Genetic Improvement of Rice for Multiple Stress Tolerance in Unfavorable Rainfed Ecology

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SUMMARY

Due to various abiotic stresses very low productivity of rice is noted from unfavourable rainfed lowland ecology, which accounts 18% of total rice growing area. Gradual change in climatic scenario including monsoon results in various abiotic stresses in this ecology frequently at various stages of crop growth in isolation or in combination. Coastal saline ecology is affected by salinity and excess water stresses. Varieties developed for this ecology through conventional and marker-assisted breeding are not significantly tolerant for either salinity or multiple abiotic stresses such as salinity and water logging at reproductive stage. Similarly for semi-deep and deep water ecology also requires biotic stress tolerance and tolerance to water logging and prolonged submergence. Anaerobic germination ability is also requisite in high yielding background for direct sowing in unfavourable lowland ecology. Validation of QTLs including those identified by National Rice Research Institute (NRRI), Cuttack for salt tolerance at reproductive stage is one of current research priorities. Conventional breeding for multiple stress (salinity and excess water) tolerance is being complemented by the efforts at NRRI, Cuttack to identify multiple stress tolerance QTLs and introgression in high yielding background suitable for coastal ecology. Research priorities for sustainable rice production in semi-deep and deep water ecology include identification of QTLs for anaerobic germination ability and prolonged submergence tolerance and pyramiding them along with genes for water logging and biotic stress tolerance. Improvement of productivity and sustainability of this ecology through our research efforts would definitely elevate the country's overall rice productivity and ensure social and economic security of people who depend on this ecology for their livelihood.

1. INTRODUCTION

of this ecology is mainly due to various abiotic stresses, which are found alone or in combination in the various crop growth stages. Therefore, enhancement of abiotic stresses tolerance can only able to improve the production, productivity and overall profitability of the system. Rice is sensitive to salinity at both seedling and reproductive stages. Although rice is sensitive to salt stress, it is a preferred crop in salt-affected coastal areas. Rice can withstand water-logging, and standing water helps in diluting and leaching salts from surface soil (Ismail et al. 2008). Therefore, despite their low yields, many landraces are still preferred by farmers in coastal areas. The degree of salt tolerance varies widely in these landraces, and the variability offers an opportunity for varietal improvement for salt tolerance. On the backdrop of lack of significant improvement of salinity tolerance in rice through traditional breeding, marker assisted breeding using major QTL (*Saltol*) for seedling stage salt tolerance was initiated in India and worldwide. Some of the introgressed high yielding varieties were showing better survivability but that could not guarantee high yield when salt stress occurs at flowering stage. Mitigation of the salt stress effects on rice growth and yield has been tried through both management practices and introduction of tolerant varieties in the affected areas; but use of fresh water supply and other management practices alone in salt-affected areas has generally proven to be uneconomical and not feasible to implement on a large scale. Thus, genetic improvement of salt tolerance in rice is the most feasible and promising strategy to provide stable food production in such a stress-prone environment. In coastal areas rise in temperature, erratic rainfall along with penetration of salty water due to sea-level rise can change the micro environment in multifaceted manners (Wassmann et al. 2009). Multiple abiotic stresses are reality in coastal plains. Addressing multiple abiotic stress tolerance, as well as the consequences of one stress followed by another could develop knowledge related to adaptation of rice crop under variable climatic conditions.

Apart from coastal ecosystem, unfavourable rainfed ecosystem facing problem of prolonged submergence and water stagnation due to flood. Therefore, development of rice varieties with flood tolerance is prime requirement. *Sub1* gene has been identified, cloned and characterized from FR13A, an *indica* rice variety, which gives 14 days (two weeks) tolerance to submergence and it has also been transferred to number of rice varieties through marker assisted introgression. But due to climate change, lowland ecology is facing problems of prolonged submergence, which is often more than 15 days. Therefore, cultivar for such situation is urgently required for sustainable production. On the other hand, early direct sowing in semi-deep water logged situation before onset of monsoon is generally practiced. Moreover, sowing seeds in standing water is an effective means of weed control and direct seeded rice in rainfed ecology is getting popularity due to reduced cultivation cost. Therefore, anaerobic germination ability in rainfed unfavorable ecology in high yielding cultivars is highly needed.

This chapter describes the research problems related to the coastal saline, waterlogging and deep water ecosystems in the backdrop of changing climatic condition and various basic, strategic and applied research carried out at international, national and NRRI level. This also deals with the future strategies and way forward on these issues.

2. PROBLEMS OF UNFAVOURABLE RAINFED ECOLOGY

Traditional approaches for introducing improved rice varieties to farmers have demonstrated significant impact in favourable ecosystems. But limited success was achieved in unfavourable ecosystems, affected by various abiotic stresses such as salinity, submergence, water logging and even drought in some cases. Through traditional breeding many varieties with high yield potential such as CR Dhan 403, CR Dhan 405, Panvel 3, Bhutnath, etc. have been developed for coastal saline areas. But none of them is tolerant to high salinity stress (>6 dS m^{-1}), especially at flowering stage. The major reasons are relatively more complexity and genotype x environment interaction, indicators of physiological traits and well accepted screening protocol for salinity tolerance at flowering stage. Therefore, robust QTLs and markers are also unavailable for marker assisted selection for this trait. Due to lack of physiological traits controlling salinity tolerance at flowering stage and simplicity in screening protocol, any major QTL is yet to be identified and used in marker assisted selection for incorporating tolerance at reproductive stage.

