



## Reverse Breeding: Re-creating Hybrid Parents from a Super Hybrid

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Reverse breeding represents a transformative strategy in plant breeding that enables the reconstruction of homozygous parental lines directly from an elite heterozygous hybrid. In contrast to conventional hybrid breeding, which proceeds from parental line development to hybrid creation, reverse breeding follows an inverted trajectory, beginning with a superior hybrid genotype and working backward to derive its parental components. This approach relies on controlled suppression of meiotic recombination to prevent genetic reshuffling, thereby allowing intact parental chromosomes to segregate into gametes. These gametes are subsequently fixed as doubled haploid lines and combined using molecular genotyping to faithfully recreate the original hybrid. Reverse breeding offers significant advantages for preserving heterosis, accelerating hybrid development and conserving elite genotypes whose parental lines are unknown, lost, or inaccessible.

**Keywords:** Reverse breeding; engineered meiosis; meiotic recombination suppression; doubled haploids; heterosis; hybrid reconstruction

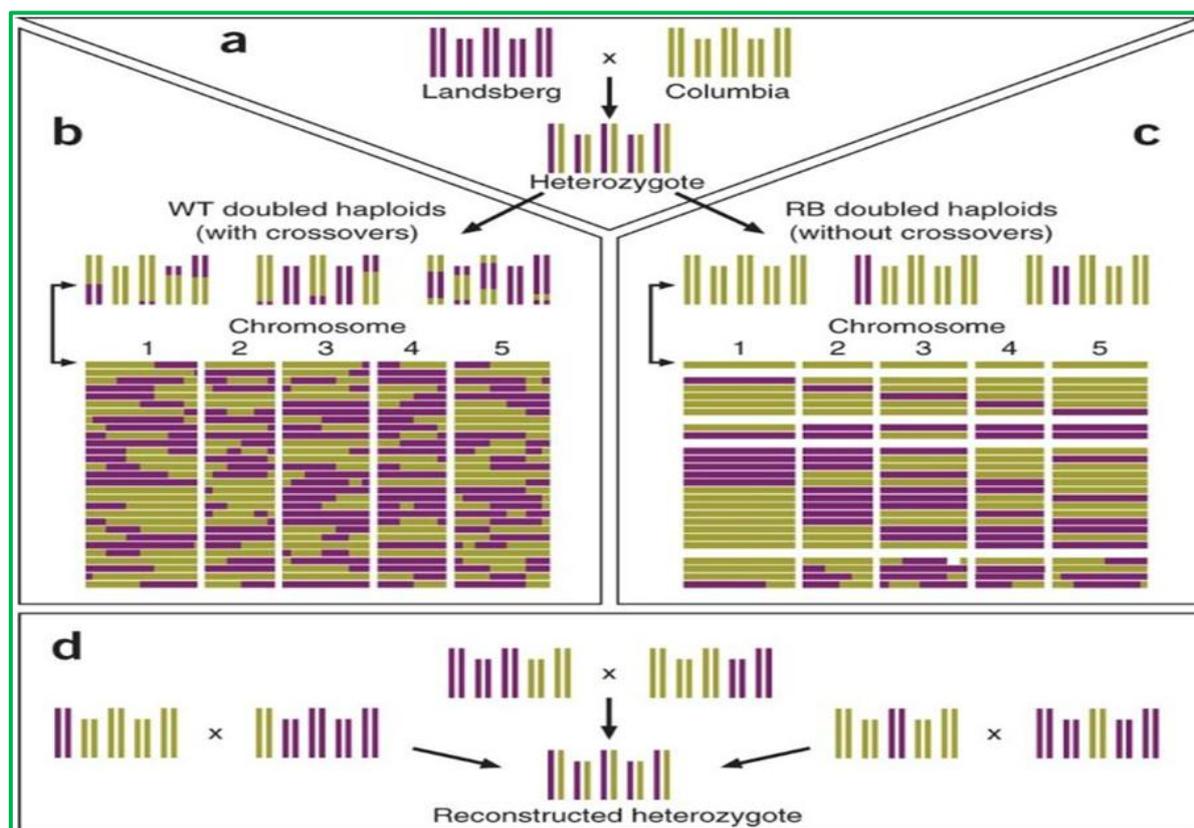
### Introduction

Hybrid breeding has played a central role in the improvement of agricultural productivity by exploiting heterosis or hybrid vigour, which manifests as superior yield, growth rate, stress tolerance and overall performance in hybrid progeny. Crops such as maize, rice, sunflower, tomato and numerous vegetable and ornamental species owe much of their productivity gains to hybrid breeding systems. Despite its success, conventional hybrid breeding is inherently time consuming and resource intensive, as it requires the development of stable, homozygous parental lines through repeated selfing and selection, followed by extensive test crossing to identify optimal parental combinations. An additional limitation of classical hybrid breeding lies in the difficulty of preserving elite hybrid genotypes when parental lines are lost, protected by intellectual property restrictions or biologically inaccessible. In some cases, superior hybrids arise serendipitously or through somatic mutation, clonal selection, or experimental crosses without systematic documentation of parentage. Under such circumstances, reproducing the elite hybrid becomes nearly impossible using traditional breeding approaches. Reverse breeding was conceptualized to address this fundamental limitation by providing a method to reconstruct parental lines directly from a selected heterozygous individual. The concept of reverse breeding was first articulated by Dirks and colleagues, who proposed that manipulation of meiotic recombination could allow breeders to recover intact parental chromosome sets from a hybrid genotype (Dirks *et al.*, 2009).

Advances in molecular genetics, meiosis research, haploid technologies and high-throughput genotyping have since made this concept experimentally feasible. Reverse breeding is now regarded as a promising, though technically demanding, addition to the modern plant breeding toolbox.

