# Backcross Breeding: Conventional & Molecular Approach

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#### Abstract

The population of the globe will increase to 9.8 billion people by the year 2050. With the ever-increasing demand of the rising population, it is very important to increase food production. Transforming the elite cultivar through backcrossing can be a vital approach. Backcrossing is a common technique in plant breeding that is most frequently used to introduce one or more traits -such as disease resistance, high yielding, etc. in to a superior or adapted variety. Genetics engineers are working on transforming their laboratories and breeders are backcrossing (MABC) proves to be the most promising approach with the use of molecular markers to identify and select genes controlling resistance in the cultivars. Now with the help of MABC, the recovery of the recurrent parent genotypes is quite possible from large population size, using only two or three backcrosses. These procedures are crucial to the development of new varieties with much higher precision and accuracy.

**Keywords:** Backcross method, Background selection, Donor parent, Foreground selection, Marker-assisted backcross, Recurrent parent.

#### I. INTRODUCTION

Backcrossing is the process of mating a hybrid (F1 or segregating generation) with either of its parents. It is a unique breeding technique where a variety that is already popular and high-yielding, is well-adapted to a location, and has other desired traits, needs to be corrected for one or two flaws.

The backcross method was frequently used for animal breeding. Harlan and Pope (1922) recognized the utility of the backcross method in crop improvement for small-grain breeding, and Briggs (1959) has since initiated an extensive backcross breeding program.

It is one of the useful plant breeding methods where the main objective is to introduce a specific number of characters from unadapted donor parents into recipient lines. This can be achieved by hybridization followed by backcrossing to the recurrent parent for several generations, followed by selecting the progenies at each generation to ensure that the desired gene(s) are present in the subsequent progenies.

This method is used for transferring major genes, in disease/plant resistance breeding, for transferring Cytoplasmic Male Sterility or alien cytoplasm, to transfer a transgene from an already developed transgenic line, and for the production of NILs (Near-Isogenic Lines), etc.

# **II. PRE-REQUISITES OF BACKCROSS BREEDING**

For a successful backcross program, the following requirement must be satisfied:

- A suitable recipient or recurrent parent: A recurrent parent can be a commercially viable variety that has to be improved for a certain trait that might increase its quality, performance, value, and range of acceptance.
- A suitable donor parent: This serves as a source of the gene(s) to be incorporated into the recurrent parent.
- Any character having high heritability can be transferred and should preferably be governed by one or a few genes.
- Enough backcrosses must be performed to completely recover the recurrent parent's genotype.

# **III. GENETIC BASIS**

In the backcross method, the hybrids and the resulting progenies in the subsequent generations are subjected to backcrossing repeatedly to one of the parents of the F1 which constitute the recurrent parent. This leads to a special type of inbreeding in which the population reverts to the genotype of the recurrent parent gradually.

Many loci will be heterozygous in the  $F_1$ , which will include the gene of interest as well as other loci containing genes for other traits. With backcrossing, there will be an increase in the homozygosity for the alleles from the recurrent parent and when several genes are considered together, the proportion of homozygotes of all the genes in the backcross generation is given by the formula  $[2^m - 1/2^m]^n$ , where m is the number of generation of backcrossing and n is the number of genes for which the parent carry contrasting alleles (Allard, 1960). Thus, a rapid increase in homozygosity and the frequency of homozygotes similar to that of selfing is the very first effect of backcrossing.

The frequency of genes from the donor parent in any backcross generation is  $(1/2)^{m+1}$ , which is bound to decrease with each additional backcross which ultimately leads to increased recovery of genes from the recurrent parent. The average genes recovered from the recurrent parent is calculated as  $[1 - (1/2)^{m+1}]$  where m = number of generations of backcrossing.

