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Review article

A REVIEW ON MARKER ASSISTED SELECTION IN CROP IMPROVEMENT

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INTRODUCTION

Marker assisted selection is the breeding strategy in which selection for a gene is based on molecular markers (DNA markers) closely linked to the gene of interest rather than the gene itself, and the markers are used to monitor the incorporation of the desirable allele from the donor source. Selection of a genotype carrying desirable gene via linked marker (s) is called Marker Assisted Selection.

Plant breeding creates novel combinations of genes and develops new crop varieties of economic value. Rate of increase in crop yield is currently declining because production potential of modern cultivars has been remained stagnant due to narrow genetic base and constraints due to biotic and abiotic stresses.

Many disease resistant varieties have been developed but they have not been widely adopted. One reason may be that these tolerant varieties lack many of desirable traits of the widely grown varieties referred to as mega varieties that are cultivated over larger areas. Replacement of these megavarieties with modern cultivars cannot be possible as farmers are well accepted these varieties. These megavarieties despite having many agronomically desirable characters often, susceptible to diseases and abiotic stresses. By correcting one or few of defects of these widely adopted cultivars, crop improvement can be achieved. Conventional breeding is time taking and laborious and even then the release of an improved variety cannot be guaranteed. Marker assisted selection speed up the creation of cultivars and shorten the development time of varieties. Measurement of resistance is laborious, and the low heritability of the trait limits the effectiveness of selection in breeding programs. Molecular markers linked to the trait would therefore provide a superior selection screen to assist in transferring resistance into improved cultivars. (Ni *et al*., 2001). Marker-assisted selection (MAS) is gaining considerable importance as it would improve the efficiency of plant breeding through precise transfer of genomic regions of interest (foreground selection) and accelerating the recovery of the recurrent parent genome (background selection). MAS has been more widely employed for simply inherited traits than for polygenic traits, although there are a few success stories in improving quantitative traits through MAS. (Babu *et al*., 2002).

Molecular marker aided selection involves scoring for the presence or absence of a desired plant phenotype indirectly based on DNA banding pattern of linked markers on a gel or on autoradiogram depending on the marker system. The rationale is that the banding pattern revealing parental origin of the bands in segregants at a given marker locus indicates presence or absence of a specific chromosomal segment which carries the desired allele. This increases the screening efficiency in breeding programmes in a number of ways.

- ✓ Selection can be carried at seedling stage
- ✓ Useful for traits that are expressed at later developmental stages, undesirable plant genotypes can be quickly eliminatd
- ✓ Individual plants can be selected based on their genotype.
- ✓ Target genotypes can be more effectively selected
- ✓ Total number of lines that need to be tested can be reduced. Since many lines can be discarded after MAS early in a breeding scheme.
- ✓ Screening of segregants for presence of genes resisance to biotic and abiotic stresses.

MAS should be able to offer significant advantages in cases where phenotypic screening is particularly expensive or difficult, including breeding projects involving multiple genes, recessive genes, late expression of the trait of interest, seasonal considerations, or geographical considerations. In addition to reducing the cost of breeding, MAS also has the potential to generate time savings. Depending on the benefits that a breeding program realizes from earlier release of its breeding products (which typically differ between the private and public sectors), the value of these time savings can be enormous—often justifying the additional cost involved in using MAS.(Dreher *et al*., 2000).

Steps in MAS procedure:

- ✓ Identification of divergent parents.
- ✓ Generation of a suitable mapping population.
- ✓ Identification of polymorphic probes.
- ✓ Analysis of marker segregation on the mapping population.
- ✓ Establishment of linkage
- ✓ Marker validation

