Title

Meta-Analysis of Public RNA Sequencing Data of Abscisic Acid-Related Abiotic Stresses in *Arabidopsis thaliana*

Author names and affiliations

Mitsuo Shintani[a], Keita Tamura[b], Hidemasa Bono[a,b*]

[a]Graduate School of Integrated Sciences for Life, Hiroshima University, 3-10-23 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-0046, Japan

[b]Genome Editing Innovation Center, Hiroshima University, 3-10-23 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-0046, Japan

*Corresponding author

Abstract

Abiotic stresses such as drought, salinity, and cold negatively affect plant growth and crop productivity. Understanding the molecular mechanisms underlying plant responses to these stressors is essential for stress tolerance in crops. The plant hormone abscisic acid (ABA) is significantly increased upon abiotic stressors, inducing physiological responses to adapt to stress and regulate gene expression. Although many studies have examined the components of established stress signaling pathways, few have explored other unknown elements. Data-driven studies have the advantage of analyzing large and independent datasets, which can lead to the identification of novel research topics. This study aimed to identify novel stress-responsive genes in plants by performing a meta-analysis of public RNA sequencing (RNA-Seq) data on *Arabidopsis thaliana* from the NCBI Gene Expression Omnibus, focusing on five ABA-related stress conditions (ABA, Salt, Dehydration, Osmotic, and Cold). The meta-analysis of 216 paired datasets from five stress conditions was conducted, and differentially expressed genes were identified by introducing a new metric, called TN (stress-treated (T) and non-treated (N))-score. Enrichment analysis was performed to characterize differentially expressed genes, and genes that were commonly regulated under different stresses were also identified. The meta-analysis revealed that 14 genes were commonly upregulated and 8 genes were commonly downregulated across all five treatments, including some that were not previously associated with these stress responses. On the other hand, some genes regulated by salt, dehydration, and osmotic treatments were not regulated by exogenous ABA or cold stress, suggesting that they may be involved in the plant response to dehydration independent of ABA. Meta-analysis revealed a list of candidate genes with unknown molecular mechanisms in ABA-dependent and ABA-independent stress responses. The identified genes could be valuable resources for selecting genome editing targets and potentially contribute to the discovery of novel stress tolerance mechanisms and pathways in plants.

Introduction

Drought and salinity are the major abiotic stressors in plants. These stresses are becoming more severe with climate change and negatively affect crop growth, leading to yield loss [1] [2] [3]. Drought, salt stress, and secondary osmotic stress induced by them reduce plant water availability, leading to dehydration stress [4] [5]. The plant hormone abscisic acid (ABA) increases significantly by these dehydration stresses and induces physiological responses to adapt to the stresses, such as stomatal closure and regulation of gene expression [6] [7] [8]. Dehydration-responsive genes can be divided into two categories: expression regulated by ABA or specifically regulated by dehydration stress, but not by ABA [9] [10]. Additionally, ABA is synthesized under cold stress conditions [11]. The pathways that respond to cold stress and their associated gene expression share similarities with those that respond to dehydration stress [9] [12]. However, the expression of some genes is regulated by stimuli specific to cold stress, such as a decrease in temperature [9] [12]. Thus, plants respond to stress through common or uncommon pathways [9] [12]. Therefore, it is important to clarify the characteristics of stress-responsive genes, such as their regulatory stress and whether they are ABA-responsive (ABA-regulated or ABA-unregulated), to understand the complex regulatory mechanisms of stress responsiveness in plants.

The core of the ABA signaling pathway consists of three types of proteins: ABA receptors (PYR/PYL/RCAR: pyrabactin resistance/PYR-like/regulatory component of ABA receptor), protein phosphatases (PP2C: protein phosphatases type-2C), and protein kinases (SnRK2: SNF1-related protein kinase 2) [13] [14]. These proteins regulate the activity of ABA signaling. ABA binds to receptors (PYR/PYL/RCAR) that inhibit phosphatases (PP2C) and activate kinases (SnRK2) that regulate various physiological responses through signaling cascades [13]. ABA-activated SnRK2 phosphorylates transcription factors such as abscisic acid-responsive element binding factors (ABFs) and ABI5, thereby controlling the expression of downstream ABA-responsive genes [8] [15]. One of the most crucial SnRK activated by

ABA is SnRK2.6/Open Stomata 1 (OST1), which regulates ABA-mediated stomatal closure [16] [17]. Although several studies have examined the core elements of these signals, few have explored other novel elements. The data-driven studies have the advantage of analyzing large and independent datasets, which can lead to the identification of novel targets, distinct from the extensively studied established factors and accelerate the development of stress-tolerant crops [18] [19] [20].

This study aimed to identify novel stress-responsive genes in plants by performing a meta-analysis of publicly available RNA sequencing (RNA-Seq) data from five ABA-related stress conditions (ABA, Salt, Dehydration, Osmotic, and Cold) in *Arabidopsis thaliana* with a focus on ABA, a factor common to many stresses. The meta-analysis revealed that 14 genes were commonly upregulated and 8 genes were commonly downregulated across all five treatments, including some that were not previously associated with these stress responses. However, some genes regulated by salt, dehydration, and osmotic treatments were not regulated by exogenous ABA or cold stress, suggesting that they may be involved in the plant response to dehydration independent of ABA.

Materials and methods

Curation of Public Gene Expression Data

RNA-Seq data relevant to stress conditions involving abscisic acid were collected from the public database, NCBI Gene Expression Omnibus (GEO) [21] (last accessed on 3 August 2022). A comprehensive search in GEO using the search query: ("ABA"[All Fields] OR "abscisic acid"[All Fields] OR "salt"[All Fields] OR "NaCl"[All Fields] OR "salinity"[All Fields] OR "dehydration"[All Fields] OR "osmotic"[All Fields] OR "mannitol"[All Fields] OR "drought"[All Fields] OR "cold"[All Fields] OR "low temperature"[All Fields]) AND "Arabidopsis thaliana"[porgn] AND "Expression profiling by high throughput sequencing"[Filter] AND ("0001/01/01"[PDAT] : "2022/08/03"[PDAT]). As a result of manual curation, the collected data were divided into five treatments: ABA, Salt, Dehydration, Mannitol, and Cold. The numbers of pairs of treated and control samples were 90, 53, 27, 12, and 34, respectively. In total, 216 pairs of samples collected in this manner were used for the meta-analysis. The RNA-Seq data used in this meta-analysis are summarized in the table available on figshare (figshare file1). The selection process of studies and samples for analysis are presented in a flowchart (Fig 1) and checklist (figshare files 2 and 3), in accordance with PRISMA 2020 guidelines [22].

Fig1. PRISMA 2020 flow diagram

Expression Quantification

We used the SRA Toolkit (v3.0.0) (https://github.com/ncbi/sra-tools) to retrieve FASTQ-formatted files for each RNA-Seq run accession number, with prefetch and fastq-dump commands, and then concatenated the files for the same experiments. Quality control of the raw reads was performed using Trim Galore (v0.6.7) (https://github.com/FelixKrueger/TrimGalore) with Cutadapt (v4.1) [23]. Transcripts were quantified using Salmon (v1.8.0) (https://github.com/COMBINE-lab/salmon) [24] with a reference cDNA sequence downloaded from Ensembl Plants (release 53)

(Arabidopsis_thaliana.TAIR10.cdna.all.fa.gz). As a result, the quantitative RNA-Seq data were calculated as transcripts per million (TPM). The transcript-level TPM were summarized to the gene level using tximport (v1.26.1) (https://bioconductor.org/packages/release/bioc/html/tximport.html). These operations were performed according to the workflow in SAQE (https://github.com/bonohu/SAQE) [19]. The TPM data are shown at figshare (figshare file4).

Calculation Score

Gene expression data from different experiments were normalized by calculating the TN-ratio, which represents the ratio of gene expression between stress-treated (T) and non-treated (N) samples.

The TN-ratio was calculated using the following equation:

TN-ratio = (stress-treated TPM +1) / (non-treated TPM +1)

If the TN-ratio was higher than the threshold, the gene was considered upregulated; if it was less than the reciprocal of the threshold, it was deemed downregulated; otherwise, the gene was unchanged. To classify the upregulated and downregulated genes, we evaluated 2-fold, 5-fold, and 10-fold thresholds and finally chose the 2-fold threshold; therefore, genes with a TN-ratio higher than 2 were classified as upregulated, whereas genes with a TN-ratio lower than 0.5 were classified as downregulated.

The TN-score of each gene was determined by subtracting the number of downregulated experiments from the number of upregulated experiments to assess the changes in gene expression under stress conditions across experiments.

