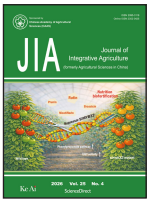
Journal of Integrative Agriculture 2026, 25(4): 1731–1735

Available online at ScienceDirect

Journal of Integrative Agriculture



LETTER

Identification and fine mapping of the male sterility gene *msg*$_{N13401}$ reveals defective pollen starch accumulation in barley

Qi Liu[1], Zhidong Qi[1], Chaofan Li[1], Zhenghan Chen[1], Wenqian Liu[1], Baoshen Liu[1], Chaozhong Zhang[2], Jiajie Wu[1], Juan Qi[1#], Fei Ni[1#]

[1] *State Key Laboratory of Wheat Improvement/College of Agronomy, Shandong Agricultural University, Tai'an 271018, China*
[2] *Department of Plant Sciences, University of California, Davis, CA 95616, USA*

Highlights

- A novel EMS-induced barley male-sterile mutant (*N13401*) was identified and characterized, and it shows defective pollen starch accumulation.
- The male sterility gene *msg*$_{N13401}$ was fine-mapped to a 576.9 kb region on chromosome 2H using a wild-cultivated barley cross to overcome the low polymorphism of cultivated barley.

Barley (*Hordeum vulgare* L.), the world's fourth most cultivated cereal crop, serves as a key raw material for the brewing industry and a significant feed source for livestock (Geng *et al.* 2022). Exploiting heterosis is a pivotal strategy for boosting crop yields (Wan *et al.* 2019). Male sterility genes and their corresponding mutants are valuable genetic resources for facilitating hybrid seed production (Li *et al.* 2020). Identifying novel male sterility genes and developing efficient hybrid seed production systems are critical for overcoming yield limitations in cereals (Gupta *et al.* 2019). To date, only a few key genes regulating barley anther development have been identified, such as the male sterility genes *HvMS1* (Fernández-Gómez *et al.* 2020), *MSG26* (Qi *et al.* 2019), *MSG38* (Amanda *et al.* 2022), and *DTT1* (Hua *et al.* 2025), as well as the fertility restorers *Rfm1* (Rizzolatti *et al.* 2017) and *Rfm3* (Bernhard *et al.* 2019). This limited number of characterized genes severely constrains germplasm innovation and hinders the advancement of molecular breeding strategies in barley. In the *HvMs1* mutant, premature programmed cell death (PCD) of the tapetum leads to non-viable pollen (Fernández-Gómez *et al.* 2020). The *msg26* mutant exhibits a complete loss-of-function phenotype at the late uninucleate microspore stage, resulting in pollenless anthers (Qi *et al.* 2019). The *dtt1* mutant is manifested as the over-proliferation of indeterminate tapetal precursor cells, absence of tapetum endomitosis, and failure of cell wall degeneration (Hua *et al.* 2025). In contrast, the *msg38* mutant displays defective starch accumulation in pollen (Amanda *et al.* 2022). Despite these advances, the genetic control of male sterility in barley remains poorly understood, highlighting the need to identify additional genes involved in anther and pollen development.

Pollen development constitutes a key biological process in flowering plants, wherein morphological differentiation and the dynamic accumulation of metabolites directly determine pollen fertility (Tang *et al.* 2018). Pollen fertility is governed by multiple interdependent factors. Premature degeneration of pollen mother cells impedes microspore formation, thereby leading to pollen abortion. Anther abortion may occur during the process from meiosis to tetrad formation (Liu *et al.* 2015). As the innermost somatic layer of the anther wall, the tapetum directly interfaces with developing meiocytes (Wang *et al.* 2020) and critically regulates pollen development through the secretion of essential components (Wang *et al.* 2025). Either delayed or premature tapetal degradation can lead to pollen abortion (Zhang *et al.* 2024; Zheng *et al.* 2025). Notably, starch is the principal energy reserve in pollen, and it provides energy and carbon skeletons for pollen development, germination, and pollen tube elongation, so it plays a decisive role in pollen biological function and fertility (Chen *et al.* 2023). Dysregulation of anther starch metabolism disrupts pollen development and causes male sterility (Lee *et al.* 2022). In barley, few genes involved in the starch deposition during pollen maturation are known, and the regulatory mechanisms governing anther starch deposition remain poorly understood.

