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Reverse Breeding and Novel Plant Breeding Technique

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SUMMARY

Reverse breeding is a one of the novel plant breeding technique designed to directly produce parental lines form any heterozygous plant. Reverse breeding also known as RB. One of the most important insights in plant breeding was the observation that hybrid (F1) progeny typically are superior in growth characteristics and yield in comparison to their homozygous parents. Reverse breeding is based on reducing genetic recombination in the selected heterozygote by eliminating meiotic crossing over.

INTRODUCTION

Humankind has been through different periods of agricultural improvement aiming at enhancing our food supply and the performance of food crops. In recent years, whole genome sequencing and deep understanding of genetic and epigenetic mechanisms have facilitated modern plant breeding approaches to meet the challenge of growing population, dwindling resources, and climate change. Reverse breeding is a one of the novel plant breeding technique designed to directly produce parental lines form any heterozygous plant. Reverse breeding also known as RB. One of the most important insights in plant breeding was the observation that hybrid (F1) progeny typically are superior in growth characteristics and yield in comparison to their homozygous parents, a phenomenon known as heterosis. Dirks *et al.* (2006, 2009) proposed the concept of reverse breeding, which is used to reconstitute elite parent plants that have different genetic constitutions from the original parents but can complement with each other to reproduce the same genotypes as the original hybrids. A favorable heterozygous genotype cannot be stably propagated through hybrid seeds because parental chromosomes may recombine when they pass on to progeny (Wijnker *et al.* 2012). Reverse breeding is based on reducing genetic recombination in the selected heterozygote by eliminating meiotic crossing over. The approach not only allows fixation of uncharacterized germplasm but provides breeders with a breeding tool that, when applied to plants of known backgrounds, allows the rapid generation of chromosome substitutions that will facilitate breeding on an individual chromosome level.

Essentiality of reverse breeding

- To overcome difficulty in maintaining hybrids stability.
- To improve hybrid performance.
- Breeding in uncharacterized heterozygotes line.
- Improvement in clonal propagated plants.

Clonal propagation (or apomixis) allows for the preservation of the parental genotype (van Dijk and van Damme 2000), but its further genetic gain can be only achieved through improvement of parental lines.

Essential Steps of Reverse Breeding

- Suppression of crossing over.
- Production of double haploids
- Selection of complementary or parental line through marker assisted selection.
- Crossing of appropriate double haploid plants.

Suppression of Crossing Over

- Gametes production from heterozygous.
- Suppression of recombination during spore formation

To stop the meiotic crossing over / recombination suppressing gene is required. Suppression of recombination done with the help of RNAi technique. Meiosis specific recombinase require for the cross over some exogenous chemicals like mirin are used to stop recombination during meiosis.

RNAi technique

RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules. American scientists Andrew Fire and Craig Mello in 1998 discovered RNAi mechanism.

- RNA with inverted repeats hairpin/panhandle constructs
- dsRNA
- miRNAs/siRNAs
- RISC (RNA-induced silencing complex)
- Destruction of target mRNA

It has been discovered that the best precursor to good RNA silencing is to have single stranded antisense RNA with inverted repeats which, in turn, build small hairpin RNA and panhandle constructs. The hairpin or panhandle constructs exist so that the RNA can remain independent and not anneal with other RNA strands. These small hairpin RNAs and/or panhandles then get transported from the nucleus to the cytosol through the nuclear export receptor called exportin-5, and then get transformed into a dsRNA, a double stranded RNA, which, like DNA, is a double stranded series of nucleotides. If the mechanism didn't use dsRNAs, but only single strands, there would be a higher chance for it to hybridize to other "good" mRNAs. As a double strand, it can be kept on call for when it is needed. The dsRNA then gets cut up by a Dicer into small (21-28 nt = nucleotides long) strands of miRNAs (microRNAs) or siRNAs (short interfering RNAs.) A Dicer is an endoribonuclease RNase, which is a complex of a protein mixed with strand(s) of RNA. Lastly, the double stranded miRNAs/siRNAs separate into single strands; the antisense RNA strand of the two will combine with another endoribonuclease enzyme complex called RISC (RNA-induced silencing complex), which includes the catalytic component Argonaute, and will guide the RISC to break up the "perfectly complementary" target mRNA or viral genomic RNA so that it can be destroyed. It means that based on a short sequence specific area, a corresponding mRNA will be cut. To make sure, it will be cut in many other places as well. (If the mechanism only worked with a long stretch, then there would be higher chance that it would not have time to match to its complementary long mRNA.) It has also been shown that the repeated-associated short interference RNAs (rasiRNA) have a role in guiding chromatin modification.

