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News and Views

Bigger meristem, higher yield? The roles of REL2 and RELK in maize meristem function and yield enhancement

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Plant development depends on the sustained yet controlled proliferation of stem cells in the meristem. The well-known CLAVATA-WUSCHEL (CLV-WUS) signaling pathway integrates various molecular and hormonal cues to maintain this balance, which determines the meristem size and organ formation. Recent studies have highlighted the potential of fine-tuning meristem size regulation to improve crop architecture and yields (Chen and Gallavotti 2021). For example, in maize, slightly enlarging the inflorescence meristem (IM) can increase the number of kernel rows, which is a critical yield trait (Je et al. 2016; Liu et al. 2021). Although the CLV-WUS signaling pathway is conserved across species, mutations in its orthologous regulators can produce different effects on meristem size, suggesting an evolutionary diversification in the control of the pathway (Wang and Jiao 2023). Therefore, targeting key regulators of meristem maintenance while overcoming genetic redundancy and pleiotropy remains a significant challenge.

The CLV-WUS signaling pathway maintains stem cell balance through a negative feedback loop: WUS, which is expressed in the organizing center, stimulates stem cell proliferation in the central zone, where it also activates CLV3, which in turn represses the WUS expression (Kitagawa and Jackson 2019) (Fig. 1A). In maize, orthologs of the CLV-WUS genes interact with various signaling peptides to regulate stem cell fate and differentiation (Chen and Gallavotti 2021). Additionally, factors like hormones and redox environments influence the CLV-WUS pathway. In these interactions, transcriptional corepressors play pivotal roles because they link the repressive machinery to control of gene expression based on developmental or environmental signals (Leydon et al. 2021). The maize transcriptional corepressor RAMOSA1 ENHANCER LOCUS2 (REL2, a co-ortholog of TOPLESS in Arabidopsis) regulates development, but its exact functions in meristem maintenance are still poorly understood (Gallavotti et al. 2010; Liu et al. 2019).

In this issue of *Plant Physiology*, Gregory et al. (2024) provide a comprehensive characterization of the maize REL2 corepressor family and its roles in plant growth and development. Through detailed genetic analyses, they found that *REL2* is partially redundant with 3 *REL-LIKE* genes (*RELKs*), exhibiting specific compensatory patterns throughout development. They found that REL2/RELKs act through the CLV-WUS pathway to control meristem size, independent of other known upstream regulators. Furthermore, the authors discovered that in addition to interacting with WUS proteins, REL2/RELKs influence meristem function by regulating hormone levels and maintaining redox balance within the meristem (Fig. 1A). Finally, through hybrid maize analysis, the authors demonstrated REL2 has the potential to significantly increase maize yield.

Maize *rel2* mutants display pleiotropic vegetative and reproductive phenotypes such as defective axillary meristem (AM) initiation and IM maintenance (Gallavotti et al. 2010). To identify the genetic modifiers of *REL2*, the authors performed an EMS mutagenesis screen for enhancers of *rel2* phenotypes and found 2 mutants both exhibiting shorter plant stature and upright tassel branches. By positional mapping and whole genome sequencing, the authors identified that both mutants carry base changes in *RELK1*, a member of the *REL2* family. About one-half of the double mutant plants failed to produce an ear, reminiscent of the *rel2* phenotype. Additionally, the *RELK1* gene was upregulated in *rel2* mutants with broad expression in various tissues, suggesting a compensatory mechanism between *REL2* and *RELK1* during development.

To further investigate *REL2/RELK* functions, the authors generated CRISPR-Cas9 knockouts of the remaining gene family members, *RELK2* and *RELK3*, and generated higher-order mutants. Although *relk2* and *relk3* single and double mutants appear normal, *rel2;relk2;relk3* triple mutants exhibit severe defects in vegetative development, including a shoot apical meristem that is not maintained through embryogenesis. These findings suggest that *REL2/RELKs* display genetic redundancy and subfunctionalization during maize development, with *REL2* serving as the primary gene.

