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Genetics of Submergence Tolerance in Rice

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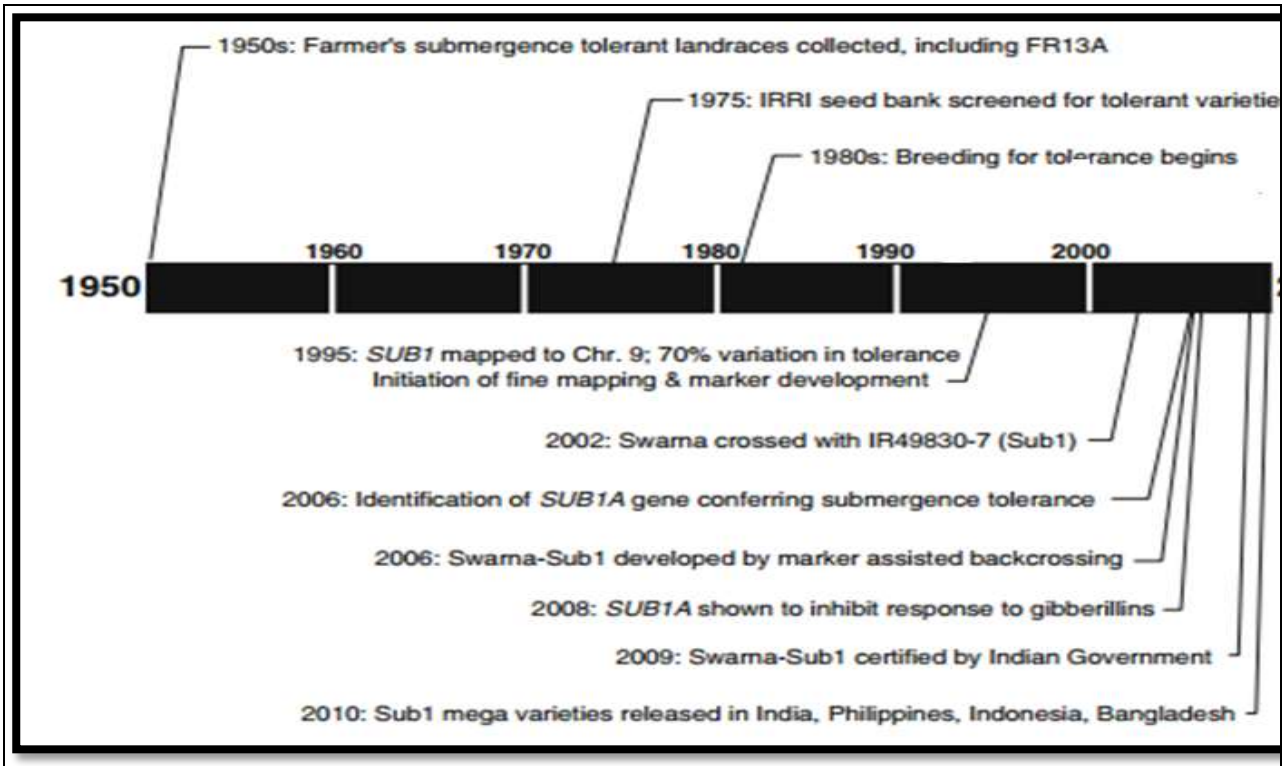
Introduction

Flooding, resulting in soil water logging and in many situations even complete submergence of plants, is an important abiotic stress in many regions worldwide. The number of floods has increased in recent decades and the severity of floods is expected to increase further in many regions of the world. Flooding reduces agricultural production, and floods shape many natural plant communities (e.g. floodplains, wetlands, salt marshes). A spectacular example of an important natural ecosystem shaped by flooding is the Amazon Floodplain forests, in which seasonal floods are deep and prolonged.

