Comparative proteomic analysis of plant responses to sound waves in Arabidopsis

Young Sang Kwon · Mi-Jeong Jeong · Jaeyul Cha · Sung Woo Jeong · Soo-Chul Park · Sung Chul Shin · Woo Sik Chung · Hanhong Bae · Dong-Won Bae

Received: 2 November 2012 / Accepted: 16 November 2012 © Korean Society for Plant Biotechnology

Abstract Environmental factors greatly influence the growth, development, and even genetic characteristics of plants. The mechanisms by which sound influences plant growth, however, remain obscure. Previously, our group reported that several genes were differentially regulated by specific frequencies of sound treatment using a sound-treated subtractive library. In this study, we used a proteomic approach to investigate plant responses to sound waves in Arabidopsis. The plants were exposed to 250-Hz or 500-Hz sound waves, and total proteins were extracted from leaves 8 h and 24 h after treatment. Proteins extracted from leaves were subjected to 2-DE analysis. Thirty-eight spots were found to be differentially regulated in response to sound waves and were identified using MALDI-TOF MS and MALDI-TOF/TOF MS. The functions of the identified proteins were classified into photosynthesis, stress and defense, nitrogen metabolism, and carbohydrate metabolism. To the best of our knowledge, this is the first report on the analysis of protein changes in response to sound waves in Arabidopsis leaves. These findings provide a better understanding of the molecular basis of responses to sound waves in Arabidopsis.

Keywords Arabidopsis, sound wave, proteomics, MALDI-TOF/TOF MS

Introduction

Plants possess highly complex sensory mechanisms to perceive dynamic surroundings and to adapt to predominant environmental conditions. Mechanical stresses, such as wind and touch, were shown to induce several physiological responses in plants, the majority of which resulted in differential growth, a reduction in growth rate, and a thickening of cell walls that resulted in enhanced resistance to subsequent mechanical stress (Jaffe and Forbes 1993). Plants have developed sensory mechanisms to detect mechanical stimulation and are capable of producing a suite of mechanically-induced responses in a process called thigmomorphogenesis (Lee et al. 2005). Physiological and developmental changes can occur in plants in response to mechanical stresses, including a rapid and dramatic fluctuation in gene expression and the synthesis of a variety of phytohormones (Yang et al. 1991). These molecular responses are thought to help plants withstand further environmental challenges (Braam et al. 1997).

Sound waves are one source of mechanical stress that affect the growth and development of plants (Braam et al., 1997; Wang et al., 2002a). The beneficial effects of sound
waves on plant physiological responses have been documented. Sound waves might encourage plant growth and change the transition temperature of cell walls, which has been shown to be strongly related to cell division (Keli and Baoshu 1999). Sound waves at 50-Hz significantly stimulated seed germination and root elongation in both rice and cucumber plants. Interestingly, this frequency barely influenced the elongation of cucumber hypocotyls, but it stimulated the elongation of rice coleoptiles (Takahashi et al. 1991). Similar results have been reported in Arabidopsis (Johnson et al. 1998; Uchida & Yamamoto 2002). Sound waves are reported to have a significant influence on membrane fluidity, the activity of H+-ATPase in plasmalemma, and the growth of callus tissue (Wang et al. 2002b). H+-ATPase activity in Chrysanthemum callus was apparently enhanced by sound stimulation. Many researchers report that sound waves with a certain frequency and strength could significantly accelerate the rates of growth and cell division, increase the contents of soluble proteins and ATP, and increase antioxidant enzyme activity in plants (Bochu et al. 2003; Xiaocheng et al. 2003; Xiujuan et al. 2003; Li et al. 2008). Bochu et al. (2004) demonstrated that IAA levels increased and ABA levels decreased in response to sound-wave stimulus in Chrysanthemum callus, suggesting that sound waves may be involved not only in the control of callus growth but also in the regulation of endogenous hormone levels. In addition, our group (Jeong et al. 2008) reported that gene expression in rice did not change in response to musical sounds, but several genes were differentially expressed after exposure to specific sound frequencies. The expression of the aldolase (AID) gene was regulated in a frequency-specific manner, increasing in response to frequencies of 125 and 250-Hz and decreasing in response to 50-Hz sound waves. The specific molecular mechanisms underlying plant responses to sound waves are still unclear.

