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Short Communication

Up-Regulated Gene Sets of Arabidopsis Thaliana in Response to Nanoparticles: An In Silico Approach Based on the Microarray Data

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Abstract

A meta-analysis on two microarray-based data was performed to identify the statistically enriched gene sets in Arabidopsis thaliana treated with nanoparticle (NPs) using Gene Set Enrichment Analysis (GSEA) based on Kyoto Encyclopedia of Genes and Genomes (KEGG). Log fold change (FC) of the gene expression under NPs treatment, compared to the control, was manually calculated in excel after data merging, to find gens with the highest expression under the treatment. GSEA analysis revealed that under NPs treatment, different pathways related to organ morphogenesis, cell adhesion molecule binding, epithelial development, immune response regulating signaling pathway, regulatory region nucleic acid binding, supramolecular complex, taxis (directed movement in response to stimulus), tube development, and vacuole were differentially expressed. Top 10 up-regulated genes under NPs treatment based on the Enrichment Score (ES) were AT1G69510, AT5G29000, AT3G17880, AT5G14590, AT5G57655, AT2G30530, AT1G55530, AT1G01770, AT2G17220, and AT2G25460. Many of these genes are involved in the response to stress and in the plant defense signaling.

Keywords: *Meta-analysis, Gene expression, Metabolic pathway, Signaling pathway.*

1. INRODUCTION

Nanoparticles (NPs) with a small size, at least one dimension less than 100 nm [1], are became venturesome for the environment due to their tremendous utilization with different applications in recent years [2, 3]. For instance, NPs can be toxic for the organisms via different mechanisms such as direct contact with the organism's cell surface resulting in the membrane integrity damage or starting a signaling pathway leading to the cell damage, releasing toxic ions before or after entering the cell, inducing oxidative stress due to the production of the harmful reactive oxygen species (ROS) [4]. Plants are more likely to interact and infect by NPs due to being sessile in nature [5]. NPs may also enter the food chain after bioaccumulation in plants [6]. This potential dangerous destiny of NPs in the environment makes it necessary to understand their different toxicity mechanism using various biological models for redesigning them with reduced environmental impact [4]. Studies based on a molecular approach, mainly with proteomic or transcriptomic methods can help to better understand the molecular basis of NPs toxicity in different organisms, such as plants [7].

Although many studies have investigated the effects of NPs on plants from different

aspects, however, few studies [7, 8-13] have used a genome-wide transcriptomic analysis for evaluating changes in gene expression of plants and green alga under NPs. However, these studies have more focused on the genes rather than gene sets. To our knowledge, the present study is the first time an effort was made to analyze changes in the gene sets of plants under NPs, by a meta-analysis. We used Gene Set Enrichment Analysis (GSEA), a powerful analytical method, for finding the NPs-induced up-regulated pathway of Arabidopsis thaliana in two microarray-based data. GSEA is a statistical method that focuses on the gene groups functioning in a common biological pathway [14]. Thus, functionally-related genes regulated by the same conditions can be statistically addressed using GSEA [15].

2. MATERIALS AND METHODS

2.1. Data Retrieval

Microarray gene expression datasets were retrieved from NCBI Gene expression omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) after searching for microarray datasets with the same platform investigating differentially expressed genes under NPs treatment in *Arabidopsis thaliana*. The details of these gene expression datasets are shown in Table 1. In an experiment conducted by Marmiroli et al. [11], wild type and two mutant lines (*atnp01* and *atnp02*) of *A. Thaliana* seedlings were exposed to 0, 40, or 80 mg L^{-1}, and to 0 or 80 mg L^{-1} cadmium sulfide quantum dots (CdS QDs) with bulk density of 4.82 g cm^{-3} and size of 5 nm for 21 days, respectively. Mutant lines were selected after screening for finding tolerant lines to CdS QDs. In another study [13], *A. thaliana* wild-type ecotype Columbia was exposed to control solution, or NPs suspensions of the titanium dioxide (TiO_2) or cerium dioxide (CeO_2) by watering from above from the day 0 to day 17 (the primary rosette stage). TiO_2 and CeO_2 had primary particle sizes of 21 nm and 33 nm, respectively.

