# Apomictic Hybrid Rice with Cloning Efficiency Exceeding 99% and Near-Normal Seed Set Rate

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## Abstract

Apomixis, as a specialized reproductive mode that achieves propagation through clonal seeds, can stably inherit heterosis and superior heterozygous genotypes in subsequent generations, thereby providing a technologically revolutionary potential approach for crop breeding and agricultural production. However, current synthetic apomixis systems still face numerous challenges in practical applications, primarily manifested as low or unstable cloning efficiency and significant reductions in seed set rate, which severely constrain their value for agricultural promotion. In this study, combined with transcriptomic analysis methods, we identified in rice a transcription factor specifically expressed in sperm cells that may function as a critical "switch" during zygote activation. Further research demonstrated that ectopic expression of this transcription factor in egg cells can efficiently induce parthenogenesis and produce haploid progeny. When this strategy was coupled with clonal gamete technology, the constructed apomixis system achieved near-complete clonal seed propagation—all obtained apomictic hybrid rice lines exhibited cloning efficiencies exceeding 99%. Most importantly, we successfully developed apomictic lines with seed set rates comparable to corresponding F1 hybrid rice and cloning efficiencies over 99%, and these superior traits could be stably maintained across consecutive generations. These achievements not only uncovered novel endogenous genes capable of efficiently inducing parthenogenesis but also established an efficient, stable apomixis technology platform with potential for large-scale application, providing a practical solution for the long-term fixation of heterosis and opening a novel technological pathway for future commercial cultivation of hybrid crops through "seed self-propagation" . Keywords

# Full Text

## Preamble

### Clonal Seed Production with Over 99% Efficiency and Near-Normal Seed Set in Apomictic Hybrid Rice

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## Abstract

Apomixis, a specialized reproductive mode that propagates through clonal seeds, offers a revolutionary approach to preserve heterosis and superior heterozygous genotypes across generations in crop breeding and production. However, current synthetic apomixis systems face critical challenges of low or unstable cloning efficiency and substantially reduced seed set, severely limiting their agricultural applicability. Here, we employed transcriptomic analyses to identify a transcription factor specifically expressed in rice sperm cells that likely functions as a key "switch" in zygote activation. Ectopic expression of this factor in egg cells efficiently induced parthenogenesis and produced haploid progeny. When coupled with clonal gamete technology, the resulting apomixis system achieved near-complete clonal reproduction—all apomictic hybrid rice lines exhibited cloning efficiencies exceeding 99%. Importantly, we successfully developed apomictic lines with seed set rates comparable to their corresponding $F_1$ hybrids while maintaining >99% cloning efficiency across multiple consecutive generations. These findings not only uncover a novel endogenous gene capable of efficiently inducing parthenogenesis but also establish a highly efficient, stable, and scalable apomixis technology platform. This work provides a practical solution for permanent fixation of heterosis and opens a new technological path for commercial cultivation of hybrid crops through "self-propagation."

**Keywords:** apomixis; clonal seeds; parthenogenesis; haploid; heterosis; rice

## Introduction

Heterosis, or hybrid vigor, describes the phenomenon where hybrid offspring exhibit superior growth, yield, and stress resistance compared to their homozygous parents, forming the biological foundation for modern crop productivity improvement. This trait has been widely exploited in major cereal crops such as rice and maize, substantially increasing global agricultural yields. Hybrid

rice, for instance, typically yields 20–30% more than conventional inbred lines, making crucial contributions to food security [2,3]. However, due to meiosis and random gamete fusion during conventional sexual reproduction, hybrid progeny undergo genetic segregation, causing rapid erosion of heterosis in $F_2$ and subsequent generations. Consequently, agricultural production requires annual parental propagation and manual hybrid seed production—a process that is technically complex, labor-intensive, and costly, severely constraining large-scale adoption and sustainable utilization of hybrid seeds [4,5].

To overcome this bottleneck, scientists have pursued a novel propagation method that could "permanently fix" heterosis: synthetic apomixis. This technology enables clonal seed reproduction through genetic engineering, allowing hybrid plants to produce genetically identical offspring without normal fertilization, thereby achieving stable transgenerational transmission of superior heterozygous genotypes. Successful implementation would dramatically simplify seed production, reduce costs, and facilitate widespread adoption of hybrid varieties among smallholder farmers and resource-limited regions [6–10]. The key challenge lies in constructing an efficient, stable, and agriculturally viable system that achieves near-100% clonal seed efficiency while maintaining normal seed set, ensuring that apomictic progeny faithfully inherit parental traits [11–14].

