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Spotlight

Creating one-line hybrid crops by synthetic apomixis

Hybrid breeding plays an important role in increasing crop yields by taking advantage of hybrid vigor, which helps crops grow with superior yields across various climates and contributes to ensuring secure food production. Hybrid seed production is relatively straightforward and simple in cross-pollinating plants such as maize but is difficult in self-pollinating plants such as rice, wheat, and soybean. Therefore, breeders have developed a three-line breeding strategy, utilizing a sterile line, a maintainer line, and a restorer line, for breeding hybrid rice (Figure 1A; Yuan, 1966). The three-line hybrid rice breeding method was further developed into a two-line hybrid system, which uses a conditionally photo-thermo-sensitive genic male sterile line instead of using a sterile line-maintainer line combination (Figure 1B). However, the three-line hybrid rice and two-line hybrid rice systems make use of hybrid vigor in rice, resulting in significantly increased yields (Chen and Liu, 2014). Moreover, the need to create sterile and restorer lines and the incompatibility between different varieties have seriously limited the exploitation of hybrid vigor across all crops. As a result, three-line and two-line hybrid breeding methods have only been applied widely in rice, and it remains difficult to produce hybrid seeds in other self-pollinating crops with them.

A one-line breeding system involving apomixis instead of sexual crosses would address the challenges imposed during the production of hybrid seeds (Figure 1C; Yuan, 1987). Apomixis is a kind of asexual reproduction through seeds, thus generating clonal offspring; apomixis differs from sexual reproduction by not requiring the fusion of male and female gametes. The term apomixis is usually used to include sporophytic apomixis (such as in *Citrus* and mangosteen) and gametophytic apomixis (such as in fountain grasses and dandelion) (Wang and Underwood, 2023). Typical apomixis is comprised of three developmental processes: (1) diplospory (apomeiosis), (2) parthenogenesis, and (3) autonomous endosperm development (Van Dijk et al., 2020). Endosperm development is necessary for apomixis because parthenogenetic embryos need endosperm cells to provide for nutrients. Although many gametophytic apomixes require the fertilization of a central cell and its subsequent development into an endosperm, autonomous endosperm development is ideal for apomixis.

In a recent study, Song et al. (2023) demonstrated that combining tissue-specific expression of the *ToPAR* gene (the *PARTHENOGENESIS* gene from *Taraxacum officinale*) in rice egg cells with the *MiMe* system could produce a high proportion of apomictic seeds without affecting fertility. The *MiMe* technology is an ideal solution to address the need for diplospory. In rice, the simultaneous knockout of three genes, *PAIR1*, *REC8*, and *OSD*, switches the development of female gametophytes from meiosis to mitosis, thus generating diploid female gametophytes in which no recombination has occurred; importantly, this modification does not affect the setting rate of rice (Mieulet et al., 2016; Khanday et al., 2019; Wang et al., 2019). In double-fertilized angiosperms, activation of the egg and central cell depends on fertilization by sperm cells, and the release of their dormancy is mediated by proteins expressed by the sperm (Figure 1D). In natural as well as synthetic apomixis, dormant egg cells self-activate and develop parthenogenetically, and central cells are fertilized by sperm (Figure 1E) or form endosperm through self-activation (Figure 1F). Although the use of apomicts avoids cost- and labor-intensive hybrid seed production, unfortunately, common crops, especially staple crops, do not undergo apomixis. There are currently two ways to achieve parthenogenesis (Underwood and Mercier, 2022): (1) overcoming the dormancy of egg cells but disturbing the double-fertilization process of plants by knocking out genes such as *CENH3*, *MTL* (*PLA1*/*NLD*), *DMPs*, etc., or (2) overexpressing activation genes, such as the *BBM-like* gene in the egg cell. However, at present, neither of these two methods can produce enough clonal seeds with hybrid vigor.