Rainfed lowland and deep-water rice together account for approximately 33% of global rice farmlands (50 million hectares of the estimated 150 million hectares of rice fields worldwide in 2004–2006 (IRRI Social Statistics Database; Huke and Huke 1997). Distribution of rice grown in upland, irrigated, rainfed lowland, and deep-water environments. Oftentimes, transient submergence is repeated or followed by a period of stagnant partial flooding. When partially or completely submerged, most rice varieties display a moderate capacity to elongate leaves and the portion of stems that are trapped underwater. This elongation growth leads to a spindly plant that easily lodges when floodwaters recede. If the flood is deep, underwater elongation growth can exhaust energy reserves, causing death within a matter of days.

Early breeding of submergence tolerant rice

Landraces with unusual flooding and submergence tolerance were first reported in the early 1950s and systematically screened in the 1970s (Fig. 4). The accessions FR13A and FR43B from Orissa, India and Kurkaruppan, Goda Heenati,



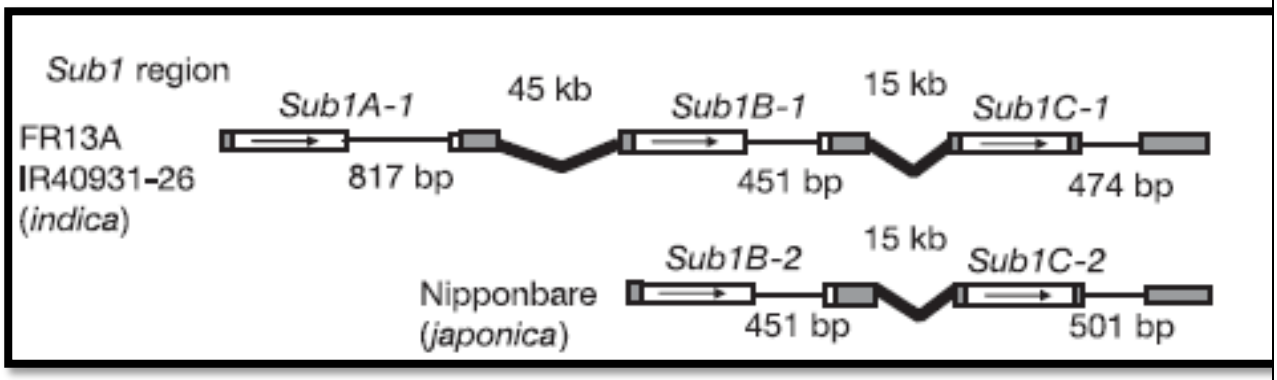
and Thavalu from Sri Lanka were recognized for their resilience to complete submergence (Vergara and Mazaredo 1975; Mackill et al. 1996). FR13A stood out as extremely submergence tolerant; 100% of 10-day-old seedlings survived 7 days of complete submergence (Vergara and Mazaredo 1975; HilleRisLambers and Vergara 1982).

However, FR13A lacks other agronomic attributes. It is photoperiod sensitive, tall, and provides low yields of poor-quality grain. Although strides towards this goal began in the 1980s (Mohanty and Khush 1985; Mohanty and Chaudhary 1986; Haque et al. 1989), it was not until the mid-1990s that submergence tolerance from the FR13A-derived breeding line IR49830- 7-1-2-2 was successfully introduced into productive short to intermediate stature lines (Mackill et al. 1993)

Fig. Sixty-year timeline of field, laboratory, and regulatory accomplishments that led to release of Sub1 mega-varieties with submergence tolerance in Asia.

