

## Approaches to breeding for salinity tolerance - a case study on *Porteresia coarctata*

By R LATHA<sup>1\*</sup>, C SRINIVAS RAO, H M SR SUBRAMANIAM, P EGANATHAN and  
M S SWAMINATHAN

*M. S. Swaminathan Research Foundation, 3<sup>rd</sup> Cross Street, Taramani Institutional Area, Chennai 600 113, India*

(Accepted 29 September 2003; Received 21 February 2003)

### Summary

Cereals are the world's major source of food for human nutrition. Among these, rice (*Oryza sativa*) is the most prominent and represents the staple diet for more than two-fifths (2.4 billion) of the world's population, making it the most important food crop of the developing world (Anon., 2000a). Rice production in vast stretches of coastal areas is hampered due to high soil salinity. This is because rice is a glycophyte and it does not grow well under saline conditions. In order to increase rice production in these areas there is a need to develop rice varieties suited to saline environments. Research has shown that *Porteresia coarctata*, a highly salt tolerant wild relative of rice growing in estuarine soils, is an important material for transferring salt tolerant characteristics to rice. It is quite possible that *Porteresia* may be used as a parent for evolving better and truly salt resistant varieties. The inadequate results and the difficulties associated with conventional breeding techniques necessitate the use of the tools of crop biotechnology in unravelling some of the characteristics of *Porteresia* that have been highlighted in this report. In view of the limited resources available for increasing salinity tolerance to the breeders to wild rice germplasm, *Porteresia* is undoubtedly one of the key source species for elevating salinity tolerance in cultivated rice.

**Key words:** Crop biotechnology, *Oryza sativa*, *Porteresia coarctata*, salt tolerance, wild rice

### Introduction

The prospect of sea level rise due to global warming brings to the popular mind the spectre of deep inundation of coastal regions. Although the present (meaning the average over the last 100 years or so) rate of rise and its interpretation are subject to a certain amount of disagreement, it is an observed fact that relative sea level is rising in most coastal regions and causing major problems just at the time when rapid coastal development is taking place (Brinkman, 1995). If the postulated global warming and associated accelerated rate of sea level rise occur in future, coastal ecology and livelihoods will be endangered. Global warming could happen twice as quickly as previously forecast over the next 100 years, and the global average temperatures could rise by between 1.4°C and 5.8°C by the end of the century, according to the report of the Intergovernmental Panel on Climate Change, made up of scientists from 100 countries and sponsored by the UN Environment Programme and the World Meteorological Organization (Raymo, 1998).

### Challenges Ahead

Among the neglected areas of potential use for cropping of rice are coastal areas. These areas have remained mostly under-utilised because of constraints such as high levels of salinity in the soil, lack of suitable germplasm and poor irrigation and water management. An increase in food production along these areas is desirable not only because of the abundance of land resources but also because over 40% of the human population lives within the 100 km-wide belt from shoreline. Further, the need for rice varieties which can perform well under conditions of coastal salinity is likely to increase if sea levels do rise. Rice is the primary staple food for more than 2 billion people in Asia, Africa and Latin America and is the most important crop in terms of both its contribution to diet and to strengthening rural livelihood. Rice production for coastal areas has been a major challenge to breeders and researchers. Though some 90 000 varieties have been screened at the International Rice Research Institute (IRRI), the donors for salt tolerance have always been traditional cultivars and non-dwarf varieties (Yeo *et al.*, 1990). Several varieties have been bred so far for salinity tolerance but the degree

\*Corresponding Author E-mail: latha\_rangan@yahoo.com;  
latharangan@hotmail.com  
© 2004 Association of Applied Biologists

<sup>1</sup>Current address: The Energy and Resources Institute, Plant Tissue Culture and Molecular Biology, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi 110003

of tolerance has often been inadequate. It is important therefore to identify new sources of tolerance. Research should focus on identifying and developing varieties that contain salt tolerance (up to  $EC = 12 \text{ dSm}^{-1}$ ) with yield ( $4.06 \text{ tonnes ha}^{-1}$ ) and grain quality.

Solutions to the increasing problem of salinity involve several different approaches such as improved water management, agricultural utilisation of halophytic species and breeding salt tolerant strains of crops. Using wild species and relatives of crop plants as sources of gene(s) for tolerance to several stresses is gaining importance in terms of sustainable agriculture and for long-standing expression of tolerance to abiotic stresses. Evaluation at IRRI has revealed that the chances of finding resistance to biotic and abiotic stresses is about 50 times greater in wild species and land races than in cultivated varieties.

