Review article

BREEDING FOR TOLERANCE TO STRESS TRIGGERED BY SALINITY IN RICE

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ABSTRACT: Rice is one of the world's most important staple crops. Although rice is considered as a sensitive crop to salinity, it is one of the most widely grown crops in coastal areas. In the present, salinity is the second most widespread soil problem in rice growing countries next to drought and considers as a serious constraint to increased rice production worldwide. Genetic Evaluation and Utilization (GEU) Program at IRRI in 1969 have been screened 138,000 rice genotypes. Of these, 17% had acceptable tolerance at electrical conductivity (EC) of 10 dS/m at the seedling stage. Traditional cultivars are the most tolerant to abiotic stresses. Cultivars Pokkali, Cheriveruppu, Nona Bokra, SR26B, Damodar and Getu are tolerant of salinity but possess poor agronomic characters. There exists tremendous variation for salt tolerance within species in rice, providing opportunities to improve crop salt-stress tolerance through genetic means. Some attempts to develop salt-tolerant genotypes were based on highly tolerant traditional rice cultivars i.e. Pokkali and Nona-Bokra (Akbar *et al.,* 1985).

Mahsuri which is saline tolerant and introduced from Malaysia. Damodar (CSR 1), Dasal (CSR 2), Getu (CSR 3), Pokkali, Vytilla 1, Vytilla 2, Vytilla 3, Vytilla 4, Vytilla 5 etc were developed through pureline selection. CSR 10, CSR13, CSR23, CSR27, CSR30, CSR36**,** CR Dhan 402, CR Dhan 403, TRY 1, TRY 2, TRY 3, White ponni, CO 43 etc. were developed through recombinant varieties. CORH 2 is developed through three line breeding method. Somaclonal variants of Pokkali with improved agronomic traits were identified. The variant (TCCP 266-2-49-B-B-3) had desirable levels of all tested characteristics and retained salinity tolerance equal to Pokkali. The variant is semi-dwarf and has vigorous growth and high yield potential without lodging. TCCP 266-2-49-B-B-3 had a white pericarp and also improved cooking quality, with medium gel consistency. High-yielding salt-tolerant AC-derived lines (IR51500-AC11-1, IR51500-AC17, IR51485- AC6534-4, IR72132-AC6-1, IR69997-AC1, IR69997- AC2, IR69997-AC3 and IR69997-AC4) had been generated in just 3 years (Senadhira *et al.,* 1994).

A major quantitative trait locus (QTL) for salt tolerance named *Saltol* was mapped on chromosome 1 using F8 recombinant inbred lines (RILs) of Pokkali/IR29 cross, which is responsible for low Na+, high K+ uptake and maintaining Na+/K+ homeostasis in the rice shoots (Nejad *et al.,* 2008).

INTRODUCTION

The Green Revolution of the 1970's resulted in remarkable increases in rice production. Since then, the rate of production in most rice-growing countries had slowed (Hossain, 1994) and has now reached a plateau. Contributing factors include a higher population growth rate and the conversion of some highly productive rice lands for industrial and residential purposes. These developments have pushed rice cultivation to less productive lands, including saline prone areas. Millions of hectares in the humid regions of South and Southeast Asia are technically suited for rice production but are left uncultivated or are grown with very low yields because of salinity and abiotic stresses (Boje-Klein, 1986). Furthermore, the problem of Salinity rarely occurs in isolation. Mineral deficiencies and toxicities frequently compound the problem of salinity. For inland salinity brought about by poor quality groundwater or irrigation, associated stresses are alkalinity, phosphorus and zinc deficiencies, and boron toxicity. In these soils, rice cannot be grown without good-quality irrigation water. For coastal salinity, on the other hand, tidal intrusion is the source of salinity, but there are associated problems.

These include: (1) acidity, where deficiencies in P, Zn, Fe and Al toxicities are prevalent; (2) acid sulfate conditions, where deficiency in phosphorus and zinc, and toxicity in aluminum and sulfides are also present; (3) peatiness, where phosphorus and zinc deficiency are always present, with accompanying iron and organic acid toxicities (DeDatta et al., 1993). Many saline soils are also prone to submergence, deep water and drought. These abiotic stresses vary in magnitude and interactions over time and place, making the long-term adaptability of a cultivar dependent on its level of tolerance for all the stresses that occur in its growing environment. Multiple abiotic stress tolerance traits must be considered as well as high yielding ability and disease resistance. The extent of salt-affected land has long been uncertain and remains so. Worldwide the estimates range from 340 to 1,200 x 10^6 ha. Despite this much area either barren or with very low productivity, there are only few instances where salt tolerant cultivar have been developed. The reason being that it is a very complex trait and has many components, which are probably controlled by polygenes. Progress in breeding for salinity tolerance and its related abiotic stresses has been slow owing to: (1) limited knowledge concerning the genetics of tolerance, (2) the involvement of several complex tolerance mechanisms (Yeo and Flowers, 1986), (3) inadequate screening techniques, (4) low selection efficiency, and (5) poor understanding of the stress and environmental interactions. Recent progress in germplasm improvement, screening techniques (Gregorio et al., 1997), genetic inheritance (Lee et al., 1996) and application of molecular-marker technology for tolerance of salinity and related abiotic stresses will accelerate breeding progress.

