

THE ROLE OF BIOTECHNOLOGY IN THE CONSERVATION, SUSTAINABLE USE AND GENETIC ENHANCEMENT OF BIORESOURCES IN FRAGILE ECOSYSTEM

Prashanth.S.Raghavan and Ajay Parida

M.S.Swaminathan Research Foundation
Third cross road, Taramani institutional area, Chennai-600113, India
Ajay@mssrf.res.in - Fax: +91-44-22541319

The coastal ecosystem is one of the most productive ecosystems. The tropical and the subtropical coastlines of the world are characterized by specialized littoral plant formations, the mangroves (1). A mangrove is a plant community, which inhabits the boundary between terrestrial and aquatic environments. These comprise about 71 species; spread over 16 families (1). All the mangrove species have the same physiognomy, physiological characteristics and structural adaptations (2). The plant species in this particular ecosystem are constantly under varied environmental stress conditions including high saline conditions and extremes of temperatures and these plants have adapted themselves to these frequent and fluctuating changes (3).

However human interference and negligence have caused rapid destruction to the mangrove forests and even caused extinction to a few of the locally existing species (4). Large areas of mangrove forests throughout the world are being converted for agriculture or being exploited for wood and other forest products (3). Indian coastline covers about 7500Km and it accounts for 8% of the world's mangrove area and the eastern coast of India accounts for about 82% of the mangrove forest cover in India (5). In the absence of any national plan for conservation and sustainable utilization, mangroves along the Indian coast have reached an alarming stage of depletion (3). A 25% reduction in mangrove forest cover has been reported along the Indian region during the last 25 years (5).

The coastal regions are also regions where there is intense agricultural activity. Increased soil erosion and water pollution caused by intensive farm practices in the inland area gets transported through the river and canal and adversely affect the coastal agro-ecosystem. The seawater intrusion and the attendant soil and water quality problems caused by the ground water depletion have already started threatening the sustainability of the agricultural ecosystem in Saurashtra region of Gujarat and Thanjavur region of Tamil Nadu in India. This has given rise to an increase in the level of abiotic stresses such as salinity, alkalinity, and drought. Climate change and consequent rise in sea level is one of the major impending dangers affecting the coastal ecosystem. The rise in sea level has been expected to be in the order of 8-29 cms due to global warming by 2025 (5). This could cause large scale inland flooding.

Salinization is posing an increasing problem in coastal and agricultural areas reducing plant productivity and yield. Salinity is one of the major abiotic stresses decreasing the plant productivity. Tolerance to salt stress is a complex trait, which involves various aspects such as osmotic, ionic stress and also secondary stress like oxidative stress. Salt stress leads to dehydration and osmotic stress with the reduced availability of water resulting in stomatal closure, reduced supply of carbon dioxide leading to a high production of reactive oxygen species in the chloroplasts (6). This effect causes an irreversible cellular damage. Similar

effects are also seen to occur during periods of high photosynthetic activity when the plants have a disturbance in photosynthetic activity and this causes an effect called photoinhibition (7). Photoinhibition and salinity stress together causes severe damage to the cellular processes in the plant. Mangroves are plants that are capable of surviving in highly saline environment coupled with high capacity to maintain active leaves in conditions, which will be expected to reduce the photosynthesis severely through photoinhibition (8). In order to combat such abiotic stress effects, we have taken up studies to conserve the mangrove genetic resources, characterize and harness the genes involved in salinity/abiotic stress tolerance from mangroves and transfer these genes to crop plants so as to generate crops with enhanced stress tolerance.

Cytological and Genetic diversity analysis of mangroves:

As an initial part of the work we studied the cytological and genetic diversity present in the mangrove plants. Mitotic chromosome analysis was carried on samples of different populations of *Acanthus illicifolius* (3) and these, revealed that the cells were characterized by 48 chromosomes resolved into 24 homomorphic pairs. There was no variation in the chromosome number seen in the plants from different populations. Chromosome analysis of ten species of the genus Rhizophoraceae (1) revealed that there were no numerical variation in the intra specific level and that the chromosome complements in these species were stable and underwent limited divergence during speciation.

Genetic diversity studies were carried out for Intra / interspecific variations in different populations of the genus *Avicennia* (9). A mangrove genetic resource centre was established in the Pichavaram mangrove area, Chidambaram, India where the endangered mangrove species are being conserved. Following the study on the genetic diversity present in *Avicennia*, seeds from all the populations that were studied were collected to represent *A. marina* in the mangrove genetic resources center and the sampling of the seeds was based on the recommendation as specified by the outcome of the study. Intra specific variation in the species *Excoecaria agallocha* was also studied (10). This study revealed that lack of morphological variation in the plants belonging to this species was not because of the lack of genetic variation.

Subsequently twenty-four species of mangroves and mangrove associates were analyzed using molecular markers. Using this study, we were able to generate species-specific restriction patterns in the genera *Rhizophora* and *Sueda*. We were able to study the intra-generic variations in three genera *Avicennia*, *Rhizophora* and *Sueda* (4). Analysis of mitochondrial DNA variation in the species of Rhizophoraceae (11) was also studied and this again resulted in species specific profiling of different species belonging to the family of Rhizophoraceae.