Coastal saline areas are normally mono-cropped with rice grown during the *Kharif* season. Agricultural productivity is low and unstable due to the frequent occurrence of abiotic stresses. During our widespread survey and demonstrations of climate resilient rice varieties in coastal region we felt that salinity and stagnant flooding in combination affecting the rice production in greater extent (Chattopadhyay et al. 2016). Due to this reason still farmers grow local land races tolerant to both salinity and stagnant flooding. Flooding with saline water is a common problem. Intrusion of sea water displaces millions of people from coastal plains and causes the direct threat of the economic security of the poor and marginal people reside along the coastal belts (Wassmann et al. 2009). Development of rice tolerant to multiple abiotic stresses especially salinity and stagnant flooding could improve the productivity and sustainability of the coastal ecosystem.

Deep and semi-deep waterlogged area is another unfavorable lowland ecosystem. Problems associated with this ecosystem are as follows.

- Several rainfed rice areas are at risk of flooding, a stress that decreases yield substantially (Ismail et al. 2013). Poor crop establishment due to deep submergence and water logging at the early stage of the crop growth is predominant. Prolonged submergence/waterlogging suppresses tillering and increases mortality. Water logging and poor drainage also leads to accumulation of toxic substance causing problems such as iron toxicity and sulphide injury.
- Incidence of pests such as stem borer, gall midge, cutworm, GLH and leaf folder and diseases such as bacterial blight, sheath blight sheath rot, tungro and false smut along with abiotic stresses are also frequent in deep and semi-deep water logged areas.
- Submergence tolerance (*Sub1*) has been incorporated in many high yielding varieties adapted to rainfed lowland ecology (Singh et al. 2016). However, additional genes are needed for imparting flood tolerance for 21 days (3 weeks) or more because of regular flooding in India and south-east Asia due to climate change. Better genotypes with submergence tolerance at early and late vegetative stages for more than 21 days are not available.

- Direct seeding is a traditional system of sowing under rainfed ecosystem and it's getting popular in irrigated areas, as the decrease in labor source and increase in labor charges. This system of sowing helps in reducing cultivation charges, a week of crop duration and maintains the physical soil structure for successive cropping. The stagnation of water in unleveled fields after irrigation or copious rainfall immediately after sowing leads to poor germination. Poor establishment and uneven growth pattern due to the difference in germination affects timely intercultural operation (weed/fertilizer, etc), that leads to poor yield. Flash flooding during sowing to harvesting is the state of problem widespread across the south to Eastern states of the country (Anandan et al. 2012). The activity of α-amylase determines the germination under water enhancing stress tolerance. No popular high yielding rice varieties possess this mechanism to germinate under anoxia. Traditional landraces have genes for this trait, but they are tall lodging type with red pericarp and susceptible to diseases and very poor phenotypic performance.

3. STATUS OF RESEARCH

During initial exposure to salinity, plants experience water stress, which in turn reduces shoot growth. As time of exposure to salinity is prolonged, plants experience ionic stress, which leads to leaf tip drying, premature senescence of leaves and mortality of plants. As salt stress is cumulative, injury symptoms increase with time. So, restricting the movements of toxic ions such as Na^+ or Cl^- to growing meristematic tissues and young photosysnthetic organs are vital for survival. The procedure of screening rice genotypes for salt tolerance at seedling stage has been well established and validated through number of experiments. But due to long growing period of rice plants and complexity in measuring tolerance level, no protocol has been found ideal for high throughput screening till date. A salt tolerant (at seedling stage) cultivar, Pokkali has Na^+ exclusion mechanism and thereby maintains low Na^+: K^+ ratio in shoot and new leaf. The *Saltol* QTL in rice was identified by employing a RIL population between the tolerant landrace Pokkali and the highly sensitive IR 29. Among more than 100 identified QTLs, this is the major QTL for seedling stage salt tolerance contributing 43% of variation for seedling shoot Na^+- K^+ ratio. A plasma membrane transporter that regulates partitioning of Na^+ between roots and shoots, *OsHKT1;5* was identified as one of the causative gene located inside the *Saltol-QTL,* which was fined mapped and cloned (Ren et al. 2005). Salinity tolerance in most of the *Indica* genotypes is generally correlated with low Na^+ ions in shoot and OsHKT1;5 is found more active in those tolerant genotypes. But some wild rice accessions of *O. glaberrima* could exclude Na^+ from shoots using a mechanism independent of OsHKT1;5 (Platten et al. 2013). Introgression lines derived from *O. rufipogon* × *O. sativa* cross revealed 15 QTLs for salinity tolerance, 13 of them derived from the tolerant *O. rufipogon* parent (Tian et al. 2011). A genotype with seedling stage salinity tolerance may not be tolerant at reproductive stage as well. For salt tolerance at reproductive stage, 16 QTLs for pollen fertility, Na^+ concentration, Na-K ratio in flag leaf in chromosome 1, 7, 8, 10 (Hossain et al. 2015) were identified. But none of them was validated.

