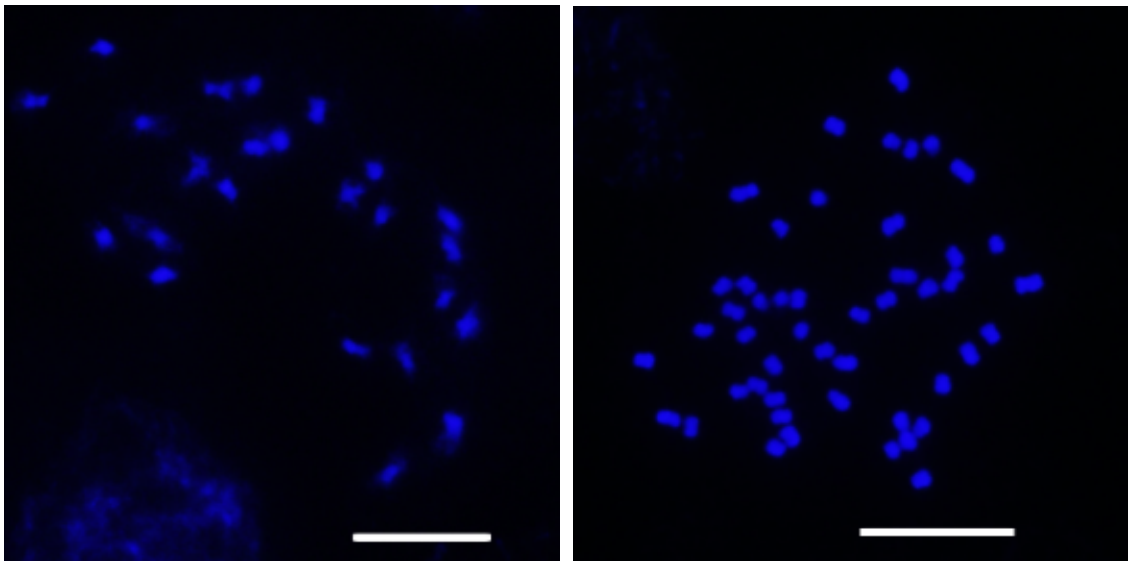

Diploid (2*n* = 2*x* = 24)

Tetraploid (2*n* = 4*x* = 48)

Fig. S1. Chromosome counts of diploid and tetraploid rice (Nipponbare). Root-tip cells were used for karyotype analysis with the chromosomes stained with DAPI (4′,6-diamidino-2-phenylindole). Scale bar = 10 μM.

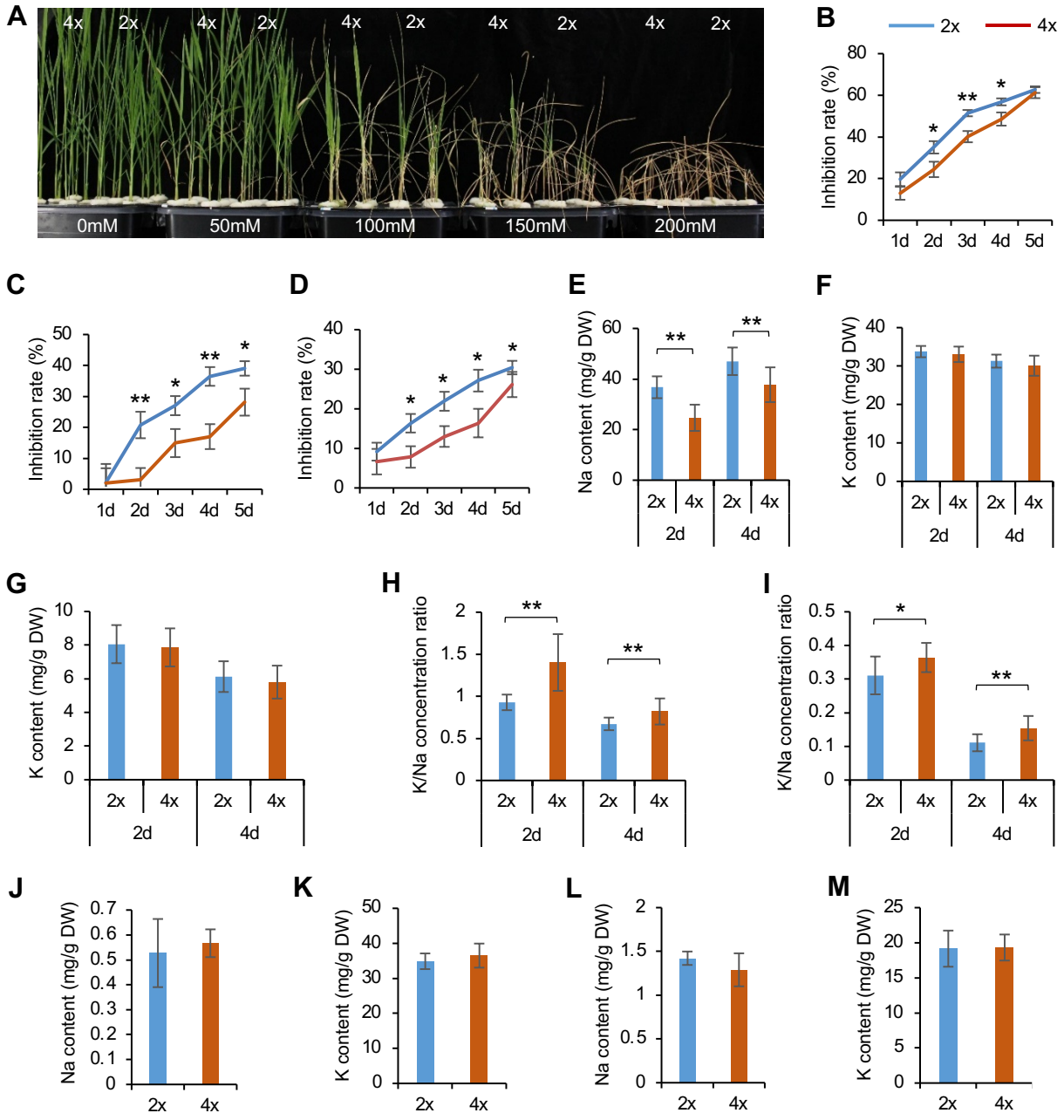

Fig. S2. Tetraploid rice of 02428 shows better salinity tolerance.
(*A*) Morphological changes in diploid and tetraploid rice under 0, 50, 100, 150 and 200 mM NaCl solution for 6 days, followed by recovery in nutrient solution for 5 days.
(*B*,*C*) Inhibition rates of shoots (*B*) and roots (*C*) after salt stress for 1 to 5 days. The fresh weight of shoots or roots was used to calculate inhibition rate (n=18 biological replicates, each replicate with 2 plants).
(*D*) Inhibition rates of shoots dry weight after salt stress for 1 to 5 days (n=18 biological replicates, each replicate with 2 plants).
(*E*,*F*) The content of sodium (*E*) and potassium (*F*) in shoots after salt stress for 2 and 4 days (n=12 biological replicates, each replicate with 2 plants).
(*G*) Potassium content in roots under the saline condition for 2 and 4 days (n=12 biological replicates, each replicate with 2 plants).
(*H*,*I*) The K/Na concentration ratios in shoots (*H*) and roots (*I*) after salt stress for 2 and 4 days.
(*J-M*) The content of sodium and potassium in shoots (*J*,*K*) and roots (*L*,*M*) without salt stress (n=3 biological replicates, each replicate with 6 plants). Single and double asterisks indicate statistical significance of $P < 0.05$ and $P < 0.01$, respectively (Student's *t* test).

