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Improvement of rice crop by Marker-assisted Backcross method

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Abstract - *MABC (Marker-assisted Backcross Method) approach plays vital role for basic research applications in rice to develop new and advance varieties with much greater precision than conventional backcrossing. MABC is used to remove abiotic stress. Main function of MABC is to reduce the chromosome size of a donor parent. It also reduces linkage drag. Different tolerance conditions like salt tolerance, phosphorous tolerance, submergence tolerance can be introduced in different rice varieties by MABC method. This will help in improving rice variety. It will help in rice cultivation even in adverse conditions like flood. Thus, MABC should be used to improve the quality of rice variety and increase its productivity.*

Keywords: Conventional backcross, MABC, tolerance, cultivar, rice yield

1. INTRODUCTION

Rice (Oryza sativa) is a monocot plant that belongs to family Poaceae. Being a part of a balanced diet and the most consumable staple food throughout the world. It is necessary to focus on the production of improved variety of rice. Following statistics suggest that rice is the one of the most demanding cereal in the world. [1]

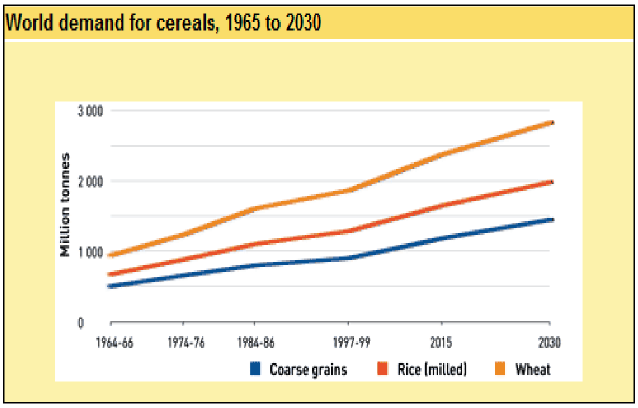

Fig1: Increase in demand of Rice [1]

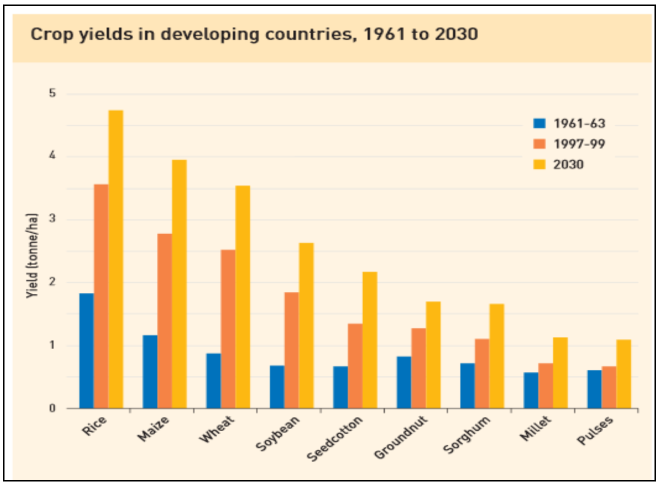

Fig2: Increase in Rice yield [2]

Rice plants if planted in a perfect environment usually grow faster and results in higher yield. By cultivation of repetitive crops it become resistant to weeds or pests and diseases, it needs less fertilizer, or thrives in saline water having enhanced levels of micronutrients. Improvement can possibly be attained through further expansion of irrigation facilities and subsidies for irrigation, fertilizer, seeds, machinery, and appropriate technologies for increasing rice productivity in both irrigated and rainy areas. [2]

1.1 Nutrient Rich: Rice

Following tables illustrates the nutritional content i.e.

- Protein, Carbohydrate, Fats,
- Amino Acids,
- Vitamins & Minerals in rice (Oryza sativa)[3]

General Information	
	Amount 130
Water	69%
Protein	2.4%g
Carbohydrates	28.7g
Fats	0.2g
Saturated	0.05g
Monosaturated	0.06g
Polyunsaturated	0.05g
Omega-3	0.01g
Omega-6	0.04g

Vitamins		
	Amount	**%DV**
Vitamin B1 (Thiamine)	0.16mg	14%
Vitamin B2 (Riboflavin)	0.02mg	1%
Vitamin B3 (Niacin)	1.49mg	9%
Vitamin B5 (Panthothenic acid)	0.4mg	8%
Vitamin B6(Pyridoxine)	0.06mg	5%
Folate	59µg	15%