Rice is unique compared to any other cereals to adapt with water stagnation. *Sub1* is the major QTL associated with submergence tolerance in rice. The locus was mapped to chromosome 9, and is composed of a cluster of ethylene response factors (ERF) genes located *in tandem*, named *SUB1A*, *SUB1B* and *SUB1C*. The tolerant *SUB1A-1* allele is derived from the *aus* subgroup of *indica* rice (Xu et al. 2006). Niroula et al. (2012) tested 109 accessions of cultivated and wild relatives, including 12 species, for submergence tolerance, and found *O. rufipogon* and *O. nivara* tolerant accessions that carry the *SUB1A-1* allele, showing that *SUB1* locus architecture determines submergence tolerance in these species, as in *O. sativa.* Rice plants adapt to very deep water stagnation (> 1 m deep) through greater elongation of stems by the action of the gene 'Snorkel' 1 and 2 (Hattori et al. 2009). On the other hand, five putative QTLs of qAG-1-2, qAG-3-1, qAG-7-2, qAG-9-1 and qAG-9-2 elucidating 17.9 to 33.5% of the PV from KhaoHlan On has been reported by Angaji et al. (2010) for anaerobic germination. Later six QTLs on chromosomes 2, 5, 6 and 7 from the landrace Ma-Zhan Red was identified by Septiningsih et al. (2013).

Traditionally cultivated local rice varieties in coastal area have tolerance to salinity and submergence but are low yielding. Some of the widely used varieties are: Vikas, Korgut, Sathi, Pichaneelu, Kuthiru, Kalundai samba, Bhurarata, Kalarata, Karekagga, Pokkali, Chettivirippu, Bhaluki, Rupsal, Nona Bokra, Kamini, Talmugur, Patnai, Getu, etc. These diverse traditional rice varieties are precious genetic resources that provide ecological balance and their conservation is crucial for future food security. Using these germplasm, many high yielding varieties such as CST1-7, Bhutnath, Panvel-3, CSR36, etc. have been developed. *Sub1* gene is attempted to transfer into popular lowland rice varieties such as Bahadur, Ranjit, Varshadhan and Savitri. Similarly, *Saltol-QTL* for salt tolerance at seedling stage is being transferred into varieties, namely, ADT 45, Savitri, Gayatri, MTU 1010, PR 114, Pusa 44 and Sarjoo 52 (Singh et al. 2016). CSR 27 (Pandit et al. 2010) was identified as tolerant variety for salt tolerance at flowering stage. Number of QTLs for salt tolerance at flowering stage were identified, but none of them were found reproducible. Due to population specificity and limited scope of using diverse tolerant germplasm in bi-parental mapping, genome wide association mapping exploiting large scale single nucleotide polymorphism have been practiced to capture natural variations in loci and allelic variations in candidate genes for complex abiotic stresses such as salinity. Using custom-designed array based on 6000 SNPs, Kumar et al. (2015) identified 20 loci associated with Na-K homeostasis. They found *Saltol* as the major salt tolerance QTL not only for seedling stage, but also for reproductive stage in relation to Na^+-K^+ ratio in leaves. New QTLs were also found on chromosomes 4, 6 and 7.

At NRRI, considerable research has been carried out on many aspects of crop improvement for unfavourable rainfed ecology.

3.1. Diversity of Saltol- QTL region and detection of Saltol introgression lines

Landraces from Sundarban region were found diverse in respect of salt tolerance. Salt tolerant cultivars from this area such as Kamini, Talmugur, etc. had allelic difference

Fig. 1. High yielding salinity tolerant line (IET 23400) from the cross Annapurna/ FL 478 carrying *Saltol* QTL.

from the widely used *Saltol*- introgression line, FL 478 in the *Saltol* - QTL region. From IR64/FL478 cross, eight F_8 tolerant and moderately tolerant lines at seedling stage (at EC= 12 dSm^{-1}) along with their parents (FL 478 and IR 64) were subjected to analysis for validation of the microsatellite markers in the *Saltol* QTL region. Four primers (RM10694, RM8094, AP3206, RM493) in *Saltol* region were found polymorphic between FL 478 and IR64. FL478 specific marker alleles for different loci situated from 11 Mb to 12.4 Mb region in chromosome 1 was found in all the lines tested in homozygous condition. These tolerant and moderately tolerant lines sharing a common segment from the donor FL 478 might carry the *Saltol* QTL in this region. Thirty seven tolerant and moderately-tolerant (SES= 3-5) F_7 lines (with 3-5 t ha^{-1} yielding ability (Fig. 1) under coastal saline situation at dry season) derived from the Annapurna × FL478 cross. These lines are sharing a common segment from the donor FL 478 might carry the *Saltol* QTL (Fig. 2) either in homozygous or heterozygous condition (Chattopadhyay et al. 2014).