### Coastal System Research in MSSRF

The work initiated at M S Swaminathan Research Foundation (MSSRF) in 1990 on Coastal Systems Research (CSR), designed to foster simultaneously the ecological security of coastal areas and the livelihood security of coastal communities, has yielded promising results. Extension of this work was made to the frontier areas of science, biotechnology and molecular genetics, now at the cutting edge, to undertake anticipatory steps to meet the challenge of a potential rise in sea level caused by global warming. The major emphasis of the ongoing programme is to characterise pre-breeding genetic material offering resistance/tolerance to salinity stress for grassroots-level breeders for developing location specific crop varieties (Anon., 2000b). The emphasis, therefore, has been on developing genetic material containing novel genes for resistance to abiotic stress, particularly salinity. One such study involves the monocot system *Porteresia coarctata* Takeota, a halophyte and a close relative of *Oryza sativa* L.

#### *Porteresia coarctata* - Asian wild rice relative

The only known source of tolerance to salt stress among the wild relatives of rice is *Porteresia coarctata*, which grows in abundance along the Eastern and Western coasts of India, Pakistan and Bangladesh. *Porteresia*, a tetraploid ( $2n = 4x = 48$ ) distant wild rice relative, is a monotypic genus occurring as an associate of mangroves along the coastal belts (Fig. 1) where the soil is inundated twice a day with saline river or seawater of  $20$  to  $40 \text{ dSm}^{-1}$ . The species can also withstand submergence with saline water for quite a long period. In deep forests of Sunderban, West Bengal, India, it is generally completely submerged by tidal water for as long as  $10$ - $11$  h every day. The mean tidal level (MTL) of

these areas is  $1.10$  m from the base and the plant grows up to a height of  $1$  m with a very hard stem and thick leathery leaves. Several useful traits are attributed to this tetraploid genus, which was poorly researched until recently. Studies into this wild relative of rice are now receiving attention due to its inherent capacity of tolerating high levels of salinity. *Porteresia* shows sustained growth despite the fact that it grows in highly saline environments. *Porteresia* helps to bind peripheral soils in mangrove forests and its distribution pattern shows a decrease in abundance in several mangrove areas because of both natural and anthropogenic pressures. It is identified as one of the pioneer species during ecological succession and grows gregariously forming the intertidal grassy meadow of the river flats and banks of the river. After *Porteresia*, mangrove species grow in the secondary stage. Unabated destruction of coastal wetlands ecosystems has alarmed environmentalists. There is a necessity to conserve this ecosystem both for the immediate purpose of cushioning both the effect of cyclones and excessive shifting of the coastline for long term benefit, considering that *Porteresia* is a potential source of genes for salinity and submergence



Fig. 1. *Porteresia* habitat

tolerance. Work at MSSRF focuses on restoration of these degraded mangrove forests, which provide an ideal habitat for this wild grass.

*Porteresia* also has an important role in socio-economic development of people of fringe areas of coastal areas of India and Bangladesh besides its important role in soil conservation (Singh & Seth, 1998). Being a wild rice relative it could serve as an intermediate agent for studying the model system in monocotyledonous plants, expression and behavioral pattern during osmotic stress. However, *Porteresia*, unlike *Oryza* spp., has recalcitrant seeds - the seeds die when they are dried making it very difficult to work with it (Probert & Longley, 1989).

#### *Morphological and anatomical features*

Many scientists have worked from time to time on the cytology, anatomy and stomatal behaviours of *Porteresia* and it was found to be a C3 species, based on CO<sub>2</sub> compensation points and PEP carboxylase activity (Garcia, 1992). Anatomical studies of the leaf of *P. coarctata* revealed that the adaxial surface possess some special unicellular salt hairs (trichomes) and a number of prominent ridges and furrows running longitudinally down the surface. The leaves are without a midrib, unlike in *Oryza* species. It was found by Tateoka (1963) that each ridge contains one small vascular bundle nearer the adaxial surface and below it a larger one; development of mechanical tissue is higher in *Porteresia* than in other *Oryza* species. These special unicellular structures are found in the furrows and are connected with the epidermal cells. Both adaxial and abaxial leaf epidermis is composed of short and long cells in rows. It was observed that the salt hairs are thin and small with blunt tips. The unicellular cells are meant for salt excretion and function only for a short period. Many mangrove species and halophytes have salt glands (Lipshitz & Waisel, 1974) but these glands do not burst like the salt hairs of wild rice.