Mapping and molecular characterization of SUB1

Submergence of plants inhibits aerobic respiration and photosynthesis, and stimulates a variety of responses that can enhance survival, such as a switch from aerobic to anaerobic respiration. In contrast to deep-water rice cultivars that avoid submergence stress by growing above the water surface and thereby restoring gas exchange, submergence-tolerant rice can survive 10–14 days of complete submergence and renew growth when the water subsides, although the duration of survival is also influenced by environmental factors such as water



turbidity, temperature and light levels. The Sub1 locus was mapped to an interval of 0.06 centimorgans on chromosome 9 using a mapping population (DX202) of 4,022 plants developed from the hybridization of a tolerant indica derivative of the FR13A cultivar (IR40931-26) and the intolerant japonica cultivar M-202. Physical coverage of this region was obtained with five overlapping bacterial artificial chromosome (BAC) clones derived from submergence-intolerant indica rice varieties and a nearly complete contig of 13 binary clones from IR40931-26. The Sub1 region, borderedby themarkers CR25K and SSR1A, physically spans over 182 kilobases (kb). This interval encodes three genes containing ethylene response- factor (ERF) domains and designated Sub1A, Sub1B and Sub1C, ten non-ERF genes including four transcribed and sixhypothetical protein-coding genes and 50% retrotransposon related sequences. The corresponding region of the japonica genome represented by the sequenced variety Nipponbare spans 142 kb and is considerably rearranged.

Notably, Sub1A is absent from the Nipponbare genome. Recombination was suppressed in this region in the mapping population, as revealed by the 10.7-fold higher-than average recombination ratio (3,030 kb/cM in the Sub1 region versus 282 kb/cM for the entire genome). This could reflect the proximity of the Sub1 locus to the centromere and/or the presence of genomic rearrangements that have altered continuity in this region in the two rice subspecies.

Fig.ERF gene structure and organization in tolerant indica (IR40931-26) and

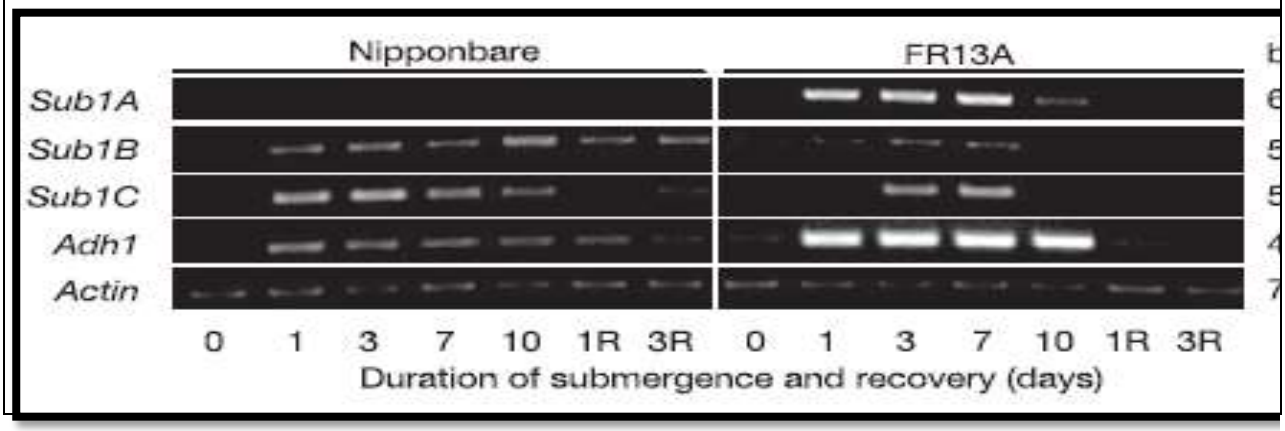
intolerant japonica (Nipponbare).

Expression profiling of genes in indica & japonica cultivars

Plant proteins that contain ERF domains are known regulators of abiotic and biotic stress responses. The accumulation of Sub1A and Sub1C messenger RNAs was strongly but transiently promoted by submergence and further reduced on de-submergence in seedling leaves of tolerant FR13A (Fig. 1b). Sub1C mRNA induction was earlier and more pronounced in intolerant Nipponbare compared with FR13A, suggesting that the rapid induction of Sub1A limits expression of Sub1C. Adh1 gene transcript levels were more strongly induced in the tolerant line, indicating that Sub1A maypositively regulate certain acclimation responses. In contrast, Sub1B transcripts increased only slightly during submergence.

