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Identification of *Glycine max* Genes Expressed in Response to *Soybean mosaic virus* Infection

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Identification of host genes involved in disease progresses and/or defense responses is one of the most critical steps leading to the elucidation of disease resistance mechanisms in plants. *Soybean mosaic virus* (SMV) is one of the most prevalent pathogen of soybean (*Glycine max*). Although the soybeans are placed one of many important crops, relatively little is known about defense mechanism. In order to obtain host genes involved in SMV disease progress and host defense especially for virus resistance, two different cloning strategies (DD RT-PCR and Subtractive hybridization) were employed to identify pathogenesis- and defense-related genes (PRs and DRs) from susceptible (Geumjeong 1) and resistant (Geumjeong 2) cultivars against SMV strain G7H. Using these approaches, we obtained 570 genes that expressed differentially during SMV infection processes. Based upon sequence analyses, differentially expressed host genes were classified into five groups, i.e. metabolism, genetic information processing, environmental information processing, cellular processes and unclassified group. A total of 11 differentially expressed genes including protein kinase, transcription factor, other potential signaling components and resistant-like gene involved in host defense response were selected to further characterize and determine expression profiles of each selected gene. Functional characterization of these genes will likely facilitate the elucidation of defense signal transduction and biological function in SMV-infected soybean plants.

Keywords : defense, differential expression, EST analysis, pathogenesis, SMV

Plants have developed complicated defense mechanism during evolution to resist the harmful pathogens they have encountered. The interaction between a plant and a pathogen often complies with gene-for-gene model involving plant resistance (*R*) gene and corresponding pathogen avirulence (*Avr*) genes (Flor, 1971). These involve changes in ion influx, generation of reactive oxygen species, production of pathogenesis-related (PR) proteins and phytoalexins, and reinforcement of the cell wall (Dixon and Lamb, 1990; Dixon et al., 1994). Induction of defense response involves an initial signal that indicating invasion by a pathogen, followed by transduction of signals activating various defense genes (Yang et al., 1997). When plants recognize the products of *Avr* genes directly or indirectly from a pathogen, the defense-related gene (DR) expression is induced and the result is resistance to pathogen attack (Keen, 1990; Staskawicz, 2001). However, when plants lack *R* genes or their products, the plant display characteristics of disease. Based on the advancements of host-pathogen interaction at the molecular level, disease resistance genes are useful for disease control strategies in agriculture.

Soybean mosaic virus (SMV), a member of the genus *Potyvirus* (Mayo and Pringle, 1998), is one of the most economically important viruses of soybean [*Glycine max* (L.) Merr]. It cause mosaic and severe necrosis in many soybean cultivars, and is present in all major soybean-growing areas, where it results in significant reductions in yield and quality. Since the first description of seven groups of SMV strains (G1-G7) based on the symptoms developed on test cultivars, viral strain variation and the resistance patterns of different soybean cultivars have been studied extensively (Cho and Goodman, 1979, 1982; Gunduz et al., 2001, 2002; Lim, 1985). Presence of many SMV strains has been reported and the continual variation is generally believed to happen in soybean fields in Korea (Cho et al., 1983). Recently, a new SMV strain, G7H, causing mosaic and necrosis on most recommended soybean cultivars that are resistant to previously prevalent SMV strains, G5 and G5H, was found in Korea (Kim et al., 2003; Lim et al., 2003).

To date, the majority of research in defense signal transduction has been performed on several model species such as tobacco and *Arabidopsis*, whereas in economically important soybean crop, our understanding of the signaling mechanisms leading to disease resistance is largely unknown (Piffanelli et al., 1999). Most characterized *R* genes can be

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classified into five classes based on their structural features and the majority fell into the TIR-NBS-LRR class (Ellis et al., 2000; Hammod-Kosack and Jones, 1997). This structural specificity makes it possible to isolate potential *R* genes by the homology-based cloning technique (Leister et al., 1996). Several cases of *R* gene clusters originally described by classical mapping have now been confirmed by sequence analysis of genomic DNA (Martin et al., 1993; Song et al., 1995; Whitham et al., 1994). As for SMV resistance genes, three SMV *R* gene loci (*Rsv1*, *Rsv3*, *Rsv4*) have been reported. *Rsv1* has been mapped in the soybean MLG F, *Rsv3* on the MLG B2 and *Rsv4* on the MLG D1b (Hayes et al., 2000; Jeong et al., 2002; Yu et al., 1994).