Received 2 September 2025; Received in revised form 23 October 2025; Accepted 3 November 2025; Available online 31 December 2025
[#]Correspondence Fei Ni, E-mail: nifei1998@sdau.edu.cn; Juan Qi, E-mail: qijuan@sdau.edu.cn

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.12.077

In this study, we isolated a completely male sterile mutant, designated *N13401*, from an ethyl methanesulfonate (EMS)-mutagenized population of the barley cultivar 'Tamalpais' (Qi *et al.* 2012). Compared to the wild type (WT), *N13401* exhibited significantly smaller anthers and produced shrunken, non-viable pollen grains, with no discernible morphological differences in other organs (Fig. 1-A). Semi-thin section analysis revealed that *N13401* undergoes normal anther development, with typical degradation of the tapetum and middle layers, similar to the WT (Fig. 1-B). However, at the mature stage, the phenotypic trajectories of *N13401* diverged significantly from those of the WT. Wild type anthers produced well-filled pollen grains rich in starch granules, whereas *N13401* developed abnormal, empty pollen grains lacking internal contents, so it displayed a typical male sterile phenotype (Fig. 1-B).

The I_2-KI staining results indicated that starch accumulation was markedly reduced in the pollen grains of *N13401* (Fig. 1-A). Starch biosynthesis from sugar substrates requires the coordinated activity of multiple enzymes (Liu *et al.* 2021). Sucrose is hydrolyzed into hexoses by invertase (INV), which are phosphorylated to glucose-6-phosphate (G6P) and transported across the amyloplast membrane by the glucose-6-phosphate transporter (GPT), followed by starch synthesis mediated by plastidic phosphoglucomutase (pPGM), ADP-glucose pyrophosphorylase (AGP), and starch synthase (Liu *et al.* 2021). To explore the cause of the reduced starch deposition in *N13401*, we compared the expression levels of six key starch-biosynthetic genes between the *N13401* mutant and the wild type (Appendix A). Only *HvINV* showed a pronounced down-regulation in the mutant, suggesting a possible reduction in its enzymatic activity (Appendix A). Because INV catalyzes the hydrolysis of sucrose into hexoses, we next quantified the sucrose levels in the anthers of both genotypes; however, no significant difference was observed (Appendix A). We therefore hypothesized that the concentrations of INV-derived hydrolysis products might be reduced. Considering that hexoses contribute to the soluble sugar pool, we also measured the soluble sugar levels in the anthers. The results revealed a significant reduction in soluble sugars in the *N13401* mutant compared with the WT (Appendix A). Collectively, these results indicate that the impaired starch accumulation observed in *N13401* is primarily caused by reduced invertase activity during pollen development.

All F_1 plants derived from a cross between *N13401* and the cultivar 'Morex' were fertile. Segregation analysis of the F_2 population (fertile:sterile=103:44, χ^2=0.90, *df*=1, *P*=0.34) confirmed that the sterility was controlled by a single recessive gene, which we named msg_{N13401}. Total RNA was extracted from a pooled sample of flag leaves collected from 25 sterile plants in the F_2 population and subsequently used for transcriptome sequencing. The BSR-Seq analysis preliminarily mapped msg_{N13401} to the distal long arm of chromosome 2H (Fig. 1-C). Initial mapping with eight insertion/deletion (InDel) markers (*pMS99M1–pMS99M8*; Appendix B) delimited the locus to a 6.8 cM interval (Fig. 1-D). Then, using five additional CAPS/dCAPS (cleaved amplified polymorphic sequences and derived CAPS) markers (*pMS99M9–pMS99M13*; Appendix B) designed from whole-genome resequencing data, a large fine-mapping population of 2,689 individuals was employed to narrow the genetic interval to 1.5 cM (Fig. 1-D). This region corresponds to a 1.38 Mb physical region (chr2H: 656.77–658.15 Mb) on the Morex V3 reference genome (Mascher *et al.* 2021) (Fig. 1-D). Polymorphism analysis within the preliminarily mapped ~6.5 Mb interval (*pMS99M5–pMS99M6*) revealed 16 G>A or C>T transitions among 18 detected variants between *N13401* and Tamalpais, confirming the authenticity of *N13401* as an EMS-induced mutant.

