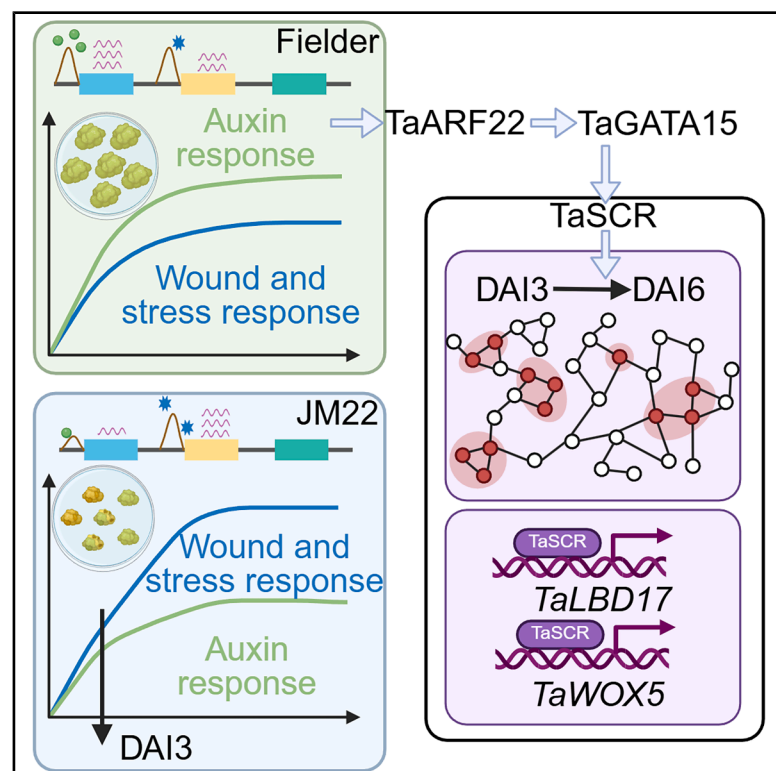


Chromatin accessibility and TaSCR-TaLBD17 circuitry shape genotypic regeneration capacity in wheat

Graphical abstract



Authors

Xiao Min Bie, Yuan Cao, Menglu Li, ..., Mengyi Zhou, Xian Sheng Zhang, Jun Xiao

Correspondence

zhangxs@sda.u.edu.cn (X.S.Z.),
jxiao@genetics.ac.cn (J.X.)

In brief

Bie et al. show that differences in chromatin accessibility, transcriptional regulatory networks, and the resulting gene-expression divergence collectively contribute to genotype-dependent regeneration efficiency in wheat. *TaSCR*, indirectly activated by auxin via *TaARF22* and *TaGATA15*, cooperates with its downstream target *TaLBD17* to promote wheat regeneration.

Highlights

- Transcriptomic and chromatin accessibility dynamics drive wheat regeneration divergence
- Complexity and centrality of transcriptional networks correlate with regenerative capacity
- *TaSCR*, indirectly induced by auxin via *TaARF22*/*TaGATA15*, regulates meristem-related genes
- The *TaSCR*-*TaLBD17* regulatory module promotes regeneration across diverse wheat genotypes



Article

Chromatin accessibility and TaSCR-TaLBD17 circuitry shape genotypic regeneration capacity in wheat

Xiao Min Bie,^{1,5} Yuan Cao,^{2,3,5} Menglu Li,^{1,5} Xuelei Lin,^{2,5} Mei Yu,¹ Xuemei Liu,^{2,3} Ying Song,¹ Xumei Luo,^{2,3} Mengyi Zhou,¹ Xian Sheng Zhang,^{1,*} and Jun Xiao^{2,3,4,6,*}

¹State Key Laboratory of Wheat Improvement, College of Life Sciences, Shandong Agricultural University, Tai'an 271018, China

²Laboratory of Advanced Breeding Technologies, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

³University of Chinese Academy of Sciences, Beijing 100049, China

⁴CAS-JIC Centre of Excellence for Plant and Microbial Science (CEPAMS), Institute of Genetics and Developmental Biology, CAS, Beijing 100101, China

⁵These authors contributed equally

⁶Lead contact

*Correspondence: zhangxs@sdaa.edu.cn (X.S.Z.), jxiao@genetics.ac.cn (J.X.)

<https://doi.org/10.1016/j.celrep.2025.116743>

SUMMARY

Genotype-dependent regeneration efficiency remains a major obstacle in wheat transformation, limiting its use in elite but recalcitrant cultivars. To elucidate the molecular basis of regeneration divergence, we compare transcriptomic profiles, chromatin accessibility dynamics, and transcriptional regulatory networks (TRNs) between the regenerable variety Fielder and the recalcitrant variety JiMai22 (JM22). Fielder displays dynamic transcriptional reprogramming and chromatin remodeling between 3 and 6 days after induction, activating morphogenic regulators such as *TaSCR*, *TaWOX5*, and *TaLBD17*. In contrast, JM22 shows limited chromatin changes and transcriptional stagnation, primarily inducing stress-responsive pathways. TRN analysis reveals a more complex, regeneration-focused network in Fielder, enriched with AP2, Dof, and GRAS transcription factor families. Among these, *TaSCR* is identified as a key regulator. Functional assays demonstrate that the *TaSCR*-*TaLBD17* regulatory cascade improves transformation efficiency across diverse wheat genotypes. These findings provide a molecular framework to overcome transformation barriers in recalcitrant cultivars.

INTRODUCTION

Plants possess remarkable regenerative potential largely attributed to the totipotency of plant cells, which enables the regeneration of whole plants under *in vitro* conditions.^{1,2} The process of plant regeneration typically involves two key stages: callus formation and shoot regeneration. Plant tissue culture is a fundamental tool widely used in genetic transformation and genome-editing applications.³ High regeneration efficiency is a fundamental prerequisite for achieving efficient genetic transformation.^{4,5} Achieving high regeneration efficiency is essential for functional genomics research and for generating genetically modified plants.⁶ However, many cereal crops, including wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and indica rice (*Oryza sativa* L. subsp. *Indica*), exhibit low regeneration capacity, making transformation particularly challenging.^{6–8} The major constraints include low regeneration efficiency, strong genotype dependency, and limited explant compatibility, which collectively hinder advancements in crop transformation.^{9,10}

The first transgenic wheat was successfully generated in the 1990s,¹¹ but the development of wheat transformation technol-

ogies has been notably slow compared to other crops.^{12,13} In recent years, however, marked progress has been achieved, particularly with the PureWheat technique, which has increased transformation efficiency in the wheat variety Fielder to 50%–90%.^{14,15} Several key regeneration factors that enhance wheat transformation efficiency have been identified, including the peptide TaREF1, the GRF4-GIF1 chimeric protein, and transcription factors (TFs), such as TaWOX5, TaDOF3.4, TaDOF5.6, and TaLAX1.^{16–20} Despite these advances, genotype dependency remains a major obstacle, particularly for difficult-to-transform wheat elite varieties such as JiMai22 (JM22), KeNong199 (KN199), and AiKang58 (AK58).^{5,19} Moreover, the effectiveness of the known regeneration factors varies among wheat varieties, indicating the need for further research into the molecular mechanisms governing variety-dependent regeneration efficiency discrepancies.

Plant regeneration is a highly complex process, involving intricate genetic interaction networks, epigenetic modifications, and tissue culture conditions, necessitating more comprehensive approaches to fully understand its regulation.^{3,21,22} Recently, multi-omics strategies have been instrumental in uncovering



the genetic and epigenetic basis of abiotic stress resilience,²³ disease resistance,²⁴ crop yield and grain quality,^{25,26} offering a novel strategy for the identification of key regulators and providing targets for precision breeding and genome editing.

In particular, the integration of RNA sequencing (RNA-seq), assay for transposase-accessible chromatin using sequencing (ATAC-seq), and chromatin immunoprecipitation sequencing (ChIP-seq) elucidated that auxin and cytokinin modulate chromatin accessibility dynamics, thereby orchestrating necessary gene activation/repression during shoot regeneration and somatic embryogenesis.^{27,28} Motif analysis of accessible chromatin regions identified that stage-specific TFs participated in distinct phases of regeneration.^{27,28} In addition, single-cell RNA-seq unveiled that the middle cell layer of callus specifically expresses quiescent center marker genes such as *WOX5*, *SCR*, and *PLT1/2* and is capable of fate transition to form shoots.²⁹ Previously, we employed time-course RNA-seq, ATAC-seq, and cleavage under targets and tagmentation (CUT&Tag) to analyze chromatin landscape dynamics during regeneration in the wheat variety Fielder, constructing a transcriptional regulatory network (TRN) governing cell fate transition for shoot regeneration, and identified 446 core TFs in Fielder regeneration.¹⁹ This provided key molecular insights into the regulation of wheat regeneration. However, the divergence in TRNs mediating varied regeneration efficiencies among different wheat varieties remains largely unexplored. Understanding how these differences contribute to regeneration efficiency disparities is crucial for optimizing wheat transformation strategies.

In this study, we compared gene expression profiles, chromatin accessibility dynamics, and TRNs in Fielder (regenerable) and JM22 (recalcitrant). Fielder showed early and dynamic activation of gene expression and chromatin remodeling during regeneration, initiating the expression of key regulatory genes such as *TaSCR*, *TaWOX5*, and *TaLBD17*. JM22, however, showed minimal transcriptional and chromatin changes, with a predominance of stress-related gene expression. The transcriptional network in Fielder was enriched for regeneration-associated TFs, especially from the AP2, Dof, G2-like, and GRAS families. Among them, *TaSCR*, a Fielder-specific highly expressed core TF, boosts regeneration by activating meristem-related genes. Furthermore, the *TaSCR*-*TaLBD17* module enhanced transformation efficiency across diverse wheat varieties, offering a strategy to overcome genotype-dependent recalcitrance.

RESULTS

Transcriptomic profiling reveals distinct molecular trajectories underlying regeneration in Fielder and JM22

To elucidate the molecular basis underlying the divergent regeneration capacities of wheat varieties Fielder and JM22, we performed comparative transcriptomic analysis at five critical time points during tissue culture: 0, 3, 6, 9, and 12 days after induction (DAI0, DAI3, DAI6, DAI9, and DAI12) (Figure 1A; Table S1). Principal component analysis (PCA) revealed a dynamic transcriptional trajectory in Fielder, while JM22 exhibited minimal transcriptional progression beyond DAI3. Notably, JM22 showed transcriptional stagnation between DAI3 and

DAI6, indicating a potential developmental arrest impeding regeneration (Figure 1A).

Differential expression analysis identified 23,313 to 26,269 differentially expressed genes (DEGs) between the two cultivars from DAI0 to DAI12, with the DEG count increasing from DAI3 to DAI12 (Figure 1B). We focused on 37,045 DEGs that showed dynamic expression in Fielder and had transcript abundance > 1 transcripts per million (TPM) in either genotype during regeneration (Figure 1C). *k*-means clustering grouped these genes into eight expression clusters (Figure 1D). Clusters C1 and C2 were enriched for genes highly expressed in the scutellum of immature embryos, such as *TaABI4*, *TaZAT10*, *TaSUS2*, and *TaSEI2*, involved in stress responses, starch biosynthesis, and lipid storage.^{30–33} Clusters C3, C4, and C5 were preferentially upregulated in Fielder during key developmental windows—DAI3, DAI6–12, and DAI9–12, respectively—and contained known regeneration regulatory genes such as *TaDOF5.6*, *TaWOX11*, *TaWOX5*, *TaSHR*, and *TaPIN1*.^{18,19,34–36} Genes in these clusters were enriched in rRNA processing, cell cycle, auxin signaling, and meristem development (Figure 1D). Meanwhile, clusters C6–C8 were dominated by JM22-biased genes and associated with stress-related processes, including cold acclimation, jasmonic acid biosynthesis, and programmed cell death (Figure 1D).

Both cultivars underwent pronounced transcriptional reprogramming from DAI0 to DAI3 (Figure 1A). However, from DAI3 to DAI6, Fielder continued to show marked transcriptional shifts, whereas JM22 remained largely static (Figure 1A). This stage-specific activation of cluster C4 genes (*n* = 4,801) highlights their potential importance in regeneration. Cluster C4 included numerous established regeneration regulatory genes, such as *TaWOX5*, *TaARF7*, *TaJMJ30*, and *TabHLH41*.^{18,37–39} (Figure 1D), along with 53 TFs previously identified among Fielder's core regulators,¹⁹ including *TaPLT5*, *TaPLT1*, *TaERF060*, and *TaSCR* (Figure 1E).

To explore the potential genetic drivers of these expression differences, we examined the coding sequence (CDS) and promoter variations in cluster C4 genes.^{40,41} Of the 4,801 genes, 36—including *TaCYCA;1*—were absent in the JM22 genome (Figure S1A). Among the remaining genes, over 93.14% shared ≥95% protein sequence similarity between cultivars (Figure S1B), suggesting differential gene regulation rather than altered protein function as the primary contributor to phenotypic divergence. Promoter analysis revealed that 34.19% of cluster C4 genes exhibited *cis*-motif variations, with greater motif divergence generally correlating with larger expression differences (Figure S1C). Notably, promoters in Fielder were enriched for binding motifs of ERF, Dof, and WRKY TF families, while JM22 promoters more frequently contained motifs recognized by NAC and bZIP TFs (Figure 1F). For instance, *TaPUP12*, *TaLUX*, and *TaPPO*, in Fielder harbored binding sites for DOF1.5 and DOF1.7; and *TaERS2*, *TaASL18*, and *TaAREB2* contained binding motifs for ERF013 and ERF015, while these motifs were absent in JM22 (Figure 1G).

In summary, Fielder's superior regeneration capacity is associated with timely transcriptional activation of developmental regulators following DAI3, whereas JM22 shows transcriptional stalling and stress-associated gene expression. These

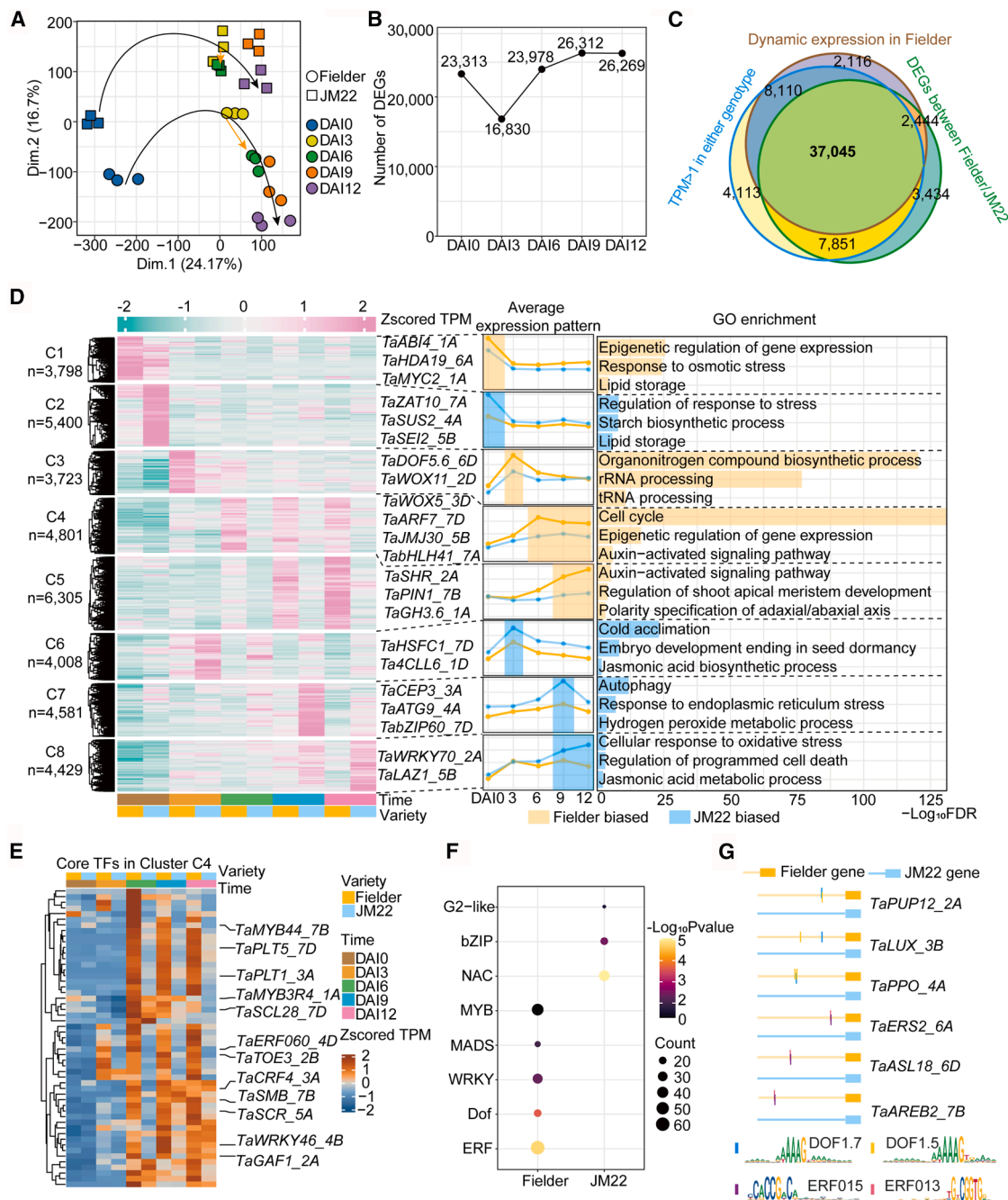


Figure 1. Atlas of differential expression genes between Fielder and JM22 during regeneration

(A) PCA of RNA-seq datasets of Fielder and JM22. Black and orange arrows indicate transcriptional trajectories from DAI0 to DAI12 and DAI3 to DAI6, respectively.