MAS Breeding Schemes

1. Marker-assisted backcrossing
2. Pyramiding
3. Early generation selection
4. 'Combined' approaches

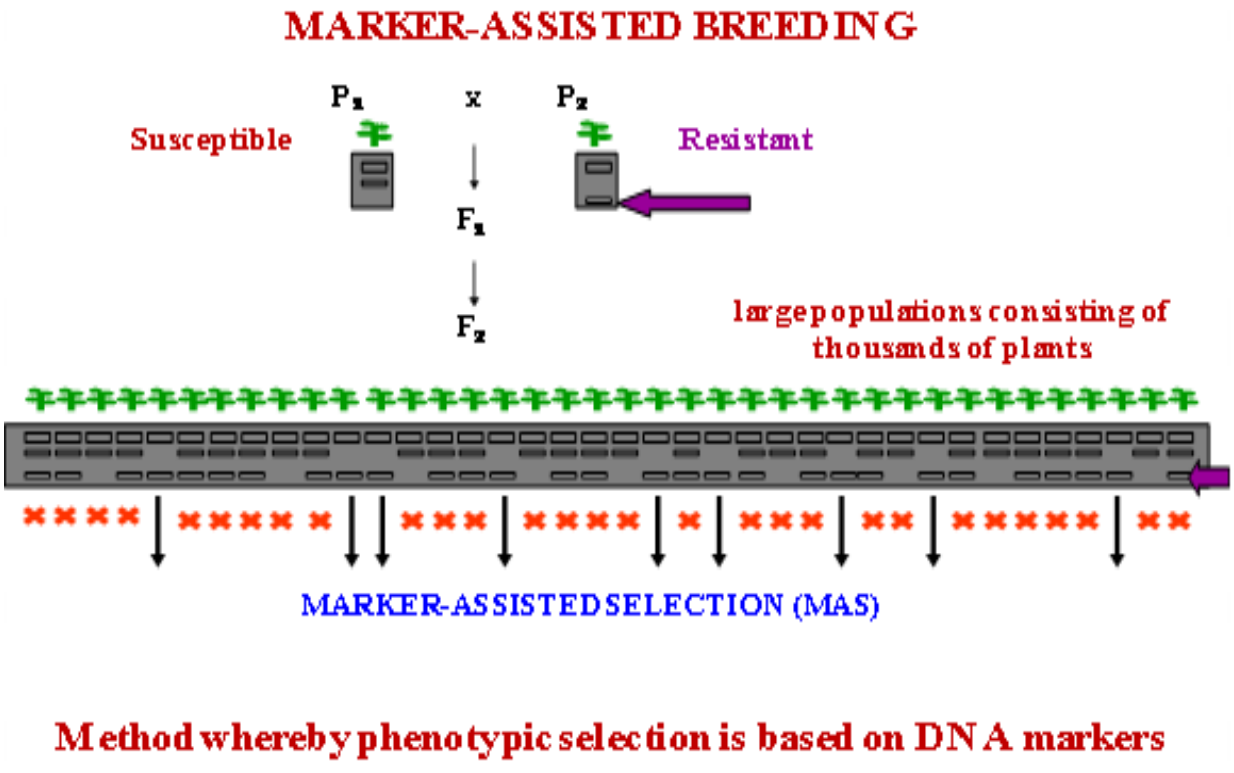

Figure 1: Marker assisted breeding

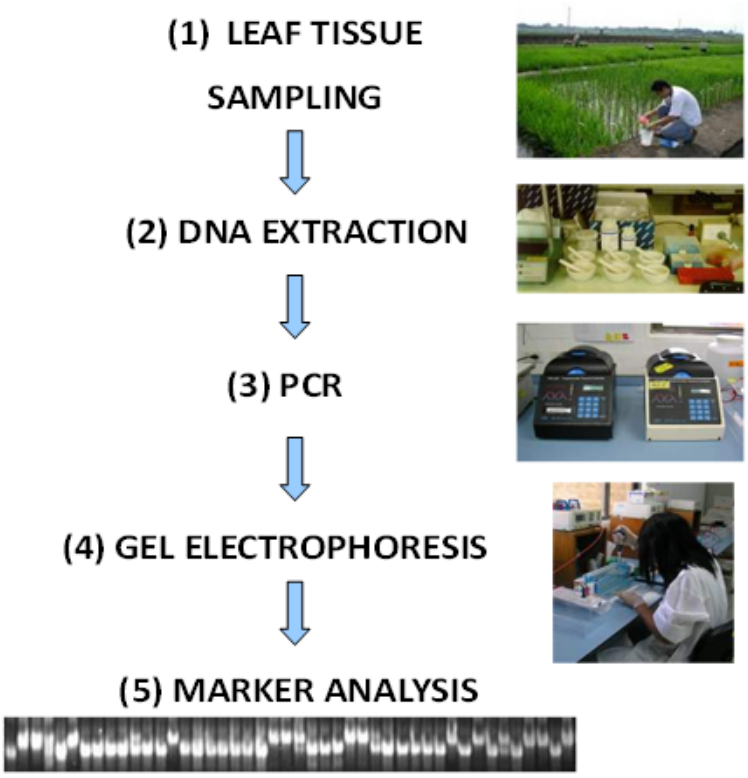

Figure 2: Overview of marker genotyping

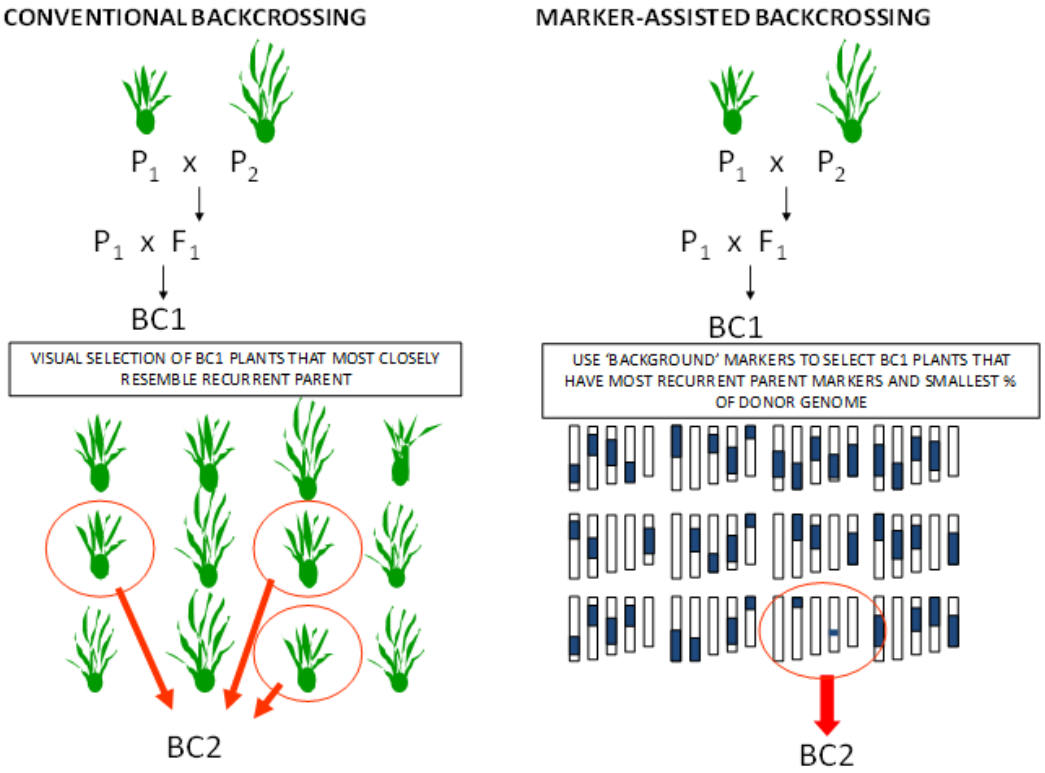

Figure 3: Comparison between conventional backcrossing and background selection during marker assisted backcrossing.

