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Supplementary Materials for

Allelic shift in cis-elements of the transcription factor *RAP2.12* underlies adaptation associated with humidity in *Arabidopsis thaliana*

Shangling Lou, Xiang Guo, Lian Liu, Yan Song, Lei Zhang, Yuanzhong Jiang, Lushui Zhang, Pengchuan Sun, Bao Liu, Shaofei Tong, Ningning Chen, Meng Liu, Han Zhang, Ruyun Liang, Xiaoqin Feng, Yudan Zheng, Huanhuan Liu*, Michael J. Holdsworth*, Jianquan Liu*

*Corresponding author. Email: liuhuanhuan85@163.com (H.L.); michael.holdsworth@nottingham.ac.uk (M.J.H.); liujq@nwipb.cas.cn (J.L.)

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The PDF file includes:

Figs. S1 to S19
Tables S1 and S4
Legends for tables S2 and S3
Legend for data S1

Other Supplementary Material for this manuscript includes the following:

Tables S2 and S3
Data S1

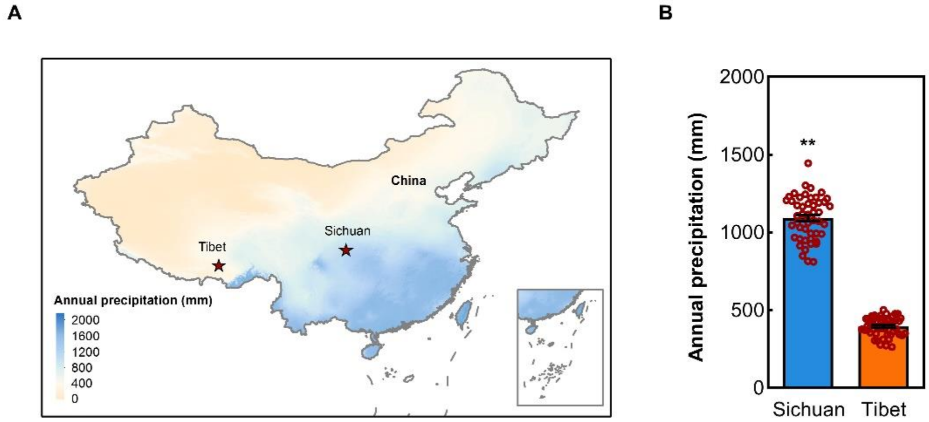


Fig. S1. Native annual precipitation in the geographical locations where Sichuan and Tibet accessions were collected is significantly different. (**A**) Annual precipitation map of Sichuan and Tibet sample sites. (**B**) Annual precipitation of the sample sites from 1970 to 2018. Data are mean ± SE. $^{**}P < 0.01$, (two-sided Student's *t*-test).

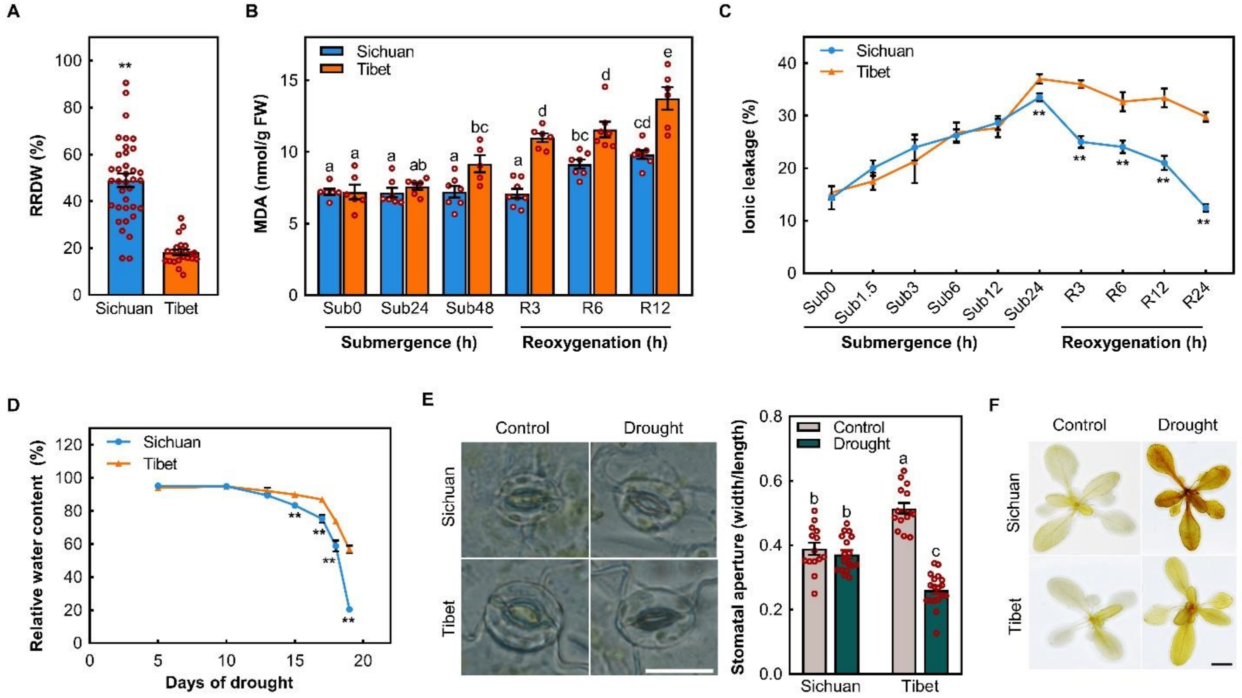


Fig. S2. Physiological responses of Sichuan and Tibet accessions to submergence and drought stress. (A) Relative rosette dry weights (RRDW) of surviving individuals in Sichuan and Tibet accessions after 7 days recovery from 64 hours of dark/submergence. Data are mean ± SE, (*n* = 23 - 36). (**B** and **C**) Malondialdehyde (MDA) (B) and ionic leakage (C) in 18-day-old rosettes of Sichuan and Tibet accessions during submergence and reoxygenation after 48 hours of submergence. Data are mean ± SE (*n* = 5 - 8). (**D**) Relative water content in rosettes of Sichuan and Tibet accessions during application of drought stress. Data are mean ± SE (*n* = 3 repeats). (**E**) Representative stomatal images and stomatal aperture after stopping watering for 18 days. Scale bars, 20 μm. Data are mean ± SE (*n* = 14 - 18). (**F**) Visualization of H_2O_2 production after 19 days of normal watering or stop watering. Shown are DAB-stained rosettes, and dark brown color indicate H_2O_2 production. Scale bars, 0.5 cm. Different letters in (B) and (E) denote significant differences according to Tukey's HSD test ($P < 0.05$). **$P < 0.01$ compared with Tibet accession by two-sided Student's *t*-test.

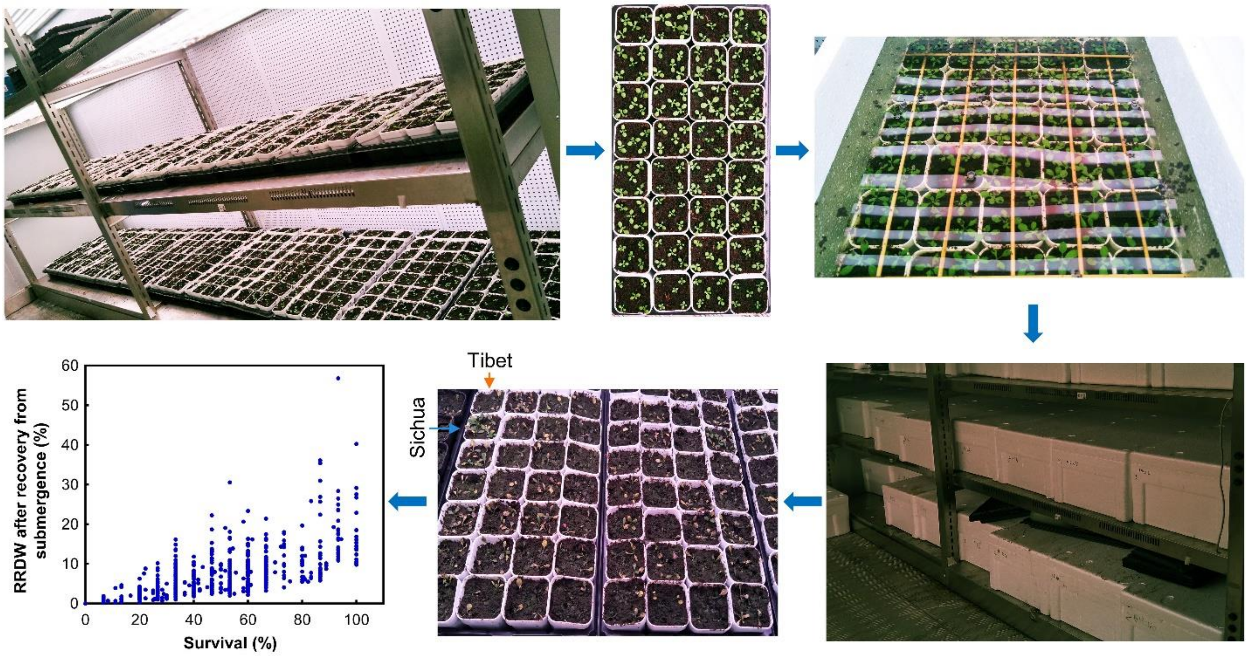


Fig. S3. Submergence process of recombinant inbred lines. A total of 623 two-week-old RILs were dark/submerged in tanks for 48, 60, and 72 (shown in this picture) hours, respectively, and followed by 7 days of recovery under normal light conditions. Average survival and RRDW of the three different treatment times have been shown in the lower-left figure.