To obtain host genes involved in SMV disease progress and host defense especially for virus resistance, we screened 40 different soybean cultivars by inoculating highly virulent SMV strain G7H and have obtained at least three resistant soybean cultivars including Geumjeong 2, Purunkong, and Myeongjunamulkong. Differential display (DD) RT-PCR and PCR-based subtractive hybridization techniques have been developed as a tool to detect and characterize altered gene expression and were applied to the study of plant-pathogen interactions (Carginale et al., 2004; Diatchenko et al., 1996; Jagoueix-Eveillard et al., 2001; Liang and Pardee, 1998). In this study, DD RT-PCR and PCR-based subtractive hybridization were employed to identify PR and DR genes from soybean seedlings using both susceptible (Geumjeong 1) and resistant (Geumjeong 2) cultivars against SMV-G7H infection and consider their potential role in the disease progresses and host defense responses.

Materials and Methods

Plant materials, plant infection and RNA extraction. Seeds of the soybean cultivars Geumjeong 1 and Geumjeong 2 were grown in the greenhouse or in pots filled with vermiculite. For virus inoculation, each leaves of 10-days soybean seedling was gently rubbed with carborundum and inoculated with a suspension of the SMV strain G7H by using a soft brush. Upper uninoculated leaves of SMV-G7H-infected soybean plants were harvested and stored at −70°C for RNA isolation at 7 days post-inoculation (dpi). Total RNAs were extracted using TriZol™ reagent (Giboco BRL, USA) according to the manufacture's protocol. Total RNAs were treated with DNase I (TaKaRa, Japan) to remove the genomic DNA contamination.

Differential display (DD) RT-PCR. Reverse-transcription was performed with 1-2 μg of total RNA in reaction volume of 50 μL containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 25 mM each dNTP, 10 pmol anchor primer, and 0.5 units M-MuLV Reverse Transcriptase (New English BioLabs, USA). Differentially expressed host genes were amplified subsequently with three one-base-anchored oligo-dT primers 5'-T11M, where M may be A, C or G, in combination with 26 different arbitrary 10 base primers (Table 1) using *Taq* DNA polymerase (TaKaRa, Japan) with [α-^{32}P] dCTP. Reactions were performed on a BioRad thermocycler using the following parameters: 5 minutes at 94°C, 15 seconds at 94°C, 30 seconds at 42°C, 15 seconds at 72°C for 30 cycles; 15 seconds at 94°C, 30 seconds at 42°C, 2 minutes at 72°C for 1 cycle. PCR products were separated on a 6% denaturing polyacrylamide gel and exposed to X-ray film. Differentially expressed PCR products larger than 200bp were cut from the dried gel, reamplified using appropriate primers, and cloned into a pGEM-T easy vector (Promega, USA) for sequence analysis.

PCR-based subtractive hybridization and cDNA library construction. Susceptible (Geumjeong 1) and resistant (Geumjeong 2) cultivars were used for PCR-based subtractive hybridization. Poly (A)+ RNAs were purified

Table 1. Primers used in DD RT-PCR and their nucleotide sequences

Primer designation	Sequence (5' to 3')
1	TACAACGAGG
2	TGGATTGGTC
3	CTTTCTACCC
4	TTTTGGCTCC
5	GGAACCAATC
6	AAACTCCGTC
7	TCGATACAGG
8	TGGTAAAGGG
9	TCGGTCATAG
10	GGTACTAAGG
11	TACCTAAGCG
12	CTGCTTGATG
13	GTTTTCGCAG
14	GATCAAGTCC
15	GATCCAGTAC
16	GATCACGTAC
17	GATCTGACAC
18	GATCTCAGAC
19	GATCATAGCC
20	GATCAATCGC
21	GATCTAACCG
22	GATCGCATTG
23	GATCTGACTG
24	GATCATGGTC
25	GATCATAGCG
26	GATCTAAGGC

with oligo (dT)-cellulose column (Qiagen, USA) using total RNA isolated from these two cultivars according to the manufacture's protocol. Double stranded cDNAs were synthesized using oligo $(dT)_{18}$ oligonucleotide primer. The synthesized cDNAs were completely digested with *Rsa*I and subjected to PCR-based subtractive hybridization by the standard protocol (Clontech, USA). cDNAs obtained from Geumjeong 1 and Geumjeong 2 were used as driver and tester, respectively. The subtracted cDNAs were finally cloned into pGEM-T easy vector (Promega, USA) and subjected to further analysis.