In this study, we hypothesized that sound signals could trigger changes in gene expression. Proteomics provides an excellent opportunity to study the response of plants to environmental stress and to identify stress-responsive proteins. Therefore, a proteomic approach using 2-DE and MALDI-TOF-MS was performed to identify differentially expressed proteins in response to sound signals in Arabidopsis. The main goal of our study was to investigate the proteome expression pattern and to identify novel proteins that are differentially regulated upon exposure to sound waves. A total of 38 proteins that were up- or down-regulated in response to sound waves were indentified and classified into multiple functional groups. Our study highlights the importance of molecular and functional characterization at the protein level.

Materials and methods

Plant materials and sound wave treatment

Arabidopsis thaliana ecotype Columbia (Col-0) seeds were surface sterilized and maintained at 4°C for 3 days to induce seed dormancy. Seedlings were cultivated on solid MS medium in a growth chamber maintained at 22°C, 60% relative humidity, under long-day conditions (16 h light/8 h dark). After 2 weeks, the plants were exposed to either 250-Hz or 500-Hz sound waves for 1 h. The speaker (Korea Scientific Technique Industry Co, Korea) and plants were placed on different shelves to prevent transfer of vibrations between samples during the sound wave treatments. A photo of the experimental set-up for sound wave loading was presented in Figure 1. Arabidopsis seedling was stimulated by sound with given intensity (80 dB) and frequencies (250 / 500 Hz) for 60 min. Control groups were cultured in the same conditions except for the sound interference. Leaf samples were harvested 8 h and 24 h after sound wave treatments. All experiments were repeated three times with reproducible results.

Protein extraction and quantification

The proteins were prepared using a modified TCA/acetone/phenol extraction method (Wang et al., 2008). Three grams of leaf material were ground into a fine powder in liquid nitrogen. Cold TCA/acetone buffer (10% TCA, 0.07% 2-ME in acetone) was added to the powder, and the mixture was shaken for 1 h before centrifugation at 8,000 rpm for 15 min at 4°C. The supernatant was washed twice with cold acetone and air-dried. Equal volumes of SDS extraction buffer [30% sucrose, 1-2% SDS, 0.1 M Tris-HCL (pH 8.8)]

![Fig. 1](image-url) Photos of experimental set-up for sound wave exposure: Chamber (A) and sound wave control system (B).
and saturated phenol were added, and the sample was mixed by vortexing. The phenol phase was transferred to a new tube, and five volumes of 0.1 M ammonium acetate in methanol were added. Proteins were pelleted by centrifugation and washed once with cold methanol and twice with acetone. The pellet was dissolved in lysis buffer composed of 7 M urea, 2 M thiourea, 4% CHAPS, 1 mM PMSF, 50 mM DTT, and 0.5% IPG buffer (Amersham Biosciences, San Francisco, CA, USA). To determine the protein concentration, a 2D-Quant kit (Amersham Biosciences) was used.

2-D gel electrophoresis and image analysis

2-D gel electrophoresis was performed as previously described (Kwon et al. 2010). Briefly, proteins (100 µg) were separated using a 17-cm pH 5-8 IPG strip in a Protean IEF cell (Bio-Rad, Hercules, CA, USA) and in-gel rehydrated for 12 h. Isoelectric focusing (IEF) was performed at 20°C for 15 min at 250 V, 3 h at 10,000 V, and 9 h at 90,000 V. The strips were equilibrated using equilibrating solution [50 mM Tris-HCL (pH 8.8), 6 M urea, 30% glycerol, 2% SDS] containing 1% DTT and then incubated with the same buffer containing 2.5% iodoacetamide. The strips were transferred onto SDS-polyacrylamide gels and stained with silver. All experiments were repeated three times. Gel images were obtained using a GS-800 Imaging Densitometer (Bio-Rad) and analyzed using PDQuest version 7.2.0 software (Bio-Rad). The volume of each spot was normalized to a relative volume, and mean values from triplicate data were compared. Gel images were obtained using a GS-800 Imaging Densitometer (Bio-Rad) and analyzed using PDQuest version 7.2.0 software (Bio-Rad).

