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Extending Mendel's legacy: Application of hawkweed *PpPAR* for inducing synthetic apomixis in hybrid rice

Dear Editor,

As Gregor Mendel conducted his groundbreaking crossbreeding experiments with peas that revealed the laws of inheritance (Mendel, 1866), he also observed an "essential difference" in the inheritance patterns between "variable hybrids" such as sexual pea and "constant hybrids" like hawkweed (*Pilosella piloselloides*), which he described in letters to the prominent botanist Carl Nägeli (van Dijk and Ellis, 2016). Subsequent investigations in hawkweed by Ostenfeld (1904) first revealed the phenomenon of apomixis. Contemporary studies have established that this highly heterozygous apomict bypasses meiosis and fertilization through the coordinated action of two dominant unlinked loci, *LOSS OF APOMEIOSIS* (*LOA*) and *LOSS OF PARTHENOGENESIS* (*LOP*) (Koltunow et al., 2011). Based on its genomic structure and expression patterns—which are strikingly similar to those of dandelion *ToPAR (Taraxacum officinale PARTHENOGENESIS)—the PpPAR (Pilosella piloselloides PARTHENOGENESIS) gene within the hawkweed LOP locus* has recently been identified as a candidate gene for apomixis (Underwood et al., 2022). Notably, *ToPAR* is specifically expressed in egg cells, and its ectopic expression has been shown to induce parthenogenesis across various species spanning Asteraceae (dandelion, lettuce) and Poaceae (foxtail millet and rice) taxa (Underwood et al., 2022; Song et al., 2023; Huang et al., 2024; Xiong et al., 2025). However, the role of *PpPAR* in inducing parthenogenesis and apomixis remains to be fully explored. In this study, we modulated the expression of *PpPAR* and successfully validated its ability to induce synthetic apomixis in hybrid rice.

To explore the potential role of *PpPAR* in parthenogenesis, we engineered a haploid induction system in hybrid rice cultivar CY84 (Figure 1A), in which egg cell-specific expression of *PpPAR* was driven by the *Arabidopsis* DD45 promoter (pDD45), previously validated for targeted transcriptional activation in rice female gametophytes (Khanday et al., 2019). After transformation, we obtained 13 independent transgenic lines, designated as *ee-PP* for *ectopic expression of PpPAR*. Though vegetative growth patterns of the *ee-PP lines* were indistinguishable from those of wild-type (WT) plants during early developmental stages (Figure 1C), seed setting was significantly reduced in *ee-PP* lines (47.1%–60.9%) compared with WT plants (71.2%) (Figure 1B; supplemental Table 1). Comprehensive genotyping using 12 polymorphic insertion-deletion (InDel) markers that distinguish the parental lines (16A and C84) was performed (supplemental Table 2), and analysis of 2435 T_1 progenies derived from 13 self-pollinated *ee-PP* T_0 plants revealed 11 individuals showing complete homozygosity across all 12 loci (supplemental Figure 1).

The ploidy status of these 11 individuals was then assessed by flow cytometry. All 11 individuals were determined to be haploid, in stark contrast to the diploid progenies from WT plants (Figure 1D). Five individuals were further subjected to whole-genome resequencing, and analysis of 1,137,240 single-nucleotide polymorphisms which exhibited variation between the two CY84 parents confirmed complete homozygosity across all chromosomes in all five individuals (Figure 1E). In total, six independent T_0 lines exhibited haploid induction rates ranging from 0.5% to 1.5% (supplemental Table 1). The 11 haploid individuals also displayed typical haploid characteristics, including reduced height, shorter panicles, smaller glume sizes, and sterile pollen (Figure 1F; supplemental Figure 2).

