Transcriptomic variations and network hubs controlling seed size and weight during maize seed development

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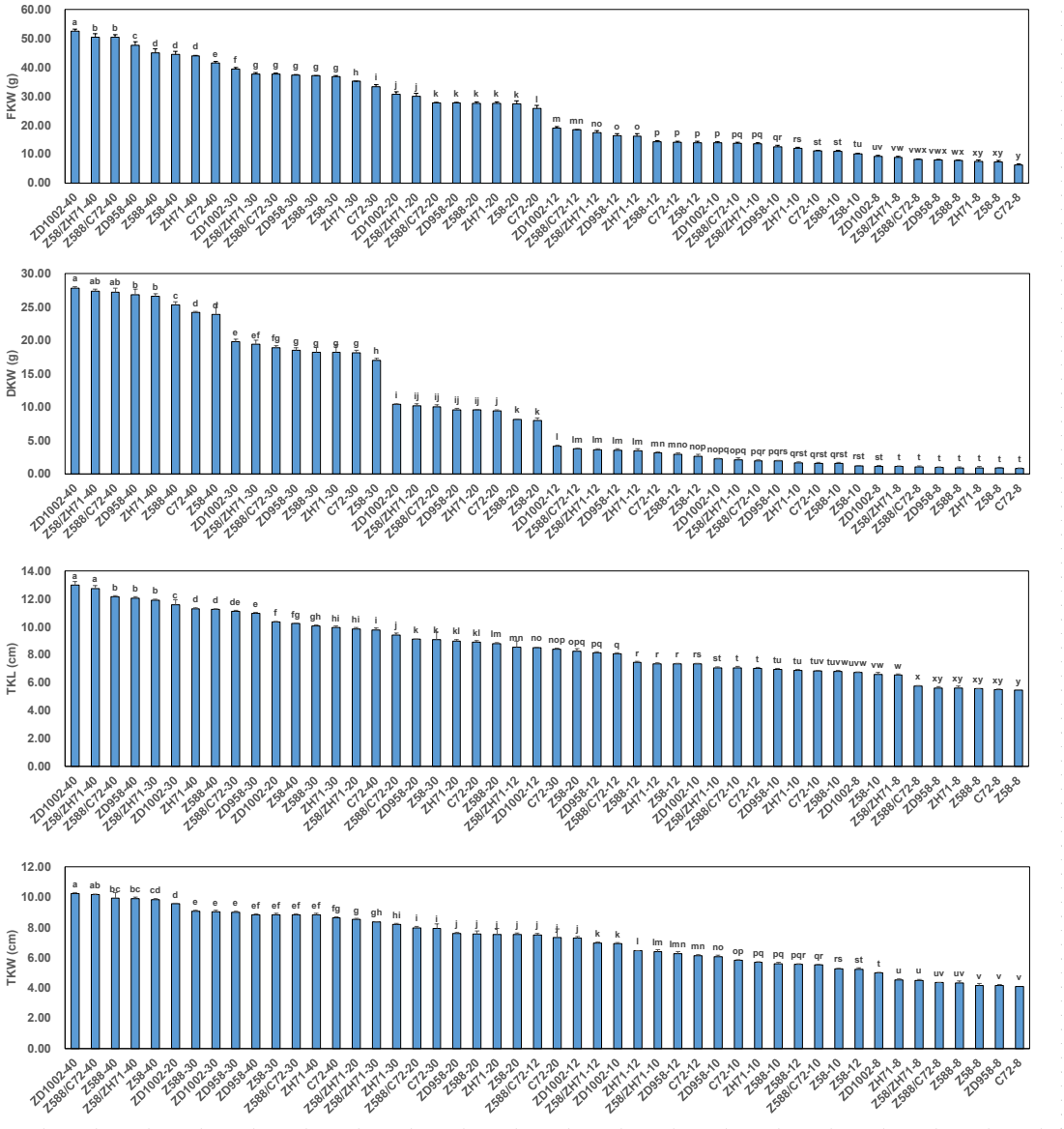
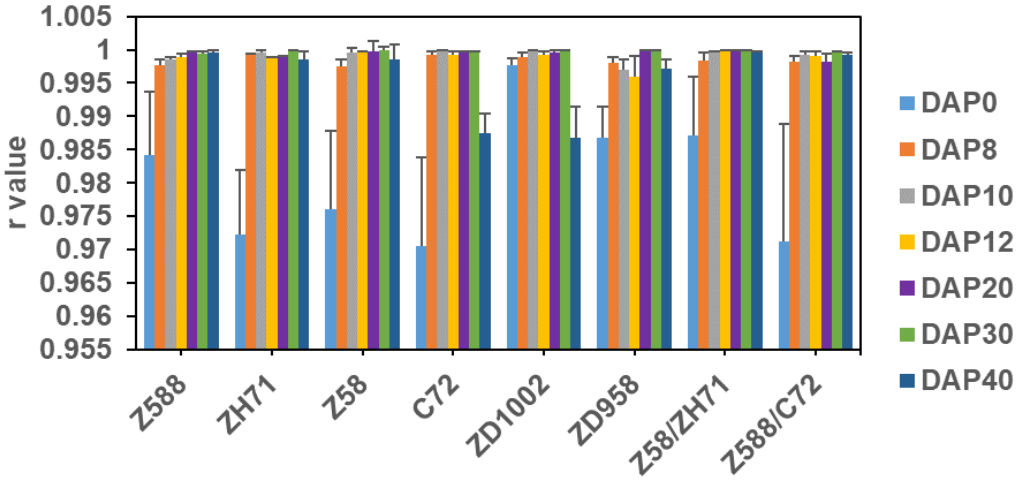
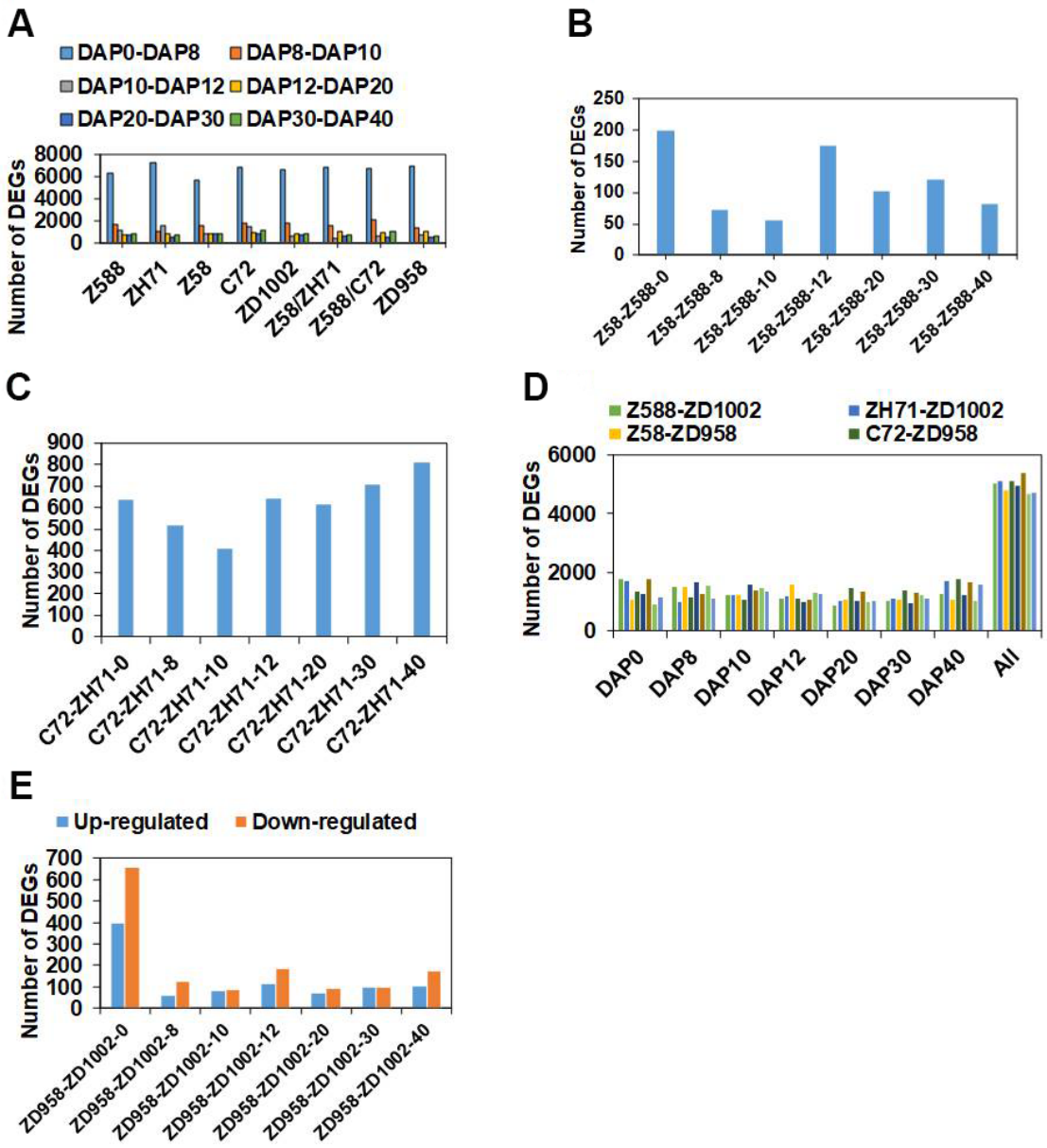