Worldwide, the research to overcome salt related problems is based on two approaches; (i) either change the growing environment (make it normal) suitable for the normal growth of plants; (ii) or select the crop and/or change genetic architecture of the plant so that it could be grown in such areas. The first approach involves major engineering and soil amelioration process which need lot of resources are often out of the reach of small and marginal farmers. The second approach i.e. breeding crop varieties with *in-built* salt tolerance is realized as the most promising, less resource consuming /economical and socially acceptable approach. So the ability of the plant to tolerate the salt stress upto an extent is of paramount importance to manage the resources optimally and this is the reason to develop the tailored crops with higher salt tolerance suited to salt stress environments. There is third approach as well which can be termed as hybrid approach as it is the combination of both environment modifying and biological approach. It is highly productive, less resource consuming and economically viable approach. Nowadays major soil reclamation programmes in different states involve both biological and hybrid approaches to combat the salt problem.

Prerequisite for the development of salt tolerant cultivars

Wide spectrum of variability in available germplasm: Existence of genetic variability for salt tolerance within species is of paramount importance in crop improvement programme. Therefore choice of germplasm in breeding programme is most crucial as the success lies on it. Extensive germplasm collection provides a useful source of genetic diversity for the studied traits.

Target environment / site characterization: Before designing any ideal plant type, it is crucial to define the soil and agroclimatic conditions of the target areas for which they are to be developed. Genotypes which are suitable for coastal areas may or may not be fit for sodic soils or inland saline soils and *vice-versa*. Therefore exact site characterization is an important aspect to meet the objective(s).

Availability of the defining traits / selection criteria: Ideally germplasm should differ as much as possible for the traits to be improved or introgressed. Other traits should not vary too much otherwise keeping all the desirable traits into one superior agronomic background become very difficult.

Repeatable screening techniques: Reliable and repeatable screening techniques are the mainstay of any successful breeding programme specifically for biotic or abiotic stress breeding. Though screening techniques vary with crop species, growth stage and type of stress imposed but ideally it should be rapid, reproducible, easy and affordable.

Manifestation of salt stress on plant: Degree of salt stress can affect the different crops differently. For rice, soil salinity beyond ECe ~ 4 dS/m is considered moderate salinity while more than 8 dS/m becomes high. Similarly pH 8.8 - 9.2 is considered as non-stress while 9.3 – 9.7 as moderate stress and ≥ 9.8 as higher stress. Extremely high salt stress conditions kills the plant but the moderate to low salt stress affect the plant growth rate and thereby manifest symptoms which could be associated with morphological, physiological or biochemical alterations.

Most of the morphological parameters like low tillering, spikelet sterility, less florets per panicle, low 1000 grain weight and leaf scorching, are affected uniformly under both sodicity and salinity; however it is not a thumb. Major symptoms are white leaf tip followed by tip burning (salinity), Leaf browning & necrosis (sodicity), stunted plant growth, low tillering, spikelet sterility, low harvest index, less florets per panicle, less 1000 grain weight, low grain yield, change in flowering duration, leaf rolling, white leaf blotches, poor root growth and patchy growth in field. It is observed that crop varieties and breeding lines do differ for their inherent capability to modify various physiological and biochemical processes in response to the salt stress.

Though numerous physiological and biochemical changes take place under altered stress environment but only few of them change very significantly and also contribute a lot to the salt tolerance mechanism. These changes control the solute and water balance and their distribution on whole plant and tissue basis. Based on the studies it was observed that most of the crop plants and varieties, physiological and biochemical manifestations under higher salt stress conditions are high Na^+ transport to shoot, preferential accumulation of Na in older leaves, high Cl^- uptake, lower K^+ uptake, lower fresh and dry weight of shoot and roots, low P and Zn uptake Change in esterase isozyme pattern, increase of non-toxic organic compatible solutes and increase in Polyamine levels.

