

Drought tolerance induced by sound in Arabidopsis plants

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ABSTRACT

We examined the responses of sound-treated arabidopsis adult plants to water deprivation and the associated changes on gene expression. The survival of drought-induced plants was significantly higher in the sound treated plants (24,8%) compared to plants kept in silence (13,3%). RNA-seq revealed significant up-regulation of 87 genes including 32 genes involved in abiotic stress responses, 31 involved in pathogen responses, 11 involved in oxidation-reduction processes, 5 involved in the regulation of transcription, 2 genes involved in protein phosphorylation/dephosphorylation and 13 involved in jasmonic acid or ethylene synthesis or responses. In addition, 2 genes involved in the responses to mechanical stimulus were also induced by sound, suggesting that touch and sound have at least partially common perception and signaling events.

Keywords

Bioacoustics, white noise, touch, mechano-stimulus, water stress

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Disclosure of Potential conflicts of interest

No potential conflicts of interests were disclosed

Declaration of Authorship

ILR performed the RNA extractions. CMV conceived and designed the experiments, analyzed the data and wrote the manuscript.

Conflict of interest

The authors declare no competing interests.

Sounds are mechanical waves of pressure that propagate through a transmission medium such as air. As a wave of pressure, the sound can be considered as a mechanical stimulus and it could have an influence on developmental and physiological plant processes (Telewski, 2006; Hassanien *et al.*, 2014; Mishra *et al.*, 2016). Different types of sound have been demonstrated to increase the growth of mung bean, rice, cucumber and arabidopsis seedlings (Takahashi *et al.*, 1992; Johnson *et al.*, 1998; Cai *et al.*, 2014), increased the leaf area in young strawberry plants (Qi *et al.*, 2010) and increased the root length in *Actinidia chinensis* and paddy rice (Bochu *et al.*, 2003; Yang *et al.*, 2004). Some sounds seem to be capable of orienting root growth (Gagliano *et al.*, 2012, 2017) and significantly increased the callus growth in *Dendranthema morifolium* (Zhao *et al.*, 2003) and *Chrysanthemum* (Xiujuan *et al.*, 2003a). There are also evidences that determinate sounds can influence the development of fruits, for example, delaying tomato fruit ripening (Kim *et al.*, 2015), has an influence in pollination, for example, in buzz-pollination the pollen from anthers is only released upon vibration at a particular frequency produced by bee buzz (De Luca & Vallejo-Marín, 2013). In a similar way, bat dependent plants have adapted to the bats' echolocation systems by providing acoustic reflectors to attract their animal partners (Schöner *et al.*, 2016). Active acoustic signaling in plants has been proposed although it is still under discussion (Gagliano, 2013).

Sound-induced changes in plants have also been observed at the physiological level. Some sounds increased the photosynthetic state of strawberry plants (Zhou *et al.*, 2010; Meng *et al.*, 2012). The photosynthetic performance index in sound-treated arabidopsis plants was lower compared to the control (Ghosh *et al.*, 2017), and the expression of different genes encoding for RuBisCO subunits was altered (Ghosh *et al.*, 2016). In arabidopsis, sound altered the expression of several enzymes involved in light reaction, Calvin cycle, glycolysis and TCA cycle, with majority of them being up-regulated (Ghosh *et al.*, 2016). Sound alters the levels of some phytohormones: increased polyamine content in chinese cabbage and cucumber (Qin *et al.*, 2003), ethylene production was lower in the sound-treated tomatoes (Kim *et al.*, 2015) and sound increased the level of indole acetic acid (IAA) and decreased the level of abscisic acid in *Chrysanthemum calli* (Bochu *et al.*, 2004). In arabidopsis, sound induced changes in the levels of gibberelins, auxins, jasmonic and salicylic acids, but not in ABA (Ghosh *et al.*, 2016). Sound has also the ability to alter antioxidant activities (Jeong *et al.*, 2008; Xiujuan *et al.*, 2003b; Mishra *et al.*, 2016; Ghosh *et al.*, 2016), and to increase oxygen uptake (Qin *et al.*, 2003), thus appears that ROS signaling is a player for sound-mediated signaling. Sound also induces the accumulation of ATP (Xiaocheng *et al.*, 2003; Yang *et al.*, 2004; Mishra *et al.*,

2016), increases plasma membrane H⁺-ATPase activity (Wang *et al.*, 2002; Yi *et al.*, 2003a), induces a higher electrolyte leakage in arabidopsis (Ghosh *et al.*, 2017), alters the calcium flux and increases the concentration of intracellular Ca²⁺ (Liu *et al.*, 2001; Wang *et al.*, 2002; Yi *et al.*, 2003a; Mishra *et al.*, 2016). Additional observed changes in cell composition induced by sound are an increase in soluble sugars (Jia *et al.*, 2003; Yi *et al.*, 2003b; Zhao *et al.*, 2003), changes in free amino acid contents (Measures & Weinberger 1973) and the induction of lipid peroxidation (Li *et al.*, 2008). Sound-induced changes in cell structure have also been observed. Sound altered the secondary structure of cell-wall proteins (Ziwei *et al.*, 1999) and the plasma membrane fluidity and permeability (Keli *et al.*, 1999; Zhao *et al.*, 2002; Yang *et al.*, 2004; Mishra *et al.*, 2016).

Sound produced no significant increase of DNA content but enhanced the synthesis of RNA and soluble proteins in *Chrysanthemum* (Xiujuan *et al.*, 2003c). A significant increase in the expression of the rice genes coding for a fructose 1,6-bisphosphate aldolase (ald) and ribulose 1,5-bisphosphate carboxylase (Rubisco) small subunit (rbcS) was observed and the ald promoter was shown to respond to specific sound frequencies (Jeong *et al.*, 2008). The expression level of several ethylene biosynthetic and ripening-regulated genes was influenced by sound in tomato fruits (Kim *et al.*, 2015). A recent microarray analysis in arabidopsis plants noted that several genes were differentially expressed after sound treatment (Ghosh *et al.*, 2016) and some of them are also induced by mechanical stimulus (Ghosh *et al.*, 2017). Changes in protein accumulation were observed in response to sound (Ghosh *et al.*, 2016). Many respiratory genes/proteins were up-regulated as well as amino acid biosynthetic enzymes, enzymes related to protein metabolism, folding and degradation and genes involved in sulfur, nitrogen and carbohydrate metabolism (Ghosh *et al.*, 2016). The arabidopsis transcriptomic and proteomic analyses showed the induction/accumulation of several stress- and pathogen defense-related genes/proteins (Ghosh *et al.*, 2016). Induction of defense mechanism by sound was previously observed (Chowdhury *et al.*, 2014). Sound enhanced the resistance of strawberry against diseases and insects (Qi *et al.*, 2010), vibration in arabidopsis leads to the accumulation of defense molecules (Appel & Cocroft 2014) and sound increased their resistance against *Botrytis* infection (Choi *et al.*, 2017). The treatment of plants with specific sound frequencies increased the disease resistance in pepper, cucumber, and tomato (Tian *et al.*, 2009).

All these data suggest the existence of molecular mechanisms for sound perception and signal transduction in plants and sound seems to induce plants defense mechanisms against pathogens and against different abiotic stresses.

Our results were focused on determining possible effects of sound on drought resistance of *Arabidopsis thaliana* plants and on the possible induced changes in gene expression. A precise description of the methods used is presented in the Supplemental File 1.

To explore a possible effect of sound in plant drought resistance, six-week-old *Arabidopsis* plants grown at 22°C with a 8/16-h light-dark photoperiod were treated with 10 h 100 dB of white noise at the middle of the dark period during one week. Control plants were kept in silence. Irrigation was stopped at the beginning of the sound treatment. After the two weeks of drought the plants were re-watered. The survival rates were calculated one week after re-watering (Supplemental File 2). Sound treated plants showed an increased drought tolerance and resulted in significantly higher survival rates compared to untreated plants (Fig.1): 24,8 % (± 3.81) of the sound-treated plants surveyed compared to 13,3 % (± 3.16) of the plants kept in silence. This difference is statistically significant and indicate that the white noise increases *arabidopsis* drought tolerance.

The possible transcriptomic changes in *arabidopsis* upon exposure to sound were investigated through RNA-seq analysis. Six week old plants at the rosette stage were exposed during 10 h to 100 db white noise during the night and the samples were collected just at the end of the sound treatment. The samples corresponded to the eight youngest leaves of the rosette. The control samples were collected in the same moment with the only difference that the plants were not exposed to sound. Total RNA was extracted, cDNA libraries constructed and sequencing runs were performed in the Illumina HiSeq 2000 platform. A total of 3.65×10^8 reads were generated. Of the clean reads, 94.4% were uniquely mapped to the *arabidopsis* reference genome sequence. The normalized FPKM was used to quantify the gene expression level (Trapnell *et al.*, 2010) to reveal differentially expressed genes. The expression of 89 genes was considered to be altered by sound (\log_2 fold change ≥ 2 and q-value < 0.05): 87 up-regulated and 2 down-regulated (Table 1). To validate RNA-seq results, RT-PCR was done in order to reveal the trend in the expression pattern of genes (Fig. 2). Expression of six genes was validated. In all the cases the RT-PCR results confirmed the patterns of expression obtained in the RNA-seq analysis.

