

Supplementary File

Integration of GWAS and transcriptome analysis to identify temperature-dependent genes involved in germination of rapeseed (*Brassica napus* L.)

Ruisen Wang¹⁺, Guangyu Wu²⁺, Jingyi Zhang², Weizhen Hu³, Shuijin Hua⁴, Xiangtan Yao^{1*}, Lixi Jiang^{2*} and Yang Zhu^{2*}

¹ Jiaxing Academy of Agricultural Sciences, Jiaxing 314000, China

² Institute of Crop Science, Zhejiang University, Hangzhou 310058, China

³ Agricultural Experiment Station, Zhejiang University, Hangzhou 310058, China

⁴ Institute of Crops and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Science, Hangzhou 310021, China

⁺Equally contributed first authors

^{*}Co-corresponding authors

^{*}E-mails:

Xiangtan Yao, yxt156@hotmail.com

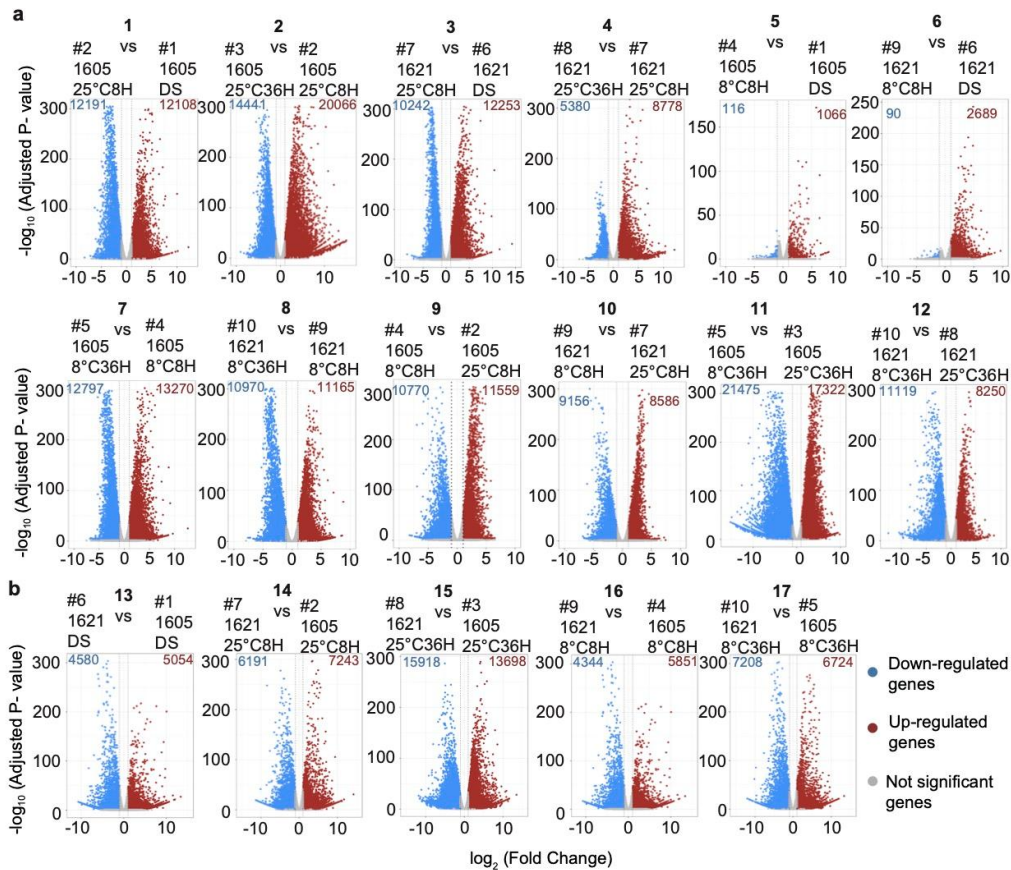
Lixi Jiang, jianglx@zju.edu.cn

Yang Zhu, zhuyang2020@zju.edu.cn