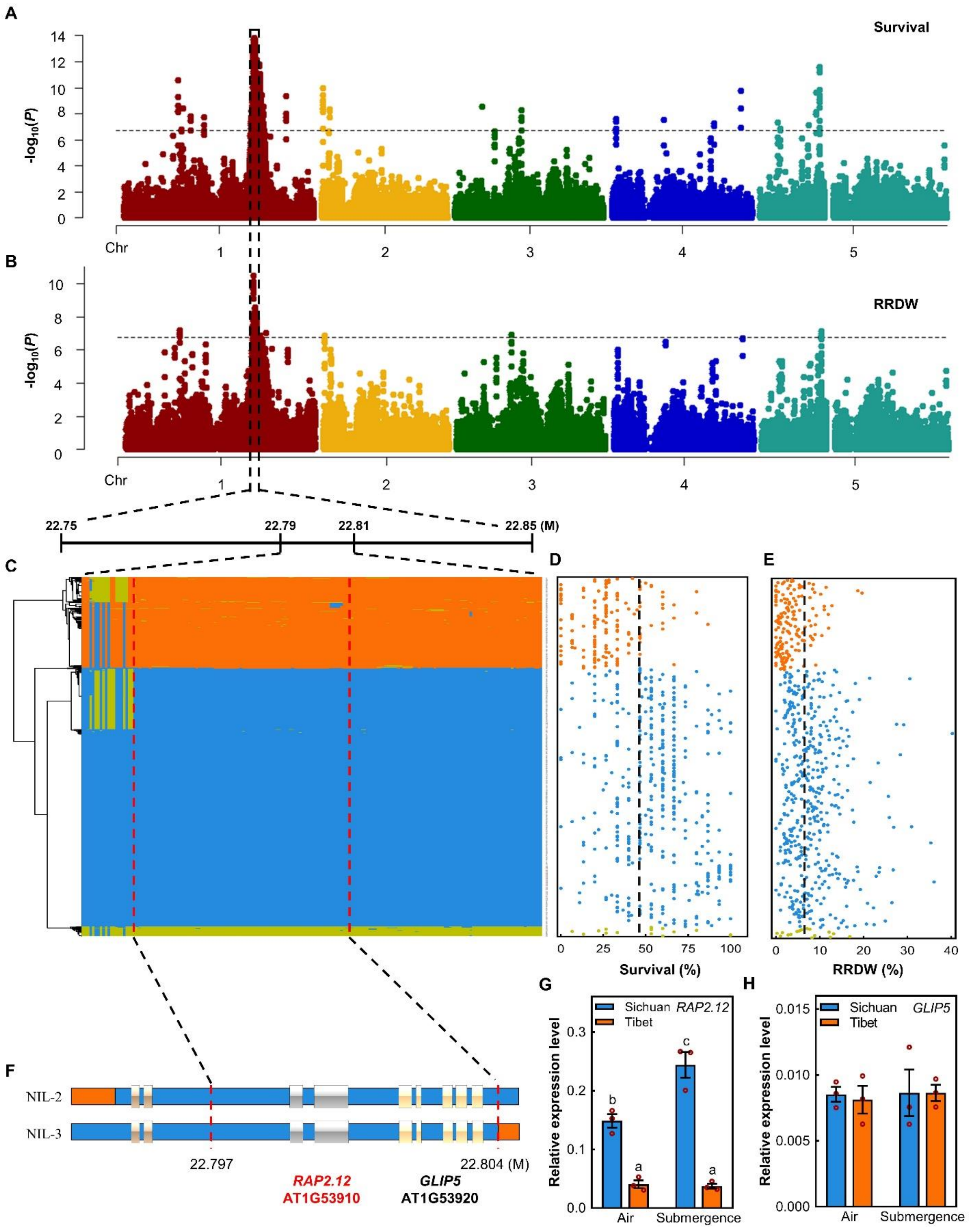


Fig. S4. GWAS and fine mapping of the major gene that underlies flooding tolerance. (**A** and **B**) Manhattan plots of GWAS for survival (A) and RRDW (B). Different chromosomes are displayed in different colors. The horizontal dash line corresponds to the Bonferroni corrected 5%

significance threshold. Vertical dotted frame indicates the significantly associated locus in both analyses. (**C**) Haplotype analysis that divides 623 RILs into three groups, Sichuan (blue), Tibet (orange), and heterozygous haplotype (green), according to 20k bp sequences around the top peak was found to be significantly associated with both survival and RRDW by GWAS. (**D** and **E**) Average survival (D) and RRDW (E) correspond to each RIL in D. (**F**) Overlapping genes for fine mapping population (NILs) and haplotype analysis. A ~7 kb interval region containing two genes (the key gene is in red) was identified by whole-genome resequencing of NILs. Blue and orange boxes represent the same nucleotide sequence as Sichuan and Tibet accessions, respectively. Boxes on nucleotide sequences in different colors represent exons. (**G** and **H**) The relative expression level of two candidate genes, *RAP2.12* (G) and *GLIP5* (H), after air or submergence treatments (20 h) in Sichuan and Tibet accessions. Data are mean ± SE relative to the average transcript levels of *ACTIN2* ($n = 3$), see also Figure 3A. Different letters denote significant differences according to Tukey's HSD test ($P < 0.05$).

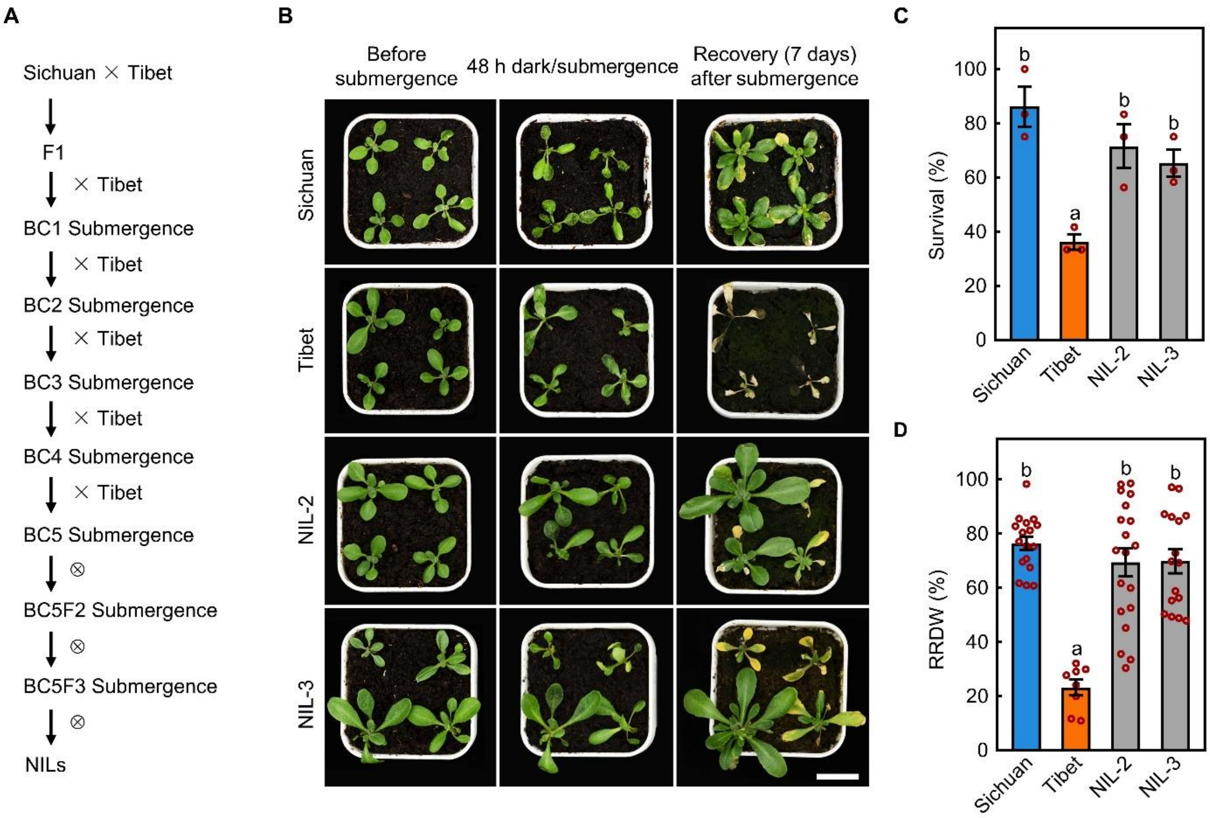


Fig. S5. The generation procedure of mapping population and its representative phenotype. (**A**) F1 generation of Sichuan and Tibet accessions was backcrossed to Tibet accession for 5 rounds, and then self-bred for three generations. Each generation was submerged for 48 hours and the survival individuals were selected out for backcrossing or self-breeding. The submergence-resistant lines were sequenced and the sequenced data were used to overlap with the results of RILs haplotype analysis. These lines with a ~7 kb overlapping interval containing Sic-RAP2.12 but Tibet accession for the rest of the genome were named NILs. (**B**) Phenotypic comparison of submergence tolerance among Sichuan accession, Tibet accession and NILs. Seedings after 48 h of dark/submergence and 7 days recovery. Scale bar, 2 cm. (**C** and **D**) Survival (C) (*n* = 3 repeats) and RRDW of survival individuals (D) in (B) (*n* = 8 - 19). Different letters in (C) and (D) denote significant differences according to Tukey's HSD test (*P* < 0.05).

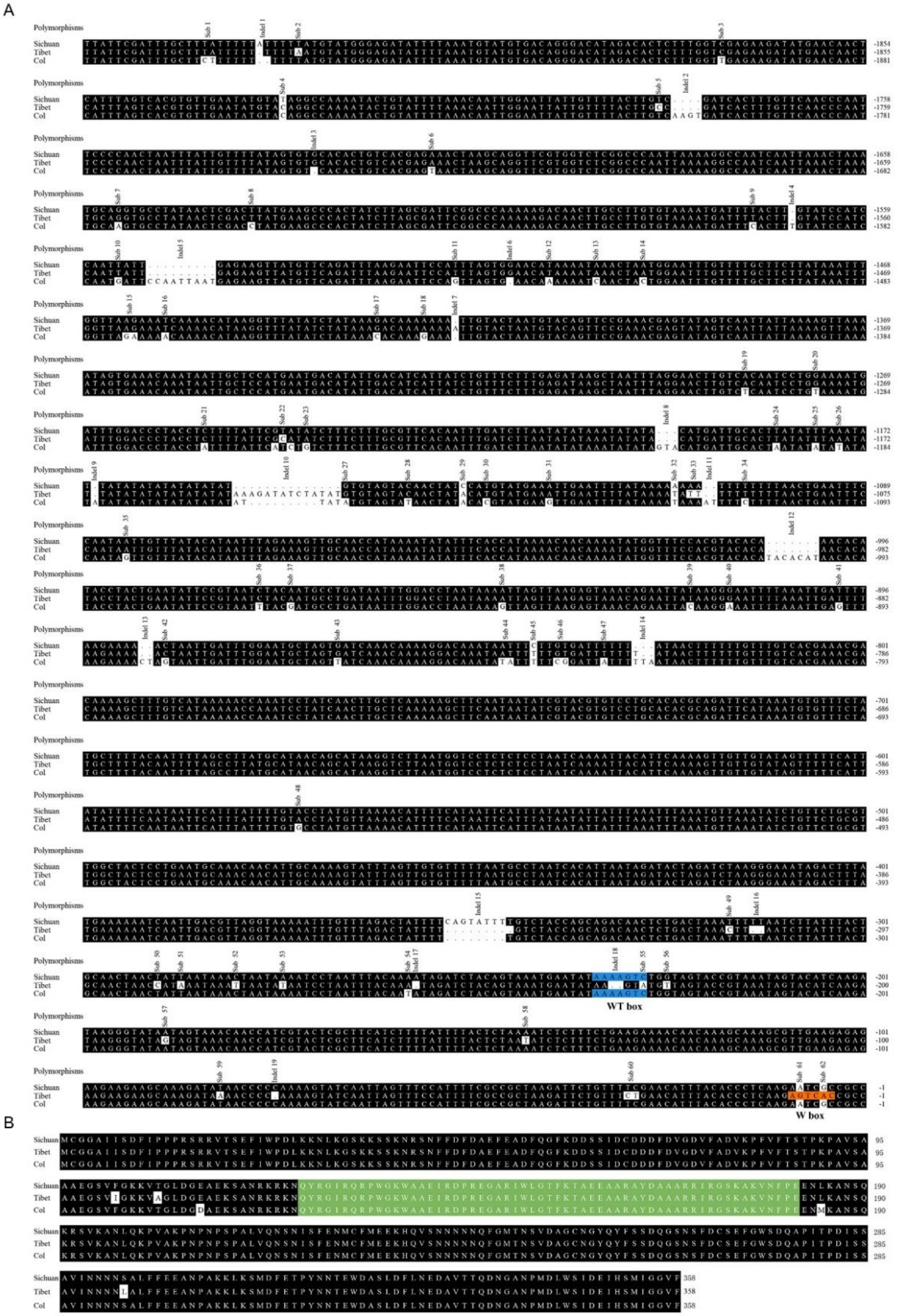
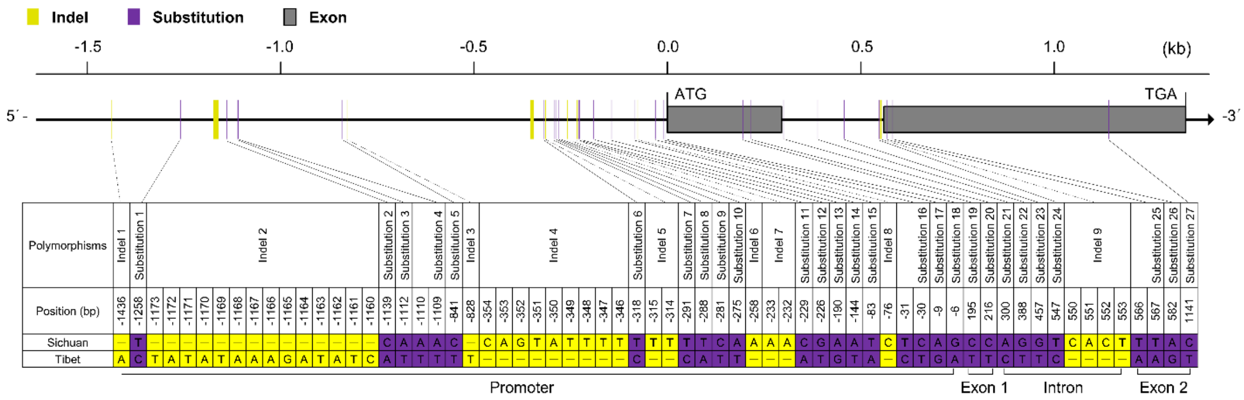


