Australian Journal of Crop Science

AJCS

AJCS 6(9):1395-1400 (2012) ISSN:1835-2707

Transcriptome analysis in response to UV-B stress in soybean [*Glycine max* (L.) Merr.]

Jun-Cheol Moon[1,2], Won Cheol Yim[1], Jae-Eun Lee[3], Young-Up Kwon[3], Kitae Song[1] and Byung-Moo Lee[1*]

[1]Plant Molecular Genetic Lab., Department of Plant Biotechnology, Dongguk University-Seoul, Seoul, 100-715 Korea
[2]Agriculture and Life Science Research Institute, Kangwon National University, Chuncheon, 200-701 Korea
[3]National Institute of Crop Science, RDA, Suwon 441-857, Korea

[*]Corresponding author: bmlee@dongguk.edu

Abstract

In order to identify the genes specifically or predominantly expressed in UV-B illuminated soybean [*Glycine max* (L.) Merr.] leaves, this study compared the differential expression levels of the mRNA fragments of untreated leaves and UV-B treated leaves using differential banding patterns in agarose gels. In the UV-B illuminated soybean leaves, the differentially expressed band patterns were determined to be 207 up-regulated bands and 214 down-regulated bands. A total of 356 differentially expressed genes (DEGs) in the UV-B illuminated soybean leaves were sequenced. Using BLASTx, the putative functions of the expressed sequence tags were identified. Most of the identified UV-B-responsive genes related to other biotic and abiotic stresses. The characterized ESTs will provide useful data in developing our understanding of the molecular bases and transcript profiles of UV-B responses.

Keywords: Soybean; Differentially expressed genes; Transcript; UV-B stress.
Abbreviations: ACP-annealing control primer; DEGs-differentially expressed genes; ROS-reactive oxygen species; V5 stage- fifth trifoliolate stage.

Introduction

Soybean is one of the major world food crops, and can be summarized, in terms of its nutritional characteristics, as providing high yields of vegetable oil and protein. Other direct uses and applications of soybean have substantial economic importance in a wide range of industrial, food, pharmaceutical, and agricultural sectors. Soybean has been used in many studies investigation various stress effects and responses. The propagation of terrestrial plant life as we know it was possible through the formation of the stratospheric ozone layer, which absorbs ultraviolet-B (UV-B) radiation (280 – 320 nm) of sunlight. Initiating in the 20th century, anthropogenic releases of chlorofluorocarbons (CFCs) and a range of other pollutants have catalyzed the depletion of this ozone shield. As a consequence, terrestrial levels of UV-B radiation have since increased, and effect a range of deleterious effects upon many organisms. As photosynthesis requires the absorption of sunlight, plants are inevitably exposed to this damaging UV-B radiation. Evolutionary adaptations in response to UV-B radiation include protective measures, such as the accumulation of UV radiation-absorbing pigments (Stapleton and Valbot, 1994; Mazza et al., 2000; Bieza and Lois, 2001), as well as damage repair mechanisms, such as the use of UV-A photons by photolyases to reverse a subset of UV radiation induced DNA lesions (Britt, 1996). Due to its absorption spectrum, DNA is a major and long-studied target of UV-B damage. Even low doses of radiation have been shown to kill plant mutants that lack specific DNA repair pathways (Britt, 1996; Landry et al., 1997), indicating both the complexity and fragility of terrestrial plant life. UV-B radiation can also damage proteins and lipids. Recently, our understanding of molecular UV-B perception and signaling was significantly advanced by Jenkins (2009). This review involved UV-B signaling (Brown et al., 2005), UV-B cell death (Jiang et al., 2009), phytochrome-mediated photoprotection (Schafer and Bowler, 2002), and the multi-functional E3-ubiquitin ligases constitutively photomorphogenic 1 (COP1; Favory et al., 2009) and COP 10 (Yanagawa et al., 2004). When subjected to UV-B irradiation, plants display diverse morphological and physiological responses that are likely to involve multiple signal transduction cascades. Changes in intracellular calcium, calmodulin, serine/threonine kinases, and phosphatase activities have been implicated in the UV-B-mediated transcriptional activation of chalcone synthase, the first gene in the flavonoid sunscreen biosynthetic pathway (Christie and Jenkins, 1996; Frohnmeyer et al., 1997). The mechanism by which UV triggers intracellular signaling pathways in plant cells remains poorly understood. Candidate triggering molecules includes ROS such as singlet oxygen, hydrogen peroxide, superoxide anion, hydroxyl radicals, and perhydroxyl radicals, all of which are increased in response to UV and hence, may be key regulators of UV-induced signaling pathways (Mackerness et al., 2001). In various studies, responses to UV-B stress in terms of plant physiology, growth, and biomass have been investigated. Intraspecific responses in wheat (Li et al., 2000a; Li et al., 200b), soybeans (D’surney et al., 1993; Li et al., 2002), and rice (Barnes et al., 1993; Dai et al., 1994) have been reported.