Northern blot analysis. About 20 μg of total RNA were electrophoretically separated on a 1.2% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0), and denatured by soaking the gel in 50 mM NaOH for 30 min at room temperature. Gels were neutralized with 1.5 M Tris-HCl (pH 7.5) containing 0.5 M NaCl for 20 min. RNAs were then transferred to a nylon membrane (Sigma, USA) by capillary blotting with 2X SSC (0.3 M NaCl, 30 mM sodium citrate, pH 7.0) for 10 min and cross-linked to the membrane by UV irradiation for 1 min. DNA probes were synthesized and labeled with [α-^{32}P]dCTP and denatured by boiling for 5 min and cooling for 5 min on ice. Prehybridization was performed for 34 hr at 42°C in hybridization buffer (50% formamide, 1X Denhart's solution, 5X SSC, 1.0% SDS, and 0.1 mg/ml denatured salmon sperm DNA). Hybridization was performed with the radioactive labeled probes for 14 hr at 42°C in hybridization buffer. Membrane was washed twice in 2X SSC for 5 min at room temperature, once in 1X SSC for 20 min at room temperature and exposed to X-ray film for autoradiography.

Subcloning, sequencing and sequence analysis. DNA sequencing for the differentially expressed cDNA clones and subtracted cDNA clones in pGEM-T vector were performed with the BigDye Terminator Cycle Sequencing Kits (PE Biosystems, USA) using an automated DNA sequencer (ABI Prism 3700 Genetic Analyzer, Perkin Elmer, USA) at the NICEM (SNU). Primers used for sequencing were M13 F (5'-TGTAAAACGACCGC-CAGT-3') and M13 R (5'-AACAGCTATGACCATG-3'). DNA sequences and deduced amino acids were analyzed using the LaserGene program (DNASTAR, USA). The sequences were analyzed with the Blast programs provided by the NCBI (http://www.ncbi.nlm.nih.gov) and further compared with sequences deposited in public database (CCGB, Soybean EST Genbank; http://soybean.ccgb.umn.edu/) that includes the annotated cDNAs of soybean plants. Gene structure identification of confirmed sequences of selected genes was conducted by using Pfam protein search and SMART (http://smart.embl-heidelberg.de/). Sequenced host cDNA frgments were classified based upon KEGG orthology (http://www.genome.ad.jp).

Results

Symptom development on susceptible and resistant soybean cultivars. To identify soybean genes that are up- or down-regulated during SMV-G7H infection on susceptible and resistant soybean cultivars, Geumjeong 1 and Geumjeong 2 that are susceptible and resistant to SMV-G7H infection, respectively, were selected and inoculated with SMV strain G7H. As shown in Fig. 1, SMV-G7H caused severe mosaic, mottling and necrosis on the leaves of Geumjeong 1. In contrast, no visible symptom was observed on the leaves of Geumjeong 2 for two weeks post-inoculation (Fig. 1). No viral cDNA was amplified when RT-PCR was conducted with total RNAs extracted from inoculated leaves of Geumjeong 2 using SMV CP-specific oligonucleotide primers (Lim et al., 2003; data not shown). These results confirm that Geumjeong 1 is truly susceptible and Geumjeong 2 is truly resistant against SMV-G7H infection.

Screening and identification of differentially expressed genes in SMV-G7H infected soybean leaves. In order to identify genes that are differentially regulated by SMV-G7H infection, the DD RT-PCR and PCR-based subtracted hybridization techniques were used to compare mRNA expression pattern. Total RNAs were extracted from upper un-inoculated leaves of control and SMV-G7H infected plants of both susceptible and resistant soybean plants at 7

Fig. 1. Soybean plants inoculated with SMV-G7H (**C** and **D**). Severe mosaic symptom was observed on susceptible (Geumjeong 1) soybean cultivar (**C**) while no distinct symptom was observed on resistant (Geumjeong 2) cultivar (**D**). Panels **A** and **B** represent healthy Geumjeong 1 and Geumjeong 2, respectively.