However, the rate of elimination of genes from the donor parent may be affected by linkage drag. Linkage is generally measured by the recombination frequencies/map distance. A reduction in the factors such as centromere location and chromosome structural abnormalities can be achieved in crossover events during meiosis. For example, if suppose an undesirable allele 'a', for lodging is linked to R (rust resistant), and selection is only for R, 'a' tends to be brought along in the F<sub>1</sub> as they are linked. However, when the R gene is being reintroduced in the recurrent parent in each backcross; the number of opportunities for crossing over between the R and 'a' loci occurs. Therefore, the probability of eliminating 'a' is:

where m = number of generations of backcrosses.

p = recombination frequency between loci. (Allard 1960)

# **IV. SELECTION OF PARENT**

- **1. Recurrent parent**: It must be a popular and high-yielding variety of the area along with possessing traits like high adaptability and desirable qualities. They must be having one or two defects that when improved can increase their suitability.
- 2. Non-recurrent parent: The parent must be having a high intensity of character to be integrated into the recurrent parent. The adaptability of yielding ability and other characteristics of this parent is not important. However, the intensity of the intended character in question is more desired in the recurrent parent as the intensity may decline during the transfer or in the new genetic background of the recurrent parent.

#### V. PROCEDURE

The general principle of the backcross method involves the hybridization of a suitable donor and recurrent parents. The  $F_1$  thus produced is crossed to the recurrent parent and the progenies having the desired characteristics are crossed in the subsequent generations, to the recurrent parent repeatedly at least four to six times. However, depending upon the nature of genetic control of the character under transfer, i.e., whether the gene being transferred is recessive or dominant, the steps for the method may vary accordingly. The procedure for transferring the dominant gene is easier as compared to the recessive genes.

#### **1.** Transfer of a Single Dominant Gene

The procedure for the transfer of a dominant gene is described below supposing that the gene confers resistance to a disease say mildew and is being transferred to high-yielding and widely adapted variety A from a variety B which is mildew resistant.

- **Hybridization**: The recurrent parent (rr) is hybridized with the donor parent (RR) to produce F<sub>1</sub> seeds. Generally, the recurrent parent is used as female as it would facilitate the identification of the selfed plant if any. However, in the case of transfer of CMS, the donor must be used as a female parent.
- **F**<sub>1</sub> generation: Since the F<sub>1</sub>s will be heterozygous for mildew resistance; no specific resistance test is required. The F<sub>1</sub> plants are crossed to recurrent parent (rr) to produce seed for the first backcross generation.
- First backcross Generation (BC<sub>1</sub>): The genetic composition of the BC<sub>1</sub> population will be 1 Rr: 1 rr which will be evaluated for its susceptibility to mildew. The resistant ones (Rr) are selected and backcrossed to variety A. Artificial epiphytotic conditions may be created, if necessary to ensure adequate disease development for distinguishing resistant and susceptible plants in BC<sub>1</sub> for further crossing. The resistant plants can be compared among themselves for resemblance with variety A. The plant that would be exhibiting maximum closeness to the recurrent parent for most of the characters may be used for crossing to enhance the rate of recovery of genes from the recurrent parent.
- $BC_2$ - $BC_5$  generation: In each backcross generation there will be segregation for resistance (Rr) and susceptibility (rr) in the ratio of 50:50, out of which resistant plants are selected and backcrossed to recurrent parents just like in the previous season.
- **BC**<sub>6</sub> generation: On average, the BC<sub>6</sub> plants will achieve 99% genes from variety A. Here selfing is done in the selected mildew-resistant plants, and their seeds are harvested separately.

- **BC**<sub>6</sub>**F**<sub>2</sub> generation: Individual plant progenies from selfed progeny are grown. Since the resistant plant in the previous generation (BC<sub>6</sub>) would be heterozygous (Rr), gene segregation of <sup>1</sup>/<sub>4</sub> RR: <sup>1</sup>/<sub>2</sub> Rr: <sup>1</sup>/<sub>4</sub> rr would be observed. Resistant plants (RR and Rr) similar to plant type of variety A are selected and their selfed seed is harvested separately. The susceptible plant (rr) is rejected.
- $BC_6F_3$ : Individual plant progenies are grown. Progenies (RR) homozygous for mildew resistance will be uniform and similar to plant type of variety A are harvested in bulk and composited to constitute the new variety. The progenies from Rr plants will show segregation and hence would be rejected.
- **Yield Trails**: The new variety is tested in replicated yield trails along with variety A as a check. The characteristics of the new variety are critically evaluated. Generally, the new variety would be similar to variety A in performance except for being resistant. Therefore, detailed yield trials are generally not conducted.

