Title

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

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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. 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*, 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. We 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. Our meta-analysis revealed a list of candidate genes with unknown molecular mechanisms in ABA-dependent and ABA-independent stress responses. These 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.

48 **Introduction**

49 Drought and salinity are the major abiotic stressors in plants. These stresses are becoming more
50 severe with climate change and negatively affect crop growth, leading to yield loss (Pereira, 2016;
51 Zhu, 2016; Mareri et al., 2022). Drought, salt stress, and secondary osmotic stress induced by them
52 reduce plant water availability, leading to dehydration stress (Dhankher and Foyer, 2018; Ma et al.,
53 2020). The plant hormone abscisic acid (ABA) increases significantly by these dehydration stresses
54 and induces physiological responses to adapt to the stresses, such as stomatal closure and regulation
55 of gene expression (Sah et al., 2016; Dar et al., 2017; Vishwakarma et al., 2017). Dehydration-
56 responsive genes can be divided into two categories: expression regulated by ABA or specifically
57 regulated by dehydration stress, but not by ABA (Shinozaki and Yamaguchi-Shinozaki, 2000;
58 Yoshida et al., 2014). Additionally, ABA is synthesized under cold stress conditions (Aslam et al.,
59 2022). The pathways that respond to cold stress and their associated gene expression share similarities
60 with those that respond to dehydration stress (Shinozaki and Yamaguchi-Shinozaki, 2000;
61 Yamaguchi-Shinozaki and Shinozaki, 2006). However, the expression of some genes is regulated by
62 stimuli specific to cold stress, such as a decrease in temperature (Shinozaki and Yamaguchi-
63 Shinozaki, 2000; Yamaguchi-Shinozaki and Shinozaki, 2006). Thus, plants respond to stress through
64 common or uncommon pathways (Shinozaki and Yamaguchi-Shinozaki, 2000; Yamaguchi-Shinozaki
65 and Shinozaki, 2006). Therefore, it is important to clarify the characteristics of stress-responsive
66 genes, such as their regulatory stress and whether they are ABA-responsive (ABA-regulated or ABA-
67 unregulated), to understand the complex regulatory mechanisms of stress responsiveness in plants.
68 The core of the ABA signaling pathway consists of three types of proteins: ABA receptors
69 (PYR/PYL/RCAR: pyrabactin resistance/PYR-like/regulatory component of ABA receptor), protein
70 phosphatases (PP2C: protein phosphatases type-2C), and protein kinases (SnRK2: SNF1-related
71 protein kinase 2) (Cutler et al., 2010; Zhang et al., 2015). These proteins regulate the activity of ABA
72 signaling. ABA binds to receptors (PYR/PYL/RCAR) that inhibit phosphatases (PP2C) and activate
73 kinases (SnRK2) that regulate various physiological responses through signaling cascades (Cutler et
74 al., 2010). ABA-activated SnRK2 phosphorylates transcription factors such as ABA-RESPONSIVE
75 EREMENT-BINDING PROTEIN (AREB)/ABRE-binding factors (ABFs) and ABA INSENSITIVE

76 5 (ABI5), thereby controlling the expression of downstream ABA-responsive genes (Furihata et al.,
77 2006; Sah et al., 2016). One of the most crucial SnRK activated by ABA is Open Stomata 1
78 (OST1)/SRK2E/SnRK2.6, which regulates ABA-mediated stomatal closure (Mustilli et al., 2002;
79 Yoshida et al., 2002; Lee et al., 2009). In addition, recent studies have revealed a crucial role for B2
80 and B3 Raf-like kinases (RAFs) as upstream regulators in the core ABA signaling pathway (Lin et al.,
81 2020; Takahashi et al., 2020). These RAFs are required for ABA signaling for the activation of
82 SnRK2 proteins, particularly OST1/SRK2E/SnRK2.6 (Takahashi et al., 2020; Lin et al., 2021).
83 Furthermore, in the downstream signaling of ABA, several genes play pivotal roles. For instance,
84 *RESPONSIVE TO DESICCATION 22 (RD22)* is recognized as a representative ABA-responsive gene
85 (Abe et al., 1997). Moreover, genes such as the *low-temperature-responsive protein 78*
86 *(LTI78)/desiccation-responsive protein 29A (RD29A)* is induced by both ABA-dependent and ABA-
87 independent pathways under conditions of drought, salinity, and cold (Yamaguchi-Shinozaki and
88 Shinozaki, 1993, 1994). Also, genes located downstream of transcription factors like dehydration-
89 responsive element binding 1s (DREB1s/CBFs) and DREB2s are known to be induced through
90 pathways independent of ABA regulation, contributing to responses against cold, salt, and
91 dehydration stresses(Yamaguchi-Shinozaki and Shinozaki, 1994, 2006; Liu et al., 1998; Lata and
92 Prasad, 2011). Although several studies have examined the core elements of these signals, few have
93 explored other novel elements. The data-driven studies have the advantage of analyzing large and
94 independent datasets, which can lead to the identification of novel targets, distinct from the
95 extensively studied established factors and accelerate the development of stress-tolerant crops (Bono,
96 2021; Ono and Bono, 2021; Tamura and Bono, 2022).
97 This study aimed to identify novel stress-responsive genes in plants by performing a meta-analysis of
98 publicly available RNA sequencing (RNA-Seq) data from five ABA-related stress conditions (ABA,
99 Salt, Dehydration, Osmotic, and Cold) in *Arabidopsis thaliana* with a focus on ABA, a factor
100 common to many stresses. The meta-analysis revealed that 14 genes were commonly upregulated and
101 8 genes were commonly downregulated across all five treatments, including some that were not
102 previously associated with these stress responses. However, some genes regulated by salt,

103 dehydration, and osmotic treatments were not regulated by exogenous ABA or cold stress, suggesting
104 that they may be involved in the plant response to dehydration independent of ABA.
105
106 **Materials and Methods**
107 **Curation of public gene expression data**
108 RNA-Seq data relevant to stress conditions involving abscisic acid were collected from the public
109 database, National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO)
110 (Barrett et al., 2013). A comprehensive search in GEO using the search query: ("ABA"[All Fields]
111 OR "abscisic acid"[All Fields] OR "salt"[All Fields] OR "NaCl"[All Fields] OR "salinity"[All Fields]
112 OR "dehydration"[All Fields] OR "osmotic"[All Fields] OR "mannitol"[All Fields] OR "drought"[All
113 Fields] OR "cold"[All Fields] OR "low temperature"[All Fields]) AND "*Arabidopsis thaliana*"[porgn]
114 AND "Expression profiling by high throughput sequencing"[Filter] AND ("0001/01/01"[PDAT] :
115 "2022/08/03"[PDAT]). As a result of manual curation, the collected data were divided into five
116 treatments: ABA, Salt, Dehydration, Mannitol, and Cold. The numbers of pairs of treated and control
117 samples were 90, 53, 27, 12, and 34, respectively. In total, 216 paired datasets collected in this
118 manner were used for the meta-analysis. In this study, a "paired dataset" refers to a set of two
119 samples: one subjected to a specific stress treatment (such as abscisic acid, salt, dehydration,
120 mannitol, or low temperature) and a corresponding control sample not subjected to the stress
121 treatment. These paired datasets provide the basis for comparing gene expression changes induced by
122 these stress conditions. The RNA-Seq data used in this meta-analysis are summarized in the table
123 available online (Supplementary Table 1; https://doi.org/10.6084/m9.figshare.22566583.v6).
124
125 **Gene expression quantification**
126 We used the SRA Toolkit (v3.0.0) (https://github.com/ncbi/sra-tools (Accessed Sep 12, 2023)) to
127 retrieve FASTQ-formatted files for each RNA-Seq run accession number, with prefetch and fastq-
128 dump commands, and then concatenated the files for the same experiments. Quality control of the raw
129 reads was performed using Trim Galore (v0.6.7) (https://github.com/FelixKrueger/TrimGalore
130 (Accessed Sep 12, 2023)) with Cutadapt (v4.1) (Martin, 2011). Transcripts were quantified using

