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**Jasmonate signaling controls negative and positive effectors of salt stress tolerance in rice**

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**Running head:** The jasmonate-dependent components of salt-stress response in rice

35 **Highlight**

36

37 Jasmonate dually impacts osmotic and ionic components of salt stress in rice. Jasmonate is  
38 required for abscisic acid-mediated water deprivation responses, but inhibits Na<sup>+</sup> retention in  
39 roots, promoting leaf damage.

40

41

42 **Abstract**

43

44 Plant responses to salt exposure involve large reconfiguration of hormonal pathways that  
45 orchestrate physiological changes towards tolerance. Jasmonate (JA) hormones are essential to  
46 withstand biotic and abiotic assaults, but their roles in salt tolerance remain unclear. Here we  
47 describe the dynamics of JA metabolism and signaling in root and leaf of rice, a plant species  
48 that is highly exposed and sensitive to salt. Roots activate the JA pathway in an early pulse,  
49 while 2<sup>nd</sup> leaf displays a biphasic JA response with peaks at 1 hour and 3 days post-exposure.  
50 Based on higher salt tolerance of a rice JA-deficient mutant (*aoc*), we examined through kinetic  
51 transcriptome and physiological analysis the salt-triggered processes that are under JA control.  
52 Profound genotype-differential features emerged that could underlie observed phenotypes.  
53 ABA content and ABA-dependent water deprivation responses were impaired in *aoc* shoots.  
54 Moreover, *aoc* accumulated more Na<sup>+</sup> in roots, and less in leaf, with reduced ion translocation  
55 correlating with root derepression of the *HAK4* Na<sup>+</sup> transporter. Distinct reactive oxygen  
56 species scavengers were also stronger in *aoc* leaf, along with reduced senescence and  
57 chlorophyll catabolism markers. Collectively, the data identify contrasted contributions of JA  
58 signaling to different sectors of the salt stress response in rice.

59

60 **Keywords:** jasmonate, rice, salt stress, signaling, tolerance, transcriptome

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## 68 1. INTRODUCTION

69

70 Upcoming global climate perturbations are imposing more frequent stressful conditions to  
71 plants, including flood, drought, or extreme temperatures episodes. Soil salinization adds as an  
72 increasing limiting factor to crop productivity in coastal agricultural areas, causing growth  
73 retardation and yield losses. Rice (*Oryza sativa*), the major staple food crop for more than half  
74 of the world's population, is particularly exposed to stress and due to its uppermost importance  
75 for food security, has been established as a model species for monocotyledonous and cereal  
76 plants. As a semi-aquatic species partly cultivated in lowland, rice is increasingly exposed to  
77 salt contaminated water, urging the need to breed and disseminate more salt tolerant varieties.  
78 The physiological responses of rice to salt have been extensively analyzed and reviewed, based  
79 on the study of a wide variety of sensitive and tolerant cultivars and wild relatives (Ponce *et al.*,  
80 2021, van Zelm *et al.*, 2020).

81

82 Salt uptake and sensing by plant roots triggers rapid osmotic stress due to a reduction in water  
83 potential, followed by a slower ionic stress phase due to sodium toxicity in tissues (Yang and  
84 Guo, 2018). Early salt-induced responses include phospholipid signaling at the plasma  
85 membrane, initiation of specific calcium waves decoded by calcium-binding proteins and  
86 kinases followed by sodium channels activation like NHX7 involved in export of Na<sup>+</sup> out of  
87 the cell (van Zelm *et al.*, 2020). Salt stress rapidly induces reactive oxygen species (ROS) in  
88 the apoplast through the activation of respiratory burst oxidase homologs (Rbohs) and ROS act  
89 together with Ca<sup>2+</sup> signaling to affect cellular pH and ion homeostasis (Hasanuzzaman *et al.*,  
90 2021). Ion distribution is orchestrated by multiple families of transporters whose members  
91 localize distinctly on the plasma membrane or endomembranes in cell specific patterns (Saddhe  
92 *et al.*, 2021) to exclude or sequester Na<sup>+</sup> ions. Continuous Na<sup>+</sup> uptake perturbs K<sup>+</sup> homeostasis,  
93 nutrient acquisition and compromises efficiency of cellular processes including photosynthesis,  
94 while inducing cell wall modifications (Liu *et al.*, 2021) and starch metabolism (Thalman and  
95 Santelia, 2017).

96

97 Immediate salt responses further transduce into the complex reconfiguration of hormone  
98 metabolism and signaling programs (Yu *et al.*, 2020). Abscisic acid (ABA) is the dominant  
99 hormone that governs cellular and metabolic responses to drought or salt exposure (Sah *et al.*,  
100 2016, Vishwakarma *et al.*, 2017). By promoting rapidly stomatal closure, ABA reduces  
101 evapotranspiration and controls overall water transport, in addition to modulating the

102 expression of arrays of genes involved in the adaptation response, including starch degrading  
103 enzymes (Thalman and Santelia, 2017). Additionally, salt triggers a decrease in osmotic  
104 pressure and water loss, that is mostly compensated by the ABA-dependent accumulation of  
105 various osmolytes such as amino acids or sugar alcohols, and the induction of water deprivation  
106 responses.

107

108 More recent evidence indicates that several other plant hormones, acting in complex synergistic  
109 or antagonistic signaling cross-talks, impact salt responses and tolerance (Choudhary *et al.*,  
110 2021, Yu *et al.*, 2020). Jasmonates (JAs) act as essential mediators of defense programs to biotic  
111 attacks (Heitz, 2021), but have also been associated with responses to various forms of abiotic  
112 stress (Kazan, 2015). In the case of salt tolerance, contrasting results and conclusions have been  
113 reported using different experimental setups or plant species, so that JA functions under salt  
114 stress are still debated (Delgado *et al.*, 2021, Riemann *et al.*, 2015). External treatments with  
115 JA were shown to attenuate salt-induced damage in some species like wheat (Qiu *et al.*, 2014)  
116 or barley (Walia *et al.*, 2007), but in *Arabidopsis*, salt triggers JA-mediated root growth  
117 inhibition (Valenzuela *et al.*, 2016) and external methyl-JA supply aggravates salt-triggered  
118 growth inhibition and senescence (Chen *et al.*, 2017). Furthermore, in tomato, a JA-deficient  
119 line exhibited enhanced oxidative stress and associated damage relative to wild-type  
120 (Abouelsaad and Renault, 2018). However, in rice, a growing body of evidence suggests that  
121 endogenous JA accumulation and signaling is detrimental to salt tolerance, at least at the  
122 seedling stage. Salt-induced inhibition of rice seminal root growth is mediated by ethylene-  
123 jasmonate interaction (Zou *et al.*, 2021). JA-deficient rice mutants *cpm2* and *hebiba* displayed  
124 less Na<sup>+</sup> accumulation and increased ROS-scavenging activity in shoots, correlated with  
125 attenuated damage upon salt exposure (Hazman *et al.*, 2015). Genes encoding catabolic  
126 enzymes acting on jasmonoyl-isoleucine (JA-Ile), the bioactive jasmonate hormone, have been  
127 identified in rice and are upregulated by salinity (Hazman *et al.*, 2019). Interestingly, a rice  
128 activation-tagging line overexpressing CYP94C2b, a cytochrome P450 oxidizing JA-Ile,  
129 displays enhanced survival rate under salt conditions (Kurotani *et al.*, 2015a). In addition, upon  
130 investigating a collection of extant rice accessions with various sensitivities, high salt-tolerance  
131 was found correlated with elevated expression of this gene (Kurotani *et al.*, 2015b). Among the  
132 known physiological and molecular responses of rice to salt, very few have so far been linked  
133 to JA signaling. Rice Salt Sensitive 3 (RSS3) is a nuclear factor that interacts with JAZ  
134 repressors to modulate the expression of JA-responsive genes and root cell elongation during  
135 adaptation to salinity (Toda *et al.*, 2013). Suppression or overexpression of JAZ9

136 antagonistically affects ion uptake with correlative impact on the expression of some ion  
137 transporter genes (Wu *et al.*, 2015). Together, these data suggest that reduced JA-Ile  
138 accumulation and/or signaling ameliorates the capacity of rice to withstand salinity.

139

140 Multiple transcriptome comparisons between sensitive and tolerant rice varieties have been  
141 reported (Formentin *et al.*, 2018, Kawasaki *et al.*, 2001, Li *et al.*, 2020, Mirdar Mansuri *et al.*,  
142 2019, Razzaque *et al.*, 2017), but the peculiar biological processes and molecular targets  
143 regulated by JA signaling upon salt stress were not genetically addressed and remain largely  
144 unknown. In the present study, we compared the phenotypic, ionic, transcriptomic and several  
145 physiological characteristics of the Kitaake variety using the wild-type (WT) and a JA-deficient  
146 mutant interrupted in *ALLENE OXIDE CYCLASE* (*aoc*), an essential JA biosynthetic gene  
147 (Nguyen *et al.*, 2020). Extensive genotype comparison of transcriptomes in roots and shoot  
148 allow to pinpoint particular biological processes that are under JA regulation. We establish that  
149 absence of JAs confers an apparent better salt tolerance phenotype in seedlings, based on a  
150 optimized regulation of Na<sup>+</sup> distribution, and a delay in onset of senescence. However, JA  
151 deficiency substantially reduces the ABA content and water deprivation responses in leaf,  
152 resulting in impaired water management. Our study provides a rationale framework to  
153 understand the specific impacts of endogenous JA signaling on rice responses to salt stress.

154

155

## 156 2. MATERIALS AND METHODS

157

### 158 2.1 Plant culture and treatments

159

160 *Oryza sativa* L. ssp. japonica cv. Kitaake was used as WT and *aoc* as jasmonate deficient mutant  
161 (Nguyen *et al.*, 2020). Jasmonate deficiency leading to male sterility, *aoc* homozygous  
162 seedlings were isolated from the progeny of AOC/*aoc* plants. For salt stress experiments,  
163 caryopses of a segregating population were first preciously rinsed, placed on Petri dishes  
164 containing water imbibed Whatman paper, then placed for 4 days at 28°C in complete darkness  
165 for germination. Skotomorphogenic development in JA-deficient mutants leads to very long  
166 mesocotyl allowing the isolation of the *aoc* homozygous mutants. WT and *aoc* seedlings were  
167 then transferred to a floating styrofoam for hydroponic culture (MS solution: 358 mg L<sup>-1</sup> basal  
168 MS buffered with MES 42 mg L<sup>-1</sup>, pH 5.8) and incubated under continuous light of ~125 μmol  
169 m<sup>-2</sup> s<sup>-1</sup> at 28°C with 60% relative humidity. After 11 days growth, the MS solution was replaced

170 by a saline solution (MS solution containing 100 mM NaCl) for the salt treatment and a new  
171 MS solution for the mock treatment.

172 For MeJA treatment, WT rice seeds were dehusked and surface-sterilized through successive  
173 baths in 70% ethanol (1 min), distilled water (1 min) and in a sodium hypochlorite solution  
174 containing ~3 % active chlorine (20 min) followed by 4 washing steps with distilled water under  
175 sterile conditions. Seeds were then sown in magenta boxes containing 0.4% phytoagar medium  
176 in MS as above and cultivated under same conditions as for the salt experiment. After 7 days,  
177 a cotton wick with 20  $\mu$ L of pure MeJA was placed in each magenta box. All samples analyzed  
178 are constituted of a pool of tissues from three plants.

179

## 180 **2.2 Stomatal conductance and relative water content (RWC)**

181

182 For stomatal conductance measurements, plants were raised in a hydroponic system as  
183 described above until the 5<sup>th</sup> leaves were fully expanded. Leaf stomatal conductance to water  
184 vapor (gsw) was measured with a LI-6800 portable photosynthesis system (LI-COR, Lincoln,  
185 NE) on the 5<sup>th</sup> leaf. Measurements were taken in an alternating manner from WT, mutant,  
186 control and stressed plants, respectively, from 1-7 h after day start (12 h days) to avoid bias  
187 imposed by the diurnal rhythm.

188 For RWC determination, leaf sheaths were collected from plants and immediately weighed to  
189 determine their fresh weight (FW). After 4 h hydration in plastic bags containing distilled water,  
190 the leaves were weighed again to measure the turgescence weight (TW). Then, leaves were dried  
191 for 24 h at 60° C and their dry weight (DW) was determined. RWC was calculated based on  
192 the following formula:  $RWC (\%) = [(FW-DW) / (TW-DW)] \times 100$  described by Barr and  
193 Weatherley (Barrs and Weatherley, 1962).

194

## 195 **2.3 Electrolyte leakage**

196

197 Leaf blades were harvested and quickly rinsed in distilled water to remove ions on the leaf  
198 surface. Leaves were then cut into small pieces (1-2 cm) and floated onto 15 ml distilled water  
199 in 50 mL tube. After 1 h gentle shaking at room temperature, water electrical conductivity of  
200 solution was determined to estimate ion leakage. Samples were then boiled for 30 min before  
201 cooling for 1 h to room temperature. Solution conductivity was re-measured to estimate the  
202 total released ions. Percentage of released ions from dead cells was calculated using the  
203 following formula:  $[\text{conductivity at } T_0 / \text{conductivity of boiled samples}] \times 100$ .

204

## 205 **2.4 Ion content measurement**

206

207 Samples were ground frozen before being dried for 48 h at 60°C. Samples were digested using  
208 0.5 mL ultrapure water, 2 mL HNO<sub>3</sub> (65% v/v, subboiled) and 0.5 mL H<sub>2</sub>O<sub>2</sub> (30% v/v, p.a.) in  
209 closed digestion tubes (Gerhardt, UK) in a heating block (DigiPrep jr, S-prep) system at 110°C  
210 for 3 h. Samples were evaporated to near dryness and after cooling final volume of each sample  
211 was adjusted to 20 mL with 1% v/v HNO<sub>3</sub> (subboiled). To check for the quality, we included  
212 blank samples and different reference materials (NIST 1573a (Tomato leaves), NCS ZC' 73013  
213 (Spinach), Spruce needles (ring test) into the digestion procedure. Potassium and sodium  
214 content in the digest were measured by inductively coupled plasma optical emission  
215 spectrometry (ICP-OES, radial mode, iCap 7000, Thermo Fisher) in the Laboratory for  
216 Environmental and Raw Materials Analysis (LERA) at KIT. The accuracy of the reference  
217 material was in a good range with ± 5.9% (K) and ±7.1% (Na) for the NIST 1573a, ± 3.2% (K)  
218 and ±3.52% (Na) for the NCS ZC' 73013 and ± 8.7% (K) for the spruce needles.

219

## 220 **2.5 Chlorophyll quantification**

221

222 Frozen leaf samples were ground with glass beads (10 s, 5500 rpm, Precellys tissue  
223 homogenizer, Bertin Instruments, France) then homogenized with 80% cold acetone to extract  
224 chlorophylls (2 x 30 s, 5500 rpm). After sedimentation of cell debris by centrifugation,  
225 supernatant absorbance at 647 and 663 nm were measured to calculate the chlorophylls content  
226 based on the equations described in Porra *et al.* (1989).

227

## 228 **2.6 RNA extraction and gene expression analysis**

229

230 Frozen roots were ground manually in a mortar and total RNA was isolated using RNeasy Plant  
231 Mini kit (Qiagen). Leaf samples were ground using the glass-bead homogenizer before RNA  
232 isolation using Trizol Reagent according to manufacturer instructions. For the purpose of  
233 RNAseq, total RNA integrity was controlled after DNase treatment using a Bioanalyzer 2100  
234 system with the RNA 6000 Nano Chip (Agilent Technologies, Santa Clara, USA). RNA with  
235 RNA integrity Number (RIN) value above 6.2 were used to construct cDNA libraries. Library  
236 construction and sequencing was performed at Novogene Europe (Cambridge, UK) on an  
237 Illumina platform (paired-end reads, 150 nucleotides). After exclusion of low quality reads and



238 reads with adaptors, clean reads were mapped to the reference genome Os-Nipponbare-  
239 Reference-IRGSP-1.0 using HISAT2. The resulting read counts were processed through the  
240 DIANE pipeline (Cassan *et al.*, 2021) for global normalization (EdgeR package), filtering of  
241 very low expressed genes (< 480 total read counts) and differential expression analysis (DeSeq2  
242 R package) for which only genes with an absolute log<sub>2</sub> Fold Change (log<sub>2</sub>FC) ≥ 1 and an FDR  
243 < 0.05 were considered as DEGs. GO enrichment analysis was performed with the PANTHER  
244 classification system (geneontology.org; (Mi *et al.*, 2019) and focused on biological processes  
245 enriched with an FDR < 0.01.

246 For RT-qPCR analysis, cDNA was synthesized with Superscript IV Reverse Transcriptase  
247 (Thermofisher) using 2 µg of RNA. qPCR was performed using 20 ng of cDNA on a  
248 LightCycler 480 II instrument (Roche Applied Science, Penzberg, Germany) as described in  
249 Berr *et al.* (2010). The expression levels of the different targets were normalized against the  
250 expression level of the reference genes *UBQ5* (Os01g0328400) and *UBQ10* (Os02g0161900).  
251 The sequences of all primers used are listed in supplemental Table S4.

252

## 253 **2.7 Hydrogen peroxide staining**

254

255 3,3'-diaminobenzidine (DAB) staining was performed as described by (Daudi and O'Brien,  
256 2012). Leaves of 5 days salt- and mock-treated rice seedling were collected, submerged in the  
257 DAB solution (1 mg mL<sup>-1</sup> DAB, 10 mM Na<sub>2</sub>PO<sub>4</sub>, 0.05% Tween). Since DAB is light sensitive,  
258 the preparation was covered with aluminum foil and incubated 4-5 h at room temperature under  
259 constant shaking (100 rpm). Leaves were then transferred to a clearing solution (ethanol/acetic  
260 acid/glycerol 3/1/1) and boiled for 20 min to bleach out the chlorophyll and reveal the H<sub>2</sub>O<sub>2</sub>  
261 staining.

