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### ► **To cite this version:**

Amandine Crabos, Yunji Huang, Thomas Boursat, Christophe Maurel, Sandrine Ruffel, et al.. Distinct early transcriptional regulations by turgor and osmotic potential in the root of Arabidopsis. *Journal of Experimental Botany*, 2023, 74 (18), pp.5917-5930. <10.1093/jxb/erad307>. <hal-04193805>

**HAL Id: hal-04193805**

**<https://hal.inrae.fr/hal-04193805v1>**

Submitted on 1 Sep 2023

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# 1 **Distinct early transcriptional regulations by turgor and osmotic**

## 2 **potential in the root of Arabidopsis**

3 Amandine Crabos\*, Yunji Huang\*, Thomas Boursat, Christophe Maurel, Sandrine Ruffel, Gabriel Krouk,  
4 Yann Boursiac

5 \*: contributed equally to the work

6 Institute for Plant Sciences of Montpellier (IPSiM), Univ Montpellier, CNRS, INRAE, Institut Agro,  
7 Montpellier, France

8 TB is also affiliated to : Laboratoire de Mécanique et Génie Civil (LMGC), Univ Montpellier, CNRS,  
9 Montpellier, France

10 Amandine Crabos: [amandine.crabos@inrae.fr](mailto:amandine.crabos@inrae.fr) [0000-0002-9746-4121](https://orcid.org/0000-0002-9746-4121)

11 Yunji Huang: [yunji.huang@supgaro.fr](mailto:yunji.huang@supgaro.fr) [0000-0001-8762-1560](https://orcid.org/0000-0001-8762-1560)

12 Thomas Boursat: [thomas.boursat@umontpellier.fr](mailto:thomas.boursat@umontpellier.fr) [0000-0001-6191-9289](https://orcid.org/0000-0001-6191-9289)

13 Christophe Maurel: [christophe.maurel@cnrs.fr](mailto:christophe.maurel@cnrs.fr) [0000-0002-4255-6440](https://orcid.org/0000-0002-4255-6440)

14 Sandrine Ruffel: [sandrine.ruffel@inrae.fr](mailto:sandrine.ruffel@inrae.fr) [0000-0002-5651-8349](https://orcid.org/0000-0002-5651-8349)

15 Gabriel Krouk: [gabriel.krouk@cnrs.fr](mailto:gabriel.krouk@cnrs.fr) [0000-0003-3693-6735](https://orcid.org/0000-0003-3693-6735)

16 Yann Boursiac: [yann.boursiac@inrae.fr](mailto:yann.boursiac@inrae.fr) [0000-0002-9545-9003](https://orcid.org/0000-0002-9545-9003)

17 **Author for correspondence:** Yann Boursiac, [yann.boursiac@inrae.fr](mailto:yann.boursiac@inrae.fr)

18 Submission date :25/07/2023

19 Word counts (excluding M&M): 4507, number of tables: 1, number of figures: 6, number of  
20 supplemental data: 4

21 **Short title:** Turgor and osmotic potentials trigger distinct transcriptional responses

22 **Highlight:** Osmotic and turgor potentials shape specific transcriptional responses in the root of  
23 Arabidopsis under osmotic challenges.

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28 **Abstract**

29 In a context of climate change, deciphering signaling pathways driving plant adaptation to drought,  
30 changes in water availability, and salt is key. A crossing point of these plant stresses is their impact on  
31 plant water potential ( $\Psi$ ), a composite physico-chemical variable reflecting the availability of water  
32 for biological processes such as plant growth and stomatal aperture. The  $\Psi$  of plant cells is mainly  
33 driven by their turgor and osmotic pressures. Here we investigated the effect of a variety of osmotic  
34 treatments in the root of Arabidopsis plants grown in hydroponics. We used, among others, a  
35 permeating solute as a way to differentiate variations on turgor from variations in osmotic pressure.  
36 Measurement of cortical cell turgor pressure with a cell pressure probe allowed to monitor the  
37 intensity of the treatments and thereby preserve the cortex from plasmolysis. Transcriptome analyses  
38 at an early time point (15min) showed specific and quantitative transcriptomic responses to both  
39 osmotic and turgor pressure variations. Our results highlight how water-related biophysical  
40 parameters can shape the transcriptome of roots under stress and provide putative candidates to  
41 explore further the early perception of water stress in plants.

42

43

44 **Keywords:** ethylene glycol, NaCl, osmotic pressure, sorbitol, transcriptional response, turgor  
45 pressure, PEG, water potential

46 **Abbreviations:**

EG	Ethylene glycol
PEG	Poly ethylene glycol
P	Turgor pressure (MPa)
$\Pi$	Osmotic pressure (MPa)
$\Psi$	Water potential (MPa)
DEPs	Differentially expressed probes
DEGs	Differentially expressed genes

47

## 48 Introduction

49 How the environment is perceived by plants is of major importance for their life cycle. This is  
50 particularly true for water deficit (Maurel and Nacry, 2020; Verslues *et al.*, 2023) which can be  
51 summarized as an imbalance between the plant's requirement and loss of water and its uptake  
52 capacity. Water deficit directly impacts the plant water status. One of the ways plant water status is  
53 assessed is via plant water potential ( $\Psi$ ), a composite variable which, in plant cells, integrates the  
54 turgor potential (or turgor pressure,  $P$ ) and the osmotic potential ( $\Pi$ ) (Haswell and Verslues, 2015).  
55 When considering the soil/plant/atmosphere continuum,  $\Psi$  can also be influenced by gravity and  
56 matric potentials.  $\Psi$  gradients allow evaluating the motive forces that generate net flows of water  
57 between different compartments of this continuum. Together with the viscoelastic properties of the  
58 cell wall,  $P$  is responsible for the elongation of cells and organs, and for the rigidity of stems and leaves,  
59 allowing them to act against gravity and optimize light interception, among others.  $\Pi$  is related to the  
60 concentration of solutes in a compartment. The presence of a  $\Pi$  gradient across a semi-permeable  
61 barrier causes osmosis: a net directional flow of water, even in the absence of any hydrostatic pressure  
62 difference (Bowler, 2017).  $\Pi$  influences biochemical reactions, and can be directly regulated by the cell  
63 through osmoticum accumulation, synthesis, and transport (Beauzamy *et al.*, 2014).

64 A critical issue in plant biology is to understand which physico-chemical parameters are  
65 perceived by plants. Terms such as osmosensing and mechanosensing are employed to describe  
66 phenomena related to perceiving the plant water status (Beauzamy *et al.*, 2014; Haswell and Verslues,  
67 2015; Hamant and Haswell, 2017; Scharwies and Dinneny, 2019). Many molecular actors, such as  
68 mechanosensitive channels and protein kinases from multiple families (detailed in the reviews cited  
69 above) are thought to be involved in this perception or are contributing to associated phenomena.  
70 However, we lack a clear picture of the early perception of water deficit. One difficulty is that water  
71 deficit translates into multiple variations in the cell status. It is still discussed, for example, whether  $\Pi$   
72 or  $P$  changes are directly sensed by plants or whether it is rather their impact on cell processes, cell  
73 wall status or cell volume (Sack *et al.*, 2018; Verslues *et al.*, 2023).

74 Another difficulty is that the links between the intensity of the stress causing the water deficit  
75 and cells parameters is hard to establish. Measuring plant physico-chemical parameters under  
76 physiological conditions is indeed difficult at cell-scale resolution.  $\Pi$  or  $P$  can be measured using a  
77 combination of challenging, low-throughput, and/or indirect techniques such as pressure chambers  
78 and pico-osmometers, cell pressure probes, picogauges or indenters (Beauzamy *et al.*, 2014;  
79 Knoblauch *et al.*, 2014; Boursiac *et al.*, 2022). Thus, there is a real need for improved tools and non-  
80 destructive techniques using, for example, chemical probes, protein reporters, or marker genes.

81 Here, we addressed the early stages of water deficit perception by considering that the drop  
82 in external  $\Psi$  would primarily provoke a change in either  $\Pi$  or P. Using hydroponically grown  
83 Arabidopsis plants that were osmotically challenged with permeating and non-permeating solutes, we  
84 first evaluated the impact of a drop in external  $\Psi$  on the P of root cortical cells. We then investigated  
85 root transcriptional regulations as a readout, to test whether  $\Pi$  or P can trigger specific and  
86 quantitative responses, a first step into the question whether  $\Pi$  or P can be genuinely perceived by  
87 plant cells.

## 88 **Material and Methods**

### 89 **Plant material and culture conditions**

90 All experiments were performed using *Arabidopsis thaliana* ecotype Col-0. Seeds were surface  
91 sterilized and kept at 4°C in dark until sowing on 1/2 Murashige and Skoog basal salt medium agar  
92 plates [2.2 g.l<sup>-1</sup> MS (Sigma), 1% sucrose (Euromedex), 0.05% MES (Euromedex), and 0.7% agar (Sigma),  
93 pH 5.7 adjusted using KOH]. For pre-germination, plates were incubated vertically in growth chamber  
94 under long-day conditions (16h/8h, 21°C, 60% humidity). After 10 days, seedlings were transferred to  
95 a hydroponic medium [1.25 mM KNO<sub>3</sub>, 0.75 mM MgSO<sub>4</sub>, 1.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 50 μM  
96 Fe-EDTA, 50 μM H<sub>3</sub>BO<sub>3</sub>, 12 μM MnSO<sub>4</sub>, 0.70 μM CuSO<sub>4</sub>, 1 μM ZnSO<sub>4</sub>, 0.24 μM MoO<sub>4</sub>Na<sub>2</sub>, 100 μM  
97 Na<sub>2</sub>SiO<sub>3</sub>] and further grown under the same culture conditions. Cell pressure probe measurements,  
98 transcriptomic analyses, and treatments for qPCR analysis were performed at 4- 8 days, 6 days, or 6-  
99 11 days after transfer, respectively.

### 100 **Osmotic treatments**

101 Osmotic stress treatments were performed using a hydroponic solution containing either 25mM-  
102 50mM-75mM-100mM NaCl (Sigma); 50mM-100mM-150mM sorbitol (Sigma); 75g.l<sup>-1</sup>-100 g.l<sup>-1</sup>-125 g.l<sup>-1</sup>-  
103 150 g.l<sup>-1</sup> polyethylene glycol 8000 (Sigma); or 50mM-100mM-150mM-200mM ethylene glycol  
104 (Sigma). Table 1 recapitulates the solutions and their respective osmotic potential.

### 105 **Cell Pressure Probe Measurements**

106 Cell pressure probe measurements were performed as described previously (Javot *et al.*, 2003). Our  
107 device uses a pulled and beveled glass microcapillary (tip external diameter: 4 to 8μm), filled with  
108 mineral oil and mounted onto a pressure probe. Primary root tip segments of ~2-3cm were excised  
109 from Arabidopsis seedlings and laid on a filter paper perfused with hydroponic or treatment solution.  
110 Measurements were performed within a distance of 1cm from the elongation of the first root hairs.  
111 Data were recorded using an especially designed software (Pfloek; Department of Plant Ecology,

112 University of Bayreuth, Germany). Due to the dead volume of the system and the maximal speed of  
113 the peristaltic pump, it took approximatively 2min to fully change the perfusion solution around the  
114 root.