Richharia & Roy (1965) found that the roots of this species show the usual structures of epidermis, cortex and stele as found in other species of *Oryza*. The mature roots, however, have strongly developed sclerotic pith. The root system of *Porteresia* is also well adapted to cope with increasing salinity leading to decreased water transport. To overcome intertidal strong flow it often forms pseudo-taproots up to a depth of 1 m and fibrous roots develop from the tip of those pseudo-taproots and internodes of a wide-spreading underground stem called a "sobole". Numerous new plants grow vegetatively after the breaking up of these soboles. Though the root system of this species does not penetrate deeply due to poor aeration of the substratum, it has an extensive rhizomatous system to cope with water starvation under high salinity.

#### *Salt tolerance mechanism in Porteresia*

Flowers *et al.* (1990) reported that *Porteresia* plants accumulated Na<sup>+</sup> and Cl<sup>-</sup> ions in leaves, but maintained a Na:K ratio as low as 0.7 even after 6 wk of growth in 25% artificial saline water (ASW) where the Na:K ratio was 34. This ability to maintain a low K:Na ratio in the leaves is in part a consequence of the secretion of ions from the leaves. They verified that the ratio of Na:K in the secreted cells was similar to that measured by X-ray microanalysis in the vacuoles of hairs found in folds of the adaxial surface of the leaf lamina, suggesting that the secretion emanates from these hairs.

The salt secreted by these hairs is an important factor in the salt-balance of the leaves. These hairs arise from the epidermis, appear to be unicellular with an electron-dense vacuole, and are covered with cuticle. The ability of *Porteresia* plants to secrete salts on to the surface of the leaf has been described previously and ascribed to the presence of "special unicellular structures" found in the furrows on the adaxial surface of the leaf (Bal & Dutt, 1986). The structures of the glands of *Porteresia* are, however, simpler than those described in the sub-family Chloroidoideae, being unicellular; there is no indication of cap and basal cells. Whether they release ions simply by collapsing as do the bladder cells of *Atriplex* species (Fahn, 1979), or whether a salt gland is able to continue secretion is as yet not known. It is clear, however, that the salt secreted by the gland is a significant proportion of the ions arriving in the leaf (Flowers *et al.*, 1990). Garcia (1992) reported the ion compartmentation between different plant parts in *Porteresia*. The maximum Na accumulation occurred in the roots and lowest in flowering parts. Leaf water status, ion content and secretion rates were balanced such that a leaf-to-leaf gradient in ion distribution did not prevail among fully expanded leaves, hence minimising osmotic potential gradients between leaves. Maintenance of a low Na:K ratio within shoots was primarily a function of the high selectivity for sodium in the secretion of ions from leaves. The positive correlation observed between leaf sodium and water status suggests good apoplastic ion control within leaves of salinised plants.

In experiments carried out at the IRRI phytotron, *Porteresia* seedlings were planted on Styrofoam frames placed over a culture solution (Latha, 2000). The treatment consisted of four salinity levels (EC 12, 15, 18 and 24 dSm<sup>-1</sup>) initiated 25 days after seedling establishment, and an unsalinised culture solution check. The salinity levels were developed by adding NaCl and CaCl<sub>2</sub> at a ratio of 1:1 by weight to the culture solution. The pH of 5.0 and the water level of the culture solution was kept constant. Salinity level significantly affected all growth parameters of *Porteresia* except the number of



panicles, as reported earlier by Akbar *et al.* (1986). Plant height at all salinity levels significantly increased relative to the control. Flowering was delayed at 18 and 24 dSm<sup>-1</sup>. The number of panicles per plant was not affected by salinity. Spikelets per plant, spikelet fertility, and grain weight increased significantly at 15 and 18 dSm<sup>-1</sup>, but not at 24 dSm<sup>-1</sup>. The results show that *Porteresia* not only tolerated high salinity but also displayed better growth characteristics at higher salinity. Akbar *et al.* (1990) reported that high concentration of P, K, Ca and Mg in the leaf and stem might serve as a source of osmotic adjustment. They also report that the lower Na concentration in the leaf than in root is an indication that either the Na was not translocated from root to shoot, or it was excluded from the leaf. Salt tolerant donors such as Nona Bokra and Pokkali are being used in tidal wetland rice breeding programmes, but their tolerance is not high as needed. Therefore, exploiting the genetic potential of wild species and the possibility of wide crosses such as that of halophyte *Porteresia* with *O. sativa* could be useful.