FIRST	NONRECURRENT	* RECURRENT
YEAR	PARENT (B)	PARENT (A)
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SECOND YEAR	F1	<b>Rr × rr</b> (Recurrent Parent A)
THIRD YEAR	BC <sub>1</sub>	, <b>Rr</b> × <b>rr</b> (Recurrent Parent A)
FOURTH YEAR	BC <sub>2</sub>	, <b>Rr</b> × <b>rr</b> (Recurrent Parent A)
FIFTH YEAR	BC3	, Rr × rr (Recurrent Parent A)
SIXTH YEAR	BC4	, <b>Rr</b> x <b>rr</b> (Recurrent Parent A)
SEVENTH YEAR	BC <sub>5</sub>	, <b>Rr × rr</b> (Recurrent Parent A)
EIGHTH YEAR	BC <sub>6</sub>	(, Rr
NINTH YEAR	BC <sub>6</sub> F <sub>2</sub>	
TENTH YEAR	BC <sub>6</sub> F <sub>3</sub>	ແມ້ແມ່ນແມ
ELEVENTH YEAR		
TWELFTH YEAR		

Figure:1 Backcross method for transfer of a dominant gene (Source: Singh B.D. 2020)

#### 2. Transfer of a Single Recessive Gene

The transfer of characters governed by a recessive gene is carried out with a slight modification to provide for the identification of plants carrying the desirable recessive allele. Hence the backcrosses can't be made one after another. After the  $BC_1$  and after every two subsequent backcrosses, the  $F_2$  generation must be grown to identify the resistant plant progenies.

- **Hybridization:** The recurrent parent (RR) is crossed as a female parent with the resistant donor parent (rr) to produce  $F_1$  seeds.
- $F_1$  generation: All the  $F_{1s}$  are susceptible but are crossed to recurrent parent (rr) to produce BC<sub>1</sub> seed.
- **BC**<sub>1</sub> generation: The BC<sub>1</sub> plants are raised which will be RR and Rr in 50:50 proportion. As a recessive gene governs the resistance, hence all the progenies will be susceptible. All these plants are self-pollinated and the seed (BC<sub>1</sub>F<sub>2</sub>) of each plant is harvested separately.
- **BC**<sub>1</sub>**F**<sub>2</sub> **generation**: The BC<sub>1</sub>F<sub>2</sub> progeny rows are grown of each BC<sub>1</sub> plant and are screened for disease reaction. The progenies from RR plants would be uniformly susceptible to the disease while those from Rr will segregate and <sup>1</sup>/<sub>4</sub> of the progenies will be resistant. Such resistant plants similar to the plant type of recurrent parents are identified and backcrossed to produce BC<sub>2</sub> seed.
- **BC**<sub>2</sub> generation: The BC<sub>2</sub> plants are grown and each is backcrossed to a recurrent parent (RR) without any verification of its genotype to produce BC<sub>3</sub> seed. No resistance test is performed.
- **BC**<sub>3</sub> generation: The BC<sub>3</sub> plants are grown which have RR: Rr genetic composition in the ratio of 50: 50. The plants are self-pollinated to raise F<sub>2</sub>. The seeds from each plant are harvested separately.
- **BC<sub>3</sub>F<sub>2</sub> generation**: The BC<sub>3</sub>F<sub>2</sub> progeny rows are grown like those of BC<sub>1</sub>F<sub>2</sub> where resistant plants are identified and backcrossed to the recurrent parent to produce BC<sub>4</sub> seed.
- **BC**<sub>4</sub> generation: The BC<sub>4</sub> plants are grown and backcrossed to the recurrent parent. No resistance test was done.
- **BC**<sub>5</sub> generation: No resistance test done. To raise F<sub>2</sub> generation, plants are self-pollinated
- **BC**<sub>5</sub>**F**<sub>2</sub>**generation:** Plants are grown which shall segregate as <sup>1</sup>/<sub>4</sub> RR: <sup>1</sup>/<sub>2</sub> rr: <sup>1</sup>/<sub>4</sub> rr. A rigid selection is done for resistance and the characteristics of the recurrent parent A. The selfed seeds from selected plants are harvested separately.
- **BC**<sub>5</sub>**F**<sub>3</sub>: Individual plant progenies are grown and are subjected to resistance tests. Seeds from several similar rust-resistant homogeneous progenies are mixed to constitute the new variety
- **Yield test and seed multiplication**: These steps are similar to that followed in case of transfer of dominant genes.