(B) DEG number dynamic between Fielder and JM22 from DAI0 to DAI12.

(C) Venn diagram showing the overlap among genes with dynamic expression during Fielder regeneration (brown), DEGs between Fielder and JM22 (green), and genes with TPM > 1 in either cultivar (blue).

(D) *k*-means clustering of 37,045 genes in (C) (left), and the bottom annotations are identical to those in (E). The expression patterns of genes in 8 clusters (middle) and Gene Ontology (GO) enrichment for each cluster (right).

(E) Heatmap showing the expression patterns of Fielder's core TFs within cluster C4.

(F) TF family enrichment of motifs preferentially occurring in Fielder and JM22 promoters. "Count" indicates gene number.

(G) DOF1.5, DOF1.7, ERF013, and ERF015 have putative specific binding sites in the promoter of genes from Fielder but not JM22.

p values were determined by Fisher's exact test (D) and hypergeometric statistical test (F) with false discovery rate (FDR) correction (D).

differences are primarily attributed to regulatory divergence at the gene expression level, likely in promoter architecture, rather than changes in protein CDSs.

Differentially accessible chromatin regions correlate with transcriptional and regeneration divergence between Fielder and JM22

While sequence variation in *cis*-regulatory elements partially explains gene expression differences between Fielder and JM22, it cannot account for the extensive transcriptional divergence observed (Figure S1C). Given the importance of chromatin accessibility in regulating transcription during regeneration,^{19,27,28} we performed ATAC-seq on JM22 samples from DAI0, DAI3, DAI6, and DAI9 and compared them with the corresponding Fielder data.

PCA revealed major chromatin remodeling from DAI0 to DAI3 in both varieties, moderate changes from DAI3 to DAI6, and minimal shifts thereafter (Figure 2A), indicating DAI3 and DAI6 as critical transition points. Inter-varietal differences in chromatin accessibility became more pronounced than intra-varietal temporal changes beyond DAI3 (Figure S1D), suggesting that the chromatin state divergence between the two genotypes intensifies over time. Across all samples, we identified 743,379 accessible chromatin regions (ACRs), the majority of which are located in intergenic regions, followed by promoters, gene bodies, and downstream sequences; among these, 222,065 are Fielder specific (Figure S1E). Comparative analysis revealed 20,320 to 26,515 (51,094 merged) differentially accessible chromatin regions (diffACRs) in proximal regions and 43,087 to 59,298 (101,297 merged) in distal regions from DAI0 to DAI9 between Fielder and JM22 (Figures 2B and S1F). The number of diffACRs increased substantially from DAI3 to DAI6, coinciding with the observed transcriptional divergence (Figures 1B, 2B, and S1F; Tables S1 and S2).

To link diffACRs to transcriptional regulation, we calculated Pearson correlation coefficients between ACR accessibility and gene expression, and focused on significantly (p value < 0.05) correlated gene-ACR pairs (Figure S1G).⁴² Of the proximal diffACRs, 13,385 showed correlations with 9,920 nearby genes, 8,121 (81.9%) of which were DEGs. Among distal diffACRs, 48,681 were correlated with 33,602 genes within 3 kb to 0.5 Mb, and 21,978 (65.4%) of these genes were DEGs (Figures 2C and S1H; Table S3). *k*-means clustering of proximal diffACRs revealed five distinct clusters (K1–K5) (Figure 2D): cluster K2, enriched in Fielder-specific peaks and regulated genes related to auxin signaling and meristem development (*TaLBD16*, *TaPLT1*, *TaWOX5*, and *TaSHR*^{18,39,43,44}) (Figure 2D). In contrast, clusters K4 and K5, highly accessible in JM22, were linked to genes involved in abiotic stress responses, including cold acclimation and abscisic acid (ABA) signaling. Similar patterns were observed for distal diffACRs (Figure S1I). These findings support a functional connection between chromatin accessibility and gene expression divergence during regeneration.

To further elucidate the chromatin accessibility differences of DEGs associated with auxin and stress responses, we extracted DEGs annotated with “auxin” or “stress” according to GO terms for further analysis. Approximately 25% of auxin-related DEGs and 18% of stress-related DEGs were regulated by ACR

(Figures S2A and S2B). Notably, ACR-regulated auxin-related DEGs were predominantly distributed in cluster K2, whereas stress-related DEGs were distributed across clusters K1–K5 (Figure S2C), even though the target genes of cluster K2 were not enriched for stress-related processes (Figure 2D). This suggests that Fielder also activates stress response pathways during regeneration, and the balance between auxin and stress signaling contributes to its high regeneration capacity, whereas JM22 appears to activate stress responses alone. For example, the auxin-related genes *PIN1_6B*, *IAA18_3D*, and *ARF8_2B* are all located within cluster K2 and specifically expressed in Fielder (Figures S2D and S2F–S2H). In contrast, *RHS18_1D*, involved in response to oxidative stress, is specifically activated in Fielder (cluster K2), whereas *SEP2_2B*, associated with response to abiotic stimulus, is specifically activated in JM22 (Figures S2E–S2H).

To identify candidate regulators of proximal diffACRs, we performed *cis*-motif enrichment analysis. Cluster K2 was particularly enriched for binding motifs from TF families implicated in regeneration, such as HD-ZIP, WOX, GRAS, and Dof (Figure 2E). Several TFs within these TF families, such as *TaWOX5*, *TaDOF5.6*, and *TaDOF3.4*,^{18,19} have been shown to promote regeneration in wheat. Notably, we identified a Fielder-specific ACR within the promoter of *TaWOX5*, a key regulator of wheat regeneration,¹⁸ containing a GRAS TF-binding motif (Figure 2F). Luciferase reporter assays demonstrated that the GRAS family TF *TaSCR* activates the *pro-TaWOX5::LUC* vector in tobacco (*Nicotiana benthamiana* L.) leaves (Figure 2G). Quantitative reverse-transcription PCR (RT-qPCR) revealed that overexpression (OE) of *TaSCR* led to the elevated expression of *TaWOX5* in four cultivars—Fielder, JM22, KN199, and AK58 (Figure 2H). This result further supports the role of *TaSCR* in activating *TaWOX5* expression and highlights the conserved function of the *TaSCR*-*TaWOX5* regulatory module across different wheat genetic backgrounds.

Collectively, these results suggest that variety-specific chromatin accessibility underpins the differential activation of regeneration programs in Fielder and JM22. Distinct TF-binding shaped by ACRs contribute to the transcriptional and phenotypic divergence in regeneration capacity between the two cultivars.

Regulatory network analysis reveals TF footprint divergence and core regulatory hubs in Fielder and JM22

To explore TF binding divergence between Fielder and JM22, we constructed a TRN for JM22 based on TF footprints—defined as TF-bound regions within accessible chromatin regions—and compared it with the TRN of Fielder.¹⁹ The JM22 TRN comprises 1,782 TFs and 33,000 target genes, encompassing a total of 7,553,706 regulatory interactions. While comparable in scale (8,856,326 in Fielder), the JM22 TRN exhibits 14.7% lower regulatory complexity than Fielder’s network (Figures 3A and S3A).¹⁹ As previously observed,²⁵ TFs from the same family often share targets, and genes with similar expression profiles tend to be co-regulated (Figures 3A and S3A). Genes highly expressed at DAI0 form a self-regulatory module within cluster J3. Genes with dynamic expression across regeneration stages follow a sequential regulatory cascade (J2 → J6 → J4 → J1 → J5), reflecting the temporal progression of regeneration (Figures 3B and S3A).

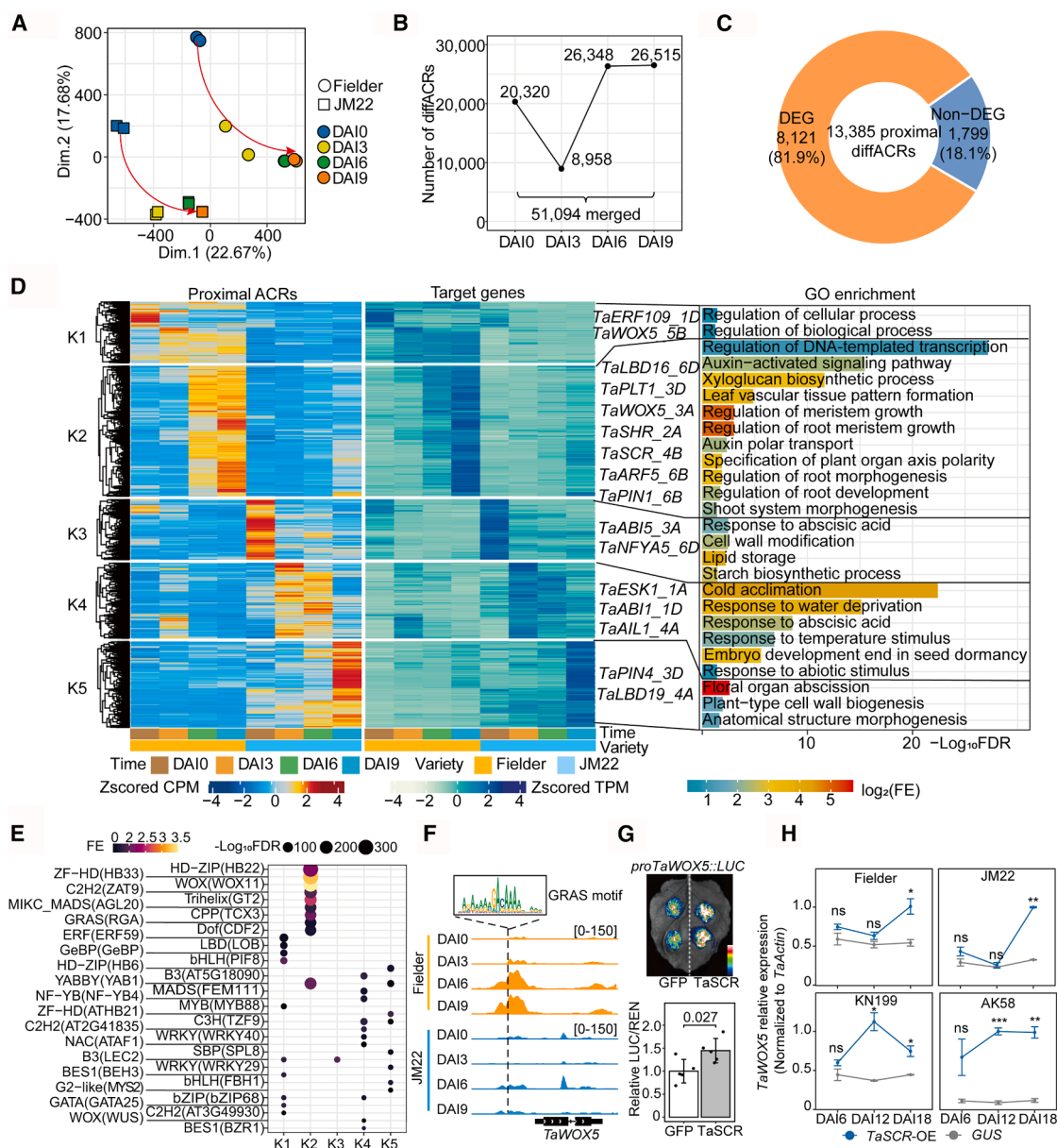


Figure 2. Differential chromatin accessibility contributes to gene expression differences between Fielder and JM22

(A) PCA of ATAC-seq profiles from Fielder and JM22 across regeneration stages. Red arrows indicate chromatin accessibility dynamics trajectories from DAI0 to DAI9.

(B) Line plot showing the number of diffACRs in proximal regions between Fielder and JM22.

(C) Pie chart showing the proportions of DEGs/non-DEGs between Fielder and JM22 among targets of proximal expression-related diffACRs.

(D) *k*-means clustering of proximal diffACR in Fielder and JM22 (left). Heatmap showing the expression of genes regulated by proximal diffACRs (middle). GO enrichment of genes regulated by different clusters of proximal diffACRs (right). FE, fold enrichment.

(E) *Cis*-motif enrichment analysis of diffACR in different clusters shown in (D). FE, fold enrichment.

(F) ATAC-seq track of *TaWOX5* and the position of GRAS family motif in Fielder and JM22.

(G) Luciferase reporter assays indicating that TaSCR activates *TaWOX5* ($n = 5$).

(H) Relative *TaWOX5* expression levels in the callus of Fielder, KN199, JM22, and AK58 transformed with *GUS* or *TaSCR*-OE vector ($n = 3$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; and ns, not significant ($p > 0.05$).

p values were calculated by Fisher's exact test (D and E) and Student's *t* test (G and H) with FDR correction (D and E). Data are mean $\pm$ SD (G and H).

