Growing rice in saline soils

Biotechnological approaches for Bangladesh

Zeba I. Seraj and M. Abdus Salam

A large part of Bangladesh's cultivable area lies in the coastal saline zone, and rice cultivation is largely hindered by the salinity. This problem can be effectively addressed by using both traditional and modern biotechnological methods, which involve identifying and developing salt-tolerant traits and species. This article discusses the application of DNA-based screening of salt-tolerant traits as well as genetic modification or transformation of rice species to enhance salt tolerance properties.

Dr. Zeba I. Seraj
(Corresponding author)
Professor, Department of Biochemistry
University of Dhaka
Dhaka 1000, Bangladesh
Tel: (+880-2) 864708, 9661900
(Ext. 6106)
Fax: (+880-2) 865583
E-mail: zseraj@fsbd.net
Mr. M. Abdus Salam
Principal Scientific Officer
Plant Breeding Division
Bangladesh Rice Research Institute
Gazipur, Bangladesh
Tel: (+880-2) 933098
E-mail: brrihq@bdonline.com

Introduction

Over 30 per cent of the net cultivable area of Bangladesh lies in the coastal zone. Out of 2.85 million hectares of coastal and off-shore land, about 1.5 million hectares are affected by varying degrees of salinity. The coastal saline soils are distributed unevenly in 64 *thanas* of 13 districts, covering portions of 8 agro-ecological zones (AEZ) of the country. The larger portions of saline land fall in the districts of Shatkhira, Khulna, Bagerhat, Barguna, Patuakhali, Pirojpur and Bhola in the west.[3] In recent years these have extended into the districts of Narail, Jessore and Magura, actually making a total of 16 districts (SRDI, 1998). The smaller portion of the saline area lies in the districts of Chittagong, Cox's bazar, Noakhali, Lakshimpur, Feni and Chandpur[3] (Table1).

Large fluctuations in salinity levels over time are observed at almost all sites in these regions. The common trend is an increase in salinity with time, from November-December to March-April, until the onset of the monsoon rains. The electrical conductivities (ECs) of the soil and water were lowest in July-August and highest in March-April at all sites (Table 2). Soil salinity, at any time, is maximum in the surface layers (0-15 cm), the salinity gradient being vertically downwards. Subsoil salinity is usually much lower than topsoil salinity. Moderately to strongly saline underground water is found within 1-2 metres below the soil surface at all locations in the dry season. The spatial and temporal variations in soil salinity indicate the need for crop production planning separately for different locations in the coastal areas. No generalization can be made in this regard.[3]

Table 1: Extent and distribution of coastal saline soils in Bangladesh

District	Area under saline soils ('000 ha)	District	Area under saline soils ('000 ha)
Satkhira	146.35	Bhola	40.33
Khulna	120.04	Chittagong	45.70
Bagerhat	107.98	Cox's Bazaar	54.70
Barguna	103.55	Noakhali	49.60
Patuakhali	115.10	Lakshimpur	19.30
Pirojpure	20.30	Feni	9.00
		Chandpur	1.50
Total			**833.45**

Source: Panaullah, 1993

Table 2: Soil salinity ranges (Ece dS/m) at some sites of six coastal districts at different times of the year

Thana	Jul-Aug	Dec-Jan	Mar-Apr
Khulna			
Paikgacha	3.5-6.2	5.0-15.2	6.1-23.3
Dumuria	1.8-4.1	4.6-10.2	6.5-14.2
Batiaghata	0.8-1.2	2.5-3.6	5.8-8.5
Dakope	2.3-6.4	6.7-17.0	8.7-23.0
Bagerhat			
Rampal	1.2-3.5	4.7-9.0	7.0-11.5
Morelganj	0.4-2.2	21-6.7	4.0-9.6
Bhola			
Daulatpur			1.3-2.2
Tazumuddin		1.4-4.4	2.5-5.7
Lalmohan		2.0-4.7	3.1-7.3
Noakhali			
Hatiya			
Noakhali		1.0-4.2	4.3-7.4
Lakshimpur		2.0-4.7	2.1-10.1
Ramgati			
Feni			3.3-71
Sonagazi		0.9-3.3	1.9-5.0

Source: Panaullah, 1993

The compositions of the soluble salts in saline soils can indicate possible management strategies for crop production. Sodium has been found to be the dominant cation, and chloride the dominant anionic species. Next in importance are magnesium and sulphate. Thus the salts are of the sodium-magnesium, chloride-sulphate type.

