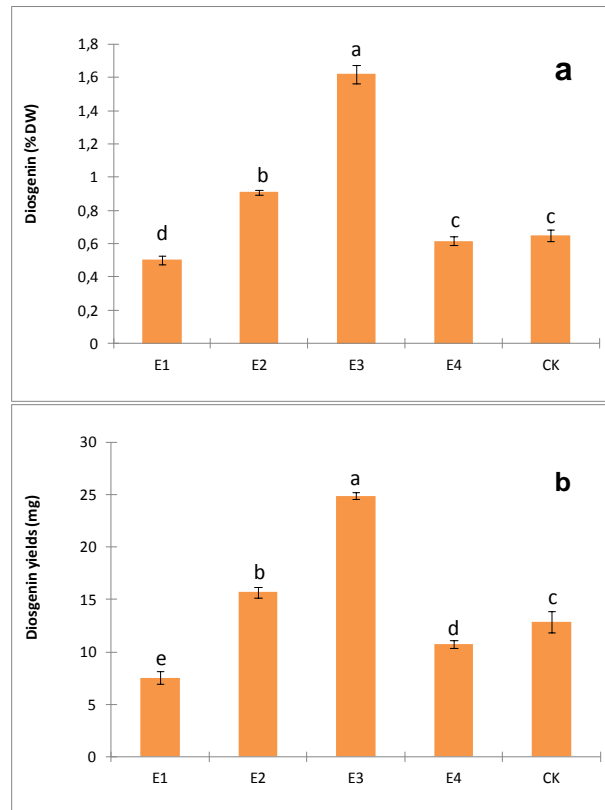
Fig. 4 Effect of ethylene on diosgenin content and diosgenin yields (10 boxes) in *in vitro* cultures of *D. zingiberensis*. Values are the means ± SD (n = 4). Values with different letters are significantly different at the $p < 0.05$ level, according to Duncan's multiple range test.

Effect of ethylene on genes expression

The results shown in Figure 6 indicated that in comparison with the control samples, the expression of cycloartenol synthase (CAS) gene was up-regulated at all time points, and HMGR gene show significant response to ≥ 8 hrs ethylene treatment. FPPS gene is only slightly up-regulated after 4 hrs elicitor application. However, ethylene treatment didn't induce any evident changes in the expression of squalene synthase (SQS) gene.

DISCUSSION

The mechanism of the ethylene effect on the diosgenin synthesis and accumulation is still not clearly understood. This study investigated enhancement effect of ethylene on the accumulation of diosgenin in *D. zingiberensis* cultures, and the gene expressions of key components involved in diosgenin biosynthesis to delineate the molecular mechanisms governing the process.

The diosgenin biosynthesis and accumulation are very complex processes which are tightly regulated by primary metabolism. Our results showed that the application of certain concentrations of ethylene can improve diosgenin biosynthesis in *in vitro* cultures of *D. zingiberensis* (Figure 4) which is consistent with existing findings (De and De, 2003). Recently, Gómez et al. (2004) reported the increase in diosgenin biosynthesis and accumulation when the fenugreek cells suspension was treated with ethephon. The diosgenin content first increased with the decrease of ethephon concentration, and then decreased. The decrease of diosgenin content induced by lower and higher concentration of ethylene may be due to the induction of other metabolic pathways whose activities can inhibit or slow down the diosgenin biosynthesis pathway. In the meantime, a decrease of biomass was observed in ethylene

treated cultures (Figure 2, Figure 3), which is in agreement with other reports in *Trigonella foenum-graecum* (De and De, 2003; Gómez et al. 2004).
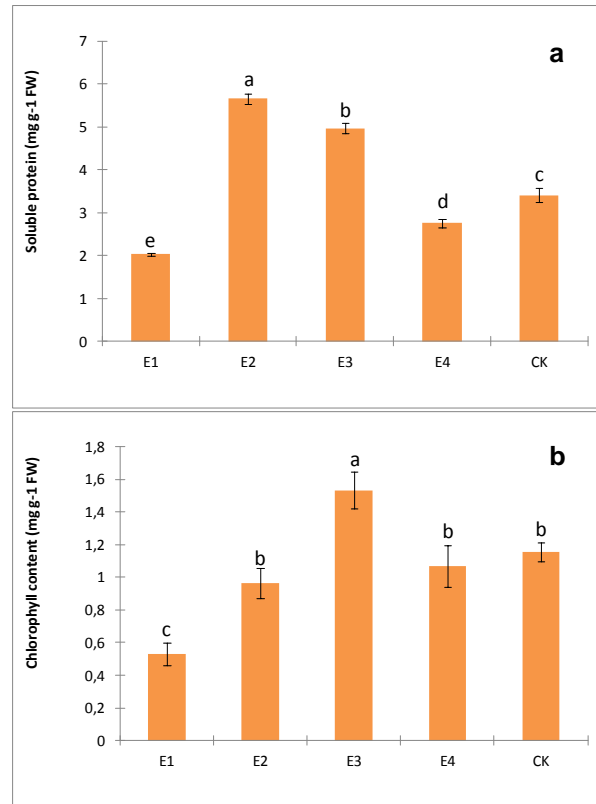

