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OsPLT7, a Non-BBM PLETHORA Factor, Drives Synthetic Apomixis Without Compromising Fertility in Rice

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Heterosis improves crop yield and stability but is not heritable due to meiotic recombination eroding parental heterozygosity. Apomixis, an asexual mode of reproduction through seeds, offers a promising strategy for fixing hybrid genotypes and enabling one-line hybrid rice. In rice, synthetic apomixis has been established by combining *MiMe* (*Mitosis instead of Meiosis*) with either haploid induction or parthenogenesis. Despite these advances, few synthetic apomixis systems simultaneously achieve normal seed set, highly efficient and stable clonal seed production and reliance solely on endogenous rice genes. Here, we explored whether other endogenous genes could be utilized to engineer synthetic apomixis in hybrid rice.

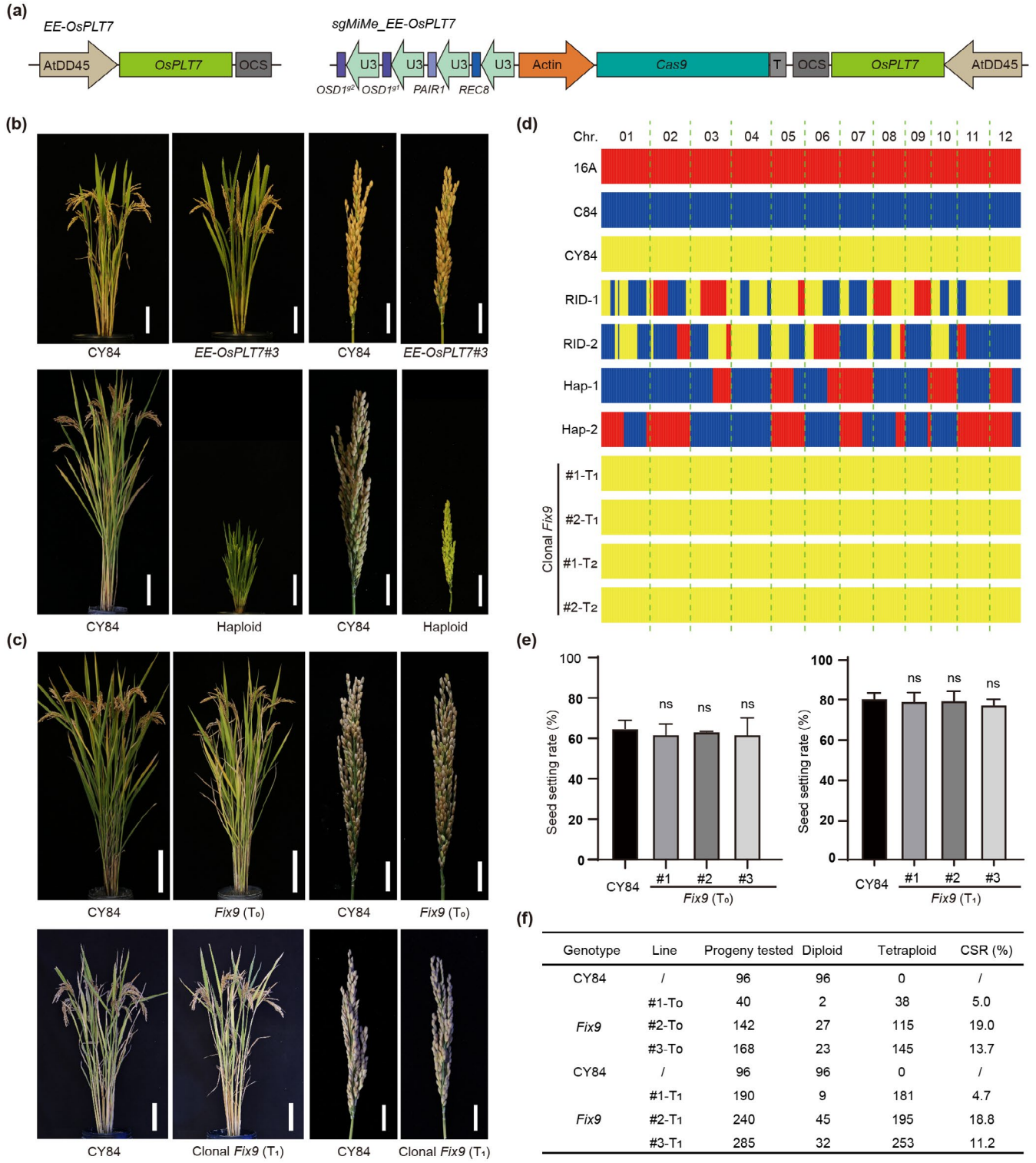
PLETHORA (PLT) transcription factors are plant-specific AINTEGUMENTA-LIKE members of the AP2/ERF superfamily that regulate cell fate, developmental plasticity and embryogenesis (Horstman et al. 2014). *PLT* genes are preferentially associated with proliferative and undifferentiated cell states, promoting meristematic programmes while preventing premature differentiation (Aida et al. 2004). PLTs also play important roles in early embryogenesis and can activate embryogenic programmes when ectopically expressed (Boutilier et al. 2002; Kerstens et al. 2024), suggesting their potential to confer embryogenic competence and promote embryo initiation. Phylogenetic analysis revealed multiple rice *PLT* clades with highly conserved AP2 domains (Figures S1 and S2). Although several *OsBBM* members have been implicated in embryogenesis and synthetic apomixis (Khanday et al. 2019; Wei et al. 2023), whether non-BBM PLT members can serve as additional endogenous inducers remains unclear. Among the non-BBM candidates, *OsPLT1* and *OsPLT7* occupy distinct evolutionary positions and are preferentially expressed in embryos, panicles and calli of wild-type plants (Figure S3).

