6. Salinity tolerance

K Chattopadhyay, B C Marndi, A K Nayak, S K Mohanty, A Poonam, P K Guru, J N Reddy, K R Mahata, D P Singh and R K Sarkar

6.1 Introduction

Both inland and coastal salinity are now becoming widespread problems for rice cultivation in India and other rice growing countries in the world. Among many abiotic stresses salinity is the most important constraint which delineates low productivity of rice in the salt affected soils. Salt affected soils have been broadly classified as i) Saline soils dominated with neutral salts and ii) Sodic soil dominated with basic salts. Rice management in salt affected soils is based on the concept that; (i) either changes the growing environment and makes it suitable for the normal growth of plants or (ii) select varieties so that they could be grown in such areas. Combination of two approaches has been the mainstay research agenda to combat the salt stress. The current chapter deals exclusively on reliable screening techniques that are repeatable and successful breeding strategies particularly on coastal saline soils. Salt tolerance is a very complex trait and reported to be of polygenic in nature that's why only few successful attempts have been made to develop salt tolerant rice varieties. Due to the rise of sea levels under the influence of increasing temperatures, many areas in the eastern coastal region of India where high-yielding rice varieties have been grown earlier are now occupied by the local landraces and in some cases, the lands have become unsuitable for rice cultivation. Sensitivity of rice crop to salinity stress varies with their growth stages. Rice is mainly susceptible to salt stress at early vegetative and from the panicle initiation to the grain filling stage. The *Saltol* is a recognized major QTL for seedling stage salt tolerance identified from population derived from IR 29/Pokkali. *Saltol* QTL linked molecular markers are complemented with phenotyping in selection for higher survivability of rice genotypes under salt stress. Conventional breeding systems could able to deliver a good number of high yielding varieties with moderate level of salt tolerance for coastal saline areas. But marker assisted selection for salt tolerance at flowering stage is not yet standardized. Lack of explicit screening protocol and selection criteria are main contributors to this bottleneck.

6.2 Manifestation of salt stress on plant

Salt stress can affect different crops differently. Plant can not survive under extremely high salt stress but under the moderate to low salt stress plant growth rate is affected manifesting symptoms which could be associated with morphological, physiological or biochemical alterations.

6.2.1 Morphological effects

There are some visible differences in plants suffering from salinity stress at field level. Major symptoms are:

- Leaf rolling
- White leaf blotches
- White leaf tip followed by tip burning (salinity)
- Stunted plant growth
- Low tillering
- Change in flowering duration
- Spikelet degeneration
- Spikelet sterility

- Low harvest index
- Less florets per panicle
- Less 1000-grain weight
- Low grain yield
- Poor root growth

6.2.2 Physiological and biochemical effects

Under altered stress environment numerous physiological and biochemical changes take place some of them also contribute to the salt tolerance mechanism. Rice crop encounters following physiological and biochemical manifestation under higher salt stress conditions.

- High Na^+ transport to shoot
- Preferential accumulation of Na^+ in older leaves
- Lower K^+ uptake
- Increase level of reactive oxygen species (ROS)
- Lower fresh and dry weight of shoot and roots
- Change in esterase isozyme pattern
- Increase of non-toxic organic compatible solutes

6.3 Screening criteria

Morphological, physiological or biochemical parameters respond differentially to salt stress necessitating for a reliable screening criterion. Salt related problems seldom occur in isolation and are coupled with many associated problems. Hence, screening criteria are grouped into three categories:

6.3.1 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 for the effective and reproducible screening.

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 high stress, it is a good selection criterion.

6.3.2 Physiological parameters

Extremely high salt concentration kills the plant but the moderate salt stress exhibits the growth differences among the crop varieties. Breeding crop varieties for saline soils, obviously tolerant to many kind of salt stresses, very often create such conditions in which visible/morphological plant traits do not provide sufficient information for selection of segregants and potential donor materials. 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. Different physiological mechanisms mentioned below responsible for salt tolerance.

- *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.
- *Tissue tolerance:* Tissue tolerance is measured in terms of LC50 which is an analogue of LD50. Dose of chemical or any toxic material which kills 50 %

individuals of a population is called lethal dose (50%) and denoted as LD50. Here, LC50 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.

6.3.3 Biochemical parameters

Accumulation or increase in level of certain osmotic constituents such as amino acids, sugars and other osmotically active organic substances in plants in response to the salt stress are indications of altered nitrogen and carbohydrate metabolisms. The increased level of osmolytes helps the plant in combating the osmotic stress tolerance. Some of the osmotic constituents are listed below:

- *Proline Content:* Proline acts as an endogenous osmotic regulant in halophytes. The accumulation of free proline in plants exposed to diverse stresses has considerable eco-physiological significance. It increases with increase in salinity and alkalinity in rice.
- *Organic solutes:* It has been well established that increase in salt stress invokes the higher concentration of organic solutes in cytoplasm and the amount of solute production varies among genotypes depending upon their tolerance level. They have two major roles (a) osmotic balance when electrolytes are lower in cytoplasm than vacuoles and (b) act as protectant to the enzymes under high electrolyte concentration in cytoplasm. Major solutes, which increase with high salt stress in crop plants, are proline, glycinebetaine and sugars *etc.*
- *Glycinebetaine:* Glycinebetaine synthesis in plant is a two-step pathway; first choline is converted into betaine aldehyde and second this betaine aldehyde is oxidized in presence of betaine aldehyde dehydrogenase (BADH) that leads to the biosynthesis of glycinebetaine. It is reported that rice plants possess the ability to take up exogenously added betaine aldehyde through the roots and convert it to glycinebetaine, resulting in an enhanced salt-tolerance of the plants (Nakamura *et al.*, 1977).
- *Sugar content:* The plant encountering salt stress often show reduction in starch and total carbohydrates and increase in reducing sugars. Limited supply of essential metabolites like carbohydrates retard the plant growth under sub-lethal salt stress. Significant decrease in the sucrose content of leaves was observed in rice genotypes under both salinity and alkalinity stresses. It decreased with increase in salinity and alkalinity.
- *Starch content:* Salt stress induces change in total leaf starch content. There is significant variation observed among the genotypes grown under stress conditions and the foliar starch decreased under all levels of stress. The accumulation of starch and sucrose content decreased with increase in level of salt stress in almost all varieties. Still it is not considered as a foolproof screening criterion (Rao, 2000).
- *Protein and enzymes:* Some of the stress responsive proteins (SRPs) and enzymes could be used as the potential criteria for screening of salt tolerant genotypes. Water soluble proteins increased in the leaves of of plant at higher salinity level.
- *Ethylene:* Role of ethylene has been indicated in plant growth response and its biosynthesis and action are strongly influenced by stress (Yang, 1985).

Among its many effects are alleviation of salt stress, high temperature and osmotic stress during germination and plant establishment (Verma *et al.*, 1973; Palevitch and Thomas, 1974; Khan *et al.*, 1987).

6.4 Screening for salinity tolerance at seedling stage

6.4.1 Screening facility- Simulation tank

6.4.1.1 Design of tank

- Dig-out cavity structures made of brick-mortar-concrete materials and fill with artificially prepared or natural transported saline soil. It has been designed for high throughput screening of rice genotypes for salinity tolerance.
- These structures are covered with poly-carbonated transparent (have >80% light transmission) sheet. The design the microplot structure is presented below (Fig. 6.1).

6.4.1.2 Preparation of tank and imposition of salinity stress

- Wall of the tank is reinforced with iron frame support. PVC delivery pipe connected with perforated laterals is placed at the bottom of the tank. The number and size of holes on the laterals are such that total cross-sectional area of holes does not exceed the internal cross-sectional area of the supply pipe.

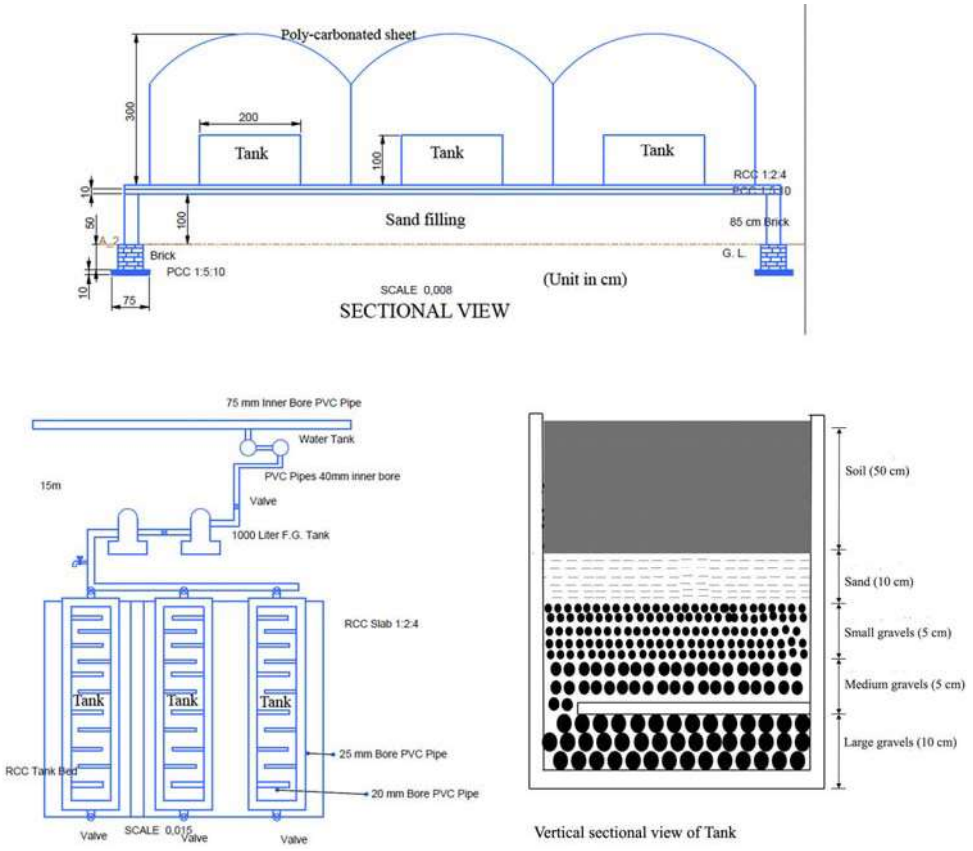


Fig. 6.1: Structural design of salinity screening facility and sectional view of salinity tank