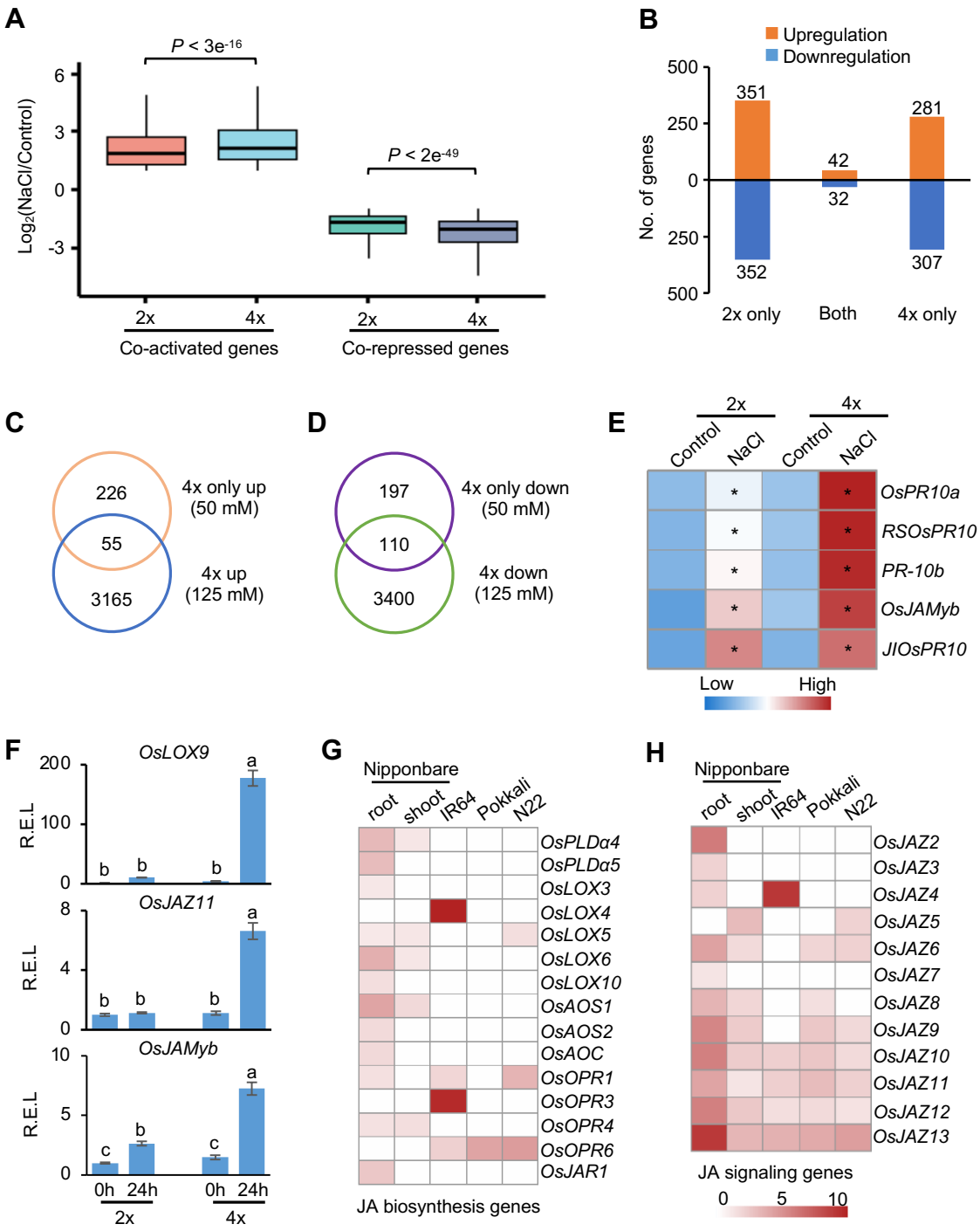

Fig. S3. Different responses between 02428 diploid and tetraploid rice after salt stress.

(*A*) Log2 expression ratios (NaCl/Control) of salt activated (co-activated) or repressed (co-repressed) genes shared between diploid and tetraploid rice.

(*B*) Number of up- or down-regulated salt responsive genes in the diploid (2x) only, tetraploid (4x) only, or shared (both) after salt stress (50mM).

(*C,D*) A portion of salt activated (B) or repressed (C) genes (50 mM) only in tetraploid rice remained up- or down-regulated under the solution with 125 mM NaCl.

(*E*) Expression levels of JA downstream related genes in the diploid and tetraploid rice without (control) or with (NaCl) salt treatment. A single asterisk indicates gene expression difference before and after the salt treatment in diploid and tetraploid rice [*FDR* < 0.05 and log2(fold change) ≥ 1].

(*F*) qRT-PCR verified the relative expression levels (R.E.L) of *OsLOX9*, *OsJAZ11* and *OsJAMyb* in diploid and tetraploid rice without (0h) or with (24h) salt stress. Different letters above each column indicate statistical significance at $P < 0.01$ (Tukey's test).

(*G,H*) Log_2(NaCl/Control) of JA biosynthesis (*G*) and signaling (*H*) related genes after salt stress in various rice varieties.