Fine mapping was restricted by the limited availability of polymorphic markers within the 1.38 Mb target region, which limited further resolution of the genetic interval. To increase polymorphism, we crossed wild barley PI 405346 (*Hordeum vulgare* ssp. *spontaneum*; Appendix C) with *N13401*, which generated an F_2 segregating population (fertile:sterile=291:106, χ^2=0.30, *df*=1, *P*=0.58). A combined analysis of the F_2 populations derived from the crosses *N13401*/Morex and *N13401*/PI 405346 narrowed msg_{N13401} to a 576.9 kb region between markers *pMS124M17* and *pMS124M16* (chr2H: 656.80–657.37 Mb; Fig. 1-D; Appendix B). We performed *de novo* assembly of the wild-type cultivar Tamalpais using PacBio Hi-Fi sequencing technology, which generated a high-quality 10.35 Mb contig (ptg000027l) spanning the target region. This contig served as the reference for subsequent sequence alignment and variant calling. Comparative analysis of the 576.9 kb target interval (flanked by *pMS124M17* and *pMS124M16*) in Morex and Tamalpais revealed a high level of genomic synteny (Fig. 1-E), with only a few SNPs and small InDels detected. The target interval contains 38 annotated genes in the Morex V3 genome, 23 of which are high-confidence genes; and among these high-confidence genes, 17 show expression in the selected developmental stages (Appendix D; Fig. 1-F). The resequencing of *N13401* and alignment against the Tamalpais reference genome revealed that only one of the 17 expressed genes harbored a sequence mutation. Additional mutations were also found in two of the 15 low-confidence genes. Consequently, these three genes were identified as candidate genes underlying the male sterility phenotype.

Among the three candidate genes, the first mutation is a G>A transition (GACGAC→AACGAC) in the promoter region of *HORVU.MOREX.r3.2HG0214540* (low-confidence gene, Appendix E). This mutation introduces a putative TGA *cis*-element, which may alter the target gene's expression pattern. *HORVU.MOREX.r3.2HG0214540* encodes pyrroline-5-carboxylate reductase (P5CR), which catalyzes the final step of proline biosynthesis by reducing Δ¹-pyrroline-5-carboxylate (P5C) to proline (Lehmann *et al.* 2010). Proline is a critical amino acid for maintaining pollen viability and fertility (Biancucci *et al.* 2015). Given that proline accumulation is required during the later stages of pollen development, altered P5CR regulation may limit pollen viability. For example, high levels of proline accumulation are required during the late microspore development and pollen grain maturation stages (Mattioli *et al.* 2018), disruption of proline metabolism can lead to pollen abortion (Ge *et al.* 2025) and proline provides both energy substrates and metabolic