Although ectopic *PpPAR* expression alone proved effective in inducing haploid offspring in rice, we engineered a combinatorial system integrating *PpPAR* expression with the *MiMe* (*Mitosis instead of Meiosis*) module—a system designed to bypass meiosis (Mieulet et al., 2016)—in hybrid rice CY84 (Figure 1G) to further explore its apomictic potential. Three of the transformed lines (designated *Fix5*, for *Fixation of hybrids 5*) exhibited concurrent mutation in *PAIR1*, *REC8*, and *OSD1*, coupled with ectopic expression of *PpPAR* (supplemental Figure 3). During the vegetative stage, the *Fix5* plants exhibited a phenotype indistinguishable from that of WT (Figure 1I). However, seed setting was notably reduced compared to WT plants (52.1%-59.6% vs. 78.4%; Figure 1H). To evaluate the clonal seed production capacity of *Fix5* plants, flow cytometry was performed to determine the ploidy level of the progeny (Figure 1J). Among the 81, 65, and 80 progenies derived from the 3 *Fix5* lines, 42, 55, and 17 were confirmed as diploid offspring, with rates ranging from 21.2% to 84.6% (Figure 1M; supplemental Table 3). To determine whether heterozygosity was fixed in these lines, whole-genome resequencing was conducted on five diploid offspring from a *Fix5* plant, revealing that these diploid progeny maintained complete heterozygosity across all chromosomes, mirroring the CY84 hybrid (Figure 1K). Collectively, these findings demonstrate the ability of *Fix5* to generate clonal seeds while maintaining the heterozygosity of F_1 hybrid rice.

Subsequently, we conducted a comprehensive examination of the phenotype of clonal offspring derived from *Fix5* in the T_1 generation. These clonal progeny displayed similar characteristics to WT plants in terms of plant structure, panicle architecture, and grain appearance (Figure 1N; supplemental Figure 4). However, they consistently exhibited a reduced seed-setting rate when compared with their WT hybrid counterparts (64.1%-72.6% vs. 84.1%; Figure 1L; supplemental Table 3). To evaluate the