Various plant species and even the variation of genotypes within species can differ greatly in their responses to UV-B. To understand the molecular basis of stress responses, the differentially expressed genes (DEGs) in stress-induced leaves must be identified. DEGs are only expressed at low levels and their transcripts are difficult to detect in the abundant mRNA within tissues. To detect DEGs transcribed at low levels, highly specific polymerase chain reaction (PCR) amplification is required. Novel annealing control primer (ACP)-based DD-PCR technology has been used to identify differentially expressed genes in tissues (Hwang et al. 2004). This method specifically targets sequence hybridization to the template via a polydeoxyinosine linker (Hwang et al. 2003). In the different progression stages of the stress response, investigations, the expression patterns of different gene pairs presented highly variable results, correlating negatively, positively, and showing no discernible correlation. This study was conducted to isolate and characterize the DEGs in response to UV-B using ACP-based differential display RT-PCR in soybean leaves. The putative functions of the DEGs using gene ontology (GO; Ashburner et al, 2000) were categorized. The characterized ESTs will serve as useful data to provide a better understanding of the molecular bases and transcript profiles of UV-B responses.

Results and discussion

Differentially expressed amplified cDNA products

To identify the genes specifically or predominantly expressed in UV-B treated soybean leaves, this study compared the differential expression levels of the mRNA fragments of treated leaves and untreated leaves, using differential banding patterns in agarose gels (Fig. 1). The differentially expressed gene (DEG) bands were detected in the untreated leaves (control) and UV-B treated leaves using a combination of 120 arbitrary primers and two anchored oligo (dT) primers from GeneFishing^{TM} DEG kits (Seegene, Korea). The PCR using ACP primer was performed twice in positive cases. A total of 421 DEGs were expressed in the UV-B treated soybean leaves (cv. Dawonkong and Jinpumkong). There were 205 up-regulated DEGs and 216 down-regulated DEGs (Table 1).

Identification of differentially expressed genes

The nucleotide sequences of the 356 DEGs in the UV-B illuminated treatments were analyzed, and their putative identification was conducted using BLASTx. As a result of homology searches, 144 DEGs (62.3%) of the UV-B responsive DEGs were matched to previously known genes with high sequence homologies. The sequenced clones of the ESTs from the UV-B treated soybean leaves included 231 unique clones (54.9%), which in turn consisted of 58 assembled sequences and 173 singletons. The sequences of 183 clones were assembled into 58 clusters, with a redundancy of 43.5% (Table 2). Transcript encoding proteins that are related to photosynthesis and CO_2 fixation, such as ribulose-1,5-bisphosphate carboxylase and proteins of both photosystems I and II were previously shown to decrease after UV-B radiation of adult leaves, and the down-regulation of photosynthetic proteins has also been documented in peas (Mackerness et al., 1997; Casati and Walbot, 2003). The DEGs responsive to UV-B included ribosomal protein genes that are regulated by direct

Table 1. Up- and down-regulated differentially expressed genes in UV-B treated soybean.

Cultivar / Regulated	Dawonkong (sensitive to UV-B)	Jinpumkong (insensitive to UV-B)
Up	116	89
Down	113	103

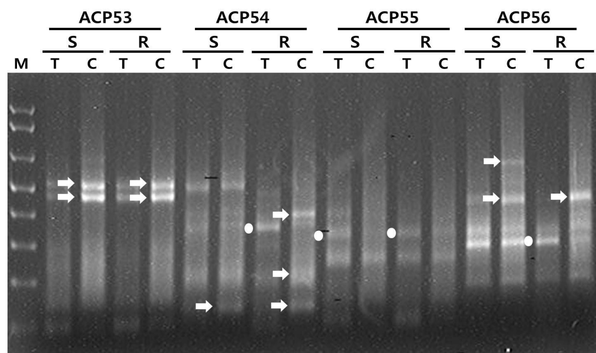

Fig 1. The bands of differentially expressed genes (DEGs) by UV-B treatment using the annealing control primer (ACP). ACP(53, 54, 55, 56): ACP series number, S: sensitive cultivar (cv. Dawonkong), R: insensitive cultivar (cv. Jinpumkong), T: UV-B treatment leaves, C: non-treatment leaves (control), M: 1 kb plus DNA ladder, white arrow: down regulated DEGs, white dot: up regulated DEGs.