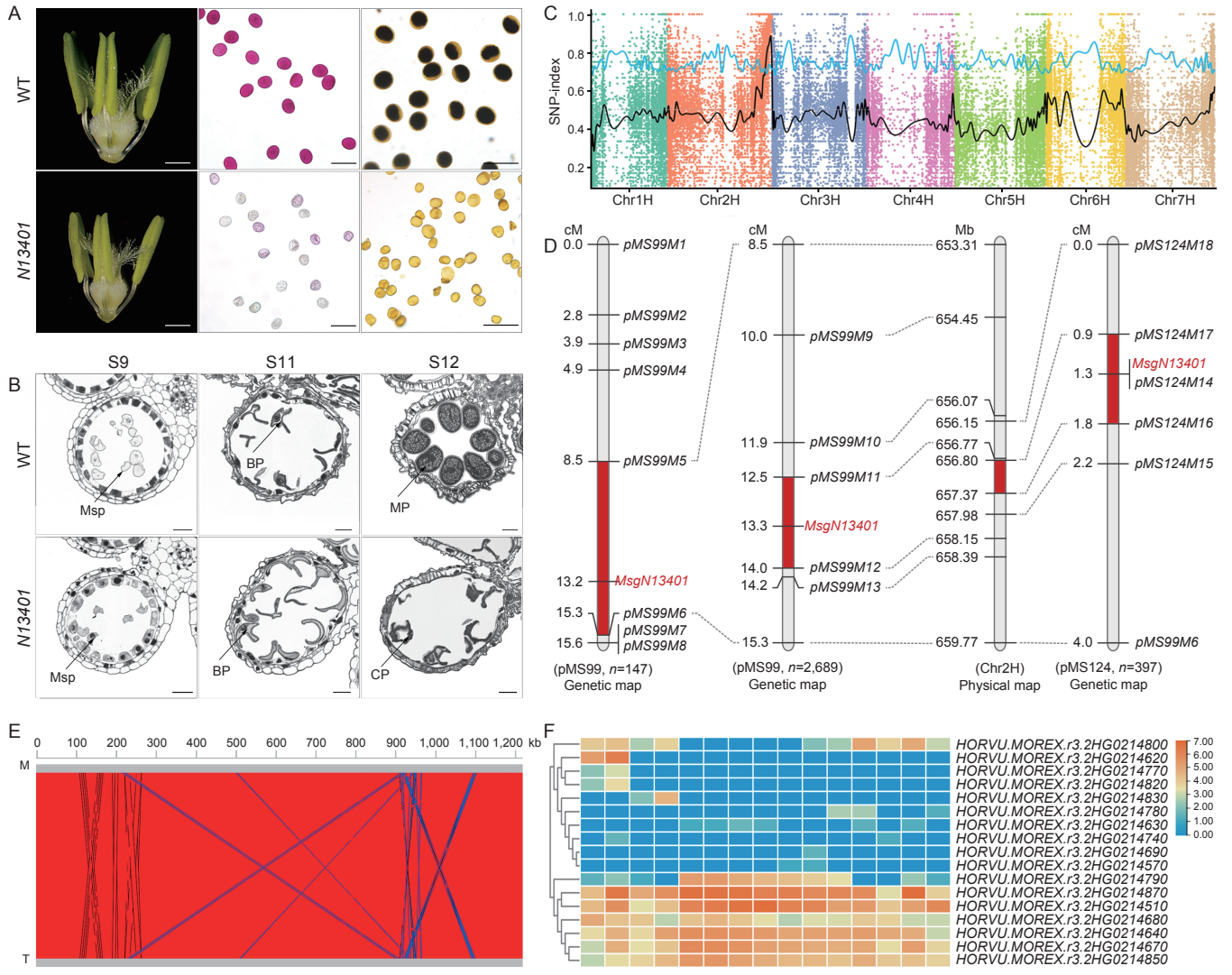


Fig. 1 Phenotypic characterization and fine mapping of the *msg*$_{N13401}$ locus with candidate gene in barley. A and B, comparison of floret morphology (stereomicroscope, scale bar=1 mm) and pollen viability (Alexander staining, scale bar=100 μm; I_2-KI, scale bar=100 μm) between wild-type (WT) and *N13401*. Scale bar=20 μm. BP, biceullar pollen; CP, collapsed pollen; MP, mature pollen; Msp, microspore. S9, microspore stage; S11, bicellular pollen stage; S12, mature pollen stage. C, BSR-Seq analysis localizes *msg*$_{N13401}$ to the distal end of chromosome 2H. D, fine mapping and physical map of *msg*$_{N13401}$. E, syntenic analysis of the target interval between barley cultivars Morex (M) and Tamalpais (T). F, expression levels of high-confidence genes within the mapped interval across developmental stages. Samples (left to right): root 10 cm; root 4 wk; shoot 10 cm; leaf 2 mon; anther 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.0, and 3.0 mm; grain 5 days post anthesis (DPA); grain 15 DPA. Expression values are $\log_2$-transformed.

precursors for pollen germination (Nepi *et al.* 2009).

Although EMS mutagenesis typically generates G>A and C>T transitions, it can also cause base deletions (Krasileva *et al.* 2017; Türkoğlu *et al.* 2023). The second mutation is a single cytosine (C) deletion in the third exon of *HORVU.MOREX.r3.2HG0214660* (low-confidence gene, Appendix E), which causes a frameshift mutation and a premature stop codon. This gene encodes a helix-hairpin-helix (HhH) motif, and such motifs typically mediate non-sequence-specific DNA binding (Kamada *et al.* 2022). While most HhH motifs are involved in protein–protein interactions or acquire enzymatic activity by incorporating catalytic residues (Shao and Grishin 2000), this gene has not been previously linked to plant male sterility.

The third mutation is a G>A missense mutation in the coding sequence of *HORVU.MOREX.r3.2HG0214850* (high-confidence gene, Appendix E), which results in a glycine (Gly) to arginine (Arg) substitution in a vacuolar iron transporter-like (VTL) protein. VTL proteins mediate critical physiological processes, including iron storage (Ram *et al.