J. Plant Biotechnology(1999) *Vol. 1(2). pp.117~121*

Journal of Plant Biotechnology

Enhanced Anthocyanin Accumulation by UV-B and JA Treatment in Cell Suspension Culture System of Grape (*Vitis vinifera* L.)

Won Yong Song, Jun Gyo In, Yong Pyo Lim[1], Kwan Sam Choi*

Division of Applied Biology, [1]Department of Horticulture, Chungnam National University, Taejon, 305-764, Korea.

Key words: anthocyanin, CHS, jasmonic acid, UV-B, *Vitis vinifera* L.

Abstract

Effects of jasmonic acid treatment, UV-B and white light treatment on the anthocyanin biosynthesis and cell growth were investigated using the cell suspension culture system of grape (*Vitis vinifera* L). Cell growth was not affected by white light irradiation, while it was remarkably suppressed by UV-B irradiation from 8 to 32 h. Anthocyanin accumulation dramatically increased after 16 h from irradiation of UV-B. Simultaneous treatment of jasmonic acid and UV-B increased anthocyanin accumulation by 10-fold. The cell division was restored when anthocyanin was abundantly accumulated after 32 h from UV-B irradiation. Optimum concentration of jasmonic acid was found to be 5 uM for maximum accumulation of anthocyanin. Application of jasmonic acid to grape suspension cells rapidly induced the expression of CHS gene after 2 h from treatment and showed maximum level at 32 h. Simultaneous treatment of jasmonic acid and light also induced CHS gene expression after 2 h, but the maximum level of CHS transcript was observed at 16 h with white light and 8 h with UV-B exposure. The synergistical effects could be explained by the defense mechanism that UV irradiation is mediated in part by alterations in JA and its signaling pathway.

Introduction

It is known that inductions of phenylpropanoid and flavonoid biosynthesis are the defense mechanism of plants against the stresses such as UV irradiation, pathogen attack and wound. Phenylalanine ammonialyase (PAL), chalcone synthase (CHS) and several enzymes involved in the phenylpropanoid and flavonoid biosynthetic pathways are induced both in intact plant and in cultured cells by environmental stresses, and play the protection against UV and phytoalexins (Beggs et al., 1985; Dixon, 1986; Landry et al., 1995).

Gene expression of PAL and CHS has been studied with connection to environmental stresses (Hahlbrock and Scheele, 1989; Lamb et al., 1989). Rapid induction of anthocyanin synthesis by UV light has been reported in serval plant species and it was reported that UV light is obligatory for flavonoid synthesis (Beggs and Wellmann, 1994; Britt et al., 1993; Hahlbrock and Scheele, 1989; Middleton and Teramura, 1994).

Recently, Creelman and Mullet (1997) reported that the jasmonic acid (JA) and its methyl ester, methyl jasmonate (Me-JA), were indentified as intracellular signal molecules that mediate the activation of gene expression in response to wounding, elicitor treatment and pathogen infection. Jasmonate application on plants caused accumulation of proteinase inhibitor, phytoalexin and defense response genes. JA also accumulated anthocyanin in soybean seedling at light condition (Creelman and Mullet, 1995; Enyedi et al., 1992; Farmer and Ryan, 1992; Pena-Cortes et al., 1995; Rhodes, 1994).

There are two kinds of pathway in the phenylpropanoid and flavonoid metabolism. One is a defensing pathway with rapid response to stress, and the other is a typical secondary metabolic pathway after cease of the primary metabolism (Ozeki, 1996).

This research was intended to investigate the ra-

* Corresponding author, E-mail; kschoi@hanbat.chungnam.ac.kr
Received May 24, 1999; accepted Jun. 21, 1999

pid response of phenylpropanoid and flavonoid metabolism that is related to the defense mechanism. This report deals with effects of JA treatment and UV-B irradiation on the anthocyanin biosynthesis and cell growth, using the cell suspension culture system of grape. We also report how CHS gene is expressed in cell suspension culture of grape under various conditions such as light, JA and simultaneous treatment of light and JA.

Materials and Methods

Plant materials

Cell suspensions prepared from callus of grape (*Vitis vinifera* L.) were subcultured per week in MS medium (Murashige and Skoog, 1962), supplemented with 3% (w/v) sucrose, 2.3×10^{-7} M 2,4-D and 9.3×10^{-7} M kinetin at 27℃ in the dark and 7-day old cells fractionated through the mesh filter (100-300 um) were inoculated into fresh medium with 1:10 dilution in 300 mL glass culture vessel that sealed with clean wrape for UV-B transmission.