### 115 **Transcriptomic analyses**

116 Osmotic treatments were performed by transferring plants for 15 min into a hydroponic or treatment  
117 solution. The whole roots were harvested after 15 min of treatment and immediately frozen in liquid  
118 nitrogen. Each sample was a pool of three plants and two sets of plants were treated independently.  
119 Frozen samples were ground using a MM 400 mixer mill (Retsch). Total RNA was extracted using TRI  
120 Reagent (Molecular Research Center, Inc), DNA contamination was removed by digestion with DNase  
121 I (Promega), and further purification of the RNAs was performed using the MinElute Cleanup Kit  
122 (Qiagen), all according to the manufacturer's instructions. Concentration and purity of the RNAs was  
123 assessed by spectrophotometry and integrity was confirmed using RNA 6000 Nanochips with a 2100  
124 Bioanalyzer (Agilent). Gene expression measurements were performed using Arabidopsis Affymetrix  
125 Gene1.1 ST array strips (Affymetrix). For each sample, 100 ng of total RNA was processed using the  
126 GeneChip WT PLUS Reagent Kit (Affymetrix) following the manufacturer's instructions. Hybridization  
127 on array strips was performed for 16h at 48°C. The arrays were washed, and stained, using GeneAtlas  
128 Hybridization, Wash and Stain Kit for WT Array Strips following the manufacturer's instructions. Array  
129 strips were scanned on the GeneAtlas system.

130 Microarrays raw data were processed with GCRMA available in the Expression Console Software  
131 package developed by Affymetrix. The Affymetrix Microarrays data have been deposited in the  
132 National Center for Biotechnology Information's Gene Expression Omnibus in compliance with  
133 Minimum Information About a Microarray Experiment standards (<http://www.ncbi.nlm.nih.gov/geo/>)  
134 and are accessible through Gene Expression Omnibus Series accession no. GSE223207.

### 135 **Transcriptomic data analyses**

136 The multi-way type II ANOVA model and one-way ANOVA analyses for genes expression correlation to  
137 II and P were run in R (v.4.2.0). Thresholds for the selection of differentially expressed probes (DEPs)  
138 were adjusted by comparison of the p-values versus FDR corrected p-values and their frequency. A  
139 general cut-off of FDR < 0.2 was ruled out, which yielded non-adjusted p-values thresholds of 0.001  
140 for the solutes (NaCl, Sorbitol, PEG and EG), and 0.0004 and 0.0012 for II and P, respectively. A few  
141 genes were removed from the II- and P-specific lists since they were associated to at least 2 probes  
142 and gave inconsistent ANOVA test results:

- 143 • Removed from “II-specific genes”: At1g72850, At1g78270, At2g24540, At2g33810,  
144 At4g13920, At4g24410, At4g25880, At4g26490,  
145 • Removed from “P-specific genes”: At1g07130, At1g07725, At1g08590, At1g51640, At1g56240,  
146 At1g72850, At2g11851, At2g22960, At3g22070, At3g54630, At3g56770, At4g24410,  
147 At4g28650, At4g36030, At4g38210, At4g38550, At5g59730.

148 Treatments clustering was obtained in RStudio (RStudio 2022.07.1+554) by calculating the  
149 Euclidean distance between treatments using the function *dist()*, then the clusters obtained by *hclust()*  
150 were plotted using *plot()*, with default values. Venn diagrams were elaborated with the nVennR  
151 package (Pérez-Silva *et al.*, 2018).

152 Semantic analysis of the clusters was performed using Genecloud (Krouk *et al.*, 2015) from  
153 m2sb.org webpage with an FDR threshold set to 1%. Gene ontology enrichment was performed in R  
154 using ClusterProfiler v.4.4.2 (Wu *et al.*, 2021) and org.At.tair.db (v3.15.1) for the Arabidopsis genome  
155 wide annotation database (Carlson, 2017). Lists overlaps scores were obtained using the Genesect  
156 algorithm from the Virtual Plant platform (Katari *et al.*, 2010).

#### 157 RT-qPCR

158 RNA extraction was performed by using the Direct-zol RNA Miniprep Plus Kits from Zymo Research  
159 (NO.2072). cDNA solution was synthesized from 1 µg RNA and oligo-DT<sub>15</sub>, dNTPs and M-MLV  
160 (Promega) according the manufacturer protocol. At1g13320 (PDF2) and At4g34270 (TIP41-like) were  
161 selected as internal normalizing genes, because of their stability in roots under abiotic stresses  
162 (Czechowski *et al.*, 2005). RT-qPCR primers were designed by using the primer3 online website (version  
163 4.1.0, Table S1). RT-qPCR reactions were performed according to the procedure recommended by the  
164 manufacturer (Takara)(0.5 µL H<sub>2</sub>O, 0.25 µL F/R, 4 µL cDNA and 5 µL TAKARA SYBR premix Ex Taq. 95°C  
165 for 30 s; 95°C for 5 s, 60°C for 30 s (40 cycles)). RStudio software was used to calculate gene expression  
166 according to Vandesompele’s method (Vandesompele *et al.*, 2002).

#### 167 mRNA decay analysis

168 The half-life time of mRNAs ( $T_{1/2}$ ) was calculated from data available in Sorenson *et al.* 2018. It is based  
169 on the decay rate ( $\alpha$ ) modeled from RNAseq data upon cordycepin treatment on *sov* mutant seedlings  
170 (i.e. Col 0), and is calculated as  $T_{1/2} = \ln(2)/\alpha$  (Sorenson *et al.*, 2018).  $T_{1/2}$  of each mRNA from genes in  
171 the clusters are presented individually and as boxplots. The values above the boxplots correspond to  
172 a non-parametric estimation of the p-value of the  $T_{1/2}$  of a given cluster being smaller than that of the  
173 whole genome. In this bootstrap-based approach, the median  $T_{1/2}$  of the cluster is compared to the  
174 median  $T_{1/2}$  of a sample (of the same size) from the whole genome data. The number of occurrences

175 where the genome sample median  $T_{1/2}$  is smaller than the cluster median  $T_{1/2}$  divided by the number  
176 of tests realized ( $10^4$  tests), aka the frequency, is reported.

## 177 **Results**

### 178 **Turgor response of root cortical cells to osmotic challenges**

179 We determined the P of root cortical cells with a cell pressure probe (Boursiac *et al.*, 2022) upon root  
180 perfusion with a standard hydroponic solution, or the same solution supplemented with various  
181 concentrations of distinct solutes: sodium chloride (NaCl), sorbitol, poly ethylene glycol 8000 (PEG), or  
182 ethylene glycol (EG) (Table 1). In contrast to others, the latter solute can significantly diffuse through  
183 cell membranes. Thus, EG is expected to concomitantly reduce the  $\Psi$  of the solution and cells, without  
184 significantly changing the  $\Psi$  gradients between compartments (Creelman and Zeevaart, 1985). Figures  
185 1A-D show cortical cell pressure measurements over the course of approximately 30 min of a perfusion  
186 with various concentrations of NaCl, sorbitol, PEG and EG. For all treatments, we observed a  
187 progressive reduction in P, which reached a minimal value within 10 min. P remained stable for at least  
188 10 additional minutes for most conditions except EG treatments, where a partial restoration of P was  
189 eventually observed. We averaged P within 10-20 min of treatment and represented it as a function of  
190  $\Pi$  of the bathing solution (Figure 1E). For all solutes except EG, we observed a linear and relatively  
191 similar relationship between  $\Pi$  of the solution and cortical cell P. In the 10-20 min time range, EG  
192 provoked a reduction in P of  $\sim 0.1$  MPa, independently of its concentration, and thereby of  $\Pi$ . Note  
193 that treatments were designed so that P remained positive in cortical cells, and hence cortical cells  
194 were not plasmolysed. These results suggest that root cortical cells behave as osmometers with the  
195 solutes except EG, and show no major osmotic regulation within the time frame of the experiment.  
196 The 15 min time-point, which corresponds to a mostly stable P, seems to be well-adapted to studying  
197 the early molecular events triggered by osmotic challenges.

### 198 **Transcriptional response of roots osmotically challenged for 15min.**

199 We treated Arabidopsis plants for 15 min using the various conditions tested above, and performed  
200 transcriptomic analyses on RNA extracted from their roots. This genome-wide investigation of gene  
201 expression in response to 15 distinct osmotic challenges (plus a control condition in which plants were  
202 transferred into an identical hydroponic solution, table 1) was recorded using Affymetrix A. thaliana  
203 genome arrays (Gene1.1 ST array strip, 2 independent biological experiments). We used the probes  
204 data from the genome array to perform a hierarchical classification of the osmotic challenges, and  
205 explore their convergence in transcriptional control (Figure 2A). A general feature is that most of the  
206 challenges were grouped by the nature of the solute (NaCl, Sorbitol, PEG or EG), suggesting that it

207 represents a main determinant of whole genome transcriptional status. EG treatments were clustered  
208 next to the hydroponic condition, which echoes to the limited effect of this solute on P. Treatments  
209 with the two highest PEG concentrations were also apart from the other challenges, which suggests  
210 that these conditions trigger responses of yet another type.

211 The data sets were then modeled through ANOVA with the following linear model:

$$212 \quad Y_i = \alpha \cdot \text{NaCl}_{\text{factor}} + \beta \cdot \text{Sorbitol}_{\text{factor}} + \gamma \cdot \text{PEG}_{\text{factor}} + \delta \cdot \text{EG}_{\text{factor}}$$

213 where  $Y_i$  is the signal intensity of an ATH1 probe,  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  are coefficients representing the effect of  
214 each of the factors, respectively, and  $\text{NaCl}_{\text{factor}}$ ,  $\text{Sorbitol}_{\text{factor}}$ ,  $\text{PEG}_{\text{factor}}$  and  $\text{EG}_{\text{factor}}$  are factors indicating  
215 the concentration of each treatment (table 1, full results provided in supplemental data S1) (Ristova *et*  
216 *al.*, 2016). Note that this model uses partial regressions against factor that are derived from the  
217 concentration of the solutes. Importantly, the factors are set to 0 when another solute is used as a  
218 treatment and, as a consequence, are negatively impacting the score of genes which could be  
219 regulated by common underlying processes (such regulations are addressed in the next section). Note  
220 also that all solutes are included in the model, despite no co-treatment was performed and thus no  
221 interaction is investigated, in a bid to increase the statistical power. We then considered a probe as  
222 differentially expressed if its ANOVA p-value was significantly different at  $p < 0.001$  ( $\text{FDR} < 0.2$ ) for any of  
223 the 4 factors. 526 differentially expressed probes (DEPs), corresponding to 436 differentially expressed  
224 genes (DEGs) were retrieved with this analysis. In order to estimate the amplitude of the transcriptomic  
225 regulation, we first separated and sorted the DEPs according to the conditions in which they were  
226 regulated (Figure 2B). Probes regulated specifically by one solute only were the most represented. PEG  
227 was the solute with most specific impact, with 182 DEPs (159 DEGs). NaCl, sorbitol and EG treatments  
228 resulted in 127 (92), 68 (60) and 14 (12) specific DEPs (DEGs), respectively. The remaining 135 DEPs  
229 were regulated significantly in 2 or more solutes treatments (Figure 2B). Because DEGs could be either  
230 up or downregulated by the treatments, we separated the genes regulated by each specific solute in  
231 two clusters based on their averaged, centered, expression signal. Figure 2C visually confirms that the  
232 DEGs identified by this approach indeed exhibit a quantitative transcriptional regulation for a particular  
233 solute mostly.