* 2021), symbiotic nitrogen fixation (Brear *et al.* 2020) and petal coloration (Momonoi *et al.* 2009). In wheat, seven VIT/VTL homologs coordinate metal transport: *TaVIT1* and *TaVIT2* facilitate iron transport to the aleurone layers (Connorton *et al.* 2017), whereas *TaVTL1/2/4/5* and *TaVIT3* may cooperatively regulate the transport of iron and manganese ions from the cytosol to the vacuole (Sharma *et al.* 2020). Considering the role of VIT/VTL in metal homeostasis, a disturbance of vacuolar sequestration may impair anther lipid metabolism and starch deposition during pollen maturation.

In summary, we characterized a novel male-sterile barley mutant, *N13401*, which exhibits defective pollen development due to insufficient starch accumulation. The proper deposition of sugars and transient starch in the anther under normal growth conditions is a critical determinant of pollen fertility (Guan *et al.* 2023). Pollen viability relies on both an adequate supply of sugars and their metabolic conversion, which is mediated by specific transport proteins within the developing anther (Goetz *et al.* 2001; Li *et al.* 2015). Although the phenotype of the *N13401* mutant is similar to that of the *msg38* mutant, the three candidate genes mentioned above differ from *msg38*. Previous studies have demonstrated that *msg38* drives starch synthesis in barley pollen, which promotes energy metabolism-related gene expression, and its loss-of-function leads to excessive sugar accumulation in anthers (Amanda *et al.* 2022). In contrast, we observed reduced *HvINV* activity in *N13401* anthers. While the sucrose content remained comparable between the mutant and WT, soluble sugars were significantly reduced in the mutant. This reduction likely reflects the lower levels of sucrose hydrolysis products resulting from diminished invertase activity, which in turn compromises starch accumulation in *N13401*. These divergent metabolic patterns indicate that msg_{N13401} and *msg38* govern two distinct pathways during barley pollen development. No previous reports on male sterility genes in barley have described a regulatory mode like the one reported here for msg_{N13401}, indicating that it is a novel male sterility locus with distinct mechanisms.

