Supporting Information

Van Ha et al. 10.1073/pnas.1322135111

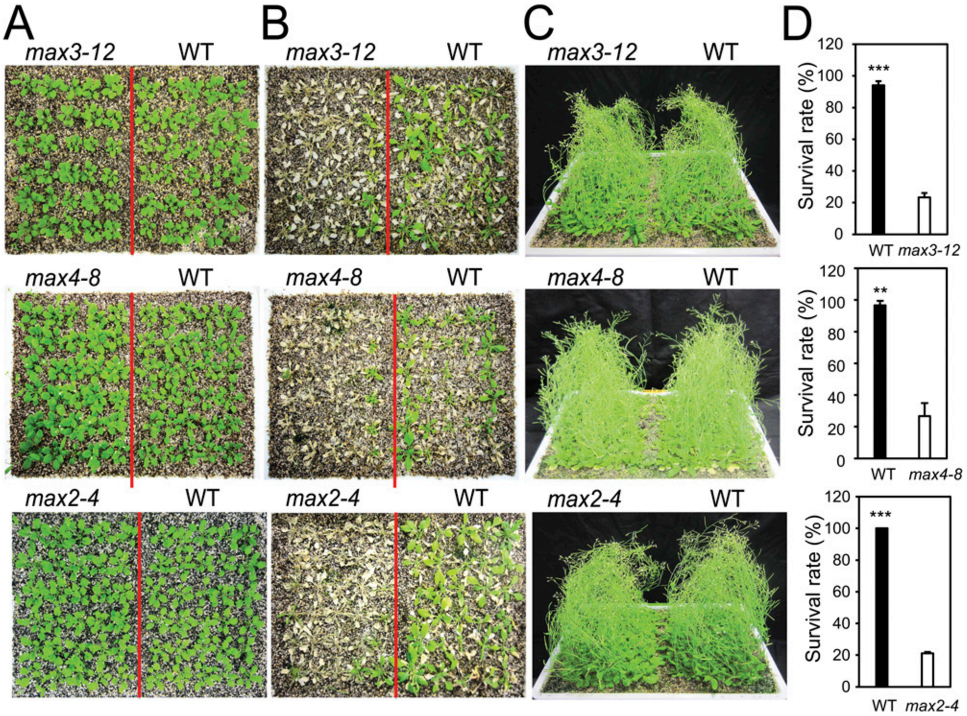


Fig. S1. Hypersensitivity of strigolactone (SL)-deficient and SL-signaling [*more axillary growth* (*max*)] mutant plants to drought stress. (*A*) Three-wk-old WT and SL-deficient *max3-12* and *max4-8* and SL-signaling *max2-4* mutant plants before being subjected to a drought stress. (*B*) Effect of drought stress on the viability of WT and mutant plants. Trays were photographed 3 d after rewatering was initiated. Inflorescences were removed from the surviving plants before photographing. (*C*) Unstressed (control) WT and *max* plants grown in parallel with the drought test. (*D*) Percent survival rates of WT and mutant plants. Data represent the mean and SE of three independent experiments ($n = 30$ per genotype per experiment). Asterisks indicate significant differences as determined by a Student *t* test (***$P < 0.001$).

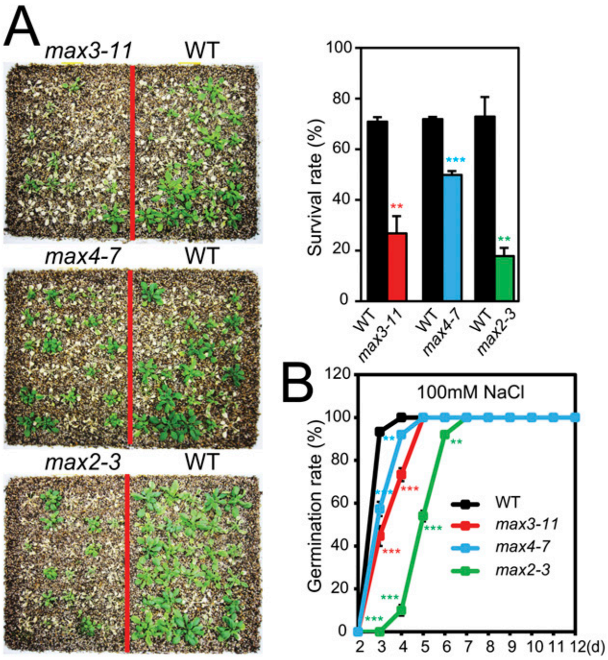


Fig. S2. Hypersensitivity of SL-deficient and SL-signaling *max* mutant plants to salt stress. (*A*) Three-wk-old WT and SL-deficient *max3-11* and *max4-7* and SL-signaling *max2-3* mutant plants were treated with a total of 2 L of 200 mM NaCl over 6 d and then watered for 4 d with plain water. Data represent the mean and SE of three independent experiments ($n = 30$ per genotype per experiment). (*B*) Percent germination of WT and *max* mutant seeds exposed to 100 mM NaCl. Data represent the mean plus SE of data pooled from three independent experiments ($n = 50$ seeds per genotype per experiment). Asterisks indicate significant differences as determined by a Student *t* test (**$P < 0.01$ and ***$P < 0.001$).