262

## 263 **2.8 Hormone profiling**

264

265 Jasmonate/ABA profiling was performed as described in Marquis *et al.* (2022)

266

## 267 **2.9 Statistical Analysis**

268

269 All statistical analysis was performed on Rstudio. Comparisons of sample means were  
270 performed by one-way analysis of variance (P < 0.05) then Tukey's HSD multiple comparisons

271 tests. Significant differences of means were determined and represented using these following  
272 significant codes: “\*”:  $P < 0.05$ ; “\*\*\*”:  $P < 0.01$  and “\*\*\*\*”:  $P < 0.001$ ).

273

## 274 **3 RESULTS**

275

### 276 **3.1 Jasmonate deficient *aoc* mutant displays reduced symptoms upon salt stress**

277

278 The Kitaake variety of rice was chosen for its short life cycle and the availability of a mutant  
279 line obtained by CrisprCas9. This line bears a disruption of the single copy *ALLENE OXIDE*  
280 *CYCLASE (AOC)* gene, encoding an essential activity for jasmonate biosynthesis (Nguyen *et*  
281 *al.*, 2020). Due to nearly full sterility, homozygous *aoc* plants were selected from a segregating  
282 population based on skotomorphogenic development after 4 days germination in the dark  
283 (Riemann, 2020), and then transferred for hydroponic cultivation for 7 days before exposure of  
284 roots to control or salt-containing solution. Salt exposure resulted in shoot growth inhibition as  
285 expected in both genotypes (Figure S1a), but reduction of seminal root length observed in WT  
286 was absent in *aoc* (Figure S1b), in accordance with previous results (Zou *et al.*, 2021). Among  
287 different concentrations of NaCl tested, 100 mM salt resulted in a differential tolerance  
288 phenotype visible from 4 days after onset of stress, and that was reminiscent of the observations  
289 of Hazman *et al.* (Hazman *et al.*, 2015) in the Nihonmasari cultivar. In WT Kitaake plants, the  
290 second (2<sup>nd</sup>) leaf first displayed severe symptoms of browning and drying starting from the leaf  
291 tip, whereas *aoc* 2<sup>nd</sup> leaf remained essentially symptom-free (Figure 1a). After longer times of  
292 incubation, the damage eventually spread to leaf 3 or even 4, while maintaining the genotype  
293 differential phenotype. Electrolyte leakage was measured as a readout of tissue damage and  
294 was found reduced in *aoc* leaves 2 and 3 relative to their WT counterparts (Figure 1b),  
295 confirming weaker disruption of tissue integrity in *aoc*.

296

### 297 **3.2 Salt stress triggers jasmonate metabolism with distinct patterns in rice roots and** 298 **shoots**

299

300 To investigate the time-resolved activation of jasmonate metabolism and signaling in WT  
301 plants, we collected separately root and 2<sup>nd</sup> leaf samples in a kinetic salt stress experiment, and  
302 submitted tissue extracts to quantitative jasmonate profiling by LC-MS/MS. Roots exhibited a  
303 rapid drop in their content in the JA precursor oxo-phytodienoic acid (OPDA) in response to  
304 salt, but JA levels remained stable throughout the experiment (Figure 2a). JA-Ile, the active

305 hormonal compound, was only occasionally detected (at very low levels in some replicates) in  
306 the early time points, whereas more significant increases were recorded at 3 and 5 days of stress.  
307 The more stable JA-Ile catabolite 12COOH-JA-Ile (Heitz *et al.*, 2012) was overaccumulated  
308 from 6 h and later on, indicating elevated metabolic flux through the JA-Ile pathway in salt-  
309 responding roots. In the second leaf, OPDA levels were low under control conditions and  
310 increased in a biphasic manner with an early peak between 1 and 6 h and a later one at 3-5 days  
311 post-salt exposure (Figure 2b), when visible tissue damage occurs. JA, JA-Ile and 12COOH-  
312 JA-Ile essentially followed similar dynamic trends than OPDA upon time, with 12COOH-JA-  
313 Ile being the highest accumulated compound. These results indicate the activation of complex  
314 jasmonate metabolism in rice seedlings in response to salt stress, with organ-specific temporal  
315 patterns and individual compound abundances in roots and in 2<sup>nd</sup> leaf.

316

### 317 **3.3 Comparative analysis of differential transcriptome in WT and JA-deficient seedlings** 318 **upon salt stress**

319

320 To generate a global overview of the influence of jasmonate signaling on salt-triggered  
321 processes, we undertook a comparative transcriptome analysis in both roots and 2<sup>nd</sup> leaf of WT  
322 and *aoc* plants, in a kinetic study collecting samples at 0, 1 h, 6 h and 3 d after salt exposure.  
323 RNAseq was conducted on 3 independent biological replicates, and the distribution of samples  
324 in a two-dimensional Principal Component Analysis (PCA) was established. Root and shoot  
325 samples were largely separated, illustrating their unique transcriptomes (Figure S2a). When  
326 datasets from each plant organ were processed separately, additional time dispersions were  
327 visible. In roots, 1 h and 6 h time points were well separated from 0 h and 72 h (Figure S2b),  
328 whereas in leaf, 1 h samples remained close to 0 h (Figure S2c). After normalizing the full  
329 dataset, we extracted from WT data the expression patterns of genes involved in JA metabolism  
330 and signaling (Table S1) relative to 0 h. This analysis revealed early upregulation of several  
331 genes at 1 and 6 h in roots such as those encoding JA-Ile catabolic CYP94 enzymes (Hazman  
332 *et al.*, 2019) or JAZ proteins, and a biphasic induction in leaf at 1 h and 72 h (Figure 3a). These  
333 transcriptional behaviors closely mirror the distinct patterns of hormone/catabolite variations  
334 (Figure 2) and further illustrate that salt stress activates distinct JA signaling dynamics in  
335 below-ground and aerial parts of the plant.

336

337 We next filtered differentially expressed genes (DEGs) within each organ, with respect to time  
338 and genotype comparisons, using a log<sub>2</sub> Fold Change (log<sub>2</sub>FC) of  $-1 < \log_2\text{FC} < 1$  and a false

339 discovery rate (FDR) < 0.05. Before salt stress application, a significant number of DEGs (179  
340 in roots, 964 in 2<sup>nd</sup> leaf) were revealed between *aoc* and WT (Figure 3b, 0 h). In time  
341 comparisons relative to their respective 0 h (Figure 3c), DEGs were most abundant at early (1  
342 h and 6 h) time points after salt exposure in roots, whereas their rise in leaf occurred later at 6  
343 h and 72 h (Figure 3c). In direct genotype comparisons (Figure 3b), except at 0 h, expression  
344 of a majority of DEGs were found reduced (labelled as down) in *aoc* mutant relative to WT at  
345 all time points. This suggests that jasmonate signaling contributes significantly to the massive  
346 gene expression reprogramming under salt stress conditions.

347

### 348 **3.3.1 Transcription factor gene dynamics and JA-dependence**

349

350 To globally estimate the extent of transcriptional changes that are under JA control, we set out  
351 to determine the dynamics of transcription factor (*TFs*) gene expression upon salt response, in  
352 particular with regards to their differential expression in both genotypes. Expression data were  
353 analyzed in time comparisons ( $-1 < \log_{2}FC < 1$ ; FDR < 0.05), and organized as UpSet diagrams  
354 (Lex *et al.*, 2014), that allow to intersect distinct expression patterns at one or several time  
355 points and visualize common or diverging behaviors in WT or *aoc*. In roots, both genotypes  
356 show about the same number of deregulated TFs at all time points after salt exposure, with the  
357 largest set of TF genes upregulated at 1 h, and their number then declined (Figure 4a left panel).  
358 Few TFs were specifically upregulated in *aoc* or WT (21 and 19 at 1 and 6 h in WT respectively)  
359 compared to the many (71 at 1 h, 23 at 6 h and 39 at 1 and 6 h) found similarly affected by salt  
360 in both genotypes. For salt-downregulated TFs in roots, genotype-specific behaviors were more  
361 common, mostly at 1 h and 72 h, indicating a stronger influence of JA signaling. In 2<sup>nd</sup> leaf,  
362 WT shows much more up-regulated TF genes at 1 h and 72 h than *aoc* but, in contrast, less  
363 down-regulated TF genes at all time points after salt exposure. These differences reflect  
364 particularly in large sets of TF genes specifically responsive in WT at 1 h (35 TFs) and 72 h  
365 (56 TFs) after salt exposure. The analysis indicates a significant influence of JA signaling on  
366 regulators of the leaf salt response, with mostly positive and few negative impacts of this  
367 hormone on TF gene expression. Finally, we crossed the lists of genotype-differential (*aoc* vs  
368 WT) TF genes in root with those in shoot (Figure S3). This highlighted the low proportion of  
369 shared TFs, suggesting that distinct JA-controlled regulators are driving transcriptional changes  
370 in below and above-ground parts of rice plants under salt.

371

372 As a case study, we next extracted the TF genes that are exclusively deregulated on one hand  
373 at 1 h and 6 h in WT root, and on the other hand at 1 h and 72 h in leaf, which are the materials  
374 where JA accumulation (Figure 2) and signaling (Figure 3a) was demonstrated. These genes  
375 are unresponsive in *aoc* and therefore likely to be activated in a JA-dependent manner and  
376 control JA-mediated processes. By doing so, we identified genes -mostly in leaf- that were  
377 previously associated to various extents with drought, dehydration, ABA, or abiotic stress  
378 responses but whose JA-dependence was largely unknown (Table 1). In addition, the filtering  
379 retrieved TF genes of the zinc finger, MYB, AP2, bHLH or WRKY families that were not  
380 previously linked to JA-dependent salt responses. This finding suggests a significant  
381 contribution of JA signaling to the water deprivation component of salt stress. Additional TF  
382 expression intersections can be filtered from Table S2 to investigate specific dynamic  
383 behaviors.

384

### 385 **3.3.2 Gene ontology analysis reveals biological processes impacted by jasmonate signaling** 386 **upon salt stress**

387

388 To sort out the biological processes affected with regard to organ, time and genotype, general  
389 DEG lists were submitted to gene ontology (GO) analysis, and GO terms were displayed as  
390 kinetic heatmaps. Two types of comparisons were utilized to extract biological information: on  
391 the one hand, were plotted time comparisons with enrichment of terms in salt-exposed samples  
392 relative to 0 h of respective genotype (Figure 5), and on the other hand genotype comparisons  
393 where *aoc* and WT enrichments were directly compared at each time point (Figure S4). Major  
394 known JA-dependent responses including genes involved in defense to biotic stress or  
395 wounding, as well as many genes encoding JAZ repressors were enriched throughout the whole  
396 kinetic in WT leaf, meaning their loss in *aoc* (Figure S4c right panel), whereas such a  
397 differential pattern was only recorded at 1 h and 6 h in roots (Figure S4a). Before stress (0 h),  
398 specific behaviors emerged for oxidative stress- and cell wall-related genes. For example, 5  
399 class III peroxidase genes were less expressed in *aoc* roots (Figure S4a), whereas in *aoc* leaf,  
400 nearly 20 peroxidase (Figure S5b) and 5 laccase genes were stronger expressed at 0 h, along  
401 with genes involved in secondary cell wall biogenesis, encoding cellulose synthases, expansins,  
402 pectin-methylesterases or hybrid prolin-rich proteins (HyPRPs) (for individual genes, see Table  
403 S3). This illustrates a significant and contrasted impact of JA signaling on the transcriptome  
404 under optimal growth conditions.

405

406 After exposure of roots to salt, numerous changes in GO term enrichment were recorded relative  
407 to non-stressed controls. Many of them had similar trends in the two genotypes, such as protein  
408 folding responses or response to heat that were shortly down at 1 h (Figure 5a bottom panel).  
409 Unexpectedly, several terms related to nitrate homeostasis, including *NRT* transporters and  
410 nitrate response genes, were downregulated at 72 h in *aoc* roots only. Among up-regulated  
411 terms, different terms related to ABA biosynthesis/responses, or water deprivation/osmotic  
412 responses showed various enrichment patterns: some transiently up in *aoc* at 1 h, related terms  
413 enriched only later in WT (Figure 5a, Table S3) (*see paragraph below*). Consistently, a number  
414 of known JA-regulated responses to biotic stress were enriched in WT (Figure S4a right panel).  
415 More surprisingly, several photosynthesis-related terms were found enriched in WT roots at 72  
416 h only. This may result from the hydroponic culture that favors greening of roots (Figure S4b);  
417 however, expression magnitude of these genes remained marginal in roots compared to shoots.  
418 Conversely, other terms were enriched at 72 h in *aoc* roots, such as some defense-related genes  
419 including several chitinases (also in ‘cell wall catabolic process’ term), *SUCROSE SYNTHASE*  
420 *7*, and *SWEET* sugar transporters. These may represent JA-repressed components of  
421 carbohydrate metabolism and transport in roots.

422  
423 The transcriptional landscape in 2<sup>nd</sup> leaf appeared largely different. Few (147) genes were  
424 downregulated by salt relative to 0 h at 1 h in WT, in contrast to *aoc* (765) (Figure 3c). In *aoc*,  
425 genes associated with lignin polymerization (7 laccases) dropped transiently. As well, most GO  
426 terms linked to cell wall biogenesis and oxidant detoxication, response to oxidative stress  
427 (mostly peroxidases) that were found enriched in *aoc* leaf at 0 h (Figure S4c left panel) were  
428 downregulated from 1 h to 72 h (Figure 5b bottom panel; Figure S5b). In addition, numerous  
429 terms related to water deprivation and ABA response were enriched in the up-response at 1 h  
430 and 72 h in WT but barely in *aoc*. These comprised for example genes encoding dehydrins,  
431 trehalose biosynthesis and cell wall biogenesis functions (Figure 4b right panel top, *see*  
432 *paragraph below*) and suggests that several processes are absent in *aoc* leaf at early time points  
433 of the salt response. In the genotype comparison, an enrichment in photosynthesis terms (‘light  
434 harvesting, pigment biosynthesis process’) in *aoc* is prominent (Figure S4c), indicating that the  
435 mutant may be prone to better maintain photosynthetic capacity under salt stress. At 6 h salt  
436 exposure, the response amplified in leaf, with a bulky group of terms that is shared between  
437 genotypes but with extended expression in *aoc*, and which relates to RNA processes, such as  
438 exosome complex components, mRNA surveillance and processing, and ribosome assembly  
439 (Figure 5b). This could be interpreted as a recovery/compensatory response of the leaf to the

440 initial rapid osmotic stress. Consistent with higher expression of photosynthesis components at  
441 1 h, *aoc* leaves exhibited an enrichment in genes related to starch synthesis, recorded at 72 h  
442 (Figure 5b, top panel ‘glycogen biosynthetic process’), which is reflective of a better energetic  
443 status of *aoc* leaf. A persistent anabolic activity is also illustrated by enrichment of primary  
444 cellular functions in *aoc* at 72 h such as protein translation or amino acid biosynthetic processes.  
445 This was accompanied by the upregulation of a large set of glutathione S-transferase genes at  
446 72 h (Table S3).

447 The global analysis therefore disclosed a significant impact of JA signaling, on root but more  
448 substantially on leaf transcriptome. Specific processes are altered in non-stressed conditions,  
449 but differential features were more prevalent upon salt stress, where JA deficiency seems to  
450 impair full induction of leaf ABA responses and oxidative stress related genes, and conversely  
451 allows for sustained maintenance of photosynthetic and carbohydrate storage components.

452

### 453 **3.4 JA signaling is required in leaves but not in roots for ABA biosynthesis and water** 454 **deprivation management under salt stress**

455

456 Given their genotype-specific GO term enrichment and importance in salt tolerance, ABA  
457 metabolism and water deficiency responses were further investigated. Genes encoding ABA  
458 biosynthetic or catabolic activities were examined, and their expression was found similarly  
459 salt-induced in roots of both genotypes (Figure 6a; Table S3). In contrast, their bi-phasic  
460 expression recorded at 1 h and 72 h in WT leaves, in particular for the induction of the rate-  
461 limiting *NCED* genes in ABA biosynthesis, was lost in *aoc*. Consistently, ABA contents in both  
462 organs supported these distinct expression patterns. In roots, ABA levels peaked similarly at 1  
463 h in WT and *aoc* and declined thereafter, whereas in leaf, ABA build-up was weaker in *aoc*  
464 compared to WT at 3 time points (Figure 6b). Concerning water deprivation response genes  
465 that are largely under ABA control, their root response remained essentially unaffected by JA  
466 deficiency, but in leaf, two groups of genes with distinct behaviors were revealed: the largest  
467 group including a number of signaling genes encoding PYL receptors, TFs or a MAP kinase  
468 and behaved similarly in *aoc* and WT (Figure 6c). This was in contrast with a smaller group of  
469 genes including *RAB* and *dehydrin* genes that were co-induced with JA pulses at 1 h and 72 h  
470 in WT, but were downregulated or unresponsive in *aoc*. Of note, expression of *OSCA1.1*  
471 (Os01g0534900), encoding a hyperosmolality-gated calcium channel (Zhai *et al.*, 2021) was  
472 slightly reduced in both *aoc* organs (Table S3). Following our observation of accelerated rolling  
473 of *aoc* detached leaf material upon symptom imaging, we determined that stomatal conductance

474 to water (gsw) dropped dramatically upon salt stress, reflecting stomatal closure, but  
475 significantly less in *aoc* (Figure 6d). Consistently, relative water content (RWC) was  
476 significantly reduced in *aoc* compared to WT upon salt stress (Figure 6d). Altogether, our  
477 results indicate that while the ABA transcriptional response remains largely functional in roots,  
478 in leaves, disrupted JA pathway prevents the full activation of ABA biosynthesis and optimal  
479 induction of osmotic stress/water deprivation responses, which in turn impinges on proper  
480 water management.

