

Supporting Information

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Genome-Wide Association Studies Reveal the Genetic Architecture of Ionomics Variation in Grains of Tartary Buckwheat

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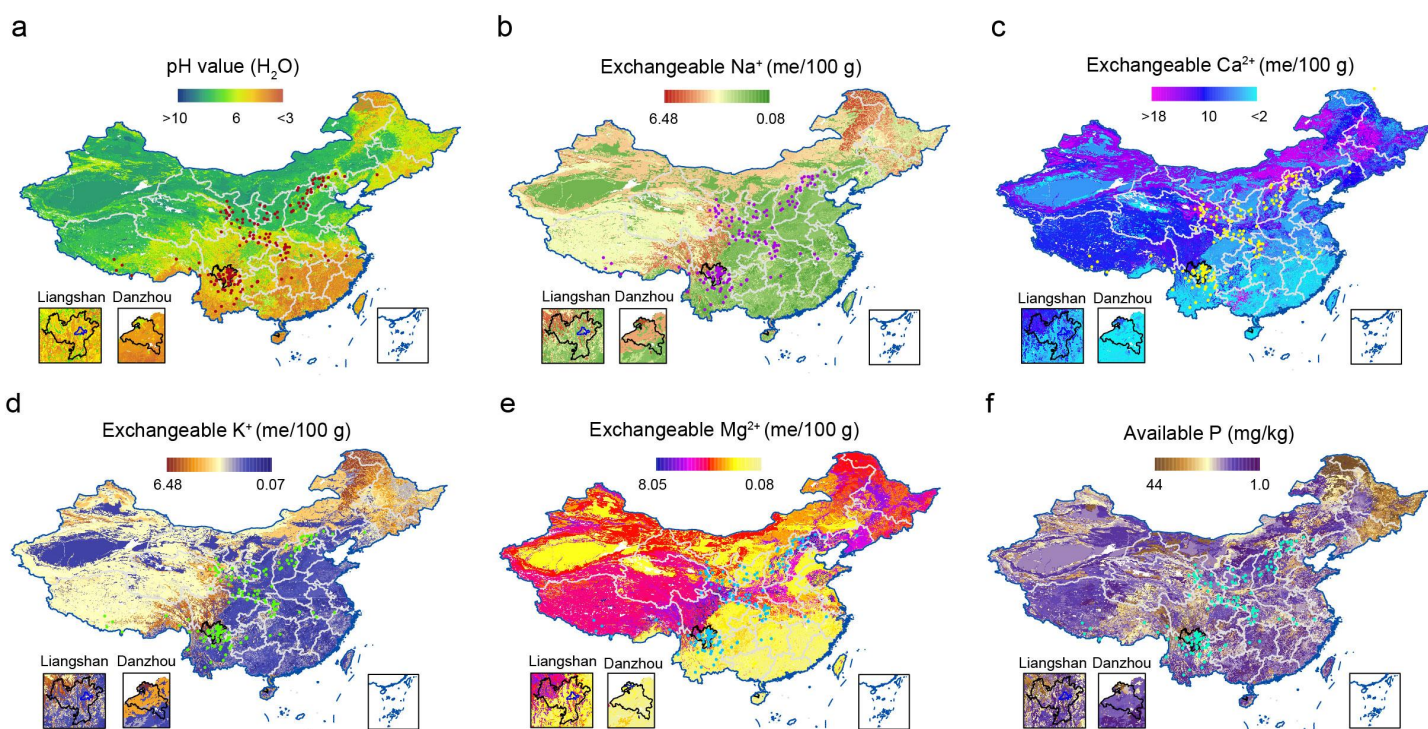


Figure S1. The soil properties of planting field. a-f. Spatial distribution of soil pH value (a), exchangeable Na^+ (me/100 g) (b), exchangeable Ca^{2+} (me/100 g) (c), exchangeable K^+ (me/100 g) (d), exchangeable Mg^{2+} (me/100 g) (e), available phosphorus (P, mg/kg) (f) in China. The circles marked with different colors represent the geographical distribution of 182 Tartary buckwheat accessions. layer (4.5-9.1 cm). The two rectangular boxes in the bottom left corner of the map represent Liangshan Yi Autonomous Prefecture and Danzhou City, respectively, with the blue box in the Liangshan map representing Zhaojue County.

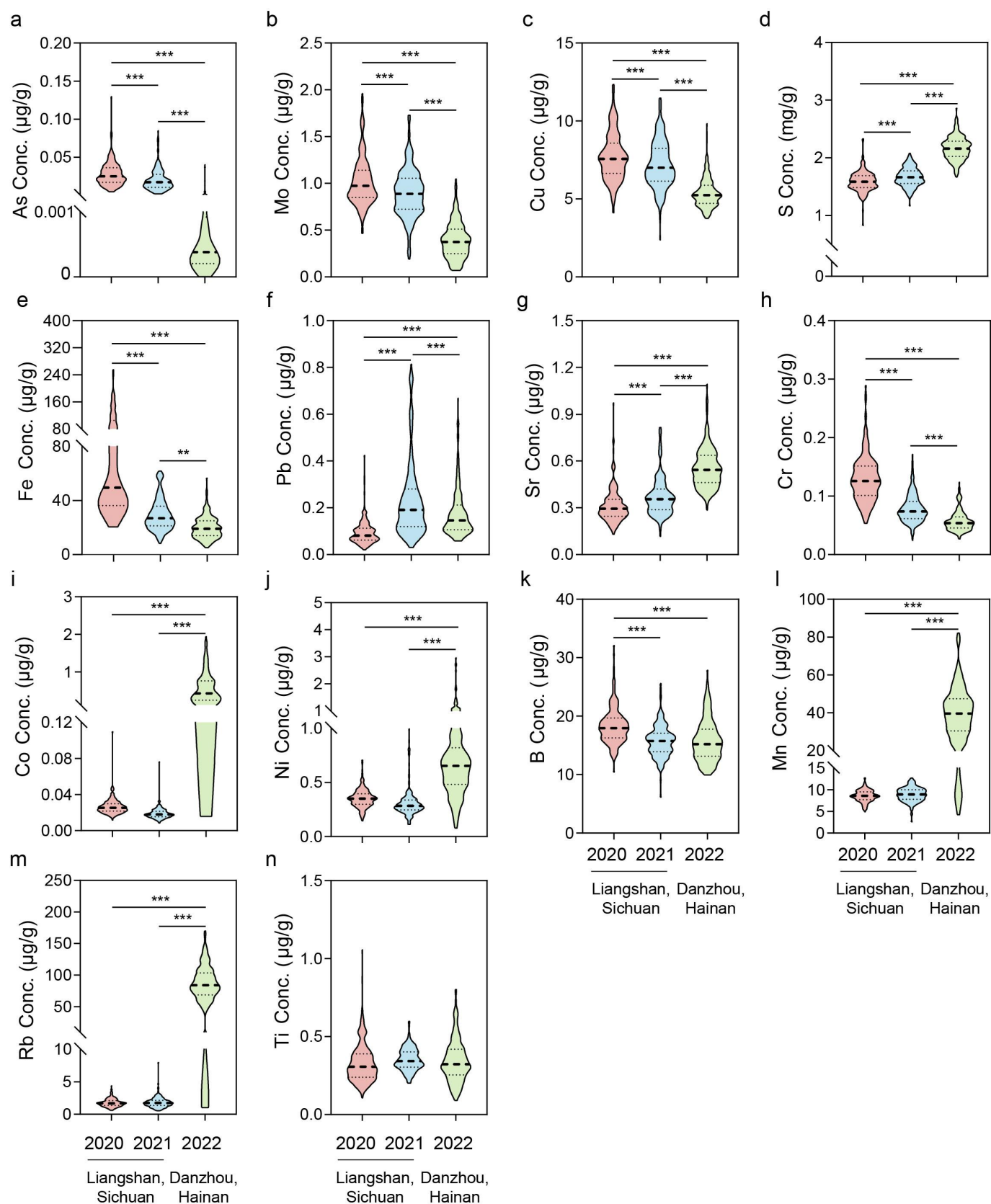


Figure S2. Violin plots of 14 elemental concentrations of Tartary buckwheat seeds among different years and locations. Asterisk (**) and (***) indicate significant difference at $P < 0.01$ and $P < 0.001$ using one-way ANOVA.

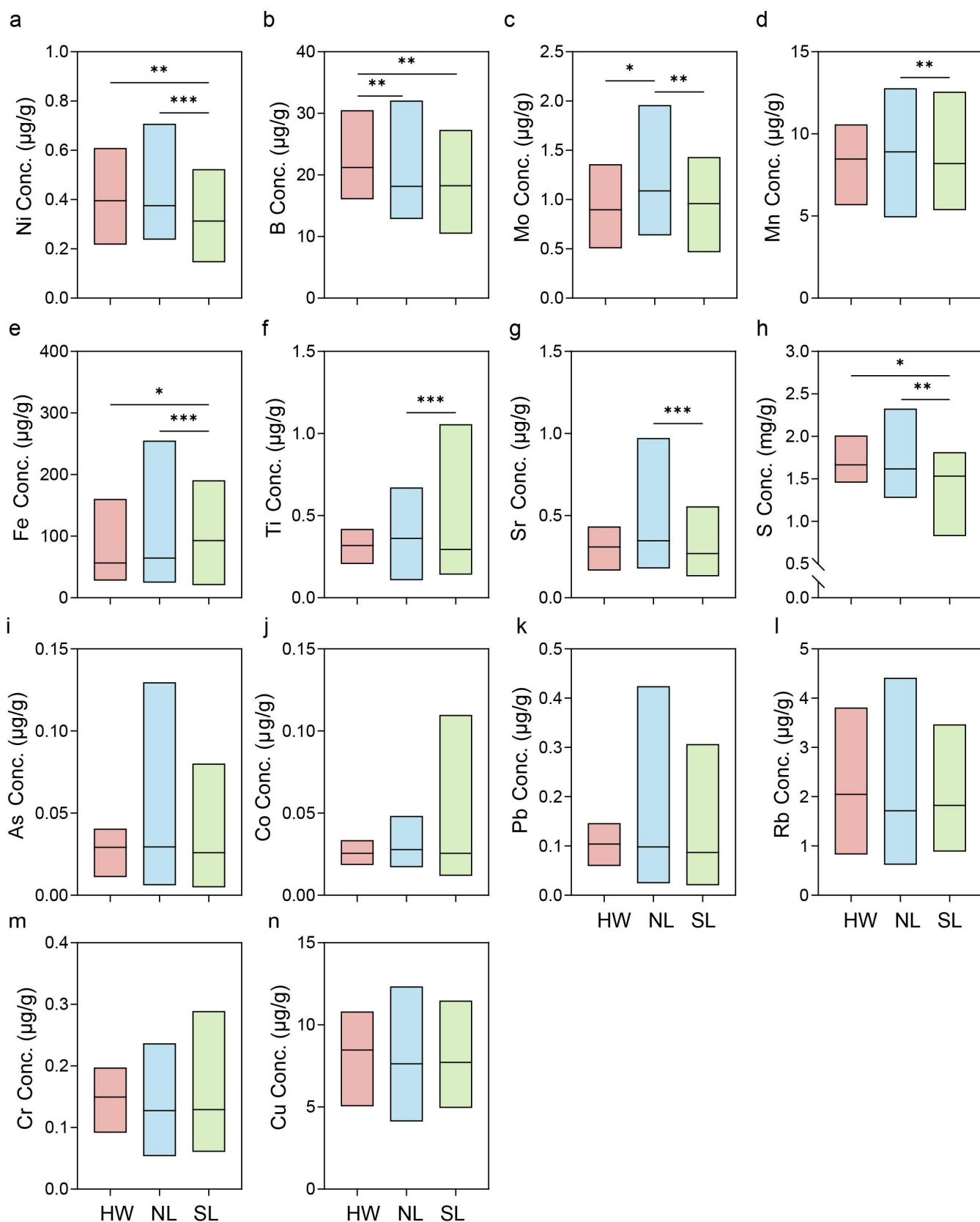
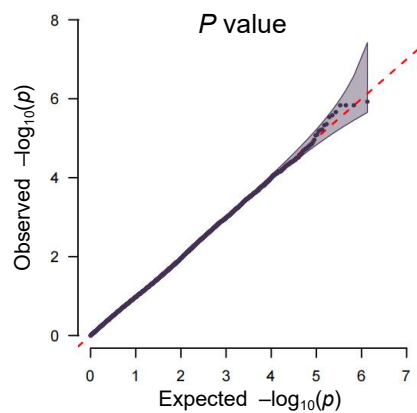
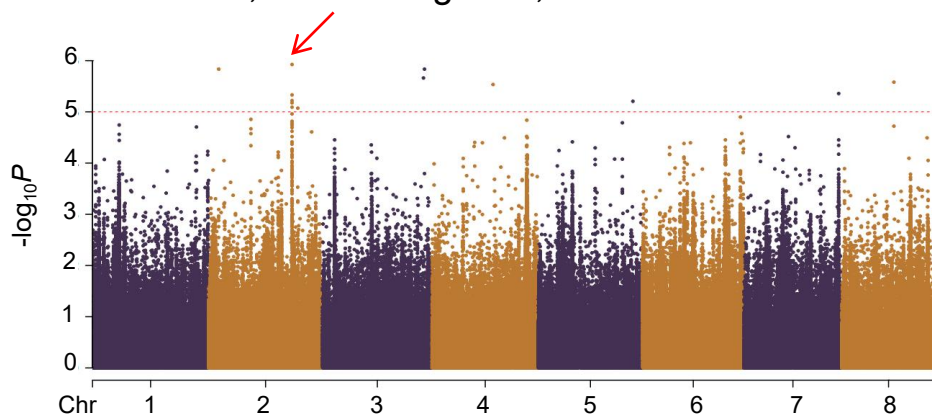
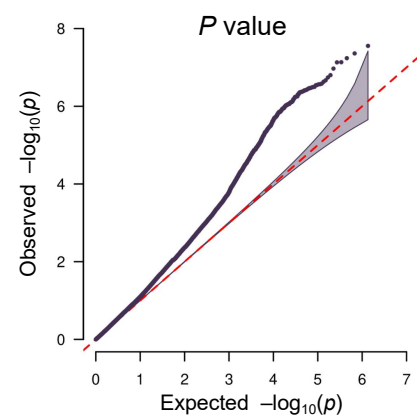
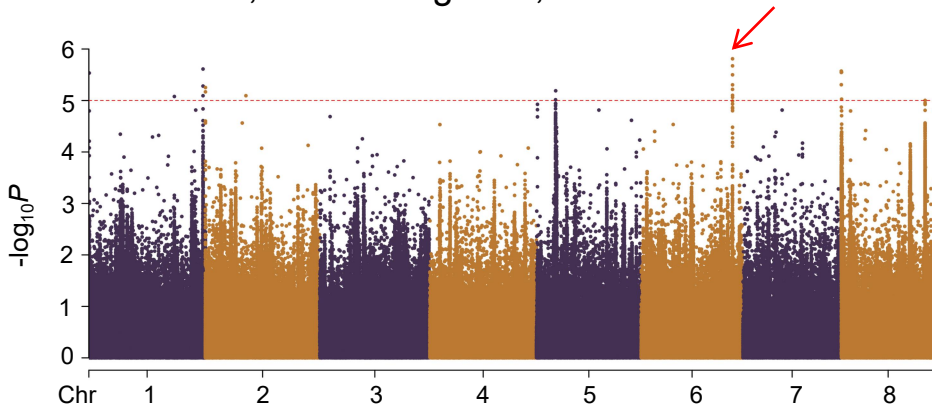


Figure S3. Box plots of 14 elemental concentrations of Tartary buckwheat seeds among three groups. Asterisk (**) and (***) indicate significant difference at $P < 0.01$ and $P < 0.001$ using one-way ANOVA.