Table 1. *Details of microarray gene expression datasets retrieved from NCBI GEO.*

GEO-ID	Platform and technology	Number of Samples	Reference
GSE80461	GPL198 [ATH1-121501] Affymetrix Arabidopsis ATH1 Genome Array	24	[13]
GSE53989	GPL198 [ATH1-121501] Affymetrix Arabidopsis ATH1 Genome Array	7	[11]

2.2. Data Analysis

All data analysis processes were performed with R software version 3.5.2. Data sets were merged based on the gene symbol. ComBat was used for removing batch effects in the merged datasets after normalizeQuantiles not being suitable. GSEA software (Broad Institute, Cambridge, MA, USA) [14] was performed on the merged dataset to identify the statistically enriched gene sets between the control and NPs -treated plants. The number of permutations and their type for GSEA running was 1000 and phenotype, respectively. Identified pathways by GSEA were based on Kyoto Encyclopedia of Genes and Genomes (KEGG) [16]. Although NPs with various physicochemical characteristics affected various genes, some different and some common [11, 13], we have focused on common genes up-regulated by different investigated NPs using meta-analysis statistical methods to calculate an overall or absolute effect. Significant up-regulated gene sets under NPs treatment were filtered based on a nominal p value < 5%. The physicochemical properties of proteins

encoded by10 genes up-regulated under NPs treatment were retrieved from Gramene [17]. Log fold change (FC) of the gene expression under NPs treatment, compared to the control, was manually calculated in excel after data merging, to find gens with the highest expression under the treatment.

3. RESULTS AND DISCUSSION

GSEA is a statistical method for finding functionally-related genes in a pathway, differentially expressed compared to the control condition [15]. GSEA analysis revealed that under NPs treatment, different gene sets (i.e. pathways) were differentially expressed including organ morphogenesis, cell adhesion molecule binding, epithelial development, immune response regulating signaling pathway, regulatory region nucleic acid binding, supramolecular complex, taxis (directed movement in response to the stimulus), tube development, and vacuole (Fig. 1).