481

### 482 **3.5 JA signaling contributes to ion homeostasis and root-to-shoot sodium translocation**

483

484 Sodium uptake by roots upon salt exposure imposes ionic stress throughout the plant that  
485 negatively affects cellular processes (Yang and Guo, 2018). Ion homeostasis that is maintained  
486 by various ion transporters is perturbed by Na<sup>+</sup> influx, and adaptation mechanisms are required,  
487 for example, to sequester Na<sup>+</sup> in vacuoles or exclude it from cells to lower cytoplasmic  
488 concentrations (Saddhe *et al.*, 2021). Very little is known to which extent JA signaling is  
489 involved in modulating ion transporter gene expression, or how it impacts long-distance Na<sup>+</sup>  
490 transport. *OsJAZ9* misexpression was reported to modulate a few transporters (Wu *et al.*, 2015),  
491 but no systematic analysis has been conducted so far. Individual ion transporters within  
492 multigene families have particular expression patterns and may encode distinct specificities,  
493 making it difficult to infer profiles from general GO analysis. Hence we undertook a more  
494 specific examination of JA impact on ion transporter expression by inspecting the profiles of  
495 about 70 rice genes (Table S1) that encode confirmed or putative Na<sup>+</sup> or K<sup>+</sup> transporters. Genes  
496 for which expression was differential in at least one time comparison (Table S3) in either  
497 genotype ( $-1 < \log_{2}FC < 1$ , FDR < 0.05) were compiled in a heatmap. This retrieved 26 and 41  
498 transporter genes from root and leaf analysis respectively (Figure S6). Contrasted expression  
499 patterns were revealed, with both down- and up-regulation of various members; most of these  
500 perturbations were comparable in parallel time-analysis of *aoc* and WT in roots and shoot,  
501 indicating that these regulations were largely JA-independent. When expression was compared  
502 on a genotype basis at each time point, more specific features emerged: in 2<sup>nd</sup> leaf, most  
503 differentially expressed transporter genes were downregulated in *aoc*, including several  
504 members of the cation/proton exchanger family (CHX) of which some were associated  
505 positively or negatively to salt tolerance (Jia *et al.*, 2021). In roots, two transporter genes were  
506 strongly differential: *HAK4* and *HAK16* were found significantly more expressed at 72 h in *aoc*  
507 roots relative to WT (Figure 7a and Figure S8b). *HAK16* is a plasma membrane-localized



508 transporter involved in K<sup>+</sup> uptake and translocation to shoots (Feng *et al.*, 2019). HAK16  
509 belongs to cluster I of HAK transporters that are involved in K<sup>+</sup> uptake and translocation to  
510 shoots (Feng *et al.*, 2019, Véry *et al.*, 2014). HAK4 belongs to the cluster IV which groups  
511 HAK transporters closely related to PpHAK13, a Na<sup>+</sup> permease identified in *Physcomitrium*  
512 *patens* (Véry *et al.*, 2014). Specificity of the rice HAK4 for Na<sup>+</sup> rather than K<sup>+</sup> transport has  
513 been recently established in a report of ZmHAK4 that was characterized in maize as conferring  
514 natural variation of shoot salt tolerance owing to its role in Na<sup>+</sup> exclusion from xylem sap  
515 (Zhang *et al.*, 2019). In the same study, the rice ortholog (*OsHAK4*) was found similarly  
516 expressed in the root stele and exhibited comparable ion transport properties. Consistent with  
517 its elevated expression in JA-deficient *aoc* roots, *OsHAK4* expression in WT was progressively  
518 repressed by external JA treatment experiment (Figure 7b).

519  
520 To examine how such differential expression patterns of transporter genes relate to actual ion  
521 accumulation, we determined Na<sup>+</sup> and K<sup>+</sup> contents in roots and leaves before and 5 days after  
522 salt exposure. In our conditions, K<sup>+</sup> content was not affected by JA deficiency (Figure 7c). In  
523 roots, salt exposure reduced K<sup>+</sup> content by half with no JA-dependence, but salt had essentially  
524 no influence on K<sup>+</sup> content in 2<sup>nd</sup> and 3<sup>rd</sup> leaves. Na<sup>+</sup> content increased significantly to higher  
525 levels in *aoc* compared to WT roots upon stress. In contrast, Na<sup>+</sup> accumulation was reduced by  
526 44% in 2<sup>nd</sup> *aoc* leaf relative to WT whereas no difference was recorded in 3<sup>rd</sup> leaf. This indicates  
527 that higher *HAK4* expression levels correlate with enhanced Na<sup>+</sup> retention in *aoc* roots and  
528 lower Na<sup>+</sup> translocation to 2<sup>nd</sup> leaf, a relationship that could sustain the better salt tolerance of  
529 *aoc* leaf.

530

### 531 **3.6 JA signaling controls discrete ROS detoxifying genes**

532

533 Salt stress comes along with oxidative stress whose degree of management is an important  
534 parameter affecting tissue survival (Hasanuzzaman *et al.*, 2021, Liu *et al.*, 2020). We previously  
535 analyzed the activity of the enzymatic ROS in *cpm2* and *hebiba* mutant lines in leaves at 72 h  
536 post salt exposure and found that total glutathione S-transferase (GST), peroxidase (POD) and  
537 superoxide dismutase (SOD) activities were elevated in JA-deficient leaves compared to WT  
538 (Hazman *et al.*, 2015). Similar to the Nihonmasari cultivar used in that study, here DAB staining  
539 of ROS in Kitaake 2<sup>nd</sup> leaf visualized stronger signal in WT relative to *aoc* in response to salt,  
540 confirming reduced H<sub>2</sub>O<sub>2</sub> accumulation in absence of JAs (Figure 8a). Concerning  
541 transcriptome data, we screened the behavior of nearly 200 genes (Table S1) encoding ROS-

542 scavenging or -consuming activities, including ascorbate (APX) and class III peroxidases,  
543 catalases, GST, POD, RBOH and SOD. A majority of salt-triggered changes were similar in  
544 both genotypes, and are thus largely JA-independent (Figure S7a). Several *GST* genes appeared  
545 however less stimulated, or downregulated by salt in *aoc*, indicating a partial JA-dependence.  
546 The genotype comparison highlighted several *GST* genes that were more expressed in *aoc*  
547 leaves at 0 h (along with many POD genes, see Figure S5b), possibly protecting tissues even in  
548 non-salty conditions, but only *GSTU16* remained upregulated at 72 h in *aoc* (Figure 8b). In  
549 addition, this comparison disclosed the sustained expression in *aoc* leaf of three *SOD* genes:  
550 the Cu/Zn-dependent *SOD2* at 1 h, and *FSD1.1* and *FSD1.2*, encoding Fe-dependent  
551 chloroplastic SODs. Normalized counts of these candidate targets were plotted in Figure S8a  
552 and illustrate lower expression in WT. FSD activity may sustain stronger superoxide  
553 dismutation into less toxic hydrogen peroxide in sodium-challenged chloroplasts and alleviate  
554 salt-induced damage. Consistently, exogenous JA treatment repressed *FSD* expression in WT  
555 plants, confirming their negative regulation by JA signaling (Figure 8c). Altogether, JA  
556 deficiency better maintains or derepresses specific genes that could underlie the amelioration  
557 of distinct ROS scavenging activities.

558  
559

### 560 **3.7 JA deficiency delays salt-induced senescence and chlorophyll degradation**

561

562 Salt intoxication along with oxidative stress are known to result in accelerated leaf senescence  
563 (Figure 1) involving mainly ABA-mediated responses, but the specific contribution of JA  
564 signaling is less characterized (Lee and Masclaux-Daubresse, 2021). We therefore examined  
565 27 rice genes (Table S1), annotated as Senescence Associated (SAG), for their transcriptional  
566 behavior in leaf. Many SAGs were salt-induced in WT, particularly at 72 h (Figure S7b),  
567 including those encoding known transcription factors acting as positive regulators of  
568 senescence, such as *ERF101* (Lim *et al.*, 2020) or *NAP* (Liang *et al.*, 2014). Both time- (Figure  
569 S7b) and genotype-comparisons (Figure 9a) showed that their expression was lower in *aoc* leaf,  
570 in terms of intensity or timeframe, along with reduced expression of senescence execution  
571 genes such as *Chloroplast vesiculation CV* (Sade *et al.*, 2018), chlorophyll degrading genes  
572 including *StayGreen (SGR)* or *NYCI*, consistent with the phenotypically delayed senescence in  
573 *aoc*. Furthermore, the SAGs *OsI43* and *NAP* were confirmed to be JA-inducible upon  
574 exogenous treatment (Figure 9b). The impaired induction of genes promoting chlorophyll  
575 degradation is consistent with the stress-resilient expression of chlorophyll and other pigment

576 biosynthesis genes in *aoc* leaf when those decline in WT (Figure S4c). Consistently, *VTE3*,  
577 encoding a methyltransferase (Os07g0179300) in the biosynthesis of the chloroplastic  
578 antioxidant  $\alpha$ -tocopherol (Muñoz and Munné-Bosch, 2019) was higher expressed in *aoc* leaf in  
579 control and early stages of the response (Table S3). Inhibition of senescence-associated  
580 catabolic processes in *aoc* leaves was supported by the stability of chlorophyll content under  
581 salt stress (Figure 9c). In contrast, WT damaged 2<sup>nd</sup> leaf reduced by 39% its chlorophyll content  
582 while content remained stable in 3<sup>rd</sup> leaves that were symptomless in this experiment. These  
583 differential expression data provide a molecular basis for a delayed salt-induced senescence in  
584 JA-deficient rice.

585

#### 586 4. DISCUSSION

587

588 Plant exposure to excessive salt affects a myriad of biological processes and triggers complex  
589 adaptive changes to maintain physiological functions. Responses to salt stress have been  
590 extensively addressed in numerous model and non-model plant species at the physiological,  
591 cellular and molecular levels, and a number of tolerance-associated genes have been identified  
592 (Arif *et al.*, 2020, Ponce *et al.*, 2021, van Zelm *et al.*, 2020, Yang and Guo, 2018). Central to  
593 adaptation responses, hormonal interactions orchestrate complex reconfigurations (Choudhary  
594 *et al.*, 2021, Yu *et al.*, 2020). Most hormonal pathways are perturbed upon plant exposure to  
595 salt, typically abscisic acid is increased and directs many beneficial responses, but negative  
596 effects on tolerance have also been reported as for cytokinins or ethylene (Yang *et al.*, 2015).  
597 Jasmonate positive impacts in adaptation to some abiotic stresses such as cold or drought are  
598 being consolidated (Kazan, 2015, Marquis *et al.*, 2022, Riemann *et al.*, 2015). In contrast,  
599 understanding JA functions in salt tolerance is still blurred by contradictory reports between  
600 exogenous JA application -which may ameliorate tolerance-, and genetic data in rice from either  
601 JA biosynthetic mutants (Hazman *et al.*, 2015) or high catabolic lines (Kurotani *et al.*, 2015a,  
602 Kurotani *et al.*, 2015b), which suggest detrimental impacts of JA on salt tolerance. To solve  
603 this paradox, a side-by-side kinetic comparison was undertaken between a recently-established  
604 mutant that is fully devoid of JAs (Nguyen *et al.*, 2020) and its wild-type in the rapidly-growing  
605 cultivar Kitaake. Our goal was less to exhaustively describe transcriptional changes triggered  
606 by salt, that are widely documented in rice (Formentin *et al.*, 2018, Kong *et al.*, 2019, Li *et al.*,  
607 2020, Zhang *et al.*, 2022), than to map biological processes -and when possible individual  
608 genes- whose activity vary in presence or in absence of a functional JA biosynthetic pathway.

609

610 We analyzed simultaneously roots and first emerged leaves (2<sup>nd</sup> and 3<sup>rd</sup>) as salt is sensed first  
611 in roots and tissue damage was recorded primarily in leaf, and also because whole plant Na<sup>+</sup>  
612 management is critical to aerial organ tolerance. Under the conditions used, that impose a  
613 limited osmotic constraint before Na<sup>+</sup> toxicity builds-up, a clear differential phenotype was  
614 obtained with WT 2<sup>nd</sup> leaf undergoing extensive damage from day 4-5 while *aoc* remained  
615 essentially symptomless, a trend that later extends to new emerging leaves. These observations  
616 comfort a previous report in Nihonmasari cultivar (Hazman *et al.*, 2015) and demonstrate that  
617 interrupted JA biosynthesis mitigates damage in rice response to salt at the seedling stage. They  
618 further suggest that some JA-controlled processes translate into suboptimal resilience to salt.

619

620 To establish a comprehensive picture of jasmonate accumulation dynamics upon salt stress,  
621 detailed hormone profiling was performed in WT rice plants, revealing distinct patterns in each  
622 organ (Figure 2). In roots, early consumption of pre-existing precursor OPDA seemed to be at  
623 the basis of JA-Ile synthesis and catabolism with low amplitude. In 2<sup>nd</sup> leaf, all four analyzed  
624 compounds exhibited bi-phasic accumulation by 1 h and later by 3-5 days, the late increase  
625 being concomitant to leaf symptom appearance. The rapid pulses recorded here were frequently  
626 missed in previous studies. These hormonal patterns were consistently matched by JA pathway  
627 gene expression dynamics and together, the data highlight the organ-specific timeframes of  
628 activation of JA metabolism and signaling: within the first hours of salt exposure in roots,  
629 whereas the leaf response expresses as an immediate pulse by 1 h followed by a longer lasting  
630 activation starting by day 3. It is tempting to interpret that the early JAs accumulation may be  
631 due to the rapid osmotic stress component of salt exposure, and the second wave to  
632 consequences of the slower Na<sup>+</sup> accumulation in tissues.

633

634 Differential expression (DEGs) was mined for each organ in two dimensions: time-resolved  
635 comparisons allowed to assess dynamics relative to untreated controls, but such readouts are  
636 impacted by expression levels at 0 h which may vary for some genes between *aoc* and WT.  
637 Data were thus also compared directly between genotypes at each time point. Overall, DEGs  
638 number was highest at 1 and 6 h in roots and more delayed in leaf, reflecting long-distance  
639 spread of signaling and organ-specific responses. TF genes could be readily filtered for peculiar  
640 behaviors throughout the kinetic. For example, a number of TFs regulated only in WT were  
641 identified, and are thus JA-dependent; a few have previously been associated with drought or  
642 ABA responses, but others are of unknown function. In a global investigation, DEG lists were  
643 submitted to GO term enrichment analysis, of which only the most prominent outcomes can be

644 discussed here. The study should be taken as a resource that can be mined for many more  
645 processes than could be addressed presently. For example, many cell wall-related terms were  
646 perturbed by JA-deficiency in various ways before or after stress. The constitutive upregulation  
647 of expansins or HyPRPs in *aoc* leaf relates to two recent findings: ectopic overexpression of  
648 *EXP7* increases salt tolerance by promoting antioxidant activity, cell elongation and ion  
649 homeostasis (Jadamba *et al.*, 2020); as well, HyPRP06 regulates salt tolerance via apoplastic  
650 ROS homeostasis (Zhao *et al.*, 2022). By extension, the elevated expression of many class III  
651 peroxidases in *aoc* leaf may alter the redox status of the apoplast and facilitate cell wall integrity  
652 maintenance under salt (Liu *et al.*, 2021).

653

654 At least four major differential features emerged from the comparative transcriptome analysis,  
655 even though additional processes in the dataset deserve attention in the future:

656

657 1. The ABA pathway, whose activation is essential to the rapid response to abiotic stresses  
658 (Raghavendra *et al.*, 2010), was selectively impaired in JA-deficient *aoc* mutant. Global  
659 assessment indicated an enrichment in ABA-related GO terms in WT leaves, meaning their  
660 under-representation in *aoc*. Upon closer examination, a contrasted picture emerged: only a few  
661 ABA-induced targets, including the TF *ZFP36* controlling antioxidant defense (Zhang *et al.*,  
662 2014) were depressed in *aoc* roots, consistent with their near-WT ABA content (Figure 6). In  
663 contrast, ABA biosynthetic gene expression and ABA hormone build-up were much lower in  
664 the early phase of the response in *aoc* leaf, correlated with a strongly reduced expression of  
665 some -but not all- targets such as several *RAB* or *DHN* genes. This impaired ABA response was  
666 reflected physiologically by an incomplete drop in stomatal conductance in *aoc* leaf in response  
667 to salt, resulting in a reduced water content due to excessive transpiration in these tissues. The  
668 importance of ABA-JA interactions under drought was recognized previously in *Arabidopsis*  
669 (de Ollas and Dodd, 2016). Our findings highlight a distinct hormone interaction in under- and  
670 above-ground organs: roots deploy a proper ABA response in absence of JA, while adaptation  
671 to water deprivation in rice leaves relies on a positive ABA-JA crosstalk, at least for a peculiar  
672 sub-branch of the response. This also coincides with the independent observation that JA  
673 signaling is required in rice to withstand osmotic stress (Tang *et al.*, 2020).