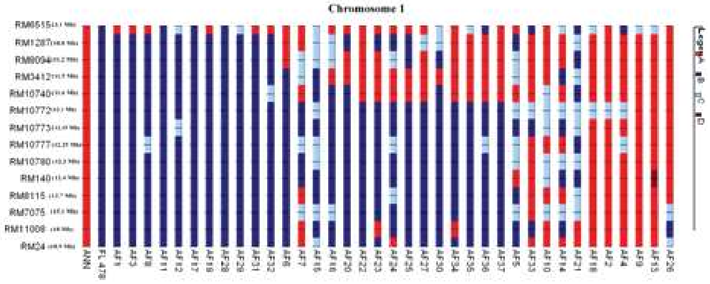

Fig. 2. Graphical genotyping of salt tolerant lines derived from Annapurna/ FL 478 carrying *Saltol* region in chromosome 1.

3.2. Parental combination for improvement of salt tolerance

Unlike Pokkali, Rahspunjar was efficient in maintaining higher level of K^+ despite high Na^+ influx in shoot and located distant from Pokkali in 3-D plot on SSR data. Morpho-physiological difference and the highest allelic difference between SR 26B and Pokkali in the *Saltol* QTL region was supported by non-significant association between *Saltol* marker RM 10745, RM 3412 with tolerance phenotype. Swarna *Sub1* × Rahspunjar and Savitri × SR 26B produced more transgression segregants for tolerance and were found ideal combination (Chattopadhyay et al. 2015).

3.3. Standardization of protocol, identification of donors and understanding the reproductive stage salinity tolerance in rice

Chattopadhyay et al. (2017a) standardized a protocol where setup was established with a piezometer placed in a perforated pot for continuous monitoring of soil EC and pH. Further, fertilized soil was partially substituted by gravels for stabilization and maintaining the uniformity of soil EC in pots without hindering its buffering capacity. The protocol having modified medium (soil:stone 4:1) at 8 dSm^{-1} salinity level was validated using seven different genotypes having differential salt sensitivity. Based on this new medium, important selection traits such as high stability index for plant yield, harvest index and number of grains/panicle and also high K^+ concentration and low Na^+-K^+ ratio in flag leaf at grain filling stage were validated and employed in the evaluation of a mapping population. The method was found remarkably efficient for easy maintenance of desired level of soil salinity for identification of genotypes tolerant to salinity at reproductive stage and evaluation of mapping population.

We have also identified tolerant germplasm with QTL linked markers for salt tolerance at flowering stage using 8 dS m^{-1} NaCl water (Chattopadhyay et al. 2013). Donors for reproductive stage tolerance viz. AC41585, AC39394 (Chattopadhyay et al. 2013), were validated with this method. Results showed that visual scoring of stress symptom and/or SPAD reading may not correspond to the stress effect. Better phenotyping technique such as chlorophyll fluorescence imaging can show clear cut differences between salt treated and untreated rice plants at reproductive stage. Gene expression analysis revealed that salt tolerant Pokkali (AC 415858) genotype showed better K^+-retention and Na^+-exclusion strategies coupled with maintenance of better membrane potential (both plasma membrane and vacuolar) by induction of ATPases and PPases activities in flag leaf (NRRI Annual report 2016-17).

Many QTLs have been identified for salt tolerance at seedling and reproductive stages. But none of them could be validated, fine mapped and cloned for using in salt tolerance breeding programme (Chattopadhyay et al. 2013). At NRRI, 180 backcross derived lines ($BC_3F_{3:5}$) from salt tolerant donor AC41585 and recurrent parent IR 64 were subjected to phenotype in saline (EC= 8 dS m^{-1}) and non-saline environments in 2014 and 2015. Normal distribution with small skewness values was found for all these significant yield attributing traits. Polymorphic 121 SSR, hyper variable-SSR and gene based primers were used and data were analysed via inclusive composite in-terval mapping and two dimensional scaling using QTL IciMapping v4.0.6. Map covered a genetic distance of 1235.53 cM. Two main effect additive QTLs for DEG-S on chromosome 2 and 4 and five additive QTLs for stress susceptibility index for sterility (SSI-STE) on chromosome 2, 3, 4 and 11 with 17-42% phenotypic variance were found common under salinity stress over the years. A main effect QTL with pleiotropic effect for SSI-STE and DEG-S in 11-15 cM region on chromosome 2 was found in marker interval HvSSR02-50 - RM13263 in 2015. Single marker analysis revealed that over the years two markers RM17016 at 64.84cM position on chromosome 1 and

HvSSR06-63 at 77.41 cM position on chromosome 6 were associated with STE-S. Functional genes (Os01g38980.1, Os06g45940.1, Os02g31910.1, Os02g33490.1) located inside or just adjacent (3 cM) to QTLs detected through composite interval mapping and single marker analysis. Functional validation is required for detection of their possible role in salt tolerance at flowering stage (Chattopadhyay et al. 2017b).