#### 3. Backcrossing for Quantitative Characters

The effectiveness of the backcross method depends on variations in the number of genes and the relative influence of the environment, in addition to the genetic control of the trait of interest. A quantitative character like grain size, plant height, flowering, days to maturity, etc., which is influenced by a large number of polygenes requires a relatively larger population to be sampled in each backcross generation. Moreover, the lower heritability of many quantitative characters, due to the environmental influence on its expression, necessitates some form of progeny testing as well. Usually, the expression of quantitative characters in the recipient parent may not be at the same level as that exhibited in the donor parent because of the involvement of several genes, each with a small individual effect. Therefore, the procedure of the backcross method in the case of such characters is modified in such a way as to accommodate a maximum number of genes for an acceptable level of character expression in the recurrent parent. To ensure that performance is restored to a satisfactory level even after a potential loss of intensity during the backcrossing procedure, the donor parent with the highest intensity for the trait of interest may be preferred.

#### **Transfer of Quantitative Characters**

- The recurrent parent is crossed with the resistant donor parent to produce  $F_1$  seeds. The nonrecurrent parent (resistant donor) must have the characters in a more intense form as compared to the new variety.
- The progeny are selfed after each backcross and 500-1000  $F_2$  plants are selected and grown.
- At the end of the backcross programme,  $F_2$  plants are handled using the pedigree method.
- The progenies similar to the recurrent parent having the characters being transferred along with the other characteristics are selected and mixed up to make a new variety.

The use of molecular markers for the QTLs governing the concerning traits also aids in the transfer of quantitative characters. Some examples may be the improvement of TSS content of *Lycopersicon pennellii* (wild tomato) advanced by the use of Marker-aided selection (MAS).

# VI.APPLICATION OF THE BACKCROSS METHOD

# 1. Intervarietal Transfer of Qualitative Characters and Quantitative Characters with High Heritability.

The qualitative characters or simply inherited characters like disease resistance, seed color, plant height, etc., are the most appropriate for transfer using the backcross method. However, failure may occur due to tight linkage of the undesirable gene with the gene being transferred, which is referred to as *linkage drag*. On the other hand, quantitative characters may only be transferred if they have high heritability like plant height, earliness, seed size, and seed shape.

#### 2. Interspecific Transfer of Qualitative Characters.

Backcross breeding has also been proven successful to transfer simply inherited characters or qualitative characters mostly disease resistance. However, the common problems associated with interspecific gene transfer are (a) the transfer of undesirable genes along with the desirable gene, and (b) there may be the differential performance of the transferred gene in the genetic environment of the new species.

#### 3. To Improve Well-Established Variety Concerning One or a Few Characters.

Backcross breeding can be used to improve the well-established variety by introducing one or a few improved genes from a resistant donor. For example, two BB resistance genes xa13 and Xa21 were introduced into the Basmati rice variety Pusa Basmati 1 and were released as "Improved Pusa Basmati 1".