From the network of JM22, we identified 375 core TFs in JM22 that play central roles in regulating regeneration-associated genes and compared them with Fielder's core TFs. Notably,

core TFs of Fielder are highly enriched in AP2, ERF, HD-ZIP, Dof, G2-like, and NAC families, while JM22 lacks core TFs from several key families involved in regeneration, including

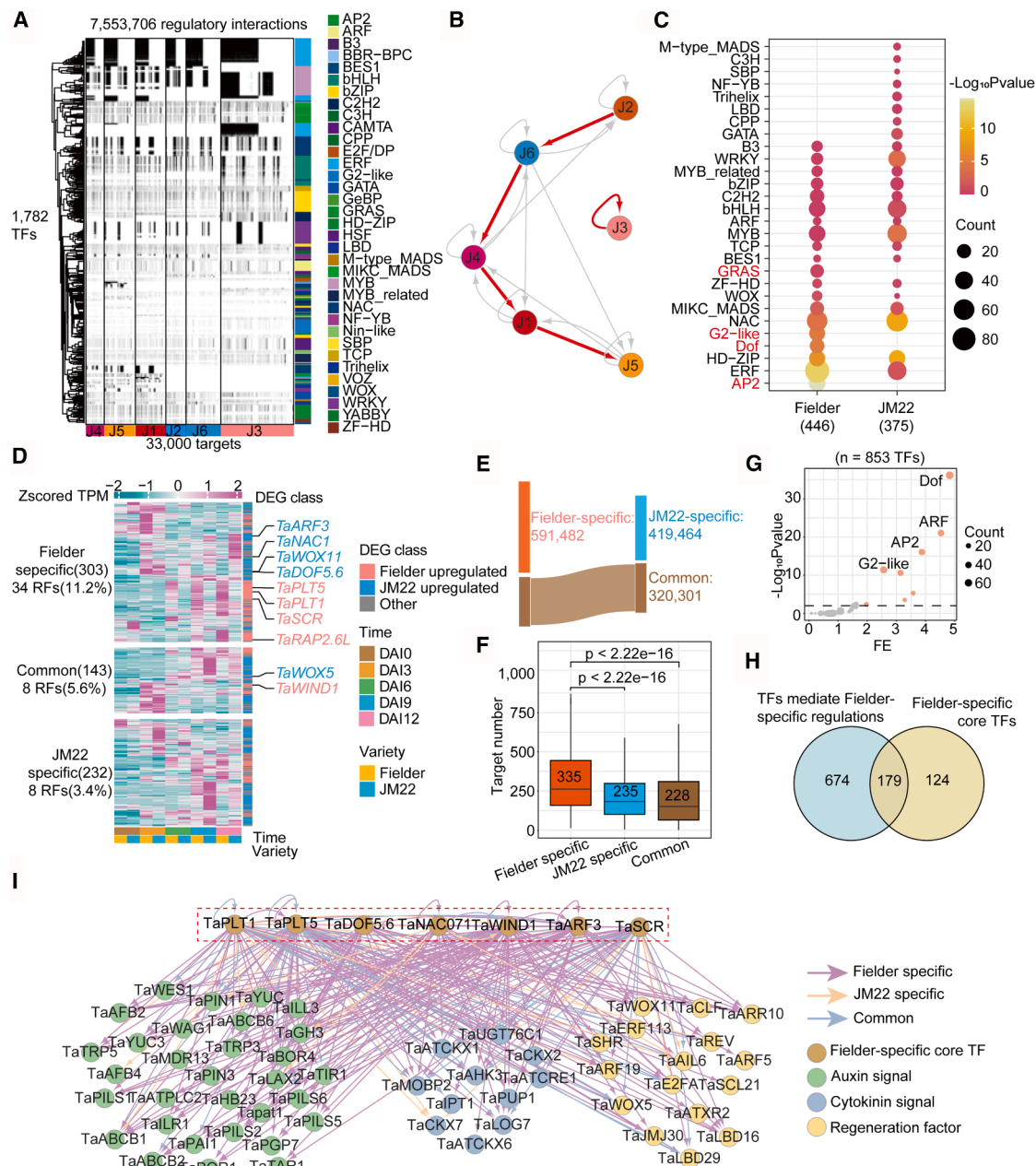


Figure 3. Reconstruction of the JM22 transcriptional regulatory network and its comparison with the TRN of Fielder

(A) TRN of JM22, rows represent TFs and columns represent target genes. Black cells indicate a regulatory relationship, and white cells indicate none.

(B) Regulatory relationships among clusters in Figure S3.

(C) Comparison between core TFs of Fielder and JM22. "Count" indicates gene number.

(D) Expression pattern of core TFs of Fielder and JM22. "Fielder upregulated"/"JM22 upregulated" indicate higher expression in each, and "Other" indicates variable higher expression at different times.

(E) Numbers of Fielder-specific, JM22-specific, and common regulatory relationships targeting TFs, cell cycle-related genes, hormone pathway genes, and epigenetic factors.

(F) Boxplot of target gene numbers per TF (target to TFs, cell cycle-related genes, hormone pathway genes, and epigenetic factors).

(G) TF family enrichment of TFs mediating Fielder-specific regulatory relationships. "Count" indicates gene number.

(H) Venn diagram showing the overlap between TFs mediating Fielder-specific relationships and Fielder-specific core TFs.

(I) Subnetwork comprising TaPLT1, TaPLT5, TaDOF5.6, TaNAC071, TaWIND1, TaARF3, and TaSCR; from 174 TFs in (H), targeting auxin, cytokinin pathway genes, and regeneration factors.

p values were calculated by Fisher's exact test (B), hypergeometric test (C and G), and two-tailed Wilcoxon test (F).

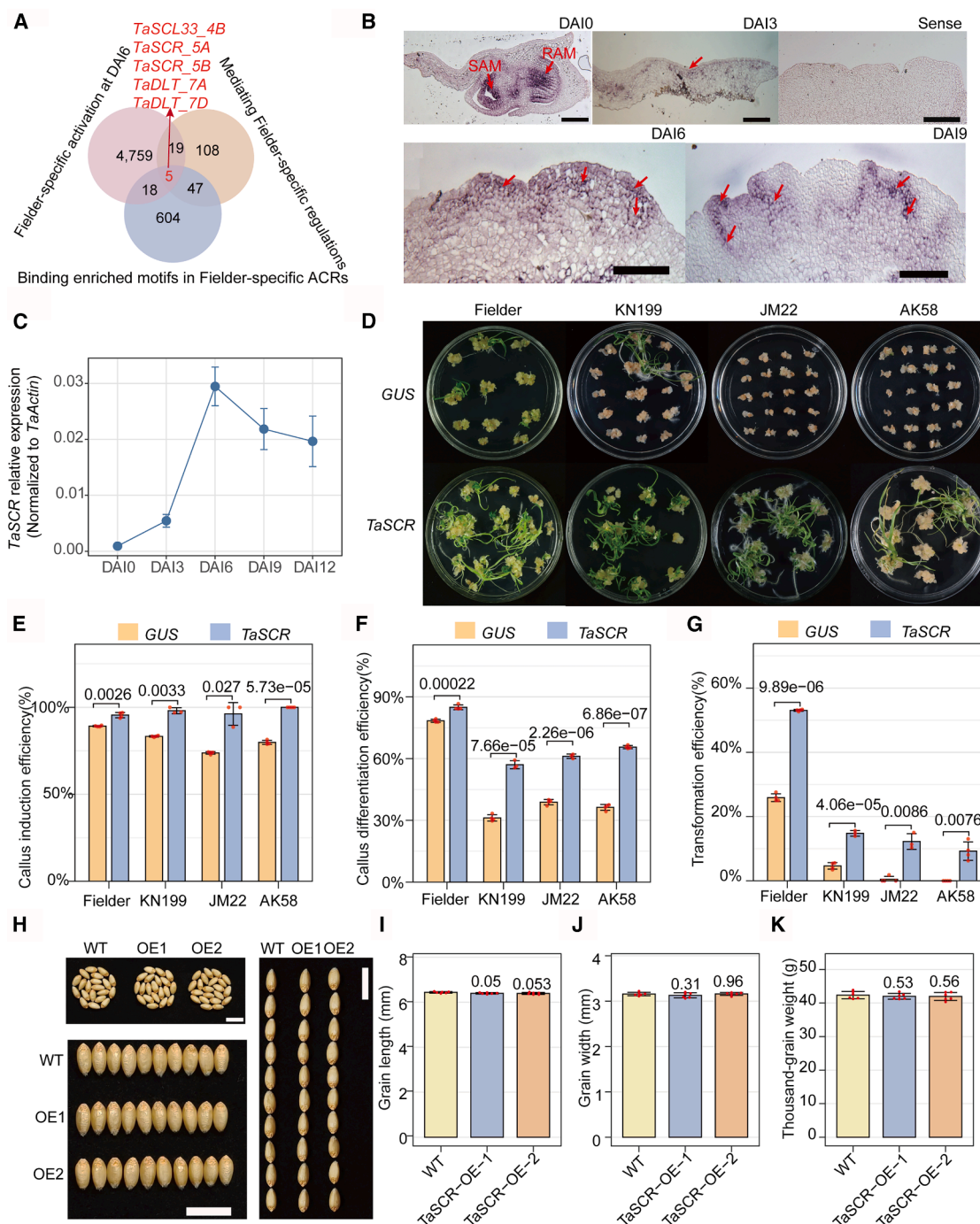


Figure 4. Overexpression of *TaSCR* improves transformation efficiency across diverse wheat varieties

(A) Venn diagram showing overlap of genes mediating Fielder-specific regulatory relationship in the TRN, having potential binding sites for the top 10 enriched motifs in Fielder-specific ACRs and specifically activated in Fielder at DAI6.

(B) *In situ* hybridization of *TaSCR* at DAI0, DAI3, DAI6, DAI9, and DAI9 sense control. SAM, shoot apical meristem; RAM, root apical meristem. Scale bars, 250 μ m. Red arrows indicate expression positions.

(C) Expression levels of *TaSCR* in Fielder analyzed by RT-qPCR ($n = 3$).

(D) Representative photographs showing *TaSCR* functional validation.

(legend continued on next page)

AP2, Dof, G2-like, and GRAS (Figure 3C).^{19,22} In addition, Fielder-specific core TFs comprise a higher proportion of known regeneration factors (such as TaWOX11, TaDOF5.6, and TaPLT1/5)—11.2% compared to 5.6% in common core TFs and only 3.4% in JM22-specific core TFs (Figure 3D). Importantly, genotype-specific upregulated DEGs are not necessarily associated with genotype-specific core TFs. For example, *TaARF3* and *TaWOX11* exhibit higher expression in JM22 but are identified as Fielder-specific core TFs, indicating that centrality within the network—rather than expression abundance—appears to be the defining feature of functional significance for these TFs (Figure 3D).

We next examined regulatory interactions specifically targeting genes involved in transcriptional regulation, hormone signaling, cell cycle, and epigenetic pathways, which are closely associated with plant developmental processes.^{45–48} Fielder exhibited the greatest number of unique interactions (591,482), followed by JM22 (419,464), with 320,301 interactions shared between the two networks (Figure 3E). Moreover, individual TFs regulated more targets in Fielder-specific interactions (mean = 335 targets) than their JM22 (mean = 235 targets) and common (mean = 228 targets) counterparts, suggesting higher regulatory reach (Figure 3F). The same conclusion applies to the total regulatory interactions. Fielder exhibited the highest number of specific interactions (5,947,577), followed by JM22-specific (4,644,957) and shared interactions (2,908,749) (Figure S3B). In Fielder-specific interactions, each TF regulated more target genes on average (mean = 3,368) than in JM22-specific (mean = 2,607) and common (mean = 2,070) interactions (Figure S3C).

To pinpoint key TFs disproportionately contributing to Fielder-specific regulatory interactions, we applied Fisher's exact test and identified 853 significantly (p value < 0.005) enriched TFs, predominantly from the Dof, auxin response factor (ARF), AP2, and G2-like families (Figure 3G). Among these, 179 TFs overlapped with Fielder-specific core TFs, including known regeneration factors, such as TaPLT1/5, TaDOF5.6, TaNAC071, and TaWIND1 (Figures 3H and 3I).^{19,49–51} These TFs regulate a suite of regeneration-associated genes in Fielder, including hormone pathway components (*TaPIN1* and *TaLOG7*) and key developmental regulators (*TaWOX5* and *TaWOX11*) (Figures 3H and 3I).

Therefore, comparative TRN analysis revealed that Fielder exhibits greater regulatory complexity and enrichment of regeneration-associated TFs than JM22, highlighting key TF families and network hubs that may underlie its superior regenerative capacity.

TaSCR enhances transformation efficiency across diverse wheat varieties without compromising agronomic traits

To further investigate key regulators underlying Fielder-specific transcriptional control, we selected TaSCR for functional anal-

ysis based on its role of mediating Fielder-specific regulatory network, its capacity to bind the top 10 enriched motifs in Fielder-specific ACRs, and expression of *TaSCR* is specifically activated at DAI6 in Fielder (Figure 4A). Notably, SCR has been previously identified as a critical regulator of *de novo* shoot regeneration in *Arabidopsis*.⁵²

In situ hybridization revealed that *TaSCR* is highly expressed in the shoot apical meristem (SAM) and root apical meristem (RAM) of immature wheat embryos at DAI0. During tissue culture, the expression of *TaSCR* was observed in one to two subepidermal cell layers of the scutellum at DAI3, in the outer and middle layers of callus at DAI6, and in part of the outer and middle layers of callus at DAI9 (Figure 4B). Moreover, the spatial expression pattern of *TaSCR* at DAI6 and DAI9 resembles that of *TaWOX5* (Figure S4A), a marker gene for the root stem cell niche that has been previously demonstrated to play a critical role in wheat regeneration.¹⁸ This indicates the role of *TaSCR* in maintaining the QC-like identity of the middle-layer cells in callus. RT-qPCR analysis showed a continuous increase in *TaSCR* expression from DAI0 to DAI6, with high levels maintained from DAI6 through DAI12 (Figure 4C), supporting its role in cell fate transition.

To evaluate the functional relevance of *TaSCR* in regeneration, we transformed *TaSCR* into the immature embryos of Fielder. Compared to the *GUS* negative control, *TaSCR* OE enhanced callus induction efficiency (from 89.18% to 95.51%), callus differentiation efficiency (from 78.36% to 84.91%), and transformation efficiency (from 25.88% to 53.05%) (Figures 4D–4G). To test whether OE of *TaSCR* could mitigate genotype dependency in wheat transformation, we extended our analysis to recalcitrant to transform cultivars JM22, KN199, and AK58. In all three varieties, OE of *TaSCR* markedly improved callus induction efficiency, callus differentiation efficiency, and transformation efficiency. In KN199, efficiencies increased from 83.30% to 98.03% (callus induction efficiency), 31.16%–57.02% (callus differentiation efficiency), and 4.64%–14.78% (transformation efficiency); in JM22, from 73.79% to 96.21%, 38.83%–61.14%, and 0.46%–12.24%, respectively; and in AK58, callus induction efficiency and differentiation efficiency rose from 79.86% to 100% and 36.30%–65.59%, respectively, while transformation efficiency reached 9.23%—in contrast to the *GUS* control, which failed to produce transgenic plants (Figures 4D–4G; Table S4). These results demonstrate that OE of *TaSCR* can enhance transformation efficiency and overcome genotype restrictions in wheat transformation.

Given the known phenotypic effects of ectopically expressing genes such as *BBM* and *WUS* in maize, which often result in developmental abnormalities,⁵³ we assessed the grain development of *TaSCR* transgenic wheat. Importantly, grains of *TaSCR*-overexpressing lines displayed normal growth and development, with no significant differences in grain length, grain width, or thousand-grain weight compared to wild-type Fielder plants (Figures 4H–4K).

(E–G) Comparison of callus induction (E), differentiation (F), and transformation (G) efficiency between callus transformed *GUS* and *TaSCR* overexpression vectors ($n = 4$; KN199-*TaSCR* and JM22-*TaSCR*, $n = 3$).

(H) Representative grain phenotypes of wild-type and *TaSCR* overexpression plants. Scale bars, 1 cm.

(I–K) Comparison of grain length (I), width (J), and thousand-grain weight (K) between wild-type and *TaSCR* overexpression plants ($n = 6$).

Data in (C), (E)–(G), and (I)–(K) are means $\pm$ SD. In (E)–(G) and (I)–(K), p values were calculated using Student's t test.

Together, these findings establish TaSCR as a promising morphogenic regulator, capable of improving transformation efficiency across diverse wheat cultivars without compromising agronomic performance.