- Delivery pipe and laterals are covered with gravels. The gravel layer is at least 10-15 cm (20 cm) thick. Gravels are then covered with a 10-cm thick coarse sand (>0.5 mm) layer. Laterals having holes are covered with nylon net sleeves to prevent clogging with suspended soil particles. Pebbles and coarse sands are washed with dilute acid.
- Soil packing started just above the coarse sand layer at the bottom of the tank. Loose dry soil is added and spread over the coarse sand till it formed a few cm thick layer. Then the soil is moistened by sprinkling water on it and brought to a soil moisture range conducive for compaction. Soil is compacted with a *dhurmus* normally used for making a floor/roof. This way the tank is packed with soil with an average bulk density of about 1.25 Mg m^{-3}.
- Salinization of the soil in the tank is brought about by releasing the saline water of desired EC value from the reservoir into the soil tank in installments at about 3-4 days interval. This time interval allowed the soil at successive depths to be saturated by capillary rise of saline water. Thus bye-pass flow of water through macro pore system is avoided to some extent. Evaporation loss of the flood water is compensated by spraying tap water with a hand sprayer. Any dilution error is corrected by releasing saline water from the reservoir (Plate 6.1)

Plate 6.1: Simulation tanks covered by polycarbonate sheet

6.4.1.3 Procedure of screening

Using the screening facility the following types of plant materials has been screened for salt tolerance at seedling and flowering stage (Plate 6.2).

- Selection of tolerant seedlings from pedigree (F_2-F_3) and bulk population (F_4-F_5) from various cross combinations involving salt tolerant donors has been done at salinity micro-plots at EC =12 dSm^{-1}. The salt tolerant donors are rescued and transplanted in the field.
- Evaluation of germplasm and mapping population at the seedling under EC of 12 dSm^{-1} has been done.
- Evaluation of germplasm at flowering stage at EC = 8 dSm^{-1} has also done.

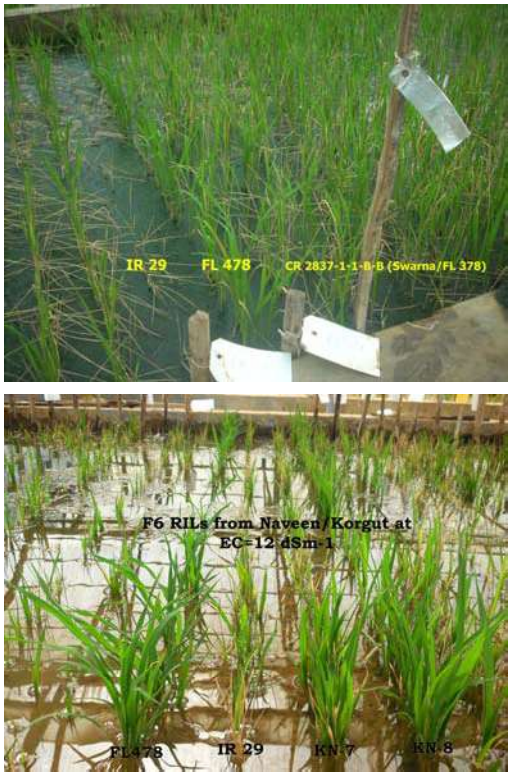


Plate 6.2: Screening breeding lines (above) and mapping population (below) for salinity tolerance at simulation tank at seedling stage

6.4.1.4 Solution culture

6.4.1.4.1 Nutrient medium

Hogland nutrient solution (Hoglang and Arnon, 1950) is found quite effective in raising seedlings in hydroponics. The following composition is found in 1.63 g of dehydrated basal salt mixture.

Table 6.1. Composition of Hogland solution

S.No.	Ingredient	Milli gram/ litre
1	Potassium nitrate	606.60
2	Calcium nitrate	656.4
3	Magnesium sulphate	240.76
4	Ammonium phosp hate monobasic	115.03
5	Manganese chloride. $4H_2O$	1.81
6	Boric acid	2.86
7	Molybdenum trioxide	0.016
8	Zinc sulphate. $7H_2O$	0.22
9	Copper sulphate. $5H_2O$	0.08
10	Ferric tartrate	5.00
	Total	1630.00

- Suspend 1.63 g of dehydrated basal salt mixture in 600 mL of distilled water and rinse media vial with small quantity of distilled water to remove traces of powder.
- Adjust the medium pH of 5.5 using 1*N* HCl/1*N* NaOH.
- Make up the final volume to 1000 mL with distilled water.
- Store the prepared medium at 2-8°C away from direct light.
- This 1 L stock solution is diluted with deionized water to make volume of 50 L.

Yoshida nutrient solution (Yoshida *et al.*, 1976) is also found effective in providing desirable nutrients to the growing seedlings. It contains the following elements in 450 mL stock solution (Table 6.2).

From the above stock solution 3600 L nutrient solution can be prepared.

Adjust the medium pH of 5-5.5 using 1*N* HCl/1*N* NaOH.