A very important aspect of the soluble salt composition of the underground waters is the large excess of magnesium relative to calcium. Thus proper measures to maintain ionic balance may be needed for good plant growth even under low salinity conditions[3]. There is a general lack of suitable salt-tolerant modern variety (MV) rice suited to different AEZ in the coastal area. Since farmers mostly grow local varieties, there is great scope for increasing land productivity through the introduction and development of MV rice.

In some regions of Khulna district, salinity is a problem even in the wet T. Aman season. According to an expert, Mr G. M. Panaullah, the development of MV rice for this region could make a major impact on rice productivity.

Help from biotechnology

Biotechnology can address the coastal salinity problem for possible rice cultivation in two major ways:

- Traditional breeding efforts at producing salt tolerant rice can be aided in terms of speed, specificity and better management by using molecular markers that indicate the presence of the salt tolerant trait without the necessity of laborious screening procedures. This technique is referred to as Marker-Aided Selection or MAS.
- Rice can be transformed or modified with foreign genes, which can confer salt tolerance properties in the modified plant.

Before explaining these biotechnological techniques, there is a need to understand how halophytes (naturally occurring salt-tolerant species) or other moderately salt-tolerant crops cope with excess salt concentrations.

Breeding salt-tolerant rice

It is difficult to breed salt-tolerant rice varieties. The salt tolerance capacity is not a single characteristic but a composite of many. Incorporation of a single character may be possible; that, however, may not be enough to confer salt tolerance.

Properties required

Crops require potassium to grow properly. However, while absorbing potassium, there might be an uptake of sodium because the structure of potassium and sodium are similar and transportation of the two ions is facilitated by the same transporter across the cell membrane. This problem is obviously acute in saline soils. In the presence of sodium, most enzymes responsible for normal cellular activities cease to function properly. In some salt-tolerant crops, this transporter has a much greater affinity for potassium and therefore, even in the presence of high external sodium, there is minimal uptake of the latter.

Some salt-tolerant crops are capable of sequestering excess sodium ions into vacuoles inside cells through special transporters. Since most of the sodium is present in vacuoles, it cannot cause harm to the enzymatic activities going on inside the cells. These transporters may also be present on the outer cell membrane and may therefore extrude sodium out of the cell. This sodium/hydrogen ion transporter, as it is called, has been characterized in the *arabidopsis* plant. This is a small plant with a very short life cycle and serves as a model for studying many plant functions.

Excess external sodium causes cellular water to be pulled out of cells, causing dehydration. Under conditions of excess external sodium, some cells can synthesize osmotically active small compounds, which are capable of retaining the water inside cells. Examples of such solutes are mannitol, pinitol, glycine betaine, trehalose, etc. For example, mangrove plants in the Sunderbans area synthesize glycine betaine.

Some halophytic plants like the wild rice called *Porteresia coarctata*, endemic to the eastern coasts of India and the coastal region of Bangladesh, have been shown to possess salt glands, and actually extrude salt outside their leaves under high external salt concentrations, e.g. in sea water. Presumably, the glands contain a kind of transporter or sodium pump in the cell wall. This pump has not yet been characterized at the molecular level.

Progress in biotechnological approaches

Marker-aided selection (MAS)

There are only two well-known and proven moderately salt-tolerant traditional rice cultivars, *Pokkali* and *Nona Bokra,* that can be used as potential donors for salt tolerance in a breeding programme to incorporate salt tolerance in commercial high yielding rice cultivars. Both are very tall varieties with low yields and are sensitive to day-length and therefore flower only once a year after a long maturation period. As a matter of fact, the International Rice Research Institute (IRRI) has released more than a hundred, salt-tolerant, high-yielding varieties (HYV) by breeding them with these salt tolerance donors. The released varieties have been made available to the various national agricultural systems in many South and South East Asian countries. In turn, these countries have used these varieties in their own breeding programmes to develop salt-tolerant varieties suited to their own particular geographical niche. For example, BR 5331 was released in Bangladesh recently after being crossed with IRRI-derived rice lines originating from *Pokkali*.

Such breeding efforts, however, take a long time, anywhere between 4 and 7 years, because of extensive phenotypic selections required at each breeding step. One way to reduce the time required is to use DNA markers, which are linked to the salinity tolerance trait. The DNA markers can be used to track genes conferring salt tolerance and to combine multiple genes effectively; since salinity tolerance is a trait controlled by multiple genes. Not only is MAS more accurate, fast and efficient, but also it can be used to screen the breeding population at an early seedling stage and under various environmental conditions.