SCREENING CRITERIA

Reproducible differential manifestation in plants with respect to their morphological, physiological or biochemical parameters in response to salt stress qualifies for a reliable screening criterion. Reliable screening is an integral part of any successful breeding programme. Salt related problems seldom occur in isolation and are coupled with many associated problems. Complexity of the salt tolerance, soil heterogeneity and various interactions are the major hurdles for the repeatability of the results. Following parameters are considered screening:

Morphological Parameters
Though there is no single definite morphological marker available for salt tolerance or sensitivity in any crop, but a combination of criteria give a good indication toward the salt response of crop plants. Therefore, several parameters are used in combination for the effective and reproducible screening.
Germination studies*: Germination percentage, coleoptile and radicle length under varying degree of salt stress for different crops is a good salt tolerance indicator at initial stages. Higher salt concentration delays or reduces the germination.
Survival of the plant: It is mainly limited to the seedling studies; however, in some of the adult plant studies it has also been considered. Under moderate stress, plant survival is not a problem but under higher stress, it is a good selection criterion.
Injury score: Individual plant or group of genotypes are scored usually on 1 to 5 or 1 to 9 scale where lower score towards 1 states tolerant and higher score denotes sensitive genotypes.
Phenotypic expression: Excessive tip burning especially in younger leaves, spikelet sterility and stunted growth are considered for the overall phenotypic expression of the genotype under stress environment.
Grain Yield: In the absence of any simple and reliable selection criteria, the 50% reduction in grain yield of the genotypes under salt stress in relation to the normal (non-stress) yield has been considered as critical limit for selection/rejection of the genotypes.
Stability of traits over environments: The genotype with high mean, near to unit regression value (bi) and uniformity of regression (Sdi^2) under multiple stress environments are adjudged as suitable, stable and adaptable genotypes for sustainable productivity in problem soils. Selection for high mean yield is a fundamental selection criterion for all the varieties while selection based on unit regression value and least deviation from regression indicates the stable performance across a range of environments (Singh and Mishra, 1997).
Mean tolerance index (MTI): It is a product of Stress Resistance Index (SRI) and Response Index (RI) of the genotype. Here SRI is the performance of a genotype for that trait averaged over all the stress environment in conjunction with performance under corresponding non-stress environment and RI is the mean of the genotype for the trait under all the stress environments divided by the mean performance of all the genotypes over all the stress locations. Beside yield, it has been used for germination, seedling screening, grains per panicle, ear bearing tillers /m^2 and many more traits for the screening of the genotypes. Similar to MTI, another criteria Stress tolerance index (STI) and Susceptibility indices were used using seed yield, main shoot length, siliqua/main shoot and primary branches / plant in Indian mustard (Sinha, 1997).

Physiological Parameters

Extremely high salt concentration kills the plant but the moderate salt stress exhibits the growth differences among the crop varieties. The tolerance to salt stresses is complex phenomena because it may require the combination of different independent and/or interdependent mechanisms and pathways. A tolerant genotype can be expected to have more than one adaptation.

Na and K Uptake: In general, tolerance of a crop variety was found to be associated with its ability to restrict potentially toxic ion uptake like Na^+ and associated with preferential uptake of the balancing ion like K^+. It is like an adaptation for the survival of plants so that the vital metabolic activities are not hampered. There are larger differences in ion (Na^+ and K^+) uptake between the species in comparison to the genotypic differences within a crop species. These are most studied parameter for the salt tolerance in crop plants.

The tolerant varieties maintained lower Na concentrations besides maintaining K concentrations under high sodicity. On the other hand, the sensitive rice varieties were unable to effectively prevent accumulation of Na^+ as well as the depletion of K^+. The success of the tolerant varieties in gaining higher fresh and dry weights at all the stages widens the differences in their Na concentrations still further by dilution. Tolerant genotypes like CSR 1 showed regulation over distribution and accumulation of Na taken up by the plants i.e. the delicate and vital organs like young and photosynthetically active leaves as well as the reproductive organs like panicles are kept relatively free of Na, besides having an assured supply of K even under higher salt concentration. No such regulation of Na and K seems to operate in case of salt sensitive genotype, which further aggravates the stress situation. Thus the differences in the distribution of ions in individual plant organs and with the age and position of these organs on plant are more important indicators of its tolerance potential than the difference in the average salt content of the plant.

Na/K ratio: Although the uptake of both Na and K is entirely independent, but lower Na/K ratio is considered as desirable trait as it maintains the ion balance. Na is transported to shoot usually through apoplastic pathways (passive transport) while K transport takes place through symplastic pathway i.e through membrane / plasmalemma (active transport). Younger leaves relatively have lower Na than K as compared to the older leaves, which in turn resulted in higher Na/K ratio in case of the older leaves. Thus Na / K ratio increased steeply with salt concentration and leaf age, and the two rice varieties behaved differently. Consequently the tolerant variety keeps their leaves relatively free of the toxic ions besides having assured K supply. This factor along with the higher number of the leaves and higher leaf area probably, contributes to its success under high salt concentration. However this parameter is also not a universal phenomena hence could be taken as a selection criteria but with cautiously.