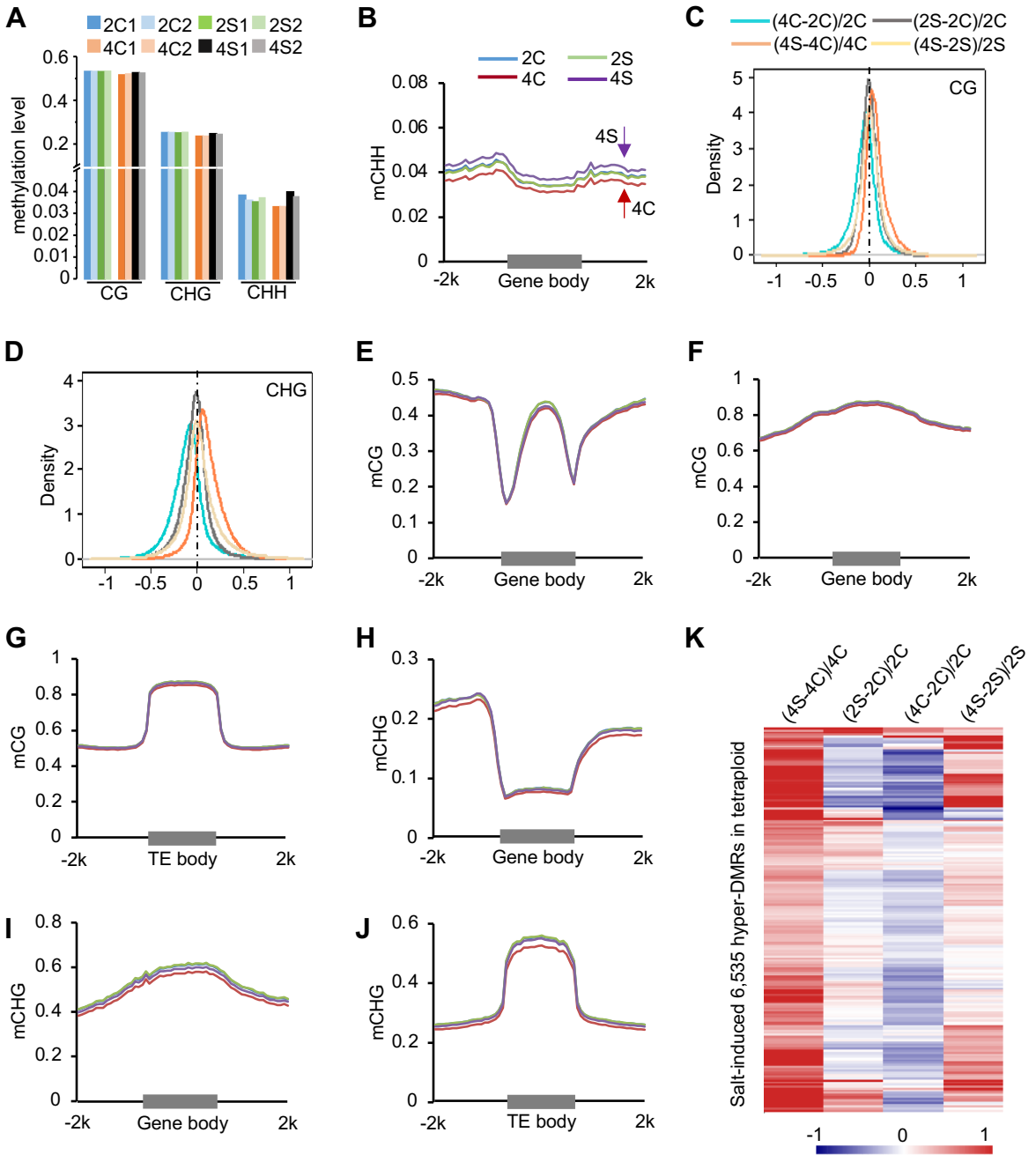

Fig. S4. DNA methylation changes in response to polyploidization and salt stress in 02428.
(A) Bulk methylation levels of diploid and tetraploid rice under the control and saline condition.
(*B*) Distributions of CHH methylation in TE genes.
(*C*,*D*) Global CG (*C*) and CHG (*D*) methylation changes after polyploidization and salt stress.
(*E*-*G*) Distributions of CG methylation in protein-coding genes (*E*), TE genes (*F*) and TEs (*G*).
(*H*-*J*) Distributions of CHG methylation in protein-coding genes (*H*), TE genes (*I*) and TEs (*J*).
(*K*) CHH methylation changes between 2S and 2C, 4C and 2C, 4S and 2S in salt-induced hyper-DMRs of tetraploid rice. 2C and 2S shows diploid rice without or with salt treatment. 4C and 4S shows tetraploid rice without or with salt stress.

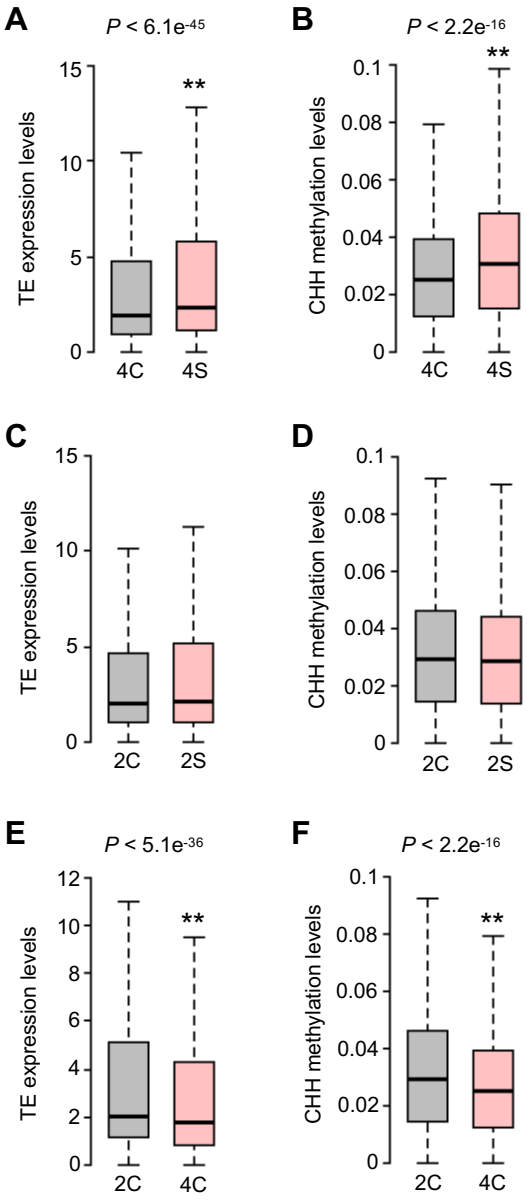

Fig. S5. TE expression and CHH methylation changes during polyploidization and salt stress in 02428.

(*A*,*B*) Changes of TE expression (*A*) and CHH methylation (*B*) levels in tetraploid rice after salt stress. The *Y*-axis indicated the values of standard transcript abundance normalization by DESeq (default methods in TEtranscripts). 4C and 4S: tetraploid rice without (4C) and with salt (4S) stress.

(*C*,*D*) Changes of TE expression (*C*) and CHH methylation (*D*) levels in diploid rice after salt treatment. 2C and 2S: diploid rice without (2C) and with salt (2S) stress.

(*E*,*F*) Changes of TE expression (*E*) and CHH methylation (*F*) levels after polyploidization. Double asterisks indicate statistical significance with shown *P* values (Wilcoxon rank sum test).