131 Salmon (v1.8.0) (https://github.com/COMBINE-lab/salmon (Accessed Sep 12, 2023)) (Patro et al.,
132 2017) with a reference cDNA sequence downloaded from Ensembl Plants (release 53)
133 (Arabidopsis_thaliana.TAIR10.cdna.all.fa.gz). As a result, the quantitative RNA-Seq data were
134 calculated as transcripts per million (TPM). The transcript-level TPM were summarized to the gene
135 level using tximport (v1.26.1) (https://bioconductor.org/packages/release/bioc/html/tximport.html
136 (Accessed Sep 12, 2023)).
137 These operations were performed according to the workflow in SAQE
138 (https://github.com/bonohu/SAQE (Accessed Sep 12, 2023)) (Bono, 2021). The TPM data are
139 available online (Supplementary Table 2; https://doi.org/10.6084/m9.figshare.22566583.v6).
140
141 **Calculation score**
142 Gene expression data from different experiments were normalized by calculating the TN-ratio, which
143 represents the ratio of gene expression between stress-treated (T) and non-treated (N) samples.
144 The TN-ratio was calculated using the following equation:
145
146 TN-ratio = (stress-treated TPM +1) / (non-treated TPM +1)
147
148 If the TN-ratio was higher than the threshold, the gene was considered upregulated; if it was less than
149 the reciprocal of the threshold, it was deemed downregulated; otherwise, the gene was unchanged. To
150 classify the upregulated and downregulated genes, we evaluated 2-fold, 5-fold, and 10-fold thresholds
151 and finally chose the 2-fold threshold; therefore, genes with a TN-ratio higher than 2 were classified
152 as upregulated, whereas genes with a TN-ratio lower than 0.5 were classified as downregulated.
153 The TN-score of each gene was determined by subtracting the number of downregulated experiments
154 from the number of upregulated experiments to assess the changes in gene expression under stress
155 conditions across experiments.
156

157 TN-ratio and TN-score were calculated using a set of scripts at
158 (https://github.com/no85j/hypoxia_code (Accessed Sep 12, 2023)) (Ono and Bono, 2021) and the data
159 are available online (Supplementary Table 3, 4; https://doi.org/10.6084/m9.figshare.22566583.v6).
160
161 **DESeq2 analysis**
162 To support the results with our TN-score method, we compared them with those generated using the
163 DESeq2 package (v 1.40.2) (https://bioconductor.org/packages/release/bioc/html/DESeq2.html), a
164 widely used tool for identifying differentially expressed genes from RNA-seq data. We set a threshold
165 for significant expression changes at a 2-fold upregulated or downregulated (log2FoldChange ≥ |1|),
166 applying multiple false discovery rate (FDR) thresholds (padj) of less than 0.05, 0.01, 0.005, and
167 0.001 to identify statistically significant changes. The analysis was conducted with ABA-treated and
168 untreated samples using read count data. In subsequent analysis, we used the FDR thresholds of less
169 than 0.05 and 0.001. The read count data and DESeq2 results are available online (Supplementary
170 Table 5, 6; https://doi.org/10.6084/m9.figshare.22566583.v6).
171
172 **Gene set enrichment analysis**
173 The web tool Metascape (https://metascape.org/ (Accessed Sep 12, 2023)) was used for gene set
174 enrichment analysis to analyze the differentially expressed gene sets. The analysis was based on a
175 queried gene list and the corresponding terms and p-values were examined.
176
177 **Visualization**
178 We used a web-based Venn diagram tool (https://bioinformatics.psb.ugent.be/webtools/Venn/
179 (Accessed Sep 12, 2023)) and the UpSet tool (https://asntech.shinyapps.io/intervene/ (Accessed Sep
180 12, 2023)) to search for and visualize overlapping genes.
181

182 **Results**

183 **Data curation of RNA-Seq from public database**

184 RNA-Seq data on abscisic acid (ABA) and stress conditions were collected from the public database
185 NCBI Gene Expression Omnibus (GEO). A comprehensive keyword search yielded a list of
186 experimental data series. In contrast to microarray data from different platforms, RNA-Seq data,
187 mostly from Illumina, are suitable for comparative analyses among studies by different research
188 groups. Therefore, only the RNA-Seq data were used in this study. Moreover, *Arabidopsis* data were
189 selected for the analysis because of the abundance of data in the database compared with other plants.
190 No mutant or transgenic lines were selected and wild type samples were used. Abscisic acid, salt
191 (NaCl), dehydration, osmotic (mannitol), and low temperature (4 °C or 10 °C) were used as stress
192 treatment conditions. The data were curated as 216 paired datasets of stress-treated and control
193 samples. A total of 216 pairs were used in the meta-analysis, and the tissue breakdown was as
194 follows: 139 seedlings, 28 leaves, 23 rosette leaves, 14 roots, 6 shoots, and 6 bud tissues (Figure 1).
195 Metadata for the curated datasets, including Sequence Read Archive (SRA) study ID, run ID, sample
196 tissue, treatment type, treatment time, and sequence library type, are available on Supplementary
197 Table 1.

198

199 **Identification of differentially expressed genes**

200 Differentially expressed genes using RNA-Seq data in *Arabidopsis* under each stress condition were
201 meta-analyzed by calculating the TN (stress-treated (T) and non-treated (N))-ratio and the TN-score
202 of each gene. After considering various thresholds, we selected a 2-fold threshold (TN2). This
203 threshold was slightly lower to provide a comprehensive analysis. More severe scores for the 5-fold
204 (TN5) and 10-fold (TN10) thresholds were also calculated and are listed in the table. The lists of
205 upregulated and downregulated genes are available online with the results of multiple thresholds
206 considered (Supplementary Table 7, 8; https://doi.org/10.6084/m9.figshare.22566583.v6). The
207 differences between the numbers of upregulated and downregulated experiments were calculated as
208 the TN-scores for each gene. Thus, a higher score indicated a trend toward increased expression over
209 the entire experiment, whereas a lower (negative) score indicated a trend toward decreased expression