Tissue tolerance: Tissue tolerance is measured in terms of LC_{50} which is an analogue of LD_{50}. Here LC_{50} is the concentration of sodium (in mmol g^{-1} ethanol-insoluble dry wt.) in the leaf tissue which causes a 50% loss of chlorophyll (Yeo and Flowers, 1983). It is taken as an indicator of metabolic damage to the tissue due to the salt load. LC_{50} values differs for different genotypes and not all the varieties in high tissue tolerant category were highly salinity tolerant and vice-versa. For example GR11 and IR2153-26-3-5-2 are highly tissue tolerant varieties but phenotypically they are not highly salt tolerant varieties. Similarly not all the low tissue tolerant varieties like CSR 10 having low tissue tolerance are highly salt tolerant variety (Mishra *et al*., 1992).

Screening methodology

In-situ Field Evaluation: Field screening is the best way to identify the most suitable genotypes because salt tolerance is very complex phenomena but spatial variability in the field sometimes give the false positive results due to escape. Therefore extensive field testing for soil salinity gradient and blocking of the same is the best way to minimize the error chances. Long rows of 10 to 30 meters with space planting depending upon the type and generation of material minimise the effect of spatial variability. It allows maximum possible exposure of all genotypes to varying soil sodicity conditions. The layout for such a test is generally incomplete block design or augmented design in which a set of check varieties is replicated many times. It increases the potential number of test varieties allowing screening of large number of genotypes at the same time. It is further possible to cut across the long plots in several parts as replicate to obtain varietal performance at varying levels of soil stresses. The involvement of more number of checks has been found better in data processing and finalizing the tolerant lines.

Screening in Microplots: Soil heterogeneity and spatial variability hinders the reliability of the response of genotypes in true and dependable way. This attracted the development of mini-field environments with varying levels of controlled salinity and sodicity environments. These devices are series of dug-out cavity structures made of brick-mortar-concrete materials and filled with artificially prepared or natural transported problem soil. It is possible to create and maintain desired levels of sodicity and salinity in these microplots in a manner very much comparable to field conditions minus the soil heterogeneity. The plot size in these pits is kept very small (single row) to 2-3 rows but because of a good control over micro-environment, it is highly representative of the genotypic performance. The microplots are being used to screen mostly early segregating and also stabilised populations besides genetic studies.

Screening in Pots: For more precise study of the individual plant response under a constant stress, round porcelain pots of 20 or 30 cm dia, with a capacity of 8 or 16 kg soil with a provision to allow or plug off leaching from bottom, are used.

Pot studies also allow for periodic uprooting of plants at a given growth stage for specific studies associated with age of plant. The edaphic environment of the pots is more or less uniform throughout the growth period of plant in respect of degree of stress. The genetics for salt tolerance is being studied in pots. Sand and soil cultures are being used in the pots for the controlled studies.

Screening in Solution Culture: A separate hand handout for this has already been provided.

Genetic Variability and Sources of Tolerance

Some degree of cultivar tolerance to salinity, submergence, Al and Fe toxicities, P and Zn deficiencies, and elongating ability is available in rice germplasm. Since the start of the Genetic Evaluation and Utilization (GEU) Program at IRRI in 1969, 138,000 rice genotypes have been screened. Of these, 17% had acceptable tolerance at electrical conductivity (EC) of 10 dS/m at the seedling stage (DeDatta et al.,1993).

Traditional cultivars are the most tolerant to abiotic stresses. Cultivars Pokkali, Cheriveruppu, Nona Bokra, SR26B, Damodar and Getu are tolerant of salinity but possess poor agronomic characters: they are tall and photosensitive, have poor grain quality and are low-yielding. These salt-tolerant cultivars are also tolerant of P deficiency but are very sensitive to pests and diseases and some are sensitive to Zn deficiency (Senadhira and Akbar, 1991). The challenge is to combine these tolerance traits through breeding to develop multiple stress-tolerant rice cultivars. This, however, requires independent selection of salt tolerance and desired plant type because early selection for agronomic characters often depletes the population of potentially salt-tolerant lines.

Salt-tolerant indica rice cultivars seem to have originated or been selected in coastal areas of India (states of Kerala and West Bengal); examples are Pokkali, Nona Bokra, Cheriviruppu and SR26B. An evaluation at IRRI of 250 traditional cultivars collected from Orissa and Tamil Nadu in India has revealed some salt-tolerant types from southeastern coastal areas of India. Among the tolerant cultivars identified, NV, Pat, Solla, and DH were found to have tolerance similar to popular Pokkali. Similar tests with coastal rice cultivars of Indonesia, Thailand, and Vietnam identified three highly tolerant types: Ketumbar (Indonesia), Khao Seetha (Thailand) and Soc Nau (Vietnam). Again, tolerance mechanisms appear similar to Pokkali. But cultivars like Pat, Ketumbar and Soc Nau have better grain quality (white fine grains, intermediate amylose) than Pokkali (red bold grain, high amylose).