Fig. S6. *RAP2.12* promoter and amino acid mutations among Tibet, Sichuan and Col accessions. (**A**) Sequences of *RAP 2.12* promoter in the alignment. Core *cis*-element sequences of WT box and W box are in blue and orange respectively. (**B**) RAP2.12 amino acid sequence in the alignment. Sequences of the functional domain (AP2 domain) are in green. The position number on the right is from ATG of each accession.



Polymorphisms	Indel 1	Substitution 1	Indel 2														Substitution 2	Substitution 3		Substitution 4	Substitution 5	Indel 3
Position (bp)	-1436	-1258	-1173	-1172	-1171	-1170	-1169	-1168	-1167	-1166	-1165	-1164	-1163	-1162	-1161	-1160	-1139	-1112	-1110	-1109	-841	-828
Sichuan	—	T	—	—	—	—	—	—	—	—	—	—	—	—	—	—	C	A	A	A	C	—
Tibet	A	C	T	A	T	A	T	A	A	A	G	A	T	A	T	C	A	T	T	T	T	T
	Promoter																					

Polymorphisms	Indel 4									Substitution 6	Indel 5		Substitution 7	Substitution 8	Substitution 9	Substitution 10	Indel 6	Indel 7		Substitution 11	Substitution 12	Substitution 13	Substitution 14	Substitution 15	Indel 8
Position (bp)	-354	-353	-352	-351	-350	-349	-348	-347	-346	-318	-315	-314	-291	-288	-281	-275	-258	-233	-232	-229	-226	-190	-144	-83	-76
Sichuan	C	A	G	T	A	T	T	T	T	T	T	T	T	T	C	A	A	A	A	C	G	A	A	T	C
Tibet	—	—	—	—	—	—	—	—	—	C	—	—	C	A	T	T	—	—	—	A	T	G	T	A	—
	Promoter																								

Polymorphisms		Substitution 16	Substitution 17	Substitution 18	Substitution 19	Substitution 20	Substitution 21	Substitution 22	Substitution 23	Substitution 24	Indel 9					Substitution 25	Substitution 26	Substitution 27
Position (bp)	-31	-30	-9	-6	195	216	300	388	457	547	550	551	552	553	566	567	582	1141
Sichuan	T	C	A	G	C	C	A	G	G	T	C	A	C	T	T	T	A	C
Tibet	C	T	G	A	T	T	C	T	T	C	—	—	—	—	A	A	G	T
	Promoter				Exon 1		Intron								Exon 2			

Fig. S7. Gene structure of *RAP2.12* and polymorphisms between Sichuan and Tibet accessions. Positions on the ruler and in the table are relative to the translation start codon of *RAP2.12* in Sichuan accession. Exons are indicated in gray squares.

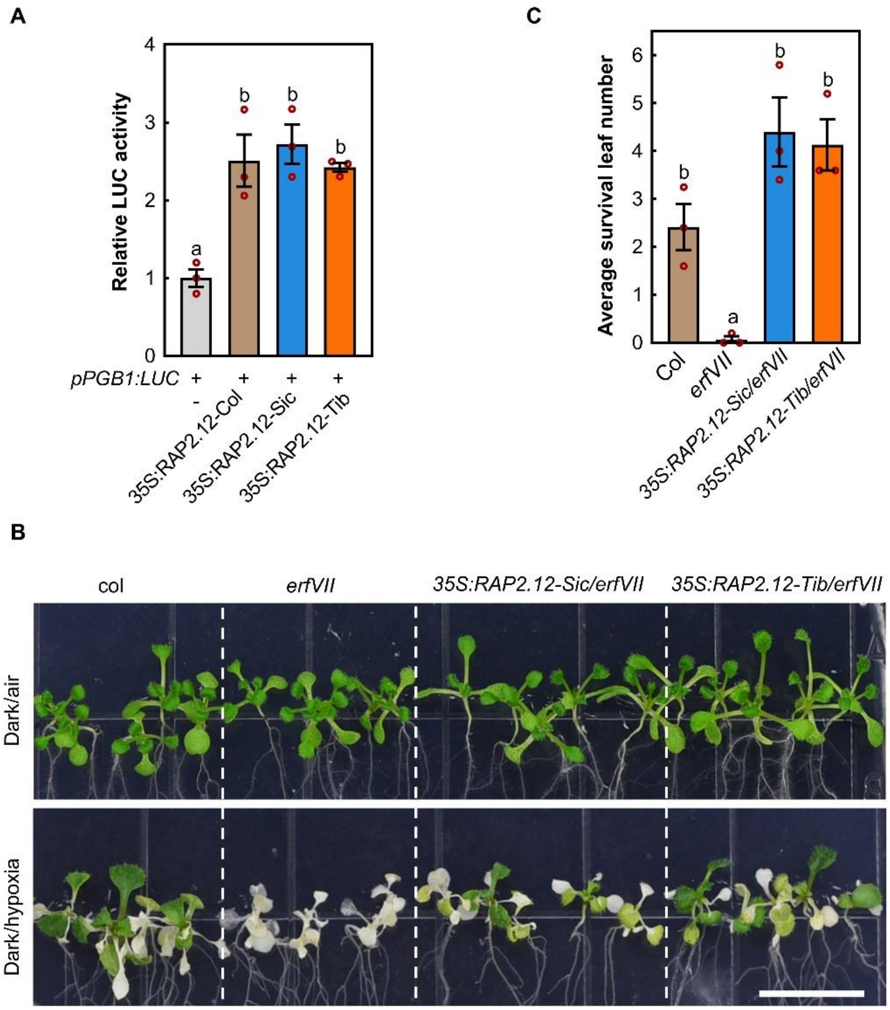


Fig. S8. The protein of RAP2.12-Sic and RAP2.12-Tib activated *PGB1* well, and rescue *erfVII*. (**A**) Transactivation of *PGB1* promoter by RAP2.12 from Col, Sichuan and Tibet accessions. The differences of RAP2.12 among the three accessions are shown in Figure S5. (**B and C**) The phenotype (B) and average survival leaf number (C) of 18-day-old *35S:RAP2.12-Sic* and *35S:RAP2.12-Tib* under *erfVII* mutant background of Col after treatment with dark/air or dark/hypoxia and followed by 3 days recovery. Data are mean ± SE ($n = 3$ repeats). Different letters denote significant differences according to Tukey's HSD test ($P < 0.05$).

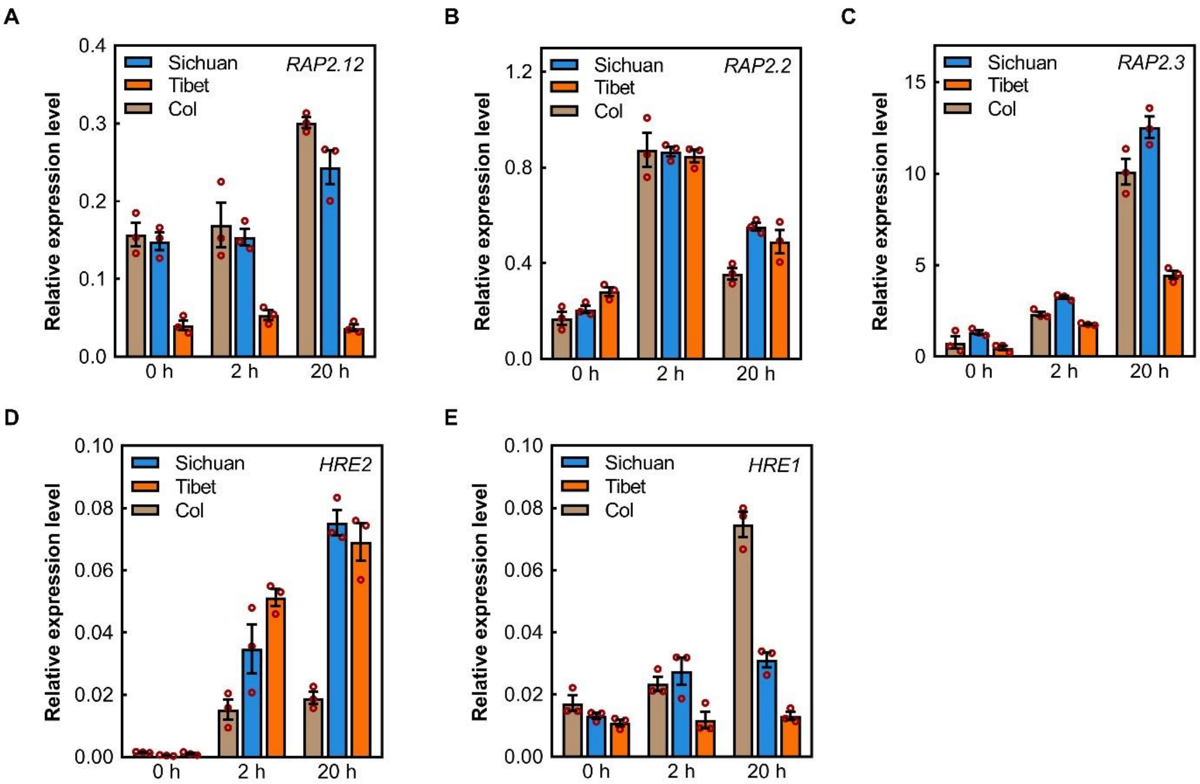


Fig. S9. The expression level of *ERFVII* in response to submergence treatment in Sichuan, Tibet, and Col accessions. Relative mRNA transcript abundance of 18-days old seedlings under different submergence treatment times as indicated. *RAP2.2*, *RAP2.3* and *HRE2* showed positive regulation in three accessions, but the *RAP2.12* and *HRE1* in Tibet lost their response to submergence. Data are mean ± SE relative to the average transcript levels of *ACTIN2* (n = 3 biologically independent samples).