Table 6.2. Composition of Yoshida solution

S. No.	Reagent	Element	Stock solution (mL)	Concentration (ppm)
1	NH_4NO_3	N	450	40
2	$NaH_2PO_4.2H_2O$	P	450	10
3	K_2SO_4	K	450	40
4	$CaCl_2.2H_2O$	Ca	450	40
5	$MgSO_4.7H_2O$	Mg	450	40
6	$MnCl_2.4H_2O$	Mn	450	0.50
7	$(NH_4)_6.Mo_7O_{24}.4H_2O$	Mo		0.05
8	$ZnSO_4.7H_2O$	Zn		0.01
9	H_3BO_3	B		0.20
10	$CuSO_4.5H_2O$	Cu		0.01
11	$FeCl_3.6H_2O$	Fe		2

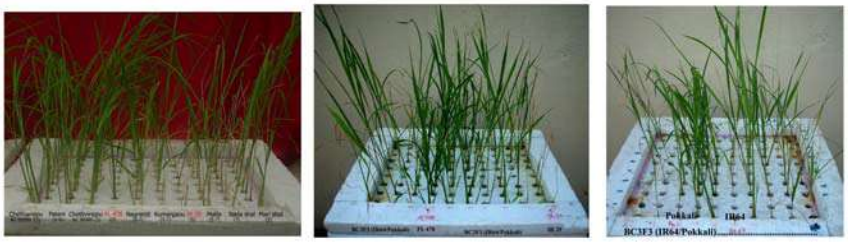
6.4.1.4.2 Procedure

The following steps are taken for screening of rice genotypes for salt tolerance at seedling stage (Plate 6.3)

- Seeds are kept at 55°C for consecutive three days to break dormancy if any and ensure uniform germination.
- The pre-germinated seeds of each genotype are placed in each of the 10 holes on the styrofoam seedling floats kept on plastic trays filled up with the Hogland or Yoshida nutrient solution (Yoshida *et al.* 1976).
- The seedlings are allowed to grow there for three days and then NaCl is added to the nutrient solution to obtain EC of 6 dS m^{-1}.
- After another three days of growth at EC 6 dS m^{-1} for acclimatization, salinity is raised to 12 dS m^{-1} by adding 6 g NaCl per litre of nutrient solution.
- When symptoms of salt-stress appeared severe in the susceptible check IR 29, all the genotypes are scored visually on 1 to 9 scale using the modified standard evaluation system (SES) of IRRI (Gregorio *et al.*, 1997).

Step-1: Placing of pre-germinated seeds on the Styrofoam float kept on plastic tray filled up with nutrient solution

Step-2: Evaluation of rice genotypes in replicated design (CRD) in nutrient solution with EC= 12 dSm^{-1} against the control (EC= 0 dSm^{-1})



Step-3: Scoring of tolerance reaction (SES score) in 1-9 scale of rice germplasms, mapping population (BC_3F_3) and its parental lines (IR 64, Pokkali) when susceptible check IR 29 attains score 9

Plate 6.3: Steps followed for evaluation of germplasm and mapping population for salt tolerance at seedling stage

- The data on shoot length (cm) and root length (cm) are recorded in 10 seedlings in each replication and the growth rate is calculated based on per cent increment of shoot length in the period starting from initiation of the stress at 12 dSm^{-1} and at the end of the experiment.
- The plant samples are oven-dried at 70° C for 5 days and then shoot and root dry weight are recorded.
- The samples are mixed together, finely ground and analyzed by flame photometry for sodium and potassium concentration after 48 h of extraction of 15 mg of samples with 30 mL of 1*N* HCl, following the procedure described by Yoshida *et al.* (1976).

Table 6.3. Modified standard evaluation score (SES) based on visual observation of rice genotypes under salt stress at seedling stage

S. No.	Observation	Score	Reaction	Sources
1.	Normal growth, no symptom	1	Highly tolerant	Nil
2.	Nearly normal growth, new leaves are coming, but leaf tips or few leaves whitish and rolled	3	Tolerant	Pokkali (AC41585, AC 39416), FL 478
3.	Growth retarded, most leaves rolled with white tips, but new leaves are coming and few leaves are elongating	5	Moderately tolerant	Rupsal, Marisal, Rahspunjar
4.	Complete cession of growth, no new leaves, most leaves dried, some plants drying	7	Moderately susceptible	Varshadhan, IR 64
5.	Almost all plants dead or drying	9	Highly susceptible	IR 29, Gayatri, Savitri

Table 6.4. Na^+/K^+ ratio of rice genotypes screning for salinity tolerance

S. No.	Rice genotypes	Na^+/K^+ ratio in shoot	Tolerance reaction
1	Pokkali (AC39416)	0.20	Tolerant
2	FL478	0.20	Tolerant
3	Chettivirippu (AC39389)	0.20	Tolerant
4	Pokkali (AC41585)	0.21	Tolerant
5	FL496	0.22	Tolerant
6	Patnai (AC43220)	0.22	Moderately tolerant
7	Kamini	0.25	Moderately tolerant
8	SR 26B	0.26	Moderately tolerant
9	Rahspunjar	0.32	Moderately tolerant
10	Matla	0.33	Moderately tolerant
11	Marishal	0.33	Moderately tolerant
12	Rupshal	0.39	Moderately tolerant
13	Kanakchur (AC43231)	0.46	Moderately susceptible
14	Nadashal (AC43225)	0.56	Moderately susceptible
15	Savitri	0.69	Highly susceptible

Na^+/K^+ ratio in shoot: Rice genotypes differ for Na^+ and K^+ concentration and Na^+/K^+ ratio in shoot during salt stress. Na^+/K^+ ratio in shoot is important indicator for salt tolerance at seedling stage. Na^+/K^+ ratio and tolerance reaction of some of the local and improved cultivars/lines (Chattopadhyay *et al.*, 2014) are presented below,

Chlorophyll fluorescence:

- The ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) plays a vital role in photosynthesis and it is an estimate of PSII maximum efûciency under salt stress conditions. Singh and Sarkar (2014) normalized the values of the chlorophyll *a* fluorescence parameters *Fv/Fm* and overall performance index (PI_{ABS}) and used in comparison among different cultivars for tolerance in salt stress.
- The decline in the values of *Fv/Fm* (Fig. 6.2) under salt stress in the susceptible cultivars can be attributed to the increasing level of energy dissipation and decreasing utilization of the absorbed energy in the photochemical processes.
- The PI_{ABS} is a key chlorophyll fluorescence parameter that provides useful and quantitative information about the state of plant vitality.
- The data shows that the highly tolerant cultivars Kamini, Paloi, Pokkali, AC39416 and FL478 maintained their vitality even after 10 days of salinity stress with 12 dSm^{-1} NaCl solution. Tolerant line, Pokkali (AC41585) maintained high Fv/Fm ratio along with low Na^+/K^+ ratio in leaves under salt stress.

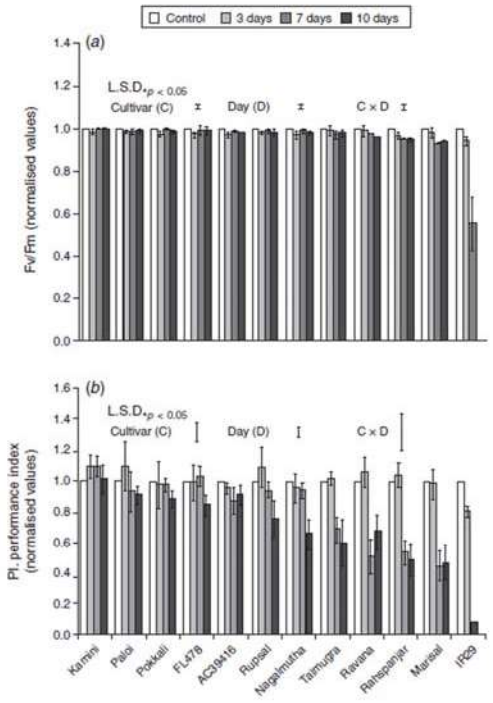


Fig. 6.2: Normalized values (salinity/control) of chlorophyll a fluorescence parameters Fv/Fm and PI_{ABS} in 1st fully expanded leaf from top on different days after salinity (12 dSm^{-1}) treatment; Data for IR29 are not available on day 10 of salinity treatment due to the complete mortality. Values are mean of wet and dry seasons. Bar represents S.E.; L.S.D.

6.4.2 Screening for salt tolerance based on *Saltol QTL* inked molecular markers

A major QTL for seedling stage salt tolerance, *Saltol*, is mapped on the short arm of chromosome 1 in between RM23 and RM140 (10.7-12.3 Mb) explaining 43% variability for shoot Na:K ratio (Bonilla *et al.*, 2002). Thomson *et al.* (2010) reported the presence of different 'Pokkali' alleles in the *Saltol* region between 11.0 and 12.2 Mb and suggested the possibility of the *Saltol* being controlled by the same gene as the SKC1 QTL located at 11.46 Mb.

Detection of Saltol introgression salt tolerant lines

- FL 478 is a *Saltol* QTL introgression line and widely used for introgression of *Saltol* QTL into high yielding background.

Plate 6.4: a. Phenotyping of introgression lines tolerant to salinity (EC= 12 dS m^{-1}) at seedling stage at salinity mocro-plot, b. Graphical genotyping of Saltol region of chromosome 1 in those Saltol introgression lines

- 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) are subjected to analysis for validation of the microsatellite markers in the *Saltol* QTL region.
- Four primers (RM10694, RM8094, AP3206 and RM493) in *Saltol* region are found polymorphic between FL 478 and IR64.

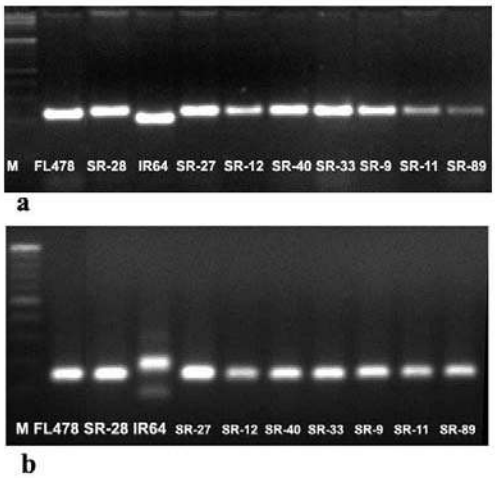


Fig. 6.3: SSR polymorphism using Saltol *markers, a. RM10694, b. RM8094 in parents and salt-tolerant introgression lines from IR64/FL478 cross*

- FL 478 specific marker alleles for different loci situated from 11 Mb to 12.4 Mb region in chromosome 1 is 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 (Plate 6.4 and Fig. 6.3)

6.5 Screening for salt tolerance at reproductive stage

To meet the need of developing of an unequivocal protocol for screening rice genotypes for salt tolerance at reproductive stage we successfully developed and validated a modified protocol for picking out proper source of tolerance and subsequent identification of QTL for this stage. We found better accuracy and better level of uniformity in our modified protocol (personal communication) over the existing medium (Gregorio *et al.*, 1997; Chattopadhyay *et al.*, 2013).

6.5.1 Design and procedure

The following steps are taken for the experimental setup.