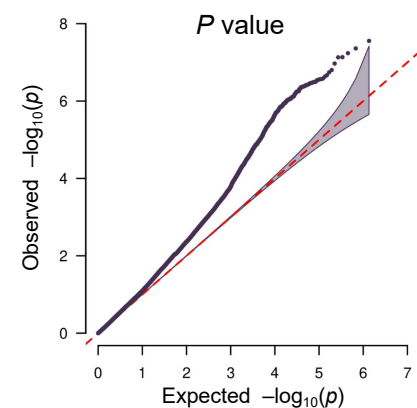
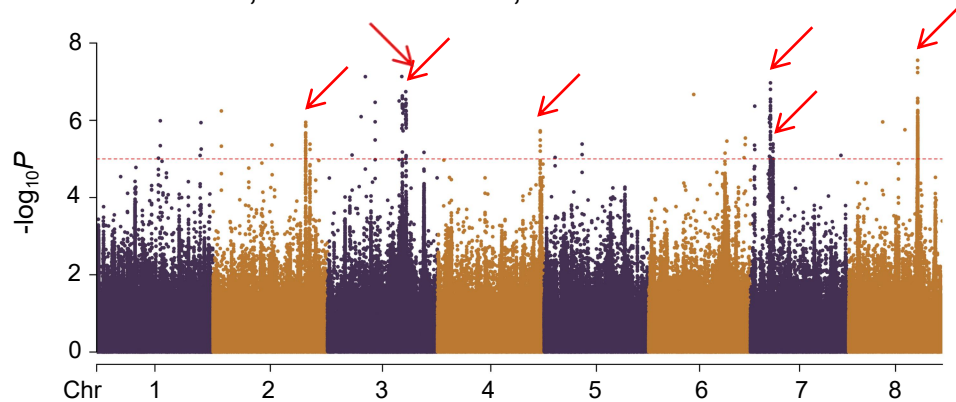
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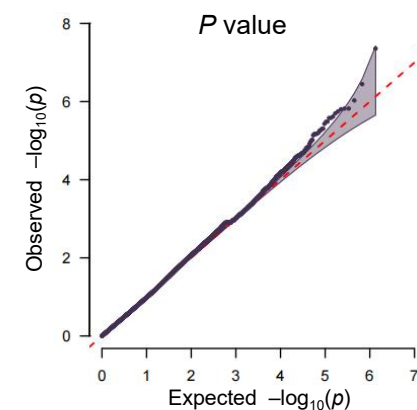
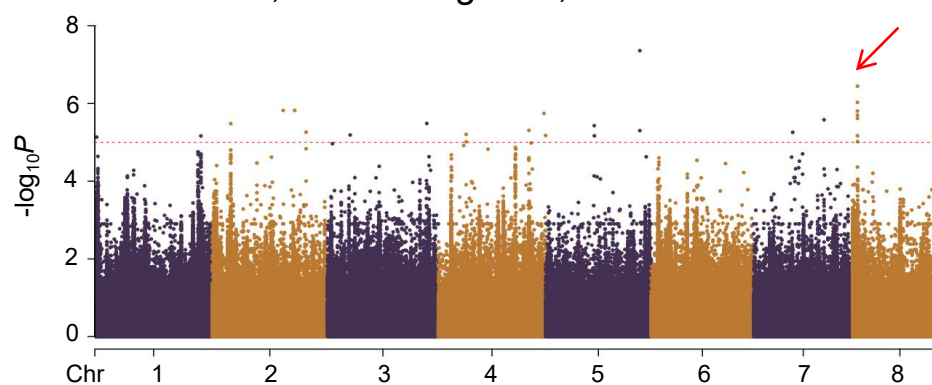
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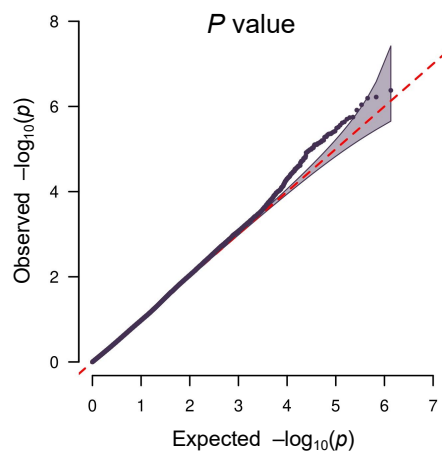
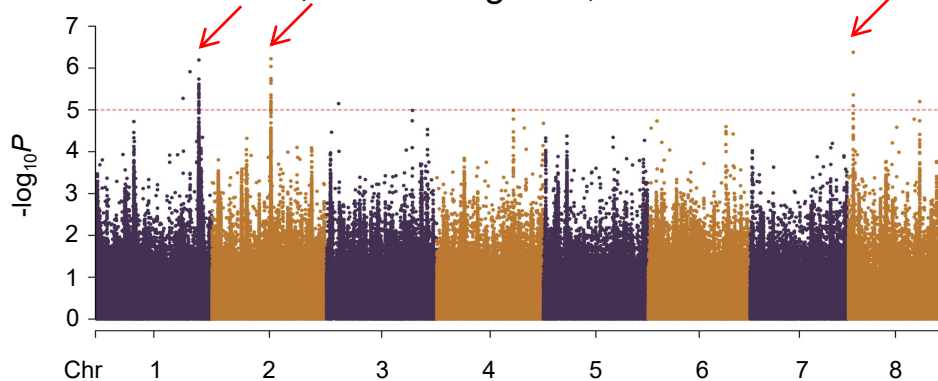
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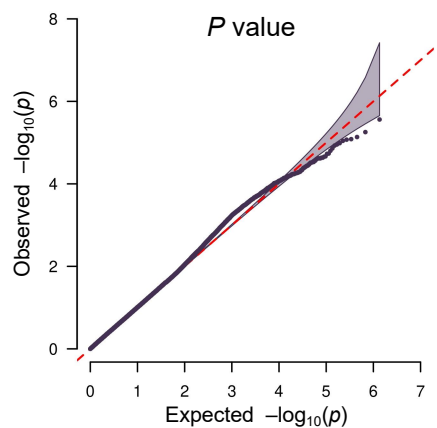
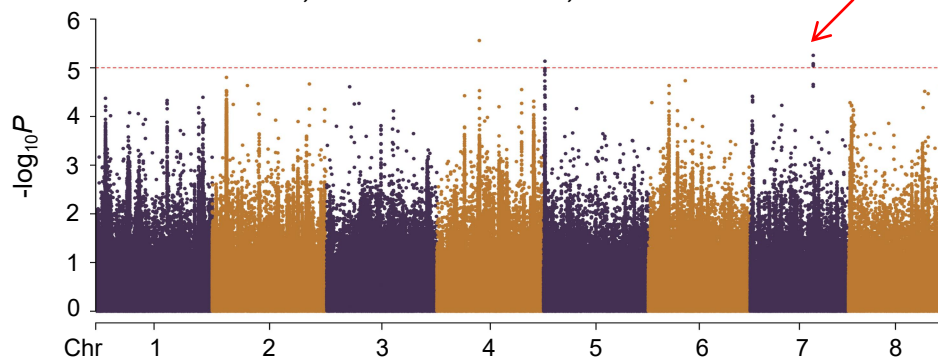
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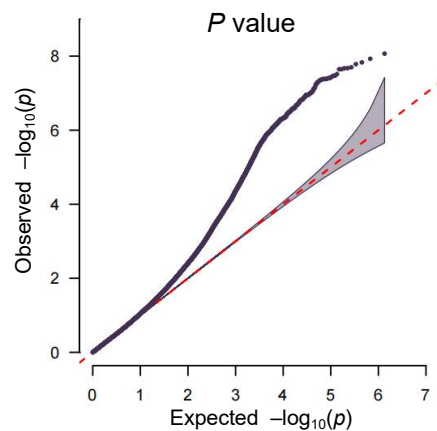
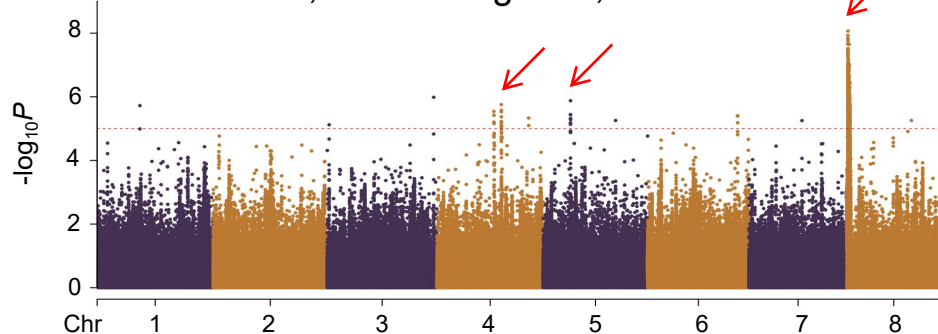
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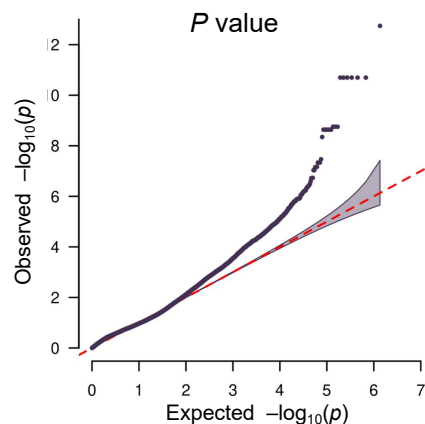
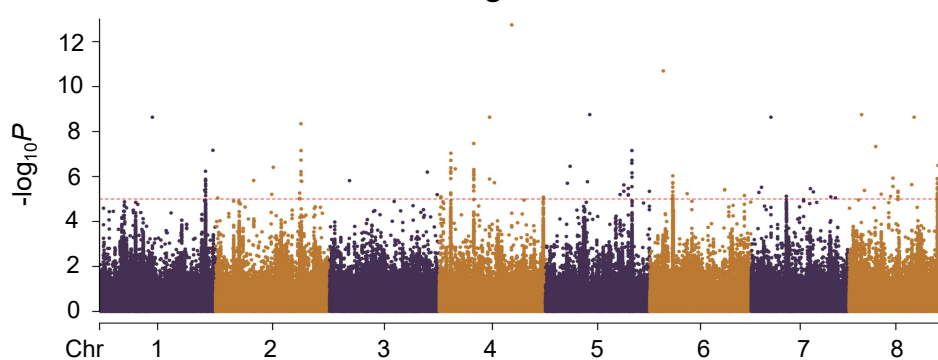
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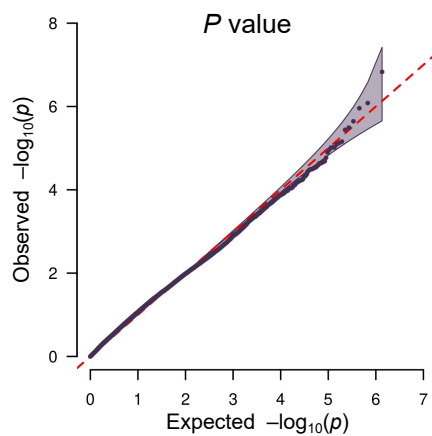
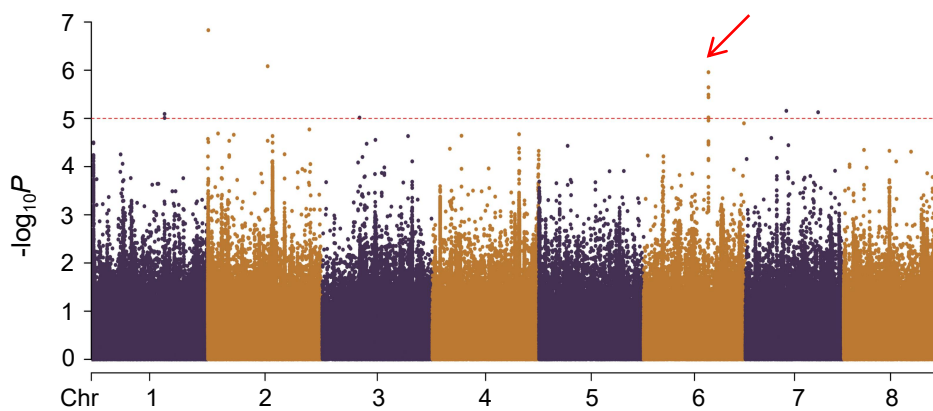
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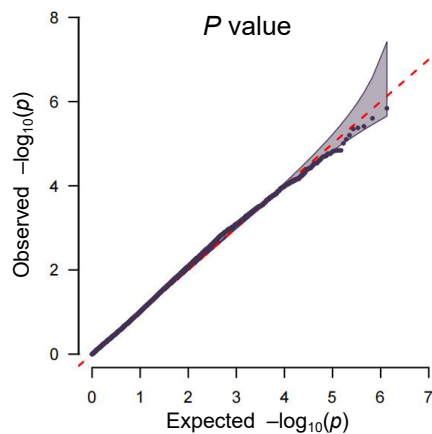
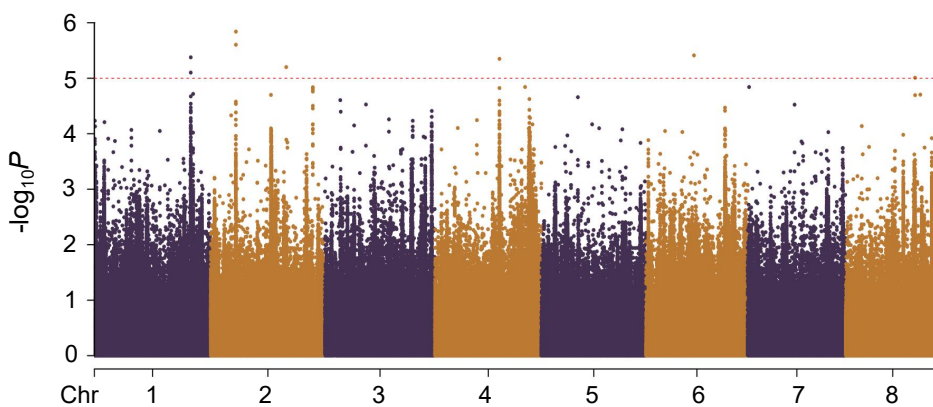
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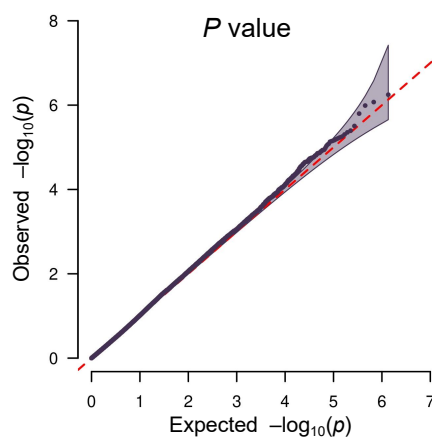
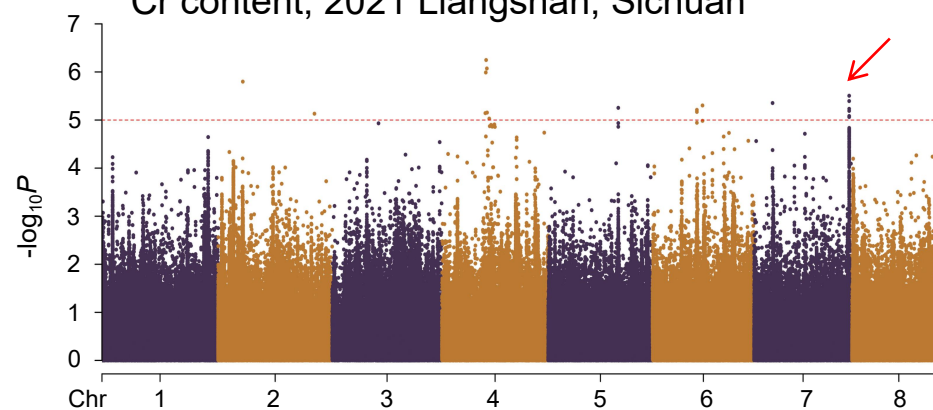
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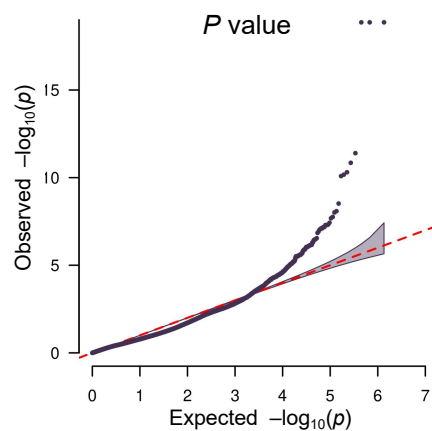
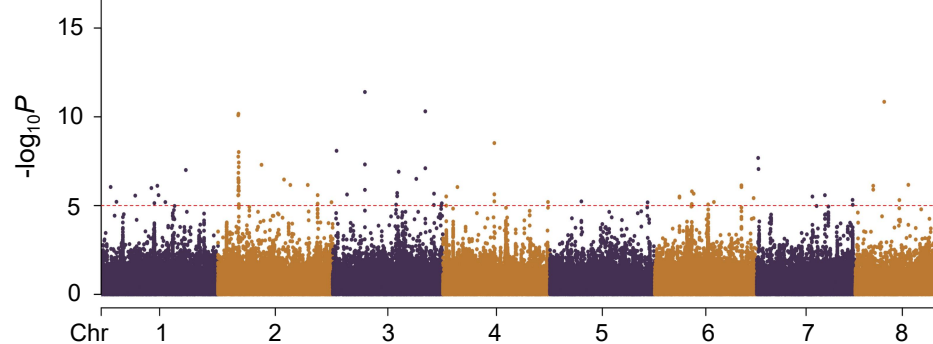
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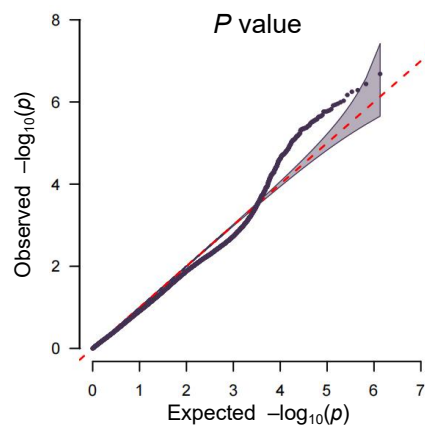
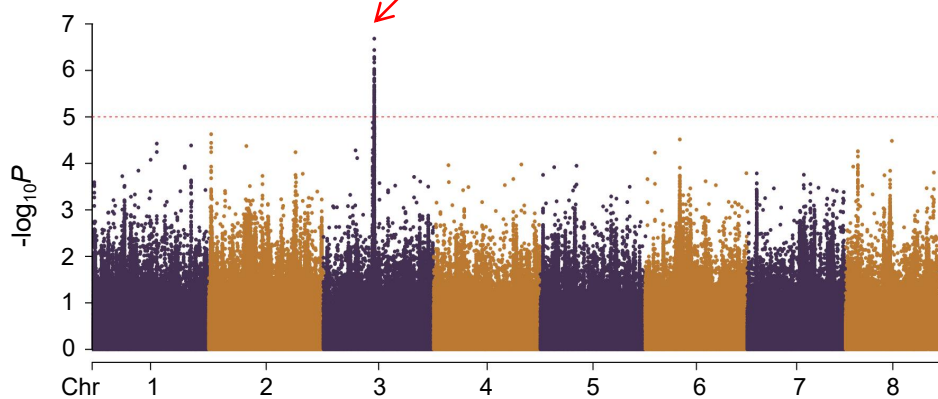
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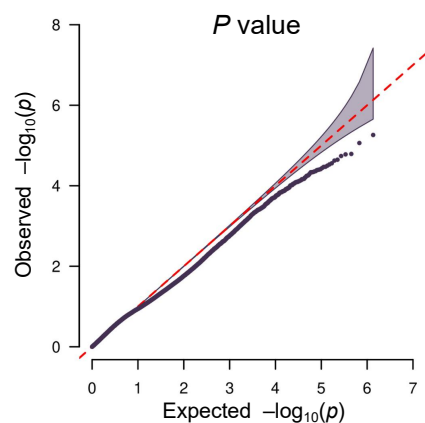
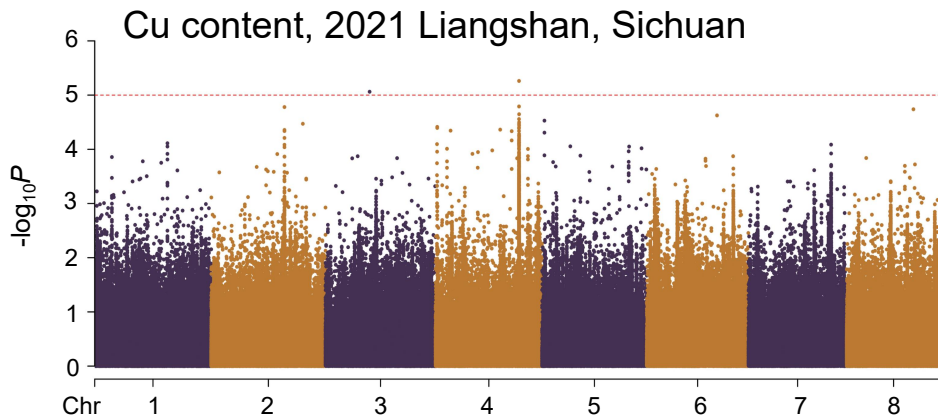
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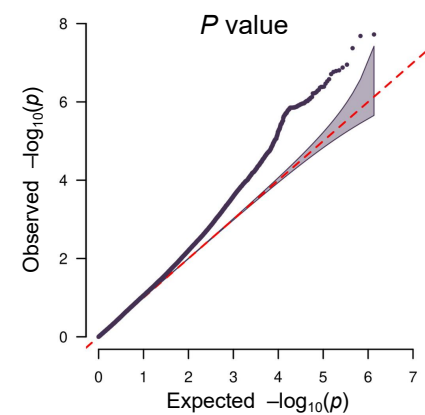
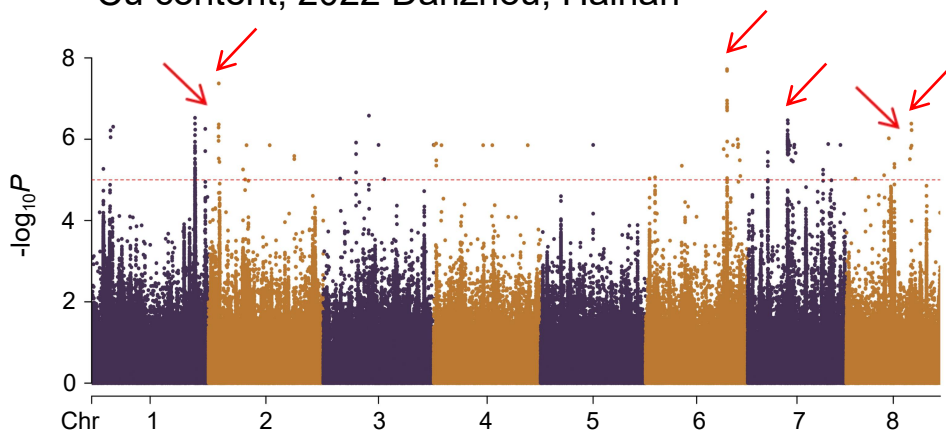
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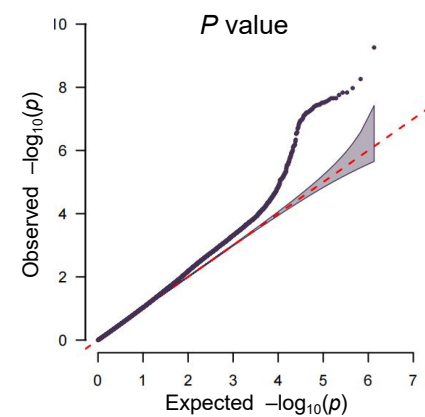
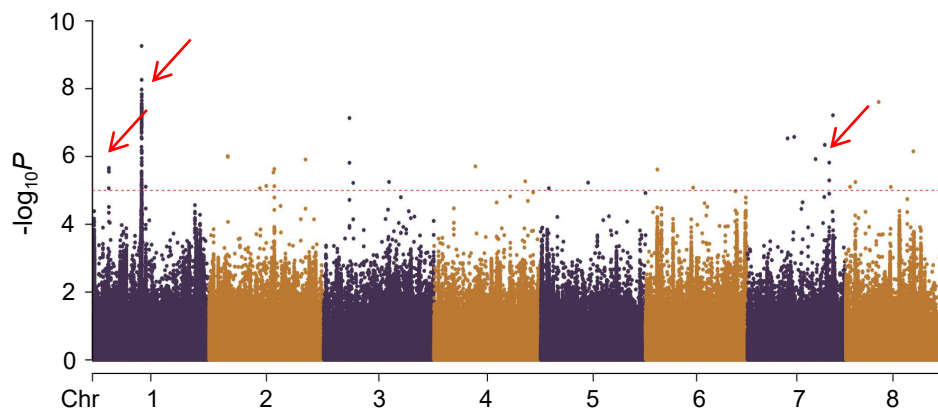
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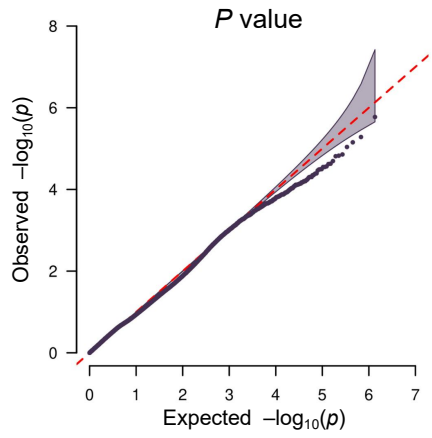
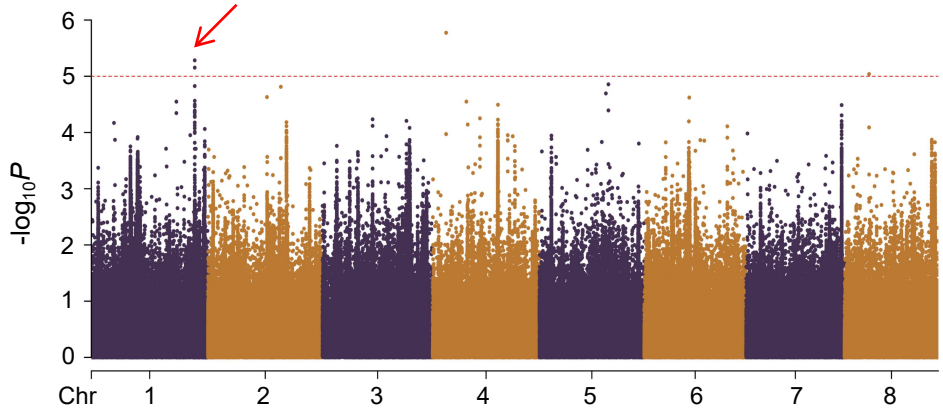
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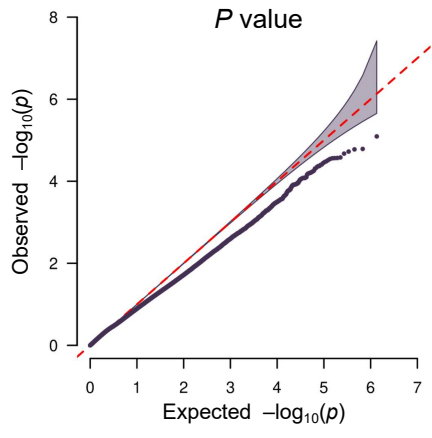
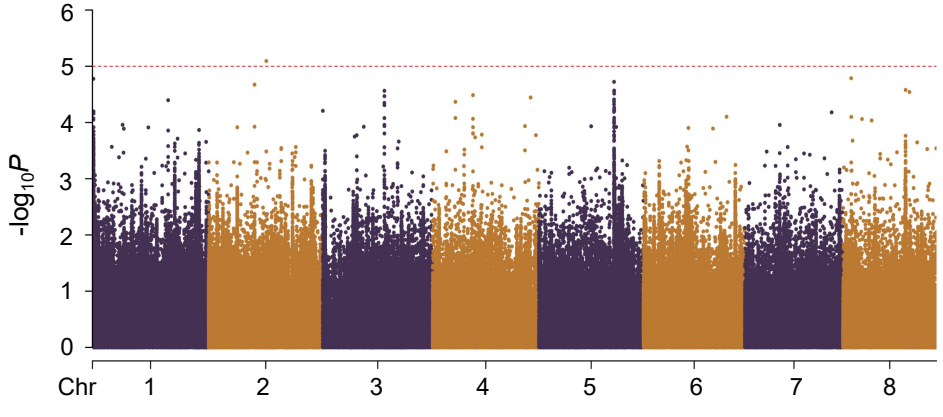
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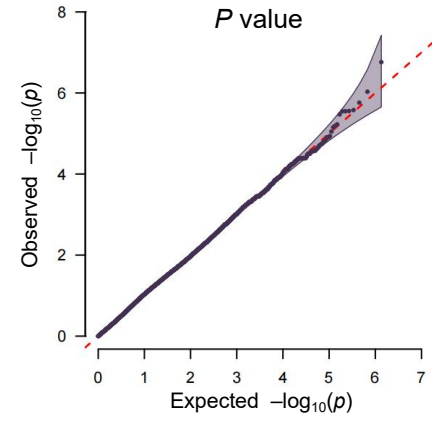
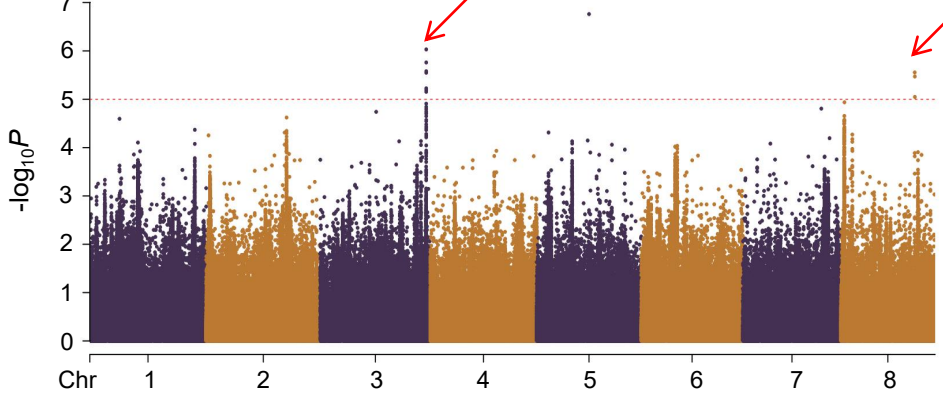
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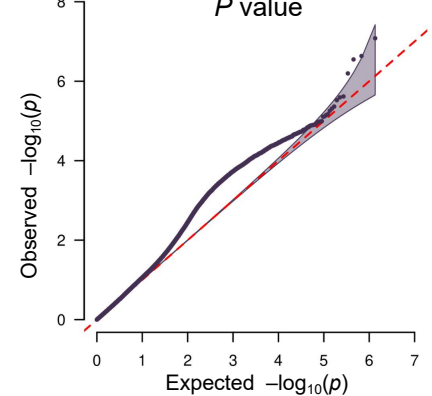
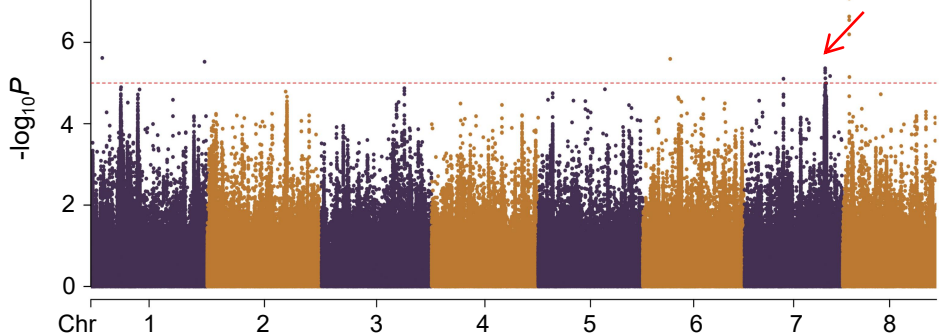
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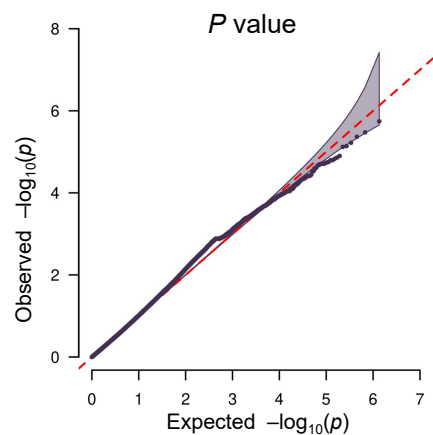
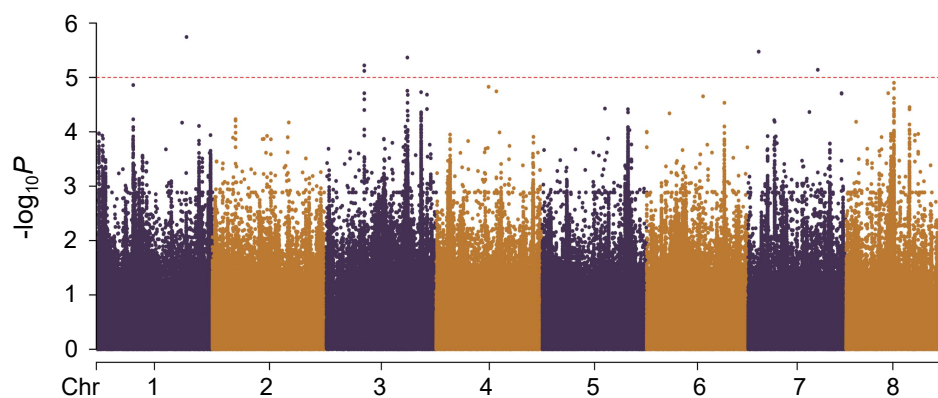
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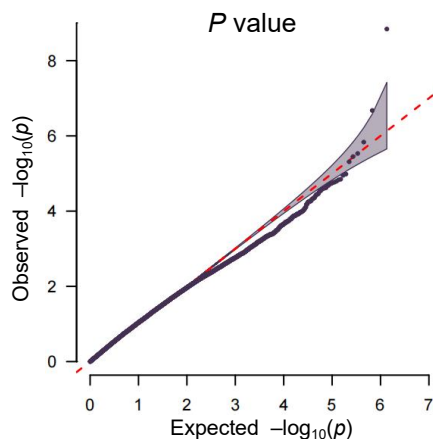
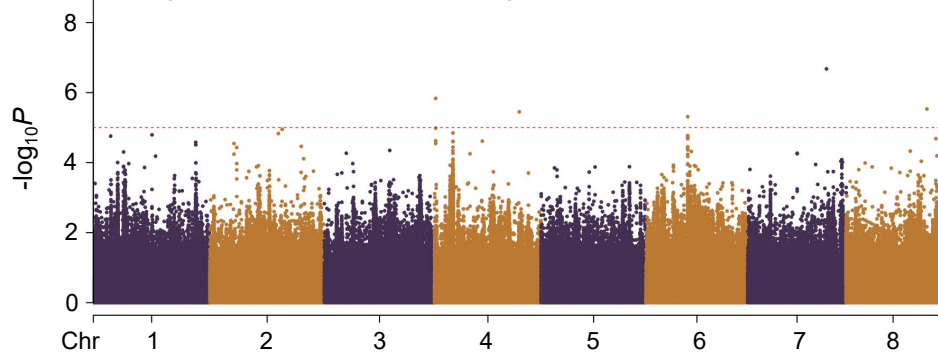
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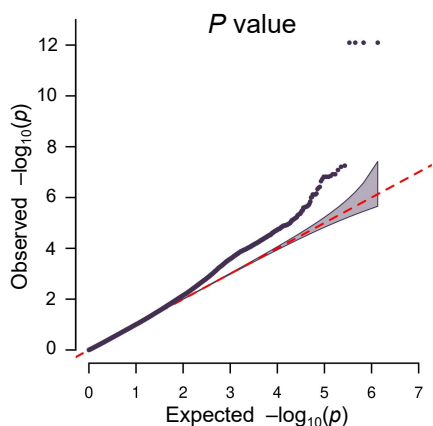
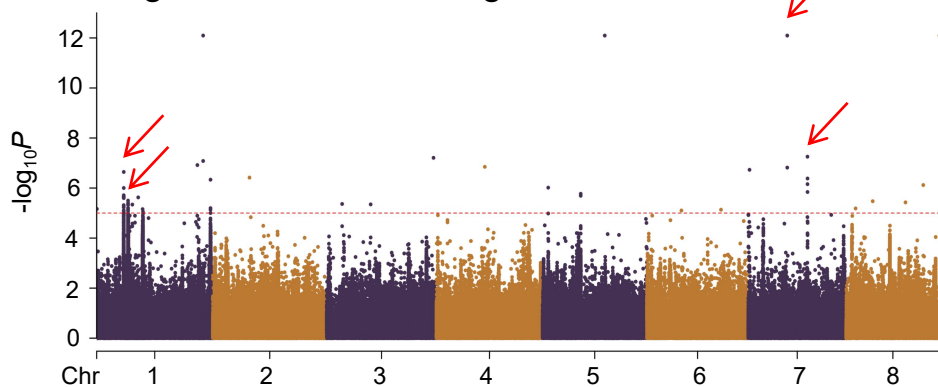
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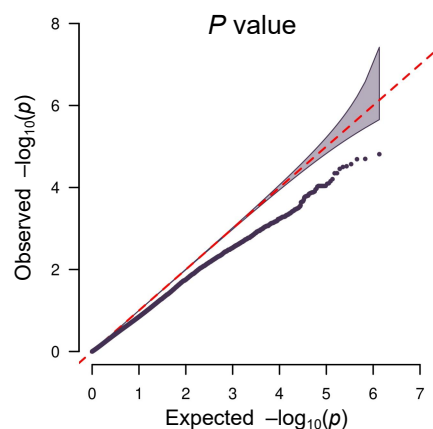
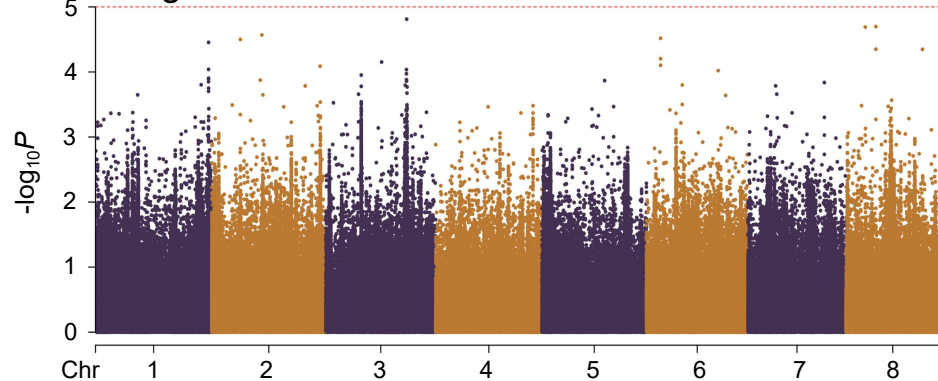
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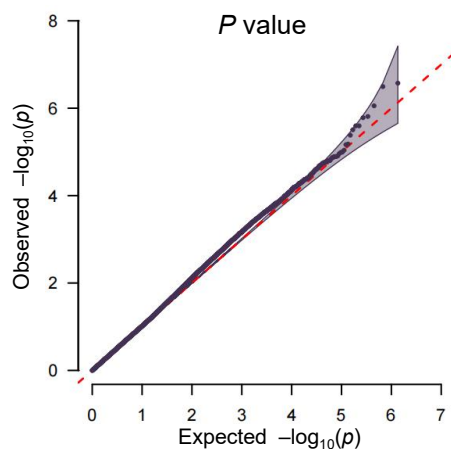
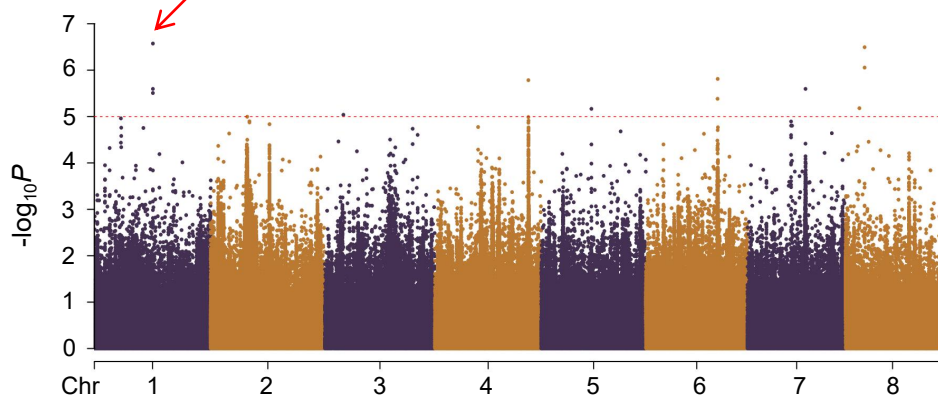
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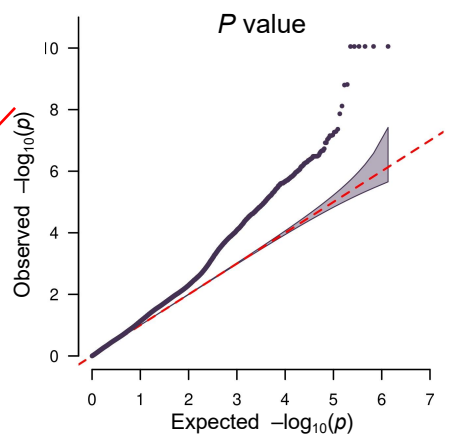
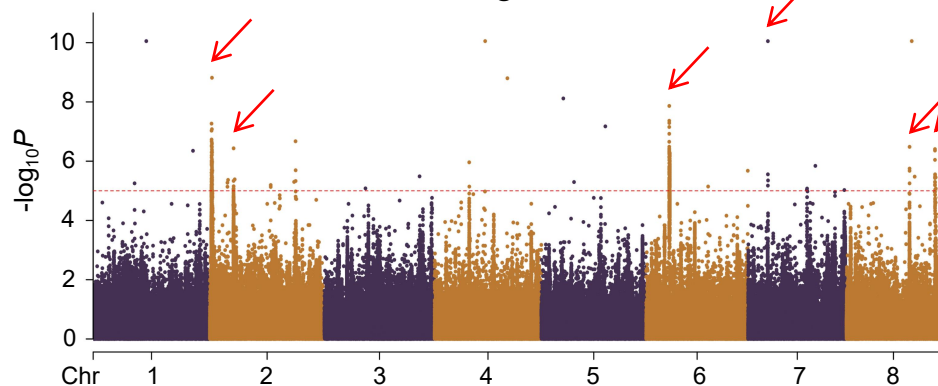
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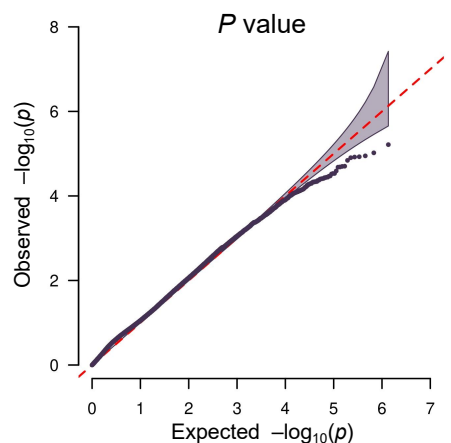
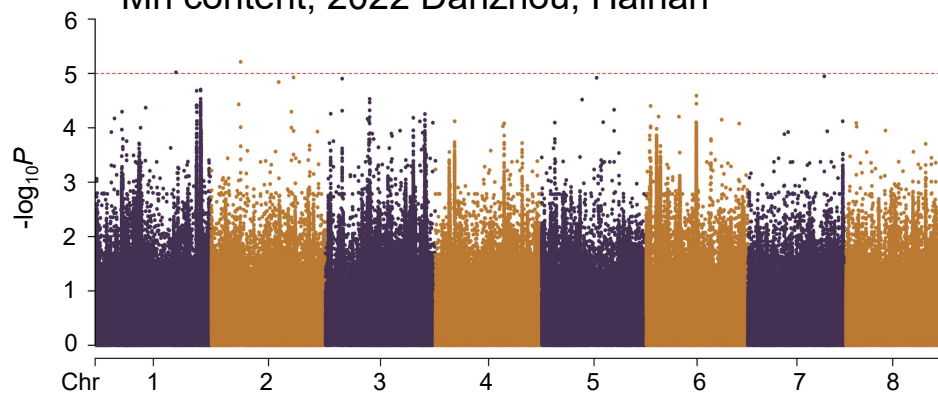
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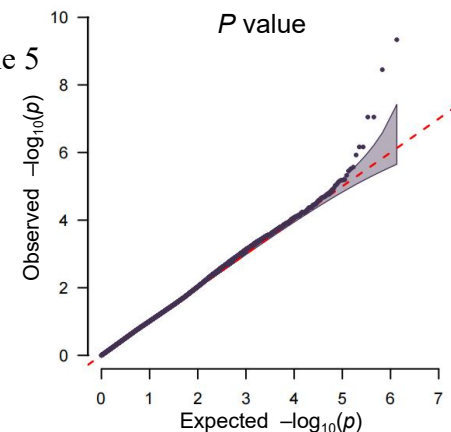
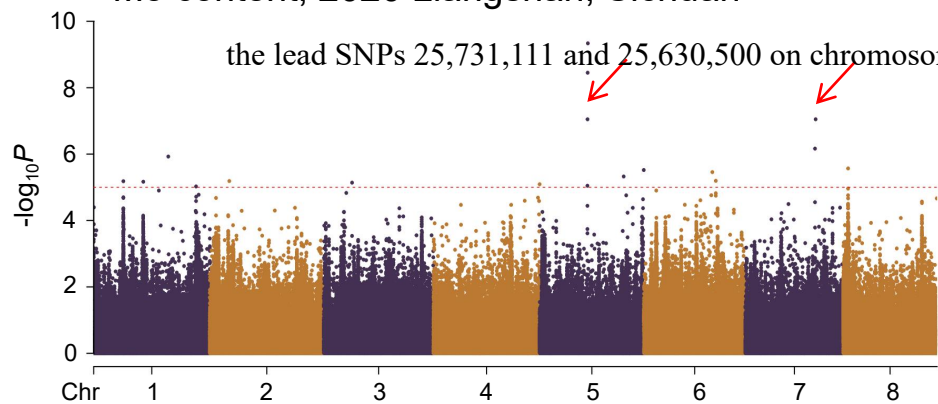
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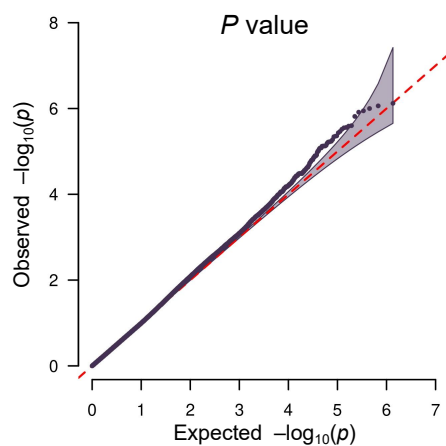
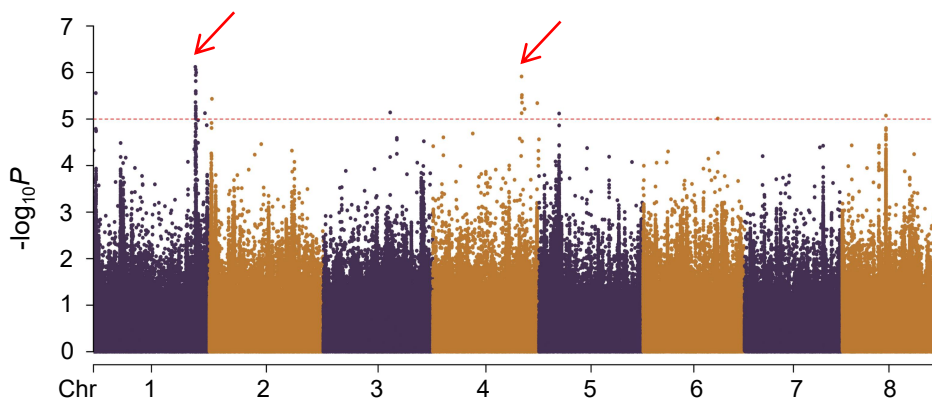
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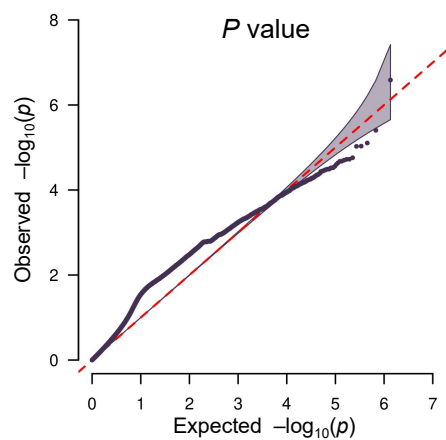
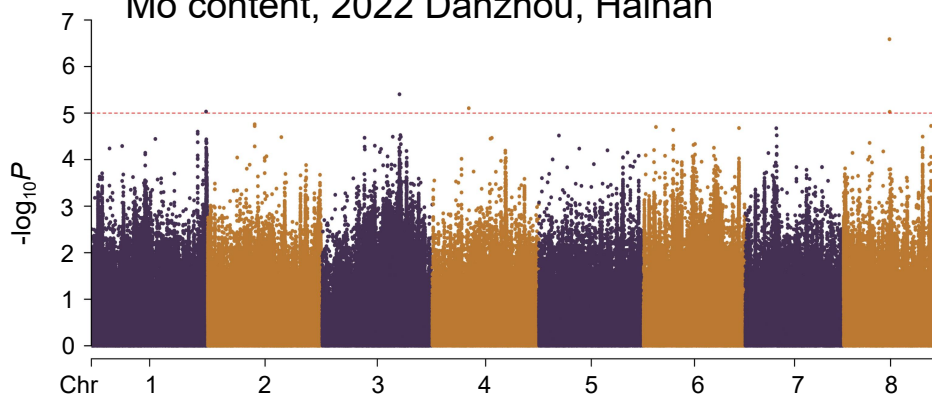
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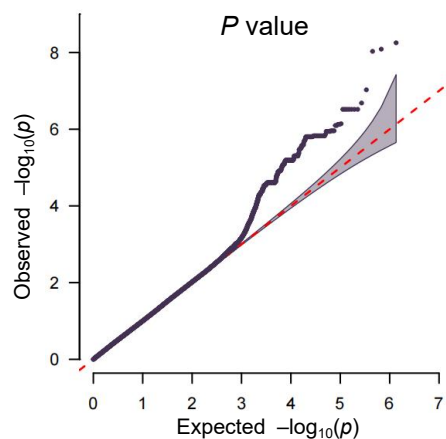
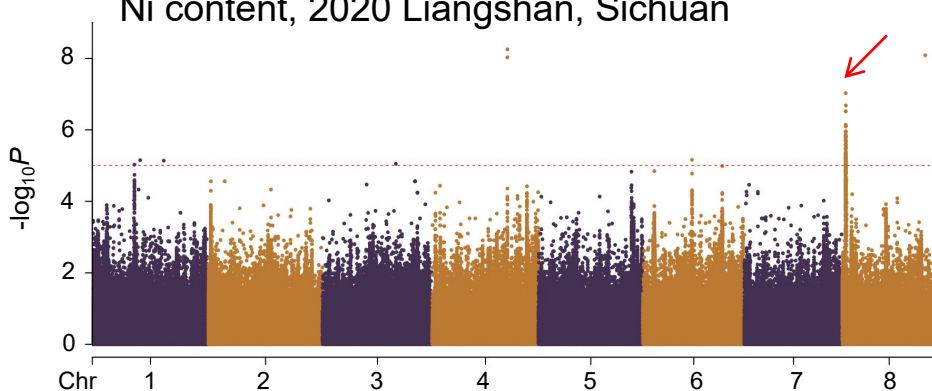
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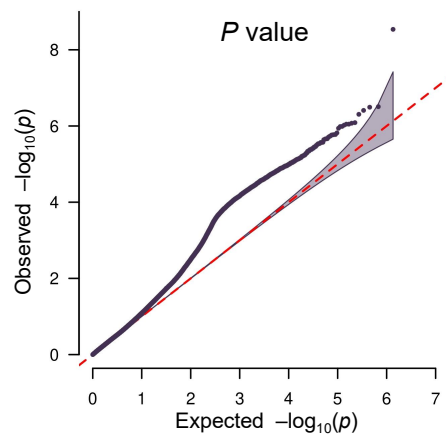
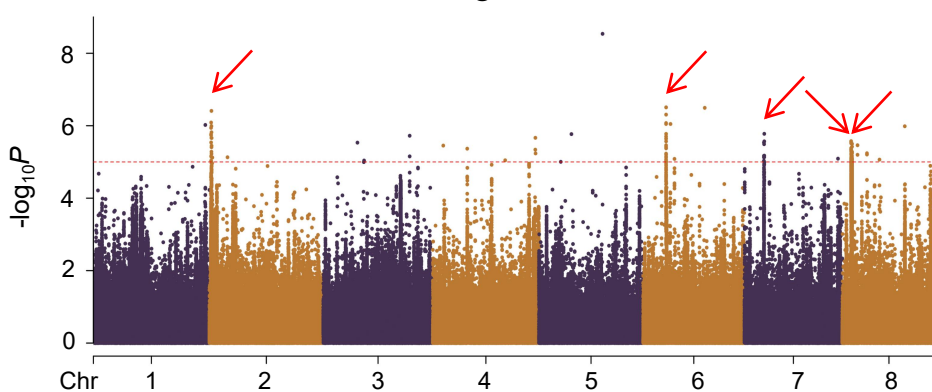
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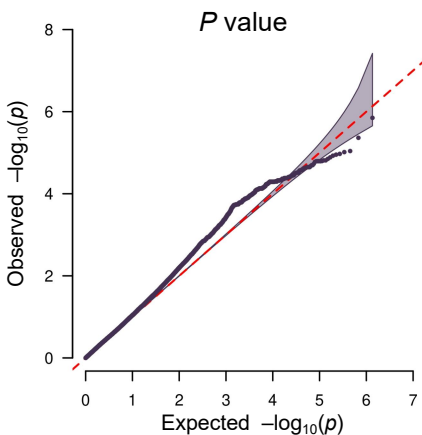
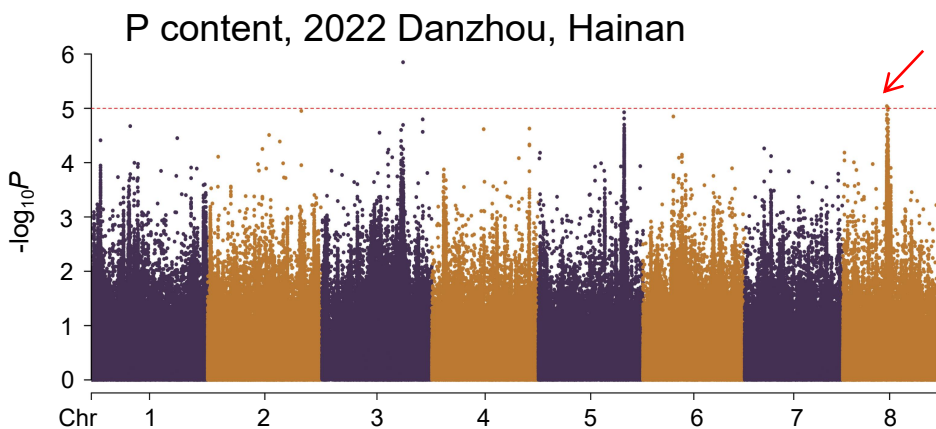
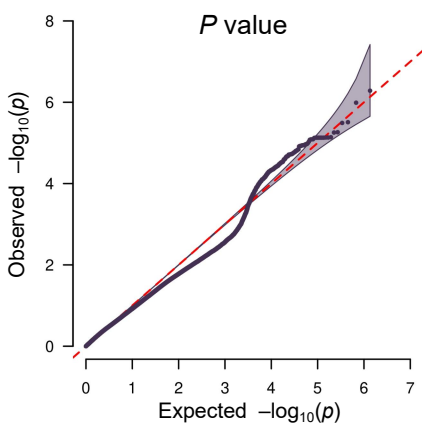
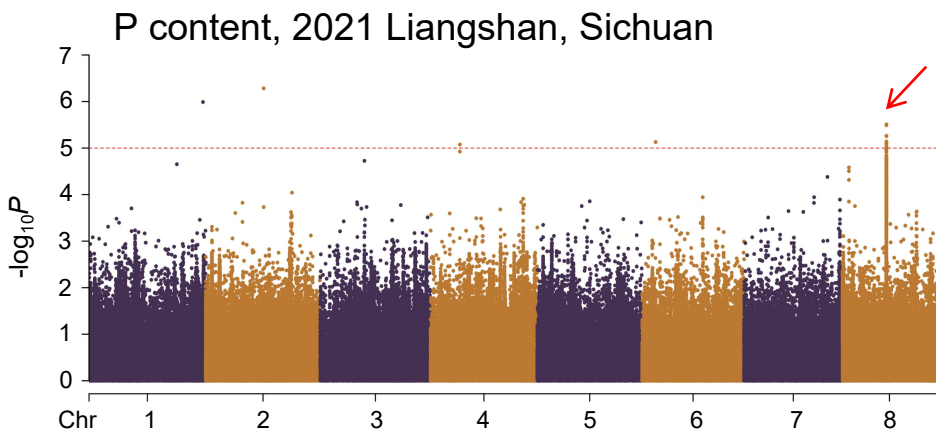
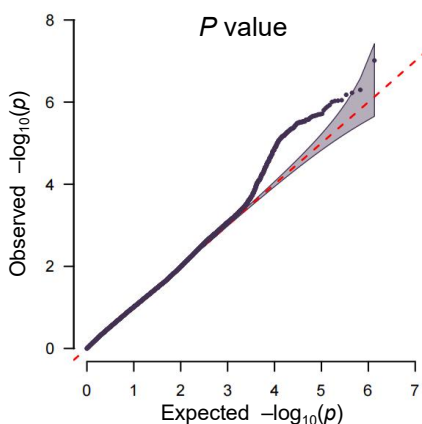
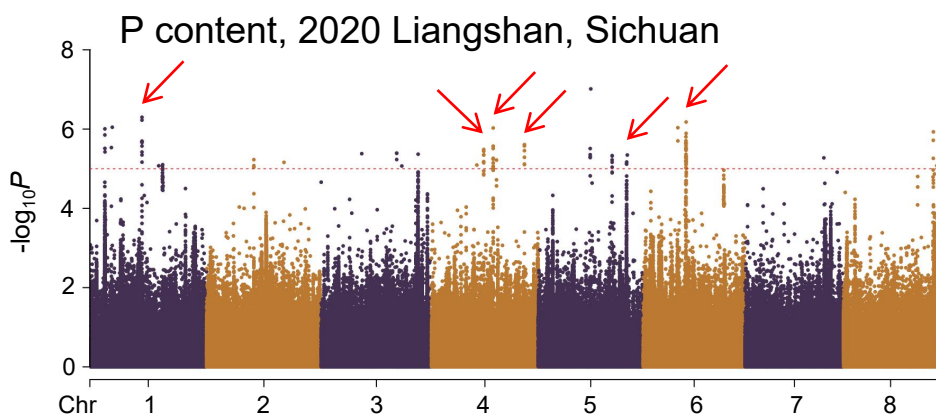
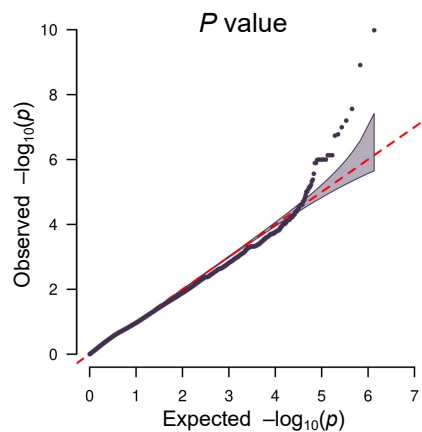
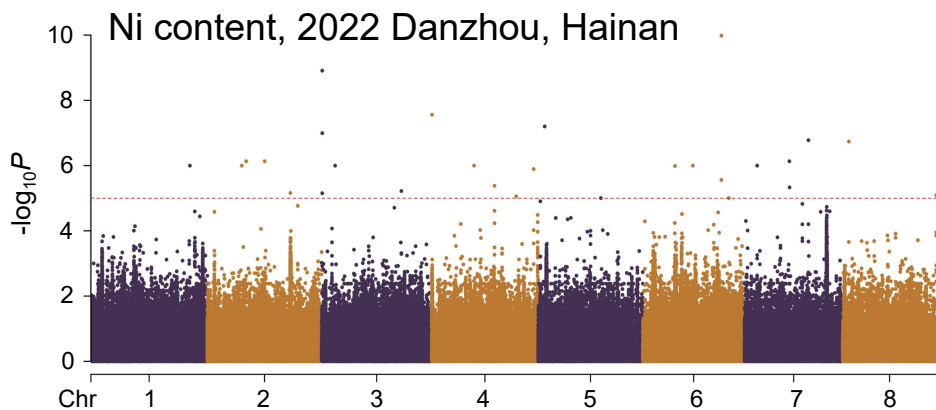