Fig. 5 Ethylene induced changes in soluble proteins and chlorophyll content. Values are the means ± SD (n = 3). Values with different letters are significantly different at the *p* < 0.05 level, according to Duncan's multiple range test.
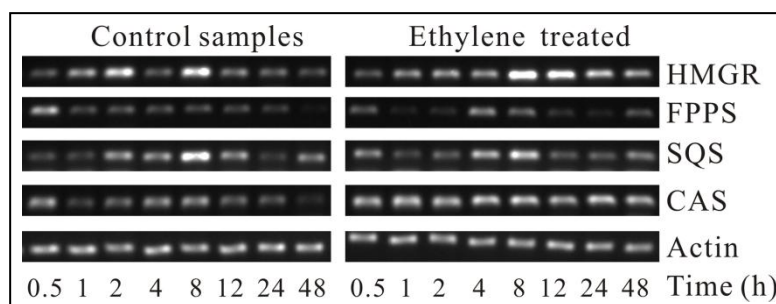

Fig. 6 Analysis of the effect of ethylene on the expression of CAS, SQS FPPS, and HMGR genes in cultures of *D. zingiberensis*.

The effect of ethylene on diosgenin content is closely related to the soluble protein and chlorophyll content (Figure 5). Diosgenin production is a kind of plant response to stress, in which de novo proteins synthesis is required. Narula et al. (2005) observed the increase of diosgenin and protein content in *D. bulbifera* callus cultures grown under abiotic stress conditions. Data for ethylene treated and control plants were grouped together to determine the possible correlations between diosgenin and other traits (Table 2). It was showed that diosgenin content was significantly and positively correlated with soluble protein (*p* < 0.01), as well as chlorophyll (*p* < 0.01). Ehmke and Eilert (1993) demonstrated a positive

correlation between solasodine level and chlorophyll content in suspension cultures of *Solanum dulcamara*, which is in agreement with our results.

Table 2. Correlations between diosgenin and other parameters. The table shows the Pearson correlation coefficient (*: $P < 0.05$; **: $P < 0.01$).

	FW	DW	Diosgenin	Diosgenin yield	Soluble Protein	Chlorophyll
FW	1					
DW	0.77**	1				
Diosgenin	0.12	-0.30	1			
Diosgenin yield	0.25	-0.12	0.98**	1		
Soluble protein	0.02	0.04	0.71**	0.77**	1	
Chlorophyll	0.53*	0.19	0.77**	0.84**	0.57*	1

Many studies show that transcriptional modulation of related genes is a common response to pathogens or elicitor signals. Inspections on DNA sequences of elicitors (for example, JA and ethylene) responsive genes have identified several elicitor response elements in these genes which are involved in the biosynthesis of secondary metabolites (Zhao et al. 2005). We provided evidence here that gene expressions of, CAS, HMGR, and FPPS in *in vitro* cultures of *D. zingiberensis* were induced by ethylene applications which led to the improvement of diosgenin biosynthesis (Figure 6).

The enzymes CAS are responsible for triterpene biosynthesis catalyzing the formation of cycloartenol which is then converted into cholesterol, the most important precursor of diosgenin (Friedman et al. 1997; Arnqvist et al. 2003). Kim et al. (2011) and Han et al. (2006) reported the elicitor MJ induced up-regulation of CAS in *Bupleurum falcatum*, and *Panax ginseng*, respectively. Lee et al. (2004) reported that overexpression of the squalene synthase gene was followed by up-regulation of CAS gene in transgenic *P. ginseng*. However, Kim et al. (2011) found that methyl jasmonate treatment reduced the expression of CAS gene in the roots of wild *Bupleurum falcatum*. HMGR, catalyzing the conversion of HMG-CoA to mevalonic acid, is considered as a rate-limiting enzyme in cholesterol synthesis, and it also plays a critical role in controlling isoprenoid derivatives related pathways (Chen et al. 2012). Cools et al. (2011) found that ethylene treatment could increase in the expression of HMGR gene in onion. HMGR gene expression was induced by elicitor in *Michelia chapensis* (Cao et al. 2011). In our study, the changes in HMGR gene expression seemed to be time-dependent, and the highest level appeared 8 hrs ethylene treatment (Figure 6). FPPS has not been identified as a key regulatory enzyme in triterpene biosynthesis (Kim et al. 2010b). In this work, its expression is time-dependent (Figure 6). Ding et al. (2008) established that FPPS gene possessed various potential regulatory elements associated with physiological and environmental factors. A down-regulation of SQS gene is observed in ethylene treated samples (Figure 6). This finding is similar with what reported by Devarenne et al. (2002), who found that absolute level of the SQS mRNA decreased approximately 5-fold in the elicitor-treated cells, suggesting decreased transcription of the SQS gene.