The authors then examined the impact of *REL2/RELK* mutations on meristem size. *rel2;relk1* double mutants show a significant increase in IM size, although the ears produced minimal seeds, suggesting that downstream developmental processes were also affected. Gene expression analysis in these mutants showed a misregulation of many genes involved in hormone balance, redox regulation, and meristem maintenance pathways. Notably, the findings suggest that the combined loss of *REL2* and *RELK1* causes broader disruptions in the stem cell regulatory network compared

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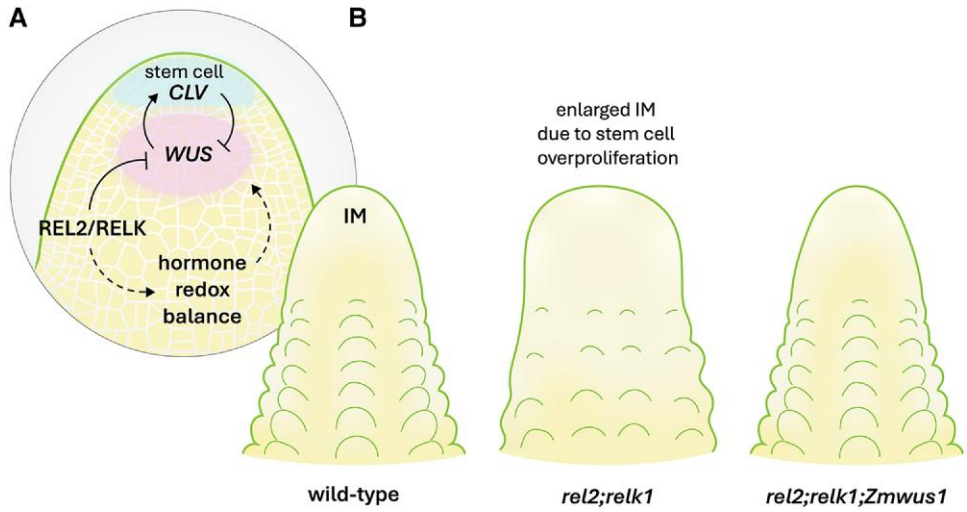

Figure 1. REL2/RELK corepressors influence meristem size by controlling meristem maintenance. **A)** A simplified model in which REL2/RELK corepressors regulate maize IM maintenance. **B)** Schematic comparison of maize inflorescences during early development depicting a wild type with a normal IM size, a *rel2;relk1* double mutant with an enlarged IM, and a *rel2;relk1;Zmwus1* triple mutant in which IM size is restored to near-normal size.

with the loss of *REL2* alone, as additional *WOX* (*WUSCHEL-like homeobox*) gene homologs were misregulated in the double mutants.

Interestingly, double and triple mutants of *rel2* and *relk1* with *Zmwus1* exhibit a more normal-looking IM, indicating a correction of meristem size in the mutants (Fig. 1B). These findings support a critical role of *REL2/RELK1* in repressing *ZmWUS1* expression during IM maintenance. How do *REL2/RELK* corepressors regulate the CLV-WUS pathway in relation to other factors? The authors analyzed double and triple mutants of *rel2 and relk1* in combination with mutations in 3 other known meristem regulators, which are associated with abnormally large and sometimes deformed IMs. The findings further support that the enlarged meristem in *rel2;relk1* mutants is likely due to WUS overexpression and stem cell overproliferation, but the precise molecular mechanism underlying this regulation requires further investigation.

In summary, REL2/RELK corepressors regulate meristem size by modulating the CLV-WUS pathway and maintaining hormone and redox balance (Fig. 1A), making them a promising target for improving maize yield. However, an overly large meristem could compromise organ development (Kitagawa and Jackson 2019). Thus, Gregory et al. (2024) investigated whether heterozygosity of *rel2* could enlarge the IM just enough to increase the kernel row number without causing detrimental effects. In maize and other crops, F_1 hybrids, produced by crossing two genetically distinct parent plants, are widely used for commercial seed production. Using diverse maize inbred lines grown in different environments, the authors produced F_1 hybrids heterozygous for the *rel2* mutation. They found that at least 40% of these hybrid lines significantly increased kernel row numbers. While further investigation is needed to fully assess the impact of *rel2* heterozygosity on yield and plant performance, this study demonstrates how targeting a key regulator can modulate plant development and potentially enhance crop yields.

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Conflict of interest statement. None declared.

Data availability

All data described in this article are available in the original publication by Gregory et al. (2024).

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