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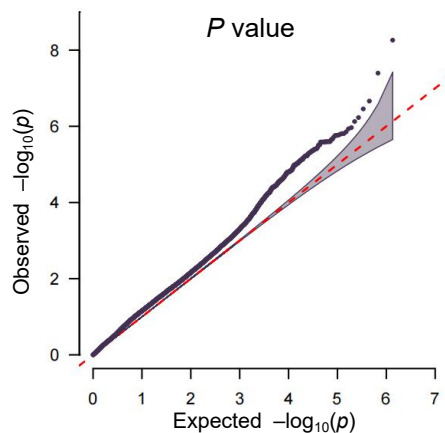
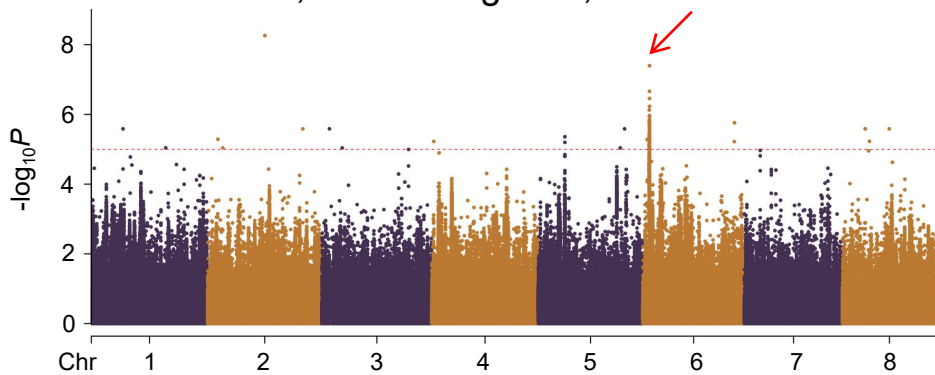