In conclusion, our results suggest that ethylene treatment enhanced diosgenin accumulation by considerably affecting the expression of CAS and HMGR gene in the present study. Further investigations will be necessary to elucidate the specific role of each gene involved in diosgenin biosynthesis regulation, and to fix the mechanism by which CAS and HMGR can be involved in triterpenes biosynthetic pathway.

REFERENCES

ARNON, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, vol. 24, no. 1, p. 1-15. **[CrossRef]**

ARNQVIST, L.; DUTTA, P.C.; JONSSON, L. and SITBON, F. (2003). Reduction of cholesterol and glycoalkaloid levels in transgenic potato plants by overexpression of a type 1 sterol methyltransferase cDNA. *Plant Physiology,* vol. 131, no. 4, p. 1792-1799. **[CrossRef]**

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of proteinutilizing the principle of protein-dye binding. *Analytical Biochemistry*, vol. 72, no. 1-2, p. 248-254. **[CrossRef]**

BROWN, G.D. (1998). The biosynthesis of steroids and triterpenoids. *Natural Product Reports*, vol. 15, no. 6, p. 653-696. **[CrossRef]**

CAO, X.Y.; LI, C.G.; MIAO, Q.; ZHENG, Z.J. and JIANG, J.H. (2011). Molecular cloning and expression analysis of a leaf-specific expressing 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase gene from *Michelia chapensis* Dandy. *Journal of Medicinal Plants Research*, vol. 5, no. 16, p. 3868-3875.

CHEN, X.; WANG, X.; LI, Z.; KONG, L.; LIU, G.; FU, J. and WANG, A. (2012). Molecular cloning, tissue expression and protein structure prediction of the porcine 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGR*) gene. *Gene,* vol. 495, no. 2, p. 170-177. **[CrossRef]**

CHENG, J.; HU, C.Y.; PANG, Z.J. and XU, D.P. (2008). Isolation and structure identification of steroidal saponin from *Dioscorea zingiberensis*. *Chinese Traditional Herb Drugs,* vol. 39, no. 2, p. 165-167.

COOLS, K.; CHOPE, G.A.; HAMMOND, J.P.; THOMPSON, A.J. and TERRY, L.A. (2011). Ethylene and 1-methylcyclopropene differentially regulate gene expression during onion sprout suppression. *Plant Physiology,* vol. 156, no. 3, p. 1639-1652. **[CrossRef]**

DE, D. and DE, B. (2003). Effect of ethephon on antioxidant enzymes and diosgenin production in seedlings *Trigonella foenum-graecum*. *Food Chemistry,* vol. 82, no. 2, p. 211-216. **[CrossRef]**

DE, D. and DE, B. (2005). Elicitation of diosgenin production in *Dioscorea floribunda* by ethylene-generating agent. *Fitoterapia,* vol. 76, no. 2, p. 153-156. **[CrossRef]**

DEVARENNE, T.P.; GHOSH, A. and CHAPPELL, J. (2002). Regulation of squalene synthase, a key enzyme of sterol biosynthesis, in tobacco. *Plant Physiology,* vol. 129, no. 3, p. 1095-1106. **[CrossRef]**

DEWICK, P.M. (2002). *Medicinal Natural Products: A Biosynthetic Approach*. Second Edition, John Wiley & Sons, Chichester, UK, p. 237-289.

DING, Z.Z.; ZHOU, X.L. and WANG, Y.C. (1991). Study of influencing factors of diosgenin in *Dioscorea zingiberensis*. *Chinese Traditional Herb Drugs,* vol. 12, p. 34-35.