Minerals		
	Amount	**%DV**
Calcium	1mg	0%
Iron	1.46mg	18%
Magnesium	8mg	2%
Phosphorous	33mg	5%
Potassium	26mg	1%
Zinc	0.4mg	4%
Copper	0.07mg	8%
Manganese	0.36mg	16%
Selenium	7.5 µg	14%

Fats	
	Amount
Saturated Fatty Acids	0.051g
14:0	1mg
16:0	46mg
18:0	3mg
Monosaturated Fatty Acids	0.058g
16:1	1mg
18:0	58mg
Polyunsaturated Fatty Acids	0.05g
18:2	41mg
18:3	9mg

Amino Acids	
	Amount
Tryptophan	27mg
Threonine	84mg
Isoleucine	102mg
Leucine	195mg
Methionine	56mg
Cytosine	48mg
Tyrosine	79mg
Valine	144mg
Arginine	197mg
Histidine	56mg
Lysine	85mg

1.2 Worldwide Consumption of Rice

Green revolution's success led to rise in rice consumption per capita in Asia from 85kg/year in '60s to almost 103kg in early '90s.

Table 1: The world wide consumption of rice in 2014/2015 (in 1,000 metric tons) [4]

Country	Consumption(in 1,000 metric tons)
China	148,400
India	99,351
Indonesia	38,600
Bangladesh	35,300
Vietnam	21,900
Philippines	13,200
Thailand	11,100
Burma	10,700
Japan	8,313
Brazil	7,900
Nigeria	6,400
South Korea	4,450
United States	4,126
Egypt	4,000

2. Methods for improvement in production and variety of crop

2.1 Backcross Method:

This is the most commonly used conventional method to insert a trait or a transgene into an elite variety. The variety that donates a gene refers to a 'donor parent' while that accepts a gene refers to a 'recipient parent'; and when this recipient gene is used repeatedly refers to a 'recurrent parent' [6] This approach can be used to transfer a disease resistant gene, from one line, often an unimproved line, to another line that is typically an elite breeding line. [7]

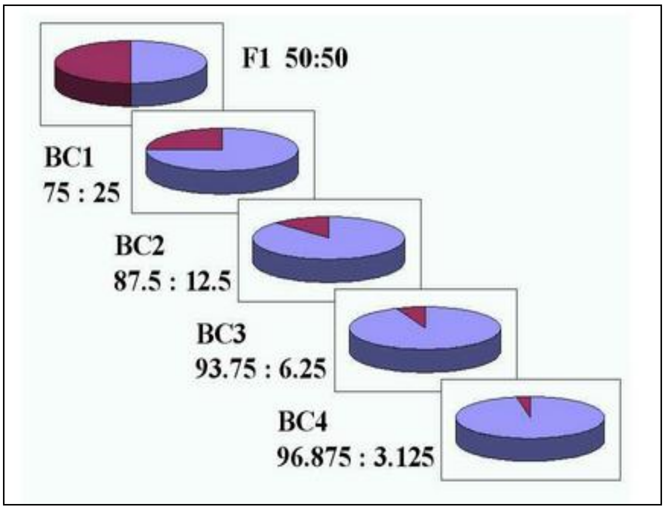

Fig 3: The contribution of the donor parent genome is reduced by half with each generation of backcrossing. Percentages of recurrent parent (light purple) are expressed as a ratio to percentages of donor parent (dark red-purple). [7]

Factors necessary for backcross method:

(i) Recurrent parent Selection: Factors such as traits, agronomic performance, target environment, and popularity with farmers are considered while choosing the recurrent parent. [8]

(ii) Screening for target trait: Phenotypic screening is done which clearly contradistinguishes between segregating progeny due to the number of rounds of crossing. High heritability traits will be more effectively incorporated compared to traits which have low heritability. [5]

(iii) Number of backcrosses: Earlier in backcross generations, breeders may visually select progeny having closely resembling characteristics with the recurrent parent while in the second generation of backcross; it may not be possible to discriminate between backcross progeny and the RP. Hence, it is believed to do the repetitive steps of backcross to attain the required/ desirable gene features from recurrent parent. [5]

2.2 Limitations of Backcross Breeding:

For transferring of quantitative traits, backcross is not an effective method. The trait should be readily identifiable and highly heritable for this method in each generation. The presence of undesirable linkages during the backcrossing may prevent the cultivar being improved from promoting the performance of the original recurrent parent. Recessive traits take more time to transfer. Loss of genetic information of recurrent parent may occur in backcross method. [5] Chances of recombination become less in backcross breeding.

