Regulation of spikelet number during wheat spike development

Chengxia Li[1, 2], Kun. Li[1, 2], Chaozhong Zhang[1, 2], Jorge Dubcovsky[1, 2*]

[1] University of California, Davis, CA 95616, USA

[2] Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA

Authors ORCIDs and emails

Chengxia Li: ORCID 0000-0003-4634-8451. Email: chxli@ucdavis.edu

Kun Li : ORCID 0000-0001-8308-1560. Email: kzli@ucdavis.edu

Chaozhong Zhang: ORCID 0000-0002-0247-3771. Email: cazhang@ucdavis.edu

Jorge Dubcovsky: ORCID 0000-0002-7571-4345. Email: jdubcovsky@ucdavis.edu

*** Corresponding author**

Jorge Dubcovsky. Email: jdubcovsky@ucdavis.edu

University of California, Davis, CA 95616, USA

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Abstract

Wheat produces unbranched inflorescences (spikes) composed of smaller inflorescences (spikelets) as their fundamental building units. The spikelet number per spike (SNS) is a major determinant of grain yield and the gene networks that regulate this trait are the focus of this review. Spikelet development starts with the transition of the shoot apical meristem into an inflorescence meristem (IM) that produces lateral spikelet meristems (SMs). The rate at which SMs are produced and the timing of the IM transition to a terminal spikelet (IM→TS) determine the final SNS. These two traits are regulated by genes expressed in the IM (e.g. meristem identity genes), as well as by the amount of FLOWERING LOCUS T1 (florigen) transported from leaves to developing spikes. The SNS can be also increased by the production of spikes with supernumerary spikelets (SS) or branch-like structures that resemble small spikes. Mutations that delay the acquisition of SM identity or that promote a reversion from SM to IM identity can induce the formation of SS. Initial efforts to incorporate these mutations into commercial wheat varieties have faced trade-offs in fertility and grain weight, which will require additional research and breeding efforts. Meanwhile, genes and allele combinations that increase SNS without affecting the number of spikelets per node have been identified and are being deployed in wheat breeding programs. Recent spatial transcriptomics, single-cell analyses, and multi-omics studies of wheat spike development are accelerating the discovery of new genes affecting SNS and enhancing our ability to engineer more productive wheat spikes.

Introduction

Inflorescences bear flowers and fruits, and their architectures affect yield in crop species. In the grasses, flowers are replaced by small inflorescences called spikelets (Fig. 1a-c), resulting in compound inflorescences or synflorescences [1]. Both spikelets and flowers are self-contained developmental modules that are expressed at different positions of the inflorescence and are commonly regulated by MADS-box genes of the SQUAMOSA clade [2,3]. Given these shared developmental characteristics, in this review we extend the simpler nomenclature used for inflorescences bearing flowers to the grass inflorescences bearing spikelets.

In many grasses, spikelets are produced on inflorescences called panicles that have branches of decreasing complexity from the base to the top (e.g. rice) [1]. In the *Triticeae*, however, genuine branches are absent, and spikelets sit directly on the rachis forming a spike (Fig. 1a). Spikelet development proceeds faster at the central part of the spike resulting in the characteristic lanceolate shape of the wheat spike (Fig. 1a, d-e). By the time the central spikelets begin forming floret meristems (FMs), the inflorescence meristem (IM) transitions into a spikelet meristem (SM) and forms a terminal spikelet (henceforth IM→TS). The terminal spikelet has different orientation from the lateral spikelets (Fig. 1a).

The timing of formation and the identity of meristems shape the inflorescence architecture. In wheat, both vegetative shoot apical meristems (vSAM) and IM produce lateral meristems in an alternate-distichous pattern, but these meristems differ in activity and identity. In the vSAM, bract meristems develop rapidly into leaves whereas their axillary meristems (AxM) are temporarily repressed before initiating tillers. By contrast, in lateral meristems generated by the IM, the lower ridges (bract meristems) are repressed, while the upper ridges (equivalent to AxM) rapidly develop into spikelets (Fig. 1d).

This review focuses on gene networks that regulate wheat spike development, from the initial vSAM→ IM transition to the IM→TS transition (Fig. 1d). It includes genes that affect the rate at which SMs are produced, the timing of the IM→TS transition, and the number of spikelets in each node. Due to space limitations, the regulation of grain number per spikelet is not covered, although its agronomic importance is recognized. This review integrates recent advances in spatial transcriptomics and single-cell expression analyses and discusses the potential contributions of this knowledge to the improvement of wheat productivity.

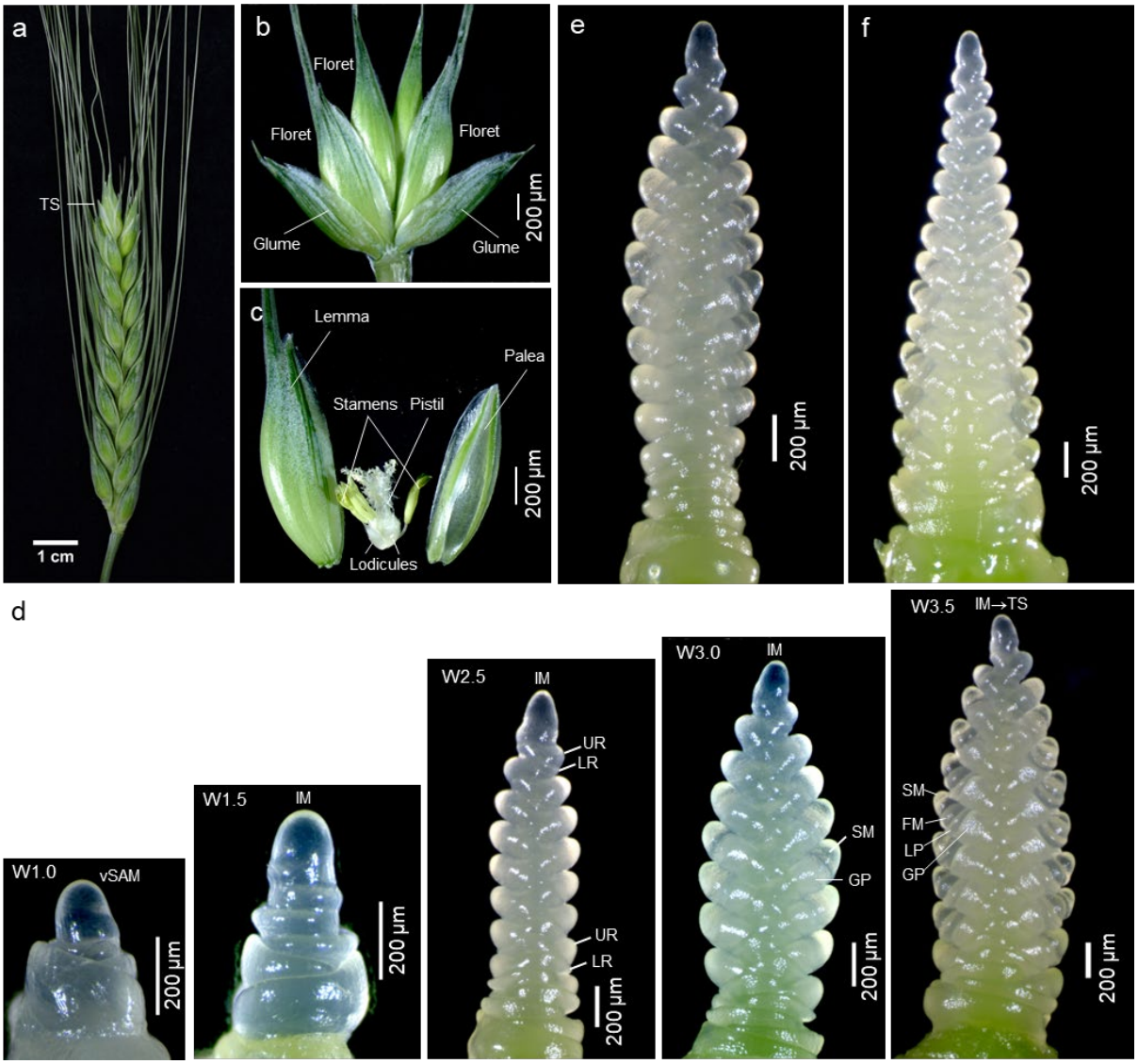

Fig.1. (a) Wheat spike showing a terminal spikelet with a different orientation relative to the lateral spikelets. **(b)** Wheat spikelet showing two basal sterile bracts (glumes) and multiple florets. **(c)** Each floret includes a bract (lemma) subtending the floral organs: the palea, two lodicules, three stamens, and a pistil. **(d)** Waddington scale of wheat spike development: W1.0= vegetative shoot apical meristem (vSAM), W1.5= initial transition of the vSAM to an inflorescence meristem (IM) that produces lateral spikelet meristems (SM). W2.5= double ridge stage. The lower ridge (LR) corresponds to a repressed bract and the upper ridge (UR) to the AxM of the repressed bract. W3.0= glume primordium (GP). W3.5= floret primordia (FM) subtended by the lemma primordium (LP). This stage is also characterized by the transition of the IM into a SM that generates a terminal spikelet (IM→TS). **(e-f)** Developing spikes at W3.0 for *vrn1 vrn2*-null mutant **(e)** and *svp1 vrt2*-null mutants **(f)** showing a higher number of SMs.

