

# Similarities and Differences in Global Gene Expression Profiles between Herbicide and Pathogen-Induced PSII Inhibition

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## Abstract

Plant pathogens, and photosynthesis inhibiting herbicides, can both damage photosystem II (PSII), causing it to be highly sensitive to damage by light energy, and to release high levels of reactive oxygen species (ROS). This photoinhibition of PSII could possibly be the source of the strong oxidative burst associated with the pathogen-induced, hypersensitive defense response (HR). To examine a possible mechanism of how the HR-associated ROS burst could originate from PSII inhibition, we compared the transcriptome responses in soybean undergoing photoinhibition induced by HR, to soybean undergoing photoinhibition induced by the herbicide bentazon, which specially stops PSII electron flow by preventing QB from binding to D1. Most genes shared similar expression patterns between HR and bentazon treatments; however, interesting differences were also observed. The most striking differences were seen with genes related to photosynthesis, where these genes were uniformly down regulated in HR, but were mostly up in response to bentazon. Another interesting difference was seen in genes of the phenylpropanoid pathway. These defense-related genes were mostly down or non-responsive to bentazon, but were generally induced in response to pathogen-induced HR, showing that soybeans activate the phenylpropanoid-based phytoalexins independent of PSII inhibition. We conclude that the PSII inhibition occurring during the HR is not being triggered simply by the inhibition of electron flow through the photosystem centers. Instead, it is more likely that the initial triggers of the HR halt the repair of damaged PSII which leads to enhancing photoinhibition and contributing the rapid production of ROS, sealing the fate of cells undergoing HR; and other triggers independently induce specific aspects of defenses such as the phenylpropanoid pathway.

**Keywords:** Bentazon; Soybean; Plant chloroplast; Protein; Photosynthesis; Gene; PSII; ROS

## Introduction

A common plant response after sensing pathogen attack is the production of reactive oxygen species (ROS) such as singlet oxygen, superoxide, and hydrogen peroxide which both harm the pathogen and signal additional defense pathways [1,2]. The hypersensitive response (HR), which often succeeds in delineating and defeating biotrophic and hemi-biotrophic pathogens, has been characterized as having two bursts of ROS. One burst, which is common to compatible and incompatible (HR) interactions, is presumably the result of NADPH oxidases, and appears within minutes of recognition of the infection [1,3-5]. The second oxidative burst (the source of which is largely speculative) is much greater than the initial burst, and is specific to HR reactions.

It has been widely reported that light can affect defense responses [6,7], and that a common plant response to pathogen infection is the rapid decreased expression of genes related to photosynthesis [8,9]. More specifically, there are several reports on the possible role of photosystem II (PSII) inhibition as an important player in plant defense and production of ROS, perhaps a major source of the ROS seen in the HR-specific oxidative burst [8,10,11]. Allen et al. [10] reported decrease in PSII efficiency in response to an elicitor of the HR, and Seo et al. [11] showed that resistance to viral infection is enhanced if replacement of damaged D1 subunit of PSII is reduced using an *FtsH* (one of the proteases responsible for D1 degradation prior to replacement) mutant. Additionally, photoinhibition was measurable as a decrease in PSII operating efficiency in soybean within 8 hours of being inoculated with HR-inducing *P. syringae* [8]. It has therefore been hypothesized that the loss of functional PSII (perhaps through the reduced replacement of photo-damaged D1 subunit) would lead

to inactivation of PSII during the HR and subsequently increase the production of ROS, leading to HR and enhanced defense [8,11]. Loss of functional D1 leads to a break in the electron transfer chain resulting in photoinhibition due to PSII inability to pass light energy through the release of electrons along the electron transfer chain, and instead releasing absorbed light energy as heat, fluorescence and ROS [12,13].

The herbicide bentazon (similar mode of action as atrazine, a well-characterized chemical that is known to induce photoinhibition), interferes with PSII function by inhibiting the ability of plastoquinone B ( $Q_B$ ) to bind to the PSII subunit D1, such that electrons cannot flow from  $Q_A$  to  $Q_B$ , effectively stopping photosynthesis and rendering the plant fatally sensitive to excess light damage [13,14-16]. Bentazon is not too damaging to soybean however, as soybean has the ability to degrade it, and there are no known secondary targets outside of a transient PSII inhibition. Based on a transcriptomic study [17] of soybean leaves treated with bentazon, soybean begins to noticeably remove bentazon toxicity between about 4-8 hours post application.

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One main reason that it is difficult to determine what is the source of the HR-specific ROS burst, is that pathogens are dynamic living organisms that provide many responses during infection. When a pathogen attacks a plant host, a wide range of signaling factors are exchanged that induce numerous host responses. For example, the bacterial pathogen *Pseudomonas syringae* releases several phytotoxins in addition to secreting about 40 different effector proteins into a host [18]. Plants also actively respond to these virulence factors, in addition to sensing and responding to numerous pathogen associated molecular patterns (PAMPs), such as bacterial flagellin and chitin oligomers from fungi [19,20]. Pathogen-associated elicitors may also include molecules of plant origin such as plant cell-wall fragments originating from pathogen-released cell-wall degrading enzymes [21,22]. On the other hand, instead of trying to untangle the responses to multiple signals, one could simply examine the effects of a chemical that specifically inhibits only one known protein-molecular interaction in plants. In this study, we used a chemical inducer of photoinhibition to gain insight into the photoinhibition induced during HR. We compared gene expression level data from a study [17] of soybean treated with bentazon, which induces PSII inhibition by a known mechanism (inhibition of electron flow at D1), to an expression study [8] on the HR induced by *P. syringae*, which induces PSII inhibition by an unknown mechanism. We hypothesize that, if the large amounts of ROS induced by *P. syringae* is due mainly to inhibition of PSII electron flow, then these two treatments will share many patterns in their global gene expression. Additionally, where differences occur, the expression patterns can help differentiate gene expression change related to PSII inhibition during HR, from those that might be due to other dynamic events occurring during host-pathogen interactions. To assist in discerning gene expression due solely to induction of cell death during the defense response, we also compared the datasets to a global expression study [23] from soybean treated with glyphosate, a shikimate pathway inhibitor. Glyphosate does not directly target photosynthesis, and the plant cell death is not caused by ROS, but by nutritional starvation [24,25].