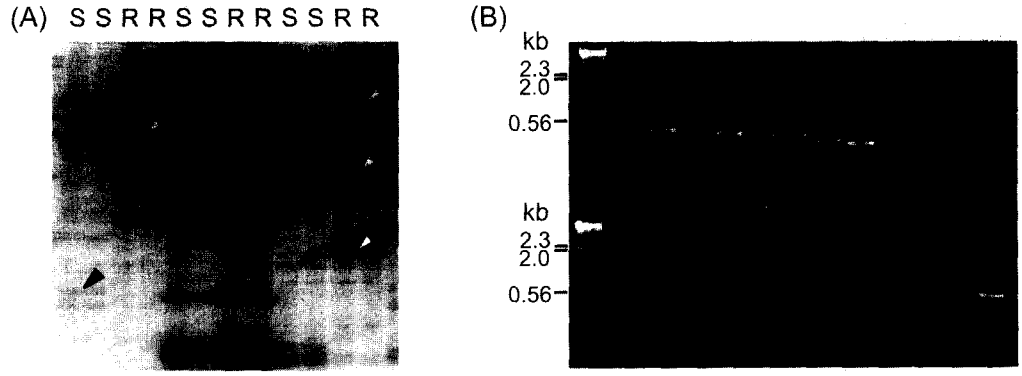

Fig. 2. A typical differential display gel and cloning of the differentially expressed cDNAs. **A**, Differential display using $T_{11}VG$ and primers #15, 16, and 17 were shown. Amplified cDNA fragments from differential display were separated by electrophoresis in 6% denaturing polyacrylanide gels. Two different total RNA samples inoculated with SMV were used for each reaction (S and R). S and R represent soybean cultivars Geumjeong 1 and Geumjeong 2, respectively. Open and closed arrowheads indicate selected cDNAs for further molecular analysis. **B**, The selected cDNAs from differential display were reamplified and cloned into pGEM-T easy vector. The first lane represent DNA molecular mass marker λ-DNA digested with *Hin*dIII.

dpi. At this time point, inoculated leaves of Geumjeong 1 usually start to show mild mosaic symptom while no distinct symptom on upper uninoculated leaves. Therefore, total RNAs extracted from upper uninoculated leaves would contain RNA transcripts likely to be enriched with genes during early stages of virus infection.

A total of 570 clones (96 clones from PCR-based subtracted hybridization and 474 clones from DD RT-PCR approaches) were selected and sequenced. To prevent isolation of false positive, all amplification experiments were performed on two different total RNAs extracted from separately inoculated plants. In DD RT-PCR, the identified DD RT-generated cDNAs were cut out of the differential display gels and reamplified (Fig. 2). The average sizes of cDNAs of obtained from Subtracted hybridization and DD RT-PCR were approximately 300 bp to 650 bp, indicating that the cDNAs contain enough information for sequence analysis. The nucleotide sequences of the differentially expressed cDNA clones were determined and were searched using the BLAST program (Altschul et al., 1997) and further compared with sequences deposited in public database (CCGB, Soybean EST GenBank; http://soybean.ccgb.umn.edu/) that includes the annotated cDNAs of soybean plants. These searches revealed that about 70% of EST clones were highly homologous to known genes in databases. Redundancy and GenBank homologues of the 10 most abundant EST sequences were summarized in Table 2.

Functional classification of genes. All sequences with matches to known genes or proteins were assigned to one of five functional classes: metabolism, genetic information processing, environmental information processing, cellular processes and unclassified group according to the KEGG Orthology (http://www.genome.ad.jp). Metabolism function includes carbohydrate metabolism, energy metabolism, biosynthesis of secondary metabolism and amino acid metabolism. Genetic information includes transcription, translation, sorting and degradation. Cellular process is

Table 2. Redundancy and GenBank homologues of the 10 most abundant ESTs obtained from SMV infected soybean plants

cDNA Clones	Length (bp)	Functional association	Redundancy
SS1-16	612	Actin (*Solanum tuberosum*)	8
SS13	450	Glycosyl hydrolase family 79 (*Arabidopsis thaliana*)	22
SS24	465	Calcium-dependent protein (*Glycine max*)	5
SS3-10	618	Protein phosphatase 2A (*Fagus sylvatica*)	5
SS3-11	431	Protein phosphatase 2C (*Medicago sativa*)	5
SS1-42	397	Putative histone H2A (*Oryza sativa*)	9
SS1-4	278	Unknown/Hypothetical protein (*A. thaliana*)	19
DSA15-4	325	Senescence-associated protein (*P. sativum*)	8
DSA21-3	228	bZIP transcription factor family (*A. thaliana*)	8
DSG19	222	DNA-binding bromodomain protein (*A. thaliana*)	21