The msg_{N13401} locus was mapped using a strategy that combined BSR-Seq with forward genetics. However, fine mapping was hindered by insufficient polymorphisms between cultivated barley varieties, resulting from domestication-induced genetic homogenization. To overcome this challenge, we exploited the extensive genetic variation that exists between undomesticated wild barley and cultivated barley and successfully constructed a new F_2 mapping population. Leveraging the abundant polymorphisms within this population, we ultimately fine-mapped msg_{N13401} to a 576.9 kb interval on chromosome 2H. This study validated an effective strategy for overcoming insufficient polymorphisms, i.e., introducing wild relatives to establish an additional mapping population. Using DNA resequencing, we successfully identified three candidate genes within the target interval which may be involved in proline biosynthesis regulation, DNA binding, or vacuolar metal transport. Notably, these candidates do not overlap with known male sterility genes, confirming msg_{N13401} as a novel locus. This discovery provides valuable genetic material for an in-depth dissection of its molecular mechanism and will accelerate the process of hybrid barley breeding.

Acknowledgements

This work was supported by the Major Scientific and Technological Innovation Program of Tai'an, Shandong Province, China (2024GZPT01), and the Youth Innovation Technology Project of Higher School in Shandong Province, China (2024KJI013).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT 4.0 to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of this article.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.12.077

References

Amanda D, Frey F P, Neumann U, Przybyl M, Šimura J, Zhang Y, Chen Z, Gallavotti A, Fernie A R, Ljung K, Acosta I F. 2022. Auxin boosts energy generation pathways to fuel pollen maturation in barley. *Current Biology*, **32**, 1798–1811.

Bernhard T, Koch M, Snowdon R J, Friedt W, Wittkop B. 2019. Undesired fertility restoration in *msm1* barley associates with two mTERF genes. *Theoretical and Applied Genetics*, **132**, 1335–1350.

Biancucci M, Mattioli R, Forlani G, Funck D, Costantino P, Trovato M. 2015. Role of proline and GABA in sexual reproduction of angiosperms. *Frontiers in Plant Science*, **6**, 680.

Brear E M, Bedon F, Gavrin A, Kryvoruchko I S, Torres-Jerez I, Udvardi M K, Day D A, Smith P M C. 2020. GmVTL1a is an iron transporter on the symbiosome membrane of soybean with an important role in nitrogen fixation. *New Phytologist*, **228**, 667–681.

Chen L, Dong X, Yang H, Chai Y, Xia Y, Tian L, Qu L Q. 2023. Cytosolic disproportionating enzyme2 is essential for pollen germination and pollen tube elongation in rice. *Plant Physiology*, **191**, 96–109.

Connorton J M, Jones E R, Rodríguez-Ramiro I, Fairweather-Tait S, Uauy C, Balk J. 2017. Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification. *Plant Physiology*, **174**, 2434–2444.

Fernández-Gómez J, Talle B, Wilson Z A, Goring D. 2020. Increased expression of the *MALE STERILITY1* transcription factor gene results in temperature-sensitive male sterility in barley. *Journal of Experimental Botany*, **71**, 6328–6339.

Ge X Y, Chen J L, Li O Q, Zou M, Tao B L, Zhao L, Wen J, Yi B, Tu J X, Shen J X. 2025. ORF138 causes abnormal lipid metabolism in the tapetum leading to Ogu cytoplasmic male sterility in *Brassica napus*. *Journal of Integrative Agriculture*, **24**, 2080–2095.

Geng L, Li M, Zhang G, Ye L. 2022. Barley: A potential cereal for producing healthy and functional foods. *Food Quality and Safety*, **6**, 1–13.

Goetz M, Godt D E, Guivarc'h A, Kahmann U, Chriqui D, Roitsch T. 2001. Induction of male sterility in plants by metabolic engineering of the carbohydrate supply. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 6522–6527.

Guan X, Zhang Y, Zhou L, Asad M A U, Zhao Q, Pan G, Cheng F. 2023. Disruptions of sugar utilization and carbohydrate metabolism in rice developing anthers aggravated heat stress-induced pollen abortion. *Plant Physiology and Biochemistry*, **202**, 107991.