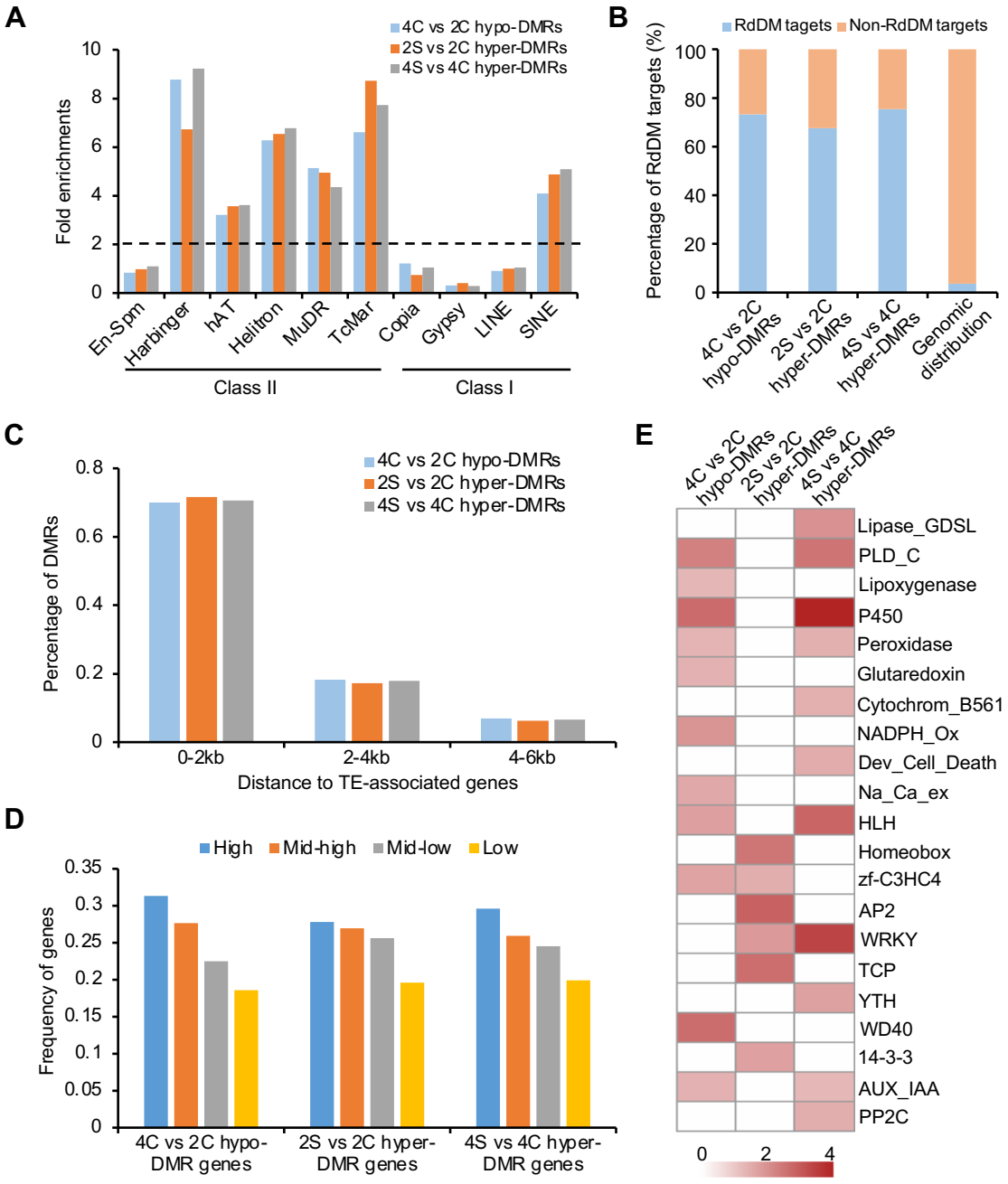

Fig. S6. Characters of stress-related DMRs and overlapping genes (02428).
(*A*) Enrichments of stress-related DMRs in TE families. Columns above the dotted lines indicating statistic significance of $P < 1e^{-5}$ (Fisher's exact test).
(*B*) Stress-related DMRs are significantly enriched in RdDM targets. RdDM targets, hypo-DMRs in *drm2* mutant ($P < 2.2e^{-16}$, hypergeometric test).
(*C*) Percentage of stress-related DMRs close to TE-associated genes with 0-2 kb, 2-4 kb and 4-6 kb distance.
(*D*) Distributions of stress-related DMR overlapping genes in regions with different densities. Genome was divided into four quartiles (High, Mid-high, Mid-low, Low) according gene densities.
(*E*) Enrichments of stress-related DMR overlapping genes in gene families. A dataset of gene families were download from Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/). 2C and 2S shows diploid rice without or with salt treatment. 4C and 4S shows tetraploid rice without or with salt stress.