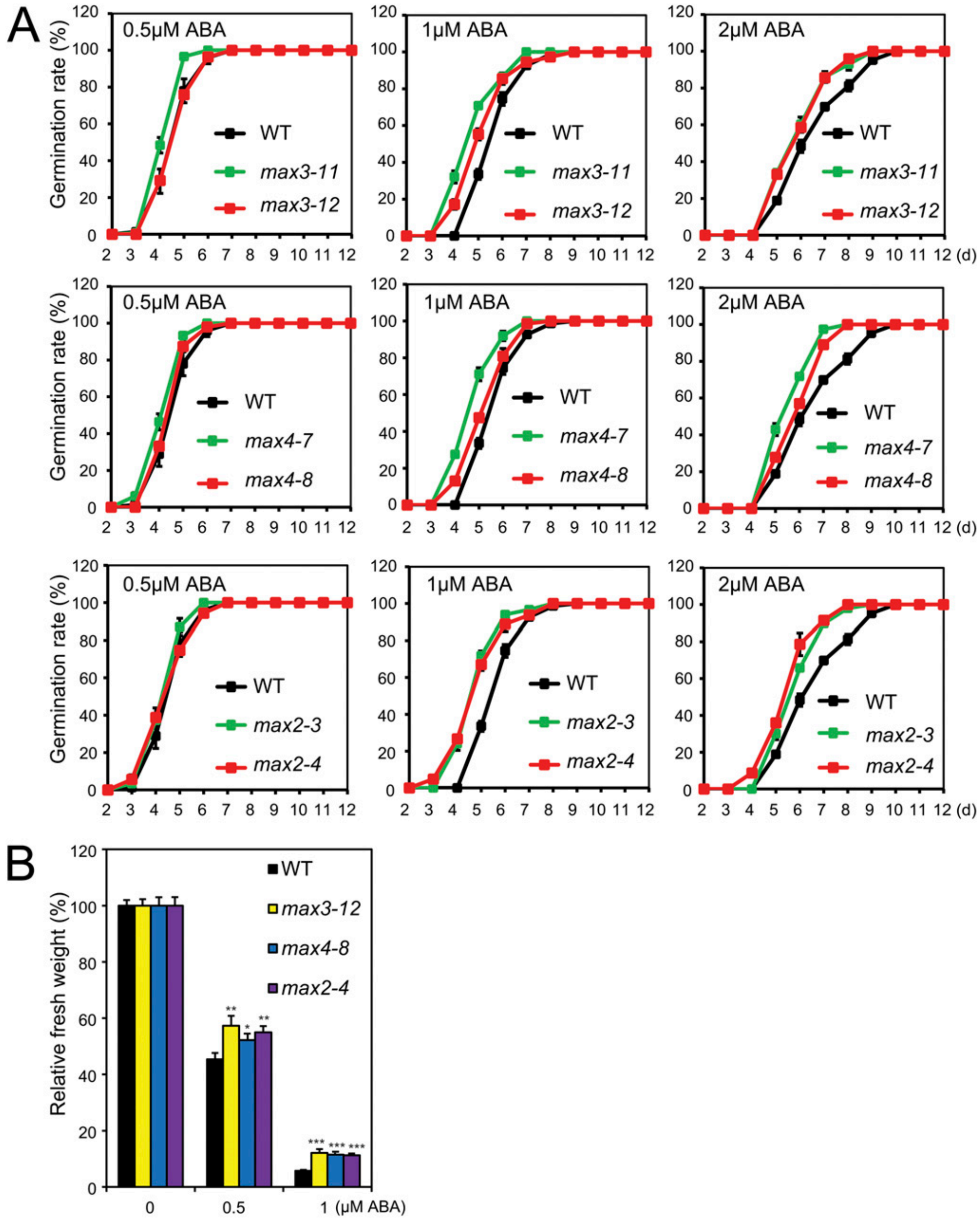


Fig. S3. Effect of abscisic acid (ABA) on seed germination and growth of young seedlings of WT and SL-deficient and SL-signaling *max* mutants. (*A*) Percent germination of WT and SL-deficient and SL-signaling *max* mutant plants treated exogenously with ABA. Data represent the mean plus SE of data pooled from three independent experiments ($n = 50$ seeds per genotype per experiment). (*B*) Relative fresh weight of WT and SL-deficient and SL-signaling mutant plants treated exogenously with ABA. Relative fresh weights of WT and *max* mutant plants were determined 14 d after application of ABA. Plants were maintained at 22 °C. Data represent the mean and SE ($n = 6$; each measurement represents the weight of seven plants). Asterisks indicate significant differences as determined by a Student *t* test ($*P < 0.05$, $**P < 0.01$, and $***P < 0.001$).

