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A review of fertility challenges in wheat–perennial rye hybrids: Insights from the successful wheat–annual rye hybrid (triticale)

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SUMMARY

Hybrids of wheat (Triticum aestivum) and perennial rye (Secale cereale x Secale strictum) have shown promising results in enhancing agronomic features such as stress tolerance, perennial growth habit, and yield potential. However, fertility issues persist, posing a significant obstacle to the efficient utilization and successful implementation of hybrid vigor in crop improvement efforts. Fertility challenges, such as sterility and low seed production, are mainly caused by chromosomal mismatches, irregularities during meiosis, and genetic differences between the parent species. These factors disrupt normal gamete development, lower pollen viability, and cause embryo failure, all of which hinder the reproductive potential of the hybrids. Scientists have used multiple approaches to restore fertility, such as using cytogenetic methods to study chromosome behavior, applying polyploidization to stabilize hybrid genomes, and utilizing molecular tools to identify fertility-related genes. This review covers the specific factors that influence fertility in wheat-perennial rye hybrids, covering cytological, genetic, and environmental components. Gaining a better knowledge of the mechanisms underlying hybrid sterility and investigating techniques to increase fertility will significantly progress hybrid breeding efforts and aid in developing hardy perennial cereal crops. The outcomes discussed in this review aim to contribute to future research and assist in creating successful strategies for enhancing hybrid fertility in perennial grain breeding projects.

Keywords: *wheat; perennial rye; hybrid fertility; sterility; polyploidization*

INTRODUCTION

Hybridization between wheat (*Triticum aestivum*) and rye (*Secale* spp.) has long been researched as a practical technique to promote the resilience and adaptation of cereal crops. In recent years, increased effort has been dedicated to finding perennial alternatives to annual food crops. Perennial cereals are of interest due to their potential for improved environmental services and reduce production costs. Soil conservation, gains in soil organic matter, water quality, and reduced reliance on fertilizer are potential benefits of replacing annual cereals with perennial analogs (Jaikumar et al., 2012).

Developing breeding programs for perennial grains, especially with perennial rye, offers chances for multipurpose and sustainable agroecological systems. A staple crop with high production potential is wheat, and perennial rye is well-known for its deep root system, which reduces erosion and enhances soil structure and its resistance to environmental factors, including cold, salinity, and drought. Hybridizing these two crops can improve wheat's resistance to disease and stress by introducing beneficial genes from rye into wheat. For instance, rye genes have improved resistance to diseases like stripe rust and powdery mildew in wheat (An et al., 2015; Li et al., 2023).

Perennial rye is a hybrid species developed as an alternative to annual cereal crops, typically diploid (2n=2x=14), combining traits from cultivated rye (*Secale cereale*) and wild rye (*Secale strictum*), it offers potential benefits such as improved stress tolerance and disease resistance, making it a viable new cereal crop (Jaikumar et al., 2012).

Hybrid sterility is one of the main challenges in intergeneric hybrids between wheat and rye. Wheat, a hexaploid species (2n=6x=42, AABBDD), differs significantly in its genomic structure, leading to fertility challenges in its hybrids. Fertility issues in wheat-rye hybrids primarily stem from chromosomal imbalances, meiotic instability, and genetic incompatibilities between the two species, which affect both male and female gametophytes. These issues limit the efficient transmission of genetic material to succeeding generations, restricting the practical application of these hybrids in agricultural systems (Tikhenko et al., 2017, 2024).

Despite these fertility barriers, researchers have made significant progress in overcoming fertility barriers in hybrids, particularly through the use of chromosome-doubling techniques such as colchicine treatment. This method has proven effective in stabilizing hybrid genomes and restoring fertility by creating amphiploids, which improve chromosome pairing and increase the likelihood of viable gamete formation (Han & Niimi, 2008; Nimura et al., 2006; Tonosaki et al., 2016; Zhang, X. et al., 2017). Additionally, advancements in molecular genetics and genome editing technologies, such as CRISPR-Cas9, offer new possibilities for manipulating hybrid genomes to enhance recombination between wheat and rye chromosomes, potentially improving fertility (Ahmar et al., 2024; Ansari et al., 2020; Shams et al., 2022).