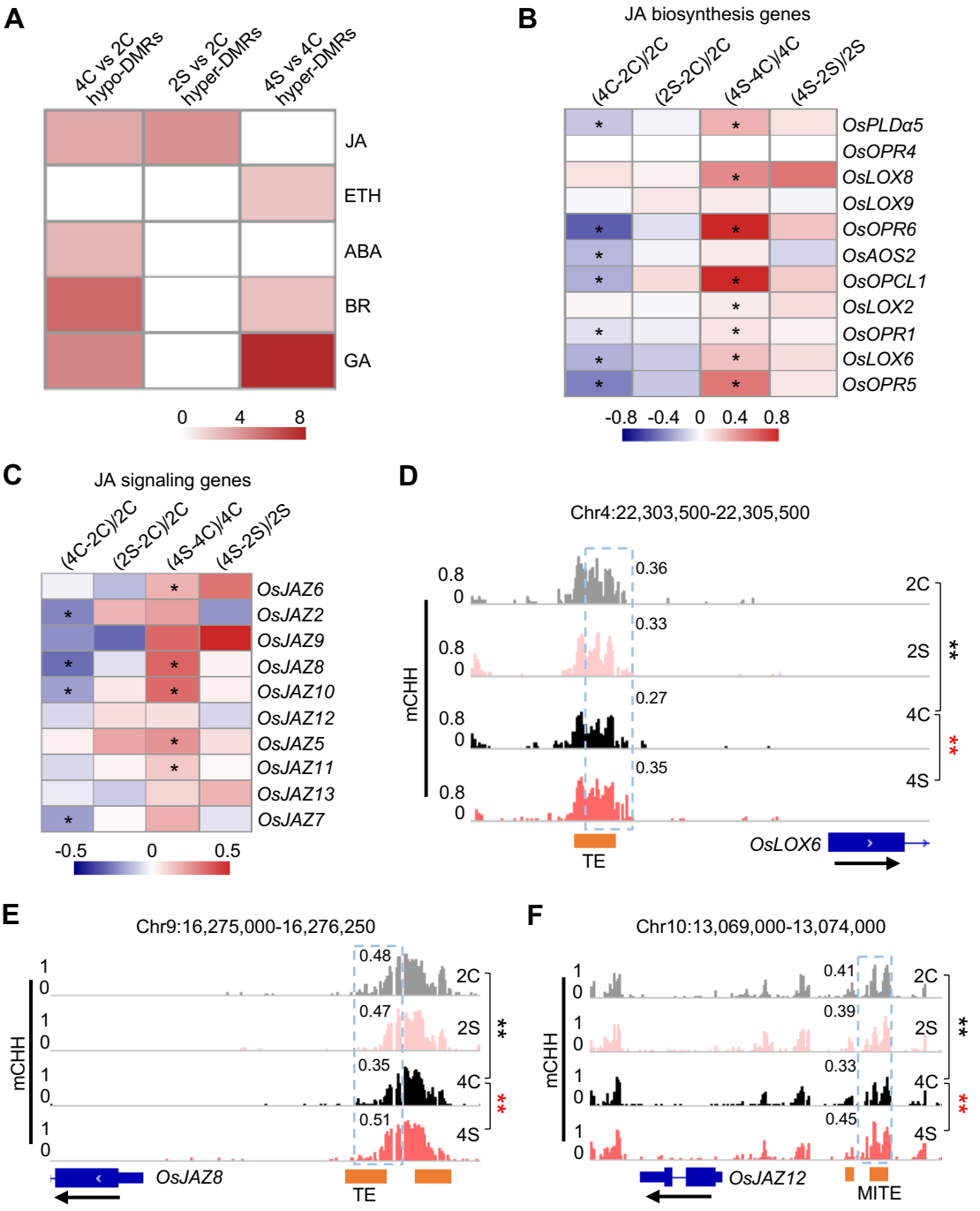

Fig. S7. CHH methylation changes fine tune activation of JA related genes and repression of TEs in 02428.

(*A*) Enriched bins of ploidy-dependent hypo- and salt-induced hyper-DMR overlapping genes.

(*B*,*C*) CHH methylation changes in promoters of JA biosynthesis (*B*) and signaling (*C*) related genes. Black asterisks indicate genes with significant CHH methylation changes with $P < 0.05$ between 4C and 2C, or between 4S and 4C (Fisher's exact test).

(*D-F*) Genome Browser snapshots showing CHH methylation changes in the promoter of *OsLOX6* (*D*), *OsJAZ8* (*E*) and *OsJAZ12* (*F*). The genes and transposable elements are shown with black arrows indicating transcriptional direction. Numbers in snapshots indicate CHH methylation levels of regions in dotted box. Double black and red asterisks indicate statistic significance of $P < 0.01$ between 4C and 2C, or between 4S and 4C, respectively (Fisher's exact tests). 2C and 2S, diploid rice without or with salt treatment. 4C and 4S, tetraploid rice without or with salt stress.

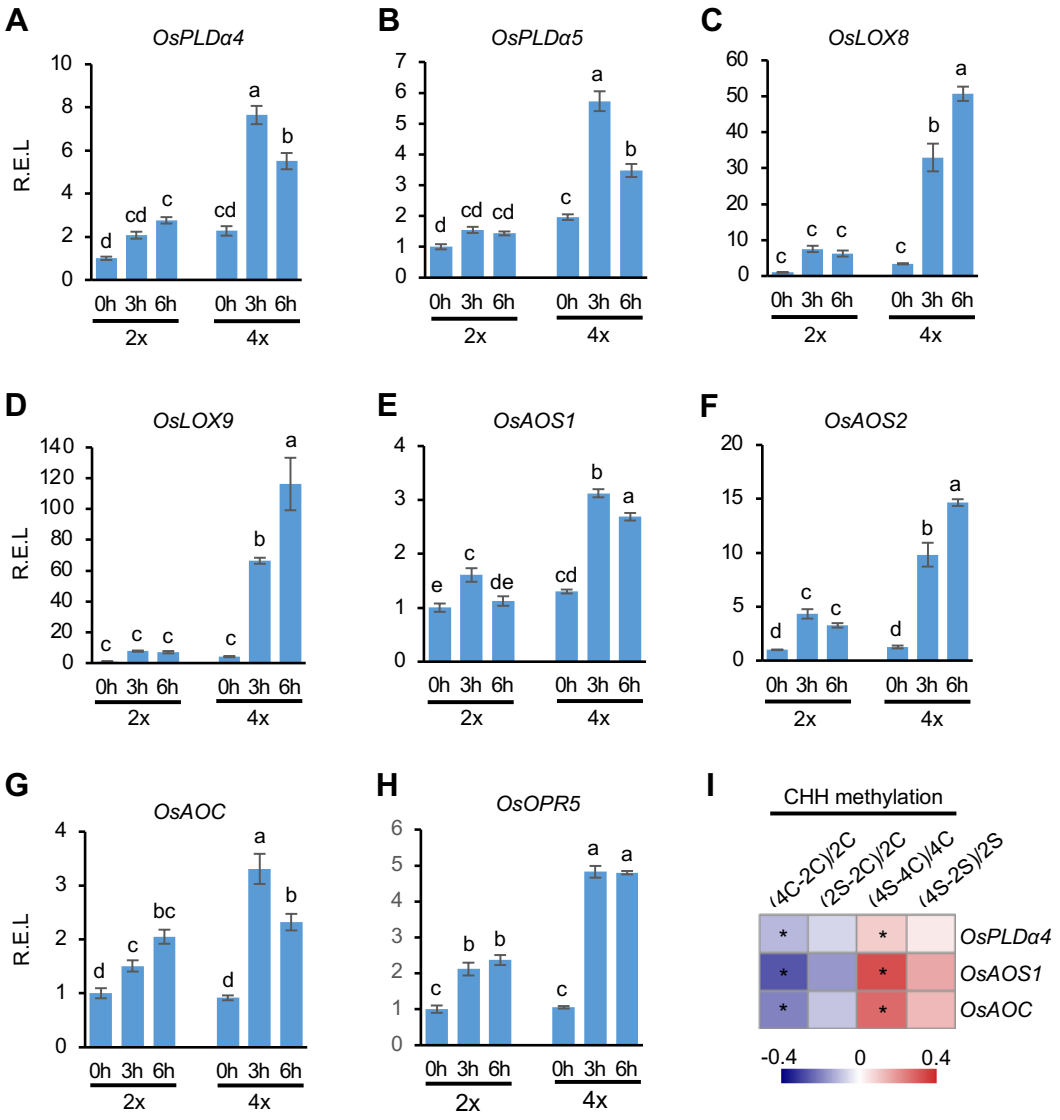

Fig. S8. Activation of JA biosynthesis related genes in the early phase of salt stress and CHH methylation changes of several genes in 02428.

(*A-H*) Relative expression levels (R.E.L) of JA biosynthesis related genes in time course of 0h, 3h and 6h after salt stress. Different letters above each column indicate statistical significance at $P < 0.01$ (Tukey's test).