Recent efforts to construct synthetic apomixis systems have integrated multiple molecular strategies. One core approach combines the "Mitosis instead of Meiosis" (MiMe) system with mutations in sperm- or pollen-specific haploid-inducing genes such as rice *OsMATL* and *OsPLA$ $2* or Arabidopsis *AtDMP8/9* [15–19]. Although theoretically T-DNA-independent and biosafe, this method alone yields insufficient cloning efficiency for practical application. A more effective strategy couples MiMe with parthenogenesis induction—triggering embryo development from unfertilized egg cells [12–14]. Studies show that ectopic expression of specific transcriptional regulators in egg cells can effectively initiate parthenogenesis. Reported inducers include endogenous genes such as *BABY BOOM 1* (*OsBBM1*), *OsBBM4*, and *WUSCHEL* (*OsWUS*) [20–22], and exogenous genes like *PpPAR* and *ToPAR* [23–26]. The *OsBBM1*-based system achieved 11–29% clonal seed efficiency in rice [20]; optimized versions can exceed 95% [27–29], but suffer from large inter-line variation (most lines range 0–90%) and severe fertility reduction [20,27–29]. Similar challenges plague *OsBBM4*, *OsWUS*, *PpPAR*, and *ToPAR*, which show improved fertility but suboptimal cloning efficiency [21–24], failing to meet commercial requirements for high efficiency and consistency.

These limitations prompted deeper investigation into zygote activation mechanisms. Conventionally, the egg cell was considered transcriptionally quiescent, requiring sperm-derived signals to initiate the first mitotic division and embryogenesis [30–34]. However, accumulating evidence reveals that plant egg cells possess a transcriptionally "pre-activated" state, enabling rapid response and developmental network activation upon fertilization [35–39]. Notably, all known endogenous parthenogenesis-inducing factors (*OsBBM1*, *OsBBM4*, *OsWUS*) are broadly expressed in roots, stems, panicles, endosperm, and embryos [20–22].

Their functional disruption causes pleiotropic developmental defects, suggesting potential interference with normal reproductive and vegetative growth [20,40–42]. This raises a key question: do transcription factors exist that are functionally specialized and specifically expressed in sperm cells to precisely trigger zygote activation during fertilization? Artificial activation of such factors in egg cells might more faithfully mimic natural fertilization initiation, enabling efficient, low-disturbance parthenogenesis induction. Success would significantly advance the development of practical, high-efficiency synthetic apomixis systems for crop improvement.

Based on this hypothesis, we employed multidimensional screening strategies including tissue-specific expression analysis, developmental stage time-series transcriptomics, and gene co-expression network analysis to identify a sperm cell-specific transcription factor in rice. Functional validation demonstrated that its ectopic expression in egg cells efficiently induced parthenogenesis and haploid progeny. Coupling this system with MiMe-mediated clonal gamete formation achieved near-complete apomixis—all hybrid rice lines exhibited >99% clonal seed efficiency. Critically, we developed apomictic lines with seed set rates comparable to $F_1$ hybrids while maintaining >99% cloning efficiency across generations. These results demonstrate that this sperm-specific transcription factor may play a key regulatory role in zygote initiation and provides a novel technical path for constructing efficient, stable, agriculturally applicable synthetic apomixis systems. In summary, this study leverages the identified parthenogenesis-inducing gene to precisely simulate critical initiation events in natural reproduction, establishing a solid theoretical and practical foundation for permanent heterosis fixation and "self-propagation" of hybrid crops.

## Identification of a Sperm-Specific Transcription Factor Delivered to the Zygote

To systematically screen candidate transcription factors that play key regulatory roles in rice fertilization, exhibit sperm cell-specific expression, and deliver transcripts to the zygote, we integrated 8,796 publicly available rice transcriptome datasets. All raw data underwent unified quality control and normalization, and TPM values were recalculated at the gene level to construct a high-consistency expression matrix with batch effects eliminated (Table S1). Using tissue-specific analysis, we identified genes significantly enriched in sperm cells with low expression elsewhere, yielding 729 genes with prominent sperm-preferential expression patterns (Figure S1) as an initial candidate set.