### Conceptual Framework of Reverse Breeding

Reverse breeding is founded on the principle that a heterozygous hybrid is composed of two complementary homozygous chromosome sets. In normal sexual reproduction, meiosis disrupts these chromosome sets through crossing over, generating new allele combinations in gametes. Reverse breeding seeks to suppress this recombination process so that chromosomes segregate without exchanging genetic material. When recombination is sufficiently reduced, gametes inherit whole parental chromosomes rather than recombinant mosaics. The conceptual framework of reverse breeding involves four major steps: selection of a superior heterozygous plant, suppression of meiotic recombination in that plant, recovery and fixation of non-recombinant gametes as doubled haploid lines and molecular identification of complementary parental line pairs that can reconstitute the original hybrid. Each step is underpinned by well-established genetic principles but requires precise technical execution to ensure success. A key theoretical insight is that complete elimination of recombination is not strictly necessary. Low levels of residual recombination can be tolerated if sufficient numbers of haploids are generated and accurately genotyped. This flexibility increases the practical feasibility of reverse breeding, particularly in species where complete recombination suppression would compromise fertility (Wijnker *et al.*, 2012).



<https://www.nature.com/articles/ng.2203>

### Genetic and Theoretical Basis

Meiosis is a specialized form of cell division that produces haploid gametes from diploid cells. During meiosis I, homologous chromosomes pair, synapse and exchange genetic material through crossing over. This process ensures proper chromosome segregation and contributes to genetic diversity. From an evolutionary perspective, recombination is advantageous however, from a breeding perspective, it represents an obstacle when the goal is to preserve specific allele combinations.

Reverse breeding exploits the predictable segregation of homologous chromosomes under conditions of reduced recombination. If crossovers are minimized, homologous chromosomes segregate largely intact, producing gametes that carry one or the other parental chromosome. These gametes can be fixed as homozygous doubled haploid lines, each representing a potential parent of the original hybrid (Dirks *et al.*, 2009). Theoretical models demonstrate that the probability of reconstructing the full hybrid genotype depends on chromosome number and the degree of recombination suppression. Species with fewer chromosomes require fewer haploid lines to recover complementary parental sets, whereas species with high chromosome numbers face exponential increases in screening requirements (Wijnker and de Jong, 2008). These considerations are critical when assessing the applicability of reverse breeding to specific crops.