This comparative study utilized data from a previously published [8] cDNA microarray survey of 27,000 genes in soybean that identified 3,898 genes as being differentially expressed due to *P. syringae* leaf infection at 2, 8, 24 hours post infection (hpi). The highest number of transcriptional changes was noted in the HR treatment at 8 hpi, including the down regulation of nearly 100 chloroplast-associated genes. The HR expression data was compared to a cDNA microarray survey of 36,000 genes on the effect of bentazon [17] on soybean leaves that recognized 6,646 genes as differentially expressed genes within 8 hours of application. These two studies were also compared to a cDNA microarray study [23] on the effect of glyphosate treatment on sensitive soybean that identified 3, 170, and 311 genes having different transcript levels at 1, 4, and 24 hours post treatment (hpt), respectively.

## Materials and Methods

### Generation of gene lists from experiments to be cross compared

Two approaches were taken to obtain an overview of clustered gene expression patterns taken from mRNA expression experiments that utilized soybean cDNA microarrays [26] developed at the University of Illinois, involving soybean response to *P. syringae* [8], bentazon [17], or glyphosate [23]. One method utilized a gene list of statistically significant genes responding to herbicide treatments, which were then used to find the corresponding expression of these genes in tissue undergoing HR induced by *P. syringae*. The top 3000 significant genes

from the bentazon experiment across the entire study were selected, as were the top 1300 significant genes from the glyphosate study at 24 hpi. Duplicate gene IDs were removed to obtain 4033 genes in total. The resulting list of 4033 genes that were highly significant in either the bentazon or glyphosate study was used to retrieve gene expression data across all the experiments of interest, regardless of significance in the HR experiment. Expression data was obtained from our in-house soybean gene expression database (SGED, <http://sged.cropsci.illinois.edu/>). The second method approached the problem from the HR perspective. The 3898 genes that were the most significant in their differentially expression in the *P. syringae* induced HR study were used to retrieve and compare expression data from the herbicide studies.

For specific comparisons between the *P. syringae* induced HR and bentazon treatments based on single functional categories, only genes that were overlapping in significance between the two studies were used for clustering. One exception was for the analysis of photosynthesis, where genes that were significant in either the HR reaction, or in response to bentazon, were used, and all duplications based on microarray match to Glyma ID (v 1.1) were removed, and the compatible (virulent isolate) *P. syringae* interaction was added.

### Clustering and imaging tool

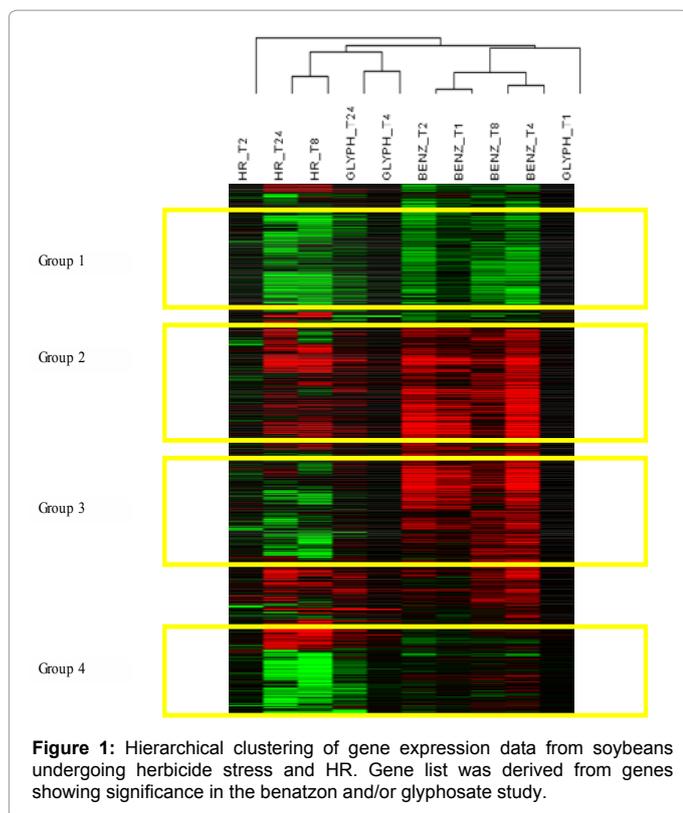
Hierarchical clustering was performed using the software Cluster [27] utilizing the average linkage method. The clustering results were visualized with Maple Tree ([http://www.eisenlab.org/eisen/?page\\_id=42](http://www.eisenlab.org/eisen/?page_id=42)). A table to convert microarray IDs with more recent GlymaIDs is provided (Supplemental Table 1).

## Results and Discussion

### Overall cross comparison of gene expression profiling from soybean treated with HR inducing pathogen, PSII inhibiting herbicide, and shikimate pathway inhibiting herbicide

The 4033 most significant differentially expressed genes from from either the effect of the herbicide bentazon on PSII inhibition [17], or the effect of the herbicide glyphosate on inhibiting the shikimate pathway [23], were compared to the gene expression response during *P. syringae*-induced HR [8]. Genes and arrays were both clustered via hierarchical clustering revealing that about 2/3 of the genes showed similar directions of expression across the three treatments (Figure 1). The clustering also indicated that the overall gene expression changes in HR were more similar to the expression patterns induced by glyphosate, than by bentazon. A similar clustering pattern was seen when using the gene list generated from the HR-significant gene list (Supplemental Figure 1).