Fig. Semi-quantitative RT–PCR assessment of gene transcript levels in shoot tissue from tolerant (FR13A) and intolerant (Nipponbare) genotypes

Haplotypes of the Sub1 locus



A haplotype is a set of DNA variation or polymorphisms that tends to inherited together. It also can refer to a set of single nucleotide polymorphisms (SNPs) found on the same chromosome.

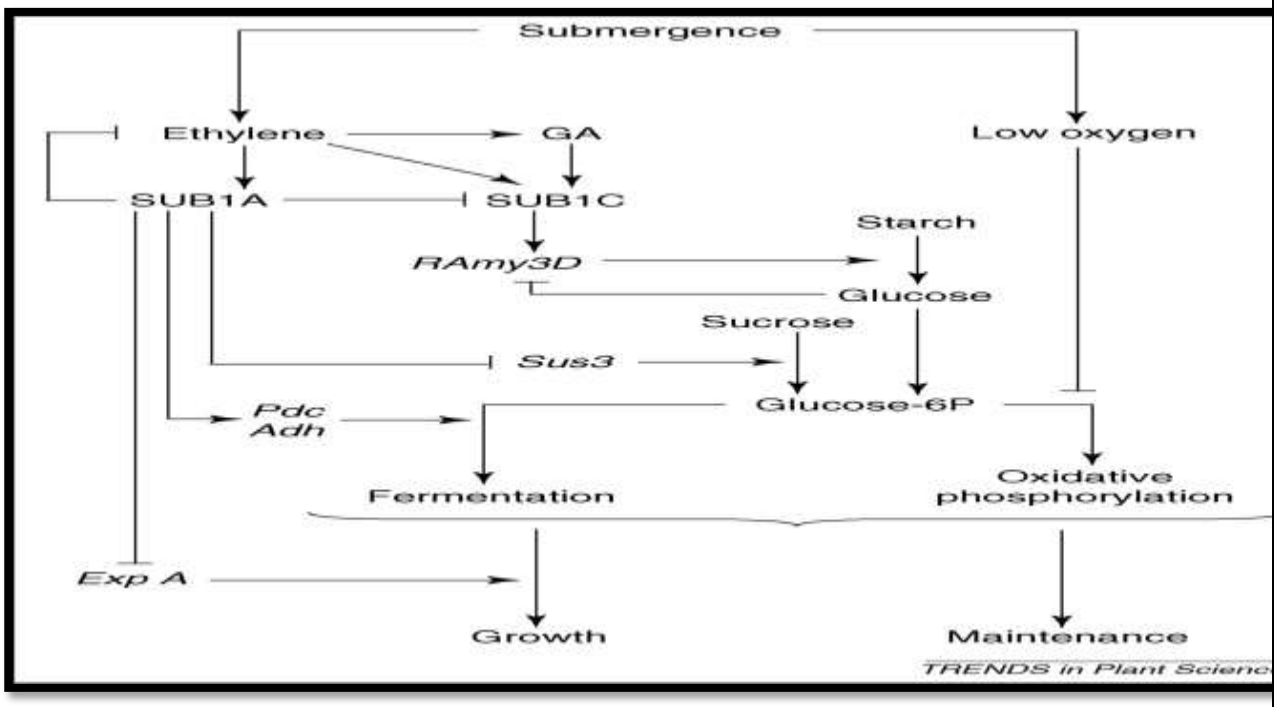
Fig. Haplotypes of the Sub1 locus based on alleles of the ERF-like genes in rice varieties

Line	Phenotype	Subspecies	Sub1A allele	Sub1B allele	Sub1C allele
FR13A	Tolerant	Indica	A1	B-1	C-1
Goda Heenati	Tolerant	Indica	A1	B-6	C-1
Kurkaruppan	Tolerant	Indica	A1	B-3	C-1
IR64	Intolerant	Indica	A2	B-1	C-3
IR24, Swarna	Intolerant	Indica	Absent	B-8	C-6
IR50	Intolerant	Indica	Absent	B-9	C-7
Nipponbare	Intolerant	Japonica	Absent	B-2	C-2
Liaogeng	Intolerant	Japonica	Absent	B-2	C-2
M-202	Intolerant	Japonica	Absent	B-2	C-2