TaSCR links upstream auxin-responsive TFs to downstream meristem-related genes to enhance wheat regeneration

To elucidate the mechanism by which TaSCR enhances transformation efficiency in wheat, we analyzed its downstream targets within Fielder's TRN. TaSCR was predicted to regulate 3,487 genes, including key genes involved in hormone signaling (*TaYUC8*, *TaPIN1*, *TaLOG7*, *TaGA1*, and *TaARR7*), epigenetic regulation (*TaDDM1* and *TaASHR2*), cell cycle control (*TaFAS1* and *TaCYCD2-1*), and transcriptional regulation (*TaARFs*, *TaBBM*, *TaDOF5.6*, *TaE2FA*, *TaLBD17*, and *TaWOX5*) (Figure S4B). GO enrichment analysis indicated that these targets are associated with developmental processes, including shoot axis formation and auxin-activated signaling pathways (Figure S4C).

To identify genes regulated by TaSCR *in vivo*, we performed RNA-seq on callus transformed with either a *GUS* control or a *TaSCR*-OE construct at DAI6 and DAI9. Differential expression analysis revealed 320 upregulated and 73 downregulated genes at DAI6, and 1,361 upregulated and 562 downregulated genes at DAI9 (Figure 5A; Table S5). These data suggest that TaSCR primarily functions as a transcriptional activator. We further overlapped TRN-predicted downstream genes with DEGs from the *TaSCR*-OE transcriptome, identifying 166 high-confidence direct targets of TaSCR. Among them, 88 genes—including *TaLBD17* and *TaPLT1*—were activated by TaSCR, while 78 genes, such as *TaHB15* and *TaOBP1*, were repressed (Figure 5B). To validate these regulatory interactions, we conducted LUC reporter assays in tobacco for four pluripotency-associated genes (*TaLBD17*, *TaFTIP3*, *TaBBM2*, and *TaGRF7*).^{54–56} The results showed that TaSCR activated *TaLBD17* and *TaFTIP3*, while repressing *TaBBM2* and *TaGRF7*, consistent with transcriptomic data (Figures 5C, 5D, S4D, and S4E).

Auxin, which is added to the culture medium, plays an important role in driving the regeneration process.²⁸ To investigate how *TaSCR* is activated by auxin during Fielder regeneration, we analyzed 288 upstream TFs predicted to regulate *TaSCR* in the TRN of Fielder. Among these TFs, 90 were highly expressed at DAI0 and 198 after DAI3. These TFs primarily belong to the WRKY, MYB, Dof, and GATA families, with no ARF family members directly regulating *TaSCR* (Figures 5E and S4F). This suggests that TaARFs may regulate *TaSCR* indirectly via intermediate TFs (Figure 5F). Expression correlation analysis identified 89 TFs co-expressed with *TaSCR*, of which 10 are potential direct targets of TaARFs, such as *TaMYB3R2*, *TaGATA15*, and *TaMYB44* (Figures 5E and S4G). To validate these regulatory interactions, we performed LUC reporter assays using five candidate TFs (*TaMYB3R2*, *TaGATA15*, *TaMYB44*, *TaWRKY57*, and *TaWRKY65*), all of which activated *TaSCR* to varying degrees (Figures 5G and S4H). Furthermore, we selected *TaARF22* and *TaGATA15*, which exhibited a higher fold change in expression between Fielder and JM22 for further study (Figure S4I). LUC

reporter assay experiments demonstrated that *TaARF22* can indeed activate the expression of *TaGATA15* (Figure 5H).

To further validate the regulatory relationship among *TaARF22*, *TaGATA15*, and *TaSCR*, we employed an RNA interference (RNAi) approach to knock down the expression of *TaARF22* and *TaGATA15* in the callus of Fielder. RT-qPCR analysis showed that silencing of *TaARF22* or *TaGATA15* led to decreased expression of their direct downstream genes, *TaGATA15* or *TaSCR*, at DAI6, DAI12, and DAI18. In addition, *TaARF22*-RNAi also resulted in reduced expression of its indirect downstream gene *TaSCR* at DAI18 (Figures 5I and 5J). Furthermore, callus transformed with *TaGATA15*-RNAi or *TaARF22*-RNAi vectors exhibited lower transformation efficiency compared with the *GUS* control, and the inhibitory effect of *TaARF22*-RNAi was stronger than that of *TaGATA15*-RNAi, further supporting that *TaARF22* acts upstream of *TaGATA15* in regulating *TaSCR* expression (Figure 5K).

Together, these results delineate a regulatory network in which *TaSCR* is indirectly activated by auxin signaling via ARF-mediated transcriptional cascades and promotes regeneration by regulating meristem-related genes (Figure 5H).^{39,55–62}

TaLBD17 functions downstream of TaSCR to promote wheat transformation

To assess whether the downstream factor of TaSCR contributes to enhanced transformation efficiency, we focused on TFs differentially expressed in *TaSCR*-OE callus. TF families such as LBD, ERF, and HSF were enriched among DEGs at both DAI6 and DAI9 (Figure S4J). Notably, members of the LBD family were identified as direct targets of TaSCR (Figures 5B and S4K). Among these, *TaLBD17* was selected for further functional and mechanistic investigation, as it was validated as a TaSCR-activated gene via LUC reporter assays (Figure 5C).

To characterize the spatial expression pattern of *TaLBD17* during regeneration, we performed *in situ* hybridization. From DAI0 to DAI3, *TaLBD17* was detected in the epidermal cells of the scutellum (Figure S4L). At DAI6, *TaLBD17* was predominantly expressed in the outer layer of callus, and by DAI9, its expression was specifically localized to the middle layer of the callus (Figure 6A), partly mirroring the expression pattern of *TaSCR*. In parallel, RT-qPCR analysis revealed that *TaLBD17* expression was progressively upregulated during tissue culture, peaking at DAI9, later than the peak expression observed for *TaSCR* at DAI6 (Figures 4C and 6B). These results further demonstrate that *TaLBD17* acts downstream in the TaSCR regulatory cascade.

Functional assays demonstrated that *TaLBD17*-OE enhanced callus induction efficiency, callus differentiation efficiency, and transformation efficiency. In Fielder, callus induction efficiency increased from 87.47% to 100%, callus differentiation efficiency from 76.44% to 88.38%, and transformation efficiency from 25.61% to 38.32%. In KN199, callus differentiation efficiency and transformation efficiency increased from 34.84% to 61.17% and from 4.66% to 11.26%, respectively. In AK58, callus induction efficiency improved from 79.89% to 91.49%, callus differentiation efficiency from 35.92% to 48.36% and transformation efficiency reached 4.59% (Figures 6C–6F; Table S4).

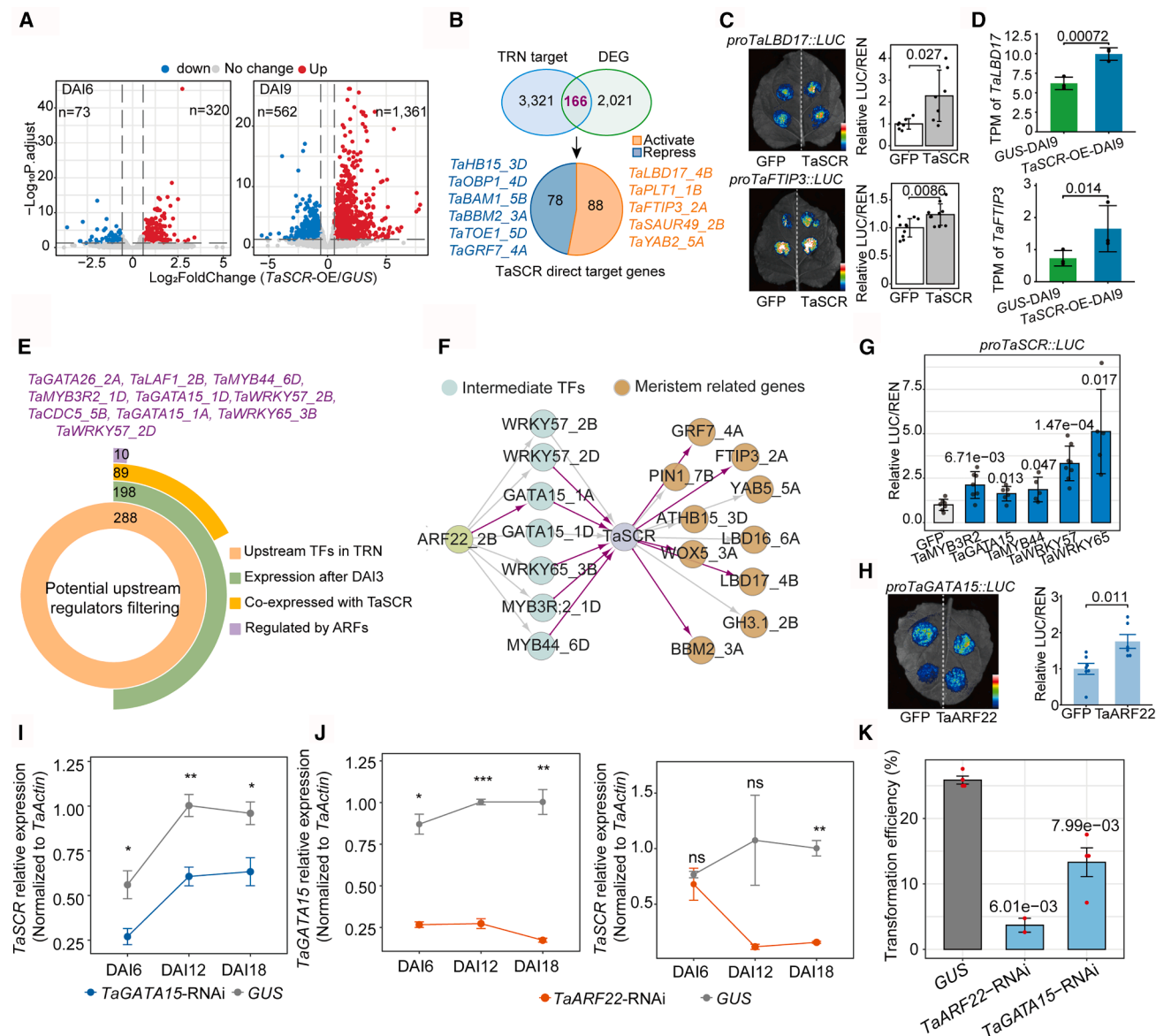


Figure 5. *TaSCR* is indirectly regulated by auxin signaling and regulates downstream meristem-related genes

(A) Volcano plots showing DEGs at DAI6 (Left) and DAI9 (Right) between *TaSCR*-OE and *GUS*.
 (B) Venn diagram showing overlap of *TaSCR* target genes in Fielder TRN and DEGs in A (top); number of upregulated and downregulated *TaSCR* direct target genes (bottom).
 (C) Luciferase reporter assays validating *TaSCR* activates *TaLBD17* (n = 7) and *TaFTIP3* (n = 10).
 (D) Boxplot showing TPM of *TaLBD17* and *TaFTIP3* in the callus transformed with *GUS* and *TaSCR*-OE vectors (n = 3).
 (E) Sunburst chart showing genes mediating auxin signaling and *TaSCR* activation.
 (F) Luciferase reporter assays validate that *TaSCR* can be activated by *TaMYB3R2* (n = 7), *TaGATA15* (n = 6), *TaMYB44* (n = 5), *TaWRKY57* (n = 8), and *TaWRKY65* (n = 5) compared to *GFP* (n = 7).
 (G) Luciferase reporter assays validating *TaARF22* (n = 6) activate *TaGATA15* (n = 7).
 (H) Network of *ARF22*-intermediate TFs-*TaSCR*-meristem related genes. Intermediate TFs refer to candidate TFs that may mediate the regulatory connection between auxin signaling and *TaSCR*. Purple arrows indicate luciferase-validated regulatory links.
 (I) Relative *TaSCR* expression in Fielder callus transformed with *GUS* or *TaGATA15*-RNAi vector (n = 3).
 (J) Relative *TaGATA15* (left) and *TaSCR* (right) expression in Fielder callus transformed with *GUS* or *TaARF22*-RNAi vector (n = 3).
 (K) Transformation efficiency of Fielder callus transformed with *TaARF22*-RNAi, *TaGATA15*-RNAi, or *GUS* vectors (n = 3).
 Data in (C), (D), and (G)–(K) are means $\pm$ SD. *p* values were determined by negative binomial distribution (A and D), Student's *t* test (C, G–K), and Benjamini–Hochberg (BH) correction for multiple comparisons (A and D). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; and ns, not significant (*p* > 0.05).

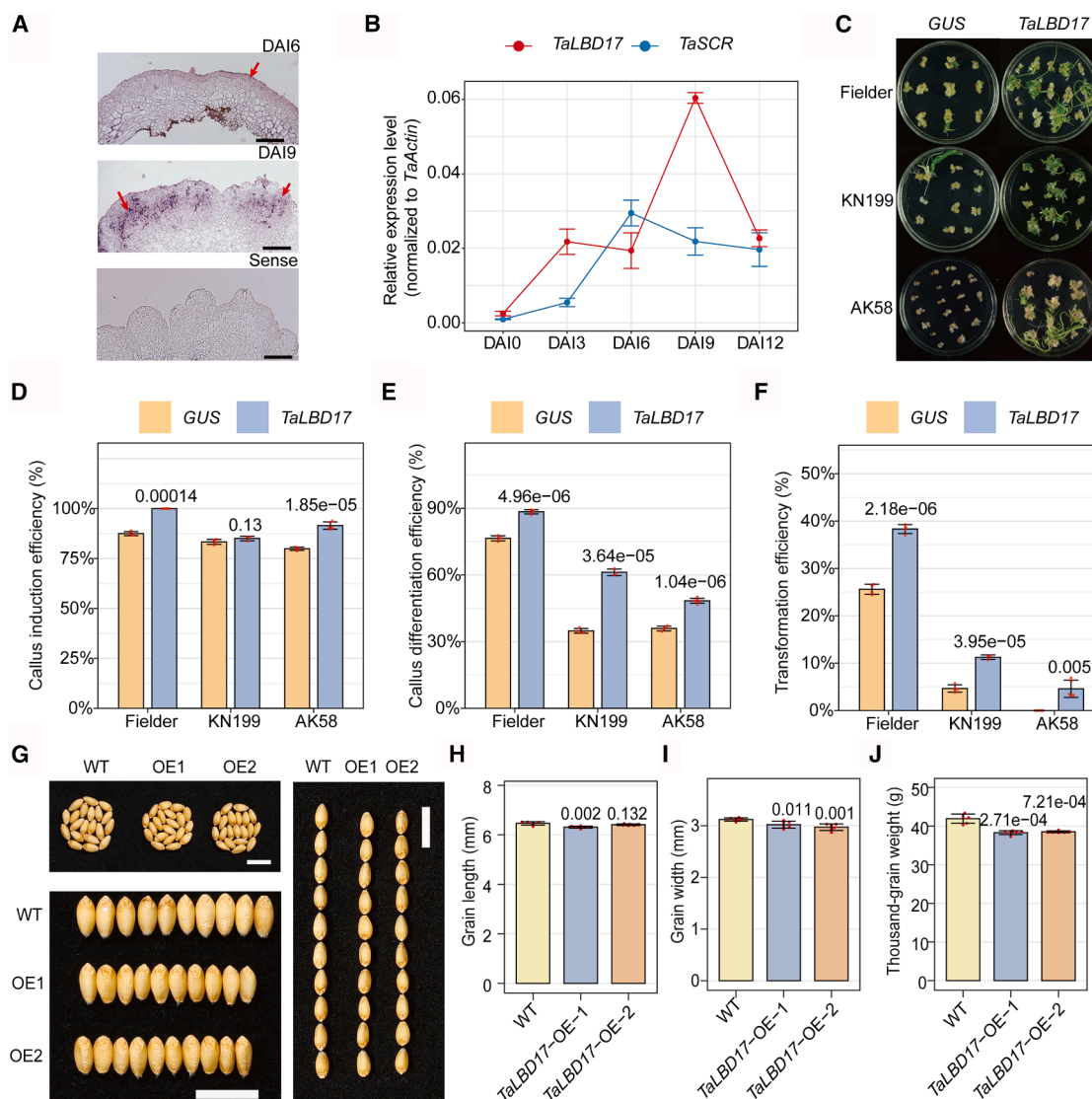


Figure 6. Overexpression of *TaLBD17* boosts transformation efficiency across different wheat varieties

(A) *In situ* hybridization of *TaLBD17* at DAI6, DAI9, and DAI9 sense control. Scale bars, 250 μ m. Red arrows indicate expression positions.
(B) Relative *TaLBD17* and *TaSCR* expression levels in Fielder ($n = 3$).
(C) Representative photographs showing *TaLBD17* functional validation.
(D–F) Comparison of callus induction (D), differentiation (E), and transformation (F) efficiency between callus transformed with *GUS* and *TaLBD17* overexpression vectors ($n = 4$; KN199-*TaLBD17*, $n = 3$; AK58-*TaLBD17*, $n = 5$).
(G) Representative photographs of grains from wild-type and *TaLBD17* overexpression plants.
(H–J) Comparison of grain length (H), width (I), and thousand-grain weight (J) between wild-type and *TaLBD17* overexpression plants ($n = 6$).
Data in (B), (D)–(F), and (H)–(J) are means $\pm$ SD. In (D)–(F) and (H)–(J), p values were determined by Student's t test.

