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GENE EXPRESSION PROFILING IN SOYBEAN SPROUTS WITH ELEVATED DISEASE RESISTANCE AGAINST *PSEUDOMONAS PUTIDA* INFECTION

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Abstract

Soybean (*Glycine max* L. Merr.) is one of the most important crops worldwide providing valuable nutrients and proteins. Previously, disease resistance against *Psuedomonas putida* infection was induced in soybean sprouts germinated under red right irradiation at 660 nm wavelength. Microarray analysis was conducted to elucidate the underlying molecular mechanisms contributing to this red-light-induced disease resistance. Four different treatments were carried out: (1) incubation in darkness without pathogen inoculation (DN), (2) incubation in darkness followed by pathogen inoculation (DI), (3) incubation under red light without pathogen inoculation (RN), and (4) incubation under red light followed by pathogen inoculation (RI). Four different comparisons were made between gene expression sets as follows: (1) DI vs. DN (DI/DN), (2) RI vs. RN (RI/RN), (3) RI vs. DI (RI/DI), and (4) RN vs. DN (RN/DN). Genes exhibiting 3-fold up- or down-regulated changes in expression were scored, which revealed 1539 and 1301 up-regulated and down-regulated genes, respectively. For the comparisons between gene expression sets DI/DN, RI/RN, RI/DI, and RN/DN, there were 1078, 651, 68, and 71 differentially regulated genes, respectively. These genes were further characterized according to their biological processes and molecular functions, and gene expression changes indicated by microarray analysis were validated by real-time PCR. Red-light irradiation regulates expression changes in many genes, whose profiles should be identified to elucidate the complex processes underlying the induced disease resistance.

Introduction

Soybean (*Glycine max* L. Merr.), a legume species native to East Asia is one of the most important crops in terms of providing oil and protein (Libault *et al.* 2010). Soy food products have significant health benefits including a reduced incidence of coronary heart disease, a reduced risk of breast and prostate cancers, improved bone health, and relief of menopausal symptoms (Xiao 2008). *Pseudomonas putida* is a rod-shaped, flagellated, Gram-negative bacterium that is found in most aerobic soil and water habitats and causes rotting disease in soybean sprouts. Reductions in soybean yield are mainly the result of events that occur during the sprout stage of development (Robertson *et al.* 2002). Many other bacteria and fungi such as *Rhizoctonia* sp., *Pseudomonas* sp., *Phytophthora* sp., and *Bradyrhizobium japonicum* also cause significant losses in soybean yield (Mendes *et al.* 2013). Therefore, microbial infection of soybean sprouts has large negative impacts on soybean production and the soybean sprout industry.

Plants exhibit higher levels of pathogen disease resistance when they are exposed to ultraviolet (UV) and red light. Soybean plants were shown to accumulate phytoalexin hydroxyphaseollin under UV-C light which led to increased disease resistance against *P. megasperma* var. sojae (Bridge and Klarman 1973). Red-light treatment of pepper, pumpkin, and tomato seedlings resulted in the development of resistance against *Phytophthora capsici* infection

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(Islam and Babadoost 2002). Furthermore, *Arabidopsis* plant irradiated with red light displayed increased systemic disease resistance against the root-knot nematode *Meloidogyne javanica* and a bacterial disease caused by *P. syringae* pv. Tomato DC 3000 (Islam *et al*. 2008).

Microarray analysis has been used to study gene expression changes in *Arabidopsis* during cyst nematode parasitism three days post-inoculation in order to gain a more detailed understanding of the molecular mechanisms underlying the basis of nematode resistance (Puthoff *et al*. 2003). In addition microarrays have been used to study gene expression in plants under abiotic stress conditions and other treatments, including drought, cold, diurnal cycling, salt, temperature, oxidative stress, small signaling molecules such as salicylic acid and methyl jasmonate, and several biotic stresses (Kiegle *et al*. 2000, Reymond *et al*. 2000). Microarray data is corroborated by the results obtained by Northern blot analysis, as has been demonstrated multiple times (Desikan *et al*. 2001).

In the previous study, present reported induced disease resistance against bacterial rotting disease in soybean sprouts germinated under red-light irradiation (Dhakal *et al*. 2015). An increase in salicylic acid was observed following red-light irradiation; however, the overall mechanism for the observed enhanced disease resistance was not fully elucidated. Therefore, a microarray analysis was conducted to gain a basal and in-depth understanding of the molecular mechanisms behind soybean sprout disease resistance induced by red-light irradiation.