1 **Supplementary figures**

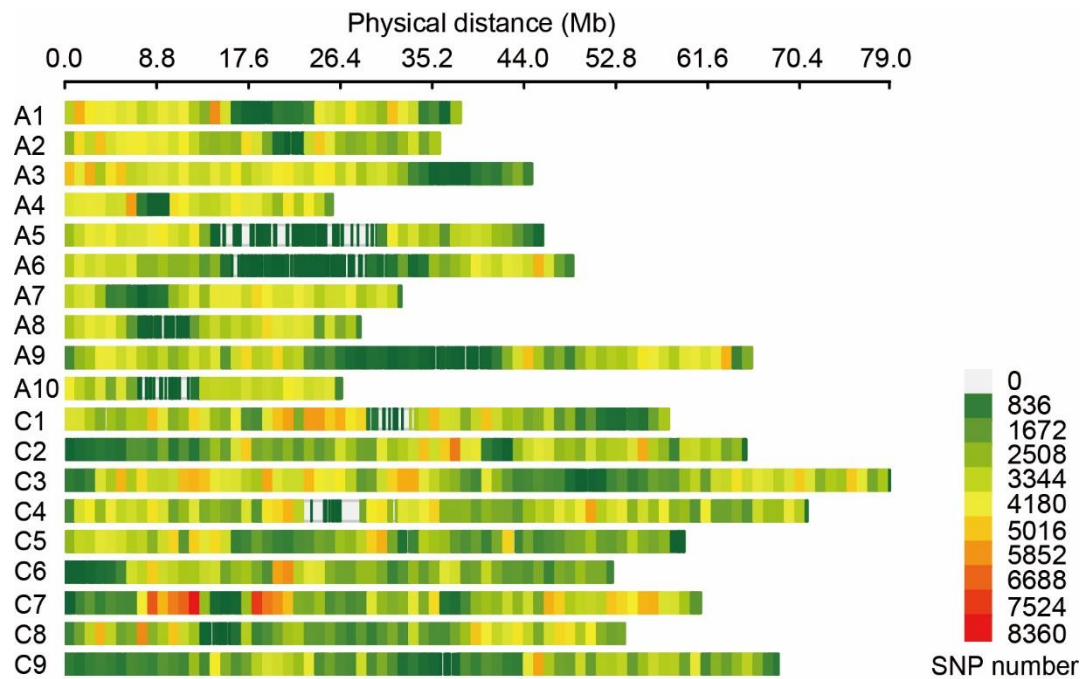


2
3 **Supplementary Fig. 1 Plant photos of JY1621 and JY1605 in different**
4 **growth stage.**
5 photos of 15-weeks-old seedlings (**a**) and silique maturation stage (**b**) of
6 JY1621 and JY1605 grown in field. The scale bar represents 10 cm.

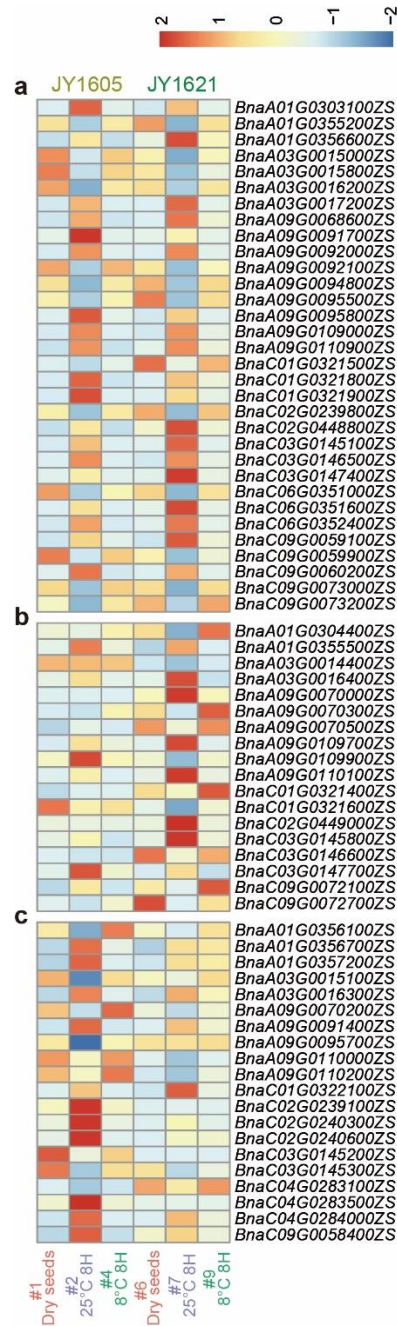


Supplementary Fig. 2 Differential gene expression analysis of normal and cold germination of JY1621 and JY1605.

a, Differentially expressed genes (DEGs) of twelve groups compared within JY1621 and JY1605. **b**, DEGs of five groups compared between JY1621 and JY1605. 1621 is short for JY1621. 1605 is short for JY1605. Down-regulated genes, up-regulated genes and genes with no biological significance in the transcriptome analysis were labeled by blue, red and grey dots, respectively.

