

Role of biotechnology in conservation and utilization of agricultural biodiversity

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Biological diversity provides the variety of life on the earth and agricultural diversity forms a part of it. Agricultural biodiversity refers to the variability among and between all forms of life relevant to food and agriculture. The Indian subcontinent is recognized as one of the important centres of origin of crop plants. There are around 811 plant species under cultivation in India with more than 900 wild relatives which are distributed over different biogeographic regions of the country. However, rapid genetic erosion of the species and surrounding ecosystem due to various factors is a big threat to this diversity. Biodiversity conservation is a global concern and all the member states of the Convention on Biological Diversity are taking measures to preserve both native and agricultural biodiversity. Biotechnological tools are proving valuable for effective and efficient conservation efforts of agricultural plant genetic resources. Among the various applications available, tissue culture, molecular marker technology, establishment of DNA banks and genotypic database for various crops and accessions have been delineated in this paper.

Keywords: Biodiversity, biotechnology, cryopreservation, database, markers, tissue culture.

AGRICULTURAL biodiversity according to Bioversity International is ‘the diversity of crops and their wild relatives, trees, animals, microbes and other species that contribute to agricultural production. This variability exists at the ecosystem, species, and genetic level and has resulted from interactions among people and environment over thousands of years’. It is believed that agriculture took roots around 12,000 years ago and using the vast diversity in plants and animals, man domesticated and bred the wild to evolve crops and livestock. A classic example is that of corn which was present as inedible wild teosinte until genetic mutations and selection brought it to the present cultivated form. With domestication, most of the cultivated plant species have met with similar fate losing their inherent traits, which helped them survive in the wild. Some of the traits lost include resistance to biotic and abiotic stresses like harsh environment, climate and soil conditions, pests and diseases. Currently the pace at which loss/extinction/endorsement of certain species is taking place in different parts of the world is alarming. It is believed that about 940 cultivated plant species are threatened globally¹ due to several reasons such as growing human population, habitat destruction, change in production system and globalization. Losing the species or diversity within the species is disadvantageous since we may lose the genes that are crucial for biotic/abiotic

stress resistance, improving crops and adaptation to changing climate. According to FAO estimate, since the beginning of commercial cultivation of crops in the 19th century, we have lost about 75% of genetic diversity. Following the principles of modern agriculture, we are growing fewer and fewer commercial, uniform varieties replacing the traditional ones, even in the areas of origin of diversity of a particular crop, leading to erosion of gene pool and bringing about a change in the ecosystem. Agricultural biodiversity has the potential to make our agricultural ecosystem more resilient and productive; it can also improve the nutritive value of crops and improve the livelihood of millions of farmers worldwide. We need interdisciplinary approach for conservation of biodiversity, and modern technologies like biotechnology along with computational tools and technologies are opening up new ways by which this daunting task of conserving Earth’s depleting biodiversity can be addressed.

According to the UN Convention on Biological Diversity, Article 2, biotechnology is ‘any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use’. Advances made in plant biotechnology especially in areas of tissue culture and molecular biology are proving valuable in characterization, evaluation, utilization and conservation of biodiversity.

Role of DNA and tissue banks

DNA technologies offer several effective strategies for long term conservation and utilization of plant genetic

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Table 1. Major world plant DNA banks

| Name | Location | Feature | Website |
|--|----------------------------|---|---|
| Royal Botanic Gardens | Kew, Surrey, UK | Contains more than 20,000 DNA specimens representative of all plant families | https://dnabank.science.kew.org |
| US Missouri Botanical Garden | Missouri, USA | Contains more than 20,000 plant tissue samples with the aim of supplying researchers with raw material for the extraction of DNA for its use in conservation research | www.welbcenter.org/dna_banking |
| Australian Plant DNA Bank | Lismore, Australia | Conserves the representative genetic information from the entire Australian flora | http://www.dnabank.com.au/ |
| Leslie Hill Molecular Systematics Laboratory of the National Botanical Institute | Kirstenbosch, South Africa | In collaboration with the Royal Botanic Gardens Kew, preserves genetic material of the South African flora | http://www.nbi.ac.za/research/dnabank.htm |
| National Institute of Agrobiological Sciences | Ibaraki, Japan | Together with the Ministry of Agriculture, Forestry and Fisheries (MAFF) in Tokyo, holds information regarding DNA and molecular functions of agricultural organisms such as rice and pig specimens | http://www.dna.affrc.go.jp/bank/index.html |
| Global Biodiversity Information | Bioversity International | Disseminate information from DNA collections made from all over the world. | www.gbif.net |

resources. The demand is rapidly increasing for professionally preserved, managed and documented samples that yield high-molecular weight DNA and RNA from large number of living organisms. The DNA bank, compared to traditional seed bank, has several advantages such as small sample size and stable nature of DNA for long-term storage. The stored DNA can be shared easily between collaborating DNA banks present worldwide such as those managed by International Rice Research Institute and South African National Biodiversity Institute. Few other important organizations managing DNA banks are listed in Table 1.