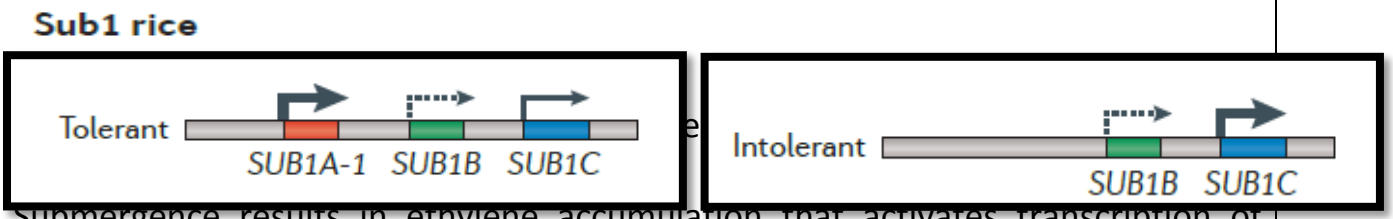
Rescuing rice from flood

Asian cultivated rice (*Oryza sativa* L.) was domesticated from wild populations of *Oryzarufipogon*and *Oryzanivara*through selection for desirable grain size and number and for reduced seed dispersal. Progenitor traits that were beneficial in local ecosystems, such as submergence survival, were retained by selection in local landraces, particularly those of the *aus*subgroup of *O. sativa* ssp. *indica*.

An example of this is the Indian landrace Dhalputtia (FR13A), the source of the *SUBMERGENCE1* (*SUB1*) locus that confers submergence tolerance by restricting underwater elongation growth, which is a quiescence strategy. Investigation of the functional role of the *SUB1* genes determined that, as floodwaters rise, the gaseous phytohormone ethylene that is trapped within cells promotes *SUB1A-1* expression, which enhances the accumulation of two transcription factors that impede the response to the phytohormone gibberellin (GA): SLENDER RICE 1 (SLR1) and SLR1-LIKE 1 (SLRL1). The outcome is a restriction of cell elongation and the less rapid consumption of energy reserves. Upon de-submergence, genotypes with *SUB1A-1* incur less tissue damage from reactive oxygen species (ROS) and the dehydration that is associated with re-aeration. Even when leaf tissue loss is substantial, the maintenance of tiller meristems enables *SUB1A-1* genotypes to



resume development when floodwaters subside.



Submergence results in ethylene accumulation that activates transcription of theSub1A gene. SUB1A represses Expansin A and sucrose synthase and, thus, represses growth. SUB1A also enhances mRNA accumulation and enzymatic activities of pyruvate decarboxylase (Pdc) and alcohol dehydroxygenase (Adh). Fermentation allows glycolysis to continue and, thus, provides the ATP needed for survival. However, the rate of ethanol production is similar in a genotype lacking Sub1A, indicating that differences in the induction of Pdcand Adhgenes might not be crucial. Starch degradation provides glucose needed for both glycolysis and growth.

In rice varieties that express Sub1A, the elongation rate is lower, enabling a pool of starch and carbohydrates, which are needed for prolonged ATP production through fermentation to be preserved. Sub1C, which appears to control the a-amylase gene Ramy3D, is repressed by Sub1A. Furthermore, gibberellins (GA) appear to be involved in the regulation of expression of Sub1C.

However, it is unlikely that regulation by GA directly affects Ramy3Dexpression given that the promoter of this gene lacks the GARE element required for GA regulation. Instead, up-regulation of Ramy3D by the lower sugar content found in Sub1A-deficient lines is likely.