A GO analysis was performed to determine the function of the identified differentially expressed genes (Supplemental Files 3 and 4). From the 87 up-regulated genes, 44 (51%) are involved in responses to different types of stresses and the function of 22 is unknown. In order to confirm the enrichment in stress-related genes a GO enrichment analysis was performed (Table 2). Most of the 15 significantly overrepresented GO terms are related to stress, defense or response to stimulus.

Our results open new aspects of the discussion on the possible effects of sound on plants. Despite the growing data on different effects of sound in plants (Hassanien *et al.*, 2014, Mishra *et al.*, 2016), the detailed molecular events triggered by sound still remain mainly unknown. In order to get some more insight in this area, we performed an RNA-seq assay and we noted differential gene expression of many genes in arabidopsis rosette leaves and we found that sound treatment increases the tolerance to water deficit of the arabidopsis plants.

Different mechanisms have been proposed to explain sound effects on plants. For example, sound stimulation might cause leaves' stomata to open (Meng *et al.*, 2012). Another theory is that sounds induce in plants a similar response as touching (thigmomorphogenesis) (Chehab *et al.*, 2009). Like sound, touch is as an external mechanical force which interacts with the plant surface. Interestingly, described thigmomorphogenetic responses include resistance to other stresses. Transcriptomic studies had identified mechanoresponsive genes, including TOUCH genes (TCH) that mainly encode calmodulins or calmodulin-like proteins and xyloglucan endo-transglycosylase/hydrolase (XTH) (Chehab *et al.*, 2009), genes encoding protein kinases (Mizoguchi *et al.*, 1996), transcription factors (Gilmour *et al.*, 1998), genes involved in jasmonic acid and ethylene synthesis (Mauch *et al.*, 1997; Arteca & Arteca, 1999), or genes involved in antioxidative responses (Gadea *et al.*, 1999). A transcriptome analysis of touch-stimulated arabidopsis rosette leaves identified over 700 differentially expressed genes (Lee *et al.*, 2005), most of them up-regulated. Up-regulated genes were specially enriched in calcium-binding proteins, cell-wall proteins, disease resistance proteins, kinases and transcription factors.

Our RNA-seq results present similarities to what was observed after touch induction, for example, we also observed a predominant up-regulation, and two genes directly involved in mechanical responses are among those up-regulated genes: TCH4, encoding a xyloglucan endotransglucosylase/hydrolase, and ACS6, encoding the 1-aminocyclopropane-1-carboxylic acid synthase 6. White-noise up-regulated genes include at least 32 genes involved in abiotic stress responses, 31 involved in pathogen responses, 11 involved in oxidation-reduction processes, five involved in transcription regulation, two genes involved in protein phosphorylation/dephosphorylation and 13 involved in jasmonic acid or ethylene synthesis or responses. Similar results were obtained in a microarray analysis based on single frequency treatments in Arabidopsis (Ghosh *et al.*, 2016, 2017).

Cell-walls play an important role in the perception of the mechanical stimulus (Telewski *et al.*, 2006). We have identified at least four genes whose function is related to cell-walls: TCH4, laccase12, GOLS8 and XTH18. TCH4 encodes a cell-wall modifying enzyme that

breaks the xyloglucan chains and make the cell-wall more elastic. The cell-wall interacts with the cell membrane, which, in turn, interacts with the cytoskeleton, which can modify the activity of ion channels, resulting in changes in the concentration of ions like calcium in the cytoplasm, which can activate several calcium binding proteins and kinases which can induce huge changes in the transcriptome and proteome, which eventually affect several vital processes, like growth, development and defense against pathogens or abiotic stresses. Supporting this hypothesis, two up-regulated genes encode calcium related proteins: a calcium-binding EF-hand family protein and a calcium uniporter. Previous results also indicate that ROS signaling is another player for sound-mediated signaling (Qin *et al.*, 2003; Jeong *et al.*, 2008; Xiujuan *et al.*, 2003b; Mishra *et al.*, 2016; Ghosh *et al.*, 2016). We identified 11 up-regulated genes involved in different aspects of oxidation-reduction processes. It thus appears that ROS signaling is an important player also for sound perception. Calcium, ROS and possibly hormonal changes may probably explain most of the effects of the sound response signaling previously observed as changes in the development and the induction of pathogen resistance, and also the induction of at least 32 genes involved in different aspects of abiotic stress, including nine genes directly involved in water stress responses, and may explain our observed increase in water deficit tolerance.

We can conclude that sound has an impact on plants probably through a perception mechanism at least partially common to that of mechanical stimuli. Previous and our experiments demonstrate that plants can respond to sounds but each experiment has been performed using different parameters, so it is difficult to systematize the results. The conditions needed may depend on each species, the tissue and the phenomenon studied, or the frequencies, intensity or duration of the sound. Another interesting question is whether sound responses are relevant in natural conditions or just a side effect of mechanoperception. The acoustic responses of plants can offer insights into innovative practical applications such as reducing the negative effects of water deficit or a reduction in the use of pesticides. In addition, the use of sound-induced promoters may have interesting biotechnological applications.

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FIGURE LEGENDS

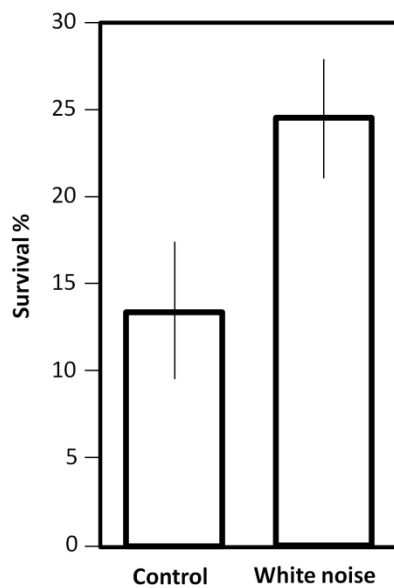


Figure 1.- Increase in the drought resistance in arabidopsis plants exposed to white noise. The percentage of plant survival is indicated. Bars represent the standard errors.

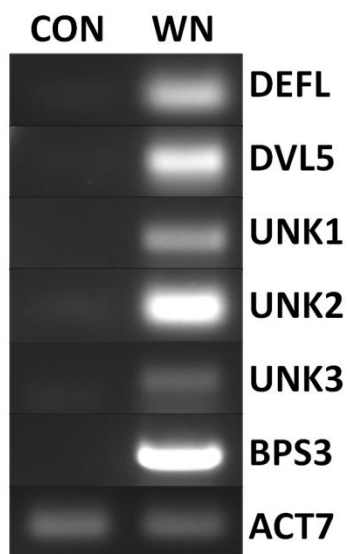


Figure 2.- RT-PCR analysis of the expression profiles of six arabidopsis genes identified as differentially expressed by RNA-seq, all them significantly over-expressed in the sound treated plants (WN) respect to the controls (CON). Ethidium bromide stained 1.5% agarose gels showing RT-PCR products. The genes are (see Table 1): DEFL, At1g13607; DVL5, At1g68825; UNK1, At2g31930; UNK2, At4g01535; UNK3, At5g38700; BPS3, At4g01360. ACT7 corresponds to actin7 (At5g09810) and was used as control of non-induced gene. In each case, the size of the band shown is those expected.

