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# Reverse breeding-Versatile Tool for Creating Homozygous Parental Lines

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**ABSTRACT**

Reverse breeding is an innovative and highly desirable plant breeding technique that aims to create homozygous parental lines directly from heterozygous plants. This method involves manipulating meiosis to minimize genetic recombination in the chosen heterozygote, thereby removing meiotic crossing over. By utilizing this approach, spores gathered from either male or female plants will possess unaltered parental chromosomes, which have not undergone any recombination. Consequently, these spores can be grown in a laboratory environment to generate homozygous doubled haploid plants (DHs). The heterozygote can be perpetually reconstituted by selecting complementary parents from these DHs. Compared to conventional plant breeding, which struggles with stabilizing unknown heterozygous genotypes, reverse breeding can revolutionize future plant breeding. In addition, reverse breeding has various applications, such as chromosome-specific breeding, that could be explored further. This approach creates offspring using only one distinct chromosome of the parent plants instead of all the chromosomes.

***Key words :*** *Reverse breeding, Homozygous parental lines, Plant breeding*

## Introduction

The concept of reverse breeding was given by Rob Dirks in 2009. A novel reverse breeding technique allows breeders to produce a new hybrid in less time than traditional methods (Dirks *et al*., 2003) and (Dirks *et al*., 2009). Reverse breeding (RB) is a novel approach to plant breeding that aims to produce homozygous lines by inhibiting meiotic crossovers and stabilizing non-recombinant chromosomes in doubled haploid lines (DHs). This method is particularly useful for heterozygous genomes (Forster *et al*., 2007). Reverse breeding is described as a process utilized in the growth of plant cell cultures, which entails producing homozygous lines from heterozygous parent lines (Dirks *et al*., 2009; Wijnker *et al*., 2012). Creating stable high-quality hybrid plants can be achieved by crossing two homozygous parent lines instead of relying on less reliable hybridisation methods. By doing so, there is a decreased likelihood of instability occurring within the resulting hybrids. This is because the characteristics of heterozygotes are not fully understood, and producing hybrid seeds could lead to the separation of desirable allele combinations in subsequent generations (Yi-Xin *et al*., 2015). A novel method of plant breeding called Reverse Breeding (RB) has been created to generate parental lines for all types of heterozygous plants. RB utilizes engineered meiosis to create completely homozygous parents, who, when bred to-

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gether, can produce identical heterozygote offspring. The gene that causes the formation of chiasmata between non-identical chromatids of homologous chromosomes is repressed by this method, which prevents genetic recombination by reducing the frequency of meiotic crossovers. Although RB can be employed in plants, fungi, and animals, it cannot be used in humans (Sinha *et al*., 2020). Reverse breeding is an innovative technique that has been developed to overcome the challenges associated with stabilizing complex heterozygous genomes. This method involves the creation of matching homozygous lines by minimizing meiotic crossovers and fixing non-recombinant chromosomes in homozygous doubled haploid lines (DHs). The primary aim of this breeding approach is to eliminate uncharacterized germplasm while also providing breeders with a helpful tool for accelerating the creation of chromosome substitutions for plants with known backgrounds. By allowing breeding on a chromosome-by-chromosome basis, reverse breeding enables plant breeders to focus on specific traits and desired characteristics, thereby increasing efficiency and productivity in the farming industry. Ultimately, this technique has revolutionized traditional plant breeding methods and offers significant potential for developing new crop varieties that are better adapted to meet the needs of modern agriculture (Singh, 2018). Reverse breeding selects a top-performing plant, creating homozygous parental lines by blocking genetic recombination. These parental lines can then reproduce the original genetics of the elite plant. A genetic constitution step is used during reverse breeding to avoid genetic recombination, resulting in intermediate plants that fall under GMO regulations. However, the chosen variety and its parental lines do not have this modification and are not subject to GMO regulations (Singh, 2018).

**Mechanism of RB**

The process of reverse breeding comprises two main steps: firstly, the suppression of crossover recombination in a selected plant, and secondly, the production of doubled haploid (DH) lines from spores that contain non-recombined chromosomes. These DH lines can be employed to mass-produce superior-quality heterozygous plants (Singh, 2018).

**Selection of heterozygote**

The chosen plant is preferred for its favourable traits without considering its origin and is then picked for having a high level of heterozygosity. The production of gametes follows from this heterozygous plant (Sinha *et al*., 2020).