DING, Y.X.; OU-YANG, X.; SHANG, C.H.; REN, A.; SHI, L.; LI, Y.X. and ZHAO, M.W. (2008). Molecular cloning, characterization, and differential expression of a farnesyl-diphosphate synthase gene from the basidiomycetous fungus *Ganoderma lucidum*. *Bioscience, Biotechnology and Biochemistry*, vol. 72, no. 6, p. 1571-1579. **[CrossRef]**

EHMKE, A. and EILERT, U. (1993). *Solanum dulcamara* L. (Bittersweet): Accumulation of steroidal alkaloids in the plant and different *in vitro* systems. In: BAJAJ, Y.P.S. ed. *Biotechnology in Agriculture and Forestry 21.* Medicinal and Aromatic Plants IV. Springer Berlin Heidelberg. p. 339-352. **[CrossRef]**

FRIEDMAN, M.; MCDONALD, G.M. and FILADELFI-KESZI, M. (1997). Potato glycoalkaloids: Chemistry, analysis, safety, and plant physiology. *Critical Reviews in Plant Sciences,* vol. 16, no. 1, p. 55-132. **[CrossRef]**

GÓMEZ, P.; ORTUÑO, A. and DEL RÍO, J.A. (2004). Ultrastructural changes and diosgenin content in cell suspensions of *Trigonella foenum-graecum* L. by ethylene treatment. *Plant Growth Regulation,* vol. 44, no. 2, p. 93-99. **[CrossRef]**

GONG, G.; QIN, Y.; HUANG, W.; ZHOU, S.; WU, X.; YANG, X.; ZHAO, Y. and LI, D. (2010). Protective effects of diosgenin in the hyperlipidemic rat model and in human vascular endothelial cells against hydrogen peroxide-induced apoptosis. *Chemico-Biological Interactions,* vol. 184, no. 3, p. 366-375. **[CrossRef]**

HAN, J.Y.; JUNG, S.J.; KIM, S.W.; KWON, Y.S.; YI, M.J.; YI, J.S. and CHOI, Y.E. (2006). Induction of adventitious roots and analysis of ginsenoside content and the genes involved in triterpene biosynthesis in *Panax ginseng*. *Journal of Plant Biology*, vol. 49, no. 1, p. 26-33. **[CrossRef]**

HUAI, Z.P.; DING, Z.Z.; HE, S.A. and SHENG, C.G. (1989). Research on correlations between climatic factors and diosgenin content in *Dioscorea zingiberensis* wright. *Acta Pharmacologica Sinica,* vol. 24, no. 9, p. 702-706.

HUANG, Z.; JIANG, K.; PI, Y.; HOU, R.; LIAO, Z.; CAO, Y.; HAN, X.; WANG, Q.; SUN, X. and TANG, K. (2007). Molecular cloning and characterization of the yew gene encoding squalene synthase from *Taxus cuspidata*. *Journal of Biochemistry and Molecular Biology,* vol. 40, no. 5, p. 625-635. **[CrossRef]**

KIEBER, J.J. (1997). The ethylene response pathway in *Arabidopsis*. *Annual Review of Plant Physiology and Plant Molecular Biology,* vol. 48, no. 1, p. 227-296. **[CrossRef]**

KIM, O.T.; BANG, K.H.; JUNG, S.J.; KIM, Y.C.; HYUN, D.Y.; KIM, S.H. and CHA, S.W. (2010a). Molecular characterization of ginseng farnesyl diphosphate synthase gene and its up-regulation by methyl jasmonate. *Biologia Plantarum,* vol. 54, no. 1, p. 47-53. **[CrossRef]**

KIM, O.T.; KIM, S.H.; OHYAMA, K.; MURANAKA, T.; CHOI, Y.E.; LEE, H.Y.; KIM, M.Y. and HWANG, B. (2010b). Upregulation of phytosterol and triterpene biosynthesis in *Centella asiatica* hairy roots overexpressed ginseng farnesyl diphosphate synthase. *Plant Cell Reports,* vol. 29, no. 4, p. 403-411. **[CrossRef]**

KIM, Y.S.; CHO, J.H.; PARK, S.; HAN, J.Y.; BACK, K. and CHOI, Y.E. (2011). Gene regulation patterns in triterpene biosynthetic pathway driven by overexpression of squalene synthase and methyl jasmonate elicitation in *Bupleurum falcatum. Planta,* vol. 233, no. 2, p. 343-355. **[CrossRef]**

LEE, M.H.; JEONG, J.H.; SEO, J.W.; SHIN, C.G.; KIM, Y.S.; IN, J.G.; YANG, D.C.; YI, J.S. and CHOI, Y.E. (2004). Enhanced triterpene and phytosterol biosynthesis in *Panax ginseng* overexpressing squalene synthase gene. *Plant and Cell Physiology*, vol. 45, no. 8, p. 976-984. **[CrossRef]**