The role that DNA banks play in PGR activities can be easily understood by considering the example of rice. Rice with its small genome size serves as a model for understanding the genomes of other cereals. Useful genomic resources such as 32,000 full-length cDNA clones of rice with full sequence information (structural information), about 50,000 Tos 17 mutant lines induced by insertion of retrotransposon Tos 17 in rice genome and genetic analysis materials are available in rice. Such genomic resources serve as an important tool for research and breeding. They also help in discovering important genetic wealth present in wild rice species. Therefore, such valuable DNA material needs to be maintained and conserved in stable fashion for long-term efficient utilization by the research community in future, facilitating rice breeding.

In vitro techniques such as tissue culture are also important for conserving plant biodiversity. Such techniques involve three basic steps: culture initiation, culture maintenance and multiplication, and storage. The strategies differ according to the period for conservation. Generally, slow growth strategies are applied for medium-term

storage (few months to few years) and cryopreservation is found to be very useful when time of storage is undefined. In cryopreservation, artificial seeds are produced by freezing plant tissues at extreme temperatures and stored at very low temperatures to slow down growth allowing 20% increase in regeneration process compared to other conservation methods. *In vitro* techniques such as shoot tip culture also help in propagation, conservation and exchange of plant germplasm among different organizations. Successful conservation of the rare and endangered species such as medicinal plants *Hedychium coronarium*, *Rauvolfia serpentina* has been made possible by using tissue culture techniques like organogenesis and somatic embryogenesis².

Characterizing the genetic diversity using molecular markers

Plant germplasm is defined as ‘the living genetic resources such as seeds or tissue that is maintained for the purpose of breeding, preservation and other research uses. These resources may take the form of seed collections stored in seed banks, trees growing in nurseries, breeding lines or gene banks, etc. Germplasm collections can range from collections of wild species to elite, domesticated breeding lines that have undergone extensive human selection³. A large germplasm collection holds information on the genetic makeup of the species. According to FAO, the number of accessions in *ex situ* gene banks has grown to more than 8 million worldwide. However, these are not being used for breeding purposes due to lack of characterization and evaluation data.

Characterization of germplasm refers to observation, measurement and documentation of heritable traits present in a collection. This helps in identification and classification of accessions, building a catalogue of descriptors which can be put to use in agriculture. Characterization is mostly done by morphological descriptors, agronomic descriptors (traits) and molecular markers. Molecular markers have a major advantage over other methods because they are not influenced by developmental differences or environmental influences⁴. They are useful in preparing genetic maps of crops and selecting favourable traits to narrow down to a better germplasm (core collection) which can be used as a pre-breeding resource by crop breeders. Molecular markers are short stretch of DNA segment present close to a gene or specific regions within the genome. Thus, they act as flag post for the genes of interest and are used for marker-assisted breeding and selection in plants. Recent advances in genomics along with the advent of next generation sequencing, research advances in proteomic and metabolomics are contributing extensively for bioprospecting of molecules and products arising out of plants in sectors such as agricultural, environmental and pharmaceutical.

Utilization of genetic resources

To illustrate the usefulness of molecular biology in utilization of genetic resources we can take the example of wheat, apple and grape which are crops of high economic potential and have genetic resources spread worldwide.

The researchers at CIMMYT have developed the system GRIS⁵ which is the portal for the genetics and breeding programmes in wheat and triticale. It holds the passport data of about 1,71,000 varieties/lines, viz. accession details, identified gene alleles, recommended regions for cultivation, reaction to biotic and abiotic stressors, sensitivity to some conditions, morphological traits, etc. It also provides pedigree information, resistance/susceptibility to diseases, identified alleles of some genes for some accessions.