1. The critical role of MADS-box genes in the initial stages of wheat spike development

Wheat spike development starts with the vSAM→IM transition, which is driven by the upregulation of the *VERNALIZATION1* (*VRN1*) gene (Fig. 2a and b). However, *VRN1* upregulation in the vSAM is not sufficient to complete spike development and stem elongation, which requires long-day (LD) induction of *FLOWERING LOCUS T1* (*FT1*) in the leaves. The FT1 protein (florigen) is transported to the developing spike, where it simultaneously up-regulates *VRN1* and gibberellin (GA) biosynthetic genes [4] (Table S1). Both GA and *VRN1* are required for the transcriptional up-regulation of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) and *LEAFY* (*LFY*), two important genes in wheat spike development [4]. Another important role of VRN1 in the leaves is to repress the LD flowering repressor *VRN2*, which negatively regulates *FT1* [5].

VRN1 and its closest paralogues *FUL2* and *FUL3* are MADS-box genes of the *SQUAMOSA* clade that are widely expressed during early spike developing (Fig. 2a-b). Spikes of plants carrying knockout mutations in all *VRN1* homeologs (*vrn1*-null) are normal [5], but further combinations with *ful2*-null and *ful3*-null mutations (*SQUAMOSA*-null) result in abnormal spikes where spikelets are replaced by vegetative tillers subtended by leaves [3]. These *SQUAMOSA*-null plants (combined with *vrn2*-null mutations to avoid extremely late heading) still undergo a vSAM→IM transition followed by a double-ridge stage, elongated stems, and AxM that develop rapidly in the "spike" region [3]. These results indicate that the *SQUAMOSA* genes play essential roles in suppressing the lower ridge and conferring spikelet meristem identity to the upper ridge, but that they are not essential for inflorescence initiation [3].

The genes responsible for the initial stages of inflorescence development in the wheat *SQUAMOSA*-null mutants are currently unknown, but in Arabidopsis MADS-box genes of the *SOC1* and *SHORT VEGETATIVE PHASE* (*SVP*) clades contribute to this initial inflorescence development [6-8]. A recent study showed that the wheat SOC1-3 protein can compete with VEGETATIVE TO REPRODUCTIVE TRANSITION 2 (VRT2) for interactions with VRN1 and can also regulate heading time [9]. In wheat, there are five *SOC1* paralogs [10] with different expression profiles (Fig. 2c) and possible different functions based on studies of the *SOC1* orthologs in rice [11]. Therefore, a more comprehensive study of the wheat *SOC1* genes would be required to elucidate their roles in heading time and spike development.

The wheat SVP transcription factors, which include SVP1, VRT2, and SVP3, physically interact with the SQUAMOSA proteins [12]. Combined loss-of-function mutations in *SVP1* and *VRT2* genes delay heading time, increase spikelet number per spike (SNS, Fig. 1e-f), and reduce plant height in a similar way as the *vrn1*-null mutants. The similar mutant effects and physical protein interactions suggest that SQUAMOSA and SVP proteins may work cooperatively to regulate early spike development [12].

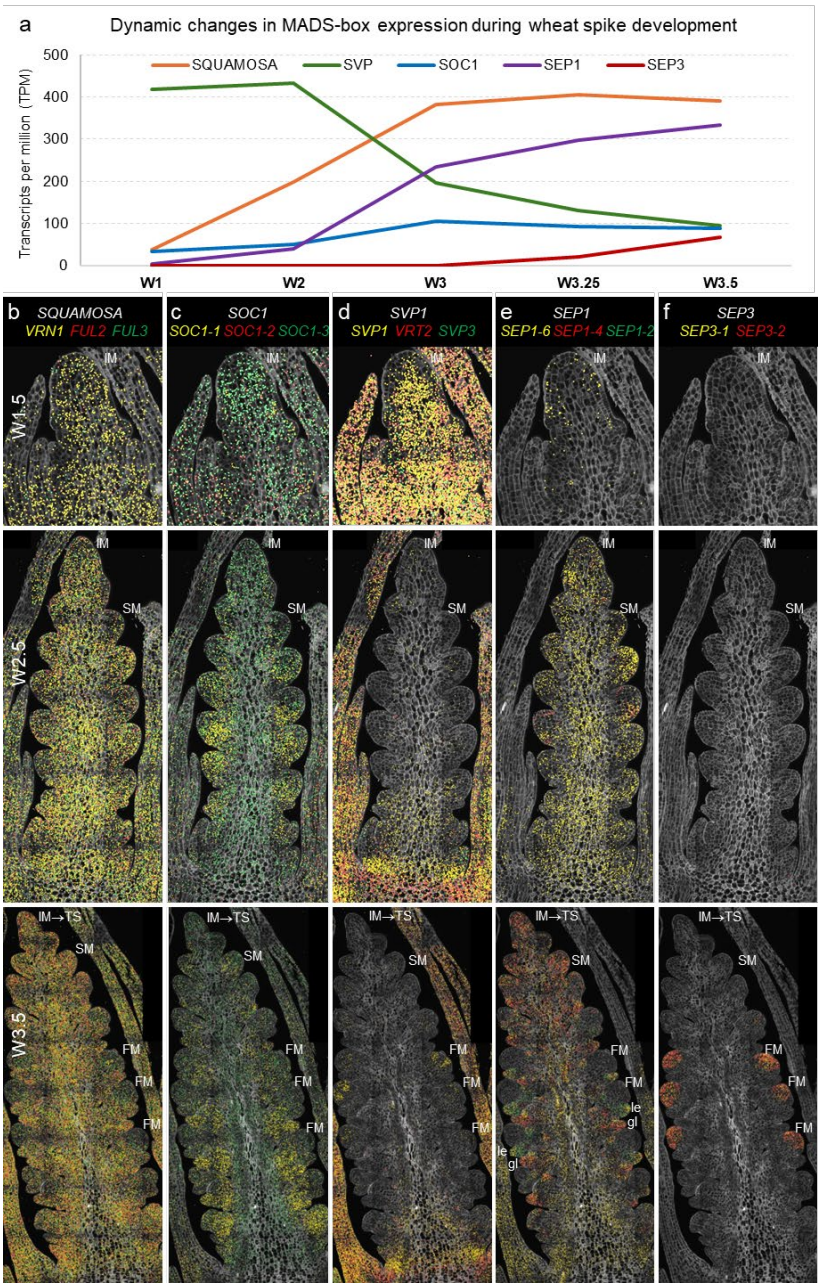

Fig. 2. Dynamic changes in MADS-box gene expression during wheat early spike development. **(a)** Curves are based on RNA-seq data of wheat spike development in tetraploid wheat Kronos [13] and individual points are the sum of transcripts per million of all homeologs and paralogs within each MADS-box group (Table S1). **(b-f)** Spatial transcriptomics of MADS-box gene families across three spike developmental stages [14]. **(b)** *SQUAMOSA*. *VRN1* is expressed earlier than the other two *SQUAMOSA* genes and all three genes are expressed at relatively lower levels at the FMs. **(c)** *SOC1*. *SOC1-2* expression is higher at W1.5 and then decreases, whereas expression of other *SOC1* genes increases during early spike development. **(d)** *SVP*. Genes are expressed in the IM at W1.5 and at the base of the spike at W2.5 and W3.5. *SVP1* is also expressed in developing anthers. **(e)** *SEP1*. *SEP1-6* is the earliest expressed gene in this clade, *SEP1-4* is enriched in the distal part of the spike and glume primordia, and *SEP1-2* is enriched in lemma primordia. **(f)** *SEP3*. *SEP3-1* and *SEP3-2* are expressed in FMs together with other floral homeotic genes (Table S1).