This review examines the main factors responsible for fertility barriers in hybrids between wheat and perennial rye, particularly emphasizing ploidy mismatches, meiotic irregularities, impaired gamete development, and embryo lethality, and also highlights

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several strategies to overcome infertility, including chromosome doubling, stimulation of homologous recombination, and the application of advanced biotechnological techniques. Understanding and addressing these fertility barriers is essential for successfully developing stable hybrids with enhanced agronomic traits.

BACKGROUND AND RATIONAL

Hybridization between annual and perennial species represents one of the most ambitious goals in modern cereal breeding. As climate change intensifies and agricultural sustainability becomes a global concern, the demand for perennial grain crops that combine yield stability with environmental resilience is rapidly increasing. Wheat–perennial rye hybrids stand at the forefront of this movement, offering a potential breakthrough toward low-input, high-efficiency farming systems. Despite numerous successful hybridization attempts, limited fertility remains the primary biological bottleneck preventing large-scale adoption. Understanding these mechanisms is not only essential for hybrid improvement but also for the broader advancement of intergeneric hybridization and polyploid stabilization in cereals.

SCOPE AND OBJECTIVES OF THE REVIEW

This review aims to critically summarize the current understanding of fertility barriers in wheat–perennial rye hybrids, focusing on cytogenetic, genomic, and epigenetic mechanisms that lead to sterility, it also highlights biotechnological and breeding strategies developed to overcome these barriers, including chromosome doubling, Ph1 suppression, embryo rescue, and modern molecular interventions such as CRISPR-based genome editing. By integrating classical and recent findings, this article provides a comprehensive framework for future research and practical breeding applications.

LITERATURE REVIEW

Meiotic instability and chromosomal aberrations in wheat-rye hybrids

Wheat (hexaploid, 2n=42, AABBDD genomes) and rye (diploid, 2n=14, RR genomes) differ significantly in ploidy level and genome organization, leading to cytogenetic incompatibilities in their hybrids. These differences contribute to meiotic irregularities, aneuploidy, and low gamete viability in hybrids. Wheat's three genomes complicate homoeologous pairing, but rye's polymorphic chromosomes with different heterochromatin impair normal segregation, resulting in hybrid sterility (Guo et al., 2019; Kussovska, 2010; Silkova et al., 2014; Xie et al., 2013).

The wheat-rye hybrid displays various chromosomal abnormalities due to complex interactions between these two genomes. These abnormalities involve structural alterations, including centromeric breaks, deletions, and changes in repeat localization patterns. While rye chromosomes are more frequently affected, wheat chromosome aberrations have also been reported (Fu et al., 2013; Silkova et al., 2018; Wan et al., 2023). These abnormalities significantly impact fertility and yield, leading to reduced fertility, abnormal meiosis, and altered yield-related traits (Nguyen et al., 2015; Tikhenko et al., 2024; Wan et al., 2023).

Wheat-rye hybrids display varied chromosome pairing behaviors during meiosis, with A-D genome pairings occurring more frequently than B-D or A-B pairings (Megyeri et al., 2013). Abnormal segregation patterns, such as lagging chromosomes and chromatid segregation, result in aneuploidy and structural changes in subsequent generations (Silkova et al., 2018; Xie et al., 2013).

Proper chromosome pairing during meiosis is essential for fertility in wheat-rye hybrids. In normal meiosis, homologous chromosomes successfully pair, aided by specific rye chromosomes that align with their wheat counterparts to form bivalents (Silkova et al., 2013). A critical feature of this process is the telomere bouquet, which initiates homologous chromosome pairing. Normal meiosis involves reduction division at anaphase I, where homologous chromosomes separate, followed by equational division at anaphase II, producing tetrads and functional gametes (Silkova et al., 2011).

However, a major challenge in wheat-rye hybrids is the absence of homologous pairing. In wheat, the Ph1 gene suppresses homoeologous chromosome pairing, often resulting in univalents during meiosis. These univalents can follow different segregation pathways, reductional, equational, or a mixed pattern, directly impacting fertility (Cifuentes et al., 2010; Silkova et al., 2013). Reductional division promotes bivalent formation and improved fertility, whereas equational division produces unreduced gametes, and mixed segregation leads to irregular chromosome distribution and aneuploidy. Specific chromosome substitutions such as 1R/1A, 2R/2D, and 6R/6A further influence meiotic behavior. For example, the 2R/2D substitution suppresses the equational division of univalents, whereas the 6R/6A substitution increases chromosome elimination, exacerbating fertility issues (Silkova et al., 2014; Tikhenko et al., 2024). Studies on microsporogenesis reveal that variations in chromosome segregation, whether reductional, equational, or mixed, contribute to the partial seed set and sterility commonly observed in these hybrids (Silkova et al., 2011).

