Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 04, Issue: 04 (JULY-AUG, 2024)
Available online at http://www.agriarticles.com

Reverse Breeding: A Novel Breeding Approach for Crop Improvement

(*Sarita Choudhary)
Ph.D. Research Scholar, Department of Genetics and Plant Breeding, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan
*Corresponding Author's email: saritachoudhary212621@gmail.com

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 (Dirks *et al.* 2009). It allows breeders to produce a new hybrid in a much shorter time than with conventional techniques. Reverse breeding starts with a heterozygous line and aims at the generation of homozygous parental lines. This innovative approach has the potential to expedite the breeding process and enhance the efficiency of plant breeding programs.

Why Reverse Breeding is needed?

- To establish breeding lines for uncharacterized hybrid
- To enhance hybrid performance by genetic improvement of parental lines
- To maintain the stability of hybrid
- To maintain a highly heterozygous plant from a homozygous parental line

Mechanism of Reverse Breeding

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

Step 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,

A. Genes responsible for meiotic recombination
   1. DMC1 gene: Disrupted Meiotic cDNA
   2. SPO1 gene: Sporulation Specific gene
   3. RecA gene: Recombinase A gene

RNAi knock down the function of these genes during spore formation

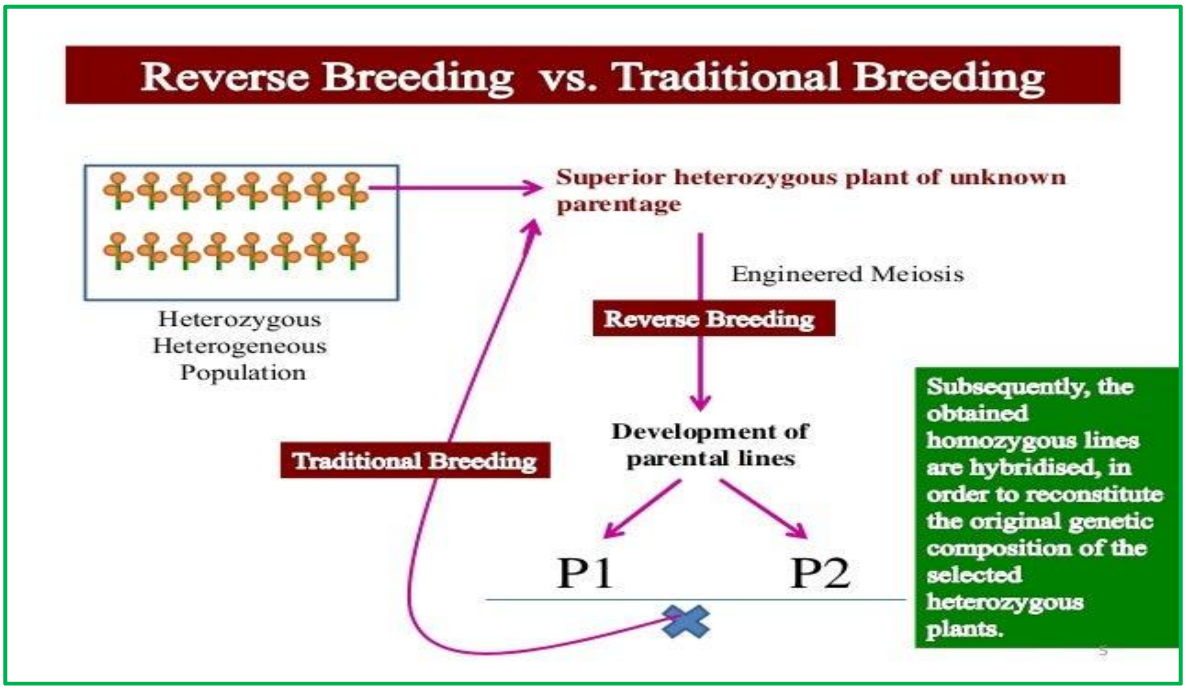
B. Exogenous application of chemical compound that cause inhibition of recombination A. E.g. Mirin (Mirin causes G2 arrest and inhibit the phosphorylation of ATM
C. Introduce genes encoding proteins that are known to interfere with crossing over
D. Endogenous mutation

Step 3. Production of Double Haploids: DH technique was included for the selection of fertile selfing lines which can produce the same hybrid genotype as produced by the original parents (Wijnker *et al.* 2012, 2014). Using pollen culture technique, resulting achaismatic gametes are grown on suitable media to develop into adult haploid plants. Selection of fertile selfing lines seeds harvested from these haploid plants are crossed to resulting into homozygous diploids.

Step 4. Selection of complimentary lines (parents) through marker assisted selection: 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). Crossing appropriate DH lines on the basis of matching molecular markers to develop superior hybrids.