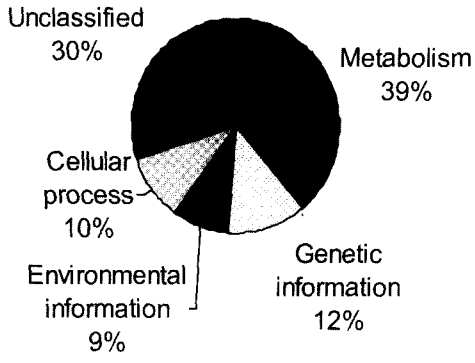

Fig. 3. Functional classification of random 570 soybean ESTs with putative biological function obtained from the KEGG Orthology (http://www.genome.ad.jp). The percentage frequency of clones in each category for the total set of random ESTs from DD RT-PCR and subtractive hybridization is shown.

related cell growth and death, cell motility and development. Environmental information processing is related membrane transport, signal transduction and ligand-receptor interaction. Unclassified group is related mostly unknown genes. In this manner, we created a functional inventory of all the ESTs in our database, which represent a partial catalog of differentially expressed genes during SMV infection processes. The most prevalent EST class was metabolism (223 ESTs, 39%), followed by genetic information processing (70 ESTs, 12%), cellular processes (57 ESTs, 10%), and environmental information processing (54 ESTs, 9%). In the analysis, all sequences with e-value $>10^{-5}$ were placed in the unknown function category (Fig. 3). This resulted to about 30% (166 ESTs) of the sequences that could not be classified.

Characterization and homologies of 12 selected soybean genes. The deduced amino acid sequences from nucleotide sequences of each EST clones were applied to BLASTX analyses to examine their homology with functional genes in the GenBank database. Several clones contained significant sequence homology to genes with interesting functions such as defender against cell death, drought induced protein, and calcium-dependent protein kinase. We arbitrarily selected 11 genes that expressed more in the resistant soybean cultivar and thus might be involved in defense responses (Table 3). We obtained full-length cDNA clones of the selected 11 genes by using BLASTX, CCGB analyses (Nadim et al., 2004), and 5' & 3' RACE approaches (TaKaRa, Japan). The amino acid sequence was deduced from nucleotide sequence and used for amino acid similarity analysis. The BLASTX analysis of the amino acid sequences of each gene led to identification of leucine-rich repeat transmembrane protein kinase, defender against cell death 1 (DAD1), dehydration stress-induced protein, drought induced protein, putative histone H2A, malate dehydrogenase, plastic aldolase NPALDP 1, oxygen-evolving enhancer protein 1, putative TFIIIA-like zink finger protein, and two unknown proteins (Table 3). Homology of genes with previously identified genes of known function was over 70% (Fig. 4).

Northern analysis of the selected genes. Northern blot analysis was performed to confirm that the differentially expressed cDNAs represented changes of mRNAs levels upon SMV infection. Increase in the accumulation of the mRNA transcripts for putative H2A, DAD1, and one hypothetical protein of unknown function was observed in the SMV-G7H infected resistant soybean than SMV-G7H infected susceptible soybean (Fig. 5). Hence, these genes were expressed differentially in SMV infection. Similar results were obtained for the other selected genes (data not shown).

Table 3. Characteristics of cloned cDNAs fragment identified by DDRT-PCR and subtracted hybridization

cDNA clones	Length (bp)	Description & accesion #[a]	E-score
DSA22	545bp	Leucine-rich repeat transmembrane protein kinase (*Arabidopsis thaliana*, NP_172244)	5e-68
DSG65	362bp	Defender against cell death 1(DAD1) (*A. thaliana*, AAC77357)	9e-51
SS15	132bp	Dehydration stress-induced protein (*A. thaliana*, AAM62648)	2e-57
SS17	619bp	Aquaporin/Drought-induced kidney (*Glycine max*, CAA11025)	1e-129
SS5	399bp	Putative H2A protein (*Oryza sativa*, AAM64788)	7e-52
SS4	568bp	Unknown protein (*A. thaliana*, AAM63262)	4e-85
SS25	529bp	Hypothetical protein (*A. thaliana*, XP_479022)	1e-16
SS22	768bp	Malate dehydrogenase (*Fagus sativa*, CAB43995)	1e-172
SS9	188bp	Plastic aldolase NPALDP1 (*N. paniculata*, BAA77603)	0
SS49	468bp	Oxygen-evolving enhancer protein1 (*A. thaliana*, P26320)	e-154
DSG57	518bp	Putative TFIIIA-like zinc finger protein (*M. sativa*, CAB77055)	2e-74

[a] Homology search was conducted with the NCBI BLAST program. The closest homology with its origin and accession number is listed for each clone. The identity is the comparison of the deduced amino acid sequences of the selected ESTs.