210 over the entire experiment. In the meta-analysis, we defined upregulated and downregulated as
211 approximately top 500 genes with the highest and lowest TN2 scores, respectively. The ranges of TN2
212 scores for the upregulated and downregulated genes are summarized in Table 1.
213
214 Similarly, for datasets comprising pairs of ABA-treated and untreated samples, we conducted
215 analyses using DESeq2 with read count data. In the DESeq2 analysis, we considered four FDR
216 (adjusted p-value, padj) thresholds: 0.05, 0.01, 0.005, and 0.001. We present the overlap between the
217 lists of genes identified as upregulated or downregulated at the highest (FDR < 0.05) and lowest (FDR
218 < 0.001) thresholds, and the list of genes obtained using the TN2 score, in UpSet plots
219 (Supplementary Figure 1). Among the 489 genes selected based on the TN2 score, 468 were
220 overlapped with the genes suggested to be significantly upregulated by DESeq2 analysis at FDR <
221 0.05, and 464 at FDR < 0.001. For the downregulated genes, out of the 486 genes selected by the TN2
222 score, 320 overlapped with those suggested to be significantly downregulated by DESeq2 at FDR <
223 0.05, and 293 at FDR < 0.001. The results of the DESeq2 analysis and the UpSet plots are accessible
224 online (Supplementary Table 6 and Supplementary Figure 1;
225 https://doi.org/10.6084/m9.figshare.22566583.v6).
226
227 **Enrichment analysis of differently expression genes**
228 We performed enrichment analysis using Metascape for five different treatment types of gene sets to
229 characterize the differentially expressed genes (Supplementary Figure 2;
230 https://doi.org/10.6084/m9.figshare.22566583.v6). The two most significantly enriched terms in each
231 gene set from the enrichment analysis are summarized in table (Table 2). The most significantly
232 enriched terms in the ABA, salt, and dehydration upregulated gene sets were all consistent with
233 "GO:0009651 response to salt stress". Focusing on the downregulated genes, the light-responsive
234 genes annotated as "GO:0009642 response to light intensity" were commonly enriched in dehydration
235 and cold treatment. The auxin-responsive genes with "GO:0009733 response to auxin" were
236 commonly enriched in dehydration and mannitol treatment. The lists of all genes and the results of

237 enrichment analysis are available online (Supplementary Table 7, 8;
238 https://doi.org/10.6084/m9.figshare.22566583.v6).
239
240 In addition, enrichment analysis was performed on all genes whose expression was suggested to be
241 upregulated by DESeq2 analysis (FDR < 0.05; 1144 genes, Supplementary Figure 3A; FDR < 0.001;
242 1018 genes, Supplementary Figure 3B) and those genes from which genes selected based on TN2
243 score were excluded (FDR < 0.05; 676 genes, Supplementary Figure 3C; FDR < 0.001; 554 genes,
244 Supplementary Figure 3D). In the enrichment analysis for all genes selected by DESeq2, the top two
245 terms, even with either threshold, were "GO:0009651 response to salt stress" and "GO:0071215
246 cellular response to abscisic acid stimulus" (Supplementary Figure 3A, 3B). This is consistent with
247 the results of the enrichment analysis for the genes selected based on the TN2 score. We also
248 performed enrichment analysis on genes exclusively selected by DESeq2 analysis, excluding those
249 selected based on the TN2 score (Supplementary Figure 3C, 3D). Despite the number of genes being
250 nearly the same as those selected by the TN2 score, the results did not include "GO:0071215 cellular
251 response to abscisic acid stimulus," and the enrichment of significant terms was not as pronounced as
252 observed in the enrichment analysis of genes selected based on the TN2 score. The lists of all genes
253 and the results of enrichment analysis are available online (Supplementary Table 9 and
254 Supplementary Figure 3; https://doi.org/10.6084/m9.figshare.22566583.v6).
255
256 **Exploring commonly regulated genes by three treatments: ABA, salt, dehydration**
257 A previous study demonstrated that some genes are involved in the regulation and response to
258 multiple stresses and that these genes have a potential role in universal stress tolerance in plants
259 (Husaini, 2022). We investigated whether such candidate genes were differentially expressed under
260 three stress conditions: ABA, salt, and dehydration. While many genes were commonly regulated
261 under the three conditions, we identified 166 upregulated (ABA_Up Salt_Up Dehydration_Up) and
262 66 downregulated genes (ABA_Down Salt_Down Dehydration_Down) under the three different
263 conditions (Figure 2). The meta-analysis also showed that the expression of *RELATED TO ABI3/VP1*
264 *1* (*RAV1*), *RAV2*, *PYR1*, *PYL4*, *PYL5*, *PYL6*, and *PCAR3* (*PYL8*) was downregulated by ABA, salt,

265 and dehydration stress, which is consistent with previous studies (Chan, 2012; Feng et al., 2014; Fu et
266 al., 2014).
267 Furthermore, we performed enrichment analysis of these commonly regulated genes to evaluate gene
268 characteristics. The results showed that the top two significant enrichment terms for the 166
269 commonly upregulated genes (ABA_Up Salt_Up Dehydration_Up) were "GO:0071215 cellular
270 response to abscisic acid stimulus" and "GO:0009651 response to salt stress". In addition, the top two
271 significant enrichment terms for the 66 commonly downregulated genes (ABA_Down Salt_Down
272 Dehydration_Down) were "ath04075 Plant hormone signal transduction - *Arabidopsis thaliana* (thale
273 cress)" and "GO:0071215 cellular response to abscisic acid stimulus". The gene sets common to the
274 three treatments were significantly enriched in terms of ABA and salt stress, indicating that these
275 genes are likely involved in ABA and salt stress. The lists of all genes overlapping in the three
276 treatments, and the results of the enrichment analysis are available online (Supplementary Table 10,
277 11; https://doi.org/10.6084/m9.figshare.22566583.v6).
278
279 **Exploring commonly regulated genes by five treatments: ABA, salt, dehydration, osmotic, cold**
280 In the previous section, we identified commonly regulated genes across ABA, salt, and dehydration
281 treatments. We then added mannitol and cold treatments to analyze gene overlap. The overlap of
282 genes commonly regulated by the five treatments (ABA, salt, dehydration, osmotic stress, and cold
283 stress) was visualized using UpSet plots and Venn diagrams (Figure 3). As a result, 14 commonly
284 upregulated genes (ABA_Up Salt_Up Dehydration_Up Mannitol_Up Cold_Up) and 8 commonly
285 downregulated genes (ABA_Down Salt_Down Dehydration_Down Mannitol_Down Cold_Down)
286 were identified. The genes included in these two groups are listed in Tables 3 and 4 respectively. The
287 genes commonly upregulated in the five treatments contained *LTI78/RD29A*, *LTI30*, *Stress-responsive*
288 *protein/Stress-induced protein 1* (*KIN1)*, *COLD-REGULATED 47* (*COR47)*, and *Alcohol*
289 *Dehydrogenase 1* (*ADH1)*, which are reported to be induced by ABA, salt, dehydration, and cold
290 stress conditions (Kurkela and Franck, 1990; Jarillo et al., 1993; Yamaguchi-Shinozaki and
291 Shinozaki, 1994; Ishitani et al., 1998; Shi et al., 2015). On the other hand, genes such as *Arabidopsis*
292 *thaliana heavy metal associated domain containing gene 1* (*ATHMAD1)*, which have not been

293 reported to have ABA-related or stress response of functions, were also identified. These genes have
294 been identified as novel stress-responsive genes involved in the ABA response. Also, the ABA-
295 independent pathway, which is specifically induced by osmotic stress has been reported in plants
296 (Nakashima et al., 2014; Yoshida et al., 2014). This pathway is hypothesized to be activated
297 independently of ABA and its closely related cold stimulus (Yamaguchi-Shinozaki and Shinozaki,
298 2006). Therefore, we examined three treatments, excluding ABA and cold, to explore genes involved
299 in the ABA-independent osmotic signaling pathway. As a result, 7 commonly upregulated genes
300 (Salt_Up Dehydration_Up Mannitol_Up) and 48 commonly downregulated genes (Salt_Down
301 Dehydration_Down Mannitol_Down), regulated by salt, dehydration, and mannitol, were also
302 identified. Notably, these genes were not included among those suggested to be regulated by ABA
303 and cold stress in the present study, and the effects of ABA and cold on the regulation of their
304 expression may not be significant. Therefore, they may be involved in the ABA-independent stress
305 response pathways. The genes included in these two groups are listed in Tables 5 and 6. The lists of
306 all genes that overlap in the five treatments are available online (Supplementary Table 12, 13;
307 https://doi.org/10.6084/m9.figshare.22566583.v6).
308