Reverse breeding v/s Traditional Breeding

- Reverse breeding is a modern plant breeding method for producing complementing parental lines for any heterozygous plant through achaismatic meiosis (meiosis without crossovers).
- Traditional Breeding achieves by crossing together plants with relevant characteristics and selecting the offspring with desired combination of characteristics, as a result of particular combinations of genes inherited from the two parents.



Applications of Reverse Breeding

Reverse breeding offers a wide range of applications within the field of plant breeding, including:

1. Construction 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

2. Breeding on the Single Chromosome Level

- Reverse Breeding explains how chromosome substitution lines can be obtained when it is applied to an F_1 hybrid of known parents.
- Offspring of plants in which just one chromosome is heterozygous, will segregate for traits present on that chromosome only.
  - It is valuable tool for study of epistatic interactions and trait mapping.
  - Development of improved breeding lines carrying introgressed traits.

3. Back Crossing in CMS Background

- In several vegetable crops such as cabbages and carrots, breeders make use of cytoplasmic male sterility (CMS).
- Gynogenesis rather than androgenesis can be used to obtain DH plants.
- This is perfectly compatible with RB in the sense that the chromosomes from the maintainer line can be recovered directly in the cytoplasm of the sterile line in one step.

4. Reverse Breeding and Marker Assisted Selection

- High throughput genotyping speeds up the process of identification of complementary parents in populations of DHs.
- The screening of populations that segregate for traits on a single chromosome allow the quick identification of QTLs, when genotyping is combined.
  - Helps in the study of gene interaction in the heterozygous inbred families.
  - Aids in generation of chromosome specific linkage maps.
  - Fine mapping of genes and alleles.

Advantages of Reverse Breeding

- RB accelerates breeding process and increase number of available genetic combinations, allowing breeder to respond much quicker to the needs of farmers with better varieties.
- Large population of plants can be generated and screened and well performing plants can be regenerated indefinitely without prior knowledge of their genetic constitutions.
- The main advantage is that using reverse breeding, elite heterozygotes plants can be produced as F_1 hybrids because of re-synthesis of parental lines.
- F_1 hybrids obtained from reverse breeding do not contain any genetic modification and will be similar to conventional breeding hybrid.
- It is free from GMO legislation.

Limitations of Reverse Breeding

- This technique is confined to those crops only where double haploid technology is common practice.
- There are some exceptions such as soybean, cotton, lettuce and tomato where DHs is barely formed.
- 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 reconstitutes the original heterozygous plant would be extremely high and practically not feasible.
- 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.

Conclusion

As a 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. Reverse Breeding could fundamentally change future plant breeding. It Increases the available genetic combinations and Production of new hybrid plant varieties in a much short time frame. Hybrid does not contain any genetic modification so it is safe for environment and used for food and feed production.

Future prospects

New possibilities for the selection and improvement of favourable genotypes by reverse breeding may contribute to increasing future crop production. The scope of reverse breeding could be envisioned for the improvement of agricultural crops, as it may enable the generation of parental lines for the recreation of hybrids. RNAi Mediated Reverse Breeding is a young work, requires extensive study to overcome technical problems. Emphasis should be given for the production of hybrids in crops like cucumber, onion, broccoli, cauliflower where seed production is problematic.

References

1. Croser, J., Lu lsdorf, M., Davies, P., Clarke, H., Bayliss, K., Mallikarjuna, N. and Siddique, K. (2006). Toward doubled haploid production in the Fabaceae: progress, constraints, and opportunities. *Crit. Rev. Plant Sci.* **25**: 139–157.
2. Dirks, R., Van Dun, K., De Snoo, C. B., Van Den Berg, M., Lelivelt, C. L., Voermans, W. and Wijnker, E. (2009). Reverse breeding: a novel breeding approach based on engineered meiosis. *Plant biotechnology journal*, **7**(9): 837-845.
3. Jain, S., Sopory, S.K. and Veilleux, R.E. (1996). In vitro haploid production in higher plants. *In Current Plant Science and Biotechnology in Agriculture, Dordrecht, Boston, London: Kluwer Academic Publishers.*
4. Kumari, P., Nilanjaya, Singh, N.K. (2018). Reverse breeding: Accelerating innovation in Plant Breeding. *Journal of Pharmacognosy and Phytochemistry,* **1**: 1811-1813.
5. Wijnker, E. and Jong, H.D. (2008). Managing meiotic recombination in plant breeding. *Trends Plant Sciences.***3**: 640–646.
6. Wijnker, E., Dun, K.V., Lelivelt, C.L.C., Joost, K.B., Naharudin, N.S., Ravi, M., Chan, W.L., de Jong, H. and Dirks, R. (2012). Reverse breeding in *Arabidopsis thaliana* generates homozygous parental lines from a heterozygous plant. *Nature genetics*. DOI: 10.1038/ng.2203.
7. Yi-Xin, G., Bao-hua, W., Yan, F. and Ping, L. (2015). Development and application of marker-assisted reverse breeding using hybrid maize germplasm. *Journal of Integrative Agriculture*, **14**(12): 2538-2546.