In order to use one-line hybrid crops created by synthetic apomixis in large-scale agricultural production, two challenges need to be addressed: all resulting seeds need to fix for hybrid vigor like the mother line, and the apomictic crops need to have superior growth and development phenotypes to ensure adequate seed-setting rates. Song et al. (2023) selected two widely cultivated *indica-japonica* hybrid rice varieties, Jiaheyou7245 (JHY7245) and Jiafengyou2 (JFY2), and used CRISPR-Cas9 genome editing technology to knockout three genes, *PAIR1*, *REC8*, and *OSD1*, thereby obtaining diploid egg cells. At the same time, the egg-cell-specific promoter *AtEC1.1* was used to overexpress the parthenogenetic *PAR* gene of common dandelion to activate the development of egg cells, thus achieving synthetic apomixis of hybrid rice. In one of five transformants derived from JHY7245, named S717, around 67% progeny were diploid, and in one of three transformants derived from JFY2, named S718, around 62.5% progeny were diploid. Further analysis showed that S717 and S718 retained the heterozygosity of JHY7245 and JFY2. Moreover, Song et al. (2023) performed whole-genome sequencing of the hybrid rice and showed that all three S717 plants were heterozygous for the JHY7245 genotype across the entire genome. These findings demonstrate that the expression of dandelion *PAR* in egg cells together with use of the *MiMe* system can successfully and effectively engineer apomixis in rice.

Current synthetic apomixis often results in a lower setting rate due to interference with the double-fertilization process. Underwood et

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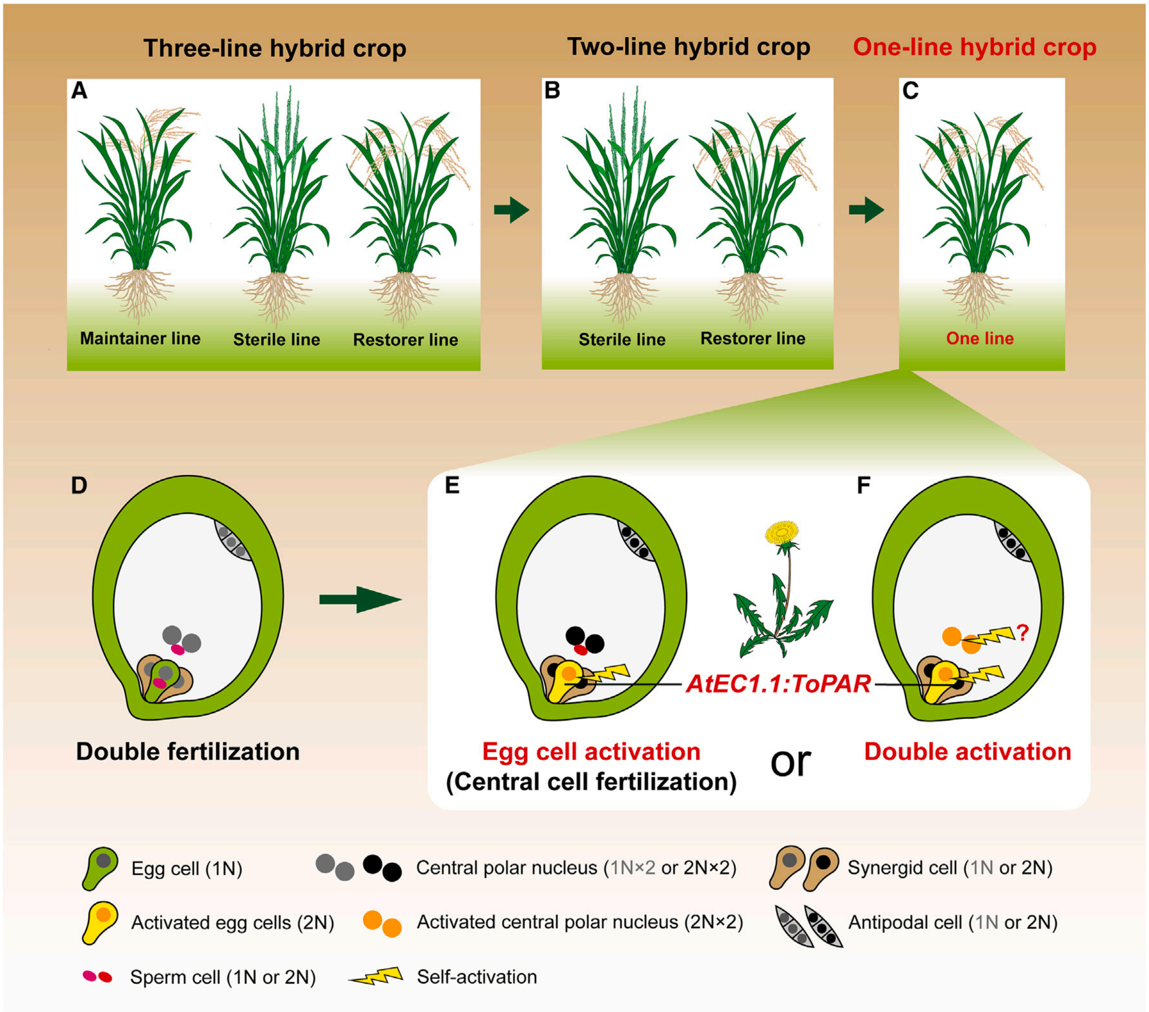