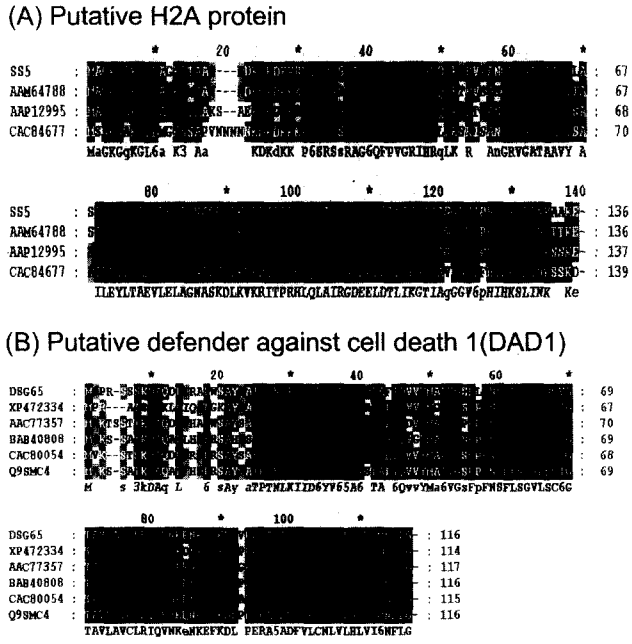

Fig. 4. Multiple alignments of amino acid sequences of selected clones, SS5 (**A**) and DSG65 (**B**). Numbers on top represent the deduced amino acid position of each protein. The amino acid sequence of the SS5 was aligned with proteins of *A. thaliana* (AAM64788), *O. sativa* (AAT12995), and *P. pinaster* (CAC84677) and DSG65 was aligned with a protein of *A. thaliana* (CAC80054), *O. sativa* (XP472334), *Pisum* (AAC77357), *Lycopersicon* (Q9SMC4), and *N. tabacum* (BAB40808).

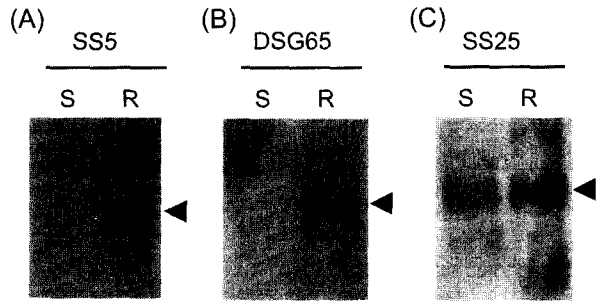

Fig. 5. Northern blot analysis of total RNAs extracted from SMV-infected Geumjeong 1 (susceptible, S) and Geumjeong 2 (resistant, R) soybean cultivars. Panels A to C represent blots probed with the insert of cDNA SS5, DSG65, and SS25, respectively. Arrowheads indicate the location of corresponding mRNA.

Discussion

It has been suggested that there are more than 63,000 genes in the soybean genome, which is higher than that of rice and tomato (van der Hoeven et al., 2003). Only a few defense-related or resistance genes, however, have been revealed on soybean (Graham et al., 2002; Kanazin et al., 1996) and the molecular mechanisms involved in the interaction between SMV and its host plant are largely unknown. The random sequencing of ESTs has been shown to be a robust approach for rapid, large-scale, identification of host genes involved in virus-host interactions (Andrejeva et al., 1999). A similar technology was employed in this study for the identification of a number of soybean genes whose expression is altered following SMV infection. Using the sensitive nature of disease resistance in soybean, we have observed remarkable changes in expression of diverse genes during the defense responses. We isolated 570 ESTs that differentially expressed during SMV infection and these include various genes involved in diverse primary and secondary metabolisms during the virus infection processes. We arbitrarily selected 11 genes that might be involved in defense responses. These selected genes were appeared to be differentially expressed as confirmed by Northern blot analysis. Full-length ORF sequence analysis revealed strong homologies to the previously reported genes of known functions of ten selected genes. Although sequences of most ESTs are incomplete and annotation inaccuracies exist, EST analyses are still powerful tool for identification of genes involved in virus-host interactions. Our data show that many soybean genes are differentially expressed in virus infected leaves and also show that DD RT-PCR and PCR-based subtracted hybridization techniques could be used for efficient identification of plant genes affected by virus infection. In addition, a significant proportion (30%) of the EST sequences had no match or homology with genes of known function in the database. This further highlights the lack of information on differentially expressed genes during virus infection.

