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Reverse Breeding: A Novel Plant Breeding Technique

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Abstract

One of the important insights in plant breeding was heterosis, the observation that hybrid progeny (F_1) typically is superior as favourable allele combination of elite heterozygote are lost in next generation due to segregation of traits. Easy preservation of heterozygous genotype is one of the greatest challenges in plant breeding. Hereby, a novel technique reverse breeding meets the challenges of fixation of complex heterozygous. Reverse Breeding generates perfectly complementing homozygous parental lines through engineered meiosis.

Introduction

Reverse breeding (RB) is a novel plant breeding technique that makes use of genetic modification to facilitate breeding of F_1-hybrids by suppression of meiotic recombination to again reproduce F_1. It starts with an elite heterozygous line and aims at the generation of homozygous parental lines. Subsequent crossing of these homozygous parental lines produces plants in which the original genetic composition of the elite heterozygous line is reconstituted. This approach offers clear advantage over existing technique due to fact that in principle any heterozygous plant can now be commercially exploited through re-synthesis of suitable parental lines (Wijnker *et al.*, 2014).

Concept

- Suppression of meiotic recombination during spore formation.
- Production of double haploids from such spores.
- Crossing appropriate DH lines having the desired set of chromosomes on the basis of matching molecular markers.

Why Reverse Breeding?

- To maintain the hybrid stability.
- To improve parental lines genetically to enhance the hybrid performance.
- To establish the breeding lines for uncharacterized heterozygote.
- To multiply a highly heterozygous plant from a homozygous parental line.

Objectives

- To provide alternative method for providing homozygous parental line for the production of hybrids.
- To provide even more flexible in combining desirable parental lines for the production of hybrids.

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• To allow generation of chromosome substitution line that facilitates breeding to study on an individual chromosome level.

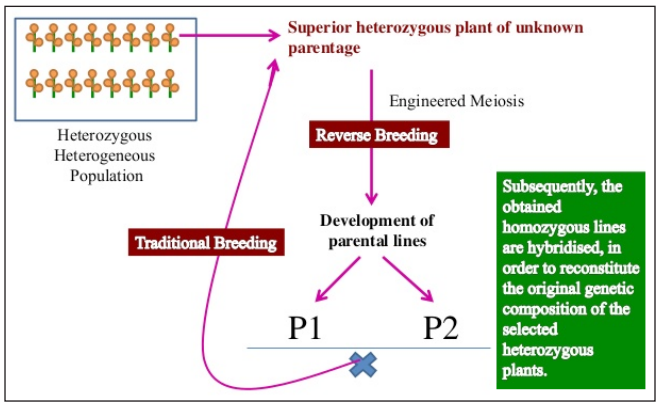

Figure 1: Differentiation between reverse and traditional breeding

Steps in Reverse Breeding

Step 1: Suppression of Meiotic Recombination

1. Produce gamete from heterozygote

• Suppressing gene required for meiotic recombination.

• Complete knockout of gene by RNAi to knock down the function of DMC1 homologue to RecA, a meiosis specific recombinase essential for the formation of crossover.

• Exogenous application of chemical compounds that cause inhibition of recombination during meiosis would speed up the application of RB. *e.g.*, Mirin.

2. Suppression of recombination during spore formation

Genes Responsible for Meiotic Recombination: RNAi knocks down the function of following genes during spore formation:

i) DMC1 gene

ii) RecA gene

iii) SPO11 gene

Step 2: Production of DH

• Tissue culture of immature pollen.

• Using tissue culture techniques referred to as "anther culture" and "isolated microspore culture", immature pollen grains grow to produce colonies of cells.

• The colonies are transferred to media with different plant growth regulators and sugars to induce growth of shoots and then roots.

Step 3: Selection of Complimentary Lines (Parents) through Marker Assisted Selection

• Achiasmatic chromosomes remains as univalent.

• Non-disjunction leads to unbalanced chromosome number (aneuploidy) in the spores.

• Consequently, achiasmatic plants are highly sterile.