Attempts have also been made to identify other sources of salt tolerance. Wild species of Oryza and the African Oryza glaberrima were screened at 2±3- leaf stage at EC 12 dS/m for 14 days. All were highly susceptible with seedling survival range 0±16% while the tolerant control, Nona Bokra, showed complete survival (Akbar et al., 1987). However, some wild rice (O. rufipogon) from Sri Lanka showed salinity tolerance at seedling stage comparable to Pokkali. A distant relative of Oryza (Porteresia coarctata), a halophyte that needs high salinity (EC of 12± 18 dS/m) to produce grain may not be a good source of tolerance in breeding programs because of the difficulty in producing fertile F1, and their requirement for high salinity. In saline-prone areas, especially in coastal lands, salinity may be low during some cropping seasons. Under these conditions, the requirement is for salt-tolerant cultivars that can yield high even under low salinity.

Research to Improve Donor Germplasm

In the mid-1970s, attempts to transfer salinity tolerance from traditional varieties to high-yielding cultivars were not successful. Several modern plant types developed at IRRI using traditional salt-tolerant parents such as Nona Bokra, Pokkali, SR26B and Kalarata do not possess the level of tolerance found in the donors. Difficulty in transferring tolerance arose because donors had too many undesirable traits, most of them linked to salinity tolerance preventing transfer to an improved plant type. Consequently, improvement of the agronomic characteristics of the donor parents was given much attention.

Somaclonal variants of Pokkali:

The Pokkali cultivar is highly salt-tolerant and low yielding despite producing vigorous seedlings (Senadhira et al., 1994) and is commonly grown in coastal areas of Kerala, India. It is a traditional, tall, photoperiod sensitive rice cultivar that is susceptible to lodging and has low tillering capacity and long, broad, dark, and droopy leaves. The leaves senesce quickly after flowering. The grain has red pericarp and poor cooking quality.

Pokkali and other tolerant donors underwent cell culture to induct somaclonal variation. Somaclonal variants of Pokkali with improved agronomic traits were identified. The traits for which the variants differed significantly from Pokkali. The variant (TCCP 266-2-49-B-B-3) had desirable levels of all tested characteristics and retained salinity tolerance equal to Pokkali. The variant has vigorous growth and, unlike the parent Pokkali, is semi-dwarf, a trait essential in increasing yield potential without lodging. TCCP 266-2-49-B-B-3 had a white pericarp and also improved cooking quality, with medium gel consistency. These characteristics make the somaclonal variant TCCP 266-2-49-B-B-3 superior to Pokkali as a donor of salt tolerance in hybridization programs. It has become a popular donor parent and has produced new high-yielding salinity-tolerant lines, some of which have been released as cultivars.

F1 anther culture-derived lines

Development of a promising salinity-tolerant line through hybridization and selection can take 8-10 years. This is aggravated by the fact that in areas where salinity or other stresses are found, only one cropping season per year is possible. Thus, generation of stable lines takes much longer. This period could be substantially reduced by applying the F1 anther culture (AC) technique.

In 1996, some high-yielding salt-tolerant AC-derived lines (IR51500-AC11-1, IR51500-AC17, IR51485-AC6534-4, IR72132-AC6-1, IR69997-AC1, IR69997-AC2, IR69997-AC3 and IR69997-AC4) had been generated in just 3 years. Most of these lines had been used as donor parents in breeding programs in Bangladesh, Dominican Republic, Egypt, Mexico, Myanmar, Philippines and Thailand (IRSTON, 1991). IR51500-AC11-1 was released as a salt-tolerant cultivar in the Philippines with the name PSBRc50 or ``Bicol'' (Senadhira et al., 2002). This is the first AC-derived line from an indica*indica cross released as a cultivar and was also the first cultivar recommended for cultivation in adverse environments. In India, IR51500-AC17 and IR51485-AC6534-4 were named as commercial cultivars CSR21 and CSR28, respectively, for cultivation on saline-alkaline soils (IRRI, 1997).

BREEDING METHODOLOGY

Conventional Breeding

Almost all the conventional breeding methods have been followed for the development of the salt tolerant materials i.e. introduction, selection, hybridization, mutation and shuttle breeding approach. Most of the initial salt tolerant rice varieties Damodar (CSR 1), Dasal (CSR 2), and Getu (CSR 3) were the pure line selections from the local traditional cultivars prevailing in the Sunderban areas in West Bengal, India. Later on other salt tolerant varietal series like CSR 10, CSR13 CSr23, CSR27, CSR30 and CSR36 were developed through recombination breeding methods like Pedigree method, Modified bulk pedigree method and Shuttle Breeding. The segregating materials are put in the long rows under salt stress with space planting in F_2 particularly. Selection pressure is gradually increased with the generation advancement simultaneously in moderate stress and high stress of sodicity and salinity. CSR 10, CSR13, CSR23, CSR27, CSR30, CSR36, CR Dhan 402, CR Dhan 403, TRY 1, TRY 2, TRY 3, White ponni, CO 43 etc. were developed through recombinant varieties.