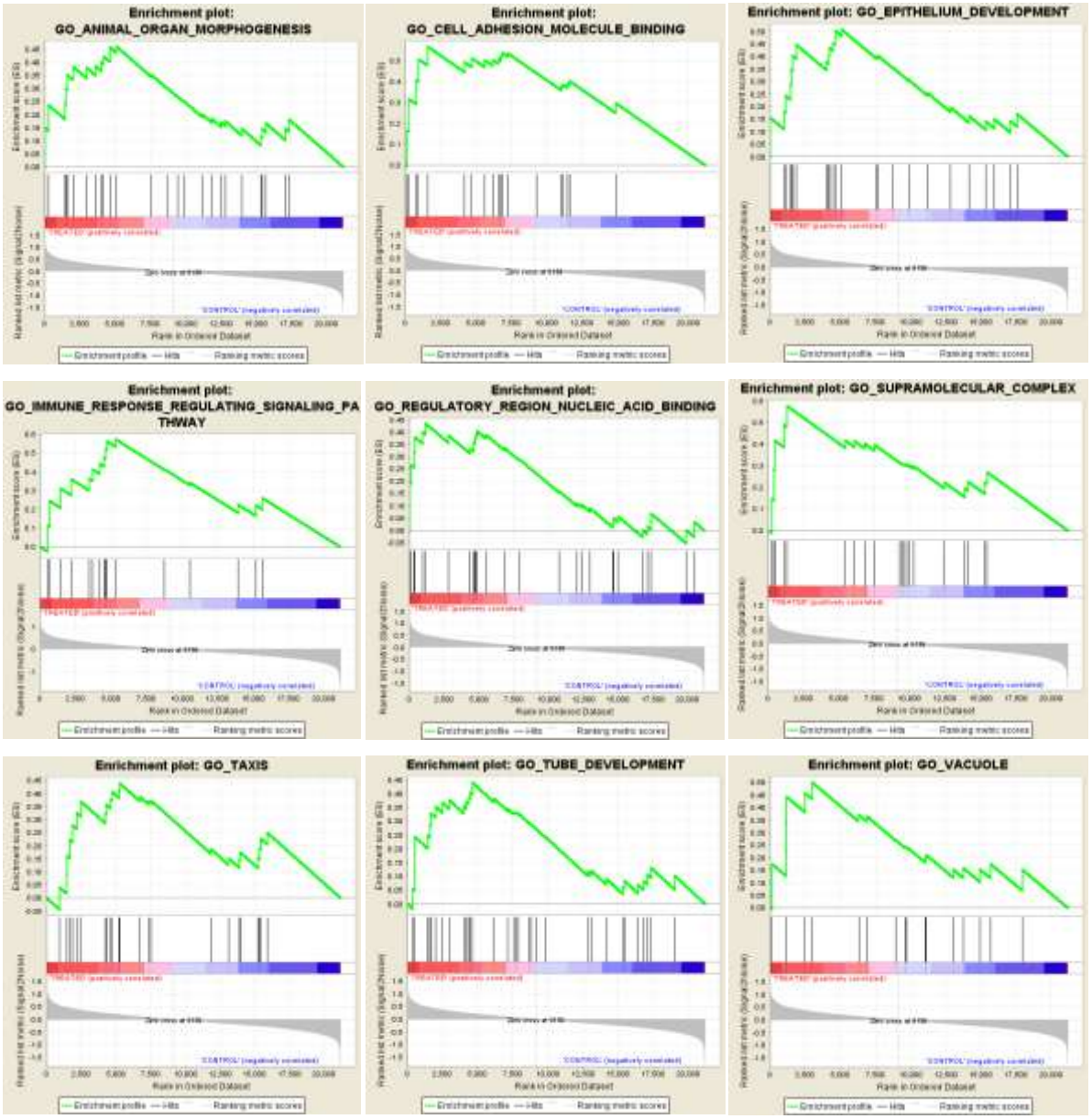

Figure 1. *GSEA Enrichment plots (score curves). Nine gene sets (enriched pathways) were significantly enriched at nominal p value < 5%. Each plot rank the genes induced (red) or repressed (blue) under NPs treatment based on "Signal-to-Noise" ratio (SNR) statistic. The green curve is related to the ES (enrichment score) curve.*

An increase in the numbers and diameters of rosette leaves under nano-ceria in one of the data-obtained studies [13] may indicate the necessity of inducing the organ morphogenesis pathway. Cell adhesion and morphogenesis are related processes in organisms due to the role of adhesion molecules in development [18]. For instance, cadherins are cell to cell adhesion molecules controlling animal morphogenesis [19]. From cadherins, E-type is the integral membrane protein of epithelial tissue [20].

Inducing immune response regulating the signaling pathway under NPs is an unavoidable and expected result, as was reported in animals [21]. GSEA analysis showed that BAG6 was the most core-enrichment gene of this pathway under NPs (Fig. 2). BAG6 encodes a calmodulin-binding protein regulating programmed cell death (PCD) under stress [22]. PCD is one of the processes involved in defense responses against abiotic and biotic stresses [23]. So far, many studies reported that NPs can induce stress and toxicity in plants [24-30].

According to GSEA analysis, the most core-enrichment gene of the supramolecular complex gene set was FSD1 (Fig. 2) which encodes superoxide dismutase (SOD). Converting superoxide, one of the toxic radicals, to hydrogen peroxide (H_2O_2) by SOD is one of the first lines of cellular defense against reactive oxygen species (ROS) [31].

The most core-enrichment gene of the vacuole gene set was SNX1 (Fig. 2). SORTING NEXIN 1 (SNX1), localized to the prevacuolar compartment (PVC), is involved in retrieving efflux auxin-carrier (PIN) from PVC back to the recycling pathways [32]. SNX1 has been reported to play a role in plant tolerance to some stresses such as salinity [33]. Therefore, it may be involved in response to NPs-induced stress by changing the auxin flow due to the changes in PINs' polar localization followed by impacting physiological and morphological processes.

Top 10 up-regulated genes under NPs treatment based on the Enrichment Score (ES) were AT1G69510, AT5G29000, AT3G17880, AT5G14590, AT5G57655, AT2G30530, AT1G55530, AT1G01770, AT2G17220, and AT2G25460 (Fig.3). The physicochemical properties of the proteins encoded by these are included in Table 2. AT1G69510 is a cAMP-regulated phosphoprotein 19-related protein. It also is reported a Glycosyltransferase in Uni ProtKB (https://www.uniprot.org/uniprot/). Transferring glycosyl groups is important in cell wall organization. It has been reported that Au NPs can increase the thickness of the outer periclinal cell wall. Cell wall thickening is a defensive reaction limiting particle penetration into the protoplast [34].