Figure 1. One-line hybridization based on synthetic apomixis.
(A) Schematic diagram of the three-line hybrid breeding system involving a maintainer line, a male sterile line, and a restorer line.
(B) Schematic diagram of the two-line hybrid breeding system involving a photo-thermo-sensitive genic male sterile line and a restorer line.
(C) Schematic diagram of one-line hybrid system.
(D) Schematic diagram of double fertilization in an angiosperm embryo sac.
(E) Schematic diagram of natural apomixis or synthetic apomixis with egg cell self-activation and central cell fertilization in angiosperms.
(F) Schematic diagram of natural apomixis or synthetic apomixis with egg cell and central cell self-activation in angiosperms.

al. (2022) showed that the heterologous expression of *ToPAR* in lettuce egg cells could induce haploid embryo-like structures without fertilization, which demonstrated that the *ToPAR* gene was a parthenogenetic gene and that it could be used for synthetic apomixis. Song et al. (2023) first expressed the heterologous *ToPAR* gene specifically in rice egg cells with the *AtEC1.1* promoter combined with the *MiMe* technology to produce apomixis embryos. Importantly, Song et al. (2023) found that the seed-setting rate of S717-1 T2 diploid plants was 74.4%–82.8%, which was not significantly different from that of JHY7245 (76.3%–84.6%), and that the seed-setting rate of S718-1 T2 diploid plants was 67.8%–74.9%, which was also not significantly different from that of JFY2 (68.9%–74.7%) under normal field conditions. Surprisingly, at low-temperature conditions, the S717 and S718 diploid plants achieved seed-setting rates of 70.39%–79.78% and 67.50%–79.31%, which are significantly higher than those of JHY7245 and JFY2, which were 35.22%–51.64% and 29.79%–59.33%, respectively. In addition, the S717 and S718 T1 plants had similar phenotypes, including leaf morphology, tiller number, panicle, and grain size, to those of JHY7245 and JFY2, respectively.

Song et al. (2023) showed that the specific expression of *ToPAR* from dandelion in egg cells could induce a high proportion of apomixis seeds (around 67%). More importantly, this method did not affect the fertility of rice because although some egg cells were not successfully activated to undergo apomictic reproduction, they could accept fertilization by pollen and produce fertile seeds, which suggests that this method did not affect the normal fertilization of rice. The self-activated development mode of egg cells reduced the dependence on pollen for fertilization, which resulted in an even higher setting rate at low-temperature conditions. Song et al. (2023) also improved the agronomic traits of the hybrid rice by incorporating gene-editing components targeting *BC1* and *SD1* to alter brittle culm and semi-dwarf. Using gene editing and apomixis combined, Song et al. (2023) successfully demonstrate that synthetic apomixis could be combined with engineering desired traits to rapidly advance agricultural breeding.

Up to now, it has been difficult to reach 100% rice seeds with fixed heterosis, possibly because genes activating egg parthenogenesis in other plants do not fully activate rice eggs. Therefore, it will be essential to further study the mechanism of apomixis and to examine the use of additional apomixis-related genes for obtaining synthetic apomixis in crops. In fact, only a few parthenogenetic genes, such as *BBM-like* and *PAR*, have been identified; it may also be possible to exploit sporophytic apomictic genes, such as *CtRWP* from *citrus* (Wang et al., 2017), to achieve synthetic apomixis. The autonomous development of self-activated central cells is essential for synthetic apomixis, as it maintains the seed-setting rate, but there have been few studies aimed at inducing central cells to form endosperm in the absence of fertilization; at the same time, there is a need to avoid influence of pollen abortion caused by various environmental factors on crop yield. The work by Song et al. (2023) has cleverly provided a solution addressing the problem of low seed-setting rates while greatly enhancing crop yields of diploid rice seed with fixed hybrid vigor. Further advances in this field of research will ensure that one-line systems for hybrid crop breeding will soon be applied across many agricultural systems.

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