The expression clusters in Figure 1 can be split into 4 major groups. Group 1 contains genes generally decreased across all three treatments, whereas group 2 contains genes that generally increased. Group 3 contains genes that tended to decrease in HR but increased in bentazon treatment, and not differentially expressed in glyphosate treatment. Group 4 contains genes sharing a similar expression pattern between HR and glyphosate treatment but weakly differentially expressed in the bentazon treatment. Groups 3 and 4 genes seem to have had the largest effect on determining that the HR expression pattern was more similar to glyphosate than bentazon treatment. In Group 4, there are about 400 genes (1/10th of the total studied) strongly decreasing in HR and glyphosate but not strongly or consistently differentially expressed in bentazon. It includes seven auxin-down-regulated genes. Interestingly, this group also contains more than 30 photosynthetic electron transport and light harvesting related genes, showing a marked difference in



how photosynthetic components are more noticeably affected in HR and glyphosate than after bentazon treatment, reflective of HR and glyphosate treated leaves destined for cell death, but battling to recover from bentazon treatment. Also in Group 4, there are around 150 genes (1/27th of total studied) strongly increased in HR and glyphosate treatment but weakly reduced or unchanging in response to bentazon. These genes include ones encoding for enzymes of the phenylpropanoid pathway, starvation associated message 22 (SAM22), genes associated with phytohormone starvation [28], adenine nucleotide translocator (ANT), a cell-death inducer [29], and phosphopyruvate hydratase, an enzyme participating in glycolysis.

Therefore, although the glyphosate experiment provoked a much weaker genomic modulation than the HR-inducing pathogen experiment (more than 10-fold fewer statistically significant genes identified in that published study), it seems that these two treatments shared many general similarities in expression patterns for genes related to photosynthesis components, phytohormone signaling, phenylpropanoid biosynthesis, and cell death. These similarities might be attributed by timing of sampling, as in both treatments the plants were headed down a path toward cell death and perhaps had already reached the point of no return [30]. The HR treatment being more intense and many cells would be dead within 24 hrs, whereas the glyphosate response would be less intense, with cells dying over a several day period. In contrast, in bentazon treatment, the cells were apparently continually attempting to recover [17].

### Specific HR vs bentazon comparisons

A detailed analysis of comparisons between genomic response to HR and the initial effects of bentazon, can help identify gene expression modulations related to PSII inhibition, and might be informative to understanding how plants differentiate biotic and abiotic stresses, as

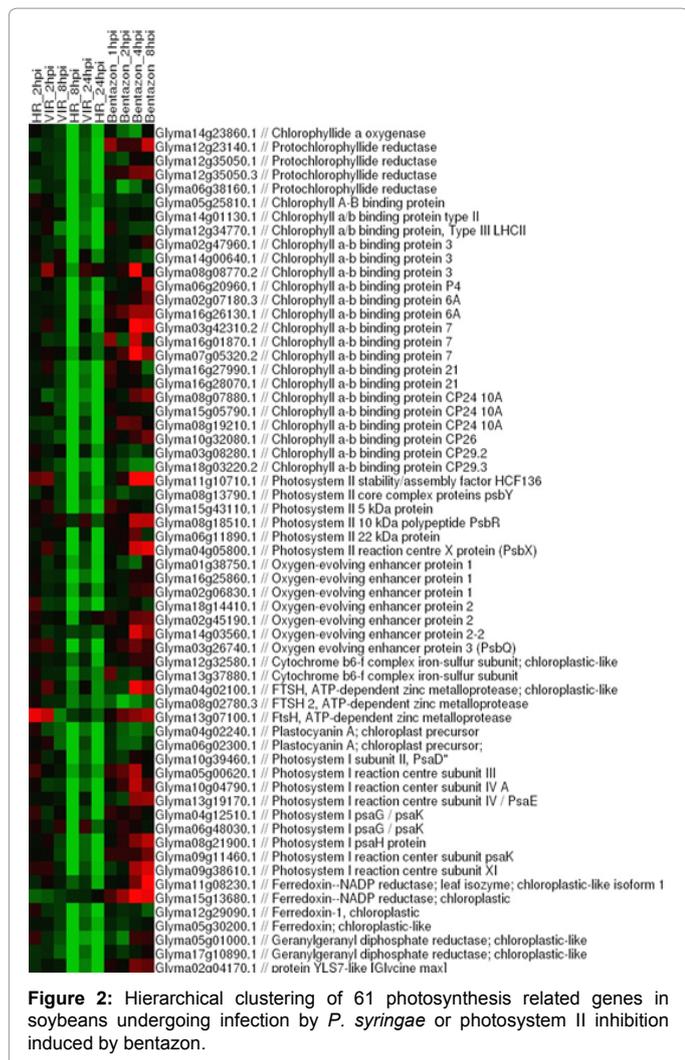
well as how plants respond to oxidative stress provoked from different possible sources. To examine more closely the events upstream of the cell death pathway that might be shared between HR and bentazon treatments, a closer focus was taken on specific comparisons involving only the studies on HR and bentazon.

### HR vs bentazon: differential expression of photosynthesis related genes

PSII is the primary target of bentazon, and the interruption of electron transport in PSII caused by bentazon resulted in modulation of 56 photosynthesis related genes [17]. The HR provoked by *P. syringae* repressed expression of 93 photosynthesis related genes within 8 hours post inoculation [8]. However, the number of genes overlapping between both HR and bentazon treatments that were deemed statistically significant in both studies was low. One cDNA of interest that was significantly differentially expressed in both studies encoded an FTSH (Gm-r1070-2561; Glyma04g02100.1), one of two proteases involved in the removal of photodamaged PSII subunit D1. This gene was significantly down 2 fold in HR at 8 hpi, and significantly up 2 fold in bentazon at 4 hpi. The other protease that teams with FtsH to degrade and remove D1 from PSII to allow for its replacement with healthy D1, is DEG2, and a gene encoding a DEG2 (Gm-r1088-1574; Glyma02g17130.2) had a very similar expression pattern as *FTSH*, and was significant in the bentazon study [17] but missed the significance cutoff in the HR study [8]. D1 is of interest in both HR and bentazon studies as both treatments are believed to induce oxidative damage to D1, which would lead to enhanced ROS production if not replaced. Seems that the genes encoding the proteases required to ensure removal of damaged D1 were actively transcribed by 4 hpi of bentazon treatment [17], but not actively transcribed in response to HR where their expression was down at 8 and 24 hpi [8].