309 **Discussion**

310 In this study, we aimed to explore uncharacterized differentially expressed genes by meta-analyzing
311 public RNA-Seq data of *Arabidopsis thaliana* to gain new insights into the effects of abscisic acid
312 (ABA) and various stress conditions on gene expression. The data were collected from the public
313 database NCBI GEO, and the study focused on RNA-Seq data because of their suitability for
314 comparative analyses among different studies. We analyzed the effects of ABA, salt, dehydration,
315 osmotic, and low-temperature stress on gene expression. In total, 216 paired datasets of stress-treated
316 and control samples were used for the meta-analysis. The central to this research is the notion that by
317 employing a comprehensive integrated analysis using large-scale data from multiple research groups,
318 we can uncover patterns and insights that might be overlooked in individual studies. We employed the
319 TN-score to identify differentially expressed genes under each stress condition. We defined
320 upregulated and downregulated genes as the top 500 genes with the highest and lowest TN-scores,
321 respectively, in the meta-analysis. Enrichment analysis was performed using Metascape to
322 characterize the differentially expressed genes. The most significantly enriched terms in the
323 downregulated genes, the light-responsive genes annotated as "GO:0009642 response to light
324 intensity" were commonly enriched in dehydration and cold treatment, and the auxin-responsive genes
325 annotated as "GO:0009733 response to auxin" were commonly enriched in dehydration and mannitol
326 treatment. Abiotic stress negatively affects various aspects of plant photosynthesis including stomatal
327 conductance, oxidative stress, RUBISCO activity, photosystems (PS I and PS II), photosynthetic
328 electron transport, and chlorophyll biosynthesis (Sharma et al., 2020). These factors reduce the
329 photosynthetic efficiency and plant growth. Therefore, it is possible that dehydration and cold
330 treatments could have downregulated the expression of light-responsive genes “GO:0009642 response
331 to light intensity” due to adjustments in light sensitivity and photosynthetic responses during the
332 process of adapting to stress. In addition, osmotic stress reduces the biosynthesis of auxin, a plant
333 growth hormone, by directly or indirectly inhibiting the auxin pathway (Naser and Shani, 2016). This
334 might contribute to reduced plant growth under stress conditions. Auxin biosynthesis may be
335 suppressed by osmotic stress conditions, resulting in downregulated auxin-responsive genes
336 "GO:0009733 response to auxin". To support the selection results of the TN2 score, we performed a

337 comparison of genes suggested to be upregulated and downregulated by DESeq2 analysis in ABA-
338 treated samples and those selected based on the TN2 score. The number of genes detected as
339 unregulated by TN2 score alone was 489, and those detected as upregulated by both DESeq2 (FDR <
340 0.05) and TN2 scores was 468, with an overlap ratio of 95.7% (Supplementary Figure 1A).
341 Furthermore, a comparison of enrichment analysis results showed that the top two GO terms for all
342 genes detected by DESeq2 and genes selected by the TN2 score were similar (Supplementary Figure
343 3A, 3B). However, the enrichment analysis of genes detected only by DESeq2 did not show a
344 significant enrichment of key terms compared to those selected by the TN2 score (Supplementary
345 Figure 3C, 3D). These suggest that employing TN-scores for gene selection not only the genes
346 detected by commonly used tools like DESeq2 but also effectively refines the overall pool of selected
347 genes. This indicates that utilizing TN- scores is an effective method for meta-analysis. Next, we
348 identified genes that were commonly regulated by ABA, salt, and dehydration stress. We also
349 performed enrichment analysis of genes commonly regulated by different stresses and verified their
350 consistency with previously reported gene expression patterns. We expanded this analysis to include
351 genes commonly regulated by all five treatments (ABA, salt, dehydration, osmotic, and cold). Several
352 genes were upregulated or downregulated in response to these stress conditions, suggesting their
353 potential roles in global plant stress tolerance mechanisms.
354
355 Fourteen upregulated genes were found to be closely involved in ABA regulation (ABA_Up Salt_Up
356 Dehydration_Up Mannitol_Up Cold_Up, Table 3), including genes that have not been previously
357 studied, one of which is ATHMAD1 (AT1G51090). ATHMAD1 contains a heavy metal-associated
358 (HMA) domain that is upregulated in response to nitric oxide (NO) (Imran et al., 2016). NO plays a
359 variety of roles in plant adaptation to abiotic stresses such as drought, salt, and cold (Fancy et al.,
360 2017; Lau et al., 2021). NO synthesis is promoted by abscisic acid (ABA) treatment and abiotic stress,
361 which contributes to stomatal closure during drought stress (Neill et al., 2008). Analysis of *athmad1*
362 mutant indicated that ATHMAD1 may play a role in regulating plant growth and immunity (Imran et
363 al., 2016). However, the function of ATHMAD1 under abiotic stress has not yet been thoroughly
364 investigated. In this study, *ATHMAD1* expression was upregulated upon treatment with ABA, salt,

365 dehydration, osmotic, and cold stress. Based on these findings, *ATHMAD1* expression may increase in
366 response to NO, which is promoted by ABA treatment and abiotic stress. Therefore, analyzing the
367 function of ATHMAD1 in plants under abiotic stress conditions is an important challenge for future
368 research.
369
370 In addition, focusing on genes downregulated by multiple stressors, we examined eight genes
371 considered to be deeply involved in ABA-dependent expression regulation (ABA_Down Salt_Down
372 Dehydration_Down Mannitol_Down Cold_Down, Table 4) and 48 genes considered to be less
373 influenced by ABA-mediated expression regulation (Salt_Down Dehydration_Down
374 Mannitol_Down, Table 6). It was found that among the eight genes, four were *SAUR (Small Auxin Up*
375 *RNA)* genes (*SAUR15, 16, 62, 63*), and among the 48 genes, five *XTH (Xyloglucan*
376 *endotransglucosylase/hydrolase)* genes (*XTH4, 6, 8, 9, 16*) were included, respectively. Although
377 SAUR and XTH gene families have distinct mechanisms and molecular targets, they play a common
378 role in controlling plant growth and cell wall expansion. Furthermore, several studies have identified
379 these genes during stress stimulation and changes in stress response phenotypes in mutants lacking
380 their functions.
381
382 The *SAUR* genes are a plant-specific gene family that plays a crucial role in plant growth and
383 development by regulating cell wall acidification in response to auxin stimulation (Ren and Gray,
384 2015; Stortenbeker and Bemer, 2019). Several *SAUR* genes were downregulated in response to ABA,
385 drought, and osmotic stress. ARABIDOPSIS ZINC-FINGER 1 (AZF1) and AZF2 were induced by
386 osmotic stress and abscisic acid, function as transcriptional repressors of 15 SAUR genes, thereby
387 inhibiting plant growth under abiotic stress conditions (Kodaira et al., 2011). Among the genes
388 repressed by AZF, AZF directly binds to the promoter region of *SAUR63* (Kodaira et al., 2011).
389 SAUR63 localizes to the plasma membrane and by inhibiting plasma membrane (PM)-associated
390 PP2C.D phosphatases, it stimulates PM H^+-ATPase proton pump activity, thereby promoting cell
391 growth (Nagpal et al., 2022). However, the functions of most SAUR genes under abiotic stress and
392 their direct relationship with stress remain unclear.