Considering that a potential "sperm-delivered factor" must satisfy three core expression features: (1) high expression in mature sperm cells; (2) minimal expression in egg cells; and (3) detectable transcripts in post-fertilization zygotes and early embryos—suggesting mRNA carriage by sperm into the egg cell for functional continuity without new transcription. We therefore integrated time-series expression profiles from sperm cells, egg cells, zygotes, and early em-

bryos to analyze dynamic expression patterns (Figure S2). Ten distinct gene expression clusters were identified based on expression trends. Clusters 1 and 2 exhibited the ideal "sperm-specific–zygote-persistent" pattern: high expression in sperm cells, silenced in egg cells, but detectable in zygotes and subsequent developmental stages (Figure 1 [Figure 1: see original paper]A and Figure S2). This trajectory strongly supports transcript delivery from male gametes to the fertilized egg, making them key candidates for initiating zygotic genome activation or early embryogenesis. We focused on the 170 genes in these two clusters.

Functional annotation identified five genes predicted to encode transcription factors, which were considered most likely to drive post-fertilization developmental programs due to their regulatory potential. To assess functional associations among these transcription factors, we constructed a weighted gene co-expression network (WGCNA) based on the normalized TPM expression matrix (Table S1). Four of the five candidate transcription factors clustered in a highly interconnected co-expression module (Figure 1B), suggesting involvement in the same biological pathway or coordinated regulatory network. One gene displayed the highest connectivity and co-expression weight, occupying the module' s core position, indicating potential function as a "hub gene." Given its likely functional importance, we named this gene **HUAXU**, inspired by the ancient Chinese mythological figure "Huaxu Shi." According to texts such as *Liezi · Huangdi*, Huaxu Shi conceived Fuxi and Nüwa after stepping in a divine footprint. This mythological narrative of "conceiving life without paternal participation"symbolically aligns with parthenogenesis or autonomous zygote activation mechanisms independent of typical biparental genetic contribution.

In summary, through multidimensional transcriptomic analysis, tissue-specific screening, and temporal expression modeling, we identified a set of sperm-specific transcription factors potentially delivered to the zygote via sperm. Among them, **HUAXU** emerged as the primary target for functional validation due to its unique network topology and ideal expression pattern.

## Ectopic HUAXU Expression in Egg Cells Efficiently Induces Parthenogenetic Embryogenesis

Previous studies suggest that sperm-derived transcription factors may participate in zygotic genome activation after gamete fusion, playing crucial roles in initiating embryogenesis [34–37]. Based on this hypothesis, artificial expression of such key regulators in egg cells could theoretically bypass fertilization and directly trigger embryonic development—i.e., parthenogenesis [32,33]. To test whether HUAXU possesses this capability, we constructed an egg cell-specific binary vector **HUAXU-ee**, utilizing the rice *OsECA* promoter with confirmed egg cell-specific activity [44] to drive HUAXU expression (Figure 1C).

Through *Agrobacterium*-mediated transformation, we introduced HUAXU-ee into callus of the hybrid rice variety Chunyou 84 (CY84, wild type, WT), obtaining four independent $T_0$ lines stably integrating the HUAXU-ee cassette (Figure

S3). To evaluate whether HUAXU can autonomously initiate embryogenesis without fertilization, we grew $T_0$ plants in the field and collected unpollinated ovaries at 3 days after emasculation (DAE) for histological analysis using eosin B staining [45]. In 30 WT ovaries, only intact egg cells and undivided central cell nuclei were observed, with no cell division or embryo formation (Figure 1D, left). In contrast, 18 of 38 HUAXU-ee ovaries (47.4%) exhibited multicellular structures morphologically resembling early embryos in the absence of fertilization, accompanied by undivided central cells—a hallmark of parthenogenesis (Figure 1D, right).

These results demonstrate that egg cell-specific ectopic expression of HUAXU efficiently triggers embryogenesis without fertilization, confirming its capacity to induce parthenogenesis and suggesting its potential function as an "initiation factor" for zygotic developmental programs.