In-gel digestion

Silver-stained protein spots from each gel were destained (Katayama et al., 2001) and subjected to in-gel trypsin digestion as previously described (Lee et al. 2004). Briefly, excised gels were destained with a mixture of 15 mM potassium ferricyanide and 50 mM sodium thiosulfate (freshly prepared) for a few minutes. Then, the gels were washed vigorously with 500 µL of 50% methanol/40% water/10% acetic acid (MWA) for 5-20 min using a micro-tube MT-360 mixer (Tomy, Tokyo, Japan). After four replacements of the MWA, the gels were mixed with 500 µL of 50 mM ammonium bicarbonate solution for 5 min, mixed with 500 µL of acetonitrile for 5 min, and dried completely in a vacuum. The destained gel particles were reduced for 45 min at 56°C using a solution of 10 mM DTT/0.1 M ammonium bicarbonate, alkylated for 30 min at room temperature in the dark in 55 mM iodoacetamide/0.1 M ammonium bicarbonate, and then completely dried in a vacuum. For in-gel digestion, dried gel pieces were swollen in 2-3 µL of a digestion buffer that contained 25 mM ammonium bicarbonate, 0.1% n-octylglucoside, and 50 ng/mL of sequencing grade trypsin (Promega, Madison, WI, USA). After rehydration, the gel slices were added to 5-15 µL digestion buffer without trypsin to keep them wet during enzymatic cleavage (at 37°C overnight) in a siliconized tube. The peptides were extracted twice with one volume of acetonitrile/water/trifluoroacetic acid (66:33:0.1, v/v/v) solution, after which the sample was sonicated and centrifuged. The supernatant was collected, speed-vacuum dried, and dissolved in a solution containing 50% ACN and 0.1% TFA.

Protein identification using MALDI-TOF MS and MALDI-TOF/TOF MS

The peptide solution was spotted onto a stainless steel MALDI and MALDI TOF/TOF target plate. MALDI-MS was performed using a Voyager DE-STR Mass spectrometer (Applied Biosystems, Framingham, MA, USA), and spectra were collected in the reflection/delayed extraction mode. Monoisotopic peptide masses were analyzed using MoverZ (http://www.proteomics.com), and the NCBInr database was searched using MASCOT software (http://www.matrixscience.com). For database searches, a mass tolerance of 50 ppm, one missed cleavage, oxidation of methionine and cysteine modified by iodoacetamide were allowed. MS and MS/MS analyses were carried out on using an ABI 4800 Plus TOF-TOF Mass Spectrometer (Applied Biosystems, Framingham, MA, USA), which uses a 200 Hz ND: YAG laser operating at 355 nm. The 10 most- and least-intense ions per MALDI spot, with signal/noise ratios >25, were subjected to subsequent MS/MS analysis in the 1 kV mode using 800-1,000 consecutive laser shots. MS/MS spectra were searched against the NCBInr database using ProteinPilot v.3.0 software (with MASCOT as the database search engine), with a peptide and fragment ion mass tolerance of 50 ppm. Carboxamidomethylation of cysteines, oxidation of methionines, and one miss were allowed during the search of the peptides. Peptide mass tolerance and fragment mass tolerance of the 38 selected proteins were set to 100 ppm. High confidence identifications had statistically significant search scores (greater than 95% confidence) consistent with the
protein’s experimental pI and MW.

Protein functional classification

For functional classification, the differentially expressed proteins were distributed into categories according to Gene Ontology (GO) analysis in the TAIR (http://www.arabidopsis.org/), UniProtKB (http://www.uniprot.org/), KEGG (http://www.genome.jp/kegg/), and PIR (Protein Information Resource) databases (http://pir.georgetown.edu/), respectively, and assigned to one of three GO groups, which represent biological processes, molecular functions, and cellular components.

Results and discussion

Protein expression profiles of Arabidopsis leaves in response to sound waves

To investigate plant responses to sound waves, proteome profiles of Arabidopsis leaves were studied using two-dimensional gel electrophoresis. Arabidopsis seedlings were exposed to sound waves of either 250-Hz or 500-Hz for 1 h. Proteins were extracted from Arabidopsis leaves 8 h and 24 h after sound wave treatment and separated by 2-DE (Fig. 2).

Image analysis of protein spots that differed between the control and sound-wave-treated samples was performed using PDQuest 2D analysis software. Proteins showing at least 1.5-fold increased or decreased expression in three biological replicates were chosen for further analysis. A total of 38 protein spots showed statistically significant differences when compared to controls (Figs. 3 and 4).