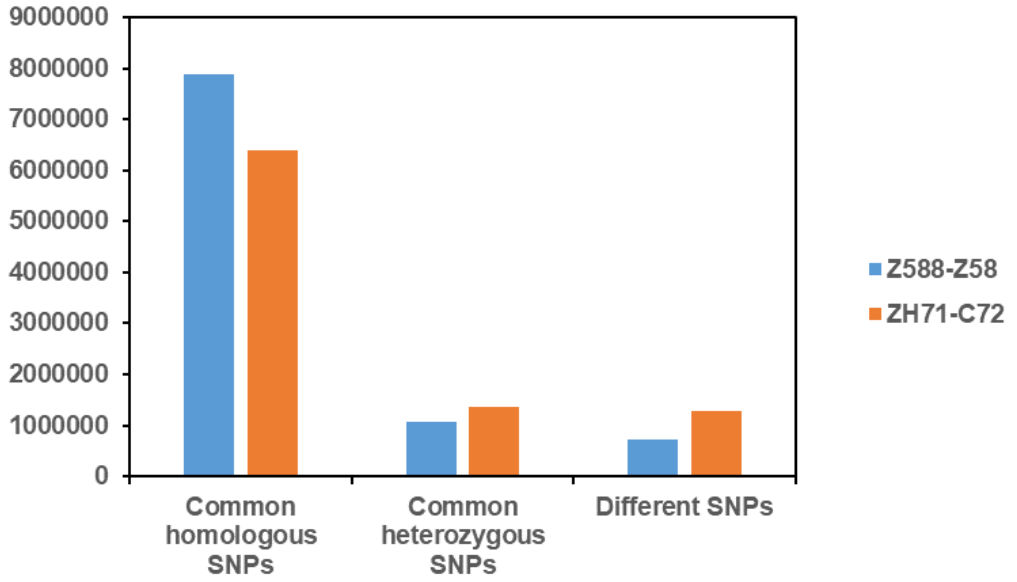
Supplementary Figure 1. Duncan's multiple comparisons of seed size and weight associated traits for eight genotypes during seed development. Data represent the means ± SD. Lower case letters above error bars represent results of Duncan's multiple comparisons at the 0.05 significant level. Means with different letters are significantly different. DAP8–40 represents 8–40 d after pollination, respectively. FKW, 100-fresh kernel weight; DKW, 100-dry kernel weight; TKL, Ten-kernel length; TKW, Ten-kernel width. Z588, Zheng588; Z58, Zheng58; ZH71, ZhengH71; C72, Chang7-2; ZD958, Zhengdan958; ZD1002, Zhengdan1002.



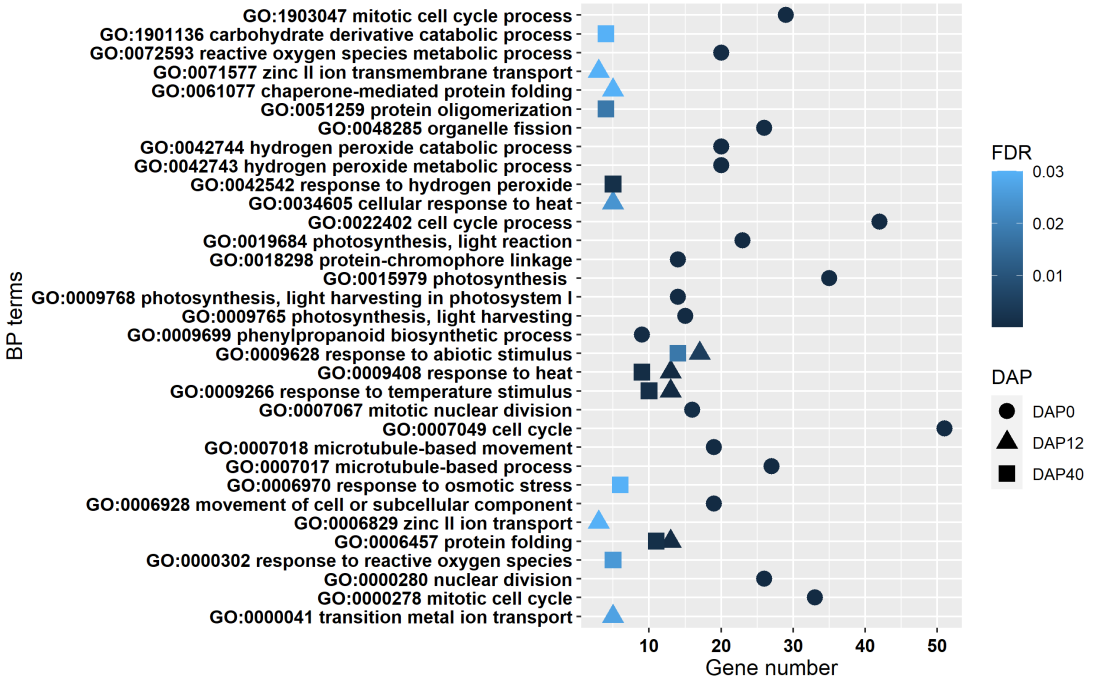
Supplementary Figure 2. The correlations of three biological replicates for eight genotypes at seven developmental stages. DAP0, DAP8, DAP10, DAP12, DAP20, DAP30, and DAP40 represent 0, 8, 10, 12, 20, 30, and 40 d after pollination, respectively. Error bars are represented by SD.



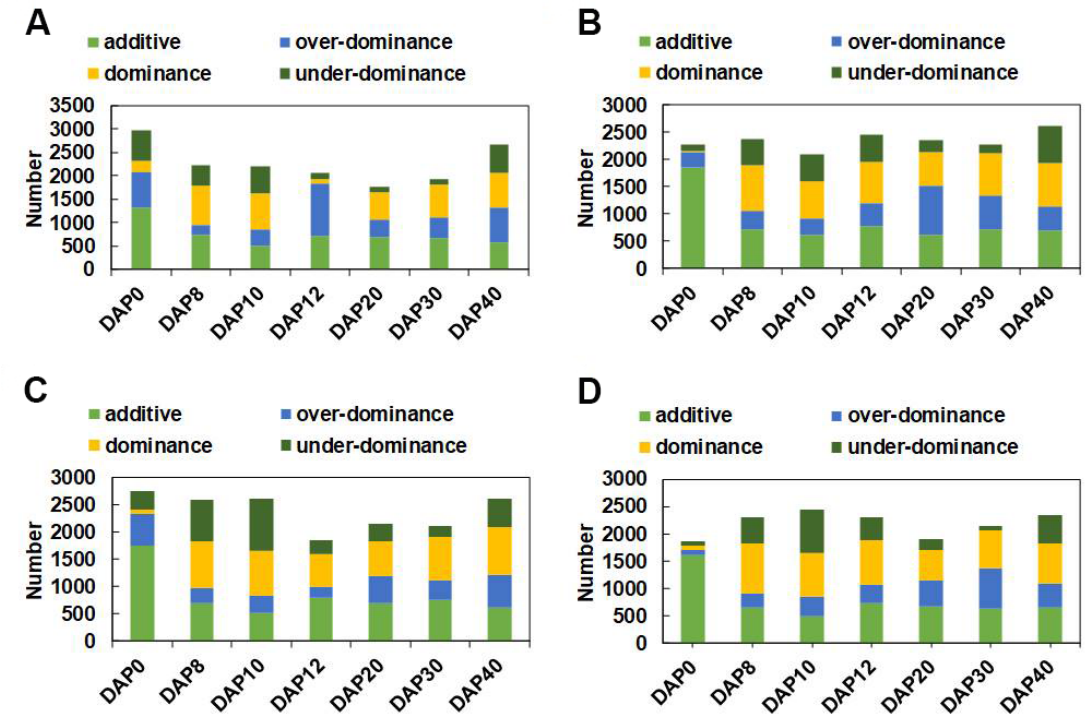
Supplementary Figure 3. Differentially expressed profiles. **(A)** The number of differentially expressed genes (DEGs) between two neighbouring stages. The previous stage is considered as CK. **(B)** The number of DEGs between Z58 and Z588. **(C)** The number of DEGs between C72 and ZH71. **(D)** The number of DEGs between F_1 and their respective parents (CK). **(E)** The number of up-regulated and down-regulated DEGs between ZD958 (CK) and ZD1002. 0, 8, 10, 12, 20, 30, and 40 denote 0–40 d after pollination (DAP). Z588, Zheng588; Z58, Zheng58; ZH71, ZhengH71; C72, Chang7-2; ZD958, Zhengdan958; ZD1002, Zhengdan1002.



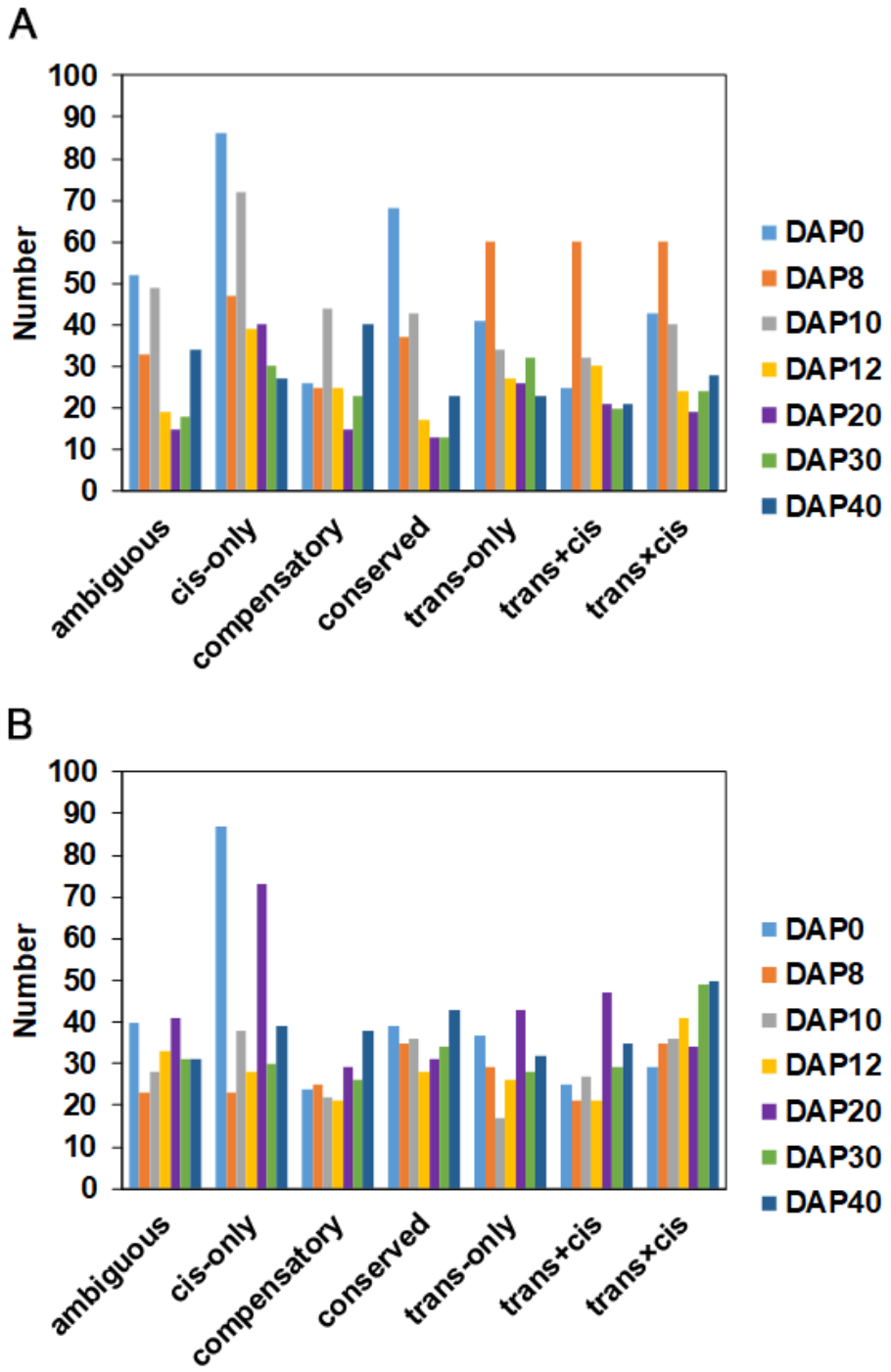
Supplementary Figure 4. The number of common and different SNPs between parents identified from DNA resequencing. Z588, Zheng588; Z58, Zheng58; ZH71, ZhengH71; C72, Chang7-2.