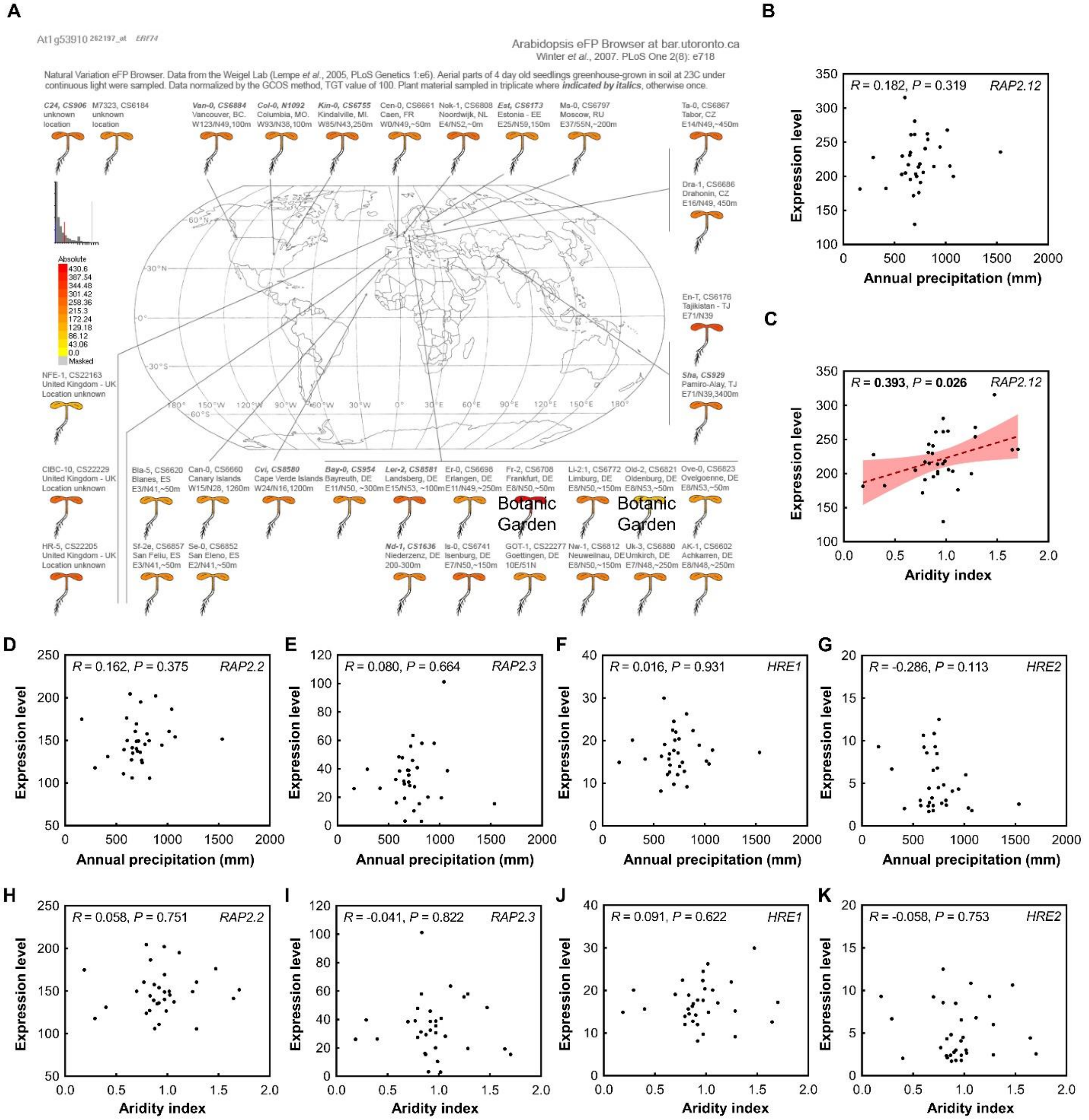


Fig. S10. The expression of *RAP2.12* increases with the increase of humidity. (**A**) Global expression pattern of *RAP2.12* in 34 *Arabidopsis* accessions from public database visualized by th*e Arabidopsis* eFP browser (*19*) (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi). Fr-2 and Old-2 from the botanic garden are marked on the seedings. (**B** - **K**) Correlation between *ERFVII* expression and precipitation and aridity index. Absolute expression levels are from the *Arabidopsis* eFP browser. The red dash line shows a linear fit, and the light red shadow indicates the 95% confidence interval of the regression. The correlation coefficients and *P* values are calculated using Pearson's correlation analysis.

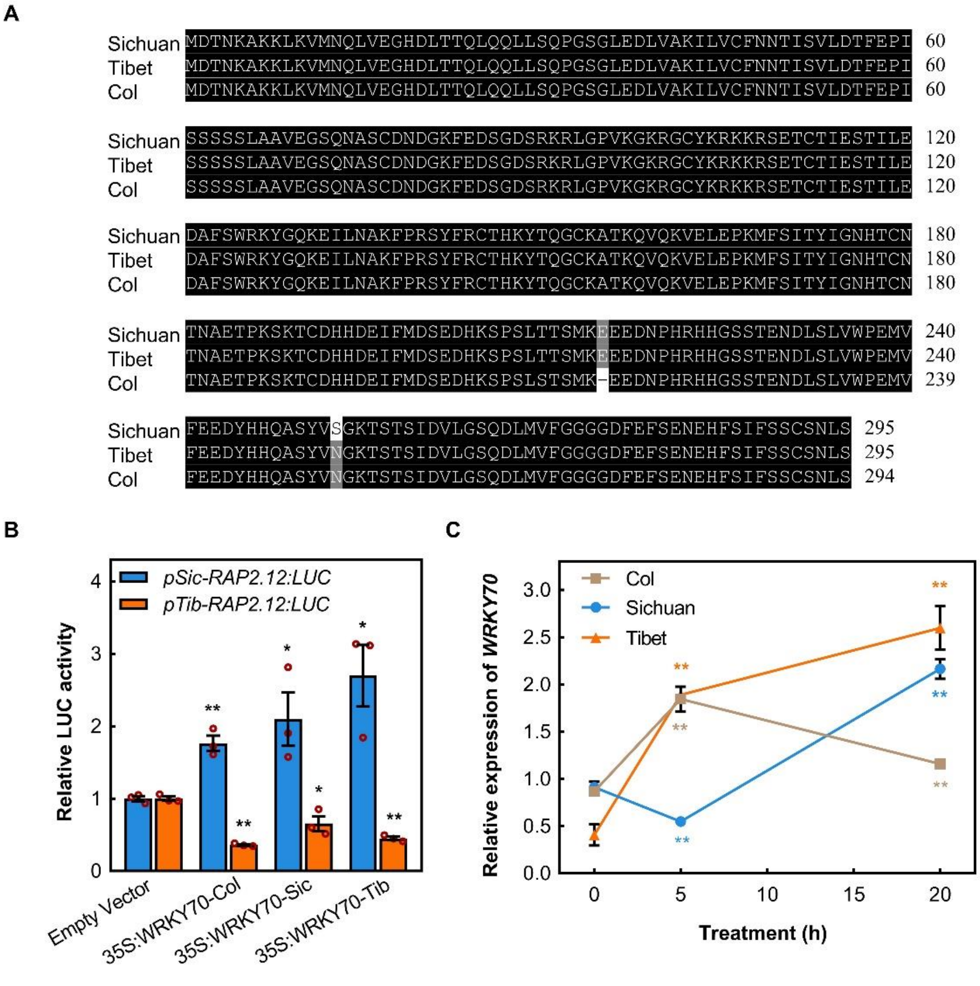


Fig. S11. WRKY70 involves submergence-induced hypoxia by directly activating the expression of *RAP2.12*. (**A**) WRKY70 amino acid sequence in the alignment. (**B**) Three types of WRKY70 protein transactivate *pSic-RAP2.12* promoter and repress *pTib-RAP2.12* promoter. Data are mean ± SE. (**C**) The relative expression level of *WRKY70* in Col, Sichuan and Tibet accessions, after air or submergence treatments for the indicated time. Data are mean ± SE relative to the average transcript levels of *ACTIN2*. $n = 3$, $^{*}P < 0.05$; $^{**}P < 0.01$, compared with empty vector (B) or air treatments (0 h) (C) by two-sided Student's *t*-test.