In apple, after development of RosBREED⁶ platform, immense progress has been achieved in the development of practical tools for breeding by evolving DNA tests for many breeding-relevant traits and collection of DNA information about breeding germplasm which in turn helps ascertain the genetic potential of parents, seedlings, selections and cultivars. Using the tools, breeders are able to select suitable parents from the germplasm that provide the highest proportion of desired progeny for valuable fruit quality traits. Genetic testing is now being routinely used to verify parentage and maintenance of breeding population. For example, under the tart cherry breeding programme at Michigan State University, 30% of the seedlings were discarded in one breeding population

because DNA markers indicated that they were derived from unintended parentage. Genetic tests for various traits like fruit texture and storability in apple, flesh type, bacterial leaf spot resistance, acidity, blush, and soluble solids concentration in peach, self-fruitfulness, ripening season, and fruit firmness and size in sweet cherry and red stele root rot resistance and the ever-bearing fruiting characteristic in strawberry are being routinely followed. Application of molecular assisted selection for discarding seedlings with undesirable traits is helping to save millions of dollars.

Worldwide the cultivated grape used for making wine, raisins and table purpose has been derived from the narrow genetic base of old traditional cultivars belonging to European wine grape – *Vitis vinifera* subsp. *Vinifera* and other cultivars/breeding lines have been developed for pest and disease resistance like phylloxera, powdery and downy mildew using wild species of Europe and Asia Minor (*Vitis vinifera* subsp. *sylvestris*), America (ca. 25 *Vitis* species) and Asia (ca. 40 *Vitis* species). Grape germplasm collections are being maintained in 130 repositories worldwide, the largest one containing more than 7000 accessions. By maintaining these collections as long-term field plantings, their evaluation and distribution become easier. The problem of occurrence of synonyms and homonyms within the germplasm collection is frequently encountered when characterization is based solely on morphological traits. Therefore, molecular characterization with suitable molecular markers has become popular to identify misnomers (incorrectly named) as well as synonyms (knowing same plant by different names) and homonyms (essentially same name applied to different plants). Another crucial aspect where molecular markers (microsatellites) are being used is for establishment of trueness to type which is of great significance when exchanging genotypes among grape germplasm collections, researchers and breeders. The *Vitis* International Variety Catalogue⁷ and other databases developed serve as a valuable tool for management of grapevine collections and research.

Another useful resource developed in Europe is the ECPGR, which aims at conservation and utilization of plant genetic resources in Europe⁸. A number of databases have been developed by individual institutes and working groups of ECPGR. The databases hold passport, characterization and primary evaluation data of the major collections of the respective crops in Europe. The germplasm accessions are also conserved in various European institutes. An online European Catalogue of *ex situ* PGRFA collections, EURISCO, has been created under this programme. It gives information on the *ex situ* plant collections established in Europe (<http://www.eurisco.ecpgr.org>). EURISCO is based on international standards for information on *ex situ* plant collections. Using this portal, a user can search for information on food crops, forages, wild species, etc. These include

breeding lines, cultivars, genetic stocks landraces, farmers' varieties and other research material. The EURISCO database contains passport data of approximately 1.1 million samples which represent almost 5,700 genera and more than 36,000 species from 43 countries as on February 2015 (ref. 8). The crop diversity samples constitute about 50% of *ex situ* accessions conserved in Europe and roughly 16% of world accessions⁸.

The Germplasm Resources Information Network (GRIN)⁹ constitutes four projects, one each for plants, animals, microbes and the invertebrates. GRIN has databases for the inventory, distribution, passport, characterization and evaluation data which aid in the management and utilization of the germplasm collection.

Genesys¹⁰ is an international portal for information on plant genetic resources in agriculture. It is the largest portal through which users can search for accessions present in the genebanks of all the countries. The portal allows integrating records from different data providers worldwide into a single interface. Hence a single search can scan genebanks around the world. By enabling access to these genetic resources, Genesys helps in their long-term conservation.

The Germplasm Collecting Missions Database¹¹ provides access to information on all germplasm-collecting missions in which Bioversity has been involved. An on-line service is now available that grants open access to detailed scientific information gathered during Bioversity (IBPGR, IPGRI) supported collecting missions over the last four decades. The samples are geo-referenced and displayed on an interactive map. This map allows for querying the database based on species, country, collector, etc. and also for downloading the selected passport and traits datasets.

At the national level, ICAR-NBPGR¹² has developed a web portal for information on the genetic resources conserved in the Genebank. This includes passport data, germplasm characterization/evaluation and core collections. The portal facilitates easy accessibility of the information about the conserved germplasm which in turn facilitates utilization of resources. Search menus have been provided for accession information or characterization and preliminary evaluation data.