Marker assisted backcrossing have several advantages over conventional backcrossing:

- Effective selection of target loci
- Minimize linkage drag
- Accelerated recovery of recurrent parent

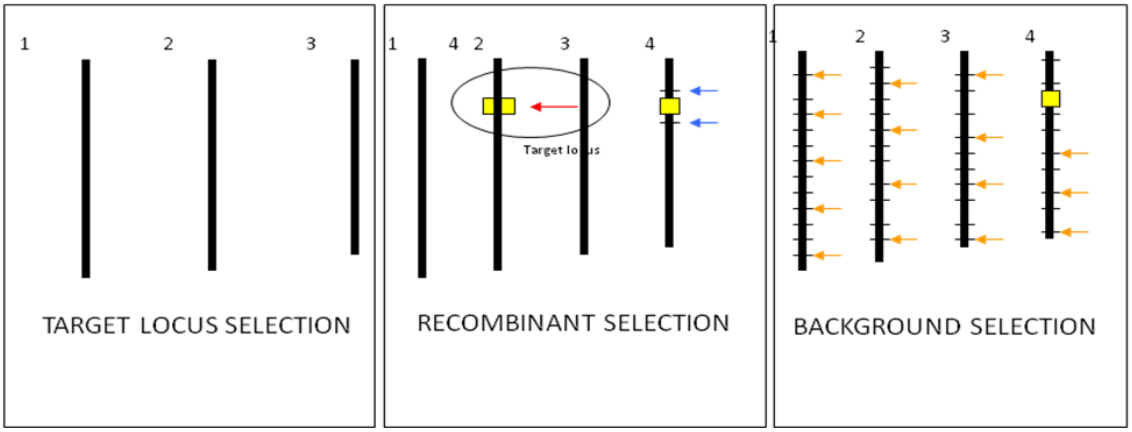

Figure 4: foreground selection (target locus selection) and background selection of marker assisted backcross breeding.

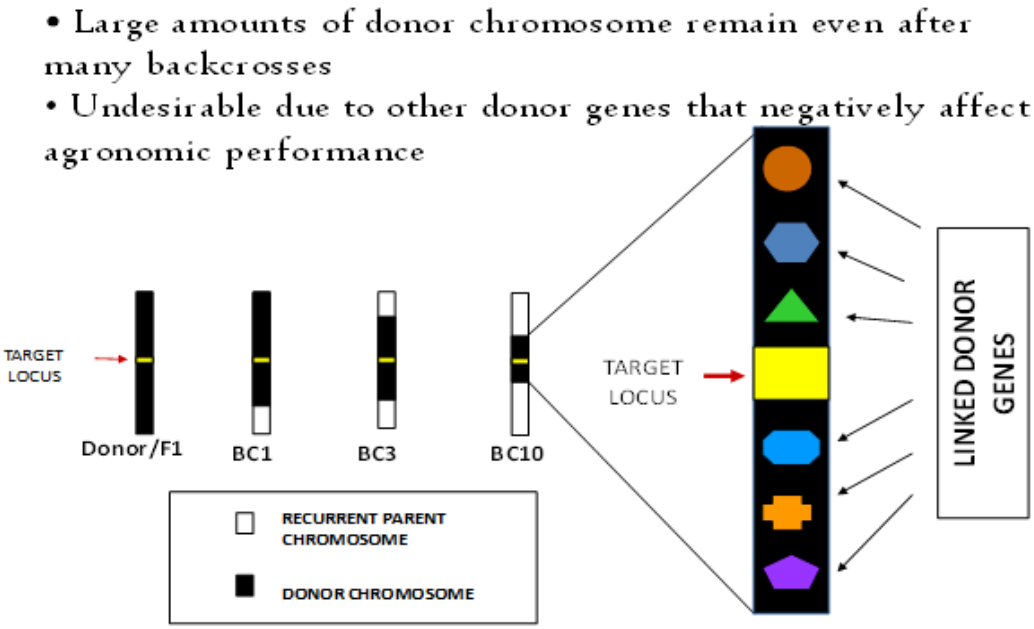

Figure 5: concept of linkage drag

Gene Pyramiding

Combining several genes together into a single genotype is known as gene pyramiding**.** Pathogens and insects are known to overcome resistance provided by single genes, which are fragile and often broken down easily. Durability of resistance has been increased by developing multilines and by pyramiding of resistance genes. Not easy to identify plants containing more than one gene through conventionl breeding.With MAS, new R gene segregation can be followed even in the presence of the existing R gene(s), and hence R genes from diverse sources can be incorporated in a single genotype for durable resistance. Large-scale and long-term cultivation of varieties carrying a single gene for resistance resulted in a significant shift in pathogen race frequency with consequent breakdown of resistance in these cultivars. To combat the problem of resistance breakdown, pyramiding of resistance genes into different cultivars is being carried out. Pyramiding of resistance genes is now possible with molecular markers that are developed for individual genes. (Kameswara Rao *et al*., 2002).