393

394 XTH is an enzyme family involved in plant cell wall remodeling that catalyzes the cleavage and
395 polymerization of xyloglucan to regulate cell elasticity and extensibility (Rose et al., 2002). XTH not
396 only plays a role in regulating cell wall structure and morphology, but also plays a crucial role in plant
397 adaptation to external stress (Ishida and Yokoyama, 2022). The expression of *AtXTH19* and *AtXTH23*
398 was induced by brassinosteroids, and the *atxth19* mutant showed lower freezing tolerance than the
399 wild type, whereas the *atxth19/atxth23* double mutant showed increased sensitivity to salt stress (Xu
400 et al., 2020; Takahashi et al., 2021). Similarly, *AtXTH30* contributes to salt tolerance (Yan et al.,
401 2019). Additionally, the loss-of-function of several *XTH* genes has been reported to affect metal ion
402 tolerance. *AtXTH31* is associated with cell wall aluminum binding capacity, and mutation of this gene
403 leads to increased aluminum tolerance (Zhu et al., 2012). Similarly, *Atxth18* mutant showed reduced
404 sensitivity to lead stress (Zheng et al., 2021). *AtXTH4* and *AtXTH9* contribute to the regulation of
405 xylem cell expansion and secondary growth, including the production of secondary xylem and the
406 accumulation of secondary walls. However, the function of stress conditions has not yet been clarified
407 (Kushwah et al., 2020). These studies suggested that XTH plays an important role in various stress
408 responses. Therefore, understanding the functions of the five XTH genes (*XTH4, 6, 8, 9, 16*), the
409 genes that were downregulated by ABA-independent stress pathways in this study, is a future
410 challenge. As discussed above, the literature information on these mutant genes reinforced the
411 correctness of our analysis.

412

413 Finally, we investigated whether the hypoxia-responsive genes identified in a previous meta-analysis
414 in *Arabidopsis thaliana* were included in the genes we focused on in this study (Tamura and Bono,
415 2022). Under hypoxic conditions, root water transport is inhibited and stomatal closure is induced.
416 This process requires aquaporins, which are proteins involved in water transport, as well as the
417 hormones ABA and ethylene (Tan et al., 2018). Furthermore, during recovery from submergence,
418 reactive oxygen species (ROS), ABA, ethylene, respiratory burst oxidase homolog D (RBOHD),
419 senescence-associated gene113 (SAG113), and ORESARA1 (ORE1) regulate ROS homeostasis,
420 stomatal opening/closing, and chlorophyll degradation (Yeung et al., 2018). These studies suggest that

421 hypoxia and ABA levels are related to each other. The analysis demonstrated that the expression of
422 several stress-regulated genes, which were the focus of this study, was regulated by hypoxia (Tables
423 3–6). These results imply that there might be an undiscovered molecular cross talk between "hypoxia"
424 and "ABA or dehydration," which has not been frequently discussed in previous research. The
425 overlap of genes commonly regulated by the six treatments (ABA, salt, dehydration, osmotic, cold,
426 and hypoxia) was visualized using UpSet plots (Figure 4). These genes are regulated by a wide range
427 of stresses and identifying their roles could contribute to further advances in our understanding of the
428 broad mechanisms of stress tolerance in plants. The lists of all genes overlapping in the six treatments
429 are available online (Supplementary Table 14, 15; https://doi.org/10.6084/m9.figshare.22566583.v6).
430
431 This study has several limitations that need to be considered. First, the identification of differentially
432 expressed genes was not subjected to statistical testing, demanding caution in the interpretation of
433 results. Second, the RNA-Seq data used in this study were collected under various growth and
434 experimental conditions, careful consideration of these variabilities. Third, experimental validation
435 using plant samples exposed to specific stress environments has not been performed, necessitating
436 further research for functional clarification of these genes.
437
438 On the other hand, the strength of this study is the integration of data from multiple research projects
439 and various stress conditions. Representative ABA-responsive or stress-responsive genes were
440 extracted using the TN-score method. Furthermore, the candidate genes with unknown function listed
441 in this study have the potential to provide new insights into the stress response mechanisms in plants.
442 These findings are expected to contribute to the broadening of the future plant stress research.
443
444 In conclusion, this meta-analysis lists candidate genes that are responsive to ABA-dependent or ABA-
445 independent stress conditions, potentially playing roles in plant responses to these stresses. In this
446 study, we focused on the gene expression commonly found under different stresses and validated our
447 analysis by confirming its consistency with previously reported patterns of gene expression. As the
448 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. We think that our research forms the foundation for revealing patterns and insights that might have been overlooked in individual studies.

Conflict of Interest

The authors declare that they have no competing interests.

Author 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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Supplementary Material

Additional data presented in this study are publicly available online (https://doi.org/10.6084/m9.figshare.22566583.v6).

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634

635 **Tables**

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

637

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

638

639

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

641

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

642

643

644 **Table 3.** The lists of commonly upregulated genes in ABA, Salt, Dehydration, Mannitol, and Cold
645 treatments
646

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

647

648

649 **Table 4.** The lists of commonly downregulated genes in ABA, Salt, Dehydration, Mannitol, and Cold
650 treatments
651

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

652

653

654

655 **Table 5.** The lists of commonly upregulated genes in Salt, Dehydration, and Mannitol treatments
656

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

657

658

659 **Table 6.** The lists of commonly downregulated genes in Salt, Dehydration, and Mannitol treatments
660

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

663 **Captions**

664 **Figure 1. Distribution of tissue types in curated RNA-Seq data**

665 Visualization of 216 paired datasets showing the distribution of tissues such as seedlings, leaves,

666 rosette leaves, roots, shoots, and bud tissues.

667

668 **Figure 2. Analysis of the overlaps of upregulated or downregulated genes in 3 different types of**
669 **treatments**
670 Venn diagrams and gene set enrichment analysis in (A-D) represent the upregulated or downregulated

671 genes in ABA, Salt, and Dehydration treatments.

672 (A): Venn diagram of the upregulated genes, (B): Venn diagram of the downregulated genes, (C):

673 Gene set enrichment analysis of the commonly upregulated genes, (D): Gene set enrichment analysis

674 of the commonly downregulated genes.

675

676 **Figure 3. Analysis of the overlaps of upregulated or downregulated genes in 5 different types of**
677 **treatments**
678 UpSet plots and venn diagrams in (A-D) represent the upregulated or downregulated genes in ABA,

679 Salt, Dehydration, Mannitol, and Cold treatments.

680 (A): UpSet plots of the upregulated genes, (B): Venn diagram of the upregulated genes, (C): UpSet

681 plots of the downregulated genes, (D): Venn diagram of the downregulated genes.