(*I*) CHH methylation changes in promoters of *OsPLDα4*, *OsAOS1* and *OsAOC*. Black asterisks indicate these genes with significantly CHH methylation changes between 4C and 2C, or between 4S and 4C ($P < 0.05$, Fisher's exact tests). 2C and 2S, diploid rice without or with salt stress. 4C and 4S, tetraploid rice without or with salt stress.

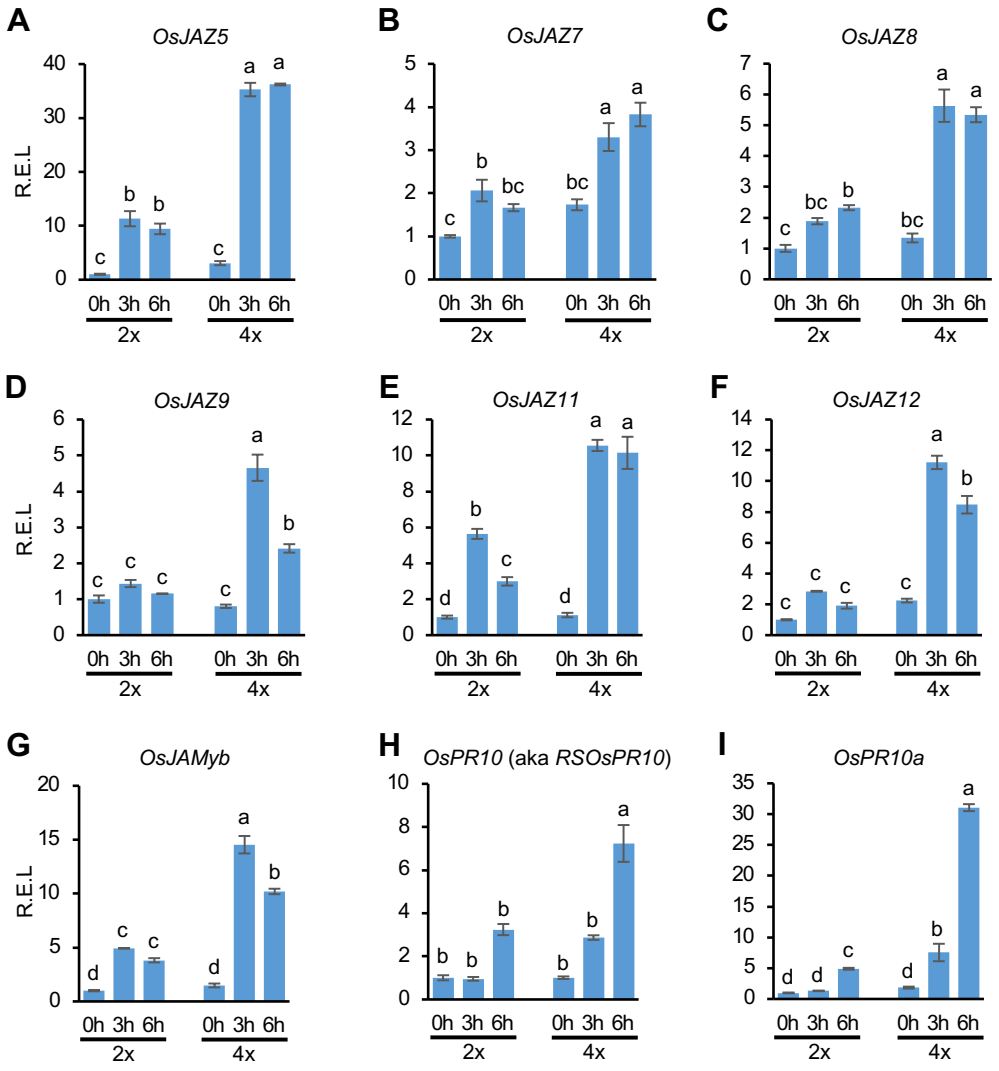

Fig. S9. Rapid activation of JA signaling and downstream related genes after salt treatment in 02428. (*A-I*) Relative expression levels (R.E.L) of JA signaling (*A-F*) and downstream (*G-I*) related genes validated by qRT-PCR after salt stress for 0h, 3h and 6h. Different letters above each column indicate statistical significance at $P < 0.01$ (Tukey's test).