Table 1.- List of the Significantly Induced Genes in arabidopsis leaves after white noise treatment (log2 fold change ≥ 2 and q-value < 0.05)

Gene_id	Gene	WN/CON	CON	WN	p_value	q_value
AT1G13607	Defensin-like	a	0,0	1,4	5,0e-05	0,00601
AT1G68825	Devil 5; Rotundifolia-like 15	a	0,0	0,4	2,0e-04	0,01862
AT2G31930	Unknown protein	a	0,0	0,9	5,0e-05	0,00601
AT4G01535	Unknown protein	a	0,0	1,4	5,0e-05	0,00601
AT5G38700	Cotton fiber protein	a	0,0	0,5	5,0e-05	0,00601
AT4G01360	BYPASS 3	41,6	0,2	7,9	5,0e-05	0,00601
AT4G30280	Xyloglucan endotransglucosylase /Hydrolase 18	26,5	0,5	12,0	5,0e-05	0,00601
AT1G35140	Exordium like 1; Phosphate-induced 1	25,1	0,5	12,0	5,0e-05	0,00601
AT5G52050	Detoxification efflux carrier 50	21,1	0,2	3,2	5,0e-05	0,00601
AT3G56790	RNA splicing factor-like	19,3	0,6	11,3	5,0e-05	0,00601
AT2G14247	Unknown protein	19,0	0,7	12,6	5,0e-05	0,00601
AT1G19210	DREB subfamily A-5 ERF/AP2 transcription factor	18,8	3,3	62,2	5,0e-05	0,00601
AT4G24580	ROP1 ENHANCER 1, Rho GTPase-activating protein	18,2	0,1	1,9	5,0e-05	0,00601
AT3G56970	Basic Helix-Loop-Helix 38; OBP3-Responsive Gene 3	15,6	0,7	11,0	5,0e-05	0,00601
AT1G50750	Aminotransferase-like mobile domain protein	14,3	0,2	3,1	5,0e-05	0,00601
AT2G17660	RPM1-interacting protein 4	12,6	0,9	10,7	5,0e-05	0,00601
AT3G02840	ARM repeat superfamily protein	11,5	11,1	126,7	5,0e-05	0,00601
AT3G44350	NAC domain containing protein 61	11,3	0,4	4,8	5,0e-05	0,00601
AT1G80840	WRKY DNA-binding protein 40	11,2	21,1	237,1	7,0e-04	0,04911
AT2G32130	Intracellular protein transporter putative (DUF641)	10,4	0,5	5,5	5,0e-05	0,00601
AT5G42380	Calmodulin like 37	10,4	4,5	47,2	5,0e-05	0,00601
AT3G23250	MYB domain protein 15	9,9	1,8	17,7	5,0e-05	0,00601
AT4G29780	Nuclease	9,9	17,8	175,2	5,0e-05	0,00601
AT3G01830	Calcium-binding EF-hand family protein	9,4	2,9	27,5	1,0e-04	0,01106
AT1G61340	F-BOX stress induced 1	8,9	4,6	40,7	5,0e-05	0,00601
AT5G22240	Ovate family protein 10	8,9	1,7	15,4	5,0e-05	0,00601
AT1G66090	Disease resistance protein TIR-NBS class	8,8	4,9	43,3	5,0e-05	0,00601
AT1G72520	Lipoxinase 4	8,3	2,5	21,0	5,0e-05	0,00601
AT2G30020	MAPK phosphatase clade B of the PP2C-superfamily	7,9	33,0	259,2	5,0e-05	0,00601
AT5G57560	TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22	7,5	20,4	153,5	5,0e-05	0,00601
AT2G35930	U-BOX 23; E3 ubiquitin ligase	7,2	7,8	55,6	5,0e-05	0,00601
AT5G45340	Cytochrome P450 family 707 Subfamily A Polypeptide 3	7,1	10,5	74,7	5,0e-05	0,00601
AT2G46400	WRKY DNA-binding protein 46	7,1	10,8	76,4	5,0e-05	0,00601
AT3G28340	Galactinol synthase 8; Galacturonosyl transferase-like 10	6,9	2,3	16,2	5,0e-05	0,00601
AT2G34600	Jasmonate-zim-domain protein 7; TIFY5B	6,9	1,2	8,6	5,0e-05	0,00601
AT3G46090	C2H2 and C2HC zinc fingers superfamily	6,8	1,6	10,9	5,0e-05	0,00601
AT1G22480	Cupredoxin superfamily protein	6,6	0,3	2,2	5,0e-05	0,00601
AT1G30135	Jasmonate-ZIM-domain protein 8	6,6	1,7	11,4	5,0e-05	0,00601
AT3G61190	BON Association protein 1	6,5	10,8	70,7	5,0e-05	0,00601
AT4G25490	C-Repeat/DRE Binding Factor 1; DRE Binding protein 1B	6,5	4,2	27,1	5,0e-05	0,00601
AT5G05390	Laccase 12	6,4	0,3	1,7	3,0e-04	0,02536
AT1G56660	MAEBL domain protein	6,4	7,2	46,1	5,0e-05	0,00601
AT3G52450	U-BOX 22 U-box domain E3 ubiquitin ligase	6,3	3,4	21,9	5,0e-05	0,00601
AT1G74930	DREB subfamily A-5 of ERF/AP2 transcription factor	6,1	43,4	263,4	5,0e-05	0,00601
AT1G17420	Lipoxynase 3	5,8	3,5	20,0	5,0e-05	0,00601
AT1G28480	Glutaredoxin GR480	5,7	5,1	29,2	5,0e-05	0,00601
AT1G76650	Calmodulin-Like 38	5,5	94,5	517,1	5,0e-05	0,00601
AT3G55980	Salt-inducible Zinc Finger 1	5,5	52,0	284,0	2,5e-04	0,02220
AT5G64870	SPFH/Band 7/PHB domain-containing membrane-associated	5,4	1,6	8,5	5,0e-05	0,00601
AT1G47400	Unknown protein	5,4	3,3	17,9	5,0e-05	0,00601
AT1G01560	MAP Kinase 11	5,4	4,1	22,3	5,0e-05	0,00601
AT5G35735	Auxin-responsive family protein	5,4	19,1	103,7	5,0e-05	0,00601

AT2G14610	Pathogenesis related 1	5,4	1,0	5,2	5,0e-05	0,00601
AT1G02400	Gibberellin 2-Oxidase 4	5,4	1,4	7,6	5,0e-05	0,00601
AT4G39670	Glycolipid transfer protein	5,3	5,4	28,6	5,0e-05	0,00601
AT5G47850	CRINKLY related 4	5,3	0,5	2,5	5,0e-05	0,00601
AT2G01180	Lipid phosphate phosphatase 1; Phosphatidic acid phosphatase 1	5,2	6,6	34,1	5,0e-05	0,00601
AT3G62260	Protein phosphatase 2C	5,1	11,6	59,2	5,0e-05	0,00601
AT1G74450	Unknown protein	5,1	24,6	124,1	5,0e-05	0,00601
AT5G66650	Calcium uniporter (DUF607)	5,0	4,2	21,0	5,0e-05	0,00601
AT2G14290	LL-diaminopimelate protein (DUF295)	5,0	0,3	1,3	5,5e-04	0,04080
AT1G18300	NUDIX Hydrolase homolog 4	4,7	92,6	438,8	5,0e-05	0,00601
AT4G11280	1-Aminocyclopropane-1-carboxylic acid synthase 6	4,7	32,3	152,8	1,0e-04	0,01106
AT4G25470	C-REPEAT/DRE binding factor 2; DRE/CRT-Binding protein 1C; Freezing tolerance QTL4	4,7	23,0	108,4	5,0e-05	0,00601
AT5G41750	Disease resistance protein (TIR-NBS-LRR class)	4,7	2,4	11,2	5,0e-05	0,00601
AT5G21960	DREB subfamily A-5 of ERF/AP2 transcription factor	4,7	7,7	36,0	5,0e-05	0,00601
AT1G66160	CYS MET PRO and GLY Protein 1	4,7	3,3	15,4	5,0e-05	0,00601
AT5G22545	Unknown protein	4,7	3,1	14,5	5,0e-05	0,00601
AT2G41640	Glycosyltransferase family 61	4,7	12,2	57,1	5,0e-05	0,00601
AT2G30040	Mitogen-activated protein kinase kinase kinase 14	4,6	9,0	41,8	5,0e-05	0,00601
AT5G64310	Arabinogalactan 1	4,6	20,7	95,7	5,0e-05	0,00601
AT2G23810	TETRASPANIN 8	4,6	39,9	182,8	5,0e-05	0,00601
AT1G47395	Unknown protein	4,5	8,6	38,7	5,0e-05	0,00601
AT1G60190	ATPUB19: U-BOX 19	4,5	14,4	64,8	5,0e-05	0,00601
AT1G72950	Disease resistance protein (TIR-NBS class)	4,5	2,0	8,9	5,0e-05	0,00601
AT2G40140	Salt-inducible Zinc Finger 2	4,5	26,5	118,3	1,0e-04	0,01106
AT1G02660	Alpha/beta-Hydrolases superfamily	4,5	1,2	5,2	5,0e-05	0,00601
AT4G24380	Dihydrofolate reductase	4,5	21,1	93,8	2,5e-04	0,02220
AT2G44840	Ethylene-responsive Element Binding Factor 13	4,4	6,9	30,5	5,0e-05	0,00601
AT5G05410	Dehydration-responsive element binding protein 2	4,4	1,1	4,8	1,5e-04	0,01528
AT1G17380	Jasmonate-ZIM-domain protein 5; TIFY11A	4,3	8,8	38,2	5,0e-05	0,00601
AT3G50930	Cytochrome BC1 Synthesis; Outer Mitochondrial Membrane Protein	4,3	8,6	36,4	5,0e-05	0,00601
AT3G10930	IDA-LIKE7, IDL7	4,2	28,0	116,9	5,0e-05	0,00601
AT1G76600	Polymerase	4,2	20,2	84,3	5,0e-05	0,00601
AT2G39650	Cruciferin (DUF506)	4,1	6,4	26,1	5,0e-05	0,00601
AT3G46620	RING and DOMAIN of unknown function 1117 1	4,1	57,1	234,8	3,0e-04	0,02536
AT5G03210	DBP-Interacting-protein 2	4,1	30,2	122,3	5,0e-05	0,00601
ATCG01130	Translocon at the inner envelope membrane of chloroplasts 214	4,3 ^b	0,7	0,2	5,0e-05	0,00601
AT4G03445	miR447A, targets several 2-phosphoglycerate kinase-related	c	1,1	0,0	6,0e-04	0,04335

a, expression was only detected in the sound treated samples

b, expression was higher in the control sample

c, expression was only detected in the control samples

Table 2- GO ontology enrichment analysis showing significant over-represented functional categories in the leaves of sound treated arabidopsis plants compared to all genes.