674

675 2. A second major aspect affecting salt tolerance is linked to variations in management of Na<sup>+</sup>  
676 that floods the successive cell layers. Global expression survey illustrated the very diverse  
677 expression changes of ion transporters, even within a given gene family. Probably various

678 subcellular, cellular, tissue and organ-specific ion transporters show distinct reactivities to the  
679 massive influx of Na<sup>+</sup>. Most of these dynamics, irrespective of their importance under normal  
680 or stress conditions, were found unaffected by the status of JA signaling (Figure S6). Because  
681 Na<sup>+</sup> management and tissue content are critical parameters for salt tolerance (Ganie *et al.*, 2021,  
682 Ponce *et al.*, 2021), and JA-deficient rice mutants were reported to accumulate less Na<sup>+</sup> in  
683 leaves (Hazman *et al.*, 2015), we sought to identify candidate ion transporter genes that were  
684 impacted by JA signaling and that could account for differential Na<sup>+</sup> homeostasis in *aoc*. By  
685 filtering the genotype comparison, a low number of *aoc* leaf-downregulated genes popped-up  
686 from the CHX and HAK families (Figure 7) and could be at the basis of distorted ion  
687 homeostasis. More specifically, *HAK4* and *HAK16* were salt-repressed in WT but not in *aoc*  
688 roots. This is in contrast to *HAK12*, *17* and *24* that were salt-induced, irrespective of the JA  
689 biosynthetic capacity (Figure S6). *HAK16* functions in K<sup>+</sup> uptake and translocation to shoots  
690 (Feng *et al.*, 2019), and its root derepression correlates with unaltered K<sup>+</sup> content in *aoc* leaf  
691 under salt conditions (Figure 7c). *HAK4* was only recently characterized as a root transporter  
692 in rice and in maize where it confers natural variation of salt tolerance. In both species, it is  
693 believed to exclude Na<sup>+</sup> from xylem sap (Zhang *et al.*, 2019). Here, its upregulation in rice *aoc*  
694 roots, indicative of a JA-repression in WT, is fully consistent with more Na<sup>+</sup> being retained in  
695 *aoc* roots and less being translocated to shoots (Figure 7c), possibly contributing to attenuate  
696 leaf damage. This result constitutes a rationale basis to investigate genetically the potentially  
697 unique function of OsHAK4 in the JA-dependent control of root-to-shoot Na<sup>+</sup> translocation.

698  
699 3. ROS production and subsequent activation of detoxication systems are integral to the build-  
700 up of salt stress. JA deficiency resulted in genotype differential expression of a number of genes  
701 encoding ROS-metabolizing activities or affecting redox status. A large number of genes  
702 encoding apoplastic H<sub>2</sub>O<sub>2</sub>-consuming class III peroxidases were stronger expressed in non-  
703 stressed *aoc* leaf, which may contribute to lower resting ROS levels and better buffering of  
704 subsequent salt-induced ROS burst. In particular, a repression by JA signaling in WT of the Fe-  
705 dependent *FSD1.1* and *FSD1.2* was uncovered and point to a better superoxide ion scavenging  
706 in chloroplasts of *aoc* leaves (Figure 8). This reinforces chloroplasts as important sites for cell  
707 death initiation under salt stress in rice (Ambastha *et al.*, 2017).

708  
709 4. More directly linked to the visual leaf damage phenotype are the processes related to  
710 senescence. While the developmental senescence-promoting activity of JAs along with other  
711 stress hormones including ABA and ethylene is well-described (Wojciechowska *et al.*, 2018),

712 the extent to which JA signaling activates salt-induced senescence pathways was largely  
713 unknown. Here, the combined analysis of SAG expression, leaf tissue integrity and chlorophyll  
714 content establishes that JA signaling is a major mediator for the execution of senescence  
715 processes under salt stress in rice. Suppressing JA biosynthesis in *aoc* impaired or delayed most  
716 of these programs, resulting in extended viability of vegetative tissue. This is in full accordance  
717 with the delayed salt-induced senescence observed in a rice line overexpressing a JA-Ile  
718 catabolic gene (Kurotani *et al.*, 2015a). Moreover, the partial impairment of ABA signaling in  
719 *aoc* leaves could also underlie delayed senescence.

720

721 In conclusion, the extensive transcriptome and physiological analysis performed in this study  
722 have disentangled some of the contradictory results reported as to negative or positive impact  
723 of JA signaling on salt tolerance (summarized in Figure 10). Salt concentrations and timing  
724 applied are important parameters as to the relative strengths and dynamics of osmotic and ionic  
725 components of the stress. While it is difficult to establish a precise timeline of JA-dependent  
726 events because many components occur simultaneously in a given organ, a common starting  
727 feature is the early (1 h) hormonal response that translates into distinct transcriptional changes  
728 in root and shoot. We demonstrate that JA is required for ABA to co-regulate positively  
729 responses limiting water loss and that JA signaling triggers several pathways leading later on  
730 to salt-triggered leaf senescence. These JA-stimulated biological processes are coordinated and  
731 converge to accelerate tissue damage when ionic toxicity culminates. Within cell wall-related  
732 perturbations, some JA-dependent upregulated pectin methyl esterases in WT could trigger a  
733 reported MeOH-JA cascade that promotes senescence (Fang *et al.*, 2016). Conversely in *aoc*,  
734 increased expression of *HAK4*, a transporter gene under negative JA regulation, correlates with  
735 higher Na<sup>+</sup> retention in roots, protecting leaves where less toxic Na<sup>+</sup> is accumulated, senescence  
736 machinery remains silent, along with increased ROS scavenging capacity in chloroplasts. These  
737 different physiological features are in accordance with similar traits recorded in a recent study  
738 with JA-defective maize seedlings (Ahmad *et al.*, 2019). The present transcriptome dataset  
739 needs to be further explored to decipher the deeper consequences of JA signaling onto responses  
740 to salt; as an example mineral nutrition would be an important target to follow throughout the  
741 plant's lifecycle. With such dual impacts, JA signaling cannot be associated strictly anymore  
742 to either salt sensitivity or tolerance.

743

744

745

746

747 **SUPPLEMENTAL DATA**

748

749 **FIGURE S1.** Developmental phenotypes of rice wild-type (WT) and jasmonate-deficient (*aoc*)  
750 seedlings exposed to control or 100 mM NaCl (salt) solutions.

751

752 **FIGURE S2.** Principal component analysis (PCA) of RNAseq data distribution.

753

754 **FIGURE S3.** Overlap of deregulated transcription factor genes in shoots and roots

755

756 **FIGURE S4.** Gene ontology (GO) analysis of differentially expressed genes (DEGs).

757 **FIGURE S5.** Kinetic expression profile of rice class III peroxidase genes displayed as  
758 heatmaps in root (a) and 2<sup>nd</sup> leaf (b).

759

760 **FIGURE S6.** Kinetic expression profile of rice ion transporter genes displayed as heatmaps in  
761 root and 2<sup>nd</sup> leaf.

762

763 **FIGURE S7.** Kinetic expression profile of rice genes encoding ROS-scavenging or –  
764 consuming activities displayed as heatmaps in root and 2<sup>nd</sup> leaf of *aoc* and WT plants.

765

766 **FIGURE S8.** Kinetic expression profile of rice genes identified as probable targets of JA  
767 signaling upon salt stress.

768

769 **TABLE S1.** Lists of selected pathway genes that were screened for salt- and JA-regulated  
770 expression.

771

772 **TABLE S2.** Matrix of differential expression for genes encoding rice transcription factors.

773

774 **TABLE S3.** Global gene expression table in WT and *aoc* rice seedlings upon salt stress.

775

776 **TABLE S4.** Primers used for RT-qPCR experiments.

777

778

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780

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785

#### 786 **AUTHOR CONTRIBUTIONS**

787 TH, SN, MR and AC collectively designed the experiments. SN, THN, EE and TH performed  
788 most of the experiments. NP, FB and SS performed LI-COR measurements. VC and DP  
789 contributed to RNAseq data processing. TH, SN, AC and MR analyzed data. TH wrote the  
790 article with input of SN, MR and AC.

791

#### 792 **CONFLICT OF INTEREST**

793

794 The authors declare having no conflict of interest

795

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802

#### 803 **DATA AVAILABILITY**

804

805 Data supporting the findings in this study are available within the paper and within its  
806 supplementary materials published online. The raw and normalized RNAseq data were  
807 submitted to the NCBI Gene Expression Omnibus repository under accession GSE206706.

808

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**Table 1**

Organ	Gene ID	TFs name	TF family	Regulation	Stress-associated function	Reference (DOI)
2 <sup>nd</sup> Leaf	<i>Os08g0481400</i>	HOX20	HALZ	down	drought tolerance	
	<i>Os01g0738400</i>	C3H10	Zinc Finger	up	drought tolerance	10.3390/plants9101298
	<i>Os03g0264600</i>	-	-	up		
	<i>Os03g0820300</i>	ZFP182	Zinc Finger	up	salt tolerance	10.1016/j.bbexp.2007.02.006
	<i>Os05g0541400</i>	bHLH119/LF	bHLH	up		10.1093/mp/sss096
	<i>Os02g0764700</i>	ERF103	AP2	up	drought responsive	
	<i>Os06g0127100</i>	DREB1C	AP2	up		
	<i>Os08g0474000</i>	ERF104	AP2	up	drought responsive	
	<i>Os01g0859300</i>	ABI5/ABF1	bZIP_1	up	promotes salt sensitivity	10.1007/s11103-008-9298-4
	<i>Os01g0192300</i>	MYB1R1	Myb_DNA-binding	up		
	<i>Os01g0874300</i>	DLN31	Myb_DNA-binding	up		
	<i>Os02g0187700</i>	MYB1	Myb_DNA-binding	up		
	<i>Os02g0618400</i>	MPS	Myb_DNA-binding	up	cell wall remodelling	10.1111/tpj.12286
	<i>Os02g0462800</i>	WRKY42	WRKY	up	promotes leaf senescence	10.14348/molcells.2014.0128
Roots	<i>Os02g0654700</i>	ERF91/AP59	AP2	up	drought/salt tolerance	10.1104/pp.109.137554
	<i>Os09g0572000</i>	ERF87	AP2	up		
	<i>Os11g0168500</i>	ERF118	AP2	up		
	<i>Os01g0108600</i>	-	bHLH	up		
	<i>Os05g0163900</i>	bHLH036	HLH	up		
	<i>Os01g0274800</i>	CSA	Myb_DNA-binding	up		
	<i>Os01g0305900</i>	-	Myb_DNA-binding	up		
	<i>Os06g0649000</i>	WRKY28	WRKY	up	represses immune responses	10.1007/s11103-013-0032-5

**FIGURE LEGENDS**

**FIGURE 1.** JA-deficient young rice plants exhibit milder symptoms than their wild-type (WT) counterparts. Seven-day old hydroponically-grown WT and *aoc* seedlings were exposed to either control or 100 mM NaCl (salt) solutions. After 5 days, representative second (2<sup>nd</sup>) and third (3<sup>rd</sup>) leaves were photographed (a). Scale bar: 1 cm. (b) Second and 3<sup>rd</sup> leaves were detached from control and salt-exposed plants and submitted to electrolyte leakage assay. Histograms show means  $\pm$  SEM (n=5). Asterisks indicate a significant difference as determined by ANOVA plus Tukey's HSD tests (\*P < 0.05).

**FIGURE 2.** Kinetic analysis of jasmonate profile in rice seedlings submitted to 100 mM NaCl stress for 5 days. Seven-day old hydroponically-grown WT and *aoc* seedlings were exposed to either control (Control) or 100 mM NaCl (Salt) and sampled at the **time points** indicated. Roots (a) and second leaf (b) were collected separately and JAs were extracted and quantified by LC-

MS. The precursor OPDA, JA, the bioactive hormone JA-Ile and its catabolite 12COOH-JA were quantified by LC-TQMS/MS and expressed as pmol g<sup>-1</sup> fresh weight (FW). Histograms represent the mean ± SEM of three biological replicates. For JA-Ile, hatched bars indicate signal has been detected only in one replicate. Asterisks indicate a significant difference (ANOVA plus Tukey's HSD tests \*P < 0.05; \*\*P < 0.01). LOQ: limit of quantification.

**FIGURE 3.** Transcriptome analysis of WT and *aoc* seedlings before and after salt stress. Plant samples generated as described in Figure 1 (three independent biological replicates) were used for RNA extraction and submitted to RNAseq analysis. Expression data (log<sub>2</sub> fold change) of selected genes involved in jasmonate metabolism or signaling were extracted and plotted as a heatmap for roots and 2<sup>nd</sup> leaf (a). Differentially expressed genes (-1 < log<sub>2</sub>FC < 1; FDR < 0.05) were filtered in both organs and displayed as histograms with numbers of down- and up-regulated genes displayed in genotype (b) or time comparisons (c).

**FIGURE 4.** Comparative analysis of expression dynamics of genes encoding transcription factors (TFs) in WT and *aoc* roots (a) and 2<sup>nd</sup> leaf (b). Left panels: upregulated genes; right panels: downregulated genes. To screen for particular temporal behaviors, UpSet diagrams (Lex *et al.*, 2014) were utilized to display shared and genotype specific expression at different time points following salt application. Total number of TFs deregulated at a given time point in each genotype are indicated by horizontal bars. Numbers of differentially expressed TF genes (-1 < log<sub>2</sub>FC < 1; FDR < 0.05) relative to 0 h are visualized as vertical bars for individual (dots) or multiple (connected dots) time points.

**FIGURE 5.** Kinetic Gene Ontology (GO) analysis of DEGs in roots (a) and 2<sup>nd</sup> leaf (b) upon salt stress. DEGs lists established in Figure 3c from each **time point** relative to 0 h were submitted to GO analysis on Panther classification system to retrieve Biological processes significantly enriched within each DEGs list (FDR < 0.01). GO terms for upregulated DEGs: upper panels, red scale; GO terms for downregulated DEGs: lower panels, blue scale)

**FIGURE 6.** Analysis of ABA pathway genes, hormone content and water management responses upon salt stress in rice. Expression heatmap of described rice ABA metabolic genes is shown for *aoc* and WT roots and 2<sup>nd</sup> leaf (a). ABA content in roots and 3<sup>rd</sup> leaf. Histograms show the mean of 3 independent biological replicates ± SEM; \*P < 0.05 (b). Expression heatmap of genes associated with GO term “response to water deprivation”. Only genes whose

expression was changed at least at one time point in *aoc* or WT ( $-1 < \log_2FC < 1$ ;  $FDR < 0.05$ ) are represented (c). Stomatal conductance to water vapor (gsw) was determined in leaf 5 of WT and *aoc* plants (d, left panel). Relative water content (RWC) was determined in 3<sup>rd</sup> leaf of WT and *aoc* plants submitted to salt stress for 4 days (d; right panel). Histograms represent the mean of 3 (gsw) or 4 (RWC) biological replicates  $\pm$  SEM. Asterisks indicate a significant difference (ANOVA plus Tukey's HSD tests, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).

**FIGURE 7.** Analysis of differential ion transporter gene expression and ion accumulation in roots and leaves upon salt stress. Expression profiles of ion transporter genes in *aoc* vs WT are represented as kinetic heatmaps (a). Only transporter genes whose expression was changed at least at one time point in *aoc* or WT ( $-1 < \log_2FC < 1$ ;  $FDR < 0.05$ ) are represented. Expression of *HAK4* transporter gene in roots of WT plants exposed to MeJA. Data are taken from RiceXpro database (b). Quantification of Na<sup>+</sup> and K<sup>+</sup> ion accumulation in roots, 2<sup>nd</sup> and 3<sup>rd</sup> leaves: means  $\pm$  SEM from 6 independent biological replicates are represented (c). Asterisks indicate a significant difference (ANOVA plus Tukey's HSD tests, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).

**FIGURE 8.** Analysis of reactive oxygen species-scavenging systems in leaves under salt stress. Second leaves of *aoc* or WT seedlings were submitted to DAB staining to visualize extent of H<sub>2</sub>O<sub>2</sub> accumulation (a). Genotype comparison (*aoc* vs WT) of differentially-expressed genes encoding ROS-scavenging activities. APX: ascorbate peroxidase; FSD: iron-dependent superoxide dismutase; GST: glutathione S-transferase; SOD: superoxide dismutase (b). Expression of *FSD1.1* and *FSD1.2* in leaves of WT plants exposed to MeJA (c). Asterisks indicate a significant difference (ANOVA plus Tukey's HSD tests, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).

**FIGURE 9.** Jasmonate deficiency delays induction of senescence-promoting genes upon salt stress. Expression of genes associated with senescence (SAGs) was compared in 2<sup>nd</sup> leaf between *aoc* and WT genotypes at four time points and genes whose expression was changed at least at one time point in either genotype ( $-1 < \log_2FC < 1$ ;  $FDR < 0.05$ ) are represented as a kinetic heatmap (a). Expression of *OsI43* and *OsNAP*, two SAGs that are strongly differential in (a), was monitored upon response to MeJA exposure (b). Histograms display means  $\pm$  SEM from 3 biological replicates. Chlorophyll contents was determined in WT and *aoc* 2<sup>nd</sup> or 3<sup>rd</sup>

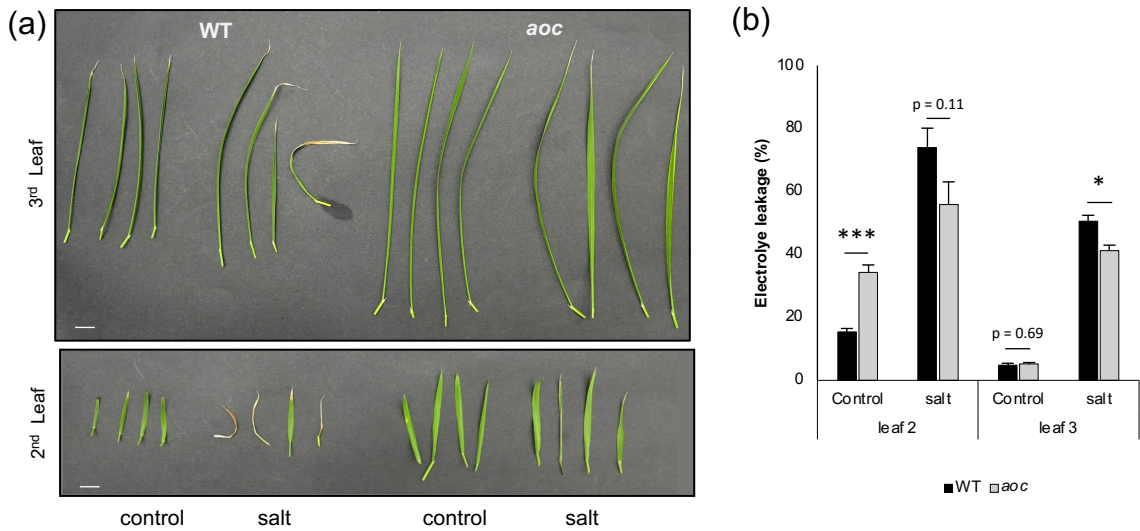


leaves after 4 days of salt stress (c). Asterisks indicate a significant difference in means (ANOVA plus Tukey's HSD tests, \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001).