Isolation of salinity tolerance genes from mangroves:

As a first step towards characterizing genes from mangroves that contribute to improving salinity stress, we constructed a cDNA library from a mangrove species *Avicennia marina*. The cDNA library was constructed from *Avicennia marina* seedlings that were treated for 48 hours with 0.5M NaCl (12). 1841 ESTs (Expressed Sequence Tags) from *A. marina* cDNA library were compared against sequences in the non-redundant database (nr) at the NCBI using the program BLASTX. Unknown genes form the largest category at 30% and followed by genes required for primary metabolism (13%). Genes involved in transcription and chromatin organization, protein synthesis and processing each represent 10% of the sequenced ESTs while those involved in membrane transport and intracellular trafficking

represent 9% of the ESTs. 8% of the ESTs relate to signal transduction while 7% are similar to previously reported stress induced genes (13).

Genetic engineering for salinity tolerance:

The Mangrove genes isolated from the mangroves were transferred into crop plants using *Agrobacterium tumefaciens* mediated transformation (14). Specific genes isolated from *A. marina* cDNA library were cloned in binary vectors. These genes were expressed under the control of constitutive promoters. These gene constructs were then transformed into *Agrobacterium tumefaciens* and used for co-cultivation with rice calli and tobacco leaf explants. Rice calli were generated from seed scutella of mature rice seeds on a callus induction medium. Callus co-cultivated with *Agrobacterium* were washed and selected on callus induction medium containing hygromycin. The selected calli were then transferred to the regeneration medium containing shoot-inducing hormones. The regenerants were transferred to the rooting medium. The plantlets were subsequently transferred to a hardening medium. Finally, these plants were transferred to the soil in pots to raise the next generation seeds. The tillers of the rice plants were bagged before the onset of flowering in order to promote self-pollination. The seeds from the selfed plants were collected and again sown for the next generation. In a similar manner leaf discs from tobacco plants were infected with *Agrobacterium* carrying the binary constructs harboring the mangrove genes. The integration and expression of the transgenes were confirmed in these plants using various molecular analyses such as PCR, Southern Hybridization, Northern hybridization, isozyme analysis and Western Blot analysis.

The homozygous lines from these transgenic plants were raised and were tested with various abiotic stresses such as salt stress and drought stress. Initial analyses in the laboratory have been promising. However, further analyses would need to be carried out to evaluate the performance of these transgenics. It was found that all the transgenic lines performed better than the control in stress conditions. This effort happens to be the first effort of it's kind in the world in terms of transferring genes from *A. marina* to crop plants. Thus this process has multiple use in terms of conserving the genetic diversity of the mangrove species which are getting destroyed very rapidly and that the crops plants are genetically engineered with genes that help them tolerate abiotic stress better.

THE ROLE OF BIOTECHNOLOGY
Villa Gualino, Turin, Italy – 5-7 March, 2005

REFERENCE LIST

1. Lakshmi M, Parani M, and Ajay Parida. (2001) Forest Genetic Resources: Status, Threats and Conservation Strategies Oxford IBH publications, New Delhi pp 31-47.
2. Yenny- Esinsine. Elements of tropical ecology (Heinemann educational books,London 1980)
3. Lakshmi M, Rajalakshmi S, Parani M, Anuratha C.S, Ajay Parida Theoretical and applied genetics (1997) 94:1121-1127.
4. Parani, M., Lakshmi, M., Zeigenhagen, B., Faldung, M., Senthilkumar, P and Ajay Parida. Theoretical and Applied Genetics (2000), 100:454-460.
5. Parida A, Parani M, Lakshmi M, Nivedita R, Elango S, Anuratha CS (1998) *IAEA TECHDOC* 1047: 95-105.
6. Tanaka, Y., Hibino, T., Hayashi, Y., Tanaka, A., Kishitani, S., Takabe, T., Yokota, S., Takabe, T. (1999) *Plant Science*. 148, 131-138.
7. Chris bowler, Marc Van Montagu, Dirk Inze (1992) *Annual review of plant physiology and plant molecular biology*. 43:83-116.
8. Cheeseman, J.M., L.B. Herendeen, A.T. Cheeseman & B.F. Clough. 1997. *Plant, Cell and Environment* 20: 579-590.
9. Parani, M., Lakshmi, M., Elango, S., Nivedita Ram, Anuratha,C.S and Ajay Parida.(1997) *Genome*, 40:487-495.
10. Lakshmi, M., Parani, M, Nivedita Ram and Ajay Parida. (2000) *Genome* 43: 110-115.
11. Lakshmi, M., Parani, M., Senthilkumar, P and Ajay Parida *Wetlands ecology and management*. (2002) 10: 355-362
12. Parani, M., Jithesh, M.N., Lakshmi, M. and Ajay Parida. *Indian Journal of Biotechnology*, (2002) Vol11: 164-169.
13. Preeti.A.M, Sivaprakash, K.R., Parani, M., Gayatri Venkataraman and Ajay Parida. (2005) *Theoretical and Applied genetics* 110: 416-424.
14. Hiei, Y., Ohta, S., Komari,T and Kumashiro, T.(1994) *The Plant journal*(1994)6(2),271-282.