The TN-score was calculated using the following equation:

TN-score = (number of upregulated)–(number of downregulated)

TN-ratio and TN-score were calculated using a set of scripts at (https://github.com/no85j/hypoxia_code) [20] and the data are available in figshare (figshare files 5 and 6).

Gene Set Enrichment Analysis

The web tool Metascape (https://metascape.org/) was used for gene set enrichment analysis to analyze the differentially expressed gene sets. The analysis was based on a queried gene list and the corresponding terms and p-values were examined.

Visualization

We used a web-based Venn diagram tool (https://bioinformatics.psb.ugent.be/webtools/Venn/) and the UpSet tool (https://asntech.shinyapps.io/intervene/) to search for and visualize overlapping genes.

Results

Data curation of RNA-Seq from public database

RNA-Seq data on abscisic acid (ABA) and stress conditions were collected from the public database NCBI Gene Expression Omnibus (GEO). A comprehensive keyword search yielded a list of experimental data series. In contrast to microarray data from different platforms, RNA-Seq data, mostly from Illumina, are suitable for comparative analyses among studies by different research groups. Therefore, only the RNA-Seq data were used in this study. Moreover, *Arabidopsis* data were selected for the analysis because of the abundance of data in the database compared with other plants. No mutant or transgenic lines were selected and wild type samples were used. Abscisic acid, salt (NaCl), dehydration, osmotic (mannitol), and low temperature (4 °C or 10 °C) were used as stress treatment conditions. The data were curated as 216 paired datasets of stress-treated and control samples. A total of 216 pairs were used in the meta-analysis, and the tissue breakdown was as follows: 139 seedlings, 28 leaves, 23 rosette leaves, 14 roots, 6 shoots, and 6 bud tissues (Fig 2). Metadata for the curated datasets, including Sequence Read Archive (SRA) study ID, run ID, sample tissue, treatment type, treatment time, and sequence library type, are available on figshare (figshare file1).

Fig 2. Distribution of tissue types in curated RNA-Seq data

Visualization of 216 paired datasets showing the distribution of tissues such as seedlings, leaves, rosette leaves, roots, shoots, and bud tissues.

Identification of differentially expressed genes

Differentially expressed genes using RNA-Seq data in *Arabidopsis* under each stress condition were meta-analyzed by calculating the TN (stress-treated (T) and non-treated (N))-ratio and the TN-score of each gene. After considering various thresholds, we selected a 2-fold threshold (TN2). This threshold was slightly lower to provide a comprehensive analysis. More severe scores for the 5-fold (TN5) and 10-fold (TN10) thresholds were also calculated

and are listed in the table. The lists of upregulated and downregulated genes are available on figshare (figshare files 7a, 7b). The differences between the numbers of upregulated and downregulated experiments were calculated as the TN-scores for each gene. Thus, a higher score indicated a trend toward increased expression over the entire experiment, whereas a lower (negative) score indicated a trend toward decreased expression over the entire experiment. In the meta-analysis, we defined upregulated and downregulated as approximately top 500 genes with the highest and lowest TN2 scores, respectively. The ranges of TN2 scores for the upregulated and downregulated genes are summarized in Table 1.

Table 1. The ranges of TN2 scores for the upregulated and downregulated genes

Treatment Type	Up or Down	TN-Score (S)	Number of Genes
ABA	upregulated	47 ≤ S ≤ 88	489
ABA	downregulated	-81 ≤ S ≤ -31	486
Salt	upregulated	18 ≤ S ≤ 41	501
Salt	downregulated	-36 ≤ S ≤ -11	446
Dehydration	upregulated	12 ≤ S ≤ 24	457
Dehydration	downregulated	-17 ≤ S ≤ -9	431
Mannitol	upregulated	7 ≤ S ≤ 12	520
Mannitol	downregulated	-11 ≤ S ≤ -6	532
Cold	upregulated	16 ≤ S ≤ 32	474
Cold	downregulated	-32 ≤ S ≤ -14	551

Enrichment analysis of differently expression genes

We performed enrichment analysis using Metascape for five different treatment types of gene sets to characterize the differentially expressed genes (Fig 3). The two most significantly enriched terms in each gene set from the enrichment analysis are summarized in table (Table 2). The most significantly enriched terms in the ABA, salt, and dehydration upregulated gene sets were all consistent with "GO:0009651 response to salt stress". Focusing on the downregulated genes, the light-responsive genes annotated as

"GO:0009642 response to light intensity" were commonly enriched in dehydration and cold treatment. The auxin-responsive genes with "GO:0009733 response to auxin" were commonly enriched in dehydration and mannitol treatment. The lists of all genes and the results of enrichment analysis are available on figshare (figshare files 7a, 7b).

Fig 3. Enrichment analysis of differentially expressed genes for 5 treatment types

Gene set enrichment analysis of the genes regulated under the five treatments is shown in (a)–(j), indicating upregulated and downregulated genes in the ABA (a, b), salt (c, d), dehydration (e, f), mannitol (g, h), and cold (i, j) treatments, respectively.

Table 2. The two most significantly enriched terms in each gene set from the enrichment analysis

Treatment Type	Up or Down	Term	Description	LogP	Log(q-value)
ABA	upregulated	GO:0009651	response to salt stress	-28.758572	-24.99
ABA	upregulated	GO:0071215	cellular response to abscisic acid stimulus	-20.943027	-17.652
ABA	downregulated	GO:0050832	defense response to fungus	-14.485652	-10.717
ABA	downregulated	GO:0001666	response to hypoxia	-7.9936821	-4.568
Salt	upregulated	GO:0009651	response to salt stress	-31.120518	-27.352
Salt	upregulated	GO:0009753	response to jasmonic acid	-18.821332	-15.402
Salt	downregulated	GO:0001666	response to hypoxia	-10.473446	-6.886
Salt	downregulated	GO:0009733	response to auxin	-6.5022334	-3.579
Dehydration	upregulated	GO:0009651	response to salt stress	-36.0593	-32.291
Dehydration	upregulated	GO:0010243	response to organonitrogen compound	-19.3438	-15.876
Dehydration	downregulated	GO:0009642	response to light intensity	-19.290212	-15.522
Dehydration	downregulated	GO:0009733	response to auxin	-17.262779	-13.795
Mannitol	upregulated	GO:0009751	response to salicylic acid	-28.659664	-24.921
Mannitol	upregulated	GO:0090693	plant organ senescence	-26.790069	-23.499
Mannitol	downregulated	GO:0033013	tetrapyrrole metabolic process	-13.988496	-10.22
Mannitol	downregulated	GO:0009733	response to auxin	-7.0652901	-3.774

Cold	upregulated	GO:0009409	response to cold	-24.0776	-20.309
Cold	upregulated	GO:0042254	ribosome biogenesis	-20.3946	-16.927
Cold	downregulated	GO:0009642	response to light intensity	-31.842859	-28.123
Cold	downregulated	ath00196	Photosynthesis - antenna proteins - Arabidopsis thaliana (thale cress)	-15.580344	-12.162

Exploring commonly regulated genes by three treatments: ABA, salt, dehydration

A previous study demonstrated that some genes are involved in the regulation and response to multiple stresses and that these genes have a potential role in universal stress tolerance in plants [25]. We investigated whether such candidate genes were differentially expressed under three stress conditions: ABA, salt, and dehydration. While many genes were commonly regulated under the three conditions, we identified 166 upregulated (ABA_Up Salt_Up Dehydration_Up) and 66 downregulated genes (ABA_Down Salt_Down Dehydration_Down) under the three different conditions (Fig 4). The meta-analysis also showed that the expression of *RAV1*, *RAV2*, *PYR1*, *PYL4*, *PYL5*, *PYL6*, and *PCAR3* (*PYL8*) was downregulated by ABA, salt, and dehydration stress, which is consistent with previous studies [26] [27] [28].

Furthermore, we performed enrichment analysis of these commonly regulated genes to evaluate gene characteristics. The results showed that the top two significant enrichment terms for the 166 commonly upregulated genes (ABA_Up Salt_Up Dehydration_Up) were "GO:0071215 cellular response to abscisic acid stimulus" and “GO:0009651 response to salt stress”. In addition, the top two significant enrichment terms for the 66 commonly downregulated genes (ABA_Down Salt_Down Dehydration_Down) were "ath04075 Plant hormone signal transduction - *Arabidopsis thaliana* (thale cress)" and "GO:0071215 cellular response to abscisic acid stimulus". The gene sets common to the three treatments were significantly enriched in terms of ABA and salt stress, indicating that these genes are likely involved in ABA and salt stress. The lists of all genes overlapping in the three treatments, and the results of the enrichment analysis are available in figshare (figshare files 8a, 8b).