#### 234 **Do $\Pi$ or P trigger specific gene regulations?**

235 Because all treatments share a common osmotic component (table 1), the transcriptional response  
236 can also be observed with the prism of a dose-dependent response to osmotic pressure. We therefore  
237 performed a one-way ANOVA on our transcriptomic data, using  $\Pi$  as the explanatory, continuous,  
238 variable (supplemental data S1). This analysis retrieved 72 DEGs. EG was also used for its capacity to

239 reduce the  $\Psi$  of the solution but, at variance of the other treatments, provoking only a limited  
240 reduction in P (Figure 1 D, E). With the aim of differentiating the effect of an osmotic treatment on the  
241 transcriptome through either the osmotic potential or its impact on P, we performed a similar one-  
242 way ANOVA analysis of the 15 min transcriptomic response to the treatments, but with P as the  
243 explanatory variable (supplemental data S1). This analysis resulted in 179 DEGs. While 53 DEGs were  
244 identified in both  $\Pi$  and P response (see discussion), 19 and 126 DEGs were specific of  $\Pi$  and P,  
245 respectively (Figure 3A). Each group of  $\Pi$  or P DEGs was split in 2 clusters, in order to account for  
246 potential up- and down-regulations. For the  $\Pi$ -specific genes, the mRNA abundance of the DEGs  
247 appeared to be regulated quantitatively for all solutes employed (Figure 3B, upper panels), while a  
248 similar regulation for NaCl, sorbitol and PEG, but not EG, was observed for the P-specific genes (Figure  
249 3B, lower panels). Most importantly, a clear quantitative correlation to  $\Pi$  or P was confirmed for the  
250  $\Pi$ -specific (Figure 3C) and P-specific (Figure 3D) DEGs, respectively. Altogether, our transcriptomic  
251 approach suggests that while cells remain turgid, at least two components of the osmotic treatment,  
252 P and the  $\Pi$  of the bathing solution, are able to provoke specific quantitative responses of the  
253 transcriptome, resulting in both up- and down-regulations.

#### 254 **Are promoter activity and mRNA decay pathways involved in the $\Pi$ or P transcriptional regulation?**

255 The 1Kb promoter regions of the DEGs were analyzed using the MEME suite (Bailey *et al.*, 2015; Grant  
256 and Bailey, 2021, Preprint). Both new and already known (O'Malley *et al.*, 2016) enriched motives were  
257 considered for  $\Pi$ -cluster 1 or the P-specific clusters (with only 4 genes, the  $\Pi$ -cluster 2 was not  
258 analyzed, supplemental data S2, S3A-C). All clusters showed an enrichment in motives (or similar  
259 motives) known to bind ABI3VP1 transcription factors (TFs). All other motives were found enriched in  
260 1 cluster only: REM, C2C2dof and BBRBPC binding elements for P-cluster 2, C2H2 and ZFHD binding  
261 motives for P-cluster 1, and an ARID binding motif for  $\Pi$ -cluster 1.

262 We also considered whether the regulation of the mRNA abundance of the DEGs could be post-  
263 transcriptional, and in particular due to their degradation. Using the transcription inhibitor cordycepin  
264 and a model-assisted RNAseq approach, Sorenson *et al.* (2018) performed a global evaluation of mRNA  
265 decay rates in Arabidopsis and evaluated the implication of the three main decay pathways (Sorenson  
266 *et al.*, 2018). As a first hint into this type of regulation for the DEGs identified herein, we used the  
267 mRNA decay rates obtained in the above-mentioned study, for the *sov* Col genotype, to calculate the  
268 mRNA half-life of our genes of interest in their growth conditions ( $T_{1/2}$ ). The median  $T_{1/2}$  of all mRNAs  
269 detected in this study was around 101 min. The median  $T_{1/2}$  calculated for genes of the  $\Pi$ -specific  
270 clusters and P-specific cluster 2 were significantly lower, with mean values of 56 min, 23 min and 69  
271 min, respectively (Figure 4), and was not different for genes of P-specific cluster 1.

## 272 **A short list of $\Pi$ or P correlated genes**

273 In a bid to confirm the robustness of the microarray approach, and select potentially good candidates  
274 that could serve as markers of P or  $\Pi$ , we ranked genes of the 4 categories ( $\Pi$ - or P-specific, up or  
275 downregulated) according to 3 parameters: the adjusted R square of a linear fit of their averaged  
276 centered expression as a function of  $\Pi$  or P, the slope of the linear fit, and the average expression level  
277 in control conditions (Supplemental data S4). We randomly selected a few genes, among the best  
278 ranked of each list, to confirm their regulation by RT-qPCR, upon 15min NaCl, sorbitol and EG  
279 treatments, and in three new, independent, biological replicates. Figure 5 shows plots of the  
280 comparison between the means of the microarray signals and of the signals obtained by RT-qPCR. 10  
281 genes out of 12 displayed a significant correlation between both signals, thus globally confirming the  
282 results obtained by the microarray approach. The  $\Pi$ -specific cluster 2 showed poor reliability, with  
283 only 1 gene out of 3 having a similar behavior upon confirmation by RT-qPCR and in new biological  
284 replicates. The P-specific cluster 1 exhibited the highest rate of confirmation, in both the p-value of  
285 the correlation and the  $R^2$  of the relationship. Among them, At1g64640 stood out with a correlation p-  
286 value below  $1e-3$  and a  $R^2 > 0.9$ . The expression of this gene is therefore robustly and quantitatively  
287 correlated to P, at 15 min after an osmotic challenge.

## 288 **What are the gene functions altered by $\Pi$ or P?**

289 A semantic analysis of the gene annotation present in the 4 clusters ( $\Pi$ - or P-specific, up or down-  
290 regulated) using Genecloud (Krouk *et al.*, 2015) revealed that the  $\Pi$ -specific clusters do not show any  
291 particular semantic enrichment. Arabinogalactan, Cys/His rich proteins, “protein kinase C” and TFs  
292 related terms were detected in the cluster 1 of P-specific genes (Figure 6A, left). Cluster 2 of P-specific  
293 genes showed enrichments in terms related to ethylene-dependent and other transcriptional  
294 regulations as well as defense responses (Figure 6A, right).

295 A Gene Ontology (GO) enrichment analysis was also performed.  $\Pi$ -specific cluster 1 was found  
296 specifically enriched in genes associated with defense responses and the cell wall (Figure 6B, left).  
297 Results for P-specific clusters were quite consistent with the semantic analysis. P-specific cluster 1 was  
298 enriched in “anchored components” which echoes to the arabinogalactan term above (not shown). P-  
299 specific cluster 2 was enriched in terms associated to ethylene and defense responses. In a bid to  
300 sharpen the above mentioned GO analysis, we also evaluated the overlap between our gene lists and  
301 the Gene Ontology list “response to NaCl” (GO:0009651) as well as two of the upstream terms:  
302 “response to abiotic stress” (GO:0009628) and “response to stimulus” (GO:0050896) (Figure 6C). The  
303 two upregulated clusters,  $\Pi$ -specific cluster 1 and P-specific cluster 2, showed a significant overlap  
304 with the genes in the categories “response to stimulus” and “response to abiotic stress”, but not with

305 “response to salt stress”, indicating the convergence of our approach to the existing knowledge.  
306 Because the specificity of the response might not prevail under short term treatment, and a general  
307 stress response (GSR) may rather be activated (Bjornson *et al.*, 2021), we also compared our lists to  
308 the genes identified as GSR from the work of Ma and Bohnert (2007). In this study, the authors  
309 analyzed a collection of transcriptome profiles of plants under various treatments, and highlighted a  
310 stress-dependent cluster that could represent cell-level stress responses (Ma and Bohnert, 2007). II-  
311 specific cluster 1 and P-specific cluster 2 showed a significant overlap with the GSR genes, with a  
312 greater Z-score than for the previous comparisons (Figure 6C). This analysis suggests that some of the  
313 upregulated genes that are quantitatively (and inversely) correlated to II or P belong to a common and  
314 early response to stresses.

315 Finally, our candidates were also compared to a list of genes encoding transcription factors  
316 (Pruneda-Paz *et al.*, 2014), and showed no significant enrichment in this category of genes (Figure 6C).

## 317 **Discussion**

318 This study was designed to improve our comprehension of which component of a water deficit can be  
319 perceived by plant roots. For this, we considered both the II of the bathing solution or the P of root  
320 cells as possible input signals (Figure 1) and used whole genome transcriptional responses as a readout.  
321 The approach was meant to unravel any quantitative relationship between the input signals and  
322 responses.

### 323 **Parameters at the origin of the transcriptional responses**

324 In addition to solute specific transcriptional responses (Figure 2D), our study unraveled 72 genes which  
325 expression correlated to II, independently of the solute used (II-specific clusters, Figure 3, A and C).  
326 These results suggest that plant cells have the capacity to sense and transduce the external osmotic  
327 potential. Measurement with a cell pressure probe allowed us to also look for correlations between  
328 gene expression and the P of root cortical cells. In this approach, the use of EG as a permeating  
329 osmoticum was critical to make a distinction between the effects of the solutes on P and II (Figure 1E).  
330 241 genes were found to be truly correlated to P (P cluster 1 and 2, Figure 3, A and D) and suggest that  
331 plant cells also have the capacity to specifically sense and respond to the internal pressure. Due to the  
332 experimental design and variability, 53 genes could not be assigned to a II- or P-specific response  
333 (Figure 3A) and would deserve more investigation, in particular with the use of other permeating  
334 solutes. Nevertheless, the identification of genes which expression is quantitatively correlated to all  
335 possible combinations (II or P, up- or down-regulation) highlights the multiplicity of water deficit  
336 responses in plant cells. Since we uncovered potentially distinct regulatory mechanisms, our results

337 will help clarifying studies on mechano- and osmo-sensing as well as our understanding of plant  
338 response to water deficit. For example, turgor recovery upon plant adaptation to low external water  
339 potential by solutes synthesis/accumulation necessarily implies an uncoupling between P and  $\Pi$ .

