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Biotechnology as a Tool for Conservation and Sustainable Utilization of Plant and Seaweed Genetic Resources of Tropical Bay Islands, India

Pooja Bohra[1,*], Ajit Arun Waman[2] and Anuraj Anirudhan[3]

*[1]Division of Horticulture and Forestry, ICAR- Central Island Agricultural Research Institute, Port Blair- 744105, Andaman and Nicobar Islands, India; [2]Division of Horticulture and Forestry, ICAR- Central Island Agricultural Research Institute, Port Blair- 744105, Andaman and Nicobar Islands, India; [3]Division of Fisheries Sciences, ICAR- Central Island Agricultural Research Institute, Port Blair- 744105, Andaman and Nicobar Islands, India;*Correspondence: poojabohra24@gmail.com; Tel.: +91-3192-250436*

Abstract: Andaman and Nicobar Islands are known to harbor large diversity of plant and seaweed species, a number of them being endemic. Micropropagation could be used as an effective tool for large scale multiplication of economically important plants and seaweeds. The technique could also help in multiplying threatened species to conserve them. *In vitro* production of pharmaceutical macromolecules could be a viable option for avoiding destructive harvesting of plant species. Somaclonal variation, *in vitro* mutagenesis and transgenic could be useful in some cases. Molecular markers could help in assessment of genetic diversity, DNA barcoding, marker assisted selection etc. The article highlights the importance and relevance of various biotechnological tools in the management of biodiversity of the fragile ecosystem of Andaman and Nicobar Islands. Various research activities undertaken to conserve species of these islands are also highlighted.

Keywords: Andaman and Nicobar Islands; Bay of Bengal; biodiversity; endemism; sustainable development

1. Introduction

Natural resources including flora and fauna have been the major associates of humankind since evolution. We are largely dependent on these resources for our existence and leading a normal day to day life. However, the increasing pressures of manmade and natural disasters have jeopardized these resources in such a way that every year the conservation status of a large number of species is pushed further in the *red* list. On the other hand, our dependence on a few species for meeting most of our requirements has worsened the situation by eliminating the so called non-useful types, which could be carrying potent genes for mitigating the stresses posed by climate change. The processes of industrialization, commercial synthetic farming, pollution, deforestation, urbanization etc. are the direct or indirect consequences of population explosion, which have largely contributed in misbalancing the resource utilization in a sustainable way. Considering the sensitivity of these issues, concerted efforts are required to protect our valuable resources so that they are available to the future generations too.

The tropical rainforests are known to harbor wide array of unique floral diversity and a number of mega biodiversity hotspots are located in these regions. The Andaman and Nicobar Islands in the Bay of Bengal (a Union Territory of the India) are strategically placed between two such biodiversity hotspots *viz*. Arakan Yoma ranges of Myanmar and the Sumatra. This has resulted in unique confluence of flora of both these regions in ANI (Pandey and Diwakar, 2008). The islands are characterized by lush green forests occupying about 81.8% of the total geographical area. There are more than 2,314 species of flowering plants reported from these islands so far (Murugan *et al*., 2016) and the number may still increase considering the larger unexplored areas. Furthermore, these islands are known to harbor a large number of endemic species in a relatively smaller geographical area of about 8,249 km^2. So far, about 300 species of endemic plants have been reported from ANI (Murugan *et al*., 2016). Majority of the diversity is still unexplored and considerable scope exists for utilizing these species for the betterment of humankind. Since, horticulture has been the major source of livelihood and nutritional security for the island dwellers (Singh *et al*., 2016), the present article focuses on the management of genetic resources of horticultural crops including their wild relatives.