Notably, *GUS* controls in AK58 failed to produce transgenic plants (Figure 6F).

To evaluate whether *TaLBD17*-OE affects plant grain development, we analyzed transgenic Fielder plants. Unlike *TaSCR*-OE lines, the *TaLBD17*-OE1 line showed reduced grain length (2.3% decreased), while both OE1 and OE2 lines exhibited decreases in grain width (3.3% and 4.8% decreased, respectively) and thousand-grain weight (8.1% and 8.6% decreased, respectively) (Figures 6G–6J).

To further investigate the importance of *TaLBD17* and the *TaSCR*-*TaLBD17* module, we examined the relative expression levels of *TaLBD17* at DAI6, DAI12, and DAI18 in the callus of Fielder, JM22, KN199, and AK58 transformed with *GUS* control and *TaSCR*-OE vectors using RT-qPCR. The results showed that *TaLBD17* expression was elevated at least at one time point in all four cultivars upon *TaSCR* OE (Figure S4M), indicating that the *TaSCR* to *TaLBD17* regulatory module functions in the regeneration process across different wheat genotypes.

Together, these findings demonstrate that *TaLBD17*, a direct downstream target of *TaSCR*, plays an important role in enhancing wheat regeneration and transformation efficiency, but slightly influences grain development.

DISCUSSION

While a robust genetic transformation system has been developed for the wheat variety Fielder,¹⁴ transformation remains inefficient in many elite cultivars, including JM22 and AK58.⁵ Here, by integrating transcriptomic profiling, chromatin accessibility mapping, and TRN comparison analysis, we uncovered key molecular mechanisms that underlie genotype-dependent regeneration capacity in wheat. These findings provide a foundation for overcoming long-standing challenges in wheat transformation.

Distinct transcriptomic and chromatin accessibility dynamics underlie regeneration divergence

Upon embryo excision (DAI0), both Fielder and JM22 activate wound response pathways. However, by DAI3, clear transcriptional divergence emerges: Fielder induces genes linked to development and regeneration, while JM22 predominantly activates genes related to stress-response pathways. Between DAI3 and DAI6, Fielder undergoes substantial transcriptional reprogramming, characterized by the upregulation of key morphogenic regulators and dynamic gene expression changes, in contrast to the transcriptional stasis observed in JM22.

Notably, most DEGs in cluster C4, which are specifically activated in Fielder from DAI3 to DAI6, encode proteins with highly conserved sequences between cultivars, indicating that regeneration divergence is primarily governed by regulatory differences rather than protein-coding variation. While differences in *cis*-regulatory motifs within promoter regions contribute to expression divergence, they are insufficient to fully explain the transcriptional discrepancies, pointing to a substantial role for epigenetic regulation.

This is supported by our chromatin accessibility analyses, which reveal that Fielder exhibits more extensive and timely chromatin remodeling, particularly at loci encoding core developmental genes such as *TaSCR*, *TaWOX5*, and *TaLBD17*.¹⁸ Such chromatin plasticity likely enables a more permissive and responsive transcriptional landscape, facilitating efficient activation of regeneration pathways. In contrast, JM22 shows limited chromatin dynamics at these critical loci, which may constrain the activation of morphogenic genes and ultimately hinder its regenerative potential.

TRN architecture and genotype-specific TFs influence regeneration potential

Comparative TRN analysis revealed that Fielder possesses a more complex and interconnected regulatory network during regeneration. Fielder-specific core TFs—particularly from AP2, Dof, G2-like, and GRAS families—are enriched in known morphogenic regulators and form key hubs in the network.²² Their absence or low connectivity in JM22's network may impair its regenerative capacity. Importantly, our analysis shows that network topology—i.e., the centrality and connectivity of TFs—is as crucial as gene expression levels in determining regenera-

tive potential. These insights pave the way for rational selection or engineering of TFs to enhance transformation in recalcitrant genotypes. By comparing TRNs across diverse varieties, key regulatory modules linked to high regeneration can be identified and targeted to improve transformation efficiency.

The *TaSCR-TaLBD17* regulatory module promotes wheat regeneration and transformation

Among the Fielder-specific TFs, *TaSCR* emerged as a central regulator of regeneration. Expressed in meristematic domains during key stages, *TaSCR* has the potential to activate numerous genes involved in hormone biosynthesis and transport (*TaYUC8* and *TaPIN1*),^{34,63} chromatin remodeling (*TaDDM1*),⁶⁴ and cell cycle (*TaCYCD2-1* and *TaFAS1*),^{44,65} Its OE improved callus induction, differentiation, and transformation efficiencies in both Fielder and recalcitrant varieties such as JM22 and AK58, without detrimental effects on grain development. This distinguishes *TaSCR* from other morphogenic regulators like *WUS* or *BBM*,⁵³ which often cause developmental abnormalities.

To elucidate how *TaSCR* confers regenerative capacity, we integrated TRN and transcriptome data. *TaSCR* is indirectly activated in response to auxin addition in the culture medium by *TaARFs* through TFs such as *TaGATA15*. In turn, *TaSCR* directly upregulates *TaWOX5*, *TaFTIP3*, and notably *TaLBD17*, a TF coding gene whose *Arabidopsis* ortholog promotes callus formation.³⁹ *TaLBD17* expression mirrors *TaSCR* in spatial localization and temporal dynamics, with peak expression in the promeristem of shoot primordia at DAI9. The OE of *TaLBD17* enhanced callus induction and transformation efficiencies across multiple genotypes. Thus, the *TaSCR* to *TaLBD17* cascade forms a robust regulatory module that coordinates regeneration-associated transcriptional and cellular events. This module offers a promising target for enhancing transformation efficiency in elite yet recalcitrant cultivars.

Collectively, by integrating transcriptomic and chromatin accessibility dynamics with TRN comparisons, we have elucidated molecular mechanisms driving differences in the regeneration capacity between Fielder and JM22. We identified *TaSCR* as a key regeneration-promoting TF that activates downstream effectors, including *TaLBD17*, to enhance transformation. The *TaSCR-TaLBD17* module exemplifies a promising strategy for improving transformation systems in wheat and possibly other cereals.

Limitations of the study

Future efforts should extend this comparative framework to a broader range of genotypes with distinct regeneration profiles.⁵ The current comparison is limited to one highly regenerable cultivar Fielder and one recalcitrant cultivar JM22. Some of the observed differences may not be directly related to regeneration efficiency diversity but rather cultivar differences per se. Expanding the analysis to groups of wheat cultivars with varying regeneration efficiency would increase statistical power, reveal the fundamental transcriptional and chromatin accessibility features underlying regeneration differences, and further facilitate the identification of broadly applicable high-efficiency regeneration factors across diverse wheat varieties.

Although OE of *TaSCR* and *TaLBD17* enhanced transformation efficiency across multiple cultivars, their effects were more pronounced in Fielder (Figures 4G and 6F). OE of *TaSCR* and *TaLBD17* in Fielder induced a larger number of DEGs enriched in auxin signaling and regeneration-related processes, such as SAM development and abaxial cell fate specification, whereas in JM22, DEGs were mainly associated with stress responses (Figures S5A–S5C). These divergent transcriptional outcomes likely reflect underlying differences in chromatin accessibility landscapes between the two cultivars, as Fielder displayed higher GRAS and LBD motif activity and direct regulation of a greater number of auxin pathway genes (Figures S5D–S5F). Therefore, the same regulatory factors may exert cultivar-specific effects on regeneration efficiency, depending on the chromatin context of each wheat genotype. Future work should investigate whether targeted modulation of chromatin accessibility, such as through chromatin remodelers, could mitigate regeneration disparities and improve transformation efficiency across recalcitrant cultivars.

The current study focuses on the functional and regulatory roles of *TaSCR-A* and *TaLBD17-B*, without addressing how polyploidy may contribute to genotype-dependent regulation or potential functional redundancy among *TaSCR/TaLBD17* homeologs. The expression level of *TaSCR-A* is slightly higher than that of *TaSCR-B* and *TaSCR-D*, whereas its chromatin accessibility is the lowest. Similarly, *TaLBD17-A* shows lower expression than its B- and D-homeologs, with *TaLBD17-D* displaying the highest chromatin accessibility (Figures S6A and S6B). Despite these regulatory differences, notable sequence divergence exists among the A, B, and D subgenome copies; however, the GRAS and LOB domains remain highly conserved, with only a single amino acid substitution found in *TaSCR-B* and *TaLBD17-A*, respectively (Figure S6C). These differences may lead to divergent contributions to the regeneration process, similar to *TaLAX1*,¹⁶ thereby adding another layer of complexity to the *TaSCR/TaLBD17* regulatory network in wheat.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources described in this study should be directed to and will be fulfilled by the lead contact, Jun Xiao (jxiao@genetics.ac.cn).

Materials availability

All unique/stable reagents generated in this study are available from the lead contact with a completed materials transfer agreement.

Data and code availability

- The Fielder and JM22 regeneration progress RNA-seq and ATAC-seq data used in this study have been deposited in the [Genome Sequence Archive](#) database under accession numbers GSA: PRJCA040308 and GSA: PRJCA012455. Quantitative results have been uploaded to the Figshare database (<https://doi.org/10.6084/m9.figshare.30632660>).
- Scripts and codes used in this study are available on GitHub (<https://github.com/caoyuan1231/Chromatin-accessibility-and-TaSCR-TaLBD17-circuitry-shape-genotypic-regeneration-capacity-in-wheat>) and Zenodo (<https://doi.org/10.5281/zenodo.17614807>).
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (32430011), the CAS Project for Young Scientists in Basic Research (YSBR-093), the Beijing Natural Science Foundation Outstanding Youth Project (JQ23026), the Shandong Provincial Natural Science Foundation (ZR2024MC155), and the Major Scientific and Technological Innovation Program of Tai'an (2024GZPT03). We thank Japan Tobacco for the Fielder seeds and *Agrobacterium* strain EHA105 and Professor Dao-Lin Fu from Shandong Agricultural University for pc186 vector.

AUTHOR CONTRIBUTIONS

J.X. and X.S.Z. designed and supervised the research; J.X., X.S.Z., Y.C., and X.M.B. wrote the manuscript; X.M.B. did the sample collection and RNA-seq experiments; Y.C. performed bioinformatics analysis with help from X. Liu; M.L. performed plasmid construction, RT-qPCR, and *in situ* hybridization; X. Lin performed ATAC-seq experiments; X.M.B., Y.S., and M.Z. performed wheat transformation and data analysis; M.L. and M.Y. conducted phenotypic analysis of transgenic plants. X. Luo conducted the LUC reporter assay; Y.C., X.M.B., M.L., and J.X. prepared all the figures; and all authors discussed the results and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **METHOD DETAILS**
 - Plant materials and growth conditions
 - Plasmid construction and wheat transformation
 - RNA-seq and ATAC-seq experiment
 - Luciferase reporter assay
 - RNA-seq analysis
 - DNA sequences comparative analysis between Fielder and JM22
 - ATAC-seq analysis
 - *Cis*-motif enrichment analysis and motif activity analysis
 - Comparative analysis of TRNs
 - *In situ* hybridization
 - Quantitative reverse-transcription PCR
 - Phenotyping of grain development
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.celrep.2025.116743>.

Received: May 27, 2025

Revised: October 31, 2025

Accepted: November 25, 2025

REFERENCES

1. Sang, Y.L., Cheng, Z.J., and Zhang, X.S. (2018). iPSCs: A Comparison between Animals and Plants. *Trends Plant Sci.* 23, 660–666. <https://doi.org/10.1016/j.tplants.2018.05.008>.
2. Marhava, P., Hoermayer, L., Yoshida, S., Marhavý, P., Benková, E., and Friml, J. (2019). Re-activation of Stem Cell Pathways for Pattern