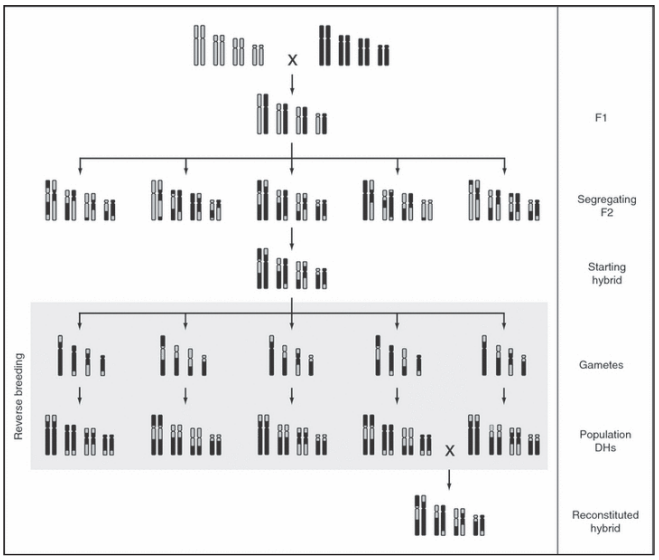

Figure 2: An idealized crossing scheme that employs RB

Reverse breeding can be used to fix unknown heterozygotes. Crossing two homozygous parents (grey and black bars) creates a heterozygous F_1; when selfed, the F_1 produces a segregating F_2 population. A starting hybrid of unknown genetic constitution is selected for its desirable characteristics, and subjected to the two steps of reverse breeding (grey box). By knocking down meiotic crossing over, whole parental chromosomes are transmitted through spores, without rearrangement. Note, in this example the four chromosomes in the hybrid can generate 16 different combinations in the gametes - only five are shown for convenience. The achiasmatic gametes are then used produce doubled haploid (DH) lines using *in vitro* culture techniques. From this population, complementary parents can be chosen that when crossed perfectly reconstitute the starting hybrid. The DH lines then serve as a permanent library that can be used to predictably generate a wide variety of defined hybrids (Dirks *et al.,* 2009).

Applications of Reverse Breeding

• Reconstruction of heterozygous germplasm: For crops where an extensive collection of breeding lines is still lacking, RB can accelerate the development of varieties. In these crops, superior heterozygous plants can be propagated without prior knowledge of their genetic constitution (Kumari and Nilanjaya, 2018).

• **For transfer of CMS**: In order to convert a desirable inbred line or a pure line into a similar line but with a CMS background, in this view CMS donor is made homozygous that confers recombination suppression. A first cross is made by pollination of the said homozygous recombination suppressed line with the pollen of desired line. The resulting F_1 progeny contains CMS and 50% of the chromosomes of the desired line. In the meiosis of the resulting F_1 plants, no recombination

occurs as a result of the invention. This resulted progeny again fertilized by pollen of desired line and the resulting seed is genetically identical for the nuclear genes to the original desired line, but now has acquired the CMS plasma. So, in the second cross with the desired line, seeds acquired the CMS plasma of donor line.

• **Breeding on the single chromosome level**: Many interesting characters in crops are based on polygenic gene interaction, very often located on different chromosomes. RB explains how chromosome substitution line can be obtained when it is applied to an F_1 hybrid of known parents. These homozygous chromosome substitution lines provide novel tool for the study of gene interaction. When crossed with one of the original parents, hybrids can be formed in which one of the chromosomes is homozygous whereas it is also possible to produce hybrids in which just one chromosome is heterozygous. The former allows the study of epistatic interaction between the background and genes contributed by substitution chromosome. Offspring of plant in which just one chromosome is heterozygous, will segregate for the traits present on that chromosome only. Selfing of plant that carry a substituted chromosome will allow the breeders to fine tune the interesting character on single chromosome scale. This could bring forth improved breeding lines carrying introgressed traits.

• Improve seed production in hybrid crops by selecting a combination of lines which allows the production of commercial seeds of both high quality & quantity.

Limitations

Development of reverse breeding is limited to those crops where double haploid technology is common practice, *e.g.*, Cucumber, Onion, Broccoli, Maize, Sugarbeat, Pea, Sorghum, Arabidopsis, *etc*.

Conclusion

The combination of crossover suppression, followed by the regeneration of haploid spores into DHs results in novel and powerful breeding applications. One important application is the production of complementary homozygous lines that can be used to generate specific F_1 hybrids. Additionally, when RB is applied to F_1 heterozygotes, it is possible to generate chromosome substitution lines that allow targeted breeding on the single chromosome scale. RB is fully compatible with commercial CMS lines that are frequently used in modern agriculture. As a plant breeding tool, reverse breeding may be regarded more versatile as its controlled deconstruction of complex genotypes into homozygous parental lines allows the further improvement of these lines by classic breeding methods.

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