International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(3): 1128-1131

Received: 04-03-2020
Accepted: 06-04-2020

Ankita Sinha
Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Ravi S Singh
Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Ujjwal Kumar
Department of Biotechnology, Tilka Manjhi Bhagalpur University, Bhagalpur, Bihar, India

Kumari Rekha
Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Apoorva Prasad
Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Ashwini kumar
Department of Horticulture, (Fruit and Fruit Tech, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Corresponding Author:
Ravi S Singh
Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Reverse breeding: A modern plant breeding approach for hybrid recreation

Ankita Sinha, Ravi S Singh, Ujjwal Kumar, Kumari Rekha, Apoorva Prasad and Ashwini kumar

DOI: https://doi.org/10.22271/chemi.2020.v8.i3o.9350

Abstract
Reverse breeding is a modern plant breeding method for producing complementing parental lines for any heterozygous plant through achaismatic meiosis (meiosis without crossovers). The achiasmatic meiosis leads to univalent segregation at meiotic metaphase-I and the generation of aneuploid gametes. These gametes are then regenerated as doubled-haploid (DH) plants. Each DH carries combinations of its parental chromosomes, and complementing DH pairs can be crossed to reconstitute the initial hybrid. In reverse breeding, the suppression of meiotic crossovers in a hybrid ensures the transmission of non-recombinant chromosomes to haploid gametes. The PAIR2 gene is required for homologous chromosome synapsis at meiosis-I in plants. An insertional mutation in the rice PAIR2 gene, the ortholog of *Arabidopsis thaliana* ASY1, results in a defect in homologous chromosome pairing during meiosis, display univalents at metaphase-I. Essentially, reverse breeding follows an approach akin to the generation of a DH population from an F_1 hybrid, carrying a dominant-acting transgene that down-regulates the expression of Disrupted Meiotic cDNA1 (*DMC1*), resulting in inhibition of crossover recombination and thereby enabling intact-chromosome inheritance. In earlier reports on reverse breeding in *A. thaliana*, a hybrid was constructed of using two of its natural ecotypes (Col-0 and Laer-0), carrying an RNAi transgene targeting the meiotic recombinase (*Rec*A homolog) DMC1 that prevented the formation of meiotic crossover recombination. This method mainly included steps: (i) the generation and selection of RNAi: DMC1 transformed lines; (ii) the generation of achiasmatic hybrids; (iii) the crossing of achiasmatic hybrids to GFP-tailswap to generate haploid chromosome substitution lines (CSLs); (iv) the generation of DHs by spontaneous doubling of haploid CSLs; and (v) the crossing of complementing CSLs to recreate the initial hybrid. The scope of reverse breeding could be envisioned for the improvement of agricultural crops, as it may enable the generation of parental breeding lines for the recreation of hybrid.

Keywords: Achiasmatic meiosis, double haploids, RNAi, *DMC1* gene

1. Introduction
The term ‘‘Reverse breeding’’ was originally introduced to describe a technique in plant cell cultures, where homozygous lines are produced from heterozygous parent lines (Dirks *et al*., 2009; Wijnker *et al*. 2012) [4, 16]. Here, the term “reverse breeding’’ includes the earlier proposed usage but goes beyond the original definition by widening the methods used to produce homozygous lines (Palmgreen *et al*. 2014). Homozygous parental lines are crossed to recreate elite hybrids afresh as the hybrids are not stable. The uncharacterized heterozygotes cannot be reproduced by hybrid seed production because it leads to loss of favorable alleles combinations due to segregation in the next generation (Yi-Xin *et al*. 2015) [17]. Reverse breeding (RB) is a novel plant breeding method designed to produce parental lines for any heterozygous plant. It generates perfectly homozygous parents, through engineered meiosis, that when mated together produce the same heterozygote. This method eliminates the phenomena of meiotic crossover by silencing the gene responsible for the formation of chiasmata between the non-sister chromatids of homologous chromosome. RB can be executed in plants, fungus, animals but not in humans (Dirks *et al*. 2008). In some genetic modifications, the residues of shuttles such as bacteria and fungus are left in the host plant but with new breeding technique like RB which makes it possible to develop homozygous lines without introduced DNA sequence (Dirks *et al*. 2009) [4]. Neither of the authorities like ACRE