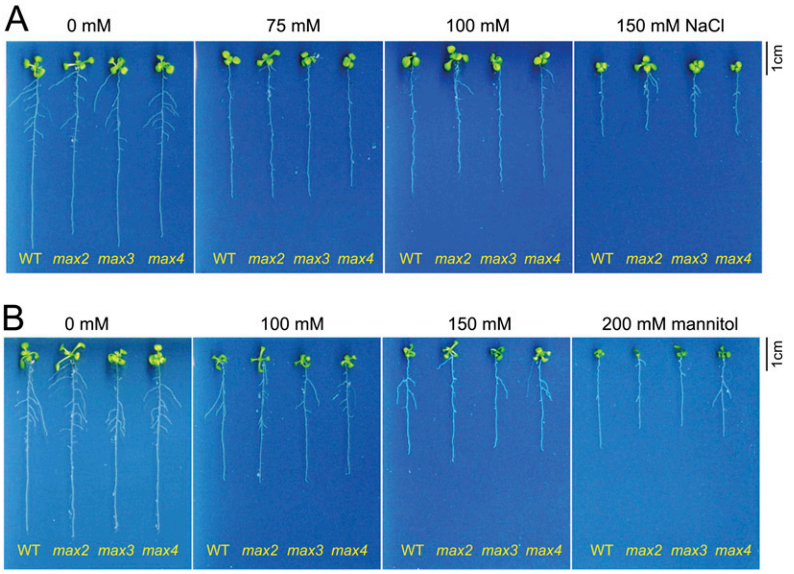


Fig. S4. Root growth in representative 11-d-old SL-biosynthetic *max3-11* and *max4-7*, SL-response *max2-3*, and WT plants grown on 0.5× Murashige–Skoog medium supplemented with various concentration of NaCl (*A*) and mannitol (*B*). Photographs were taken after 7 d of incubation at 22 °C.

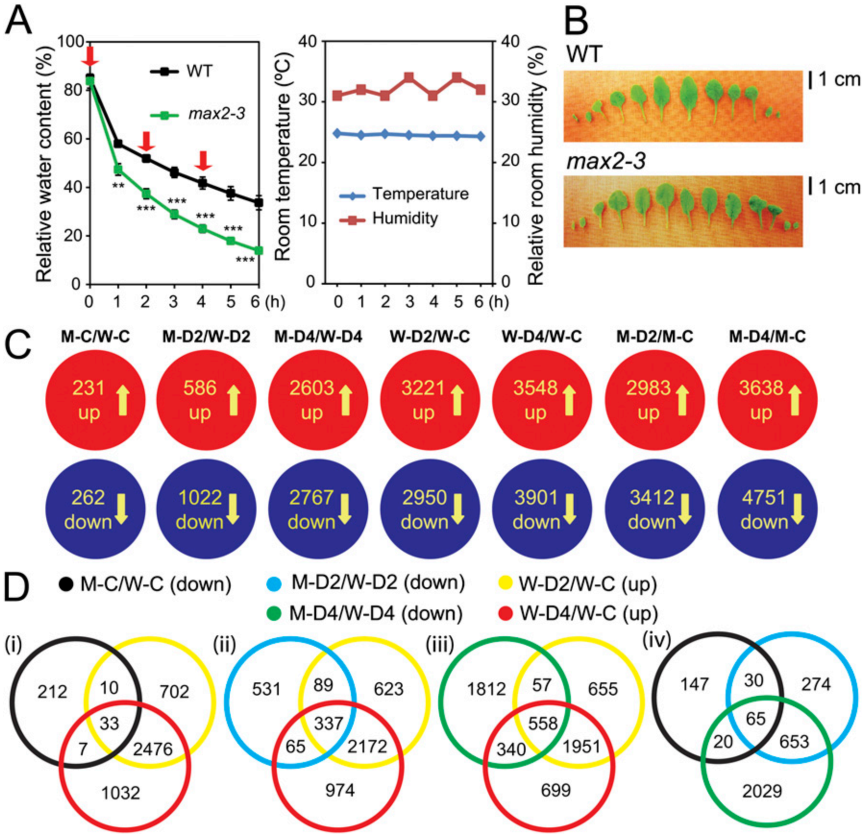
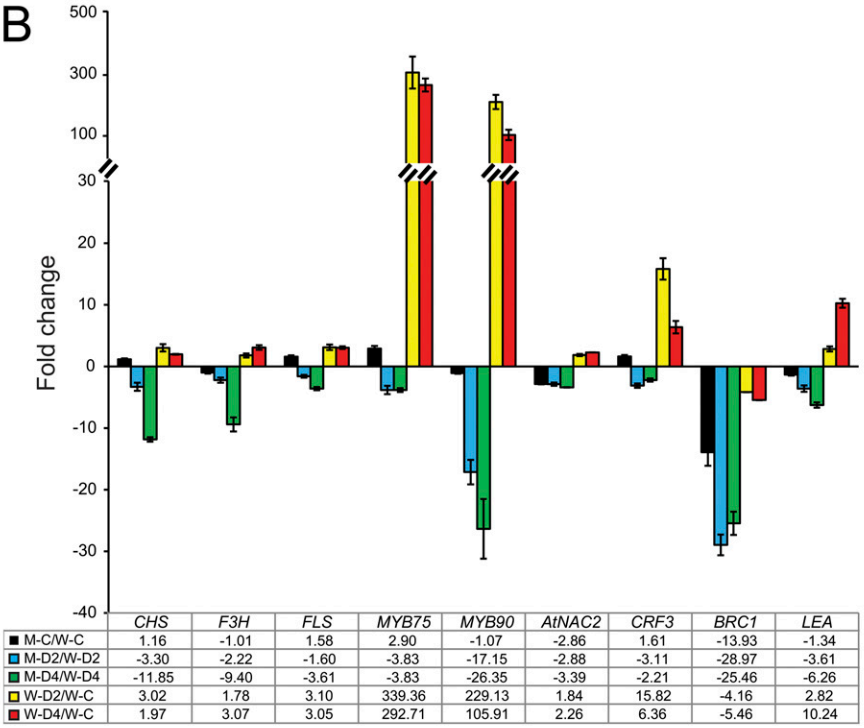


