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Mini Review

Molecular markers assisted DNA polymorphism: Implications in mangrove research

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Abstract

Mangroves are defined as woody, evergreen group of plant community; grow on the swampy substrate at tropical and sub-tropical habitats, adjusted to high salinity, periodical tidal influence, strong winds, high temperatures, high precipitation and anaerobic soils. They possess unique morphological and physiological adaptive features to cope with these extreme conditions. Mangrove vegetation is the cradle of several marine fauna and provides first line of defense against devastating sea surges, typhoon, tsunami, etc. However, since industrial era, many of the mangrove members were affected by several environmental constrains and anthropogenic activities that raised the sea level, lowered sweet water influx from the adjacent rivers and encroachment for the new settlement formation, increasing salinity. Hence, mangrove restoration program is the front line topic of interest to the plant biologists across the tropical and subtropical world since it has a productive and protective role for the inhabitants. A detailed study of DNA polymorphism of the individual taxa will be provide an advantage for this initiative as the wide genetic plasticity is a prerequisite for sustainability in changed environment. Recent advancement in molecular markers assisted PCR technique will provide the information regarding genetic background of each individual taxon, ultimately leading to valid guided references towards the understanding the inherent nature of the plant itself and beneficial to proper restoration program.

Keywords

DNA polymorphism; isozyme; mangroves; molecular markers; PCR technique

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Introduction

Mangroves are an assemblage of plant community of several unrelated genera which are well adopted in hostile environment like high substrate salinity, periodic inundation, maximum precipitation and high irradiance. They are best suited in the swampy

estuaries of the tropical and subtropical world. The Indo-Malaysian area has the highest mangrove population, as this zone is the cradle of initial mangrove formation, which later spread across the tropical and sub-tropical world over the time scale (1). According to a report (2) total mangrove coverage in the world is approximately 137,760 km²,

among which 60% coverage is in Asia. Mangrove provides immense support, both ecological and economical, for the fauna as well as human settlements in the coastal areas all over the globe. Their total worth including the commercial importance, timber and plant products, coastal protection, biodiversity and tourism is estimated at \$ 186 million per year approximately (3). Mangroves, due to their dense population, acts as the primary guard against sea surges, storms; which was evident during 2004 Tsunami in some of the Asian countries (4). But due to some natural edaphic factors and different anthropogenic activities, the mangrove forests all over the globe are decreasing at an alarming level. According to a report, the mangrove forest area loss in the south Asia is approximately 1% every year (5). Sea level rise and increase in substrate salinity are the two major abiotic factors initiating the natural depletion of mangroves all over the world (6, 7).

Sundarbans, being the overlapping border of two countries, India and Bangladesh, is the largest continuous stretch of mangrove vegetation (2152 km²) in the world (8). This forest also reports the highest species richness in the world comprising of 36 true mangroves, 28 associate and 7 obligatory mangroves which represents 29 families and 49 genera (9). Adaptability to high saline environment varies to a great extent among the different genera. As such, due to various natural and anthropogenic activities salinity of Indian Sundarbans is increasing rapidly and it reaches up to 27 parts-per-thousand (ppt) (10, 11). This phenomenon is proving to be lethal for some of the important mangrove species, like *Aegialitis rotundifolia*, *Xylocarpus granatum*, *X. mekongensis*, *Nypa fruticans* and *Heritiera fomes* (12, 13).

Molecular markers and their implication

Various morphological, anatomical, physiological and molecular characteristics are being used to study the differential adaptability of a species, and molecular markers have an edge over its counterparts as it is not influenced by environmental factors (14). Genetic plasticity has a key role in any species to cope up with the changing environments or sustainability (15, 16). A species with high genetic diversity has always more probability to adapt to hostile or rapid changing surroundings, than a species with reduced genetic diversity (17). Molecular marker mediated PCR based study of DNA polymorphism is considered as a prime utility tool for understanding the genetic background of a plant, leading to undertake efficient regeneration or restoration program.

Mangroves doesn't represent a single genetic group, rather they represent the convergent genetic adaptation of a large number of plant families to a specific environment. Because of their uniqueness, physiological, floristic, ecological

and taxonomic studies have been reported on Mangrove plant community's time to time (18-20). The development of molecular methods has offered opportunities to develop mangrove research in new directions and also to address unsolved problems of salt management in mangrove studies. Molecular markers can be broadly classified into two ways, PCR based (*e.g.* Inter Simple Sequence Repeats (ISSR), Random amplified Polymorphic DNA (RAPD)) and based on hybridization (*e.g.* Restriction Fragment Length Polymorphism (RFLP)). The simplicity in use, low cost, no prior information of DNA sequence and requirement of small amount of DNA, make RAPD marker considered as the most basic and widely used among the all molecular markers assisted study along with ISSR (21). Molecular markers may be dominant and co-dominant, where in dominant markers can only generate information about the presence or absence of a specific trait or loci and co-dominant markers can provide higher level of resolution and information per locus.