and COEGM see any justification that the resultant product produced by RB is GMOs (ACRE, 2013) [1] and transgenesis is just an intermediate step to pave path for breeding and selection (Kuligowska *et al*. 2013) [7] and with knockdown constructs such as GFP-Tailswap, on different chromosomes, multiple transgenic lines can be used to generate a full array of complementary DHs not having transgenes (Wijnker and de Jong. 2008) [13] but the question arises whether the product obtained, should be considered GMOs even in the absence of insert (Parisi. 2013) [10]. However, according to the European legislations, the progeny of GMO should be considered genetically modified whether the concerned gene is present is the succeeding generations (Hartung and Schiemann. 2014) [6]. RB is a new breeding technique which allows for production of new hybrid plant varieties in a much shorter time frame and ambient numbers compared to conventional plant breeding. Another reconstruction technique has been proposed called Near Reverse Breeding, in polyploids or species with high chromosome numbers that is based on the omission of the second meiotic division, which give way to unreduced second division restitution (SDR) spores. These SDR spores facilitate the near reconstruction of desired phenotypes, and also provide the possibility of obtaining CSLs (Van Dun and Dirks, 2006) [12].

Following goals could be achieved through RB:

- 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

Applications of RB

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

2. Mechanism of RB

2.1 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.

2.2 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 (Dupree *et al*., 2008).

2.3 Generation 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) [15]. Using pollen culture technique, the resulting achaismatic gametes are grown on suitable media to develop into adult haploid plants and the seeds harvested from these haploid plants are crossed to Cenh3-1 GFP-Tailswap resulting into homozygous diploids shown in Fig:1. (Wijnker *et al*. 2014) [15].

2.4 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) [4]. The hybrid obtained through RB does not carry the transgene and hence they should not be considered as GM.

2.5 Marker Assisted Reverse Breeding in Maize

In maize, chip-based SNP genotyping was done for the selection of homozygous plants similar to the two parents, so it is named as marker-assisted reverse breeding (MARB). This breeding procedure took four crop seasons each with a cycle of marker-assisted selection completed in a year. The maternal and paternal inbreds developed look phenotypically similar to those from two standard US heterotic groups, Lancaster and Reid, respectively. RMRB and MARB take same span of time due to population development and chip screening. Both RB methods are more efficient than that of conventional breeding which take six–ten years to produce homozygous parental lines (Yi-Xin *et al*. 2015) [17].

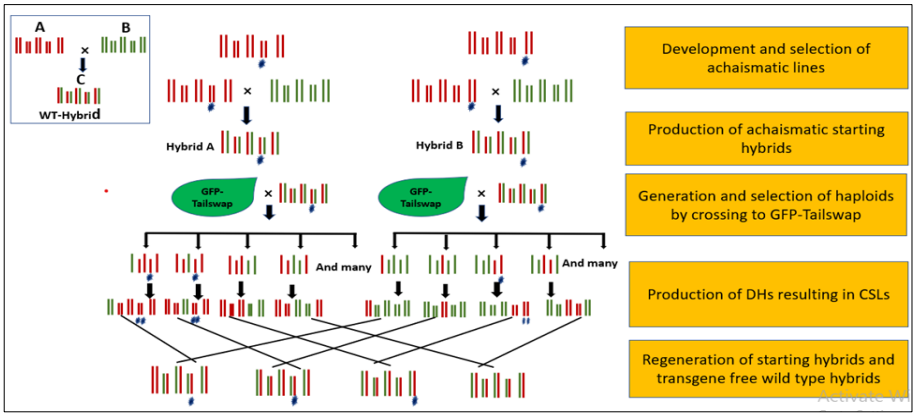

Fig 1: Flow chart of RB for regeneration of starting hybrids (adopted from Wijnker and Jones. 2014) [15]. Firstly, two transgenic lines at different chromosomes *viz.* DMC1: RNAi are developed; crossing of resulting achaismatic lines with second accession to develop starting hybrids; haploids are generated using haploids constructs like GFP-Tailswap; generation of DHs and intercrossing of chromosome substitution lines (CSLs) to regenerate starting hybrids and WT- type hybrid with no transgene.