AT5G29000 (PHL1) encodes PHR1-LIKE 1 which is a transcription factor (TF) being important in the positive or negative control of phosphate starvation responses [35]. It has been reported that exposure to NPs can lead to the down-regulating of phosphate-starvation genes [12], probably by inducing the expression of PHR1-LIKE 1.

Due to its thioredoxin domain, AT3G17880 (AtTDX) is a protein-disulfide reductase that its amino-terminal domain is closely related to the co-chaperone Hsp70-interacting protein (HIP). Chaperones have an important role in renaturing proteins following denaturing under a variety of stresses [36].

Other strongly up-regulated genes under NPs treatment have a variety of functions, some of which are involved in the plant defense signaling (AT2G17220) or in the response to oxidative stresses (AT5G14590) according to UniProtKB.

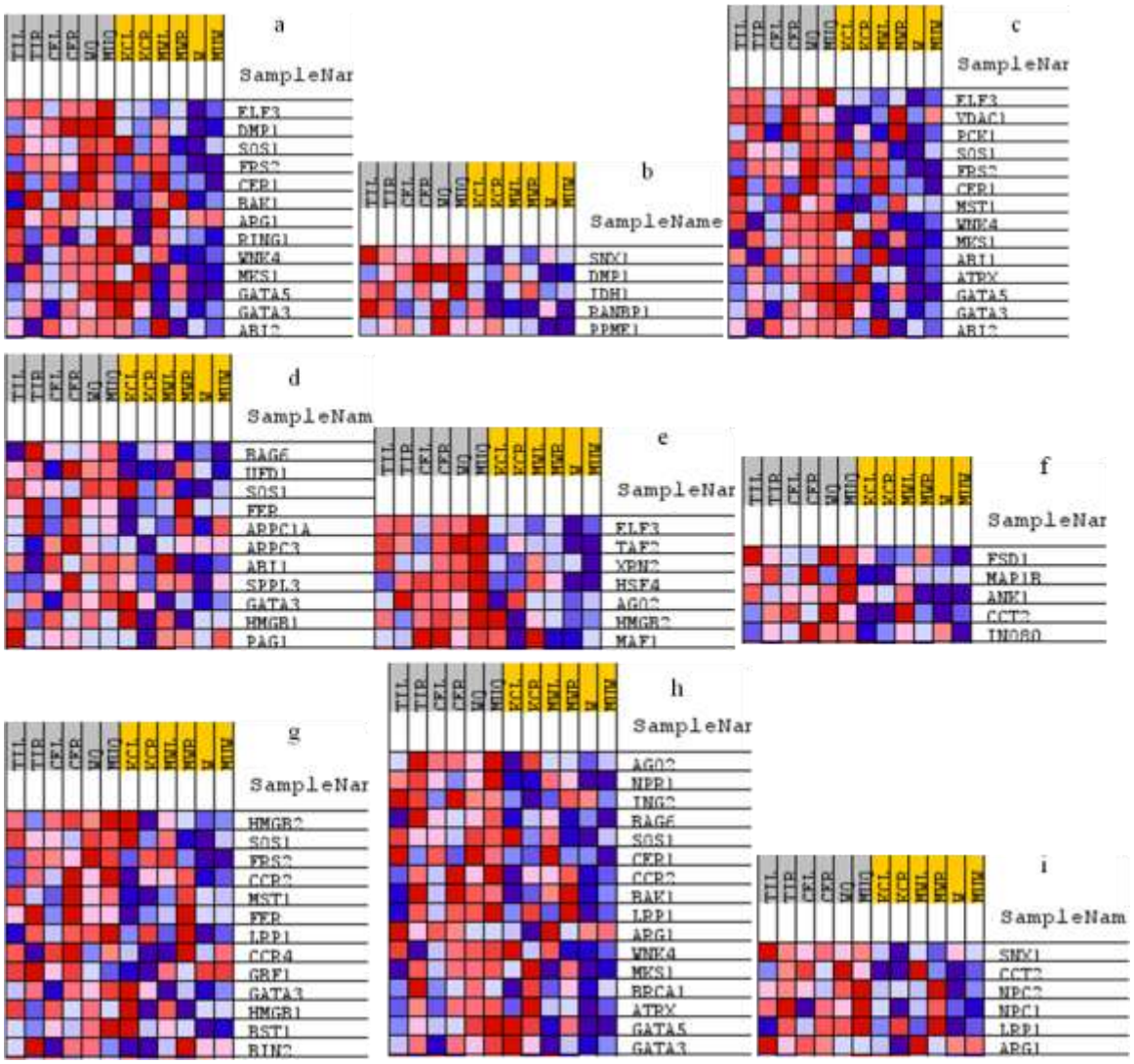

Figure 2. *Core-enrichment genes under NPs in 9 gene sets significantly enriched at nominal p value < 5%. (a) organ morphogenesis, (b) cell adhesion molecule binding, (c) epithelial development, (d) immune response regulating