Fig 4. Analysis of the overlaps of upregulated or downregulated genes in 3 different types of treatments

Venn diagrams and gene set enrichment analysis in (a)-(d) represent the upregulated or downregulated genes in ABA, Salt, and Dehydration treatments.

(a) Venn diagram of the upregulated genes; (b) Venn diagram of the downregulated genes; (c) Gene set enrichment analysis of the commonly upregulated genes; (d) Gene set enrichment analysis of the commonly downregulated genes.

Exploring commonly regulated genes by five treatments: ABA, salt, dehydration, osmotic, cold

In the previous section, we identified commonly regulated genes across ABA, salt, and dehydration treatments. We then added mannitol and cold treatments to analyze gene overlap. The overlap of genes commonly regulated by the five treatments (ABA, salt, dehydration, osmotic stress, and cold stress) was visualized using UpSet plots and Venn diagrams (Fig 5). As a result, 14 commonly upregulated genes (ABA_Up Salt_Up Dehydration_Up Mannitol_Up Cold_Up) and 8 commonly downregulated genes (ABA_Down Salt_Down Dehydration_Down Mannitol_Down Cold_Down) were identified. The genes included in these two groups are listed in Tables 3a and 3b respectively. The genes commonly upregulated in the five treatments contained *LTI78/RD29A*, *LTI30*, *KIN1*, *COR47*, and *ADH1*, which are reported to be induced by ABA, salt, dehydration, and cold stress conditions [29] [30] [31] [32] [33]. On the other hand, genes such as *ATHMAD1*, which have not been reported to have ABA-related or stress response of functions, were also identified. These genes have been identified as novel stress-responsive genes involved in the ABA response. Also, the ABA-independent pathway, which is specifically induced by osmotic stress has been reported in plants [10] [34]. This pathway is hypothesized to be activated independently of ABA and its closely related cold stimulus [12]. Therefore, we examined three treatments, excluding ABA and cold, to explore genes involved in the ABA-

independent osmotic signaling pathway. As a result, 7 commonly upregulated genes (Salt_Up Dehydration_Up Mannitol_Up) and 48 commonly downregulated genes (Salt_Down Dehydration_Down Mannitol_Down), regulated by salt, dehydration, and mannitol, were also identified. Notably, these genes were not included among those suggested to be regulated by ABA and cold stress in the present study, and the effects of ABA and cold on the regulation of their expression may not be significant. Therefore, they may be involved in the ABA-independent stress response pathways. The genes included in these two groups are listed in Tables 3c and 3d. The lists of all genes that overlap in the five treatments are available on figshare (figshare files 9a, 9b).

Fig 5. Analysis of the overlaps of upregulated or downregulated genes in 5 different types of treatments

UpSet plots and venn diagrams in (a)-(d) represent the upregulated or downregulated genes in ABA, Salt, Dehydration, Mannitol, and Cold treatments.

(a) UpSet plots and (b) Venn diagram of the upregulated genes; (c) UpSet plots and (d) Venn diagram of the downregulated genes.

Table 3. The lists of commonly regulated genes in different stress treatments

Table 3a

Gene ID	Gene Name	Description	Regulated by Hypoxia in previous meta-analysis
AT1G11210	-	cotton fiber protein, putative (DUF761)	No
AT1G16850	-	transmembrane protein	No
AT1G73810	-	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein	No
AT4G01985	-	hypothetical protein	No
AT5G11110	SPS2F	sucrose phosphate synthase 2F	No
AT1G51090	ATHMAD1	Heavy metal transport/detoxification superfamily protein	No
AT5G17460	DFR1	glutamyl-tRNA (Gln) amidotransferase subunit C	No
AT1G67360	LDAP1	Rubber elongation factor protein (REF)	Induced

AT1G77120	ADH1	alcohol dehydrogenase 1	Induced
AT1G20440	COR47	cold-regulated 47	No
AT5G15960	KIN1	stress-responsive protein (KIN1) / stress-induced protein (KIN1)	No
AT1G01470	LEA14	Late embryogenesis abundant protein	No
AT3G50970	LTI30	dehydrin family protein	No
AT5G52310	RD29A	low-temperature-responsive protein 78 (LTI78) / desiccation-responsive protein 29A (RD29A)	No

Table 3b

Gene ID	Gene Name	Description	Regulated by Hypoxia in previous meta-analysis
AT3G23880	-	F-box and associated interaction domains-containing protein	No
AT5G19190	-	hypothetical protein	No
AT4G38850	SAUR15	SAUR-like auxin-responsive protein family	No
AT4G38860	SAUR16	SAUR-like auxin-responsive protein family	Repressed
AT1G29430	SAUR62	SAUR-like auxin-responsive protein family	Repressed
AT1G29440	SAUR63	SAUR-like auxin-responsive protein family	Repressed
AT1G74670	GASA6	Gibberellin-regulated family protein	Repressed
AT4G36570	RAD-LIKE 3	RAD-like 3	Repressed

Table 3c

Gene ID	Gene Name	Description	Regulated by Hypoxia in previous meta-analysis
AT1G17830	-	hypothetical protein (DUF789)	Induced
AT1G76590	-	PLATZ transcription factor family protein	No
AT5G42050	-	DCD (Development and Cell Death) domain protein	No
AT3G22370	AOX1A	alternative oxidase 1A	No
AT2G21590	APL4	Glucose-1-phosphate adenylyltransferase family protein	No
AT1G02850	BGLU11	beta glucosidase 11	No
AT4G37370	CYP81D8	cytochrome P450, family 81, subfamily D, polypeptide 8	No

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Gene ID	Gene Name	Description	Regulated by Hypoxia in previous meta-analysis
AT4G30610	BRS1	alpha/beta-Hydrolases superfamily protein	No
AT1G55330	AGP21	arabinogalactan protein 21	Repressed
AT4G16980	-	arabinogalactan-protein family	Repressed
AT4G12980	-	Auxin-responsive family protein	Repressed
AT2G41240	BHLH100	basic helix-loop-helix protein 100	No
AT2G42380	BZIP34	Basic-leucine zipper (bZIP) transcription factor family protein	Repressed
AT3G58120	BZIP61	Basic-leucine zipper (bZIP) transcription factor family protein	Repressed
AT2G10940	-	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	Repressed
AT5G48485	DIR1	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	No
AT2G45180	-	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	No
AT5G48490	-	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	No
AT1G23480	CSLA03	cellulose synthase-like A3	Repressed
AT5G15350	ENODL17	early nodulin-like protein 17	No
AT4G12730	FLA2	FASCICLIN-like arabinogalactan 2	Repressed
AT5G44130	FLA13	FASCICLIN-like arabinogalactan protein 13 precursor	No
AT2G45470	FLA8	FASCICLIN-like arabinogalactan protein 8	Repressed
AT1G10020	-	formin-like protein (DUF1005)	Repressed
AT5G20630	GER3	germin 3	No
AT1G72610	GER1	germin-like protein 1	No
AT1G33240	GTL1	GT-2-like 1	No
AT5G44020	-	HAD superfamily, subfamily IIIB acid phosphatase	Repressed
AT1G04040	-	HAD superfamily, subfamily IIIB acid phosphatase	No
AT2G23690	-	HTH-type transcriptional regulator	No
AT4G37240	-	HTH-type transcriptional regulator	No
AT4G00950	MEE47	hypothetical protein (DUF688)	Repressed
AT4G04840	MSRB6	methionine sulfoxide reductase B6	Repressed
AT1G24170	LGT9	Nucleotide-diphospho-sugar transferases superfamily protein	No
AT1G12500	-	Nucleotide-sugar transporter family protein	No
AT4G23820	-	Pectin lyase-like superfamily protein	No
AT4G08950	EXO	Phosphate-responsive 1 family protein	No
AT2G42870	PAR1	phy rapidly regulated 1	Repressed
AT5G44420	PDF1.2	plant defensin 1.2	No
AT1G66100	-	Plant thionin	Repressed
AT4G36850	-	PQ-loop repeat family protein / transmembrane family protein	No

AT5G02760	-	Protein phosphatase 2C family protein	Repressed
AT2G04790	-	PTB domain engulfment adapter	No
AT3G23805	RALFL24	ralf-like 24	Repressed
AT1G22330	-	RNA-binding (RRM/RBD/RNP motifs) family protein	Repressed
AT1G72430	-	SAUR-like auxin-responsive protein family	No
AT5G08330	TCP11	TCP family transcription factor	No
AT5G03120	-	uncharacterized protein	Repressed
AT5G35480	-	uncharacterized protein	No
AT2G01755	-	uncharacterized protein	No
AT3G23730	XTH16	xyloglucan endotransglucosylase/hydrolase 16	No
AT2G06850	XTH4	xyloglucan endotransglucosylase/hydrolase 4	Repressed
AT5G65730	XTH6	xyloglucan endotransglucosylase/hydrolase 6	Repressed
AT1G11545	XTH8	xyloglucan endotransglucosylase/hydrolase 8	No
AT4G03210	XTH9	xyloglucan endotransglucosylase/hydrolase 9	Repressed

(a) Commonly upregulated in ABA, Salt, Dehydration, Mannitol, and Cold treatments; (b) Commonly downregulated in ABA, Salt, Dehydration, Mannitol, and Cold treatments; (c) Commonly upregulated in Salt, Dehydration, and Mannitol treatments; (d) Commonly downregulated in in Salt, Dehydration, and Mannitol treatments.