- Restoration in Plant Wound Healing. *Cell* 177, 957–969.e13. <https://doi.org/10.1016/j.cell.2019.04.015>.
3. Chen, C., Hu, Y., Ikeuchi, M., Jiao, Y., Prasad, K., Su, Y.H., Xiao, J., Xu, L., Yang, W., Zhao, Z., et al. (2024). Plant regeneration in the new era: from molecular mechanisms to biotechnology applications. *Sci. China Life Sci.* 67, 1338–1367. <https://doi.org/10.1007/s11427-024-2581-2>.
4. Wang, P., Si, H., Li, C., Xu, Z., Guo, H., Jin, S., and Cheng, H. (2025). Plant genetic transformation: achievements, current status and future prospects. *Plant Biotechnol. J.* 23, 2034–2058. <https://doi.org/10.1111/pbi.70028>.
5. Wang, K., Liu, H., Du, L., and Ye, X. (2017). Generation of marker-free transgenic hexaploid wheat via an Agrobacterium-mediated co-transformation strategy in commercial Chinese wheat varieties. *Plant Biotechnol. J.* 15, 614–623. <https://doi.org/10.1111/pbi.12660>.
6. Chen, Z., Debernardi, J.M., Dubcovsky, J., and Gallavotti, A. (2022). Recent advances in crop transformation technologies. *Nat. Plants* 8, 1343–1351. <https://doi.org/10.1038/s41477-022-01295-8>.
7. Anjanappa, R.B., and Gruissem, W. (2021). Current progress and challenges in crop genetic transformation. *J. Plant Physiol.* 261, 153411. <https://doi.org/10.1016/j.jplph.2021.153411>.
8. Klocke, E., Nothnagel, T., and Schumann, G. (2010). Vegetables. In *Genetic Modification of Plants: Agriculture, Horticulture and Forestry*, F. Kempken and C. Jung, eds. (Springer), pp. 499–550. https://doi.org/10.1007/978-3-642-02391-0_25.
9. Delporte, F., Jacquemin, J.-M., Masson, P., and Watillon, B. (2012). Insights into the regenerative property of plant cells and their receptivity to transgenesis. *Plant Signal. Behav.* 7, 1608–1620. <https://doi.org/10.4161/psb.22424>.
10. Liu, S., Wang, K., Geng, S., Hossain, M., Ye, X., Li, A., Mao, L., and Kogel, K.-H. (2024). Enemies at peace: Recent progress in Agrobacterium-mediated cereal transformation. *Crop J.* 12, 321–329. <https://doi.org/10.1016/j.cj.2023.12.009>.
11. Vasil, V., Redway, F., and Vasil, I.K. (1990). Regeneration of Plants from Embryogenic Suspension Culture Protoplasts of Wheat (*Triticum aestivum* L.). *Nat. Biotechnol.* 8, 429–434. <https://doi.org/10.1038/nbt0590-429>.
12. Vasil, I.K. (2005). The story of transgenic cereals: The challenge, the debate, and the solution—A historical perspective. *Vitro Cell Dev. Biol. Plant* 41, 577–583. <https://doi.org/10.1079/IVP2005654>.
13. Harwood, W.A. (2012). Advances and remaining challenges in the transformation of barley and wheat. *J. Exp. Bot.* 63, 1791–1798. <https://doi.org/10.1093/jxb/err380>.
14. Ishida, Y., Tsunashima, M., Hiei, Y., and Komari, T. (2015). Wheat (*Triticum aestivum* L.) Transformation Using Immature Embryos. In *Methods Mol. Biol.*, K. Wang, ed. (Springer New York), pp. 189–198. https://doi.org/10.1007/978-1-4939-1695-5_15.
15. Hayta, S., Smedley, M.A., Demir, S.U., Blundell, R., Hinchliffe, A., Atkinson, N., and Harwood, W.A. (2019). An efficient and reproducible Agrobacterium-mediated transformation method for hexaploid wheat (*Triticum aestivum* L.). *Plant Methods* 15, 121. <https://doi.org/10.1186/s13007-019-0503-z>.
16. Yu, Y., Yu, H., Peng, J., Yao, W.J., Wang, Y.P., Zhang, F.L., Wang, S.R., Zhao, Y., Zhao, X.Y., Zhang, X.S., and Su, Y.H. (2024). Enhancing wheat regeneration and genetic transformation through overexpression of TaLAX1. *Plant Commun.* 5, 100738. <https://doi.org/10.1016/j.xplc.2023.100738>.
17. Yang, W., Zhai, H., Wu, F., Deng, L., Chao, Y., Meng, X., Chen, Q., Liu, C., Bie, X., Sun, C., et al. (2024). Peptide REF1 is a local wound signal promoting plant regeneration. *Cell* 187, 3024–3038.e14. <https://doi.org/10.1016/j.cell.2024.04.040>.
18. Wang, K., Shi, L., Liang, X., Zhao, P., Wang, W., Liu, J., Chang, Y., Hiei, Y., Yanagihara, C., Du, L., et al. (2022). The gene TaWOX5 overcomes genotype dependency in wheat genetic transformation. *Nat. Plants* 8, 110–117. <https://doi.org/10.1038/s41477-021-01085-8>.
19. Liu, X., Bie, X.M., Lin, X., Li, M., Wang, H., Zhang, X., Yang, Y., Zhang, C., Zhang, X.S., and Xiao, J. (2023). Uncovering the transcriptional regulatory network involved in boosting wheat regeneration and transformation. *Nat. Plants* 9, 908–925. <https://doi.org/10.1038/s41477-023-01406-z>.
20. Debernardi, J.M., Tricoli, D.M., Ercoli, M.F., Hayta, S., Ronald, P., Palatnik, J.F., and Dubcovsky, J. (2020). A GRF–GIF chimeric protein improves the regeneration efficiency of transgenic plants. *Nat. Biotechnol.* 38, 1274–1279. <https://doi.org/10.1038/s41587-020-0703-0>.
21. Liu, X., Zhu, K., and Xiao, J. (2023). Recent advances in understanding of the epigenetic regulation of plant regeneration. *aBIOTECH* 4, 31–46. <https://doi.org/10.1007/s42994-022-00093-2>.
22. Ikeuchi, M., Favero, D.S., Sakamoto, Y., Iwase, A., Coleman, D., Rymer, B., and Sugimoto, K. (2019). Molecular Mechanisms of Plant Regeneration. *Annu. Rev. Plant Biol.* 70, 377–406. <https://doi.org/10.1146/annurev-arplant-050718-100434>.
23. Zhou, Y., Wang, D., Wang, H., Qiao, Y., Zhao, P., Cao, Y., Liu, X., Yang, Y., Lin, X., Xu, S., et al. (2025). Integrative omics of the genetic basis for wheat WUE and drought resilience reveal the function of TaMYB7-A1. *Nat. Commun.* 16, 8622. <https://doi.org/10.1038/s41467-025-63642-5>.
24. Nobori, T., Monell, A., Lee, T.A., Sakata, Y., Shirahama, S., Zhou, J., Nery, J.R., Mine, A., and Ecker, J.R. (2025). A rare PRIMER cell state in plant immunity. *Nature* 638, 197–205. <https://doi.org/10.1038/s41586-024-08383-z>.
25. Zhao, L., Chen, J., Zhang, Z., Wu, W., Lin, X., Gao, M., Yang, Y., Zhao, P., Xu, S., Yang, C., et al. (2024). Deciphering the Transcriptional Regulatory Network Governing Starch and Storage Protein Biosynthesis in Wheat for Breeding Improvement. *Adv. Sci.* 11, 2401383. <https://doi.org/10.1002/adv.202401383>.
26. Lin, X., Xu, Y., Wang, D., Yang, Y., Zhang, X., Bie, X., Gui, L., Chen, Z., Ding, Y., Mao, L., et al. (2024). Systematic identification of wheat spike developmental regulators by integrated multi-omics, transcriptional network, GWAS, and genetic analyses. *Mol. Plant* 17, 438–459. <https://doi.org/10.1016/j.molp.2024.01.010>.
27. Wang, F.-X., Shang, G.-D., Wu, L.-Y., Xu, Z.-G., Zhao, X.-Y., and Wang, J.-W. (2020). Chromatin Accessibility Dynamics and a Hierarchical Transcriptional Regulatory Network Structure for Plant Somatic Embryogenesis. *Dev. Cell* 54, 742–757.e8. <https://doi.org/10.1016/j.devcel.2020.07.003>.
28. Wu, L.-Y., Shang, G.-D., Wang, F.-X., Gao, J., Wan, M.-C., Xu, Z.-G., and Wang, J.-W. (2022). Dynamic chromatin state profiling reveals regulatory roles of auxin and cytokinin in shoot regeneration. *Dev. Cell* 57, 526–542.e7. <https://doi.org/10.1016/j.devcel.2021.12.019>.
29. Zhai, N., and Xu, L. (2021). Pluripotency acquisition in the middle cell layer of callus is required for organ regeneration. *Nat. Plants* 7, 1453–1460. <https://doi.org/10.1038/s41477-021-01015-8>.
30. Kodaira, K.-S., Qin, F., Tran, L.-S.P., Maruyama, K., Kidokoro, S., Fujita, Y., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2011). Arabidopsis Cys2/His2 Zinc-Finger Proteins AZF1 and AZF2 Negatively Regulate Abscisic Acid-Repressive and Auxin-Inducible Genes under Abiotic Stress Conditions. *Plant Physiol.* 157, 742–756. <https://doi.org/10.1104/pp.111.182683>.
31. Cai, Y., Goodman, J.M., Pyc, M., Mullen, R.T., Dyer, J.M., and Chapman, K.D. (2015). Arabidopsis SEIPIN Proteins Modulate Triacylglycerol Accumulation and Influence Lipid Droplet Proliferation. *Plant Cell* 27, 2616–2636. <https://doi.org/10.1105/tpc.15.00588>.
32. Delvallé, D., Dumez, S., Wattedled, F., Roldán, I., Planchot, V., Berbezy, P., Colonna, P., Vyas, D., Chatterjee, M., Ball, S., et al. (2005). Soluble starch synthase I: a major determinant for the synthesis of amylopectin in Arabidopsis thaliana leaves. *Plant J.* 43, 398–412. <https://doi.org/10.1111/j.1365-313X.2005.02462.x>.
33. Penfield, S., Li, Y., Gilday, A.D., Graham, S., and Graham, I.A. (2006). Arabidopsis ABA INSENSITIVE4 Regulates Lipid Mobilization in the Embryo and Reveals Repression of Seed Germination by the Endosperm. *Plant Cell* 18, 1887–1899. <https://doi.org/10.1105/tpc.106.041277>.

34. Gordon, S.P., Heisler, M.G., Reddy, G.V., Ohno, C., Das, P., and Meyero-witz, E.M. (2007). Pattern formation during de novo assembly of the Arabi-dopsis shoot meristem. *Development* 134, 3539–3548. <https://doi.org/10.1242/dev.010298>.
35. Xu, J., Hofhuis, H., Heidstra, R., Sauer, M., Friml, J., and Scheres, B. (2006). A Molecular Framework for Plant Regeneration. *Science* 311, 385–388. <https://doi.org/10.1126/science.1121790>.
36. Wan, Q., Zhai, N., Xie, D., Liu, W., and Xu, L. (2023). WOX11: the founder of plant organ regeneration. *Cell Regen.* 12, 1. <https://doi.org/10.1186/s13619-022-00140-9>.
37. Xu, C., Chang, P., Guo, S., Yang, X., Liu, X., Sui, B., Yu, D., Xin, W., and Hu, Y. (2023). Transcriptional activation by WRKY23 and derepression by removal of bHLH041 coordinately establish callus pluripotency in Arabi-dopsis regeneration. *Plant Cell* 36, 158–173. <https://doi.org/10.1093/plcell/koad255>.
38. Lee, K., Park, O.-S., and Seo, P.J. (2018). JMJ30-mediated demethylation of H3K9me3 drives tissue identity changes to promote callus formation in Arabidopsis. *Plant J.* 95, 961–975. <https://doi.org/10.1111/tpj.14002>.
39. Fan, M., Xu, C., Xu, K., and Hu, Y. (2012). LATERAL ORGAN BOUNDARIES DOMAIN transcription factors direct callus formation in Arabidopsis regeneration. *Cell Res.* 22, 1169–1180. <https://doi.org/10.1038/cr.2012.63>.
40. Sato, K., Abe, F., Mascher, M., Haberer, G., Gundlach, H., Spannagl, M., Shirasawa, K., and Isobe, S. (2021). Chromosome-scale genome assembly of the transformation-amenable common wheat cultivar ‘Fielder’. *DNA Res.* 28, dsab008. <https://doi.org/10.1093/dnares/dsab008>.
41. Jiao, C., Xie, X., Hao, C., Chen, L., Xie, Y., Garg, V., Zhao, L., Wang, Z., Zhang, Y., Li, T., et al. (2025). Pan-genome bridges wheat structural variations with habitat and breeding. *Nature* 637, 384–393. <https://doi.org/10.1038/s41586-024-08277-0>.
42. Zhang, H., Jin, Z., Cui, F., Zhao, L., Zhang, X., Chen, J., Zhang, J., Li, Y., Li, Y., Niu, Y., et al. (2023). Epigenetic modifications regulate cultivar-specific root development and metabolic adaptation to nitrogen availability in wheat. *Nat. Commun.* 14, 8238. <https://doi.org/10.1038/s41467-023-44003-6>.
43. Shen, S.-Y., Ma, M., Bai, C., Wang, W.-Q., Zhu, R.-B., Gao, Q., and Song, X.-J. (2024). Optimizing rice grain size by attenuating phosphorylation-triggered functional impairment of a chromatin modifier ternary complex. *Dev. Cell* 59, 448–464.e8. <https://doi.org/10.1016/j.devcel.2023.12.013>.
44. Cockcroft, C.E., den Boer, B.G., Healy, J.M., and Murray, J.A. (2000). Cyclin D control of growth rate in plants. *Nature* 405, 575–579. <https://doi.org/10.1038/35014621>.
45. Feng, S., Liu, Z., Chen, H., Li, N., Yu, T., Zhou, R., Nie, F., Guo, D., Ma, X., and Song, X. (2024). PHGD: An integrative and user-friendly database for plant hormone-related genes. *iMeta* 3, e164. <https://doi.org/10.1002/imt2.164>.
46. Coudert, E., Gehant, S., de Castro, E., Pozzato, M., Baratin, D., Neto, T., Sigrist, C.J.A., Redaschi, N., and Bridge, A.; UniProt Consortium (2023). Annotation of biologically relevant ligands in UniProtKB using ChEBI. *Bioinformatics* 39, btac793. <https://doi.org/10.1093/bioinformatics/btac793>.
47. Tian, F., Yang, D.-C., Meng, Y.-Q., Jin, J., and Gao, G. (2020). PlantReg-Map: charting functional regulatory maps in plants. *Nucleic Acids Res.* 48, D1104–D1113. <https://doi.org/10.1093/nar/gkz1020>.
48. Gutierrez, C. (2009). The Arabidopsis cell division cycle. *Arabidopsis Book* 7, e0120. <https://doi.org/10.1199/tab.0120>.
49. Iwase, A., Harashima, H., Ikeuchi, M., Rymer, B., Ohnuma, M., Komaki, S., Morohashi, K., Kurata, T., Nakata, M., Ohme-Takagi, M., et al. (2017). WIND1 Promotes Shoot Regeneration through Transcriptional Activation of ENHANCER OF SHOOT REGENERATION1 in Arabidopsis. *Plant Cell* 29, 54–69. <https://doi.org/10.1105/tpc.16.00623>.
50. Kareem, A., Durgaprasad, K., Sugimoto, K., Du, Y., Pulianmackal, A.J., Trivedi, Z.B., Abhayadev, P.V., Pinon, V., Meyerowitz, E.M., Scheres, B., and Prasad, K. (2015). PLETHORA Genes Control Regeneration by a Two-Step Mechanism. *Curr. Biol.* 25, 1017–1030. <https://doi.org/10.1016/j.cub.2015.02.022>.
51. Asahina, M., Azuma, K., Pitaksaringkarn, W., Yamazaki, T., Mitsuda, N., Ohme-Takagi, M., Yamaguchi, S., Kamiya, Y., Okada, K., Nishimura, T., et al. (2011). Spatially selective hormonal control of RAP2.6L and ANAC071 transcription factors involved in tissue reunion in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 108, 16128–16132. <https://doi.org/10.1073/pnas.1110443108>.
52. Kim, J.Y., Yang, W., Forner, J., Lohmann, J.U., Noh, B., and Noh, Y.S. (2018). Epigenetic reprogramming by histone acetyltransferase HAG1/AtGCN5 is required for pluripotency acquisition in Arabidopsis. *EMBO J.* 37, e98726. <https://doi.org/10.15252/embj.201798726>.
53. Lowe, K., Wu, E., Wang, N., Hoerster, G., Hastings, C., Cho, M.-J., Sce-longe, C., Lenderts, B., Chamberlin, M., Cushatt, J., et al. (2016). Morpho-genetic Regulators Baby boom and Wuschel Improve Monocot Transfor-mation. *Plant Cell* 28, 1998–2015. <https://doi.org/10.1105/tpc.16.00124>.
54. Horstman, A., Li, M., Heidmann, I., Weemen, M., Chen, B., Muino, J.M., Angenot, G.C., and Boutilier, K. (2017). The BABY BOOM Transcription Factor Activates the LEC1-ABI3-FUS3-LEC2 Network to Induce Somatic Embryogenesis. *Plant Physiol.* 175, 848–857. <https://doi.org/10.1104/pp.17.00232>.
55. Kim, J.H. (2019). Biological roles and an evolutionary sketch of the GRF-GIF transcriptional complex in plants. *BMB Rep.* 52, 227–238. <https://doi.org/10.5483/BMBRep.2019.52.4.051>.
56. Liu, L., Li, C., Song, S., Teo, Z.W.N., Shen, L., Wang, Y., Jackson, D., and Yu, H. (2018). FTIP-Dependent STM Trafficking Regulates Shoot Meristem Development in Arabidopsis. *Cell Rep.* 23, 1879–1890. <https://doi.org/10.1016/j.celrep.2018.04.033>.
57. Boutilier, K., Offringa, R., Sharma, V.K., Kieft, H., Ouellet, T., Zhang, L., Hattori, J., Liu, C.-M., van Lammeren, A.A.M., Miki, B.L.A., et al. (2002). Ectopic Expression of BABY BOOM Triggers a Conversion from Vegeta-tive to Embryonic Growth. *Plant Cell* 14, 1737–1749. <https://doi.org/10.1105/tpc.001941>.
58. Green, K.A., Prigge, M.J., Katzman, R.B., and Clark, S.E. (2005). CORONA, a Member of the Class III Homeodomain Leucine Zipper Gene Family in Arabidopsis, Regulates Stem Cell Specification and Organogenesis. *Plant Cell* 17, 691–704. <https://doi.org/10.1105/tpc.104.026179>.
59. Stahle, M.I., Kuehlich, J., Staron, L., von Arnim, A.G., and Golz, J.F. (2009). YABBYs and the Transcriptional Corepressors LEUNIG and LEUNIG_HOMOLOG Maintain Leaf Polarity and Meristem Activity in Ara-bidopsis. *Plant Cell* 21, 3105–3118. <https://doi.org/10.1105/tpc.109.070458>.
60. Vernoux, T., Kronenberger, J., Grandjean, O., Laufs, P., and Traas, J. (2000). PIN-FORMED 1 regulates cell fate at the periphery of the shoot apical meristem. *Development* 127, 5157–5165. <https://doi.org/10.1242/dev.127.23.5157>.
61. Wang, Q., De Gernier, H., Duan, X., Xie, Y., Geelen, D., Hayashi, K.-i., Xuan, W., Geisler, M., ten Tusscher, K., Beeckman, T., and Vanneste, S. (2023). GH3-mediated auxin inactivation attenuates multiple stages of lateral root development. *New Phytol.* 240, 1900–1912. <https://doi.org/10.1111/nph.19284>.
62. Zhang, Y., Jiao, Y., Liu, Z., and Zhu, Y.-X. (2015). ROW1 maintains quies-cent centre identity by confining WOX5 expression to specific cells. *Nat. Commun.* 6, 6003. <https://doi.org/10.1038/ncomms7003>.
63. Won, C., Shen, X., Mashiguchi, K., Zheng, Z., Dai, X., Cheng, Y., Kasahara, H., Kamiya, Y., Chory, J., and Zhao, Y. (2011). Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 108, 18518–18523. <https://doi.org/10.1073/pnas.1108436108>.
64. Liu, H., Zhang, H., Dong, Y.X., Hao, Y.J., and Zhang, X.S. (2018). DNA METHYLTRANSFERASE1-mediated shoot regeneration is regulated by cytokinin-induced cell cycle in Arabidopsis. *New Phytol.* 217, 219–232. <https://doi.org/10.1111/nph.14814>.