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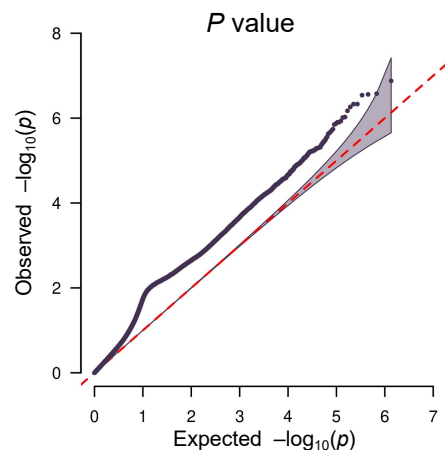
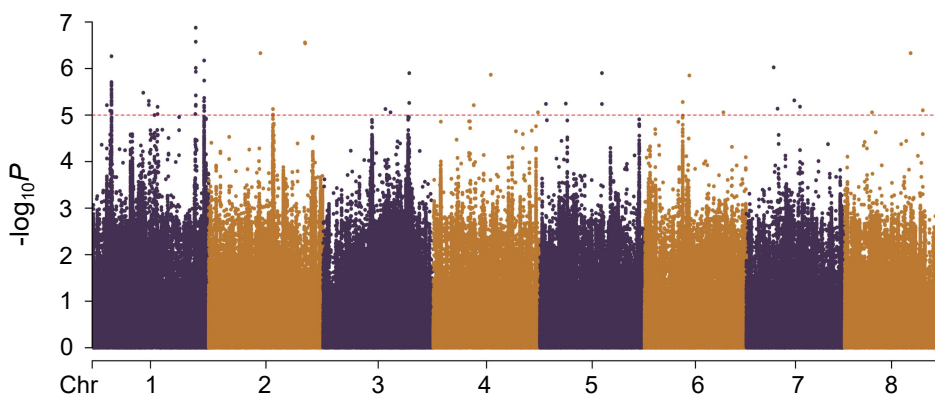




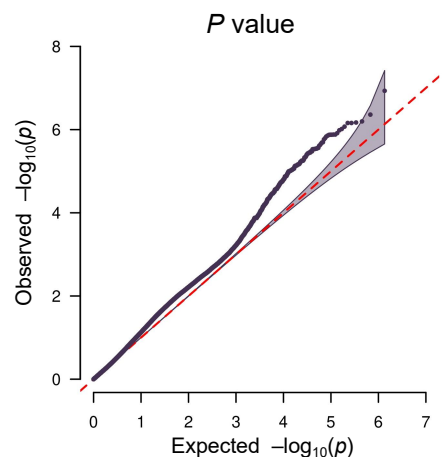
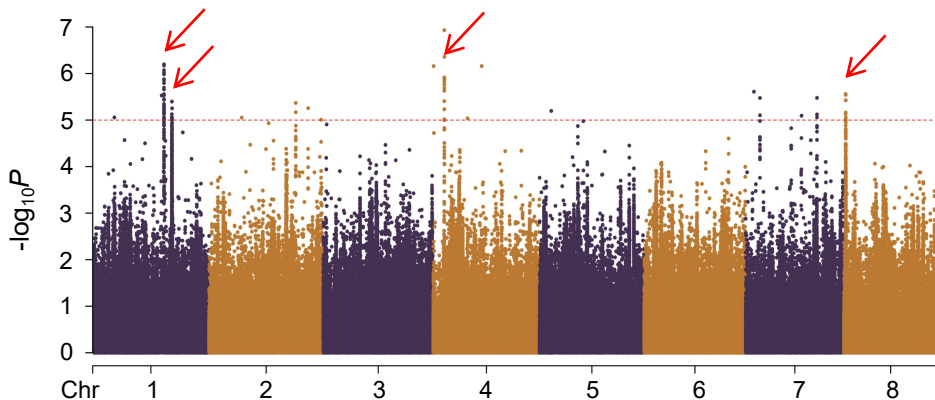
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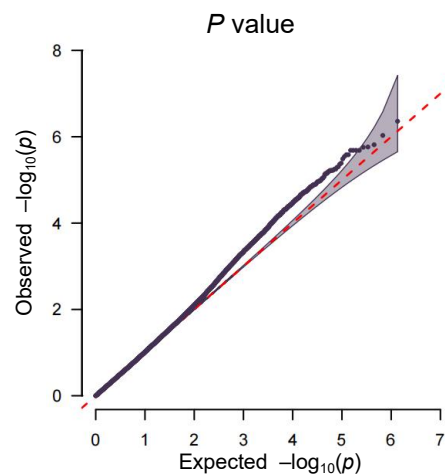
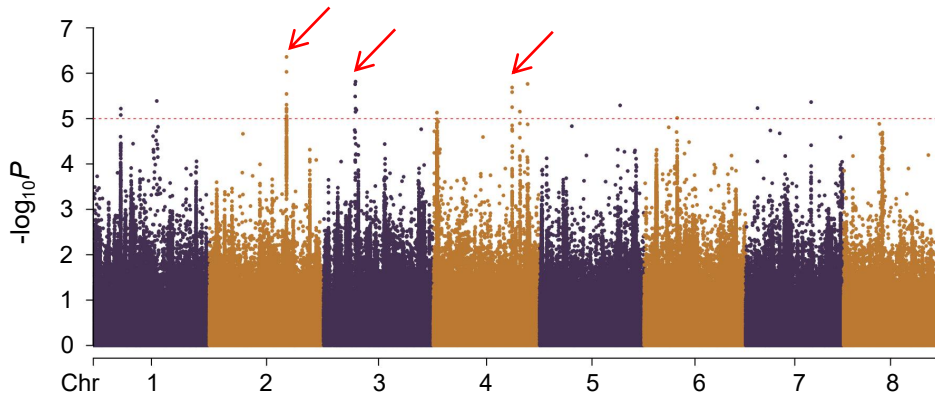
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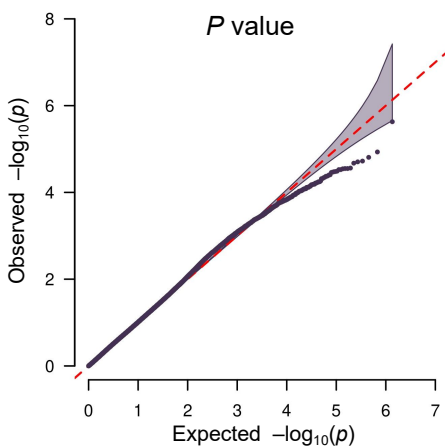
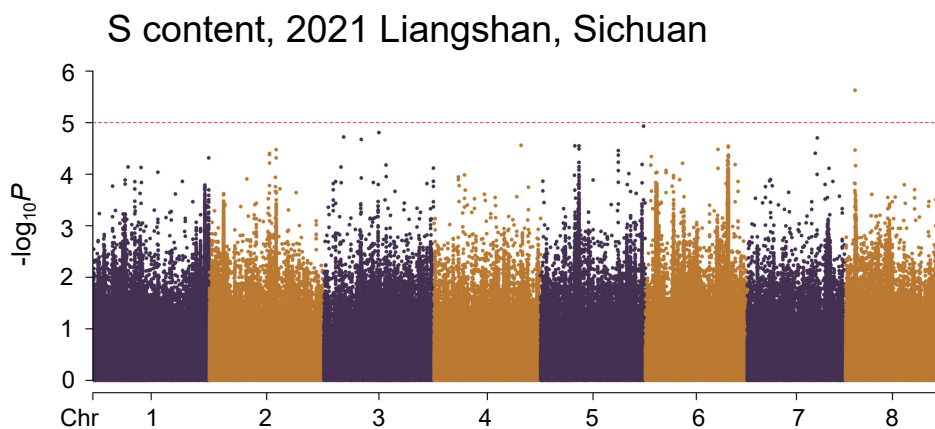
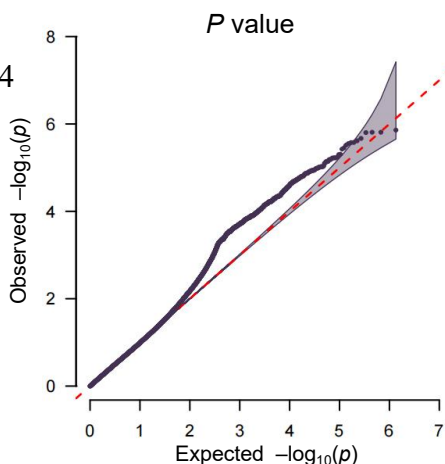
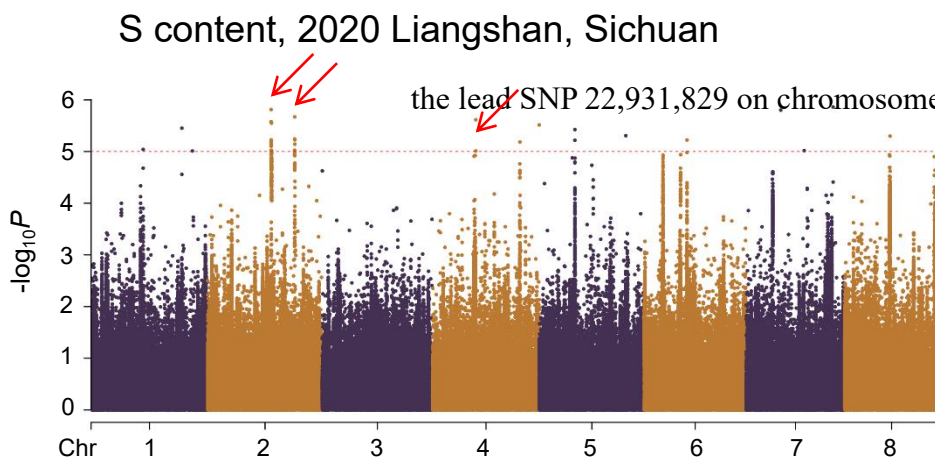
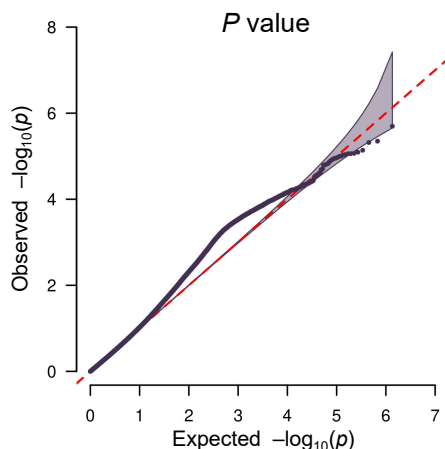
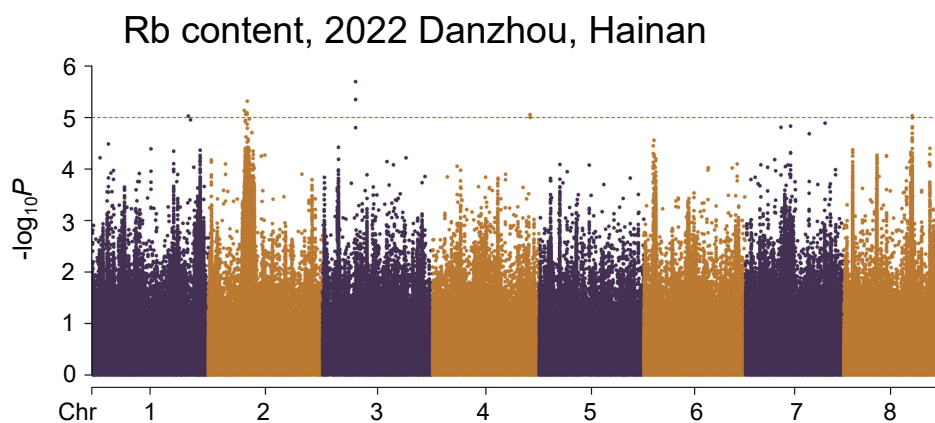
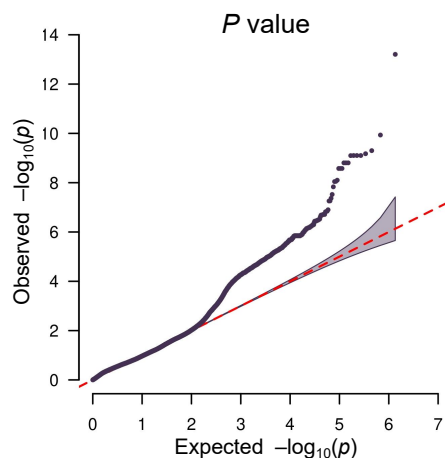
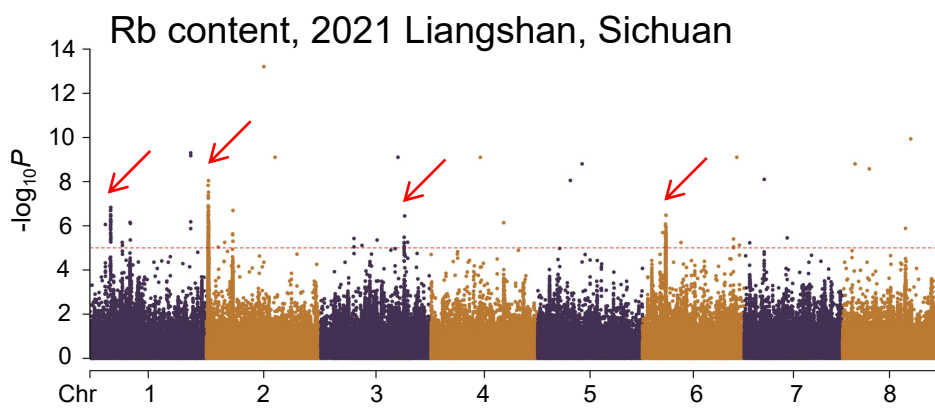


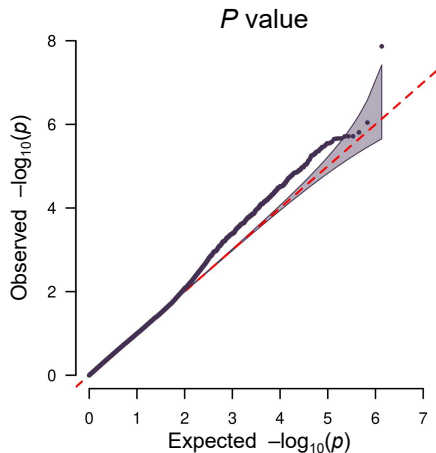
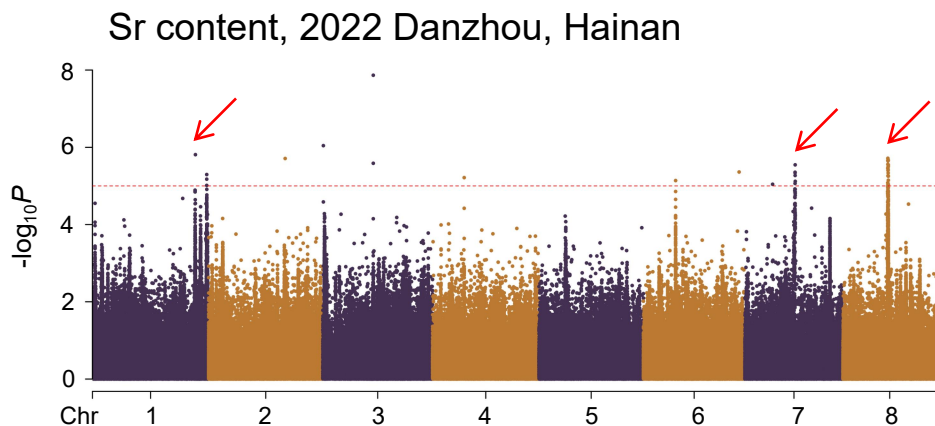
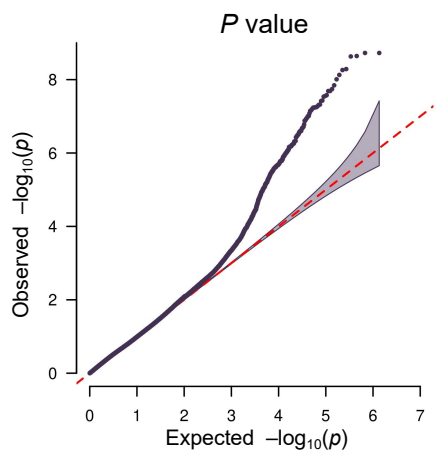
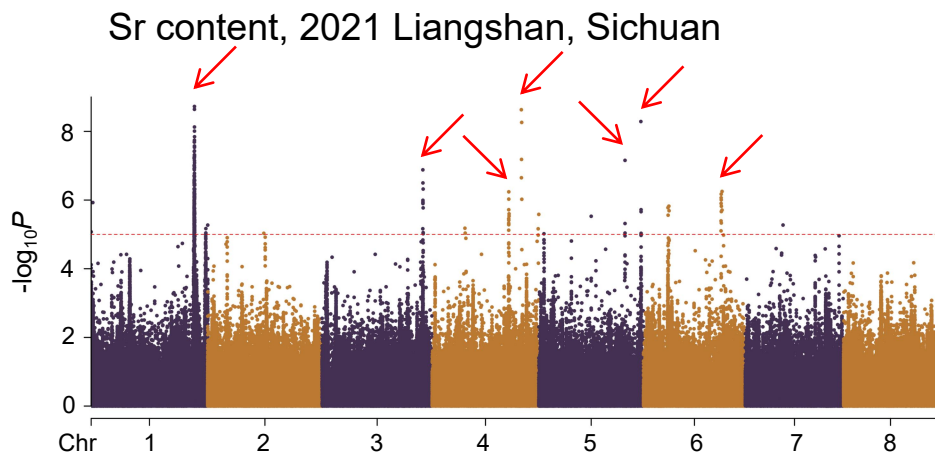
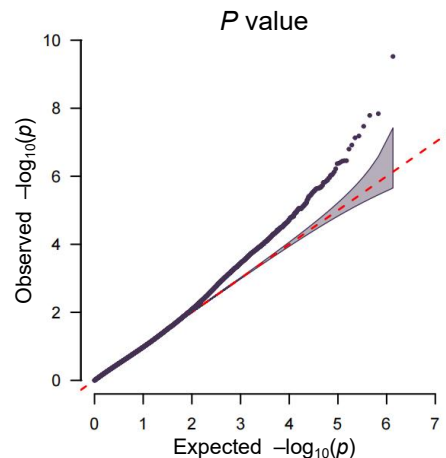
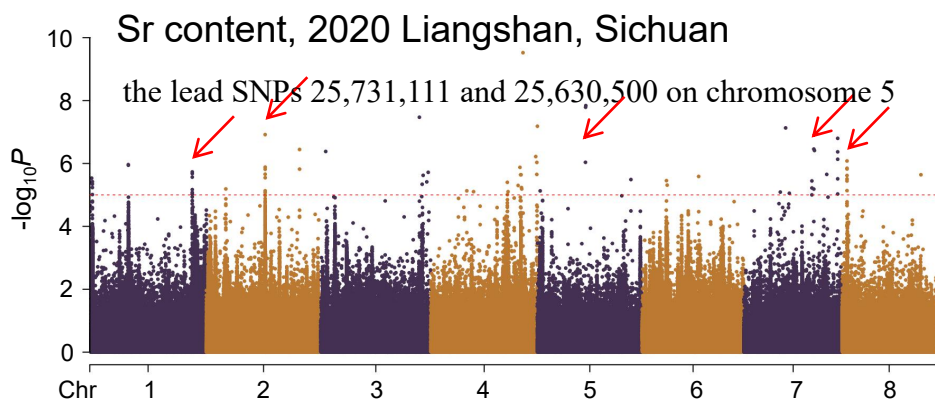
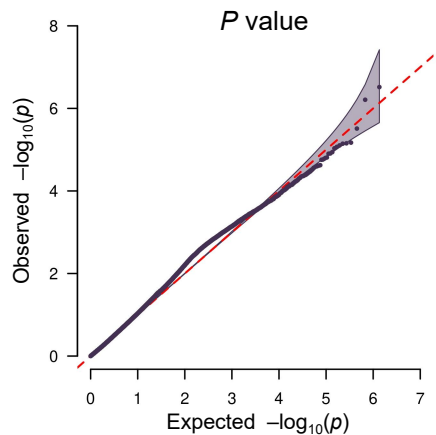
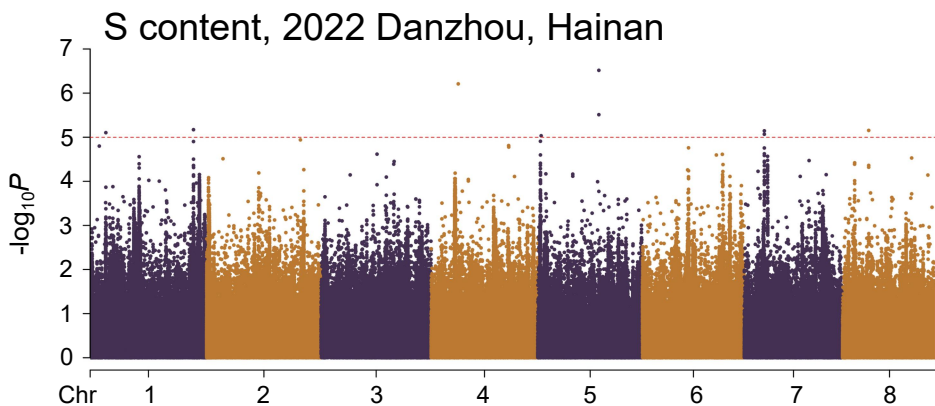
Pb content, 2022 Danzhou, Hainan



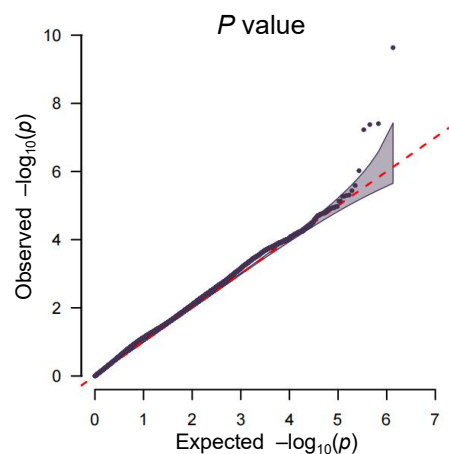
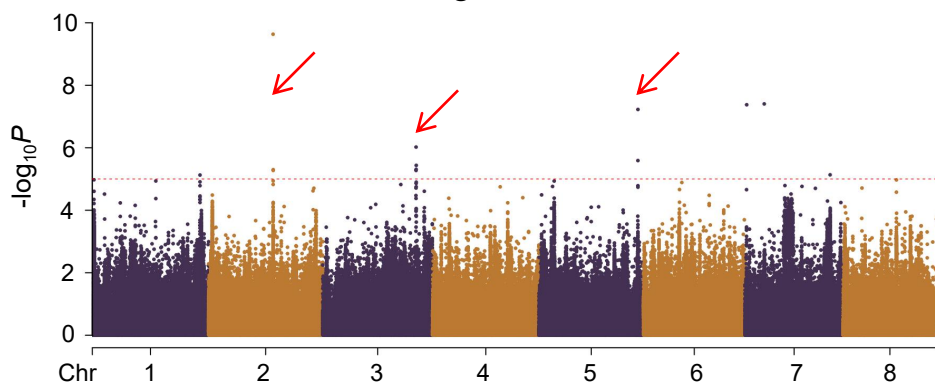
Rb content, 2020 Liangshan, Sichuan



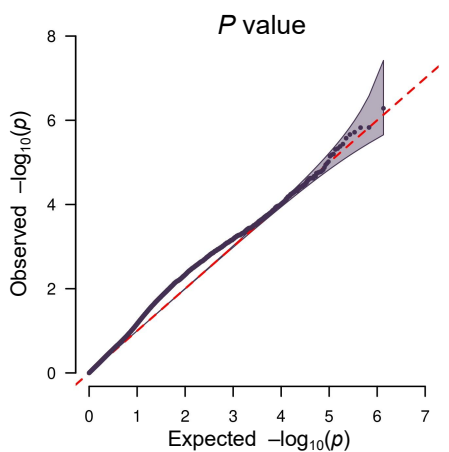
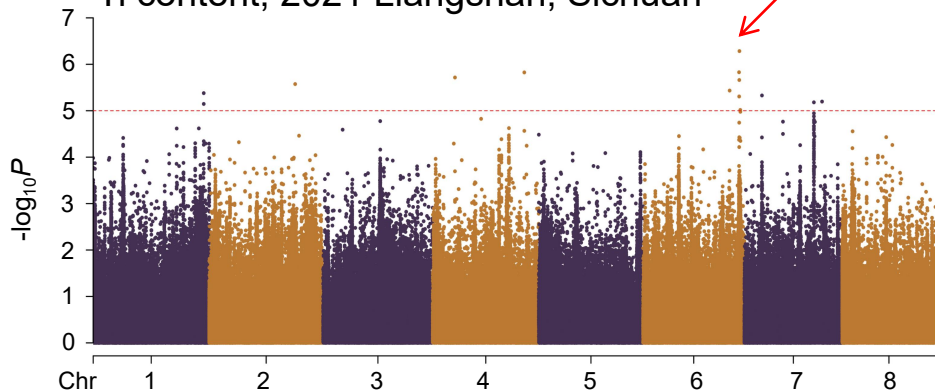