Researching DNA markers

Much progress has been made at IRRI[1] to locate the genes controlling the salt tolerance trait of the traditional salt-tolerant donor *Pokkali*. This is an arduous task involving generating a mapping population from a cross between *Pokkali* and a salt-sensitive yet high-yielding rice. After the initial cross and production of F_1, these are allowed to self-propagate, producing an F_2 population, which should have a permutation and combination of various characteristics from both parents. Each plant from F_2 is allowed to self-propagate up to 7-8 generations, with seed collections from each plant being kept separate. This process is called single-seed descent. Differences in DNA patterns in the original parents are then correlated with salt tolerance or salt sensitivity of individuals of the F_8 population which are called recombinant inbred lines. Once these markers are found, however, these can be used in any breeding programme in which Pokkali has been used as a donor.

Application of identified markers

As mentioned, BR5331 is an advanced salt-tolerant rice variety released by Bangladesh Rice Research Institute (BRRI) whose salt tolerance donor was *Pokkali*. BR5331 is a *T. Aman* or monsoon season variety, which means it is sensitive to day length, flowers only during October and has a relatively long maturation time. It would be of benefit if BR5331 could be further adapted to make it insensitive to day-length, which could automatically reduce its maturation time. This would have important implications for improved crop yield because salinity levels start gradually increasing from late September. A shorter duration rice variety could be harvested earlier and hence would be able to avoid the deleterious effects of salt toxicity on the yield, says G. M. Panaullah.

Since DNA markers linked to the salinity tolerance trait of *Pokkali* have been identified, and salt tolerant BR5331 has been derived from *Pokkali*, the same markers are being used in breeding programmes to further improve BR5331. One condition has to be fulfilled, however. The identified linked markers have to be quite close to the salinity tolerance locus. Otherwise they may segregate or separate from salt-tolerant gene neighbours during the various breeding processes they have undergone. To ensure that the marker is closely linked, one simply has to confirm that the marker showing the difference between salt-tolerant *Pokkali* and a salt-sensitive variety also shows the same difference in a gel between BR5331 and its salt-sensitive breeding partner. At the moment, IRRI is involved in identifying very tightly linked salinity tolerance markers to the salt tolerant gene locus of *Pokkali*, according to Glen B Gregorio.

Genetic modification or transformation

Traditional or pre-biotechnological era efforts at producing improved crops for, say, disease resistance, were based on crossing the commercial crop with a closely related wild cultivar having the desired resistance. The crossing step would then be followed by back-crossing and selection steps to eliminate the undesirable characteristics of the wild cultivar. All this could take from five to seven years. With the advent of biotechnological advances such as genetic engineering, useful genes from diverse sources such as bacteria or totally unrelated plant species can be isolated and the specific gene inserted into the crop being improved by transformation.

Genetically modified plants are very much a reality in many developed and developing countries. Already about 70 different cultivated plants have been genetically modified and tested in over 3,600 field tests on more than 15,000 individual agricultural tracts without any negative occurrences. These crops include rice, maize, wheat, oats and barley among cereals; soybean, sunflower pea and bean among food legumes and oilseeds; cotton, sugarbeet, tobacco, cocoa and coffee among industrial crops; tomato, papaya, many citrus fruits, and other fruits among horticultural crops; and poplar and aspen among trees. The modifications include introduction of genes for virus, fungus and pest resistance, herbicide tolerance, and improved product quality.

Genetic modification of plants involves simple techniques. A natural genetic engineer called *Agrobacterium tumefaciens* exists in nature. This bacteria is capable of transferring a portion of its genes contained within specific regions of its DNA into plants which it infects. Molecular biologists have constructed several strains of bacteria in which a scientist can insert desirable genes within the specific regions. When such strains are used to infect plant tissues, the desirable genes are inserted and subsequently integrated into the plant genome. The bacteria are used to infect plant tissues. Thereafter, using tissue culture techniques, the plant tissues are regenerated to give back the whole plant, which is now modified with the desirable genes that the scientist had inserted.

Monocots like rice are not natural hosts of *Agrobacterium tumefaciens*. Efficiencies of rice transformation using other methods like DNA transfer in rice cells using electrical charge or microprojectile DNA bombardment of rice cells has been low (Figure1).

Figure 1: Stages in production of a transformed rice plant using the microprojectile mode of plant transformation. Rice embryos are removed from seeds and induced to give undifferentiated cells called *callus*. These are bombarded with microscopic beads coated with foreign DNA, in this case with a gene producing an enzyme which gives a blue colour after incubation with chromogenic substrate. The blue indicates that transformation has taken place. Transformed cells are selected in special media where only they can grow. These cells then are manipulated to regenerate rice plants, which look the same as non-transgenic rice.