signaling pathway, (e) regulatory region nucleic acid binding, (f) supramolecular complex, (g) taxis, (h) tube development, and (i) vacuole. TIL: TiO_2 NPs-treated, leave; TIR: TiO_2 NPs-treated, root; CEL: CeO_2 NPs-treated, leave; CER: CeO_2 NPs-treated, root; WQ: wild plant CdS QDs-treated; MUQ: mutant plant CdS QDs-treated; KCL: KCl-treated, leave; KCR: KCl-treated, root; MWL: Millipore water-treated, leave; MWR: Millipore water-treated, root; W: wild plant untreated; MUW: mutant plant untreated.*

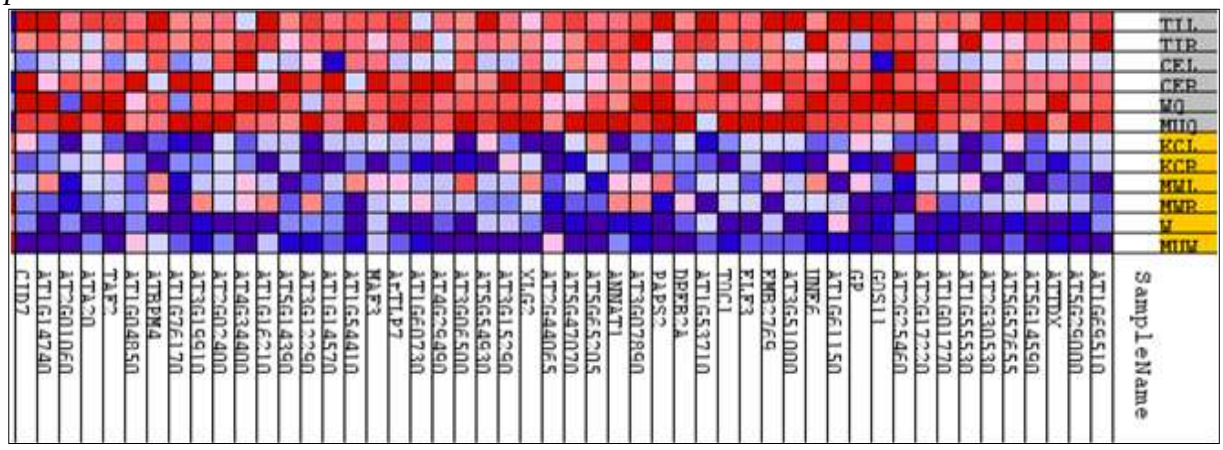

Figure 3. *Heat Map of the top 50 differentially expressed genes under NPs. TIL: TiO2 NPs-treated, leave; TIR: TiO2 NPs-treated, root; CEL: CeO2 NPs-treated, leave; CER: CeO2 NPs-treated, root; WQ: wild plant CdS QDs-treated; MUQ: mutant plant CdS QDs-treated; KCL: KCl-treated, leave; KCR: KCl-treated, root; MWL: Millipore water-treated, leave; MWR: Millipore water-treated, root; W: wild plant untreated; MUW: mutant plant untreated.*

Table 2. *The physicochemical properties of proteins encoded by the top 10 up-regulated genes under NPs based on the Enrichment Score (ES).*

