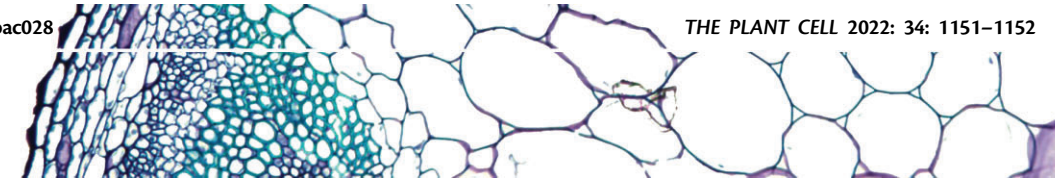
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Fantastic four: bHLH factors and the making of the pollen

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In Brief

Sexual reproduction has helped to fuel the extraordinary diversity underlying the evolution of animal and plant life on Earth. Like animals, flowering plants produce haploid sperm and egg cells that combine their genetic information to produce a diploid zygote, which will develop into a new multicellular organism. Plant reproductive success relies not only on the correct formation of sexual organs containing functional male and female gametes, but also on the proper development of the seed protecting the embryo upon fertilization. Besides the perpetuation of the species, plant fertility represents a key agronomic trait for crops such as maize that are cultivated worldwide for their grains. Nonetheless, male sterility turns out to be a powerful tool in breeding programs to produce hybrid seeds with increased vigor. Compared with mechanical emasculation, the use of male sterile parental lines is more efficient and less invasive, thus facilitating hybrid seed production and reducing its cost.

In maize, hundreds of *Male sterile* (*Ms*) mutants have been identified and used to dissect the regulatory pathways controlling reproductive development (Skibbe and Schnable, 2005). Several mutants show defects in the formation of the four layers of somatic cells that constitute anther lobes, and particularly in the differentiation of the tapetum—the innermost layer that provides nutrients and exine components to the developing pollen grains. For example, the historic *ms23* mutant exhibits a double layer of aberrant pre-tapetal cells (instead of the single function layer) that results in pollen abortion. The *ms23* phenotype is caused by a large deletion in a gene encoding an anther-specific bHLH transcription factor (TF), considered as the master regulator of tapetal cell specification (Nan et al., 2017). MS23 acts upstream of additional *bHLH* genes (namely *Ms32*, *bHLH122*, and *bHLH51*) but how these four factors interact with each other throughout anther development remains to be clarified.

In this issue, **Guo-Ling Nan and colleagues (Nan et al., 2022)** used CRISPR-Cas9 technology to generate frameshift mutations that cause early translation termination in bHLH122 and bHLH51. Similar to previously described *ms23* and *ms32* mutants, genome-edited plants showed defects in tapetal cell proliferation although at later stages of anther development (see Figure). The temporal order of morphological alterations in male sterile mutants suggested that the sequential action of the four bHLH factors guides the specification and differentiation of the tapetum, which ultimately influences the production of viable pollen grains.

Newly generated *bhlh122* and *bhlh*51 knockout lines together with historic *ms23* and *ms32* mutants were also used to determine dynamic changes in gene expression at different stages of anther development, from pre-meiosis to post-meiosis. At earlier stages, *ms23*, *ms32*, and *bhlh121* mutants showed strong downregulation of *Dicer-like* 5 (*DCL5*) and depletion of 24-nt phased secondary small interfering RNAs (phasiRNAs), whose abundance peaks in 1.5- and 2-mm developing anthers of fertile plants. At later stages, *bhlh122* and *bhlh51* mutants showed upregulation of negative regulators of programmed cell death—an essential process for tapetum senescence. Altogether, patterns of differential expression in the male sterile mutants compared with the fertile counterpart suggested that the four bHLH TFs orchestrate transcriptional reprogramming that defines the progression of tapetal cell differentiation.