Recombination Strategy

Grouping of the genotypes based on the inherent physiological mechanism responsible for salinity tolerance, inter-mating of the genotypes with high degree of expression of the contrasting salinity tolerance mechanism and identifying / screening of the recombinants for pooling of the mechanisms is being followed to enhance the further level of salt tolerance. The genotypes are grouped into different categories based on the physiological mechanism for salt tolerance. Crosses are made between the parents/donors possessing contrasting physiological traits like tissue tolerance, Na^+ exclusion, K^+ uptake and Cl^- exclusion to pyramid the genes governing or contributing for salinity tolerance into one agronomically superior background. The ideal high yielding salinity tolerant variety should posses' traits of able to withstand high amount of Na^+ (tissue tolerant), per day uptake of Na^+ is minimum (takes more number of days for LC_{50} stage), high uptake of K^+ per day, good initial vigour, agronomically superior with high yield potential.

Recent Strategies to supplement the conventional breeding programs:

Marker Assisted Selection (MAS)

DNA MAS has been seen as a means of improving the speed and efficiency of plant breeding programs because it is growth stage independent, unaffected by environment; no dominance effect and efficient to use in early generations. Most widespread use of MAS to date has done in the marker assisted backcrossing (MAB) of major genes to into already established varieties, mega-varieties (which occupies a large area within the country on across the countries) or elite cultivars. These markers could reduce the linkage may around the target gene, and also recover the recurrent parent background within less number of generation in comparison to conventional breeding

Marker-Assisted Selection in early generation: MAS in early generation is most useful for relatively less number of genes but which are affecting the important traits and difficult to phenotype. Two important factor need to be satisfied for effective MAS strategy, first: the markers are tightly linked (1-2 cM) to loci with large effects on trait which are difficult or costly or appear lately (maturity) for accurate phenotyping. Second, specific marker alleles are associated with desired alleles at target loci consistently across the different breeding populations. But unfortunately both of these two situations are not applicable for most traits and most populations. MAS for soybean *cystx* nematode is a good example where maker alleles (2 flanking marks) are consistent with desired allele (*rhg1*) across the populations and it is difficult to phenotype (Cregan *et al*., 1999).

Marker Assisted Backcrossing: Most wide use of markers in conventional breeding have been in back crossing purpose where previously evolved varieties or elite material through conventional breeding is augmented with selected alleles with major effects for which they are lacking. Young and Tanksley (1989), demonstrated that large amount of DNA from the donor can remain around the target gene even after many generation of backcrossing. This surrounding material contributes toward "linkage drag" especially if the donor parent is a wild relative. So markers are used to select the same progeny in which recombination near the target gene have as little chromosome segment as possible. This is called "fore-ground selection".

MAS for Polygenic traits: Most of the traits of economic importance like yield and stresses are controlled by polygenes and considerably influenced by environment and g x e interaction for their expression. These traits are most difficult to breed conventionally and using non-conventional techniques like MAS as well. DNA markers could be of great importance to plant breeding if they are used to aid selection for quantitative traits. Now a days microsatellites or simple sequence repeats (SSR) markers are the first choice of molecules biologist while single nucleotide polymorphism (SNPs) are going to be the most preferred markers of the future. After the completing the draft sequence of rice genome with >99% accuracy, 18,838 new class I SSR markers are reported on its physical map.

Major difficulty in the employing MAS for polygenic traits is the limited phenotyping accuracy of the quantitative trait loci (QTL) because QTLs do not have direct phenotypic variation hence their chromosomal location is typically inferred by calculating the LOD (likelihood of odds) value. The chromosomal location with maximum LOD value has the likelihood for the QTLs of the trait but there is always a good possibility that the QTL is not located precisely at the maximum likelihood position. This precision could be increased by increasing size of mapping population. (Zamir, 2001).