Supplementary Figure 5. Significant biological process (BP) terms of differentially expressed genes between Zhengdan1002 and Zhengdan958 at 0 d after pollination (DAP0), DAP12, and DAP40. Top20 terms are shown at DAP0 stage, and all significant BP terms are shown at DAP12 and DAP40 stages.

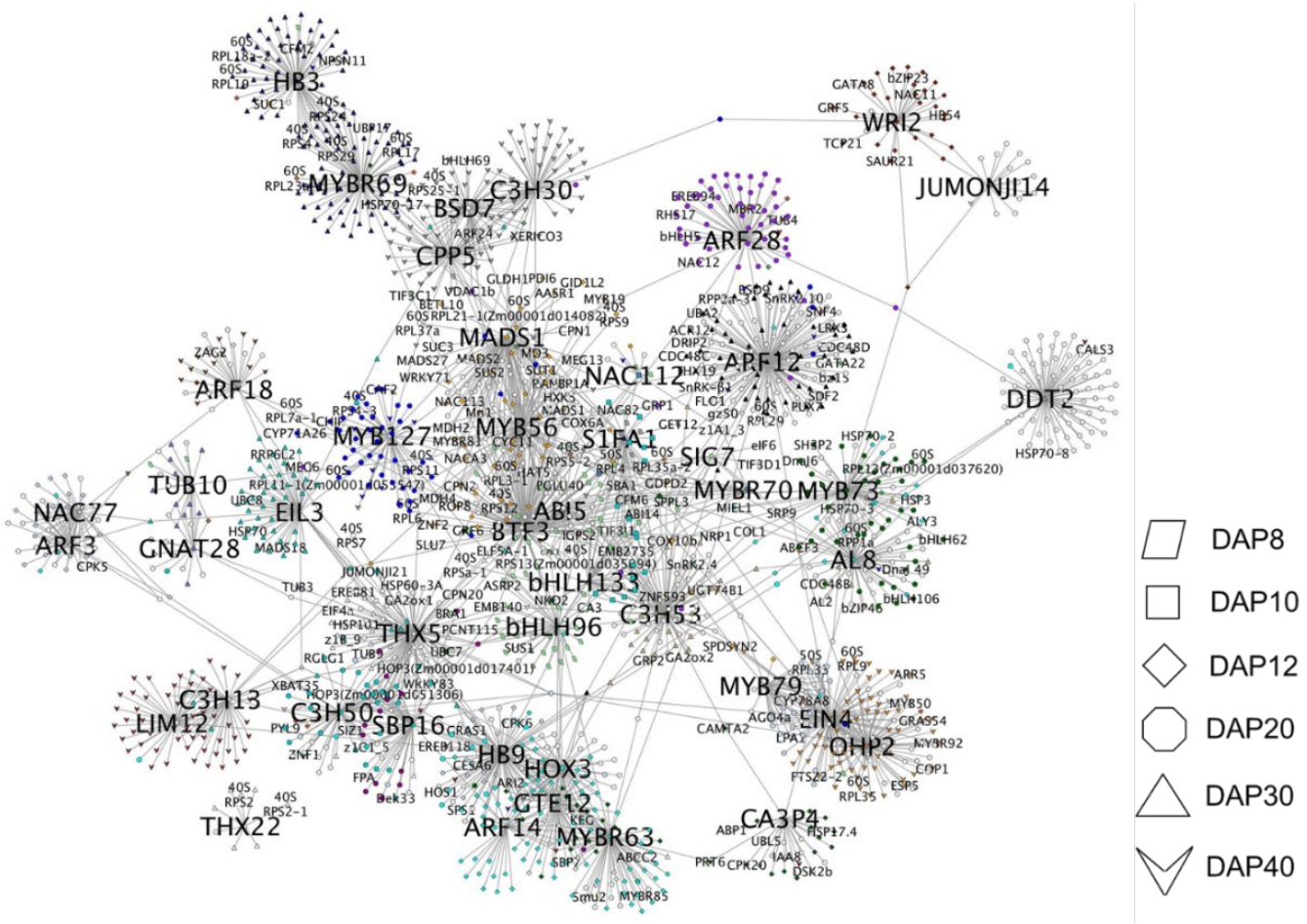


Supplementary Figure 6. Classification of additive and non-additive patterns in Zhengdan1002 **(A)**, Zhengdan958 **(B)**, Zheng588/Chang7-2 **(C)**, and Zheng58/ZhengH71 **(D)** at seven developmental stages. DAP0, DAP8, DAP10, DAP12, DAP20, DAP30, and DAP40 represent 0, 8, 10, 12, 20, 30, and 40 d after pollination, respectively.

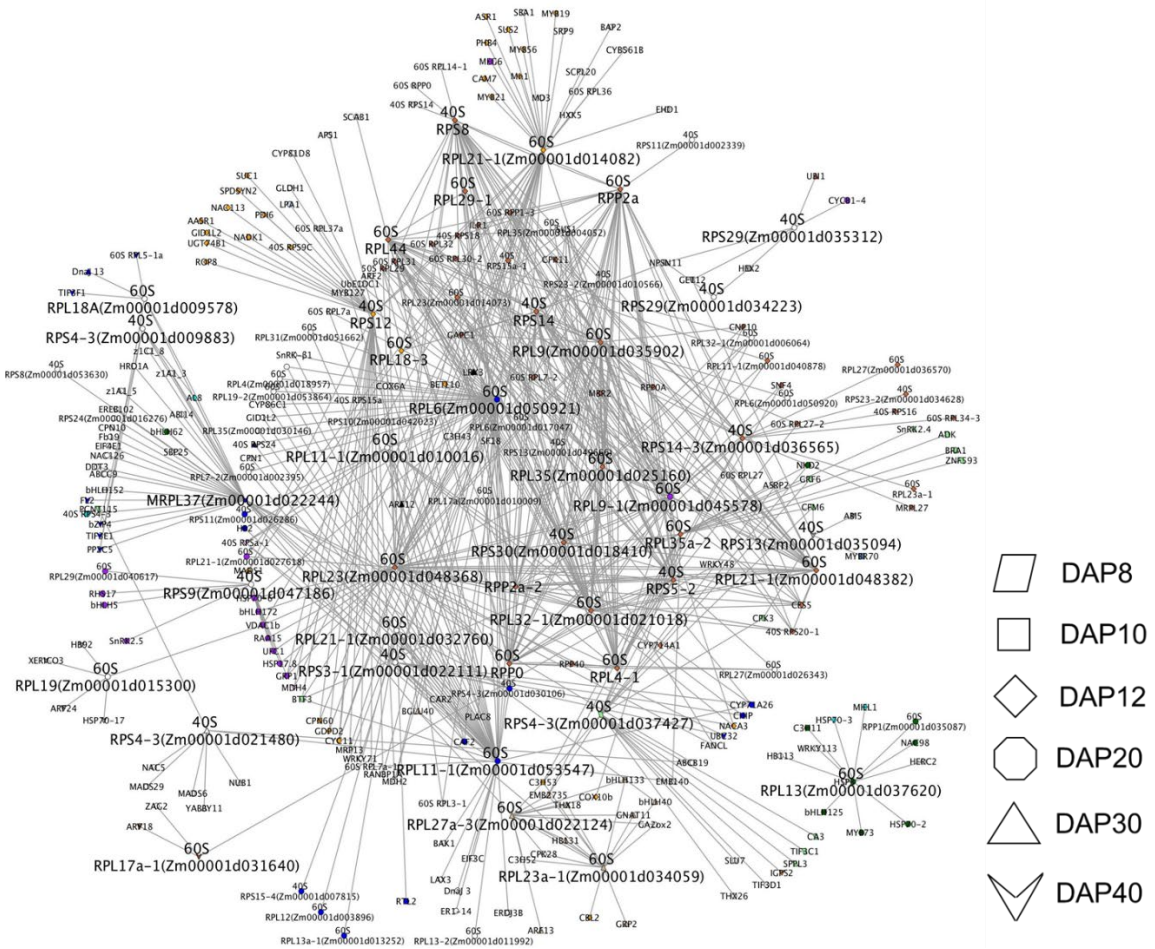


Supplementary Figure 7. Number of seven regulatory patterns at seven developmental stages in Zhengdan1002 **(A)** and Zhengdan958 **(B)**. DAP0, DAP8, DAP10, DAP12, DAP20, DAP30, and DAP40 represent 0, 8, 10, 12, 20, 30, and 40 d after pollination, respectively.