Production of double haploids

Double haploids are produced from the immature pollen through tissue culture. Double haploid (DH) technology was integrated into the breeding process to select fertile selfing lines that can produce the same hybrid genotype as the original paternal and maternal parents (Wijnker *et al.* 2012, 2014). Van Dun *et al.* (2008) proposed another method called near reverse breeding. The basic idea was to obtain diploid spores with partial segregation and recombination, and then double haploid technology was used to select complementary fertile selfing lines in the offsprings. Cell or colonies are transferred to different media which contains different combination of growth regulators as well as sugar or energy source for the formation of shoots and roots (whole plant). This will lead to haploid microspores from which the genome will subsequently be doubled. The diploid microspores will eventually be developed into embryos and subsequently into homozygous plants using tissue culture techniques. These double haploid plants are used for the further investigation.

Selection of complementary or parental line through marker assisted selection.

In this step compare the plant genotypes at molecular level. Select the complementary plants from above developed double haploid plant with the help of marker assisted selection by using different molecular markers. Complementary plant are select as parent material of heterozygous hybrid as well as selected complementary plants / genotype are used for the further hybridization to develop hybrid or to develop novel combination for high yielding as well as resistance to biotic and abiotic stress.

Crossing of appropriate double haploid plants.

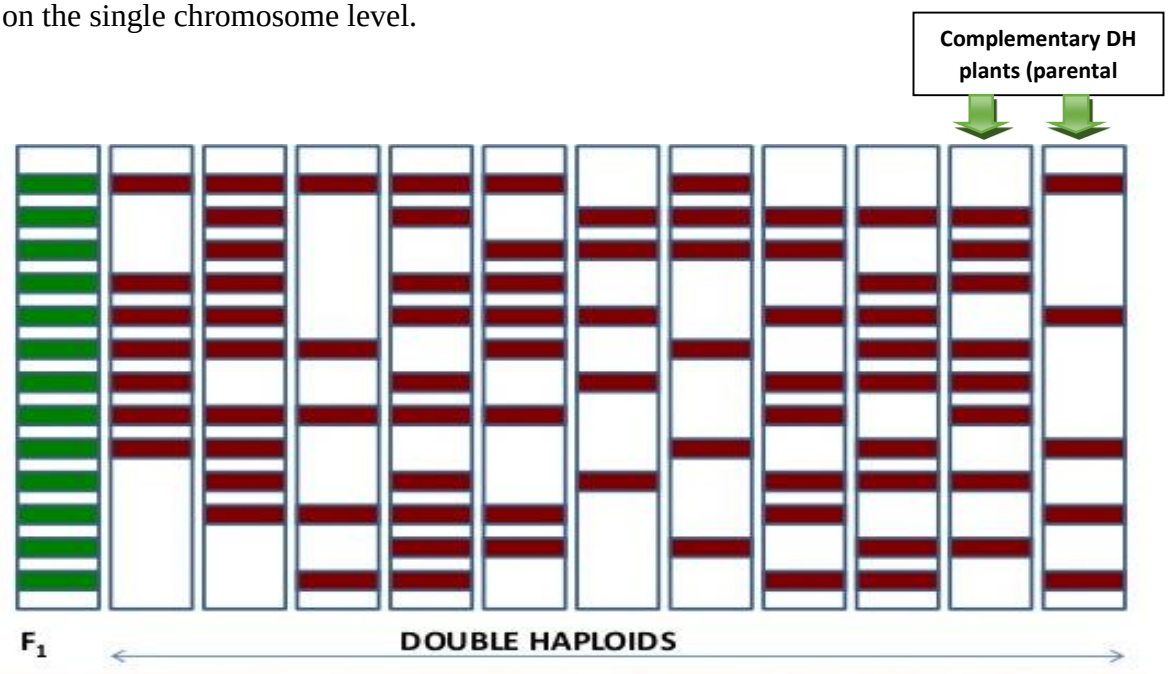
Complementary parents can be chosen that when crossed perfectly reconstitute the starting hybrid. The DH lines then serve as a permanent library that can be used to predictably generate a wide variety of defined hybrids.

Futures of reverse breeding

- End product of the reverse breeding is similar to parental lines obtained by conventional breeding.
- RNAi restrict only meiotic crossing over only no any change in the DNA sequence in reverse bred plant
- Resulting offspring are regarded as non-genetically modified.

Applications of reverse breeding

- Production of parental lines form any unknown heterozygous plant.
- Reconstruction of heterozygous germplasm.
- Breeding on the single chromosome level.



Limitation of reverse breeding

It is only useful to those crops where double haploid technology is commonly practiced. e.g. sorghum, pea, onion. The technique is limited to only crops with haploid chromosome number 12 or less.

CONCLUSION

This technique is based on reducing genetic recombination in the selected heterozygote by eliminating meiotic crossing over. It's helpful for Production of parental lines form any unknown heterozygous plant, reconstruction of heterozygous germplasm, breeding on the single chromosome level.

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