To obtain a clearer overview of activity of other photosynthetic genes in the two experiments, all photosynthesis related genes printed on soybean cDNA microarrays that were significant in either study, minus duplications realized by the more recent genome sequencing project [31] were compiled and their corresponding gene expression data from the two experiments were compared. The heatmap (Figure 2) of expression from these 61 genes shows that virtually all of these photosynthetic genes were reduced in the HR by 8 hpi, but in the bentazon treatment half (30 of 61) of these genes were generally increasing, with the other half either not changing much or tending to be down-regulated. That the only increases in expression were in the bentazon treatment, the 2 hpi *P. syringae* treatments, or from the compatible (vir) interaction, supports that photosynthesis repair is not happening in HR, but it is being attempted in the bentazon treatment. This data also supports that this possible lack of repair is much weaker in the compatible (vir) than for the incompatible (HR) strain.

The genes in Figure 2 were grouped by functional annotation, and one can see that for each group, the trend was for the genes to be down in both the compatible and incompatible interactions (but much more reduced in incompatible HR), and generally up in response to bentazon. One of the gene groups was FtsH. There were two FtsH genes following this pattern, and a third FtsH homolog was down in all samples. An increase in FtsH expression would imply the need of more FtsH for the degradation and repair of damaged D1. That these genes are not being induced at 8 and 24 hpi for HR would be consistent with damaged D1 not being replaced. Interestingly, these *FtsH* that were up in bentazon treatment, appeared to be up in expression in the 2 hpi *P. syringae* samples, implying that the cells might have been attempting



D1 replacement in the early stage of pathogen defense, but this expression stopped by 8 hpi. A similar pattern of expression was also seen for another gene involved in PSII repair, a photosystem stability/assembly factor HCF-135 (Glyma1g10710.1). Western blotting of D1 in response to *P. syringae* also showed that D1 is not being noticeably degraded during the HR [32]. These differences in gene expression indicate recovery from bentazon, but no such recovery in HR, and are consistent with the hypothesis proposed by van Doorn [30] that it is the health of the chloroplast, and whether or not injured/non-functional ones are repaired or replaced, that determines whether or not a plant cell will undergo autophagic programmed cell death (PCD). The gene expression is consistent with the chloroplast becoming non-functional during the *P. syringae* induced HR and PCD is eminent, whereas under bentazon treatment in soybean, the chloroplast were being repaired, and recovering without initiating massive PCD.

**HR vs bentazon: similar oxidative stress and antioxidant accumulation**

There were 130 and 206 genes related to oxidative stress that were differentially expressed in soybean treated with bentazon and HR-inducing bacteria, respectively; 41 of which were statistically significant in both treatments. The hierarchical cluster of these 41 significant

genes displayed three distinct patterns between the HR-inducing and bentazon treatments (Figure 3). The most striking feature of this cluster was that 12 of the 19 genes that were strongly induced by both HR and bentazon were various glutathione S-transferases (GSTs), which are critical for quenching of free radicals and often involved in conjugation of herbicides, leading to herbicide resistance [33,34]. Furthermore, GSTs are effective antioxidants against oxidative stresses generated from toxins, ozone, and pathogen attack [35,36]. As these GSTs tend to modify a range of substrates, including foreign molecules such as some herbicides, it might be of benefit to the plant to simply express as many of these enzymes as possible during biotic or toxic stresses, increasing the odds that one will be able to modify and detoxify any introduced toxins.

Another noticed similarity in this group of oxidative-stress-related genes was that, of the 11 genes that were down-regulated in both treatments, five were peroxidases and two were genes encoding a respiratory burst NADPH oxidase homolog. Although NADPH oxidase has been shown to be needed for the initial oxidative burst observed within the first hour or two of compatible and incompatible interactions, it is also believed to be a negative regulator of PCD in some cases [37].

In addition to these 30 genes that had shared expression patterns between HR and bentazon treatments, there were 11 of the 41 genes showing opposite expression patterns, with eight (two of which were lipoxygenases) being down in HR and up in bentazon, and three (two of which were peroxidases) were up in HR and down in bentazon.

**HR vs bentazon: similar regulation of signaling components**

There were a total of 71 signaling related genes that were differentially expressed in both the HR and bentazon treatments with statistical significance. The majority of these genes shared very similar expression patterns as shown in Figure 4. This similarity is emphasized by the reduction of a variety of signaling genes in both experiments such as protein kinase, gibberellic acid related proteins, and myo-inositol-1-phosphate synthase (MIPS). The most prominent co-reduction of signaling-related genes was seen with the six cDNAs encoding MIPS. MIPS catalyze the reaction from glucose-6-phosphate to 1-myoinositol-1-phosphate, which is the rate-limiting step for the synthesis of inositols [38]. Inositols play critical roles in signal transduction for a variety of mammalian hormones and growth factors [39], and in plants have been reported to decreased upon pathogen infection [40].

Three jasmonic acid (JA) related genes encoding 12-oxophytodienoate reductase (OPR) were significantly increased in abundance in both experiments. 12-oxophytodienoate reductases are enzymes involved in JA biosynthesis [41,42] and the expression of the OPR3 gene was shown to be induced by a variety of stresses including UV-light, touch, wind, wounding, and the application of detergent. Additionally, two ethylene genes were also up in response both HR and bentazon. Although it is well documented that JA and ethylene play important roles in signaling when plants are attacked by many necrotrophic pathogens and insects [43], the general induction of some genes related to these pathways in both bentazon treated or *P. syringae* infected leaf tissue, supports that in these plants, JA may also function as signals during general oxidative stress caused by PSII inhibition.