**FIGURE 10.** Proposed model of JA-regulated functions in the rice response to salt stress. The study links newly-defined JAs gene targets to specific impacts of JA signaling on the salinity response physiology. Salt stress through its successive osmotic and ionic components stimulates JA metabolism which leads to waves of transcriptional changes with organ-specific temporal patterns. Root response is immediate-early while in shoot a bi-phasic response is discerned at hormonal and transcriptional levels. ABA responses are largely JA-independent in root, but JA signaling is critical for full induction of ABA biosynthetic genes and ABA accumulation in shoot. Both hormones synergistically activate ABA-regulated responses including dehydrins and RAB genes to boost water retention, including reduction of stomatal conductance and water loss in rice leaves. On the other hand, JA signaling, through transcriptional repression of *HAK4* in roots and *FSD* genes in leaves, impairs Na<sup>+</sup> exclusion from root xylem and ROS detoxification in leaves respectively, which aggravates Na<sup>+</sup> toxicity in photosynthetic tissues. This set of responses, associated with the induction by JAs of *NAP*, a transcriptional activator of leaf senescence, can explain the severe necrotic symptoms observed in WT leaves after salt stress. The findings establish JAs as multifaceted regulators of the rice salt stress response, where JA signaling can no longer be uniformly associated with salt sensitivity or tolerance. Black arrows and red lines indicate transcriptional activation and repression of JA-target genes respectively; compiled “greater than” symbols indicate positive regulation of key pathways involved in rice salt stress response. h: hours. d: days.

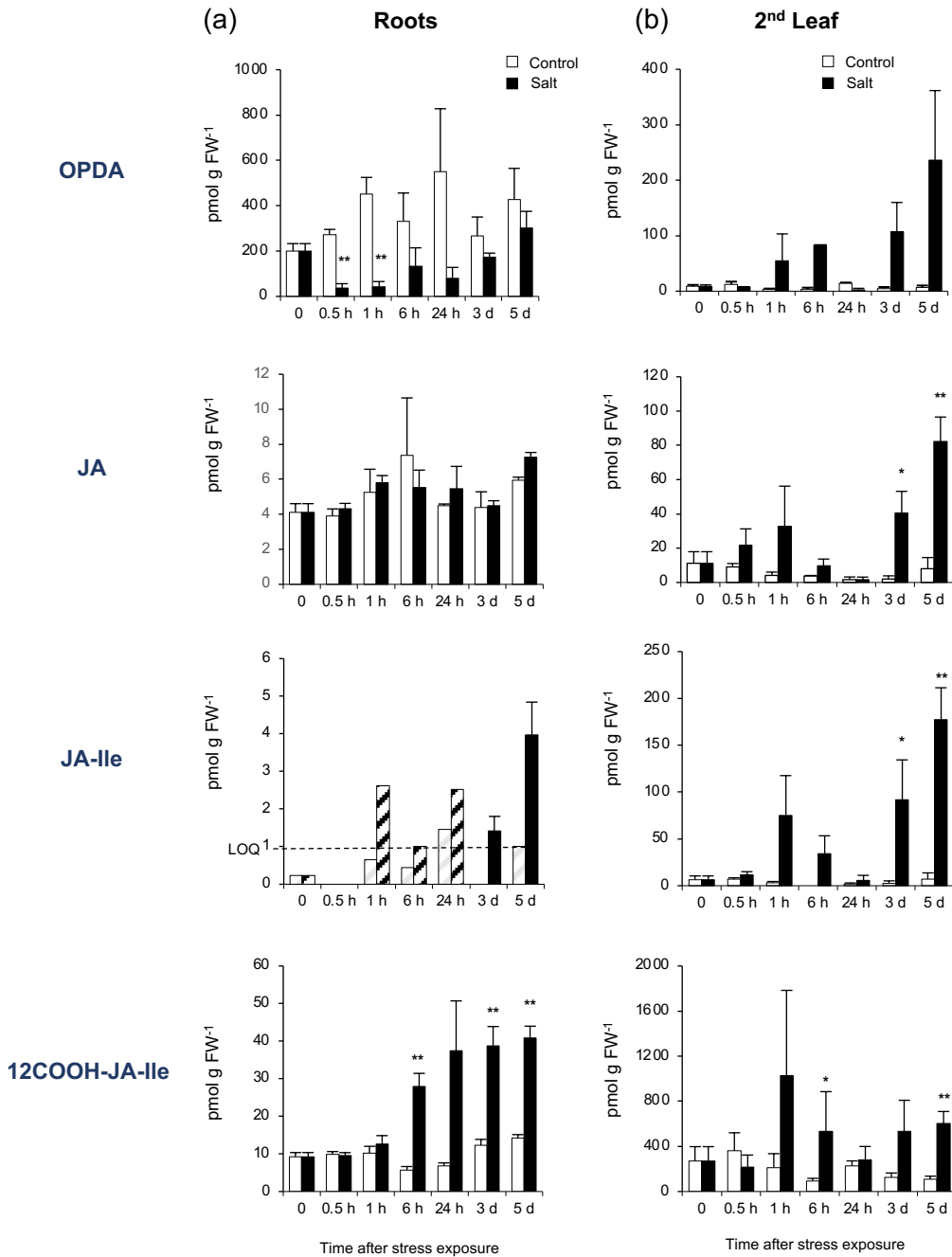
**TABLE 1:** List of transcription factor (TF) genes coregulated with JA pathway genes in rice salt stress response. Genes were selected from data in Figure 4 on the basis of their exclusive regulation in WT at 1 h and 6 h for roots, or 1 h and 72 h for shoot, and their absence of response in *aoc* ( $-1 < \log_2FC < 1$ ; FDR < 0.05).

**Figure 1**



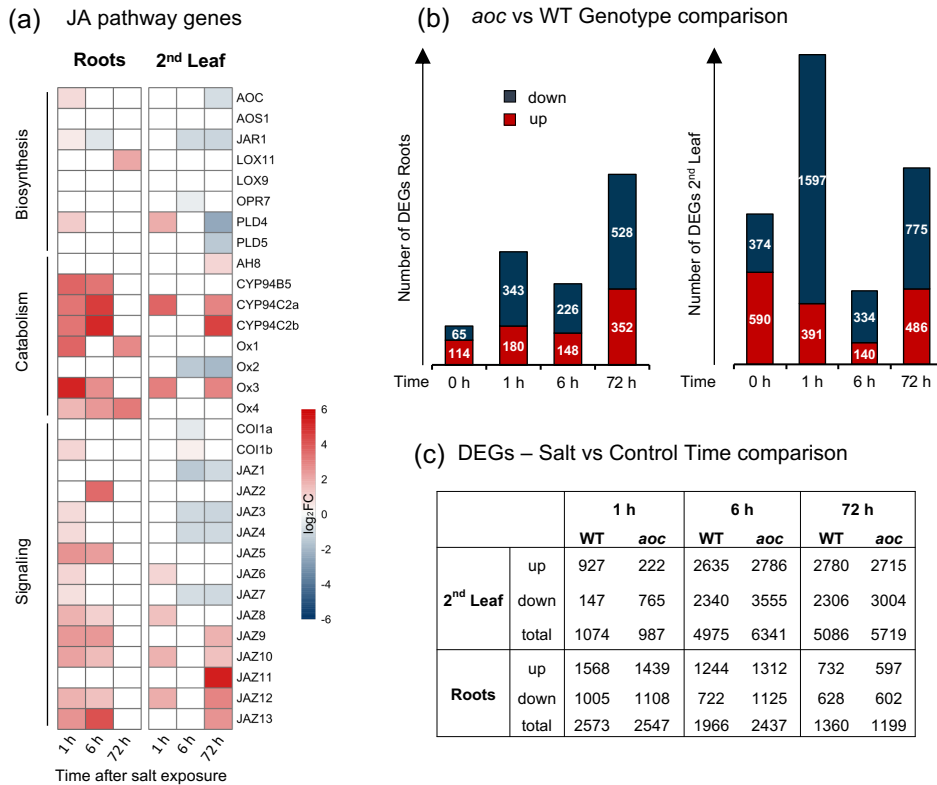
**FIGURE 1.** JA-deficient young rice plants exhibit milder symptoms than their wild-type (WT) counterparts. Seven-day old hydroponically-grown WT and *aoc* seedlings were exposed to either control or 100 mM NaCl (salt) solutions. After 5 days, representative second (2<sup>nd</sup>) and third (3<sup>rd</sup>) leaves were photographed (a). Scale bar: 1 cm. (b) Second and 3<sup>rd</sup> leaves were detached from control and salt-exposed plants and submitted to electrolyte leakage assay. Histograms show means  $\pm$  SEM (n=5). Asterisks indicate a significant difference as determined by ANOVA plus Tukey's HSD test (\*P < 0.05).

**Figure 2**



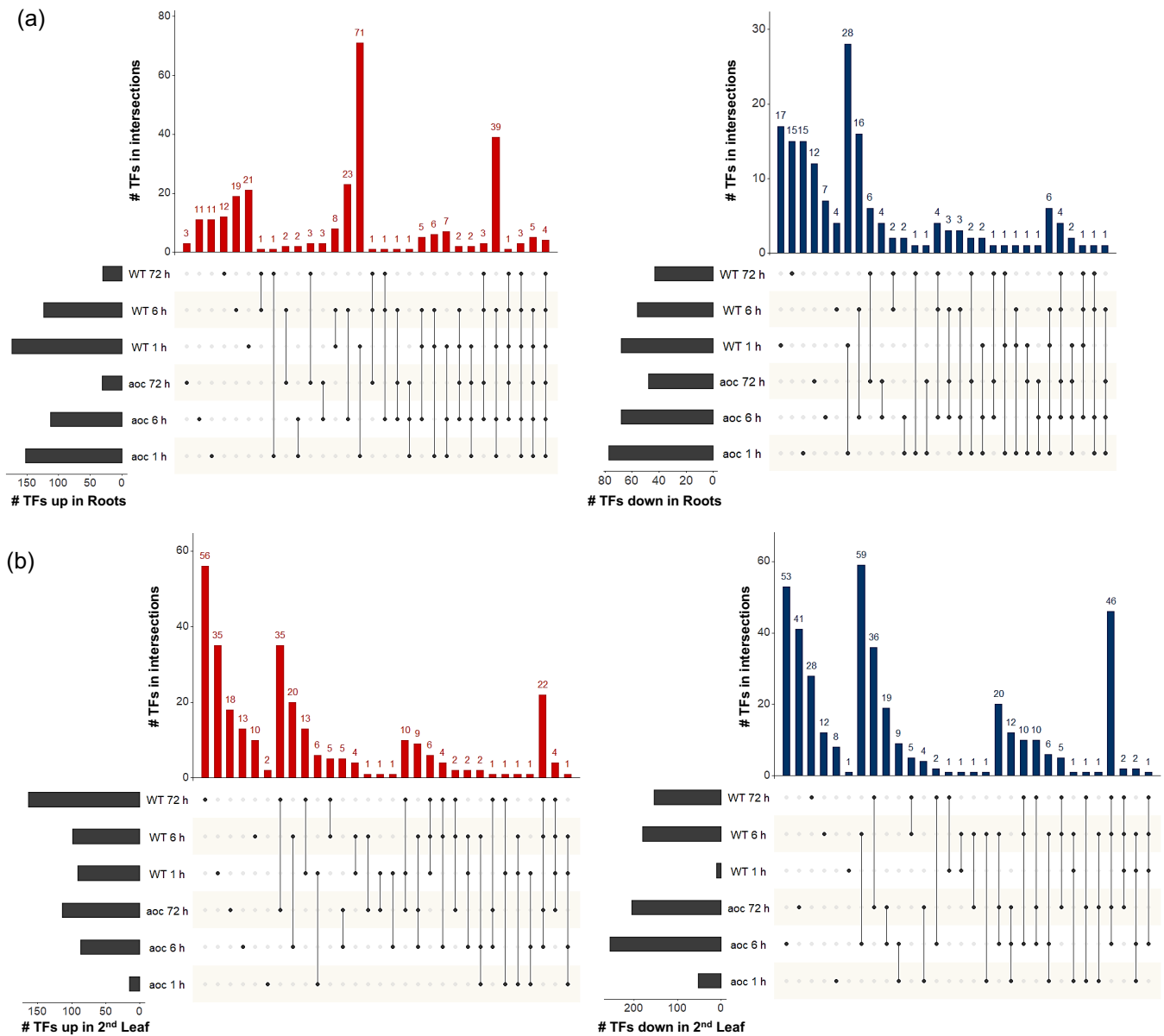
**FIGURE 2.** Kinetic analysis of jasmonate profile in rice seedlings submitted to 100 mM NaCl stress for 5 days. Seven-day old hydroponically-grown WT and *aoc* seedlings were exposed to either control (Control) or 100 mM NaCl (Salt) and sampled at the time points indicated. Roots (a) and second leaf (b) were collected separately and JAs were extracted and quantified. The precursor OPDA, JA, the bioactive hormone JA-Ile and its catabolite 12COOH-JA were quantified by LC-MS/MS and expressed as pmol g<sup>-1</sup> fresh weight (FW). Histograms represent the mean  $\pm$  SEM of three biological replicates. For JA-Ile, hatched bars indicate signal has been detected only in one replicate. Asterisks indicate a significant difference (ANOVA plus Tukey's HSD tests \*P < 0.05; \*\*P < 0.01). LOQ : limit of quantification.

**Figure 3**



**FIGURE 3.** Transcriptome analysis of WT and *aoc* seedlings before and after salt stress. Plant samples generated as described in Figure 1 (three independent biological replicates) were used for RNA extraction and submitted to RNAseq analysis. Expression data ( $\log_2$  fold change) of selected genes involved in jasmonate metabolism or signaling were extracted and plotted as a heatmap for roots and 2<sup>nd</sup> leaf (a). Differentially expressed genes ( $-1 < \log_2FC < 1$ ; FDR < 0.05) were filtered in both organs and displayed as histograms with numbers of down- and up-regulated genes displayed in genotype (b) or time comparisons (c).

Figure 4



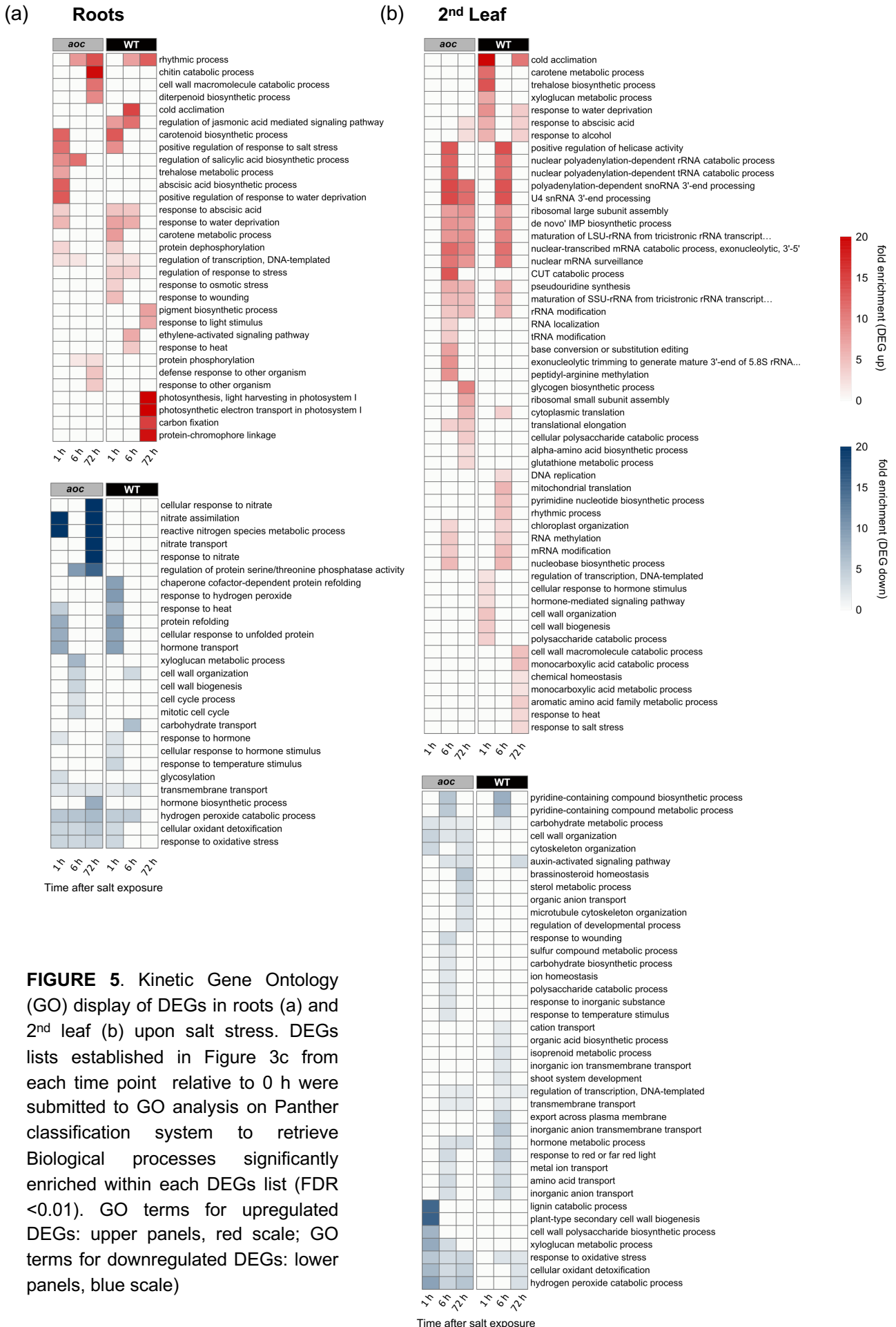
**FIGURE 4.** Comparative analysis of expression dynamics of genes encoding transcription factors (TFs) in WT and *aoc* roots (a) and 2<sup>nd</sup> leaf (b). Left panels: upregulated genes; right panels: downregulated genes. To screen for particular temporal behaviors, UpSet diagrams (Lex et al., 2014) were utilized to display shared and genotype specific expression at different time points following salt application. Total number of TFs deregulated at a given time point in each genotype are indicated by horizontal bars. Numbers of differentially expressed TF genes ( $-1 < \log_2FC < 1$ ; FDR < 0.05) relative to 0 h are visualized as vertical bars for individual (dots) or multiple (connected dots) time points.