JA and Light treatments

White light was obtained from warm-white fluorescent tubes (20W FL, Korea) and UV-B from G 30T8 30W UV fluorescent tubes (Sankyo, Japan) covered with cellulose acetate to remove UV-C wavelength. The intensity was measured with a spectroradiometer (Q101, Macam, Photometrics, UK). The intensities of white and UV-B source were 3.5 $Wm^{-2}sec^{-1}$ and 0.25 $Wm^{-2}sec^{-1}$, respectively.

Determination of cell number and anthocyanin contents

Freshly harvested cells from 1 mL of suspension were suspended in 1mL of enzyme solution, which contained 1% cellulase, 0.05% macerozyme and 0.6 M mannitol, and incubated for 30 min on a shaker at 60 rpm at 30℃. Cell numbers were estimated by counting protoplast with a haemocytometer after enzymatic maceration of cultured cells. For anthocyanin measurment cells were collected by centrifugation at 4,000×g for 5 min from 1mL of the suspension and anthocyanin was extracted with 1mL of 1% HCl-methanol for 24 h at 4℃ and the absorbance was measured at 530 nm.

RNA extraction and northern hybridization

Total RNA was extracted from grape suspension cells as described by Sparvoli et al. (1994). Twenty μg of total RNA were separated on a agarose gel and transferred onto membrane. Hybridization was carried out in 6×SCP, 1% sarcosin, 1% dextran sulfate, 1% non-fatmilk and 50% formamide at 42℃ with ^{32}P-labeled grape CHS cDNA. Membranes were washed with 0.2×SSC, 0.1% SDS and reprobed with the 28S ribosomal RNA of grape.

Results and Discussion

Effects of JA, UV-B, and white light on cell growth and anthocyanin accumulation

Choi et al. (1994) previously reported that maximum accumulation of anthocyanin was observed at the stationary phase under white light, whereas accumulation of anthocyanin was not observed under dark.

In order to investigate the response of plant cell to stresses such as UV-B, white light and JA, kinetics of cell division and anthocyanin accumulation under UV-B and white light were measured using the grape suspension culture systems. Cell division was not affected by white light, while it was remarkably suppressed by UV-B from 8 to 32 h after irradiation (Figure 2). Anthocyanin accumulation, however, was dramatically increased from 16 h after UV-B irradiation (Figure 3B) and cell division was resumed when anthocyanin was abundantly accumulated after 32 h from UV-B exposure (Figure 2). As shown in Figure 3, anthocyanin was not accumulated in the dark, while anthocyanin accumulation was moderate under light condition. The most dramatic increase was observed in UV-B and JA treated suspension cells during from 16 to 64 h (Figure 3B).

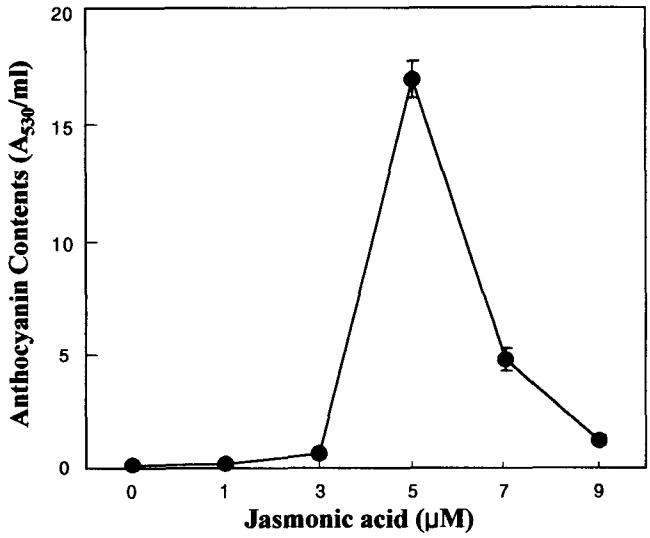

Figure 1. The effect of jasmonic acid on anthocyanin accumulation in the cell suspension culture of grape. Cells treated with JA were grown for 7 days in the dark and collected to measure the anthocyanin contents.