#### In vitro studies

Micropropagation of wild species of rice provides an opportunity to maintain a disease-free, clonal population of vegetative material which does not require any attention between subcultures and which can be multiplied and rooted readily for the production of mature plants (Finch *et al.*, 1992). Some wild species have been used extensively as donors of gene(s) controlling resistance to biotic and abiotic stresses in addition to contributing to qualitative characteristics. Several wild species are being researched as possible sources of useful traits, with the advent of wide hybridisation techniques (Jena, 1994; Khush *et al.*, 1977; Stich *et al.*, 1990) and by somatic hybridisation experiments (Finch *et al.*, 1990).

Resources of wild rice germplasm are in some cases scarce, and the advancement of wide hybridisation technology and associated techniques, such as development of species specific DNA probes and genetic maps of wild *Oryzae* and related genera, will rely on the ready availability of these species for distribution to laboratories world wide (D Vaughan, personal communication).

As *Porteresia* is a good source of gene(s) for salt and submergence tolerance, attempts have been made to transfer the salt-tolerant character to cultivated rice, through wide hybridisation procedures (Jena, 1994). But the difficulty has been to establish the regenerating material in a suitable medium (K K Jena, personal communication). Protoplast fusion was therefore investigated in a study of somatic hybridisation between the two species (Finch *et al.*, 1990; Jelodar *et al.*, 1999).

However the production of somatic hybrids of rice and related wild species has been reported only where suspension cultures are available for both parents (Hayashi *et al.*, 1988), which was difficult to achieve in *Porteresia*. Even attempts at anther culture (F L Zapata, personal communication) have faced problems at the multiplication stage. Another inherent problem with *Porteresia* is its association with a fungal endophyte, *Acremonium* sp., that caused considerable problems in establishing axenic cultures (Ramanan *et al.*, 1996). We therefore attempted to develop a clonal protocol with the following objectives: (1) to mass multiply and reintroduce *Porteresia* into mangrove areas successfully; (2) to use the protocol in wide hybridisation experiments and (3) to establish disease-free material of *Porteresia* for further studies (Latha *et al.*, 1998).

In our study we investigated the effect of Woody Plant medium (WP medium; Lloyd & McCown, 1980), so far traditionally used in multiplication of tree species, for multiplying *Porteresia*. Our previous attempts to use MS basal medium (Murashige & Skoog, 1962) with a suitable hormonal combination to achieve multiple shoot production, rooting, and establishment did not yield responses that were as good as WP-based medium. The possible explanation for this could be that the amount of ammoniacal nitrogen in WP medium corresponds to the mineral composition of the mangrove native environment (Joshi *et al.*, 1975) where *Porteresia* occurs. Perhaps the habitat of *Porteresia* being saline and the natural availability of ammoniacal nitrogen being rather low help the plant to respond to a similar situation in culture. This may explain the greater response of *Porteresia* in WP medium compared with that in MS-based medium. Further, an association with an endophytic fungus may not have been conducive to protoplast isolation and fusion studies. With the current micropropagation protocols for *Porteresia* we have overcome this problem.

There are no reports on chromosome complementation studies in *P. coarctata* except for the chromosome number which is  $2n = 48$ . Somatic hybrid plants were obtained following the electrofusion of rice (*O. sativa* cv. Taipei) cell suspension-derived protoplast with non-dividing leaf protoplasts of *Porteresia* (Jelodar *et al.*, 1999). One of the somatic hybrid lines had an allohexaploid complement of  $2n = 6x = 72$  following examination of its vegetative clones by genomic *in situ* hybridisation. It was confirmed as an amphiploid somatic hybrid with 72 chromosomes containing full chromosome complement of both *O. sativa* and *P. coarctata*. These studies indicate the divergent origins of chromatin within the nuclei of the respective species.

To date there are no reports of the successful anther culture of *Porteresia*. Anther culture could be an

important tool in this species, since it offers the possibility of obtaining genetically diverse haplodiploids, which can be used in wide hybridisation experiments. It allows early expression of recessive genes and increased selection efficiency as the number of plants required to obtain desirable recombinants is less than in conventional breeding (Torriso & Zapata, 1986). In our laboratory, attempts were made to obtain callus from anthers of *Porteresia*. However, the frequency of callusing from anthers was very low and was habitat specific (H M SR Subramaniam, R Latha and C Srinivasa Rao, unpublished).