Similarly, seaweeds are important component of the diversity of these islands. The macro algae mainly belonging to Chlorophtya, Phaeophtya and Rhodophyta are found attached to the substratum in benthic zone. They are non-flowering plants with true roots, stem and leaves, and are known to contribute substantially to the primary production in the marine environment. Seaweeds have been used for centuries as food either in raw or processed form in many of the South East Asian countries and the trend is picking up in the western countries as well. Seaweeds are the only known natural sources of hydrocolloids viz. agar, algin and carrageenan. These multipurpose products find application in industrial, pharmaceutical and medicinal fields. Besides, seaweeds are used as animal feed and biofertilizers in crop production. The current research on seaweeds is centering on bioactives which are of great interest in the medical field. Andaman and Nicobar Islands, with 1/3rd of India's coastal line, supports good diversity of seaweeds and so far about 206 species including commercially important agarophytes and alginophytes have been reported from here.

Interestingly, different parts of these islands are inhabited by six native tribes since centuries. Two Mongoloid tribes, Shompen and Nicobarese, reside in the Nicobar groups of islands, while the tribes of Negrito origin i.e. Jarawa, Great Andamanese, Onge and Sentinelese, are residing in the Andaman islands. These tribes differ in most of their cultures and habits. Some of the local species are presently being used by the native tribes for food, medicine, fodder, fuel and other purposes. Similarly, the settler population migrated from different parts of mainland India are utilizing these plants for variety of purposes. Underutilized fruits, indigenous leafy vegetables and tuber crops have immensely contributed in the livelihood and nutritional security of the island dwellers. Further, a large number of wild relatives of cultivated crop plants have been reported to occur in these islands. This diversity needs to be assessed and utilized sustainably to strengthen our resource base, while striking the fine balance between development and ecological soundness (Waman and Bohra, 2016).

Biotechnology could be an effective tool for achieving this target through the application of techniques namely micropropagation, *in vitro* production of secondary metabolites, *in vitro* mutation, *in vitro* conservation, marker assisted selection, genetic diversity assessment, development of trait specific markers *etc*. (Waman *et al*., 2015; Waman and Bohra, 2016). Present chapter concerned exploring the possibility of utilizing various biotechnological tools for management of biodiversity of the tropical Bay Islands of India.

2. Relevance of biotechnological approaches and tools for island ecosystem

2.1. Micropropagation and in vitro conservation

Andaman and Nicobar Islands are home to many endemic species belonging to different botanical families, which are of potential economic/ ecological significance and require timely attention for their conservation. A number of species belonging to rare, endangered and threatened (RET) category are also found distributed in these islands. Some of these species have problems in natural regeneration owing to the damage caused by birds/animals, poor seed viability, anthropogenic pressure etc. For example, *Myristica andamanica* is a vulnerable wild nutmeg species endemic to the islands and micropropagation could help to multiply it in large number. Similarly, natural populations of an underutilized fruit species – blood fruit (*Haematocarpus validus*) are dwindling (Bohra *et al*., 2016a) and micropropagation could help in saving the species from extinction from the region. Experiments are in progress to standardize micropropagation protocol for this species.

Secondly, banana is a major crop of the islands covering more than half of the area under fruit crops cultivation. However, the islands are largely dependent on the planting material supplies from mainland India. This has probably resulted in inadvertent introduction of dreadful banana bunchy top virus in the pristine islands. Developing protocols for *in vitro* multiplication of locally suitable varieties would help in production of their quality planting material. The importance of tissue culture technology for the island farmers has been emphasized earlier (Bohra *et al*., 2016b). Considering this, experiments have been initiated at authors' institute for optimization of protocols for locally popular banana varieties of the islands. Other commercializable crops of the islands include variety of orchids and ornamental plants, which need further attention. Use of low cost options including concurrent ex vitro rooting cum hardening has been emphasized in *low price-high value* crops, especially medicinal plants (Waman and Bohra, 2016).

In vitro conservation is a technique, wherein plant tissues are cultured *in vitro* under sub-optimal growth conditions in order to reduce the frequency of sub-culturing. The technique has proven to be very efficient for short to medium term storage of a number of species. Considering vulnerability of the islands to natural disasters, the endemic species could be conserved under *in vitro* conditions and copies of the same could be maintained at other laboratories in mainland India. Some horticulturally important endemic species of islands have been listed in Table 1.