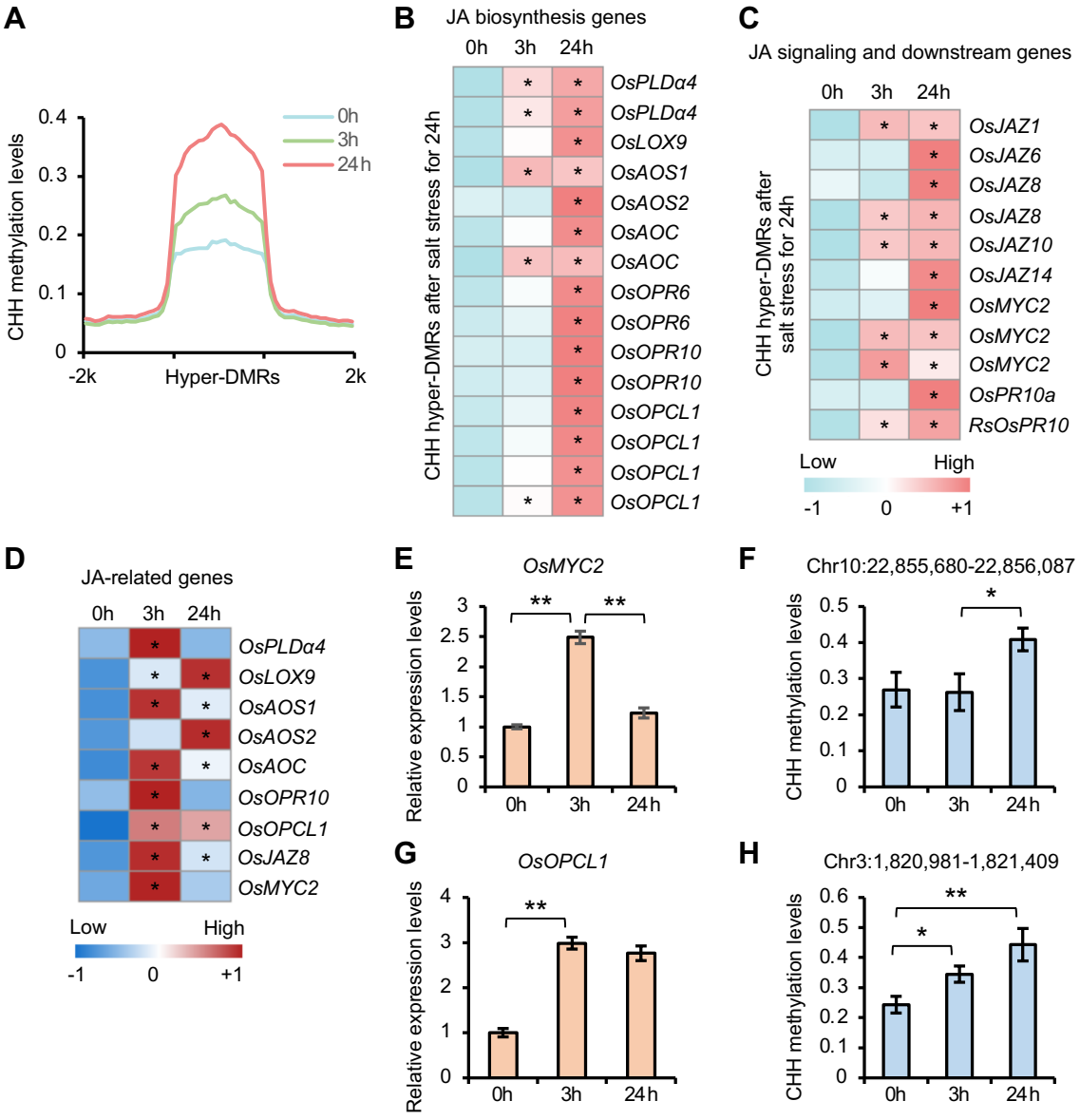

Fig. S10. Salt-induced gene activation preceded CHH methylation level increase in 02428 tetraploid rice.
(*A*) CHH methylation changes in salt-induced hyper-DMRs (after stress for 24h) of tetraploid after salt stress for 0, 3 and 24 hours.
(*B*, *C*) CHH methylation level (*FDR* < 0.05 and absolute methylation difference over 0.1) changes in salt-induced hyper-DMRs proximal to JA biosynthesis (*B*), signaling and downstream (*C*) pathway genes in tetraploid rice after salt stress for 0, 3 and 24 hours. A single asterisk indicates methylation level difference between 0h and 3h or between 0h and 24h, respectively (Fisher's exact test).
(*D*) qRT-PCR analysis (relative expression levels) of nine JA-related genes after salt stress for 0, 3 and 24 hours. A single asterisk indicates gene expression level difference between 0h and 3h or between 0h and 24, respectively ($P < 0.01$, Student's *t* test).
(*E*) Relative expression levels of *OsMYC2* after salt stress for 0, 3 and 24 hours. Single and double asterisks indicate statistical significance of $P < 0.05$ and $P < 0.01$, respectively (Student's *t* test).
(*F*) CHH methylation level changes after salt stress for 0, 3 and 24 hours in a promoter region of *OsMYC2*, a key transcriptional factor in JA signaling transmission.
(*G*) Relative expression levels of *OsOPCL1* after salt stress for 0, 3 and 24 hours.
(*H*) CHH methylation level changes after salt stress for 0, 3 and 24 hours in a promoter region of *OsOPCL1*.

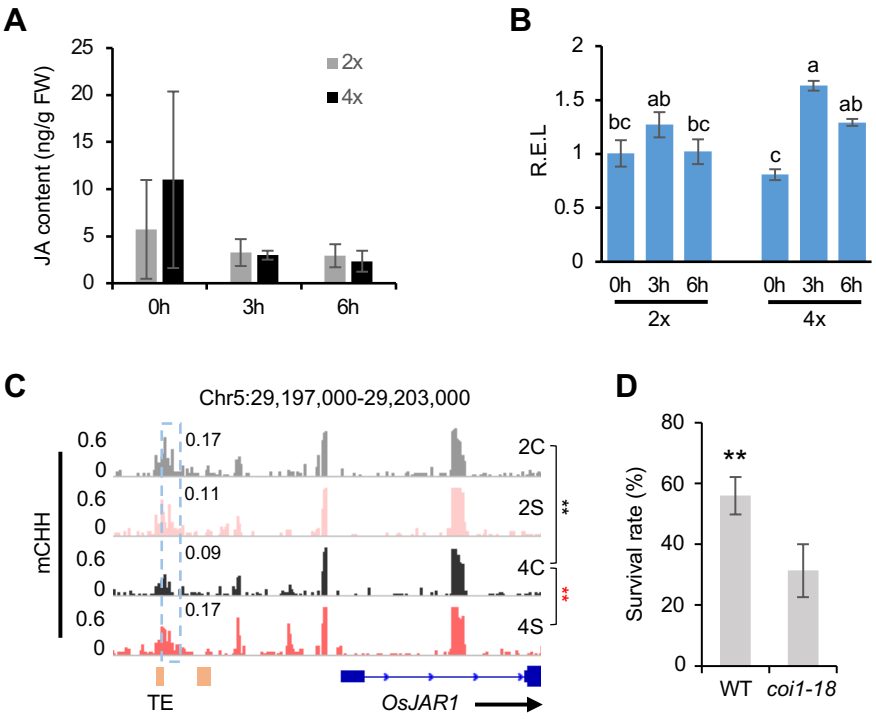

Fig. S11. Stronger activation of *OsJAR1* in 02428 tetraploid rice after salt treatment depends on ploidy-induced hypomethylation.

(*A*) Content of JA in diploid and tetraploid rice after salt stress for 0h, 3h and 6h (n=3 biological replicates).

(*B*) Relative expression levels (R.E.L) of *OsJAR1* after salt stress for 0h, 3h and 6h. Different letters above each column indicate statistical significance at $P < 0.01$ (Tukey's test).

(*C*) Genome Browser view showing CHH methylation changes in the promoter of *OsJAR1*. Black arrow indicates transcriptional orientation of genes. Numbers in snapshot indicate CHH methylation levels of regions in dotted box. Double black and red asterisks indicate statistic significance of $P < 0.01$ between 4C and 2C, or between 4S and 4C, respectively (Fisher's exact tests). 2C and 2S, diploid rice without or with salt treatment. 4C and 4S, tetraploid rice without or with salt stress.

(*D*) Survival rates were reduced in the *coi1* mutant relative to the wild type (WT) under the salt stress (n = 4 biological replicates, each replicate with 30 plants). *coi1-18*: RNAi silencing lines for *CORONATINE INSENSITIVE 1a* and *1b (COI1a and b)*, encoding an F-box protein and identified as a JA receptor. Double asterisks indicate statistical significance of $P < 0.01$ (Student's *t* test).