**Generation of Double haploids**

The doubled haploidy (DH) technique has been employed to carefully pick out the most optimal selfing lines equipped with the necessary capacity to produce an identical hybrid genotype as their original parents. This is a crucial process in plant breeding, where genetic uniformity and stability are key factors in maintaining desirable traits across successive generations. By utilizing DH, breeders can streamline the selection of these lines and ensure greater accuracy and efficiency in preserving genetic characteristics that are important for successful crop production (Wijnker *et al*., 2012; Wijnker *et al*., 2014). Usingthe pollen culture method, achaismatic gametes are matured into adult haploid plants by cultivating them on suitable media. The resultant seeds from these haploid plants are later bred with Cenh3-1 GFP-Tailswap to generate homozygous diploids (Wijnker *et al*., 2014).

**Complementary parent crossing**

Marker Assisted Selection (MAS)is a widely used technique in plant breeding that involves identifying parents with complementary traits through genotyping. This technique is particularly useful in producing original hybrids by crossing these complementary parents. To genotype each Doubled Haploid (DH) plant, only one polymorphic molecular marker per chromosome is required if there is no meiotic recombination. In this case, since the entire chromosome acts as a single linkage block, one marker can suffice for genotyping. However, if residual crossovers are present between the chromosomes of the DHs, two markers are needed per chromosome for accurate genotyping (Dirks *et al*., 2009). The hybrid created through RB lacks transgene, so it should not be considered genetically altered (Sinha *et al*., 2020).

**Reverse Breeding in Maize using Markers**

A marker-assisted reverse breeding (MARB) technique was developed using chip-based SNP genotyping in maize. This method involved four crop seasons, each with a cycle of marker-assisted selection, completed within a year to select homozygous plants resembling both parents. The maternal and paternal inbreds that resulted exhibited pheno-

typic similarities to the standard US heterotic groups Lancaster and Reid. RMRB and MARB required an equivalent amount of time to develop populations and chip screening. Compared to traditional breeding methods, which can take up to ten years to produce homozygous parental lines, RB methods are more efficient (Yi-Xin *et al*., 2015).

**Differences in end product of conventional and reverse bred crops.**

The parental lines produced through traditional breeding methods are very similar to the end outcomes of reverse breeding crops. The RNAi silencing technique only affects meiotic crossover and does not affect the DNA sequence, making the resulting products safe for utilization. Since reverse breeding crops are not genetically modified, bioethical concerns are not involved (Sinha *et al*., 2020).

**Applications**

In 2009, a new method called reverse breeding was introduced, and although it has yet to be commercially used, many breeders have already started using it for research. The technique has the potential to be very useful for maintaining superior hybrid crop plants even when access to parental lines is limited. Breeders have been seeking a dependable method like this for many years, so interest in reverse breeding is growing. It is expected to positively affect the breeding of critical agricultural crops such as cucumber, onion, broccoli, cauliflower, watermelon, tomato, and eggplant, among others.

**Heterozygous germplasm reconstruction**

Accelerating the creation of new crop varieties is possible through reverse breeding, particularly in crops where there is a deficiency of breeding lines. This technique enables the production of high-quality heterozygous plants without prior knowledge of their genetic composition (Anand, 2020). Agricultural varieties can be developed faster using reverse breeding in cases with a restricted collection of breeding lines. It enables the propagation of excellent heterozygous plants without prior knowledge of their genetic characteristics. Even at varying probability levels, the number of doubled haploid plants needed to recreate the original plant can be surprisingly low (Dirks *et al*., 2009).

**Breeding at the single chromosome level**

Many desirable traits in crops are caused by interactions between multiple genes, known as polygenic gene interactions. However, these genes are often found on different chromosomes, making them difficult to breed for. Reverse breeding can be used to create homozygous chromosome substitution lines by applying it to an F1 hybrid with known parents. These lines can be used to study gene interactions, such as epistatic interactions between background and substitution chromosomes. Hybrids can be formed when these chromosome substitution lines are crossed with one of the original parents. Breeders can exercise complete control over homozygosity or heterozygosity by using Reverse Breeding to adjust desirable characteristics on a single chromosome level finely. However, identifying specific substitution lines in crops with numerous chromosomes can be challenging. In these cases, it may be beneficial to backcross a DH line that carries the desired substitution with one of the original parents. Marker-assisted breeding can also simplify the process of obtaining the desired chromosome substitution (Dirks *et al*., 2009). Hybrids can then be produced by crossing these lines with one of the original parents, resulting in offspring where one or more chromosomes are either homozygous or heterozygous (Singh 2018).