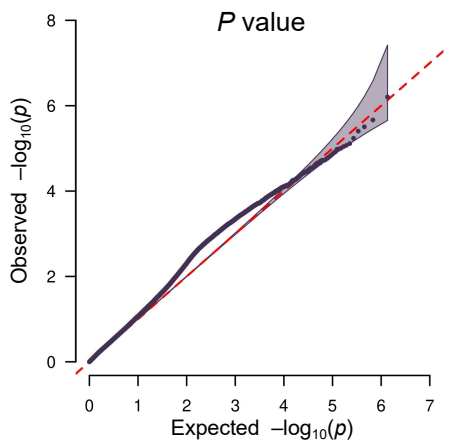
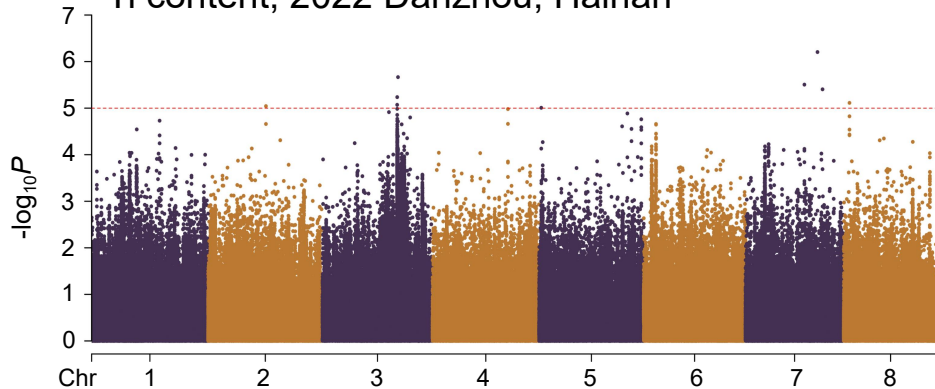
Ti content, 2020 Liangshan, Sichuan



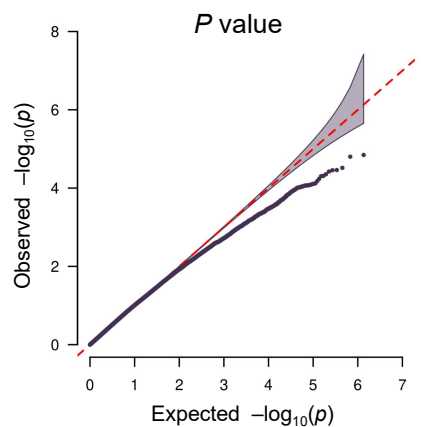
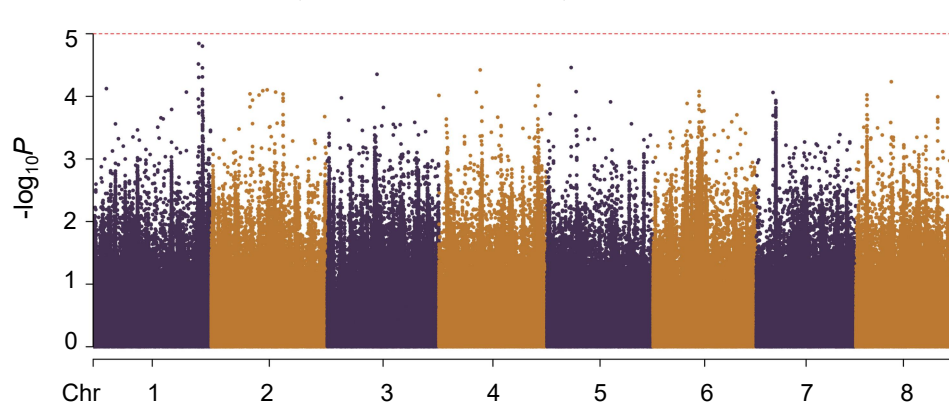
Ti content, 2021 Liangshan, Sichuan



Ti content, 2022 Danzhou, Hainan



Na content, 2022 Danzhou, Hainan



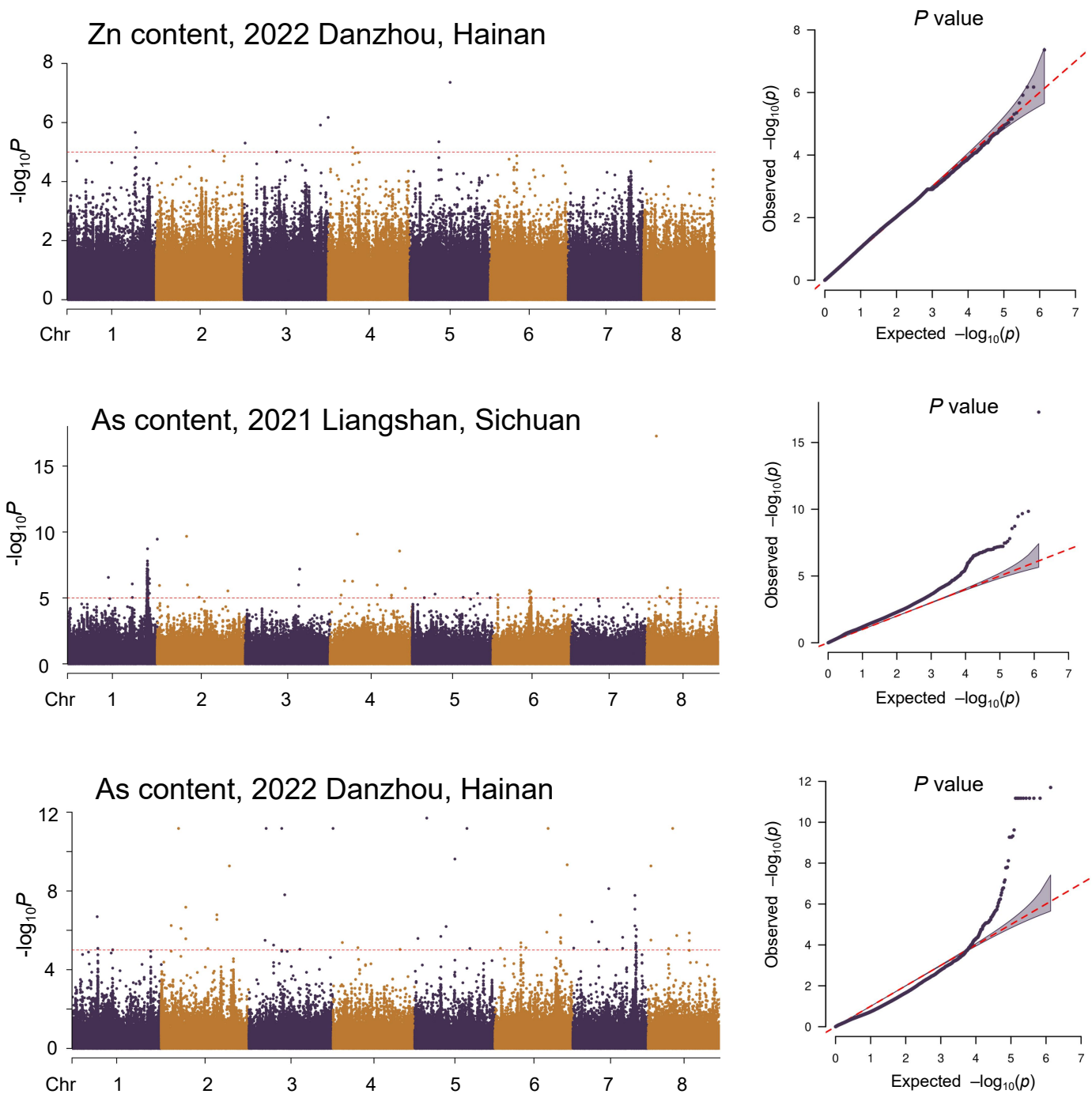


Figure S4. Manhattan and QQ plots of GWAS results of 20 ion elements.

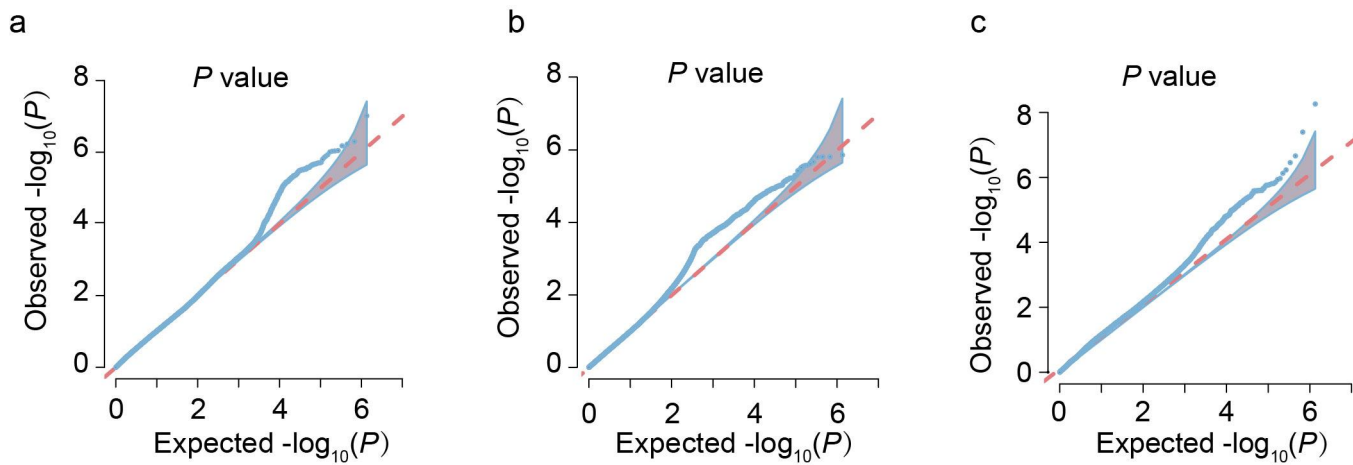


Figure S5. QQ plots of GWAS results of P (a), S (b) and Pb (c) ion elements.

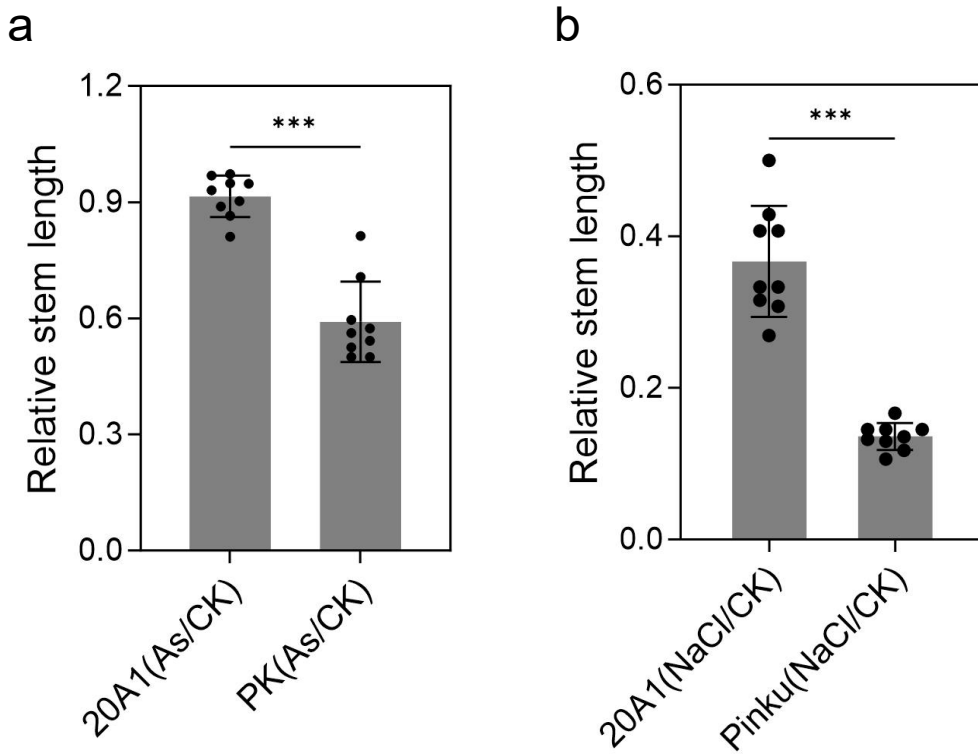


Figure S6. a. The relative stem length of 20A1 and Pinku under control and 0.6 $\mu\text{g/ml}$ As conditions. $n=9$. b. The relative stem length of 20A1 and Pinku under control and 150 mM NaCl conditions. $n=9$. Data is presented as mean $\pm$ SD. Significant differences are indicated by asterisks: *** $P < 0.001$, two-tailed t -test.

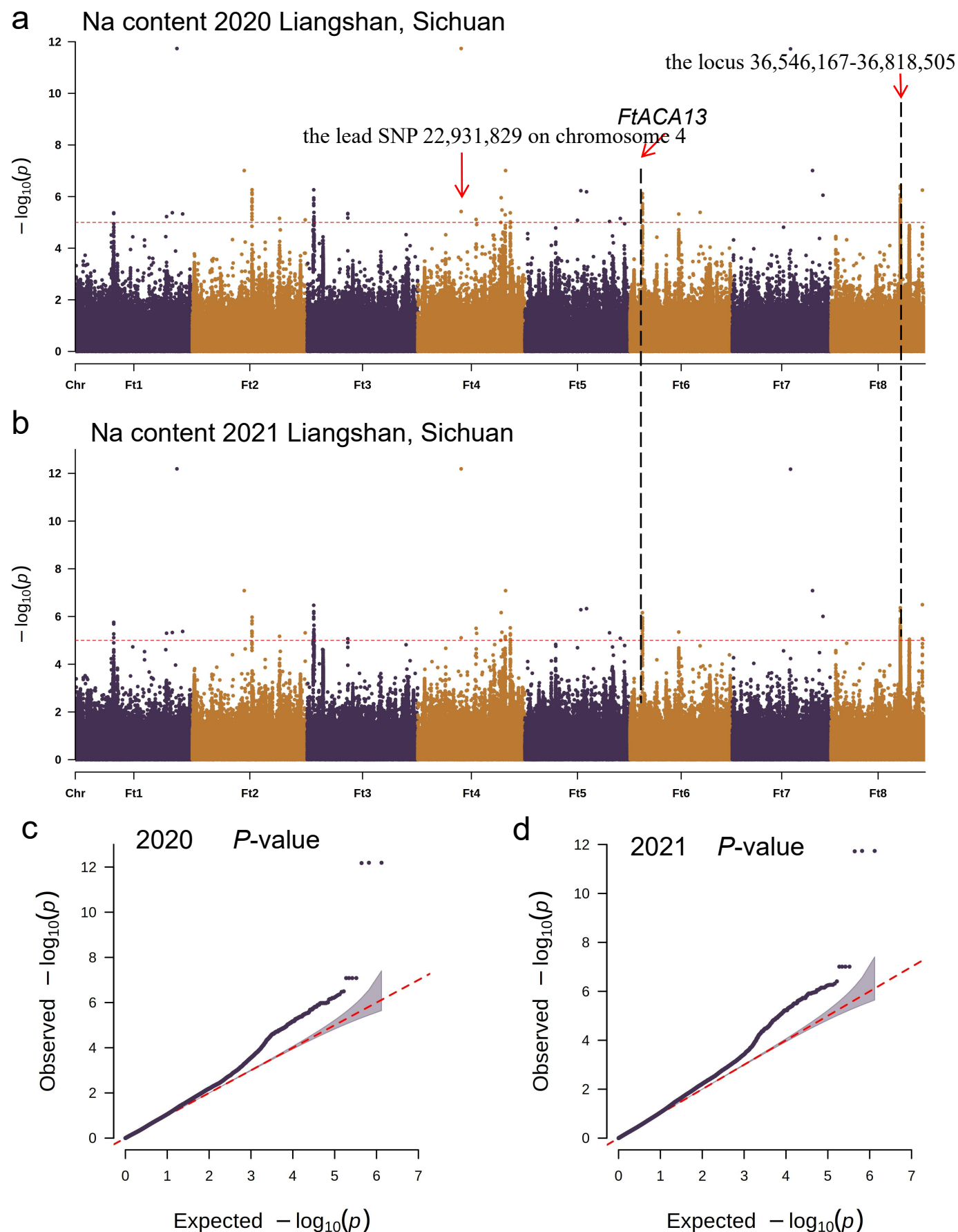


Figure S7. Manhattan and QQ plots of GWAS results of Na content of Tartary buckwheat.

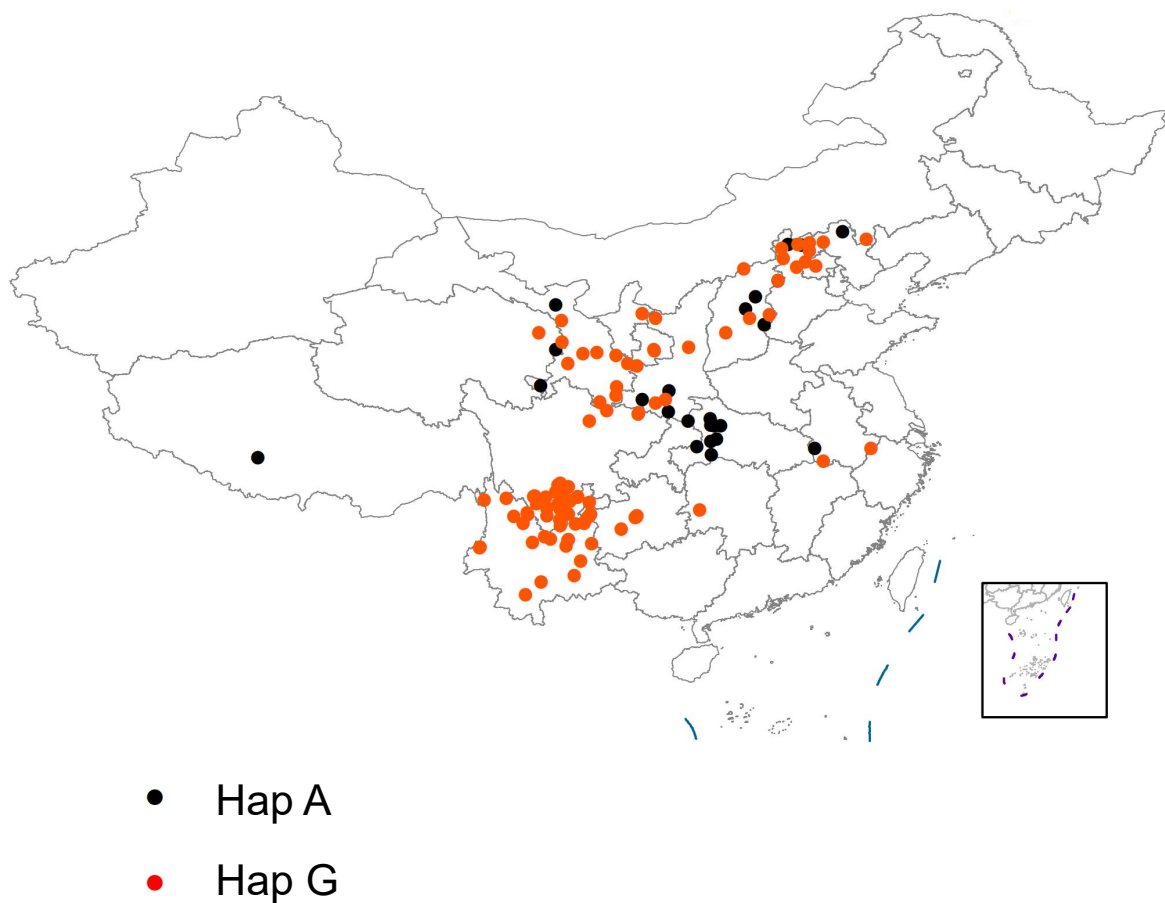


Figure S8. Geographic distribution of the Hap G (red) and Hap A (black) located in the promoter of *FtACA13* in Tartary buckwheat accessions.

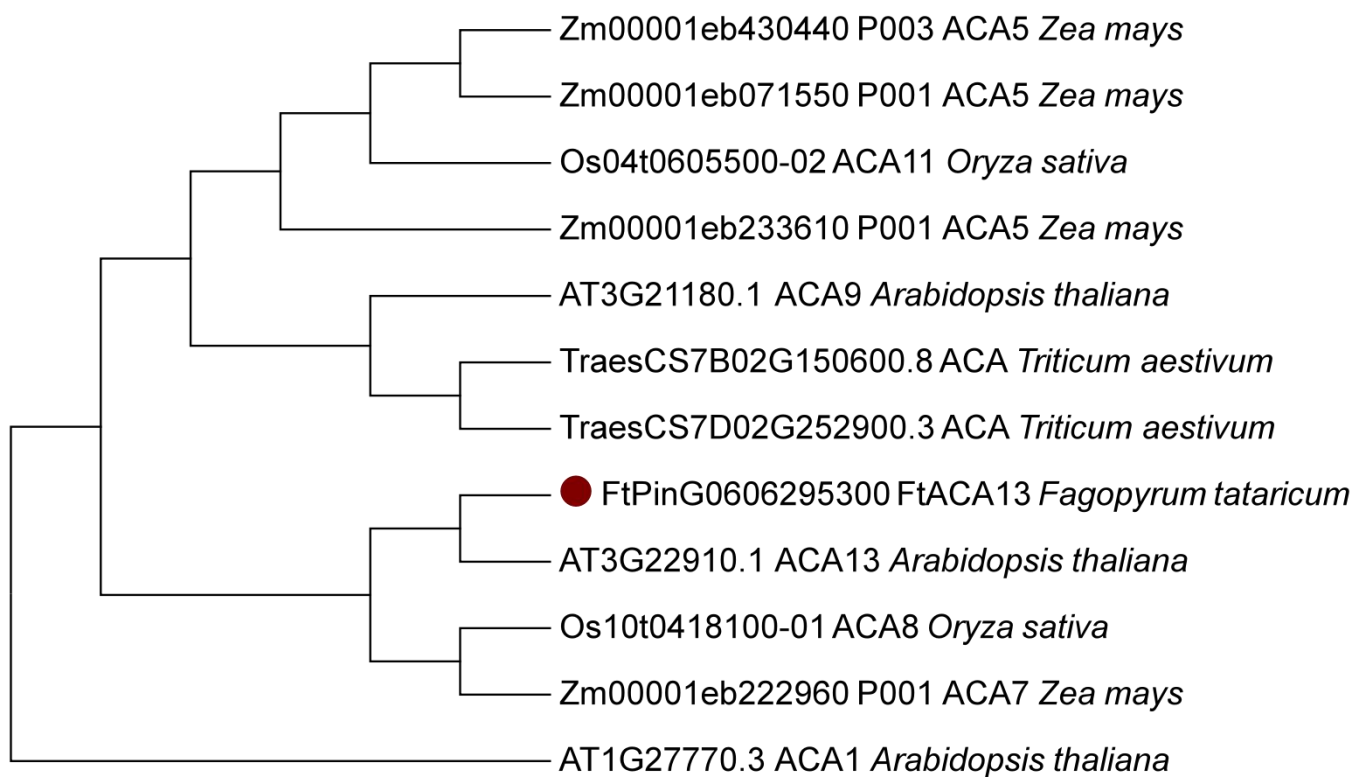


Figure S9. FtACA13 phylogenetic tree based on the neighbor joining method tree using full-length amino acid sequences of orthologues genes in Tartary buckwheat and other plants.

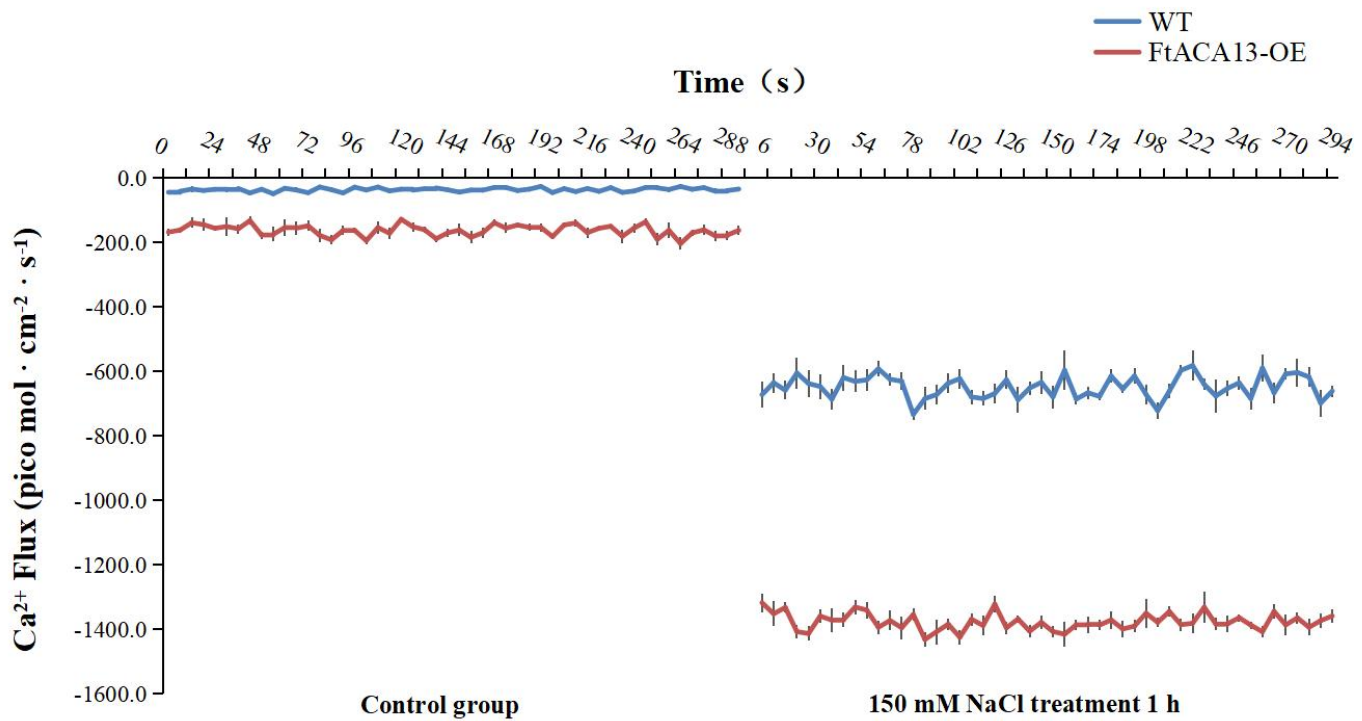


Figure S10. Comparison of extracellular calcium flux in living roots of *FtACA13* overexpression hairy roots and wild type. The control group indicates no treatment. The error bars indicate mean $\pm$ SD from six biological replicates.