Discussion

In this study, we aimed to explore uncharacterized differentially expressed genes by meta-analyzing public RNA-Seq data of *Arabidopsis thaliana* to gain new insights into the effects of abscisic acid (ABA) and various stress conditions on gene expression. The data were collected from the public database NCBI GEO, and the study focused on RNA-Seq data because of their suitability for comparative analyses among different studies. We analyzed the effects of ABA, salt, dehydration, osmotic, and low-temperature stress on gene expression. In total, 216 paired datasets of stress-treated and control samples were used for the meta-analysis. We employed the TN-score to identify differentially expressed genes under each stress condition. We defined upregulated and downregulated genes as the top 500 genes with the highest and lowest TN-scores, respectively, in the meta-analysis. Enrichment analysis was performed using Metascape to characterize the differentially expressed genes. The most significantly enriched terms in the downregulated genes, the light-responsive genes annotated as "GO:0009642 response to light intensity" were commonly enriched in dehydration and cold treatment, and the auxin-responsive genes annotated as "GO:0009733 response to auxin" were commonly enriched in dehydration and mannitol treatment. Abiotic stress negatively affects various aspects of plant photosynthesis including stomatal conductance, oxidative stress, RUBISCO activity, photosystems (PS I and PS II), photosynthetic electron transport, and chlorophyll biosynthesis [35]. These factors reduce the photosynthetic efficiency and plant growth. Therefore, it is possible that dehydration and cold treatments could have downregulated the expression of light-responsive genes “GO:0009642 response to light intensity” due to adjustments in light sensitivity and photosynthetic responses during the process of adapting to stress. In addition, osmotic stress reduces the biosynthesis of auxin, a plant growth hormone, by directly or indirectly inhibiting the auxin pathway [36]. This might contribute to reduced plant growth under stress conditions. Auxin biosynthesis maybe suppressed by osmotic stress conditions, resulting in downregulated auxin-responsive genes "GO:0009733 response to auxin". Next, we identified genes that were commonly regulated by ABA, salt, and dehydration stress. We

also performed enrichment analysis of genes commonly regulated by different stresses and verified their consistency with previously reported gene expression patterns. We expanded this analysis to include genes commonly regulated by all five treatments (ABA, salt, dehydration, osmotic, and cold). Several genes were upregulated or downregulated in response to these stress conditions, suggesting their potential roles in global plant stress tolerance mechanisms.

Fourteen upregulated genes were found to be closely involved in ABA regulation (ABA_Up Salt_Up Dehydration_Up Mannitol_Up Cold_Up, Table 3a), including genes that have not been previously studied, one of which is ATHMAD1 (AT1G51090). ATHMAD1 contains a heavy metal-associated (HMA) domain that is upregulated in response to nitric oxide (NO) [37]. NO plays a variety of roles in plant adaptation to abiotic stresses such as drought, salt, and cold [38] [39]. NO synthesis is promoted by abscisic acid (ABA) treatment and abiotic stress, which contributes to stomatal closure during drought stress [40]. Analysis of *athmad1* mutant indicated that ATHMAD1 may play a role in regulating plant growth and immunity [37]. However, the function of ATHMAD1 under abiotic stress has not yet been thoroughly investigated. In this study, *ATHMAD1* expression was upregulated upon treatment with ABA, salt, dehydration, osmotic, and cold stress. Based on these findings, *ATHMAD1* expression may increase in response to NO, which is promoted by ABA treatment and abiotic stress. Therefore, analyzing the function of ATHMAD1 in plants under abiotic stress conditions is an important challenge for future research.

In addition, focusing on genes downregulated by multiple stressors, we examined eight genes considered to be deeply involved in ABA-dependent expression regulation (ABA_Down Salt_Down Dehydration_Down Mannitol_Down Cold_Down, Table 3b) and 48 genes considered to be less influenced by ABA-mediated expression regulation (Salt_Down Dehydration_Down Mannitol_Down, Table 3d). It was found that among the eight genes, four were SAUR genes (*SAUR15, 16, 62, 63*), and among the 48 genes, five XTH genes

(*XTH4, 6, 8, 9, 16*) were included, respectively. Although SAUR and XTH gene families have distinct mechanisms and molecular targets, they play a common role in controlling plant growth and cell wall expansion. Furthermore, several studies have identified these genes during stress stimulation and changes in stress response phenotypes in mutants lacking their functions.

The *SAUR* (*Small Auxin Up RNA*) genes are a plant-specific gene family that plays a crucial role in plant growth and development by regulating cell wall acidification in response to auxin stimulation [41] [42]. Several *SAUR* genes were downregulated in response to ABA, drought, and osmotic stress. ARABIDOPSIS ZINC-FINGER 1 (AZF1) and AZF2 were induced by osmotic stress and abscisic acid, function as transcriptional repressors of 15 SAUR genes, thereby inhibiting plant growth under abiotic stress conditions [43]. Among the genes repressed by AZF, AZF directly binds to the promoter region of *SAUR63* [43]. SAUR63 localizes to the plasma membrane and by inhibiting plasma membrane (PM)-associated PP2C.D phosphatases, it stimulates PM H^+-ATPase proton pump activity, thereby promoting cell growth [44]. However, the functions of most SAUR genes under abiotic stress and their direct relationship with stress remain unclear.

Xyloglucan endotransglucosylase/hydrolase (XTH) is an enzyme family involved in plant cell wall remodeling that catalyzes the cleavage and polymerization of xyloglucan to regulate cell elasticity and extensibility [45]. XTH not only plays a role in regulating cell wall structure and morphology, but also plays a crucial role in plant adaptation to external stress [46]. The expression of *AtXTH19* and *AtXTH23* was induced by brassinosteroids, and the *atxth19* mutant showed lower freezing tolerance than the wild type, whereas the *atxth19/atxth23* double mutant showed increased sensitivity to salt stress [47] [48]. Similarly, *AtXTH30* contributes to salt tolerance [49]. Additionally, the loss-of-function of several *XTH* genes has been reported to affect metal ion tolerance. *AtXTH31* is associated with cell wall aluminum binding capacity, and mutation of this gene leads to increased aluminum tolerance [50].

Similarly, *Atxth18* mutant showed reduced sensitivity to lead stress [51]. *AtXTH4* and *AtXTH9* contribute to the regulation of xylem cell expansion and secondary growth, including the production of secondary xylem and the accumulation of secondary walls. However, the function of stress conditions has not yet been clarified [52]. These studies suggested that XTH plays an important role in various stress responses. Therefore, understanding the functions of the five XTH genes (*XTH4, 6, 8, 9, 16*), the genes that were downregulated by ABA-independent stress pathways in this study, is a future challenge.

Finally, we investigated whether the hypoxia-responsive genes identified in a previous meta-analysis in *Arabidopsis thaliana* were included in the genes we focused on in this study [18]. Under hypoxic conditions, root water transport is inhibited and stomatal closure is induced. This process requires aquaporins, which are proteins involved in water transport, as well as the hormones ABA and ethylene [53]. Furthermore, during recovery from submergence, reactive oxygen species (ROS), ABA, ethylene, respiratory burst oxidase homolog D (RBOH D), senescence-associated gene113 (SAG113), and ORESARA1 regulate ROS homeostasis, stomatal opening/closing, and chlorophyll degradation [54]. These studies suggest that hypoxia and ABA levels are related to each other. The analysis demonstrated that the expression of several stress-regulated genes, which were the focus of this study, was regulated by hypoxia (Table 3a-d). These results imply that there might be an undiscovered molecular cross talk between “hypoxia” and “ABA or dehydration,” which has not been frequently discussed in previous research. The overlap of genes commonly regulated by the six treatments (ABA, salt, dehydration, osmotic, cold, and hypoxia) was visualized using UpSet plots (Fig 6). These genes are regulated by a wide range of stresses and identifying their roles could contribute to further advances in our understanding of the broad mechanisms of stress tolerance in plants. The lists of all genes overlapping in the six treatments are available in figshare (figshare files 10a, 10b).