3. Marker-Assisted Backcross Method

MABC is an accurate and efficient method of breeding preserving the important features of recurrent parent. In this method, a marker is used that may be based on morphological characters, biochemical or on DNA/RNA variation for indirect selection of a genetic consideration or consideration of a trait of interest (e.g., disease resistance, abiotic stress tolerance, productivity and quality). This process is used in plant and animal breeding. [5].

The principle of MABC is to integrate the targeted gene obtain from donor parent with the defined locus of recurrent parent. MABC helps in reducing the linkage drag that prevents the loss of traits of recurrent parent. It also get positive results for the recovery of characteristics of recurrent parent by minimizing the chromosome size of the donor parent. Markers that are used may be they are simple sequence markers or may microsatellite markers. [5]

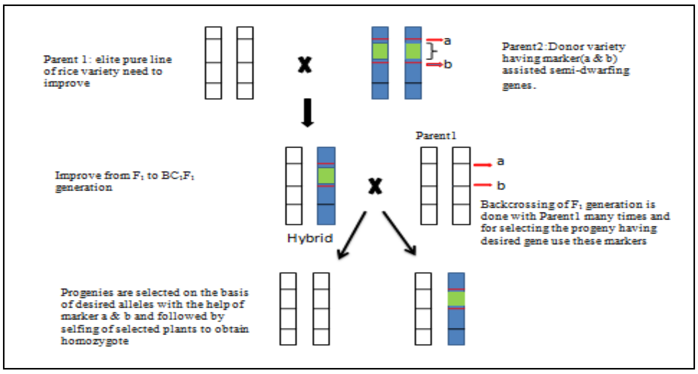

Fig 4: Marker assisted back cross

Factors responsible for the efficiency of MABC are-

- Size of population for each backcross generation.
- Markers distance from locus.
- Number of markers is used in each selection process.[9]

(i) Gene/Target/QTL/Foreground selection

In this step, the selection of the plants having the marker allele of the donor parent at the target locus. The objective of foreground selection is before final backcross is completed there should be prevention of target locus in a heterozygous state (one is the RP allele and another one is donor allele). After this the self-pollination of selected plants can be done and progeny plants identified that are homozygous for the donor allele. Target gene that is tightly linked with markers and those markers have already been developed or QTL are used for the selection of plants that having the target gene in early progenies by selecting the target locus. This is referred to as 'foreground selection' [10] although referred to 'positive selection' [11]. Tanksley proposed Marker-assisted foreground selection [12]. Some screening procedures are time-consuming and laborious for some traits, there this method help us to overcome the problem. It can also be used to select for reproductive-stage traits and allowing the best plants to be identified for backcrossing in seedling stage. [5]

(ii) Recombinant selection

In second level selecting the BC progeny having not only the target gene but also showing the phase of recombination between linked flanking markers and target locus which is termed as 'recombinant selection'[13]. This selection is done to reduce the donor chromosome segment size having the target locus (i.e. size of the introgression). This is important because sometimes many objectionable genes and unlinked regions get linked to the target locus coming from donor parent that provide adverse affect to the performance of growing crop plants and these move more faster than the donor segment [14]. Basically two BC generations are considered for recombinant selection [15]. It must be emphasized that this is only possible for genes or QTLs for which the map position has been well defined. Before the recombination selection fine and accurate mapping or we can say high resolution mapping of all the genes or QTLs should be done that help us for easily selection of BC generation carrying the target gene. Furthermore, recombinant selection can minimize the size of the donor chromosome segment, thus reducing ***'linkage drag'*** of the plant breeder [16] as mentioned in ***Figure 5.***

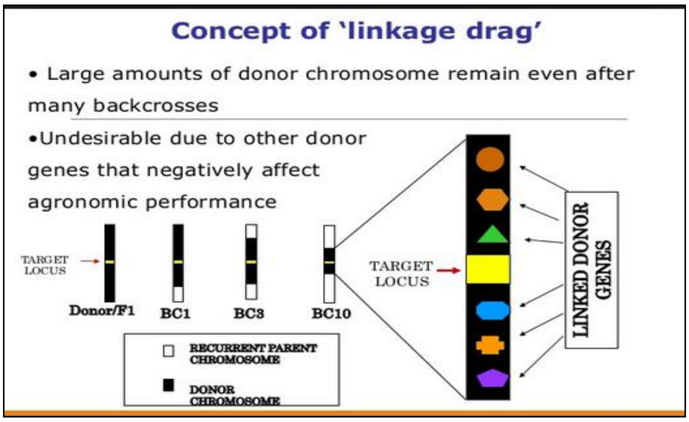

Fig 5: Schematic representation of transferring undesirable genes with targeted gene [17]

(iii) Background Selection

In the third level of MABC approach, all genomic regions can be selected in background selection using RP marker alleles and on phenotypic screening of rice plant one can select the plant having target locus. This assortment is important in order to diminish unwanted genes incorporated from donor parent.