2.2. In vitro production of secondary metabolites

A large number of species are valued for their medicinal properties. However, the yield of bioactive molecules is very low in most of the cases. At times, complete plants are destroyed for obtaining the desired active ingredients. Such practice of destructive harvesting has been a cause of concern as it tends to threaten the natural populations to a great extent (Waman and Bohra, 2013). Biotechnological tools could help in large scale quality production of secondary metabolites under *in vitro* conditions. Induction of callus and extraction of active ingredients from them has been suggested as an important alternative for obtaining the desired molecules without disturbing the wild populations (Waman *et al*., 2015).

2.3. Creation of variability

There are a few species e.g. mangosteen (*Garcinia mangostana*), which are economically important for the islands but have narrow genetic base. For improvement of such crops, creation of variability is possible through the induction of somaclonal variations in tissue culture or using the technique of *in vitro* mutation

Table 1: List of selected endemic species of horticultural importance reported from ANI

Family	Species
Anacardiaceae	*Mangifera andamanica, M. nicobarica, Semecarpus kurzii*
Apocyanaceae	*Carissa andamanensis*
Arecaceae	*Phoenix andamanensis*
Clusiaceae	*Garcinia andamanica, G. cadelliana, G. calycina, G. dhanikhariensis, G. kingii, G. kurzii, G. microstigma*
Dilleniaceae	*Dillenia andamanica*
Dioscoreaceae	*Dioscorea vexans*
Musaceae	*Musa bulbisiana var. andamanica, M. indandamanensis, M. sabuana, M. paramjitiana*
Myristicaceae	*Myristica andamanica, Knema andamanica*
Myrtaceae	*Syzygium andamanicum, S. manii*
Pandanaceae	*Pandanus lerum var. lerum*
Tiliaceae	*Grewia indandamanica*
Orchidaceae	*Vanilla andamanica, Eulophia nicobarica*
Zingiberaceae	*Kaempfaria siphonantha*

breeding. Being cornerstone of breeding activity, variability created will also be useful in development of island suitable varieties.

2.4. Estimation of genetic diversity

The ANI is considered as a centre of origin or diversity for a number of species e.g. wild populations of *Piper betle* are present in these islands. Both inter and intra specific diversity occurs for a number of species of ecological and economic importance (Singh *et al*., 2016). This diversity needs to be tapped in such a way that commercially viable types are identified for the benefit of island farmers. Selection of such elite types needs systematic characterization. Molecular characterization is one of the most reliable methods of estimating such diversity. Further, available diversity could also be compared with their counterparts occurring in mainland India or other parts of the world. Through this information, the studied population could be categorized in a way to pave the way for future breeding programmes. A few attempts were initiated at the authors' Institute or other organizations in country in this direction, which have been summarized in Table 2. The similar technique could also be employed for diversity estimation and further studies in non-traditional and underutilized species, which play pivotal role in the lives of island dwellers. The generated information from such studies could be useful for selection of parents for carrying out conventional crop improvement programmes.

2.5. Evolutionary studies

From time to time, a number of new species have been reported by various research workers in the ANI, however, absence of requisite scientific information about their genetic relationship with their existing commercial counterparts could delay the process of their commercial utilization. Molecular phylogenetic studies could help in this regard. For example the presence of Indo-Myanmarese as well as Indonesian forms of *Erianthus arundinaceus* (Retz.) Jeswiet, a wild relative of sugarcane (*Saccharum officinarum*) was confirmed through the use of molecular markers (Nair and Mary, 2006). They concluded that collections from North Andaman were more similar to Indo-Myanmarese form, while that from Nicobar were of Indonesian form. This report supports the fact that ANI harbor confluence of flora of two different regions. Similarly, genetic diversity between the collections of *Musa balbisiana* from mainland India and the islands suggested that ANI is one of