**Table 1**

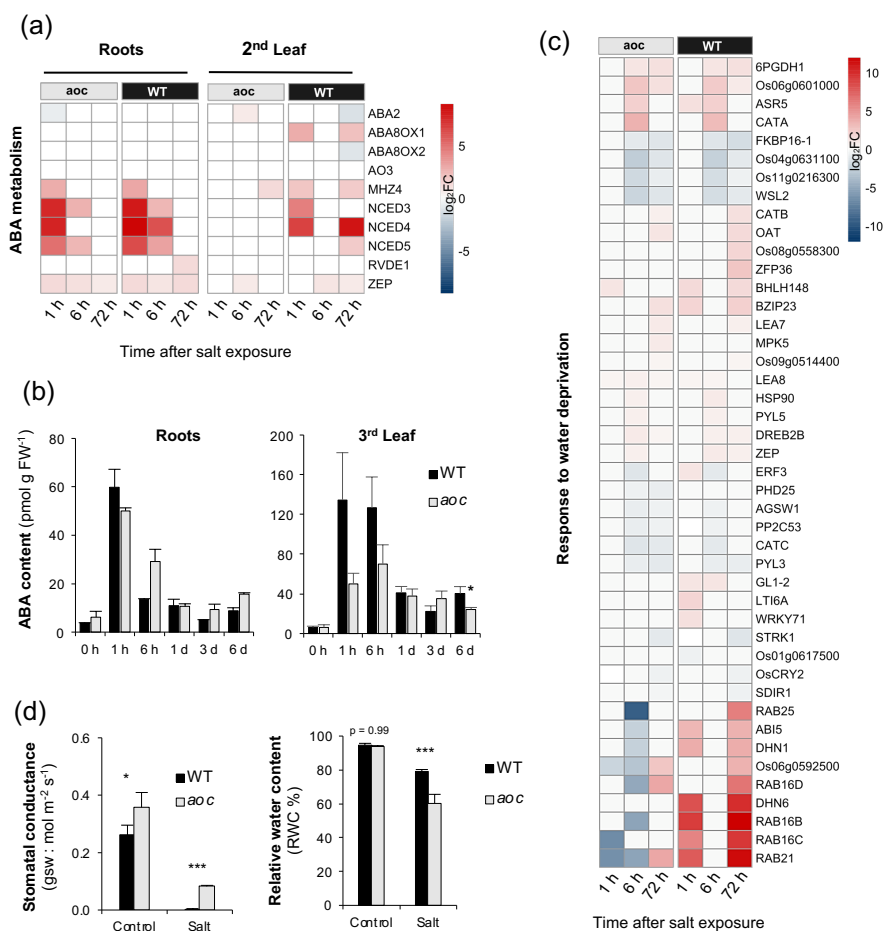
Organ	Gene ID	TFs name	TF family	Regulation	Stress-associated function	Reference (DOI)
2 <sup>nd</sup> Leaf	Os08g0481400	HOX20	HALZ	down	drought tolerance	
	Os01g0738400	C3H10	Zinc Finger	up	drought tolerance	10.3390/plants9101298
	Os03g0264600	-	-	up		
	Os03g0820300	ZFP182	Zinc Finger	up	salt tolerance	10.1016/j.bbaexp.2007.02.006
	Os05g0541400	bHLH119/LF	bHLH	up		10.1093/mp/sss096
	Os02g0764700	ERF103	AP2	up	drought responsive	
	Os06g0127100	DREB1C	AP2	up		
	Os08g0474000	ERF104	AP2	up	drought responsive	
	Os01g0859300	ABI5/ABF1	bZIP_1	up	promotes salt sensitivity	10.1007/s11103-008-9298-4
	Os01g0192300	MYB1R1	Myb_DNA-binding	up		
	Os01g0874300	DLN31	Myb_DNA-binding	up		
	Os02g0187700	MYB1	Myb_DNA-binding	up		
	Os02g0618400	MPS	Myb_DNA-binding	up	cell wall remodelling	10.1111/tpj.12286
	Os02g0462800	WRKY42	WRKY	up	promotes leaf senescence	10.14348/molcells.2014.0128
Roots	Os02g0654700	ERF91/AP59	AP2	up	drought/salt tolerance	10.1104/pp.109.137554
	Os09g0572000	ERF87	AP2	up		
	Os11g0168500	ERF118	AP2	up		
	Os01g0108600	-	bHLH	up		
	Os05g0163900	bHLH036	HLH	up		
	Os01g0274800	CSA	Myb_DNA-binding	up		
	Os01g0305900	-	Myb_DNA-binding	up		
	Os06g0649000	WRKY28	WRKY	up	represses immune responses	10.1007/s11103-013-0032-5

**TABLE 1:** List of transcription factor (TF) genes coregulated with JA pathway genes in rice salt stress response. Genes were selected from data in Figure 4 on the basis of their exclusive regulation in WT at 1 h and 6 h for roots, or 1 h and 72 h for shoot, and their absence of response in *aoc* ( $-1 < \log_2FC < 1$ ;  $FDR < 0.05$ ).

**Figure 5**



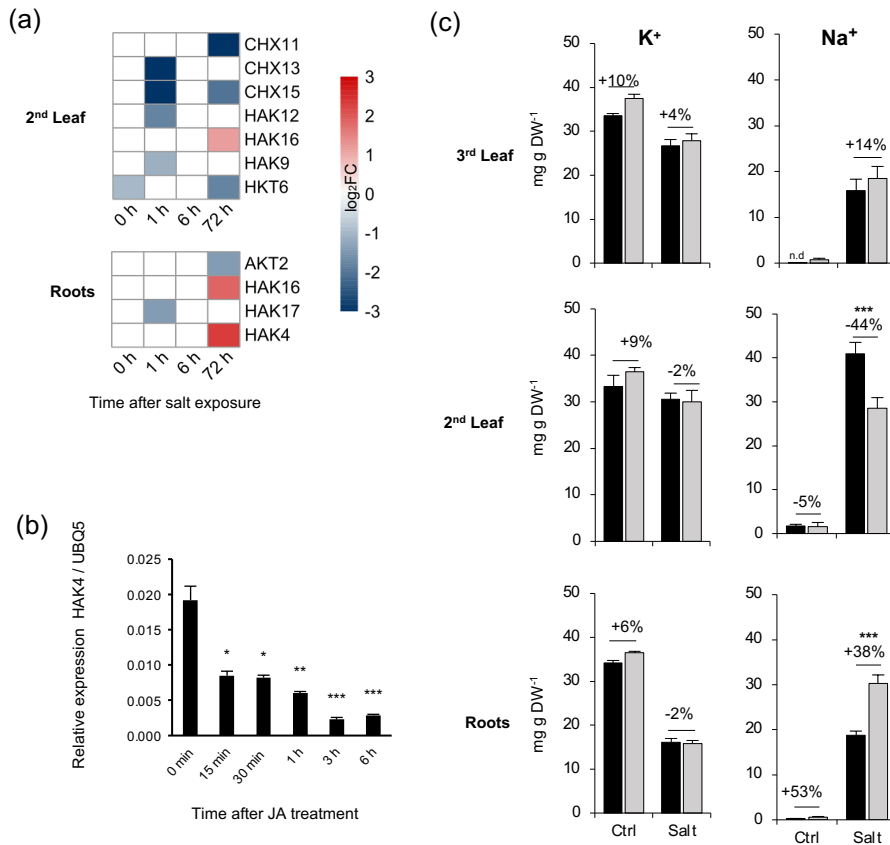
**Figure 6**



**FIGURE 6.** Analysis of ABA pathway genes, hormone content and water management responses upon salt stress in rice. Expression heatmap of described rice ABA metabolic genes is shown for *aoc* and WT roots and 2<sup>nd</sup> leaf (a). ABA content in roots and 3<sup>rd</sup> leaf. Histograms show the mean of 3 independent biological replicates  $\pm$  SEM; \*P < 0.05 (b). Expression heatmap of genes associated with GO term "response to water deprivation". Only genes whose expression was changed at least at one time point in *aoc* or WT ( $-1 < \log_2FC < 1$ ; FDR < 0.05) are represented (c). Stomatal conductance to water vapor (gs<sub>w</sub>) was determined in leaf 5 of WT and *aoc* plants (d, left panel). Relative water content (RWC) was determined in 3<sup>rd</sup> leaf of WT and *aoc* plants submitted to salt stress for 4 days (d; right panel). Histograms represent the mean of 3 (gs<sub>w</sub>) or 4 (RWC) biological replicates  $\pm$  SEM. Asterisks indicate a significant difference (ANOVA plus Tukey's HSD tests, \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001).

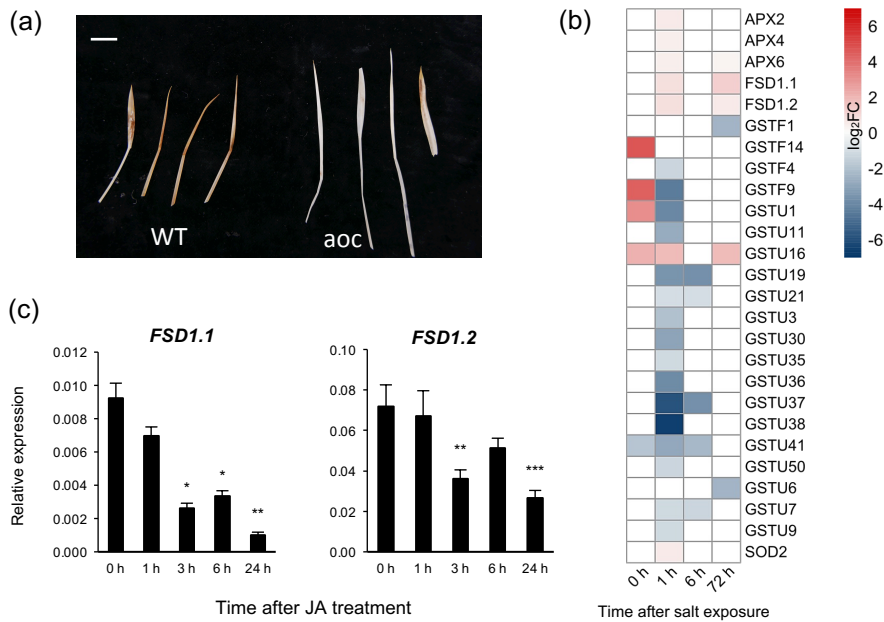


**Figure 7**



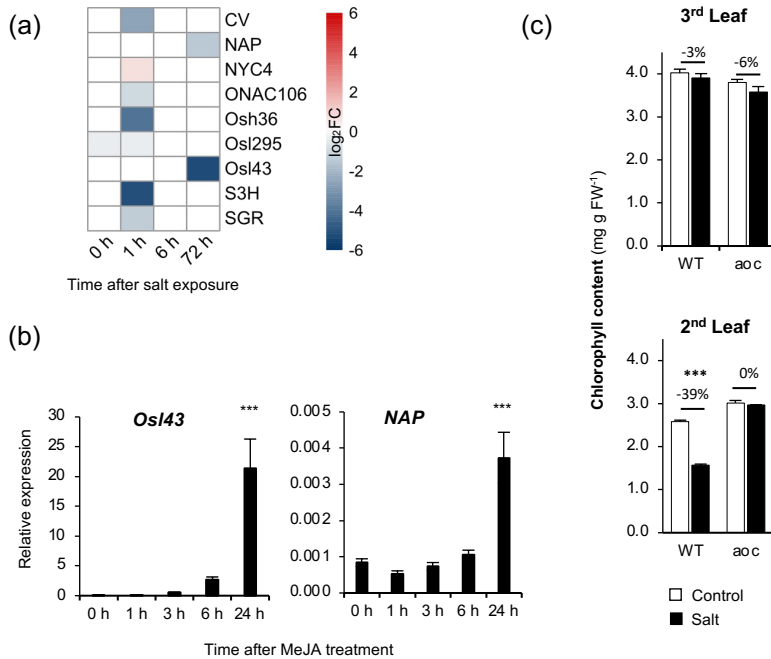
**FIGURE 7.** Analysis of differential ion transporter gene expression and ion accumulation in roots and leaves upon salt stress. Expression profiles of ion transporter genes in *aoc* vs WT are represented as kinetic heatmaps (a). Only transporter genes whose expression was changed at least at one time point in *aoc* or WT ( $-1 < \log_2FC < 1$ ;  $FDR < 0.05$ ) are represented. Expression of *HAK4* transporter gene in roots of WT plants exposed to MeJA. Data are taken from RiceXpro database (b). Quantification of Na<sup>+</sup> and K<sup>+</sup> ion accumulation in roots, 2<sup>nd</sup> and 3<sup>rd</sup> leaves : means  $\pm$  SEM from 6 independent biological replicates are represented (c). Asterisks indicate a significant difference (ANOVA plus Tukey's HSD tests, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).

**Figure 8**



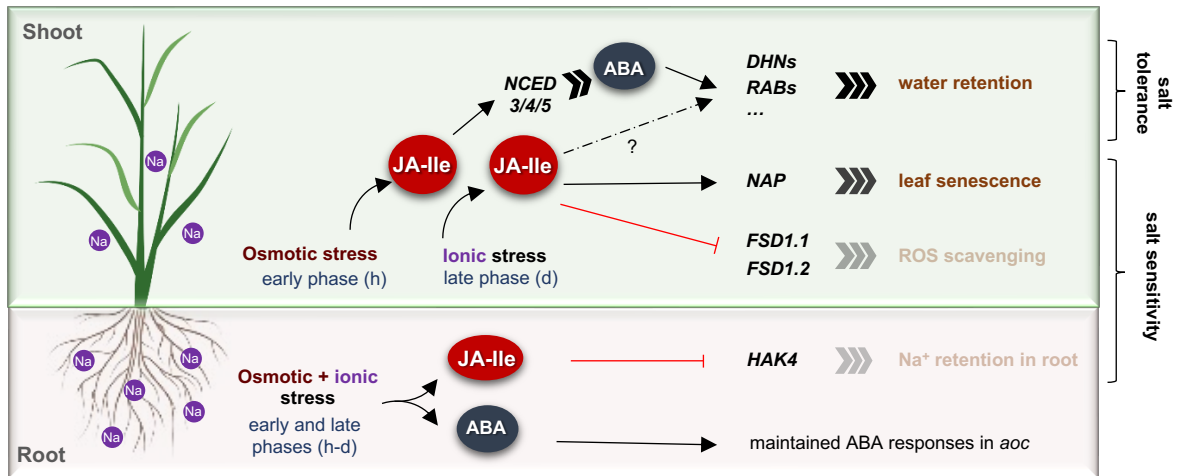
**FIGURE 8.** Analysis of reactive oxygen species-scavenging systems in leaves under salt stress. Second leaves of *aoc* or WT seedlings were submitted to DAB staining to visualize extent of H<sub>2</sub>O<sub>2</sub> accumulation. Scale bar: 1 cm (a). Genotype comparison (*aoc* vs WT) of differentially-expressed genes encoding ROS-scavenging activities. APX : ascorbate peroxidase; FSD: iron-dependent superoxide dismutase; GST: glutathione S-transferase; SOD: superoxide dismutase (b). Expression of *FSD1.1* and *FSD1.2* in leaves of WT plants exposed to MeJA (c). Asterisks indicate a significant difference (ANOVA plus Tukey's HSD tests, \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001).