Some of the other important germplasm information systems existing world-wide are – The AVRDC Vegetable Genetic Resources Information System (AVGRIS)¹³, Crop Wild Relative Global Portal, PGR Forum, Mansfield's World Database for Agricultural and Horticultural Crops, World Information and Early Warning System (WIEWS), Millenium Seed Bank Seed Lists, etc. In addition to these, many countries have their own databases.

Plant genetic resources in agriculture are vital for world food security. These resources form the basis of crop improvement, for ensuring food security under environmental challenges like climate change and increasing world population. These challenges require urgent atten-

tion for public policy interventions which promote both safeguarding of biodiversity and progress in biotechnology to meet the requirements of the world. The concern for conservation of biodiversity is no longer limited to a specific country and a global forum like the Convention on Biological Diversity (CBD) is taking measures on behalf of member countries to preserve both native and agrobiodiversity. The Nagoya Protocol on access to genetic resources and fair benefits sharing is a step in this direction. This protocol has provided an essential legal framework for biotechnology industries for using the available genetic diversity in a way which is just and equitable in sharing the benefits between the industry and local communities which have been the custodians of this diversity since centuries.

India's strategy for conservation and sustainable utilization of biodiversity

It is well known that biodiversity is not uniformly distributed across the world. Several countries in the tropical region are blessed with rich resources in species, many of which are endemic to the region. India is considered one of the centres of origin of cultivated plants and has been divided into 22 agro-biodiversity hotspots (Table 2). This region is the centre of crop diversity for approximately 375 closely related wild species of economically important crops such as rice, few important pulses, millets, vegetables, fruits and fibre plants (Table 3). The conservation goal in this region has been accomplished by protecting such areas under the name of ecologically fragile/sensitive areas, national parks, wildlife sanctuaries and biosphere reserves. Roughly 5% of the total geographical area is safeguarded in this manner and it helps in the *in situ* conservation of habitats and ecosystems.

The first international event on environment issues, the Stockholm Conference on Human Environment and Development, was held in 1972. India has been actively participating in all such meetings and has endorsed various important multilateral agreements on environment issues. Another important event is the CBD. As a follow-up to the CBD and a widespread consultative process, a 'National Policy and Macrolevel Action Strategy on Biodiversity' was framed in 1999 to strengthen existing strategies and programmes relating to biodiversity. The Biological Diversity Act was enacted in 2002 giving effect to the provisions of Article 15 of the Convention, allowing equitable sharing of benefits arising out of use of traditional knowledge and biological resources present in the country. India is one of the few countries to have enacted such legislation. The Government has also publicized the Biological Diversity Rules in 2004.

In order to implement India's Biological Diversity Act (2002), the National Biodiversity Authority (NBA)