Fig 6. Analysis of the overlaps of upregulated or downregulated genes in 6 different types of treatments

UpSet plots and venn diagrams in (a) and (b) represent the upregulated or downregulated genes in ABA, Salt, Dehydration, Mannitol, Cold, and Hypoxia treatments.

(a) UpSet plots of the upregulated genes; (b) UpSet plots of the downregulated genes.

Conclusions

In conclusion, this meta-analysis lists candidate genes involved in novel molecular mechanisms under ABA-dependent and ABA-independent stress conditions. In this study, we focused on the gene expression commonly found under different stresses and validated our analysis by confirming its consistency with previously reported patterns of gene expression. As the data in the database increases, it is expected that new findings will be revealed by updating and reanalyzing the datasets. Although further studies are required to reveal the role of the genes identified in this study, the list obtained in this study can be a useful decision criterion for the selection of genome editing targets and the discovery of novel molecular mechanisms.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data presented in this study are publicly available in figshare (https://doi.org/10.6084/m9.figshare.22566583.v2).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MS and HB conceived and designed the study. MS performed the experiments and collected data. KT helped the analysis. Writing—original draft: MS. All authors discussed the data and helped with manuscript preparation. HB supervised the project. All authors read and approved the final manuscript.

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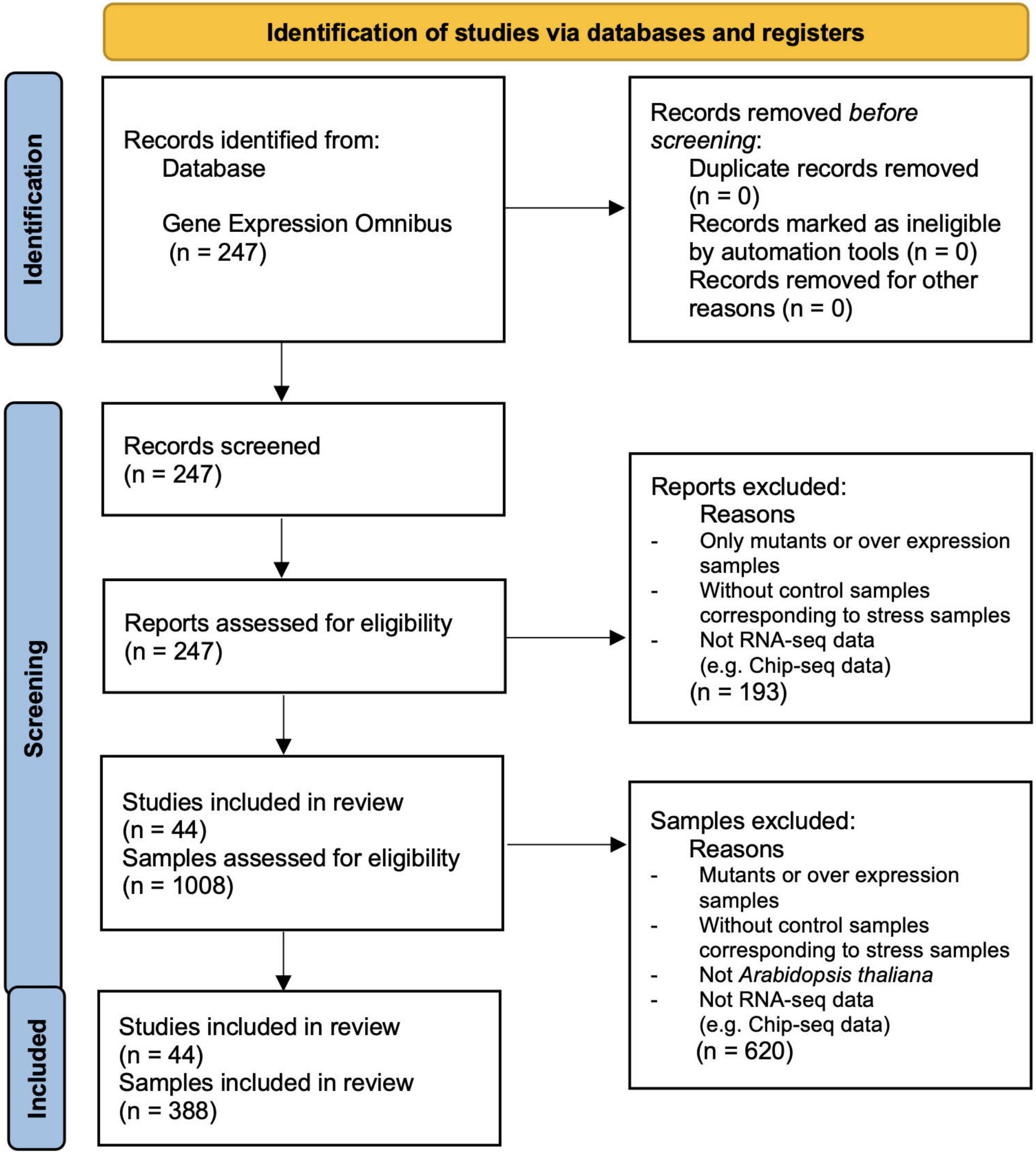
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Gene Expression Omnibus
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Duplicate records removed
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Reports assessed for eligibility
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Reports excluded:
Reasons
- Only mutants or over expression samples
- Without control samples corresponding to stress samples
- Not RNA-seq data
(e.g. Chip-seq data)
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Reasons
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- Not *Arabidopsis thaliana*
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Studies included in review
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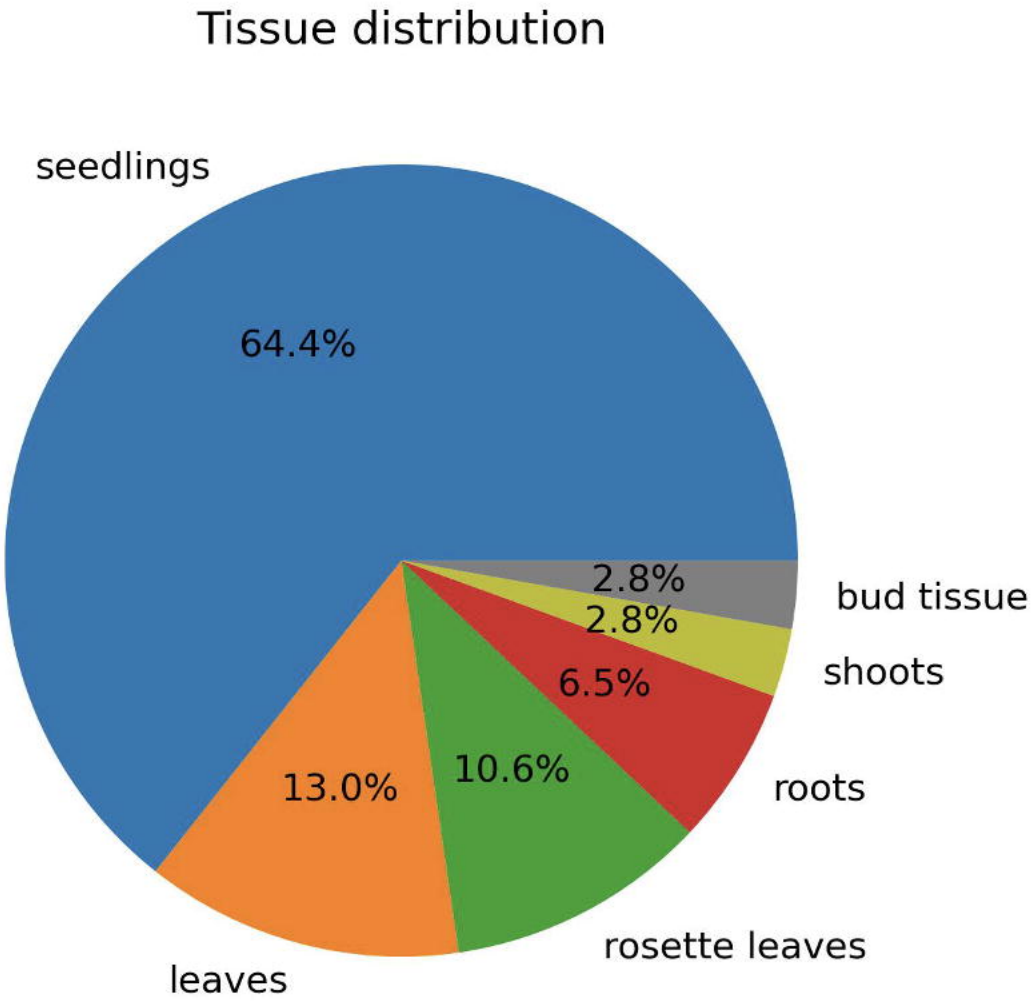
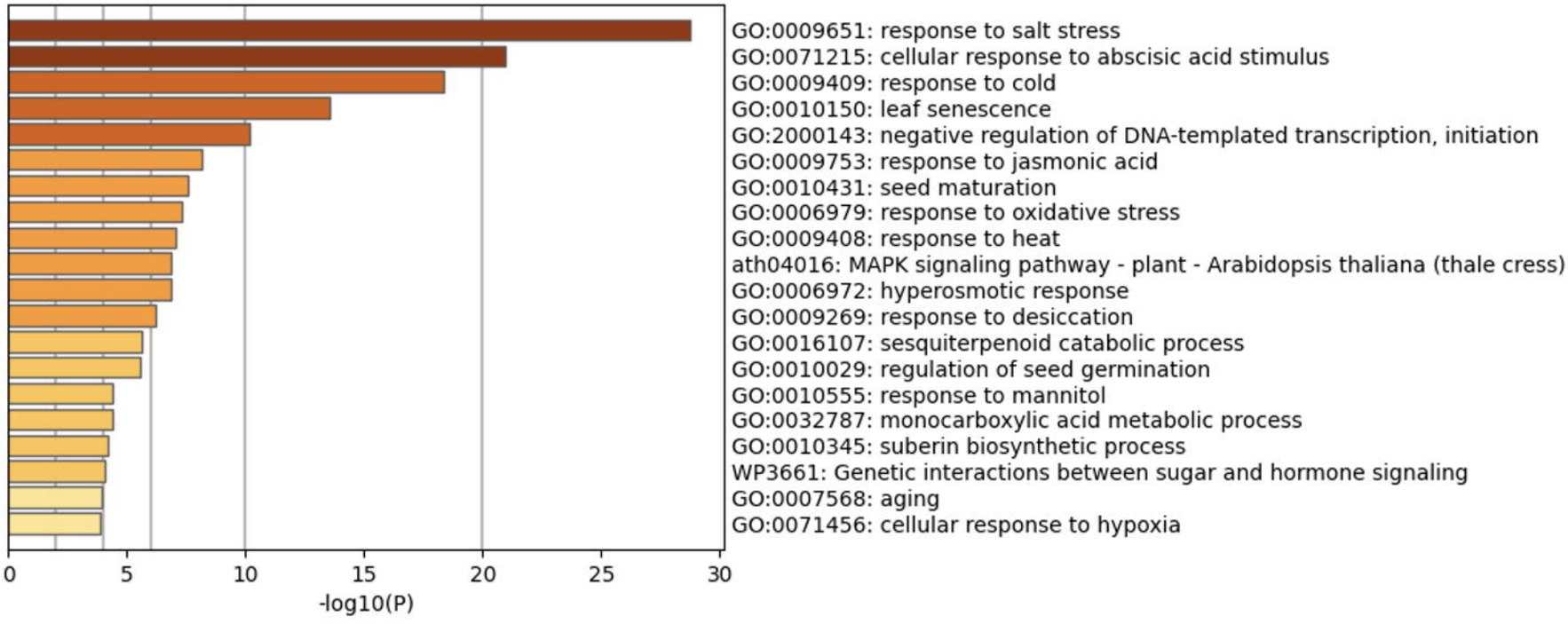
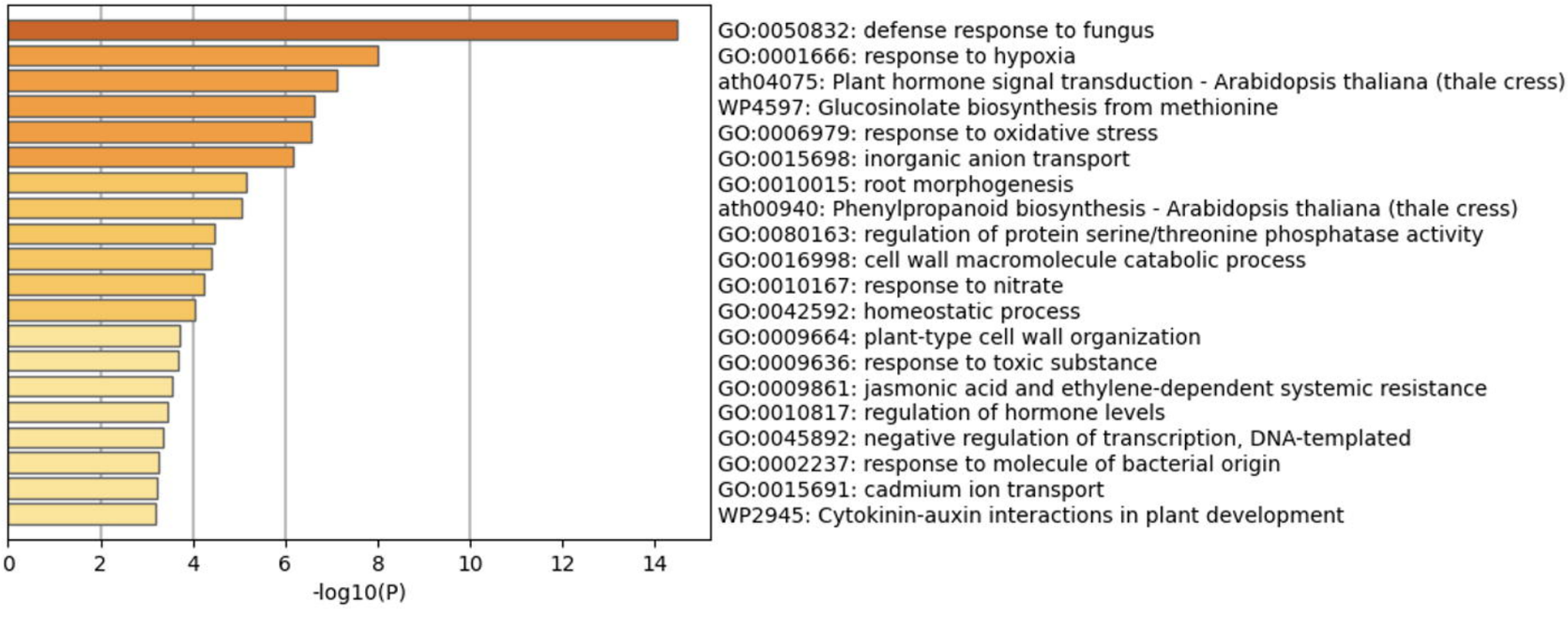