The wheat *SVP* genes are co-expressed with the *SQUAMOSA* genes in the early IM at W1.5, but their expression is restricted to the base of the spike and to the leaves at later stages (Fig. 2d). The transcriptional downregulation of the *SVP* genes by the *SQUAMOSA* genes is important for the normal progression of spike development, as SVP proteins can interfere with the SQUAMOSA-SEPALLATA1 (SEP1) protein interactions [12]. Plants carrying high-expressing natural *VRT2* alleles or transgenic plants overexpressing this gene exhibit more vegetative traits such as larger glumes and lemmas [12,15]. By contrast, the vegetative characteristics of the *vrn1 ful2* spikes are mitigated when combined with *vrt2* mutations [12], highlighting the importance of the downregulation of the *SVP* genes for normal spike development.

High *VRT2* levels are also associated with a higher number of basal rudimentary spikelets [16], suggesting that *VRT2* can delay the development of basal spikelets and contribute to the lanceolate shape of the wheat spikes [16]. This hypothesis is supported by the triangular shape of the *svp1 vrt2* mutant spike at W3.0 (Fig. 1f), where basal spikelet development is not delayed relative to the central spikelets as in the wildtype (Fig. 1d).

The *SEP1* and *SVP* genes show opposite expression profiles (Fig. 2a). Among the *SEP1* genes, *SEP1-6* expression is initiated the earliest (Fig. 2e), and its overexpression in wheat results in reduced SNS [17]. Combined mutations in the rice homologs of *SEP1-6* (*OsMADS34*) and *SEP1-4* (*OsMADS5*) lead to enhanced branching in rice panicles [18], but have limited effects on barley spikes [19,20]. Mutations in the *SEP1-2* ortholog in rice (*OsMADS1*) result in floral organs with leaf-like characteristics [21], whereas mutations in the barley ortholog lead to smaller lemmas and awns at room temperature [22] and branched spikes at high temperatures [19]. Given the different effects of the *SEP1* mutants in rice and barley, it is not possible to infer their function in wheat without dedicated research efforts. The *SEP3* genes are co-expressed in the FM (Fig. 2f) with other floral homeotic genes [14] and contribute to floral organ identity [23].

MADS-box proteins form quaternary complexes of varied compositions, each with specific DNA binding specificities [24]. Therefore, the early upregulation of *VRN1* in the IM of wheat (Fig. 2b) and the gradual changes in the relative expression of other MADS-box genes (Fig. 2a) likely affect the composition of these quaternary complexes and the sequential morphological changes observed during the early stages of wheat spike development.

2 Regulation of wheat spike determinacy and spikelet number per spike (SNS)

Both wheat and barley produce spikes with a genetically regulated number of spikelets, but they differ in the fate of the IM. In the determinate wheat spike, the IM transitions into a SM, and forms a terminal spikelet (Fig. 1a), whereas in the indeterminate barley spike the IM is exhausted and degenerates. In barley, loss-of-function mutations in *GRASSY TILLERS1* (*GT1*) delay IM degeneration and increase SNS [25], whereas mutations in *APETAL2-LIKE5* (*ap2l5*-null) result in a determinate spike with extra florets per spikelet [26]. In wheat, mutations in *AP2L5* also lead to extra florets per spikelet and are associated with a 21% reduction in SNS [27]. Increased expression of *AP2L5* in miR172 resistant alleles increases SNS by 5-10% [28], but neither the mutants nor the *AP2L5* overexpressing alleles affect wheat spike determinacy.

By contrast, wheat *vrn1 ful2*-null and *vrn1 ful2 ful3*-null mutants have indeterminate spikes, demonstrating overlapping and essential roles of *VRN1* and *FUL2* in the IM→TS transition [3]. Individual loss-of-function mutations in *VRN1* or *FUL2* significantly increase SNS, but the effects are more pronounced in *vrn1*-null (58%) than in *ful2*-null mutants (10%) [3]. Both *vrn1*-null and *svp1 vrt2*-null mutants show delayed spike and spikelet development associated with a reduced rate of SM production and a significantly delayed IM→TS transition (Fig. 3a-b).

The rate of SM production is also reduced in *lfy* and *wapo1* mutants relative to the wildtype, but the timing of the IM→TS transition is not significantly altered [29] (Fig. 3c). Loss-of-function mutations in *LFY*, *WAPO1*, or both result in similar reductions in SNS, suggesting that they work cooperatively to regulate this trait. This hypothesis is supported by the physical interaction between their encoded proteins and their co-expression in the early IM at W1.5 [29]. Natural alleles with positive effects on SNS have been identified for *WAPO1* [30] and *LFY* and both show evidence of positive selection and a significant genetic interaction for SNS [31].

The IM→TS transition at W3.5 is characterized by the upregulation of *SQUAMOSA* genes *VRN1* and *FUL2* and the downregulation of both *LFY* [29] and the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* gene *SPL14* in the IM region [14]. Loss-of-function mutations in *SPL14* result in a premature IM→TS transition and a 42% reduction in SNS, demonstrating an important role of this gene in spike development [14]. This hypothesis is further supported by the discovery of natural alleles for *SPL14* and its close paralog *SPL17* with positive effects on wheat SNS [32,33].

Another group of genes with significant effects on wheat SNS includes the *PHOTOPERIOD1* (*PPD1*) gene and its downstream targets *FT1* and *FT2*. Plants carrying the *PPD1* photoperiod-sensitive (PS) allele have lower *FT1* expression and higher SNS than those with photoperiod-insensitive (PI) alleles. By contrast, wheat plants with loss-of-function mutations in *PPD1* show reduced *FT1* expression and higher SNS [34,35]. Shorter days also reduce *FT1* transcript levels, delay spike and spikelet development, and increase SNS (Fig. 3d) [35]. These effects are attenuated in the *vrn2*-null mutants, which exhibit higher *FT1* expression and a faster rate of SM formation (Fig. 3d). The dominant role of florigen proteins in the regulation of SNS is also supported by the low SNS observed in plants overexpressing FT1 [36], FT2 [37], or FT3 [38]. Based on these results, we hypothesize that the reduced rate of SM-production and delayed IM→TS transition in the *vrn1 vrn2*-null and *svp1 vrt2*-null mutants (Fig. 3a-b) could be associated with the downregulation of *FT1* observed in the leaves of these mutants [3,12].