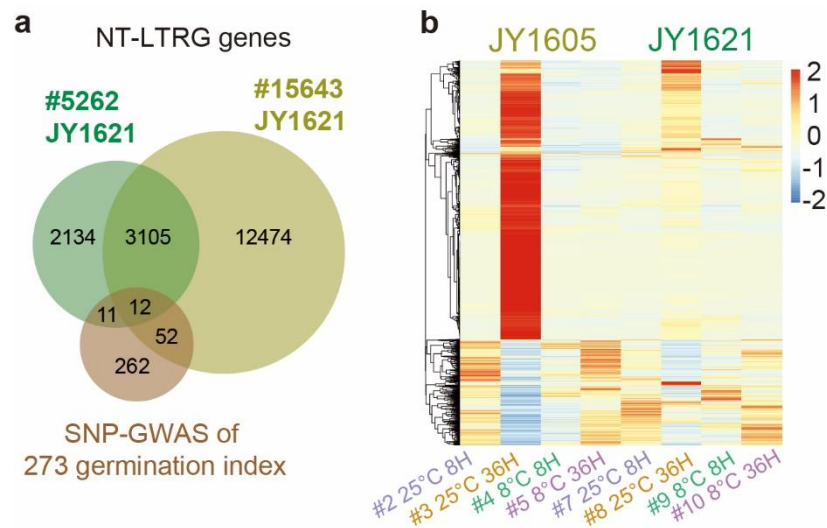


Supplementary Fig. 3 Distribution of SNPs within 1Mb window size on 19 chromosomes of 273 rapeseed accessions. The color bar on the right indicates the number of SNPs distributed in different regions.



Supplementary Fig. 4 Heatmaps showing the expression levels of 70 genes that overlap between early NT-ETRG genes and potential germination genes from SNP-GWAS.

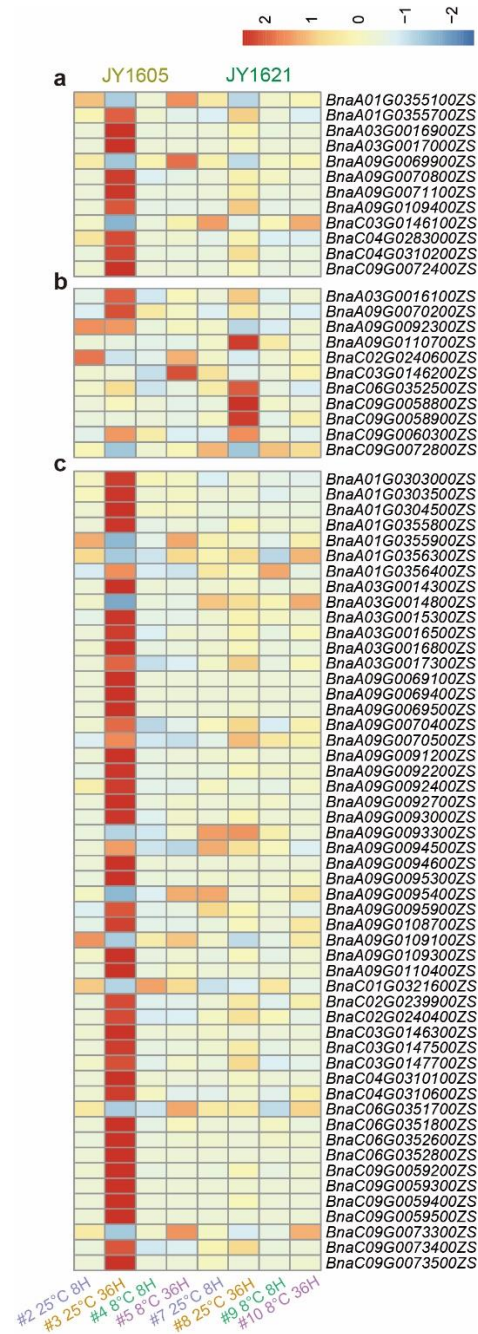
a, 32 genes that overlap between GWAS and NT-ETRG genes shared by both JY1621 and JY1605. **b**, 18 genes that overlap between GWAS and NT-ETRG genes in the JY1621 dataset. **c**, 20 genes that overlap between GWAS and NT-ETRG genes in the JY1605 dataset.