*Use of bridge species for transferring salinity tolerance traits from Porteresia to O. sativa*

Detailed knowledge about genetic relationships among species of *Oryza* is an important factor in the success of plant breeding and genetic manipulation programmes and it will enhance the utility value of the wild species. Molecular markers are becoming important and useful tools for clarifying phylogenetic relationships among wild species and for providing a useful method of studying polymorphism. The use of genes from wild germplasm requires repeated backcrossing to the female cultivated parents to eliminate undesirable traits from the wild germplasm.

Many of the problems associated with plant breeding programmes based on phenotypic estimation can now be eliminated by use of DNA-based diagnostic assay. These approaches are expected to be particularly advantageous in breeding programmes involving quantitative traits, which include salinity resistance. With the availability of the inherently tolerant rice relative, *Porteresia*, it should be possible to understand better mechanisms of tolerance. For any future analysis using *Porteresia* it may be useful to have information regarding the genome relationship with cultivated and wild species of rice. This is now possible with the advent of several molecular techniques. New technological developments (Rafalski *et al.*, 1996) have expanded the range of DNA polymorphism assays for genetic mapping, marker assisted plant breeding, genome fingerprinting and investigating genetic relatedness. Molecular markers such as AFLPs and RAPDs were used to assess the genetic relationship between cultivated and wild rice species and for *Porteresia*. An assessment of the genetic distance between this species and other members of the genus *Oryza* may have practical implications, including the identification of a possible candidate for the successful attainment of an intermediate hybrid (Jena & Khush, 1989) which could then be crossed with cultivated rice to impart relevant agronomic characters, namely salt and submergence tolerance. By generating and comparing two nuclear gene

(*Adh1* and *Adh2*) trees and a chloroplast gene (*matK*) tree of all rice species, Ge *et al.* (1999) inferred phylogenetic relationships among the rice genomes. Origins of all the allotetraploid species, which constitute more than one third of rice species diversity, were reconstructed based on the *Adh* gene phylogenies, and the genome type HHKK was recognised for *P. coarctata*, suggesting its inclusion in *Oryza* species. Molecular analysis has helped in positioning *Porteresia* in the vicinity of the other wild rice species and to understand better the pattern of species differentiation in *Oryza* (Latha *et al.*, 2002).

Principally the study addressed the following questions: how is *Porteresia* positioned in the *Oryza* group, which are its nearest relatives and what possible future trends may be postulated. The dendrogram suggests that *Porteresia* is more similar to some species of *Oryza* than some other diploid species of *Oryza* are to one another; this could be taken as evidence to support the inclusion of *Porteresia* in *Oryza*. The results based on the two different marker systems revealed that the closest related species to *P. coarctata* based on genetic distance is *O. australiensis* at the diploid level. The similarities in the genetic distance between *P. coarctata* and *O. sativa* and that of *O. australiensis* and *O. sativa* suggest that a possible candidate for wide hybridisation with *P. coarctata* is *O. australiensis* and thus *O. australiensis* may represent an effective “bridge” species in transferring genetic traits from *P. coarctata* to *O. sativa*.

Grouping of *O. australiensis* into the tetraploid group along with *Porteresia* was not expected. However, the results based on RFLP data (Wang *et al.*, 1992) postulates that since the D genome is unknown in *Oryza* at the diploid level, the next most closely related diploid species to the CC-genome species is *O. australiensis* (EE). Based on the AFLP and RAPD clustering it can now be assumed that the E genome is related to the D genome, and that an E-genome ancestor may have played a role in the formation of CCDD species and other tetraploids. The results are partly in accordance with phylogenetic reconstruction of rice genomes as ascertained by Ge *et al.* (1999), which clearly shows that the closest ancestral species to KK (*P. coarctata*) genome are DD and HH genomes.

Fig. 2 gives the diagrammatic presentation of interlinking *Oryza* species with *Porteresia*. Crosses involving tetraploid species as is the case with *Porteresia* also require repeated backcrossing to return the hybrid progeny to the diploid level. A carefully planned prebreeding programme is necessary to transfer useful genes from wild species to an improved plant type before the breeder can use the germplasm. The development of several monosomic alien addition lines for various species

is one such prebreeding approach.