Previous reports have shown that flavonoids play an important role in the protection of plant against UV-B through the attenuation of UV in plant (Caldwell et al., 1983; Landry et al., 1995; Li et al., 1993). While irradiation of high energetic UV causes the inhibition of plant growth, irradiation of low energetic UV-B promote the growth of plants (Middleton and Teramura, 1994; Tezuka et al., 1993). Cell division was resumed with a large amount of anthocyanin accumulation after 32 h from UV-B irradiation (Figure 3). This may imply anthocyanin play a defense mechanism against UV-B irradiation.

JA also plays an important role on insect and disease resistance of plant. JA accumulates both in wounded plants and in cultured cells treated with elicitors. Addition of JA to soybean suspension increased mRNA levels of wound-responsive genes (Creelman and Mullet, 1997). JA induced anthocyanin biosynthesis in light grown soybean, whereas it did not at the dark condition (Franceschi and Grimes, 1991). Optimum concentration of JA was found to be 5 μM for maximum accumulation of anthocyanin, when grape cells cultured at dark for 7 days were treated with JA and cultured at 27℃ in the dark. The optimum concentration of JA was determined at 5 μM (Figure 1) while JA became toxic to grape cells at higher concentrations (Figure 1). The microscopic observation indicated that the cells became necrotic (data not shown). Furthermore, JA and lights may affect differently on the anthocyanin biosynthesis. As shown in Figure 3, highly synergistical effects were observed by simultaneous treatment of JA and lights, especially by JA with UV-B irradiation. After 32 h from UV-B treatment with JA, anthocyanin production was dramatically increased ca. 10-fold compared to either JA or UV-B only. Accumulation of anthocyanin by exogenous JA suggests that JA may induce directly or indirectly the transcription of genes related to flavonoid biosynthesis.

CHS gene expression by simultaneous treatment with JA and lights

PAL and CHS played key roles in the regulation of anthocyanin synthesis. In order to investigate the CHS gene expression at the early stage of grape suspension cells in response to stress such as JA and lights irradiation, northern analysis was performed. CHS expression level in white light irradiated cells was very low, whereas the expression level of UV-B was induced after 16 h exposure (Figure 4A). Application of JA in grape suspension cells, however, rapidly induced the expression of CHS gene after 2 h treatment and showed the maximun level at 32 h exposure (Figure 4B). Interestingly, CHS gene was also induced at 2 h after JA treatment with UV-B or white light, but at the treatment of JA with UV-B or white light the maximum levels of CHS transcripts were observed at 16 h with white light and 8 h with UV-B light irradiation (Figure 4B). It is interesting that the levels at the 64 h with JA and white light were disappeared.

Ozeki (1996) proposed that two PAL genes, TRN-PAL and ANT-PAL, existed at the carrot cell suspension culture. TRN-PAL expressed at the early stage in response to stress when carrot cells transferred into fresh medium and ANT-PAL for anthocyanin production. The expression of CHS gene in carrot

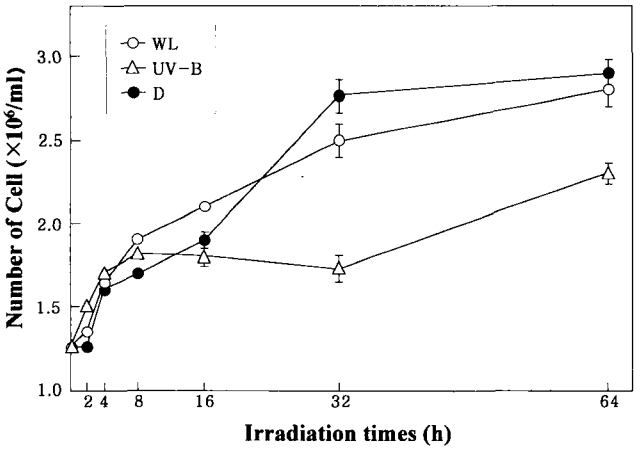

Figure 2. Effect of lights on the cell growth in the cell suspension culture of grape. Cells were irradiated with UV-B (0.08 $Em^{-2}sec^{-1}$) or white light (1.2 $Em^{-2}sec^{-1}$) at the 4th day after subculture in the dark. Dark cultured cells were used as a control. The data are mean values of five independent experiments. D: dark; WL: white light; UV-B: ultraviolet-B.