Supplementary Fig. 5 The expression profiling of NT-LTRG genes.

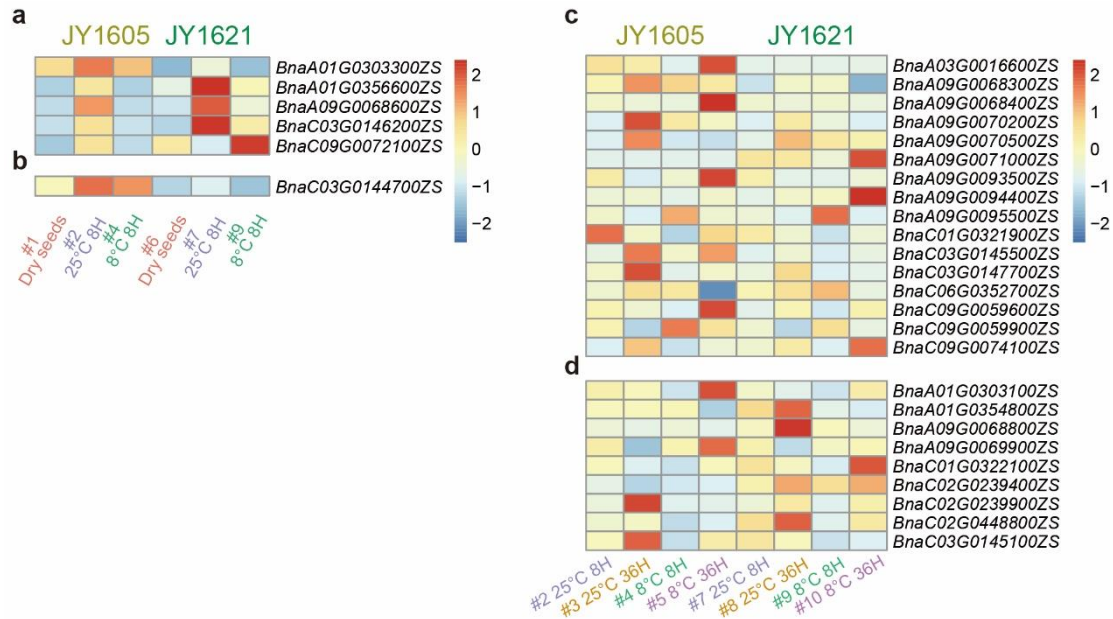
a, The Venn diagram represents the overlap between potential germination genes identified by SNP-GWAS and NT-LTRG genes of JY1621 and JY1605.

b, The clustered heatmap showing expression levels of totally 17788 NT-LTRG genes.