Materials and Methods

Seeds of soybean (cv. Pungsan) were soaked and germinated under either darkness or red-light irradiation for 5 days as described by Dhakal *et al*. (2015). *P. putida* 229 was prepared and inoculated as previously reported (Dhakal *et al*. 2015). Total RNA from the treatment was extracted using Trizol^{TM} reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer protocol and stored at -80°C use in microarray and quantitative real-time PCR (qRT-PCR) analysis. The quantity and quality of total RNA was assessed using an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Biotinylated cRNA were prepared according to the standard Affymetrix protocol from 100 ng total RNA (Expression Analysis Technical Manual, 2001, Affymetrix, Santa Clara, CA, USA). Following fragmentation, 5 µg of cRNA was hybridized for 16 hrs at 4°C on the GeneChip® Soybean Genome Array (Affymetrix, Santa Clara, CA, USA), which was comprised of over 37,500 probe sets. For each gene, eleven pairs of oligonucleotide probes were synthesized *in situ* on the arrays. The microarray chips were washed and stained in the Affymetrix Fluidics Station 450 (Affymetrix, Santa Clara, CA, USA).

The Gene Chips were scanned using the Affymetrix Gene Chip Scanner 3000 7G (Affymetrix, Santa Clara, CA, USA). Data were analyzed with Microarray Suite version 5.0 (MAS 5.0) using Affymetrix default analysis settings and global scaling as the normalization method. The trimmed mean target intensity of each array was arbitrarily set to 100. The normalized and log-transformed intensity values were then analyzed using GeneSpring GX 11.5.1 (Agilent technologies, Santa Clara, CA, USA). Fold-change filters were applied including the requirement that genes be present in at least 200% of controls for up-regulated genes and in less than 50% of controls for down-regulated genes. Hierarchical clustering data were clustered groups that behave similarly across experiments as determined using GeneSpring GX 11.5.1. The clustering algorithm was Euclidean distance, average linkage. Excel files with statistically relevant up-regulated and down-regulated genes and their signal Log ratios were provided by the analysis company. The microarray data used to support the findings of the present study are available from the corresponding author upon request.

The sequences of those differentially expressed genes identified by microarray analysis were collected from NCBI-EST (http://www.ncbi.nlm.nih.gov/est). Gene sequences were compared with annotated sequences using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) at NCBI to determine if there was significant homology to known gene products. Results of the BLAST analysis were then categorized based on different functional categories, such as biological process and molecular function, using GO analysis (http://www.geneontology.org).

To validate the microarray data, representative genes that were considered differentially expressed were selected for quantitative real-time PCR (qRT-PCR) analysis. Primers were designed using the Primer3 program and the *G. max* gene sequences in either Samuel Roberts Noble Foundation database (http://plantgrn.noble.org/LegumeIP/) or GenBank (http://www.ncbi.nlm.nih.gov) (Table 1). Total RNA 100 ng were used for qRT-PCR using the One Step SYBR® PrimeScript™ RT-PCR kit (Perfect Real Time; Takara Bio Inc., Japan). qRT-PCR was performed using a Rotor-Gene 2000 (Corbett Research, Sydney, Australia). The qRT-PCR conditions were as follows: 42°C for 5 min for synthesis of first strand cDNA; 1 min denaturation at 95°C; 35 cycles of 95°C for 15 sec, 55°C for 40 sec, and 72 °C for 40 sec. Signal was detected corresponding to the amount of synthesized DNA.

Results and Discussion

For microarray analysis of soybean sprouts, four different treatment conditions were used: (1) incubation in darkness without pathogen inoculation (Dark Non-inoculated, DN), (2) incubation in darkness followed by pathogen inoculation (Dark Inoculated, DI), (3) red-light irradiation without pathogen inoculation (Red light Non-inoculated, RN), and (4) red-light irradiation followed by pathogen inoculation (Red light Inoculated, RI). Four different comparisons were made between gene expression sets as follows: (1) DI vs. DN (DI/DN), (2) RI vs. RN (RI/RN), (3) RI vs. DI (RI/DI), and (4) RN vs. DN (RN/DN).