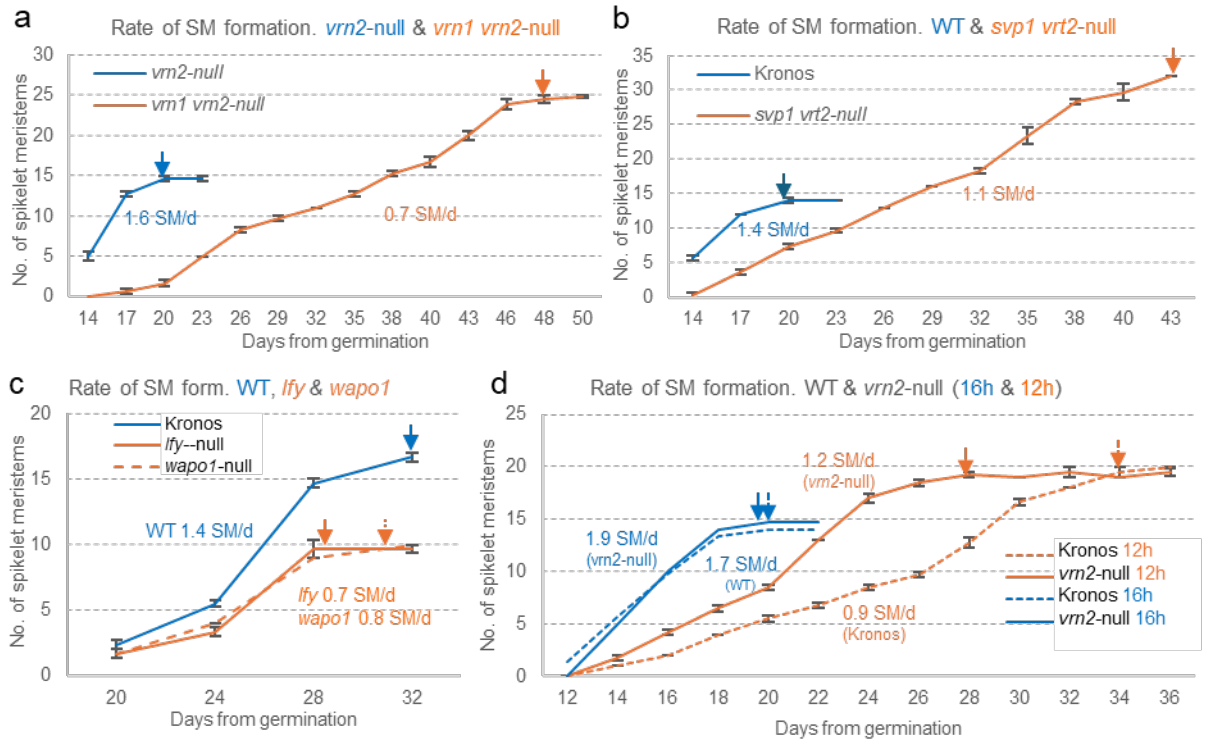

Fig. 3. Rate of spikelet meristem (SM) formation and IM→TS transition across mutant backgrounds and photoperiods. **(a)** *vrn1 vrn2*-null mutant versus *vrn2*-null control. **(b)** *svp1 vrt2*-null mutant versus Kronos control. **(c)** *lfy*-null and *wapo1*-null mutants versus Kronos control [29]. **(d)** Effect of photoperiod on SM production rate: 16h light /8 h darkness (blue) vs. 12h light / 12h darkness (orange) in Kronos (dotted lines) and Kronos *vrn2*-null mutant (solid line). Longer photoperiods [35] and absence of *vrn2* [5] both result in increased transcript levels of *FT1* and a faster rate of SM production. Arrows indicate the approximate time of the IM→TS transition. Rates are expressed as number of SM formed per day. Data points represent the averages of three dissected apices and error bars indicate standard errors of the means (SEM). Data is available on Table S3.

The *FT2* gene also affects SNS, and its encoded protein physically interacts with bZIPC1, a homeodomain-leucine zipper transcription factor [35,39]. The two genes affect SNS in opposite directions, with loss-of-function mutations in *FT2* increasing SNS and those in *bZIPC1* reducing SNS. Natural alleles with positive effects on SNS have been identified for both genes, which show evidence of positive selection and significant genetic interactions for SNS [39,40]. The genetic interactions for SNS discovered for *FT2-bZIPC1* and *WAPO1-LFY* have both led to the identification of optimal allele combinations that maximize SNS [31,40]. These results illustrate the practical value of understanding the gene networks that regulate spike development.

An increase in SNS does not guarantee an increase in grain number per spike (GNS) because floret degeneration can offset the gains in SNS. Fortunately, a natural allele of the *GRAIN NUMBER INCREASE 1* gene (*GNI1*, or *VRS1* in barley) provides a tool to increase spikelet fertility and reduce premature floret abortion [41]. Even when increases in SNS are translated into more GNS, reduced grain weight can offset the gains in grain yield. When plants cannot produce sufficient resources to fill the extra grains, a negative correlation is usually observed between GNS and grain weight. For example, the favorable *WAPO1* allele for increased SNS showed positive effects on grain yield only when introgressed into high-biomass genotypes and when the lines were grown under well-fertilized and well-watered conditions [30,42]. These experiments suggest that balanced increases in both sink and source, together with favorable environmental conditions, are necessary to translate increases in SNS into higher grain yields.

3. Regulation of branching in the wheat spike

An alternative strategy to improve wheat SNS is to increase the number of spikelets produced per node, generating supernumerary spikelets (SS). In mutants such as 'Miracle-Wheat' and 'Compositum-Barley' (*com2*), some basal spikelets are replaced by branch-like structures resembling smaller secondary spikes [43,44]. In these mutants, the basal branch-like structures resemble small spikes rather than panicle branches, suggesting that those SMs acquired IM identity rather than branch meristem (BM) identity.

Branched-like phenotypes and SS have been associated with hypomorphic mutations in the APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) domain transcription factor

FRIZZY PANICLE (*FZP*) [43-45]. However, in more severe *FZP* mutants in rice [46] and durum wheat [14]**,** secondary axes emerge from the axils of the glumes forming new bracts/glumes that produce tertiary axes and repeat the process. These recurrent glume structures suggest that, in addition to its effect on the conversion of basal SMs into secondary IMs, an important function of *FZP* is to suppress the glume's AxM. This result is consistent with the restricted expression of *FZP* in the axils of wheat glume primordia (Fig. 4a-b).

Studies in wheat and barley suggest that *FZP* transcript levels are positively regulated by two transcription factors: wheat MULTI-FLORET SPIKELET1 (TaMFS1, referred to as DUO in [47]) and barley RAMOSA2 (HvRA2, synonymous VRS4) [44]. In wheat, MFS1 directly promotes the expression of *FZP* [47] and the expression domains of these two genes show significant overlap at early stages of spikelet development (Fig. 4b). Consistent with its effect on *FZP*, the wheat *mfs-B1* mutant also produces spikes with SS [47].

In barley, loss-of-function mutations in *HvRA2* (*VRS4*) are also associated with reduced *FZP* transcript levels. These mutants also promote lateral spikelets, occasional branch-like formation [44], and reduced expression of barley *VRS1* and *SISTER OF RAMOSA3* [48]. In the wheat spike, *RA2* is first induced in the abaxial part of initiating SMs, then surrounds the growing SM (Fig. 4c), and later delimits the base of the developing spikelet, overlapping with the *FZP* expression domain in the axil of the second glume (Fig. 4d).

Wheat MFS1 [47] and maize RA2 [49] are also positive regulators of *TCP24,* which corresponds to *COMPOSITUM1* (*COM1*) in barley and *BRANCH ANGLE DEFECTIVE 1* (*BAD1*) in maize [49] (Fig. 4e). Loss-of-function mutations in *COM1* result in branched barley spikes, suggesting that the active COM1 promotes SM identity and contributes to branch suppression [50,51]. The interactions among *MFS1*, *RA2*, and *TCP24* in wheat are also supported by significant correlations among their expression profiles in a single-cell RNA-seq (scRNA-seq) study of early wheat spike development [14] and by their overlapping expression domains (Fig. 4d). Moreover, increased expression of all three *TCP24* homeologs is observed in the wheat *mfs-B1* mutant [47]. A similar interaction has been observed in maize, where *RA2* operates upstream of *BAD1* [49].