3.4. Rice varieties for coastal saline areas

Lunishree, the first high yielding variety for coastal saline area was developed by NRRI, Cuttack. In recent years (for the last six years) high yielding varieties such as Luna Sampad, Luna Suvarna and Luna Barial has been developed by ICAR-NRRI for wet season and Luna Sankhi (Fig. 3) has been developed by ICAR-NRRI in collaboration with IRRI, Philippines for dry season cultivation in coastal saline areas. CR Dhan 402 (Luna Sampad, IET 19470) and CR Dhan 403 (Luna Suvarna, IET 18697) were developed at the National Rice Research Institute (CRRI), Cuttack and released by the Odisha State Sub-Committee on Crop Standards (State Varietal Release Committee) in 2010. They were found promising in testing under the All India Coordinated Rice Improvement Programme (AICRIP), participatory varietal selection (PVS) and other on-farm trials conducted in rainfed coastal saline areas of Odisha. The average grain yield of Luna Suvarna recorded over the four years of testing in Jagatsinghpur, Kendrapara and Puri districts of Odisha was 4.6 t ha^{-1}. It had shown an average 17% yield superiority over the national check CST 7-1 in the All India Coordinated trial. The variety was also found promising in Gosaba, Basanti and Sandeshkhali blocks in the Sundarban area of West Bengal. Luna Sampad also out yielded CST 7-1, the national check and Lunishree in all India Coordinated trial. It is well accepted by farmers of Basudevpur of Bhadrak, Marshaghai of Kendrapara and Puri districts of Odisha with average yield with 3.6 to 4.2 t ha^{-1}. Another rice variety, Luna Barial (CR Dhan 406, IET 19472), developed at the NRRI, Cuttack was released by the Odisha State Sub-Committee on Crop Standards in 2012. This variety was ranked first (3908 kg/ha) in eastern zone in the All India Coordinated trial. Under multilocation trials, it has shown yield superiority with 3.7-4.5 t ha^{-1} over national and local checks in Ganjam, Cuttack, Balasore and Khurda districts of Odisha. All the three varieties can be grown along the coastal belt of eastern India with medium salinity stress (EC 5-7 dS m^{-1}). The parentage and important features of these varieties are listed in Table 1.

Fig. 3. Field view of Luna Sankhi (CR Dhan 405) developed for coastal saline areas in dry season in Odisha.

Table 1. High yielding rice varieties released by NRRI, Cuttack for semi-deep water logged, deep water and coastal saline ecologies.

Sl No	Variety	Ecology	Parentage	Year of release	Released by CVRC/ SVRC	Duration (PS)	Reaction to biotic and abiotic stresses
1	Utkalprabha	Medium/ Semi-deep	Waikyaku/ CR 1014	1983	Odisha	155	MR or field tolerance to major pest and diseases
2	CR 1014	Medium/ Semi-deep	T 90/Urang Urangan	1988	Odisha	160	MR to Sh. B. MR or field tolerance to all pest and diseases
3	Gayatri	Medium/ Semi-deep	Pankaj/ Jagannath	1988	Odisha, West Bengal, Bihar	160	MR to Sh. B. MR or field tolerance to all pest and diseases
4	Kalashree	Medium deep	CR 151-79/ CR 1014	1988	Odisha	160	Tolerant to Blast and GM
5	Panidhan	Medium/ Semi-deep	CR 151-79/ CR 1014	1988	Odisha	180	Tolerant to Blast and GM
6	Tulasi	Medium/ Semi-deep	CR 151-79/ CR 1014	1988	Odisha	170	Field tolerance to major pests and diseases
7	Sarala	Medium/ Semi-deep	CR 151/ CR 1014	2000	Odisha	150	Intermediate, non-lodging, Photosensitive
8	Durga	Medium/ Semi-deep	Pankaj/ CR 1014	2000	Odisha	155	Resistant to RTD and suitable for late planting
9	Varshadhan	Medium/ Semi-deep	IR 31432-8-3-2/IR 31406-3-3-3-1//IR 26940-3-3-3-1	2006	Odisha	160	Non lodging and suitable for water logging situation
10	Hanseswari (CR Dhan 70)	Medium/ Semi-deep	Pure line selection in composite cross	2008	Odisha	150	MR- Blast, Sh B, Tol-False Smut, RTV
11	CR Dhan 501	Medium/ Semi-deep	Savitri/ Padmini	2010	UP, Assam	152	R- Neck blast
12	CR Dhan 500	Deep water	Ravana / Mahsuri	2011	Odisha, UP	160	MR to leaf blast, neck blast, brown spot, gall midge biotype 1&5, stem borer dead heart and white-ear head damageand leaf folder attack

Contd....