Genomic, cytogenetic, and epigenetic challenges

In certain wheat-rye combinations, embryo lethality has been associated with the interaction of incompatible alleles at the Eml loci in wheat (Eml-A1) and rye (Eml-R1b), which disrupts shoot apical meristem formation (Tikhenko et al., 2008, 2010). This is compounded by differences in centromere-specific histone variant CENH3, which causes centromere dysfunction and uniparental chromosome elimination (Comai et al., 2017; Marimuthu et al., 2021).

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Additionally, endosperm malfunction due to ploidy differences between wheat (hexaploid) and rye (diploid) further contributes to seed abortion (Lafon-Placette & Köhler, 2016). These genetic incompatibilities create strong post-fertilization barriers that prevent normal embryo development.

At the cytogenetic level, chromosome elimination represents a major challenge, with rye chromosomes being preferentially lost during cell division in early-generation hybrids (Fu et al., 2013; Tang et al., 2012). This elimination results from failed chromosome pairing during meiosis, leading to univalent formation, micronuclei, and chromosomal fragments (Loginova & Silkova, 2014; Xie et al., 2013). The process is exacerbated by irregularities in spindle fiber formation, which cause chromatid lagging and abnormal segregation. These meiotic errors frequently produce aneuploid gametes with unbalanced chromosome numbers, severely impacting both male (pollen sterility) and female (abnormal embryo sacs) fertility (Evtushenko et al., 2019; Tikhenko et al., 2017).

Another layer of complexity related to hybrid infertility is epigenetic modifications. Differential DNA methylation patterns between wheat and rye genomes alter the expression of critical genes, including those encoding ribosomal RNA (Carvalho et al., 2013; Silva et al., 2008). Wheat-rye addition lines demonstrate that introgressed rye chromosomes can induce widespread epigenetic changes, potentially disrupting gene regulatory networks essential for normal development (Zheng et al., 2016). These epigenetic differences may contribute to nucleolar dominance and the observed genomic instability in hybrids. Together, these multi-level barriers, such as genomic incompatibilities, cytogenetic instability, and epigenetic dysregulation, create significant challenges for wheat-rye hybridization.

Environmental and physiological factors

Environmental and physiological conditions have a major impact on the fertility of wheat–perennial rye hybrids. Temperature variation during meiosis can disturb chromosome pairing, spindle formation, and segregation, leading to partial or complete pollen sterility. Low temperatures in wheat and rye prolong meiotic division and increase the frequency of sterile anthers due to irregular chromosome behavior (Bennett et al., 1972). Rey et al. (2017) found that temperature regulates homoeologous chromosome pairing in wheat–rye hybrids lacking the Ph1 locus, with cooler conditions (~13 °C) enhancing crossover formation and higher temperatures (~30 °C) reducing it. This demonstrates that temperature influences both meiotic timing and recombination frequency between the parental genomes. Photoperiod is another important environmental factor affecting reproduction. Short-day conditions and limited light slow anther development and lower pollen viability, as observed in triticale and wheat–*Thinopyrum* hybrids. Silkova et al. (2014) reported that long exposure to low temperatures during meiosis increases univalent and lagging chromosome formation, which reduces fertility. Conversely, moderate heat stress can accelerate cell division but create asynchronous meiosis and defective gamete formation (Guo et al., 2019).

Nutrient balance and hormone levels also play vital roles. Proper nitrogen and boron availability are necessary for normal anther and pollen development, while deficiencies reduce pollen tube growth and embryo sac function. Internal hormones, especially auxins and gibberellins, control anther opening and ovule development, and any imbalance may cause incomplete fertilization or embryo abortion (An et al., 2019). In perennial rye hybrids, long vegetative growth before flowering can delay reproductive organ formation, causing poor synchronization between male and female gametes and a lower seed set (Tikhenko et al., 2024).