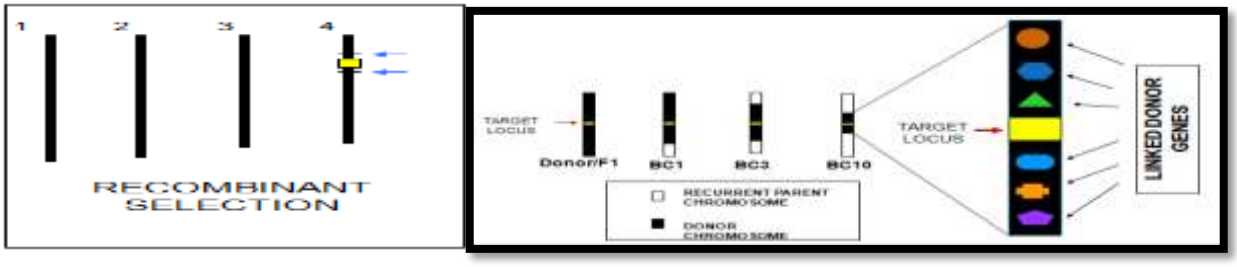
Marker-assisted backcrossing (MABC)

MABC is a precise and an effective method to introgress a single locus controlling a trait of interest while retaining the essential characteristics of the RP. It is known that MABC is effective for genes or quantitative trait loci (QTLs) with large variations in phenotype. MABC is the process of using markers to select for target loci, minimize the length of the donor segment containing a target locus and/or accelerate the recovery of the RP genome during backcrossing.

The main objective of MABC is to integrate a targeted gene from agronomical substandard sources (the donor parent) into an exclusive breeding line (the RP). MABC is superior to conventional backcrossing in precision and efficiency. The three selection steps are as follows.

Foreground selection

Marker-assisted foreground selection was proposed by Tanksley and investigated in the context of introgression of resistance genes by Melchinger. The objective is to maintain the target locus in a heterozygous state (one donor allele and one RP allele) until the final backcross is completed. Then, the selected plants are self-pollinated and progeny plants identified that are homozygous for the donor allele. Those markers which have already been developed and they are tightly linked to the target gene or QTL should be used to select the target locus of donor parent in early (BC) progenies for the selection of plants that having the target gene. This is referred to as ‘foreground selection’, although referred to ‘positive selection’.



Recombinant selection

The second level involves selecting BC progeny with the target gene and recombination events between the target locus and linked flanking markers is termed as 'recombinant selection'. The purpose of recombinant selection is to reduce the size of the donor chromosome segment containing the target locus (i.e. size of the introgression).

This is important because the rate of decrease of this donor fragment is slower than for unlinked regions and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent (i.e. as linkage drag).

Background Selection

Except target locus, all genomic regions can be selected in background selection using RP marker alleles and the selection of target locus is done on the basis of phenotype. This selection is important in order to reduce unnecessary genes (linkage drag, introduced from donor.

By using molecular markers, it is easy to delete the unwanted donor alleles in the same genomic region as the target locus. The third level of MABC involves selecting BC progeny with the greatest proportion of RP genome, using markers that are unlinked to the target locus, we refer to this as 'background selection'.

Fig. Schematic representation of MABC procedure for the transfer of major QTLs for submergence tolerance into popular rice varieties

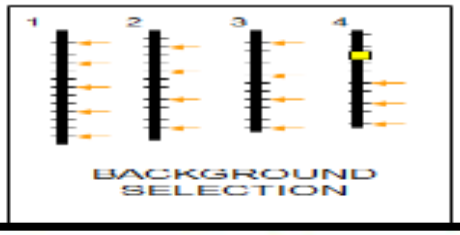
Advantages of MABC over conventional backcrossing