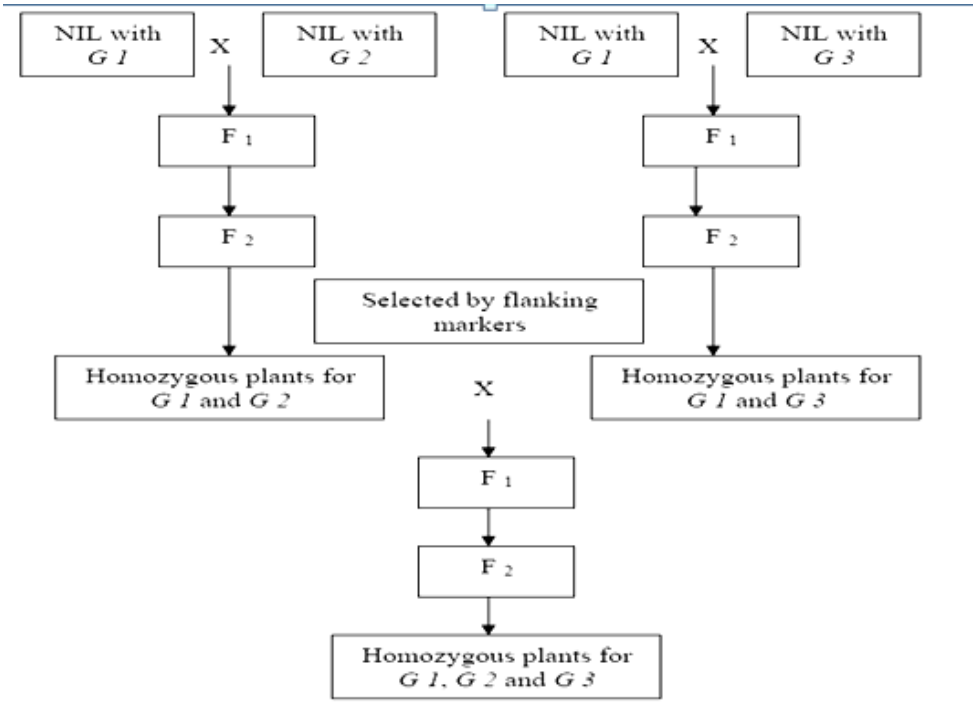

Figure 6: schematic representation of marker assisted pyramiding of major genes

Combining bacterial blight resistance and basmati quality characteristics by marker assisted selection in rice (Case study by Joseph *et al*, 2004 **)**

Basmati rice from the Indian subcontinent is highly priced in the international market for its unique quality. (desirable kernel dimension, appealing aroma, palatability, easy digestibility and longer shelf-life).The traditional basmati cultivars are tall, prone to lodging, photoperiod and temperature sensitive and low yielding. Pusa Basmati 1 (PB1) was the first semi-dwarf, high yielding Basmati quality rice variety. This variety became susceptible to BB disease caused by *Xanthomonas oryzae* pv. oryzae (Xoo) .

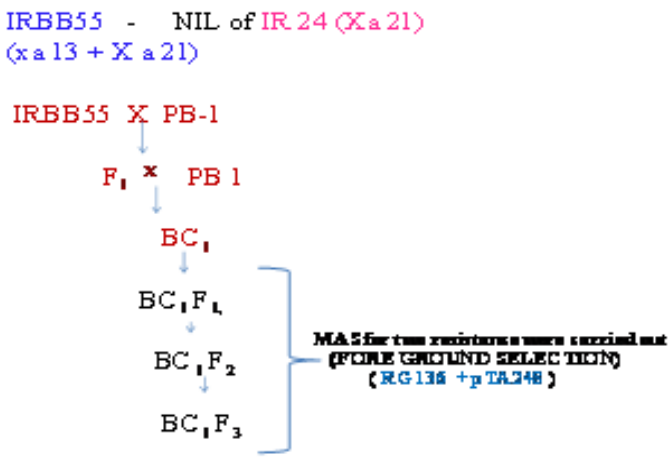

Figure 7: schematic representation of foreground selection of MABB.

MABB approach was used to incorporate BB resistance in PB1 using IRBB 55 (an isogenic line of IR 24) as a donor for BB resistance genes xa 13 and xa 21 .The CAPS marker RG 136 linked to *xa* 13, and STS marker pTA 248 linked to *Xa 21* were used for foreground selection. MAS for foreground genes *xa* 13 and *Xa 2*1 was coupled with phenotypic selection for agronomic , grain and cooking quality traits in BC_1 F_1, BC_1 F_2 and BC_1 F_3 generations in figure 8.