Based on the above, the fertility in wheat–perennial rye hybrids depend not only on genetic compatibility but also on environmental and physiological stability. Careful management of temperature, light, and nutrition during meiosis and flowering can help reduce meiotic abnormalities and improve reproductive success. Combining such environmental optimization with molecular and cytogenetic breeding methods will be essential for developing stable, fertile, and high-yielding perennial cereal hybrids.

OVERCOME REPRODUCTIVE BARRIERS

Chromosome doubling and embryo rescue

Chromosome doubling has proven highly effective in restoring fertility to wheat-rye hybrids by compensating for genomic imbalances. Colchicine-induced amphiploidization stabilizes meiosis by providing homologous chromosome pairs, addressing the sterility caused by divergent wheat (AABBDD) and rye (RR) genomes. Early studies on octoploid triticale demonstrated that spontaneous chromosome doubling could restore fertility (Evtushenko et al., 2019), while later work showed that targeted colchicine treatment further improved meiotic regularity and seed set in synthetic hybrids (Tikhenko et al., 2017; Zhang, L. et al., 2008). This approach rescues fertility and enhances genomic stability, enabling the retention of desirable rye-derived traits like disease resistance (An et al., 2019; Rabanus-Wallace et al., 2021). Natural chromosome elimination in subsequent generations refines hybrid genomes by removing deleterious material, improving developmental stability (Tikhenko et al., 2017, 2024).

Embryo rescue techniques are used to overcome the postzygotic barriers caused by the Eml gene interactions discussed in the previous section. Immature hybrid embryos are cultured in vitro, where colchicine is often applied to induce chromosome doubling during callus formation, generating fertile amphidiploids (Rogo et al., 2023; Tikhenko et al., 2017). Success depends on optimizing the embryo's developmental stage, media composition, and growth conditions (Rogo et al., 2023). While rescued plants may initially exhibit sterility due to gametophytic defects, successive generations allow natural selection to eliminate incompatible alleles and improve fertility

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(Tikhenko et al., 2024). These combined strategies, chromosome doubling and embryo rescue, address both pre- and postzygotic barriers, enabling the development of fertile, agronomically viable wheat-rye hybrids.

Cytoplasmic male sterility in wheat-rye hybrids

Cytoplasmic male sterility (CMS) is a natural system that prevents pollen formation, forcing plants to rely on cross-pollination, a valuable trait for hybrid breeding. It is primarily caused by the interaction between the nuclear genome and the cytoplasm, resulting in non-viable pollen and male sterility in female lines (Martín et al., 2018). This sterility poses significant challenges to hybrid breeding programs by preventing the natural production of fertile gametes.

The system works through a balance between sterilizing cytoplasm and restorer genes. CMS systems rely on restorer of fertility (Rf) genes to counteract the sterilizing effects. Key Rf genes identified in rye include Rfg1 on 4RL and minor genes like Rfg2 on 3R and Rfg3 on 6R, essential for fertility restoration (Vendelbo et al., 2021). The recent discovery of novel non-PPR Rf gene RfNOS1 on 3RL provides further insight into restoration mechanisms.

A successful application exists in triticale breeding, where wheat with rye cytoplasm (inducing sterility) is crossed with rye-carrying Rf genes to produce fertile, high-yielding hybrids. At the cellular level, CMS induces pollen developmental abnormalities through mitochondrial-nuclear genome incompatibility (Shahinnia et al., 2020; Vendelbo et al., 2021).

Despite its utility, CMS systems face critical limitations, including dependence on strong Rf genes, as weak restorers can result in partial sterility, and genetic vulnerability arising from overreliance on single cytoplasm types, also environmental sensitivity where stress conditions disrupt sterility, and complex implementation (Abdullah et al., 2025).