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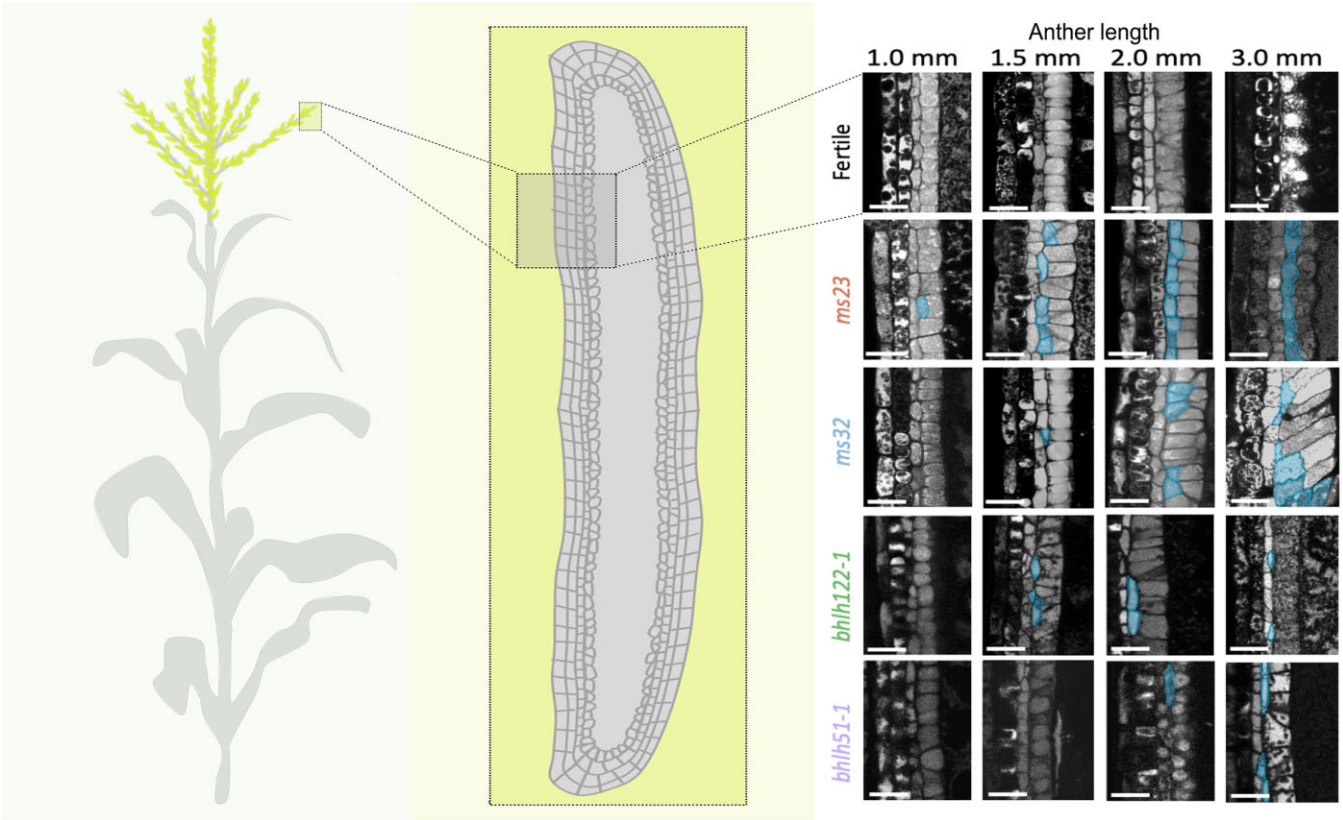


Figure Specific stages of anther development in fertile maize plant versus male sterile mutants. Temporal order of developmental defects in tapetal cell proliferation in the four loss of function mutants examined in this article (bottom) when compared with the fertile counterpart (top). Aberrant cell types in the innermost layer of anther lobes are highlighted in blue. Figure credit: M.O., longitudinal confocal images from Nan et al. (2022), Figure 2.

Based on transcriptomics analyses and protein–protein interaction studies, the authors proposed a regulatory framework underlying the temporal development of the tapetum and redefine the hierarchy of the four bHLH factors. Additional experiments identified a specific role of MS23 and MS32 in the biogenesis of 24-nt phasiRNAs through activation of *DCL5*, previously shown to be essential for male fertility under optimal growth temperature for maize cultivation (Teng et al., 2020). Nevertheless, the mode of action of this class of small RNAs is still unresolved despite their importance for anther functionality.

To conclude, this study reinforces the virtues of maize as model species for investigating the molecular basis of fertility in cereal crops, a century after the first male sterile mutant had been described. Novel findings reported here support the use of technological advances (e.g. CRISPR-Cas9 system) and OMICS approaches in reproductive biology to generate valuable genetic resources for crop improvement and decipher gene networks controlling developmental processes fundamental for sexual reproduction.

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