PCR amplification of BC1F2 plants with the marker pTA248 linked to *Xa21, Top row: M: Gene ruler 100 bp ladder plus; Lane* 1: PB-1; Lane 2: IRBB21; Lane 3: IRBB55; Lane 4: IRBB59; Lane 5: IR 24; Lanes 6-31: BC1F2 plants 1 to 26, Bottom row: M: Gene ruler 100 bp ladder plus; Lanes 32-62: BC1F2 plants 27 to 57. Amplification was repeated for lane 44. Lanes having 950bp fragment only are homozygous for *Xa21;*

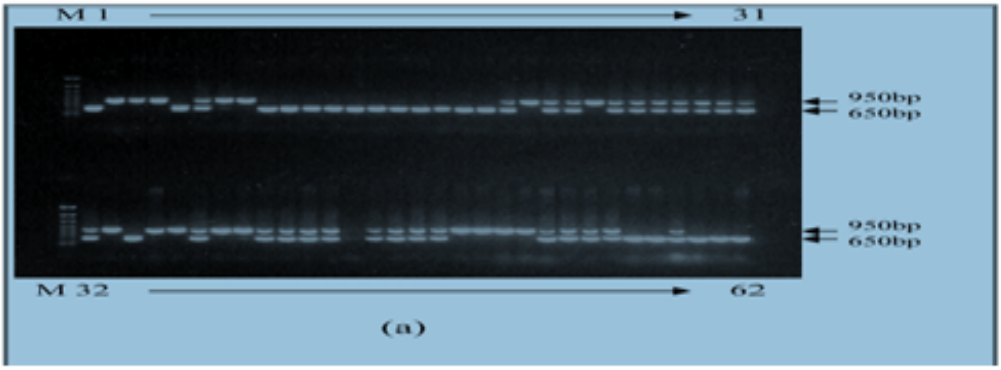

Figure 8: PCR amplification of BC1F2 plants with the marker p TA248 linked to Xa 21.

CAPS analysis of BC1F2 plants for xa 13. Top row: M: Gene ruler 100 bp ladder plus; Lane 1: PB-1; Lane 2: IRBB13; Lane 3: IRBB55; Lane 4: IRBB59; Lane 5: IR 24; Lanes 6-33: BC1F2 plants 1 to 29. Amplification was repeated for lanes 20 and 23, Bottom row: M: Gene ruler 100 bp ladder plus; Lanes 34-62: BC1F2 plants 30 to 57, Lanes showing 500, 450 and 150bp fragments are homozygous for *xa 13.*

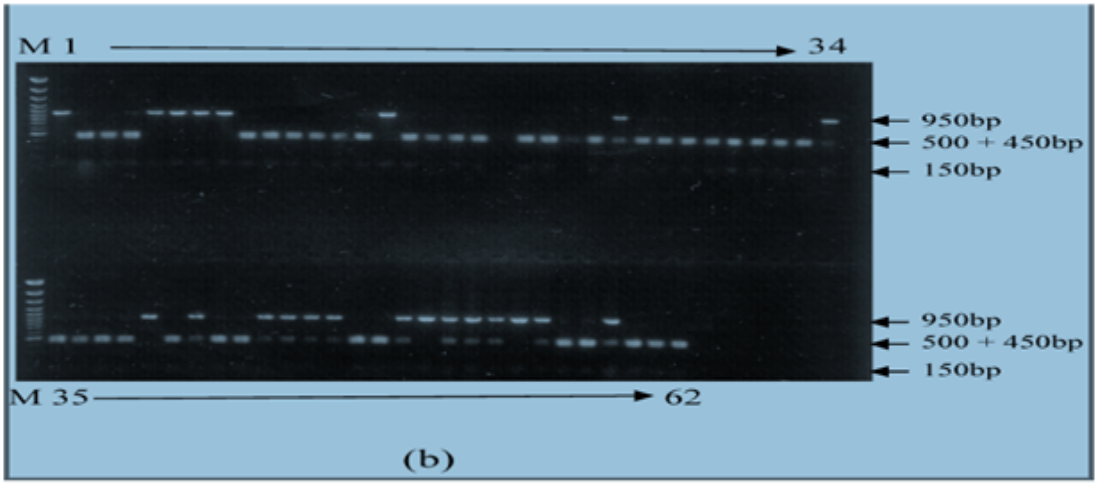

Figure 9: CAPS analysis of BC1F2 plants for xa 13

Improvement of other traits in Basmati rice varieties: (Singh *et al.*, 2011)

- Improvement of parental lines of **Pusa RH 10** for BB resistance.
- Development of blast resistant isogenic lines of **PRR 78**
- Development of near isogenic lines carrying major blast resistance genes in the background of **Pusa Basmati 1**

- Pyramiding of genes for resistance to BB (xa 13 and Xa 21), blast (Piz 5 and Pi54) and brown plant hopper (Bph3, Bph17, Bph18, Bph 20 and Bph 21) into Basmati rice varieties **Pusa Basmati 1121** and **Pusa Basmati 6** is under way.
- Improvement of **Pusa Basmati 1121** and **Pusa Basmati 6** for salt tolerance
- Molecular mapping and MAS for fertility restorer genes in Basmti rice (RM 258 and RM 6100 in **PRR 78**).
- QTL mapping for grain dimension traits.
- Validation and use of fragrance gene linked markers.

Marker assisted introgression of bacterial Leaf Blight resistance in Samba Mahsuri (Case study by Sundaram *et al.*, 2008)

Samba Mahsuri (also called BPT5204) is a leading rice variety of Andhra Pradesh and has been developed by scientists at the ANG Ranga Agricultural University. This variety is being cultivated in many other parts of India because of its exceptional yield and quality characteristics. Susceptible to several diseases including Bacterial Leaf Blight (BLB). BLB Resistance genes are introduced into Samba Mahsuri background without loss of its unique quality and yield characteristics. Bacterial Leaf Blight (BLB) is a serious disease of rice caused by *Xanthomonas oryzae pv. oryzae* (Xoo), especially severe on rice grown under irrigated conditions. The damage from natural epidemics of bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae pv. oryzae* was examined in 400 farmers' fields in four rice-growing districts of India during the wet seasons between 1995 and 1998. One hundred contiguous farm fields were rated for BLB on a 0–9 scale. Disease prevalence at levels above the economic threshold (score 45) ranged from 7 to 39% (Rajarajeswari and Muralidharan, 2006). Effective bactericides are not available for controlling the disease. Bacterium can overcome rice cultivars containing single genes for resistance against the disease. Rice cultivars should be developed that contain multiple genes for Bacterial Leaf Blight Resistance without impairing grain characteristics.
SS1113 is resistant to Bacterial Leaf Blight disease but scores low on characteristics like yield and quality that are favored by farmers and consumers. It is used as the donor for developing disease resistant improved version of Samba Mahsuri.