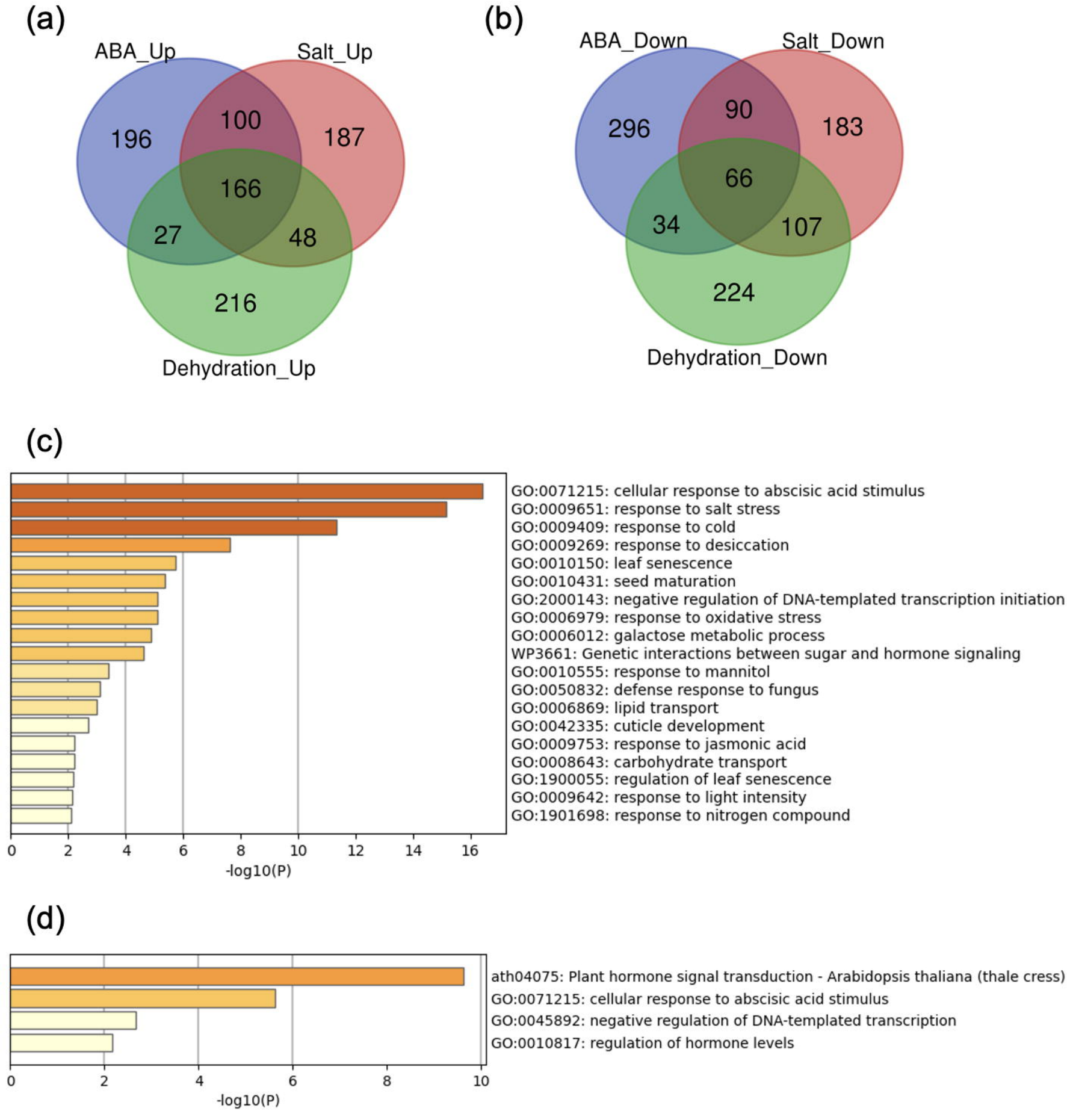
Sample tissues	Counts
seedlings	139
leaves	28
rosette leaves	23
roots	14
shoots	6
bud tissue	6