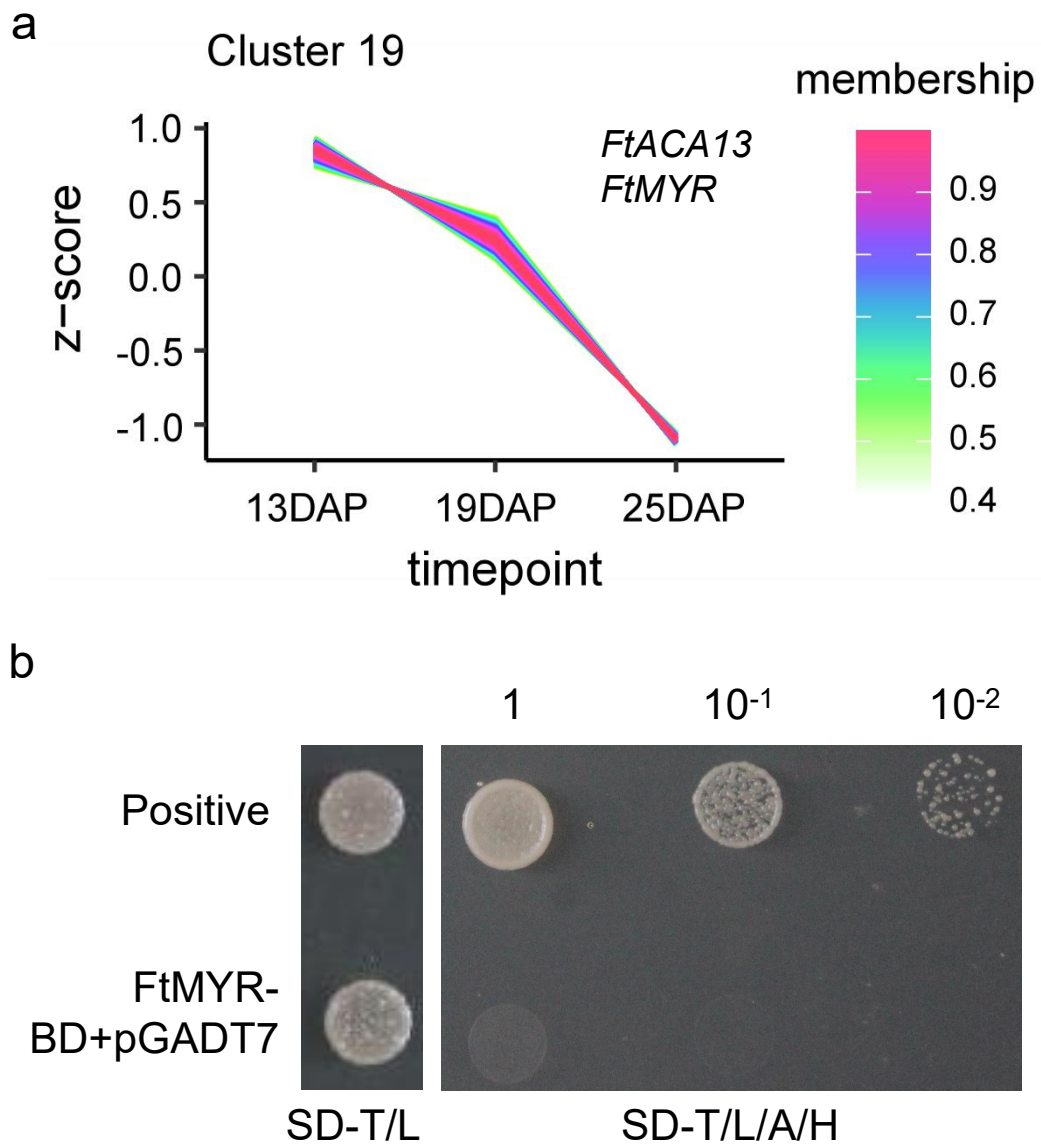


Figure S11. a. Analysis of the co-expression patterns of *FtACA13* and *FtMYR* transcription factors in the transcriptome of buckwheat seed development. b. Transcriptional activation assay of FtMYR protein in yeast. pBD-53 was used as a positive control.

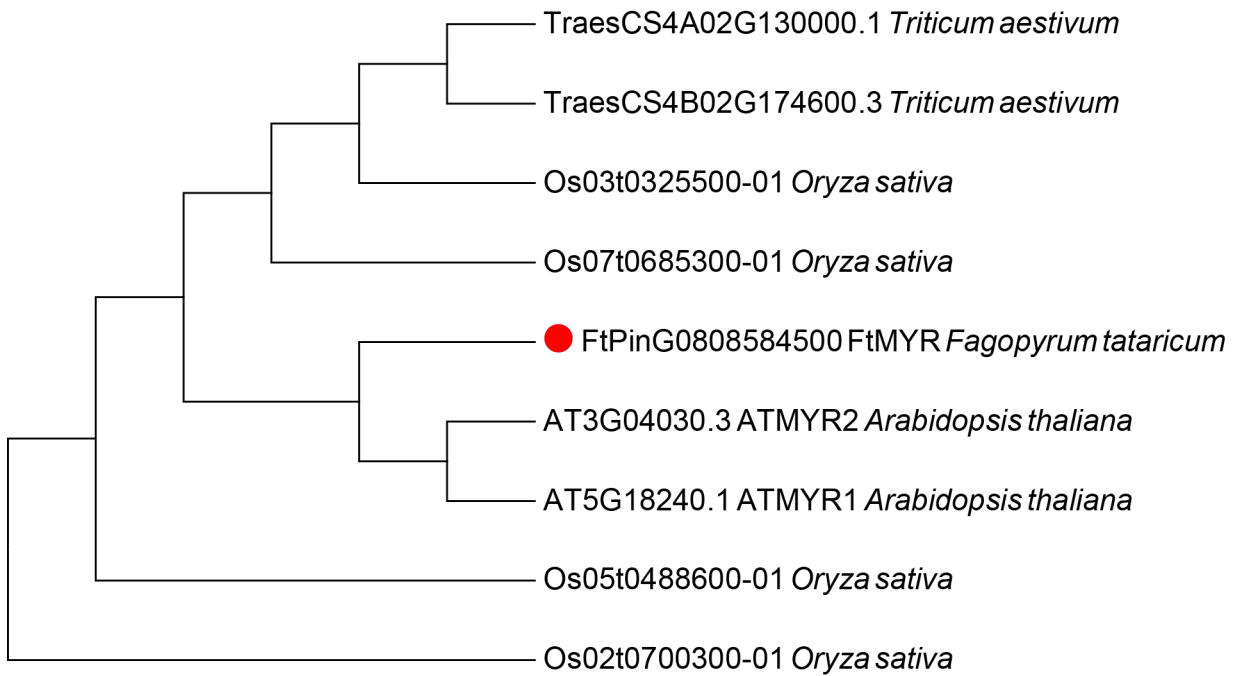


Figure S12. FtMYR phylogenetic tree based on the neighbor joining method tree using full-length amino acid sequences of orthologues genes in Tartary buckwheat and other plants.

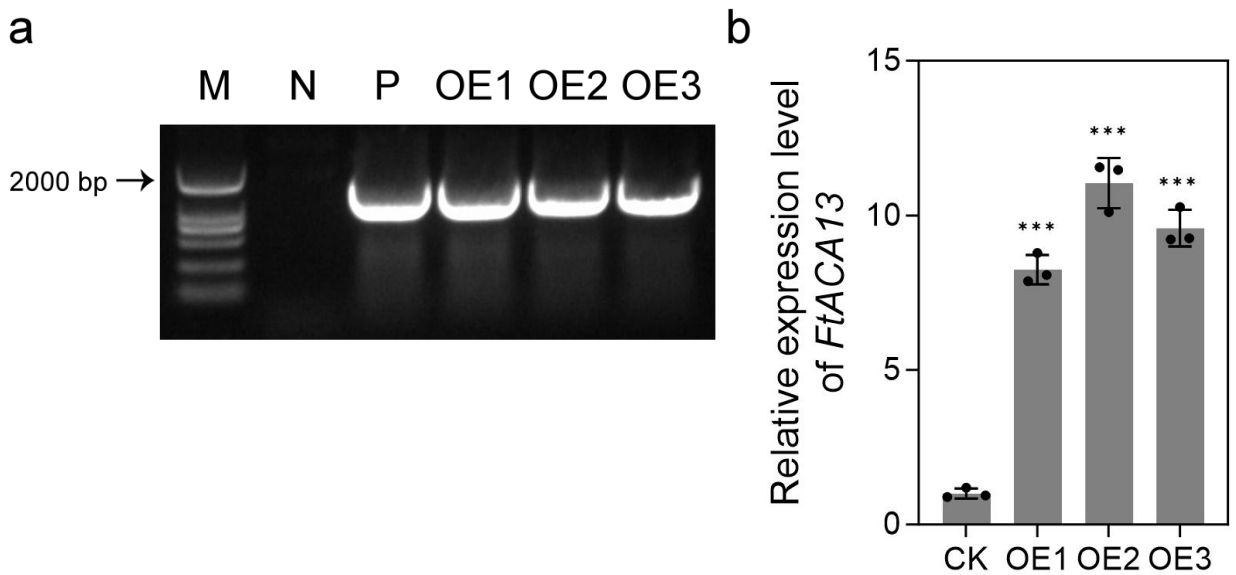


Figure S13. PCR (a) and RT-qPCR (b) analysis of hairy root lines expressing *FtACA13*. The hairy roots from wild type were used as negative control. N: negative control; P: positive control; M: DNA marker. The error bars indicate mean $\pm$ SD from three biological replicates. *** $P < 0.001$, two-tailed t -test.

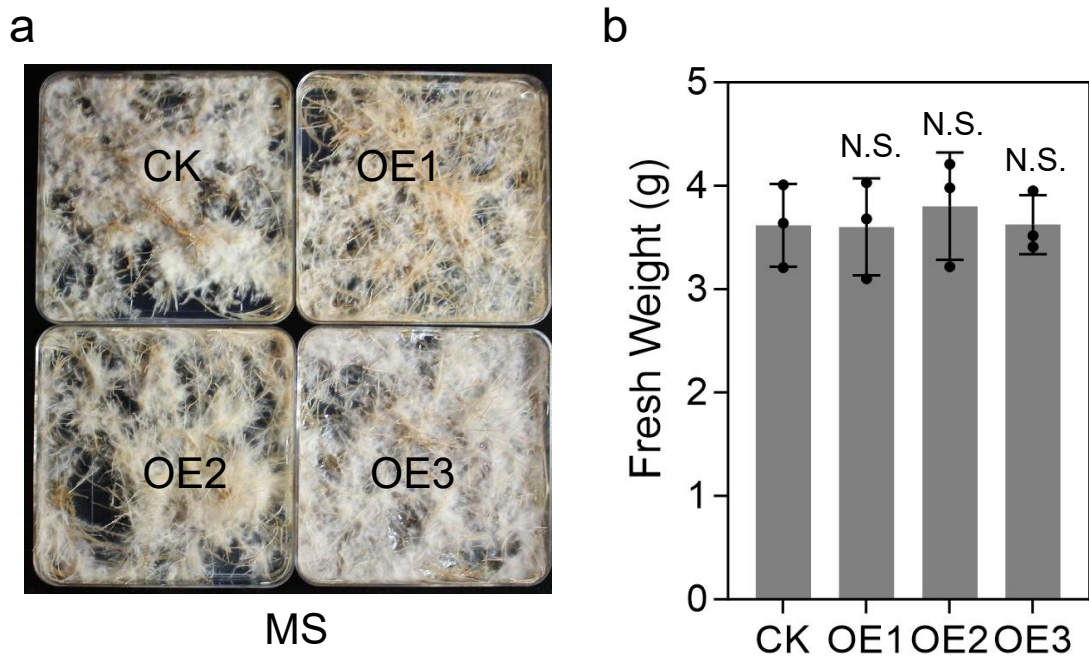


Figure S14. a. The hairy root phenotype grown on MS medium for 3 weeks. b. The fresh weight of different genotype hairy roots. The error bars indicate mean $\pm$ SD from three biological replicates. N.S. no significance, two-tailed *t*-test.

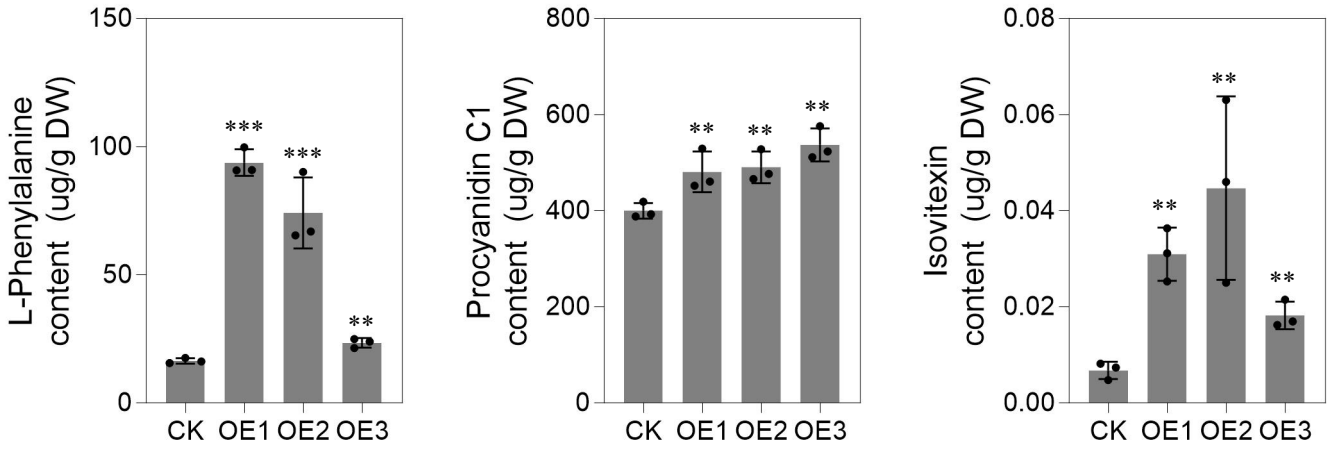


Figure S15. L-phenylalanine, procyanidin, and isovitexin contents in different genotype hairy roots. The error bars indicate mean $\pm$ SD from three biological replicates. Asterisk (***) and (**) indicate significant difference at $P < 0.001$ and $P < 0.01$ using two-tailed t -test.

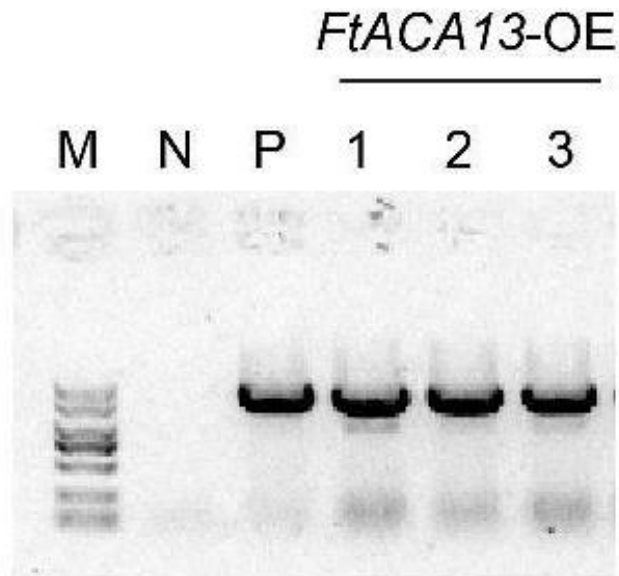


Figure S16. PCR analysis of *Arabidopsis* lines heterologously expressing *FtACA13*. The leaves from wild type (Col-0) were used as negative control. N: negative control; P: positive control; M: DNA marker.

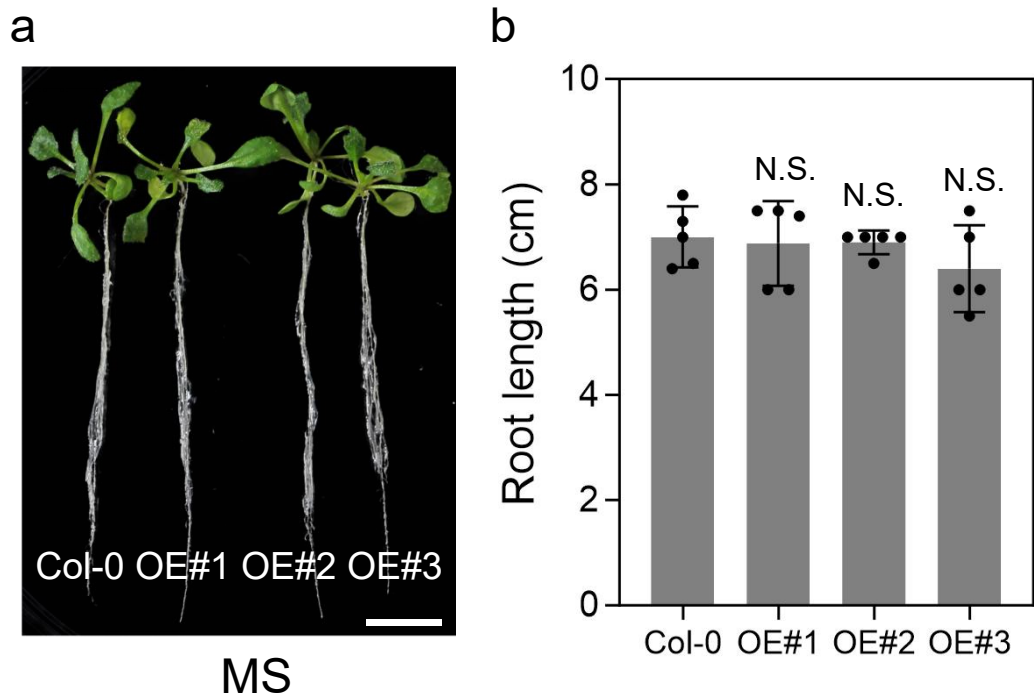
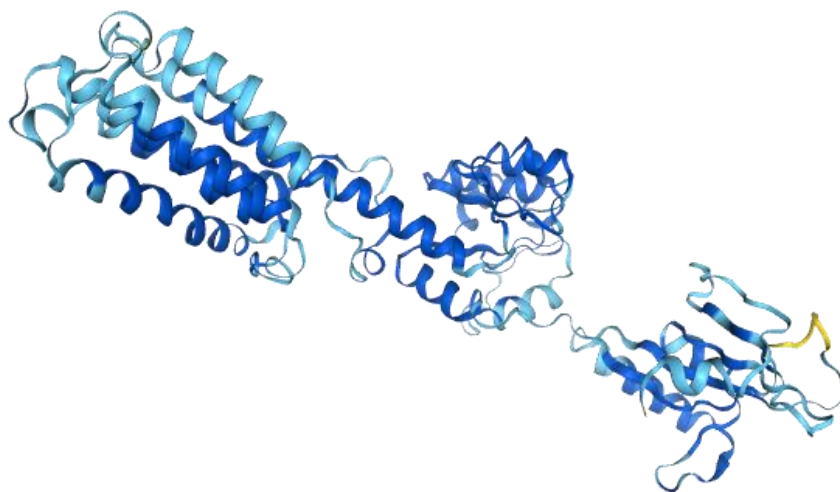


Figure S17. a. The phenotype of different genotype *Arabidopsis* lines grown on MS medium for 3 weeks. b. The root length of *Arabidopsis* lines. The error bars indicate mean $\pm$ SD from five biological replicates. N.S. no significance, two-tailed *t*-test.

a



Very high (pLDDT > 90)

Confident (90 > pLDDT > 70)

low (70 > pLDDT > 50)

Very low (pLDDT < 50)

b

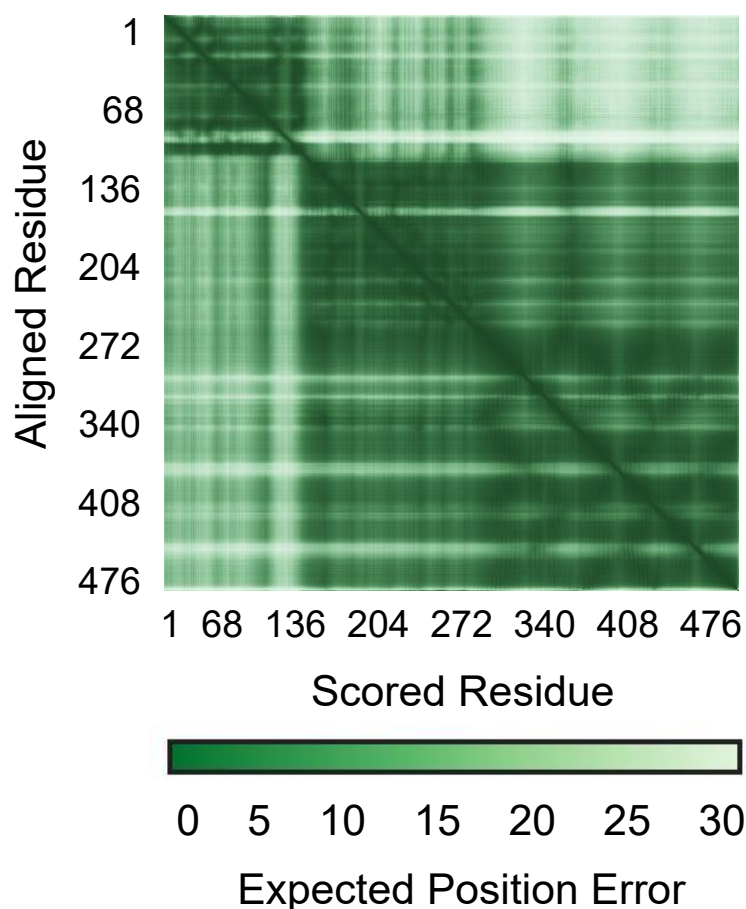


Figure S18. a. Predicted structure of FtACA13 coloured by pLDDT (orange: 0-50, yellow: 50-70, cyan 70-90, and blue 90-100). b. Predicted Aligned Error (PAE) matrix of same prediction (darker is more confident).

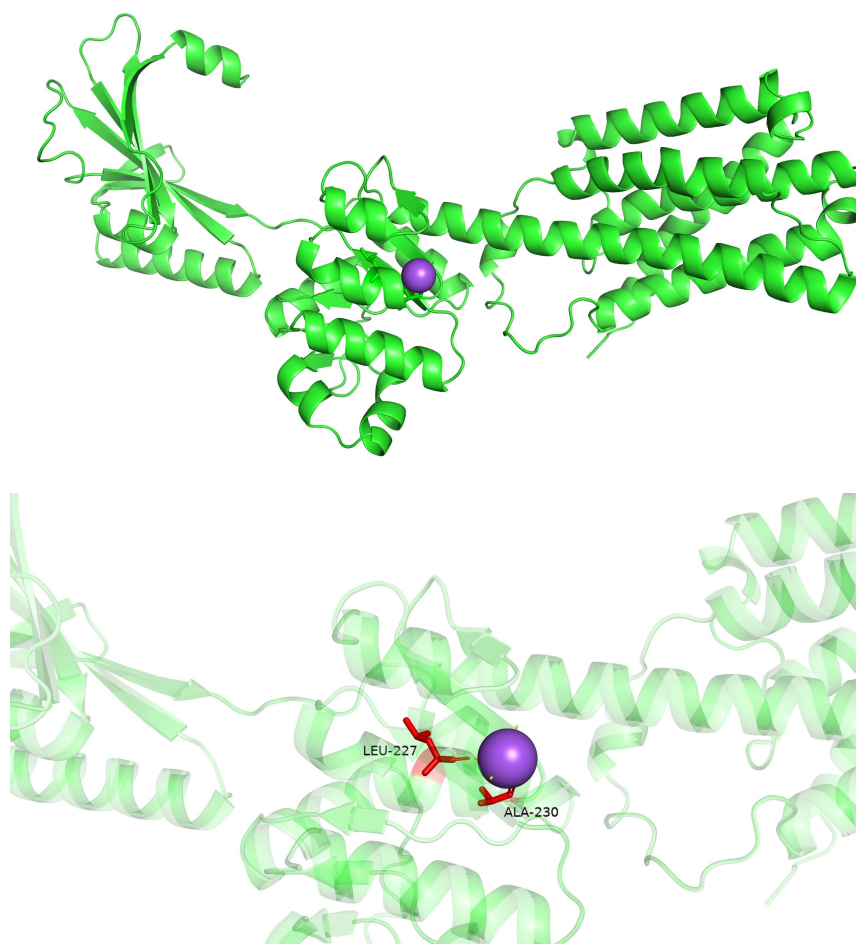


Figure S19. AlphaFold 3 predictions of FtACA13 protein interaction with sodium ion.