A total of 2840 genes were identified with significant changes in expression level. Among these, 1539 and 1301 genes were significantly up-regulated and down-regulated, respectively (Fig. 1). In the comparisons DI/DN, RI/RN, RI/DI, and RN/DN, the numbers of genes with expression up-regulated more than 3-fold were determined as 1078, 651, 68, and 71, respectively (Fig. 1A). Only a single gene displayed consistently up-regulated expression in the comparisons DI/DN, RI/DI, and RI/RN whereas two genes displayed consistently up-regulated expression in the comparisons DI/DN, RI/RN, and RN/DN. The highest number of up-regulated genes (total 299 genes) was observed in both DI/DN and RI/RN comparisons. The numbers of genes with consistently up-regulated expression between the comparisons DI/DN and RI/DI, DI/DN and RN/DN, RI/DI and RI/RN, RI/DI and RN/DN were determined as 1, 3, 14, and 6, respectively.

In the comparisons DI/DN, RI/RN, RI/DI, and RN/DN, the numbers of genes with expression down-regulated more than 3-fold were determined as 739, 443, 28, and 320, respectively, and only a single gene exhibited down-regulated expression in each of the four comparisons (Fig. 1B). In the DI/DN, RI/DI, and RI/RN comparisons, two genes displayed consistently down-regulated expression levels whereas five genes displayed consistently down-regulated expression levels in the DI/DN, RI/RN, and RN/DN comparisons. The highest number of down-regulated genes (113 in total) was observed in both DI/DN and RI/RN comparisons. The numbers of genes with consistently down-regulated expression between the comparisons DI/DN and RN/DN, RI/DI and RI/RN, RI/RN and RN/DN, and RI/DI and RN/DN were determines as 90, 4, 1, and 4, respectively.

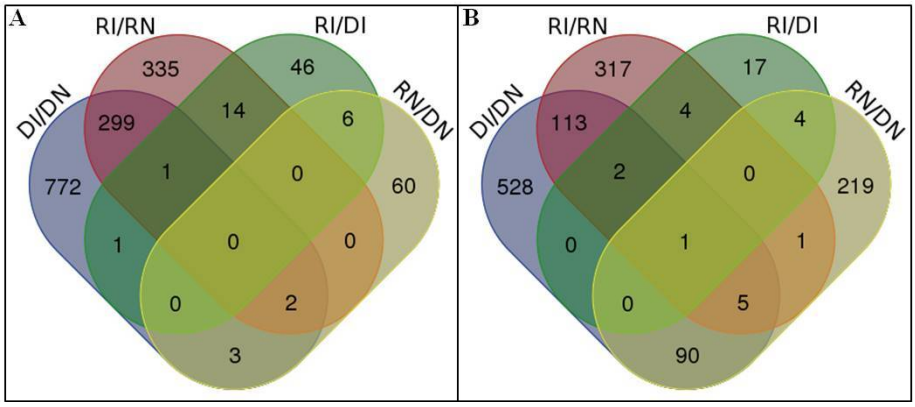

Fig. 1. Venn diagram depicting the overlap of differentially regulated genes, defined as those exhibiting more than three-fold up- or down-regulated expression, in different comparisons. (A) Up-regulated genes and (B) down-regulated genes. The number outside the overlapping areas (circles) denotes the total number of genes up- or down-regulated in each comparison. The number within one circle or more than two circles denotes the specific genes or overlapped genes, respectively. DI/DN, Darkness Inoculated vs. Darkness Non-inoculated; RI/RN, Red light Inoculated vs. Red light Non-inoculated; RI/DI, Red light Inoculated vs. Darkness Inoculated; RN/DN, Red light Non-inoculated vs. Darkness Non-inoculated.

In the DI/DN comparison, the total number of genes with expression changes was 1817. The functional and annotation analysis revealed that 997 genes had known functions while 820 genes were classified as having unknown function (Fig. 2). Functional analysis revealed that these up- and down-regulated genes were categorized into 20 biological process and 15 molecular function categories based on the gene ontology (GO) analysis test. The largest biological process and molecular function categories represented by up-regulated genes were “stress response” and “binding activity”, respectively. These categories accounted for 35.16 and 26.91% of all up-regulated genes, respectively (Fig. 2). Interestingly, the same two categories were also found to be those most represented by down-regulated genes, which included 27.43 and 29.06% of all down-regulated genes in the comparison, respectively (Fig. 2).