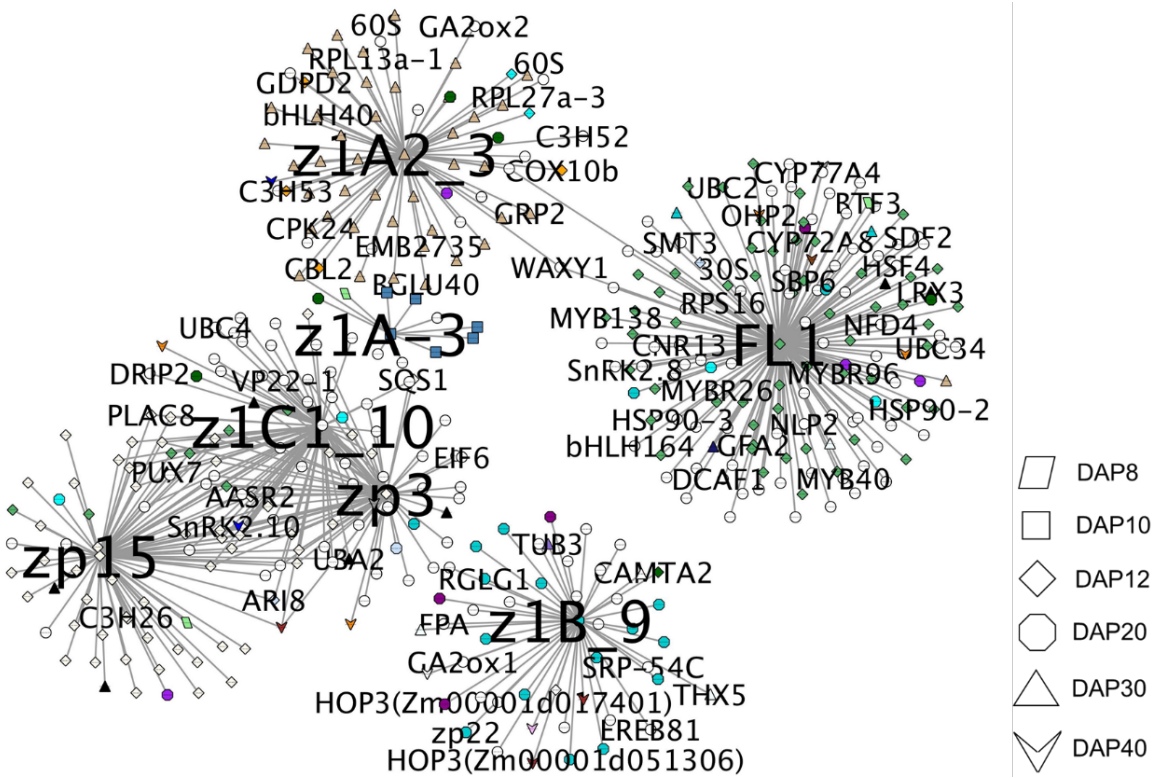
Supplementary Figure 8. Module-trait relationship of the module eigengene correlation with seed size and weight at different development stages using WGCNA. **(A)**: 8 d after pollination (DAP8). **(B)**: DAP10. **(C)**: DAP12. **(D)**: DAP20. **(E)**: DAP30. **(F)**: DAP40. Modules are represented by colors. Shown are correlation values, and * and ** represent 0.05 and 0.01 significant level, respectively. FKW, 100-fresh kernel weight; DKW, 100-dry kernel weight; TKL, ten-kernel length; TKW, ten-kernel width.



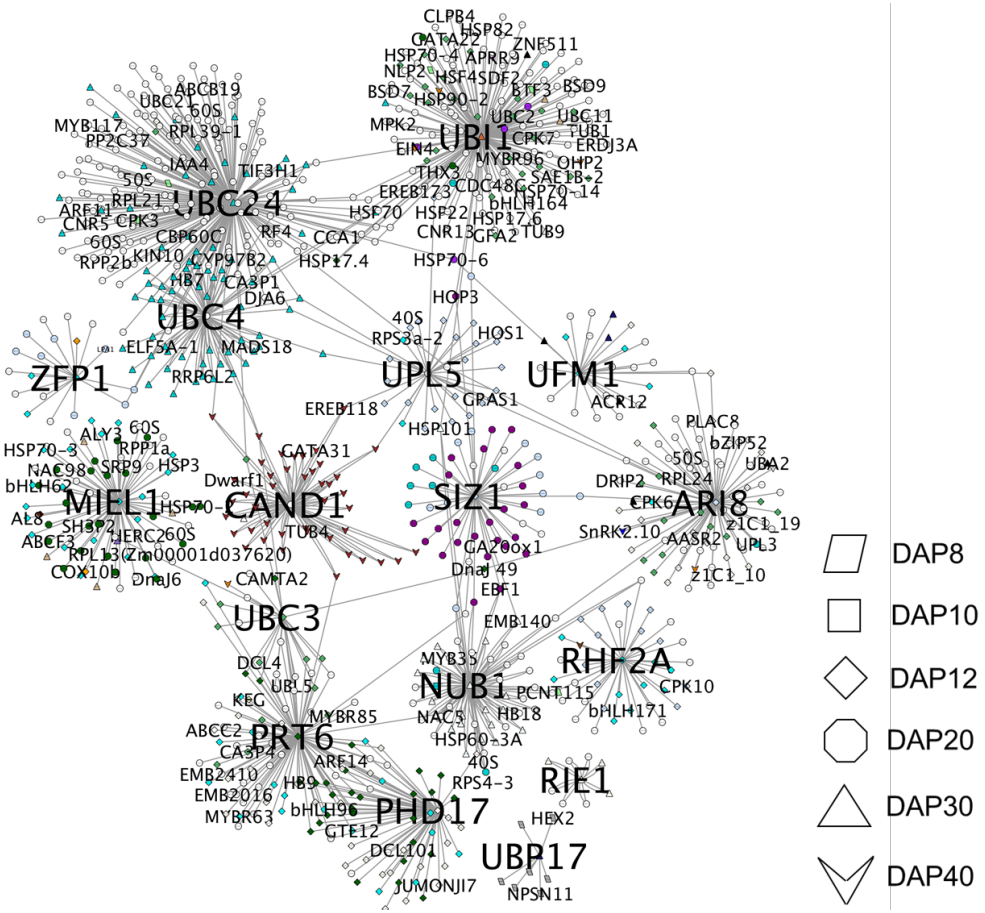
Supplementary Figure 9. Gene networks of hub genes associated with transcription factors at DAP8–40 stages. Top 10% genes are shown according their weight value with these hub genes. Different colors represent different module. Circle and geometric figures with no colors denote genes are appeared more than two stages or modules. DAP8–40 represents 8–40 d after pollination, respectively.



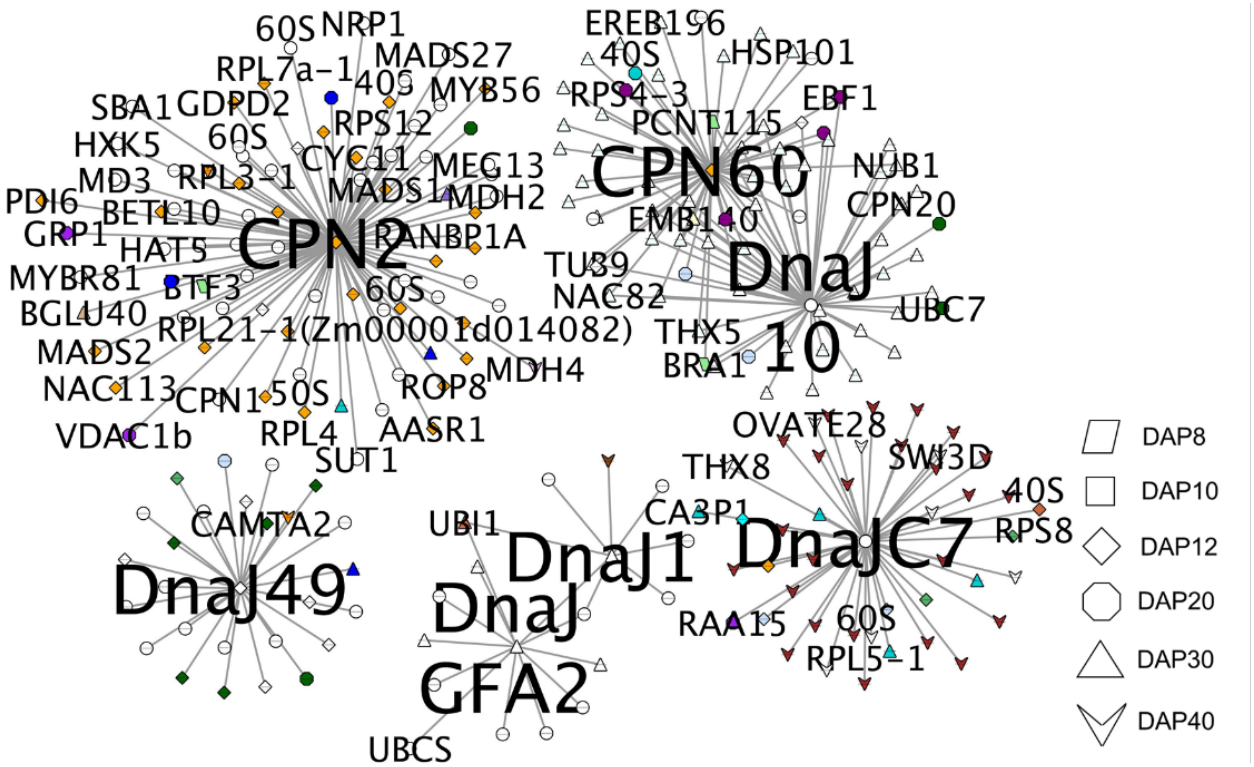
Supplementary Figure 10. Gene networks of hub genes associated with ribosomal proteins at DAP8–40 stages. Top 10% genes with annotations are shown according their weight value with these hub genes. Different colors represent different module. Circle and geometric figures with no colors denote genes are appeared more than two stages or modules. DAP8–40 represents 8–40 d after pollination, respectively.