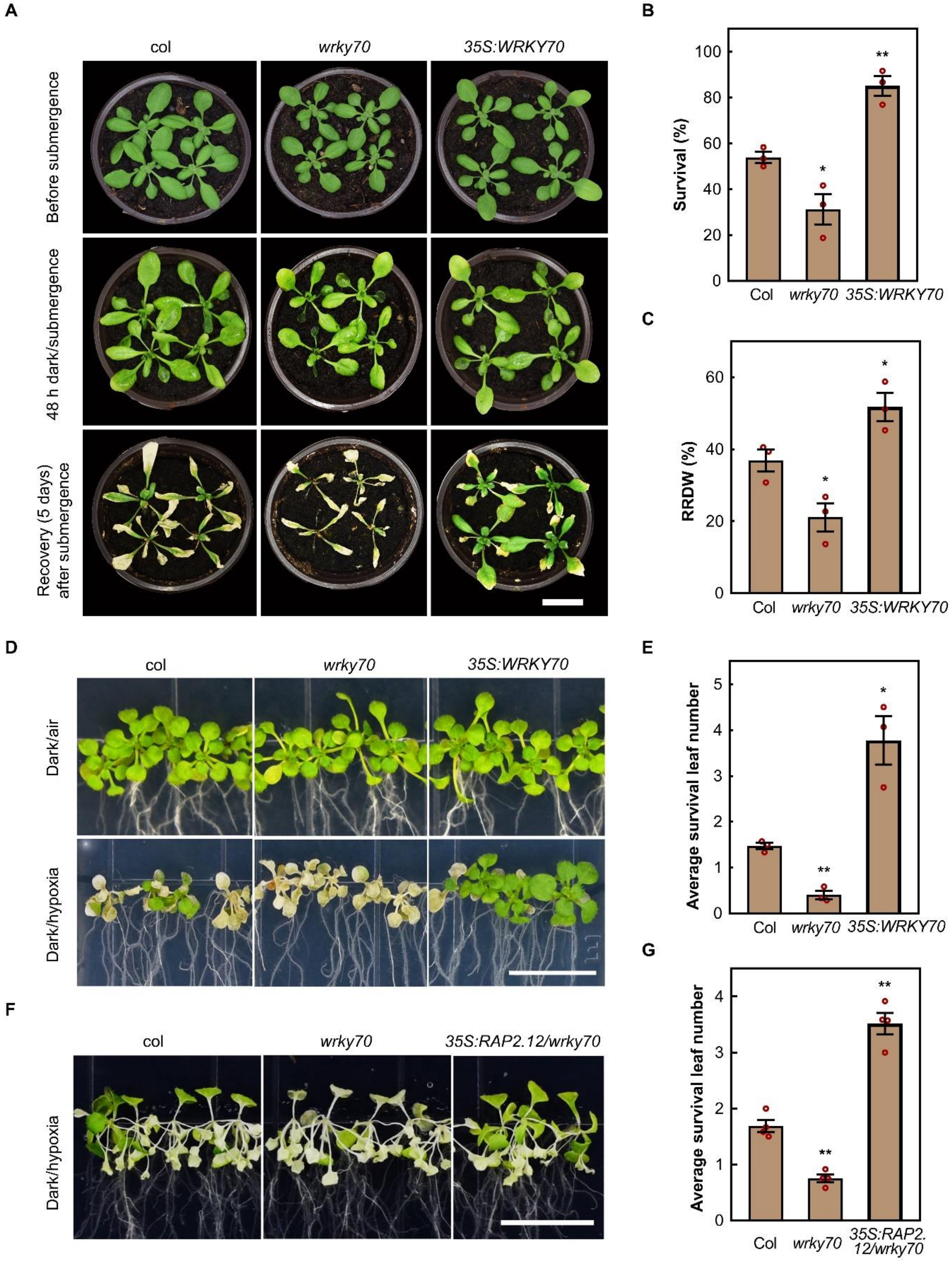


Fig. S12. WRKY70 acts upstream of *RAP2.12* in controlling submergence-induced hypoxic stress. (**A**) Wild-type (Col), *wrky70* mutant, and *35S:WRKY70* plants after dark submergence treatment for 48 h, followed by 5 days recovery. (**B** and **C**) Survival (B), and RRDW (C) of survival individuals in (A). (**D**) Phenotype of Wild-type (Col), *wrky70* mutant, and *35S:WRKY70* plants after treatment with dark/air or dark/hypoxia (5% (v/v) oxygen for 24 h and lower than 0.01% for 16 h), followed by 3 days recovery. (**E**) Average survival Leaf number in (D). (**F**) Overexpression of RAP2.12 (*35S:RAP2.12)* under wrky70 mutant background of Col-0 restored the hypoxic resistance with the same treatment in (D). (**G**) Average survival leaf number in (D). Data are mean ± SE (*n* =3 for 12 individuals in (B) and (C) and 10 individuals in (E) and (G)). Scale bars, 2 cm in (A), and 1 cm in (D) and (F). $*P < 0.05$; $**P < 0.01$, compared with Col by two-sided Student's *t*-test.

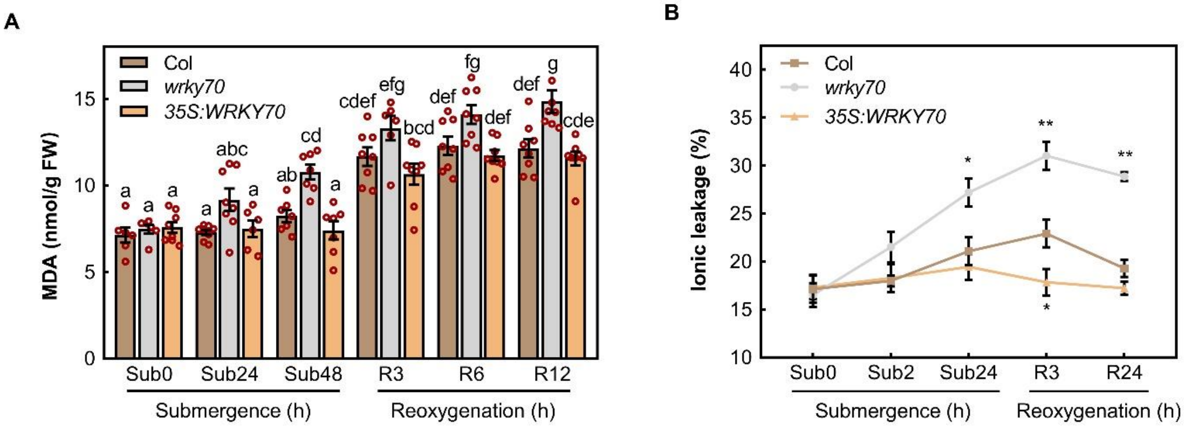


Fig. S13. Analyses of MDA and electrolyte leakage in Col, *wrky70*, and *35S:WRKY70* during submergence and reoxygenation. (**A**) MDA response during submergence and reoxygenation after 48 hours of submergence in 18-day-old rosettes. Data are mean ± SE (n = 6 - 8). Different letters denote significant differences according to Tukey's HSD test ($P < 0.05$). (**B**) Ionic leakage during submergence and reoxygenation after 24 hours of submergence in 18-day-old rosettes. Data are mean ± SE (n = 4). *$P < 0.01$; **$P < 0.01$, compared with wild type (Col) by two-sided Student's t-test.

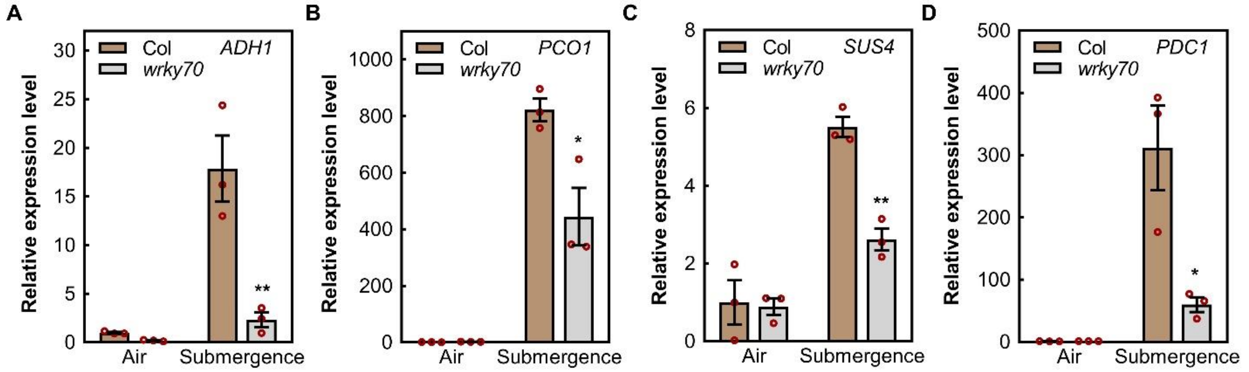


Fig. S14. Relative expression levels of hypoxia-responsive marker genes in response to submergence. Relative mRNA transcript abundance of four randomly selected marker genes (*ADH1*, *PCO1*, *SUS4* and *PDC1*) under air or 2 hours of submergence treatments using 18-days old shoots. Data were normalized to air treatments and are mean ± SE; $n = 3$ biologically independent samples. $*P < 0.05$; $**P < 0.01$, compared with wild type (Col) by two-sided Student's *t*-test.

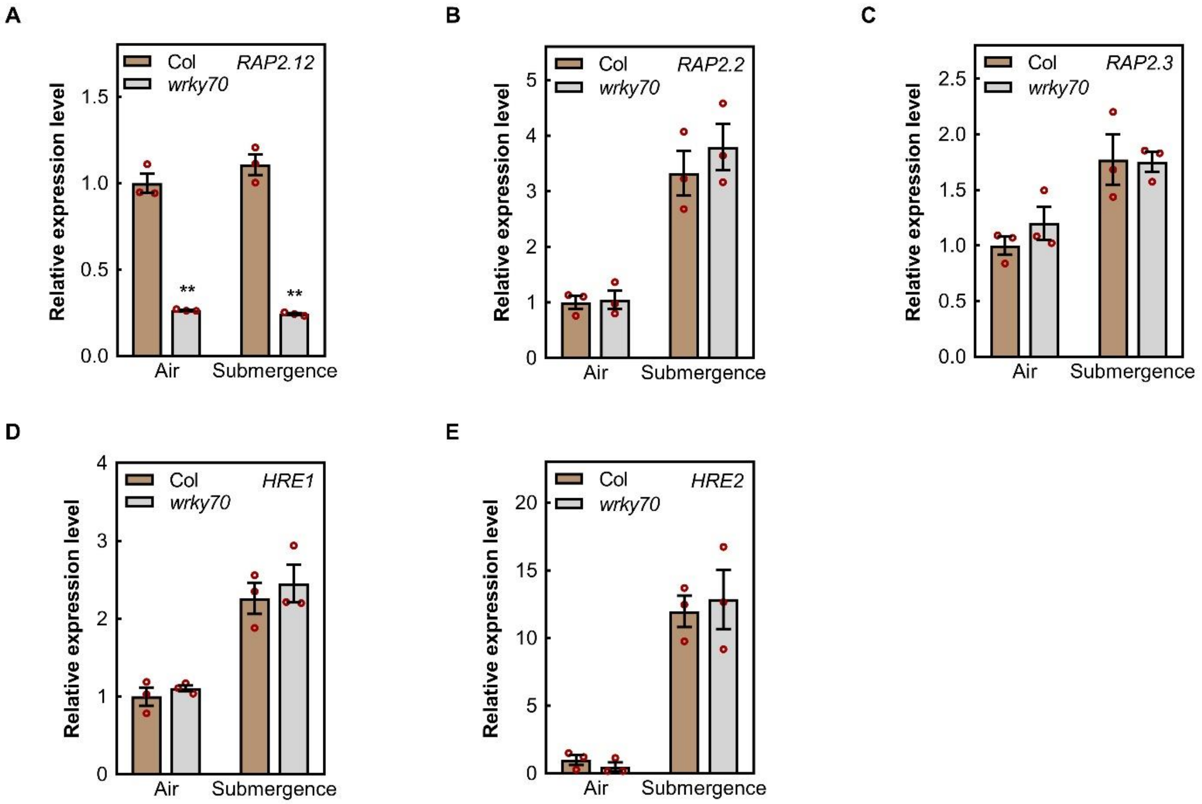


Fig. S15. Relative expression levels of *ERFVII* members in response to submergence in Col and *wrky70* mutant. Relative mRNA transcript abundance of *ERFVII* under air or 2 hours of submergence treatments using 18-days old shoots. Data were normalized to air treatments and are mean ± SE; $n = 3$ biologically independent samples. $^{**}P < 0.01$, compared with wild type (Col) by two-sided Student's t-test.