exposure to UV-B in tissues. In previous studies, most of the genes regulated by UV-B were involved in translation (Casati and Walbot, 2003). Because RNA strongly absorbs UV photons, *in vitro* irradiation causes the formation of crosslinks in ribosomal RNA and between mRNA, tRNA, rRNA and proteins (Noah et al., 2000). The metallothionein-like proteins are down-regulated in response to UV-B treatment. In a previous report, the up-regulation of many genes encoding glycine-rich proteins, ABA and stress-induced protein, metallothionein-like proteins, glutathione S-transferase, and ascorbate peroxidase in salt-tolerant rice (Kawasaki et al., 2001). For the identified DEGs, 21 up-regulated DEGs and 33 down-regulated DEGs were overlapped in both sensitive and insensitive cultivars from the UV-B-treated leaves (Fig. 2). The overlapping up- and down-regulated genes were not determined in terms of their molecular function category in the Gene Ontology database. DNA binding protein, S1FA, and Ribulose-1,5-bisphosphate carboxylase small subunit were overlapped in the down-regulated DEGs. MSS4 (*Saccharomyces cerevisiae* protein) and metalloendorproteinase were overlapped in the up-regulated DEGs. For the UV-B responsive genes, chlorophyll a/b binding protein type III was overlapped in the up-regulated DEGs, and chlorophyll a/b binding protein type II was overlapped in the down-regulated DEGs. S1FA is an unusual small plant peptide of only 70 amino acids with a basic domain that contains a nuclear localization signal and a putative DNA binding helix. S1FA is highly conserved between dicotyledonous and monocotyledonous plants, and may be a DNA-binding protein that specifically recognizes the negative promoter element S1F. S1FA mRNA accumulates more in roots and etiolated seedlings than in green leaves (Zhou et al., 1995).

Functional classification of differentially expressed genes

The putative functions of the UV-B-regulated DEGs were categorized according to data from two databases: Gene Ontology and MIPS (suppl. Table 1). The functions fewer than half of the UV-B-responsive DEGs were determined. For their molecular functions, transporter activity was the major class in the UV-B-responsive DEGs of the insensitive cultivar, but catalytic activity was the major class in those of the sensitive cultivar. The major classes for biological processes were cellular and metabolic processes in the UV-B-responsive DEGs. Cytoplasmic parts of cellular components were the major category for the UV-B-responsive DEGs. The UV-B-responsive DEGs were distributed between all classes within the MIPS database (Fig. 3). The major classes of the up-regulated DEGs by UV-B-response included protein fate (30.2%), biogenesis of cellular components (28.0%), cellular communication/signal transduction mechanism (20.8%), protein with binding function of cofactor requirement (25.9%), metabolism (20.2%), cellular transport, transport facilities and transport routes (24.7%), and subcellular localization (25.4%). The other classes were distributed as unclassified proteins (34.7%), energy (10.1%), cell cycle and DNA processing (11.5%), transcription (13.1%), protein synthesis (10.9%), regulation of metabolism and protein function (11.2%), cell rescue, defense and virulence (5.8%), interaction with the environment (15.0%), transposable elements, viral and plasmid proteins (4.0%), and cell fate (8.2%). In the UV-B responsive down-regulated DEGs, biogenesis of cellular components (36.1%), protein with binding function of cofactor requirement (30.5%), energy (26.3%), and protein fate (24.8%) were the major classes. The other classes were distributed as unclassified proteins (34.2%), metabolism (16.5%), cell cycle and DNA processing (6.8%), transcription (17.0%), protein synthesis (13.2%), regulation of metabolism and protein function (16.1%), cellular transport, transport facilities and transport routes (18.2%), cellular communication/signal transduction mechanism (17.2%), cell rescue, defense and virulence (11.0%), interaction with the environment (4.2%), transposable elements, viral and plasmid proteins (0.6%), cell fate (8.1%), and subcellular localization (19.3%). Previously, a study reported that most of the UV-B regulated genes in maize are organ-specific (Casati and Walbot, 2004). The regulated genes in response to UV-B were widely distributed among the main functional categories of FunCat.