## Ectopic HUAXU Expression in Egg Cells Efficiently Induces Haploid Progeny

In sexually reproducing plants, artificial parthenogenesis offers a promising route to produce haploid embryos directly from unfertilized egg cells, providing an important tool for haploid breeding [46–47]. To assess whether HUAXU ectopic expression in egg cells can trigger haploid formation, we first observed $T_0$ plants carrying the HUAXU-ee construct in the field. HUAXU-ee plants showed no obvious differences from wild-type (WT) controls in vegetative growth or reproductive development (Figure S4A and S4B). However, seed set rates among the four independent lines varied from 50.3 ± 4.2% to 74.6 ± 4.3%, compared to 74.3 ± 2.5% in WT (Figure S4B and S4C), suggesting partial fertility reduction in some lines.

To further investigate haploid induction, we self-pollinated the four HUAXU-ee $T_0$ lines, grew $T_1$ progeny, and analyzed ploidy levels in leaf tissue via flow cytometry. Among 54–70 $T_1$ individuals per line, haploid proportions ranged from 20.0% to 57.4%, while all 96 WT progeny were diploid (Figure 1E, 1F and Figure S5). This confirms that HUAXU expression in egg cells can induce haploid progeny.

Since haploids originate from unfertilized egg cells, their genomes should be fully maternally derived and completely homozygous [48]. To verify this, we genotyped 12 InDel polymorphic markers across the 12 rice chromosomes that differentiate the parental lines of CY84 (Table S2). While recombinant inbred diploid (RID) progeny from conventional selfing retained heterozygous genotypes at multiple loci, all flow cytometry-identified haploids exhibited complete homozygosity with single parental alleles at all 12 loci (Figure S6), consistent with parthenogenetic origin. Whole-genome resequencing of representative haploids at ~20× depth confirmed complete absence of heterozygous SNPs genome-wide, unlike RID controls with extensive heterozygous regions (Figure 1G). Additionally, HUAXU-ee-induced haploids displayed typical haploid phenotypes:

significantly dwarfed stature, smaller panicles, reduced grain size, and complete sterility (Figure 1H and 1I), further supporting ploidy identification.

In summary, egg cell-specific ectopic expression of HUAXU in hybrid rice successfully induced fertilization-independent embryogenesis, yielding progeny with complete genetic homozygosity and typical haploid morphology, demonstrating efficient parthenogenesis induction.

## Achieving 100% Cloning Efficiency in Synthetic Apomixis

Combining engineered parthenogenesis with clonal gamete formation is a core strategy for synthetic apomixis in sexual plants [12–14]. To evaluate HUAXU' s potential for inducing synthetic apomixis, we constructed a **HUAXU-ee_{sgMiMe}** vector integrating the HUAXU-ee cassette with a previously reported "MiMe" (Mitosis instead of Meiosis) system (Figure 2 [Figure 2: see original paper]A). This design uses CRISPR/Cas9 to simultaneously knock out three key meiotic genes—*OSD1*, *PAIR1*, and *REC8*—replacing meiosis with mitosis to produce diploid gametes without recombination [16,17]. Using *Agrobacterium*-mediated transformation, we introduced HUAXU-ee_{sgMiMe} into hybrid rice line CY84 (WT) and identified $T_0$ lines via high-throughput mutation tracking (Hi-TOM) [49]. Five independent $T_0$ lines were obtained with frameshift mutations in *OSD1*, *PAIR1*, and *REC8* (Figure S7A), all containing the egg cell-specific HUAXU-ee fragment (Figure S7B). These lines were designated **Fixation of Hybrids 8 (Fix8)** #1-#5.

Phenotypically, Fix8 $T_0$ plants showed no significant differences from WT CY84 in growth or panicle architecture (Figure 2B and 2C), indicating minimal developmental disruption from multiple gene mutations and transgene expression. However, seed set rates varied among lines ($50.4 \pm 3.9\%$ to $74.3 \pm 2.4\%$), lower than WT ($74.9 \pm 2.4\%$) (Figure 2C and 2D), though Fix8 #3 showed no statistical difference (two-tailed t-test, $P > 0.05$). To confirm clonal reproduction, we analyzed progeny ploidy. Pure MiMe mutants (lacking HUAXU-ee) should produce tetraploid offspring after normal fertilization due to unreduced gametes [15–17,20]. Indeed, all 96 progeny from MiMe-only controls were tetraploid (Figure 2E, 2G and Figure S8), validating the system. In stark contrast, all progeny from the five Fix8 lines (80, 112, 96, 80, and 96 individuals analyzed) were stable diploids with no tetraploids detected (Figure 2F, 2G and Figure S8), suggesting non-fusion or clonal mechanisms.