A variety of genes including genes classified in metabolism, genetic information processing, environmental information processing, and cellular processes that probably involved in disease progresses and/or defense responses against SMV infection were obtained. Pathogen defense entails a major shift in metabolic activity rather than altering a few defense-related genes (Rushton and Somssich, 1998). The induction of genes that contain receptor-like serine/threonine kinase (Leucine rich repeat family kinase, LRR domain), however, has been previously described in plant treated with either elicitors or an incompatible pathogen (Ellis, 2000). Therefore, we selected 11 host genes including leucine-rich repeat transmembrane protein kinase, DAD1, dehydration stress-induced protein, drought induced protein, putative histone H2A, malate dehydrogenase, plastic aldolase NPALDP 1, oxygen-evolving enhancer protein 1, putative TFIIIA-like zink finger protein, and two unknown proteins that might involve in defense response against SMV infection. As expected, stronger induction of mRNA transcript in resistant cultivar was observed than in susceptible cultivar suggesting that these genes might relate with soybean defense mechanism. Various studies have

elucidated functions of eukaryotic translation initiation factors during virus disease progresses (Browning, 2004; Gao, 2004). The cDNA DSG57 encodes a part of putative TFIIIA (or kruppel)-like zinc finger protein of *Medicago sativa*. The deduced amino acid sequences showed 70% similarity while the deduced amino acid sequence of cDNA DSG65 showed 89% similarity to DAD 1 of *Arabidopsis thaliana*. Zinc finger protein and DAD1 have been reported to function in plants defense against diseases and in controlling plant programmed cell death through the N-linked glycosylation (Crosti et al., 2001; Klug, 1999; Rushton and Somssich, 1998). The amino acid sequences of cDNA clones SS15 and SS17 showed 80% and 88% similarities to dehydration stress-induced protein of *A. thaliana* and aquaporin protein of *Brassica rapa*, respectively. These genes were shown to be primary related with plant-environment and with the water-channel activity during tomato-microbes interaction (Werner et al., 2001).

The cDNA SS5 showed 94% similarity with putative histone H2 protein of *Oryza sativa*. A recent report also showed that histone H2A is related with resistance of *Petroselinum crispum* against fungal infection (Logemann et al., 1995). The cDNA SS49 encodes part of oxygen evolving enhancer protein 1 precursor of *Bruguiera gymnorrhiza*. The deduced amino acid sequence showed 83% similarity. This gene usually induces a long-lasting, broad-based, systemic resistance to secondary pathogen attack. Many studies implicate salicylic acid as an essential signal in the development of such systemic acquired resistance in several plant species (Dempsey et al., 1994). The cDNA DSA22 encodes part of a receptor-like serine/threonine kinase (Leucine rich repeat family kinase) of *A. thaliana*. The deduced amino acid sequence showed 70% similarity. It has been reported that receptor-like serine/threonine kinase gene was differentially regulated during various plant-pathogen interactions (Czernic et al., 1999). In tomato, the *Pto* gene, which is involved in resistance to *Pseudomonas syringe* pv. *tomato*, encodes a serine-threonine protein kinase for signal transduction pathway (Czernic et al., 1999). Therefore, it is possible that the putative receptor-like serine-threonine protein kinase might function as a signal receptor for SMV infection.

The complete ORF sequences and the deduced amino acid sequences of two clones revealed no significant matches in GenBank database. The lack of knowledge about such unidentified genes, however, does not reduce their values in the disease progresses and/or plants defense responses. The full characterization of these unknown proteins may reveal their critical role(s) in virus-host interactions. Although it remains to be seen what roles these selected genes play in the defense responses of soybean, our study could potentially provide information on mechanisms of susceptible and resistant soybean as well as the identity of genes whose expression is induced. Further characterization and functional analysis of these selected genes should enhance our understanding of defense signal transduction and biological function in soybean plants.

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