2.6 Difference of end product of conventional and reverse bred crops

- The end products of reverse bred crops are as similar as parental lines obtained by conventional breeding.
- RNAi silencing is confined to only meiotic crossover; there will be no change in the DNA sequence. The products are safe to use.
- Reverse bred crops are non-genetically modified so, there is no bioethical issue.

3. Limitations of RB

- 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 (Croser *et al*. 2006) [2].
- 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 (Lusser *et al.* 2011) [8].
- 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 (Van Dun and Dirks, 2008).

4. Future prospects

- New possibilities for the selection and improvement of favorable genotypes by RB may contribute to increasing future crop production.
- 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.

5. Conclusions

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 of 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.

6. References

1. Advice ACRE, ACRE: New techniques used in plant breeding, 2014.
2. Croser J, Lu¨ lsdorf M, Davies P, Clarke H, Bayliss K, Mallikarjuna N. Toward doubled haploid production in the Fabaceae: progress, constraints, and opportunities. Critical Reviews in Plant Sciences. 2006; 25:139-157.
3. Dirks RHG, Van Dun CMP, Reinink K, De Wit JPC. U.S. Patent 8,242,327 No. Washington, DC: U.S. Patent and Trademark Office, 2012.
4. Dirks R, Van Dun K, De Snoo CB, Van Den Berg M, Lelivelt CL, Voermans W. Reverse breeding: a novel breeding approach based on engineered meiosis. Plant biotechnology journal. 2009: 7(9):837-845.
5. Dupré A, Boyer-Chatenet L, Sattler RM, Modi AP, Lee JH, Nicolette ML *et al*. A forward chemical genetic screen reveals an inhibitor of the Mre11–Rad50–Nbs1 complex. Nature chemical biology. 2008; 4(2):119.
6. Hartung F, Schiemann J. Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. The Plant Journal. 2014; 78(5):742-752.
7. Kuligowska K, Lütken H, Hegelund JN, Müller R. Future perspectives of *in vitro* culture and plant breeding. In VIII International Symposium on *In Vitro* Culture and Horticultural Breeding. 2013; 1083:27-34.
8. Lusser M, Parisi C, Plan D, Rodriguez-Cerezo E. Deployment of new biotechnologies in plant breeding. Nature biotechnology. 2012; 30(3):231.
9. Palmgren MG, Edenbrandt AK, Vedel SE, Andersen MM, Landes X, Østerberg JT *et al*. Are we ready for back-to-nature crop breeding? Trends in plant science. 2015; 20(3):155-164.
10. Parisi C. New plant breeding techniques: state of the art, potential and challenges, 2013.
11. Van Dun CMP, Dirks RHG. U.S. Patent No. 9,332,697. Washington, DC: U.S. Patent and Trademark Office, 2016.
12. Van Dun CMP, Dirks RHG. Rijk Zwaan Zaadteelten Zaadhandel BV Near Reverse Breeding, 2006. WO⁄2006⁄094773

13. Wijnker E, de Jong H. Managing meiotic recombination in plant breeding. Trends in plant science. 2008; 13(12):640-646.
14. Wijnker E, de Jong H. Managing meiotic recombination in plant breeding. Trends in plant science. 2008; 13(12):640-646.
15. Wijnker E, Deurhof L, Van De Belt J, De Snoo CB, Blankestijn H, Becker F and De Jong H. Hybrid recreation by reverse breeding in *Arabidopsis thaliana*. Nature protocols. (2014); *9*(4), 761.
16. Wijnker E, van Dun K, de Snoo CB, Lelivelt CL, Keurentjes JJ, Naharudin NS *et al*. Reverse breeding in *Arabidopsis thaliana* generates homozygous parental lines from a heterozygous plant. Nature genetics. 2012; 44(4):467.
17. Yi-Xin GUAN, Wang BH, Yan FENG, Ping LI. Development and application of marker-assisted reverse breeding using hybrid maize germplasm. Journal of Integrative Agriculture. 2015; 14(12):2538-2546.