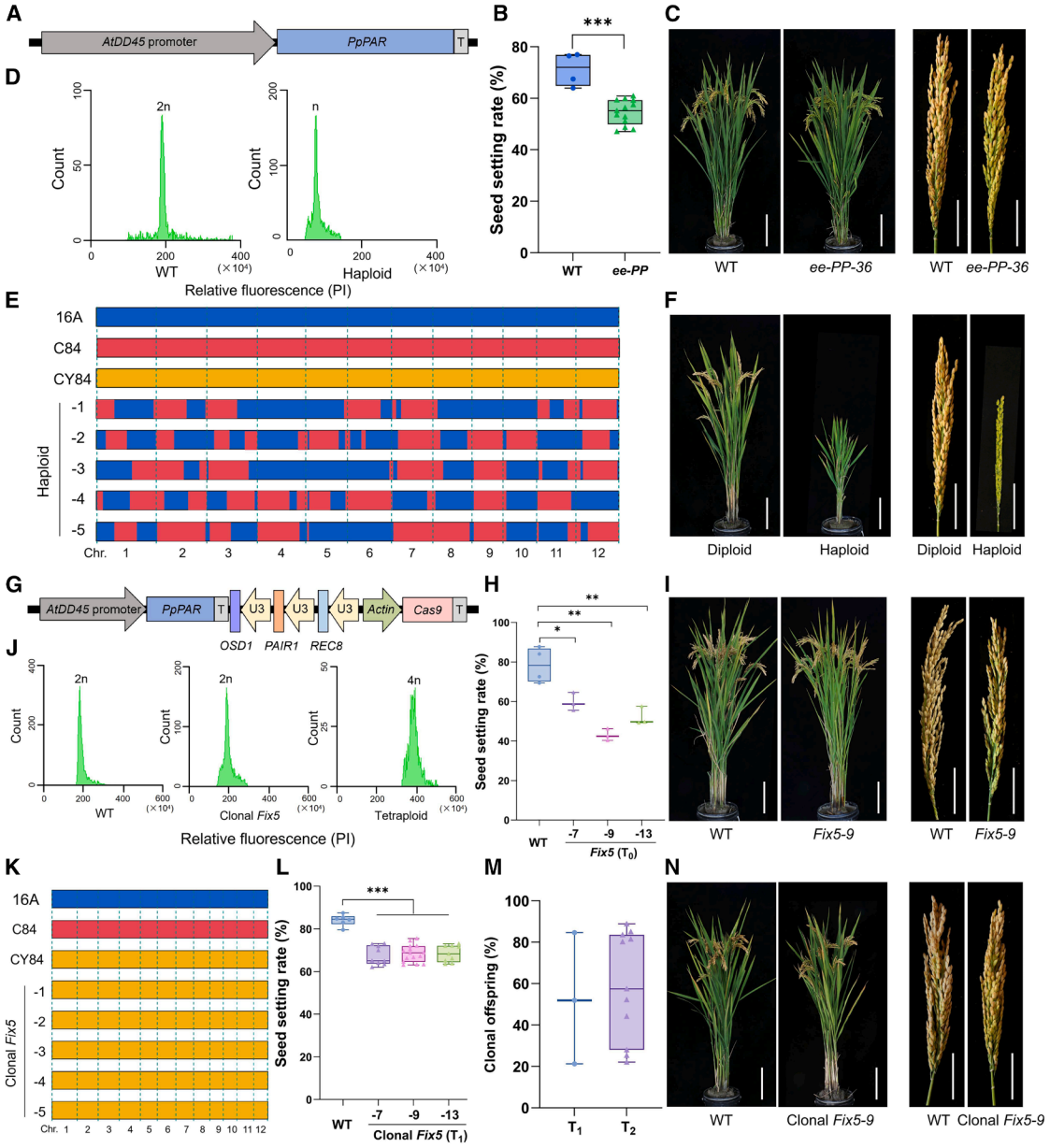

Figure 1. Ectopic expression of *PpPAR* induces apomixis in hybrid rice.

(A) Schematic diagram of the vector designed for the ectopic expression of *PpPAR* in egg cells. T, terminator.

(B) Comparison of the seed setting rates between WT and *ee-PP* plants. ***$P < 0.001$.

(C) Comparison of the plant morphology and panicles between WT and *ee-PP-36*. Scale bars correspond to 20 cm for plants and 5 cm for panicles.

(D) Flow cytometric analysis of ploidy levels in the offspring of *ee-PP*. PI, propidium iodide.

(E) Whole-genome sequencing of haploids. Highlighted SNPs of 16A, C84, and concurrent presence are shown in red, blue, and yellow, respectively, across the 12 chromosomal blocks. Parental lines 16A and C84 of the hybrid rice CY84 were used as reference controls.

(F) Morphological comparison of diploid and haploid offspring, including overall plant morphology and panicles. Scale bars correspond to 20 cm for plants and 5 cm for panicles.

(G) Schematic diagram of the vector integrating *MiMe* with the ectopic expression of *PpPAR*.

(H) Evaluation of the seed setting rate in WT and *Fix5* T_0 plants. **$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

(I) Comparison of plant morphology and panicles between WT and *Fix5-9* plants. Scale bars correspond to 20 cm for plants and 5 cm for panicles.

(J) Flow cytometric analysis of ploidy levels in the offspring of *Fix5* plants. PI, propidium iodide.

(K) Whole-genome sequencing results for clonal *Fix5* plants. The diploid progeny of clonal *Fix5* plants are heterozygous and identical to WT CY84.

(L) Evaluation of the seed setting rate in WT and *Fix5* T_1 plants. ***$P < 0.001$.

(M) Clonal offspring rate in *Fix5* T_1 and T_2 generations.