To exclude chromosomal recombination artifacts and verify maternal heterozygous genome inheritance, we genotyped progeny using 12 chromosome-wide InDel markers. While $F_2$ RID controls showed homozygous genotypes at multiple loci indicating segregation, all Fix8 progeny maintained complete heterozygosity identical to the original CY84 hybrid at all 12 loci (Figure S9), confirming absence of meiotic recombination. Whole-genome resequencing at ~20× depth of representative Fix8 progeny revealed genome-wide heterozygosity matching the parental CY84, unlike $F_2$ RID plants with extensive recombination and ho-

mozygous blocks (Figure 2H). This demonstrates that Fix8 progeny arise not from conventional sexual reproduction but through recombination-free clonal reproduction.

In conclusion, Fix8 $T_0$ plants produce diploid progeny that retain the full maternal $F_1$ heterozygous genotype genome-wide. Multiple lines of evidence confirm that Fix8 achieves apomixis with direct clonal offspring from $F_1$ hybrids. Importantly, no exceptions were found among all progeny analyzed, indicating 100% clonal reproduction efficiency across all five independent lines—a milestone representing the first efficient and stable synthetic apomixis in a major cereal crop.

## Stable Inheritance of Clonal Reproduction and Agronomic Traits in Apomictic Hybrid Rice Fix8

Stable inheritance of seed set and cloning efficiency is critical for agricultural application of synthetic apomixis. To evaluate whether Fix8 diploid progeny (hereafter "clonal Fix8" ) maintain $F_1$ heterosis, we grew $T_1$ generation plants under field conditions and assessed key agronomic traits. Except for one dwarf line possibly due to T-DNA insertion position effects, the four other independent lines showed no significant differences from WT $F_1$ hybrid CY84 in plant height, tiller number, or panicle length (Figure 2I, 2J and Figure S10), demonstrating effective preservation of elite phenotypes through apomictic transmission. $T_1$ clonal Fix8 seed set rates ranged from $50.9 \pm 3.5\%$ to $72.2 \pm 3.0\%$, equivalent to 68.0–96.5% of parallel-grown WT $F_1$ controls ($74.8 \pm 2.7\%$) (Figure 2J and 2K). Notably, Fix8 #3 showed no statistical difference from WT $F_1$ plants, exhibiting near-normal fertility.

Given Fix8 #3' s stable morphology and high seed set across $T_0$ and $T_1$ generations, we selected it for further assessment of cloning efficiency in larger populations and its agricultural potential. We grew $T_2$ populations derived from three independent $T_1$ plants and analyzed ploidy composition. Among 1,104, 1,180, and 806 $T_2$ individuals, 1,101, 1,176, and 804 were diploid, respectively (Figure 3 [Figure 3: see original paper]A–3C), corresponding to cloning efficiencies of 99.7%, 99.7%, and 99.8%. This demonstrates near-complete penetrance of clonal reproduction that remains highly stable across generations without obvious decline.

To further validate stability under diverse field conditions, we parallel-planted Fix8 #3 $T_2$ and WT $F_1$ hybrid rice in multiple plots with varying soil types, water management, terrain, and cultivation practices. Morphological observations showed that clonal Fix8 $T_2$ plants maintained high consistency with WT $F_1$ across different ecological conditions in plant architecture and panicle morphology (Figure 3A–3D). Critically, all clonal Fix8 individuals were highly uniform, showing no intra-line variation and comparable population uniformity to $F_1$ hybrids, without the trait segregation typical of $F_2$ generations (Figure 3D). Seed set rates for Fix8 #3 $T_2$ ranged from $76.8 \pm 6.1\%$ to $79.4 \pm 3.8\%$ across plots, showing no significant difference from parallel WT $F_1$ controls ($77.3 \pm 3.2\%$

to 80.7 ± 2.9%) (Figure 3E), confirming normal or near-normal fertility under variable field environments.

