PBL Technology

Innovation in life sciences

Institute of Genetics and Developmental Biology
Chinese Academy of Sciences

CNRRI
China National Rice Research Institute

Evaluation and Licensing Opportunities

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Patent Literature

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Novel Hybrid Seed Production System

Seed sorting based on size differentiation

Proven in rice and applicable in other seeded species

Tractable by mutation or editing of GSE3 alleles

The challenges of effective and economically viable field-production of hybrid seed are well known and in many crop species these critically limit the adoption of hybrids and the productivity benefits they bring. To keep male parent seed out of the harvested F1 seed crop seed, male-line pollinators have to be planted in separate rows, and either not harvested or culled (perhaps by hand) before harvesting the F1 seed, wasteful enough in itself but also, particularly for poor out-pollinators, the fertilization of F1-bearing female plants is often restricted, resulting in reduced production of F1 seed. Interplanting of male and female lines is a possible strategy but requires the subsequent separation of the bulk harvest to remove seeds set on the male plants from F1 seed on the females. Various separation techniques have been proposed, including those based on seed coloration, herbicide-resistance and seed size, but they are difficult to implement in practice.

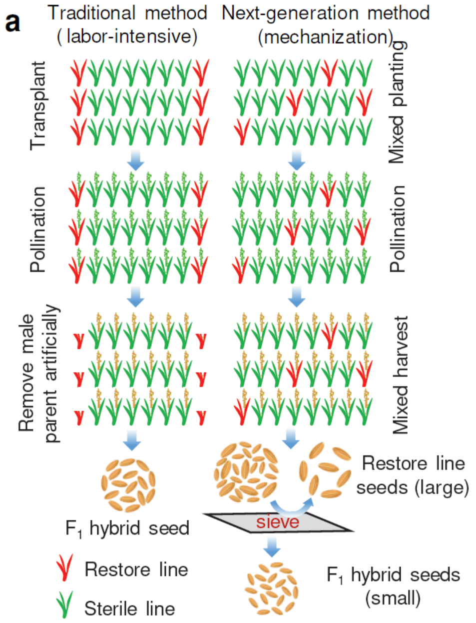
Now, a collaboration between The Institute of Genetics and Developmental Biology (Beijing) and the China National Rice Research Institute (Hangzhou) has developed a seed-size sorting strategy that enables fully mechanized hybrid rice breeding, and that should be readily applicable in other crop species. The work is published in Nature Plants.

The CNRRI group has previously demonstrated the potential of seed-size sorting in the context of hybrid rice production. A decade ago, they showed that (small-grained) F1 seed can be mechanically separated from (large-grained) male parent seed harvested in bulk from an interplanted production field with high efficiency and good resulting hybrid purity. However, the genetics and performance aspects of the seed-size differentiation phenotype are crucial. Ideally, the seed-size trait should be **simply inherited (single locus), recessive, and maternally determined**. There must be no collateral penalty to the numbers of F1 seed produced or the eventual yield performance of the F1 seed crop itself. So, while many genes are known to influence seed size, none hitherto have been shown to fit all necessary criteria.