### **Molecular Biology of Meiotic Recombination**

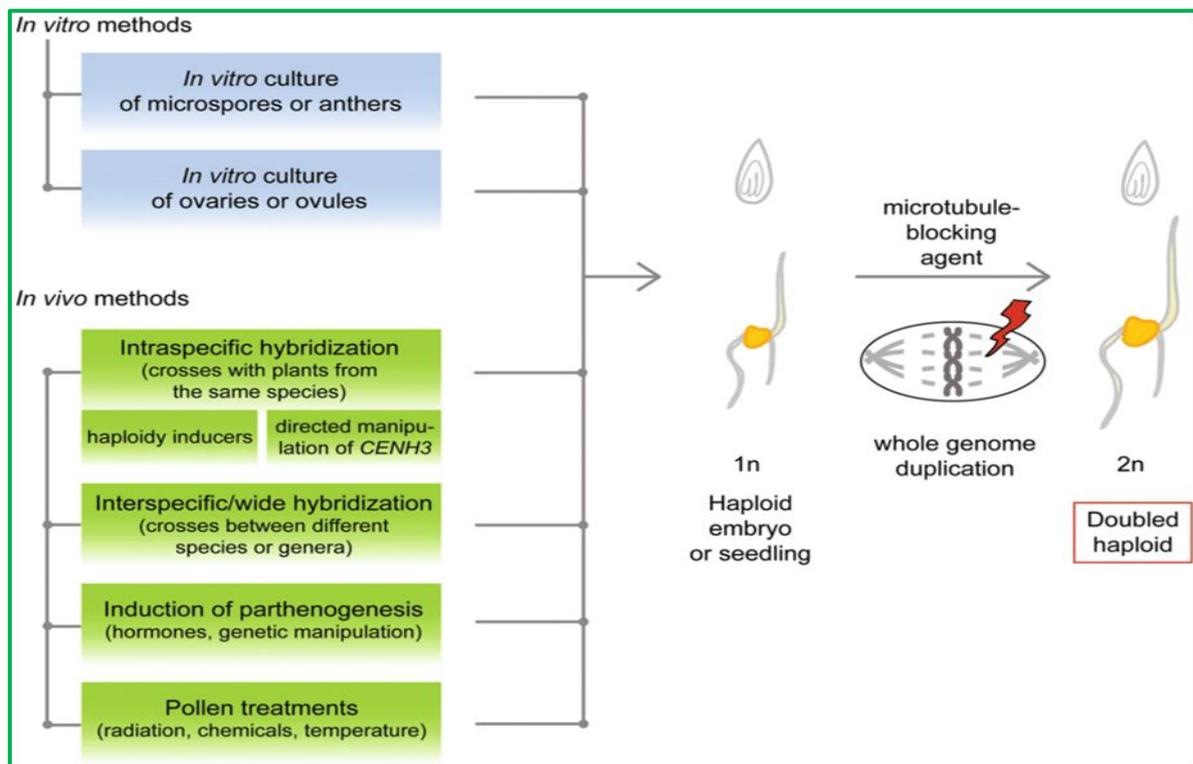
Meiotic recombination is initiated by programmed DNA double-strand breaks generated by the conserved enzyme SPO11. These breaks are repaired through homologous recombination pathways involving RAD51, DMC1 and associated proteins, resulting in the formation of crossovers and non-crossover events (Keeney *et al.*, 1997; Mercier *et al.*, 2015). Crossovers create physical connections between homologous chromosomes, ensuring accurate segregation during meiosis I. In most plant species, at least one crossover per homologous chromosome pair is required for proper segregation, a phenomenon known as the obligate crossover. Excessive suppression of recombination can therefore lead to meiotic failure and sterility. Reverse breeding requires partial rather than complete suppression of crossover formation, striking a balance between chromosome integrity and reproductive viability. Molecular targets for recombination suppression include genes involved in double-strand break formation, strand invasion and crossover resolution. Experimental studies have shown that down-regulation of such genes can significantly reduce crossover frequency without abolishing meiosis, thereby enabling the production of viable non-recombinant gametes (Mercier *et al.*, 2015).

### **Engineered Meiosis in Reverse Breeding**

Engineered meiosis refers to deliberate manipulation of meiotic processes to achieve specific breeding outcomes. In reverse breeding, engineered meiosis is used to suppress recombination and preserve parental chromosome sets. RNA interference was among the first molecular tools proposed for this purpose, as it allows targeted and reversible suppression of gene expression (Dirks *et al.*, 2006). Experimental validation of engineered meiosis was achieved in *Arabidopsis thaliana*, where RNAi-mediated suppression of SPO11 homologs resulted in a dramatic reduction in crossover events (Wijnker *et al.*, 2012). Gametes produced under these conditions were largely non-recombinant and could be recovered and fixed as doubled haploid lines. While effective, stable RNAi constructs introduce transgenic elements that may complicate regulatory approval. To address this issue, transient suppression strategies such as virus-induced gene silencing have been developed. VIGS enables temporary down-regulation of meiotic genes during gametogenesis and is subsequently lost, producing doubled haploid lines that are free of foreign DNA (Calvo-Baltanás *et al.*, 2018). This innovation significantly enhances the practical and regulatory appeal of reverse breeding.