Quantitative Trait Loci (QTL) and candidate genes for salt stress: Identification of molecular markers tightly linked to salt tolerant genes can serve as land marks for the physical localization of such genes facilitating marker assisted selection (MAS). In rice a major gene for salt tolerance has been mapped to chromosome 7 using RFLP markers (Zhang *et al.* 1995). Random amplified polymorphic DNA markers were also shown to be linked to salt tolerance using the same population (Ding *et al.* 1998). Several QTLs for salinity tolerance have also been identified (Prasad *et al.* 2000). Lang *et al.*, 2001 detected one microsatellite marker, RM 223 associated with salt tolerance at vegetative stage. Based on RFLP markers, Koyama *et al.* (2001) have identified the QTLs for Na and K uptake and Na: K ratio. Major QTLs were found on chromosome 6, 4, 1 and 9. IRRI (1997) also reported major gene for salinity tolerance on chromosome 1 which could be similar to the *Sal*T gene of the rice Cornell map. Glenn *et al.* (2002) also tried to construct an AFLP map with the help of 206 markers and found major QTLs for high K absorption, low Na uptake and low Na : K ratio on 5 chromosomes (no. 4, 12, 3, 1 and 10) including the big affect QTL i.e. *Saltol* on chromosome 1. Molecular markers based approach (MAS) has enormous potential to be used as reliable tool to the breeders for the effective, fool proof and early generation screening. Ren *et al.* (2005) mapped eight QTLs responsible for the K^+ and Na^+ content of F_2 RILs of cross Nonabokra and Koshikari in rice. *SKC1* was mapped as a major QTL for shoot K^+ content on chromosome 1. They used BC_2F_2 populations of 2,973 plants to generate high resolution map and also to find out the Koshikari background with clone of *SKC1* from salt tolerant Nonabokra. Under salt stress, the shoot K^+ content in the NIL (*SKC1*) containing small Nonabokra region between caps markers K159 and K 061 was substantially higher than that in the isogenic control Koshikari, hence regulating the K^+/Na^+ homeostasis under salt stress.
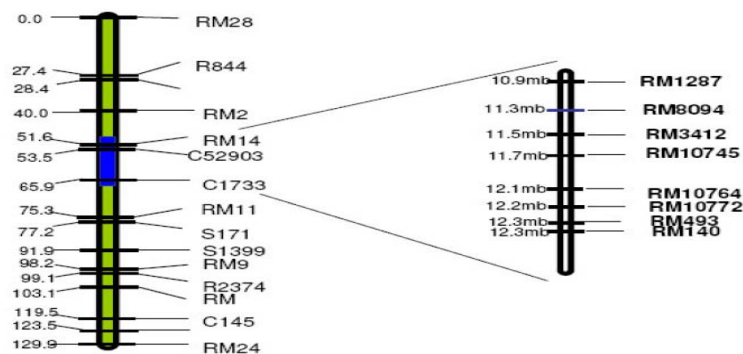

Fig: Saltol segment on chromosome 1 of rice

Practical Application of MAS: Major research program on MAS at International Rice Research Institute (IRRI) are concentrated on introgressing *Sub1* and *Saltol* QTLs for submergence and salinity tolerance respectively into the improved germplasm or adapted varieties. The major QTL for salinity tolerance i.e. *Saltol* is also being transferred to the improved background as well as adapted rice varieties. *Saltol* is located on chromosome 1 and linked with SSR markers like RM 8094, RM 493 and RM 3412. However, in both the cases, these markers are population specific which limits the scope of the MAS application across the diverse material. Flowers *et al.* (2005), while reporting the putative AFLP markers for ion transport and selectivity for salinity tolerance from a custom made mapping population of rice, also cautioned against any expectation of general applicability of markers for these physiological traits.

Transgenic rice for salt tolerance

Breeders around the world are trying to create transgenic plants for solving varied problems like improving yield, stress tolerance, increase in shelf life etc., Transgenic plants which can improve salt tolerance in rice have been attempted. Hoshida *et al.* (2000) have reported on enhanced tolerance to salt stress in transgenic rice that over expresses chloroplast glutamine synthetase gene. Salt tolerance of transgenic rice with *mtlD* gene and *gutD* gene were analysed by Huizhong Wang *et al.* 2000. Analysis of sugar alcohol showed that transgenic rice plants could produce and accumulate mannitol and sorbitol. The salt tolerance of transgenic plants was much higher than that of their controls. Fengyun Zhao *et al.* (2006) have reported on the expression of yeast Na+/H+ antiporter *SOD2* in transgenic rice. These transgenic plants accumulated more K+, Ca2+, Mg2+ and less Na+ in their shoots compared with those of non-transformed controls. Xujun Chen and Zejian Guo (2008) have reported that tobacco OPBP1 enhances salt tolerance and disease resistance of transgenic rice.

Genetics of salt tolerance:

Genetic studies at IRRI indicated that both additive and dominance effects are important in the inheritance of almost all characters associated with salt tolerance (Lee, 1996). At the seedling stage under saline condition, characters associated with salinity tolerance such as shoot length, low Na‡ and high K‡ content in the shoots, and large dry weights of shoots and roots showed highly significant additive effects as the of heritability in these characters is low. Characters at the maturing stage such as plant height and yield per plant showed highly significant additive effects, suggesting the greater importance of additive gene action in the inheritance of these characters (Mishra et al., 1990).