Name of the annotation data category	Total ¹	Induced ²	Expected ³	Fold Enrichment ⁴	P value ⁵
Response to chitin	109	9	0,35	25,66	2.35E-07
Response to organonitrogen compound	154	10	0,50	20,18	2.08E-07
Response to nitrogen compound	228	11	0,73	15,00	5.15E-07
Response to wounding	182	8	0,59	13,66	3.31E-04
Response to jasmonic acid	172	7	0,55	12,65	3.40E-03
Response to acid chemical	886	21	2,85	7,37	1.37E-09
Response to oxygen-containing compound	1144	26	3,68	7,06	3.55E-12
	356	8	1,15	6,98	4.51E-02
Response to inorganic substance	690	13	2,22	5,86	7.38E-04
Response to organic substance	1466	22	4,72	4,66	2.25E-06
Defense response	1253	18	4,03	4,47	1.87E-04
Response to chemical	2084	28	6,70	4,18	8.04E-08
Response to stress	2639	31	8,49	3,65	1.50E-07
Response to abiotic stimulus	1491	17	4,80	3,54	1.05E-02
Response to stimulus	4612	40	14,84	2,70	8.66E-07

1 Total number of genes in each annotation data category in *Arabidopsis thaliana* .

2 The number of induced genes in each annotation data category.

3 The number of genes you would expect in each annotation data category in *Arabidopsis thaliana* according to the total number of induced genes supposing a random distribution.

4 Number of induced genes in each annotation data category divided by the expected number. If it is greater than 1, it indicates that the category is overrepresented.

5 The p-value determined by the binomial statistic. This is the probability that the number of genes you observed in this category occurred by chance (randomly).

SUPPLEMENTAL FILES LEGENDS

Supplemental File 1.- Materials and methods

Plant materials and drought treatment

Arabidopsis thaliana (Col-0 ecotype) seeds were placed in 5.5 cm diameter pots containing the same quantity of soil and stratified for 3 days in the dark at 4°C for homogenous germination. Subsequently, pots were transferred to the growth room at 22°C with 8/16-h light-dark photoperiod. Each pot contained one plant.

Water deficit stress assays were performed with six-week-old plants at rosette stage. Irrigation was stopped for two weeks maintaining the same environmental conditions. After this period the plant were watered again. The survival rates of plants were assessed one week after rehydration. It was considered that a plant had survived when it was able to produce new leaves. The results were calculated based on five independent experiments with 21 plants per treatment. The statistical analyses were done using the t test for 2 independent means. Significance level was tested at $p < 0.05$.

Sound treatment

Sound treatments were performed over six-week-old plants at rosette stage growing at 22°C with a 8/16-h light-dark photoperiod. They consisted in 10 h of 100 dB white noise (a random signal having equal intensity at all the frequencies between 35 and 22000 Hz) at the middle of the dark period. To prevent the mechanical vibrations during the sound treatment the speaker and plants were placed on different platforms. Control plants were kept in similar conditions without sound. The Audacity 2.0.3 software was used for sound generation. For RNA-seq experiments After the sound treatment, the eight youngest rosette leaves were harvested for RNA extraction.

RNA-seq

Total RNA was extracted with Maxwell RSC Plant RNA kit (Promega) according to the manufacturer's instructions and purified with Maxwell RSC Instrument. The cDNA library for Illumina HiSeq 2000 sequencing was constructed using an mRNA-seq sample preparation kit (TruSeq® RNA Sample Prep Kit v2, Cat.8207217, Illumina Inc., San Diego, CA, USA). The cDNA libraries were pooled at equal molar concentration for loading onto two lanes of an Illumina flow cell and then merged for data analysis. Sequencing runs were performed in the Illumina HiSeq 2000 platform at aScidea Computational Biology Solutions, S.L (Sant Cugat del Valles, Barcelona, Spain). Overall experiment quality was determined using FastQC and initial data processing was

performed using Fastx toolkit (Bullard et al. 2010). The sequence reads were mapped to the arabidopsis reference genome (TAIR10) using TopHat 2.1.1 (Trapnell et al. 2009) and Bowtie (Langmead 2010) under default parameters, except for inner-distance parameter which was set to 50bp. The Cuffdiff 2.2.1 program (Trapnell et al. 2010) was used to test statistically significant differences in transcript expression. Genes with $\text{Log}_2\text{FC} > 2$ and $\text{Log}_2\text{FC} < -2$ (Log_2 Fold-Change) and a q value < 0.05 were selected as genes differentially expressed (DEG). Selected genes were surveyed using Gene Ontology (GO) (<http://amigo.geneontology.org/amigo/search/ontology?q=UV-B>) and The Arabidopsis Information Resource (TAIR) (<https://www.Arabidopsis.org/>) database. Enrichment of GO categories among the selected genes was assessed using Panther GO ontology enrichment analyses (release 20170413; <http://pantherdb.org/webservices/go/overrep.jsp>) (Mi et al. 2017).

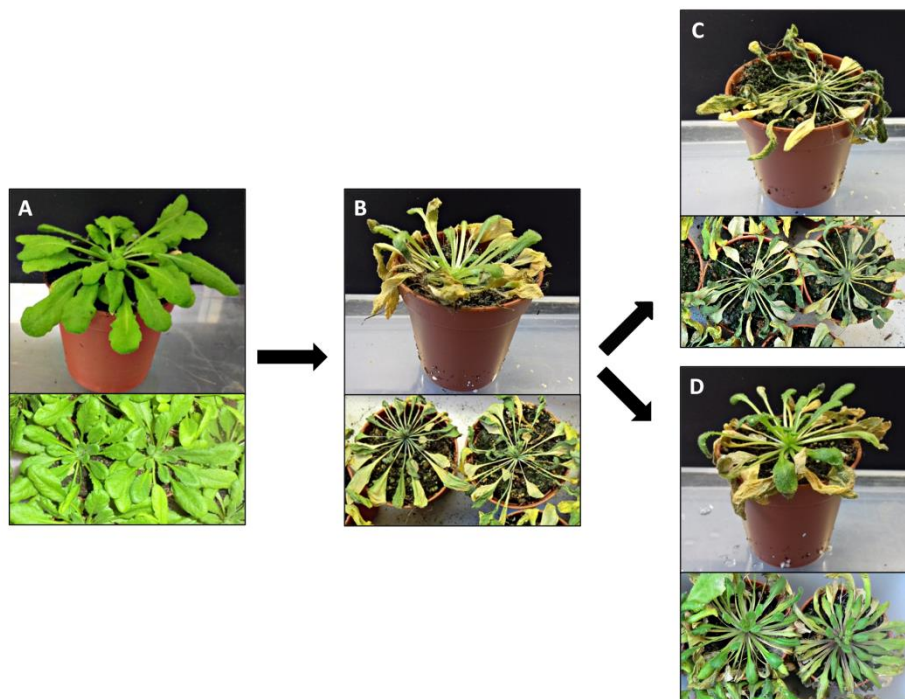
Validation of RNA-seq analysis by RT-PCR analysis

Total RNAs were treated with DNase I (RNase-free DNaseI, Promega) and 2 μg was reverse transcribed with the Omniscript RT kit (Qiagen) using an oligo-dT primer. cDNAs were amplified with specific primers (Supplemental Table 1). Controls with non-reverse transcribed RNA were also used to detect gDNA contamination. Actin 7 gene (At5g09810) was used as a control for RNA loading. PCR reactions were performed using 0.2 mM each dNTP, 360 $\mu\text{g ml}^{-1}$ BSA and 1 $\text{pmol } \mu\text{l}^{-1}$ of each primer in a final volume of 50 μl . The reaction mixtures were heated to 95 $^{\circ}\text{C}$ for 5 min, followed by cycles of 94 $^{\circ}\text{C}$ for 30 s, 55 $^{\circ}\text{C}$ for 30 s, and 72 $^{\circ}\text{C}$ for 90 s. Reactions were completed with incubation at 72 $^{\circ}\text{C}$ for 10 min. The amounts of template cDNA and the number of PCR cycles were determined for each gene to ensure that amplification occurred in the linear range and allowed good comparison of the amplified products. Three independent analyses were carried out on each sample.