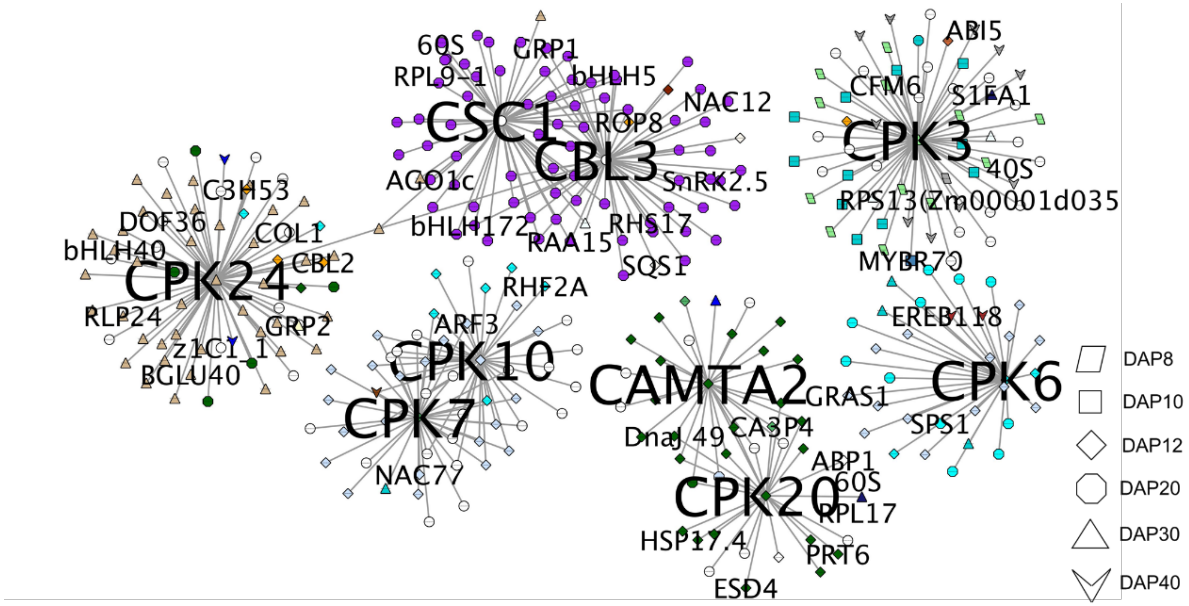
Supplementary Figure 11. Gene networks of hub zein genes at DAP8–40 stages. Top 10% of genes with weight value >= 0.1 are shown according their weight value with these hub genes. Different colors represent different module. Circle and geometric figures with no colors denote genes are appeared more than two stages or modules. DAP8–40 represents 8–40 d after pollination.



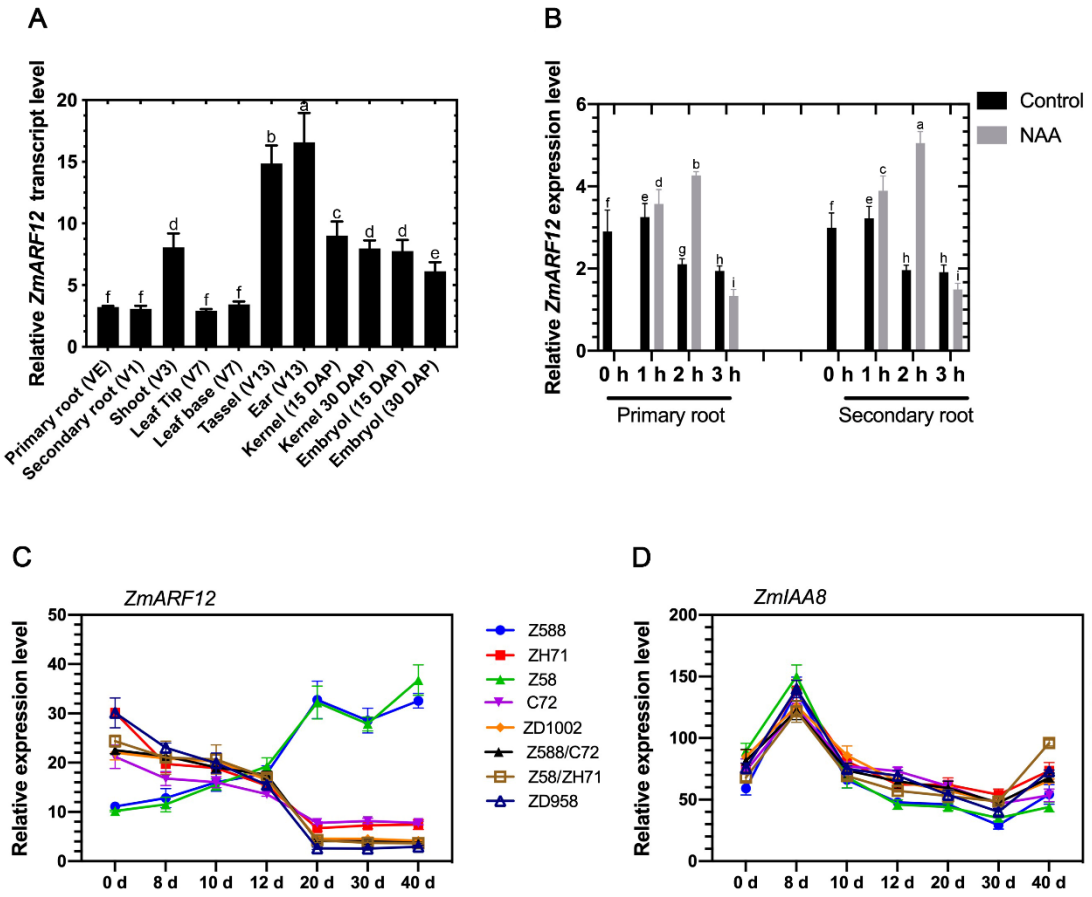
Supplementary Figure 12. Gene networks of hub genes associated with ubiquitin proteins at DAP8–40 stages. Top 10% of genes with weight value >= 0.1 are shown according their weight value with these hub genes. Different colors represent different module. Circle and geometric figures with no colors denote genes are appeared more than two stages or modules. DAP8–40 represents 8–40 d after pollination.



Supplementary Figure 13. Gene networks of hub genes associated with chaperone proteins at DAP8–40 stages. Top 10% of genes with weight value >= 0.1 are shown according their weight value with these hub genes. Different colors represent different module. Circle and geometric figures with no colors denote genes are appeared more than two stages or modules. DAP8–40 represents 8–40 d after pollination.

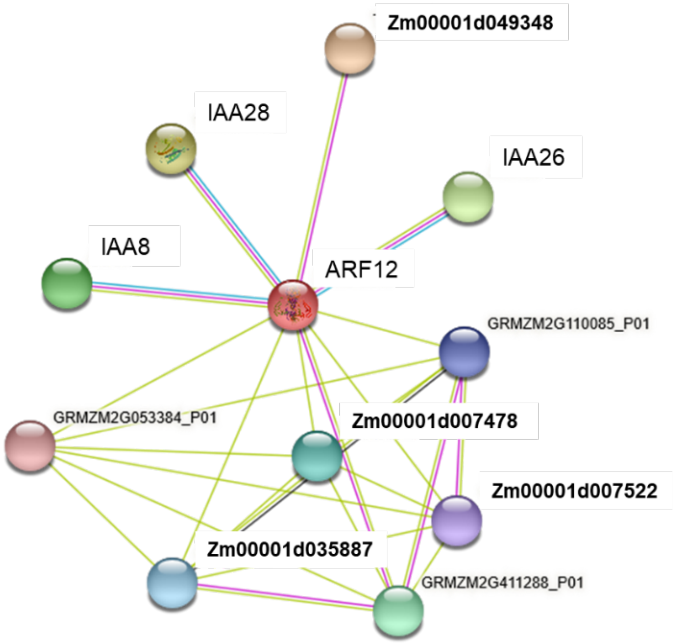


Supplementary Figure 14. Gene networks of hub genes associated with calcium-dependent protein kinases at DAP8–40 stages. Top 10% of genes with weight value >= 0.1 are shown according their weight value with these hub genes. Different colors represent different module. Circle and geometric figures with no colors denote genes are appeared more than two stages or modules. DAP8-40 represents 8–40 d after pollination.