340  $\Pi$  or P may not be the exact physico-chemical parameters that are genuinely perceived by  
341 plants. It has been suggested that, in leaves, accumulation of ABA is triggered by a drop in relative  
342 water content (RWC) rather than variations in P or  $\Pi$  (Jia *et al.*, 2001; Sack *et al.*, 2018). This distinction  
343 was made possible by experiments of leaf dehydration beyond the turgor point loss. In our  
344 experiments, turgor was preserved because we anticipated that plasmolysis could trigger distinct  
345 responses and, on a crude assumption, the relative change in cell volume ( $\Delta V/V$ ) is linearly correlated  
346 to the variation in P ( $\Delta P$ ) according to  $\Delta P = \varepsilon \cdot \Delta V/V$  where  $\varepsilon$  stands for the cell wall elastic modulus  
347 (Hüsken *et al.*, 1978). Thus, we cannot differentiate P or RWC and may use them interchangeably in  
348 our interpretations. It would also be interesting to establish whether the internal (intracellular)  
349 osmotic potential ( $\Pi_{int}$ ) can be sensed and trigger specific transcriptional responses. Because cells  
350 behave as osmometers in the presence of NaCl, sorbitol and PEG,  $\Pi_{int}$  can be expressed, at equilibrium,  
351 as  $\Pi_{int} = \Pi + P$ . Since the effects on  $\Pi$  and P were close (Figure 1E), those solutes do not allow to  
352 distinguish  $\Pi_{int}$  from  $\Pi$ . EG flux was not completely equilibrated after a 15min treatment (Figure 1D),  
353 and  $\Pi_{int}$  could be calculated based on equations that describe P variations in cells perfused with a  
354 permeating solute (Steudle, 1989). However, experimental variations did not allow us to reach a  
355 sufficient resolution of the hydraulic and solute relaxation phases in cells under EG treatment. Thus,  
356 our current study cannot conclude on the ability of root cells to respond to changes in  $\Pi_{int}$ .

357 Finally, we would like to point other kinds of avenues for interpreting our data. Firstly,  $\Pi$   
358 changes are isotropic in the hydroponic solution, so that all root parts were somewhat homogeneously  
359 challenged. In contrast, P, which was only measured in resting cortical cells, close to root tip, may be  
360 different in other cell types. For example, epidermal cells of Arabidopsis roots usually show a P that is  
361 about 0.1MPa lower than that of cortical cells (Javot *et al.*, 2003). This difference translates into a shift  
362 in the response curve of P to  $\Pi$  and could eventually lead to plasmolysis, in a limited number of cell  
363 types, and under the most severe osmotic challenges. Secondly, gene expression data were obtained  
364 from a whole root mRNA extraction, and could mask cell-type specific regulations which are known to  
365 exist (Ma and Bohnert, 2007; Dinneny *et al.*, 2008). Finally, the transcriptome status at 15min is the  
366 consequence of regulatory mechanisms that were activated within the first 10 min, where turgor  
367 pressure was in a transient status. A more detailed kinetic of the early events would shed light on the  
368 gene regulatory networks (Krouk *et al.*, 2010) activated very early by  $\Pi$  or P variations. All these aspects

369 deserve more investigations at the cell, gene and genome levels, for which our current work provide a  
370 well-defined framework.

### 371 **Mechanisms of mRNA abundance regulation by II or P**

372 In this study, mRNA abundance, as monitored by microarrays or RT-qPCR, was employed as a readout  
373 of water deficit signaling. We realize that changes in a mRNA abundance can be due to many molecular  
374 aspects acting on their synthesis or decay. We gathered informations for two of them: the  
375 corresponding promoter activity and its RNA degradation.

376 We first analyzed the promoters of genes in the II– or P-specific clusters for the presence of  
377 binding sites for putative TFs that could regulate their expression at the transcriptional level (Sup data  
378 S3 and S4). We mostly identified binding sites for TFs belonging to the C2C2dof, ABI3VP1, BBR/BPC,  
379 C2H2, ZFHD, and REM families. Members of these families have been involved in a broad range of  
380 processes but are not specific of water deficit (Yamaguchi-Shinozaki and Shinozaki, 2006; Coutand *et al.*,  
381 2009; Noguero *et al.*, 2013; Mantegazza *et al.*, 2014; Taylor-Teeple *et al.*, 2015; Perrella *et al.*,  
382 2018; Lai *et al.*, 2021; Yan *et al.*, 2021; Wang *et al.*, 2022). This result corroborates the idea that a  
383 multiplicity of TFs can regulate each gene. It also indicates that the short-term responses to both II  
384 and P likely occur through transcriptional regulation, for which we could not identify representative  
385 motives nor the critical role of specific TFs.

386 To address a possible role of mRNA degradation, we referred to a previous work that studied  
387 mRNA decay, but under control conditions (Sorenson *et al.*, 2018). With a median  $T_{1/2}$  of >100 min at  
388 the whole genome level, we hypothesized that a short  $T_{1/2}$  in resting conditions might be a prerequisite  
389 for genes we identified as down-regulated within 15 min by the osmotic treatments (II cluster 2 and  
390 P cluster 1, Figures 3 and 4). Indeed, the relatively short  $T_{1/2}$  calculated for genes of II-specific cluster  
391 2, together with a promoter activity arrest, is compatible with the regulation we observed. Conversely,  
392 this may not be the case for genes of P cluster 1 which showed a median  $T_{1/2}$  similar to that of the  
393 whole genome. Here, we speculate that on top of a down-regulation of their promoter activity, a  
394 reduction in their  $T_{1/2}$  should be induced by the osmotic challenges, thereby leading to their rapid  
395 downregulation. Indeed, phosphorylation of proteins of the mRNA decapping complex is regulated by  
396 osmotic stresses (Sieburth and Vincent, 2018). The multiplicity of II or P responses that we identified  
397 therefore seems to translate into a similar complexity of mRNA regulation mechanisms, and provides  
398 an interesting avenue for further investigation.

### 399 **Functions regulated by II or P**

400 Our approach could possibly identify genes which function in II or P signaling. Semantic and gene  
401 ontology enrichments were performed on the gene lists and identified complementary terms. Generic  
402 terms retrieved by this approach were mainly associated to transcriptional regulation, responses to  
403 abiotic or biotic stimuli, and the cell wall (Figure 6A and B). It is difficult to extract any precise signaling  
404 pathway here since many annotations of these genes are inferred, and some of the terms define  
405 diverse functions. For example, arabinogalactan proteins are involved in many processes in roots  
406 including biotic and abiotic responses (Hromadová *et al.*, 2021), and genes of the C1-like domain  
407 superfamily have been associated to various biological/developmental processes, including root  
408 epidermal cell differentiation (Bruex *et al.*, 2012). It is also somehow surprising to extract terms related  
409 to biotic stresses and defense responses. However, this may result from genes whose annotation  
410 originates from, but not necessarily restricts to, “biotic” conditions, or whose function was only  
411 indirectly inferred. Indeed, our approach uncovers genes associated to the “short-term”, less specific,  
412 general stress response (Figure 6C)(Bjornson *et al.*, 2021). Importantly, we introduce here the notion  
413 that there is a quantitative relationship between the mRNA abundance of these genes and physico-  
414 chemical parameters (Figure 3C).

#### 415 **Quantitative responses to physico-chemical parameters**

416 Our study integrates into earlier works focused on the perception of the physico-chemical conditions  
417 of cells. We investigated here the dose dependent effects of physico-chemical parameters on the root  
418 transcriptome. Such an approach has been successfully applied in poplar, where it was shown that the  
419 abundance of ZFP2 mRNA is correlated to the sum of strains upon stem bending (Coutand *et al.*, 2009),  
420 and which initiated great advances on the understanding of thigmomorphogenesis. With respect to  
421 water, a study was performed in sunflower where a generalized linear model fed by the expression  
422 level of 3 genes was developed in order to compute integrated parameters such as the pre-dawn water  
423 potential or the soil water content (Marchand *et al.*, 2013). There is a gap between obtaining  
424 correlations between physico-chemical parameters and gene expression –such as what we present  
425 here, and creating biomarkers or biosensors (Jones, 2014). Nevertheless, the genes identified in the 4  
426 clusters could serve as molecular reporters to investigate the perception and signaling of II or P.  
427 Indeed, this has been successfully achieved for temperature sensing, where the promoter of HSP70  
428 was used as a quantitative reporter of ambient temperature, and allowed to discover the role of H2A.Z  
429 proteins in the temperature-dependent modulation of transcription (Kumar and Wigge, 2010).

#### 430 **Conclusion**

431 Thanks to a combination of physiological techniques and a transcriptome approach, we showed the  
432 existence of rapid, specific transcriptional responses to water-related physico-chemical parameters.

433 We propose herein a list of early responsive genes whose mRNA abundance in quantitatively  
434 correlated to external II or to cell P. This list provides potential reporter genes that could serve to  
435 elaborate biomarkers of the plant cells water status. This study also paves the way for future dissection  
436 of the molecular perception of water deficit in plants, through the identification of how their mRNA  
437 abundance is regulated.

## 438 **Supplementary data**

439 Table S1: primers sequences for the RT-qPCR analysis

440 Supplemental data S1: p-values of the ANOVA analyses and probe/AGI correspondence for the  
441 Gene1.1 ST array

442 Supplemental data S2: summary of TFs binding sites enrichment in the II and P clusters

443 Supplemental data S3 A, B, C: output of the promoter analysis for each cluster

444 Supplemental data S4: gene list of each cluster, highlighting the genes that were tested further by  
445 RT-qPCR

446

## 447 **Acknowledgements**

448 We thank Alexandre Martinière, François Parcy and François Tardieu for helpful discussions, and Cécile  
449 Fizames for help in the promoter analysis. We thank 2 anonymous reviewers for helping us improve  
450 this manuscript.

## 451 **Authors contributions**

452 YB and GK conceptualized the research. AC and YH performed the research with contributions from TB  
453 for physiological measurements. YH, GK and YB analyzed the data with contributions from SR and CM.  
454 YB wrote the article with insights from CM. All authors contributed to reviewing the manuscript and  
455 agreed to its content.

## 456 **Funding**

457 This project was funded through Labex AGRO 2011-LABX-002, project number 1403-012 to YB, in the  
458 I-Site Muse framework, coordinated by Agropolis Fondation, and by the CNRS through the MITI  
459 interdisciplinary programs (“Turgomap” to YB). YH is supported by the China Scholarship Council. TB is  
460 supported by the LabEx NUMEV (ANR-10-LABX-0020) within the I-Site MUSE (ANR-16-IDEX-0006) and  
461 by Region Occitanie.

## 462 **Conflict of interest**

463 None declared

464 **Data availability**

465 The transcriptomic data that support the findings of this study are openly available in Gene Expression  
466 Omnibus Series (<http://www.ncbi.nlm.nih.gov/geo/>) GSE223207.

467 The code and source files (besides transcriptomic data) used to analyze the data and/or to generate  
468 the figures 1 to 5 of this study can be downloaded from  
469 [https://github.com/ybinrae/Watermarker\\_paper1.git](https://github.com/ybinrae/Watermarker_paper1.git).