(N) Comparison of plant morphology and panicles between WT and clonal *Fix5* plants. Scale bars correspond to 20 cm for plants and 5 cm for panicles.

cloning efficiency of the T_1 generation, we employed flow cytometry and whole-genome resequencing, as described previously. The cloning efficiency of the T_1 generation was consistent with that of T_0, with rates ranging from 22.1% to 88.9% (Figure 1M; supplemental Table 3). To determine whether cloning efficiency correlated with the expression level of *PpPAR*, quantitative reverse transcription PCR (qRT-PCR) was performed on three *Fix5* lines in the T_1 generation (supplemental Figure 5). Notably, *Fix5-9*, which exhibited the highest clonal rate, showed 2.3-fold stronger *PpPAR* expression compared with *Fix5-13*, indicating a possible association between cloning efficiency and *PpPAR* expression level. Collectively, these results demonstrate the efficacy of ectopic expression of the parthenogenesis gene *PpPAR*, in combination with *MiMe*, in generating clonal progeny through apomictic reproduction across generations in rice.

Although Mendel is best known for his work on seven pea genes—the last three of which have recently been cloned (Feng et al., 2025)—which revealed foundational genetic principles, his parallel investigations on reproduction in *Phaseolus* (beans), *Cucumis* (cucumbers), *Dianthus* (carnations), and *Fuschia* species revealed different breeding patterns in *Hieracium* (hawkweed) (van Dijk and Ellis, 2016). This botanical enigma was ultimately resolved through the characterization of apomixis, a reproductive paradox wherein reproduction circumvents both meiosis and fertilization. Contemporary molecular dissection of hawkweed's apomictic machinery has identified a 654 kb *LOP* locus containing the *PpPAR* gene, whose sequence suggests potential parallel evolution via independent transposable element insertions in *cis*-regulatory elements (Underwood et al., 2022; Bicknell et al., 2023). In this study, we have demonstrated that the ectopic expression of *PpPAR* can induce haploid offspring in rice, thereby establishing its function as a bona fide parthenogenesis gene. Although the observed haploid induction rate was modest, it aligns with observations from other parthenogenesis genes, suggesting functional constraints on the parthenogenetic activation of egg cells (Xiong et al., 2023). Moreover, we have successfully established the *Fix5* apomixis system by co-expressing *PpPAR* with *MiMe*, achieving a notably high cloning efficiency. Crucially, the high clonal propagation rate is stably inherited in subsequent generations. The *Fix5* apomixis system still exhibits certain fertility deficiencies, and it will be essential to enhance both the seed setting rate and induction efficiency of *Fix5* for its potential commercialization in rice. This could be addressed through the use of more robust and specific promoters or by integrating *PpPAR* with additional parthenogenetic factors.

Notably, marked differences in parthenogenesis efficiency were observed between *ee-PP* and *Fix5* lines, which also occur in rice *ToPAR* systems but are less apparent in rice *OsBBM1* systems (Khanday et al., 2019; Vernet et al., 2022; Song et al., 2023; Xiong et al., 2025). These differences may originate from variable sensitivity to egg cell ploidy. In non-*MiMe* parthenogenetic lines, reproduction involves standard meiosis and gametogenesis, yielding haploid egg cells. Conversely, the *MiMe* system leads to diploid clonal egg cells due to meiosis bypass. This fundamental divergence in ploidy status likely accounts for the enhanced embryonic viability observed in diploid embryos compared with their haploid counterparts. In conclusion, our findings validate *PpPAR* as a functional parthenogenesis gene, providing a molecular explanation for a conundrum first encountered by Mendel and highlighting the potential for generating apomictic clonal progeny through genetic engineering.

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AUTHOR CONTRIBUTIONS

K.W. and Q.Q. developed and led the project. J.X., Y.J., and S.Y. performed the experiments. Y.H. and X.Q. analyzed the data. C.J.U. provided input on apomixis in Asteraceae plants. J.X. wrote the manuscript. K.W. and C.J.U. revised the manuscript.

SUPPLEMENTAL INFORMATION

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