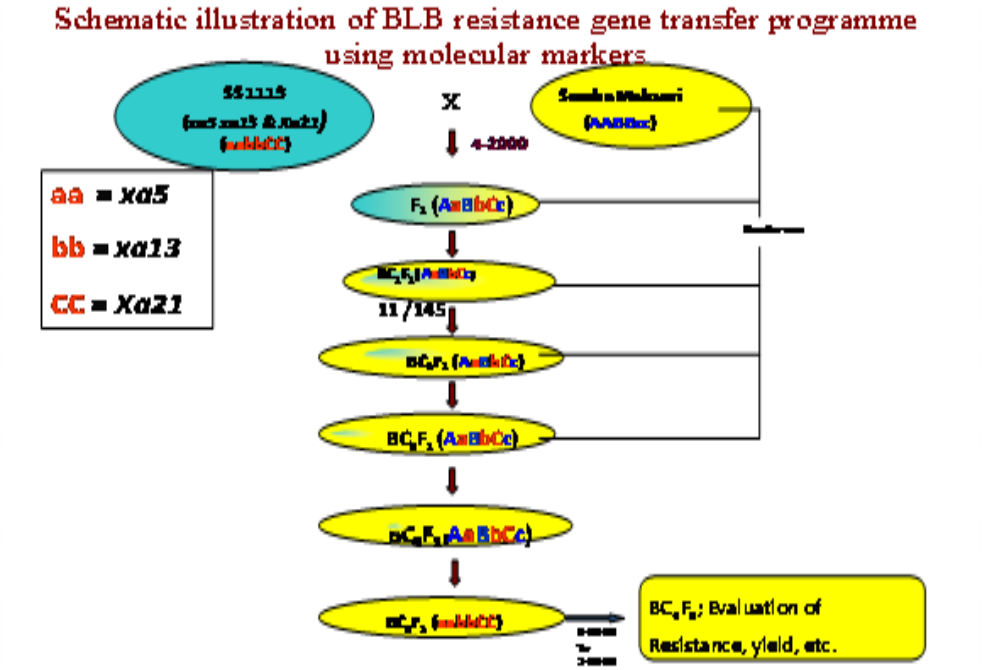

Figure 10: schematic representation of BLB resistance gene transfer using molecular markers.

F_1 plants were confirmed for the presence of *Xa 21* in heterozygous condition by using markers linked with resistance gene *Xa 21*. The plants with *Xa 21* plants in heterozygous condition only selected and remaining plants were discarded. Selected plants were checked for presence of marker linked to *xa13* in heterozygous condition. Double positive plants were screened for presence of marker linked to *xa5*, this type of selection which involves the detection of target gene by using flanking or tightly linked markers are called foreground selection.