The study of Gregorio and Senadhira (1993) using a 9 parent complete diallel analysis of Na/K ratio in shoots showed that both additive and dominance gene effects govern salinity tolerance (low Na/K ratio). The trait exhibited over-dominance and is controlled by at least two groups of dominant genes. Environmental effects were large and narrow-sense heritability was low (19%). These findings suggest that: (1) early generation breeding populations must be large and replicated, and (2) selection for tolerance must be made in a later generation and under controlled conditions to minimize environmental effects. This further suggests the use of modified-bulk and single-seed descent (SSD) as breeding methods to develop salinity-tolerant rice varieties. A genetic combining ability study (Gregorio and Senadhira, 1993) showed the significance of both general combining ability (GCA) and specific combining ability (SCA) effects in the inheritance of salinity tolerance in rice. Moderately tolerant parents (e.g., IR4595-4-1-13 and IR98884-54-3-1E-P1) were the best combiners for salt-tolerant progenies. Most of the best specific cross-combinations for salt tolerance had susceptible but high-yielding parents such as IR28 and IR29. The presence of reciprocal effects among crosses necessitates the use of high-yielding susceptible parents as males in a hybridization program. Further, the large heterotic effects that were observed suggest the potential of a hybrid rice program for salt-affected lands.

The Inheritance of sodicity tolerance was inferred from the phenotypes observed in F_1 and bulked F_3 generation of 3 crosses between two tolerant parent (CSR10 and CSR11) and two sensitive parents (Basmati 370 and Pakistani basmati) under artificially created sodic soils (pH 9.8) in lysimeters. All the F3 plants, scored based on IRRI scoring method at adult stage, showed continuous variation suggesting that sodicity tolerance is controlled by polygenic trait acting additively in most of the cases along with interactions between the alleles at some loci. A similar inheritance study for salinity was conducted involving same populations. Results indicated the role of few major genes along with numerous minor genes involved for salinity tolerance. It was also inferred that salinity tolerance trait is polygenic in nature and lacks maternal influence (Mishra *et al*., 1998).

Future and research needs

Recent progress in breeding for rice for salinity tolerance and other associated stresses includes the development of rapid and reliable techniques of screening for elongation ability and tolerance for salinity, submergence, iron toxicity, aluminum toxicity, and phosphorus efficiency.

These new screening methods allowed us to identify germplasm with tolerance for all major abiotic stresses. The inheritance of these traits is now known and breeding procedures for incorporating abiotic stress tolerance into high yielding rice cultivars ware formulated. Some prototypes of new plant types for deepwater, salt-tolerant lines released as varieties, and elite germplasm with multiple stress tolerance, but breeding for tolerance to abiotic stresses is very costly and time consuming. The present strategy is to develop MAS techniques which will accelerate the breeding process, increase selection efficiency and affordable to NARES partners. Conventional breeding methods in developing tolerance to salinity and other related abiotic stresses should not be reduced but be supplemented with studies on soil and water management, and biotechnological techniques. Through recent developments in biotechnology, breeders can now review their wish list of desirable traits to be incorporated through gene pyramiding. The challenge is how rice breeders adopt and harness these novel techniques.

CONCLUSION

Salinity is wide spread problem and particularly important for rice in Asia (> 15 m ha are affected). In India, 6.73 million ha are affected by salinity. Substantial effect on rice productivity (0.0 -2.5 t ha^{-1}). Prevalence of salinity is on the rise because of global climate changes, with increased sea level excessive irrigation / improper drainage, underlying rocks rich in salts. Rice is salt sensitive with a threshold of about 2-3 dSm-1 being most sensitive at seedling and reproductive stage. Salinity tolerance is complex trait, involving numerous mechanisms - early seedling vigour, salt exclusion, salt compartmentation, tissue tolerance, salt translocation, osmo-protectants and efficient active oxygen-scavenging system. Among the approaches to combat salinity stress in rice, the most promising , economical and eco-friendly approach is genetic manipulation and exploitation of genetic variability. Wide variability in level of tolerance to salt stress exists among germplasm accessions. They are mostly tall, photosensitive traditional cultivars (poor yielders) from India, Indonesia, Thailand, and Vietnam eg., Pokkali, Chetiviruppu, NV, Pat, Solla, DH, Ketumbar, Khao Seetha and Soc Nau. Pokkali from Kerala is the world renowned donor for tolerance to salinity. Both conventional breeding approaches and innovative approaches are being employed for breeding varieties tolerant to salt stress. *Saltol* gene and other QTLs associated with salt tolerance have been mapped and are being used for breeding slat tolerant lines eg., BRRI dhan 28 and BR11.

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