The variations in protein expression levels after 250-Hz and 500-Hz treatments are displayed in Venn diagrams (Fig. 5). Significant changes in protein expression patterns between control and treated plants could be observed at both time points (8 h and 24 h). Twenty-seven and 30 protein spots were differentially regulated after 250-Hz and 500-Hz sound wave treatments, respectively. Among the spots, 17 and nine spots were up-regulated 8 h and 24 h after exposure to 250-Hz sound waves, respectively, and seven spots and one spot were down-regulated at 8 h and 24 h, respectively (Fig. 5A). Furthermore, 22 spots and three spots were significantly up-regulated 8 h and 24 h after exposure to 500-Hz sound waves, respectively, while four spots were down-regulated 8 h and 24 h after sound wave treatments, respectively (Fig. 5B). There were more significant differences in protein

Fig. 2 Representative 2-DE gel images of Arabidopsis leaves exposed to sound waves. Two-week-old seedlings of Arabidopsis control plants (A and D), and plants treated with 250-Hz (B and E) or 500-Hz (C and F) sound waves, were assessed after 8 h and 24 h. A total of 100 µg protein was loaded onto a 17 cm IPG strip with a linear gradient of pH 5-8. After isoelectric focusing, 12% SDS-PAGE gels were used for second-dimension separation. Proteins were visualized by silver staining.
Fig. 3 Enlarged images (A) and graphical representation (B) of differentially expressed proteins identified from Arabidopsis plants 8 h after sound wave treatment. The relative intensities of three biological replicates (±SD) are presented. All identified spots showed statistically significant differences ($P < 0.05$), according to Student’s t-test. The spot numbers are the same as those specified in (A). Asterisks indicate significant differences ($P \leq 0.05$) between control and sound wave-treated plants.
Protein identification and functional classification of sound wave-responsive proteins

Thirty-eight proteins responsive to sound waves were successfully identified by MALDI-TOF/MS analysis, as shown in Table 1. We also validated the identities of 38 proteins using peptide sequencing by MS/MS analysis (Supplemental Table 1). The functional distribution of proteins is shown in Fig. 6. The proteins were classified into categories including cellular components, molecular function, and biological process. Proteins involved in cellular components were subdivided into cytoplasm (24%), chloroplast (24%), mitochondrion (18%), plastid (11%), peroxisome (8%), cytosol (5%), thylakoid (5%), and others (5%) (Fig. 6A). Proteins associated with molecular function were divided into eight sub-groups, namely antioxidant (18%), oxidoreductase (18%), binding (13%), lyase (13%), isomerase (13%), hydrolase (12%), transferase (8%), and unknown (5%) (Fig. 6B). Proteins involved in biological processes were divided into six sub-groups, including stress and defense (26%), photosynthesis (21%), nitrogen metabolism (13%), carbohydrate metabolism (8%), transport/protein folding (8%), and other metabolic processes (16%) (Fig. 6C).

In this study, we focused on four areas that are closely related to the response of plants to sound wave stimulation, including photosynthesis, stress and defense, nitrogen metabolism, and carbohydrate metabolism. The roles of these proteins are discussed below with respect to their functional distributions.
Fig. 5 Venn diagram analysis of proteins that were differentially expressed as a result of sound wave treatment. The numbers of differentially expressed proteins from *Arabidopsis* treated with 250-Hz (A) or 500-Hz (B) sound waves are presented in Venn diagrams.

Fig. 6 Functional distribution of identified proteins. Using TAIR, UniProtKB, KEGG, and PIR database Go annotation, differentially expressed proteins were classified into three main groups. Identified proteins were categorized as either cellular components (A), molecular function proteins (B), or proteins involved in biological processes (C). Pie chart values represent the percentages of each subgroup of protein that comprises each of the three protein categories.
Table 1: Identification of proteins differentially expressed after sound wave treatment in *Arabidopsis*