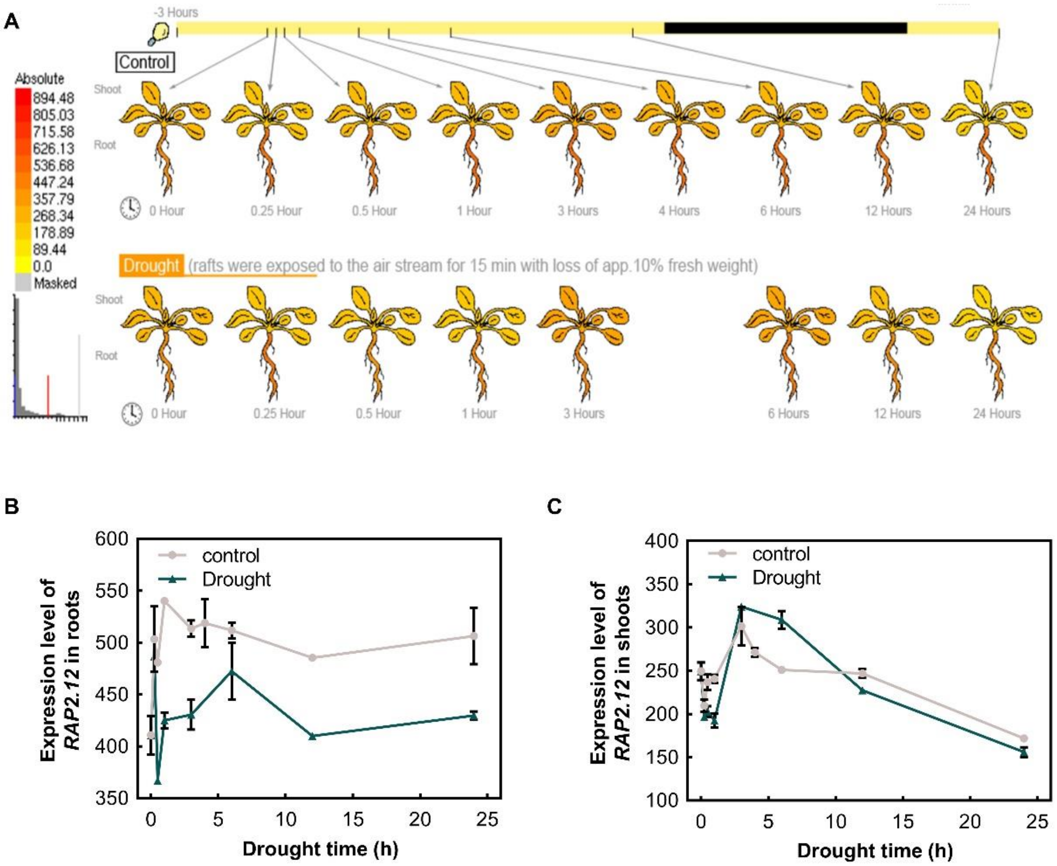


Fig. S16. Drought response pattern of *RAP2.12* expression in Col accession. (**A**) Drought treatment-dependent expression pattern of *RAP2.12* in Col from public database visualized by th*e Arabidopsis* eFP browser (*19*) (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi). (**B** and **C**) The absolute expression level of *RAP2.12* in roots (B) and shoots (C) respond to drought treatment in (A). Data are obtained from the *Arabidopsis* eFP browser.

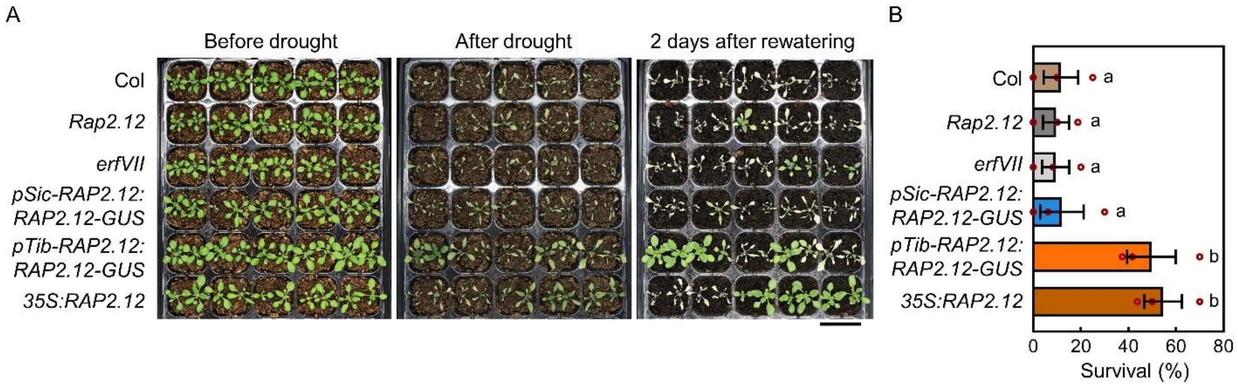


Fig. S17. *RAP2.12* promoter in Tibet accession with W box *cis*-element promotes drought resistance. (**A**) Comparison of drought resistance among Col-0, *rap2.12*, *erfVII*, *pSic-RAP2.12:RAP2.12-GUS*, *pTib-RAP2.12:RAP2.12-GUS* and *35S:RAP2.12* (all in Col-0 background). Phenotypes after stopping watering for 10 days (Before drought), 14 days (After drought), and 2 days after rewatering. Scale bar, 5 cm. (**B**) Survival of drought treatments in (B). Data are mean ± SE (*n* = 3 for 10 - 16 individuals). Different letters denote significant differences according to Tukey's HSD test (P < 0.05).

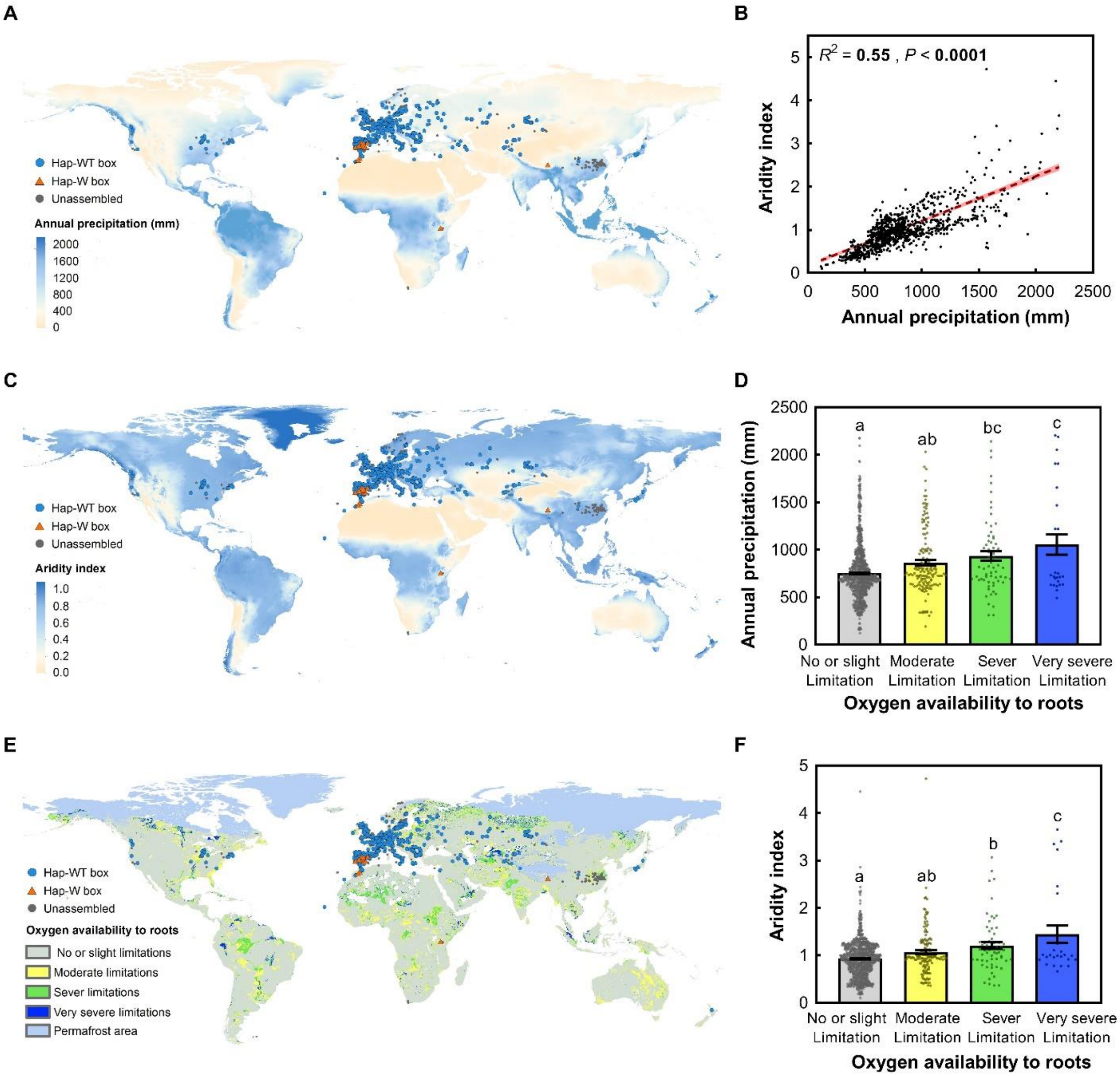


Fig. S18. Aridity stress, oxygen availability to roots and precipitation were significantly associated in 1,475 global non-repetitive *A. thaliana* accessions. (**A**, **C** and **E**) Non-repetitive sites of 1,475 natural inbred lines of *A. thaliana* on annual precipitation (A), aridity index (C), and oxygen availability to roots (E) around the world. (**B**) Regression between annual precipitation and aridity index. The red dash line shows a linear fit, and the light red shadow indicates the 95% confidence interval of the regression. (**D** and **F**) Annual precipitation (D) and aridity index (F) in different classes of oxygen availability to roots. Different letters denote significant differences according to Tukey's HSD test ($P < 0.05$).