- Plastic pots (with 12 inch diameter) are perforated at regular intervals using a drilling machine. The diameter of each hole is approximately 0.5 cm and intervals of these holes are 2-3 cm. One tightly netted nylon mess or cotton cloth is fitted inside the pot (Plate 6.5)
- In steed of filling pot with only soil, 20% of the soil (v/v) is substituted by gravels. Three different sizes of gravels are used. They had small (2-3 mm), medium (6-8 mm) and large (10-15 mm) diameters. After using nylon mess/cotton cloth in perforated pot, one layer of large followed by one layer of medium and small gravels are placed one after another at the bottom of the pot.
- One layer of sand is given after gravels and the porous pipe (piezometer) is placed over it. The rest of the pot is filled with well-ground soil mixed with gravels of medium size.
- Flat plastic frame is kept at the bottom of the plastic water tank of 0.5 m height and 1 m diameter and perforated pots are kept over it to ensure free water flow from the bottom to the top of the pots.
- Standard procedure (Gregorio *et al.*, 1997; Bhowmik *et al.*, 2007) with requisite modifications is followed to salinize potted plants. For salinization, NaCl is dissolved in tank water to make water EC of 8 dSm^{-1} and salt water is allowed to enter the porous pots to saturate soil.
- Salt stress is imposed on plants before booting. One perforated pipe (piezometer) is placed inside the soil with its opening outside the soil surface. Water from saturated soil is collected inside this pipe. Regular monitoring of pH and salinity level of water in saturated soil inside this pipe is done using a handheld EC cum pH meter.

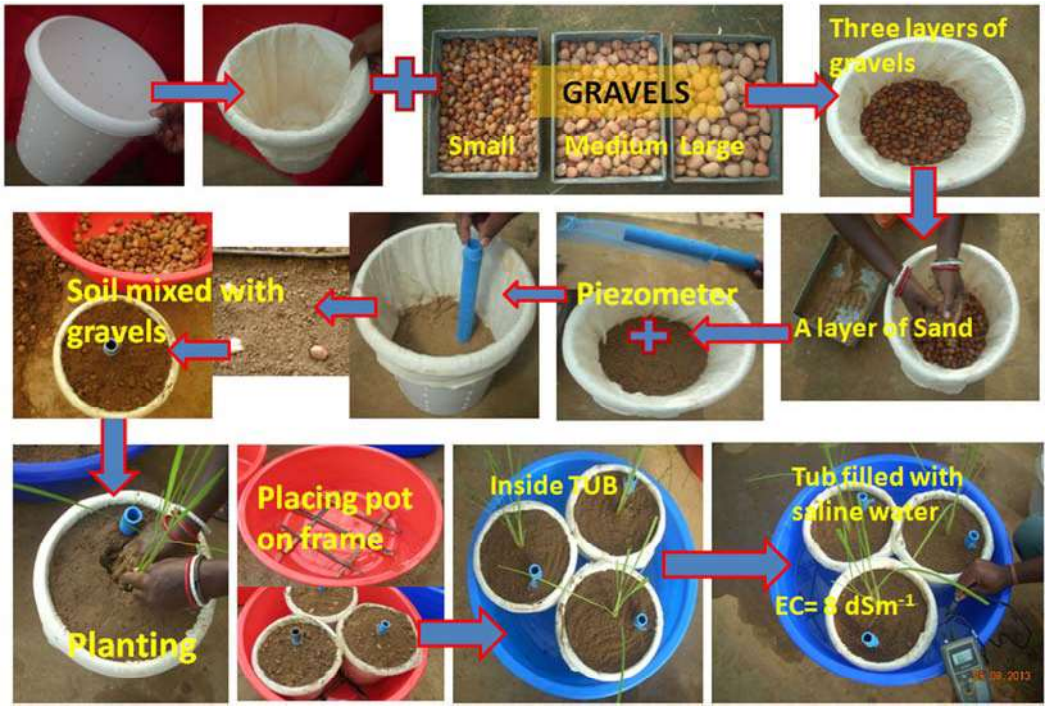


Plate 6.5: Stepwise layout for salt tolerance in rice screened at flowering stage

- Seedlings at the age of 20-25 days are planted in these perforated pots. The level of water in plastic bath is maintained at 2 cm below the soil surface of the perforated pots.
- The N:P:K is applied in pots at the rate of 100:50:50. The required amount of ammonium sulphate (NH_4SO_4) and potassium hydrogen phosphate (KH_2PO_4) are dissolved in water to fertilize each pot with 30 mL solution. Half of the amount of NH_4SO_4 is applied at the time of planting and the rest amount is applied before panicle initiation.
- One set of potted seedlings is salinized and the other set is allowed to grow in normal condition in the net-house till the grain filling stage.

6.5.2 Advantage

The following improvements are observed in our protocol of screening for salt tolerance at flowering stage over the pot experiments with only soil medium (Fig. 6.4).

- Attaining in the desired salinity level is little faster.
- Stabilization of salt stress is relatively improved. The average mean fluctuation of EC level, calculated taking absolute values of differences of readings, is found significantly lower in soil: stone (4:1) medium than the soil medium (Fig. 6.4).
- Regular monitoring of EC and pH through piezometer follows quick intervention.
- In order to maintain the EC in perforated pots, plain water (ECodsm^{-1}) is applied in plastic water bath. The EC level in soil: stone (4:1) medium is recovered a bit faster than the soil medium.