Fig. S5. Relative water content (RWC) of leaves of WT and SL-signaling *max2-3* mutant plants exposed to a dehydration stress and analysis of differential gene expression analysis in leaves of WT and SL-signaling *max2-3* mutant plants under well-watered and dehydrative conditions. (*A*) Time course of RWC of aerial portions of WT and *max2-3* plants exposed to a dehydration stress. Data represent the mean and SE ($n = 5$). Asterisks indicate significant differences as determined by a Student *t* test ($**P < 0.01$ and $***P < 0.001$). Rosette leaf samples collected at 0, 2, and 4 h (arrows) were used for microarray analysis. Room temperature and relative room humidity were recorded during the dehydration treatment. (*B*) Detached representative leaves from well-watered WT and *max2-3* plants. (*C*) Diagrams showing the compilation of genes with altered expression in each comparative expression analysis. Data were obtained from the results of three independent microarray experiments. (*D*) Venn diagram analysis showing the overlapping and nonoverlapping up-regulated gene sets. M-C/W-C, *max2-3* well-watered control 0 h vs. WT well-watered control 0 h; M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D2/W-C, WT dehydrated 2 h vs. WT well-watered control 0 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h; M-D2/M-C, *max2-3* dehydrated 2 h vs. *max2-3* well-watered control 0 h; M-D4/M-C, *max2-3* dehydrated 4 h vs. *max2-3* well-watered control 0 h.

A

AGI	Gene description	M-C/W-C		M-D2/W-D2		M-D4/W-D4		W-D2/W-C		W-D4/W-C	
		Fold change	q-value	Fold change	q-value	Fold change	q-value	Fold change	q-value	Fold change	q-value
AT5G13930	*CHS*	1.00	0.9992	-2.04	0.0502	-11.04	0.0044	3.67	0.0221	4.76	0.0154
AT3G51240	*F3H*	-1.24	0.4150	-1.91	0.0234	-7.33	0.0076	2.72	0.0100	4.25	0.0073
AT5G08640	*FLS1*	1.10	0.7997	-1.06	0.6422	-2.44	0.0413	3.69	0.0175	3.99	0.0303
AT1G56650	*MYB75*	2.51	0.0792	-2.49	0.0337	-3.07	0.0199	396.15	0.0001	340.25	0.0003
AT1G66390	*MYB90*	-1.06	0.2608	-20.35	0.0349	-25.12	0.0107	43.95	0.0001	33.59	0.0033
AT5G39610	*AtNAC2*	-2.86	0.0133	-2.02	0.0314	-2.08	0.0041	2.53	0.0007	3.06	0.0008
AT5G53290	*CRF3*	1.53	0.2257	-2.22	0.0287	-2.13	0.0186	32.09	0.0003	13.23	0.0010
AT3G18550	*BRC1*	-31.71	0.0117	-20.65	0.0244	-9.89	0.0073	-3.76	0.0093	-5.18	0.0005
AT1G54890	*LEA*	1.86	0.2879	-2.91	0.2257	-7.06	0.0044	12.19	0.0023	55.52	0.0003



	CHS	*F3H*	*FLS*	*MYB75*	*MYB90*	*AtNAC2*	*CRF3*	*BRC1*	*LEA*
■ M-C/W-C	1.16	-1.01	1.58	2.90	-1.07	-2.86	1.61	-13.93	-1.34
🟦 M-D2/W-D2	-3.30	-2.22	-1.60	-3.83	-17.15	-2.88	-3.11	-28.97	-3.61
🟩 M-D4/W-D4	-11.85	-9.40	-3.61	-3.83	-26.35	-3.39	-2.21	-25.46	-6.26
🟨 W-D2/W-C	3.02	1.78	3.10	339.36	229.13	1.84	15.82	-4.16	2.82
🟥 W-D4/W-C	1.97	3.07	3.05	292.71	105.91	2.26	6.36	-5.46	10.24

Fig. S6. Real-time quantitative PCR (RT-qPCR) confirmation of microarray data. Nine genes were selected and their expression profiles were assessed by RT-qPCR in leaf samples obtained from nonstressed and dehydrated *max2-3* and WT plants to validate the microarray data. (*A*) Fold changes were obtained from microarray analysis. (*B*) Fold changes obtained by RT-qPCR of three independent biological replicates. *UBQ10* was used as reference gene. Data represent the mean plus SE (n = 3). M-C/W-C, *max2-3* well-watered control 0 h vs. WT well-watered control 0 h; M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D2/W-C, WT dehydrated 2 h vs. WT well-watered control 0 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h.