#### 4. Near-Isogenic Lines Production

NILs are lines that are identical in genotypes except for one gene. NILs can be easily produced using backcross breeding.

### 5. It may Help in the Recovery of Transgressive Segregants.

Introgressive segregants can be obtained by backcrossing the  $F_1$  to the recurrent parent once or twice followed by allowing the appearance of enough heterozygosity for transgressive segregants.

### VII. ACHIEVEMENTS

The backcross method has been developed, described, and widely used for the last more than half a century for the transfer of genes from one variety to another including related species, and also the transfer of cytoplasmic genes. Some notable achievement has been mentioned in the table below:

Sl. No	Crops	Variety evolved	Traits for BCB	Recurrent parent	Donor parent
	Wheat	Kharchia 65 <sup>4</sup>	Salinity and sodicity stress	Kharchia local	EG953
1.		NP852 <sup>5</sup>	Leaf blight resistance	NP761	EG953
		HUW234	Stem rust resistance	HUW234	PMBWIR4
2.	Cotton	Vijay	High ginning outturn	Broach Desi 8 (BD 8)	Goghari A-26
		Digvijay	Increased fiber length and early maturity	Vijay	1027A L-F
3.	Pearl millet	Tift23A	Downy mildew resistant	Tift23A	MS521, MS541A, MS570A

Table.1	Examples	of remarkable	achievements	through	backcross	breeding
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# VIII. MERITS

- Extensive field testing for the performance of the new variety is not required as it is the improved version of the already popular variety. This will reduce up to 3-5 years and considerably reduce the expenses.
- The outcome of a backcross program can be predicted and it can be reproduced anytime in the future.
- It is an ideal method to utilize unadapted germplasms which are generally known to be unproductive but serve as an important source of genes for resistance, quality, or even yield attributing characters.
- It is the only method for the correction of defects.
- A much smaller population is handled as opposed to the pedigree method.
- The only method was useful for gene transfer from related species, and transfer of the cytoplasm.
- Modifications could be done so that transgressive segregation could occur for quantitative' characters.

# **IX. DEMERITS**

- Except for the transferred characters, the new variety generally cannot be superior to the recurrent parent,
- Requires hybridization to be done in each backcross which is often time-consuming, labor-intensive, costly, and difficult.
- Undesirable genes closely linked to the transfer character may cause a nuisance.
- By the time the new variety is developed by the backcross method, the recurrent parent may have been replaced by other varieties which might be superior in yielding ability and other characteristics.

# X. COMPARATIVE STUDY

Table.2 Comparative study of different breeding methods in reference to backcross breeding

Particulars	Pedigree Method	Bulk Method	<b>Backcross Method</b>	
Application	Used in self and cross- pollinated crops	Used in self-pollinated crop	Used in self and cross- pollinated crops.	
Hybridization	Done only once	Done only once	Done repeatedly with the recurrent parent.	
Selection	Artificial selection plays a key role	Artificial selection plays a key role Both artificial and natural selection are involved		
Size of the F2 population	Smaller than the bulk method	Larger than the pedigree method	Smaller than both pedigree and bulk method	
Maintenance of records	Pedigree records maintained	No such records maintained	No such records maintained	
Time taken	Takes 14-15 years for the release of a new variety	More time as compared to the pedigree method	Takes 7 to 8 years for the release of a new variety	
Efficiency	Equally effective with both oligogenic and polygenic traits	Equally effective with both oligogenic and polygenic traits	More effective with oligogenic than polygenic traits	
Testing	Extensive testing is done before the release of a variety	Extensive testing is done before the release of a variety	Exclusive testing is not required as the new variety is an improved version of the old variety for the defects present in it	
Breeding procedure	Same for both dominant and recessive traits	Same for both dominant and recessive traits	Differ for both dominant and recessive traits	
Adaptation	Variety released has a narrow adaptation	Variety released a wide adaptation	Adaptation similar to the parent variety	
Popularity	Widely used	Less popular	Widely used	
Features of a new variety	Different from both parent	Different from both parent	Identical to parent variety except for the character transferred.	