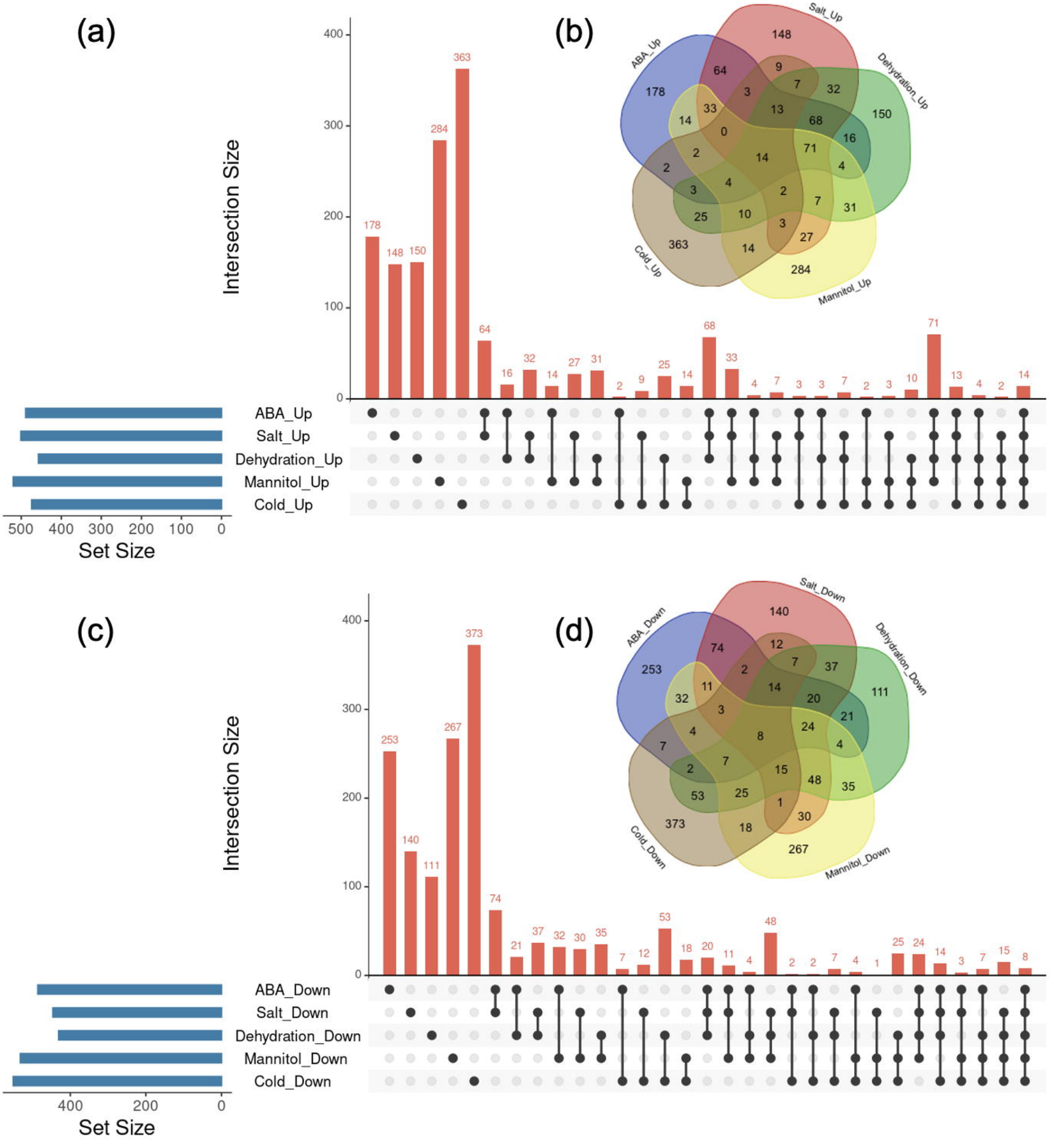
(a)


GO:0009651: response to salt stress
GO:0071215: cellular response to abscisic acid stimulus
GO:0009409: response to cold
GO:0010150: leaf senescence
GO:2000143: negative regulation of DNA-templated transcription, initiation
GO:0009753: response to jasmonic acid
GO:0010431: seed maturation
GO:0006979: response to oxidative stress
GO:0009408: response to heat
ath04016: MAPK signaling pathway - plant - Arabidopsis thaliana (thale cress)
GO:0006972: hyperosmotic response
GO:0009269: response to desiccation
GO:0016107: sesquiterpenoid catabolic process
GO:0010029: regulation of seed germination
GO:0010555: response to mannitol
GO:0032787: monocarboxylic acid metabolic process
GO:0010345: suberin biosynthetic process
WP3661: Genetic interactions between sugar and hormone signaling
GO:0007568: aging
GO:0071456: cellular response to hypoxia
0
5
10
15
20
25
30
-log10(P)

(b)


GO:0050832: defense response to fungus
GO:0001666: response to hypoxia
ath04075: Plant hormone signal transduction - Arabidopsis thaliana (thale cress)
WP4597: Glucosinolate biosynthesis from methionine
GO:0006979: response to oxidative stress
GO:0015698: inorganic anion transport
GO:0010015: root morphogenesis
ath00940: Phenylpropanoid biosynthesis - Arabidopsis thaliana (thale cress)
GO:0080163: regulation of protein serine/threonine phosphatase activity
GO:0016998: cell wall macromolecule catabolic process
GO:0010167: response to nitrate
GO:0042592: homeostatic process
GO:0009664: plant-type cell wall organization
GO:0009636: response to toxic substance
GO:0009861: jasmonic acid and ethylene-dependent systemic resistance
GO:0010817: regulation of hormone levels
GO:0045892: negative regulation of transcription, DNA-templated
GO:0002237: response to molecule of bacterial origin
GO:0015691: cadmium ion transport
WP2945: Cytokinin-auxin interactions in plant development
0
2
4
6
8
10
12
14
-log10(P)


(a)
ABA_Up
Salt_Up
Dehydration_Up
196
100
187
166
27
48
216
(b)
ABA_Down
Salt_Down
Dehydration_Down
296
90
183
66
34
107
224
(c)
GO:0071215: cellular response to abscisic acid stimulus
GO:0009651: response to salt stress
GO:0009409: response to cold
GO:0009269: response to desiccation
GO:0010150: leaf senescence
GO:0010431: seed maturation
GO:2000143: negative regulation of DNA-templated transcription initiation
GO:0006979: response to oxidative stress
GO:0006012: galactose metabolic process
WP3661: Genetic interactions between sugar and hormone signaling
GO:0010555: response to mannitol
GO:0050832: defense response to fungus
GO:0006869: lipid transport
GO:0042335: cuticle development
GO:0009753: response to jasmonic acid
GO:0008643: carbohydrate transport
GO:1900055: regulation of leaf senescence
GO:0009642: response to light intensity
GO:1901698: response to nitrogen compound
0
2
4
6
8
10
12
14
16
-log10(P)
(d)
ath04075: Plant hormone signal transduction - Arabidopsis thaliana (thale cress)
GO:0071215: cellular response to abscisic acid stimulus
GO:0045892: negative regulation of DNA-templated transcription
GO:0010817: regulation of hormone levels
0
2
4
6
8
10
-log10(P)