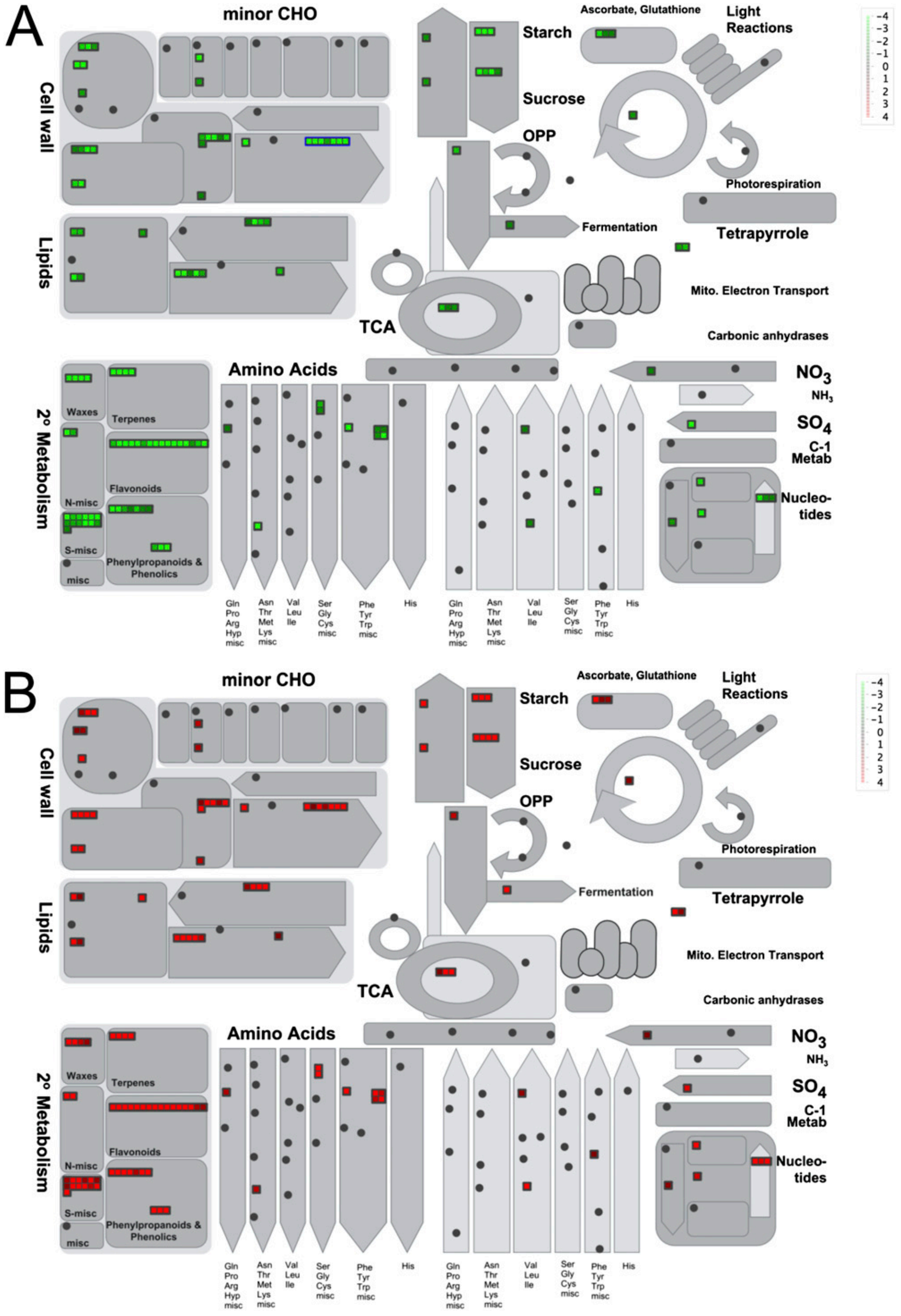


Fig. S7. Overview of genes involved in different metabolic processes that were down-regulated in M-D4/W-D4 (*A*) and up-regulated in W-D4/W-C (*B*). Green and red colors indicate repression and induction, respectively. Colored bars in each panel indicate fold changes in gene expression. MapMan software was used to illustrate the different functional categories that were involved. M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h.