Contributions of Molecular Markers for the Mangrove Research

Codominant Markers

Isozymes: Peroxidase (PRX), Superoxide Dismutase (SOD) and Catalase are antioxidant enzymes which play major role in combating stressful environment apart from plant growth and development. Cheeseman et al. (22) found the ascorbate peroxidase and SOD synthesis to be relatively higher in field-grown mangroves. Ge and Sun (23) reported a reduced genetic polymorphism both at inter and intra population level, as revealed by Isozymes analysis, in the mangrove species *Kandelia candel*. While studying the suitability of peroxidase enzyme as an indicator of heavy metal accumulation in plants, Macfarlane and Burchett (24) reported that the leaf tissue metal concentrations and Peroxidase activity created a positive linear relationship in *Avicennia marina* (24). Ye, Tam (25) reported that the level of PRX and SOD increased during water logging stress in *Kandelia candel* and *Bruguiera gymnorhiza*. Parida (26, 27) reported differential changes in the levels of various antioxidant enzymes in an *in vitro* experiment with varied NaCl treatment on *Bruguiera parviflora*, indicating towards the antioxidant enzymes being a potential marker for salt tolerance in mangroves. The authors commented that the high levels of the antioxidant enzymes enhanced the defensive ability of the plants against reactive oxygen species (ROS) which in turn avoids lipid peroxidation during salt stress. Dasgupta et al. (7, 28) worked with some mangrove species from Indian Sundarbans and reported an increase in concentration of two antioxidant enzymes PRX and SOD; and two stress-related hydrolyzing enzymes, Acid phosphatase and Esterase with the

rise of salinity in *in situ* condition. Qualitative study with gel electrophoresis also indicated the density and/or number of bands increase (isoforms) for all the four enzymes with increased salinity. They also reported decrease in the concentration of total protein with salinity hike, as the high molecular weight proteins get degraded to produce low molecular weight proteins which need to be accumulated in the cell sap to restore the required osmotic potential (Ψ) during salt stress.

RFLP: Restriction Fragment Length Polymorphism (RFLP) is a non PCR based molecular marker, in which the DNA is digested with restriction enzymes and the resulting fragments are separated depending on their molecular weight in agarose gel electrophoresis. Parani et al. (29) had reported 66% variation in *Avicennia marina*, collected from two different locations and described it as high degree of divergence among the populations. Later, Parani et al. (30) had also reported a high level of intra-specific as well as inter-generic genetic diversity in 11 true mangroves, 3 true minor mangroves and 2 mangrove associates. Moreover, the genetic diversity of *Avicennia marina* was reported to be higher among 24 true mangroves and mangrove associates using PCR based RFLP of *trnS-psbC* and *rbcL* gene regions using *HaeIII* restriction enzyme (31) and showed Intra-generic variation in mangrove genera *Avicennia*, *Suaeda* and *Rhizophora*.

SSR: Simple Sequence Repeats (SSR) or Microsatellite is a stretch of DNA in which a specific motif, usually 3 to 5 nucleotide long is repeated 5-50 times (32). Their abundance in genome as well as high mutation rates has made them the codominant markers of choice for studying genetic diversity. The initial cost is high as it involves sequencing the PCR products, but once the loci are selected, it is highly reproducible. ISSR on the other hand, is the region flanking the microsatellite loci. They are more reproducible than RAPD and their cost is less than AFLP. Maguire, Peakall (33) studied *Avicennia marina* and reported higher level of genetic polymorphism using SSR marker than AFLP. Intra and interspecific genetic polymorphism of 10 mangrove and non-mangrove populations of *Heritiera littoralis* from three sites of China and one site of Australia using ISSRs was reported by Jian et al. (34). Chen et al. (35) developed and characterized 9 SSR markers for the mangrove species *Sonneratia caseolaris*. Jian et al. (36) reported reduced level of SSR polymorphism in their study in *Nypa fruticans* collected from six natural populations in China, Thailand and Vietnam.