Another instance where HR and bentazon showed similar regulation of signaling components included the induction of ethylene responsive elements such as beta-cyanoalanine synthase and ethylene response factor 1. An ACC oxidase increased especially at early time

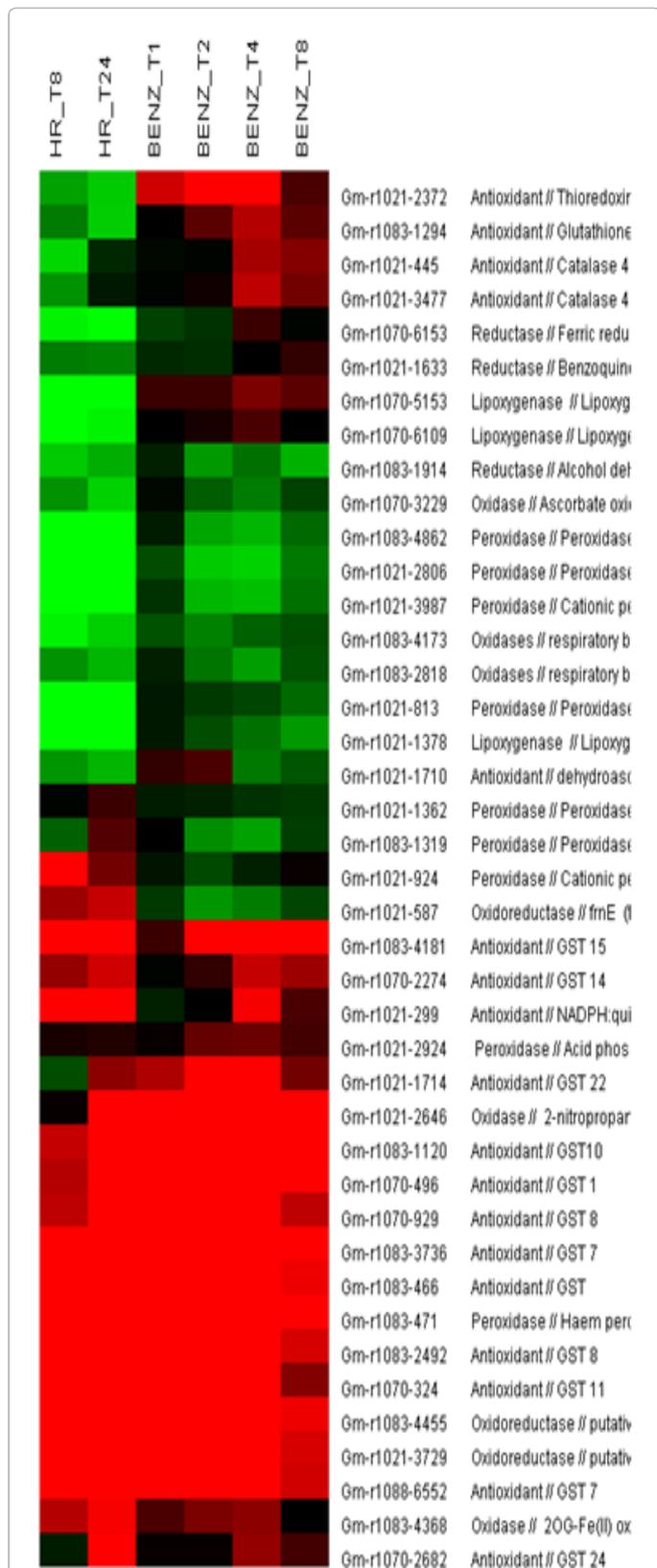


Figure 3: Hierarchical clustering of 41 redox related genes in soybeans undergoing HR and photosystem II inhibition by bentazon.

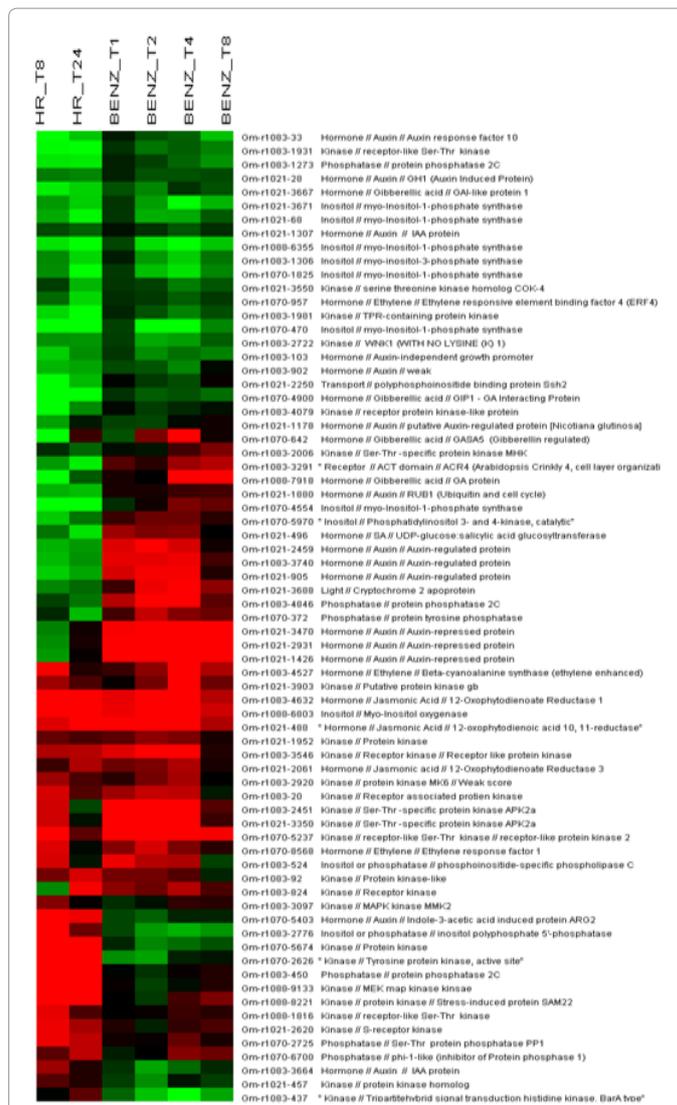


Figure 4: Hierarchical clustering of 71 signaling related genes in soybeans undergoing HR and photosystem II inhibition by bentazon.

points and then incrementally decreased at 8 hours in bentazon treatment, but this gene was not differentially expressed in the HR study. However, another ACC oxidase was overexpressed in HR. This result may indicate that the ethylene-mediated pathway was induced in both HR and bentazon treatment.