682

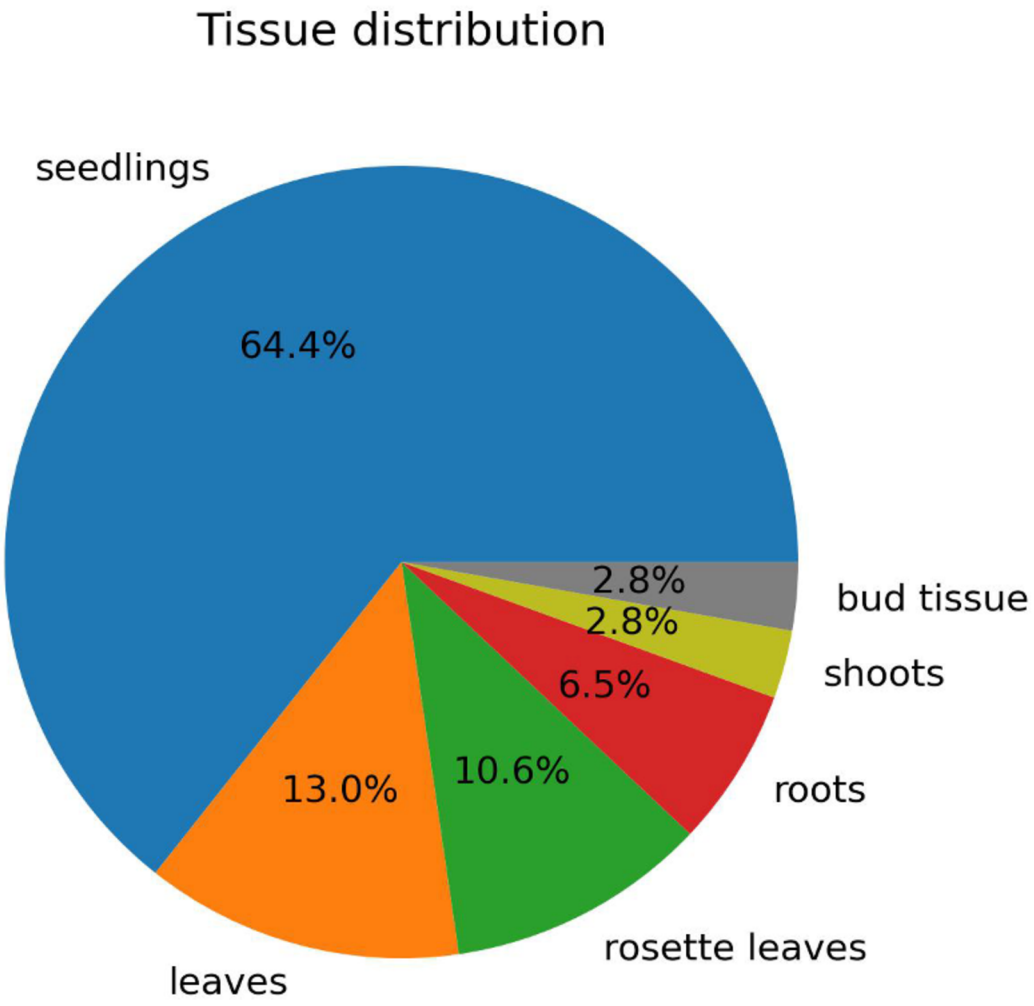
683 **Figure 4. Analysis of the overlaps of upregulated or downregulated genes in 6 different types of**

684 **treatments**

685 UpSet plots in (A, B) represent the upregulated or downregulated genes in ABA, Salt, Dehydration,

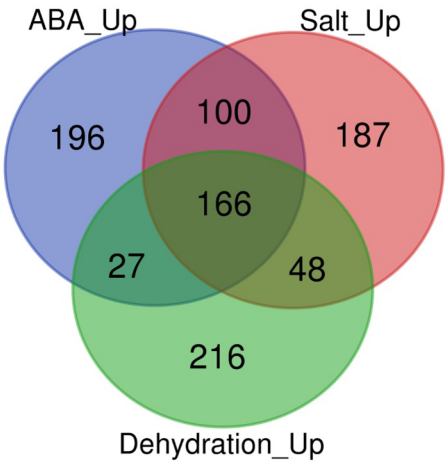
686 Mannitol, Cold, and Hypoxia treatments.

687 (A): UpSet plots of the upregulated genes, (B): UpSet plots of the downregulated genes.

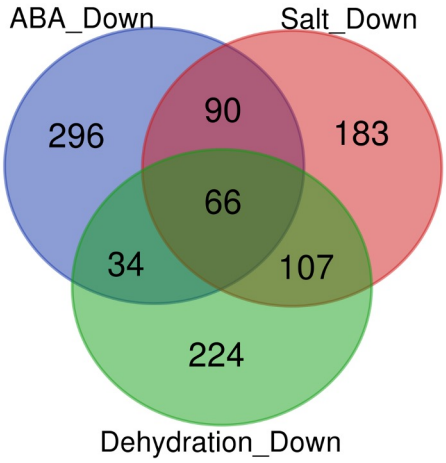


Sample tissues	Counts
seedlings	139
leaves	28
rosette leaves	23
roots	14
shoots	6
bud tissue	6