#### Biochemical changes in response to salt stress

*Porteresia* has been suggested as a model for salt tolerance in *Oryza* species and has an ability to withstand sudden changes in salt concentrations (e.g. 150 mM NaCl) in the medium that is detrimental not only to salt-sensitive rice cultivars (IR29) but also to traditional salt-tolerant varieties (Pokkali and Nona Bokra). So far, attempts to transfer the salt tolerance trait from this wild relative of rice to cultivated rice through a traditional rice breeding programme has been unsuccessful because of resultant hybrid sterility. Hence, an alternative to circumvent this problem could be transferring appropriate genetic traits that confer salt tolerance in *Porteresia* to modern rice varieties through transformation. Therefore, in order to exploit the genetic potential of *Porteresia* for biotechnological purposes, it is essential to understand the biochemical and molecular basis of salt tolerance in this wild rice species.

*In vitro* studies by Ghosh *et al.* (2001) showed that purified Fructose-1,6-Bisphosphatase (FruP2ase) from salt tolerant *P. coarctata* was more highly tolerant of NaCl, KCl and NH<sub>4</sub>Cl than salt-sensitive rice cultivars like IR26. FruP2ase catalyses an important reaction pertaining to regeneration of RuBP in the Calvin cycle which enables photosynthesis to occur under water stress while RuBisCo levels decrease. The tolerance might be due to the potential role of a number of osmolytes in protecting FruP2ase under salt stress, or due to the inherent protein structure of the enzyme. It will be interesting to envisage the role of this enzyme in salt-sensitive rice cultivars by transformation procedures.

In an attempt to study salt stress responsive proteins in *Porteresia* we imposed salinity at 150 mM under controlled conditions (Latha & Swaminathan, 2002). Leaf samples were harvested from salinised and control plants for proteome

analyses on the tenth day of the stress. Around a thousand spots were detected on single 18 cm gel. In addition to some proteins, which showed qualitative changes (absent or present and positional shifts), many proteins changed quantitatively. Expression of many proteins was not significantly affected by salt stress, whereas some proteins were up-regulated or down-regulated under salt stress. Induced proteins may be the result of increased transcription or activation, while those repressed may be due to decreased transcription, activated degradation or inactivation through post-translational modifications. Induced proteins could be important in the cellular response to a given environmental change (J Bennett, personal communication). It will be interesting to select some of these proteins for their identification by MALDI-TOF and Q-TOF from the preparative gels and then to search the peptide mass against SWISS-PROT and TREMBL using PeptIdent. This is the first report on 2D gel analyses from the leaves of salinised *Porteresia* plants showing considerable quantitative and qualitative changes. Partial amino acid sequencing of these proteins will help both in predicting the function of the proteins and for molecular cloning for future experiments.

#### Conclusion

In view of limited genetic resources available to breeders in wild rice germplasm for increasing tolerance to salinity (Akbar *et al.*, 1986) *Porteresia* is undoubtedly one of the key source-species for elevating salinity tolerance in cultivated rice. As a long-term goal, work has been initiated at MSSRF where the emphasis is to develop and to characterise pre-breeding genetic material of value in the development of location-specific crop varieties. The case study reported here has both theoretical and applied significance. Micropropagation methods have opened up opportunities for the restoration of lost *Porteresia* populations from their original habitat, of the mangrove ecosystems and of pure cultures for somatic hybridisation studies. Molecular phylogenetic analysis has helped in positioning *Porteresia* in the vicinity of the other wild rice species and to understand species differentiation in *Oryza*. It also has important implications for germplasm utilisation. Molecular analysis will also help to enhance our capacity to undertake precision breeding in rice.

Plant improvement research has now entered an exciting period in which genomics and proteomics will play a significant role. Development of varieties with the desired agronomic and culinary characteristics will however require an integration of Mendelian and molecular breeding techniques. Also, through a combination of pre-breeding and