Table 2. Summary of ESTs from soybean leaves treated with UV-B.

	Number	Percentage by definition (%)[a]
Total clones	421	
Sequenced clones	356	84.6
Non-sequenced clones	65	15.4
Non-redundant sequence	231	54.9
Cluster[b]	58	
Redundancy of assembled clones	183	43.5
Singlet	173	
Blast match	144	62.3
Blast no-match	87	37.7

[a]Each number/total clones number x 100, [b]This was indicated the group of assembled clones.

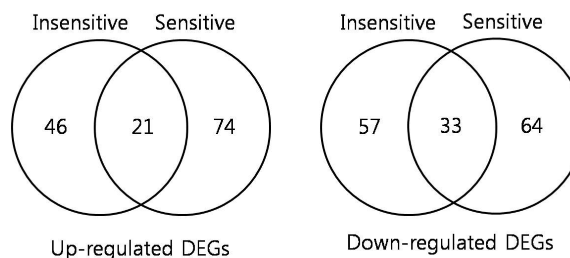

Fig 2. Distribution of the DEGs according to type of UV-B response. In UV-B treated soybean leaves, 21 of the up-regulated DEGs and 33 of the down-regulated DEGs were overlapped between sensitive and insensitive cultivars.

Materials and methods

Plant materials and UV-B treatment

Soybeans [*Glycine max* (L.) Merr] were grown in a greenhouse at the National Institute of Crop Science (NICS) in Korea, and UV-B stress testing was performed at the fifth trifoliolate (V5) stage. For UV-B treatment, sensitive and insensitive soybean cultivars were selected by according to previous reports (Lee et al., 2006). UV-B-sensitive (cv. Dawonkong) and UV-B-insensitive (cv. Jinpumkong) soybeans were illuminated with 10 kJ/m^2/day of UV-B for 4 h, on each of 5 consecutive days in a greenhouse under normal light conditions.

Total RNA extraction and annealing control primer (ACP)-based differential display

The total RNA was isolated from 200 mg of soybean leaves using TRIZOL reagent (Invitrogen), as instructed by the manufacturer, and was then employed in the synthesis of first-strand cDNA by reverse transcriptase, as previously described (Hwang et al., 2003). First-strand cDNA synthesis was performed using the dT-ACP1 primer (GeneFishing^{TM} DEG kits, Seegene, Korea) and the total RNA (2 µg) was incubated with 2 µl of dT-ACP1 (10 µM) at 80 °C for 3 min. Sebsequently, reverse transcription was performed for 1.5 h at 42 ℃ in 20 µl of final reaction buffer containing purified RNA, 4 µl of 5x first strand buffer (Invitrogen), 5 µl dNTPs (each 2 mM), 0.5 µl of RNasin Plus RNase Inhibitor (20 U/ µl, Promega), and 1 µl of SuperScript^{TM}II RNase H- Reverse Transcriptase (200 U/ µl, Invitrogen). First-strand cDNA samples were diluted by the addition of 80 µl DNase-free water. The diluted first-strand cDNAs were then subjected to second-strand cDNA synthesis by random PCR amplification using dT-ACP2 and one of 120 arbitrary ACPs (GeneFishing^{TM} DEG kits, Seegene, Korea) as primers. Second-strand cDNA synthesis was performed in final reaction volume of 20 µl containing diluted first-strand cDNA (50 ng), 2 µl of 5 µM arbitrary ACP primer, 1 µl of 10 µM dT-ACP2, and 10 µl of 2x SeeAmp^{TM}ACP^{TM}Master Mix (GeneFishing^{TM} DEG kits, Seegene, Korea). The PCR condition for second-strand synthesis included one cycle at 94 °C for 1 min, followed by 50 °C for 3 min, and 72 °C for 1 min. After the completion of second-strand DNA synthesis, 40 cycles were performed. Each cycle involved denaturation at 95 °C for 20 sec, annealing at 65 °C for 40 sec, extension at 72 °C for 40 sec, and a final extension step of 5 min at 72 °C to complete the reaction.