During MABC, background selection helps to hasten the development of recurrent parent's feature/factor in further generation has been referred to as 'variety development or enhancement [18] and 'conversion of complete line' [19].

In **figure 4**, for clarity each vertical bar represents the entire genome of the plant. The front resistant (FR) trait can be screened phenotypically only at the end of growing season, but the FR gene is linked with a DNA marker and PCR is the most convenient method to identify it. To move the FR gene into established elite line, the seedlings in each backcross generation are screened by PCR for the marker, indicating the presence of the FR trait thereby increasing efficiency of backcross method [5].

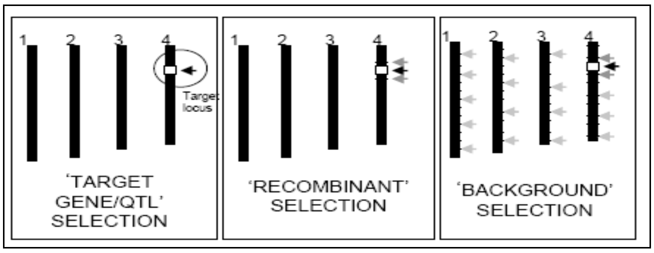

Fig 6: Three Levels of Selection during Marker assisted backcrossing

3.1 Marker-Assisted Breeding helps to overcome the problems related to abiotic stress

3.1.1 Submergence tolerant high yielding in Bangladesh

Scientists worked on Submergence tolerant high yielding rice variety that was developed by using BR11 as a recipient parent and making approaches for selection by using the methods- foreground, phenotypic and background selection. Recombinant selection was found essential to minimize linkage drag by BC2F2 generation. For growing rice in flash flood prone environment submergence is one of the important factor [21]. At Bangladesh Rice Research Institute the BRLs were found submergence tolerance compared to the check varieties under complete submergence for two weeks, and produced higher yield where they compared to the isogenic Sub1-line under controlled submerged condition [22]. In 2010, BRL IR85260-66-654-Gaz2 was released as BRRI dhan52, which was the first high yielding submergence tolerant variety in Bangladesh. The trait is not only represented by the QTL region of SUB1 [23]. This study demonstrated the efficiency of better adaptability and recombinant selection and of the newly released submergence tolerant high yielding variety in flash flood prone areas of the country with respect to submergence tolerance and yield potential. [24]

3.1.2 Improved Submergence tolerance of Vietnamese Rice

It is reported that four different field trials had been done to assess the levels of submergence tolerance ability of the imported rice cultivars. [25]
Two rice varieties OM1490 and AS996 are first successfully improved varieties by MABC method and make them tolerance against submergence [26, 27]. IR64-Sub1 has the highest submergence tolerance with stable character and high yield was taken as donor plant and Bacthom7, Vietnamese rice as a recipient plant. Closely linked markers for Sub1 are used for the foreground selection, background selection in the backcrossing generation by monitoring the locus [28]. For foreground and recombination selection sequence information and mapping of Sub1 locus is done [29]. Collard and Mackill (2008) introduced previously the two markers out of five that are used to link with *Sub1* [30]. Improved rice variety helps to come out the problems produced by nature as for growing in submerge areas.

3.2 Phosphorous Tolerance

To make the advance variety of rice one more factor is considered i.e. phosphorous tolerance. The gene model were predicted targeted introgression of rice variety and mapped the QTL region of Pup1 gene based on marker that are evenly distributed over the map. Genetic markers are very important for the validation of map. In more than 80 diverse rice varieties accession was tested. Pup1 is effective genetic backgrounds and environmental factors under field condition were confirmed by first phenotypic evolution of introgression line [31].

3.3 Semi- Dwarfing

Gene sd1 was contributed the semi-dwarfing characteristic to rice variety *(japonica)* [32]. MABC is used for the introgression of donor gene to the recipient plant that helps to adopt the characteristic of semi dwarfing. [33]

3.4 Improved Salt Tolerance of Rice by Molecular Breeding

In BT7 cultivar rice improve the salt tolerance, QTL region of BT7 was introgrased by donor plant FL 478. From *Saltol* to F478 into BT7 transfer successfully the alleles carrying the positive gene after three backcrosses. The plant IL32 and IL34 in BC_3 F1 population accepted recurrent genome recovery of up to 99.2% and 100% respectively [34]. Microsatellite markers or simple sequence repeat (SSR) or were preferred for the study of molecular markers [35]. All improved lines showing the alleles of *Saltol*. QTL for salt tolerance was Saltol that was identified in Pakkali cultivar and presence of this QTL shows confirmation of salinity tolerance [36]. We have the successful breeding lines for the salt tolerance by MABC. In North Vietnam OMC52000 rice cultivar improve by applying Marker assisted selection (MAS) for salt tolerance. [37]