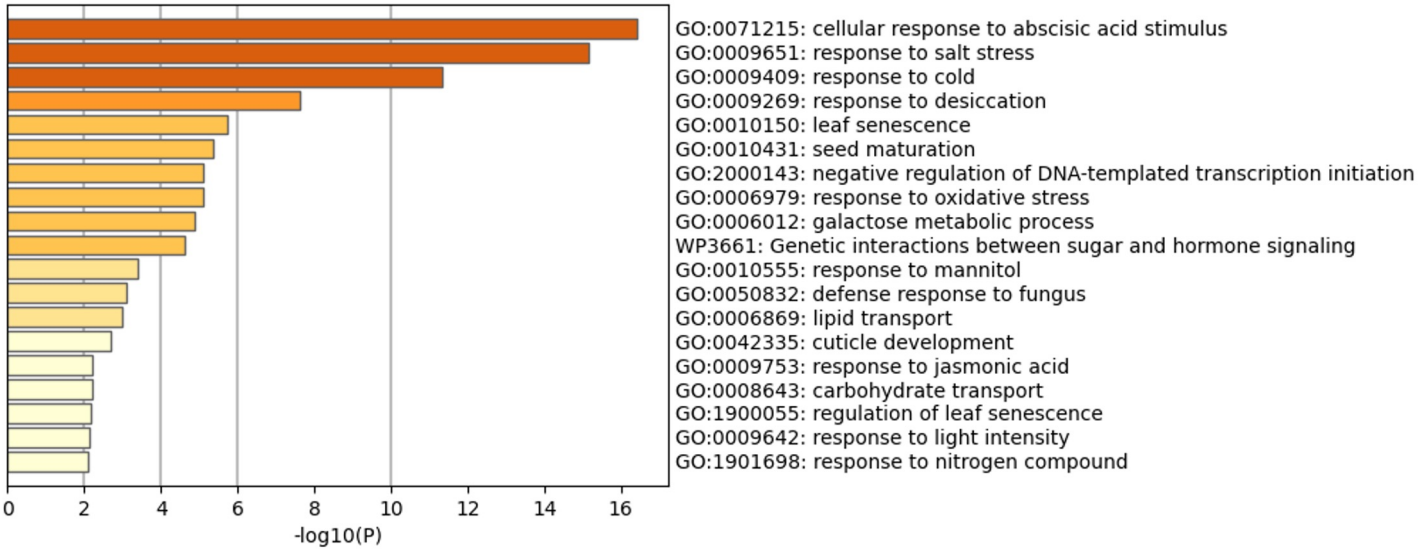
A


ABA_Up
Salt_Up
Dehydration_Up
196
100
187
166
27
48
216

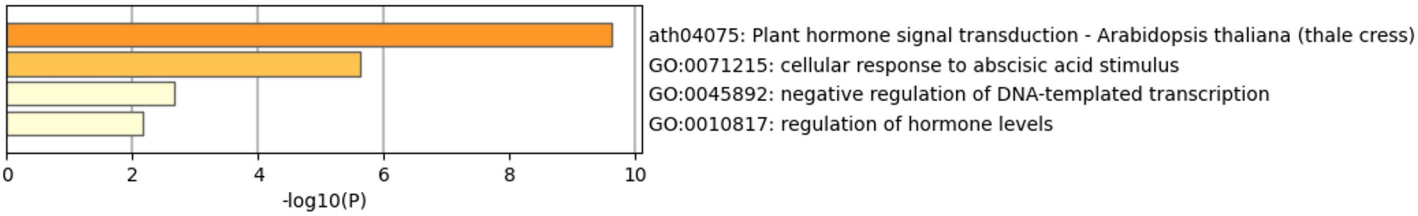
B

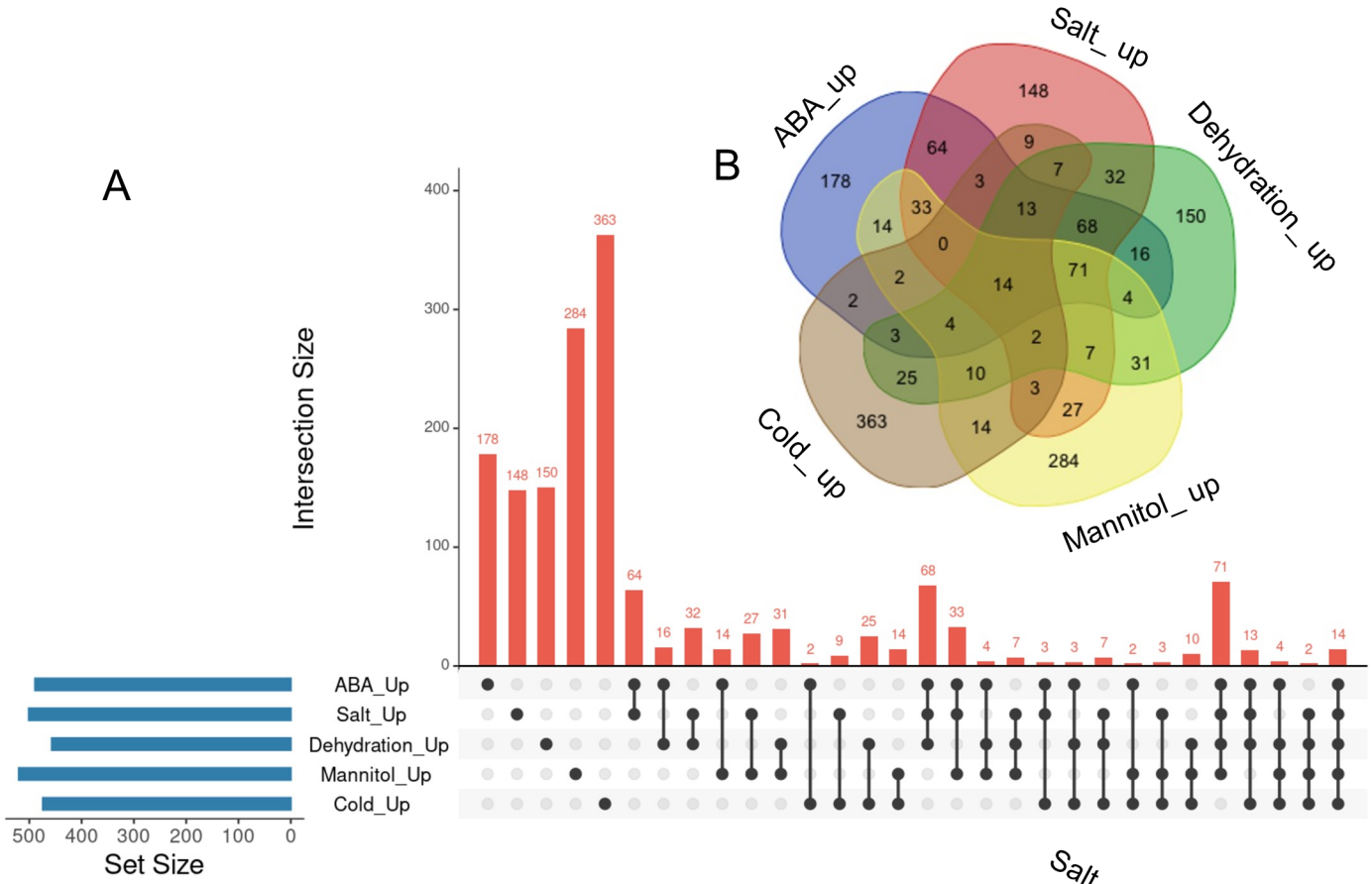

ABA_Down
Salt_Down
Dehydration_Down
296
90
183
66
34
107
224

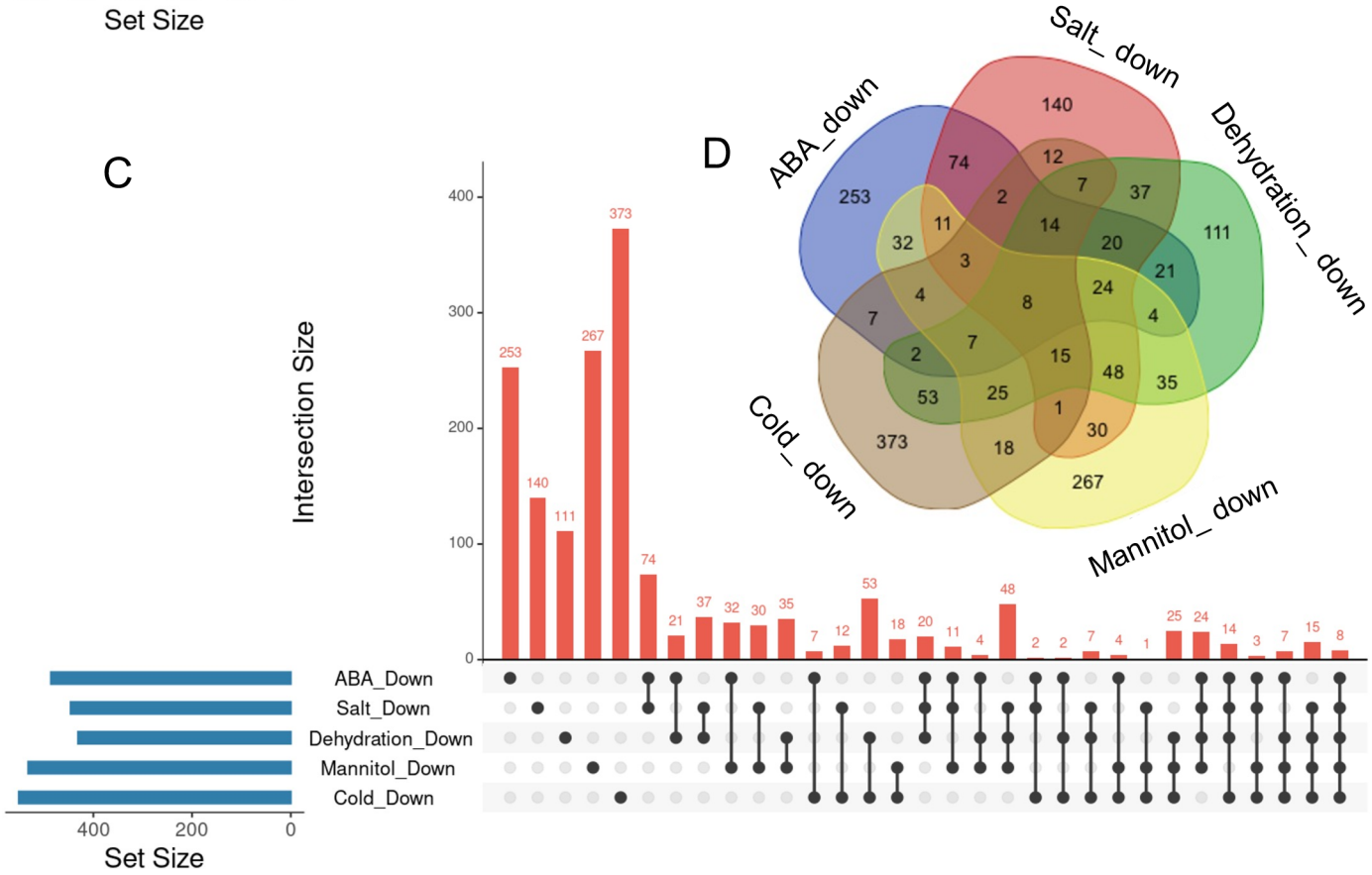
C

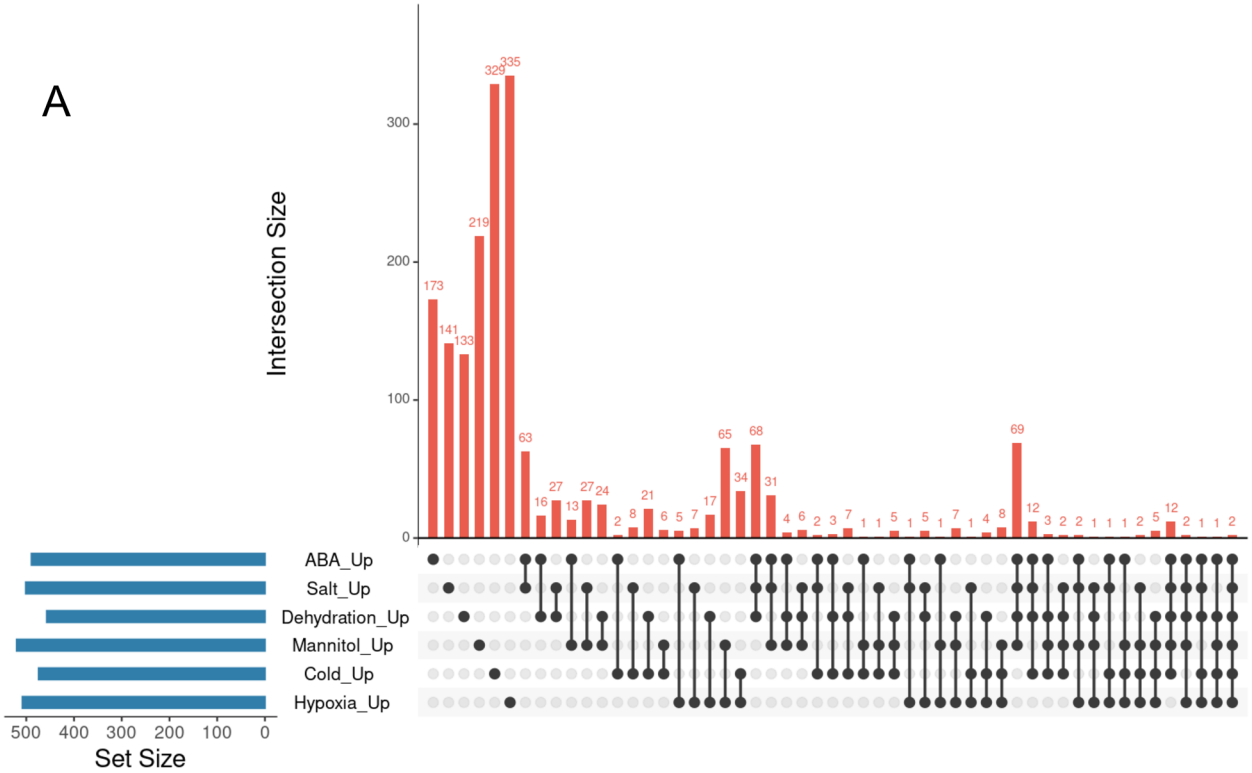

GO:0071215: cellular response to abscisic acid stimulus
GO:0009651: response to salt stress
GO:0009409: response to cold
GO:0009269: response to desiccation
GO:0010150: leaf senescence
GO:0010431: seed maturation
GO:2000143: negative regulation of DNA-templated transcription initiation