Ph1 suppression or deletion

Suppressing or deleting the Ph1 locus is a key strategy to restore fertility in wheat-rye hybrids by enabling homoeologous chromosome pairing and recombination. The Ph1 (Pairing homoeologous 1) locus, located on chromosome 5BL, is the most effective suppressor of homoeologous pairing in wheat (Rey et al., 2017). Additional suppressors include Ph2 (3DS) and a weaker suppressor on 3AS, but their effects are less pronounced than Ph1 (Türkösi et al., 2022). The Ph1 gene in wheat-rye hybrids inhibits chromosomal pairing between the parental genomes, resulting in hybrid sterility, the introduction of the ph1b deletion mutation allows for homoeologous pairing and recombination (Fan et al., 2021). This mutation has been instrumental in wheat-rye hybrid breeding, allowing the introgression of beneficial rye traits into wheat (Türkösi et al., 2022). Mutations in TaZIP4-B2, a gene within the Ph1 locus, have induced high levels of homoeologous crossovers, offering a potentially more stable alternative (Rey et al., 2017). Furthermore, meiotic restitution, which leads to unreduced gametes, can enhance fertility in Ph1-deficient hybrids (Silkova et al., 2013).

Despite its benefits, Ph1 manipulation presents challenges, including poor agronomic performance in mutant lines (e.g., the original ph1b mutant in Chinese Spring wheat) and genomic instability due to large-scale chromosome rearrangements (Türkösi et al., 2022). Backcrossing with adapted wheat cultivars is often necessary to improve hybrid viability (Tikhenko et al., 2024).

Translocation, introgression, and backcrossing strategies

Fertility restoration and agronomic traits in wheat-rye hybrids rely on translocation lines (e.g., 1RS.1BL, 1RS.1AL, 6RS.6AL) and introgression breeding to improve disease resistance and yield, though some translocations may affect quality or productivity (Jung & Seo, 2014; Kaur et al., 2022). Introgression breeding further restores fertility by incorporating rye genes into wheat, leveraging CMS systems and restorer genes like Rfp1 and Rfp3 to overcome sterility (Hackauf et al., 2017). Integrating molecular marker and marker-assisted selection (MAS) with large-scale chromosome rearrangements significantly enhances breeding efficiency by stabilizing hybrid genomes and minimizing linkage drag (Jung & Seo, 2014; Kaur et al., 2022; Tikhenko et al., 2024). Furthermore, the application of these tools within cytoplasmic male sterility (CMS) systems provides a streamlined approach to overcoming genetic barriers in wheat-rye hybridization (Tikhenko et al., 2024).

Backcrossing with wheat or triticale also aids fertility restoration by improving meiotic stability and leveraging fertility restoring loci, such as the Rf locus on chromosome 6AS (Shahinnia et al., 2020), achieving stable and fertile wheat-rye hybrids often requires multiple backcrossing generations to overcome cytoplasmic incompatibility issues (Orlovskaya et al., 2023; Pershina et al., 2012).

Molecular and biotechnological approaches (gene editing, CRISPR)

Recent advances in fertility restoration, particularly through molecular and biotechnological approaches such as CRISPR/Cas9 genome editing, provide promising solutions for overcoming reproductive barriers in wheat-perennial rye hybrids. CRISPR/Cas9 is a highly efficient and precise tool for modifying plant genomes in polyploid species (Awan et al., 2022; Das et al., 2022). Its multiplex editing capability allows the simultaneous targeting of multiple gene copies, which is particularly important in polyploid crops such as wheat, where homoeologous genes are present across different genomes (Miroshnichenko et al., 2023). For instance, mutations in the Ms45 gene in wheat can induce male sterility, which can be reversed by introducing a wild-type copy from another species, such as rice (Singh et al., 2018). Similar strategies could be applied to wheat-rye hybrids by modifying or eliminating incompatibility alleles (Tikhenko et al., 2024).

DOI: 10.34101/ACTAAGRAR/1/16431

Beyond fertility restoration, CRISPR/Cas9 significantly enhances breeding programs by improving key agronomic traits, disease resistance, and abiotic stress tolerance (Awan et al., 2022; Yigider et al., 2023). The editing efficiency of CRISPR systems has been significantly enhanced through improvements in single-guide RNA (sgRNA) scaffolds and promoter optimization, allowing for more precise genetic modifications in wheat (Li et al., 2020).

CRISPR/Cas9-induced male sterility is crucial in hybrid seed production. For example, wheat mutations in the TaNP1 gene lead to complete male sterility, streamlining hybrid seed production (Li et al., 2020). CRISPR/Cas9 can also be used to develop stress-resilient and high-yielding crops, supporting sustainable agriculture and global food security (Das et al., 2022).