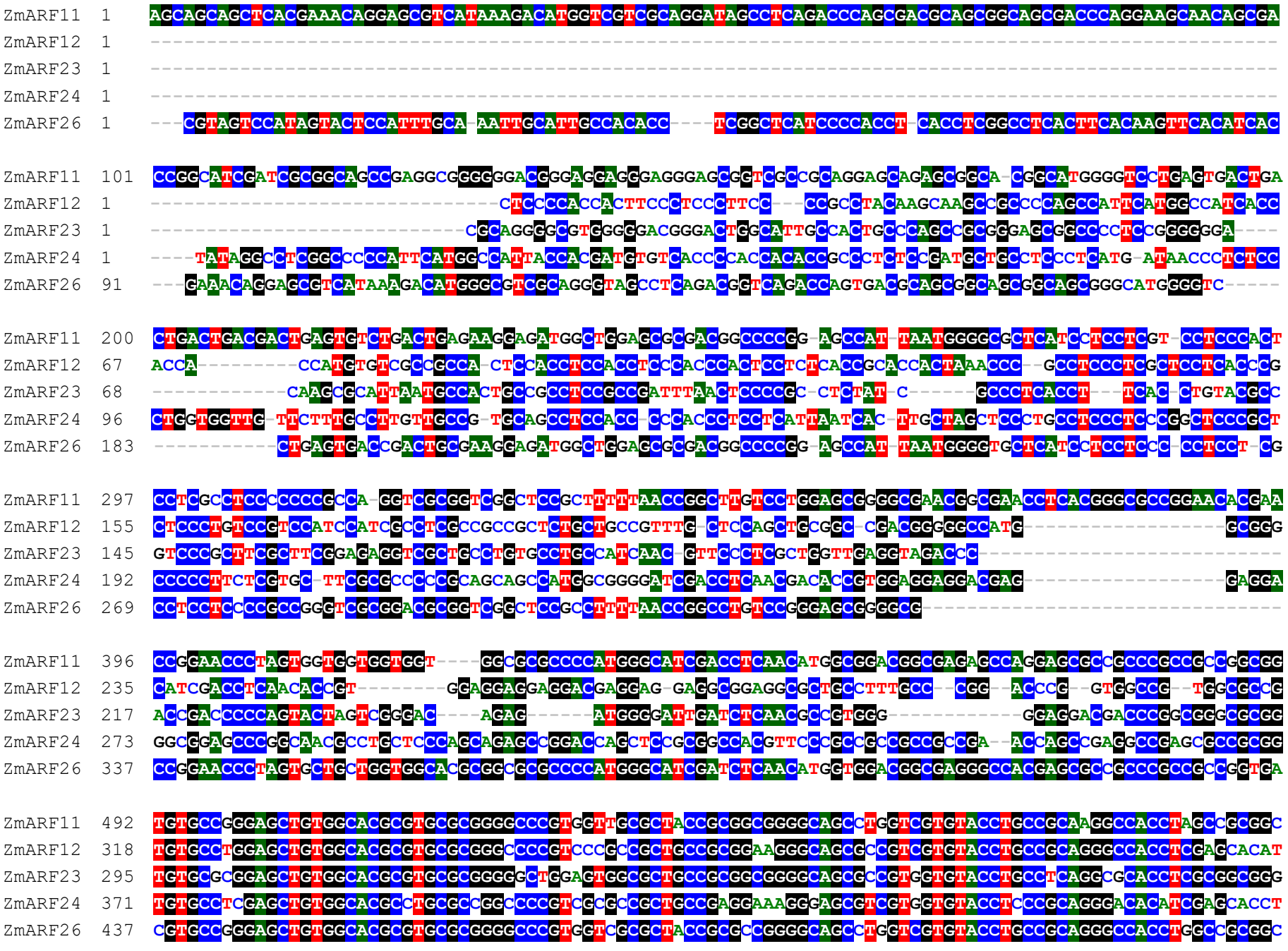


Supplementary Figure 15. The expression levels of *ZmARF12* and its potential interactor in different tissues, under auxin stimuli, and different time points post fertilization. **(A)** Real-time RT-PCR analysis of *ZmARF12* in different tissues. For each sample, the primary root and secondary root was cut from seedlings that had germinated for 8 d (vegetative emergency, VE) and V1 stage, respectively; the shoots were collected from plants at the V3 collar stage; the

leaf tip and leaf base were cut from plants at the V7 growth stage; and the tassel and ear were cut from plants at the V13 stage. Kernels were peeled off the ears at 15 d after pollination (DAP15) and DAP30, and the embryos were excised from the ears at 15 DAP and 30 DAP. **(B)** Fold change of *ZmARF12* conditioning exogenous auxin treatment. The primary and lateral roots of 8-day-old maize seedlings were isolated after 0 h, 1 h, 2 h and 3 h of incubation in 5 μM αNAA and Hoagland's solution control and subjected to real-time PCR analysis. **(C)** and **(D)** The expression profiles of *ZmARF12* and its potential interacting factor *ZmIAA8* during ear fertilization. The kernels of four hybrids (ZD1002, Zhengdan1002; Z588/C72, Zheng588/Chang7-2; Z58/ZH71, Zheng58/ZhengH71; ZD958, Zhengdan958) and their parental inbred lines (Z588, Zheng588; ZH71, ZhengH71; Z58, Zheng58; C72, Chang7-2) were harvested 0 d, 8 d, 10 d, 12 d, 20 d, 30 d, and 40 d after pollination and used for real-time RT-PCR. Relative mRNA abundance of each tested gene was averaged for triplicate reactions and the values were normalized according to the *Ct* of the internal control of ubiquitin gene by using the $2^{-\Delta\Delta Ct}$ method. All bars represent means ± SD (n = 6 repeats). Different letters indicate significant difference ($P < 0.05$) as determined by Tukey-Kramer test.