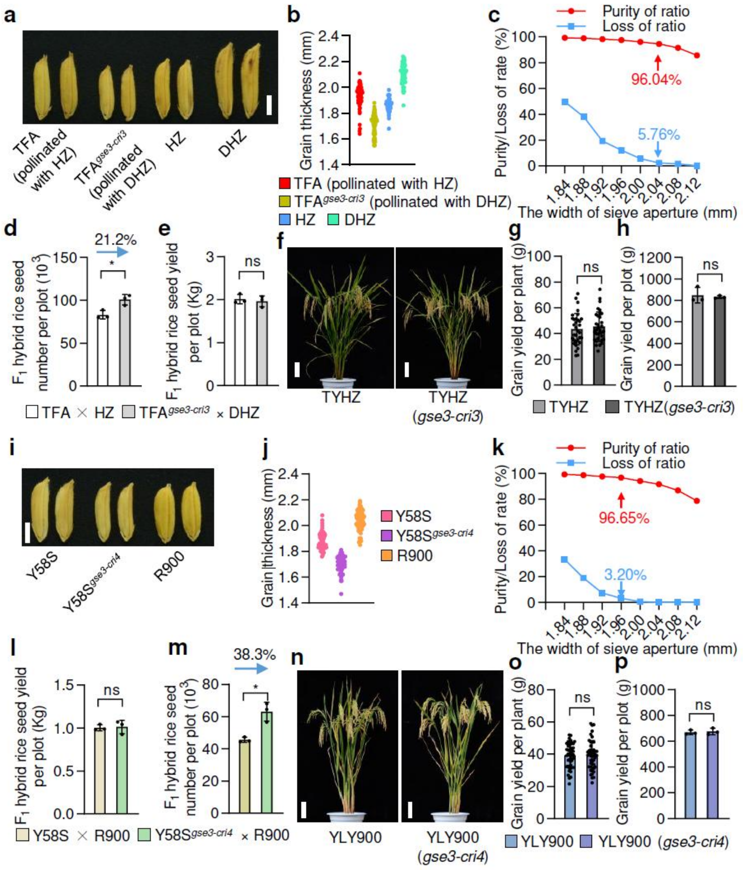
The IGDB/CNRRI collaboration has now identified the gene **GSE3** as an "ideal" source of genetics for the **hybrid production strategy to work very successfully**, with high F1 seed purity. GSE3, importantly, also **increases the number of F1 hybrid seeds produced** (by 21-38% in rice field trials), which is a vital determinant of the economic viability of F1 seed production. , although the F1 seeds are smaller the total yield (weight) of F1 seed is the same as with non-gse3 hybrids. And crucially, the yield of crop they produce is identical to the equivalent non-gse3 hybrids generated using conventional hybrid production, i.e. there is **no penalty of small-grained F1 seed** to the resulting hybrid crop. For the separation of small F1 from large male-parent seed, the group use a mechanical sifter with adjustable rectangular hole-size. Once calibrated, F1 hybrid seed purity of over 96% is readily achievable, meeting standards for commercial hybrid seed production. The partners have demonstrated the utility of GSE3 in hybrid rice breeding and field-based F1 seed production using, separately, all three of **conventional breeding, mutagenesis and gene-editing approaches**.

To locate potential sources of "ideal" seed-size genetics, near-isogenic rice lines for various small seed-size sources were crossed to large grain lines and the progenies studies and the loci responsible mapped. **GSE3 emerged as a candidate gene** from this investigation and was confirmed by genomic complementation in transgenic rice. A very few other rice lines were found to contain the *GSE3*$^{\text{-small grain allele}}$, so it is relatively rare in rice germplasm, with small-grain likely having been selected against. In a separate but parallel study screening M2 EMS mutant populations yielded over 600 small-grain mutants. Of these, most had defects in various productivity traits, but just one had small grains combined with elevated grain number. This turned out to be a **mutant in GSE3.** It differs from the predominant GSE3 allele by a **single base substitution**.

In the **Nature Plants (2024)** paper, the IGDB group show that GSE3 encodes a GCN5-related N-acetyltransferase-like protein that binds to histones and influences acetylation levels. In rice, GSE3 is a nuclear-localised and its expression is detected in developing spikelet hulls, panicles young seedlings and roots. It is recruited by the transcription factor GS2 to the promoters of their co-regulated genes that control seed size. Overexpression of GSE3 increases grain size, but tiller number and grain number per panicle are reduced, the converse of the loss-of-function phenotype. GSE3 is shown to interact with GS2, and also with GRF3/4 to control grain size/weight, described in detail in the Nature Plants paper. The *GSE3*$^{\text{-small grain}}$ allele is a loss-of-function allele with a variant C-terminal region. **Highly conserved homologues exist in many species such as maize, sorghum, wheat, rye, barley, ryegrass, soybean, sunflower, rapeseed, sugar beet and pea**.



Sorting F1 seed from male pollinator seed using seed-size allows mixed planting of pollinator and female lines and fully mechanized, economically enhanced hybrid seed production



Two independent gene-edited versions of male-sterile lines – TFA (a-h) and Y58 (i-p) – show increased F1 hybrid seed numbers of 20-30%, but with the same overall weight of F1 seeds produced (d/e and l/m). No penalty on F1 crop yield (g/h and o/p). Both gene-editing approaches introduced a single base to cause a frame shift in GSE3.

The GSE3 hybrid production technology is the subject of patent applications filed by PBL on behalf of IGDB and CNRRI. Please inquire with PBL for further information.

References:

Ke Huang *et al* (2024). Modulation of histone acetylation enables fully mechanized hybrid rice breeding. *Nature Plants*. https://doi.org/10.1038/s41477-024-01720-0