Sl No	Variety	Ecology	Parentage	Year of release	Released by CVRC/ SVRC	Duration (PS)	Reaction to biotic and abiotic stresses
13	Jayanti (CR Dhan 502)	Deep water	Samson Polo/ Jalanidhi	2012	Odisha	160	MR- leaf blast, neck blast, sheath bight, sheath rot, rice tungro virus and gall midge biotype1 R- stem borer , leaf folder, rice thrips, and whorl maggot.
14	Jalamani (CR Dhan 503)	Deep water	Panikekoa/ Ambika	2012	Odisha	160	MR- leaf folder, green leaf hopper, leaf blast, neck blast, brown spot, gall midge, dead heartand stem borer
15	CR Dhan 505	Deep water	CRLC 899/ Ac.38606	2014	Odisha and Assam	162	MR-blast, neck blast, sheath rot, sheath blight and rice tungro virus, stem borer, leaf folder, whorl maggot, submergence tolerance, elongation ability
16	CR Dhan 506	Semideep	CRLC 899/ Warda2	2017	Assam, Andhra Pradesh & Karnataka	165	-
17	Prasant (CR Dhan 507)	Semideep	Gayatri/ Sudhir// Varshadhan	2016	Odisha	160-165	MR-NBL, BS, ShB, ShR,SB, LF
18	CR Dhan 508	Deep water	CRLC 899/ Warda2	2017	Odisha, West Bengal, Assam	187	MR-sheath blight, brown spot and sheath rot
19	Lunishree	Coastal saline	Nonasail Gamma Irradiated Mutant	1992	Odisha/ CVRC	145	Tolerant to coastal salinity
20	Luna Sampad (CR Dhan 402)	Coastal saline	Mahsuri / Chakrakanda	2010	Odisha	140	Tolerant to coastal salinity
21	Luna Suvarna (CR Dhan 403)	Coastal saline	Mahsuri / Ormundakan	2010	Odisha	150	Tolerant to coastal salinity
22	Luna Barial (CR Dhan 406)	Coastal saline	Jaya / Lunishree	2012	Odisha	155	Tolerant to coastal salinity
23	Luna Sankhi (CR Dhan 405)	Coastal saline (dry season)	IR31142-14-1-1-3-2/ IR71350	2012	Odisha	120	Tolerant to coastal salinity

3.5. Enhancing excess water tolerance and biotic stress tolerance for unfavourable rainfed lowland ecology

By screening of thousands of traditional landraces at ICAR-NRRI, several tolerant sources form different genetic background was identified and several rice varieties suitable for deep and semi-deep water ecosystem have been developed. Varshadhan (Fig. 4), Sarala, Gayatri, CR Dhan 500 and CR Dhan 505 are popular among them (Table 1). Molecular marker integrated backcross breeding program has been employed to transfer three major BB resistance genes (Xa21, xa13 and xa5) into Jalmagna variety. The three major BB resistance genes pyramided lines exhibited high level of resistance and provided durable resistance under deep water situation (Pradhan et al. 2015). The ICAR-NRRI, Cuttack identified a genotype, AC 20431B, which gives submergence tolerance up to 21 days. A mapping population was developed by crossing of Swarna-Sub1 and AC 20431B for mapping and identification of novel genes responsible for submergence tolerance other than *Sub1* gene. An attempt was also made for identification of linked marker(s) for 21 days submergence tolerance. The well characterized *Sub1* gene gives submergence tolerance for 14 days (2 weeks). Therefore, Swarna-Sub1 was used as one of the parent with AC 20431B (donor parent) for mapping and identification of 21 days submergence tolerance genes other than *Sub1*. A set of 568 F_2 plants were submerged for 21 days. Based on selective genotyping results, a marker RM27322, located on chromosome 11 was found to be linked with 21 days submergence tolerance. This marker will be further validated by genotyping of fixed population (NRRI Annual Report 2017).

Fig. 4. Popular rice variety, Varshadhan in water logged condition at Sandeshkhali-1 block of Sundarban.

3.6. A transcriptomic study to understand the combined effect of waterlogging and salinity stress in rice

In coastal-saline belts rice often faces combined stresses of waterlogging and salinity during different phases of growth. To assess the physiological and metabolic changes in rice associated with waterlogging and salinity stresses, a transcriptome profiling was performed in two waterlogging tolerant rice genotypes, Varshadhan (salinity susceptible) and Rahspunjor (salinity tolerant). Transcriptome analysis in leaf sheath at reproductive stage revealed that in response to waterlogging stress a total of 1489 and 1028 genes were differentially expressed in Varshadhan and Rahspunjor, respectively. Interestingly, combined stress of waterlogging and salinity (WS) resulted in fewer numbers of differentially expressed genes (748 and 840 in Varshadhan and Rahspunjor, respectively) in both the genotypes. Although both the studied genotypes were tolerant to waterlogging stress, but the transcriptome data primarily indicated existence of differential tolerance mechanisms in them. Varshadhan showed up-regulation of hormonal biosynthesis pathway genes (ethylene and gibberellic acid) and triggers NADPH oxidase activity pointing towards ethylene dependent aerenchyma formation, while Rahspunjor showed up-regulation of genes related plant growth (SPL 8, SPL 16 etc.) as stress induced response. The combined stress (WS) showed up-regulation of Ca^{2+}-dependent signalling (Ca^{2+}-ATPase, CAX etc.) in both the genotypes, but the induction was more pronounced in Rahspunjor. Changes in the expression level of key K^+-transporters (up-regulation of HAK5 and down-regulation of AKT1) emphasized better K^+-retention ability in Rahspunjor under salinity stress contributing towards its salt-tolerant behaviour as compared to Varshadhan (Chakraborty et al. 2017).