Primers used:

Gene_id	Gene	Abbr.	bp	Ind.	Primer 1	Primer 2
AT1G13607	DEFL; Defensin-like	DEFL	180	SO	AGCCGTTCTTGCTTCACCA	GGCATGCATTTACAGGTCCA
AT1G68825	DVL5; Devil 5; RTFL15; Rotundifolia-like 15	DVL5	126	SO	GACCGGTTCCGAGTGTAGGAG	TGCCAACAAAGAAGCATGACA
AT2G31930	Unknown protein (UNK1)	UNK1	165	SO	ACATTTTCGAGGATGGCGAGG	TTAAACCGAAAGACCGGCCGA
AT4G01535	Unknown protein (UNK2)	UNK2	155	SO	GGCAAGGGAAGTCACTCCAG	GATTGCGAAGATCTGGGCCT
AT5G38700	Cotton fiber protein (UNK3)	UNK3	106	SO	AACAGCGTTGACAACAACCG	TCATGGATGTAGGGCGATGC
AT4G01360	BPS3; BYPASS 3	BPS3	244	41,62	ACGCGGGACTTAAAGTAGCGATTG	GGTTCCGAGAGCGGTACGTTG
AT5G09810	Actin7	ACT7	1100	ND	GGCCGATGGTGAGGATATTC	CTGACTCATCGTACTCACTC

Supplemental File 2.- Example pictures of arabidopsis plants at different moments of the drought treatment. A, before the treatment; B, after 15 days of drought; C, after 15 days of re-watering some plants were didn't survive; D, after 15 days of re-watering some plants survived and produced new green leaves. The appearance of the treated and untreated plants was similar except that the percentage of survivors was higher in the sound treated plants.



Supplemental File 3.- The GO terms in the differentially expressed genes.

Keyword ID	GO definition	Differentially expressed genes in this category
GO:0000209	Protein polyubiquitination	RING and DOMAIN of unknown function 1117 1 (AT3G46620)
GO:0000271	Polysaccharide biosynthetic process	Galactinol synthase 8; Galacturonosyl transferase-like 10 (AT3G28340)
GO:0001666	Response to hypoxia	Exordium like 1; Phosphate-induced 1 (AT1G35140)
GO:0001944	Vasculature development	DREB subfamily A-5 of ERF/AP2 transcription factor (AT1G74930) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0002213	Defense response to insect	DREB subfamily A-5 of ERF/AP2 transcription factor (AT1G74930)
GO:0002237	Response to molecule of bacterial origin	WRKY DNA-binding protein 40 (AT1G80840) Cytochrome BC1 Synthesis; Outer Mitochondrial Membrane Protein (AT3G50930)
GO:0002679	Respiratory burst involved in defense response	U-BOX 23; E3 ubiquitin ligase (AT2G35930) U-BOX 22 U-box domain E3 ubiquitin ligase (AT3G52450)
GO:0003674	Molecular function	Unknown protein (AT2G31930) Unknown protein (AT4G01535) Cotton fiber protein (AT5G38700) RNA splicing factor-like (AT3G56790) Unknown protein (AT2G14247) Aminotransferase-like mobile domain protein (AT1G50750) Intracellular protein transporter putative (DUF641) (AT2G32130) Nuclease (AT4G29780) MAEBL domain protein (AT1G56660) SPFH/Band 7/PHB domain-containing membrane-associated (AT5G64870) Unknown protein (AT1G47400) LL-diaminopimelate protein (DUF295) (AT2G14290) NUDIX Hydrolase homolog 4 (AT1G18300) Unknown protein (AT5G22545) Glycosyltransferase family 61 (AT2G41640) Arabinogalactan 1 (AT5G64310) Unknown protein (AT1G47395) Dihydrofolate reductase (AT4G24380) Polymerase (AT1G76600) Cruciferin (DUF506) (AT2G39650) miR447A, targets several 2-phosphoglycerate kinase-related (AT4G03445)

GO:0005096	GTPase activator activity	ROP1 ENHANCER 1, Rho GTPase-activating protein (AT4G24580)
GO:0005509	Calcium ion binding	Calcium-binding EF-hand family protein (AT3G01830)
GO:0005886	Plasma membrane	Cupredoxin superfamily protein (AT1G22480)
GO:0006351	Transcription, DNA-templated	DREB subfamily A-5 ERF/AP2 transcription factor (AT1G19210) Basic Helix-Loop-Helix 38; OBP3-Responsive Gene 3 (AT3G56970) NAC domain containing protein 61 (AT3G44350) WRKY DNA-binding protein 40 (AT1G80840) MYB domain protein 15 (AT3G23250) Ovate family protein 10 (AT5G22240) WRKY DNA-binding protein 46 (AT2G46400) Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) C2H2 and C2HC zinc fingers superfamily (AT3G46090) Jasmonate-ZIM-domain protein 8 (AT1G30135) C-Repeat/DRE Binding Factor 1; DRE Binding protein 1B (AT4G25490) DREB subfamily A-5 of ERF/AP2 transcription factor (AT1G74930) C-REPEAT/DRE binding factor 2; DRE/CRT-Binding protein 1C; Freezing tolerance QTL4 (AT4G25470) DREB subfamily A-5 of ERF/AP2 transcription factor (AT5G21960) Ethylene-responsive Element Binding Factor 13 (AT2G44840) Dehydration-responsive element binding protein 2 (AT5G05410) Jasmonate-ZIM-domain protein 5; TIFY11A (AT1G17380)
GO:0006355	Regulation of transcription, DNA-templated	DREB subfamily A-5 ERF/AP2 transcription factor (AT1G19210) Basic Helix-Loop-Helix 38; OBP3-Responsive Gene 3 (AT3G56970) NAC domain containing protein 61 (AT3G44350) WRKY DNA-binding protein 40 (AT1G80840) WRKY DNA-binding protein 46 (AT2G46400) Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) C2H2 and C2HC zinc fingers superfamily (AT3G46090) Jasmonate-ZIM-domain protein 8 (AT1G30135) Salt-inducible Zinc Finger 1 (AT3G55980) C-REPEAT/DRE binding factor 2; DRE/CRT-Binding protein 1C; Freezing tolerance QTL4 (AT4G25470) DREB subfamily A-5 of ERF/AP2 transcription factor (AT5G21960) MYB domain protein 15 (AT3G23250) DREB subfamily A-5 of ERF/AP2 transcription factor (AT1G74930) Salt-inducible Zinc Finger 2 (AT2G40140) Ethylene-responsive Element Binding Factor 13 (AT2G44840) Dehydration-responsive element binding protein 2 (AT5G05410) Jasmonate-ZIM-domain protein 5; TIFY11A (AT1G17380)
GO:0006357	Regulation of transcription from RNA polymerase II promoter	Basic Helix-Loop-Helix 38; OBP3-Responsive Gene 3 (AT3G56970) MYB domain protein 15 (AT3G23250)
GO:0006468	Protein phosphorylation	CRINKLY related 4 (AT5G47850)
GO:0006470	Protein dephosphorylation	MAPK phosphatase clade B of the PP2C-superfamily (AT2G30020) Protein phosphatase 2C (AT3G62260)
GO:0006629	Lipid metabolic process	Alpha/beta-Hydrolases superfamily (AT1G02660)
GO:0006644	Phospholipid metabolic process	Lipid phosphate phosphatase 1; Phosphatidic acid phosphatase 1 (AT2G01180)
GO:0006855	Drug transmembrane transport	Detoxification efflux carrier 50 (AT5G52050)
GO:0006952	Defense response	Disease resistance protein TIR-NBS class (AT1G66090) Lipoxygenase 4 (AT1G72520) U-BOX 23; E3 ubiquitin ligase (AT2G35930) Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) Jasmonate-ZIM-domain protein 8 (AT1G30135) BON Association protein 1 (AT3G61190) U-BOX 22 U-box domain E3 ubiquitin ligase (AT3G52450) Lipoxygenase 3 (AT1G17420) Pathogenesis related 1 (AT2G14610) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280) Disease resistance protein (TIR-NBS-LRR class) (AT5G41750) Disease resistance protein (TIR-NBS class) (AT1G72950) Ethylene-responsive Element Binding Factor 13 (AT2G44840) Jasmonate-ZIM-domain protein 5; TIFY11A (AT1G17380)
GO:0006970	Response to osmotic stress	F-BOX stress induced 1 (AT1G61340)
GO:0006979	Response to oxidative stress	Galactinol synthase 8; Galacturonosyl transferase-like 10 (AT3G28340) C2H2 and C2HC zinc fingers superfamily (AT3G46090) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0007165	Signal transduction	Disease resistance protein TIR-NBS class (AT1G66090) MAP Kinase 11 (AT1G01560) Disease resistance protein (TIR-NBS-LRR class) (AT5G41750) Disease resistance protein (TIR-NBS class) (AT1G72950)
GO:0007275	Multicellular organism development	NAC domain containing protein 61 (AT3G44350) Cytochrome P450 family 707 Subfamily A Polypeptide 3 (AT5G45340)
GO:0007346	Regulation of mitotic cell cycle	Mitogen-activated protein kinase kinase kinase 14 (AT2G30040)
GO:0007568	Ageing	TETRASPANIN 8 (AT2G23810)
GO:0008219	Cell death	Cytochrome BCl Synthesis; Outer Mitochondrial Membrane Protein (AT3G50930)
GO:0009055	Electron carrier activity	Cupredoxin superfamily protein (AT1G22480)
GO:0009266	Response to temperature stimulus	BON Association protein 1 (AT3G61190)
GO:0009408	Response to heat	TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22 (AT5G57560) BON Association protein 1 (AT3G61190) Dehydration-responsive element binding protein 2 (AT5G05410)