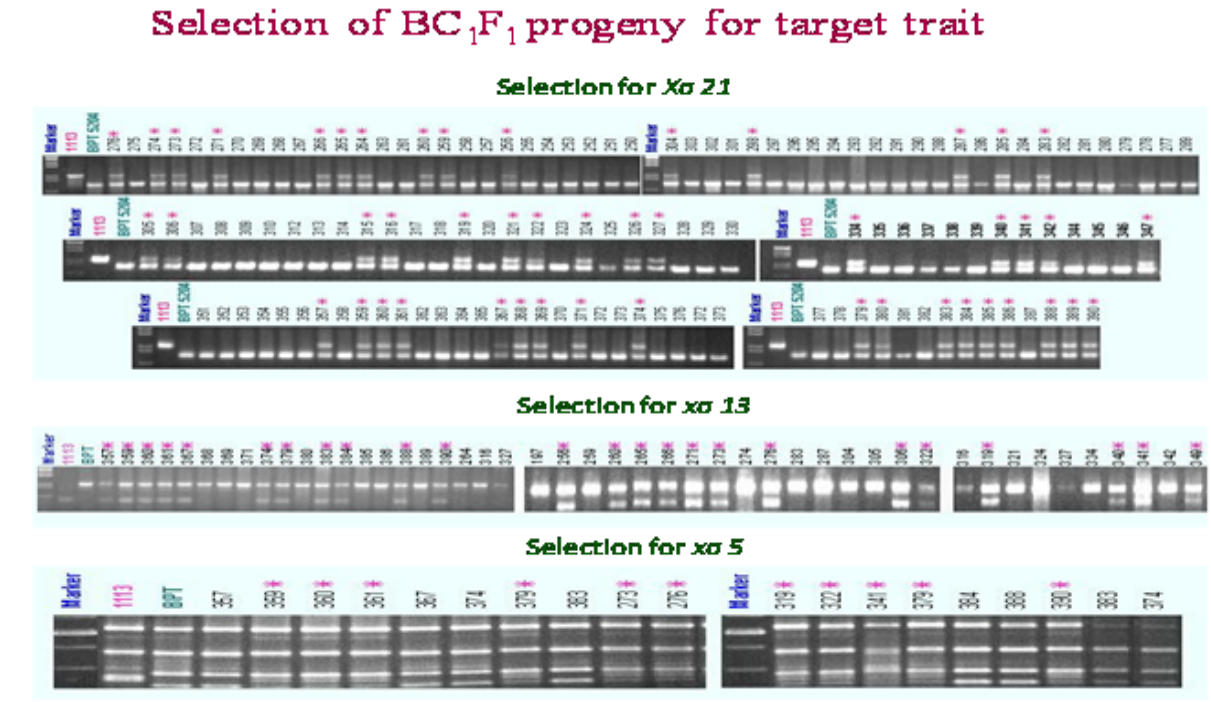

Figure 11: Fore ground selection for targeted traits

11 plants out of 145 BC_1F_1 plants were found to be heterozygotes for R gene linked markers. These 11 plants only subjected to background selection.

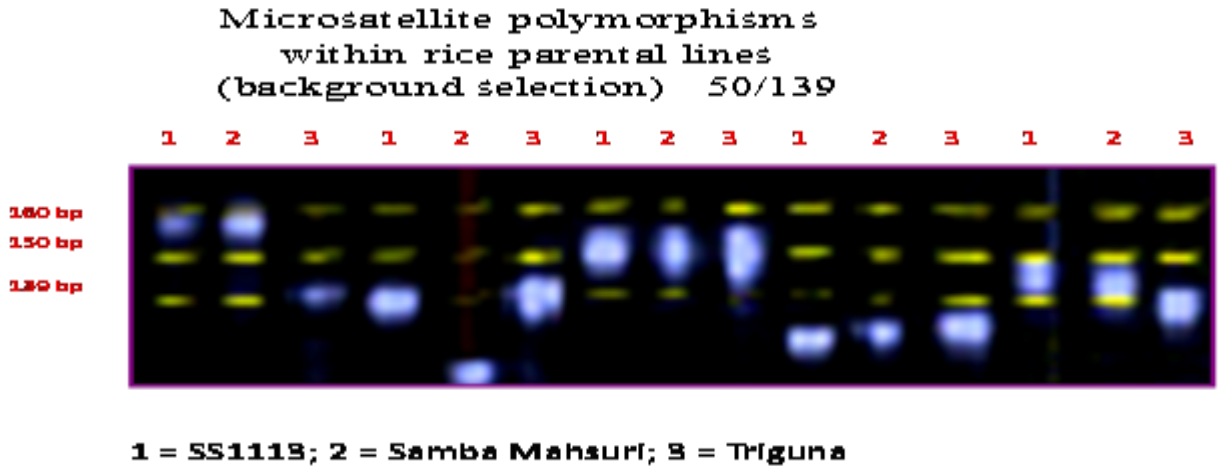

Figure 12: Back ground selection for recovery of parent genome

139 rice microsatellite markers were screened. 50 were found to be polymorphic between parents. The selected plants having the maximum recurrent parent genome contribution was backcrossed to generate BC_2F_1 plants and process was continued upto BC_4F_1 stage.

Table 1: Number of lines with multiple R gene combinations

Number of lines with multiple 'R' gene combinations

Ser. #	Gene combinations	SS1113 X Samba Mahsuri BC_4F_2
1	*Xa21/Xa21 xa13/xa13 xa5/xa5*	5
2	*Xa21/Xa21 xa13/xa13 Xa5/Xa5*	5
3	*Xa21/Xa21 Xa13/Xa13 xa5/xa5*	4
4	*xa21/xa21 xa13/xa13 xa5/xa5*	2
5	*Xa21/Xa21 Xa13/Xa13 Xa5/Xa5*	4
6	*xa21/xa21 xa13/xa13 Xa5/Xa5*	4
7	*xa21/xa21 Xa13/Xa13 xa5/xa5*	2

At BC_4F_1 generation plants having maximum contribution from recurrent parent was selfed to obtain BC_4F_2 lines. Then plants were screened using R gene linked markers to identify plants homozygous for different R genes. Those plants only were selected and seed was multipled to release as a variety. Thus Samba Mahsuri was improved with the objective of developing BB resistant lines without changing the yield and quality characteristics in 4 generations using marker assisted selection.

Marker assisted backcross approach for developing Submergence tolerant rice cultivars

(Neeraja *et al*., 2007**)**

Submergence tolerant version of the widely grown cultivar swarna has been developed in 3 backcross generations within 2-3 years by using marker assisted backcross breeding. MABC offers the prospect of conversion of a mega-variety into a new variety with the same characteristics except for the new trait for submergence tolerance conferred by a major gene or QTL. FR 13A derived submergence tolerant breeding line IR 49830 used as donor of sub1 and it is crossed with Swarna (vasista x mahsuri). Backcross between a submergence tolerant donor X recurrent parent. Foreground selection of sub1 locus was done (RM 464A) using tightly linked markers in BC_1F_1 generation, individual plants that were heterozygous at the sub1 locus were identified to reduce population size for further screening. From individual plants that were heterozygous for sub1, those that were homozygous for the recipient allele at one marker locus RM219, distantly flanking the sub1 locus (recombinant selection) was identified. From these recombinant plants, individuals with fewest numbers of markers from the donor genome were selected

In the second backcross generation the same strategy was followed for selection of individuals, with the desired allele combination at the target loci including selection for recombinants between sub1 and the nearest RM 316. Microsatellite markers unlinked to sub1 covering all the chromosomes including the sub1 carrier chromosome 9, that were polymorphic between the two parents , were used for background selection to recover recipient genome. In the selected plant of BC_2F_2, the tip of chromosome 9 was introgressed. An enhanced mega variety of rice have been developed by using a marker assisted backcross approach to incorporate submergence tolerance which was controlled by a major QTL. Obtaining smaller donor region within only a few backcross generations would be impossible using conventional methods.