In summary, Fix8 is a synthetic apomictic hybrid rice line with highly efficient and stable clonal reproduction. It achieves near-100% cloning efficiency while preserving elite agronomic traits and good seed set across generations, representing a critical breakthrough for translating "fixed heterosis" breeding strategies from theory to practical application.

## Discussion

Genetic uniformity in crop populations is fundamental to sustainable modern agriculture, affecting not only consistent field management and predictability but also yield stability and mechanization feasibility. This requirement is reflected in national and international seed quality standards. For rice, Chinese national standards mandate >99% seed purity for conventional varieties and >97% for hybrids. Therefore, any novel breeding technology must ensure high genetic uniformity in progeny while maintaining good agronomic performance and reproductive capacity. Synthetic apomixis, a frontier technology promising to fix heterosis and enable hybrid "self-cloning," faces the core challenge of achieving efficient, stable clonal seed production while maintaining normal seed set. Previous studies could induce apomixis but often with reduced fertility or unstable cloning efficiency, failing to meet dual requirements for seed purity and yield.

Our HUAXU-based synthetic apomixis system overcomes this bottleneck. It achieved >99% clonal seed ratios across multiple independent transgenic lines and consecutive generations, demonstrating high consistency even in large-scale populations. This efficiency not only meets but substantially exceeds current seed purity standards, showing clear commercial potential.

Importantly, HUAXU demonstrates superior parthenogenesis induction compared to previously reported factors like *OsBBM1*, *OsBBM4*, and *OsWUS*. While these can activate embryogenesis when ectopically expressed, their broad spatiotemporal expression patterns often cause unstable induction or reduced overall fertility. In contrast, HUAXU, as a naturally sperm-localized transcriptional regulator, may more precisely mimic fertilization signals to specifically initiate embryonic development pathways in unfertilized egg cells, achieving a more physiologically coordinated parthenogenesis. This hypothesis is supported by experimental data: Fix8 lines carrying HUAXU achieve near-complete clonal reproduction (>99%) while maintaining normal plant morphology and near-WT seed set—a rare combination in previous synthetic apomixis systems, marking a critical step toward practical application. Moreover, the system stably transmits and maintains genome-wide heterozygosity across generations, effectively achieving permanent heterosis fixation. This capability significantly reduces the high annual costs and resource consumption of repeated seed production, providing a feasible path toward "perpetual hybrids."

Although the HUAXU-based system shows exceptional cloning fidelity, occasional tetraploid progeny appear. These individuals typically exhibit reduced tillering, delayed flowering, larger glumes, awned grains, and near-sterility—characteristic polyploid traits easily identified at anthesis and removable during field management to maintain genetic purity. Moreover, due to their extremely low fertility, tetraploid Fix8 progeny have limited capacity for gene flow under natural pollination, posing minimal risk to system stability. Nevertheless, achieving complete diploid penetrance (100% cloning efficiency) remains an important future optimization goal. Potential strategies include fine-tuning promoter-effector combinations, optimizing spatiotemporal expression control, or screening for more specific and potent embryogenesis triggers to eliminate tetraploid events entirely.

In conclusion, our HUAXU-based synthetic apomixis system demonstrates significant advantages in cloning efficiency, seed set, genetic stability, agronomic trait preservation, and cross-generational consistency, representing a major breakthrough in the field. These results deepen our understanding of plant embryogenesis initiation and provide powerful technological support for revolutionizing crop breeding paradigms based on apomixis.

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## Figure Legends

**Figure 1. Ectopic expression of HUAXU in egg cells efficiently induces parthenogenesis and haploid progeny formation.**
(A) Cluster analysis showing expression patterns characteristic of sperm-delivered zygotic factors, based on time-series data from sperm cells, egg cells, zygotes, and embryos.
(B) Gene co-expression weights within the cluster containing four sperm-specific candidate transcription factors. These four genes are highlighted in red. Among them, HUAXU exhibits the highest weight, indicating its role as a major hub gene.