In the RI/RN comparison, the total of genes with expression changes was 1094. The functional and annotation analysis revealed that 704 genes had known functions while 390 genes were classified as having unknown function (Fig. 3). Functional analysis revealed that these up- and down-regulated genes were categorized into 15 biological process and 14 molecular function categories based on the GO analysis test. The largest biological process and molecular function categories represented by up-regulated genes were “stress response” and “binding activity” respectively. These categories accounted for 35.16 and 24.28% of all up-regulated genes, respectively (Fig. 3). As was observed in the DI/DN comparison, the same two categories were also found to be those most represented by down-regulated genes, which included 25.64 and 30.49% of all down-regulated genes, respectively (Fig. 3).

In the RI/DI comparison, the total of genes with expression changes was 96. The functional and annotation analysis revealed that 54 genes had known functions while 42 genes were classified as having unknown function (Fig. 4). Functional analysis revealed that these up- and down-regulated genes were distributed into 7 biological process and 6 molecular function categories based on the GO analysis test. The largest biological processes categories represented by up-regulated genes were “cell wall organization” and “stress response”, which, when combined, accounted for 33.33% of all up-regulated genes; whereas the largest molecular function

category represented by up-regulated genes was “oxidoreductase activity”, which accounted for 50.0% all up-regulated genes (Fig. 4). The largest biological process category represented by down-regulated genes was “stress response”, which accounted for 63.64% of all down-regulated genes; whereas, the largest molecular function categories represented by down-regulated genes were “binding activity” and “transcription factor activity”, which, when combined, accounted for 33.33% of all down-regulated genes (Fig. 4).

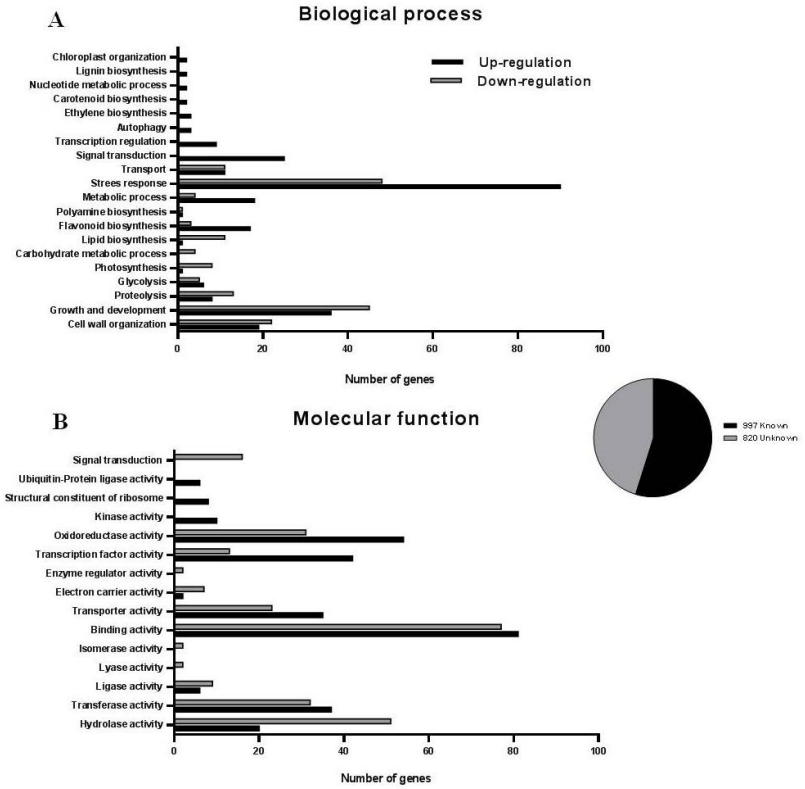

Fig. 2. Functional categories of differentially expressed genes in the Darkness Inoculated vs. Darkness Non-inoculated (DI/DN) comparison. Distribution of genes in (A) biological process and (B) molecular function categories. Only the genes up- and/or down-regulated more than 3-fold were used for the microarray analysis.

In the RN/DN comparison, the total of genes with expression changes was 391. The functional and annotation analysis revealed that 234 had known functions while 157 genes were classified as having unknown function (Fig. 5). Functional analysis revealed that these up- and down-regulated genes were distributed into 10 biological process and 9 molecular function categories based on the GO analysis test. The largest biological process category represented by up-regulated genes was “stress response”, which accounted for 38.78% of all up-regulated genes; whereas the largest molecular function categories represented by up-regulated genes were “binding activity” and “oxidoreductase activity”, which, when combined, accounted for 30.43% of all up-regulated genes (Fig. 5). The largest biological process and molecular function categories represented by down-regulated genes were “stress response” and “binding activity”, respectively. These categories accounted for 26.92% and 30.91% of all down-regulated genes, respectively.