LEE, J.; JUNG, K.; KIM, Y.S. and PARK, D. (2007). Diosgenin inhibits melanogenesis through the activation of phosphatidylinositol-3-kinase pathway (PI3K) signaling. *Life Sciences,* vol. 81, no. 3, p. 249-254. **[CrossRef]**

LEE, S. and POULTER, C.D. (2008). Cloning, solubilization, and characterization of squalene synthase from *Thermosynechococcus elongatus* BP-1. *Journal of Bacteriology,* vol. 190, no. 11, p. 3808-3816. **[CrossRef]**

LI, J.; YANG, D.; YU, K.; HE, J. and ZHANG, Y. (2010). Determination of diosgenin content in medicinal plants with enzyme-linked immunosorbent assay. *Planta Medica,* vol. 76, no. 16, p. 1915-1920. **[CrossRef]**

MALDONADO-MENDOZA, I.E.; VINCENT, R.M. and NESSLER, C.L. (1997). Molecular characterization of three differentially expressed members of the *Camptotheca acuminate* 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) gene family. *Plant Molecular Biology,* vol. 34, no. 5, p. 781-790. **[CrossRef]**

MARKER, R.E.; TSUKAMOTO, T. and TURNER, D.L. (1940). Sterols. C. Diosgenin. *Journal of the American Chemical Society,* vol. 62, no. 9, p. 2525-2532. **[CrossRef]**

MURASHIGE, T. and SKOOG, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum,* vol. 15, no. 3, p. 473-479. **[CrossRef]**

NAMDEO, A.G. (2007). Plant cell elicitation for production of secondary metabolites: A review. *Pharmacognosy Reviews,* vol. 1, no. 1, p. 69-79.

NARULA, A.; KUMAR, S. and SRIVASTAVA, P.S. (2005). Abiotic metal stress enhances diosgenin yield in *Dioscorea bulbifera* L. cultures. *Plant Cell Reports,* vol. 24, no. 4, p. 250-254. **[CrossRef]**

ONCINA, R.; DEL RÍO, J.A.; GÓMEZ, P. and ORTUÑO A. (2002). Effect of ethylene on diosgenin accumulation in callus cultures of *Trigonella foenum-graecum* L. *Food Chemistry,* vol. 76, no. 4, p. 475-479. **[CrossRef]**

PARK, H.; DENBOW, C.J. and CRAMER, C.L. (1992). Structure and nucleotide sequence of tomato *HMG2* encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Plant Molecular Biology,* vol. 20, no. 2, p. 327-331. **[CrossRef]**

RADMAN, R.; SÁEZ, T.; BUCKE, C. and KESHAVARZ, T. (2003). Elicitation of plants and microbial cell systems. *Biotechnology and Applied Biochemistry,* vol. 37, no. 1, p. 91-102. **[CrossRef]**

TADA, Y.; KANDA, N.; HARATAKE, A.; TOBIISHI, M.; UCHIWA, H. and WATANABE, S. (2009). Novel effects of diosgenin on skin aging. *Steroids,* vol. 74, no. 6, p. 504-511. **[CrossRef]**

WANG, Y.; GUO, B.; ZHANG, F.; YAO, H.; MIAO, Z. and TANG, K. (2007). Molecular cloning and functional analysis of the gene encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase from hazel (*Corylus avellana* L. Gasaway). *Journal of Biochemistry and Molecular Biology,* vol. 40, no. 6, p. 861-869. **[CrossRef]**

YAN, L.L.; ZHANG, Y.J.; GAO, W.Y.; MAN, S.L. and WANG, Y. (2009). *In vitro* and *in vivo* anticancer activity of steroid saponins of *Paris polyphylla* var. yunnanensis. *Experimental Oncology,* vol. 31, no. 1, p. 27-32.

ZHAO, J.; DAVIS, L.C. and VERPOORTE, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances,* vol. 23, no. 4, p. 283-333. **[CrossRef]**

How to reference this article:

DIARRA, S.T.; HE, J.; WANG, J. and LI, J. (2013). Ethylene treatment improves diosgenin accumulation in *in vitro* cultures of *Dioscorea zingiberensis* via up-regulation of CAS and HMGR gene expression. *Electronic Journal of Biotechnology*, vol. 16, no. 5. http://dx.doi.org/10.2225/vol16-issue5-fulltext-9