Table 2: Selected examples of application of molecular markers for diversity assessment in various horticultural crops of ANI

Species	Molecular marker used	Salient findings	Reference
Morinda citrifolia	RAPD, ISSR	Distinct clustering of collections from ANI islands	Singh *et al*., 2012
Morus laevigata	RAPD, ISSR	Significant genetic divergence between collection from mainland India and Andaman Is.	Naik *et al*., 2015
Carica papaya	ISSR	Geographical clustering of collections from various islands	Sudha *et al*., 2013
Bouea oppositifolia, Mangifera andamanica	SSR	Genetic similarity of 43% between both the species of Anacardiaceae family	Damodaran *et al*., 2013
Cocos nucifera	RAPD	Two distinct clusters based on morphological and nut parameters	Sankaran *et al*., 2012
	SSR	High genetic diversity among the collections, separate clustering of tall and dwarf types	Rajesh *et al*., 2008
Syzygium cuminii	RAPD, ISSR	Genotype collected from Car Nicobar was distinct amongst 21 island genotypes and 2 mainland genotypes	Ahmad *et al*., 2012
Colocasia esculenta	RAPD, ISSR	Island collections were distinctly different from 3 released varieties used as reference genotypes	Singh *et al*., 2012
Mangifera indica	SSR	Separate clustering of monoembryonic, polyembryonic and wild mango species	Damodaran *et al*., 2012
Orchid species	RAPD	Distinct clustering of green orchid species	Singh and Srivastava, 2010
Costus speciosus	RAPD	Intra-specific variations to the extent of 35%	Mandal *et al*., 2007

the centres of diversity of the species (Uma *et al*., 2005). This new piece of information would go a long way in devising conservation strategy of *Musa* spp. from ANI.

2.6. Development of trait linked markers

As previously mentioned, a number of perennial dioecious species are known to occur in the islands. Important being *Myristica andamanica, Knema andamanica, Horsfieldia glabra, Piper betle, Carica papaya, Garcinia spp., Momordica spp.* etc. Development of sex linked markers would be a boon for the conservation and utilization of these species as the desired plants could be identified during early stages of development. In case of medicinal plants, identification of markers linked to the presence of their active ingredients would be very useful. Flowering behavior related markers in mango have been identified, which could be of practical utility (Damodaran *et al*., 2006).

3. Applications of biotechnological interventions in seaweeds

In the wake of increasing demand for seaweeds for various applications, collections from the natural habitats are not sufficient to meet the requirement. Artificial culture of commercially exploited seaweeds still remains the major means of raw material supply to the industries. However, on farm culture of seaweeds using vegetative fragments generally results in reduced growth rate and productivity over time. In order to overcome these problems and to develop strains with improved growth and yield parameters, biotechnological techniques such as micropropagation, transgenics and molecular markers have been tried in seaweed breeding and genetic studies. Micropropagation is a tool to produce large number of seeding material from explants of seaweed possessing desirable traits. Among the three cellular organization types in seaweeds, most of the work on micropropagation has been reported on parenchymatous and pseudo-parenchymatous types (Aguirre-Lipperheide *et al.*, 1995). Although Gibor attempted to cultivate seaweeds axenically in as early as 1950, the first successful attempt is considered to be made during 1978 by Chen and Taylor in *Chondrus crispus* (Yokoya and Valentin, 2011). Seaweed micropropagation protocols have been developed in similar lines with that of higher plants and so far micropropagation protocols have been developed in about 85 species (Reddy *et al*, 2008). Considering the diversity present and diversified applications of seaweeds, the technique is considered to be still in nascent stage. *In vitro* derived calli of economically important seaweeds have been commonly used for maintenance and clonal propagation of seed stock for mariculture (Dawes and Koch, 1991; Reddy *et al.*, 2003; Rajakrishna *et al.*, 2004; Reddy *et al*, 2008). Studies suggested that growth and quality of carrageenan obtained from tissue cultured *Kappaphycus alvarezii* were superior when compared with conventional vegetative fragments (Rajakrishna *et al.*, 2007). Non-availability of standardized protocols for obtaining viable axenic cultures from wild, lack of knowledge about the role of culture incubation conditions, media supplements, explanting season etc. on callus induction are the major limiting factors in the development of seaweed micropropagation. Table 3 represents list of selected seaweed species reported from ANI in which micropropagation has been attempted elsewhere.