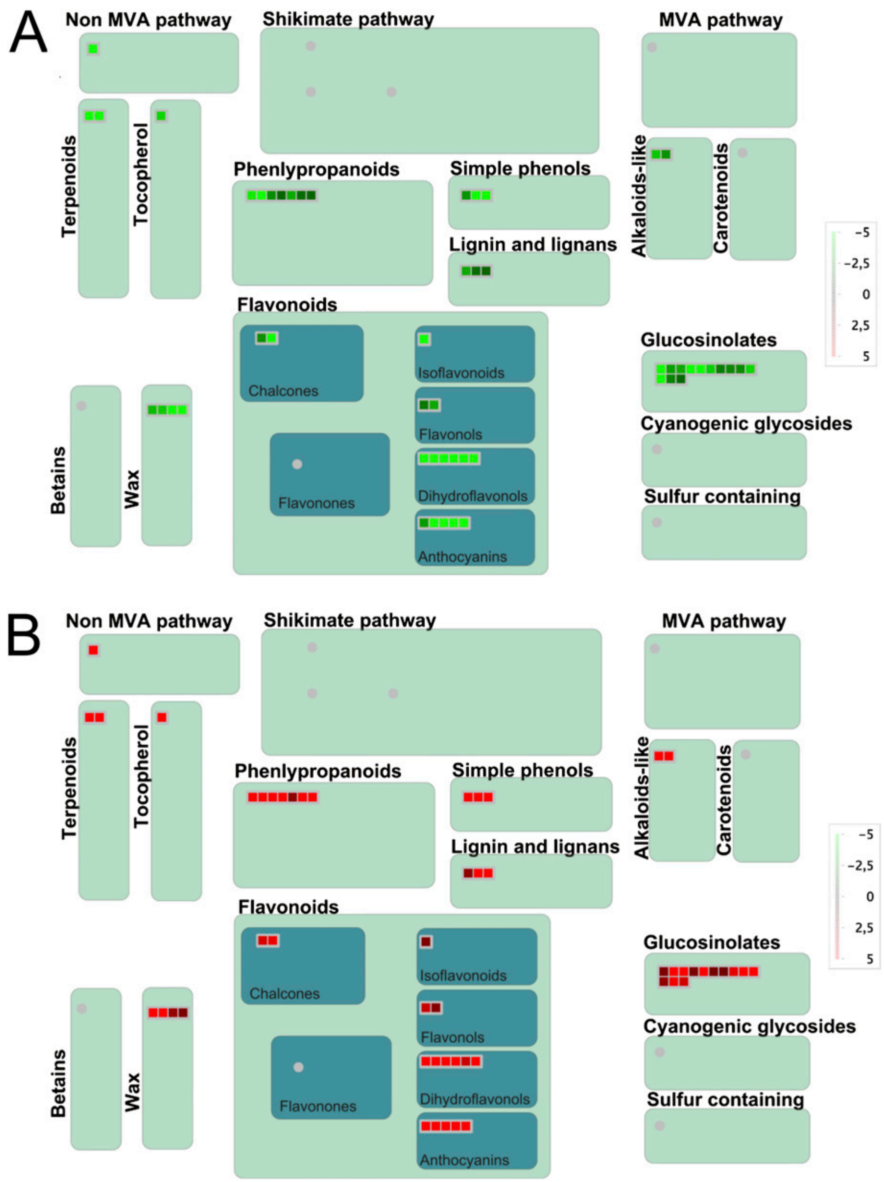


Fig. S8. Diagrammatic representation of genes associated with secondary metabolism that were down-regulated in M-D4/W-D4 (*A*) and up-regulated in W-D4/W-C (*B*). Green and red colors indicate repression and induction, respectively. Colored bars in each panel indicate fold changes in gene expression. MapMan software was used to illustrate the different functional categories that were involved. M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h.