3.7. *Breeding for multiple abiotic stress tolerance for coastal ecology*

Few elite breeding lines with salt and waterlogging tolerance were performing well in multilocational testing (Fig. 5).

- Salt tolerant lines, CR 2459-23-1-1-S-B1-2B-1 (Gayatri/Rahspunjar) (IET 25101) and CR 2839-1-S-10-B2-B-43-3B-1 (Swarna/ FL 496) (IET 25078) were also performed well in waterlogged situation with estimated grain yield of 4 t/ha. These lines were also promoted to AVT-1 in CSTVT trial.
- Salinity tolerant lines with Waterlogging tolerance were identified 2016-17
  - CR 2851-S-1-B-4-1-1-1-1 (Gayatri/SR 26B)- 160 days MS yield- 4403 kg ha^{-1}
  - CR 2850-S-2B-12-1-1-2-1-1 (Gayatri/FL 496) 160 days- MS- 4616 kg ha^{-1}

Fig. 5. IET 25078 (CR 2839-1-S-10-B2-B-43-3B-1) performed well in salinity and stagnant flooding affected Basanti block of Sundarban, West Bengal in 2017.

3.8. *Researches on agronomic practices for coastal ecosystem*

On-farm trials were conducted in the Ersama block of Jagatsinghpur district (Odisha) using rice varieties were selected nutrient management practices were evaluated and the most promising options were validated in participatory farmer-managed trials during 2004-2007 at six to eight locations. In the shallow lowlands, the findings suggest that under both the shallow and intermediate lowlands, *Sesbania* for the wet season and *Azolla* biofertilizer for the dry season are promising organic nutrient sources that can improve soil quality and contribute to enhancing and sustaining crop productivity in coastal areas. Among different integrated nutrient management practices, *Sesbania* green manuring (GM) for intermediate lowlands (0-50 cm water depth), *Sesbania* GM + prilled urea (PU) and *Sesbania* GM + *Azolla* for shallow lowlands (0-30 cm water depth) in the wet season, and *Azolla* + PU in the dry season were found to be promising (Singh et al. 2009).

Poor crop stand and low fertilizer inputs are the important causes of poor and unstable rice yields in coastal saline ecosystem with multiple stresses. Appropriate crop establishment and nutrient management technology options were validated through farmers' participatory on-farm trials. Use of robust aged (50-day old) seedlings raised with nursery fertilization and closer planting (15x10 cm) in the wet season, and early planting (January 1st fortnight) in the dry season significantly improved the crop survivability and yield. However, substantial yield improvements (91% in wet and 75% in dry season) could be achieved by combining salt-tolerant varieties with improved crop management (Saha et al. 2008).

Studies on water management for dry season rice indicated that marginally-saline (EC 2.4-3.1 dS m^{-1}) water could be used safely for two weeks during the vegetative stage under high salinity condition. Providing fresh water irrigation 2 days after disappearance of standing water during the vegetative stage produced as much yield as continuous ponding. These approaches would help in substantial saving of precious fresh water and expanding the cropping area, leading to enhanced land and water productivity. For non-rice crops, the highest yields of sunflower and groundnut were obtained with 4 cm irrigation at 15 day intervals.

4. KNOWLEDGE GAPS

The following questions are to be addressed in the future research programme for improvement of abiotic stress tolerance in unfavourable rainfed ecology.

- What is the robust QTL for salt tolerance at reproductive stage in rice?
- Is it possible to identify multiple stress tolerance QTLs in rice?
- Can we incorporate both salinity and waterlogging tolerance to a high yielding background in coastal ecology through traditional and marker-assisted breeding?
- What is the robust QTL for anaerobic germination ability required for direct sowing in lowland rainfed ecology?
- Can we utilize source for improvement of tolerance for prolonged submergence for more than 2 weeks?
- Can we combine biotic stress tolerance (Bacterial blight and Stem borer) with abiotic stress tolerance in unfavourable rainfed system?

5. RESEARCH AND DEVELOPMENT NEEDS AND RESEARCH PRIORITIES

5.1. Breeding for desirable plant characters of rice for water logged and deep water ecologies

- *Medium/semi-deep water logged lowlands:* Desirable plant characters for this ecology include 115-130 cm plant height, stiff culm, erect leaves, low to moderate tillering ability, high N-use efficiency at low N level, early seedling vigour, drought tolerance at seedling stage, prolonged submergence (beyond 15 days) tolerance with less elongation and without culm elongation, photoperiod-sensitive, thermo-insensitive, heavy panicle weight type and strong seed dormancy.

- *Deep water ecology*: Desirable plant characters for this ecology include 130-160 cm plant height, stiff culm, erect leaves, low tillering ability, high N- use efficiency at low N level, early seedling vigour, drought tolerance at seedling stage, submergence tolerance with leaf sheath and culm elongation, photoperiod-sensitive, thermo-insensitive, heavy panicle weight type, strong seed dormancy and kneeing ability.

5.2. Breeding scheme for water logging condition

- For multiple abiotic stress tolerance breeding for areas where submergence at early stage of crop growth was followed by the stagnant flooding at the later stage was frequently occurred, F_2-F_3 breeding lines are screened for submergence followed by screening for pest-diseases and evaluation for stagnant flooding tolerance. The tolerant check for screening for submergence tolerant is Swarna Sub1 and for stagnant water are IRRI 154 and Khoda.