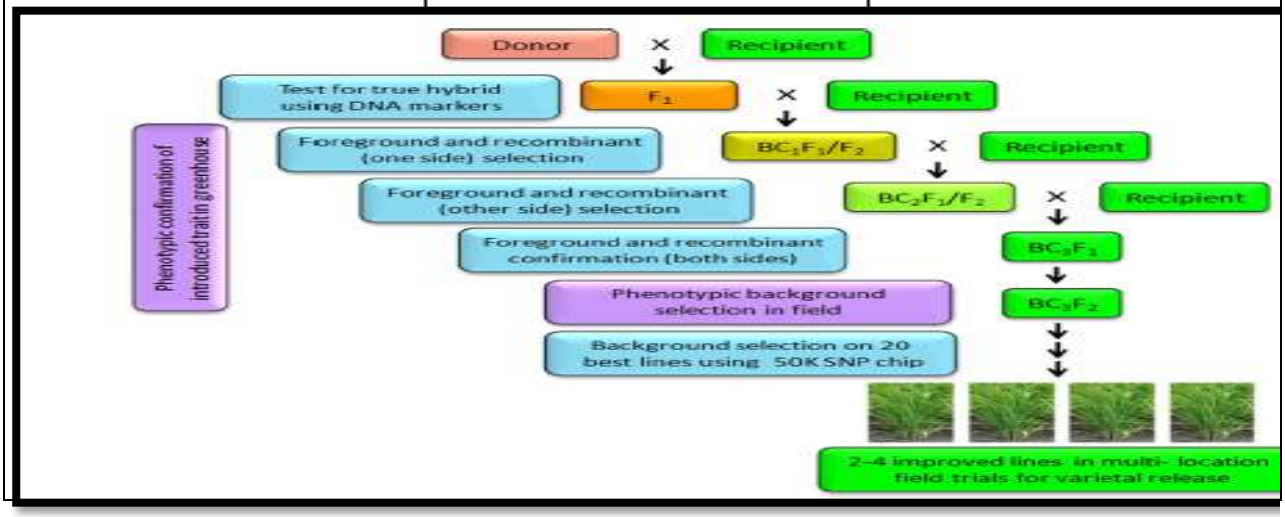
1. Consistency

Environmental factors are of great concern and may hamper the expression of plant characteristics. But molecular markers are consistent from any significant impact of environmental stresses, which presents great opportunities for selecting molecular markers for MABC.

2. Minimize the linkage drag

To reduce the large number of donor





chromosome, minimum six backcross generations are needed whether MABC may need two or three backcross generations. Linkage drag requires many additional backcross generations, and if the undesirable genes are really tightly linked to the target locus it may be difficult to eliminate these genes using conventional backcrossing.

3. Efficiency

By sorting of breeding lines in few generations with the application of molecular markers, it is easy to discard all the progenies from the programme except our targeted lines.

Conclusion

The marker-assisted introgression of the SUB1 region has successfully improved submergence tolerance in a wide range of mega-varieties without any penalties on development, yield, and grain quality (Sarkar et al. 2006, 2009; Neeraja et al. 2007; Singh et al. 2009). These new lines endure submergence, as long as the flood occurs after the seedling stage but before flowering and the flood completely subsides within 10 to 20 days, depending on floodwater conditions (Das et al. 2009).

Although vegetative growth is restricted in some SUB1 varieties until the water level drops to 10–15 cm, mainly because of short stature, our recent studies showed that this is not the case when SUB1 is transferred into taller varieties or those with better tolerance of partial stagnant flooding (20–50 cm). The yield advantage provided by Sub1 introgression lines is anticipated to greatly stabilize production in rainfed lowland environments that experience flash flooding.

References (if any)

Septiningsih, E.M., Pamplona, A.M., Sanchez, D.L., Neeraja, C.N., Vergara, G.V., Heuer, S., Ismail, A.M. and Mackill, D.J. (2009). Development of submergence tolerant rice cultivars: the *Sub1* locus and beyond*. Annals of Botany***103**: 151–160.

Xu, K., Xu, X., Fukao, T., Canlas, P., Maghirang-Rodriguez, R., Heuer, S. ...&Mackill, D. J. (2006). Sub1A is anethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature*, ***442***: 705-708.