Dominant Markers

RAPD: This is relatively the most basic and quick PCR based technique for assessing the genetic

diversity. Parani et al. (29) reported both intra- as well as interspecific genetic variability in the *Avicennia* genus based on RAPD markers. In another experiment Dasgupta et al. (30) reported 96.5% similarity of *Rhizophora apiculata* and *R. mucronata* to that of their hybrids as revealed by RAPD analysis. Intraspecific genetic polymorphism in mangrove species *Excoecaria agallocha* was reported by Lakshmi et al. (37). Genetic polymorphism of *Avicennia alba* was tested by Teixeira et al. (38) using 20 primer pairs for 16 individuals from the Mekong Delta, Vietnam. The authors reported satisfactory polymorphism with six primer pairs; while six primer pairs did not produce adequate polymorphism and seven failed to produce any polymorphism. The molecular source of divergence of the species *Ceriops zippeliana* and *C. decandra* from Southeast Asia using RAPDs was reported by Sheue et al. (39). High level of genetic polymorphism among the *Avicenniaceae* species i.e. *Avicennia marina*, *A. alba* and *A. officinalis* was also reported by Kader et al. (40). Genetic diversity of *Xylocarpus granatum*, *X. moluccensis* and *X. mekongensis* collected from different locations of Tamil Nadu and Andhra Pradesh of India was assessed by Pawar et al. (41) using RAPD technique, where they reported low level of genetic similarity between two species *X. granatum* and *X. mekongensis*. Dasgupta et al. (42, 43) worked with some Indian mangroves and reported lower level of genetic polymorphism as exists in two plant species *Heritiera fomes* and *Xylocarpus granatum*, which are considered to be on the verge of extinction from Indian Sundarbans. On the other hand, relatively higher level of genetic diversity exist among three other taxa, *Bruguiera gymnorrhiza*, *Phoenix paludosa* and *Excoecaria agallocha*; which in turn pointed out to their sustainable existence in the same regime.

ISSR: ISSR is another widely used molecular marker, which has relatively higher reproducibility than RAPD molecular markers (44). Li and Chen (45) reported a high genetic polymorphism value of 77.44% while estimating the genetic diversity of *Sonneratia alba* from China using ISSR marker. Tan et al. (46) used ISSR marker to assess the genetic diversity in populations of a mangrove *Ceriops decandra* collected from 10 different locations in Malay Peninsula and North Australia. Genetic diversity of *Lumnitzera racemosa* and *Lumnitzera littorea* (Combretaceae), an endangered mangrove as reported by Su et al. (47, 48) from the Indo West Pacific zone used ISSR marker. High level of diversity was reported at the intraspecific level, leading to be focused on planning a proper conservation strategy for this species. Chen et al. (49) assessed the genetic diversity of 7 populations of *Kandelia obovata* from China using ISSR

marker. Dasgupta et al. (42, 43) reported relatively higher level of ISSR polymorphism in *Bruguiera gymnorrhiza*, *Phoenix paludosa* and *Excoecaria agallocha*, and reduced ISSR polymorphism in *Heritiera fomes* and *Xylocarpus granatum* expressing feebleness adaptability than those of earlier taxa.

AFLP: Amplified Fragment Length Polymorphism (AFLP) is a popular molecular marker in which the DNA is digested with Restriction enzymes which is followed by PCR amplification. Maguire et al. (33) commented that AFLPs to be competent at revealing polymorphic loci, despite of their lower average heterozygosity. Mukherjee et al. (50) used AFLP markers to perform molecular characterization of three *Heritiera* species. These authors postulated that *Heritiera littoralis* is better suited as an associate mangrove than a true one. Giang et al. (51) reported a high level of polymorphism in *Avicennia marina* collected from coastal area of Vietnam. Genetic diversity of *Merope angulata*, a mangrove associate was assessed by Jena et al. (52) The authors reported low level of genetic polymorphism as revealed by AFLP among the samples collected from two different mangrove forests in India.

Future Prospects

The molecular level study of the Mangrove Genetics is still at very initial stage. Although some work on the molecular characterization of mangroves is being done in different parts of the world, the information available is still very limited. Adequate molecular data is a primary prerequisite for preparing the proper conservation strategies for mangrove species. Quantitative real time PCR analyses of the different mangrove taxa collected from different saline zones can provide important information regarding the differential adaptive features of the plant species. With the recent development of Next Generation Sequencing it will be possible to generate a huge amount of data, regarding functional genomics, Expressed Sequence Tags (ESTs), Single Nucleotide Polymorphism (SNP) etc., if proper initiatives are taken into account. This technique of research would be highly beneficial for mangrove research, in order to understand the differential salt management ability of different taxa leading to proper restoration and Conservation strategy.

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Competing interests

The authors declare that they have no competing interests.

Authors contributions

ND begin the work and carried out manuscript writing. SS and AH contributed in manuscript writing. SD contributed in manuscript writing, corrections and final checking.

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