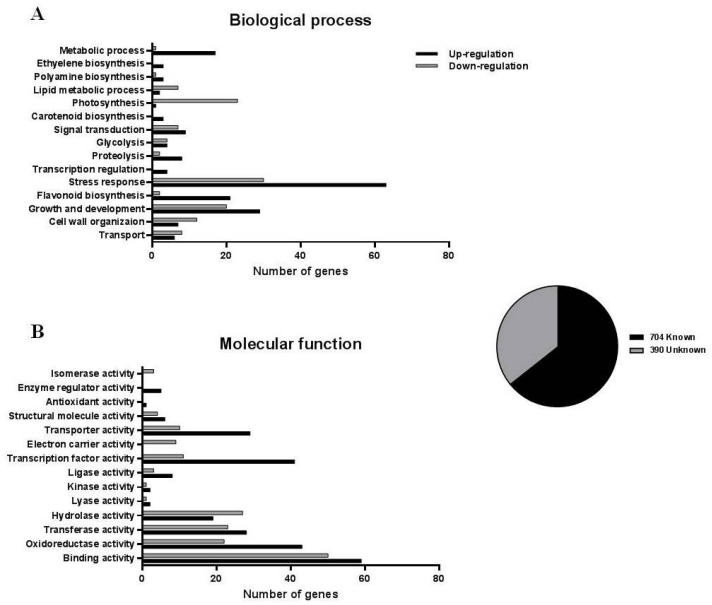

Fig. 3. Functional categories of differentially expressed genes in the Red light Inoculated vs, Red light Non-inoculated (RI/RN) comparison. Distribution of genes in (A) biological process and (B) molecular function categories. Only the genes up- and/or down-regulated more than 3-fold were used for the microarray analysis.

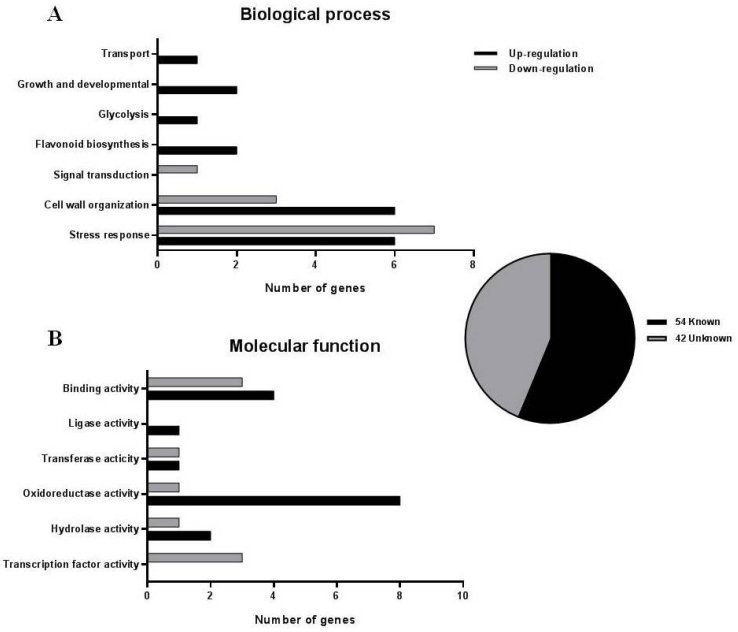

Fig. 4. Functional categories of differentially expressed genes in the Red light Inoculated vs Darkness Inoculated (RI/DI) comparison. Distribution of genes in (A) biological process and (B) molecular function categories. Only the genes up- and/or down-regulated more than 3-fold were used for the microarray analysis.