To test whether *AtDD45*-driven expression of *OsPLT1* or *OsPLT7* could induce haploid formation in rice, we introduced each coding sequence into the hybrid rice Chunyou 84 (CY84; Figures 1a and S4a). Both *EE-OsPLT1* and *EE-OsPLT7* plants exhibited normal vegetative growth comparable to wild-type CY84, whereas seed-setting rates varied: 42.1%–78.8% for *EE-OsPLT1* and 34.4%–78.1% for *EE-OsPLT7*, relative to 77.0% ± 1.6% and 79.7% ± 5.2% in wild-type controls, respectively (Figures 1b, S4b, S7a and Table S1). To assess haploid-induction efficiency, self-pollinated progeny were genotyped with 12 InDel markers. In total, 1 of 752 *EE-OsPLT1* progeny and 23 of 569 *EE-OsPLT7* progeny were marker-homozygous (Figures S5c and S6). Flow cytometry confirmed their haploid status (Figures S5a and S7b), and these plants showed reduced height, smaller glumes and complete sterility (Figures 1b and S5b). Haploid-induction frequencies ranged from 1.8% to 6.5% across four independent *EE-OsPLT7* lines, compared with approximately 0.5% in the single haploid-producing *EE-OsPLT1* line (Figure S7c, Table S1). Notably, *EE-OsPLT1* lines #9 and #13 showed reduced seed set but no detectable haploids (Table S1), indicating that reduced fertility alone does not account for haploid induction. Whole-genome sequencing and chromosome bin-map analysis further confirmed the single-gamete origin of haploids (Figures 1d and S5d). Further

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(f)

Genotype	Line	Progeny tested	Diploid	Tetraploid	CSR (%)
CY84	/	96	96	0	/
	#1-To	40	2	38	5.0
Fix9	#2-To	142	27	115	19.0
	#3-To	168	23	145	13.7
CY84	/	96	96	0	/
	#1-T1	190	9	181	4.7
Fix9	#2-T1	240	45	195	18.8
	#3-T1	285	32	253	11.2

FIGURE 1 | *OsPLT7*-driven synthetic apomixis in hybrid rice. (a) Schematic of the binary vector for *AtDD45*-driven *OsPLT7* expression (left) and the binary construct combining *MiMe* with ectopic expression of *OsPLT7* (right). (b) Plant morphology and panicles of wild-type CY84, *EE-OsPLT7* T_0 plants and haploid progeny. Scale bars, 10 cm for plants and 3 cm for panicles. (c) Plant morphology and panicles of wild-type CY84 and *Fix9* plants (T_0 and T_1). Scale bars, 10 cm for plants and 3 cm for panicles. (d) Whole-genome sequencing of 16A, C84, CY84, the RIDs (recombinant inbred diploids), haploids and the clonal *Fix9* (T_1 and T_2). (e) Seed-setting rates of *Fix9* plants (T_0 and T_1). $n = 3$ panicles; Student's *t*-test (ns, not significant). (f) Clonal seed rate (CSR) of *Fix9* plants (T_0 and T_1).

supporting the weak parthenogenetic capacity of *OsPLT1*, an independent *sgMiMe_OsECA1::OsPLT1* system produced clonal diploid seeds at frequencies of 0.4%–2.4% (Figure S8). Together, these results identify *OsPLT7* as a previously unrecognized rice parthenogenesis inducer, with substantially higher haploid-inducing capacity than *OsPLT1*.