470

accepted manuscript

## References

- 471 **Bailey TL, Johnson J, Grant CE, Noble WS.** 2015. The MEME Suite. *Nucleic Acids Research* **43**, W39-49.
- 472 **Beauzamy L, Nakayama N, Boudaoud A.** 2014. Flowers under pressure: ins and outs of turgor regulation in  
473 development. *Annals of Botany* **114**, 1517–1533.
- 474 **Bjornson M, Pimprikar P, Nürnberg T, Zipfel C.** 2021. The transcriptional landscape of *Arabidopsis thaliana*  
475 pattern-triggered immunity. *Nature Plants* **7**, 579–586.
- 476 **Boursiac Y, Protto V, Rishmawi L, Maurel C.** 2022. Experimental and conceptual approaches to root water  
477 transport. *Plant and Soil* **478**, 349–370.
- 478 **Bowler MG.** 2017. The physics of osmotic pressure. *European Journal of Physics* **38**, 055102.
- 479 **Bruex A, Kainkaryam RM, Wieckowski Y, et al.** 2012. A Gene Regulatory Network for Root Epidermis Cell  
480 Differentiation in *Arabidopsis*. *PLOS Genetics* **8**, e1002446.
- 481 **Carlson M.** 2017. [org.At.tair.db](http://org.At.tair.db).
- 482 **Coutand C, Martin L, Leblanc-Fournier N, Decourteix M, Julien J-L, Moulia B.** 2009. Strain Mechanosensing  
483 Quantitatively Controls Diameter Growth and PtaZFP2 Gene Expression in Poplar. *Plant Physiology* **151**, 223–  
484 232.
- 485 **Creelman RA, Zeevaart JAD.** 1985. Abscisic Acid Accumulation in Spinach Leaf Slices in the Presence of  
486 Penetrating and Nonpenetrating Solutes. *Plant Physiology* **77**, 25–28.
- 487 **Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible W-R.** 2005. Genome-Wide Identification and Testing  
488 of Superior Reference Genes for Transcript Normalization in *Arabidopsis*. *Plant Physiology* **139**, 5–17.
- 489 **Dinneny JR, Long TA, Wang JY, Jung JW, Mace D, Pointer S, Barron C, Brady SM, Schiefelbein J, Benfey PN.**  
490 2008. Cell Identity Mediates the Response of *Arabidopsis* Roots to Abiotic Stress. *Science* **320**, 942–945.
- 491 **Grant CE, Bailey TL.** 2021. XSTREME: Comprehensive motif analysis of biological sequence datasets. [bioRxiv](https://doi.org/10.1101/2021.03.10.434814).
- 492 **Hamant O, Haswell ES.** 2017. Life behind the wall: sensing mechanical cues in plants. *BMC Biology* **15**, 59.
- 493 **Haswell ES, Verslues PE.** 2015. The ongoing search for the molecular basis of plant osmosensing. *Journal of*  
494 *General Physiology* **145**, 389–394.
- 495 **Hromadová D, Soukup A, Tylová E.** 2021. Arabinogalactan Proteins in Plant Roots – An Update on Possible  
496 Functions. *Frontiers in Plant Science* **12**.
- 497 **Hüsken D, Steudle E, Zimmermann U.** 1978. Pressure Probe Technique for Measuring Water Relations of Cells  
498 in Higher Plants. *Plant Physiology* **61**, 158–163.
- 499 **Javot H, Lauvergeat V, Santoni V, et al.** 2003. Role of a Single Aquaporin Isoform in Root Water Uptake. *Plant*  
500 *Cell* **15**, 509–522.
- 501 **Jia W, Zhang J, Liang J.** 2001. Initiation and regulation of water deficit-induced abscisic acid accumulation in  
502 maize leaves and roots: cellular volume and water relations. *Journal of Experimental Botany* **52**, 295–300.
- 503 **Jones HG.** 2014. The use of indirect or proxy markers in plant physiology. *Plant, Cell & Environment* **37**, 1270–  
504 1272.

- 505 **Katari MS, Nowicki SD, Aceituno FF, et al.** 2010. VirtualPlant: A Software Platform to Support Systems Biology  
506 Research. *Plant Physiology* **152**, 500–515.
- 507 **Knoblauch J, Mullendore DL, Jensen KH, Knoblauch M.** 2014. Pico Gauges for Minimally Invasive Intracellular  
508 Hydrostatic Pressure Measurements. *Plant Physiology* **166**, 1271–1279.
- 509 **Krouk G, Carré C, Fizames C, Gojon A, Ruffel S, Lacombe B.** 2015. GeneCloud Reveals Semantic Enrichment in  
510 Lists of Gene Descriptions. *Molecular Plant* **8**, 971–973.
- 511 **Krouk G, Mirowski P, LeCun Y, Shasha DE, Coruzzi GM.** 2010. Predictive network modeling of the high-  
512 resolution dynamic plant transcriptome in response to nitrate. *Genome Biology* **11**, R123.
- 513 **Kumar SV, Wigge PA.** 2010. H2A.Z-Containing Nucleosomes Mediate the Thermosensory Response in  
514 Arabidopsis. *Cell* **140**, 136–147.
- 515 **Lai W, Zhu C, Hu Z, Liu S, Wu H, Zhou Y.** 2021. Identification and Transcriptional Analysis of Zinc Finger-  
516 Homeodomain (ZF-HD) Family Genes in Cucumber. *Biochemical Genetics* **59**, 884–901.
- 517 **Ma S, Bohnert HJ.** 2007. Integration of Arabidopsis thaliana stress-related transcript profiles, promoter  
518 structures, and cell-specific expression. *Genome Biology* **8**, R49.
- 519 **Mantegazza O, Gregis V, Mendes MA, Morandini P, Alves-Ferreira M, Patreze CM, Nardeli SM, Kater MM,  
520 Colombo L.** 2014. Analysis of the arabidopsis REM gene family predicts functions during flower development.  
521 *Annals of Botany* **114**, 1507–1515.
- 522 **Marchand G, Mayjonade B, Varès D, et al.** 2013. A biomarker based on gene expression indicates plant water  
523 status in controlled and natural environments. *Plant, Cell & Environment* **36**, 2175–2189.
- 524 **Maurel C, Nacry P.** 2020. Root architecture and hydraulics converge for acclimation to changing water  
525 availability. *Nature Plants* **6**, 744–749.
- 526 **Noguero M, Atif RM, Ochatt S, Thompson RD.** 2013. The role of the DNA-binding One Zinc Finger (DOF)  
527 transcription factor family in plants. *Plant Science* **209**, 32–45.
- 528 **O'Malley RC, Huang SC, Song L, Lewsey MG, Bartlett A, Nery JR, Galli M, Gallavotti A, Ecker JR.** 2016. Cistrome  
529 and Epicistrome Features Shape the Regulatory DNA Landscape. *Cell* **165**, 1280–1292.
- 530 **Pérez-Silva JG, Araujo-Voces M, Quesada V.** 2018. nVenn: generalized, quasi-proportional Venn and Euler  
531 diagrams. (J Wren, Ed.). *Bioinformatics* **34**, 2322–2324.
- 532 **Perrella G, Davidson MLH, O'Donnell L, Nastase A-M, Herzyk P, Breton G, Pruneda-Paz JL, Kay SA, Chory J,  
533 Kaiserli E.** 2018. ZINC-FINGER interactions mediate transcriptional regulation of hypocotyl growth in  
534 Arabidopsis. *Proceedings of the National Academy of Sciences* **115**, E4503–E4511.
- 535 **Pruneda-Paz JL, Breton G, Nagel DH, Kang SE, Bonaldi K, Doherty CJ, Ravelo S, Galli M, Ecker JR, Kay SA.** 2014.  
536 A Genome-Scale Resource for the Functional Characterization of Arabidopsis Transcription Factors. *Cell Reports*  
537 **8**, 622–632.
- 538 **Ristova D, Carré C, Pervent M, et al.** 2016. Combinatorial interaction network of transcriptomic and  
539 phenotypic responses to nitrogen and hormones in the Arabidopsis thaliana root. *Science Signaling* **9**, rs13–  
540 rs13.
- 541 **Sack L, John GP, Buckley TN.** 2018. ABA Accumulation in Dehydrating Leaves Is Associated with Decline in Cell  
542 Volume, Not Turgor Pressure. *Plant Physiology* **176**, 489–495.
- 543 **Scharwies JD, Dinneny JR.** 2019. Water transport, perception, and response in plants. *Journal of Plant Research*  
544 **132**, 311–324.

- 545 **Sieburth LE, Vincent JN.** 2018. Beyond transcription factors: roles of mRNA decay in regulating gene expression  
546 in plants. *F1000Research* **7**, F1000 Faculty Rev-1940.
- 547 **Sorenson RS, Deshotel MJ, Johnson K, Adler FR, Sieburth LE.** 2018. *Arabidopsis* mRNA decay landscape arises  
548 from specialized RNA decay substrates, decapping-mediated feedback, and redundancy. *Proceedings of the*  
549 *National Academy of Sciences* **115**, E1485–E1494.
- 550 **Steudle E.** 1989. Water flow in plants and its coupling to other processes: An overview. *Biomembranes Part U:*  
551 *Cellular and Subcellular Transport: Eukaryotic (Nonepithelial) Cells. Methods in Enzymology.* Academic Press,  
552 183–225.
- 553 **Taylor-Teeple M, Lin L, de Lucas M, et al.** 2015. An *Arabidopsis* gene regulatory network for secondary cell  
554 wall synthesis. *Nature* **517**, 571–575.
- 555 **Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F.** 2002. Accurate  
556 normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.  
557 *Genome Biology* **3**, research0034.1.
- 558 **Verslues PE, Bailey-Serres J, Brodersen C, et al.** 2023. Burning questions for a warming and changing world: 15  
559 unknowns in plant abiotic stress. *The Plant Cell* **35**, 67–108.
- 560 **Wang Z, Wong DCJ, Chen Z, Bai W, Si H, Jin X.** 2022. Emerging Roles of Plant DNA-Binding With One Finger  
561 Transcription Factors in Various Hormone and Stress Signaling Pathways. *Frontiers in Plant Science* **13**.
- 562 **Wu T, Hu E, Xu S, et al.** 2021. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *The*  
563 *Innovation* **2**, 100141.
- 564 **Yamaguchi-Shinozaki K, Shinozaki K.** 2006. Transcriptional regulatory networks in cellular responses and  
565 tolerance to dehydration and cold stresses. *Annual Review of Plant Biology* **57**, 781–803.
- 566 **Yan J, Liu Y, Yang L, He H, Huang Y, Fang L, Scheller HV, Jiang M, Zhang A.** 2021. Cell wall  $\beta$ -1,4-galactan  
567 regulated by the BPC1/BPC2-GALS1 module aggravates salt sensitivity in *Arabidopsis thaliana*. *Molecular Plant*  
568 **14**, 411–425.

569 **Tables**

570 **Table 1: Summary of the osmotic treatments applied to the roots, and the factors of the ANOVA**  
 571 **model.**

572 Solutes were dissolved in the hydroponic solution. The osmotic potential of the solution was measured  
 573 at 20°C with an osmometer (Wescor), 3 digits after the decimal point are shown. The osmotic potential  
 574 is the opposite of the osmotic pressure.