3.5 Advantages of MABC over conventional backcross breeding

To recover recurrent parent genome need six backcrosses in conventional method but we get earlier in MABC so it saves our time. [10, 15, 39, 40]
Consistency is maintained in MABC approached as environmental stresses hamper the expression plant characteristics in conventional methods [5].

For the biosafety purpose as without inoculation of pathogen, the definite character for disease resistant can be conducted by using molecular markers of specific characteristic and linked it with the target gene.
Selection of polygenic characters can be identified more accurately. [5]

3.6 Lucrative of MABC Program

Inheritance of the trait, field/glasshouse and labour costs method of phenotypic evaluation, and the cost of resources are such factors which influence the cost of MABC utilization. In some cases, MABC is more costly than phenotypic screening methods [41, 42]. But on the other hand, phenotypic screening is more time consuming and cannot be easily done. By using markers it, may be low cost and more suitable [42]. It is considered from some cases that MABC have improved the selection efficiency of recurrent parent over the phenotypic selection from conventional breeding methods. Evolution backcross progenies may be considered expensive by using DNA markers. So cost estimation should be clear before performing the experiment.
It is very necessary to minimize the cost effect that may be done by standardization and optimization of markers during genotypic mapping. MABC is very effective method for plant breeders because it improved varieties and get result as soon as possible. [5]

4. Comparison of conventional and Marker-Assisted backcrossing

With conventional backcrossing, it takes a minimum of five to six generations to recover the recurrent parent. Data from simulation studies suggests that at least two but possibly three or even four backcross generations can be saved by using markers.

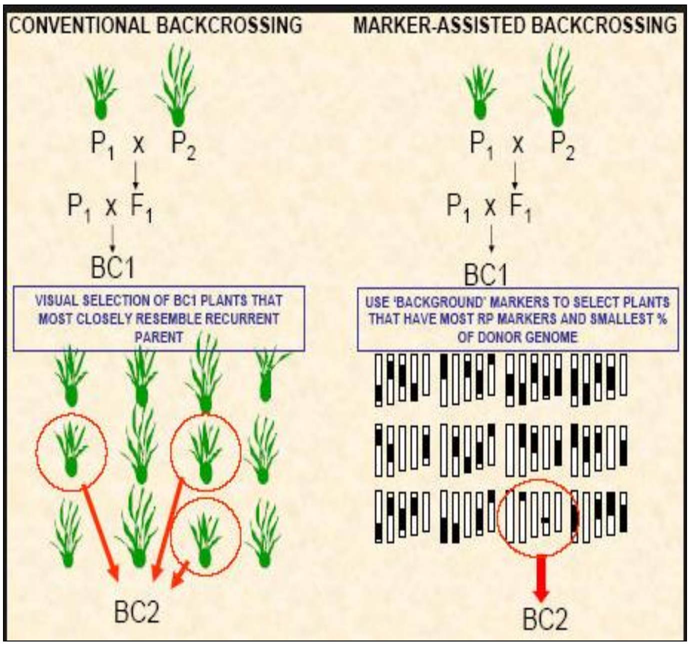

Fig 7: Conventional Vs Marker Assisted Backcross [44]

Table 2: Comparing the expected recovery of recurrent parent in conventional and Marker-Assisted backcrossing in subsequent generation. [11]

		% of recurrent parent (RP)	
Backcross generation	Number of Generation	Marker-Assisted backcross	Conventional backcross
BC1	70	79.0	75.0
BC2	100	92.2	87.5
BC3	150	98.0	93.7
BC4	300	99.0	96.9

CONCLUSION AND FUTURE PROSPECTS

With the growing technologies and approaches MABC is more popular to rice researcher as a potential and simple techniques, because the major benefit of the MABC techniques is to use the varieties that a already well accepted by farmers so that the improved variety that are already grown offer to develop new traits in it by using this approach. In addition, the MABC approach plays a vital for basic research application in rice to enhance new varieties with much greater accuracy rather than using conventional backcrossing method.
MABC has generated good deal of expectation, which in some cases let to over/optimize and in other to disappointment because many of the expectation have not been realized. The most significant cost prior MABC is the development of genetic linkage map for the species of interest. [5]

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