**Figure 9**



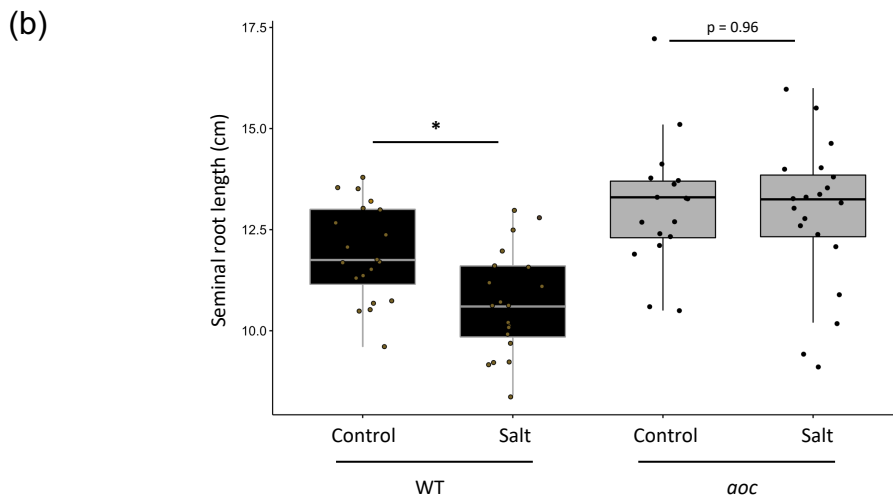
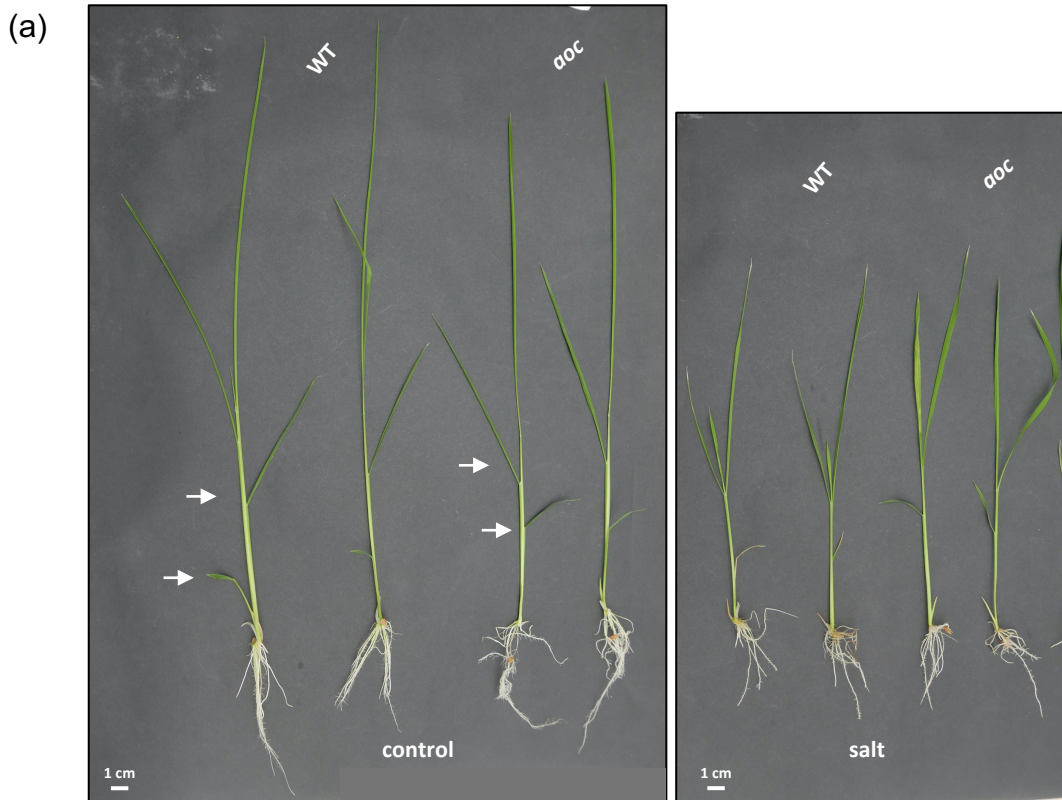
**FIGURE 9.** Jasmonate deficiency delays induction of senescence-promoting genes upon salt stress. Expression of genes associated with senescence (SAGs) was compared in 2<sup>nd</sup> leaf between *aoc* and WT genotypes at four time points and genes whose expression was changed at least at one time point in either genotype ( $-1 < \log_2FC < 1$ ; FDR < 0.05) are represented as a kinetic heatmap (a). Expression of *Osl43* and *OslNAP*, two SAGs that are strongly differential in (a), was monitored upon response to MeJA exposure (b). Histograms display means  $\pm$  SEM from 3 biological replicates. Chlorophyll contents was determined in WT and *aoc* 2<sup>nd</sup> or 3<sup>rd</sup> leaves after 4 days of salt stress (c). Asterisks indicate a significant difference in means (ANOVA plus Tukey's HSD tests, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).

Figure 10



**FIGURE 10. Proposed model of JA-regulated functions in the rice response to salt stress.** The study links newly-defined JAs gene targets to specific impacts of JA signaling on the salinity response physiology. Salt stress through its successive osmotic and ionic components stimulates JA metabolism which leads to waves of transcriptional changes with organ-specific temporal patterns. Root response is immediate-early while in shoot a bi-phasic response is discerned at hormonal and transcriptional levels. ABA responses are largely JA-independent in root, but JA signaling is critical for full induction of ABA biosynthetic genes and ABA accumulation in shoot. Both hormones synergistically activate ABA-regulated responses including dehydrins and RAB genes to boost water retention, including reduction of stomatal conductance and water loss in rice leaves. On the other hand, JA signaling, through transcriptional repression of *HAK4* in roots and *FSD* genes in leaves, impairs  $\text{Na}^+$  exclusion from root xylem and ROS detoxification in leaves respectively, which aggravates  $\text{Na}^+$  toxicity in photosynthetic tissues. This set of responses, associated with the induction by JAs of *NAP*, a transcriptional activator of leaf senescence, can explain the severe necrotic symptoms observed in WT leaves after salt stress. The findings establish JAs as multifaceted regulators of the rice salt stress response, where JA signaling can no longer be uniformly associated with salt sensitivity or tolerance. Black arrows and red lines indicate transcriptional activation and repression of JA-target genes respectively; compiled “greater than” symbols indicate positive regulation of key pathways involved in rice salt stress response. h: hours. d: days.

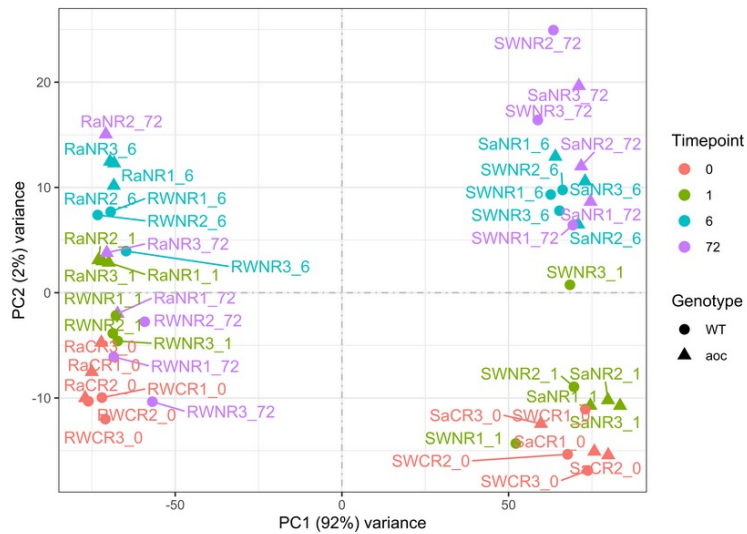
Figure S1



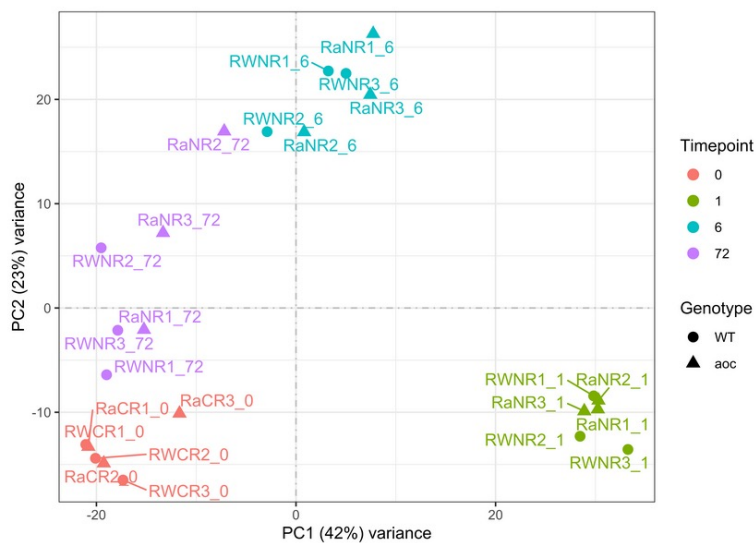
**FIGURE S1.** Developmental phenotypes of rice wild-type (WT) and jasmonate-deficient (*aoc*) seedlings exposed to control or 100 mM NaCl (salt) solutions. Two representative plants from each condition were photographed 5 days after exposure. Arrows on control plants designate second (lower) and third leaves (a). Seminal root length was measured and represented in boxplots with  $n = 20$  (except *aoc*-control,  $n = 17$ ) (b). Asterisk \* indicates significant difference (ANOVA plus Tukey's HSD tests,  $*P < 0.05$ ).

**Figure S2**

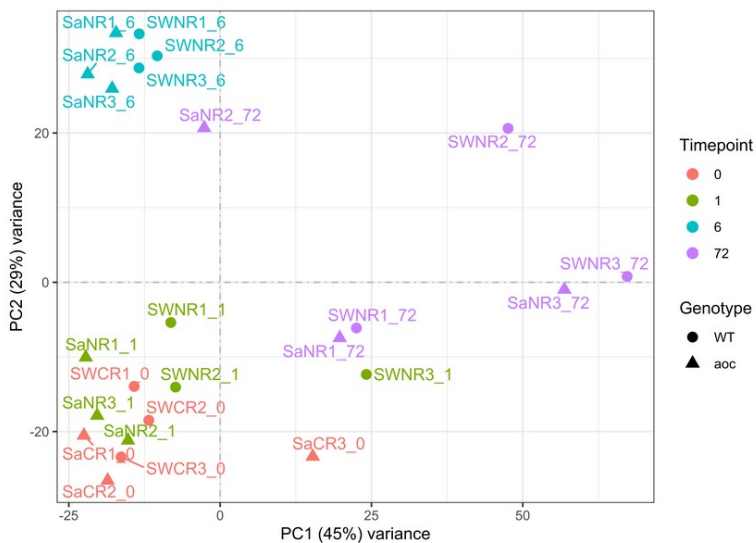
(a) Global



(b) Roots



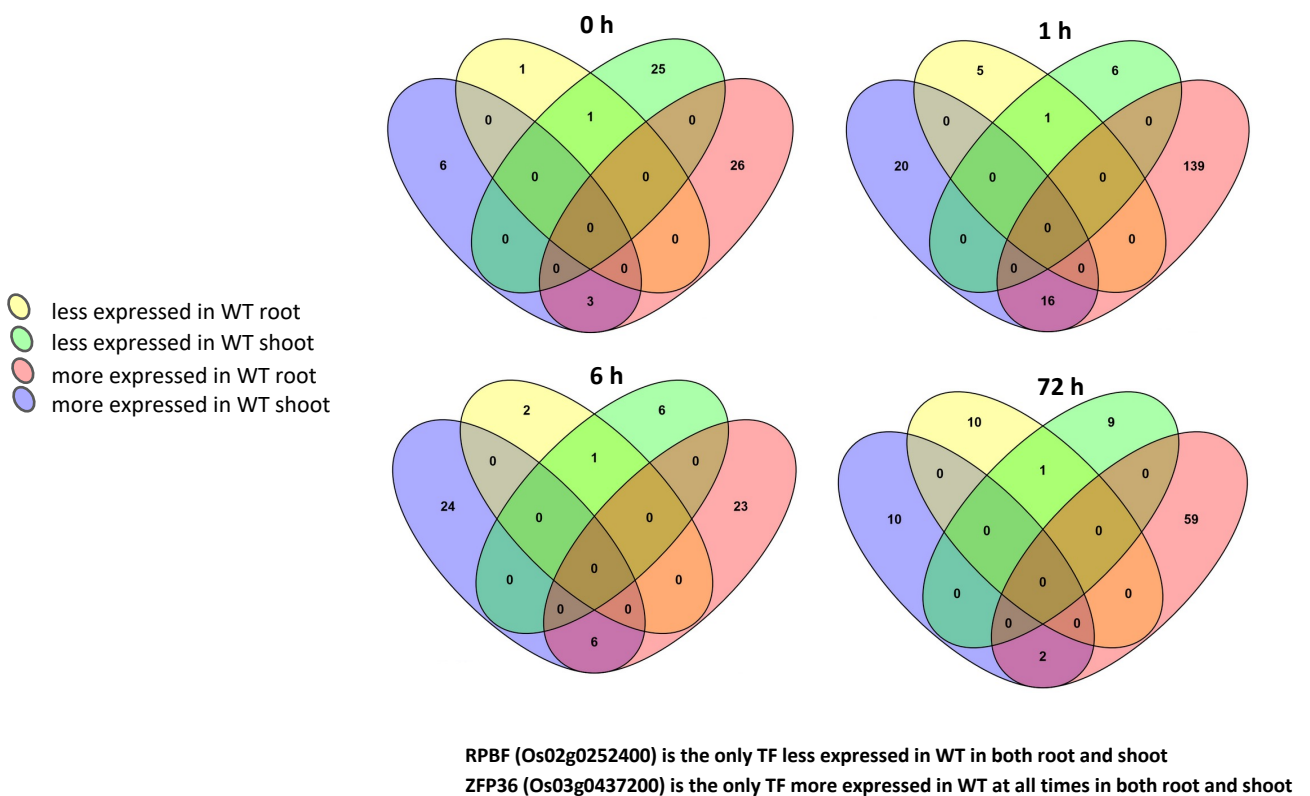
(c) 2<sup>nd</sup> Leaf



**FIGURE S2.** Principal component analysis (PCA) of RNAseq data distribution. Global normalization was applied to root and 2<sup>nd</sup> leaf samples (Total of 48 samples with each biological condition in 3 independent replicates) (a). Sample designation: R or S = Root or Shoot; W or a = WT or aoc; Rx = replicate number. Root samples only (24) were normalized and analyzed in (b). 2<sup>nd</sup> leaf samples only (24) were normalized and analyzed in (c).

**Figure S3**

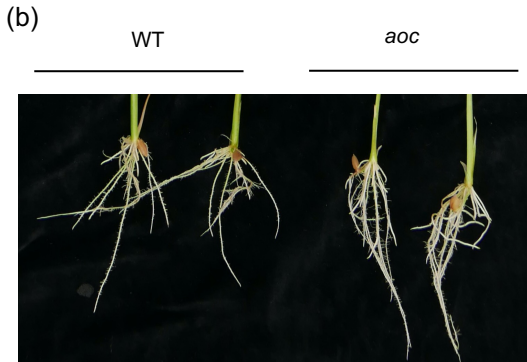
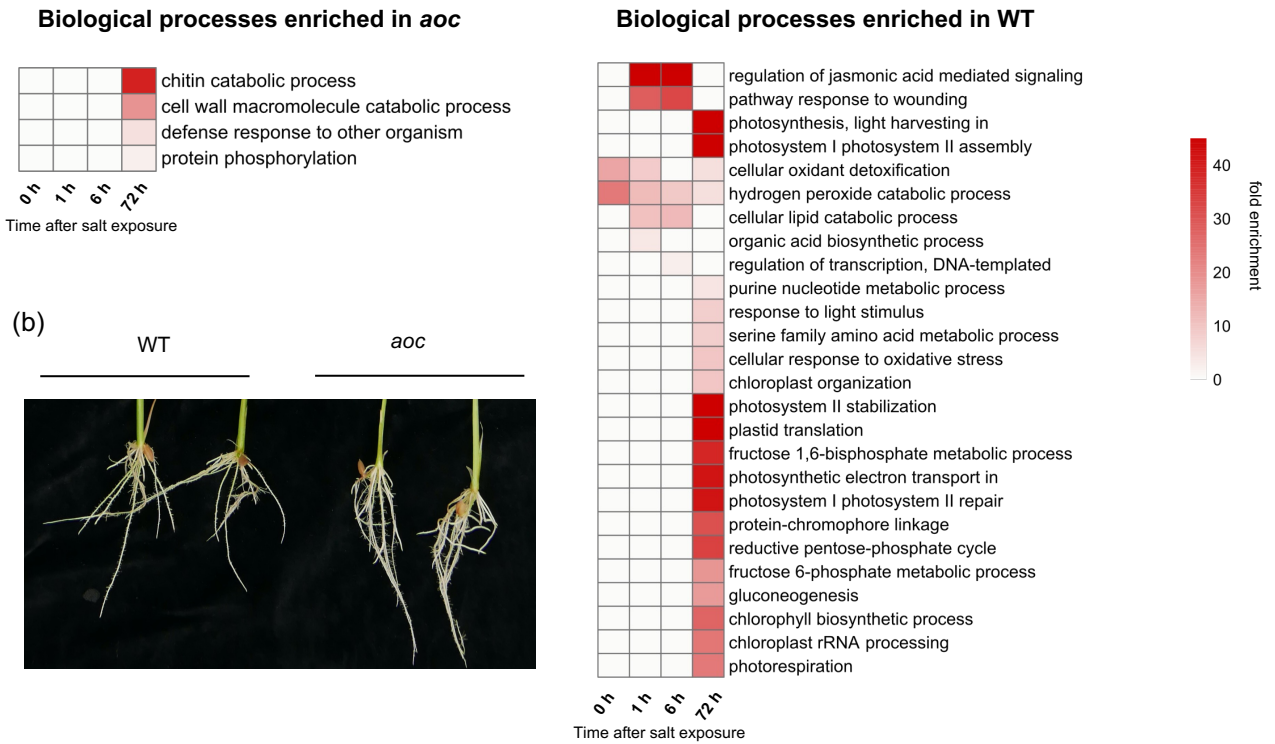
**Venn Diagrams of deregulated TFs - *aoc*/WT**



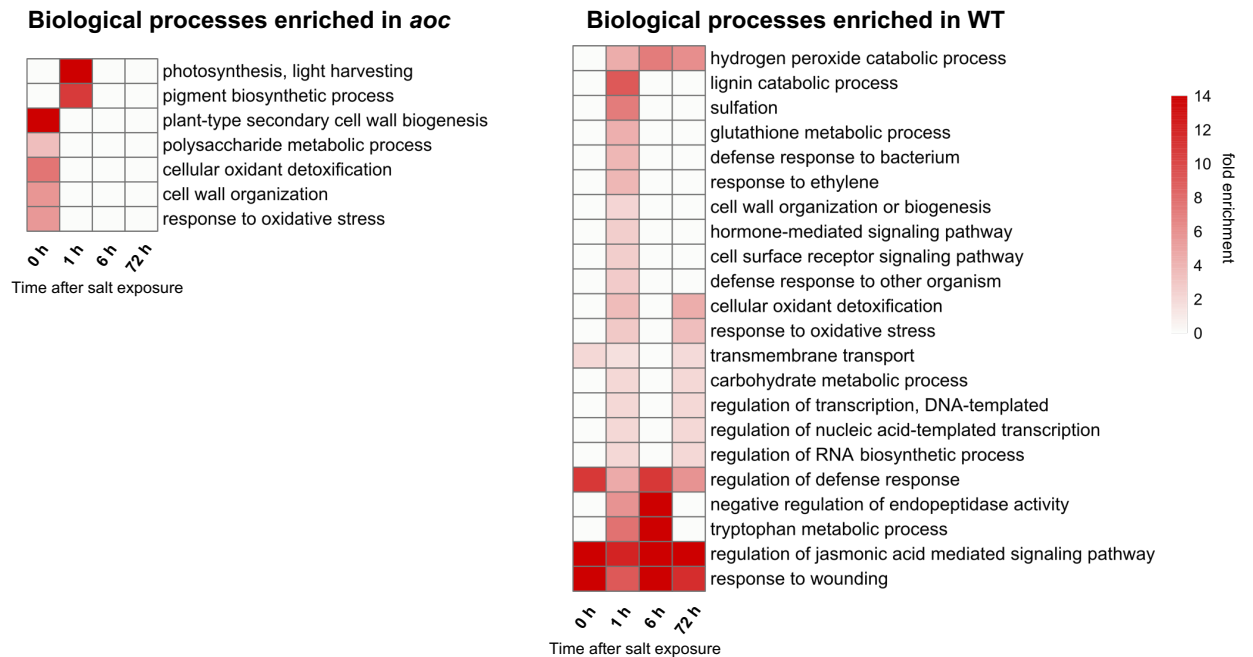
**FIGURE S3.** Analysis of extent of overlap between deregulated transcription factor (TF) genes in shoot and root. Lists of genes showing differential expression in each organ in the *aoc*/WT comparison were crossed for each of the 4 timepoints (h) of salt response. Number of genes in common between the two organs comparisons are represented in Venn diagrams.