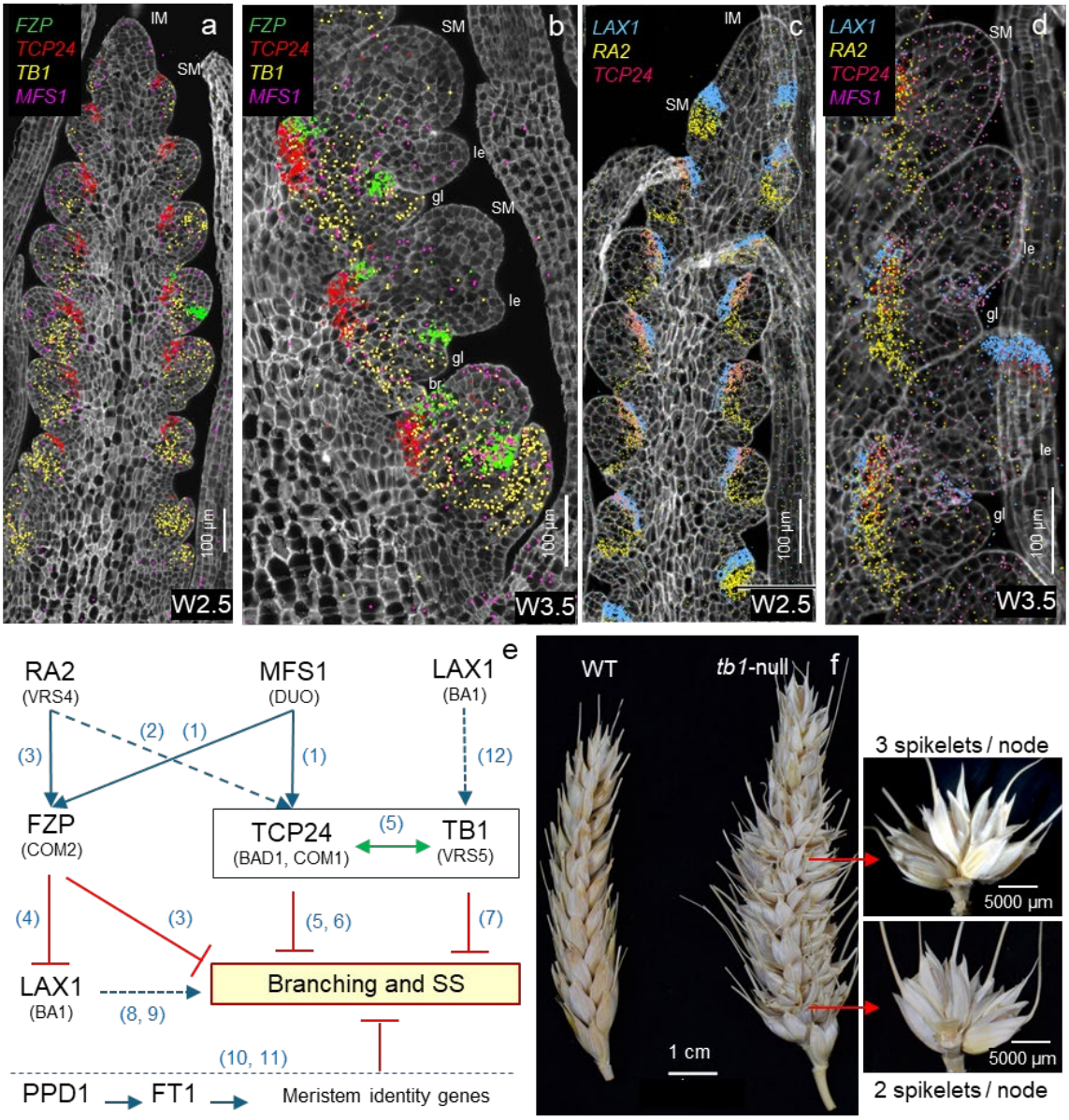

Fig. 4. Genes and gene network regulating branching. (**a-d**) Single-molecule fluorescence *in situ* hybridization of Kronos developing spikes at W2.5 (a and c) and W3.5 (b and d). Cell walls are stained with calcofluor white. gl= glume, le= lemma, br= suppressed bract, SM= spikelet meristem, IM= inflorescence meristem. (**a-b**) Expression profiles of *FZP, TCP24, TB1* and *MFS1* based on [14] (**c-d**) Expression profiles of *LAX1*, *RA2*, *TCP24* and *MFS1* in Kronos spikes based on a 10X-Xenium spatial transcriptomics study. (**e**) Working model of a gene network regulating branching in wheat. Blue arrows indicate promotion, red T-shaped symbols indicate repression, and green double arrows represent protein-protein interactions. Solid lines are supported by wheat or barley studies, whereas dotted lines are supported by studies in the more distantly related rice or maize. Gene names and IDs are included in Table S1. Numbers in parenthesis indicate references: 1:[47], 2:[49], 3:[44], 4:[45], 5:[50], 6:[51], 7:[53], 8:[55], 9:[56], 10:[57], 11:[54], 12:[56]. (**f**) Left: Spikes of wildtype Kronos and *tb1*-null CRISPR mutant showing SS. Right: nodes bearing two or three spikelets. Note the different orientation of the terminal and lateral spikelets, their similar development, and the higher frequency of triplets at the center of the spike.

TCP24 belongs to the CYC/TB1 clade of TCP transcription factors, which also includes TCP22, TEOSINTE BRANCHED1 (TB1), and TB2. In barley, TB1 is known as INTERMEDIUM SPIKE-C (INT-C or VRS5), and its mutant contributes to the six-rowed phenotype [52]. In wheat, *TB1* expression is detected in the SMs at W2.5 and becomes restricted to the base of the spikelets at W3.5. At both stages it is more abundant at the base of the spike (Fig. 4a-b). Knockout mutations of both *TB1* homeologs in tetraploid wheat (*tb1*-null) result in frequent SS (Fig. 4f, Table S2), as reported previously [53]. These results suggest that *TB1/INT-C* modulates SM timing or identity, thereby contributing to the repression of SS in both barley and wheat. Surprisingly, *TB1* duplications or transgenic overexpression were also associated with SS [54], suggesting that balanced levels of *TB1* may be required to prevent SS formation.

In maize, *TB1* transcription is regulated by *BARREN STALK1* (*BA1*), the ortholog of rice *LAX PANICLE1* (*LAX1*) [56]. *BA1* acts downstream of auxin signaling to position boundary regions for AxM formation [58]. Loss-of-function mutations in *BA1* or *LAX1* result in drastic reductions in panicle branching and lateral spikelet formation in both maize and rice [55,56], whereas mutations in the wheat ortholog have milder effects [59]. Wheat plants with mutations in the three *LAX1* homeologs have a more compact spike [59] that resembles gain-of-function mutations in the domestication gene *Q* (*AP2L5*) [28,60]. By contrast, overexpression of wheat *LAX1* reduces spike threshability and spikelet density, similar to the pre-domestication *q* allele [59]. LAX1 and Q proteins physically interact with each other, providing a potential mechanism for their opposite effects on these domestication traits [59]. In wheat, FZP has been reported to directly repress *LAX1* transcription by binding to its regulatory regions [45]. However, the functional significance of this interaction remains unclear, given that *FZP* is expressed later than *LAX1* in developing wheat spikes (Fig. 4a-c). From the early stages of wheat spike development, *LAX1* is expressed at the adaxial boundary layer of the SMs (Fig. 4c), similar to observations in rice [55] and maize [56], suggesting a conserved function.

In hexaploid wheat, a particular class of SS, designated as paired spikelets (PS), is characterized by the formation of a secondary spikelet immediately adjacent to and below (abaxial) the more advanced main spikelet. At maturity, PSs appear parallel to each other and are more frequent at the central nodes of the spike. PS are affected by genes in the photoperiod pathway, with shorter

photoperiods, mutations in *PPD1*, and reduced expression of *FT1* all increasing PS frequency [57]. Since these changes are associated with reduced expression of meristem identity genes (*VRN1*, *FUL2*, and *FUL3*), the authors of [57] hypothesized that this reduction may contribute to the delayed initiation of the SM, providing the time for PS formation.

In summary, none of the known mutants in barley and wheat form genuine branches as observed in the panicles of other grasses, suggesting that these species have lost the ability to generate functional branch meristems. However, multiple mutants generate SS or branch-like structures by modifying the timing or identity of the SMs, providing the time for the formation of one or more lateral spikelets. It remains unclear why some mutations induce higher SS frequencies at the center of the spike (e.g. *Tb1*), whereas others result in branch-like structures with an elongated secondary rachis (e.g. *FZP*). Studying branching in the un-branched *Triticeae* spikes can provide valuable insights into our understanding of grass inflorescence development and evolution.

Conclusions and perspectives

Increasing spikelet number through SS or branching has been an attractive strategy to increase grain yield, but initial efforts have been limited by trade-offs in fertility and grain size. The *GNI1* allele for high fertility [41] may help mitigate the reductions in SS fertility, whereas high-biomass varieties with higher source resources may limit negative correlations between grain number and grain size. A recent study using a cross between a spike-branching landrace and the elite durum cultivar CIRNO showed that a better balance of source–sink traits can reduce yield penalties associated with branched phenotype [61]. However, developing higher yielding wheat varieties with SS or branched spikes will require a dedicated breeding effort to fine-tune the multiple epistatic interactions within the complex gene network regulating branching.

Meanwhile, rapid progress has been made in the identification of genes that control grain number by modifying the rate of SM production, the timing of the IM →TS transition, and/or the number of fertile florets. Favorable alleles and allele combinations are being identified and incorporated into breeding programs through marker-assisted selection or by integrating functional SNPs into high-throughput marker platforms used for genomic selection. The impressive number of

spikelets and grains produced by high yielding triticale varieties suggest that there is still room for improvement of the SNS trait in wheat.

The identification and functional characterization of genes regulating wheat spike development have been greatly accelerated in recent years, fueled by new tools including CRISPR, sequenced mutant populations, improved wheat transformation efficiency, and sequenced genomes. These resources have been complemented by single-cell RNA-seq and spatial transcriptomic studies, which provide cellular resolution to the rapid changes in gene expression that occur during spike development [14,62]. In parallel, multi-omics studies are integrating expression profiles, chromatin accessibility, binding motifs, GWAS, and evolutionary information into comprehensive networks that will help prioritize the functional characterization of novel regulators of wheat spike development [63,64]. The knowledge generated by these new tools can enhance our ability to engineer changes in spike development.

Each year, more than 200 trillion wheat spikes are harvested globally, providing one fifth of the calories and proteins consumed by the human population. Therefore, every small improvement in wheat spike productivity is worth the effort.

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References

1. Kellogg EA: **Genetic control of branching patterns in grass inflorescences**. *Plant Cell* 2022, **34**:2518-2533.

2. Ferrándiz C, Gu Q, Martienssen R, Yanofsky MF: **Redundant regulation of meristem identity and plant architecture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER***. *Development* 2000, **127**:725-734.