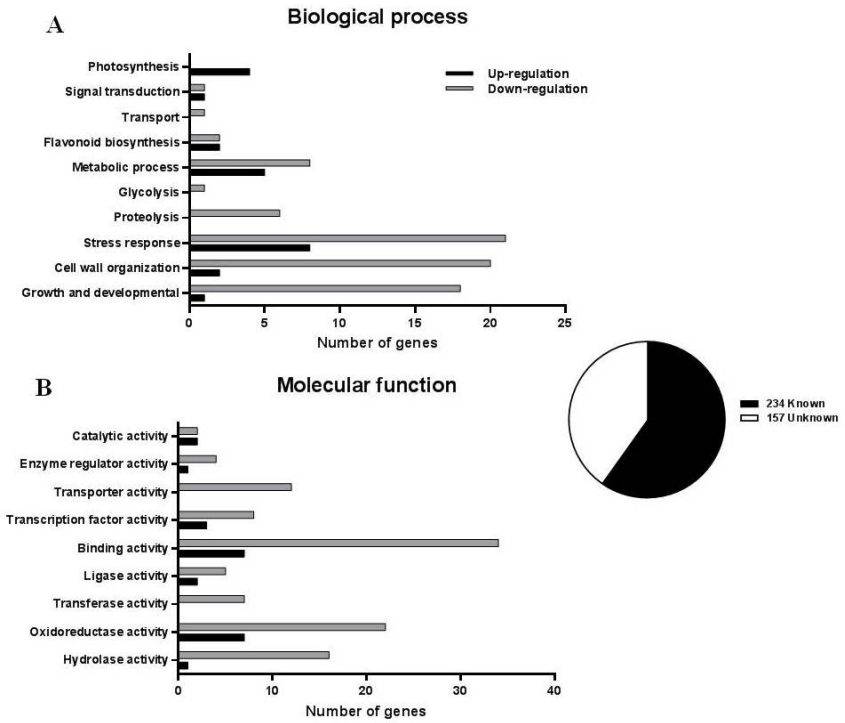

Fig. 5. Functional categories of differentially expressed genes in the Red light Non-inoculated vs. Darkness Non-inoculated (RN/DN) comparison. Distribution of genes in (A) biological process and (B) molecular function categories. Only the genes up- and/or down-regulated more than 3-fold were used for the microarray analysis.

In order to validate the gene expression changes identified by microarray analysis, 10 differentially-expressed genes from the microarray data were selected (Table 1), which were those identified to vary in gene expression level in each of the microarray comparisons (Table 2). The primer sequences used for each gene are described in Table 1. The selected genes included 6 genes with increased expression levels; namely Glutathione Peroxidase 6 (*GPX6*), chalcone and stilbene synthase family protein (*TT4*), WRKY family transcription factor 53 (*WRKY53*), Glutathione S-Transferase TAU 15 (*GSTU15*), and Heat Shock Protein 70B (*HSP70B*); and 5 genes with decreased expression levels; namely Expansin-like B1 (*EXLB1*), Kunitz Trypsin Inhibitor 1 (*KTI1*), Xyloglucan endotransglycosylase 6 (*XTR6*), Expansin A8 (*EXPA8*), and Myb domain protein 40 (*MYB40*).

In the microarray data, *WRKY53*, *GPX6*, *TT4*, *GSTU15*, and *EXLB1* expression was up-regulated in the DI/DN comparison (Table 2). Although the absolute gene expression levels detected by qRT-PCR did not match those determined by microarray analysis exactly, the trends were similar and those genes that exhibited increased expression levels in the DI/DN comparison based on microarray data (Table 2). The same result was observed for the RI/RN comparison, with *GPX6*, *TT4*, *GSTU15*, and *EXLB1* exhibiting expression up-regulation in both microarray data and following qRT-PCR analysis. In the RI/DI comparison, *HSP70B* expression was found to be up-regulated in both microarray data and following qRT-PCR analysis. In the RN/DN comparison, *HSP70B* and *WRKY53* also exhibited expression up-regulation in both microarray data and following qRT-PCR analysis.

Table 1. List of primers used for the validation of the microarray data by quantitative real-time PCR analysis.