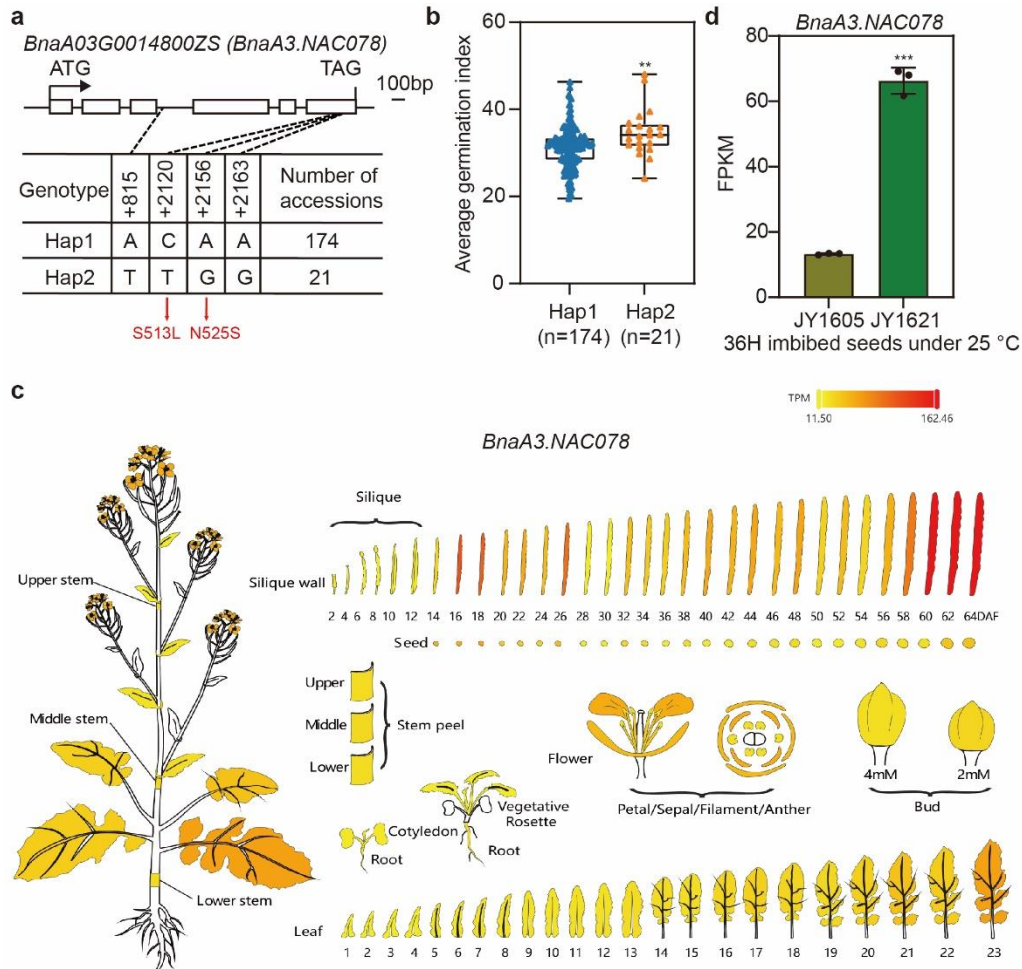
Supplementary Fig. 6 Heatmaps showing the expression levels of 75 genes that overlap between NT-LTRG genes and potential germination genes identified by SNP-GWAS.

a, 12 genes that overlap between GWAS and NT-LTRG genes shared by both JY1621 and JY1605. **b**, 11 genes that overlap between GWAS and NT-LTRG genes in the JY1621 dataset. **c**, 52 genes that overlap between GWAS and NT-LTRG genes in the JY1605 dataset.



Supplementary Fig. 7 Heatmaps displaying the expression levels of genes overlapping among *BnCDTs*, *BnTPIs*, and potential germination genes identified by SNP-GWAS.

a and **b**, Expression patterns of 8H *BnCDTs* (**a**) and 8H *BnTPIs* (**b**) overlapping with candidate genes identified by GWAS. **c** and **d**, Expression patterns of 36H *BnCDTs* (**c**) and 36H *BnTPIs* (**d**) overlapping with candidate genes identified by GWAS.



Supplementary Fig. 8 Haplotype analysis and expression patterns of *BnaA3.NAC078*. **a**, Diagrammatic illustration showing the gene structure (top) and two main haplotypes (bottom) of *BnaA3.NAC078* using ZS11 as the reference. S513L and N525S represented two amino acid changes caused by nonsynonymous mutations. **b**, Comparison of average germination indices under normal-temperature conditions between accessions with two different haplotypes of *BnaA8.NAC078*. **c**, The tissue-specific expression pattern of *BnaA3.NAC078*. Data of expression levels in various tissues were obtained from BnIR (<https://yanglab.hzau.edu.cn/BnIR/>) (Yang et al. 2023). **d**, Comparison of expression levels of *BnaA8.NAC078* in 36H imbibed seeds under normal-temperature conditions between JY1605 and JY1621. In **b** and **d**, *P*-values were calculated by the two-tailed Student's *t* test. ** indicates *P* < 0.01, *** indicates *P* < 0.001.

63 **References in the Supplementary File**

64 Yang Z, Wang S, Wei L, Huang Y, Liu D, Jia Y, Luo C, Lin Y, Liang C, Hu Y,
65 Dai C, Guo L, Zhou Y, Yang Q-Y (2023) BnIR: A multi-omics database with
66 various tools for Brassica napus research and breeding. Molecular Plant
67 16:775-789