Supplementary Figure 16. Protein-protein interaction networks of ARF12 revealed by String database. The protein-protein interaction enrichment *P*-value was 5.21E-05.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ZmARF23 1
ZmARF24 1
ZmARF26 1
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ZmARF24 1
ZmARF26 91
ZmARF11 200
ZmARF12 67
ZmARF23 68
ZmARF24 96
ZmARF26 183
ZmARF11 297
ZmARF12 155
ZmARF23 145
ZmARF24 192
ZmARF26 269
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ZmARF12 235
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ZmARF23 295
ZmARF24 371
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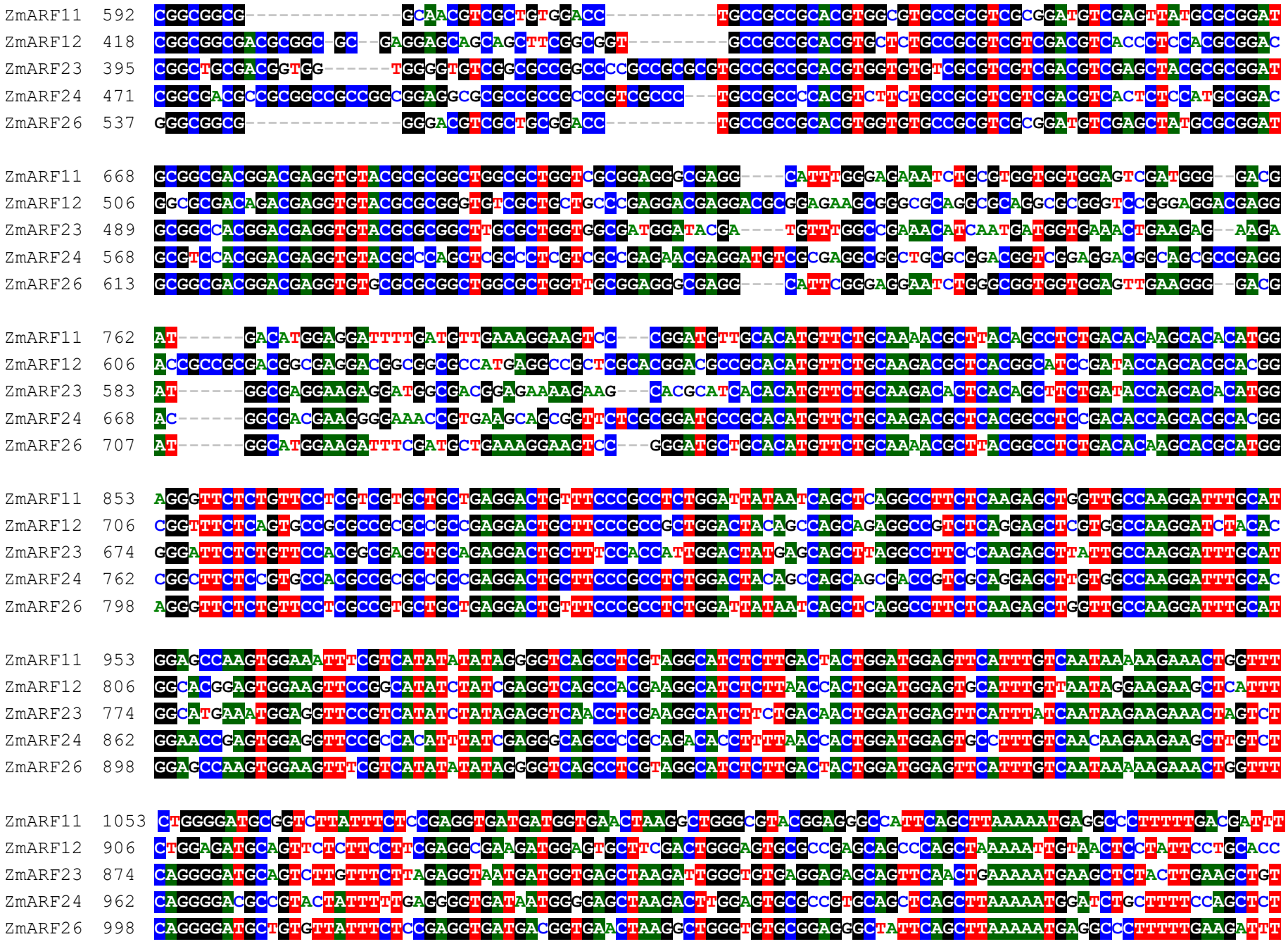
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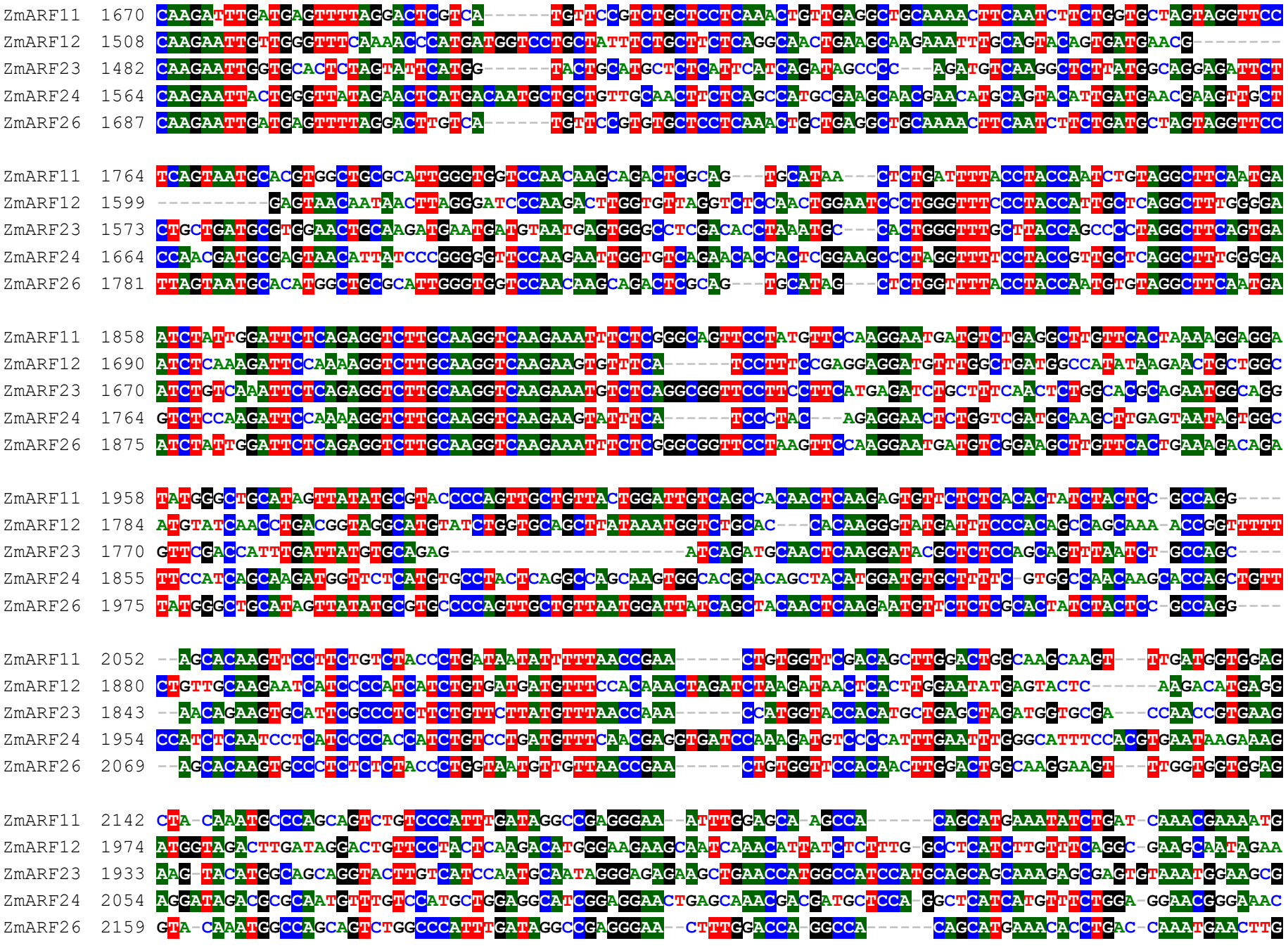
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ZmARF11 2231
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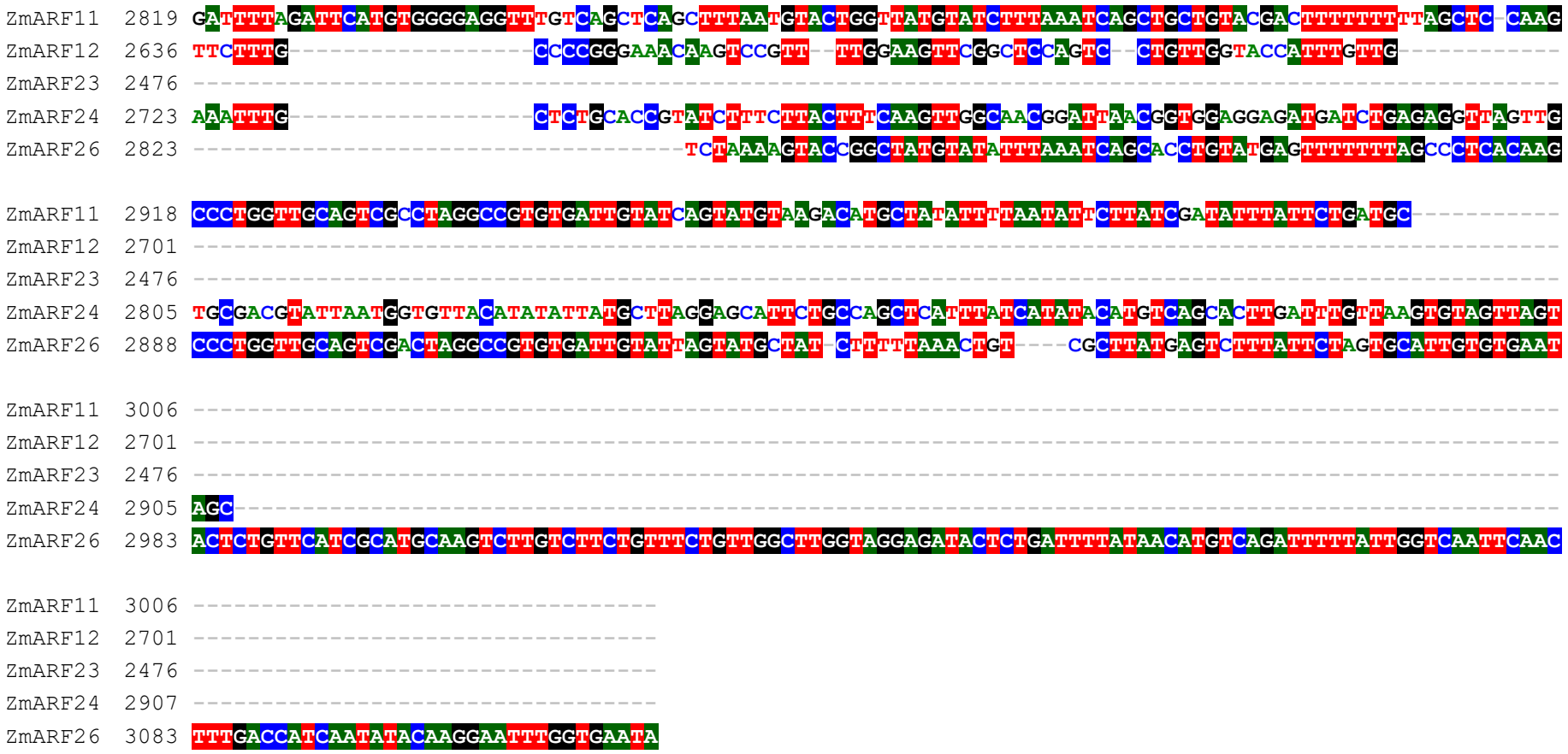
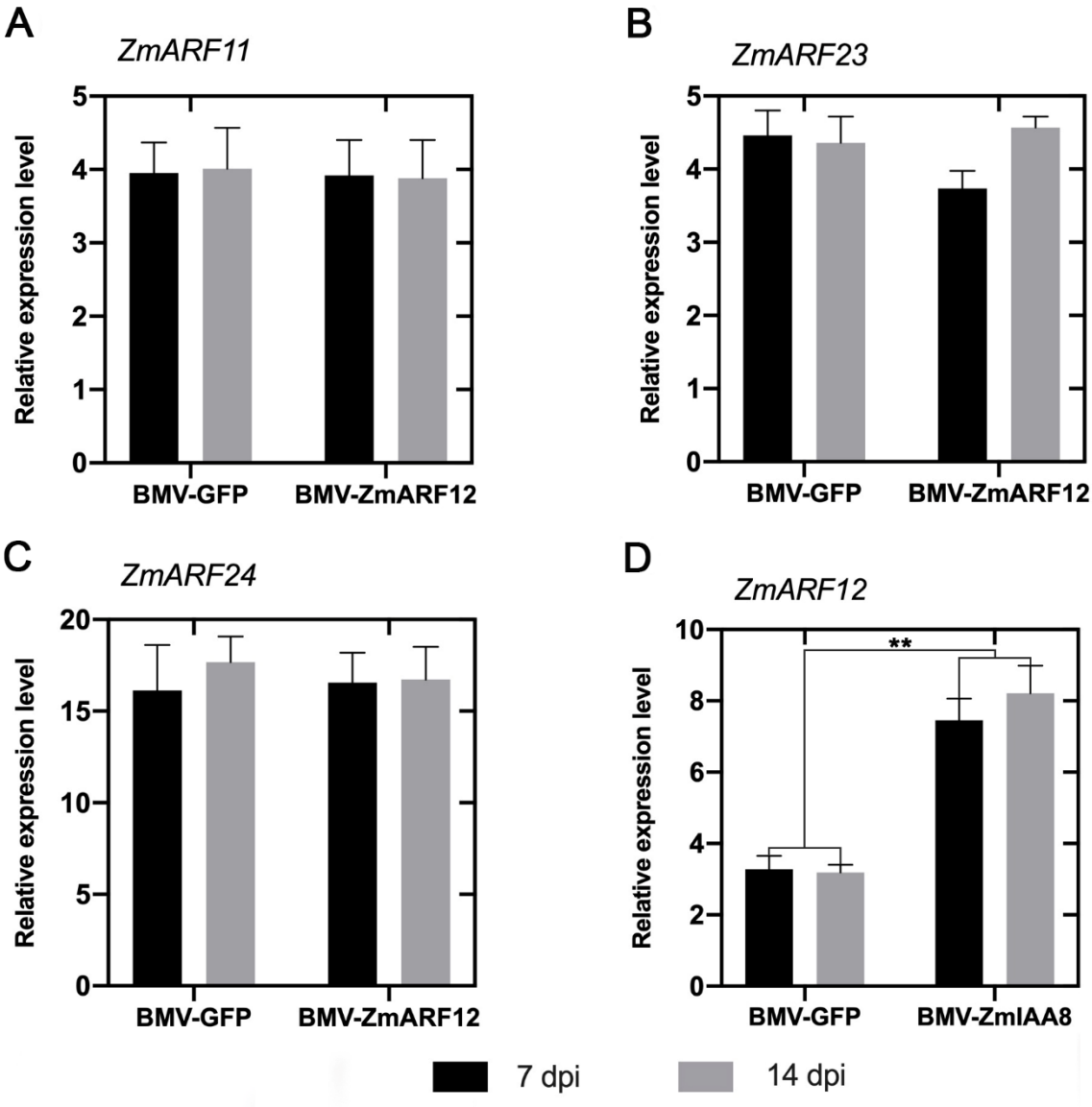
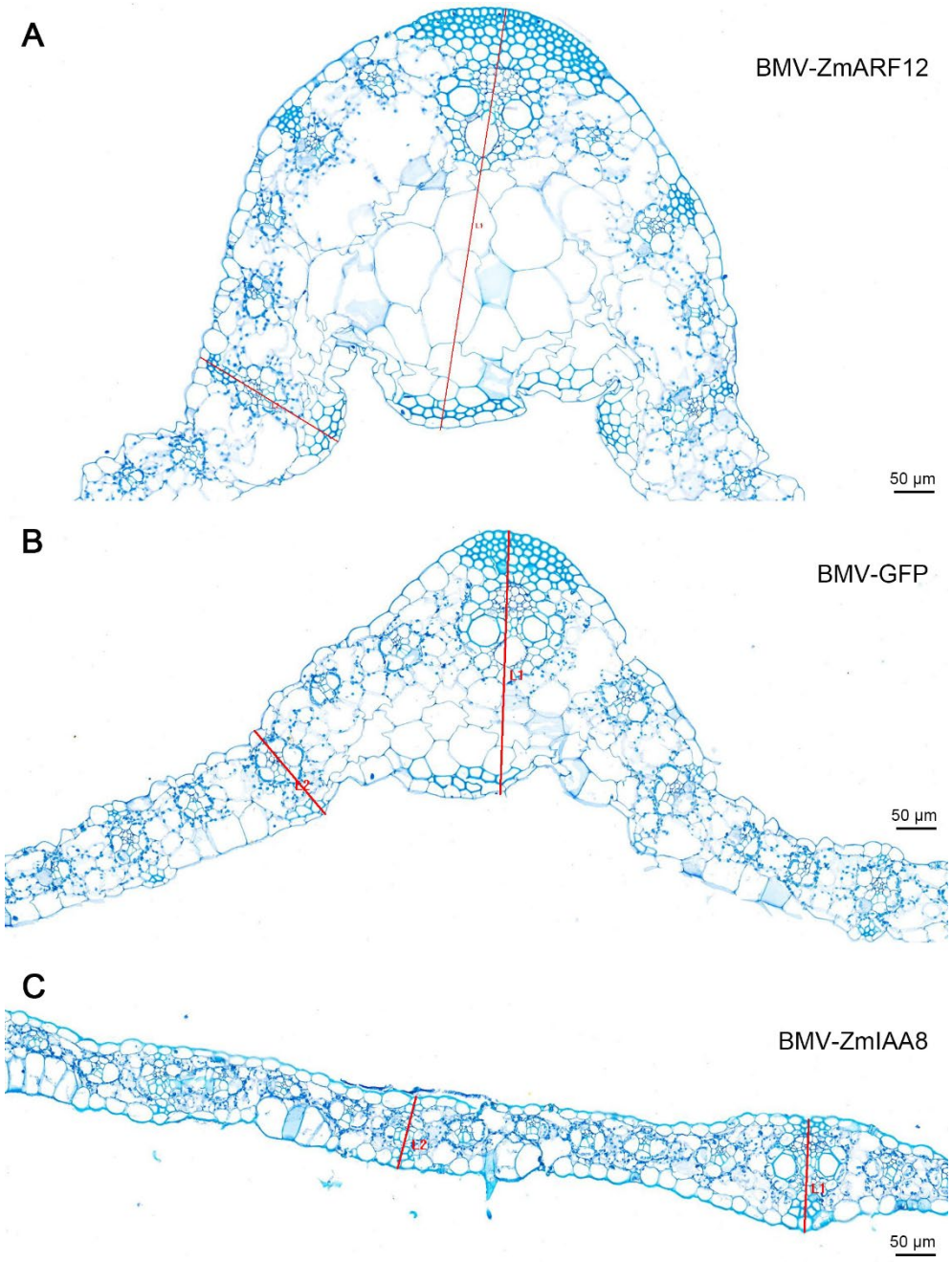