GenBank Accession No.	Description	Primer sequence (5′–3′)	Product length (bp)
NM_001249966	Glutathione peroxidase 6 (*GPX6*)	CGCTTCAAAGCTGAGTTTCCC GCTGAGAGGAGAAGTTGTGGG	195
XM_003517481	Chalcone and stilbene synthase family protein (*TT4*)	CACCTCCTCAAGGATGTTCCC CACAAGCACTTGACATGTTCCC	229
XM_003530331	WRKY family transcription factor (*WRKY53*)	CCTATACCAAACACACCTGTTATCC GAAATTTGGATCAATTTCCACCGG	214
NM_001304515	Glutathione S-transferase TAU 15 (*GSTU15*)	CTGCAGTTATTGCATTCTTCCTTAGC CATCTTCCAGGACCTTCGC	222
XR_137282	Heat shock protein 70B (*HSP70B*)	GAGATGAGGAGGATGGTGAGAG CCAACTCCTGCTTCTTGTACTC	217
NM_001254980	Expansin-like B1 (*EXLB1*)	GTGTGCAAGTTACTGGACCG CTAAGTCGTATGTTTGTCCAGCC	175
GQ168589	Kunitz trypsin inhibitor 1 (*KTI1*)	CAAGTGGACTATCGTTGAGGGTC CCTTACCATCGGTACGAATCCC	215
XM_003533702	Xyloglucan endotransglycosylase 6 (*XTR6*)	CATACTCCGTCCAATGGAATCC GTTTCTGTATGAGGCTGTGAATGG	218
XM_003544882	Expansin A8 (*EXPA8*)	GTTTTGATCACCAACGTCGCG GTGACAGTTCTACCGTCACTGG	176
XM_003554964	Myb domain protein 40 (*MYB40*)	CAAGGTGACTTGGGATGATACC CTGAAGGTAAGAGGATTCACCCAC	181
U60500.1	*Glycine max* actin gene	GAGAGAGGATACTCCTTCAGC GAACAGTACTTCTGGGCAAC	204

Table 2. Microarray validation of some selected up-regulated genes under all treatment conditions by quantitative real time PCR analysis.

Genes		Fold change	
GenBank Accession No.	Description	Microarray analysis	Real-time PCR analysis
Dark Inoculated/Dark Non-inoculated (DI/DN)			
XM_003530331	WRKY family transcription factor (*WRKY53*)	6.9	6.37
NM_001249966	Glutathione peroxidase 6 (*GPX6*)	33.1	8.32
XM_003517481	Chalcone and stilbene synthase family protein (*TT4*)	111.4	80.78
NM_001304515	Glutathione S-transferase TAU 15 (*GSTU15*)	143.6	396.49
NM_001254980	Expansin-like B1 (*EXLB1*)	109.9	104.67
Red light Inoculated/Red light Non-inoculated (RI/RN)			
NM_001249966	Glutathione peroxidase 6 (*GPX6*)	17.6	5.27
XM_003517481	Chalcone and stilbene synthase family protein (*TT4*)	70.6	115.80
NM_001304515	Glutathione S-transferase TAU 15 (*GSTU15*)	108.6	496.53
NM_001254980	Expansin-like B1 (*EXLB1*)	95.5	83.34
Red light Inoculated/Dark Inoculated (RI/DI)			
XR_137282	Heat shock protein 70B (*HSP70B*)	4.1	1.96
Red light Non-inoculated/Dark Non-inoculated (RN/DN)			
XR_137282	Heat shock protein 70B (*HSP70B*)	3.0	1.25
XM_003530331	WRKY family transcription factor (*WRKY53*)	3.5	3.01

There were a number of genes whose expression was down-regulated in most of the comparisons according to microarray data. In the DI/DN, RI/RN, RI/DI, and RN/DN comparisons, *XTR6*, *KTI1*, and *EXPA8*; *XTR6*, *KTI1*, *EXPA8*, and *MYB40*; *XTR6* and *KTI1*; and *XTR6*, *EXPA8* and *MYB40* expression was down-regulated, respectively, in both microarray data and qRT-PCR analysis (Table 3).

Table 3. Microarray validation of some selected down-regulated genes under all treatment conditions by quantitative real-time PCR analysis.