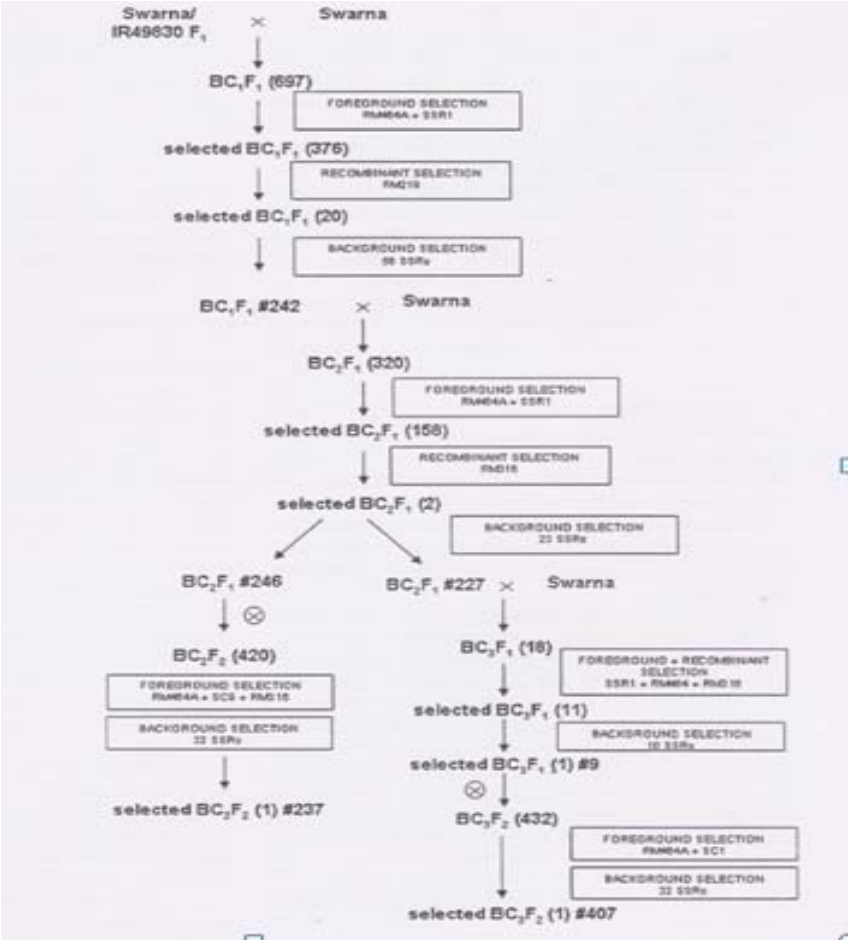

Figure 13: development of the submergence tolerant Swarna-*Sub* 1with details of markers used for foreground, reocombinant and background selection. The numbers of plants selected in each generation are indicated in parenthesis.

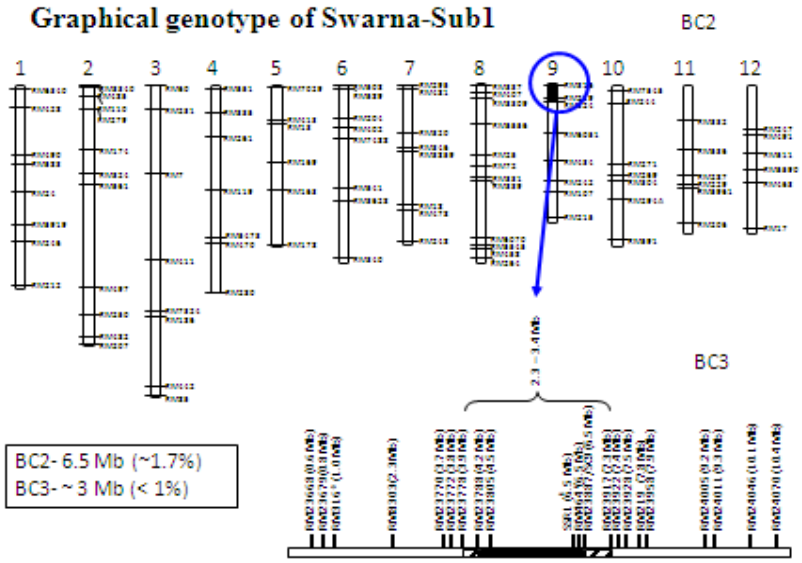

Figure 14: graphical genotype of Swarna-Sub 1

CONCLUSIONS

Breeding improved cultivars for resistance to biotic and abiotic stresses has been going on for more than 3 decades. However, improved cultivars have not been generally adopted by farmers due to undesirable characteristics. So, developing disease resistance versions of popular varieties or megavarieties which cannot be replaced by any other varieties and this improvement of trait is without losing its desirable characteristics. Adoption of a completely new variety by farmers could take considerable time, whereas chances of acceptability of converted popular varieties are relatively higher. Improvement of these traits illustrates the superiority of using marker assisted selection in crop improvement compared to conventional breeding.

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