Gupta P K, Balyan H S, Gahlaut V, Saripalli G, Pal B, Basnet B R, Joshi A K. 2019. Hybrid wheat: Past, present and future. *Theoretical and Applied Genetics*, **132**, 2463–2483.

Hua M, Yin W, Tidy A C, Gómez J F, Li H, Shi S, Xing G, Zong J, Wilson Z A. 2025. The bHLH transcription factor *DTT1* is part of a paired key that unlocks the tapetum transition in barley anther development. *The Plant Cell*, **37**, koaf230.

Kamada R, Sakaguchi S, Kura S, Takamatsu K, Yamamoto T, Imagawa T, Sakaguchi K. 2022. Stereoselective interaction of helix–hairpin–helix motif peptides with DNA structure. *Chemistry Letters*, **51**, 1029–1032.

Krasileva K V, Vasquez-Gross H A, Howell T, Bailey P, Paraiso F, Clissold L, Simmonds J, Ramirez-Gonzalez R H, Wang X, Borrill

P, Fosker C, Ayling S, Phillips A L, Uauy C, Dubcovsky J. 2017. Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, E913–E921.

Lee S K, Lee J, Jo M, Jeon J S. 2022. Exploration of sugar and starch metabolic pathway crucial for pollen fertility in rice. *International Journal of Molecular Sciences*, **23**, 14091.

Lehmann S, Funck D, Szabados L, Rentsch D. 2010. Proline metabolism and transport in plant development. *Amino Acids*, **39**, 949–962.

Li J, Wang Z, He G, Ma L, Deng X W. 2020. CRISPR/Cas9-mediated disruption of *TaNP1* genes results in complete male sterility in bread wheat. *Journal of Genetics and Genomics*, **47**, 263–272.

Li X, Lawas L M F, Malo R, Glaubitz U, Erban A, Mauleon R, Heuer S, Zuther E, Kopka J, Hincha D K, Jagadish K S V. 2015. Metabolic and transcriptomic signatures of rice floral organs reveal sugar starvation as a factor in reproductive failure under heat and drought stress. *Plant*, *Cell and Environment*, **38**, 2171–2192.

Liu H Z, Zhang G S, Zhu W W, Ba Q S, Niu N, Wang J W, Ma S C, Wang J S. 2015. Relationship between male sterility and β-1,3-glucanase activity and callose deposition-related gene expression in wheat (*Triticum aestivum* L.). *Genetics and Molecular Research*, **14**, 574–584.

Liu S, Li Z, Wu S, Wan X. 2021. The essential roles of sugar metabolism for pollen development and male fertility in plants. *The Crop Journal*, **9**, 1223–1236.

Mascher M, Wicker T, Jenkins J, Plott C, Lux T, Koh C S, Ens J, Gundlach H, Boston L B, Tulpová Z, Holden S, Hernández-Pinzón I, Scholz U, Mayer K F X, Spannagl M, Pozniak C J, Sharpe A G, Šimková H, Moscou M J, Grimwood J, *et al.* 2021. Long-read sequence assembly: A technical evaluation in barley. *The Plant Cell*, **33**, 1888–1906.

Mattioli R, Biancucci M, El Shall A, Mosca L, Costantino P, Funck D, Trovato M. 2018. Proline synthesis in developing microspores is required for pollen development and fertility. *BMC Plant Biology*, **18**, 356.

Momonoi K, Yoshida K, Mano S, Takahashi H, Nakamori C, Shoji K, Nitta A, Nishimura M. 2009. A vacuolar iron transporter in tulip, TgVit1, is responsible for blue coloration in petal cells through iron accumulation. *The Plant Journal*, **59**, 437–447.

Nepi M, von Aderkas P, Wagner R, Mugnaini S, Coulter A, Pacini E. 2009. Nectar and pollination drops: How different are they? *Annals of Botany*, **104**, 205–219.