Particulars	Pedigree Method	Bulk Method	Backcross Method	
Production of Substitution or addition lines or a gene transfer from related species	Not suitable	Not suitable	Suitable	
Breeder's involvement	Demands close attention from the breeder from F2 onward.	Does not require much attention from the breeder during the period of bulking	Require breeder's attention during the subsequent backcrosses	

#### XI. MOLECULAR APPROACH TO BACKCROSSING

Conventional backcrossing has several drawbacks, such as the need for a large number of plants for selection in each generation of backcrossing, the difficulty of introgressing quantitative traits, the limited effectiveness of recovering the genome of the recurrent parent, and the difficulty of negative selection of undesirable genes or avoiding linkage drag problems. Moreover, in the case of the transfer of a recessive gene, there is a requirement for more generations after alternative backcross generation to obtain a genotype with a target gene having the maximum recurrent parent genomic background. By combining molecular methods with backcrossing, these issues are eliminated. Molecular markers can greatly minimize the number of donor chromosomes in a considerable amount of time as compared to the conventional method.





#### XII. MARKER-ASSISTED BACKCROSSING (MABC)

A backcross program based on markers is known as marker-assisted backcrossing (MABC). It plays a vital role in the manipulation of 'classical' genes between elite lines or from wild species/ wild relatives or other genetic resources. It is the simplest form of MAS, and the main goal is to incorporate genes of interest from the suitable donor(s) into the genetic background of an elite cultivar or breeding line.

With the selection criteria changing from the selection of phenotypes by the naked eye to the selection of genes that control the trait of interest, either directly or indirectly, MABC is an approach that has been used to overcome the issue associated with the conventional backcrossing breeding method. The molecular markers are less affected by environmental vagaries, not tissue or stage-specific, and are more precise which can help in indirect selection provided the marker are tightly associated with the trait of interest.

MABC uses molecular tests to aid the selection of individuals that will be included in the next generation for the genetic improvement of various crops. The success of MABC depends on the number of target genes to be transferred, genetic control of the trait, the distance between the closest marker and target gene, the genetic background to which the gene is being transferred and the type of molecular marker used. In MABC, the markers can be used for:-

- Target gene selection indirectly with the help of markers associated closely with that gene (foreground selection).
- The individual plants with the highest proportion of the recurrent parent genome (background selection) are selected.
- Avoid linkage drag (recombinant selection).

When markers are utilized for foreground as well as background selections, the backcross programme is often called full MAS, or simply MABC.

# **Foreground Selection**

Tanksley (1983) proposed indirect selection of the target gene based on linked marker genotype, and Hospital and Charcosset coined the term "foreground selection" (1997)<sup>7</sup>. This selection is more effective than simple phenotypic selection as the phenotypic evaluation of many traits is either tedious, time-consuming, destructive, or might be dependent on specific threshold requirements. In this selection process, the breeder identifies plants carrying the desirable allele from the donor parent. To achieve this, the linked marker is used to genotype BC1F1 plants for the target gene. The genetic distance between the marker and the target gene determines the effectiveness of this process. The closer the marker; the more will be the effectiveness of foreground selection. The probability that a particular genotype can be obtained through the selection of marker genotype, i.e., the probability of selecting the correct individuals, is

 $P = (1 - r)^2$ 

Where r = recombination frequency of marker and gene

#### **Background selection**

Tanksley and colleagues (1983) advocated the use of genetic markers to aid in the recovery of recurring parent genomes, while Hospital and Charcosset coined the phrase "Background Selection" (1997). In this process, the breeder goes for genotyping of the progenies using a genome-wide marker that is considered distributed over the whole genome and is polymorphic between the donor parent and recurrent parent. The markers can be selected against the donor genome. The recurrent parent genotype can be restored with two or four backcrosses combined with background selection as compared with six to seven generations of backcrosses with phenotypic selection as is done in the conventional backcross breeding method.