65. Kaya, H., Shibahara, K.-i., Taoka, K.-i., Iwabuchi, M., Stillman, B., and Araki, T. (2001). FASCIATA Genes for Chromatin Assembly Factor-1 in Arabidopsis Maintain the Cellular Organization of Apical Meristems. *Cell* 104, 131–142. [https://doi.org/10.1016/S0092-8674\(01\)00197-0](https://doi.org/10.1016/S0092-8674(01)00197-0).
66. Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34, i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
67. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25, 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
68. Kim, D., Paggi, J.M., Park, C., Bennett, C., and Salzberg, S.L. (2019). Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat. Biotechnol.* 37, 907–915. <https://doi.org/10.1038/s41587-019-0201-4>.
69. Ramírez, F., Ryan, D.P., Grüning, B., Bhardwaj, V., Kilpert, F., Richter, A.S., Heyne, S., Dündar, F., and Manke, T. (2016). deepTools2: a next generation web server for deep-sequencing data analysis. *Nucleic Acids Res.* 44, W160–W165. <https://doi.org/10.1093/nar/gkw257>.
70. Zhang, Y., Liu, T., Meyer, C.A., Eeckhoute, J., Johnson, D.S., Bernstein, B.E., Nusbaum, C., Myers, R.M., Brown, M., Li, W., and Liu, X.S. (2008). Model-based Analysis of ChIP-Seq (MACS). *Genome Biol.* 9, R137. <https://doi.org/10.1186/gb-2008-9-9-r137>.
71. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
72. Ross-Innes, C.S., Stark, R., Teschendorff, A.E., Holmes, K.A., Ali, H.R., Dunning, M.J., Brown, G.D., Gojis, O., Ellis, I.O., Green, A.R., et al. (2012). Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature* 481, 389–393. <https://doi.org/10.1038/nature10730>.
73. Li, Z., Schulz, M.H., Look, T., Begemann, M., Zenke, M., and Costa, I.G. (2019). Identification of transcription factor binding sites using ATAC-seq. *Genome Biol.* 20, 45. <https://doi.org/10.1186/s13059-019-1642-2>.
74. Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30, 923–930. <https://doi.org/10.1093/bioinformatics/btt656>.
75. McLeay, R.C., and Bailey, T.L. (2010). Motif Enrichment Analysis: a unified framework and an evaluation on ChIP data. *BMC Bioinf.* 11, 165. <https://doi.org/10.1186/1471-2105-11-165>.
76. Robinson, J.T., Thorvaldsdottir, H., Turner, D., and Mesirov, J.P. (2023). igv.js: an embeddable JavaScript implementation of the Integrative Genomics Viewer (IGV). *Bioinformatics* 39, btac830. <https://doi.org/10.1093/bioinformatics/btac830>.
77. Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504. <https://doi.org/10.1101/gr.1239303>.
78. Yu, G. (2024). Thirteen years of clusterProfiler. *Innovation* 5, 100722. <https://doi.org/10.1016/j.xinn.2024.100722>.
79. Shumate, A., and Salzberg, S.L. (2021). Liftoff: accurate mapping of gene annotations. *Bioinformatics* 37, 1639–1643. <https://doi.org/10.1093/bioinformatics/btaa1016>.
80. Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M., and Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience* 10, giab008. <https://doi.org/10.1093/gigascience/giab008>.
81. Grant, C.E., Bailey, T.L., and Noble, W.S. (2011). FIMO: scanning for occurrences of a given motif. *Bioinformatics* 27, 1017–1018. <https://doi.org/10.1093/bioinformatics/btr064>.
82. Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797. <https://doi.org/10.1093/nar/gkh340>.
83. Wang, Q., Li, M., Wu, T., Zhan, L., Li, L., Chen, M., Xie, W., Xie, Z., Hu, E., Xu, S., and Yu, G. (2022). Exploring Epigenomic Datasets by ChIPseeker. *Curr. Protoc.* 2, e585. <https://doi.org/10.1002/cpz1.585>.
84. Schep, A.N., Wu, B., Buenrostro, J.D., and Greenleaf, W.J. (2017). chromVAR: inferring transcription-factor-associated accessibility from single-cell epigenomic data. *Nat. Methods* 14, 975–978. <https://doi.org/10.1038/nmeth.4401>.
85. Gu, Z., Eils, R., and Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 32, 2847–2849. <https://doi.org/10.1093/bioinformatics/btw313>.
86. Zhao, L., Yang, Y., Chen, J., Lin, X., Zhang, H., Wang, H., Wang, H., Bie, X., Jiang, J., Feng, X., et al. (2023). Dynamic chromatin regulatory programs during embryogenesis of hexaploid wheat. *Genome Biol.* 24, 7. <https://doi.org/10.1186/s13059-022-02844-2>.
87. Zhang, Z., Lin, X., Yue, J., Xu, Y., Miao, L., Tang, W., Guo, W., and Xiao, J. (2025). Reshaping epigenomic landscapes facilitated bread wheat speciation. *Plant Physiol.* 199, kiaf399. <https://doi.org/10.1093/plphys/kiaf399>.
88. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., et al. (2000). Gene Ontology: tool for the unification of biology. *Nat. Genet.* 25, 25–29. <https://doi.org/10.1038/75556>.
89. Quinlan, A.R., and Hall, I.M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842. <https://doi.org/10.1093/bioinformatics/btq033>.
90. Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T.L. (2009). BLAST+: architecture and applications. *BMC Bioinf.* 10, 421. <https://doi.org/10.1186/1471-2105-10-421>.
91. Meng, W.J., Cheng, Z.J., Sang, Y.L., Zhang, M.M., Rong, X.F., Wang, Z.W., Tang, Y.Y., and Zhang, X.S. (2017). Type-B ARABIDOPSIS RESPONSE REGULATORS Specify the Shoot Stem Cell Niche by Dual Regulation of WUSCHEL. *Plant Cell* 29, 1357–1372. <https://doi.org/10.1105/tpc.16.00640>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
GV3101	N/A	N/A
EHA105	Coolaber	Cat#CC403
Trans T1	TransGen Biotech	Cat#CD501-02
Chemicals, peptides, and recombinant proteins		
FastDigest Apal	Thermo Fisher	Cat#FD1414
FastDigest Eco32I	Thermo Fisher	Cat#FD0304
DNase I	Thermo Fisher	Cat#EN0521
Protector RNase inhibitor	Roche	Cat#3335399001
ChamQ Universal SYBR qPCR Master Mix	Vazyme	Cat#Q711-02
2 × Phanta Max Master Mix	Vazyme	Cat#P515-01
2 × Rapid Taq Master Mix	Vazyme	Cat#P222-01
DIG RNA Labeling Mix	Roche	Cat#11277073910
T7 RNA Polymerase	Roche	Cat#10881767001
NBT/BCIP Stock Solution	Roche	Cat#11681451001
Critical commercial assays		
Ultrapure RNA Kit	CWBIO	Cat#CW0581S
ChamQ Universal SYBR qPCR Master Mix	Vazyme	Cat#Q711-02
FastPure Gel DNA Extraction Mini Kit	Vazyme	Cat#DC301-01
FastPure Plasmid Mini Kit	Vazyme	Cat#DC201-01
pEASY®-Blunt3 Cloning Kit	TransGen	Cat#CB301-01
pENTR™/D-TOPO™ Cloning Kit	TransGen	Cat#K240020SP
Dual Luciferase Reporter Assay System Kit	Promega	Cat#E1910
PCR purification kit	QIAGEN	Cat#28106
AMPure XP Beads	Beckman	Cat#A63881
TruePrep DNA Library Prep Kit V2 for Illumina	Vazyme	Cat#TD501-01
Deposited data		
Raw data of RNA-sequencing		GSA: PRJCA012455 ; GSA: PRJCA040308
Raw data of ATAC-sequencing	https://ngdc.cncb.ac.cn/	GSA: PRJCA012455
Scripts and codes used in this study	This paper	Zenodo: https://doi.org/10.5281/zenodo.17614807
Experimental models: Organisms/strains		
<i>Triticum aestivum</i> cv. Fielder: TaSCR-OE-1	This paper	N/A
<i>Triticum aestivum</i> cv. Fielder: TaSCR-OE-2	This paper	N/A
<i>Triticum aestivum</i> cv. Fielder: TaLBD17-OE-1	This paper	N/A
<i>Triticum aestivum</i> cv. Fielder: TaLBD17-OE-2	This paper	N/A
<i>Triticum aestivum</i> cv. Fielder	This paper	N/A
<i>Triticum aestivum</i> cv. KN199	This paper	N/A
<i>Triticum aestivum</i> cv. JM22	This paper	N/A
<i>Triticum aestivum</i> cv. AK58	This paper	N/A
<i>Nicotiana benthamiana</i>	This paper	N/A
Oligonucleotides		
Primers used in this study	This paper	Table S6
Recombinant DNA		
CP461-LUC-TaSCR _{pro}	This paper	N/A

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
CP461-LUC- <i>TaLBD17pro</i>	This paper	N/A
CP461-LUC- <i>TaFTIP3pro</i>	This paper	N/A
CP461-LUC- <i>TaBBM2pro</i>	This paper	N/A
CP461-LUC- <i>TaGRF7pro</i>	This paper	N/A
PTF101-TaSCR	This paper	N/A
PTF101-TaMYB3R2	This paper	N/A
PTF101-TaGATA15	This paper	N/A
PTF101-TaMYB44	This paper	N/A
PTF101-TaWRKY57	This paper	N/A
PTF101-TaWRKY65	This paper	N/A
PTF101-TaARF22	This paper	N/A
Software and algorithms		
fastp	Chen ⁶⁶	https://github.com/OpenGene/fastp
bwa	Li ⁶⁷	https://github.com/lh3/bwa
Hisat2	Kim et al. ⁶⁸	https://daehwankimlab.github.io/hisat2/
picard	N/A	https://github.com/broadinstitute/picard
deeptools	Ramírez et al. ⁶⁹	https://github.com/deeptools/deepTools
MACS2	Zhang et al. ⁷⁰	https://github.com/macs3-project/MACS/wiki/Install-macs2
R	R-Project	https://www.r-project.org/
DESeq2	Love et al. ⁷¹	https://github.com/thelovelab/DESeq2
DiffBind	Ross-Innes et al. ⁷²	https://github.com/nshanian/DiffBind
HINT	Li et al. ⁷³	https://reg-gen.readthedocs.io/en/latest/hint/introduction.html
featurecount	Liao et al. ⁷⁴	https://subread.sourceforge.net/featureCounts.html
ame	McLeay & Bailey ⁷⁵	https://meme-suite.org/meme/doc/ame.html
Integrative Genomics Viewer	Robinson et al. ⁷⁶	https://igv.org/
Cytoscape	Shannon et al. ⁷⁷	https://cytoscape.org/
clusterProfiler	Xu et al. ⁷⁸	https://github.com/YuLab-SMU/clusterProfiler
Liftoff	Shumate et al. ⁷⁹	https://github.com/agshumate/Liftoff
samtools	Danecek et al. ⁸⁰	https://www.htslib.org/
fimo	Grant et al. ⁸¹	https://web.mit.edu/meme_v4.11.4/share/doc/fimo.html
MUSCLE	Edgar ⁸²	https://drive5.com/muscle/
ChIPseeker	Wang et al. ⁸³	https://github.com/YuLab-SMU/ChIPseeker
chromVAR	Schep et al. ⁸⁴	https://github.com/GreenleafLab/chromVAR
PlantTFDB	Tian et al. ⁴⁷	https://planttfdb.gao-lab.org/
ComplexHeatmap	Gu et al. ⁸⁵	https://jokergoo.github.io/ComplexHeatmap-reference/book/
Adobe Illustrator CC 2023	Adobe	https://www.adobe.com/cn/

METHOD DETAILS

Plant materials and growth conditions

The hexaploidy wheat varieties, Fielder, JM22, KN199 and AK58 were used in our experiment. Seeds of the spring wheat cultivar Fielder, along with transgenic seeds of *TaSCR*-OE1/2 or *TaLBD17*-OE1/2, were directly sown in pots (21 cm height × 23 cm diameter). As winter wheat varieties, the grains of KN199, JM22 and AK58 were germinated at 25°C for 2 days and then vernalized in incubator at 6°C for 35 days. All wheat materials were grown in a climate chamber (manufactured by Beijing Yisheng Taihe CTRL Tech Co., Ltd.) with 300 μmol m⁻² s⁻¹ light intensity, 24°C 16 h light/16°C 8 h dark, and 45% humidity. The cultivation substrate was a mixture of nutrient soil, seedling substrate, and vermiculite at a volume ratio of 2:2:1.

Plasmid construction and wheat transformation

Full-length complementary DNA of *TaSCR*, *TaLBD17* and *GUS*, along with the specific sequences 87–176 bp within the 5' untranslated region (5' UTR) of the *TaARF22* and 102–295 bp within the coding sequence (CDS) region *TaGATA15* were cloned into *pEASY-Blunt3* Cloning Vector (TransGen, CB301) for sequencing. Subsequently, the pENTR/D-TOPO Vector (Thermo Fisher, 2502110) was digested with restriction endonucleases, and then inserted cDNA of *TaSCR*, *TaLBD17*, or *GUS* into the overexpressed pc186 vector (containing *ZmUbi* promoter and *Nos* terminator), while the specific cDNA sequence of the *TaARF22* and *TaGATA15* into the RNAi pc336 vector. Vector pc186-*TaSCR*, pc186-*TaLBD17*, pc186-*GUS*, pc336-*TaARF22* and pc336-*TaGATA15* was introduced into *Agrobacterium* strains EHA105. All constructed vectors were prepared for wheat transformation. Primers used are listed in Table S6.