One of the noteworthy observations in the signaling category were the high number of genes related to auxin, and that although six showed a common direction of expression (down in both treatments), nine auxin related genes had opposite directions of expression, with seven of the nine being down in HR but up after bentazon treatment. Auxins are naturally synthesized plant hormones that regulate growth and development, and have also been implicated in several defense responses. As an important signal transduction component, auxins have shown the ability to either induce or repress various genes. In soybean, three families of auxin down-regulated genes were characterized and their expression was found to be regulated in a tissue/organ-specific manner by the level of auxin as well as light [44]. The overexpression

of auxin repressed genes is presumably caused by a reduction of auxin in the leaf tissue in bentazon treatment, whereas the reduction of auxin repressed genes in HR would indicate that auxin accumulated.

### HR vs bentazon: general induction of WRKY transcription factors

Cluster analysis identified 28 genes with homology to transcription factors that were statistically significantly expressed in both HR and bentazon treatment (Figure 5). The most impressive data was that, even though WRKYs are often thought of as being specific to biotic stresses, all the transcripts that encoded for WRKY transcription factors that were significant in both treatments, increased in abundance. WRKYs have been found in many plant species [45], and function by binding to conserved WRKY domains in the promoters of numerous defense

related plant genes [46,47]. Therefore, many WRKYs are considered to regulate the response to pathogen infection. The induction of WRKYs due to herbicide treatment was reported in soybean treated with glyphosate [23], but the role for WRKY transcription factors in plants treated with bentazon is unknown. However, WRKYs were found to be significantly expressed on the onset of leaf senescence [48], which could be indicative of regulation related to senescing or dying tissue. Therefore, we suspect that the induction of WRKYs could be caused by a provocation similar to leaf senescence induced by herbicide treatment in support of a discovery that WRKY can be induced by both pathogen infection and leaf senescence [49]. Alternatively, there might be a common signal triggered in both herbicide treatment and pathogen infection that leads to increased WRKY transcripts, such as oxidative stress. It was also found that certain WRKYs act upstream of NPR1 and positively regulate its expression during the activation of plant defense responses [50]. The induction of NPR transcript in our herbicide study is consistent with this hypothesis. Other increased transcription factors included ones homologous to MYB and NAM, which are also transcription factors induced by various stress [50].

### HR vs bentazon: differential regulation of phenylpropanoid biosynthesis

It is well recognized that many components in the phenylpropanoid pathway are induced at the transcript level upon pathogen infection, as they act as potent antimicrobial compounds [51]. The clustering of phenylpropanoid biosynthesis related genes between HR and bentazon treatments revealed both similarities and major differences (Figure 6). In order to obtain a clearer image of the activities of individual genes in this complex pathway, the three main branches of the phenylpropanoid pathway: isoflavonones, anthocyanins and lignin biosynthesis, were drawn and the increased or decreased modulation of each gene was indicated (Figures 7 and 8). During the HR, the isoflavonones biosynthetic branch was up regulated as reflected by increased transcript abundance for a series of genes specifically expressed within the pathway such as: isoflavone synthase (IFS), isoflavone reductase (IFR), isoflavone-O-methyltransferase (IOMT), and 2-hydroxyisoflavanonone dehydratase (2HID). Enzymes at the start of the pathway, such as phenylalanine ammonia-lyase (PAL), 4-Coumarate--CoA ligase (4CL), chalcone synthase (CHS), and chalcone isomerase (CHI) likewise showed increased transcript levels during HR. Conversely, the anthocyanins biosynthesis branch was attenuated as genes involved in this pathway were reduced at the transcript level, reflected in the reduced expression of: flavanone 3-hydroxylase (F3H), flavonoid-3', 5'-Hydroxylase (F3'5'H), flavonol synthase (FLS), dihydroflavonol-4-reductase (DFR) and UDP-glycose: flavonoid glycosyltransferase (UFGT). The repressed anthocyanin pathway indicates that the anthocyanin and flavonol products are not necessary for plant defense against *P. syringae* infection and that their repression could divert the chalcone molecules for the synthesis of isoflavonones and phytoalexins, which are important for defense. In the lignin biosynthesis branch, genes displayed a mixed expression pattern. Cinnamoyl-CoA reductase (CCR) was under-expressed and caffeic acid O-methyltransferase (COM), cinnamyl-alcohol dehydrogenase (CAD) and laccase showed both increased and decreased levels of gene expression for different cDNA clones within the microarray. The mixed pattern of gene expression could be attributed to family members with different subcellular locations or tissue specific regulation [52].

In contrast to the HR, the entire phenylpropanoid pathway in leaves treated with bentazon was generally repressed (Figures 6 and 7). In addition to the reduction of pathway entry enzymes such as PAL,