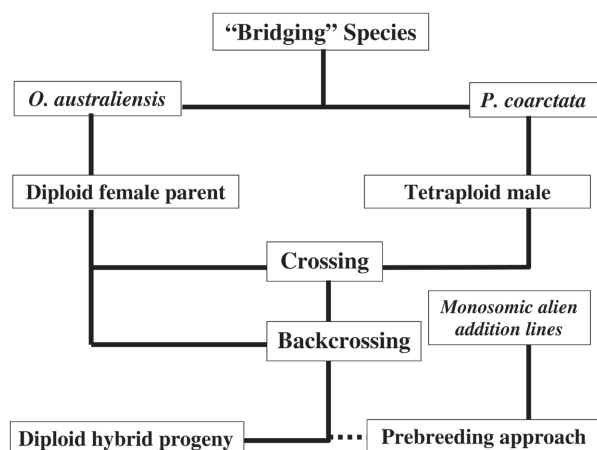


Fig. 2. *Oryza*-interlinking



participatory breeding with farm families, it will be possible to combine genetic efficiency with genetic diversity. This is likely to be the pathway for environmentally sustainable advances in the productivity of rice. However, before an applied breeding programme is undertaken, basic information generated of the type gathered during the present study is essential. Such studies will pave the way for designing a molecular breeding programme for salinity tolerance in rice.

### Acknowledgements

R Latha thanks University Grants Commission, New Delhi, for a fellowship. Thanks go to the Forest Department officials of Bhitarknika, Orissa, India and the Genetic Resources Center (GRC), International Rice Research Institute, Philippines for kind supply of the germplasm material.