		*	20	*	40	*		
ACA13_pro_20A1	:	TTTAGCTTAATATGACATCATGCGAGCGAAAGTTATTTCA					AAAAATAATA	: 50
ACA13_pro_Pinku	:	TTTAGCTTAATATGACATCATGCGAGCGAAAGTTATTC					AAAAATAATA	: 48
		TTTAGCTTAATATGACATCATGCGAGCGAAAGTTATT					AAAAATAATA	
		60	*	80	*	100		
ACA13_pro_20A1	:	TGGCTAATTCATGTTGAATGATTTTGTATATCGTATACAGC					CTCAAGCTTA	: 100
ACA13_pro_Pinku	:	TGGCTAATTCATGTTGAATGATTTTGTATATCGTATACAG					CTCAAGCTTA	: 98
		TGGCTAATTCATGTTGAATGATTTTGTATATCGTATACAG					CTCAAGCTTA	
		120	*	140	*			
ACA13_pro_20A1	:	AAGAGTTTTGTTTGAAAAAGTTTGTGTTTCATAAGTTTGTACAATTGCA						: 150
ACA13_pro_Pinku	:	AAGAGTTTTGTTTGAAAAAGTTTGTGTTTCATAAGTTTGTACAATTGCA						: 147
		AAGAGTTTTGTTTGAAAAAGTTTGTGTTTCATAAGTTTGTACAATTGCA						
		160	*	180	*	200		
ACA13_pro_20A1	:	CACTTTTGCCTTTATTTATTACTCTTTCAGTTTCTC					CATAGATATTTT	: 200
ACA13_pro_Pinku	:	CACTTTTGCCTTTATTTATTACTCTTTCAGTTTCTC					CATAGATATTTT	: 197
		CACTTTTGCCTTTATTTATTACTCTTTCAGTTTCTC					CATAGATATTTT	
		220	*	240	*			
ACA13_pro_20A1	:	GAGTAAAAAATACACATCTTAAGAAATTTAAATATAGAAAAATATAATAT						: 250
ACA13_pro_Pinku	:	GAGTAAAAAATACACATCTTAAGAAATTTAAA-GTAAAAAATATAATAT						: 246
		GAGTAAAAAATACACATCTTAAGAAATTTAAA					TA AAAAAATATAATAT	
		260	*	280	*	300		
ACA13_pro_20A1	:	TTAATGATGTATAATAATTTTAAAAAGTGTAATAAAAAATGGTAGAGTGTC						: 300
ACA13_pro_Pinku	:	TTAATGATGCATAATAA-----						: 263
		TTAATGATG ATAATAA						
		320	*	340	*			
ACA13_pro_20A1	:	GAAAATTGTATGAGAAAATGAAATATTGCTTTGAAAATACAAACAATAAA						: 350
ACA13_pro_Pinku	:	-----						: -
		360	*	380	*	400		
ACA13_pro_20A1	:	TAAAAAGGTAGTTACTATTATATTTATAGAAAGTTTGAGTATTAATAACA						: 400
ACA13_pro_Pinku	:	-----					A	: 264
		-----					A	
		420	*	440	*			
ACA13_pro_20A1	:	CTTATAAGTCTAAATATTATGAAATAAAATTTTAGAGTTCAAATAACAAA						: 450
ACA13_pro_Pinku	:	TCCATAAGGTCAAATATTATGAAAT-AAATTTTAGAGTCAAATGACAAA						: 313
		ATAAG AAATATTATGAAAT AAATTTTAGAG TCAAAT ACAA						
		460	*	480	*	500		
ACA13_pro_20A1	:	TATTTAAAAACAGATGAGTAATAAAAAGGGCTTACATCATTTAATAAAAA						: 500
ACA13_pro_Pinku	:	TATTTGAAAACAGATGAGTAATAAAAAGGGCTTACATCATTTAATAAAAA						: 363
		TATTT AAAACAGAT GAGTAATAAAAAGGGCTTACAT ATTTAATAAAAA						

Figure S20. SV in the promoter of *FtACA13* between 20A1 and Pinku.

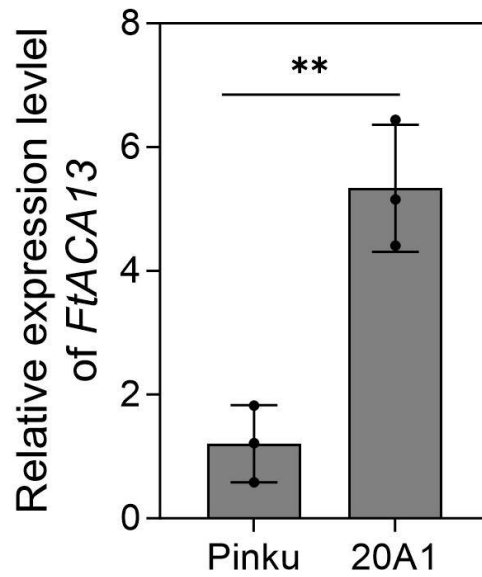


Figure S21. The expression level of *FtACA13* between Pinku and 20A1 Tartary buckwheats. The error bars indicate mean $\pm$ SD from three biological replicates. ** $P < 0.01$, two-tailed t -test.

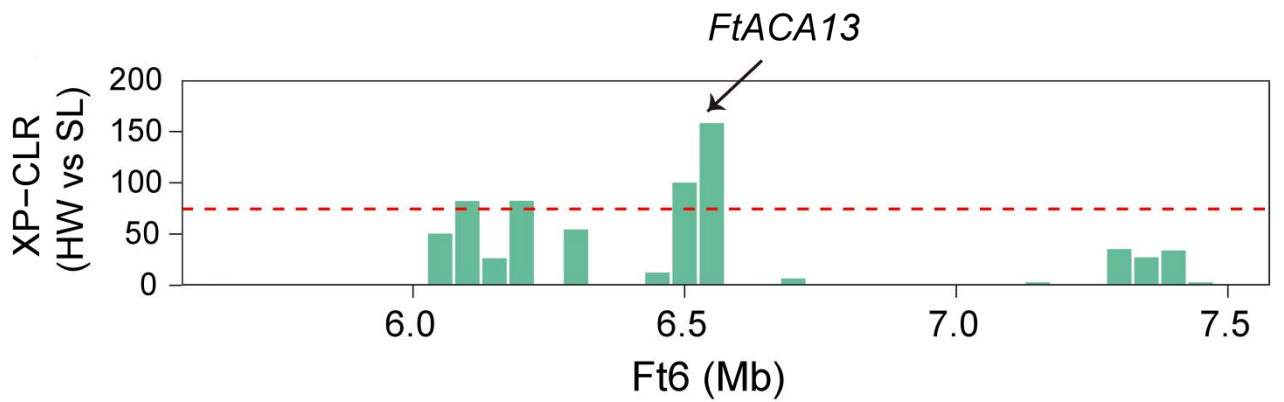


Figure S22. Selective signals identified through comparisons between HW and SL using XP-CLR on the 6 chromosomes. The dashed line represents the top 5% of values therefore scores in these regions were regarded as selective sweeps.

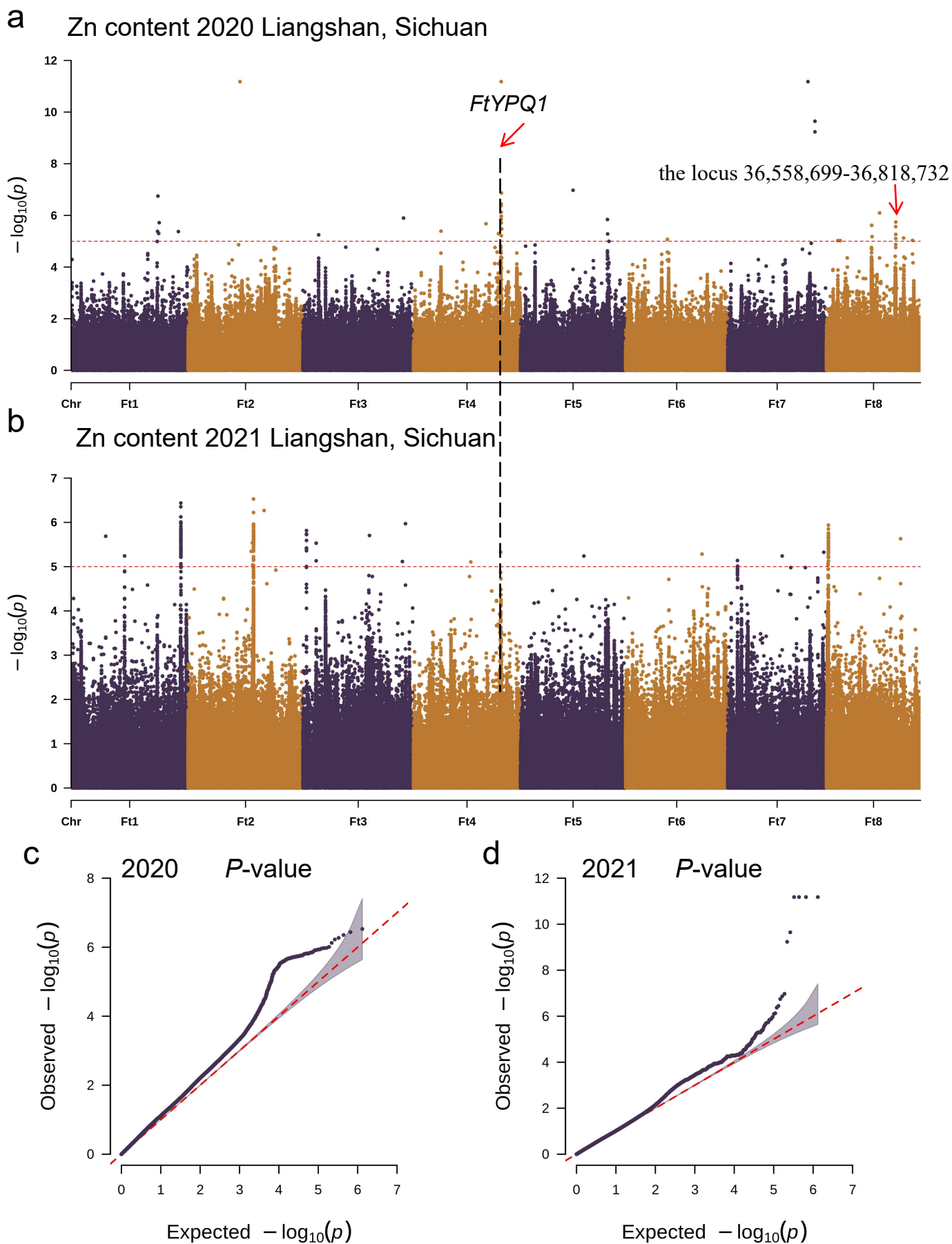


Figure S23. Manhattan and QQ plots of GWAS results of Zn content of Tartary buckwheat.

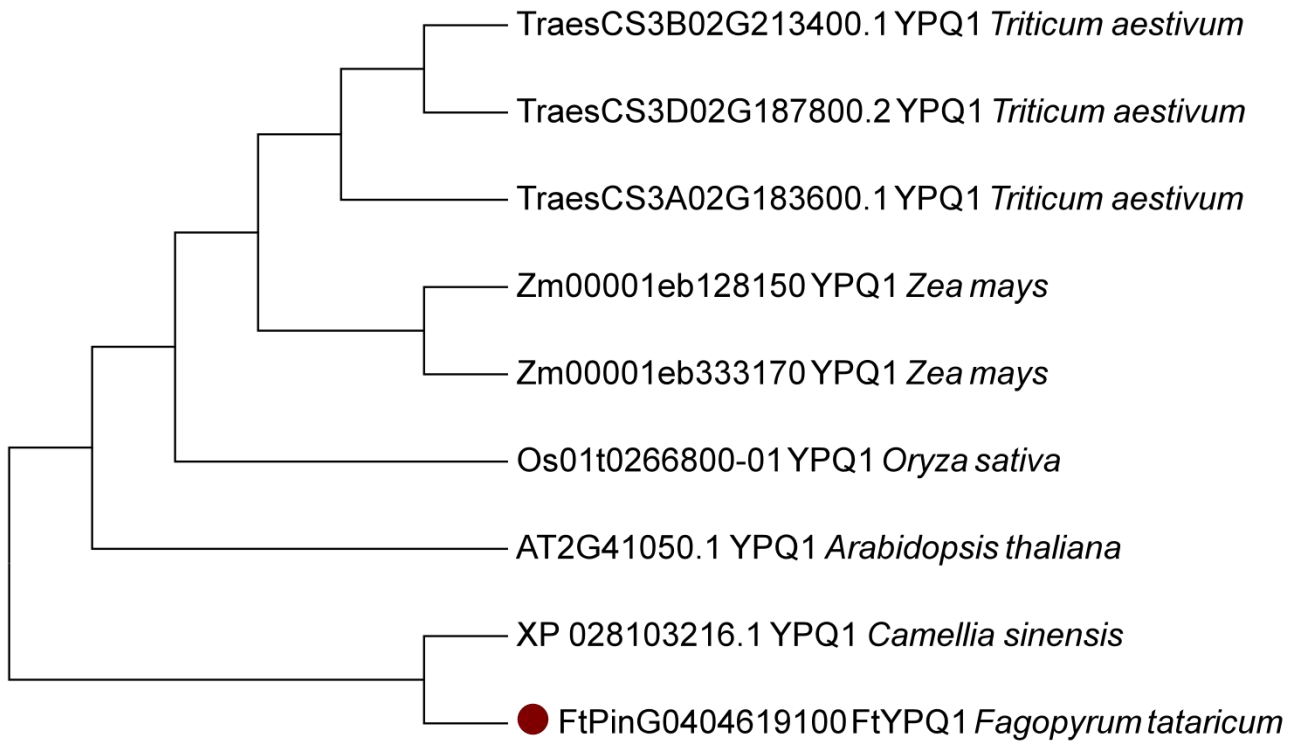


Figure S24. FtYPQ1 phylogenetic tree based on the neighbor joining method tree using full-length amino acid sequences of orthologues genes in Tartary buckwheat and other plants.

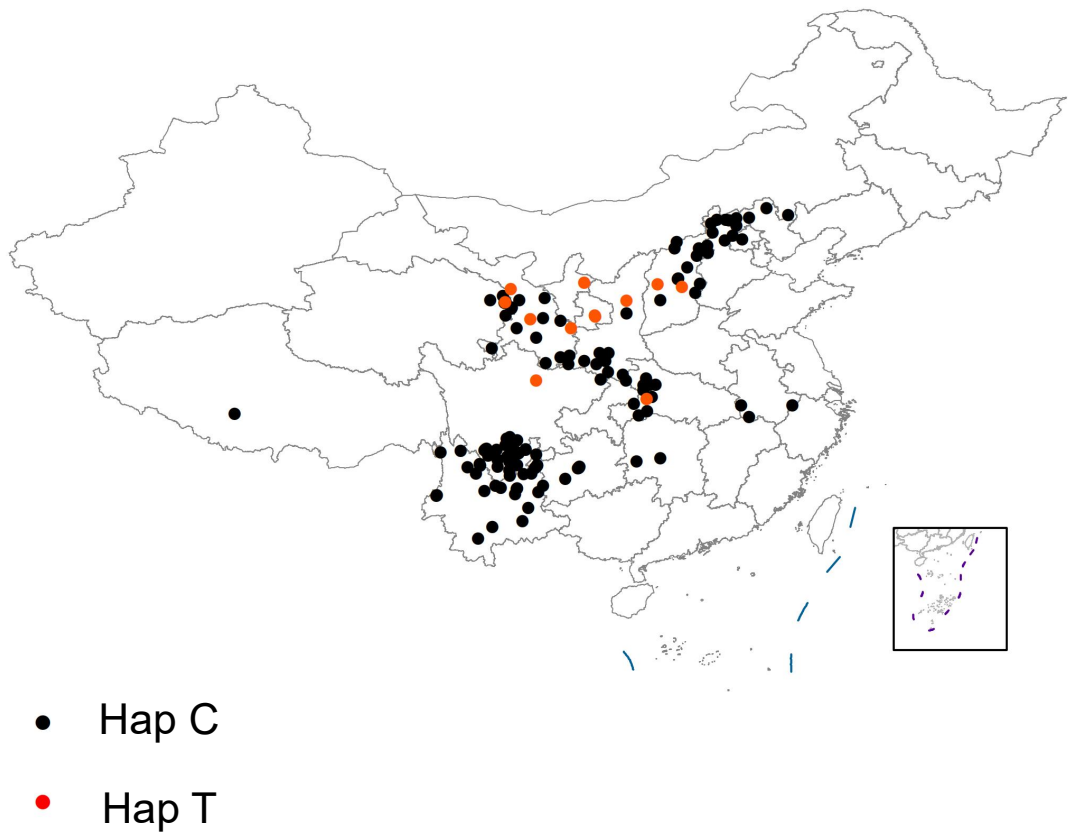
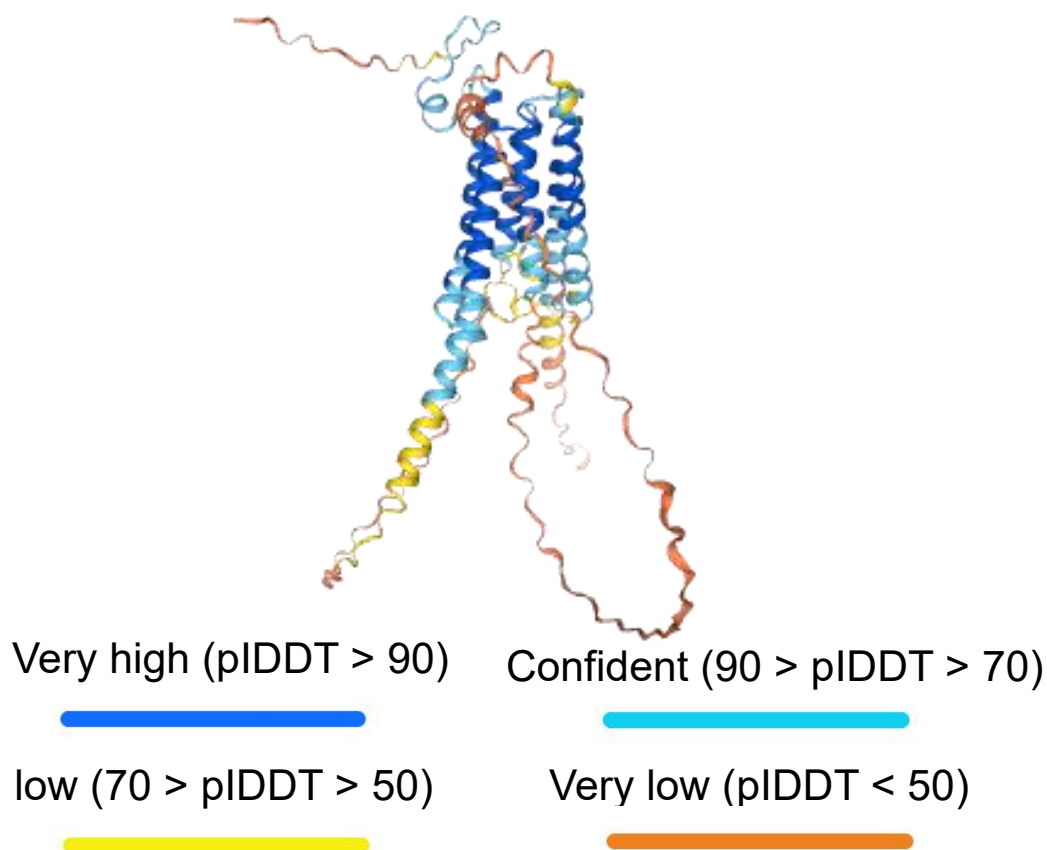


Figure S25. Geographic distribution of the Hap T (red) and Hap C (black) located in the promoter of *FtYPQ1* in Tartary buckwheat accessions.

a



b

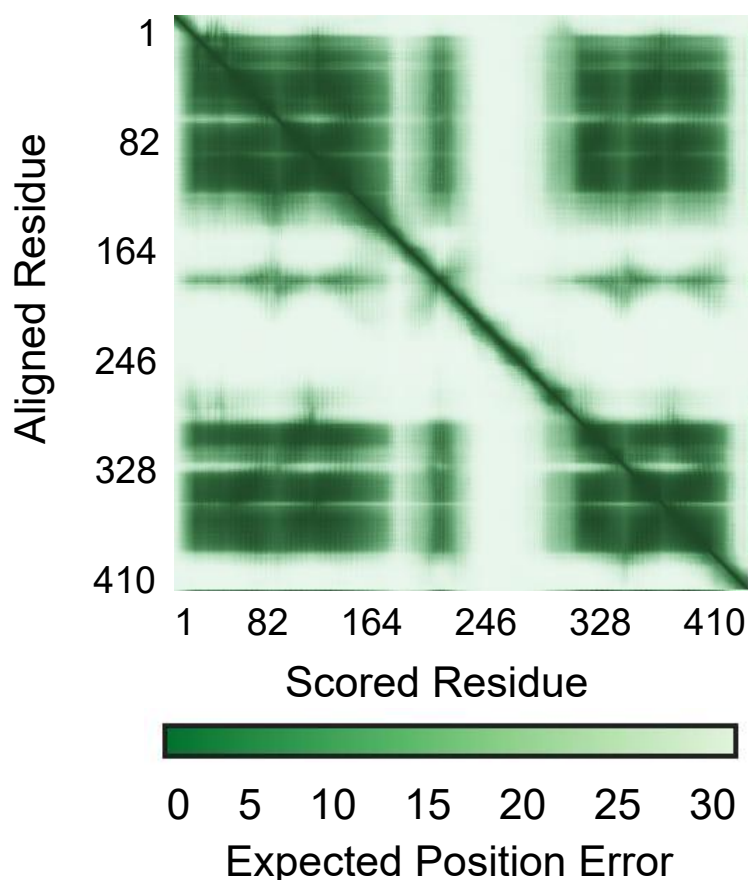


Figure S26. a. Predicted structure of FtYPQ1 colored by pLDDT (orange: 0-50, yellow: 50-70, cyan 70-90, and blue 90-100). b. Predicted Aligned Error (PAE) matrix of same prediction (darker is more confident).

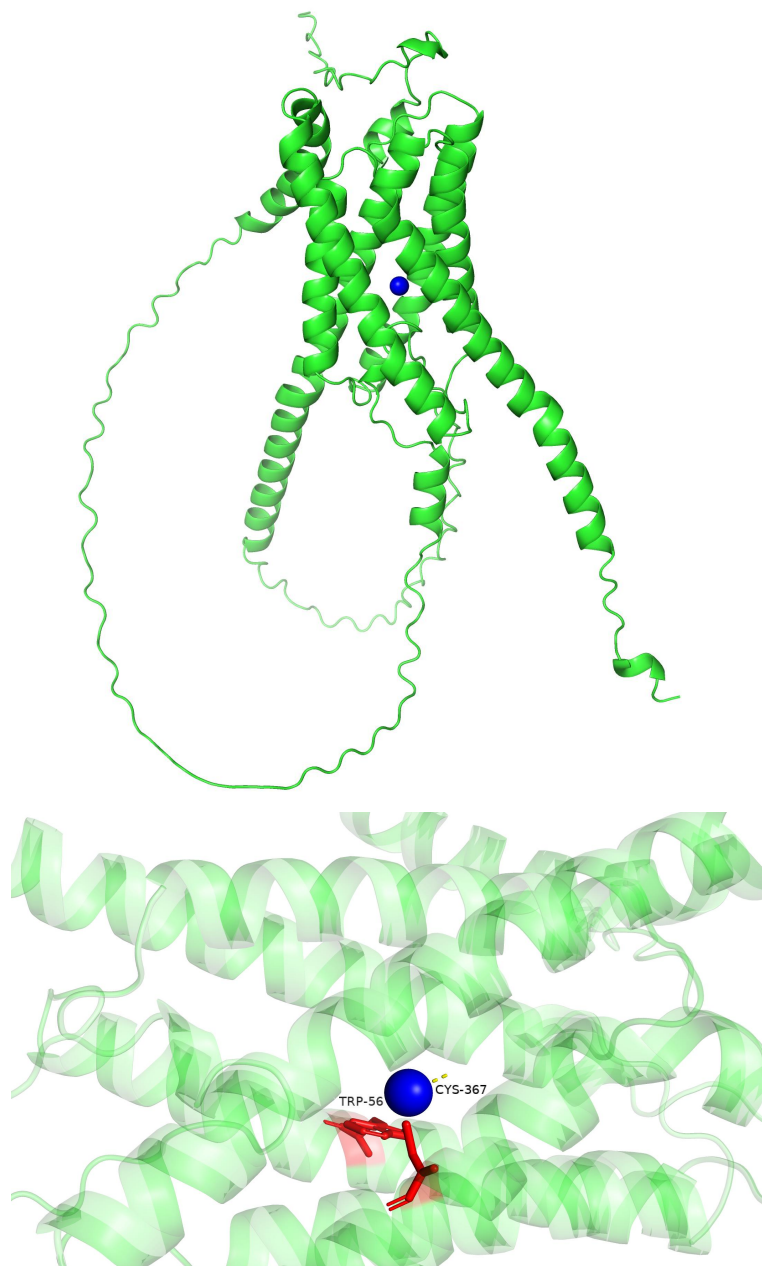


Figure S27. AlphaFold 3 prediction of FtYPQ1 protein interaction with zinc ion.

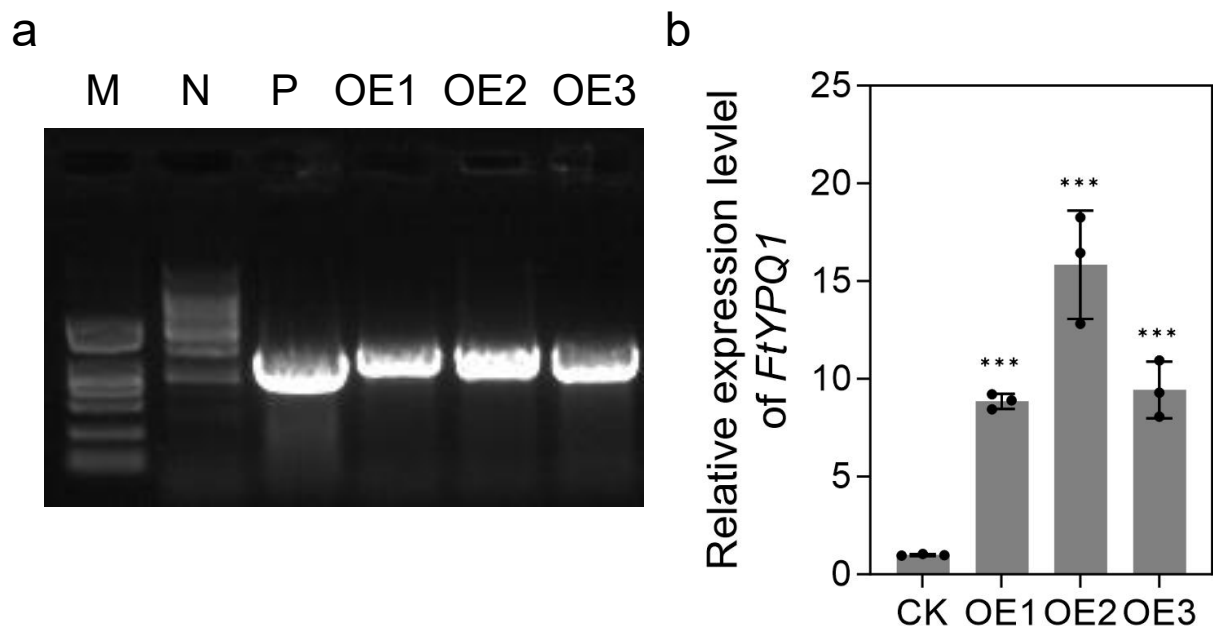


Figure S28. PCR (a) and RT-qPCR (b) analysis of hairy root lines expressing *FtYPQ1*. The hairy roots from wild type were used as negative control. N: negative control; P: positive control; M: DNA marker. The error bars indicate mean $\pm$ SD from three biological replicates. *** $P < 0.001$, two-tailed t -test.