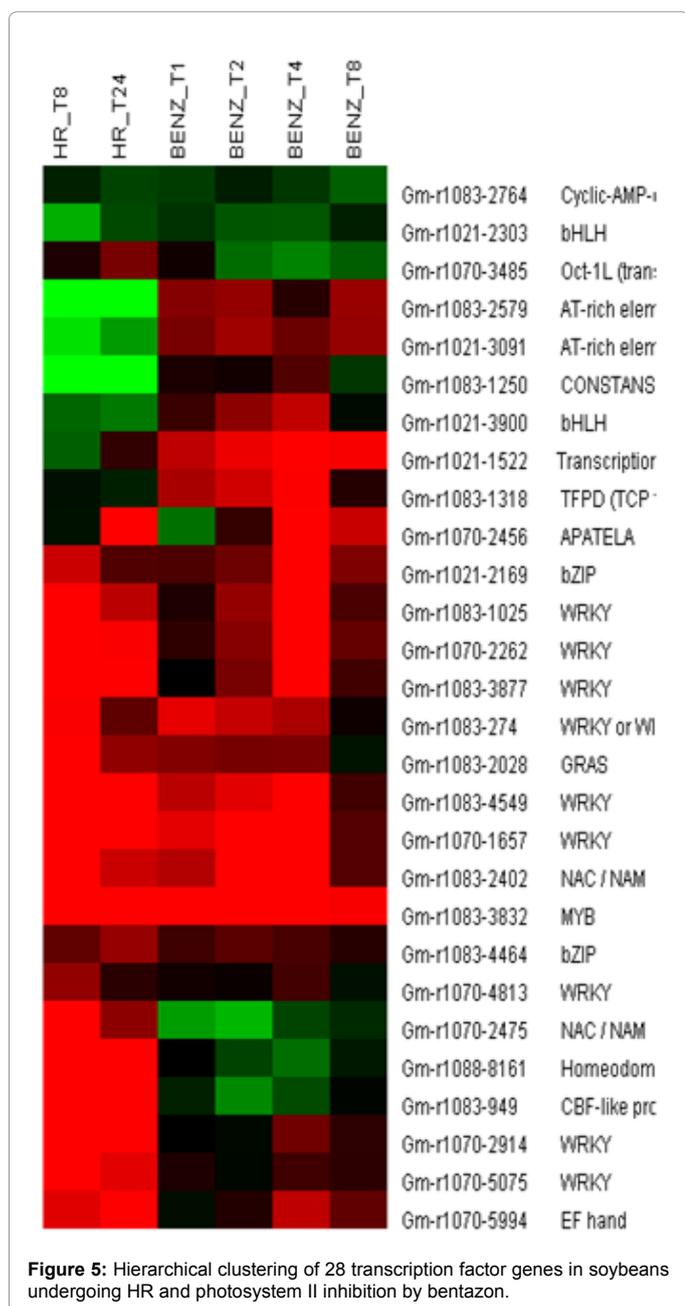
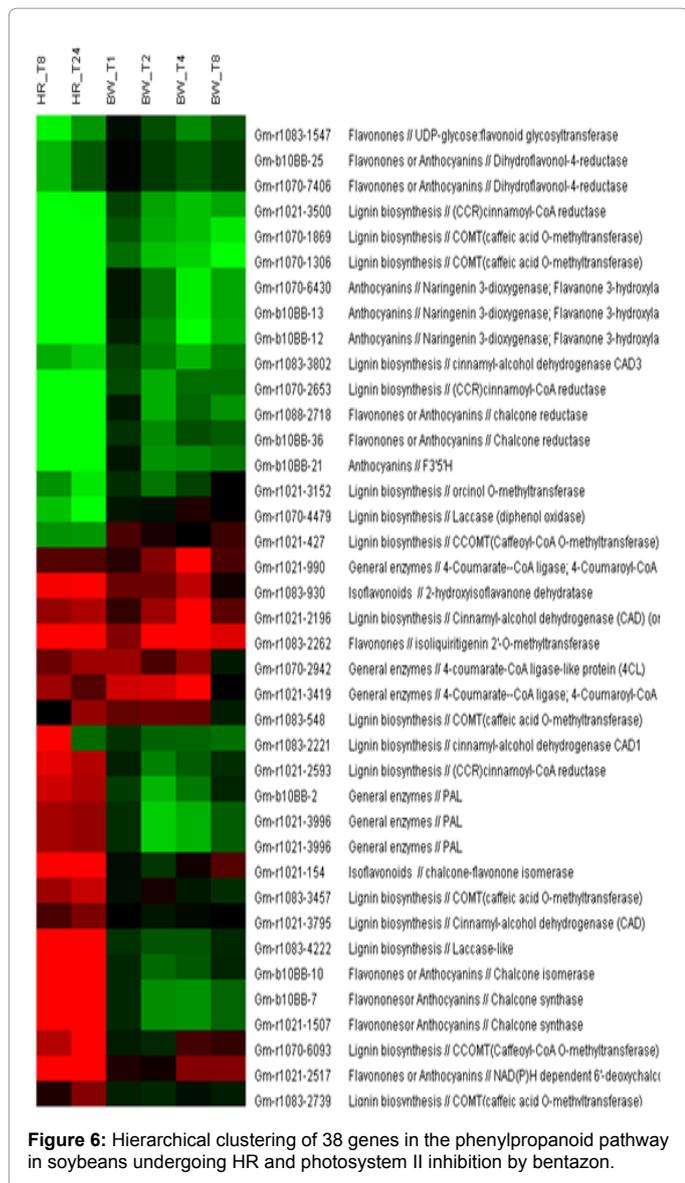


Figure 5: Hierarchical clustering of 28 transcription factor genes in soybeans undergoing HR and photosystem II inhibition by bentazon.



CHS, CHI and CHR, genes specially functioning in the isoflavones biosynthesis pathway branch including IFS, IFR, IOMT and 2'HID were all repressed. Likewise, the anthocyanins and flavonol biosynthesis were reduced concurrently as enzymes F3H, F3'H, F3'5'H, FLS, DFR and UFGT decreased significantly in transcript levels. Furthermore, expression of genes related to lignin biosynthesis was reduced including genes homologous to the key enzyme CCR, COMT and laccase. These genes were all repressed at all time points examined. It is suggested that induction of the phenylpropanoids is advantageous for plants to eliminate oxidative stress caused by pathogen infection, in addition to their antimicrobial toxicity [51]. The reduction of phenylpropanoid products in herbicide stress indicates that phenylpropanoid products are probably not essential antioxidant sources for oxidative stress caused by PSII inhibiting herbicide bentazon, and that perhaps the phenolic-based substrates for these pathways were diverted for other needs. These striking differences in expression of the multiple branches of the phenylpropanoid pathway, is one of the clearer differences in how plants responded to the *P. syringae* attack versus PSII inhibition induced by bentazon and shows that the induction of these genes in soybean is independent of PSII inhibition.