Gene		Fold change	
GenBank Accession No.	Description	Microarray analysis	Real-time PCR analysis
Dark Inoculated/Dark Non-inoculated (DI/DN)			
XM_003533702	Xyloglucan endotransglycosylase 6 (*XTR6*)	-12.6	-22.55
GQ168589	Kunitz trypsin inhibitor 1 (*KTI1*)	-4.2	-4.58
XM_003544882	Expansin A8 (*EXPA8*)	-14.3	-5.65
Red light Inoculated/Red light Non-inoculated (RI/RN)			
XM_003533702	Xyloglucan endotransglycosylase 6 (*XTR6*)	-3.1	-3.33
GQ168589	Kunitz trypsin inhibitor 1 (*KTI1*)	-9.7	-17.66
XM_003544882	Expansin A8 (*EXPA8*)	-5.2	-2.33
XM_003554964	Myb domain protein 40 (*MYB40*)	-5.6	-7.56
Red light Inoculated/Dark Inoculated (RI/DI)			
XM_003533702	Xyloglucan endotransglycosylase 6 (*XTR6*)	-3.8	-3.00
GQ168589	Kunitz trypsin inhibitor 1 (*KTI1*)	-4.3	-5.13
Red light Non-inoculated/Dark Non-inoculated (RN/DN)			
XM_003533702	Xyloglucan endotransglycosylase 6 (*XTR6*)	-14.0	-20.30
XM_003544882	Expansin A8 (*EXPA8*)	-3.7	-4.08
XM_003554964	Myb domain protein 40 (*MYB40*)	-5.5	-4.26

Microarray analysis was performed to obtain a global understanding of the differentially expressed genes in soybean sprouts grown in darkness or under red-light irradiation, as well as these light conditions combined with pathogen inoculation. This resulted in identification of 2840 genes in total with significant gene expression changes, defined as those with expression more than three-fold up- or down-regulated. These differentially expressed genes were subjected to further statistical and biological analysis, and comparisons were made between experimental treatments. To determine the differentially expressed candidate genes, multivariate outlier detection was also performed. This approach provided fold-change values; therefore, 1539 and 1301 genes were identified with expression up-regulated and down-regulated, respectively, using a three-fold change as a criterion.

There were considerable overlaps between the sets of genes with up- and down-regulated expression in each of the expression-set comparisons performed. Bifunctional inhibitor/lipid-transfer protein (Glyma17g14910.1) displayed gene expression up-regulation in each of the DI/DN, RI/DI, and RI/RN comparisons. Calmodulin-like 41 (Glyma11g25660.1) and peroxidase superfamily protein (Glyma15g05820.1) displayed gene expression up-regulation in the each of the DI/DN, RI/RN, and RN/DN comparisons. Among those genes with down-regulated expression, xyloglucan endotransglycosylase 6 (Glyma09g07070.1) displayed down-regulated expression in all comparisons. Kunitz trypsin inhibitor 1 (Glyma08g45610.1) and gibberellin-regulated family protein (Glyma19g01590.1) displayed down-regulated expression in DI/DN, RI/DI, and RI/RN comparisons, whereas plant invertase (Glyma08g04880.1), IQ-domain 9

(Glyma15g08660.1), glycosyl hydrolase 9B13 (Glyma10g02130.1), expansin A8 (Glyma14g38430.1), and phosphoglycerate mutase family protein (Glyma08g14650.1) displayed down-regulated expression in DI/DN, RI/RN, and RN/DN comparisons. Each of the chosen genes from the different comparisons yielded consistent results, albeit with low or high levels of variation, indicating that the quality of the microarray data were valid as verified by qRT-PCR analysis (Table 2, 3).

Microarray analysis has been used to identify and compare different stress- and development-related genes (Rohrmann *et al.* 2011). The greatest number of differentially expressed genes encoded stress response proteins (Fig. 2A) and, in almost all comparisons, the expression of genes involved in stress response were increased. Pathogen inoculation of soybean sprouts led to expression induction of those genes involved in the response to transcription binding activity. MYB transcription factor family proteins, zinc-binding family proteins, and integrase-type DNA-binding superfamily protein were represented by the highly-expressed transcription-related genes (Shinde *et al*. 2015). Plant genomes encode a large number of MYB proteins which play important roles in many developmental processes and in various defense responses (Yanhui *et al*. 2006). WRKY genes encode transcription factors that control the plant's response to several stresses and are involved in the defense response (Robatzek and Somssich 2001, Dong *et al*. 2003).

Different categories of genes displayed significantly differentially expression in response to dark- and red-light incubation followed by pathogen inoculation. The greatest number of differentially expressed genes encoded stress response proteins. However, a large number of genes encoding transcriptional regulatory proteins and proteins involved in metabolism, transport, photosynthesis, signal transduction, and energy transfer were differentially expressed in response to dark- and red-light incubation followed by pathogen inoculation.

Overall, the microarray analysis revealed global transcription changes that were associated with red-light irradiation and involved in pathogen defense responses. Further experimentation and manipulation of the identified candidate genes could lead to further understanding of plant-microbial interactions that underlie elevated pathogen resistance.

Acknowledgments

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