3.** Li C, Lin H, Chen A, Lau M, Jernstedt J, Dubcovsky J: **Wheat *VRN1*, *FUL2* and *FUL3* play critical and redundant roles in spikelet development and spike determinacy**. *Development* 2019, **146**:dev175398.

This study demonstrates that the SQUAMOSA-clade MADS-box genes *VRN1*, *FUL2*, and *FUL3* in wheat play critical and redundant roles in spikelet development. These genes are essential for the suppression of the lower leaf ridge and the establishment of spikelet meristem identity. Moreover, loss-of-function mutations in *VRN1* or *FUL2* lead to a significant increase in spikelet number per spike, and the inflorescence meristem of the *vrn1 ful2* double mutant becomes indeterminate, indicating both genes are required for spike determinacy in wheat.

4. Pearce S, Vanzetti LS, Dubcovsky J: **Exogenous gibberellins induce wheat spike development under short days only in the presence of *VERNALIZATION1***. *Plant Physiology* 2013, **163**:1433-1445.

5. Chen A, Dubcovsky J: **Wheat TILLING mutants show that the vernalization gene *VRN1* down-regulates the flowering repressor *VRN2* in leaves but is not essential for flowering**. *PLoS Genetics* 2012, **8**:e1003134.

6. Lee J, Oh M, Park H, Lee I: **SOC1 translocated to the nucleus by interaction with AGL24 directly regulates leafy**. *Plant J* 2008, **55**:832-843.

7. Perez-Ruiz RV, Garcia-Ponce B, Marsch-Martinez N, Ugartechea-Chirino Y, Villajuana-Bonequi M, de Folter S, Azpeitia E, Davila-Velderrain J, Cruz-Sanchez D, Garay-Arroyo A, et al.: **XAANTAL2 (AGL14) is an important component of the complex gene regulatory network that underlies *Arabidopsis* shoot apical meristem transitions**. *Molecular Plant* 2015, **8**:796-813.

8. Liu C, Xi W, Shen L, Tan C, Yu H: **Regulation of floral patterning by flowering time genes**. *Developmental Cell* 2009, **16**:711-722.

9. Luo X, Liu B, Xie L, Wang K, Xu D, Tian X, Xie L, Li L, Ye X, He Z, et al.: **The TaSOC1-TaVRN1 module integrates photoperiod and vernalization signals to regulate wheat flowering**. *Plant Biotechnol J* 2024, **22**:635-649.

10. Schilling S, Kennedy A, Pan S, Jermiin LS, Melzer R: **Genome-wide analysis of MIKC-type MADS-box genes in wheat: pervasive duplications, functional conservation and putative neofunctionalization**. *New Phytologist* 2020, **225**:511-529.

11. Ryu CH, Lee S, Cho LH, Kim SL, Lee YS, Choi SC, Jeong HJ, Yi J, Park SJ, Han CD, et al.: ***OsMADS50* and *OsMADS56* function antagonistically in regulating long day (LD)-dependent flowering in rice**. *Plant Cell Environ* 2009, **32**:1412-1427.

12.* Li K, Debernardi JM, Li C, Lin H, Zhang C, Jernstedt J, von Korff M, Zhong J, Dubcovsky J: **Interactions between SQUAMOSA and SVP MADS-box proteins regulate meristem transitions during wheat spike development**. *The Plant Cell* 2021, **33**:3621-3644.

The SVP-clade MADS-box proteins, including SVP1, VRT2, and SVP3, physically interact with the SQUAMOSA proteins, and these interactions can interfere with SQUAMOSA-SEPALLATA interactions. Loss-of-function mutations in *VRT2* and *SVP1* result in delays in heading time and increases in spikelet number per spike like those observed in *SQUAMOSA* mutants, suggesting that these genes play overlapping roles in the regulation of heading time and SNS during the early reproductive phase. As spike development progresses, the *SVP* genes are repressed by the *SQUAMOSA* genes. This repression is important for enabling the interactions between SQUAMOSA and SEPALLATA1 MADS-box proteins, which are required for the initial stages of spikelet development. Therefore, SQUAMOSA-SVP interactions are key regulators of meristem transitions in wheat spike development.

13. VanGessel C, Hamilton J, Tabbita F, Dubcovsky J, Pearce S: **Transcriptional signatures of wheat inflorescence development**. *Scientific Reports* 2022, **12**:17224.

14.* Xu X, Lin H, Zhang J, Burguener G, Paraiso F, Li K, Tumelty C, Li C, Liu Y, Dubcovsky J: **Spatial and single-cell expression analyses reveal complex expression domains in early wheat spike development**. *Genome Biology* 2025, **26**:352.

This study integrated single-molecule fluorescence in situ hybridization (smFISH) and single-cell RNA sequencing (scRNA-seq) during three early stages of spike development in a tetraploid spring wheat to generate an atlas of cell clusters and expression domains at high spatial and cellular resolution. The study used scRNA-seq information to impute the expression of 74,464 genes into the spatially anchored cells, extending spatiotemporal resolution to a genome-wide scale. The study provides a public website to visualize the smFISH and imputed gene expression (https://dubcovskylab.ucdavis.edu/spatial-transcriptomic). Functional validation of key genes, including *LFY*, *SPL14* and *FZP* documented the value of the spatial transcriptomics data for inferring gene function. The study was performed in a tetraploid spring wheat variety.

15. Adamski NM, Simmonds J, Brinton JF, Backhaus AE, Chen Y, Smedley M, Hayta S, Florio T, Crane P, Scott P, et al.: **Ectopic expression of *Triticum polonicum VRT-A2* underlies elongated glumes and grains in hexaploid wheat in a dosage-dependent manner**. *The Plant Cell* 2021, **33**:2296-2319.

16. Backhaus AE, Lister A, Tomkins M, Adamski NM, Simmonds J, Macaulay I, Morris RJ, Haerty W, Uauy C: **High expression of the MADS-box gene VRT2 increases the number of rudimentary basal spikelets in wheat**. *Plant Physiol* 2022, **189**:1536-1552.

17. Wang YG, Yu HP, Tian CH, Sajjad M, Gao CC, Tong YP, Wang XF, Jiao YL: **Transcriptome association identifies regulators of wheat spike architecture**. *Plant Physiology* 2017, **175**:746-757.

18. Zhu W, Yang L, Wu D, Meng Q, Deng X, Huang G, Zhang J, Chen X, Ferrandiz C, Liang W, et al.: **Rice *SEPALLATA* genes *OsMADS5* and *OsMADS34* cooperate to limit inflorescence branching by repressing the *TERMINAL FLOWER1-like* gene *RCN4***. *New Phytologist* 2022, **233**:1682-1700.

19. Li G, Kuijer HNJ, Yang X, Liu H, Shen C, Shi J, Betts N, Tucker MR, Liang W, Waugh R, et al.: **MADS1 maintains barley spike morphology at high ambient temperatures**. *Nature Plants* 2021, **7**:1093-1107.

20. Shen C, Yang X, Wang D, Li G, Tucker MR: **Functional retrogression of LOFSEPs in specifying floral organs in barley**. *aBIOTECH* 2025, **6**:1-11.

21. Wu D, Liang WQ, Zhu WW, Chen MJ, Ferrandiz C, Burton RA, Dreni L, Zhang DB: **Loss of LOFSEP transcription factor function converts spikelet to leaf-like structures in rice**. *Plant Physiology* 2018, **176**:1646-1664.

22. Zhang Y, Shen C, Li G, Shi J, Yuan Y, Ye L, Song Q, Shi J, Zhang D: ***MADS1*-regulated lemma and awn development benefits barley yield**. *Nature Communications* 2024, **15**:301.

23. Shen C, Zhang Y, Li G, Shi J, Wang D, Zhu W, Yang X, Dreni L, Tucker MR, Zhang D: **MADS8 is indispensable for female reproductive development at high ambient temperatures in cereal crops**. *Plant Cell* 2023, **36**:65-84.

24. Theissen G, Melzer R, Rumpler F: **MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution**. *Development* 2016, **143**:3259-3271.

25. Shanmugaraj N, Rajaraman J, Kale S, Kamal R, Huang Y, Thirulogachandar V, Garibay-Hernandez A, Budhagatapalli N, Tandron Moya YA, Hajirezaei MR, et al.: **Multilayered regulation of developmentally programmed pre-anthesis tip degeneration of the barley inflorescence**. *Plant Cell* 2023, **35**:3973-4001.