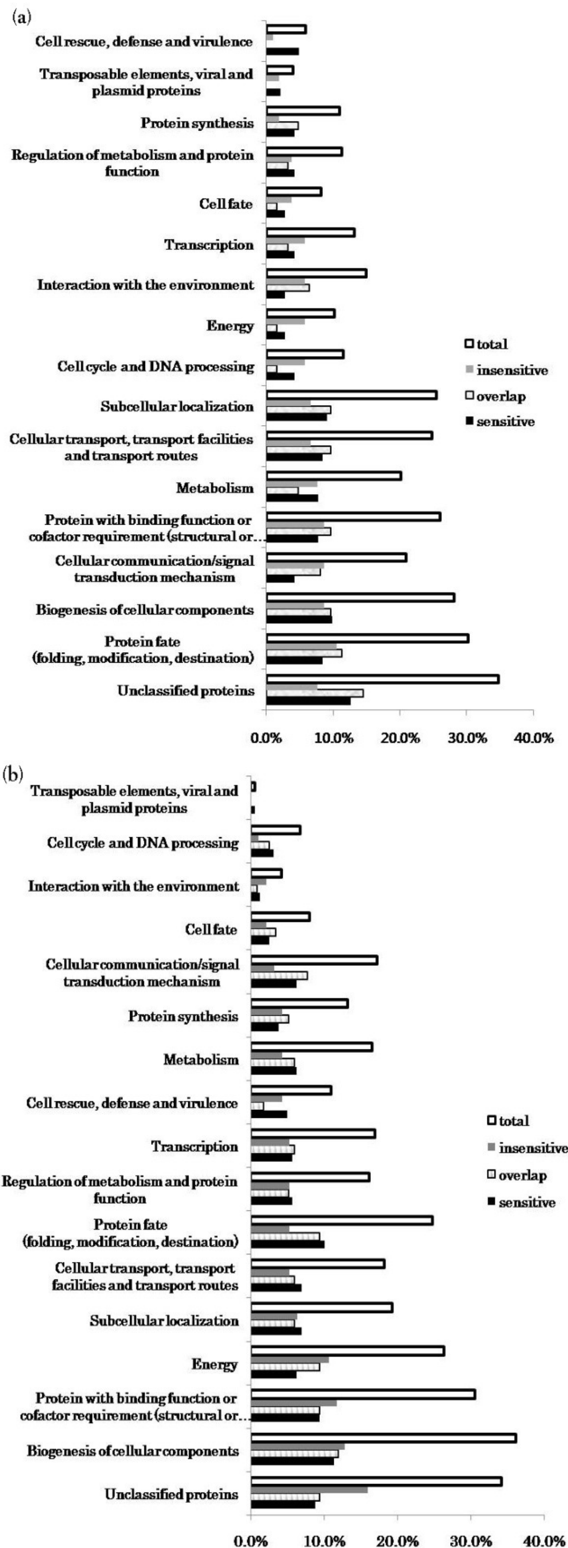

Fig 3. Functional distribution of the UV-B regulated DEGs. (a): the rate of up-regulated DEGs, (b): the rate of down-regulated DEGs. *X axis: the putative functional categories of the DEGs were determined using the MIPS database.

Amplified products were separated on 2% agarose gels, and stained with ethidium bromide. For ensure the accuracy of the experiment the ACP-based DD-PCR was performed two times using same primer.

Cloning, transformation and sequencing of the expressed sequence tags (ESTs)

The differentially expressed transcripts were eluted and cloned into a T&A cloning vector (Real Biotech Corp., Taiwan), as instructed by the manufacturer. The ligated vector was transformed into competent *E. coli* (strain: DH5α) cells. Transformed cells were plated onto LB agar plate containing 100 μg ml^{-1} ampicillin, 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and 80 μg ml^{-1} X-gal, and incubated at 37 ℃ overnight for blue/white screening. White colonies were grown overnight in LB liquid media supplemented with 100 μg ml^{-1} ampicillin and plasmid DNA was isolated by a commercial kit (Invitrogen, USA). To verify the identity of insert DNA, isolated plasmids were sequenced automatically. DNA sequencing was performed by dyeterminator cycle sequencing using the BigDyeTM Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, USA), followed by ethanol precipitation of the extension products. Both procedures were performed according to the manufacturer's instructions. The M13 primers were used to generate sequences for all isolated cDNAs.