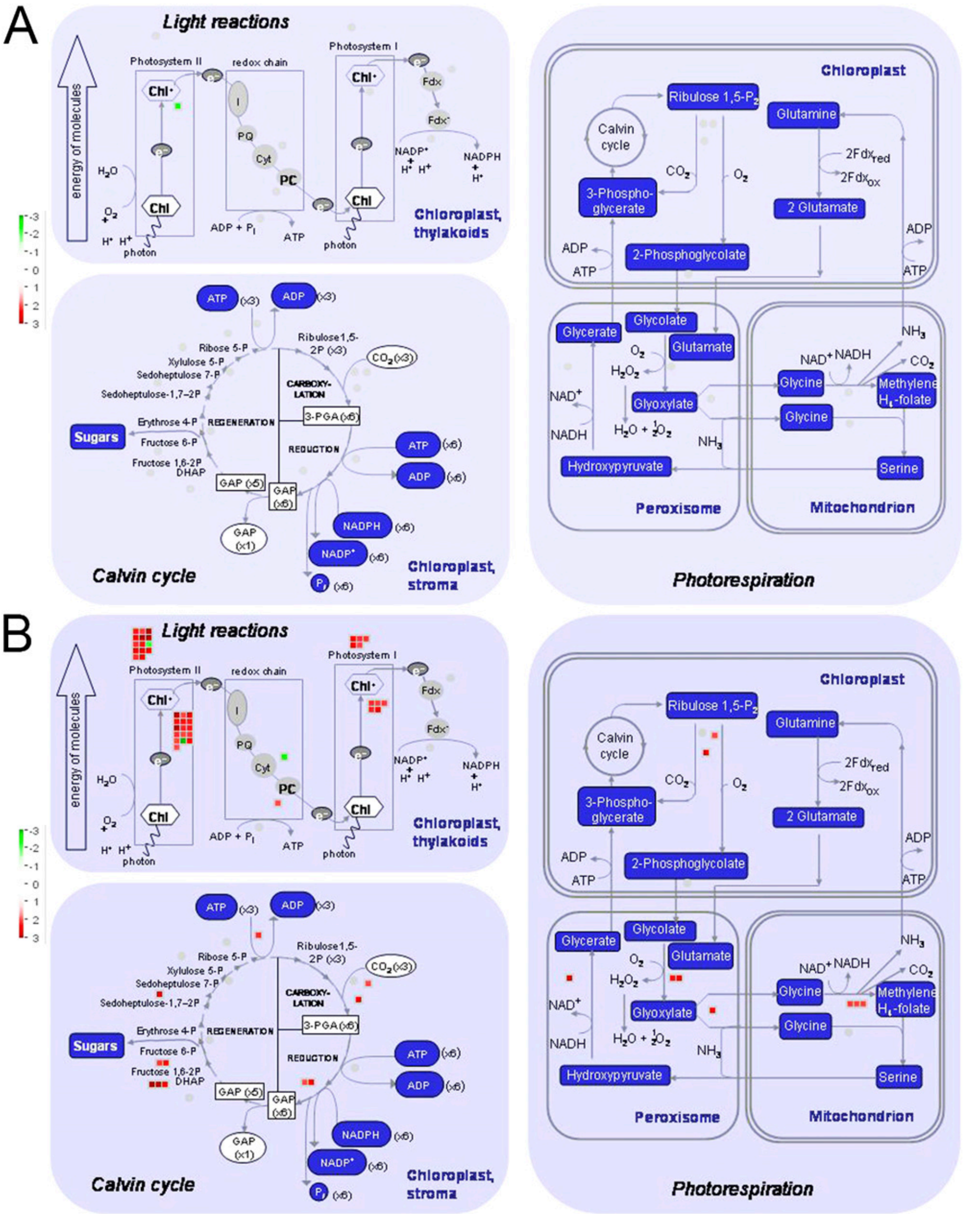


Fig. S9. Diagrammatic representation of genes involved in photosynthesis related processes that have altered expression in *max2* vs. WT plants under nonstress (M-C/W-C) (*A*) and stress conditions (M-D2/W-D2 and/or M-D4/W-D4) (*B*). Green and red colors indicate down-regulated and up-regulated genes, respectively. Colored bars in each panel indicate fold changes in gene expression. MapMan software was used in the analysis. M-C/W-C, *max2-3* well-watered 0 h vs. WT well-watered control 0 h. M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h.

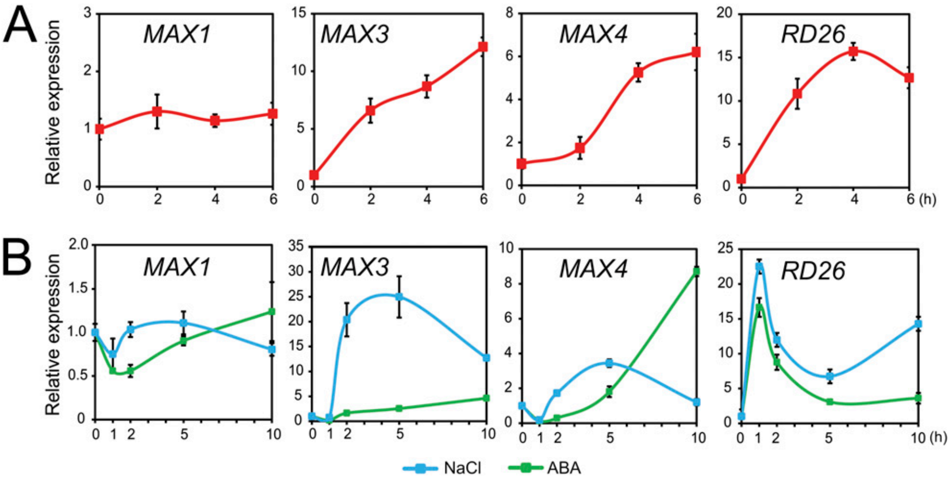


Fig. S10. Expression of SL-biosynthetic *MAX1*, *MAX3*, and *MAX4* genes in WT plants under various stress and ABA treatments. (*A*) Expression of the SL-biosynthetic *MAX* genes in leaves of WT plants exposed to a dehydration stress. (*B*) Expression of the SL-biosynthetic *MAX* genes in WT plants exposed to 250 mM NaCl or 100 μM ABA. Relative expression levels were normalized to a value of 1 in the respective mock control plants. Data represent the mean and SE of three independent biological replicates. The stress-inducible *RD26* gene was used as a marker to confirm the efficacy of the treatments. *UBQ10* was used as reference gene.

Dataset S1. Microarray analysis of differential expression of WT and *max2-3* plants

Dataset S1

Microarray analysis of differential expression of WT and *max2-3* plants under well-watered and dehydrative conditions. M-C/W-C, *max2-3* well-watered control 0 h vs. WT well-watered control 0 h; M-D2/M-C, *max2-3* dehydrated 2 h vs. *max2-3* well-watered control 0 h; M-D4/M-C, *max2-3* dehydrated 4 h vs. *max2-3* well-watered control 0 h; M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D2/W-C, WT dehydrated 2 h vs. WT well-watered control 0 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h.