GO:0009409	Response to cold	MYB domain protein 15 (AT3G23250) TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22 (AT5G57560) BON Association protein 1 (AT3G61190) C-Repeat/DRE Binding Factor 1; DRE Binding protein 1B (AT4G25490) C-REPEAT/DRE binding factor 2; DRE/CRT-Binding protein 1C; Freezing tolerance QTL4 (AT4G25470) Salt-inducible Zinc Finger 2 (AT2G40140)
GO:0009411	Response to UV	Cytochrome BC1 Synthesis; Outer Mitochondrial Membrane Protein (AT3G50930)
GO:0009414	Response to water deprivation	MYB domain protein 15 (AT3G23250) U-BOX 23; E3 ubiquitin ligase (AT2G35930) Cytochrome P450 family 707 Subfamily A Polypeptide 3 (AT5G45340) C-Repeat/DRE Binding Factor 1; DRE Binding protein 1B (AT4G25490) U-BOX 22 U-box domain E3 ubiquitin ligase (AT3G52450) Pathogenesis related 1 (AT2G14610) Dehydration-responsive element binding protein 2 (AT5G05410) RING and DOMAIN of unknown function 1117 1 (AT3G46620)
GO:0009555	Pollen development	Lipoxygenase 4 (AT1G72520) Lipoxygenase 3 (AT1G17420) Unknown protein (AT1G74450)
GO:0009611	Response to wounding	WRKY DNA-binding protein 40 (AT1G80840) F-BOX stress induced 1 (AT1G61340) Lipoxygenase 4 (AT1G72520) MAPK phosphatase clade B of the PP2C-superfamily (AT2G30020) Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) Jasmonate-ZIM-domain protein 8 (AT1G30135) BON Association protein 1 (AT3G61190) DREB subfamily A-5 of ERF/AP2 transcription factor (AT1G74930) Lipoxygenase 3 (AT1G17420) Calmodulin-like 38 (AT1G76650) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280) Jasmonate-ZIM-domain protein 5; TIFY11A (AT1G17380)
GO:0009612	Response to mechanical stimulus	TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22 (AT5G57560) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0009617	Response to bacterium	F-BOX stress induced 1 (AT1G61340) Lipoxygenase 4 (AT1G72520) Cytochrome BC1 Synthesis; Outer Mitochondrial Membrane Protein (AT3G50930)
GO:0009620	Response to fungus	MAPK phosphatase clade B of the PP2C-superfamily (AT2G30020) Lipoxygenase 3 (AT1G17420)
GO:0009626	Plant-type hypersensitive response	Lipid phosphate phosphatase 1; Phosphatidic acid phosphatase 1 (AT2G01180) Cytochrome BC1 Synthesis; Outer Mitochondrial Membrane Protein (AT3G50930)
GO:0009627	Systemic acquired resistance	Pathogenesis related 1 (AT2G14610)
GO:0009631	Cold acclimation	C-Repeat/DRE Binding Factor 1; DRE Binding protein 1B (AT4G25490) C-REPEAT/DRE binding factor 2; DRE/CRT-Binding protein 1C; Freezing tolerance QTL4 (AT4G25470)
GO:0009639	Response to red or far red light	Cytochrome P450 family 707 Subfamily A Polypeptide 3 (AT5G45340) Gibberellin 2-Oxidase 4 (AT1G02400)
GO:0009644	Response to high light intensity	Lipoxygenase 3 (AT1G17420)
GO:0009651	Response to salt stress	MYB domain protein 15 (AT3G23250) F-BOX stress induced 1 (AT1G61340) IDA-LIKE7, IDL7 (AT3G10930)
GO:0009658	Chloroplast organization	Mitogen-activated protein kinase kinase kinase 14 (AT2G30040)
GO:0009664	Plant-type cell wall organization	TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22 (AT5G57560)
GO:0009686	Gibberellin biosynthetic process	Gibberellin 2-Oxidase 4 (AT1G02400)
GO:0009693	Ethylene biosynthetic process	1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0009695	Jasmonic acid biosynthetic process	Lipoxygenase 4 (AT1G72520) Lipoxygenase 3 (AT1G17420)
GO:0009723	Response to ethylene	MYB domain protein 15 (AT3G23250) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0009733	Response to auxin	MYB domain protein 15 (AT3G23250) TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22 (AT5G57560) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0009737	Response to abscisic acid	Detoxification efflux carrier 50 (AT5G2050) F-BOX stress induced 1 (AT1G61340) MAP Kinase 11 (AT1G01560) RING and DOMAIN of unknown function 1117 1 (AT3G46620)
GO:0009738	Abscisic acid-activated signaling pathway	MAPK phosphatase clade B of the PP2C-superfamily (AT2G30020) RING and DOMAIN of unknown function 1117 1 (AT3G46620)
GO:0009741	Response to brassinosteroid	TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22 (AT5G57560)
GO:0009751	Response to salicylic acid	WRKY DNA-binding protein 40 (AT1G80840) F-BOX stress induced 1 (AT1G61340) BON Association protein 1 (AT3G61190) Glutaredoxin GR480 (AT1G28480)
GO:0009753	Response to jasmonic acid	MYB domain protein 15 (AT3G23250) F-BOX stress induced 1 (AT1G61340) Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) Lipoxygenase 3 (AT1G17420) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280) Jasmonate-ZIM-domain protein 5; TIFY11A (AT1G17380)
GO:0009790	Embryo development	BYPASS 3 (AT4G01360)
GO:0009835	Fruit ripening	1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0009863	Salicylic acid mediated signaling pathway	Glutaredoxin GR480 (AT1G28480) Cytochrome BC1 Synthesis; Outer Mitochondrial Membrane Protein (AT3G50930)
GO:0009867	Jasmonic acid mediated signaling pathway	Glutaredoxin GR480 (AT1G28480)