26.* Zhong J, van Esse GW, Bi X, Lan T, Walla A, Sang Q, Franzen R, von Korff M: ***INTERMEDIUM-M* encodes an HvAP2L-H5 ortholog and is required for inflorescence indeterminacy and spikelet determinacy in barley**. *Proceedings of the National Academy of Sciences of the United States of America* 2021, **118**:e2011779118.

This study identifies the causal gene for the *intermedium-m* (*int-m*) and *double seed1* (*dub1*) mutant phenotypes, which convert the indeterminate inflorescences of barley into wheat-like determinate inflorescences characterized by a terminal spikelet and spikelets with multiple florets. *INT-M/DUB1* encodes an APETALA2-like transcription factor (HvAP2L-H5) that suppresses ectopic and precocious spikelet initiation and maintains inflorescence meristem activity. This study further demonstrates that HvAP2L-H5 inhibits the identity shift of the inflorescence meristem to a terminal spikelet meristem in barley. Null mutations in AP2L-5 result in fewer spikelets per spike but additional florets per spikelet.

27. Debernardi JM, Greenwood JR, Jean Finnegan E, Jernstedt J, Dubcovsky J: ***APETALA 2-like genes AP2L2* and *Q* specify lemma identity and axillary floral meristem development in wheat**. *Plant Journal* 2020, **101**:171-187.

28. Greenwood JR, Finnegan EJ, Watanabe N, Trevaskis B, Swain SM: **New alleles of the wheat domestication gene *Q* reveal multiple roles in growth and reproductive development**. *Development* 2017, **144**:1959-1965.

29. Paraiso F, Lin H, Li C, Woods DP, Lan T, Tumelty C, Debernardi JM, Joe A, Dubcovsky J: **LEAFY and WAPO1 jointly regulate spikelet number per spike and floret development in wheat**. *Development* 2024, **151**:dev202803.

30. Kuzay S, Lin H, Li C, Chen S, Woods DP, Zhang J, Lan T, von Korff M, Dubcovsky J: ***WAPO-A1* is the causal gene of the 7AL QTL for spikelet number per spike in wheat**. *PLoS Genetics* 2022, **18**:e1009747.

31. Zhang J, Burguener GF, Paraiso F, Dubcovsky J: **Natural alleles of *LEAFY* and *WAPO1* interact to regulate spikelet number per spike in wheat**. *Theoretical and Applied Genetics* 2024, **137**:257.

32. Chen H, Zhang X, Xu SH, Song CX, Mao HL: ***TaSPL17*s act redundantly with *TaSPL14*s to control spike development and their elite haplotypes may improve wheat grain yield**. *Frontiers in Plant Science* 2023, **14**:1229827.

33. Liu Y, Chen J, Yin C, Wang Z, Wu H, Shen K, Zhang Z, Kang L, Xu S, Bi A, et al.: **A high-resolution genotype-phenotype map identifies the *TaSPL17* controlling grain number and size in wheat**. *Genome Biology* 2023, **24**:196.

34. Shaw LM, Turner AS, Herry L, Griffiths S, Laurie DA: **Mutant alleles of *Photoperiod-1* in wheat (*Triticum aestivum* L.) that confer a late flowering phenotype in long days**. *PLoS ONE* 2013, **8**:e79459.

35. Gauley A, Boden SA: **Stepwise increases in *FT1* expression regulate seasonal progression of flowering in wheat (*Triticum aestivum*)**. *New Phytologist* 2021, **229**:1163-1176.

36. Lv B, Nitcher R, Han X, Wang S, Ni F, Li K, Pearce S, Wu J, Dubcovsky J, Fu D: **Characterization of *FLOWERING LOCUS T1* (*FT1*) gene in *Brachypodium* and wheat**. *Plos One* 2014, **9**: e94171.

37. Shaw LM, Lyu B, Turner R, Li C, Chen F, Han X, Fu D, Dubcovsky J: ***FLOWERING LOCUS T2* regulates spike development and fertility in temperate cereals**. *Journal of Experimental Botany* 2019, **70**:193-204.

38. Gauley A, Lacey I, Gonzalez-Suarez P, Taylor H, Hirsz D, Hayta S, Smedley M, Harwood W, Griffiths S, Cheng S, et al.: **Fine-tuning wheat development for the winter to spring transition**. *Plant Commun* 2025, **6**:101501.

39. Glenn P, Zhang J, Brown-Guedira G, DeWitt N, Cook JP, Li K, Akhunov E, Dubcovsky J: **Identification and characterization of a natural polymorphism in *FT-A2* associated with increased number of grains per spike in wheat**. *Theoretical and Applied Genetics* 2022, **135**:679-692.

40. Glenn P, Woods DP, Zhang J, Gabay G, Odle N, Dubcovsky J: **Wheat bZIPC1 interacts with FT2 and contributes to the regulation of spikelet number per spike**. *Theoretical and Applied Genetics* 2023, **136**:237.

41. Sakuma S, Golan G, Guo Z, Ogawa T, Tagiri A, Sugimoto K, Bernhardt N, Brassac J, Mascher M, Hensel G, et al.: **Unleashing floret fertility in wheat through the mutation of a homeobox gene**. *Proceedings of the National Academy of Sciences of the United States of America* 2019, **116**:5182-5187.

This study reports that Grain Number Increase 1 (GNI1), a homeodomain leucine zipper class I (HD-Zip I) transcription factor, is an important contributor to floret fertility in both wheat and barley (where it is known as VRS1). GNI1 inhibits rachilla growth and apical floret development; therefore, a single amino acid substitution in the HD domain of GNI1 results in a reduced-function allele, which increases grain number compared to the ancestral wild type allele, thereby leading to higher yield in field conditions. Harnessing natural and induced variation in GNI1 and its orthologs that favor increased grain production represents a promising strategy for crop improvement.

42. Kuzay S, Xu Y, Zhang J, Katz A, Pearce S, Su Z, Fraser M, Anderson JA, Brown-Guedira G, DeWitt N, et al.: **Identification of a candidate gene for a QTL for spikelet number per**

spike on wheat chromosome arm 7AL by high-resolution genetic mapping. *Theoretical and Applied Genetics* 2019, **132**:2689–2705.

43. Dobrovolskaya O, Pont C, Sibout R, Martinek P, Badaeva E, Murat F, Chosson A, Watanabe N, Prat E, Gautier N, et al.: ***FRIZZY PANICLE* drives supernumerary spikelets in bread wheat**. *Plant Physiology* 2015, **167**:189-199.

44.** Poursarebani N, Seidensticker T, Koppolu R, Trautewig C, Gawronski P, Bini F, Govind G, Rutten T, Sakuma S, Tagiri A, et al.: **The genetic basis of composite spike form in barley and 'Miracle-Wheat'**. *Genetics* 2015, **201**:155-165.

This study reports the identification of the wheat *FRIZZY PANICLE* (*FZP*) gene, which encodes a member of the APETALA2/Ethylene Response Factor (AP2/ERF) transcription factor family, as the causal gene responsible for the branch-like structures form at the spikes of the barley *COMPOSITUM2* mutants and in 'Miracle Wheat'. Mutants in both barley and wheat showed amino acid changes either in the AP2/ERF domain or in the conserved terminal region but not loss-of-function mutations. In barley, *COM2* transcripts are significantly down-regulated in the *vrs4.k* mutant suggesting that *VRS4* (synonymous *HvRA2*) may function upstream of *COM2*.

45. Du DJ, Zhang DX, Yuan J, Feng M, Li ZJ, Wang ZH, Zhang ZH, Li XT, Ke WS, Li RH, et al.: ***FRIZZY PANICLE* defines a regulatory hub for simultaneously controlling spikelet formation and awn elongation in bread wheat**. *New Phytologist* 2021, **231**:814-833.

46. Komatsu M, Chujo A, Nagato Y, Shimamoto K, Kyozuka J: ***FRIZZY PANICLE* is required to prevent the formation of axillary meristems and to establish floral meristem identity in rice spikelets**. *Development* 2003, **130**:3841-3850.

47.* Wang YG, Du F, Wang J, Wang K, Tian CH, Qi XQ, Lu F, Liu XG, Ye XG, Jiao YL: **Improving bread wheat yield through modulating an unselected AP2/ERF gene**. *Nature Plants* 2022, **8**:930-939.

