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Reversed Breeding: A Novel Approach for Development of Homozygous Plants

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Reverse breeding (RB) is a novel plant breeding technique designed to directly produce parental lines for any heterozygous plant, one of the most sought after goals in plant breeding. RB generates perfectly complementing homozygous parental lines through engineered meiosis. The method is based on reducing genetic recombination in the selected heterozygote by eliminating meiotic crossing over. Male or female spores obtained from such plants contain combinations of non-recombinant parental chromosomes which can be cultured in vitro to generate homozygous doubled haploid plants (DHs). From these DHs, complementary parents can be selected and used to reconstitute the heterozygote in perpetuity. Since the fixation of unknown heterozygous genotypes is impossible in traditional plant breeding, RB could fundamentally change future plant breeding.

Introduction

A novel plant breeding technique designed to directly produce homozygous parental lines from any heterozygous plant. It was proposed by Dirks et al. (2009) in Arabidopsis thaliana. Homozygous parental lines are produced from selected plants by suppressing meiotic recombination. Gametes are directly converted into adult plants, which after chromosome doubling are used as homozygous parental lines. The main objective of reverse breeding is to generate homozygous parental lines (complementing parents) that can be mated to recreate a desired heterozygous genotype (i.e. the initial hybrid; Winker et al. 2012). It has not been commercialized yet and very limited work has been done.

Mechanism of Reverse breeding

1. Selection of heterozygote: A highly heterozygous plant with favourable trait combination is chosen whether its parentage is known or not. Gamete from the heterozygote is produced.

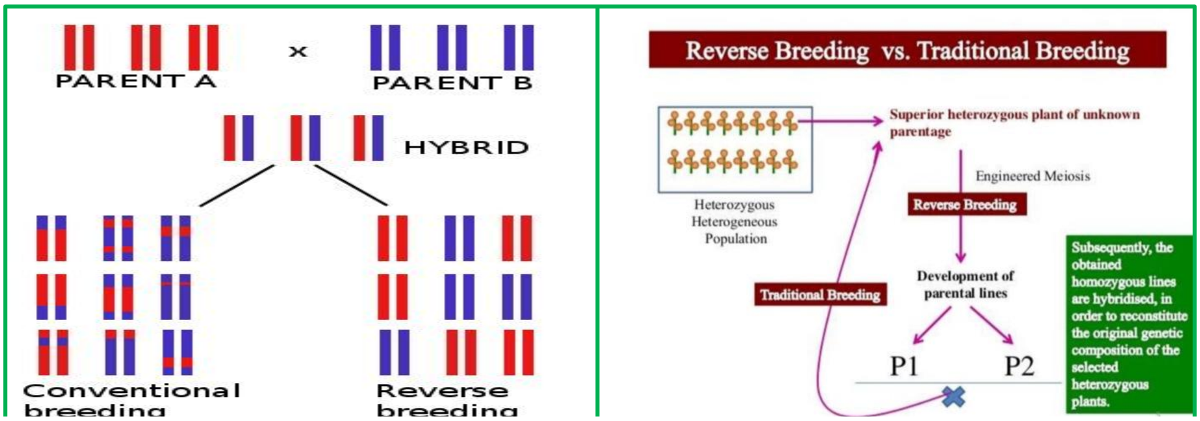
2. Suppression of meiotic recombination during spore formation: This is best achieved by dominant suppression of one of the several genes required for meiotic recombination. Recombination can be prevented or repressed by several ways, particularly through dominant transgenic accesses, dominant negative mutation or chemical treatment. RNA interference which is a post transcriptional gene silencing (PTGS) tool, is used for silencing of genes responsible for recombination. DMC1 gene which encodes the meiotic recombination protein DISRUPTED MEIOTIC cDNA1 in hybrids of *A. thaliana*, so that non-recombined parental chromosomes segregate during meiosis. RNA silencing being genetically dominant approach, it makes easy to obtain progeny devoid of the RNA cassette. Brassica carinata DMC1 is 91.1 percent identical to A. thaliana DMC1. Genes required for the happening of meiotic recombination are following:

1. DMC1 gene: Disrupted Meiotic cDNA
2. SPO1 gene: Sporulation Specific gene
3. RecA gene: Recombinase A gene

Suppression of meiotic recombination is also achieved by chemical compounds like MIRIN, an inhibitor of Mre11-Rad50-Nbs1 complex. It arrests G2 stage and inhibits phosphorylation of ATM i.e. Ataxia Telangiectasia Mutated=serine/threonine protein kinase (Sinha et al., 2020).

3. Generation of Double haploids: Anther or pollen culture are used for production of haploid plant. By doubling of these haploids by colchicine treatment double haploids produced.

4. Crossing of complementary parents: Using Marker Assisted Selection (MAS), the complementary parents are detected and they are crossed to regenerate the initial hybrid. In the condition of complete deprivation of meiotic recombination one polymorphic molecular marker per chromosome would be sufficient to genotype every DH as the entire chromosome would behave as a single linkage block and if there is a presence of any residual crossovers, two markers are required per chromosome (Dirks et al. 2009). The hybrid obtained through RB does not carry the transgene and hence they should not be considered as GM.



Applications of RB

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1. As RB can construct homozygous parental lines, when mated perfectly constitute the selected heterozygous hybrid plant afterwards.
2. These homozygotes parents can be propagates indefinitely by breeders.
3. The technical feasibility in A. thaliana suggests that it, might be possible to apply this technique in crop improvement.
4. Backcrossing in CMS background.

Limitations of RB

1. This technique is confined to those crops only where double haploid technology is common practice.
2. There are some exceptions such as soybean, cotton, lettuce and tomato where DHs is barely formed.
3. It is confined to crops having haploid chromosome no. of 12 or less than it or in which spores can be regenerated into DHs. In the plants having higher number of chromosomes, the number of non-recombinant double haploids needed for searching the complementary pair that reconstitute the original heterozygous plant would be extremely high and practically not feasible.

4. Due to the complete homozygosity of the received plants. There is no room for further selections which limits the genetic variation wanted in plant breeding.

Future prospectus

New possibilities for the selection and improvement of

1. New possibilities for the selection and improvement of favourable genotypes by RB may contribute to increasing future crop production.
2. The scope of RB could be envisioned for the improvement of agricultural crops, as it may enable the generation of parental lines for the recreation of hybrids.

Future prospectus

Though, RB is used as an intermediate step of the breeding process, but it has huge implication in crop breeding as it generates homozygous parental lines from complex genotypes. Transgenesis and marker-assisted selection techniques behind many commercial varieties of agricultural crops produced in the last two decades are now have new tools derived from modern biotechnology. Now-days it is believed that the extent of the adoption and the application the techniques will depend on factors such as the need to increase the technical efficiency of some processes and the decisions on related-regulatory status.

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