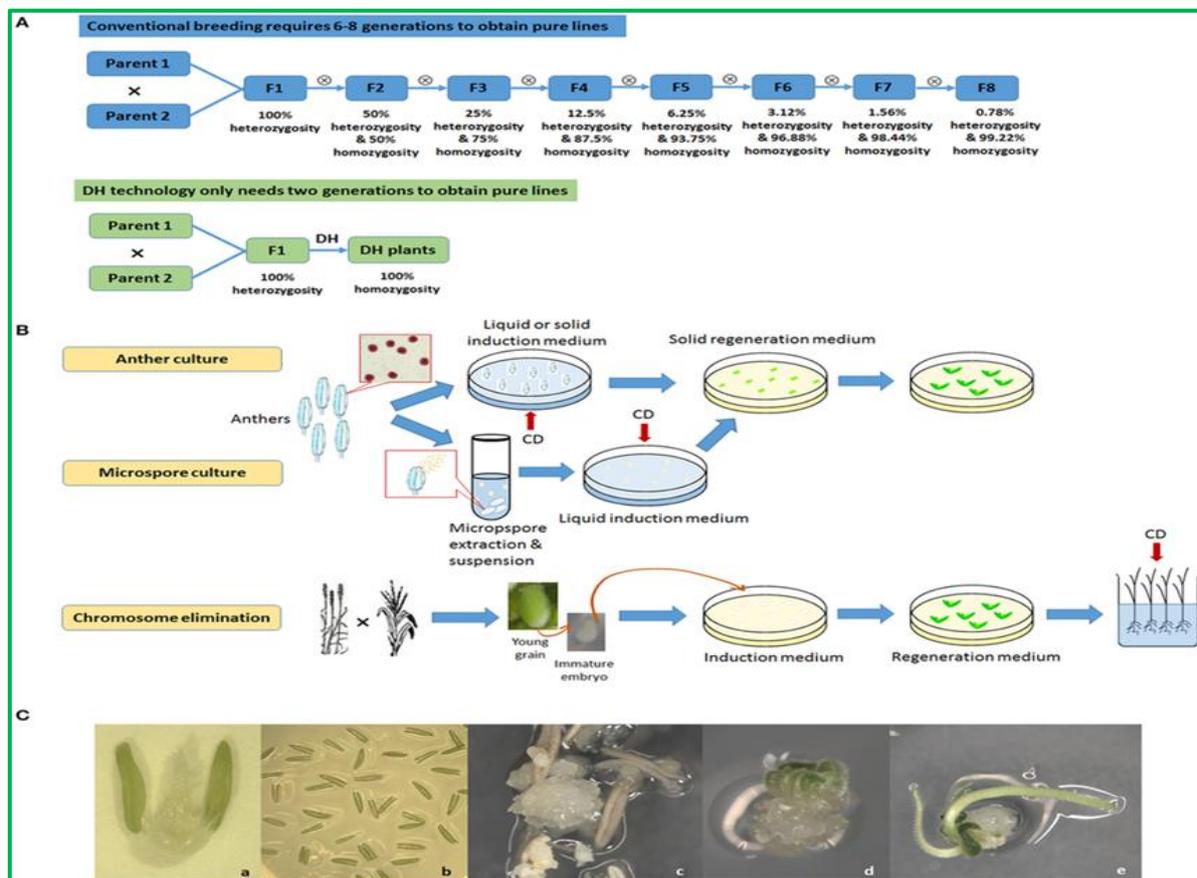
### **Haploid Induction and Doubled Haploid Technology**

Haploid and doubled haploid technologies are indispensable components of reverse breeding because they allow rapid fixation of gametic genomes. Haploid plants can be produced through anther culture, microspore culture, ovule culture or genetic haploid induction systems, depending on the species. These haploids represent single meiotic products and therefore capture the chromosomal composition of individual gametes.