Solute	Concentration (mM, g/l for PEG)	Π	P <sub>cort</sub>	NaCl <sub>factor</sub>	Sorbitol <sub>fac</sub> tor	PEG <sub>factor</sub>	EG <sub>fac</sub> tor
None	0	0.021	0.41	0	0	0	0
NaCl	25	0.117	0.32	25	0	0	0
	50	0.238	0.25	50	0	0	0
	75	0.355	0.14	75	0	0	0
	100	0.425	0.05	100	0	0	0
Sorbitol	50	0.134	0.32	0	50	0	0
	100	0.255	0.18	0	100	0	0
	150	0.370	0.1	0	150	0	0
Polyethylene glycol 8000	75	0.073	0.31	0	0	75	0
	100	0.131	0.26	0	0	100	0
	125	0.209	0.2	0	0	125	0
	150	0.280	0.14	0	0	150	0
Ethylene Glycol	50	0.151	0.3	0	0	0	50
	100	0.268	0.29	0	0	0	100
	150	0.394	0.29	0	0	0	150
	200	0.516	0.26	0	0	0	200

## 577 **Figure legends**

### 578 **Figure 1: Osmotic treatments reduce the P of root cortical cells in Arabidopsis.**

579 A portion of ~3cm of root of 21 day-old plants, laid on a perfused Whatman paper, was treated with  
580 various solutes at different concentrations. Cortical cell P was measured with a cell pressure probe. **A**  
581 **to D:** measurement kinetics performed on plants treated with NaCl at concentrations of 0, 25, 50, 75,  
582 100mM (the darker the color, the more concentrated,  $n > 2$  for each treatment), sorbitol at 50, 100 and  
583 150mM, PEG8000 at 75, 100, 125 and 150  $\text{g.l}^{-1}$ , and EG at 50, 100, 150 and 200mM, respectively. Zero  
584 in the time axis indicates the change in perfusion from hydroponic solution to the same solution  
585 complemented with treatments. A lowess smoothing was added in order to highlight the general  
586 behavior of P after each treatment **E:** plot recapitulating the measurements of P within the 10-20min  
587 time frame as a function of the osmotic pressure of the solution (average value  $\pm$  SEM,  $n \geq 2$ , blue:  
588 sorbitol, pink: NaCl, green: PEG, red: EG).

589

### 590 **Figure 2: Features of the early transcriptomic response to osmotic treatments.**

591 **A:** Dendrogram illustrating the effects of the solute nature and concentration on the regulation of gene  
592 expression. **B:** number of DEPs classified according to the solute used for the treatment. The matrix  
593 below indicates if those DEPs were found for a single or for various solute(s). **C:** Gene expression  
594 signals in the different treatments. Genes regulated by one solute only were selected from B and split  
595 in 2 clusters. The average, centered, expression value for each gene is plotted against the combination  
596 of solutes and concentrations used in the transcriptomic approach.

597

### 598 **Figure 3: Correlations between gene expression and osmotic or turgor pressure.**

599 **A:** Venn diagram showing the number of genes, in the same transcriptomic approach as in figure 2,  
600 which expression is significantly correlated to  $\Pi$ , P, or both. **B:** The 137 “ $\Pi$ -specific” genes and 401 “P-  
601 specific” genes were split in 2 clusters each, and their average centered expression is expressed as a  
602 function of the solute/treatment combination corresponding to the biological assays of the  
603 transcriptome approach. **C:** The 137 “ $\Pi$ -specific” genes were separated into 2 clusters and their  
604 average centered expression is expressed as a function of the osmotic potential of the solution of  
605 treatment, or the cortical cell turgor pressure. **D:** same representation as in C but for the 401 “P-  
606 specific” genes.

607

### 608 **Figure 4: mRNA half-life time is reduced in 3 clusters in control conditions.**

609 The half-life time of mRNAs ( $T_{1/2}$ ) was calculated from Sorenson et al. 2018, based on the decay rate  
610 ( $\alpha$ ) modeled upon cordycepin treatment on *sov* mutant seedlings (i.e. Col 0,  $T_{1/2} = \ln(2)/\alpha$ ) (Sorenson  
611 *et al.*, 2018).  $T_{1/2}$  of each mRNA from genes in the clusters are presented individually and as boxplots.  
612 The numbers above the boxplots correspond the p-value of a bootstrap based test of  $T_{1/2}$  of the cluster  
613 being smaller than the whole genome median  $T_{1/2}$  (see M&M).

614

### 615 **Figure 5: RT-qPCR validation of osmotic or turgor pressure clusters.**

616 The expression of three candidates per list: II or P, clusters 1 and 2 were investigated by RT-qPCR in 3  
617 independent biological replicates. The plants were harvested 15min after transfer into a hydroponic  
618 solution, or the solution complemented with 25, 50,75, 100mM NaCl, or 50, 100, 150mM Sorbitol, or  
619 50, 100, 150, 200g.l<sup>-1</sup> EG. For each gene, normalized RT-qPCR signal ( $\pm$  SEM) is expressed as a function  
620 of the averaged signal ( $\pm$  SEM) obtained from the transcriptome analysis. The p-value of a correlation  
621 test (Pearson) as well as the linear fit (with its R<sup>2</sup> value) between the average values are indicated for  
622 each gene.

623

624 **Figure 6: classification of the II and P correlated genes.**

625 **A:** output of the Genecloud semantic analysis for the 2 clusters showing a significant enrichment. **B:**  
626 Output of a gene ontology analysis for the 2 clusters showing significant enrichments in GO terms. The  
627 GO terms, gene counts for each GO term and adjusted p-value of the enrichment are presented. **C:**  
628 Degree of overlap (Z-score) between our genes lists and public genes lists related to osmotic stress,  
629 the general stress response, and a list of transcription factors, using the Genesect algorithm.

630

accepted manuscript

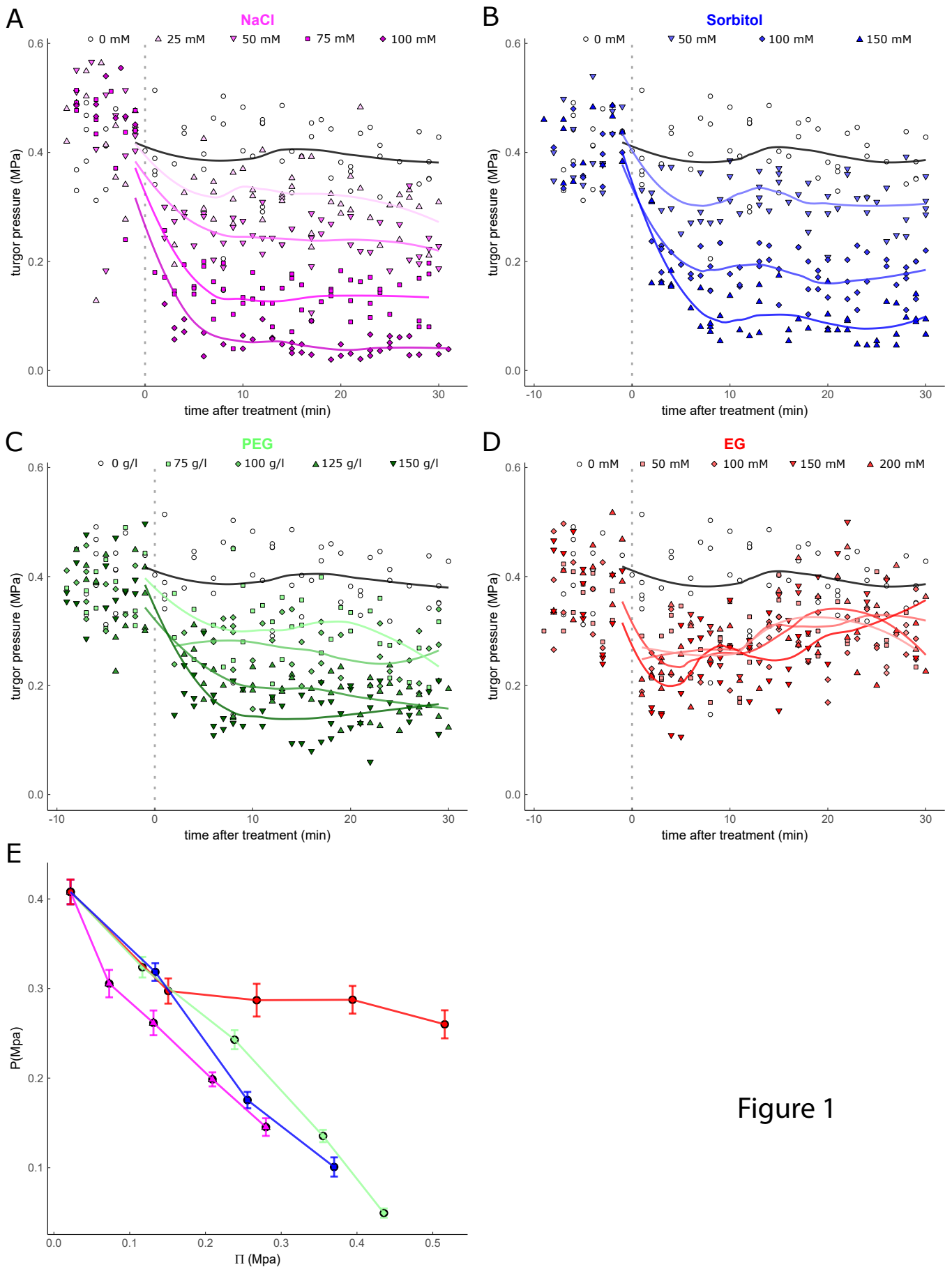


Figure 1

A portion of ~3cm of root of 21 day-old plants, laid on a perfused Whatman paper, was treated with various solutes at different concentrations. Cortical cell P was measured with a cell pressure probe. A to D: measurement kinetics performed on plants treated with NaCl at concentrations of 0, 25, 50, 75, 100mM (the darker the color, the more concentrated, n>2 for each treatment), sorbitol at 50, 100 and 150mM, PEG8000 at 75, 100, 125 and 150 g.l<sup>-1</sup>, and EG at 50, 100, 150 and 200mM, respectively. Zero in the time axis indicates the change in perfusion from hydroponic solution to the same solution complemented with treatments. A lowess smoothing was added in order to highlight the general behavior of P after each treatment E: plot recapitulating the measurements of P within the 10-20min time frame as a function of the osmotic pressure of the solution (average value  $\pm$  SEM, n  $\geq$  2, blue: sorbitol, pink: NaCl, green: PEG, red: EG).