<table>
<thead>
<tr>
<th>No</th>
<th>Protein</th>
<th>ID (NCBI)</th>
<th>MW/pI, theor.</th>
<th>MP/TP</th>
<th>SC (%)</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 h</td>
<td>24 h</td>
<td>250 Hz</td>
<td>500 Hz</td>
</tr>
<tr>
<td>1</td>
<td>Photosystem I reaction center subunit II-2</td>
<td>5732201</td>
<td>22335/9.81</td>
<td>7/10</td>
<td>29</td>
<td>-2.38</td>
</tr>
<tr>
<td>2</td>
<td>Hypothetical protein</td>
<td>2961342</td>
<td>20195/5.23</td>
<td>5/10</td>
<td>22</td>
<td>-2.56</td>
</tr>
<tr>
<td>3</td>
<td>Putative protein</td>
<td>7413654</td>
<td>19243/6.07</td>
<td>4/8</td>
<td>26</td>
<td>-2.23</td>
</tr>
<tr>
<td>4</td>
<td>2-Cys peroxiredoxin</td>
<td>15229806</td>
<td>29074/6.92</td>
<td>7/11</td>
<td>33</td>
<td>+2.93</td>
</tr>
<tr>
<td>5</td>
<td>Fe superoxide dismutase 1</td>
<td>15234913</td>
<td>23776/6.06</td>
<td>5/10</td>
<td>12</td>
<td>+2.30</td>
</tr>
<tr>
<td>6</td>
<td>Manganese superoxide dismutase 1</td>
<td>79313181</td>
<td>25329/8.47</td>
<td>6/11</td>
<td>30</td>
<td>+2.40</td>
</tr>
<tr>
<td>7</td>
<td>Glutathione S-transferase</td>
<td>20197312</td>
<td>24062/6.09</td>
<td>10/11</td>
<td>52</td>
<td>+1.74</td>
</tr>
<tr>
<td>8</td>
<td>Glutathione S-transferase PM24</td>
<td>15235401</td>
<td>24114/5.92</td>
<td>12/16</td>
<td>35</td>
<td>+1.85</td>
</tr>
<tr>
<td>9</td>
<td>Glutathione S-transferase</td>
<td>21555418</td>
<td>24036/6.08</td>
<td>5/10</td>
<td>40</td>
<td>+1.86</td>
</tr>
<tr>
<td>10</td>
<td>Carbonic anhydrase 1</td>
<td>30678347</td>
<td>29485/5.54</td>
<td>8/12</td>
<td>49</td>
<td>+4.00</td>
</tr>
<tr>
<td>11</td>
<td>Chlorophyll a/b binding protein</td>
<td>15222446</td>
<td>28602/5.29</td>
<td>6/11</td>
<td>21</td>
<td>-3.84</td>
</tr>
<tr>
<td>12</td>
<td>Chlorophyll a/b binding protein</td>
<td>15224465</td>
<td>28602/5.29</td>
<td>7/10</td>
<td>15</td>
<td>-4.36</td>
</tr>
<tr>
<td>13</td>
<td>Chlorophyll a/b binding protein</td>
<td>18403546</td>
<td>28036/5.28</td>
<td>9/17</td>
<td>28</td>
<td>-2.32</td>
</tr>
<tr>
<td>14</td>
<td>Triosephosphateisomerase</td>
<td>15226479</td>
<td>33325/7.67</td>
<td>11/12</td>
<td>36</td>
<td>+2.92</td>
</tr>
<tr>
<td>15</td>
<td>Triosephosphateisomerase</td>
<td>15226479</td>
<td>33325/7.67</td>
<td>11/14</td>
<td>34</td>
<td>+2.90</td>
</tr>
<tr>
<td>16</td>
<td>Triosephosphateisomerase</td>
<td>15233272</td>
<td>27152/5.39</td>
<td>11/17</td>
<td>41</td>
<td>+2.04</td>
</tr>
<tr>
<td>17</td>
<td>NADH dehydrogenase flavoprotein 2</td>
<td>18411985</td>
<td>28370/8.09</td>
<td>8/14</td>
<td>25</td>
<td>-3.56</td>
</tr>
<tr>
<td>18</td>
<td>Glutathione S-Transferase</td>
<td>2554769</td>
<td>23983/5.93</td>
<td>6/11</td>
<td>41</td>
<td>+2.00</td>
</tr>
<tr>
<td>19</td>
<td>Adenylatekinase 1</td>
<td>15242753</td>
<td>26915/6.91</td>
<td>10/12</td>
<td>52</td>
<td>-2.03</td>
</tr>
<tr>
<td>20</td>
<td>Carbonic anhydrase 2</td>
<td>42573371</td>
<td>28326/5.36</td>
<td>7/10</td>
<td>33</td>
<td>+2.23</td>
</tr>
<tr>
<td>21</td>
<td>AT2G37660</td>
<td>22720445</td>
<td>26280/5.29</td>
<td>12/16</td>
<td>50</td>
<td>+1.80</td>
</tr>
<tr>
<td>22</td>
<td>Carbonic anhydrase 2</td>
<td>42573371</td>
<td>28326/5.36</td>
<td>11/14</td>
<td>62</td>
<td>+2.53</td>
</tr>
<tr>
<td>23</td>
<td>Putative p-nitrophenylphosphatase</td>
<td>26450878</td>
<td>34034/5.09</td>
<td>10/17</td>
<td>26</td>
<td>+1.93</td>
</tr>
<tr>
<td>24</td>
<td>Thiazole biosynthetic enzyme, chloroplastic</td>
<td>61679812</td>
<td>30030/5.88</td>
<td>6/8</td>
<td>14</td>
<td>+2.63</td>
</tr>
<tr>
<td>25</td>
<td>THI1; protein homodimerization</td>
<td>15239735</td>
<td>36641/5.82</td>
<td>12/18</td>
<td>37</td>
<td>+2.90</td>
</tr>
<tr>
<td>26</td>
<td>PYK10-BINDING PROTEIN 1</td>
<td>15228198</td>
<td>32138/5.46</td>
<td>8/13</td>
<td>36</td>
<td>+5.44</td>
</tr>
<tr>
<td>27</td>
<td>Ferredoxin-NADP+ reductase 1</td>
<td>145334919</td>
<td>26695/5.91</td>
<td>7/11</td>
<td>19</td>
<td>-4.89</td>
</tr>
<tr>
<td>28</td>
<td>Ferredoxin-NADP+ reductase 2</td>
<td>15223753</td>
<td>41142/8/51</td>
<td>9/13</td>
<td>25</td>
<td>+2.02</td>
</tr>
<tr>
<td>29</td>
<td>Arginase, putative</td>
<td>15236635</td>
<td>37957/5.90</td>
<td>9/15</td>
<td>30</td>
<td>+1.68</td>
</tr>
<tr>
<td>30</td>
<td>ATP synthase gamma chain 1</td>
<td>18412632</td>
<td>40886/8.13</td>
<td>7/10</td>
<td>22</td>
<td>+1.92</td>
</tr>
<tr>
<td>31</td>
<td>Cysteine synthase C1</td>
<td>15233111</td>
<td>39902/8/71</td>
<td>8/12</td>
<td>20</td>
<td>+2.23</td>
</tr>
<tr>
<td>32</td>
<td>Glutamine synthetase cytosolic isozyme 1-2</td>
<td>15219598</td>
<td>39183/5.14</td>
<td>8/12</td>
<td>18</td>
<td>+1.79</td>
</tr>
<tr>
<td>33</td>
<td>Ribulosebisphosphate carboxylase/oxygenaseactivase</td>
<td>18405145</td>
<td>51948/5.87</td>
<td>13/16</td>
<td>27</td>
<td>+1.96</td>
</tr>
<tr>
<td>34</td>
<td>Elongation factor Tu</td>
<td>15237059</td>
<td>51598/5.84</td>
<td>9/18</td>
<td>23</td>
<td>+3.85</td>
</tr>
<tr>
<td>35</td>
<td>Isocitrate dehydrogenase</td>
<td>15218869</td>
<td>45717/6.13</td>
<td>10/13</td>
<td>22</td>
<td>+2.08</td>
</tr>
<tr>
<td>36</td>
<td>Monodehydroascorbatereductase</td>
<td>15231702</td>
<td>46458/6.41</td>
<td>12/12</td>
<td>26</td>
<td>+2.44</td>
</tr>
<tr>
<td>37</td>
<td>beta glucosidase 18</td>
<td>15218992</td>
<td>60421/6.74</td>
<td>13/15</td>
<td>33</td>
<td>+2.91</td>
</tr>
</tbody>
</table>