To determine whether *OsPLT7* enables synthetic apomixis, we introduced a construct into CY84 combining *AtDD45*-driven *OsPLT7* expression with simultaneous editing of the three *MiMe* genes, *OSD1*, *PAIR1* and *REC8* (Figure 1a). Three independent lines carrying homozygous or biallelic mutations in all three *MiMe* genes and the *OsPLT7* expression cassette were designated *Fix9* (*Fixation of hybrids 9*; Figure S9). *Fix9* plants displayed normal growth and panicle architecture comparable to wild-type CY84 (Figure 1c). Confocal microscopy showed that fertilized wild-type ovules developed both embryo and endosperm (10/10), whereas emasculated wild-type ovules retained only an unfertilized egg cell and two polar nuclei (11/11). By contrast, 16.7% (3/18) of emasculated *Fix9* ovules formed embryo-like structures without endosperm (Figure S10), demonstrating fertilization-independent embryo initiation. We next examined clonal seed production in self-pollinated *Fix9* plants. Seed-setting rates in *Fix9* plants ranged from 61.8% to 63.3%, comparable to wild-type CY84 (64.7% ± 4.2%) (Figure 1e). Flow-cytometric analysis of T_1 progeny identified diploid frequencies of 5.0% (2/40), 19.0% (27/142) and 13.7% (23/168) in three independent *Fix9* lines (Figures 1f and S11). These diploids were phenotypically indistinguishable from CY84 in plant height, tiller number, panicle length, seed setting, grain morphology and pollen viability (Figures 1c,e and S12–S14). Similar diploid frequencies were recovered in T_2 generation, reaching 4.7% (9/190), 18.8% (45/240) and 11.2% (32/285) in the three lines, demonstrating stable inheritance across generations (Figure 1f). Whole-genome resequencing further confirmed that both T_1 and T_2 diploids retained a CY84-like heterozygous genotype across the genome (Figure 1d). Collectively, these results establish *OsPLT7* as a rice parthenogenesis inducer that supports synthetic apomixis, enabling fertilization-independent embryo initiation, stable clonal seed production and near-wild-type fertility.

Our findings identify *OsPLT7* as a non-BBM PLT factor with substantially greater parthenogenesis-inducing capacity than *OsPLT1*, extending the repertoire of *PLT* regulators capable of supporting synthetic apomixis beyond the *BBM* branch. Despite their highly conserved AP2 domains, OsPLT1 and OsPLT7 exhibit substantial sequence divergence in their N- and C-terminal regions (Figure S2), which may contribute to their differential capacities to induce parthenogenesis. Previous *BBM*-based systems have demonstrated a trade-off between clonal induction efficiency and fertility (Vernet et al. 2022; Wei et al. 2023). Notably, *OsPLT7* supports stable clonal seed production while maintaining near-normal fertility when combined with *MiMe*. Moreover, *OsPLT7* expression levels were positively associated with clonal seed frequencies among *Fix9* lines (Figure S15), suggesting that expression level may influence clonal induction efficiency. Further optimization of egg cell-specific promoters and *OsPLT7* expression, potentially in combination with complementary endogenous regulators, could enhance clonal seed production. Collectively, these findings broaden the genetic toolkit for engineering synthetic apomixis and provide a promising route towards practical one-line hybrid rice breeding.

Author Contributions

K.W. and Y.H. managed the project; S.X., Y.H., X.Z., Y.X. and J.M. performed the experiments and analysed data; Y.R. and X.L. contributed to discussion and manuscript revision; S.X. and Y.H. wrote the manuscript; K.W. revised the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in NCBI Sequence Read Archive (SRA) at https://www.ncbi.nlm.nih.gov/sra, reference number PRJNA1495391.

References

Aida, M., D. Beis, R. Heidstra, et al. 2004. "The *PLETHORA* Genes Mediate Patterning of the *Arabidopsis* Root Stem Cell Niche." *Cell* 119: 109–120. https://doi.org/10.1016/j.cell.2004.09.018.

Boutilier, K., R. Offringa, V. Sharma, et al. 2002. "Ectopic Expression of *BABY BOOM* Triggers a Conversion From Vegetative to Embryonic Growth." *Plant Cell* 14: 1737–1749.

Horstman, A., V. Willemsen, K. Boutilier, and R. Heidstra. 2014. "AINTEGUMENTA-LIKE Proteins: Hubs in a Plethora of Networks." *Trends in Plant Science* 19: 146–157. https://doi.org/10.1016/j.tplants.2013.10.010.

Kerstens, M., C. Galinha, H. Hofhuis, et al. 2024. "PLETHORA Transcription Factors Promote Early Embryo Development Through Induction of Meristematic Potential." *Development* 151: dev202527. https://doi.org/10.1242/dev.202527.

Khanday, I., D. Skinner, B. Yang, R. Mercier, and V. Sundaresan. 2019. "A Male-Expressed Rice Embryogenic Trigger Redirected for Asexual Propagation Through Seeds." *Nature* 565: 91–95. https://doi.org/10.1038/s41586-018-0785-8.

Vernet, A., D. Meynard, Q. Lian, et al. 2022. "High-Frequency Synthetic Apomixis in Hybrid Rice." *Nature Communications* 13: 7963. https://doi.org/10.1038/s41467-022-35679-3.

Wei, X., C. Liu, X. Chen, et al. 2023. "Synthetic Apomixis With Normal Hybrid Rice Seed Production." *Molecular Plant* 16: 489–492. https://doi.org/10.1016/j.molp.2023.01.005.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figures S1–S15** and **Table S1–S3**.