(a)
(b)
(c)
(d)
Intersection Size
Set Size
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Salt_Up
Dehydration_Up
Mannitol_Up
Cold_Up
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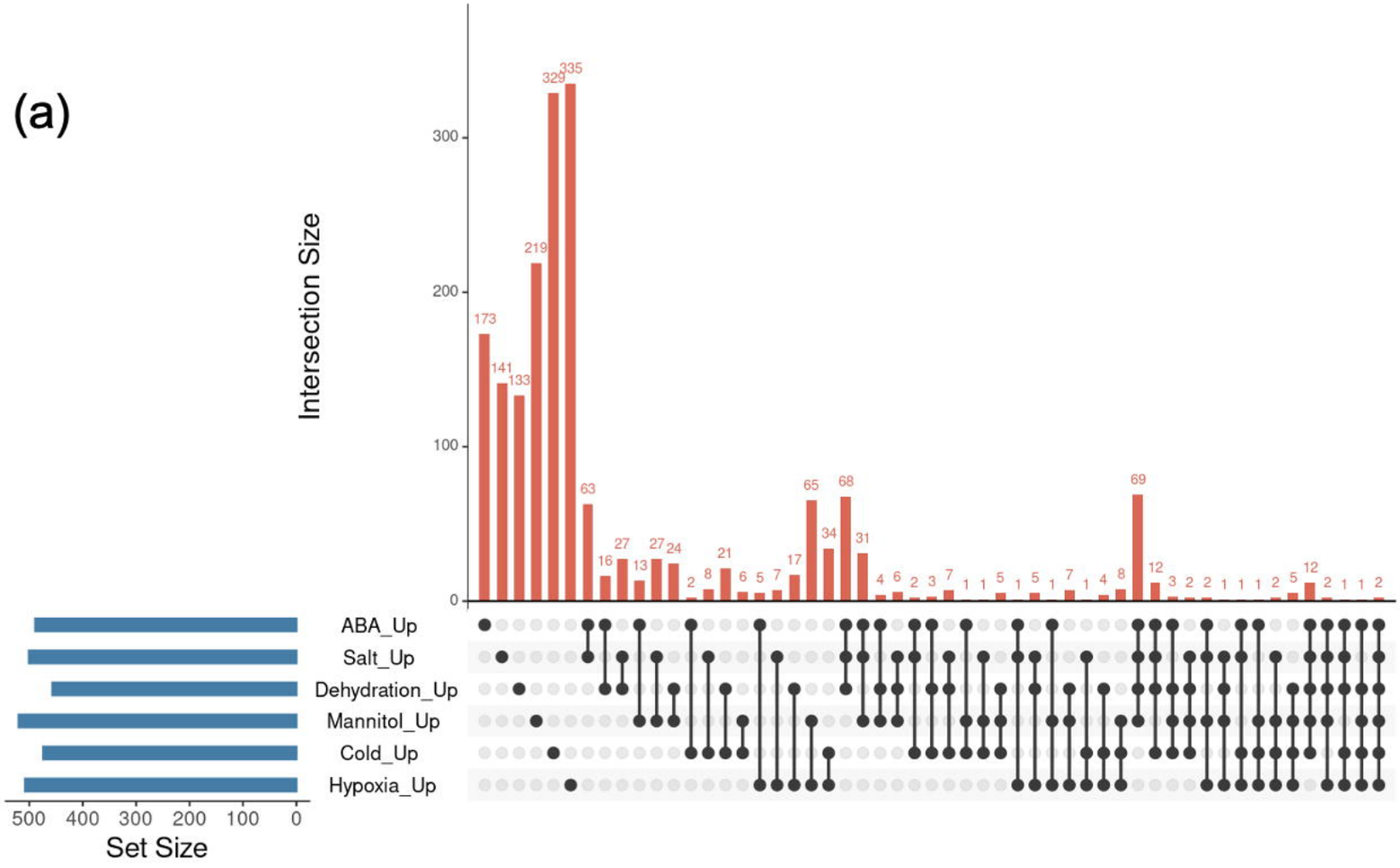
Dehydration_Down
Mannitol_Down
Cold_Down


(a)
Intersection Size
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Salt_Up
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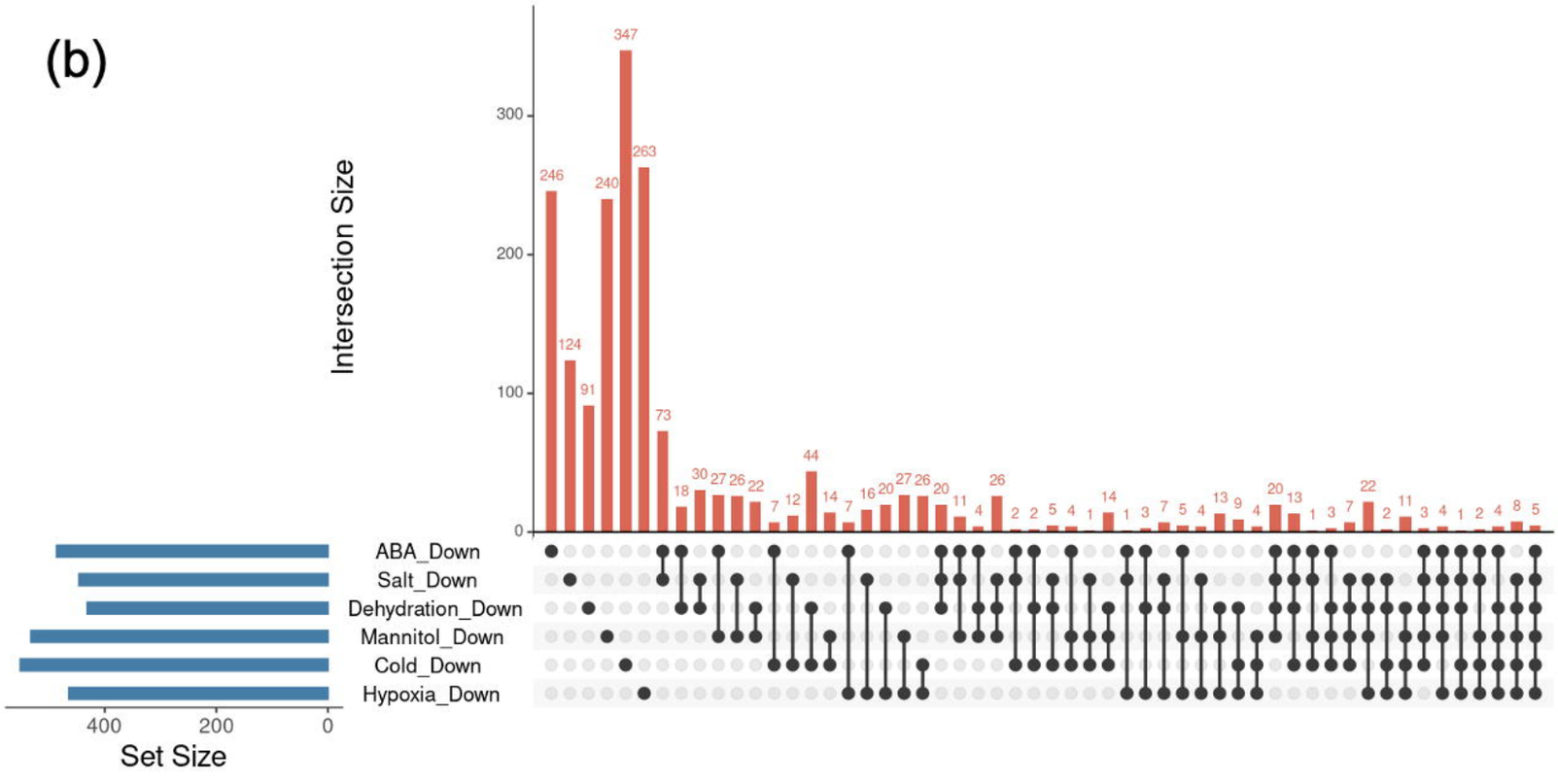
Cold_Up
Hypoxia_Up
Set Size


(b)
Intersection Size
ABA_Down
Salt_Down
Dehydration_Down
Mannitol_Down
Cold_Down
Hypoxia_Down
Set Size