* Numbers correspond to the 2-DE gels shown in Fig. 1.
* Theoretical MW (Da) and pl values.
* Ratio of matched peptides/detected peptides.
* Sequence coverage.
* '+' up regulation; '-' down regulation after sound-wave treatment.
Photosynthesis-related proteins

Generally, plants are known to use light energy to convert carbon dioxide into organic compounds through a process known as photosynthesis. The functions of eight identified proteins (spots 1, 11, 12, 13, 27, 28, 30, and 34) were associated with photosynthesis (Table 1 and Fig. 5). Seven of eight proteins involved in photosynthesis were differentially regulated 8 h after sound wave exposure, but not after 24 h, suggesting that photosynthesis-related proteins could be regulated at early stages during the sound wave response in plants. Photosystem I reaction center subunit II-2 (spot 1) and chlorophyll a/b binding protein (spots 11, 12, and 13) were down-regulated 8 h after sound wave treatment. On the other hand, two ferredoxin-NADP⁺ reductases (spots 27 and 28), ATP synthase gamma chain 1 (spot 30), and ribulosebisphosphate carboxylase/oxygenaseactivase (spot 34) were up-regulated. Interestingly, only ATP synthase gamma chain 1 (spot 30) decreased 24 h after treatment with 500-Hz sound waves.