### References

- Akbar M, Khush G S, Hillerislambers D. 1986.** Genetics of salt tolerance in rice. *Proceedings of the International Rice Genetics Symposium, IRRI Philippines*, pp. 399-409.
- Akbar M, Seshu D V, Senadhira D. 1990.** Salinity tolerance of *Porteresia coarctata* Tateoka and its possible use in rice varietal improvement. *Paper presented at the International Conference on Current Development in Salinity and Drought Tolerance of Plants, AEARC, Tandojam, Pakistan.*
- Anon. 2000a.** Food security and poverty alleviation. *Proceedings of the 23<sup>rd</sup> International Rice Research Conference, IRRI, Philippines.*
- Anon. 2000b.** *Tenth Annual Report of M. S. Swaminathan Research Foundation, 1999-2000.*
- Bal A R, Dutt S K. 1986.** Mechanisms of salt tolerance in wild rice (*Oryza coarctata* Roxb.). *Plant and Soil* **92**:399-404.
- Brinkman R. 1995.** Impact of climatic change on coastal agriculture. In *Climate Change Impact on Coastal Habitation*, pp. 235-245. Ed. Doeke Eisma. Lewis Publications.
- Fahn A. 1979.** *Secretory Tissues in Plants*. London: Academic Press. 302 pp.
- Finch R P, Baset A, Slamet I H, Cocking E C. 1992.** *In vitro* shoot culture of wild *Oryzae* and other grass species. *Plant Cell, Tissue and Organ Culture* **30**:31-39.
- Finch R R, Slamet I H, Cocking E C. 1990.** Production of heterokaryons by the fusion of mesophyll protoplasts of *Porteresia coarctata* and cell suspension-derived protoplasts of *Oryza sativa*: a new approach to somatic hybridization in rice. *Journal of Plant Physiology* **136**:592-598.
- Flowers T J, Flowers S A, Hajibagheri M A, Yeo A R. 1990.** Salt tolerance in the halophytic wild rice, *Porteresia coarctata* T. *New Phytologist* **114**:675-684.
- Garcia A. 1992.** *Salt tolerance in the halophytic wild rice, Porteresia coarctata Tateoka*. Ph.D. Thesis, University of Sussex.
- Ge S, Sang T, Lu B R, Hong De-Yong. 1999.** Phylogeny of rice genomes with emphasis on origins of allotetraploid species. *Proceedings of National Academy of Sciences of the United States of America* **96**:14400-14406.
- Ghosh S, Bagchi S, Majumder A L. 2001.** Chloroplast fructose-1,6-bisphosphatase from *Oryza* differs in salt tolerance property from *Porteresia* enzyme and is protected by osmolytes. *Plant Science* **160**:1171-1181.
- Hayashi Y, Kyojuka J, Shimamoto K. 1988.** Hybrids of rice (*Oryza sativa* L.) and wild *Oryza* species obtained by cell fusion. *Molecular and General Genetics* **214**:6-10.
- Jelodar N B, Blackhall N W, Hartman T P V, Brar D S, Khush G S, Davey M R, Cocking E C, Power J B. 1999.** Intergeneric somatic hybrids of rice (*Oryza sativa* L. + *Porteresia coarctata* (Roxb.) Tateoka). *Theoretical and Applied Genetics* **99**:570-577.
- Jena K K. 1994.** Production of intergeneric hybrid between *Oryza sativa* L. and *Porteresia coarctata* T. *Current Science* **67**:744-746.
- Jena K K, Khush G S. 1989.** Monosomic alien addition lines of rice: production, morphology, cytology, and breeding behaviour. *Genome* **32**:449-455.
- Joshi G V, Jamale B B, Bhosale S. 1975.** Ion regulation in mangroves. In *Proceedings of International Symposium on Biology and Mangroves* **2**:595-607.
- Khush G S, Ling R C, Aquino R C, Aguiro V M. 1977.** Breeding for resistance to grassy stunt virus in rice. *Proceedings of 3<sup>rd</sup> International Rice Congress, SABRAO, Canberra, Australia. Plant Breeding Papers* **1-4(6)**:3-9.
- Latha R. 2000.** *Studies on propagation, genetic relationship and characterization of salinity tolerance in Porteresia coarctata (Roxb.) Tateoka, a wild relative of rice*. Ph.D. Thesis, University of Madras, India.
- Latha R, Swaminathan M S. 2002.** Protein changes in response to salt stress in *Porteresia coarctata* Tateoka. *Journal of Biochemistry and Biotechnology* **11**:49-52.
- Latha R, Anand A K, Rao C S, Eganathan P, Balakrishna P. 1998.** *In vitro* propagation of salt tolerant wild rice relative, *Porteresia coarctata* Tateoka. *Plant Growth Regulation* **17**:231-235.
- Latha R, Shankaramasubramaniam H M S, Radha R, Swaminathan M S. 2002.** Genetic relationships of *Porteresia coarctata* Tateoka using molecular markers. *Plant Biosystems* **136**:339-348.
- Liphshitz N, Waisel Y. 1974.** Existence of salt glands in various genera of the Gramineae. *New Phytologist* **73**:507-513.
- Lloyd G, McCown B. 1980.** Use of microculture for production and improvement of *Rhododendron* sp. *HortScience* **15**:416 (abstract 321).
- Murashige T, Skoog F. 1962.** A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**:473-494.
- Probert R J, Longley P L. 1989.** Recalcitrant seed storage physiology in three aquatic grasses (*Zizania palustris*, *Spartina anglica* and *Porteresia coarctata*). *Annals of Botany* **63**:53-63.
- Rafalski J A, Morgante M, Powell W, Vogel J M, Tingey S V. 1996.** Generating and using DNA markers in plants. In *Analysis of Non-Mammalian Genomes: A Practical Guide*, pp. 75-134. Eds B Birren and E Lai. Boca Raton, FL: Academic Press.
- Ramanan B V, Balakrishna P, Suryanarayanan T S. 1996.** Search for seed borne endophytes in rice (*Oryza sativa*) and wild rice (*Porteresia coarctata*). *Rice Biotechnology Quarterly* **27**:7-8.
- Raymo M E. 1998.** Paleoclimate: Glacial puzzles. *Science* **281**:1467-1468.
- Richharia R H, Roy J K. 1965.** Anatomical studies in the genus *Oryza*-I. Anatomy of *Oryza coarctata* in relation to its systematic position in the genus. *Oryza* **2**:1-9.
- Singh K K, Seth S. 1998.** Socio-economic importance of *Porteresia coarctata* Tateoka. *Indian Forester* **124**:578-579.
- Stich L A, Dalmacio R D, Elloran E, Romero G O, Amante A D, Leung H, Nelson R, Khush G S. 1990.** Wide hybridization for rice improvement (abstract). *Proceedings of the 4<sup>th</sup> Annual Meeting of the Rockefeller Foundations International Program on Rice Biotechnology, IRRI, Philippines.*

- Tateoka T. 1963.** Taxonomic studies of *Oryza* III. Key to the species and their enumeration. *Botany Magazine* **76**:165-173.
- Torrizo L B, Zapata F J. 1986.** Anther culture in rice: IV. The effect of abscisic acid on plant regeneration. *Plant Cell Reports* **5**:136-139.
- Wang Z Y, Second G, Tanksley S D. 1992.** Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. *Theoretical and Applied Genetics* **83**:565-581.
- Yeo A R, Yeo M E, Flowers S A, Flowers T J. 1990.** Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theoretical and Applied Genetics* **79**:377-384.