5.3. Breeding for desirable plant architecture for coastal saline ecology

- *Coastal salinity at wet season:* Desirable plant characters for this ecology include 120-130 cm plant height, more than 145 days duration, stiff culm, erect leaves, moderate tillering ability, heavy panicle weight, early vigour, salinity tolerant at seedling and reproductive stages, submergence and/ or water logging tolerance with minimum elongation, photoperiod sensitive and strong seed dormancy.

- *Coastal salinity at dry season:* Desirable plant characters for this ecology include 100-110 cm plant height, 100-110 days duration, stiff culm, erect leaves, moderate to high tillering ability, early vigour, tolerant to salinity at both seedling and reproductive stages.

5.4. Breeding scheme for coastal saline condition

Wet season in eastern coastal area is affected not only by salinity but also by submergence at vegetative stage and water logging at different crop growth stages. Standardization of multiple stress tolerance breeding strategy and management practices to be given priority. The suitable breeding scheme for multiple abiotic stress tolerance in coastal saline areas in wet season is presented below with a schematic diagram (Fig. 6).

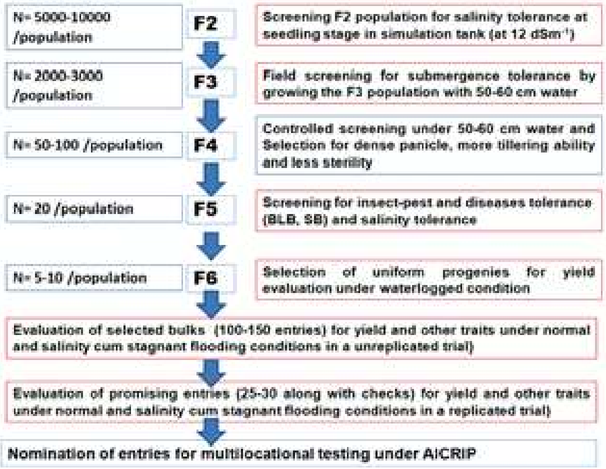

Fig. 6. Breeding scheme for multiple abiotic stress tolerance in coastal saline areas in wet season

5.5. Use of wide genetic base in rice improvement

- Evaluation of wild accession can open up the possibility of getting better tolerance for abiotic (salinity and water logging) and biotic stress (stem borer, BB, etc.) required in unfavourable rainfed lowland ecology. Effort is needed to utilize these tolerance sources in developing agronomically superior elite rice lines. Introduction of wider genetic base in pre-breeding lines and identification of new QTLs associated with multiple abiotic stresses would help in developing more robust varieties for this unfavourable ecology.

5.6. Molecular breeding approaches for improvement of rice for unfavourable lowland ecology

- *Saltol-QTL* explained 46% variation for Na^+-K^+ homeostasis leading to salt tolerance at seedling stage. QTLs other than *Saltol* to be identified and pyramided in tolerant lines along with *Saltol* QTL.
- Lot of scope for identification of better donors, their physiology and gene expression study in relation to salt tolerance at flowering stage. QTLs for reproductive stage salt tolerance are to be validated.
- Pyramiding of genes/QTLs for tolerant to submergence (*Sub1*), salinity (*Saltol*) and water logging in popular rice varieties of coastal saline areas are required.
- Pyramiding of genes/QTLs for anaerobic germination ability, tolerance to excess water submergence during germination and vegetative stages for getting successful crop at direct seeded rainfed lowland areas.
- Promising QTLs can be introgressed into popular rice varieties through MAB approach to improve their performance under flash flooded condition. If

pyramiding of different tolerant mechanism together into a single background would be beneficial to get tolerance under different microclimatic condition.

6. WAY FORWARD

The climate resilient varieties for rainfed unfavourable ecosystem should have multiple stress tolerance. The orientation of research is in that direction. Identification of new sources of multiple abiotic stress tolerance and development of mapping populations (Swarna/Rahspunjar, Savitri/AC39416a) for identification of QTL for multiple abiotic stress tolerance (salinity and waterlogging) is in progress. The salinity and waterlogging tolerant germplasm from cultivated and wild (*O. rufipogon* and *O. nivara*) rice collection are also being used for development of elite pre-breeding lines (BC_2F_2). On the other hand, research is focused on the identification of the robust QTLs other than Sub1 for excess water tolerance at various stages of crop growth and pyramiding them along with genes of important biotic stress tolerance. The use of community participatory approaches in the design, validation and dissemination of technologies is required to address problems of rice cultivation in unfavourable ecology. It is also required to anticipate and address constraints to the widespread adoption of new salt and water logging tolerant varieties and evaluate additional crops adapted to unfavourable ecology. Further studies will be carried out in the ICAR-NRRI-Regional Coastal Rice Research Station, Naira on the effects of soil salinity changes in the coastal districts on spatial and temporal scale and their consequences of production on the economics of high yielding and salt tolerant genotypes of rice.

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