Photosynthesis in green plants is mediated by two types of reaction centers, photosystems I and II, to engender proton motive force and reducing power (NADPH or NADH). Photosystem I reaction center subunit II-2 (spot 1) and chlorophyll a/b binding protein (spots 11, 12, and 13), which are related to the light reactions of photosynthesis, were significantly down-regulated. Like various biotic stresses (Bilgin et al. 2010), sound wave stress resulted in the down-regulation of several photosynthesis-related proteins. Ferredoxin-NADP⁺ reductase (spots 27 and 28) and ATP synthase gamma chain 1 (spot 30) were up-regulated (Table 1 and Fig. 5). Ferredoxin-NADP⁺ reductase is known to be a plastidial enzyme that participates in cellular defense against oxidative damage and catalyzes the reduction of ferredoxin by NADPH in heterotrophic plant cells (Bilgin et al. 2010). Tobacco plants overexpressing ferredoxin-NADP⁺ reductase showed an augmented tolerance to photo-oxidative damage and redox-cycling oxidants (Rodriguez et al. 2007). Thus, the increased expression of ferredoxin-NADP⁺ is thought to be related to photo-oxidative protection (Cortleven et al. 2011). We detected a 3.14-fold increase in the level of ATP synthase gamma chain 1 (spot 30) 8 h after the 500-Hz treatment, but the level of this protein was slightly decreased (1.69-fold) 24 h after the 500-Hz treatment. Rubiscoactivase has been reported to play an important role in plant responses to various stress conditions including light stress (Liu et al. 1996), cold stress (Goulas et al. 2006), and stress induced by jasmonic acid (Shan et al. 2010). In our study, Rubiscoactivase (spot 34) was up-regulated 1.96-fold 8 h after 250-Hz sound wave treatment.

Stress- and defense-related proteins

Eight of the differentially expressed protein spots were stress- and defense-related proteins: glutathione S-transferase (spots 7, 8, 9, and 18), 2-Cys peroxiredoxin (spot 4), Fe superoxide dismutase 1 (spot 5), manganese superoxide dismutase 1 (spot 6), and beta glucosidase 18 (spot 38). All of these proteins were up-regulated at 8 h in response to 250- and 500-Hz sound waves (Table 1 and Fig. 5).

Sound waves are one type of mechanical and environmental stress. Environmental stress is known to generate reactive oxygen species (ROS), causing oxidative damage to cellular components. Various biotic and abiotic stresses can generate and accumulate ROS, which may act as signaling molecules that trigger plant stress responses (Herbette et al. 2011). The levels of seven proteins with antioxidant functions were increased by sound wave stress. For example, 2-Cys peroxiredoxin (spot 4), Fe superoxide dismutase 1 (spot 5), and glutathione S-transferase (spot 18) were significantly up-regulated after sound wave treatment. In addition, the levels of three glutathione S-transferases (spots 7, 8, and 9), and manganese superoxide dismutase 1 (spot 6), increased after sound wave treatment. Glutathione S-transferase (spots 7, 8, and 9) is an important enzyme that can scavenge toxic compounds and regulate ROS level in plant cells. Glutathione S-transferase is reported to be involved in diverse aspects of biotic and abiotic stresses, especially detoxification processes (Jiang et al. 2010). A previous study showed that different organs of Dendrobiumcandidum accumulated ROS, and activities of SOD, CAT, POD, and APX in leaves, stems, and roots of D. candidum significantly increased after sound wave stress treatment (Bochu et al. 2004). Two superoxide dismutases, Fe superoxide dismutase 1 (spot 5) and manganese superoxide dismutase 1 (spot 6), were also up-regulated by sound waves in Arabidopsis leaves. The level of 2-Cys peroxiredoxin (spot 4), an antioxidant enzyme primarily found in developing shoots and photosynthesizing leaves, also increased in response to sound wave treatment. Thus, antioxidant enzymes induced by sound wave treatments are thought to play an important role in protecting plant cells from oxidative damage generated from sound waves.