Molecular marker assisted breeding based on quantitative trait loci (QTLs) offers several advantages over traditional phenotypic based breeding. Development of markers linked with genes of desirable traits could increase accuracy and efficiency of the breeding process. Several molecular markers such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Se-

Table 3: List of seaweed species reported from ANI in which cell and tissue culture has been accomplished elsewhere

	Seaweed	Reference
Chlorophyta	*Boergesenia forbessi*	Enomoto and Hirose, 1972
	Bryopsis plumosa	Tatewaki and Nagata, 1970
	E. intestinalis	Polne-Fuller and Gibor, 1987; Russig and Cosson, 2001
Rhodophyta	*Gracilaria corticata*	Subbaraju *et al.*, 1981; Rajakrishna *et al.*, 2007
	G. verrucosa	Gusev*et al.,* 1987; Kaczyna and Megnet, 1993
	Gelidiella acerosa	Rajakrishna *et al.*, 2004
Phaeophyta	*Grateloupia filipina*	Huang and Fujita, 1997; Baweja and Sahoo, 2009
	Hypnea musciformis	Rajakrishna *et al.*, 2007
	Sargassum tenerrimum	Rajakrishna *et al.*, 2007
	Turbinaria conoides	Rajakrishna *et al.*, 2007

quence Characterized Amplified Region (SCAR), Amplified Fragment Length Polymorphism (AFLP), Sequence Tagged Site (STS), Microsatellites etc. have been developed in seaweeds (Lin *et al.*, 2012). Molecular markers have also been used for DNA barcoding, which could be used for assessing genetic diversity and taxonomic identification of seaweeds species, which lack reliable morphological characters for identification. For this purpose, RAPD, Restriction Fragment Length Polymorphism (RFLP), AFLP, Microsatellites, Single Nucleotide Polymorphism (SNP) etc. have been used (Liu and Cordes, 2004). Further, molecular markers could also be used for identification of invasive seaweed species, which could pose threats to the pristine biodiversity of these Islands. Transgenic technology has been mainly focused on genetic engineering of economically important seaweeds (Lin *et al.,* 2012) like *Gracilaria, Kappaphycus, Porphyra* and *Ulva*. As with any other transgenic technology, the safety issues associated with culture of genetically modified seaweeds should be of prime consideration. Transgenic technology has been attempted in Chlorophyta (Huang *et al*., 1996), Rhodophyta (Wang *et al.*, 2010) and Phaeophyta (Zhang *et al.,* 2008). Production of valuable products in an indoor cultivation system with proper biosafety from transgenic kelp sporophyte has been successfully demonstrated (Qin *et al.*, 2005).

The general perception that compounds and chemicals produced from natural resources are safe and pose less risk to health has brought seaweeds in the limelight for extracting environmental friendly natural compounds and chemicals. The development of species-specific *in vitro* cell and tissue culture technology is essential for use of other biotechnological tools for genetic improvement and production of high value compounds from seaweeds. With plethora of problems faced in seaweed cultivation, the intervention of biotechnological tools is essential for overall growth of this sector.

4. Perspectives

The tropical islands in the Bay of Bengal are known to harbor considerable diversity of flowering plants as well as seaweed species. Most of these species are ecologically important, while a large number of species could be utilized for their commercial potential. The diversified applications of biotechnology could be helpful in carrying out the major activities related to management of this unique diversity including conservation, regeneration, characterization, utilization etc. Though some efforts have been initiated in this direction, there is vast scope for employing biotechnological tools for sustainable development of such fragile ecosystem in near future.

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