GO:0009873	Ethylene-activated signaling pathway	DREB subfamily A-5 ERF/AP2 transcription factor (AT1G19210) DREB subfamily A-5 of ERF/AP2 transcription factor (AT1G74930) DREB subfamily A-5 of ERF/AP2 transcription factor (AT5G21960) Ethylene-responsive Element Binding Factor 13 (AT2G44840)
GO:0009901	Anther dehiscence	Lipoxinase 4 (AT1G72520) Lipoxygenase 3 (AT1G17420)
GO:0009961	Response to 1-aminocyclopropane-1-carboxylic acid	F-BOX stress induced 1 (AT1G61340)
GO:0010015	Root morphogenesis	Detoxification efflux carrier 50 (AT5G52050)
GO:0010029	Regulation of seed germination	ATPUB19; U-BOX 19 (AT1G60190)
GO:0010106	Cellular response to iron ion starvation	Basic Helix-Loop-Helix 38; OBP3-Responsive Gene 3 (AT3G56970)
GO:0010167	Response to nitrate	RPM1-interacting protein 4 (AT2G17660)
GO:0010193	Response to ozone	ARM repeat superfamily protein (AT3G02840) Calmodulin like 37 (AT5G42380) Lipoxygenase 4 (AT1G72520)
GO:0010200	Response to chitin	NAC domain containing protein 61 (AT3G44350) WRKY DNA-binding protein 40 (AT1G80840) MYB domain protein 15 (AT3G23250) U-BOX 23; E3 ubiquitin ligase (AT2G35930) WRKY DNA-binding protein 46 (AT2G46400) Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) C2H2 and C2HC zinc fingers superfamily (AT3G46090) U-BOX 22 U-box domain E3 ubiquitin ligase (AT3G52450) Salt-inducible Zinc Finger 1 (AT3G55980) CYS MET PRO and GLY Protein 1 (AT1G66160) Salt-inducible Zinc Finger 2 (AT2G40140) Ethylene-responsive Element Binding Factor 13 (AT2G44840) RING and DOMAIN of unknown function 1117 1 (AT3G46620)
GO:0010224	Response to UV-B	Lipid phosphate phosphatase 1; Phosphatidic acid phosphatase 1 (AT2G01180) Dehydration-responsive element binding protein 2 (AT5G05410)
GO:0010266	Response to vitamin B1	Pathogenesis related 1 (AT2G14610)
GO:0010268	Brassinosteroid homeostasis	Cytochrome P450 family 707 Subfamily A Polypeptide 3 (AT5G45340)
GO:0010286	Heat acclimation	Dehydration-responsive element binding protein 2 (AT5G05410)
GO:0010411	Xyloglucan metabolic process	Xyloglucan endotransglucosylase/Hydrolase 18 (AT4G30280) TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22 (AT5G57560)
GO:0010468	Regulation of gene expression	MAP Kinase 11 (AT1G01560)
GO:0010629	Negative regulation of gene expression	IDA-LIKE7, IDL7 (AT3G10930)
GO:0015031	Protein transport	Translocon at the inner envelope membrane of chloroplasts 214 (ATCG01130)
GO:0016051	Carbohydrate biosynthetic process	Galactinol synthase 8; Galacturonosyl transferase-like 10 (AT3G28340)
GO:0016125	Sterol metabolic process	Cytochrome P450 family 707 Subfamily A Polypeptide 3 (AT5G45340)
GO:0016132	Brassinosteroid biosynthetic process	Cytochrome P450 family 707 Subfamily A Polypeptide 3 (AT5G45340)
GO:0016567	Protein ubiquitination	U-BOX 23; E3 ubiquitin ligase (AT2G35930) U-BOX 22 U-box domain E3 ubiquitin ligase (AT3G52450) CYS MET PRO and GLY Protein 1 (AT1G66160) ATPUB19; U-BOX 19 (AT1G60190)
GO:0019725	Cellular homeostasis	BON Association protein 1 (AT3G61190)
GO:0023014	Signal transduction by protein phosphorylation	Mitogen-activated protein kinase kinase kinase 14 (AT2G30040)
GO:0030154	Cell differentiation	MYB domain protein 15 (AT3G23250)
GO:0031098	Stress-activated protein kinase signaling cascade	Mitogen-activated protein kinase kinase kinase 14 (AT2G30040)
GO:0031225	Anchored component of membrane	Cupredoxin superfamily protein (AT1G22480)
GO:0031347	Regulation of defense response	WRKY DNA-binding protein 40 (AT1G80840) Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) Jasmonate-ZIM-domain protein 8 (AT1G30135) Jasmonate-ZIM-domain protein 5; TIFY11A (AT1G17380)
GO:0031348	Negative regulation of defense response	BON Association protein 1 (AT3G61190)
GO:0031408	Oxylipin biosynthetic process	Lipoxinase 4 (AT1G72520) Lipoxygenase 3 (AT1G17420)
GO:0031640	Killing of cells of other organism	Defensin-like (AT1G13607)
GO:0032147	Activation of protein kinase activity	Mitogen-activated protein kinase kinase kinase 14 (AT2G30040)
GO:0034440	Lipid oxidation	Lipoxinase 4 (AT1G72520) Lipoxygenase 3 (AT1G17420)
GO:0040007	Growth	Exordium like 1; Phosphate-induced 1 (AT1G35140) Lipoxygenase 4 (AT1G72520) Lipoxygenase 3 (AT1G17420)
GO:0042542	Response to hydrogen peroxide	Dehydration-responsive element binding protein 2 (AT5G05410) IDA-LIKE7, IDL7 (AT3G10930)
GO:0042546	Cell wall biogenesis	Xyloglucan endotransglucosylase/Hydrolase 18 (AT4G30280) TOUCH 4; Xyloglucan endotransglucosylase/hydrolase 22 (AT5G57560)
GO:0042742	Defense response to bacterium	WRKY DNA-binding protein 40 (AT1G80840) IDA-LIKE7, IDL7 (AT3G10930)
GO:0042787	Protein ubiquitination involved in ubiquitin-dependent protein catabolic process	RING and DOMAIN of unknown function 1117 1 (AT3G46620)

GO:0043161	Proteasome-mediated ubiquitin-dependent protein catabolic process	RING and DOMAIN of unknown function 1117 1 (AT3G46620)
GO:0045037	Protein import into chloroplast stroma	Translocon at the inner envelope membrane of chloroplasts 214 (ATCG01130)
GO:0045454	Cell redox homeostasis	Glutaredoxin GR480 (AT1G28480)
GO:0045487	Gibberellin catabolic process	Gibberellin 2-Oxidase 4 (AT1G02400)
GO:0045489	Pectin biosynthetic process	Galactinol synthase 8; Galacturonosyl transferase-like 10 (AT3G28340)
GO:0045892	Negative regulation of transcription, DNA-templated	WRKY DNA-binding protein 40 (AT1G80840) Ovate family protein 10 (AT5G22240)
GO:0045893	Positive regulation of transcription, DNA-templated	C-Repeat/DRE Binding Factor 1; DRE Binding protein 1B (AT4G25490) Mitogen-activated protein kinase kinase kinase 14 (AT2G30040) Dehydration-responsive element binding protein 2 (AT5G05410)
GO:0045926	Negative regulation of growth	C2H2 and C2HC zinc fingers superfamily (AT3G46090)
GO:0046274	Lignin catabolic process	Laccase 12 (AT5G05390)
GO:0046345	Abscisic acid catabolic process	Cytochrome P450 family 707 Subfamily A Polypeptide 3 (AT5G45340)
GO:0046686	Response to cadmium ion	MYB domain protein 15 (AT3G23250)
GO:0046777	Protein autophosphorylation	Mitogen-activated protein kinase kinase kinase 14 (AT2G30040)
GO:0048367	Shoot system development	Devil 5; Rotundifolia-like 15 (AT1G68825)
GO:0048527	Lateral root development	WRKY DNA-binding protein 46 (AT2G46400)
GO:0048653	Anther development	Lipoxygenase 4 (AT1G72520) Lipoxygenase 3 (AT1G17420)
GO:0050691	Regulation of defense response to virus by host	WRKY DNA-binding protein 40 (AT1G80840)
GO:0050829	Defense response to Gram-negative bacterium	TETRASPANIN 8 (AT2G23810)
GO:0050832	Defense response to fungus	Defensin-like (AT1G13607) WRKY DNA-binding protein 40 (AT1G80840) MAPK phosphatase clade B of the PP2C-superfamily (AT2G30020) Salt-inducible Zinc Finger 2 (AT2G40140)
GO:0051301	Cell division	DREB subfamily A-5 of ERF/AP2 transcription factor (AT1G74930) 1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0051607	Defense response to virus	DBP-Interacting-protein 2 (AT5G03210)
GO:0051707	Response to other organism	ARM repeat superfamily protein (AT3G02840)
GO:0051865	Protein autoubiquitination	U-BOX 23; E3 ubiquitin ligase (AT2G35930) U-BOX 22 U-box domain E3 ubiquitin ligase (AT3G52450) RING and DOMAIN of unknown function 1117 1 (AT3G46620)
GO:0055072	Iron ion homeostasis	Basic Helix-Loop-Helix 38; OBP3-Responsive Gene 3 (AT3G56970)
GO:0055114	Oxidation-reduction process	Cytochrome P450 family 707 Subfamily A Polypeptide 3 (AT5G45340) Laccase 12 (AT5G05390) Glutaredoxin GR480 (AT1G28480) Auxin-responsive family protein (AT5G35735) Gibberellin 2-Oxidase 4 (AT1G02400)
GO:0070588	Calcium ion transmembrane transport	Calcium uniporter (DUF607) (AT5G66650)
GO:0071281	Cellular response to iron ion	1-Aminocyclopropane-1-carboxylic acid synthase 6 (AT4G11280)
GO:0071555	Cell wall organization	Xyloglucan endotransglucosylase/Hydrolase 18 (AT4G30280) Galactinol synthase 8; Galacturonosyl transferase-like 10 (AT3G28340)
GO:0080086	Stamen filament development	Lipoxygenase 4 (AT1G72520) Lipoxygenase 3 (AT1G17420)
GO:0080168	Abscisic acid transport	Detoxification efflux carrier 50 (AT5G52050)
GO:0120009	Intermembrane lipid transfer	Glycolipid transfer protein (AT4G39670)
GO:1902883	Negative regulation of response to oxidative stress	IDA-LIKE7, IDL7 (AT3G10930)
GO:1903507	Negative regulation of nucleic acid-templated transcription	Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) Jasmonate-ZIM-domain protein 8 (AT1G30135) Jasmonate-ZIM-domain protein 5; TIFY11A (AT1G17380)
GO:2000022	Regulation of jasmonic acid mediated signaling pathway	Jasmonate-zim-domain protein 7; TIFY5B (AT2G34600) Jasmonate-ZIM-domain protein 8 (AT1G30135) Jasmonate-ZIM-domain protein 5; TIFY11A (AT1G17380)
GO:2000070	Regulation of response to water deprivation	Detoxification efflux carrier 50 (AT5G52050)

Supplemental File 4.- GO ontology terms of the Significantly Induced Genes after white noise treatment in arabidopsis leaves (log2 fold change ≥ 2 and q-value < 0.05)