### HR vs bentazon: mixed patterns of defense related genes

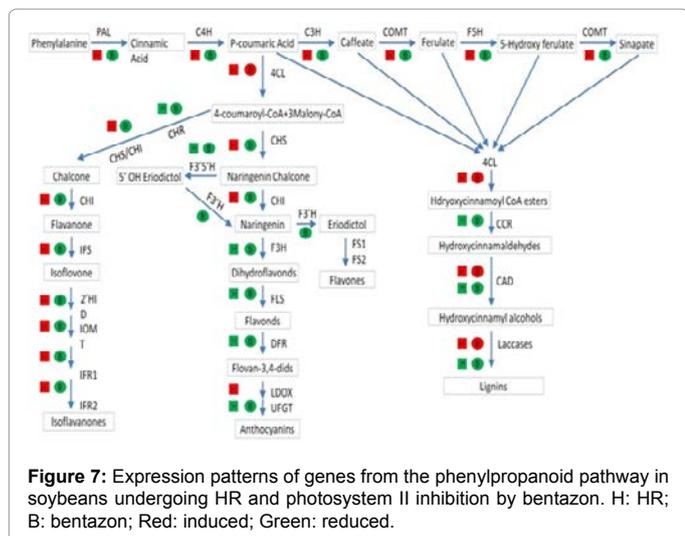
The clustering of defense related genes across the two experiments revealed both similar and contrasting expression patterns (Figures 5 and 6). Defense related genes that increased in both HR and bentazon treatments included two non-expression of pathogenesis resistance (NPR) homologs. NPR plays an essential role in salicylic acid (SA)-mediated local resistance and systemic acquired resistance when plants are infected by pathogens [53-55]. In addition to plant defense, SA also plays a role when plants are under adverse environmental stresses, such as salt and osmotic stress, by potentiating the production of ROS [56]. The main downstream component of SA is the NPR1 gene product, which is an activator of some defense-related transcription factors such as TGA [55]. The induced expression of the NPR gene in bentazon treatment suggests SA mediated signaling pathways may play a role in xenobiotic stresses as well as biotic. Other genes that increased in both experiments include several pathogenesis-related (PR) proteins.

Transcripts encoding beta-1,3-glucanase were increased in the HR but decreased in bentazon treatment. Beta-1,3-glucanases can release cell-wall fragments that serve as defense activating signals in defense response [57], but the reason for beta-1,3-glucanase repression by herbicide is unclear. Similarly, induction of PR-5 (thaumatin) is pathogen specific in this study. The adenine nucleotide translocator (ANT) was also induced in HR but reduced in the bentazon treatment. ANTs are mitochondrial proteins cooperating with BAX, an apoptosis molecule, to increase mitochondrial membrane permeability and trigger cell death [29]. The reduced levels of ANT transcripts observed in the bentazon treatment may reduce programmed cell death (PCD), compared to the increased ANT transcripts in HR which may reflect the effort of plants to induce PCD for defense to this hemi-biotrophic pathogen.

Several defense-related genes were repressed in both experiments. These genes included homologs to dirigent-like protein and class I chitinases. Interestingly, the class III acidic chitinases were regulated differentially, as they increased in HR but decreased in bentazon treatment.

### Conclusion

Expression patterns for these two studies overlapped greatly, roughly 67%, but there were still a lot of inconsistencies in expression



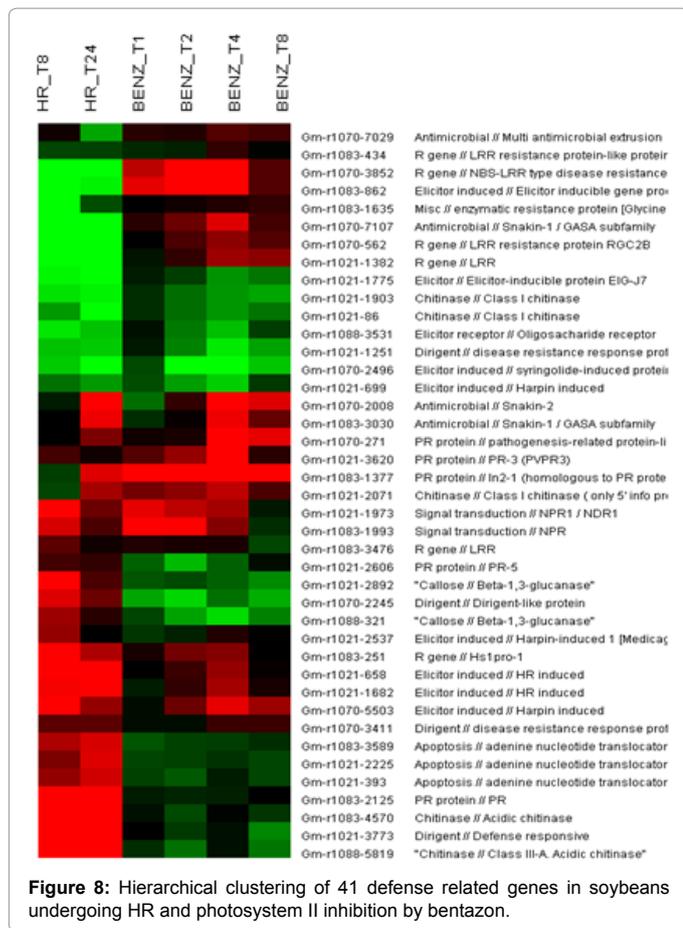


Figure 8: Hierarchical clustering of 41 defense related genes in soybeans undergoing HR and photosystem II inhibition by bentazon.

between the two treatments. The best possible explanation for why the gene expression correlation between the two treatments was not higher, even though both involve photoinhibition, would be that the dynamic interaction during HR involves too many other signaling events that also alter host gene expression. Plants make a multitude of responses and adjustments to an infection by a living pathogen (a pathogen that is also responding and making adjustments to the plant) as they both battle for life. Examples of important defense genes that were not induced by PSII inhibition, and yet were induced by the pathogen, were genes of the phenylpropanoid pathway. Perhaps these important genes were induced by other mechanisms, such as plant response to PAMPS.

Several studies support a role PSII inhibition in plant defense, including the measurement of reduced PSII efficiency at 8 hpi [8]. The observation that reducing levels of FtsH, a protease that degrades the D1 subunit of PSII prior to its replacement with healthy D1, enhanced resistance to TMV, pointed to a role of D1. One possibility was that damaged D1 would lead to blocked electron flow from  $Q_A$  to  $Q_B$ , and that this would be enough to enhanced defense. However, based on the gene expression comparisons here, of PSII inhibition induced by *P. syringae*, versus PSII inhibition induced by herbicide treatment that blocks  $Q_A$  to  $Q_B$  electron transfer in D1, one has to conclude that the enhanced ROS in HR defense, is most likely not solely the result of blocking D1 function, but more likely due to inefficient repair and replacement of photodamaged components.

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