Annotation of EST genes and function of soybean

The EST sequences were decoded to FASTA format by the PHRED program, using default parameters (Ewing et al., 1998). These sequences were filtered to remove contaminants such as vector and bed quality sequences using the LUCY2 tool (Li and Chou, 2004). The CAP3 program was used to cluster each EST so that they directly assembled to contig and siglet (Huang and Madan. 1999). The sequence homologies were determined by BLASTx of the NCBI nr database (http://www.ncbi.nlm.nih.gov), using the default options. Non-significant results were removed using a cut-off e-value of 10^{-4}. The functions were assigned using the Gene Ontology database (http://www.geneonotology. org) and the Munich Information Center for Protein Sequence (MIPS) database (http://mips.gsf.de/proj/funcatDB). All the EST data are publicly available through the NCBI, with GenBank dbEST accession nos. EV554905~ EV555198.

Conclusion

This study was conducted for the analysis of DEGs that were differentially expressed in response to UV-B illumination in soybean leaves. The categorization of DEGs expressed in response to UV-B was conducted based on putative identification using GO and MIPS databases. Many of the UV-B regulated DEGs were matched to previously known genes with high degree of confidence. Using Gene Ontology database, the category of transporter activity was found to be a major class of the UV-B regulated DEGs of insensitive cultivars, and catalytic activity a major class of sensitive cultivars in terms of their molecular function. Major classes of biological processes constituted those of cellular and metabolic processes in UV-B responded DEGs. Cytoplasmic part of cellular component was the most frequently observed association for UV-B responded DEGs. In the MIPS database, the major classes of up-regulated

DEGs in UV-B-response were protein fate, biogenesis of cellular components, cellular communication/signal transduction mechanism, protein with a binding function of cofactor requirement, metabolism, cellular transport, transport facilities and transport routes, as well as subcellular localization. Biogenesis of cellular components, protein with binding function of cofactor requirement, energy, and protein fate were the major classes in down-regulation of UV-B-responded DEGs. The results showed various functions for the DEGs by UV-B-response, and inferred that the DEGs might affect a vast number of cellular factors. This DEG profiling by UV-B treatment outlines the possible signaling pathways and molecular biological processes for future research. Investigations relating to the regulatory pathways that offer such diverse acclimation responses to UV-B, as well as the interrelationships between UV-B stress and other types of abiotic stress are the next step towards fully understanding past and current genetic modifications in response to changing environments.

Acknowledgment

This work was conducted with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project No. 20110401-030-612-001-03-00 (PJ907047)), Rural Development Administration, Republic of Korea.

References

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM and Sherlock G (2000) Gene ontology; tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25: 25-29.

Barnes PW, Maggard S, Holman SR and Vergara B (1993) Intraspecific variation in sensitivity to UV-B radiation in rice. Crop Sci 33: 1041-1046.

Bieza K and Lois R (2001) An *Arabidopsis* mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolic. Plant Physiol 126: 1105-1115.

Brown BA, Cloix C, Jiang GH, Kaiseril E, Herzyk P, Kliebenstein DJ and Jenkins GI (2005) A UV-B-specific signaling component orchestrates plant UV protection. P Natl Acad Sci USA 102: 18225-18230.

Britt AB (1996) DNA damage and repair in plants. Annu Rev Plant Phys 4: 75-100.

Casati P and Walbot V (2003) Gene expression profiling in response to ultraviolet radiation in *Zea mays* genotypes with varying flavonoid content. Plant Physiol 132: 1739-1754.

Casati P and Walbot V (2004) Rapid transcriptome responses of maize (*Zea mays*) to UV-B in irradiated and shielded tissues. Genome Biol 5: R16.

Christie JM and Jenkins GI (1996) Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in *Arabidopsis* cells. Plant Cell 8: 1555-1567.

Dai QJ, Peng SB, Chavez AQ and Vergara BS (1994) Intraspecific responses of 188 rice cultivars to enhanced UV-B radiation. Environ. Exp. Bot. 34: 422-433.

D'surney SJ, Tschaplinski TJ, Edwards NT and Shugart LR (1993) Biological responses of two soybean cultivars exposed to enhanced UV-B radiation. Environ Exp Bot 46: 40-52.

Ewing B and Green P (1998) Basecalling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8: 186-194.

Favory JJ, Sec A, Gruber H, Rizzini L, Oravecz A, Funk M, Albert A, Cloix C, Jenkins GI, Oakeley EJ, Seidlitz HK, Nagy F and Ulm R (2009) Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis. EMBO J 28: 591-601.

Frohnmeyer H, Bowler C and Schafer E (1997) Evidence for some signal transduction elements involved in UV-light dependent responses in parsley protoplasts. J Exp. Bot. 48: 739-750.