This study reports that the *DUO-B1* gene (referred to as *MFS-B1* in this review using the original name from rice) encodes an AP2/ERF transcription factor that regulates spike architecture in bread wheat. Mutations in DUO-B1 lead to a mild supernumerary spikelet (SS) phenotype and increased grain yield under field conditions. This study further demonstrates that DUO-B1 regulates spike architecture by

promoting *FZP* and *TCP24* (*TraesCS5A02G207300* and *TraesCS5B02G205600*) which was incorrectly referred to in this study as *TB1*.

48. Koppolu R, Anwar N, Sakuma S, Tagiri A, Lundqvist U, Pourkheirandish M, Rutten T, Seiler C, Himmelbach A, Ariyadasa R, et al.: ***Six-rowed spike4* (*Vrs4*) controls spikelet determinacy and row-type in barley**. *Proceedings of the National Academy of Sciences of the United States of America* 2013, **110**:13198-13203.

49. Bai F, Reinheimer R, Durantini D, Kellogg EA, Schmidt RJ: **TCP transcription factor, BRANCH ANGLE DEFECTIVE 1 (BAD1), is required for normal tassel branch angle formation in maize**. *Proceedings of the National Academy of Sciences of the United States of America* 2012, **109**:12225-12230.

50. Moraes TD, van Es SW, Hernández-Pinzón I, Kirschner GK, van der Wal F, da Silveira SR, Busscher-Lange J, Angenent GC, Moscou M, Immink RGH, et al.: **The TCP transcription factor HvTB2 heterodimerizes with VRS5 and controls spike architecture in barley**. *Plant Reproduction* 2022, **35**:205-220.

51. Poursarebani N, Trautewig C, Melzer M, Nussbaumer T, Lundqvist U, Rutten T, Schmutzer T, Brandt R, Himmelbach A, Altschmied L, et al.: **COMPOSITUM 1 contributes to the architectural simplification of barley inflorescence via meristem identity signals**. *Nature Communications* 2020, **11**:5138.

52. Ramsay L, Comadran J, Druka A, Marshall DF, Thomas WT, Macaulay M, MacKenzie K, Simpson C, Fuller J, Bonar N, et al.: ***INTERMEDIUM-C*, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene *TEOSINTE BRANCHED 1***. *Nature Genetics* 2011, **43**:169-172.

53. Hale CO: **Genetic optimization of seed size, plant height, and tillering traits for enhanced yield in Montana wheat**. Edited by. Bozeman, Montana: Montanta State University; 2025. vol PhD.]

54. Dixon LE, Greenwood JR, Bencivenga S, Zhang P, Cockram J, Mellers G, Ramm K, Cavanagh C, Swain SM, Boden SA: ***TEOSINTE BRANCHED1* regulates inflorescence architecture and development in bread wheat (*Triticum aestivum*)**. *Plant Cell* 2018, **30**:563-581.

55. Komatsu K, Maekawa M, Ujiie S, Satake Y, Furutani I, Okamoto H, Shimamoto K, Kyozuka J: ***LAX* and *SPA*: Major regulators of shoot branching in rice**. *Proceedings of the National Academy of Sciences of the United States of America* 2003, **100**:11765-11770.

56. Gallavotti A, Zhao Q, Kyozuka J, Meeley RB, Ritter MK, Doebley JF, Pe ME, Schmidt RJ: **The role of *barren stalk1* in the architecture of maize**. *Nature* 2004, **432**:630-635.

57.** Boden SA, Cavanagh C, Cullis BR, Ramm K, Greenwood J, Jean Finnegan E, Trevaskis B, Swain SM: ***Ppd-1* is a key regulator of inflorescence architecture and paired spikelet development in wheat**. *Nature Plants* 2015, **1**:14016.

This study shows that *PHOTOPERIOD1* (*PPD1*) is a key regulator of spike architecture in wheat. It further demonstrates that *PPD1* inhibits paired spikelet formation by promoting *FT1* expression. Reduced *FT1* expression in wheat is associated with paired spikelets. Unlike the supernumerary spikelet phenotype observed in "Miracle Wheat", paired spikelets are characterized by the formation of a secondary spikelet directly adjacent to and below the primary spikelet, which is usually more advanced in its development than the secondary spikelet. At maturity, the PS appear parallel and are more frequent at the center of the spike. The study hypothesizes that low levels of *FT1* lead to reduced expression of the meristem identity genes, which delay the formation of the SM and provides the time necessary for the formation of the lateral meristem that originates the secondary spikelet.

58. Galli M, Liu Q, Moss BL, Malcomber S, Li W, Gaines C, Federici S, Roshkovan J, Meeley R, Nemhauser JL, et al.: **Auxin signaling modules regulate maize inflorescence architecture**. *Proceedings of the National Academy of Sciences of the United States of America* 2015, **112**:13372-13377.

59. He G, Zhang Y, Liu P, Jing Y, Zhang L, Zhu Y, Kong X, Zhao H, Zhou Y, Sun J: **The transcription factor TaLAX1 interacts with Q to antagonistically regulate grain threshability and spike morphogenesis in bread wheat**. *New Phytologist* 2021, **230**:988-1002.

60.* Debernardi JM, Lin H, Chuck G, Faris JD, Dubcovsky J: **microRNA172 plays a crucial role in wheat spike morphogenesis and grain threshability**. *Development* 2017, **144**:1966-1975.

This study shows that a nucleotide substitution in the miR172 target site of the *Q* allele (*AP2L5*) disrupts the ability of miR172 to cleave the mRNA. This results in increased levels of *AP2L5* contributing to more

compact spikes and free-threshing grains. Increased levels of *AP2L5* favor the partial conversion of glumes to lemmas by reducing sclerenchymatic tissue, increasing awn length and reducing the axillary meristem repression. By contrast, reduced levels of *AP2L5* favor the partial conversion of lemmas to glumes by increasing sclerenchymatic tissue, reducing awn length and repressing flower development.

61. Abbai R, Golan G, Longin CFH, Schnurbusch T: **Grain yield trade-offs in spike-branching wheat can be mitigated by elite alleles affecting sink capacity and post-anthesis source activity**. *Journal of Experimental Botany* 2024, **75**:88-102.

62. Long KA, Lister A, Jones MRW, Adamski NM, Ellis RE, Chedid C, Carpenter SJ, Liu X, Backhaus AE, Goldson A, et al.: **Spatial transcriptomics reveals expression gradients in developing wheat inflorescences at cellular resolution**. *bioRxiv* 2024, https://doi.org/10.1101/2024.12.19.629411.

63. Chen Y, Guo Y, Guan P, Wang Y, Wang X, Wang Z, Qin Z, Ma S, Xin M, Hu Z, et al.: **A wheat integrative regulatory network from large-scale complementary functional datasets enables trait-associated gene discovery for crop improvement**. *Molecular Plant* 2023, **16**:393-414.

This study presents a genome-wide wheat integrative gene regulatory network (wGRN) that combines an updated genome annotation, large-scale gene expression profiles, transcription factor (TF) binding motifs (DAP-seq), chromatin accessibility data, and evolutionary conserved regulatory regions. Using these datasets, the study built seven independent regulatory networks and integrated them into a single large-scale network that comprises ~7.2 million TF–target interactions, linking 5,947 TFs to 127,439 target genes. The authors demonstrated the applicability of wGRN in uncovering previously unknown gene functions and pathways, interpreting new datasets, and prioritizing candidate genes from GWAS and map-based cloning studies. By integrating the wGRN with a spike transcriptome time-series dataset, this study constructed high-resolution gene networks that enabled the identification of novel regulators of SNS and enhanced the power of spike phenotypic trait prediction. An interactive webserver (http://wheat.cau.edu.cn/wGRN) is provided to explore gene regulation and discover trait-associated genes.

64. Lin X, Xu Y, Wang D, Yang Y, Zhang X, Bie X, Gui L, Chen Z, Ding Y, Mao L, et al.: **Systematic identification of wheat spike developmental regulators by integrated multi-omics, transcriptional network, GWAS, and genetic analyses**. *Molecular Plant* 2024, **17**:438-459.

The study used a multi-omics approach to analyze and integrate the transcriptome and epigenome profiles at eight early stages of the spike development in winter wheat. It combines RNA-seq, ATAC-seq, and histone modification data to construct a transcriptional regulatory network (TRN) of spike development. Further integration of this TRN with genome-wide association studies (GWAS) identified numerous transcription factors potentially involved in spike development. Mutant analysis confirmed spike phenotypes for 36 of these transcription factors and identified MYB30 as a direct target of *FZP.* The datasets generated in this study are available in the open access Wheat Spike Multi-Omic Database (http://39.98.48.156:8800/#/).

References and recommended reading Papers of particular interest, published within the period of review, have been highlighted as: * of special interest and ** of outstanding interest