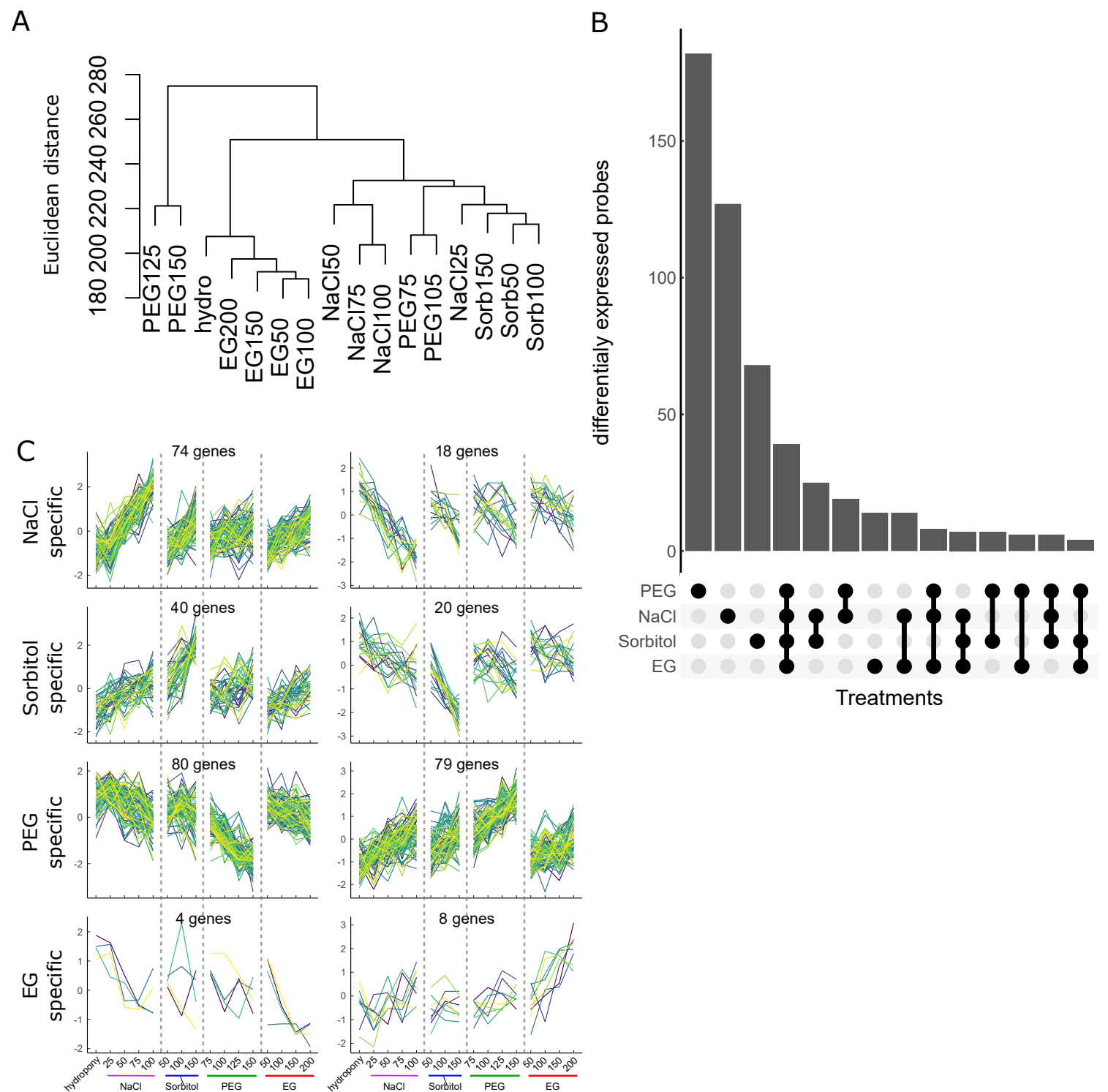


Figure 2

A: Dendrogram illustrating the effects of the solute nature and concentration on the regulation of gene expression. B: number of DEPs classified according to the solute used for the treatment. The matrix below indicates if those DEPs were found for a single or for various solute(s). C: Gene expression signals in the different treatments. Genes regulated by one solute only were selected from B and split in 2 clusters. The average, centered, expression value for each gene is plotted against the combination of solutes and concentrations used in the transcriptomic approach.

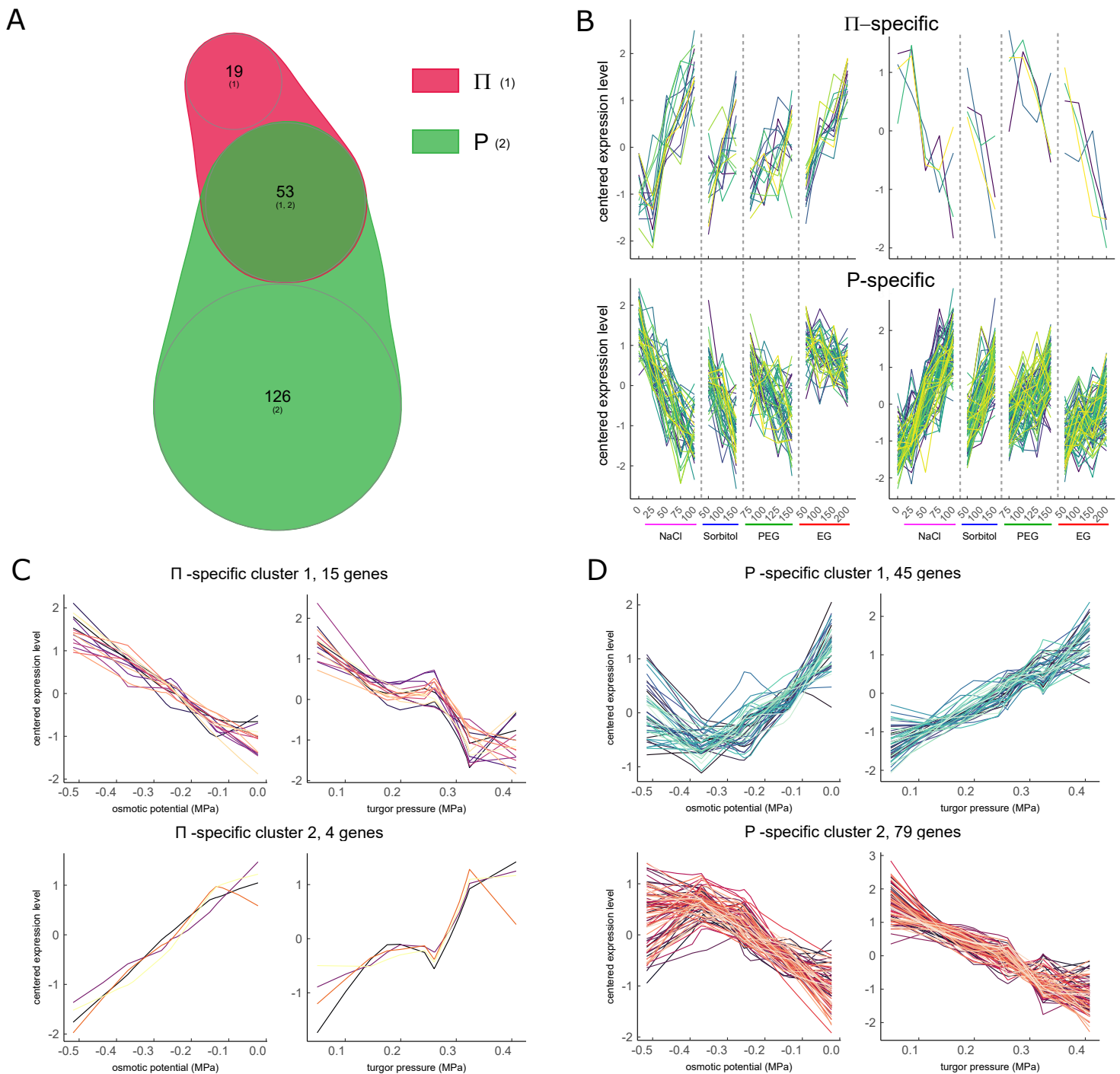


Figure 3

A: Venn diagram showing the number of genes, in the same transcriptomic approach as in figure 2, which expression is significantly correlated to  $\Pi$ , P, or both. B: The 137 “ $\Pi$ -specific” genes and 401 “P-specific” genes were split in 2 clusters each, and their average centered expression is expressed as a function of the solute/treatment combination corresponding to the biological assays of the transcriptome approach. C: The 137 “ $\Pi$ -specific” genes were separated into 2 clusters and their average centered expression is expressed as a function of the osmotic potential of the solution of treatment, or the cortical cell turgor pressure. D: same representation as in C but for the 401 “P-specific” genes.

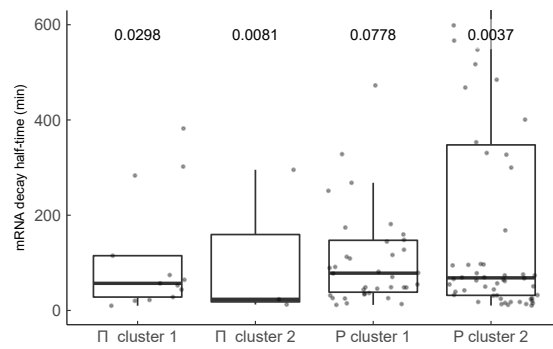
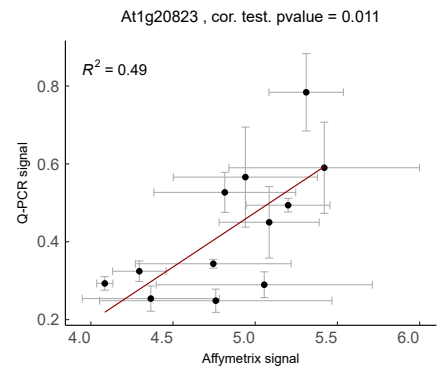
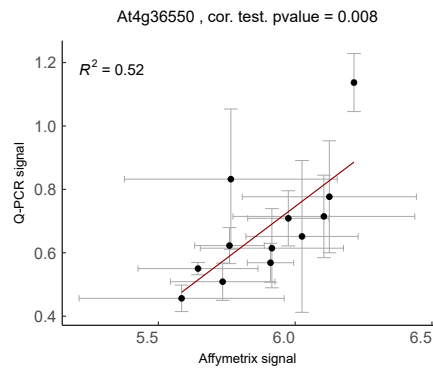
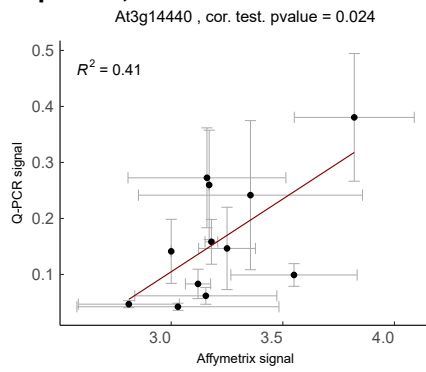