Huang X and Madan A (1999) CAP3: A DNA sequence assembly program. Genome Res 9: 868-877.

Hwang IT, Kim YJ, Kim SH, Kwak CI, Gu YY and Chun JY (2003) Annealing control primer system for improving specificity of PCR amplification. BioTechniques 35: 1180-1184.

Hwang K-C, Cui X-S, Park S-P, Shin M-R, Park S-Y, Kim E-Y and Kim N-H (2004) Identification of differentially regulated genes in bovine blastocysts using an annealing control primer system. Mol Reprod Dev 69:43-51.

Jenkins KI (2009) Signal transduction in responses to UV-B radiation. Annu Rev Plant Biol 60: 407-431.

Jiang L, Wang Y, Bjorn LO and Li SS (2009) Arabiopsis RADICAL-INDUCED CELL DEATH1 is involved in UV-B signaling. Photoch Photobio Sci 8: 838-846.

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D and Bohnert HJ (2001) Gene expression profiles during the initial phase of salt stress in rice. Plant Cell 13: 889-906.

Landry LG, Stapleton AE, Lim J, Hoffman P, Hays JB, Walbot V and Last RL (1997) An *Arabidopsis* photolyase mutant is hypersensitive to ultraviolet-B radiation. P Natl Acad Sci USA 94: 328-332.

Lee J-E, Kwon Y-U, Lee C-K, Shin J-C and Lee B-M (2006) Physiological response of soybean to enhanced ultraviolet-B radiation. Korean J Crop Sci 52 (suppl. 1) PB-23.

Li S and Chou HH (2004) LUCY2: an interactive DNA sequence quality trimming and vector removal tool. Bioinformatics 20: 2865-2866.

Li Y, Zu YQ, Chen HY, Chen JJ, Yang JL and Hu ZD (2000a) Intraspecific responses in crop growth and yield of 20 wheat cultivars to enhanced ultraviolet-B radiation under field conditions. Field Crop Res 67: 25-33.

Li Y, Zu YQ, Chen JJ, Chen HY, Yang JL and Hu ZD (2000b) Intraspecific differences in physiological responses of 20 wheat cultivars to enhanced ultraviolet-B radiation under field conditions. Environ Exp Bot 44: 95-103.

Li Y, Zu YQ, Chen JJ and Chen HY (2002) Intraspecific responses in crop growth and yield of 20 soybean cultivars to enhanced ultraviolet-B radiation under field conditions. Field Crops Res 78: 1-8.

Mackerness SA, John CF, Jordan BR and Thomas B (2001) Early signaling components in ultraviolet-B responses: distinct roles for different reactive oxygen species and nitric oxide. FEBS Lett 489: 237-242.

Mackerness SA, Tomas HB and Jordan BR (1997) The effect of supplementary ultraviolet-B radiation on transcripts, translation and stability of chloroplast proteins and pigment formation in *Pisum sativum* L. J Exp Bot 48: 729-738.

Mazza CA, Boccalandro HE, Giordano CV, Battista D, Scopel AL and Ballaré CL (2000) Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. Plant Physiol 122: 117-125.

Noah JW, Shapkina T and Wollenzien P (2000) UV-induced crosslinks in the 16S rRNAs of *Excherichia coli*. *Bacillus subtillis* and *Thermus aquaticus* and their implications for ribosome structure and photochemistry. Nucleic Acids Res 28: 3785-3792.

Schafer E and Bowler C (2002) Phytochrome-mediated photoperception and signal transduction in higher plants. EMBO Rep 3: 1042-1048.

Stapleton AE and Walbot V (1994) Flavonoids can protect maize DNA from the induction of ultraviolet-radiation damage. Plant Physiol 105: 881-889.

Yanagawa Y, Sullivan JA, Komatsu S, Gusmaroli G, Suzuki G, Yin J, Ishibashi T, Saijo Y, Rubio V, Kimura S, Wang J and Deng XW (2004) Arabidopsis COP10 forms a complex with DDB1 and DET1 in vivo and enhances the activity of ubiquitin conjugation enzymes. Gene Dev 18: 2172-2181.

Zhou DX, Cordelia BS and Régis M (1995) Molecular cloning of a small DNA binding protein with specificity for a tissue-specific negative element within the *rps*1 promoter. Nucleic Acids Res 23: 1165-1169.