The immature embryos of Fielder, JM22, AK58 and KN199 were tissue cultured generally following a previously described protocol.^{14,19} Briefly, immature embryos at 14 days after anthesis were isolated from the immature grains and inoculated with *Agrobacterium tumefaciens* strains EHA105 contained constructed vector for 2 days. After co-cultivation, embryonic axes were excised and the scutella were transferred to resting medium at 25°C for 5 days. Each callus was transferred to selection medium for 2 weeks, and then was cut into 2 pieces and continued culture for 3 weeks. Proliferated callus was differentiated at 25°C with light for 10 days. Transformed plants were transferred on the root induction medium for elongation and root formation.

When wheat callus tissue cultured on root induction medium, we counted explants, explants forming callus, callus differentiating into shoot or root, and callus producing at least one confirmed shoot and calculated the callus induction efficiency, callus differentiation efficiency and transformation efficiency. We identified the candidate transgenic wheat plants by PCR. Primers used for the identification of transgenic plants are listed in Table S6.

Callus induction efficiency, callus differentiation efficiency and transformation efficiency are calculated as follows:

Callus induction efficiency (%):

$$(\text{the number of explants forming callus} / \text{the number of explants incubated}) \times 100 \quad (\text{Equation 1})$$

Callus differentiation efficiency (%):

$$(\text{the number of callus differentiating into shoot or root} / \text{the number of explants incubated}) \times 100 \quad (\text{Equation 2})$$

Transformation efficiency (%):

$$(\text{the number of callus producing at least one confirmed shoot} / \text{the number of explants incubated}) \times 100 \quad (\text{Equation 3})$$

RNA-seq and ATAC-seq experiment

Calli transformed *TaSCR*, *TaLBD17* or *GUS* control at DAI6, DAI9, DAI12 and DAI18 of Fielder and at DAI6, DAI12 and DAI18 of JM22 were collected for RNA-seq. Total RNA were extracted using Ultrapure RNA kit (CWBIO, CW0581M) according to manufacturer instructions. Three biological replicates were performed. The RNA seq data for this experiment was measured using DNBSEQ77.

ATAC-seq experiments were done following a previously described method.⁸⁶ The samples were soaked in HBM buffer, dissected with a razor blade, and then filtered through a 40 μm cell strainer. The crude nuclei suspension obtained was washed twice and the nuclei were counted using a haemocytometer. Approximately 5,000 isolated nuclei per reaction were incubated with Tn5 transposase in tagmentation buffer at 37°C for 30 min (Vazyme Biotech, TD501-01). DNA was then purified using a PCR purification kit (QIAGEN, 28106). The sequencing libraries were generated by PCR amplification for 9–12 cycles under the following conditions: 72°C for 5 min; 98°C for 30 s; and thermocycling at 98°C for 15 s, 63°C for 30 s and 72°C for 40 s; followed by 72°C for 5 s. After amplification, the libraries were purified using AMPure beads (Beckman, A63881). The final sequencing step was carried out on the Illumina Novaseq platform (Annoroad Gene Technology).

Luciferase reporter assay

For LUC analyses, we generally followed the methodology described in previously published studies.⁸⁷ Full-length coding sequences of *TaSCR*, *TaMYB3R2*, *TaGATA15*, *TaMYB44*, *TaWRKY57*, *TaWRKY65* and *TaARF22* from Fielder were cloned into PTF101 vector to generate the effector vectors p35S: *TaSCR*-eGFP, p35S: *TaMYB3R2*-eGFP, p35S: *TaGATA15*-eGFP, p35S: *TaMYB44*-eGFP, p35S: *TaWRKY57*-eGFP, p35S: *TaWRKY65*-eGFP and p35S: *TaARF22*-eGFP. About 3 Kb promoter fragment of *TaSCR*, *TaLBD17*, *TaFTIP3*, *TaBBM2*, *TaGRF7*, *TaWOX5* and *TaGATA15* were amplified and fused in-frame with the CP461-LUC vector to generate the reporter construct *proTaSCR::LUC*, *proTaLBD17::LUC*, *proTaFTIP3::LUC*, *proTaBBM2::LUC*, *proTaGRF7::LUC*. The plasmids were transformed into *Agrobacterium* GV3101. Then these strains were injected into *N. benthamiana* leaves in different combinations. Dual luciferase assay reagents with the Renilla luciferase gene as an internal control were used for luciferase imaging. The Dual-Luciferase Reporter Assay System Kit (Promega, E1910) was used to quantify fluorescence signals. Relative activity was calculated by the ratio of LUC/REN. Primers used for luciferase reporter assay are listed in Table S6.

RNA-seq analysis

To remove adapters and filter out low quality reads, fastp⁶⁶ (v0.23.4) was applied with the parameter “–detect_adapter_for_pe”. Cleaned reads were mapped to IWGSC Refseq v1.0 using hisat2⁶⁸ (v2.2.1) and the resulting bam files were sorted with samtools⁸⁰ (v1.16.1). Gene counts were quantified by featureCounts⁷⁴ (v2.0.3) command provided by subread with the parameter “–t exon –p –P

-B -C -g gene_id" and normalized to transcripts per million (TPM) for further PCA and K-means cluster analysis. Differentially expressed genes (DEGs) were identified using DESeq2⁷¹ (v1.34.0) with a threshold of "p.adjust <0.05 and |FoldChange|>1.5". GO enrichment was performed using the online website <http://geneontology.org/>.⁸⁸ Heatmap were generated by "k-means" function in R and ComplexHeatmap⁸⁵ (v2.10.0) package.

DNA sequences comparative analysis between Fielder and JM22

To identify the corresponding sequences of genes in cluster C4 in Fielder and JM22 genomes, we used Liftoff⁷⁹ (v1.6.3) to identify their genomic coordinates in both assemblies.

To compare protein sequence similarity of homologous genes between the two cultivars, we aligned protein sequences of every gene using MUSCLE⁸² (v3.8.1551), and calculated the proportion of differing amino acids relative to the length of the longer protein.

To analyze the *cis*-element differences in promoter (-2Kb ~ TSS) of these genes between Fielder and JM22, we used fimo⁸¹ (v5.4.1) to scan for potential TF binding sites (threshold = 1e-5), based on 619 motifs from PlantTFDB.⁴⁷ For each gene, we quantified the difference in motif occurrence between the two varieties. A motif was defined as variety-preferential if it was predicted to bind to more genes in one variety than the other. Finally, we performed TF family enrichment analysis on the preferential motifs identified in Fielder and JM22 using the enricher function in the clusterProfiler⁷⁸ (v4.2.2) package.

ATAC-seq analysis

To remove adapters and filter out low quality reads, fastp (v0.23.4) was applied with the parameter "-detect_adapter_for_pe". Cleaned reads were mapped to IWGSC Refseq v1.0 using bwa mem⁶⁷ (v0.7.18) and the resulting bam files were sorted and filtered with the "-F 1804 -f 2 -q 30" parameter using samtools (v1.16.1). Then picard (v2.27.2) (<https://broadinstitute.github.io/picard/>) was used to remove the duplicated reads and we merged the two biological replicates using samtools from the de-duplicated bam files. The merged bam files were used to convert to bigwig files using bamCoverage (deeptools⁶⁹ v3.5.1) with the parameters "-bs 10 -effectiveGenomeSize 14600000000 -normalizeUsing RPKM -smoothLength 50" and call peaks using macs2⁷⁰ (v2.2.9.1) with the parameters "-q 0.05 -f BAMPE -nomodel -extsize 200 -shift -100 -g 14600000000". The bigwig files were visualized using the Integrative Genomics Viewer.⁷⁶ ChIPseeker⁸³ (v1.32.0) was used to annotate peaks: promoter (-3000 to +500 of TSS), genic (from +500 of TSS to TES), downstream (from TES to +300 of TES) and distal (other).

To merge a specific set of ACRs, we first sorted the regions using "bedtools sort"⁸⁹ command, and then merged them using the "bedtools merge" command.

To quantify the ATAC-seq data, we first merged the peaks from all Fielder and JM22 samples to generate reference ACRs. DiffBind⁷² (v3.4.11) was used with the parameter "DBA_SCORE_TMM_READS_EFFECTIVE_CPM, bUseSummarizeOverlaps = TRUE, summits = FALSE, peaks = referenceACRs.bed" to get count and CPM of each peak. And counts of peaks were used to identify diffACRs using DESeq2 (v1.34.0) with the threshold of "p.adjust <0.05 and |FoldChange|>2".

To assess the correlation between chromatin accessibility and gene expression, we referenced methodologies described in previously published studies.⁴² Briefly, we calculated the Pearson correlation coefficients (PCCs) between each chromatin accessibility peak (measured in CPM) and the expression levels (TPM) of genes located within ± 0.5 Mb of the peak.

To establish a null model, we computed PCCs between randomly selected peaks and randomly chosen genes located on different chromosomes, then calculated the mean and standard deviation of the null distribution. Using these parameters, we calculated *p*-values for the observed peak-gene correlations within the ± 0.5 Mb window with the pnorm function in R. Regulatory region-gene pairs with *p*-values <0.05 were considered significant.

Cis-motif enrichment analysis and motif activity analysis

To identify *cis*-regulatory elements enriched in different clusters of diffACR shown in Figure 2D, we used AME⁷⁵ (v5.4.1) tool from the MEME Suite. The sequences of proximal ACRs associated with all genes were used as control. A total of 619 *Arabidopsis* TF motifs, obtained from PlantTFDB (<https://planttfdb.gao-lab.org/>), were used as candidate motifs for enrichment analysis.

For the motif activity analysis, we employed the chromVAR⁸⁴ (1.20.0) package to assess the activity of all GRAS and LBD family motifs in PlantTFDB within promoter regions, and motif activity was normalized using the deviationScores function.

Comparative analysis of TRNs

The construction of the TRN for JM22 was based on previously published methodologies.¹⁹ Briefly, we identified DEGs during the JM22 regeneration process using the threshold of "p.adjust <0.05 and |FoldChange| > 1.5", and subsequently clustered them into six clusters using K-means (J1-J6). Next, target genes with TPM <1 in all samples were first filtered out. We used HINT⁷³ to identify footprints within ACRs of JM22 and detect associated motifs (619 motifs obtained from PlantTFDB), thereby establishing motif-target gene associations. Subsequently, *Arabidopsis* motifs were mapped to wheat transcription factors using blastp⁹⁰ (v2.10.1) (e-value < 1e-5, identity >30%, and belonging to the same TF family). The wheat TF annotations were obtained from PlantTFDB.

We next retained those TF-target gene pairs that were co-expressed and connected via motifs enriched specific clusters. Regulatory relationships between clusters ($J2 \rightarrow J6 \rightarrow J4 \rightarrow J1 \rightarrow J5$) were determined using Fisher's exact test, with the following formula:

$$Pr(x = q) = \frac{\left(\frac{Q}{q}\right) \left(\frac{M-Q}{m-q}\right)}{\left(\frac{M}{m}\right)}. \quad (\text{Equation 4})$$

For example, when calculated the significance of regulations from clusters J1 to J2, Pr represents the probability, x represents each regulatory relationship, M represents the total number of regulations, Q represents the total number of regulations from TFs in cluster J1, m represents the total number of regulations targeting Cluster J2, q represents the number of regulations from cluster J1 to cluster J2 and the general form $\left(\frac{c1}{c2}\right)$ is a binomial coefficient. A threshold of $p < 1 \times 10^{-6}$ was applied to infer regulatory regulations between clusters. Networks were visualized in Cytoscape.⁷⁷

Finally, transcription factors were defined as core TFs in JM22 if their motifs are enriched in the promoters of genes within their own cluster or in the downstream cluster they regulate.

To determine whether a TF is enriched in mediating Fielder-specific regulations, we first merged the TRNs of Fielder and JM22 and focus on the regulations targeting to TFs, hormone pathways, epigenetic regulatory factors and cell cycle regulators, and identified Fielder-specific, JM22-specific, and common regulatory interactions. Next, we performed a Fisher's exact test using the Equation 4. Pr represents the probability, x represents each TF, and M represent the total number of regulatory interactions in the merged TRN. Q denotes the total number of Fielder-specific interactions across the merged network. m represents the number of regulatory interactions mediating by this TF. q is the number of Fielder-specific regulations mediating by this TF. A threshold of $p < 5 \times 10^{-3}$ was used to define significant enrichment.

In situ hybridization

Immature embryos at 14 days after anthesis in Fielder and callus with tissue cultured at DAI3, DAI6 and DAI9 were fixed in 0.1 M phosphate buffer (pH 7.0) and 4% v/v paraformaldehyde (Sigma, 30525-89-4) overnight at 4°C. The samples were dehydrated by ethanol and transparent by dimethylbenzene, then embedded in paraplast (Leica, 39601006). We synthesized antisense and sense RNA probes of *TaSCR* and *TaLBD17* using DIG RNA labeling kit (Roche, 11175025910). Detailed experimental protocol of *in situ* hybridization and detection of hybridized signals were performed as described.⁹¹ Primers used for *in situ* hybridization are listed in Table S6.

Quantitative reverse-transcription PCR

The leaves of *TaSCR* or *TaLBD17* transgenic plants and callus of the same period as *in situ* hybridization were used to extract the total RNA using Ultrapure RNA kit (CW BIO, CW0581M). 2 µg of DNase I-treated total RNA was reverse-transcribed into cDNA using Hi-Script II Q RT SuperMix for qPCR (Vazyme, R223-01). 1 µg cDNA of each sample was used to perform the first RT-PCR using SYBR qPCR Master Mix (Vazyme, Q711-02). The Cq mean value was used to dilute cDNA of all samples to the same concentration. Subsequently, a second RT-PCR and data analysis were conducted. Primers used for RT-qPCR are listed in Table S6.

Phenotyping of grain development

Grains were collected from transgenic plants for weight and size measurements. Thousand-grain weight was determined by randomly selecting ≥ 100 grains per replicate, weighing them, and then scaling to thousand-grain weight. Grain length and grain width were measured using an automated seed phenotyping system (TPKZ-1-G; Zhejiang Top Cloud-Agri Technology Co., Ltd., China) with a measurement accuracy of 0.01 mm by randomly selecting ≥ 100 grains per replicate. All data were calculated based on six independent replicates.

QUANTIFICATION AND STATISTICAL ANALYSIS

If not specified, R (<https://cran.r-project.org/>; version 4.1.3) was used to compute statistics. The correlation between two groups of data was conducted with the Pearson analysis (cor function in R) (Figures S1C, S1D, S3B, S3G, and S4G). For the p -values shown in Figure S1G, A one-sided (right-tailed) test was performed for each observed correlation coefficient to assess its significance relative to the empirical null distribution (pnorm function in R). For comparisons between two groups of data (Figures 2G and 2H, 4E–4G, 4I–4K, 5C, 5G–5K, S4D, 6D–6F, 6H–6J, and S4M), Student's two tailed t test was used (t.test function in R). For the enrichment analysis (Figures 1F, 3C, 3G, and S4J), p -values were calculated using the hypergeometric test (enricher function in R). The FDR of GO enrichment for Figures 1D and 2D, S1I, S4C, and S5C was calculated using the website <https://geneontology.org/>. The FDR for Figure 2E was calculated using AME (5.4.1). For two-group comparison of data that did not conforming to a normal distribution, the Wilcoxon rank-sum test was used (wilcox.test function in R) (Figures 3F and S3C).