A

Species	gene	Genomic location	Data source/ GenBank accession number
Arabidopsis thaliana(Col)	*AT1G53910(RAP2.12)*	1:20134988-20135241	http://plants.ensembl.org/Multi/Tools/Blast?db=core
Arabidopsis thaliana(Sichuan)	*RAP2.12*	-254-0 from ATG	sequence
Arabidopsis thaliana(Tibet)	*RAP2.12*	-251-0 from ATG	sequence
Arabidopsis lyrata	*scaffold_105235.1*	1:28877650-28877905	http://plants.ensembl.org/Multi/Tools/Blast?db=core
Arabidopsis halleri	*g24906*	FJVB01000147.1:286768-287021	http://plants.ensembl.org/Multi/Tools/Blast?db=core
Arabidopsis arenosa	*AaRAP2.12*	1:22090461-22090724	NCBI:PRJEB42625
Capsella rubella	*CrRAP2.12*	1:18963344-18963583	https://phytozome.jgi.doe.gov
Camelina sativa	*Csa03g061220*	3:27434238-27434507	http://plants.ensembl.org/Multi/Tools/Blast?db=core
Lepidium meyenii	*LmRAP2.12*	scaffold216:4109567-4109829	NCBI:SRP049292
Arabis alpina	*AALP_AA4G024200*	4:2465754-2465978	http://plants.ensembl.org/Multi/Tools/Blast?db=core
Eutrema salsugineum	*EUTSA_v10011606mg*	KI517809:796566-796832	http://plants.ensembl.org/Multi/Tools/Blast?db=core
Brassica oleracea	*Bo3g184310*	C3:63832343-63832590	http://plants.ensembl.org/Multi/Tools/Blast?db=core
Brassica napus	*BnaA08g00990D*	LK031935:433910-434158	http://plants.ensembl.org/Multi/Tools/Blast?db=core
Brassica rapa	*Bra030919*	A08:784672-784920	http://plants.ensembl.org/Multi/Tools/Blast?db=core

B

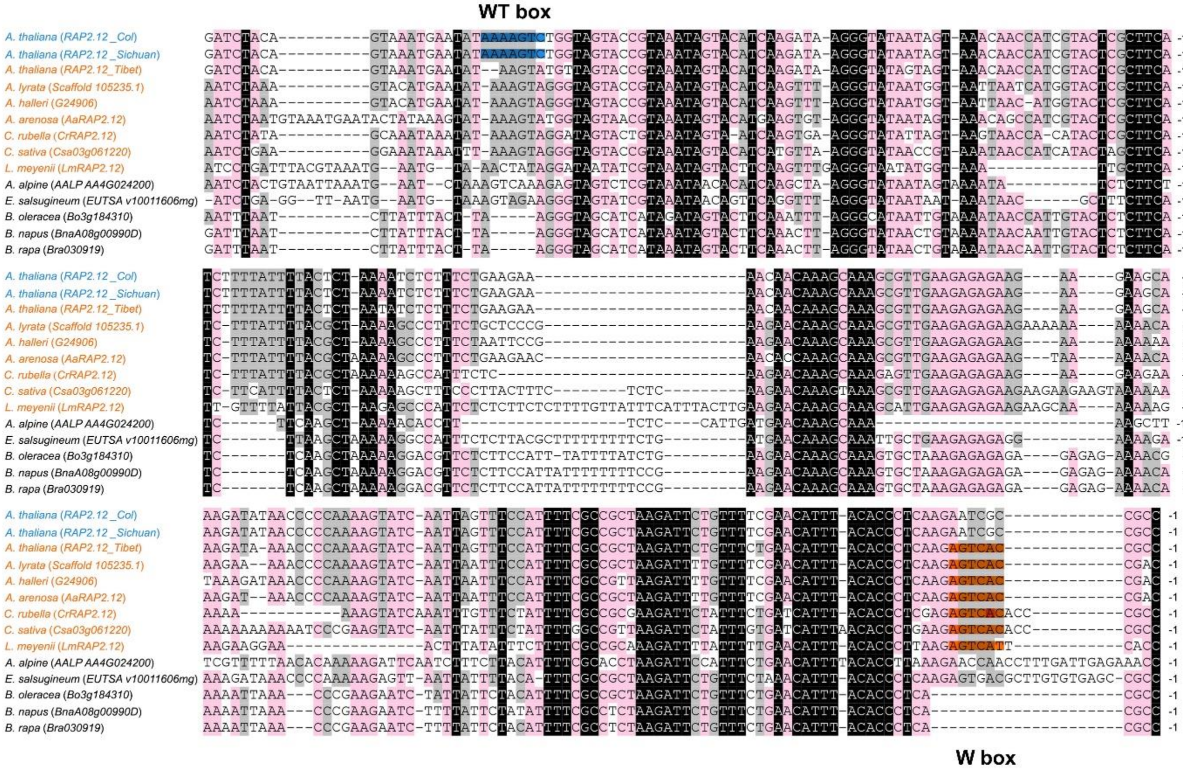


Fig. S19. Sequences of RAP2.12 orthologous genes used for Phylogenetic tree among different Brassicaceae species. (**A**) About 250 bp promoter sequences from ATG were used for the phylogenetic tree. (**B**) Sequence details in the alignment. Core *cis*-element sequences of WT box and W box are in blue and orange respectively.

Table S1. Expression pattern of ERFVIIs in 32 global distributed *Arabidopsis* accessions. Data are from the *Arabidopsis* eFP browser.

Accession	CS_number	Latitude	Longitude	precipitation	AI	RAP2.12_expression	RAP2.2_expression	RAP2.3_expression	HRE1_expression	HRE2_expression
Ak-1	CS6602	48.0683	7.62551	1075.213257	0.9184	199.85	154	38.65	17.75	1.8
Bay-0	CS954	49	11	780.4552612	1.0237	205.83	145.41	40.83	14.88	2.66
Bla-5	CS6620	41.6833	2.8	685.5853271	0.7693	171.55	160.44	39.1	22.45	3.3
C24	CS906	40.2077	-8.42639	945.2876587	0.8301	242.98	144.35	57.96	18.93	4.33
Can-0	CS6660	29.2144	-13.4811	161.5827179	0.1871	181.4	174.9	26.05	14.9	9.3
Cen-0	CS6661	49	0.5	733.2858276	0.9163	213.7	136.05	45.85	12	8.5
CIBC-10	CS22229	51.4083	-0.6383	696.7718506	0.9722	261.5	169.25	38.9	9.75	1.8
Col-0	CS1092	38.3	-92.3	1041.571289	0.8342	215.15	186.6	101.23	14.5	2.11
Cvi-1	CS8580	15.1111	-23.6167	292.1398315	0.2911	227.76	117.75	39.69	20.11	6.68
Dra-1	CS6686	49.4167	16.2667	575.31604	0.8663	229.7	139.1	16.14	16.3	2.4
En-T	CS6176	39	71	601.2260742	1.4735	315.5	176.3	48.4	29.95	10.65
Er-0	CS6698	49.5955	11.0087	752.8178711	0.7937	191	123.9	27.35	13.95	12.5
Est	CS6173	58.6656	24.9871	716.2064209	1.2467	199.9	149.36	55.95	22.01	9.31
GOT-1	CS22277	51.5338	9.9355	653.7061768	0.8761	195.45	106.05	29.35	16.89	1.7
HR-5	CS22205	51.4083	-0.6383	696.7718506	0.9722	280.8	140.25	35.5	17.7	2.4
Is-0	CS6741	50.5	7.5	820.2348022	1.0178	262.2	149.69	2.95	26.25	3
Kin-0	CS6755	44.46	-85.37	799.9605103	0.8719	240.68	157.6	15.35	12.78	4.81
Ler-2	CS8581	47.984	10.8719	1014.838318	1.2847	267.83	160.54	19.56	15.18	6
Li-2:1	CS6772	50.3833	8.0666	648.7250366	0.8261	230.85	127.2	31.2	15.65	2.35
M7323S	CS6184	59.3324	18.0656	569.8093262	0.9179	202.75	110.8	32.5	8.15	3
Ms-0	CS6797	55.7522	37.6322	706.6424561	1.0643	203.35	137.3	28.15	20.1	10.85
Nd-1	CS1636	50	10	634.1703491	0.7944	217.03	204.56	47.85	12.05	8.58
NFE-1	CS22163	51.4083	-0.6383	696.7718506	0.9722	129.6	148.94	30.65	24.5	6.55
Nok-1	CS6808	52.24	4.45	825.0281982	1.2845	254.05	105.65	57.95	9.15	2.45
Nw-1	CS6812	50.5	8.5	747.0148926	0.9892	218.25	126.7	10.4	20.39	4.5
Ove-0	CS6823	53.3422	8.42255	737.317749	1.1158	176	195	63.6	17.1	6.8
Se-0	CS6852	38.3333	-3.53333	416.2077942	0.3988	182.25	131.05	26.3	15.65	2.04
Sf-2	CS6857	41.7833	3.03333	605.6061401	0.6997	204.95	149.9	38.5	19.1	9.25
Sha	CS929	37.29	71.3	655.486145	1.6468	235.05	141.43	19.23	12.63	4.43
Ta-0	CS6867	49.5	14.5	660.043335	0.8992	261	135.05	3.25	14.25	2.75
Uk-3	CS6880	48.0333	7.7667	884.7943115	0.9686	214.45	202.1	20.05	22.35	4.09
Van-0	CS6884	49.2655	-123.206	1535.476807	1.7035	235.68	151.54	15.36	17.21	2.56

Table S4. List of Primer Sequences Used in the Study.

Primers for linkage mapping, the intervals of single nucleotide polymorphism (SNP) markers are shown in brackets

Primer name	Forward primer sequence	Reverse primer sequence
POLY1.(22791833-792483)	TGGAGAGAATCGGCTTCGTT	CCACAATGCAGAGATTACTCGG
POLY1.(22792686-793619)	GAGTCCTGCTCAAATCGTAGC	TATCTGGCCTTCCCTCTGT
POLY1.(22793680-794561)	TCGATTCAGCCGTGGCCTAT	CACTACACTGCAATAATTGC
POLY1.(22794634-796015)	GCATGAGAAACGTGATAGAA	CAAAATCCCAAATCTCCTCTG
POLY1.(22796666-797636)	GTGACAGGGACATAGACACTC	GGGCCGAGACCACGAACCTGC
POLY1.(22798005-798587)	AGGAACTTGTCACAATCCTGG	GCAGGACACGTACGATATTATTG
POLY1.(22798815-799697)	TGGCTACTCCTGAATGCAAAC	GGTATCTATGAACAGAGCT
POLY1.(22799774-800718)	GAGTGATGAGACTTGATTTGTGTG	TGATAAACGAACGATGCGGGAA
POLY1.(22800795-801368)	GTCTCTCTCAGGAGTTCCTGTT	TAAAGAAGCAGAACTGTCCAG
POLY1.(22801829-802528)	AGGAGAACTATCTCCTCCTTCA	CTCAAAACCCACTTCCATATGCAA
POLY1.(22802532-803530)	TGACCCGATTATTGTGGCT	AGCTGAATACTCCTCTCCAC
POLY1.(22803593-804615)	TGGGATTCACTTCATCTCA	AACTCGAAGATGGTCTCACTT
POLY1.(22804616-805596)	GGTCCACCGGGATGCCTTGTT	AGACAAGATAGAACCTCTTCC
POLY1.(22805806-806902)	AGTCACTTGGTTTCATCTCTGTG	ACGAACTATGTTACAAGAGGG
POLY1.(22806971-807804)	TGCACGTACGCAATGACCAC	TCCGACTAAAGCTCCGGCAC
POLY1.(22807806-808654)	CCTTTCCCGGAATGGTAAT	TTCCACAACATGCCATTTTCCC

Primers for T-DNA insertion identification

Primer name	Forward primer sequence	Reverse primer sequence	Germplasm Name	Reference
wrky70-LP	TGATCTTCGGAATCCATGAAG	CAAACCACACCAAGAGGAAAG	wrky70(SALK_025198)	(34)
LBb1.3-BP(T-DNA)		ATTTTGCCGATTTCGGAAC		
rap2.12-LP	TCTTCGATTTTGACGCTGAGT	AGGGTTTGCACCATTGTCCTGAG	rap2.12(SAIL_1215_H10)	(21)
LB3-BP(T-DNA)		GAATTTCATAACCAATCTCGATACAC		