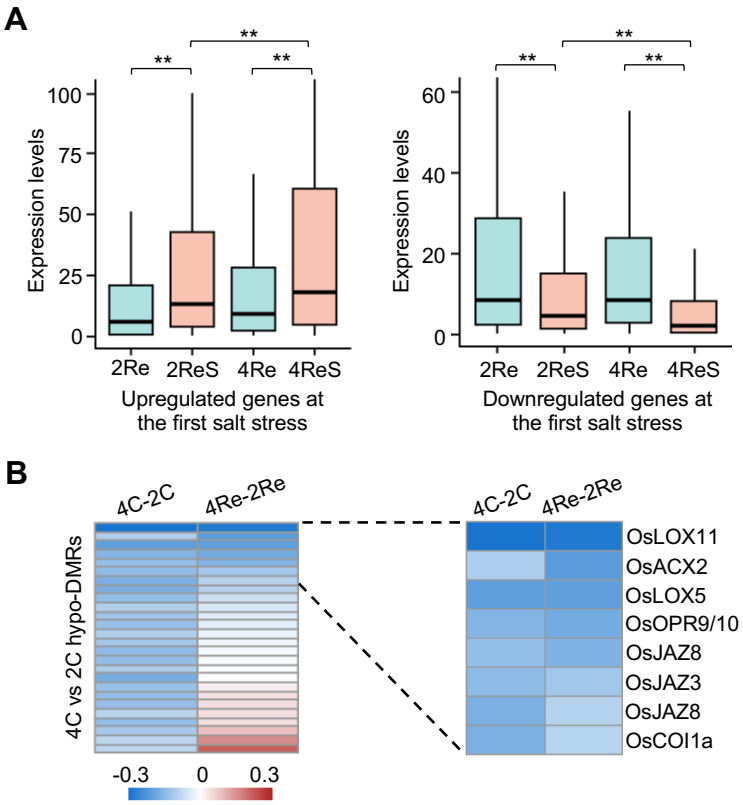

Fig. S12. Gene expression and CHH methylation changes in 02428 after a second round of salt stress.
(A) Expression changes of upregulated or downregulated genes (after the first salt treatment) in diploid and tetraploid response to a second round of salt stress. Double asterisks indicate statistic significance of $P < 2.2e^{-6}$ (Wilcoxon rank-sum test).
(*B*) CHH methylation changes between diploid and tetraploid rice recovery in nutrient solution (4Re vs 2Re) in ploidy-induced hypo-DMRs (4C vs 2C) around JA-related genes. 2C and 4C, diploid and tetraploid rice under control condition; 2Re and 4Re, diploid and tetraploid recovery in nutrient solution; 2ReS and 4ReS, diploid and tetraploid after retreatment by salt stress.

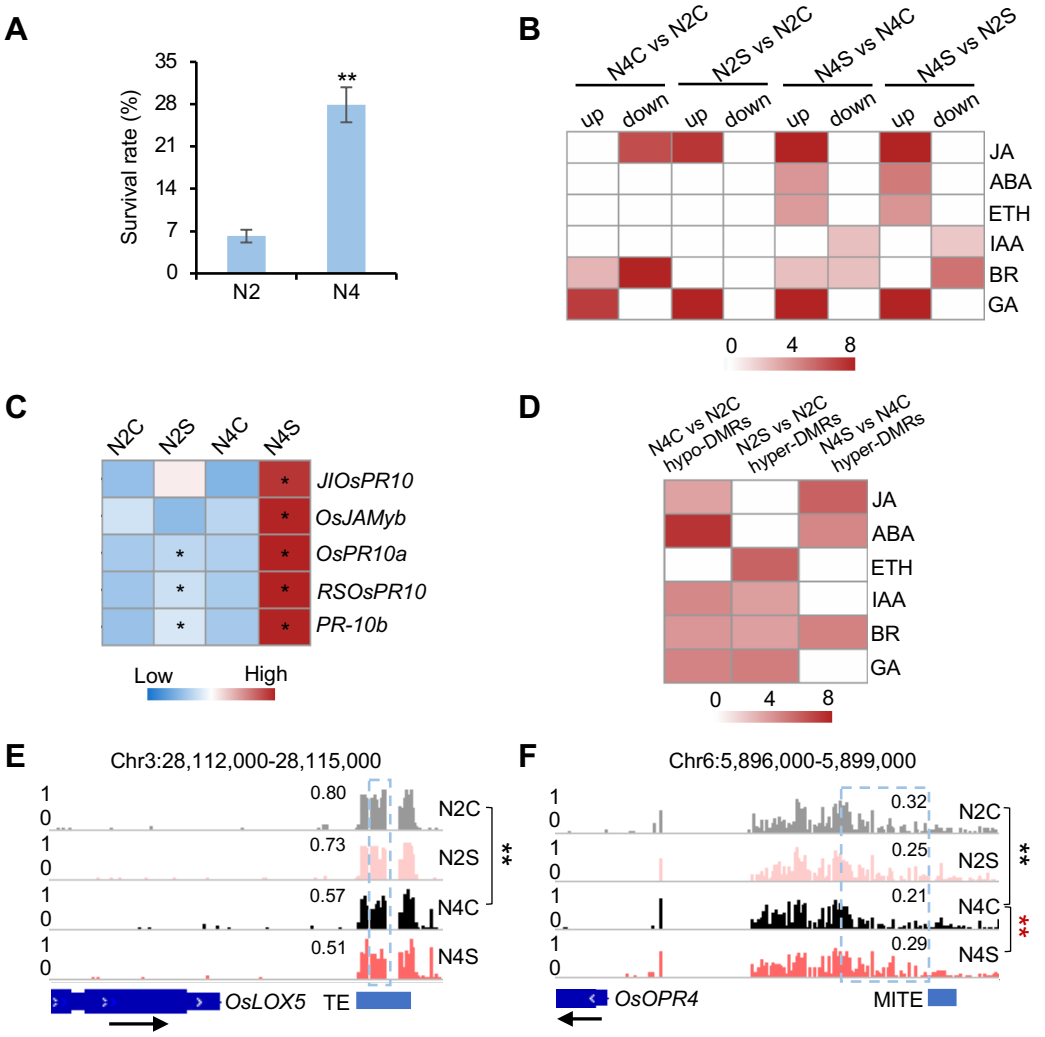

Fig. S13. Activation of JA-related genes and DNA methylation changes in diploid and tetraploid of Nipponbare after salt stress.

(*A*) Survival rates of Nipponbare diploid (N2) and tetraploid (N4) rice after salt treatment for 7 days, following recovery in water for 5 days (n=5 biological replicates). Double asterisks indicate statistical significance of $P < 0.01$, Student's *t* test.

(*B*) Enrichments of phytohormone-related genes in ploidy-dependent and salt responsive genes in diploid or tetraploid rice without (N2C and N4C) or with (N2S and N4S) salt stress.

(*C*) Expression levels of JA downstream related genes in diploid and tetraploid rice without or with salt treatment. A single asterisk indicates gene expression level difference before and after salt stress in tetraploid and diploid rice, respectively [$P < 0.05$ and log2(fold change) ≥ 1].

(*D*) Enriched bins of ploidy-induced hypo- and salt-induced hyper-DMR overlapping genes.

(*E,F*) Genome browser views showing CHH methylation changes of *OsLOX5* (*E*) and *OsOPR4* (*F*). The genes and TEs are shown with black arrows indicating transcriptional orientations. Numbers in snapshot indicate CHH methylation levels of regions in dotted box. Double black and red asterisks indicate statistic significance of $P < 0.01$ between N4C and N2C, or between N4S and N4C, respectively (Fisher's exact tests).