6.5.3 Procedure and screening criteria

Yield reduction under salt stress at flowering stage is the main indicator of susceptibility. Tolerant genotypes are supposed to be capable of reducing yield loss under stress. The following criteria can be set to classify genotypes based on their tolerance to salt stress at flowering stage at EC of 8 dSm^{-1} (Plate 6.6 & Table 6.5).

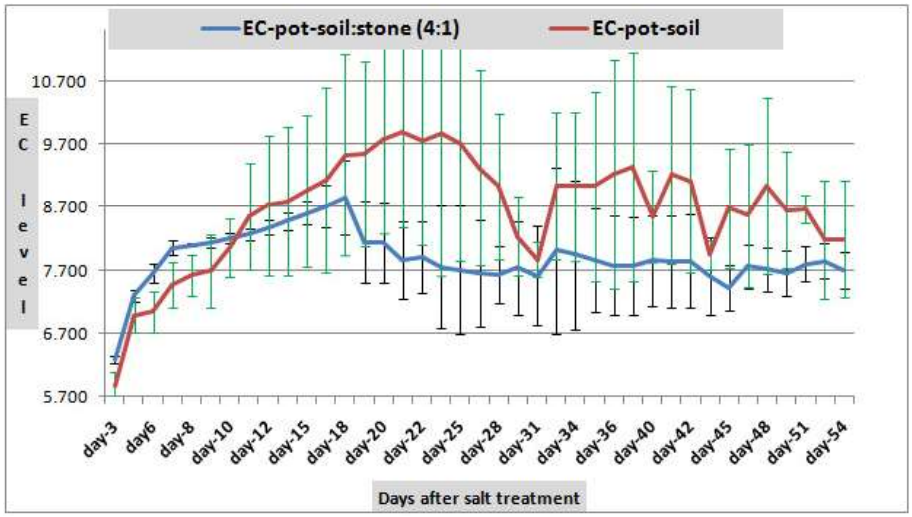


Fig. 6.4: Temporal changes of EC_w in experimental system during 2014

Table 6.5. Classification of genotypes based on salt tolerance

Sl No	Reaction to salt stress	Percentage of yield loss	Probable sources	Tolerant Score
1	Highly tolerant	<10.00	Nil	1
2.	Tolerant	<25.00	Pokkali (AC41585)	3
3	Moderately tolerant	<50.00	Chettivirippu (AC39389), Luna Sankhi, Binadhan 10	5
4.	Moderately susceptible	<60.00	Dhinkiasali	7
5.	Susceptible	>60.00	Swarna, Savitri	9

Plate 6.6: Tolerant (AC41585) and susceptible (Naveen) lines for salt tolerance at flowering stage. Symptoms of susceptibility are Leaf rolling, spikelet sterility and degeneration, reduced panicle length and plant yield

Other parameters- Apart from grain yield panicle length, spikelet per panicle, K^+ concentration, and spikelet fertility are important contributors to sustain yield under salt stress (Shereen *et al.* 2005; Zeng *et al.* 2004; Chattopadhyay *et al.* 2013, Hossain *et al.* 2015). Recently, it was observed that high K^+ concentration and low Na^+-K^+ ratio in flag leaf seemed to be good indicators of tolerance at flowering stage under salt stress condition (unpublished).

6.6 Marker assisted breeding for salt tolerance in rice

Introgression of salinity tolerant QTL '*Saltol*' into popular high yielding variety Gayatri using marker assisted backcross breeding is in progress in a DBT funded project "QTL to Variety" at NRRI, Cuttack. At present, the material is in BC_2F_3 stage. Promising progenies are being screened for salinity tolerance, yield and other traits during *Kharif*, 2016. A schematic diagram has been provided here in developing salinity tolerant Gayatri with *Saltol* (Fig. 6.5).

6.7 Breeding procedure for improvement of salt tolerance in rice

Bulk pedigree breeding is suitable for traits influenced by environmental variation. Yield and its component traits are influenced significantly by salinity stress and governed by many QTLs (mostly small) with large environmental effects. Therefore, selection for salt tolerance in F_4 generation onwards is effective after fixing of genotypes. On the other hand screening for salt tolerance at early seedling stage can be started at early generation (F_2-F_3) as it is significantly governed by fairly major QTL, *Saltol* along with other minor QTLs. Therefore, this method with little modification is found suitable to breed varieties with salinity

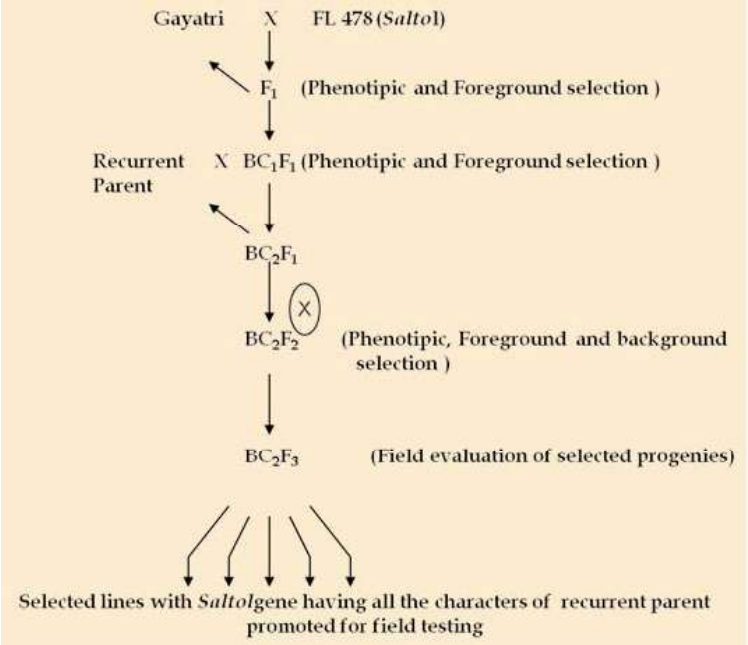


Fig. 6.5: Scheme for marker assisted backcross breeding for salt tolerance in rice