Further refinement of CRISPR/Cas9 systems, including base and prime editing, could enhance precision and reduce off-target effects in polyploid crops (Miroshnichenko et al., 2023). Exploring epigenetic modifications and synthetic biology approaches may offer additional strategies to stabilize hybrid fertility and improve stress resilience (Das et al., 2022).

CONCLUSION AND FUTURE DIRECTION

Fertility restoration in wide hybrid systems remains a multifaceted challenge involving cytoplasmic-nuclear incompatibilities, meiotic irregularities, and genetic barriers like the Ph1 locus. While traditional approaches such as CMS systems with Rf genes, ph1b-induced homoeologous pairing, and introgression breeding have enabled progress, significant limitations persist. These include genomic instability in translocation lines, environmental sensitivity of CMS systems, and fundamental trade-offs between fertility restoration and agronomic performance (Fan et al., 2021; Kaur et al., 2022; Martín et al., 2018; Rey et al., 2017; Tikhenko et al., 2024; Türkösi et al., 2022; Vendelbo et al., 2021)

Overcoming fertility barriers in wheat-rye hybrids faces three persistent technical challenges. First, chromosome doubling protocols exhibit inconsistent success due to variable tissue response to antimitotic agents and hybrid genomic instability (Tikhenko et al., 2017). Second, embryo rescue outcomes remain unpredictably genotype-dependent, suggesting underlying genetic/epigenetic incompatibilities that particularly affect early hybrid development (Rogo et al., 2023; Shahinnia et al., 2020). Third, insufficient understanding of centromere stabilization mechanisms hinders meiotic control, as demonstrated by persistent pairing errors in hybrids lacking the Ph1 gene (Fan et al., 2021; Rey et al., 2017). These interconnected limitations demand targeted research combining cytogenetic and molecular approaches to achieve reliable hybrid fertility. Future research should focus on developing more reliable chromosome manipulation protocols while advancing our fundamental knowledge of meiotic control systems (Shahinnia et al., 2020; Tikhenko et al., 2024).

Precision genome engineering approaches, particularly CRISPR-Cas9 systems, offer transformative potential for overcoming these challenges. Strategic targeting of key incompatibility alleles and the development of synthetic CMS systems could bypass the current limitations of natural Rf genes (Das et al., 2022; Tikhenko et al., 2024). These efforts should be combined with meiotic control innovations, including exploiting TaZIP4-B2 mutants and epigenetic regulators of crossover frequency, to stabilize chromosome pairing in hybrids (Fan et al., 2021; Rey et al., 2017).

For practical implementation, breeding programs must adopt integrated systems-level approaches. This includes combining genomic selection with high-throughput phenotyping to accelerate rye chromatin introgression while reducing linkage drag (Jung & Seo, 2014; Kaur et al., 2022). Simultaneously, diversifying the genetic basis of CMS systems and Rf gene resources will be crucial for maintaining genetic resilience (Vendelbo et al., 2021). Ultimately, successfully translating these technologies will require rigorous field testing under real-world conditions to bridge the gap between laboratory innovation and agricultural application (Awan et al., 2022; Tikhenko et al., 2024).

Bridging the gap between research and practical application, developing high-yielding wheat-rye hybrids demand a carefully balanced integration of modern genetic technologies and precision breeding approaches. The ultimate goal is to create stable, fertile hybrids that deliver superior field performance while meeting the rigorous demands of commercial agriculture. Multiple scientific disciplines are required to achieve this objective, with geneticists optimizing chromosome stability, breeders selecting desirable traits, and agronomists ensuring field adaptability, all through coordinated efforts across research and applied science. Only through such comprehensive, collaborative science can we fully harness the potential of these innovative cereal hybrids to enhance global food production systems.

Future progress in wheat–perennial rye hybrid fertility will depend on integrating classical cytogenetics with emerging molecular tools. Combining FISH/GISH cytogenetic mapping with high-throughput sequencing will reveal hidden chromosomal interactions, while transcriptomic profiling can identify gene expression changes associated with meiosis. Applying genome editing to modify hybrid-specific regulatory elements may stabilize fertility without compromising agronomic traits. Multi-disciplinary collaborations among geneticists, molecular biologists, and breeders will be crucial to translate lab-based advances into field-ready hybrids that combine perenniality with fertility and yield.

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