Recently some scientists established protocols for *agrobacterium*-mediated rice transformation by using very high concentrations of the bacteria and using external chemical inducers, which rice does not naturally produce. Efforts are underway to establish protocols for the successful transformation of Bangladeshi rice varieties. For establishment of such transformation protocols, marker genes are first used. The successful incorporation of marker genes can be monitored by simple assays, which give a blue colour. Transformed cells also have to be separated from non-transformed cells. This is done through transformation by a gene, which confers on the cells the ability to grow on certain selection agents like antibiotics. Once the marker gene insertion protocol is established, useful genes can be inserted into the plants using the same protocols. For establishment of protocols for transformation, such rice varieties are first used that are easy to manipulate in tissue culture, such as the traditional rice cultivar *binnatoa*.[4]

Transfer of genes

The most common strategy that has been so far used is to transfer the genes of enzymes producing osmoprotective compounds into an easily manipulated plant like tobacco and to see the effect of the transformed gene. Scientists have manipulated tobacco plants to produce mannitol, glycine betaine, proline and trehalose.[2] In all cases, the transgenic tobacco plants were more tolerant to salinity and drought stress compared to controls. Most of these plants produced osmo-protective compounds all the time and not as a stimulus to stress conditions. Production of these compounds, even when not required, caused some problems such as stunting of plants. Such problems can be avoided by adding on controls or promoters (which drive expression of genes so that eventually the required enzyme/protein is produced) in front of the genes in question, which can respond to stress. Such promoters have already been identified.

As yet, however, there are no published reports of transgenic cereal crops that can produce osmo-protective compounds. There have been reports from Myongji University in Korea of developing salt-tolerant rice after insertion of trehalose-producing genes, and efforts are underway in Bangladesh to insert mannitol-producing genes into rice with the gene provided by IRRI.

Osmoprotective compounds (except for proline) are not produced in rice after salt stress. Therefore, if the existing moderately salt-tolerant IRRI and BRRI varieties are transformed with osmolyte-producing genes, their salt tolerance is expected to be enhanced. Most of these HYV have been derived from *Pokkali* or *Nona Bokra*. Their mechanism of salt tolerance is, most likely, salt exclusion. Some salt-tolerant HYV rice varieties from IRRI and BRRI have therefore been tested for their efficiency of transformation by using marker gene assays, and some varieties chosen for further transformation studies with mannitol-producing genes.

In another approach, the *arabidopsis* plant was transformed with an over-expressing sodium/hydrogen ion transporter and the plants were more salt-tolerant than their controls. It will be recalled that these transporters can sequester excess sodium in vacuoles and also extrude sodium out of the cell.

Conclusion

Abiotic stresses such as salinity and drought are serious threats to sustainable food production. In rice, abiotic stresses affect cultivation more than biotic stresses caused by fungal or bacterial pathogens or insect pests. It is a challenge for plant biotechnologists to generate crops which can withstand, reproduce and set seeds in at least milder levels of abiotic stress, if not in extremes. There have been many reports stating that an increased level of salinity stress has been achieved. The concern now is to consolidate these advancements through collaborative research in different crops, including rice.

There is also need for a continued effort at achieving a complete understanding of all the reactions/processes/ events that occur during salt stress. The primary and secondary effects of salt stress have also to be distinguished. This is important because not all the metabolic changes accompanying salt stress would have an adaptive value.

References

1. Gregorio, Glenn B, 'Tagging salinity tolerance genes in rice, using amplified fragment length polymorphism (AFLP)', Ph. D. Thesis, 1997, University of Philippines, Los Banos, The Philippines.
2. Grover, A, Sahi, C, Sanan, N and Grover, A, 'Taming abiotic stresses in plants through genetic engineering: current strategies and perspective', 1999, *Plant Science,* 143:101-111.
3. Panaullah, GM, 'Soil salinity and associated problems in connection with crop production in the coastal regions of Bangladesh', 1993, Proceedings of the workshop on, 'Coastal salinity and crop production in Bangladesh', February 17, 1993, Bangladesh Rice Research Institute publication, pp 1-30.
4. Rasul, NM, Ali, KM, Islam, R and Seraj, ZI, ' Transformation of an indica rice cultivar Binnatoa with *Agrobacterium tumefaciens*', 1997, *Plant Tissue Cult*, 7: 71-80. ❑