Dataset S2. List of up-regulated genes with at least twofold change

Dataset S2

List of up-regulated genes with at least twofold change in the (*A*) M-C/W-C comparison; (*B*) M-D2/W-D2 comparison; (*C*) M-D4/W-D4 comparison; (*D*) W-D2/W-C comparison; (*E*) W-D4/W-C comparison; (*F*) M-D2/M-C comparison; and (*G*) M-D4/M-C comparison. M-C/W-C, *max2-3* well-watered control 0 h vs. WT well-watered control 0 h; M-D2/M-C, *max2-3* dehydrated 2 h vs. *max2-3* well-watered control 0 h; M-D4/M-C, *max2-3* dehydrated 4 h vs. *max2-3* well-watered control 0 h; M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D2/W-C, WT dehydrated 2 h vs. WT well-watered control 0 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h.

Dataset S3. List of down-regulated genes with at least twofold change

Dataset S3

List of down-regulated genes with at least twofold change in the (*A*) M-C/W-C comparison; (*B*) M-D2/W-D2 comparison; (*C*) M-D4/W-D4 comparison; (*D*) W-D2/W-C comparison; (*E*) W-D4/W-C comparison; (*F*) M-D2/M-C comparison; (*G*) and M-D4/M-C comparison. M-C/W-C, *max2-3* well-watered control 0 h vs. WT well-watered control 0 h; M-D2/M-C, *max2-3* dehydrated 2 h vs. *max2-3* well-watered control 0 h; M-D4/M-C, *max2-3* dehydrated 4 h vs. *max2-3* well-watered control 0 h; M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D2/W-C, WT dehydrated 2 h vs. WT well-watered control 0 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h.

Dataset S4. Overlapping genes between gene sets

Dataset S4

Overlapping genes between gene sets derived (*A*) from two comparisons, M-C/W-C (down) and W-D2/W-C (up); (*B*) from two comparisons, M-C/W-C (down) and W-D4/W-C (up); (*C*) from three comparisons, M-C/W-C (down), W-D2/W-C (up), and W-D4/W-C (up); (*D*) from two comparisons, M-D2/W-D2 (down) and W-D2/W-C (up); (*E*) from two comparisons, M-D2/W-D2 (down) and W-D4/W-C (up); (*F*) from three comparisons, M-D2/W-D2 (down), W-D2/W-C (up), and W-D4/W-C (up); (*G*) from two comparisons, M-D4/W-D4 (down) and W-D2/W-C (up); (*H*) from two comparisons, M-D4/W-D4 (down) and W-D4/W-C (up) ; (*I*) from three comparisons, M-D4/W-D4 (down), W-D2/W-C (up), and W-D4/W-C (up); (*J*) from two comparisons, M-C/W-C (down) and M-D2/W-D2 (down); (*K*) from two comparisons, M-C/W-C (down) and M-D4/W-D4 (down); and (*L*) from three comparisons, M-C/W-C (down), M-D2/W-D2 (down), and M-D4/W-D4 (down). M-C/W-C, *max2-3* well-watered control 0 h vs. WT well-watered control 0 h; M-D2/M-C, *max2-3* dehydrated 2 h vs. *max2-3* well-watered control 0 h; M-D4/M-C, *max2-3* dehydrated 4 h vs. *max2-3* well-watered control 0 h; M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D2/W-C, WT dehydrated 2 h vs. WT well-watered control 0 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h.

Dataset S5. Down-regulated genes

Dataset S5

Down-regulated genes in the (*A*) M-C/W-C comparison; (*B*) M-D2/W-D2 comparison; and (*C*) M-D4/W-D4 comparison. These genes were identified as up-regulated by both dehydration and ABA. M-C/W-C, *max2-3* well-watered control 0 h vs. WT well-watered control 0 h; M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h.

Dataset S6. Z-scores for overlaps between comparisons

Dataset S6

Z-scores for overlaps between comparisons of M-C/W-C (down), W-D2/W-C (up) and W-D4/W-C (up), between comparisons of M-D2/W-D2 (down), W-D2/W-C (up) and W-D4/W-C (up), and between comparisons of M-D4/W-D4 (down), W-D2/W-C (up) and W-D4/W-C (up). M-C/W-C, *max2-3* well-watered control 0 h vs. WT well-watered control 0 h; M-D2/M-C, *max2-3* dehydrated 2 h vs. *max2-3* well-watered control 0 h; M-D4/M-C, *max2-3* dehydrated 4 h vs. *max2-3* well-watered control 0 h; M-D2/W-D2, *max2-3* dehydrated 2 h vs. WT dehydrated 2 h; M-D4/W-D4, *max2-3* dehydrated 4 h vs. WT dehydrated 4 h; W-D2/W-C, WT dehydrated 2 h vs. WT well-watered control 0 h; W-D4/W-C, WT dehydrated 4 h vs. WT well-watered control 0 h.

Dataset S7. Changes in the level of expression of genes

Dataset S7

(*A*) Changes in the level of expression of genes involved in flavonoid biosynthesis in *max2-3* plants relative to WT plants under well-watered and dehydrative conditions; and (*B*) changes in the level of expression of genes involved in cytokinin biosynthesis in *max2-3* plants relative to WT plants under well-watered and dehydrative conditions.

Dataset S8. List of photosynthesis-related genes up-regulated in *max2-3* plants relative to WT plants under dehydrative conditions

Dataset S8

Dataset S9. Primers used to amplify specific genes in the RT-qPCR analyses

Dataset S9