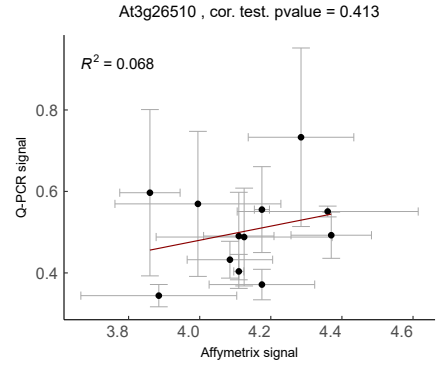
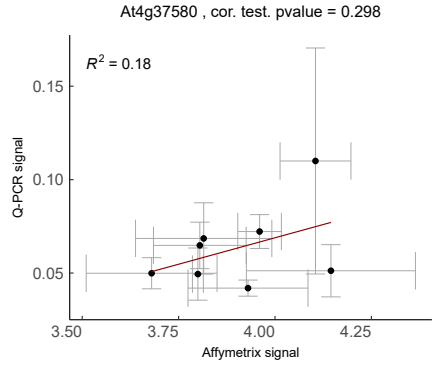
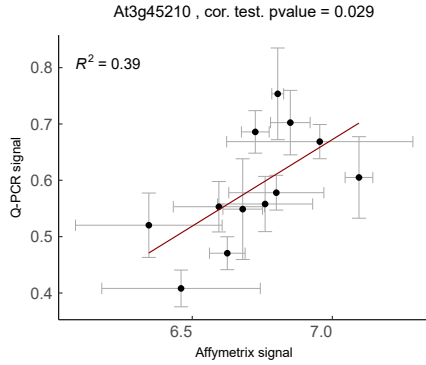
Figure 4

The half-life time of mRNAs ( $T_{1/2}$ ) was calculated from Sorenson et al. 2018, based on the decay rate ( ) modeled upon cordycepin treatment on sov mutant seedlings (i.e. Col 0,  $T_{1/2} = \ln(2) /$ ) (Sorenson et al., 2018).  $T_{1/2}$  of each mRNA from genes in the clusters are presented individually and as boxplots. The numbers above the boxplots correspond the p-value of a bootstrap based test of  $T_{1/2}$  of the cluster being smaller than the whole genome median  $T_{1/2}$  (see M&M).

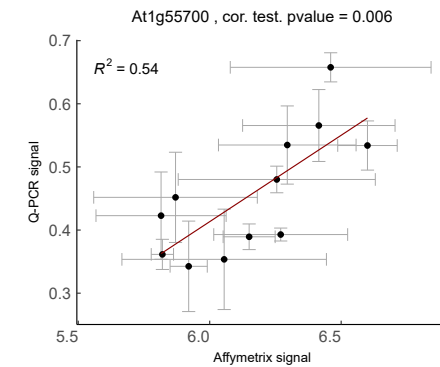
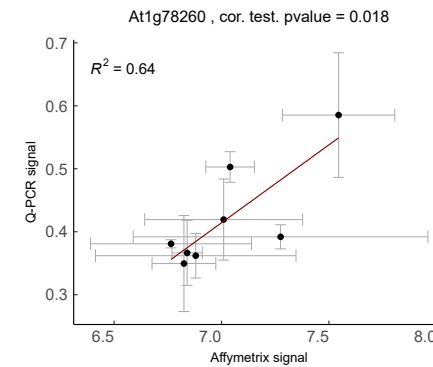
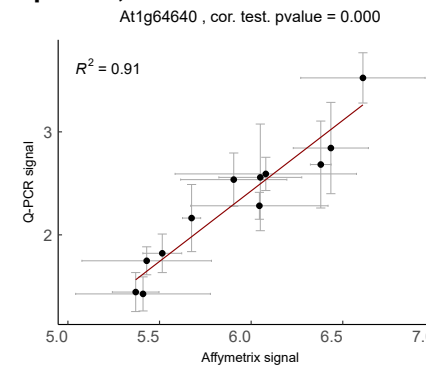
### $\Pi$ -specific, cluster 1



### $\Pi$ -specific, cluster 2



### P-specific, cluster 1



### P-specific, cluster 2

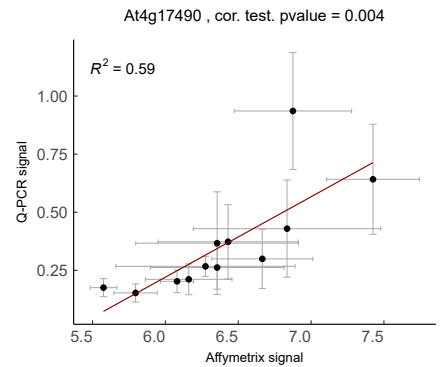
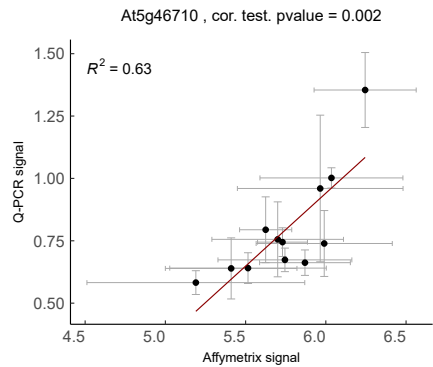
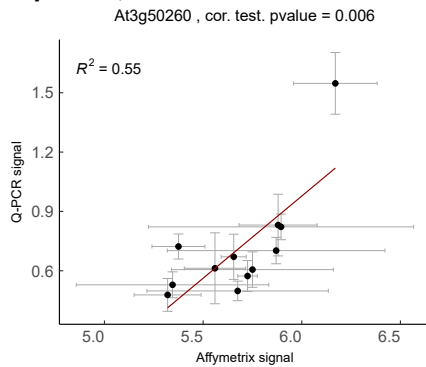
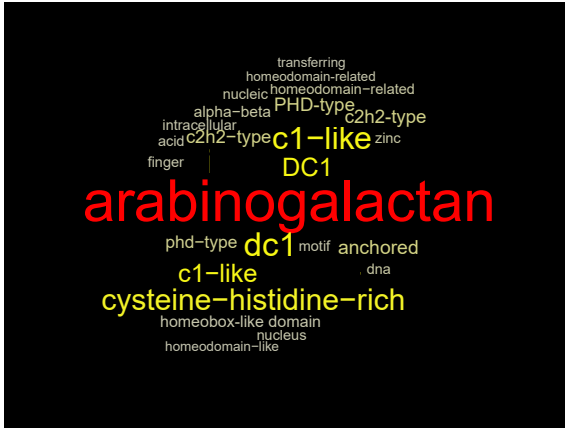


Figure 5

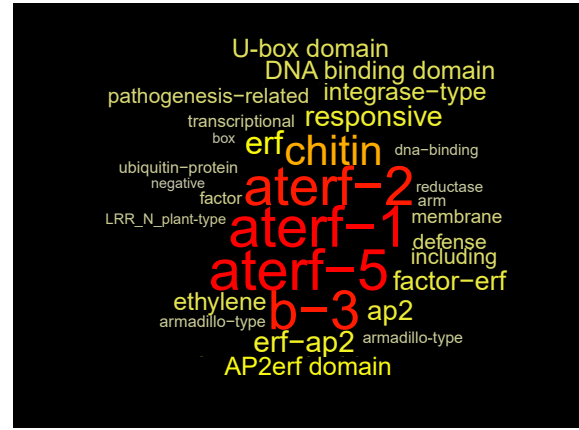
The expression of three candidates per list:  $\Pi$  or P, clusters 1 and 2 were investigated by RT-qPCR in 3 independent biological replicates. The plants were harvested 15min after transfer into a hydroponic solution, or the solution complemented with 25, 50, 75, 100mM NaCl, or 50, 100, 150mM Sorbitol, or 50, 100, 150, 200g.l-1 EG. For each gene, normalized RT-qPCR signal ( $\pm$  SEM) is expressed as a function of the averaged signal ( $\pm$  SEM) obtained from the transcriptome analysis. The p-value of a correlation test (Pearson) as well as the linear fit (with its  $R^2$  value) between the average values are indicated for each gene.

A

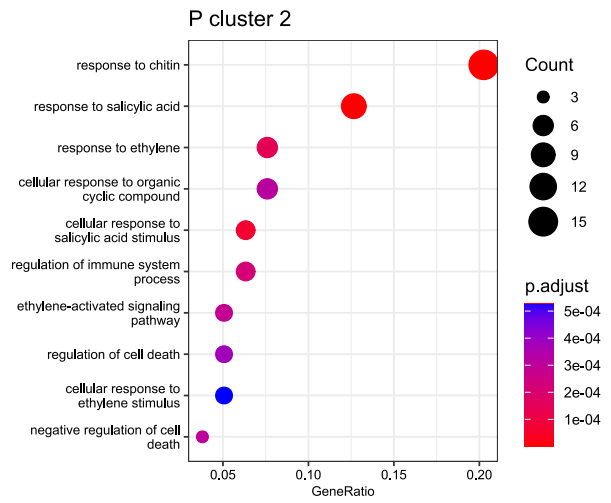
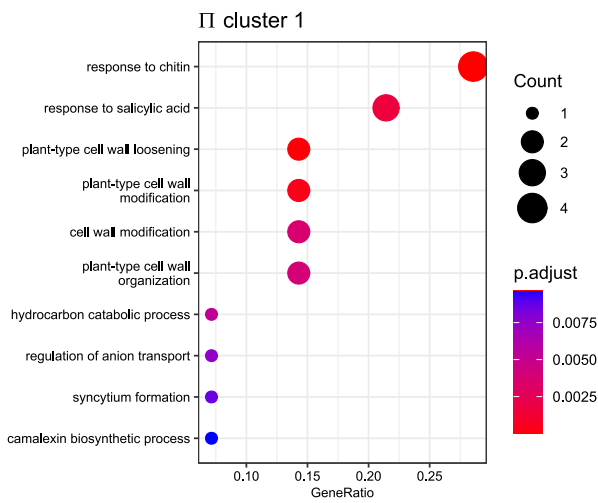
P-specific, cluster 1



P-specific, cluster 2



B



C

	GO response to stimulus	GO response to abiotic stimulus	GO response to salt stress	general stress response	transcription factor	II-specific cluster 1	II-specific cluster 2	P-specific cluster 1	P-specific cluster 2
GO response to stimulus	83.3	31.2	10	16.5	2.5			1.6	4.2
GO response to abiotic stimulus		59.2	14.6	17.6	3.1			0.8	4.2
GO response to salt stress			8.7	4.9	1.5				0.7
general stress response				5.2	9.7				7.7
transcription factor					2				3.3
II-specific cluster 1							-0.4	-0.7	-0.9
II-specific cluster 2								-0.5	-0.5
P-specific cluster 1									-1
P-specific cluster 2									

Figure 6

A: output of the Genecloud semantic analysis for the 2 clusters showing a significant enrichment. B: Output of a gene ontology analysis for the 2 clusters showing significant enrichments in GO terms. The GO terms, gene counts for each GO term and adjusted p-value of the enrichment are presented. C: Degree of overlap (Z-score) between our genes lists and public genes lists related to osmotic stress, the general stress response, and a list of transcription factors, using the Geneset algorithm.