#### **Recombinant selection**

Collard and Mackill (2008) gave the term 'Recombinant selection' and it is a special type of background selection that aims in reducing the size of an integrated gene i.e. the size of the donor chromosome segment containing the target gene. In other words, the transfer of the target gene into the recurrent parent with a minimum of the donor genome to reduce the risk of linkage drag is achieved through recombinant selection. The elimination of the undesirable effect of linkage drag is very difficult with the conventional method. The marker used for recombinant selection must flank and close to the target gene for foreground selection.



Figure 4:- Types of selection in MABC (Source:- IRRI knowledge bank)

# MABC for Gene Pyramiding

Nelson (1978) proposed the concept of gene pyramiding to develop durable resistance in crop varieties by bringing together a few to several different oligogenic from the same or different sources into the same plant. Gene pyramiding, in its broadest sense, refers to the incorporation of two or more genes that govern the same feature in a single line or variety. The genes controlling two or more distinct traits are introgressed into a single recurrent parent; this is known as multi-trait introgression rather than gene pyramiding.

#### Strategy for gene pyramiding

It is relatively simple to transfer two or more genes from a single donor parent; the donor parent needs to be crossed with the recipient parent and the  $F_1$  and subsequent progeny are repeatedly backcrossed. However, if the desired genes are present in different donor parents, the following strategies may be utilized:-

- Separate backcross program: By crossing each donor parent with the recurrent parent individually, the target gene from each donor parent is first recovered into the genetic background of the recurrent parent, either in the heterozygous or homozygous condition. The improved versions of recurrent parents obtained from different backcrosses are then crossed together to produce a complex hybrid. And finally, by selfing coupled with selection, the pyramided version of the recurrent parent having all the target genes is recovered from this hybrid.
- **Single backcross program**: -In this approach, all the donor parents are accommodated into a single backcross program according to a suitable mating scheme. The different mating schemes are as follows;-
  - Symmetrical mating scheme: Different donor parents are crossed to the recurrent parent in pairs, the F<sub>1</sub>s are then crossed to obtain double-cross progenies which are ultimately crossed to obtain the complex hybrid.

> Tandem mating scheme: The complex hybrid is created by first mating the recurrent parent with one of the donor parents, then mating the resulting  $F_1$  to the second donor parent, and so on, until all the donors have been successively mated.

The complex hybrid obtained from either of the schemes is backcrossed with the recurrent parent to recover the pyramided version of the recurrent parent.





Figure 5: Strategies for gene pyramiding (Source: Singh B.D. and Singh A.K. 2014)

#### Applications of MABC

MABC offers significant advantages in cases: -

- It can be used to assist in the selection of such traits for which phenotypic screening is difficult or impossible and expensive to carry out by conventional backcrossing.
- If the trait of interest is expressed late in the plant development (like fruit and flower characters or adult characters), the use of MABC can hasten the process of selection as markers are not stage-specific.
- It can facilitate the introduction of characters with low heritability—that is, characters that are significantly influenced by their environment.
- For incorporation of genes for resistance to diseases and insect pests effectively and without even meeting the threshold requirements. Screening can easily be done in the laboratory with the help of molecular technologies.
- In comparison to the conventional method, MABC can be utilized for the transfer of genes with recessive genetic control of a particular trait, without much difficulty.
- Multiple genes for one or more traits within the same cultivar can be accumulated, which is referred to as gene pyramiding.