**Figure S4**

**(a) Roots**



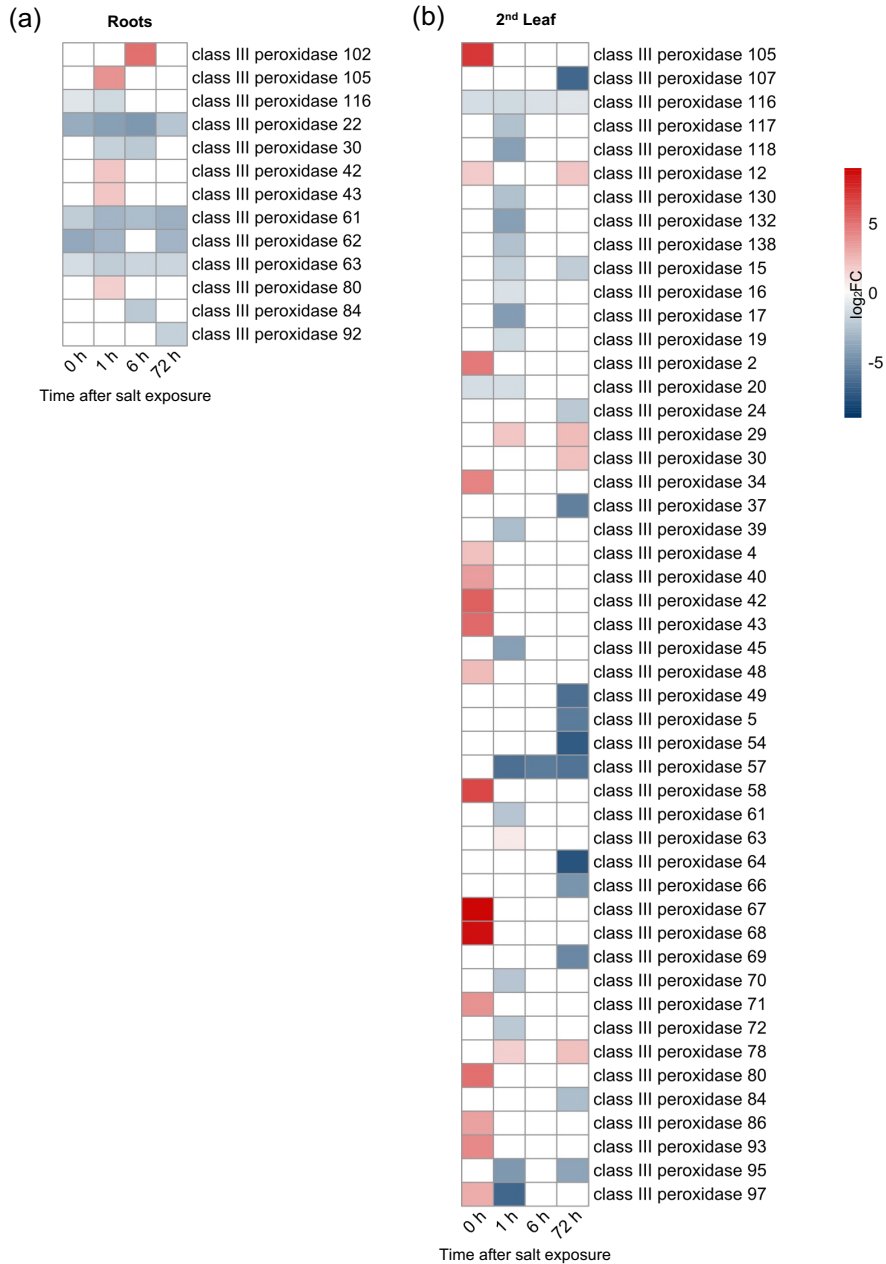
**(c) 2<sup>nd</sup> Leaf**



**FIGURE S4.** Gene ontology (GO) analysis of differentially expressed genes (DEGs). DEG lists at each time point relative to 0 h were submitted to GO analysis and enrichment of biological processes (FDR < 0.01) in *aoc* or WT was displayed as heatmaps for roots (a) and 2<sup>nd</sup> leaf (c). Roots were photographed at 6 days after exposure (b).

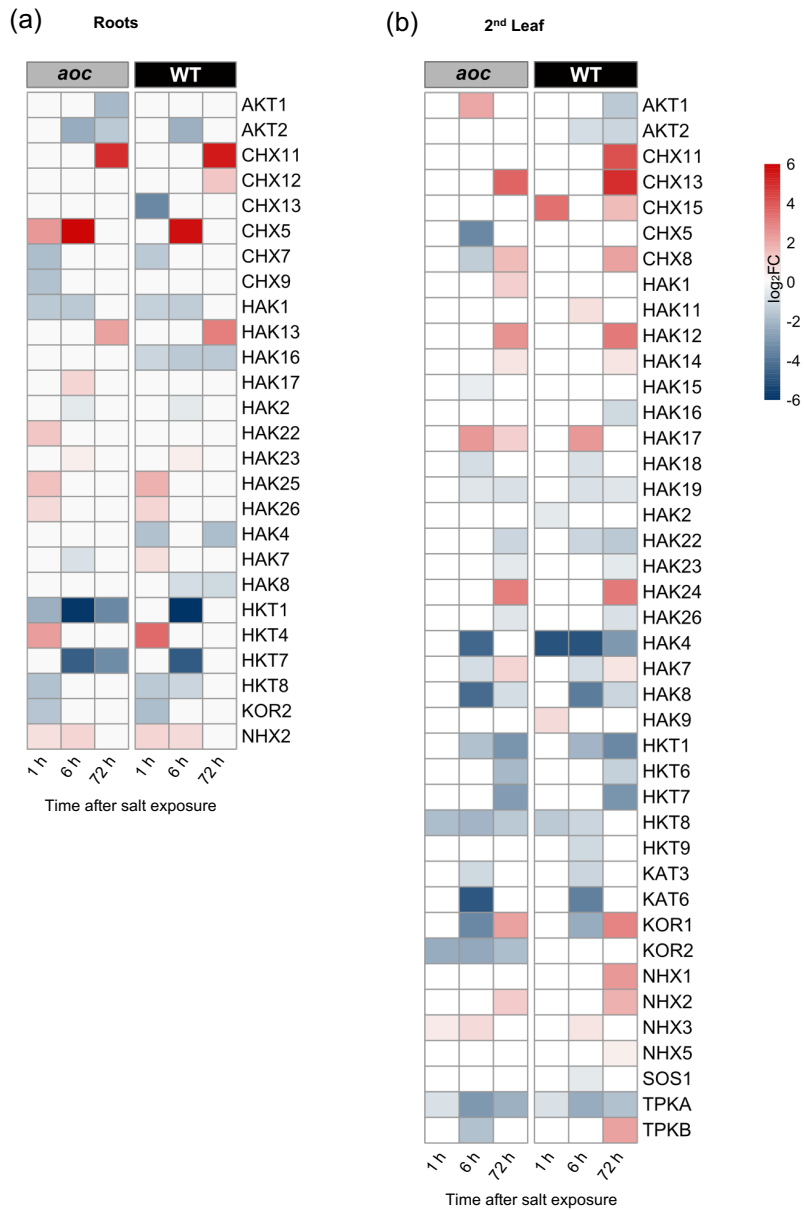


**Figure S5**



**FIGURE S5.** Kinetic expression profile of rice class III peroxidase genes displayed as heatmaps in root (a) and 2<sup>nd</sup> leaf (b).

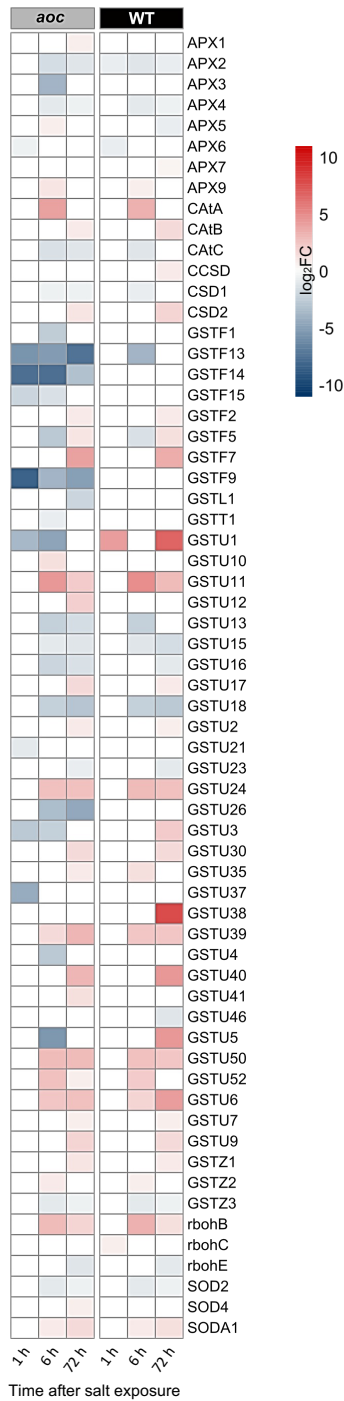
**Figure S6**



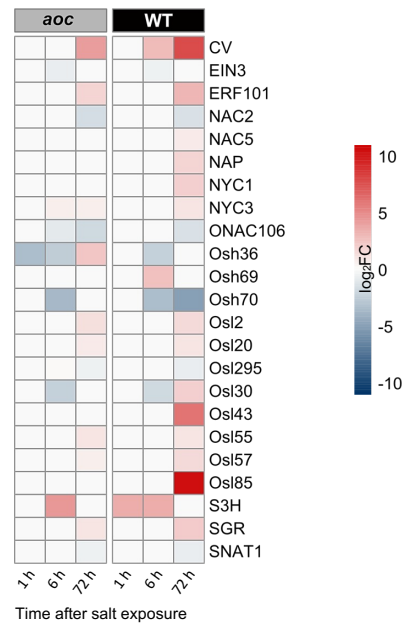
**FIGURE S6.** Kinetic expression profile of rice ion transporter genes displayed as heatmaps in root (a) and 2<sup>nd</sup> leaf (b).

**Figure S7**

**(a) ROS detoxification genes**



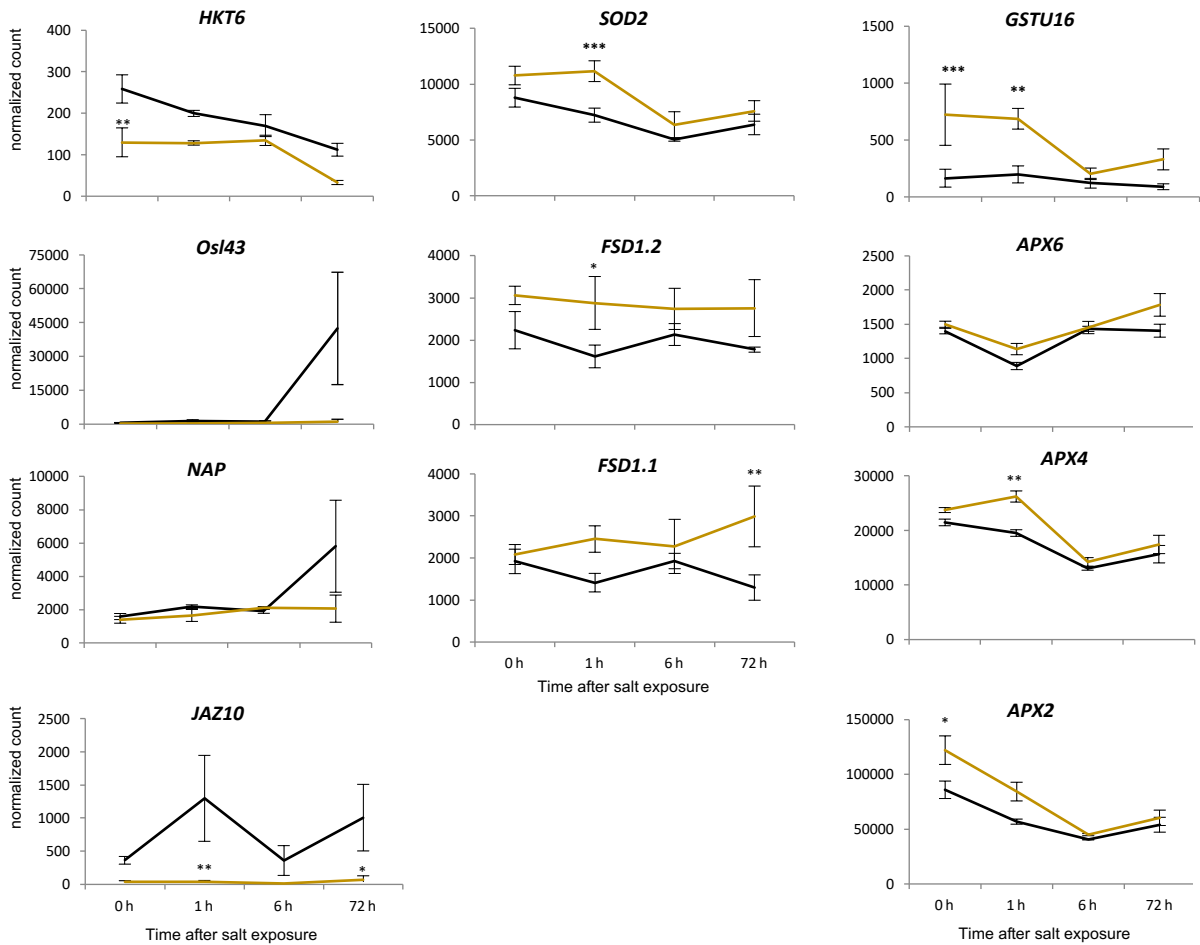
**(b) Senescence associated genes (SAG)**



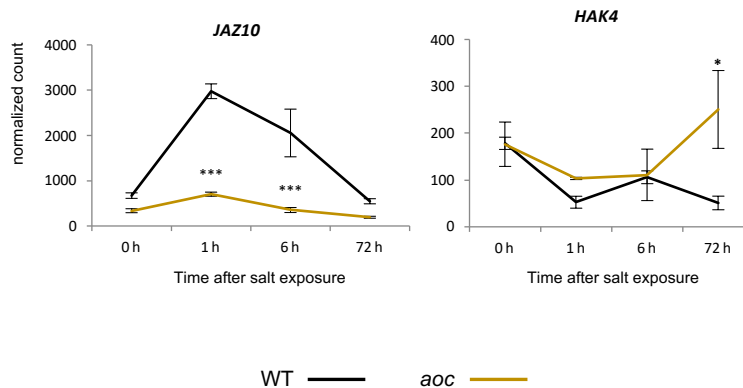
**FIGURE S7.** Kinetic expression profile of rice genes encoding ROS-scavenging or –consuming activities displayed as heatmaps in root (a) and 2<sup>nd</sup> leaf (b) of *aoc* and WT plants.

**Figure S8**

(a) Expression profile of shoot targets in RNAseq data



(b) Expression profile of Roots targets in RNAseq data



**FIGURE S8.** Kinetic expression profile of rice genes identified as probable targets of JA signaling in shoots (a) and roots (b) upon salt stress. Asterisks indicate a significant difference in means (ANOVA plus Tukey's HSD tests, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  Student's t test \*\*\* $P < 0.001$ ).

Target	Gene ID	Forward primer	Reverse primer
UBQ5	Os01g0328400	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
UBQ10	Os02g0161900	GAGCCTCTGTTCTGCAAGTA	ACTCGATGGTCCATTAAACC
FSD1.1	Os06g0115400	TCACGTGTACTCCAGTGTGC	GCATCGGAAGCGGTTTCATC
FSD1.2	Os06g0143000	ACAACGGCAACCCATTACCA	TGGCTGCATTGATTCCCAGA
NAP	Os03g0327800	AGTTCCGCAACACCTCCA	CTGCTCGTGGTCGGAGAG
OsI43	Os01g0348900	AGGCGTGACAATCTACAG	GGTTCAGAAATCTCCTTGA

**Table S4:** Primers used for RT-qPCR experiments.