GO:0006979: response 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)

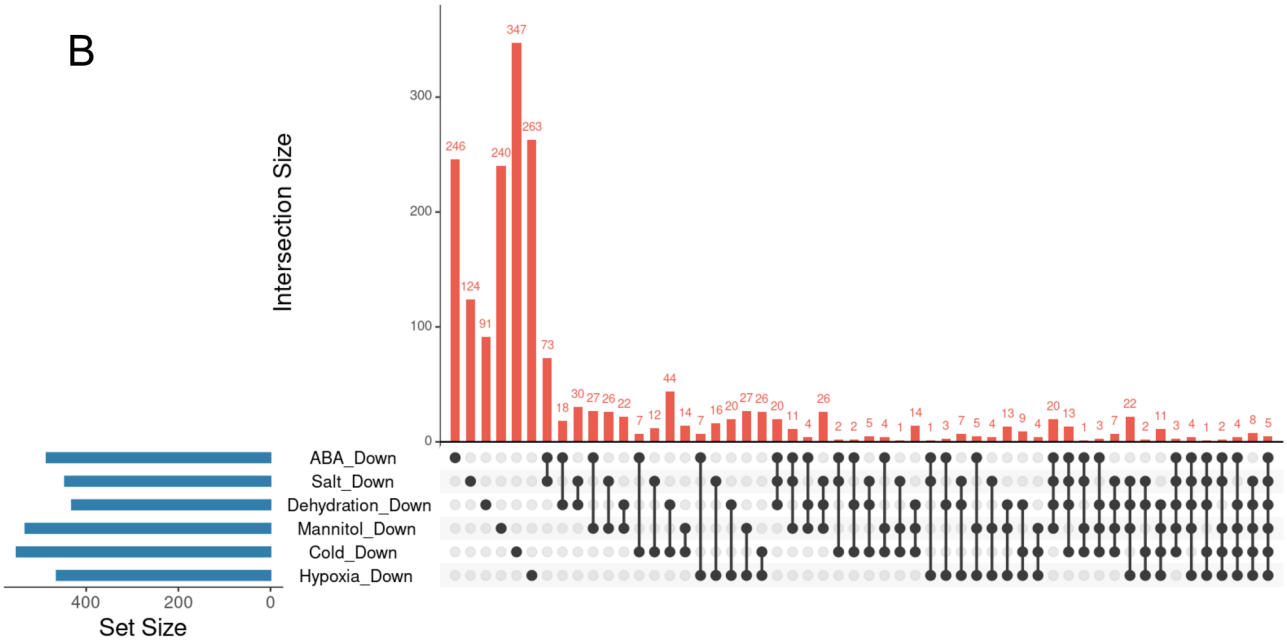
D


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
Intersection Size
Set Size
ABA_Up
Salt_Up
Dehydration_Up
Mannitol_Up
Cold_Up
ABA_up
Salt_ up
Dehydration_ up
Cold_ up
Mannitol_ up


C
D
Intersection Size
Set Size
ABA_Down
Salt_Down
Dehydration_Down
Mannitol_Down
Cold_Down
ABA_down
Salt_ down
Dehydration_ down
Cold_ down
Mannitol_ down


A
Intersection Size
Set Size
ABA_Up
Salt_Up
Dehydration_Up
Mannitol_Up
Cold_Up
Hypoxia_Up


B
Intersection Size
Set Size
ABA_Down
Salt_Down
Dehydration_Down
Mannitol_Down
Cold_Down
Hypoxia_Down