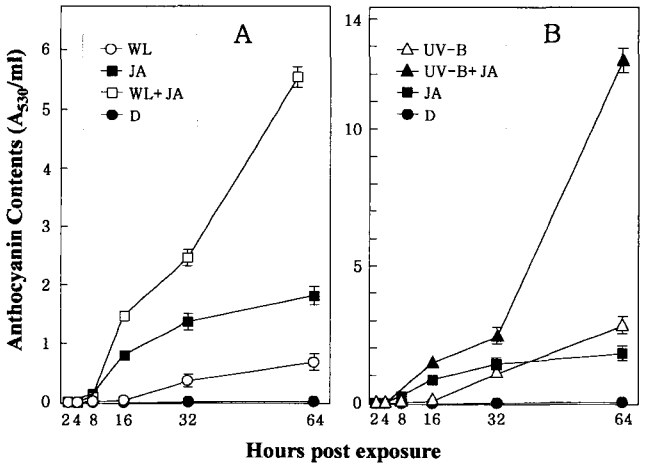

Figure 3. Time course of anthocyanin accumulation by treatment of WL, JA, WL + JA (A) and UV-B, UV-B + JA, JA (B). Cells grown for three days in the dark were treated with 5 μM JA or without JA, and cultured up to 64 h. D: dark; JA: jasmonic acid; UV-B: ultraviolet-B; WL: white light.

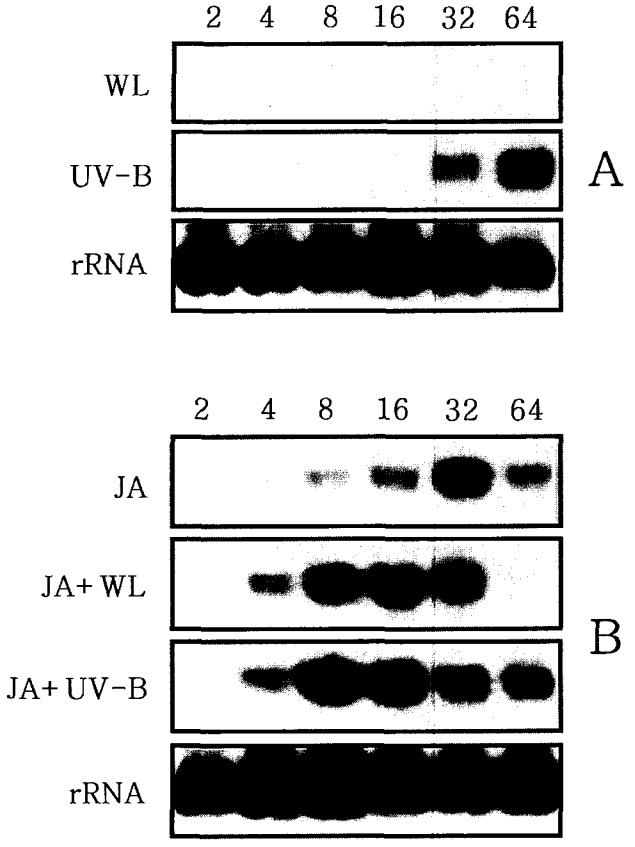

Figure 4. Northern blot analysis of CHS mRNA in grape cell suspension cultures in response to UV-B and WL (A), JA and simultaneous UV-B or WL exposure (B). Cells grown for three days in the dark were cultured for each time (h) and then harvested for total RNA extraction. The blots were hybridized with the CHS cDNA isolated from grape. WL: white light; UV-B: ultraviolet-B.

cell suspension cell was correlated with ANT-PAL for anthocyanin production at the log phase of growth. Whereas in suspension cell of alfalfa, Ni et al. (1996) reported that elicitor treatment rapidly activated PAL and CHS genes and increased to measurable transcription within 10-20 min after elicitation. Inducible plant defenses to UV light may be in part by alterations in JA levels (Conconi et al., 1996). Treatment of tomato leaves with UV-B or UV-C irradiation induced the accumulation of defense gene transcripts encoding the protease inhibitor Pin1 and Pin2. No accumulation of *Pin*1 or *Pin*2 mRNA, however, appeared treated with salicylic acid, a strong inhibitor of JA biosynthesis and its action (Doares et al., 1995). The UV-mediated induction of these genes was also blocked in *def*1, a tomato mutant with a defect in the JA biosynthetic pathway (Conconi et al., 1996).

The synergistical effects could be explained that some defense responses to UV irradiation are mediated in part by alterations in JA and its signaling pathway. Thus, JA treatment causes the enhanced induction of anthocyanin synthesis coupled by white light and UV-B.