#### Achievements

MABC has been used to develop many crop varieties with improved quality characters and nutrient content. In the year 2006, Monsanto, USA developed the first variety of maize hybrid and offered it for commercial cultivation in the USA. Some notable achievement of MABC has been discussed in the table below:

SI. No	Сгор	Variety Involved / Target Variety	Variety evolved	Gene(s) / QTL (s)	Traits evolved	Reference
1	Rice	Swarna and Samba Mahsuri	Swarna Sub 1	Sub-1	Submergence tolerance	Toojinda <i>et al.</i> 2005
		Ranjit	Ranjit Sub 1	Sub-1	Submergence tolerance	Chetia <i>et al.</i> 2018
		Samba Mahsuri	Improved Sambha Mahsuri	xa5, xa13, Xa21	Bacterial Blight resistance	Sundaram <i>et al.</i> 2008
		Pusa Basmati 1	Improved Pusa Basmati 1	xa13, Xa21	Bacterial Blight resistance; 11.9% higher yield than PB 1	Joseph <i>et al.</i> 2004, Gopalkrishnan <i>et al.</i> 2008
		Taraori Basmati and Basmati 386	Improved Taraori Basmati and Improved Basmati 386	Xa21 and Xa13	Bacterial Blight resistance	Pandey <i>et al.</i> 2013
			KhaoDawk Mali 105	bph3	Brown Plant Hopper resistance	Jairin <i>et al.</i> 2009

Table:3 Examples of Marker-assisted breeding in crop improvement

		PRR78, Basmati rice		Pi5 and Pi54	Blast resistance	Singh <i>et al.</i> 2012
		Q5DB		Saltol	Salt tolerance	Huyen <i>et al.</i> 2013
		Thai Rice		Pup1	Phosphorous tolerance	Chin <i>et al.</i> 2011
		Shengdao 15, Shengdao 16 and Xudao 3		Stv-bi	Rice stripe resistance	Xu <i>et al</i> . 2013
		Improved variety Samba Mashuri		Gm8	Gall midge resistance	Sama <i>et al.</i> 2012
		Japonica Varieties		sd1	Semi-dwarfing	Negrao <i>et al.</i> 2010
2	Barley		Sloop SA and Sloop Vic		Multiple disease resistance	Singh B.D. 2020
3	Maize		Vivek QPM 9	<i>o</i> 2	Extra early- quality protein	Semagn <i>et al.</i> 2006
4	Pearl millet		ННВ 67-2		Downy mildew (Sclerospora graminicola) resistance	Singh B.D. 2020

#### Advantages of MABC over conventional backcrossing

- The recovery of the recurrent parent can be achieved by BC4 or BC3, or even BC2 with the help of MABC; whereas conventional backcrossing needs six backcrosses to recover the recurrent parent.
- Environmental factors cannot affect the molecular markers and offer great potential for the selection of molecular markers for MABC.
- MABC reduces the linkage drag of donor chromosomes within two or three backcross generations which may be difficult using conventional backcrossing.
- The characteristics of disease resistance can be introgressed without inoculation of a pathogen with the help of tightly linked target genes of molecular markers.
- With the help of MABC, it is possible to select the lines using marker gene expression, which is difficult through conventional breeding techniques.

#### Conclusion

With the recent advancement of technologies in the field of genomics, plant breeders have challenges to meet the ever-increasing demand for producing novel varieties. Without a doubt, conventional backcross breeding has proven to be an important tool in improving the defects of many commercial varieties in order to improve their economic prospects, however, this progress can be accelerated by the use of molecular techniques. Several backcrossed varieties are now frequently produced and preferred by farmers.

The development of commercially viable improved cultivars can be achieved through molecular marker-assisted breeding in different crops. More effective selection criteria based

on marker data are sought, leading to the better genetic composition of plants, a sharper description of the genetic architecture of quantitative characters, a better understanding of the epistasis of QTL, and a broader understanding of genotype by environment interactions. With the consideration of bright future possibilities and potential effects of MABC, the cost of utilization of the markers is the possible limiting factor in its implementation on a large scale. An efficient cost-effective MABC in combination with the traditional breeding programs will aid in its wider application internationally, particularly in developing countries. Plant breeders must now wisely make use of the novel techniques in MAB to create commercially viable enhanced cultivars that will assure food and nutritional security in the face of changing climatic conditions. This goal will be met only if MAS is integrated into traditional breeding practices rather than being viewed as a substitute.

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