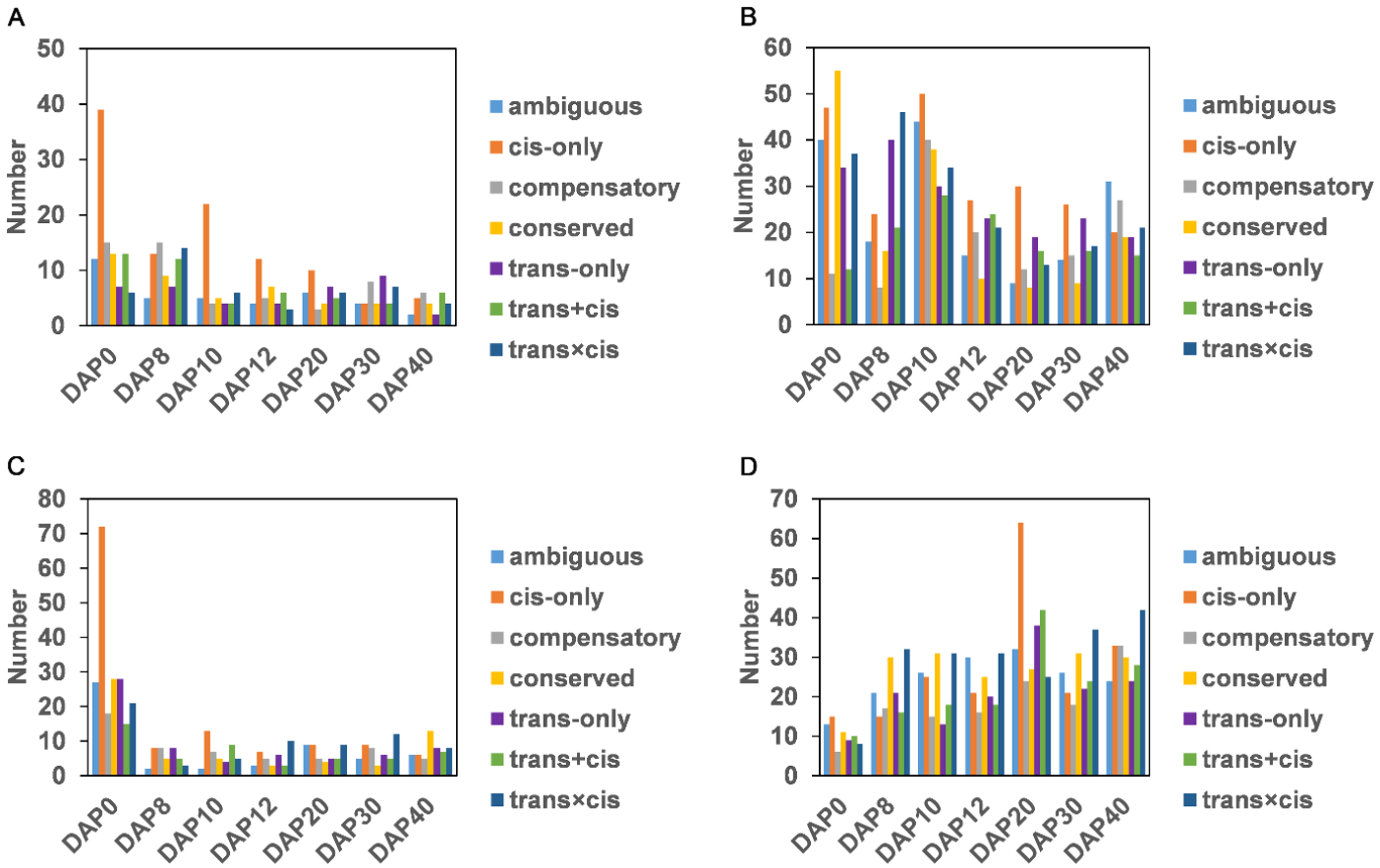
Supplementary Figure 17. The alignment of different *ZmARF* orthologues. The alignment was performed using BioEdit (v7.2.5, http://www.mbio.ncsu.edu/bioedit/bioedit.html). Sequences of *ZmARF* genes analyzed in the alignment were obtained from MaizeGDB. Accessions were as follows: *ZmARF11*, GRMZM2G056120; *ZmARF12,* GRMZM2G437460; *ZmARF23*, GRMZM2G441325; *ZmARF24,* GRMZM2G030710*; and ZmARF26,* GRMZM5G874163.



Supplementary Figure 18. The expression level of *ZmARF11*, *ZmARF12*, *ZmARF23*, and *ZmARF24* in *ZmARF12*- and *ZmIAA8*-silenced maize seedlings. **(A)** to **(C)** The silencing specificity of *ZmARF12* was evaluated by detecting the expression levels of *ZmARF11*, *ZmARF23* and *ZmARF24*, which share high identities with *ZmARF12*, in the relevant systemic leaves using qRT-PCR at 7 days and 14 days post inoculation (dpi), respectively. **(D)** The relative transcript level of *ZmARF12* in the *ZmIAA8* transient knocked-down plants was evaluated by using real-time RT-PCR at 7 dpi and 14 dpi, respectively. Values are means ± SD, * $P < 0.05$, ** $P < 0.01$ (paired Student's *t*-test); ns, not significant.



Supplementary Figure 19. Microscopic analysis of the leaf disk of the *ZmARF12*- and *ZmIAA8*-silenced plants. The leaf disks were dissected from the second systemic leaves above the inoculated leaves of the *ZmARF12* transient silencing, *ZmIAA8*-silenced and control plants, respectively. The cells along the two red lines (L1 and L2) were calculated, respectively. The scale bar = 50 μm.



Supplementary Figure 20. Regulatory category assignments of additive **(A)**, **(C)** and nonadditive **(B)**, **(D)** differentially expressed genes in Zhengdan1002 **(A)**, **(B)** and Zhengdan958 **(C)**, **(D)**. DAP0, DAP8, DAP10, DAP12, DAP20, DAP30, and DAP40 represent 0, 8, 10, 12, 20, 30, and 40 d after pollination, respectively.