Qi J, Ni F, Wang X, Sun M, Cui Y, Wu J, Caplan A, Fu D. 2019. The anther-specific CYP704B is potentially responsible for *MSG26* male sterility in barley. *Theoretical and Applied Genetics*, **132**, 2413–2423.

Qi X, Xu Z, Pei H, Hu X, Wu J, Li X, Wang T, Fu D. 2012. Construction and functional evaluation of an EMS-induced mutant population in barley. *Journal of Triticeae Crops*, **32**, 846–852. (in Chinese)

Ram H, Sardar S, Gandass N. 2021. Vacuolar iron transporter (like) proteins: Regulators of cellular iron accumulation in plants. *Physiologia Plantarum*, **171**, 823–832.

Rizzolatti C, Bury P, Tatara E, Pin P A, Rodde N, Bergès H, Budar F, Mireau H, Gielen J J L. 2017. Map-based cloning of the fertility restoration locus *Rfm1* in cultivated barley (*Hordeum vulgare*). *Euphytica*, **213**, 276.

Shao X, Grishin N V. 2000. Common fold in helix–hairpin–helix proteins. *Nucleic Acids Research*, **28**, 2643–2650.

Sharma S, Kaur G, Kumar A, Meena V, Ram H, Kaur J, Pandey A K. 2020. Gene expression pattern of vacuolar-iron transporter-like (VTL) genes in hexaploid wheat during metal stress. *Plants*, **9**, 229.

Tang H, Song Y, Guo J, Wang J, Zhang L, Niu N, Ma S, Zhang G, Zhao H. 2018. Physiological and metabolome changes during anther development in wheat (*Triticum aestivum* L.). *Plant Physiology and Biochemistry*, **132**, 18–32.

Türkoğlu A, Haliloğlu K, Tosun M, Bujak H, Eren B, Demirel F, Szulc P, Karagöz H, Selwet M, Özkan G, Niedbała G. 2023. Ethyl methanesulfonate (EMS) mutagen toxicity-induced DNA damage, cytosine methylation alteration, and iPBS-retrotransposon polymorphisms in wheat (*Triticum aestivum* L.). *Agronomy*, **13**, 1767.

Wan X, Wu S, Li Z, Dong Z, An X, Ma B, Tian Y, Li J. 2019. Maize genic male-sterility genes and their applications in hybrid breeding: Progress and perspectives. *Molecular Plant*, **12**, 321–342.

Wang B, Fang R, Zhang J, Han J, Chen F, He F, Liu Y G, Chen L, Wilson Z. 2020. Rice LecRK5 phosphorylates a UGPase to regulate callose biosynthesis during pollen development. *Journal of Experimental Botany*, **71**, 4033–4041.

Wang R, Sun Y, Liu W, Chen X, Xu J, Yuan Z, Liang W, Zhang D. 2025. TIP2-UDT1-OsUPEX1/2 module regulates tapetum development and function in rice. *New Phytologist*, **247**, 651–668.

Zhang S, An X, Jiang Y, Hou Q, Ma B, Jiang Q, Zhang K, Zhao L, Wan X. 2024. Plastid-localized ZmENR1/ZmHAD1 complex ensures maize pollen and anther development through regulating lipid and ROS metabolism. *Nature Communications*, **15**, 10857.

Zheng H, Zhao Z, Zhu S, Ren Y, Shen J, Xun Z, Yu X, Wang C, Yao B, Cheng S, Hu Y, Zhang S, Wang Q, Lu J, Xie Z, Lei D, Jian A, Chen M, Chen K, Liu S, *et al.* 2025. OsBRK1-mediated phosphorylation of OsPFN2 regulates meiotic spindle actin assembly and rice fertility. *Plant Communications*, **6**, 101417.

Executive Editor-in-Chief Xueyong Zhang
Managing Editor Ning Wang