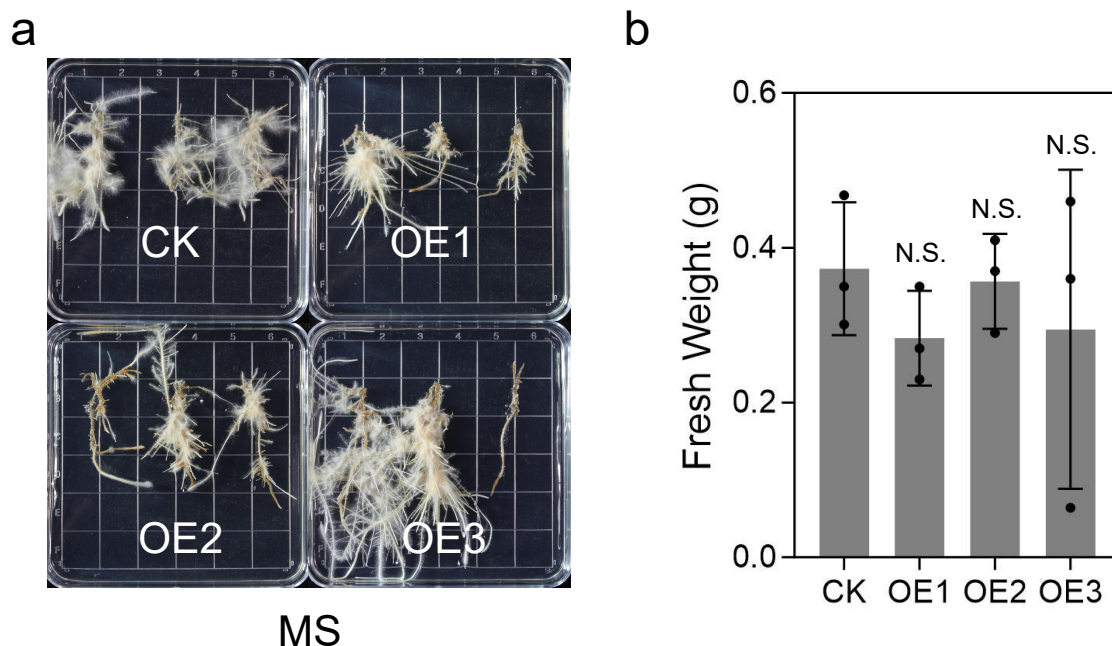


Figure S29. a. The hairy root phenotype grown on MS medium for 3 weeks. b. The fresh weight of different genotype hairy roots. The error bars indicate mean $\pm$ SD from three biological replicates. N.S. no significance, two-tailed *t*-test.

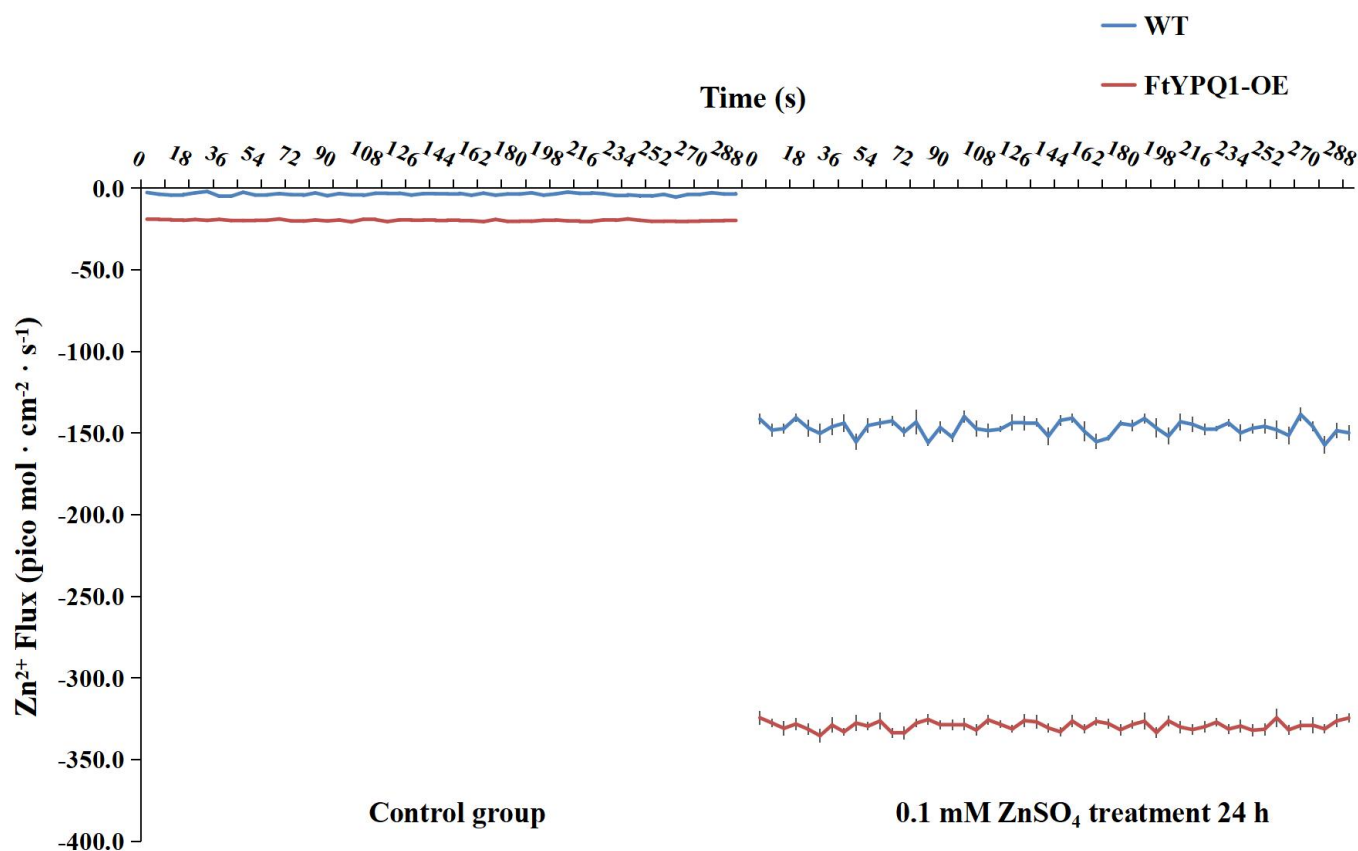


Figure S30. Comparison of extracellular zinc flux in living roots of *FtYPQ1* overexpression hairy roots and wild type. The control group indicates no treatment. The error bars indicate mean $\pm$ SD from six biological replicates.

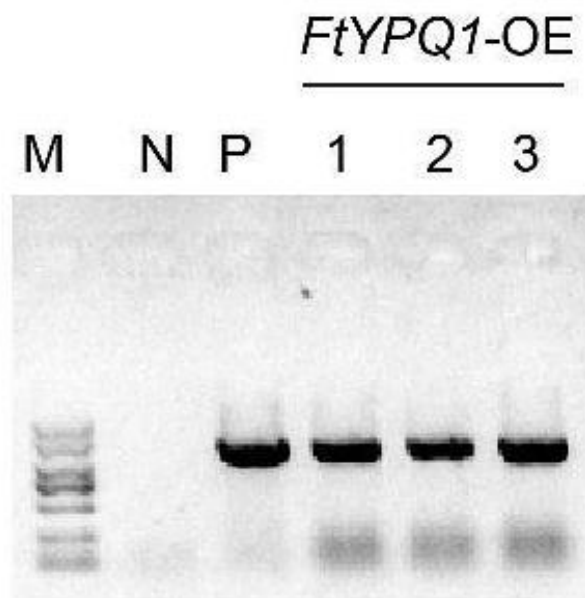


Figure S31. PCR analysis of *Arabidopsis* lines heterologously expressing *FtYPQ1*. The leaves from wild type (Col-0) were used as negative control. N: negative control; P: positive control; M: DNA marker.

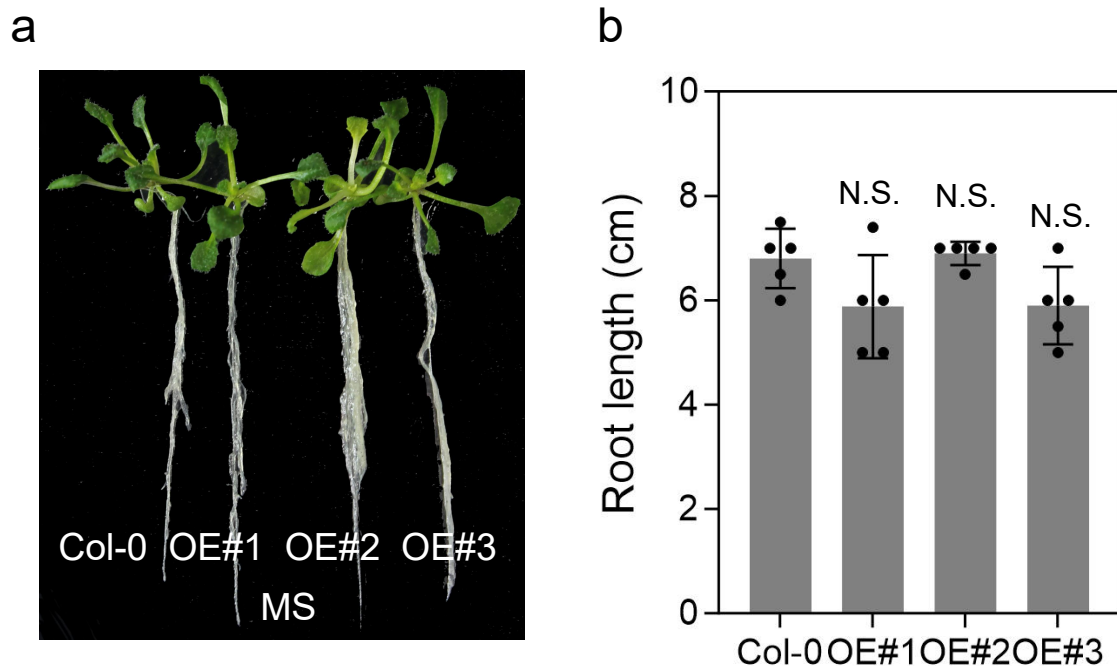


Figure S32. a. The phenotype of different genotype *Arabidopsis* lines grown on MS medium for 3 weeks. b. The root length of *Arabidopsis* lines. The error bars indicate mean $\pm$ SD from five biological replicates. N.S. no significance, two-tailed *t*-test.

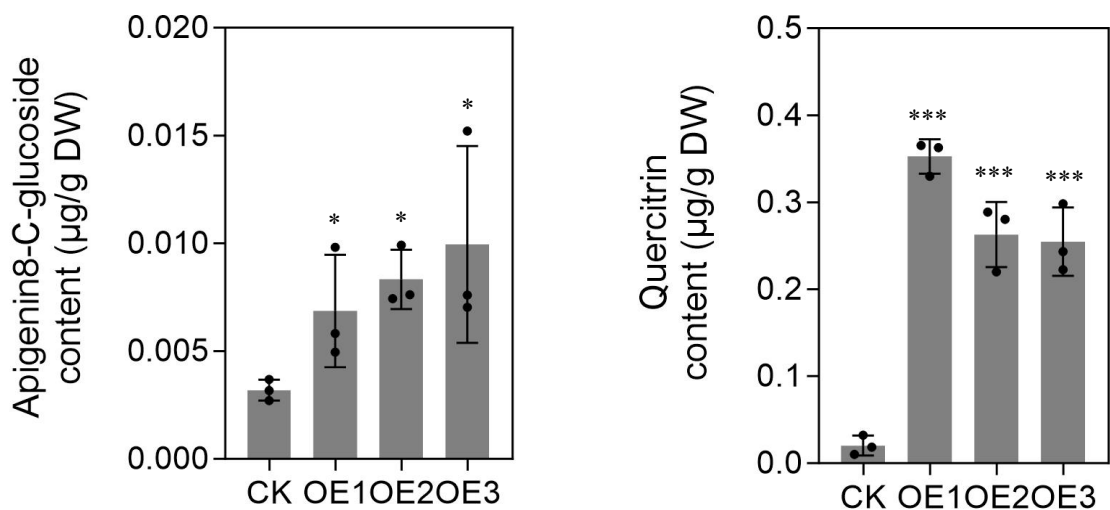


Figure S33. Apigenin-8-C-glucoside and quercitrin contents in different genotype hairy roots. The error bars indicate mean $\pm$ SD from three biological replicates. Asterisk (*) and (***) indicate significant difference at $P < 0.05$ and $P < 0.001$ using two-tailed t -test.

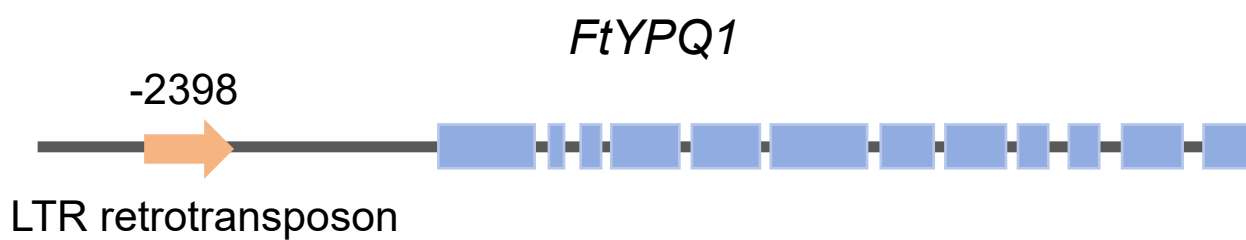


Figure S34. The position of LTR retrotransposon located in the promoter of *FtYPQ1* in Pinku.

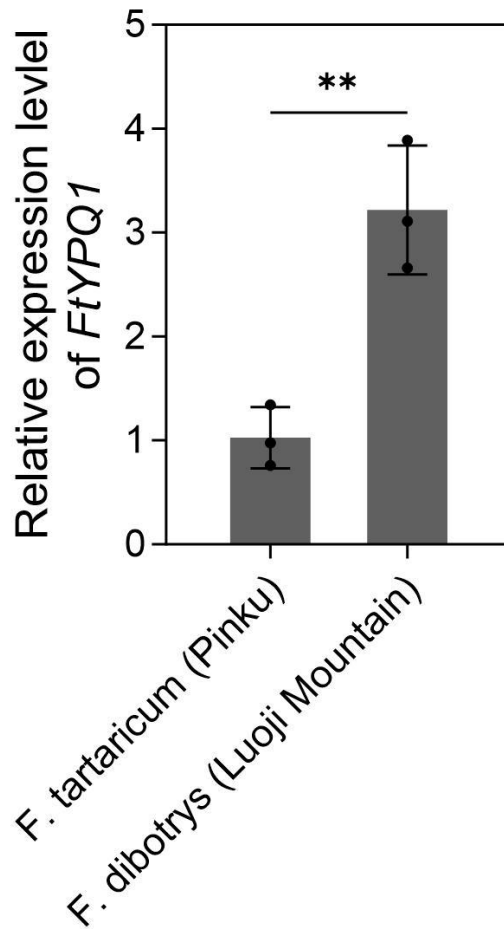


Figure S35. The expression level of *FtYPQ1* between *F. tataricum*-Pinku and *F. dibotrys*-Luoji Mountain. The error bars indicate mean $\pm$ SD from three biological replicates. ** $P < 0.01$, two-tailed *t*-test.

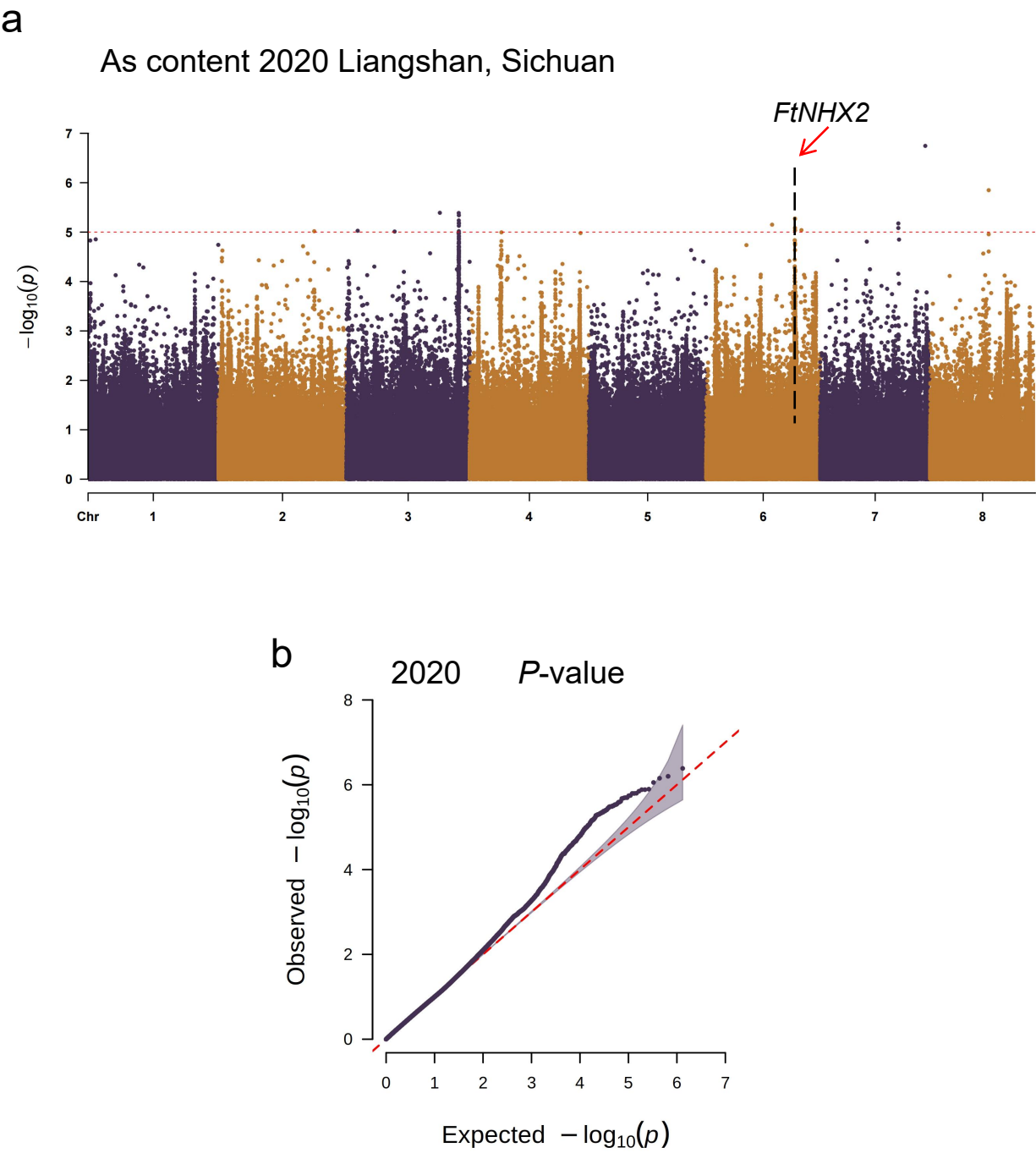
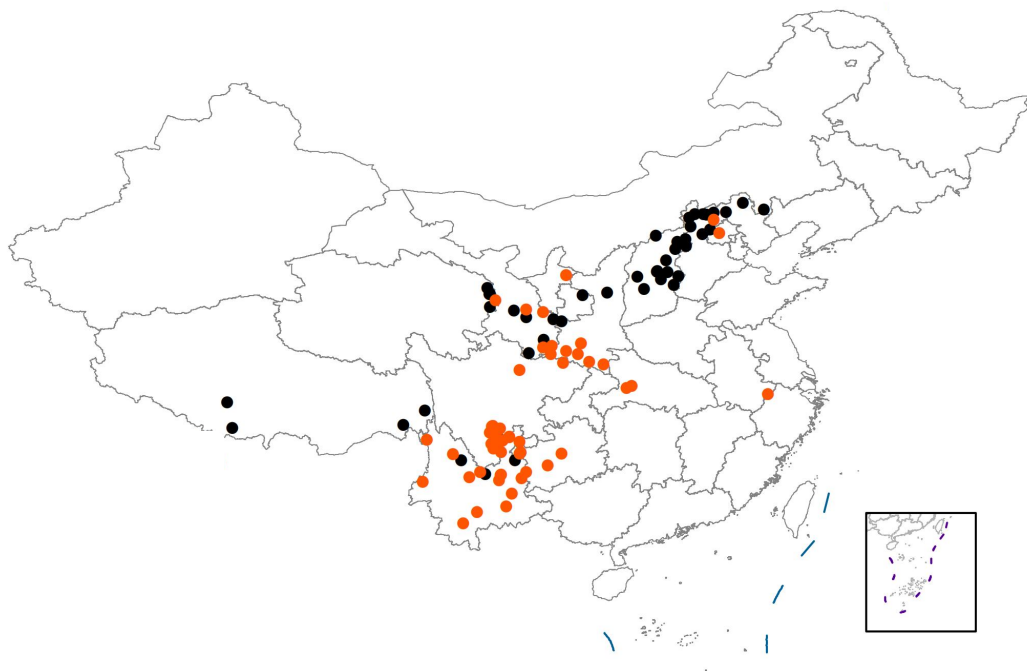


Figure S36. Manhattan and QQ plots of GWAS results of As content of Tartary buckwheat.



- Hap 1
- Hap 2

Figure S37. Geographic distribution of the Hap 2 (red) and Hap 1 (black) located in the promoter of *FtNHX2* in Tartary buckwheat accessions.

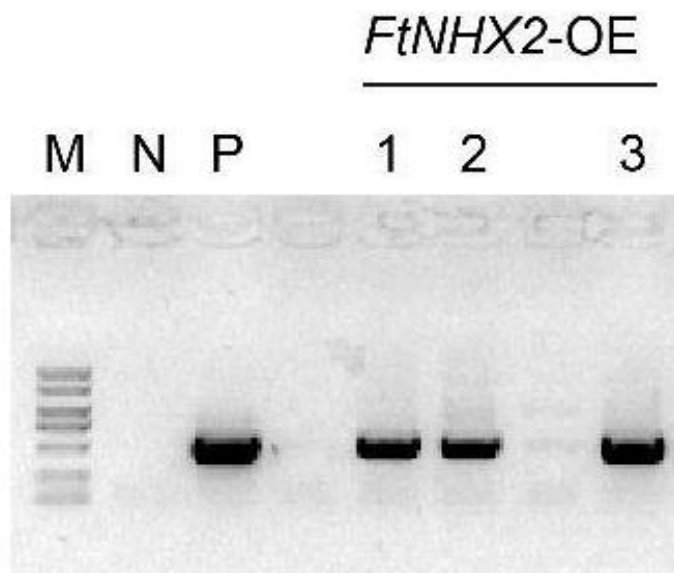


Figure S38. PCR analysis of *Arabidopsis* lines heterologously expressing *FtNHX2*. The leaves from wild type (Col-0) were used as negative control. N: negative control; P: positive control; M: DNA marker.

a



b

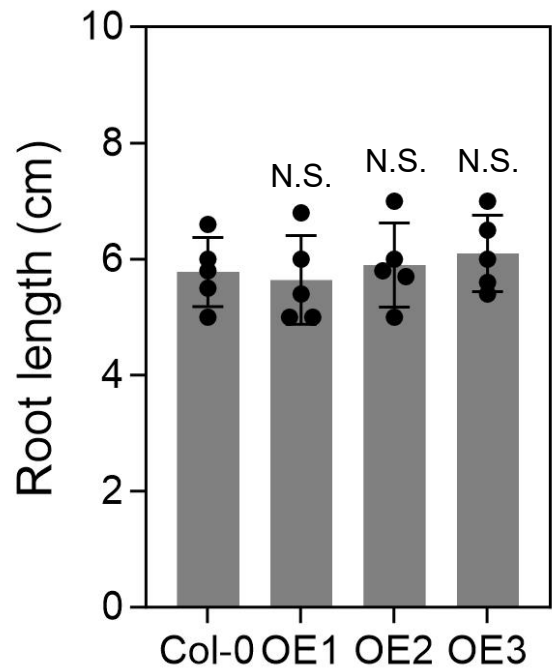


Figure S39. a. The phenotype of different genotype *Arabidopsis* lines grown on MS medium for 3 weeks. b. The root length of *Arabidopsis* lines. The error bars indicate mean $\pm$ SD from five biological replicates. N.S. no significance, two-tailed *t*-test.

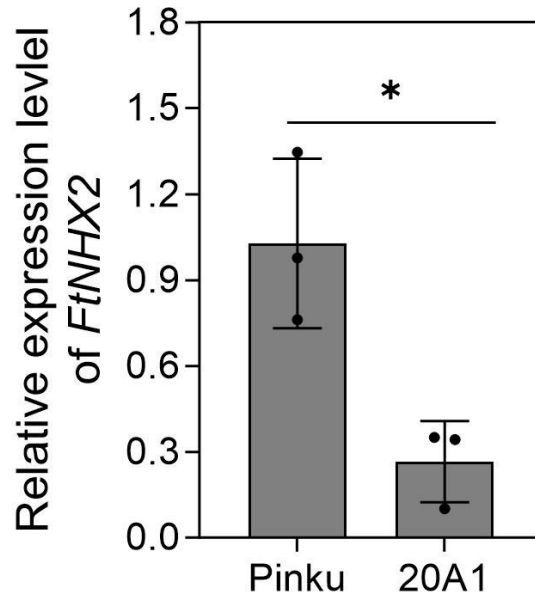


Figure S40. The expression level of *FtNHX2* between Pinku and 20A1 Tartary buckwheats. The error bars indicate mean $\pm$ SD from three biological replicates. * $P < 0.05$, two-tailed *t*-test.