(C) Schematic of the binary vector construct (HUAXU-ee) designed for egg cell-specific ectopic expression of HUAXU. HYGR, hygromycin resistance gene.
(D) Parthenogenetic embryos (orange asterisks) induced by egg cell-specific HUAXU expression in ovaries of an emasculated HUAXU-ee plant at 3 days after emasculation (DAE; n = 18/38). In wild-type (WT) ovaries, no multicellular embryo structures are observed, with only egg cells present (n = 30). In the absence of fertilization, no endosperm development is observed, and only central cell polar nuclei (white arrows) are detected in both WT and HUAXU-ee ovaries. Embryos (em; orange asterisks), central nuclei (cn; white arrows), and egg cells (ec; white asterisks) are indicated. Scale bars, 20  m.
(E) Flow cytometry analysis of HUAXU-ee progeny showing both haploid (left) and diploid (right) individuals. PI, propidium iodide.
(F) Ploidy analysis of progeny from WT and HUAXU-ee $T_0$ lines. Ploidy was assessed by flow cytometry. HI rate represents haploid induction rate.
(G) Whole-genome sequencing of haploid progeny derived from HUAXU-ee $T_0$ lines. SNPs specific to the 16A allele are shown in red, C84 in blue, and mixed alleles in yellow. Haploid progeny display genome-wide homozygosity from either 16A or C84, with no heterozygous regions. By contrast, an $F_2$ recombined inbred diploid (RID) of CY84 shows recombination and partial heterozygosity.
(H–I) Morphology of plants (H) and panicles (I) of WT (left) and haploids derived from HUAXU-ee (right). Scale bars, 20 cm (H) and 2 cm (I).

**Figure 2. Full penetrance of apomictic hybrid rice induced by combining MiMe with egg cell-ectopic expression of HUAXU.**
(A) Schematic of the HUAXU-ee_{sgMiMe} construct, designed for egg cell-specific HUAXU expression and simultaneous knockout of OSD1, PAIR1, and REC8 to induce MiMe and clonal gamete formation. Two sgRNAs targeted OSD1 to enhance homozygous mutation efficiency.
(B–C) Plant (B) and panicle (C) morphology of WT and Fix8 $T_0$ plants. Scale bars, 20 cm (B); 2 cm (C).
(D) Seed setting rate of Fix8 $T_0$ plants compared to WT. Data represent the mean ± SD from three primary panicles per plant (n=3). **$p < 0.01$; *$0.01 \leq p < 0.05$; ns, not significant ($p \geq 0.05$; two-tailed Student' s t-test).
(E–F) Ploidy determination of progeny from Fix8 (E) and MiMe (F) lines by flow cytometry. PI, propidium iodide.
(G) Ploidy analysis of the progeny from WT, MiMe, and Fix8 lines.
(H) Whole-genome sequencing of diploid Fix8 $T_0$ progeny. SNPs specific to parental alleles 16A (red), C84 (blue), or both (yellow) across the 12 rice chromosomes. Fix8 diploid progeny exhibited genome-wide heterozygosity, closely matching the CY84 hybrid $F_1$, whereas CY84 $F_2$ recombinant inbred lines (RIDs) showed recombination and partial homozygosity.
(I–J) Plant (I) and panicle (J) morphology of WT and clonal Fix8 progeny grown in paddy fields. Scale bars, 20 cm (I); 2 cm (J).
(K) Seed setting rate of clonal Fix8 $T_1$ plants compared to WT. Data represent the mean ± SD from six plants (three primary panicles per plant). **$p < 0.01$;

ns, not significant ($p \geq 0.05$; two-tailed Student' s t-test).

**Figure 3. Stable inheritance of nearly complete penetrance of apomixis and normal seed set in Fix8.**
(A) Population performance of WT and clonal Fix8 line #3 in the $T_2$ generation.
(B) Clonal diploid progeny and rare induced tetraploids from Fix8 line #3 in the $T_2$ generation. Scale bar: 20 cm.
(C) Panicles of the progeny from Fix8 line #3 in the $T_2$ generation. Scale bar, 2 cm.
(D) Morphological comparison of $F_1$ (Hybrid CY84, WT), $F_2$ (RIDs of CY84), and clonal Fix8 $T_2$ plants. Scale bar, 20 cm.
(E) Seed-setting rate of Fix8 line #3 in the $T_2$ generation versus WT grown in parallel plots. Data are mean ± SD from six randomly selected plants. ns, not significant ($p \geq 0.05$; two-tailed Student' s t-test).

*Note: Figure translations are in progress. See original paper for figures.*