Beta glucosidase 18 is induced by various stresses, such as cold and osmotic stress (Ahn et al. 2009), and is the key enzyme involved in the abscisic acid signaling pathway (Lee et al. 2006; Kato-Noguchi et al. 2008). Induction of this enzyme under sound wave stress suggests that the
particular sound wave frequency that was tested may induce the plant defense system in *Arabidopsis*.

Nitrogen metabolism-related proteins

The levels of two enzymes related to nitrogen metabolism (arginase; spot 29 and glutamine synthetase cytosolic isozyme 1-2; spot 32) were differentially changed by sound-wave treatment (Table 1 and Fig. 2).

The level of glutamine synthetase cytosolic isozyme 1-2 (spot 32), which catalyzes the synthesis of glutamine from glutamate, was increased at 24 h after 500-Hz sound wave treatment. As a critical nutritional compound required by plants, assimilated nitrogen could support the use of CO₂ to produce the basic building blocks of plants, including sugars, organic acids, amino acids, nucleotides, chlorophylls, and numerous other metabolic components (Nunes-Nesi et al. 2010). Members of the cytosolic glutamine synthetase gene family are regulated in response to plant nitrogen status and environmental cues, including nitrogen availability and biotic/abiotic stresses (Bernard and Habash 2009).

Arginase (spot 29), an enzyme involved in the polyamine biosynthesis pathway, was induced in the 8 h samples treated with either 250-Hz or 500-Hz sound waves (Table 1 and Fig. 5). Arginase expression in plants is regulated by developmental or stress-related cues. A previous report showed that arginase activity increased in response to wounding, jasmonic acid treatment, and bacterial pathogen attack in tomato leaves (Chen et al. 2004).

Carbohydrate metabolism-related proteins

Carbohydrate metabolism is an important function in plants that serves to capture photosynthetic energy and provide the carbon needed for the production of new tissues (Sivaci 2006). Many genes are involved in major carbohydrate metabolic pathways that include the Calvin cycle, glycolysis, and the glycogenesis pathway. Stressing plants with dehydrative treatments (e.g., low-temperatures, heat, drought, salinity, and mechanical wounding) induce profound changes in carbon metabolism systems including carbon fixation (Calvin cycle) and carbohydrate metabolism (Kosova et al. 2011).

Three homologs of triosephosphateisomerase (spot 14, 15, and 16) involved in the Calvin cycle and glycolysis were up-regulated in *Arabidopsis* plants analyzed 8 h after the 500-Hz sound wave treatment. Triosephosphateisomerase levels have also been reported to be responsive to many environmental stresses imposed on plants including drought, salt, and metal stress (Riccardi et al. 1998; Salekdeh et al. 2002; Fukao et al. 2009). Isocitrate dehydrogenase is involved in the tricarboxylic acid cycle. The stimulation of both of these enzymes is thought to be required for the production of metabolic energy and redox power to fulfill the demands of cells in response to sound stress. Isocitrate dehydrogenase was also found to be up-regulated by cadmium stress in *Arabidopsis* (Sarry et al. 2006).

Carbonic anhydrase levels rapidly increase in response to treatment with 200 mM NaCl, increasing the carboxylation rate of RuBisCO (Badger and Price 1994; Fan et al. 2011). Carbonic anhydrase 1 and 2 (spot 10, 20, and 21) were dramatically increased by the sound wave treatment, suggesting that carbonic anhydrase may be involved in increasing the carboxylation rate of RuBisCO in response to sound wave stress.

Conclusion

To date, there have been no reports describing a comparative proteomic analysis of *Arabidopsis* in response to sound waves. We performed proteomic analysis to monitor physiological and biochemical changes in *Arabidopsis* induced by sound waves. A total of 38 protein spots responsive to sound waves were identified by MALDI-TOF/MS. Our data showed that proteins belonging to different functional groups including photosynthesis, stress and defense, carbon and nitrogen metabolism, and carbohydrate metabolism were significantly affected by sound waves, meaning that plants actively respond to sound signals in various biological ways. This is the first proteome report focused on the protein expression profile in *Arabidopsis* exposed to sound waves. Our study provides a better understanding of the molecular mechanisms underlying responses to sound waves in plants.

Acknowledgments

This work was carried out with the support of the Cooperative Research Program for Agriculture Science & Technology Development (PJ907054) of the Rural Development Administration, Korea. YSK was supported by scholarships from the BK21 program funded by MOEST in Korea.

References


early responses of *Arabidopsis thaliana* cells to cadmium exposure explored by protein and metabolite profiling analyses. Proteomics 6:2180-2198