Acknowledgments

This work was supported in part by a grant from the Korea Science and Engineering Foundation (Project No. 96-0401-02-01-3)

References

Beggs CJ, Stdzer-Jehle A, Wellman E (1985) Isoflavonoid formation as an indicator of UV stress in bean (*Phaseolus vulgaris* L.) leaves. Plant Physiol 79: 630-634

Beggs CJ, Wellmann E (1994) Photocontrol of flavonoid biosynthesis. In RE Kendrick, GHM Kronenberg, eds, Photomorphogenesis in plant, Ed 2. Kluwer Academic, Dordrecht, The Netherlands, pp 733-750

Britt AB, Chen JJ, Wykoff D, Mitchell D (1993) A UV-sensitive mutant of *Arabidopsis* defective in the repair of Pyrimidine Pyrimidinone(6-4) dimers. Science 261: 1571-1574

Caldwell MM, Robberecht R, Flint SD (1983) Internal filters: Prospects for UV-acclimation in higher plant. Physiol Plant 58: 445-450

Choi KS, In JG, Lee YB (1994) Effects of light on production of anthocyanin and betacyanin through cell suspension culture systems in *Vitis vinifera* L. and *Phytolacca americana* L. Korean J Plant Tissue Culture 22: 59-64

Conconi A, Smerdon MJ, Howe GA, Ryan CA (1996) The octadecanoid pathway in plants mediates a response to ultraviolet radiation. Nature 383: 827-829

Creelman RA, Mullet JE (1995a) Jasmonic acid distribution and action in plants: Regulation during development and response to biotic and abiotic stress. Proc Natl Acad Sci USA 92: 4114-4119

Creelman RA, Mullet JE (1997b) Oligosaccharins, brassinolides, and jasmonates: Nontraditional regulators of plant growth, development, and gene expression. Plant Cell 9: 1211-1223

Dixon RA (1986) The phytoalexins response: elicitation, signalling and control of host gene expression. Biol Rev 61: 239-291

Doares SH, Narvaez-Vasquez J, Conconi A, Ryan CA (1995) Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol 108: 1741-1746

Enyedi AJ, Yalpani N, Silverman P, Raskin I (1992) Signal molecules in systemic plant resistance to pathogens and pests. Cell 70: 879-886

Farmer EE, Ryan CA (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 4: 129-134

Franceschi VR, Grimes HD (1991) Induction of soybean vegetative storage proteins and anthocyanins by low-level atmosmopheric methyl jasmonate. Proc Natl Acad Sci USA 88: 6745-6749

Hahlbrock K, Scheele D (1989) Physiology and molecular biology of phenylpropanoid metabolism. Ann Rev Plant Physiol Plant Mol Biol 40: 347-364

Lamb CJ, Lawton MA, Dron M, Dixon RA (1989) Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell 56: 215-224

Landry LG, Chapple SCS, Last RL (1995) *Arabidopsis* mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiol 109: 1159-1166

Li J, Ou-Lee TM, Raba R, Amundson RG, Last RL (1993) *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation. Plant Cell 5: 171-179.

Middleton EM, Teramura AH (1994) Understanding photosynthesis, pigment and growth responses induced by UV-B and UV-A irradiances. Photochem Photobiol 60: 38-45

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15: 473-497

Ni W, Fahrendorf T, Ballance GM, Lamb CJ, Dixon RA (1996) Stress response in alfalfa (*Medicago sativa* L.). XX. Transcriptional activation of phenylpropanoid pathway genes in elicitor-induced cell suspension cultures. Plant Mol Biol 30: 427-438

Ozeki Y (1996) Regulation of anthocyanin synthesis in carrot suspension cultured cells. J Plant Res 109: 343-351

Pena-Cortes H, Fisahn J, Willmitzer L (1995) Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. Proc Natl Acad Sci USA 92: 4106-4113

Rhodes MJC (1994) Physiological roles for secondary metabolites in plants: Some progress, many outstanding problems. Plant Mol Biol 24: 1-20

Sparvoli F, Martin C, Scienza A, Gavazzi G, Tonelli C (1996) Cloning and molecular analysis of structural genes involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinifera* L.). Plant Mol Biol 24: 743-755

Tezuka T, Hotta T, Watanabe I (1993) Growth promotion of tomato and radish plants by solar UV radiation reaching the Earth's surface. Photochem Photobiol 19: 61-66