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

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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 investigating 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 Vallbot, 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 include 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., 2000b), 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™ 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 BLASTXs. 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 CO2 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 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, SIF1, and Ribulose-1,5-bisphosphate carboxylase small subunit were overlapped in the down-regulated DEGs. MSS4 (Saccharomyces cerevisiae protein) and metalloendoproteinase 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. SIF1 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. SIF1 is highly conserved between dicotyledonous and monocotyledonous plants, and may be a DNA-binding protein that specifically recognizes the negative promoter element SI1. SIF1 mRNA accumulates more in roots and etiolated seedlings than in green leaves (Zhou et al., 1995).

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

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Dawonkong (sensitive to UV-B)</th>
<th>Jinpumkong (insensitive to UV-B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulated</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td></td>
<td>116</td>
<td>113</td>
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<tr>
<td></td>
<td>89</td>
<td>103</td>
</tr>
</tbody>
</table>

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.
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 (28.8%), 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 (34.2%), metabolism (16.5%), 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).

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 trifoliate (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/m²/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™ 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. Subsequently, reverse transcription was performed for 1.5 h at 42 °C 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™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™ 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™ACP™Master Mix (GeneFishing™ 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.

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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.
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.

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References