Gene_id	WN/CON	GO Biological processes
AT1G13607	a	Defense response to fungus; Killing of cells of other organism
AT1G68825	a	Shoot system development
AT2G31930	a	
AT4G01535	a	
AT5G38700	a	
AT4G01360	41,62	Embryo development
AT4G30280	26,45	Cell wall biogenesis and organization; xyloglucan metabolism

AT1G35140	25,05	Growth; response to hypoxia
AT5G52050	21,08	Abscisic acid transport; drug transmembrane transport; regulation of response to water deprivation; response to abscisic acid; root morphogenesis
AT3G56790	19,34	
AT2G14247	18,96	
AT1G19210	18,83	Ethylene-activated signaling pathway; regulation of transcription; DNA-templated
AT4G24580	18,15	Activation of GTPase activity; negative regulation of Rho protein signal transduction; pollen germination; pollen tube adhesion; pollen tube development; signal transduction
AT3G56970	15,58	Cellular response to iron ion starvation; iron ion homeostasis; regulation of transcription from RNA polymerase II promoter; DNA-templated
AT1G50750	14,29	
AT2G17660	12,63	Response to nitrate
AT3G02840	11,45	Response to ozone; response to other organism
AT3G44350	11,25	Multicellular organism development; regulation of transcription; DNA-templated; response to chitin
AT1G80840	11,22	Defense response to bacterium and fungus; negative regulation of transcription; DNA-templated; regulation of defense response; regulation of defense response to virus by host; response to chitin; response to molecule of bacterial origin; response to salicylic acid; response to wounding; transcription
AT2G32130	10,44	
AT5G42380	10,44	Response to ozone
AT3G23250	9,94	Cell differentiation; regulation of transcription from RNA polymerase II promoter; DNA-templated; response to auxin; response to cadmium ion; response to chitin; response to ethylene; response to jasmonic acid; response to salt stress
AT4G29780	9,86	
AT3G01830	9,35	
AT1G61340	8,91	Response to 1-aminocyclopropane-1-carboxylic acid; response to abscisic acid; response to bacterium; response to jasmonic acid; response to osmotic stress; response to salicylic acid; response to salt stress; response to wounding
AT5G22240	8,88	negative regulation of transcription; DNA-templated
AT1G66090	8,80	Defense response; signal transduction
AT1G72520	8,26	Anther dehiscence and development; defense response; growth; jasmonic acid biosynthetic process; lipid oxidation; oxylipin biosynthetic process; pollen development; response to bacterium; response to ozone; response to wounding; stamen filament development
AT2G30020	7,85	Abscisic acid-activated signaling pathway; protein dephosphorylation; response to fungus; response to wounding
AT5G57560	7,51	Cell wall biogenesis; plant-type cell wall organization; response to auxin; response to brassinosteroid; response to cold; response to heat; response to mechanical stimulus; xyloglucan metabolic process
AT2G35930	7,16	Defense response; protein autoubiquitination; protein ubiquitination; respiratory burst involved in defense response; response to chitin; response to water deprivation
AT5G45340	7,14	abscisic acid catabolic process; brassinosteroid biosynthetic process; brassinosteroid homeostasis; multicellular organism development; oxidation-reduction process; response to red or far red light; response to water deprivation; sterol metabolic process
AT2G46400	7,06	Lateral root development; regulation of transcription; DNA-templated; response to chitin
AT3G28340	6,90	Carbohydrate biosynthetic process; cell wall organization; pectin biosynthetic process; polysaccharide biosynthetic process; response to oxidative stress
AT2G34600	6,89	Defense response; negative regulation of nucleic acid-templated transcription; regulation of jasmonic acid mediated signaling pathway; regulation of transcription; DNA-templated; response to chitin; response to jasmonic acid; response to wounding
AT3G46090	6,82	Negative regulation of growth; regulation of transcription; DNA-templated; response to chitin; response to oxidative stress
AT1G22480	6,63	
AT1G30135	6,60	Defense response; negative regulation of nucleic acid-templated transcription; regulation of jasmonic acid mediated signaling pathway; regulation of transcription; DNA-templated; response to wounding
AT3G61190	6,54	Cellular homeostasis; defense response; negative regulation of defense response; response to cold; response to heat; response to salicylic acid; response to temperature stimulus; response to wounding
AT4G25490	6,47	Cold acclimation; positive regulation of transcription; DNA-templated; response to cold; response to water deprivation
AT5G05390	6,42	Lignin catabolic process; oxidation-reduction process
AT1G56660	6,41	
AT3G52450	6,34	Defense response; protein autoubiquitination; protein ubiquitination; respiratory burst involved in defense response; response to chitin; response to water deprivation
AT1G74930	6,06	Cell division; defense response to insect; ethylene-activated signaling pathway; regulation of transcription; DNA-templated; response to wounding; vasculature development
AT1G17420	5,79	Anther dehiscence; anther development; defense response; growth; jasmonic acid biosynthetic process; lipid oxidation; oxylipin biosynthetic process; pollen development; response to fungus; response to high light intensity; response to jasmonic acid; response to wounding; stamen filament development
AT1G28480	5,68	Cell redox homeostasis; jasmonic acid mediated signaling pathway; oxidation-reduction process; response to salicylic acid; salicylic acid mediated signaling pathway
AT1G76650	5,47	Response to wounding
AT3G55980	5,46	Regulation of transcription; DNA-templated; response to chitin
AT5G64870	5,44	
AT1G47400	5,43	
AT1G01560	5,42	Intracellular signal transduction; regulation of gene expression; response to abscisic acid; signal transduction
AT5G35735	5,42	Oxidation-reduction process
AT2G14610	5,40	Defense response; response to vitamin B1; response to water deprivation; systemic acquired resistance
AT1G02400	5,36	Gibberellin biosynthetic process; gibberellin catabolic process; oxidation-reduction process; response to red or far red light
AT4G39670	5,30	Glycolipid transport
AT5G47850	5,26	Protein phosphorylation
AT2G01180	5,19	Phospholipid metabolic process; plant-type hypersensitive response; response to UV-B
AT3G62260	5,12	Protein dephosphorylation
AT1G74450	5,05	Pollen development
AT5G66650	4,99	Calcium ion transmembrane transport
AT2G14290	4,95	
AT1G18300	4,74	Nucleoside catabolic process
AT4G11280	4,74	Cell division; cellular response to iron ion; defense response; ethylene biosynthetic process; fruit ripening; response to auxin; response to ethylene; response to jasmonic acid; response to mechanical stimulus; response to oxidative stress; response to wounding; vasculature development
AT4G25470	4,72	Cold acclimation; regulation of transcription; DNA-templated; response to cold
AT5G41750	4,71	Defense response; signal transduction
AT5G21960	4,70	Ethylene-activated signaling pathway; regulation of transcription; DNA-templated
AT1G66160	4,69	Protein ubiquitination; response to chitin

AT5G22545	4,69	
AT2G41640	4,68	
AT2G30040	4,64	Chloroplast organization; positive regulation of transcription; DNA-templated; protein autophosphorylation
AT5G64310	4,62	
AT2G23810	4,58	Aging; defense response to Gram-negative bacterium
AT1G47395	4,52	
AT1G60190	4,49	Protein ubiquitination; regulation of seed germination
AT1G72950	4,48	Defense response; signal transduction
AT2G40140	4,47	Defense response to fungus; regulation of transcription; DNA-templated; response to chitin; response to cold
AT1G02660	4,46	Lipid metabolic process
AT4G24380	4,45	
AT2G44840	4,40	Defense response; ethylene-activated signaling pathway; regulation of transcription; DNA-templated; response to chitin
AT5G05410	4,38	Heat acclimation; positive regulation of transcription; DNA-templated; regulation of transcription; response to UV-B; response to heat; response to hydrogen peroxide; response to water deprivation
AT1G17380	4,33	Defense response; negative regulation of nucleic acid-templated transcription; regulation of defense response; regulation of jasmonic acid mediated signaling pathway; regulation of transcription; DNA-templated; response to jasmonic acid; response to wounding
AT3G50930	4,26	Cell death; plant-type hypersensitive response; response to UV; response to bacterium; response to molecule of bacterial origin; salicylic acid mediated signaling pathway
AT3G10930	4,18	
AT1G76600	4,17	
AT2G39650	4,11	
AT3G46620	4,11	Abscisic acid-activated signaling pathway; proteasome-mediated ubiquitin-dependent protein catabolic process; protein autoubiquitination; protein polyubiquitination; protein ubiquitination involved in ubiquitin-dependent protein catabolic process; response to abscisic acid; response to chitin; response to water deprivation
AT5G03210	4,05	Defense response to virus
ATCG01130	4,25 ^b	Protein import into chloroplast stroma; protein transport
AT4G03445	c	

a, expression was only detected in the sound treated samples; b, expression was higher in the control sample; c, expression was only detected in the control samples.

b, expression was higher in the control sample

c, expression was only detected in the control samples