Primers for qRT-PCR

Primer name	Forward primer sequence	Reverse primer sequence	Purpose
ACTIN2-RT	GAACCAGAAGGATGCATATGTTGGTG	GCTCATTGTAGAAAGTGTGATGCCAG	Internal standard
GLIP5-RT	GCCGTTAATTCCGCCGTTTTTG	GAACCTTGGAACGTTTCCACA	qRT-PCR for gene expression
RAP2.12-RT	TGCTGGATGTAATGGGTATCAG	CAGAAGAGATGTCGGGAGTTATC	qRT-PCR for gene expression
RAP2.2-RT	CATGGAAGAGAAGCCTCAGATG	GCCCTGATCGGAACTGAAATA	qRT-PCR for gene expression
RAP2.3-RT	CCATCCCACCAACCAAGTTA	ATTCTTCCTCTTCCTCCGTTTC	qRT-PCR for gene expression
HRE1-RT	ACGAATCCTCTGGAAAGAGGA	TCCGGATTATTCTCCTCCCACA	qRT-PCR for gene expression
HRE2-RT	GAAGAAGCCGATACTAAACCAGG	CTCATGTAATCCTCCAATGCC	qRT-PCR for gene expression
WRKY70-RT	GAGATCGGAGACGTGTACTATAGAGTCG	GCACCCTTGGGTATACTTGTGTGTG	qRT-PCR for gene expression
ADH1-RT	TATTCGATGCAAAGCTGCTGTG	CGAACTTCGTGTTTCTGCGGT	qRT-PCR for gene expression
PDC1-RT	CGATTATGGCACTAACCGGATT	TGTTCACCACCGCCTGATAAC	qRT-PCR for gene expression
SUS4-RT	CGCAGAACGTGTAATAACGCG	CAACCCTTGAGAGCAAAGCAAA	qRT-PCR for gene expression
PCO1-RT	ATTGGGTGGTTGATGCTCCAATG	ATGCATGTTCCCGCCATCTTCC	qRT-PCR for gene expression

Plasmid construction for luciferase activity assay and production of transgenic *Arabidopsis*

Primer name	sequence	Purpose	Vector
RAP2.12cds-KpnI-F	acgaacgatagccatGGTACCatgtgtggaggagctataatatccgat	p35S:RAP2.12-Col p35S:RAP2.12-Sic p35S:RAP2.12^{Tibet}	pCAMBIA1300
RAP2.12cds-XbaI-R	gcgctcagttggaatTCTAGAtcagaagactcctccaatcatggaatgaatt		
pPGB1-HindⅢ-F	gtcgacggtatcgatAAGCTTcctcttagatatgaaagccaca	pPGB1:LUC	pGreenII-0800-LUC
pPGB1-BamHI-R	cgctctagaactagtGGATCCaatatttcacaacctctaaatgat		
WRKY70cds-KpnI-F	acgaacgatagccatGGTACCatggatactaataaagcaaaa	p35S:WRKY70-Col p35S:WRKY70-Sic p35S:WRKY70^{Tibet}	pCAMBIA1300
WRKY70cds-XbaI-R	gcgctcagttggaatTCTAGAtcaagatagattcgaacatgaactga		
pRAP2.12-HindⅢ(Sic)-F	gtcgacggtatcgatAAGCTTgcgattcggcccaaaaagaca	pSic-RAP2.12:LUC	pGreenII-0800-LUC
pRAP2.12-BamHI(Sic)-R	cgctctagaactagtGGATCCggcgg**cgat**tcttgagggtgt		
pRAP2.12-HindⅢ(Sic)-F	gtcgacggtatcgatAAGCTTgcgattcggcccaaaaagaca	pSic-RAP2.12(WT$_M$):LUC	pGreenII-0800-LUC
pRAP2.12-WTm-R	tttactgtagatctattttgtaaattagg		
pRAP2.12-WTm-F	tagatctacagtaaatgaatat**aagta**tggtagtaccg		
pRAP2.12-BamHI(Sic)-R	cgctctagaactagtGGATCCggcgg**cgat**tcttgagggtgt		
pRAP2.12-HindⅢ(Sic)-F	gtcgacggtatcgatAAGCTTgcgattcggcccaaaaagaca	pSic-RAP2.12(WT$_{M+W}$):LUC	pGreenII-0800-LUC
pRAP2.12-WTm-R	tttactgtagatctattttgtaaattagg		
pRAP2.12-WTm-F	tagatctacagtaaatgaatat**aagta**tggtagtaccg		
pRAP2.12-BamHI(Tib)-R	cgctctagaactagtGGATCCggcgg**tgac**tcttgagggtgt		
pRAP2.12-HindⅢ(Tib)-F	gtcgacggtatcgatAAGCTTgcgattcggcccaaaaagaca	pTib-RAP2.12:LUC	pGreenII-0800-LUC
pRAP2.12-BamHI(Tib)-R	cgctctagaactagtGGATCCggcgg**tgac**tcttgagggtgt		
pRAP2.12-HindⅢ(Tib)-F	gtcgacggtatcgatAAGCTTgcgattcggcccaaaaagaca	pTib-RAP2.12(W$_M$):LUC	pGreenII-0800-LUC
pRAP2.12-BamHI(Sic)-R	cgctctagaactagtGGATCCggcgg**cgat**tcttgagggtgt		
pRAP2.12-XcmI(Sic)-F	gactctagaggatccCCAATACTTGTATGGgcgattcggcccaaaaagaca	pSic-RAP2.12:RAP2.12-GUS	pCXGUS-P
pRAP2.12-RAP2.12cds(Sic)-R	agctcctccacacatggcgg**cgat**tcttgagggtgt		
RAP2.12cds-F	atgtgtggaggagctataatatccgat		
RAP2.12cds-XcmI-R	acgtaacatggatccCCAATACTTGTATGGgaagactcctccaatcatgg		
pRAP2.12-XcmI(Tib)-F	gactctagaggatccCCAATACTTGTATGGgcgattcggcccaaaaagaca	pTib-RAP2.12:RAP2.12-GUS	pCXGUS-P
pRAP2.12-RAP2.12cds(Tib)-R	agctcctccacacatggcgg**tgac**tcttgagggtgt		
RAP2.12cds-F	atgtgtggaggagctataatatccgat		
RAP2.12cds-XcmI-R	acgtaacatggatccCCAATACTTGTATGGgaagactcctccaatcatgg		
pRAP2.12-XcmI(Sic)-F	gactctagaggatccCCAATACTTGTATGGgcgattcggcccaaaaagaca	pSic-RAP2.12:GUS	pCXGUS-P
pRAP2.12-XcmI(Sic)-R	acgtaacatggatccCCAATACTTGTATGGggcgg**cgat**tcttgagggtgt		
pRAP2.12-XcmI(Tib)-F	gactctagaggatccCCAATACTTGTATGGgcgattcggcccaaaaagaca	pTib-RAP2.12:GUS	pCXGUS-P
pRAP2.12-XcmI(Tib)-R	acgtaacatggatccCCAATACTTGTATGGggcgg**tgac**tcttgagggtgt		
pRAP2.12-XcmI(Sic)-F	gactctagaggatccCCAATACTTGTATGGgcgattcggcccaaaaagaca	pSic-Tib$_{(-125-0)}$-RAP2.12:GUS	pCXGUS-P
pRAP2.12(-125)-R	cttcttctctcttcaacgctttgc		
pRAP2.12(-125)-F	gcaaagcgttgaagagagaagaag		
pRAP2.12-XcmI(Tib)-R	acgtaacatggatccCCAATACTTGTATGGggcgg**tgac**tcttgagggtgt		
pRAP2.12-XcmI(Sic)-F	gactctagaggatccCCAATACTTGTATGGgcgattcggcccaaaaagaca	pSic-Tib$_{(-250-0)}$-RAP2.12:GUS	pCXGUS-P
pRAP2.12(-250)-R	ttatattcatttactgtagatctat		
pRAP2.12(-250)-F	atagatctacagtaaatgaatataa		

pRAP2.12-XcmI(Tib)-R	acgtaacatggatccCCAATACTTGTATGGggcgg**tgac**tcttgagggtgt		
pRAP2.12-XcmI(Sic)-F	gactctagaggatccCCAATACTTGTATGGgcgattcggcccaaaaagaca	pSic-Tib$_{(-500-0)}$-RAP2.12:GUS	pCXGUS-P
pRAP2.12(-500)-R	tgcattcaggagtagccaacgcag		
pRAP2.12(-500)-F	ctgcgttggctactcctgaatgca		
pRAP2.12-XcmI(Tib)-R	acgtaacatggatccCCAATACTTGTATGGggcgg**tgac**tcttgagggtgt		

Oligonucleotides and plasmid construction for EMSA

Primer name	sequence	Purpose	Vector
pRAP2.12-WT-F from Sichuan with biotin	Biotin-TAAATGAATAT**AAAAGTC**TGGTAGTACCGT	Probe with Biotin for EMSA	-
pRAP2.12-WT-R from Sichuan with biotin	Biotin-ACGGTACTACCA**GACTTTT**ATATTCATTTA	Probe with Biotin for EMSA	-
pRAP2.12-WT-F from Sichuan	TAAATGAATAT**AAAAGTC**TGGTAGTACCGT	Probe without Biotin for EMSA	-
pRAP2.12-WT-R from Sichuan	ACGGTACTACCA**GACTTTT**ATATTCATTTA	Probe without Biotin for EMSA	-
pRAP2.12-WTm-F from Tibet	TAAATGAATAT**AAGTA**TG**T**TAGTACCGTAA	Probe without Biotin for EMSA	-
pRAP2.12-WTm-R from Tibet	TTACGGTACTA**A**CA**TACTT**ATATTCATTTA	Probe without Biotin for EMSA	-
pRAP2.12-W-F from Tibet with biotin	Biotin-TACACCCTCAAGA**GTCA**CCGCCATGTGTGG	Probe with Biotin for EMSA	-
pRAP2.12-W-R from Tibet with biotin	Biotin-CCACACATGGCGG**TGAC**TCTTGAGGGTGTA	Probe with Biotin for EMSA	-
pRAP2.12-W-F from Tibet	TACACCCTCAAGA**GTCA**CCGCCATGTGTGG	Probe without Biotin for EMSA	-
pRAP2.12-W-R from Tibet	CCACACATGGCGG**TGAC**TCTTGAGGGTGTA	Probe without Biotin for EMSA	-
pRAP2.12-Wm-F from Sichuan	TACACCCTCAAGA**ATCG**CCGCCATGTGTGG	Probe without Biotin for EMSA	-
pRAP2.12-Wm-R from Sichuan	CCACACATGGCGGCGATTCTTGAGGGTGTA	Probe without Biotin for EMSA	-
WRKY70cds-BamHI-F	gatctggttccgcgtGGATCCatggatactaataaagcaaaaaagcttaaag	Prokaryotic expression of GST-WRKY70-Sic/GST-WRKY70-Tib	pGEX4T-1
WRKY70cds-EcoRI-R	ctcgagtcgacccggGAATTCtcaagatagattcgaacatgaactgaagatag	Prokaryotic expression of GST-WRKY70-Sic/GST-WRKY70-Tib	pGEX4T-1

Table S2. World-wide *RAP2.12* promoter sequence classification in *Arabidopsis* used in haplotype network analysis, related to figure 4, B and C.

Table S3. Annual precipitation, aridity index, and oxygen availability to roots in 1475 non-repetitive *Arabidopsis* accessions around the world.

Data S1. (separate file)
Fasta format sequence of *RAP2.12* promoter used in haplotype network analysis, related to figure 4B.