**Breeding methods such as marker-assisted breeding and reverse breeding**

By utilizing high throughput genotyping, RB can be a versatile tool for identifying appropriate parents in doubled haploids (DHs) populations and expediting the process. In addition, RB can also assist in studying gene interactions among different heterozygous inbred families (HIFs) that are produced through crossing and backcrossing RB products. Through screening populations segregating for single chromosome traits, QTLs can be quickly identified when combined with transcriptome or metabolome profiling. HIFs are also useful in creating chromosome-specific linkage maps and finely mapping genes and alleles. All of these factors provide valuable insights into the nature of heterotic effects through the use of RB (Singh, 2018).

**Backcrossing in CMS background**

Breeders have adopted and implemented cytoplasmic male sterility (CMS) as a standard technique across different types of vegetable crops, including but not limited to cabbages and carrots (Chase, 2007). A distinct strategy is required to introduce RB

in crops with cytoplasmic male sterility (CMS). In lieu of androgenesis, gynogenesis can be employed to produce DH plants. Chromosomes are acquired from the maintainer line and transferred into the cytoplasm of the sterile line in one go with this technique. Gynogenesis has been effective in different crops such as Brassica, maize, sugar beet, cucumber, melon, rice, onion, sunflower and barley (Keller and Korzun, 1996). Gynogenesis protocols for several species were frequently abandoned after introducing anther and microspore culture techniques. If gynogenesis proves inefficient, it is feasible to mate the male sterile (A) lines with maintainer lines (B) containing a single restorer gene copy. The AB combination will be fertile, allowing for RB. Successful transformation of restorer genes in rice has been demonstrated (Wang *et al*., 2006). Performing RB is possible in a setting where both CMS and suppression of crossover happen at the same time by utilizing a vector that includes a restorer gene and a gene for crossover suppression as transgenes. This technique allows RB to be executed in a "double suppressed" background.

**Limitations of RB**

- Reverse breeding is a technique restricted to plant cultivation where double haploid technology has already been established as a regular practice (Sinha *et al*., 2020).
- The technique of reverse breeding applies only to crops that regularly use double haploid technology, including maize, onion, cucumber, sugar beet, and pea. It can only be used for crops with a chromosome number of 12 or less and where spores can be regenerated into double plants
- Double haploid production is uncommon for crops such as cotton, soybean, lettuce, and tomato (Croser *et al*., 2006).
- It is not practically possible to attain the original heterozygous plant by generating a large number of non-recombinant double haploids to match the pair for plants with a high number of chromosomes (Lusser *et al*., 2012).
- The genetic diversity in plant breeding may be limited due to the restricted potential for further selection resulting from the full homozygosity of plants obtained through RB. (Van Dun and Dirks, 2006).

## Conclusion

RB is a useful breeding technique that permits the controlled breakdown of complex genotypes into homozygous parental lines, making them more suitable for further enhancement using traditional breeding approaches. This approach can perfectly match homozygous parental lines by manipulating meiosis to reduce genetic recombination in selected heterozygotes and eliminate meiotic crossing over. The male or female spores resulting from these plants contain combinations of non-recombinant parental chromosomes that may be cultured in vitro to generate homozygous doubled haploid plants (DHs). These DHs can then be employed to select complementary parents that can be perpetually reconstituted into the heterozygote. Since unknown heterozygous genotypes cannot be fixed through conventional plant breeding methods, Reverse Breeding has the potential to revolutionize future plant breeding techniques.

**Future prospects**

In the future, crop production could be increased by utilizing reverse breeding techniques to identify and improve advantageous genotypes (Sinha *et al*., 2020). The combination of reverse breeding and high-through put genotyping can expedite the process of identifying compatible parents in populations of double haploids during early stages, making it a valuable technique. It has the potential to introduce favorable traits from ancestral sources into crop species (Dirks *et al*., 2009; Palmgren *et al*., 2015). MARB is a molecular breeding technique that can quickly and easily convert any maize hybrid into inbred lines (Yi-Xin *et al*., 2015). Recreating hybrids through reverse breeding is a modern technique for crop breeding that shows considerable potential (Sinha *et al*., 2020).

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