Gene name	Protein Description	Number of amino acids	Ave. residue weight (g/mol)	Charge	Molecular weight (g/mol)	Isoelectric point
AT1G69510	cAMP-regulated phosphoprotein 19-related protein	137	110.188	-5.5	15,095.76	4.6625
AT5G29000	Homeodomain-like superfamily protein (Protein PHR1-LIKE 1)	370	111.954	-6.5	41,423.05	5.4340
AT3G17880.1	TPR repeat-containing thioredoxin TDX	380	112.753	-6.0	42,846.19	5.4160
AT5G14590	Isocitrate dehydrogenase [NADP], chloroplastic/mitochondrial	485	111.745	7.0	54,196.12	7.9854
AT5G57655	Xylose isomerase	287	112.920	4.0	32,408.02	7.7009
AT2G30530	zinc finger CCCH domain protein	371	111.092	-3.5	41,215.06	5.4125
AT1G55530	RING/U-box superfamily protein	351	111.006	-40.5	38,963.24	3.9652
AT1G01770	propionyl-CoA carboxylase	632	109.177	-5.5	69,000.05	5.9552
AT2G17220	Probable serine/threonine-protein kinase	414	110.028	17.5	45,551.68	9.8730
AT2G25460	EEIG1/EHBP1 protein amino-terminal domain protein	423	110.814	7.5	46,874.16	8.6044

Eleven genes including AT5G12030, AT1G07400, AT1G19530, AT2G05510, AT5G06730, AT5G39110, AT4G11650, AT2G42540, AT3G46230, AT2G26020, and AT5G24780 had a log FC > 10. The physicochemical properties of the proteins encoded by these are included in Table 3. AT5G12030 (AT-HSP17.6A), AT1G07400 (HSP17.8) and AT3G46230 (HSP17.4) encode heat-shock protein (smHSPs). HSPs, including smHSPs, are involved in stress tolerance. For instance, AT-HSP17.6A can be induced by heat and osmotic stress [37].

According to UniProtKB, AT5G06730 (PER54) encodes a Peroxidase enzyme involved in response to environmental stresses by removing H_2O_2 as reactive oxygen species (ROS). NPs have been shown that could induce oxidative stress by an increase in ROS, resulting in disturbing some physiological functions related to redox [38].

AT4G11650 (ATOSM34) encodes an osmotin protein. The osmotin protein belongs to the pathogenesis-related (PR)-5 family, those induced under various biotic and abiotic stresses for stress tolerance [39]. AT2G26020 (PDF1.2b) is Predicted to encode a PR gene from the plant defensin (PDF) family protein [40]. Finally, AT5G24780 encoding a vegetative storage protein is reported to be induced by wounding, herbivory, and jasmonic acid (JA) [41].

Table 3. *The physicochemical properties of proteins encoded by up-regulated genes with log FC > 10.*

Gene name	Protein Description	Number of amino acids	Ave. residue weight (g/mol)	Charge	Molecular weight (g/mol)	Isoelectric point	log FC
AT5G12030	heat shock protein 17.6A	156	113.368	-1.5	17,685.37	5.3946	10.5
AT1G07400	HSP20-like chaperones superfamily protein	157	113.555	-0.5	17,828.16	6.2656	10.3
AT1G19530	DNA polymerase epsilon catalytic subunit A	117	116.720	5.0	13,656.27	9.8248	12.4
AT2G05510	Glycine-rich protein family	127	93.045	6.0	11,816.68	7.2596	11.1
AT5G06730	Peroxidase 54	358	104.163	-10.5	37,290.41	4.2747	11.5
AT5G39110	RmlC-like cupins superfamily protein	222	107.718	0.5	23,913.39	6.7115	12.2
AT4G11650	osmotin 34	244	109.151	0.0	26,632.76	6.3900	10.6
AT2G42540	cold-regulated 15a	127	105.921	0.5	13,451.93	7.0202	13.2
AT3G46230	17.4 kDa class I heat shock protein	156	111.792	-4.0	17,439.59	4.9420	10.5
AT2G26020	PDF1.2b	80	108.002	3.0	8,640.16	7.8233	10.2
AT5G24780	vegetative storage protein 1	270	112.080	-3.0	30,261.57	5.3870	14.3

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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