<https://www.researchgate.net/figure/Overview-of-methods-for-haploid-induction-in-plants-In-vitro-methods-for-haploid>

Chromosome doubling converts haploids into fully homozygous doubled haploid lines, typically using antimitotic agents such as colchicine. Doubled haploids are genetically uniform and stable, making them ideal candidates for parental line reconstruction and molecular analysis (Forster *et al.*, 2007).



<https://www.researchgate.net/figure/Doubled-haploid-DH-technology-A-Comparison-between-conventional-breeding-and-DH>

The efficiency of haploid induction and regeneration is highly species dependent. Crops with established doubled haploid protocols, such as maize, barley and wheat are more amenable to reverse breeding. In contrast, species with recalcitrant tissue culture responses present significant technical challenges.

### **Molecular Genotyping and Hybrid Reconstruction**

Genome-wide molecular genotyping is essential for identifying complementary parental line pairs among doubled haploid populations. Single nucleotide polymorphism markers, simple sequence repeats and genotyping-by-sequencing platforms provide high-resolution insight into chromosomal composition and integrity (Collard and Mackill, 2008). The goal of genotyping in reverse breeding is to identify pairs of doubled haploid lines whose combined genomes match the heterozygous hybrid. Computational tools can be used to optimize parent selection by maximizing genome coverage and minimizing redundancy. Residual recombination events can be detected and excluded, ensuring faithful reconstruction of the hybrid genotype (Dirks *et al.*, 2009).

### **Experimental Proof of Concept**

The first experimental demonstration of reverse breeding was reported in *Arabidopsis thaliana*. Wijnker *et al.* (2012) successfully generated homozygous parental lines from a heterozygous plant by suppressing meiotic recombination and producing doubled haploids. Crossing selected parental lines recreated the original hybrid genotype and phenotype, thereby validating the theoretical framework of reverse breeding. This study provided critical insights into recombination thresholds, chromosome number effects and haploid recovery requirements. Importantly, it demonstrated that low levels of residual recombination can be managed through marker-assisted selection, increasing the robustness of the approach.

### **Crop-Specific Feasibility and Applications**

Reverse breeding holds particular promise for crops where hybrid performance is paramount and haploid technologies are well developed. Maize is a leading candidate due to the availability of efficient haploid inducer lines and extensive molecular marker resources. Vegetable crops such as cucumber, onion and tomato, which have relatively low chromosome numbers, are also considered suitable candidates. In addition to food crops, reverse breeding has potential applications in ornamentals, where elite hybrids with unique aesthetic traits are highly valued. The approach allows preservation and reproduction of superior phenotypes without lengthy parental line development.

### **Advantages of Reverse Breeding**

Reverse breeding offers several advantages over conventional breeding. It enables rapid reconstruction of elite hybrids, preserves heterosis, reduces breeding timelines and allows exploitation of superior genotypes with unknown parentage. When combined with molecular breeding tools, reverse breeding supports precision breeding and genetic conservation.

### **Limitations and Challenges**

Despite its advantages, reverse breeding faces several challenges. Effective recombination suppression without inducing sterility remains technically demanding. Haploid induction efficiency varies widely among species and high chromosome numbers reduce the probability of recovering complementary parental sets. Regulatory and intellectual property considerations may also influence adoption (Mercier *et al.*, 2015).

### **Regulatory and Intellectual Property Considerations**

The regulatory status of reverse-bred products depends on whether transgenic elements remain in the final parental lines. Transient approaches such as VIGS may facilitate regulatory acceptance by producing transgene-free lines. Intellectual property issues related to patented methodologies and reconstructed parental lines must be addressed for commercial deployment.

## Future Perspectives

Future research should focus on improving transient recombination suppression methods, enhancing haploid induction efficiency and integrating reverse breeding with genome editing and genomic selection. Advances in molecular delivery systems and computational tools are expected to expand the applicability of reverse breeding across diverse crops.

## Conclusion

Reverse breeding represents a paradigm shift in plant breeding by enabling the reconstruction of parental lines from elite hybrids. By integrating engineered meiosis, haploid technologies and molecular genotyping, reverse breeding offers a powerful strategy for preserving and exploiting heterosis. Continued technological refinement and empirical validation will determine its broader adoption in sustainable crop improvement programs.

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