tolerance. The following bulk-pedigree schemes are proposed to develop elite breeding lines using salt tolerant donors (Fig. 6.6).

Back-cross method of breeding is useful in stress tolerance breeding to reveal 'hidden' diversity, to break linkage with undesirable traits and to generate mapping population with introgression lines in the background of high yielding recurrent parent (Fig. 6.7).

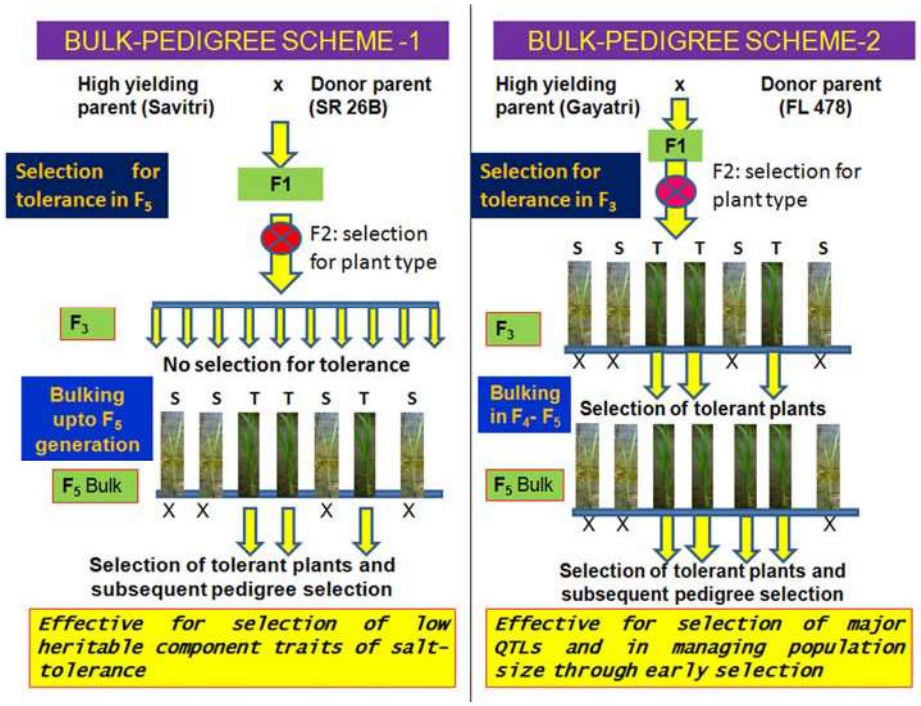


Fig. 6.6: Schematic diagram of bulk-pedigree method of breeding

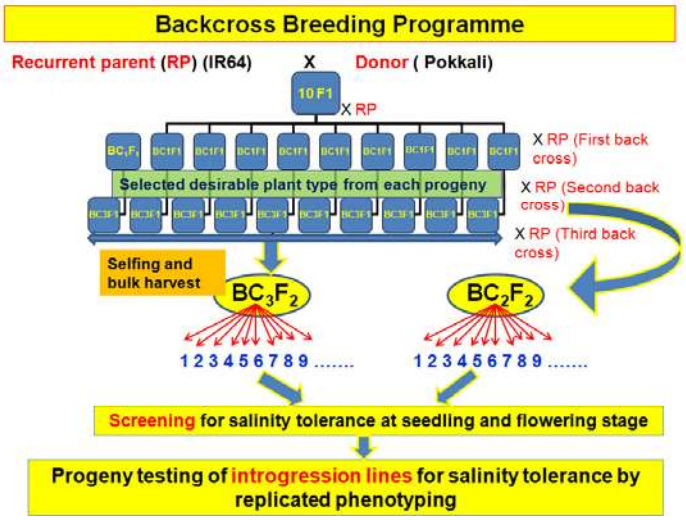


Fig. 6.7: Schematic diagram of backcross method of breeding in rice improvement for salinity stress tolerance.

6.8 Germplasm and varieties for coastal saline areas

- *Germplasm screened:* 1050 (seedling stage) and 280 (reproductive stage)
- *Number of tolerant lines:* 25 (seedling stage, *e.g.*, AC39416, AC 41585, AC 39394, AC 39389 and Kamini) and 4 (reproductive stage, *e.g.*, AC 41585 and AC 39394)
- *Number of moderately tolerant lines:* 82 (seedling stage, *e.g.*, Talmugur, Rahspunjar and Matchal) and 8 (reproductive stage, *e.g.*, AC 39389, Binadhan 10 and Luna Sankhi)
- *Varieties:* Lunishree, Luna Sampad, Luna Suvarna, Luna Barial and Luna Sankhi

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