Table 2. Agro-biodiversity hotspots of India

| Hotspot region | Areas covered |
|-------------------------------------|---|
| Cold Desert | Western Himalayas covering Ladakh and Kargil. Upper reaches of Lahaul–Spiti districts of Himachal Pradesh. |
| Western Himalayan | Districts of Srinagar, Anantnag, Udhampur, Reasi, Kathna in Jammu & Kashmir, all the districts of Himachal Pradesh except the cold arid region and all the districts of Uttarakhand. |
| Eastern Himalayan | All the districts of Arunachal Pradesh, Sikkim and Darjeeling district of West Bengal. |
| Brahmaputra Valley | Dhubri, Kokrajhar, Bongaigaon, Barpeta, Nalbari, Goalpara, Kamrup, Golaghat, Darrang, Morigaon, Nagaon, Sonitpur, Jorhat, Lakhimpur, Sibsagar, Dibrugarh, Dhemaji and Tinsukia. |
| Khasia-Jaintia-Garo Hills | All the seven districts of Meghalaya, i.e. East Garo Hills, West Garo Hills, South Garo Hills, East Khasi Hills, West Khasi Hills, Jaintia Hills and Ri-Bhoi. |
| North-Eastern Hills | All the districts of Manipur, Mizoram, Nagaland, Tripura and the adjoining Cachar and North Cachar districts of Assam. |
| Arid Western | Sikar, Nagaur, Pali, Hanumangarh, Ganganagar, Jalore, Sirohi, Jodhpur, parts of Jaisalmer and Bikaner, Udaipur, Dungarpur, Churu and Jhunjhunun districts of Rajasthan. |
| Malwa Plateau and Central Highlands | Malwa plateau, Central highlands, the Mewar plateau and semi-arid south-eastern Rajasthan. Shadel, Raisen, Bhopal, Sehore, Shajapur, Indore, Ujjain, Mandasaur, Rajgarh, Hoshangabad, Narsinghpur, Jabalpur, Mandla, Umaria districts. |
| Kathiawar | Ahmedabad, Surendranagar, Jamnagar, Rajkot, Porbandar, Junagadh, Amreli, Bhavnagar, Bharuch, Surat, Navsari, Valsad, Banaskantha and Anand districts of Gujarat. |
| Bundelkhand | Districts of Jhansi, Banda, Chitrakoot, Hamirpur, Jalaun and Lalitpur in Uttar Pradesh and Damoha, Datia, Panna, Sagar, Tikamagarh and Chattarpur in Madhya Pradesh. |
| Upper Gangetic Plains | Districts of Hardoi, Sitapur, Barabanki, Lucknow, Unnao, Rae Bareilly, Kanpur, Kannuj of Central Uttar Pradesh and the districts of Maharajganj, Sidharatnagar, Kushinagar, Deoria, Sant Kabir Nagar, Gorakhpur, Basti of North-eastern Uttar Pradesh. |
| Lower Gangetic Plains | Districts of Paschim Champaran, Purbi Champaran, Gopalganj, Siwan, Sitamarhi, Muzaffarpur, Saran, Buxar, Bhojpur, Patna, Rohatas, Jahanabad, Vaishali, Samastipur, Darbhanga, Madhubani, Sheohar in North Bihar. |
| Gangetic Delta | Broadly includes the deltaic 24-Parganas districts, and the districts of Hoogly, Howrah, Nadia, Bardhaman, Birbhum and Murshidabad. |
| Chotanagpur | Districts of Singhbhum, Gumla, Ranchi, Lohardaga, Palamau and Hazaribagh and Santhal Pargana in Jharkhand and Mayurbhanj district in Orissa. |
| Bastar | Districts of Bastar, Bilaspur, Durg, Jashpur, Kabirdham, Kanker, Kirba, Korba, Mahasamund, Kondaigaon and Rajnandgaon of Chattisgarh. |
| Koraput | Districts of Malkangiri, Sonabeda, Jeypore, Koraput, Nabrangpur, Kalahandi, Bolangir, Rayagada of Orissa and districts of north eastern Andhra Pradesh, i.e. Srikakulam, Vizianagaram and Vizagapatnam. |
| Southern Eastern Ghats | Districts of Chittoor, Ananthapur, Cuddapah and Kurnool in Andhra |
| Kaveri | Districts of Chengalput, South Arcot, North Arcot, Thiuvannamalai, Tiruchirapalli, Pudukottai, Thiruarur, Vellore, Kanchipuram, Dharmapuri, Salem, Namakkal, Karur and Dindigal. |
| Deccan | Districts of Jalna, Hingoli, Parbhani, Beed, Nanded, latur, Osmanabad, Solapur, Sangli, Gondia, Gadchiroli in Maharashtra, districts of Adilabad, Karimnagar, Warangal and Khamman in Andhra Pradesh and districts of Bidar and Gulbarga in Karnataka. |
| Konkan | Coastal districts of Thane, Raigad, Ratnagiri, Sindhudurg and part of Sahyadri districts of Pune, Satara and Kolhapur of Maharashtra, all the districts of Goa and Uttar Kannda district of Karnataka. |
| Malabar | Districts of Kasargod, Kannur, Wayanad, Kozikode, Malappuram, Palakkad, Thrissur, Idukki, Ernakulam, Alappuza, Kollam, Kottayam, Pathanamthitta and Thiruvananthapuram in Kerala, Udhamandalam (Nilgiri) and Kanyakumari districts of Tamil Nadu and districts of Dakshin Kannada, Kodagu and Udupi in Karnataka. |
| Islands | Andaman and Nicobar Islands and Lakshadweep |

Source: PPVFRA, New Delhi.

Table 3. Wild relatives of crop plants in India

| Crop | Number of wild relatives |
|-----------------------|--------------------------|
| Cereals and millets | 46 |
| Pulses | 81 |
| Fruits | 91 |
| Spices and condiments | 28 |
| Vegetables | 76 |
| Fibre crops | 15 |
| Oilseeds | 14 |
| Miscellaneous plants | 28 |
| Total | 379 |

Source: Faunal Resources of India, 2007.

(<http://nbaindia.org>) was established in 2003. NBA is a statutory body under Government of India with headquarters in Chennai (Tamil Nadu). It looks after all the issues related to conservation, utilization of biological resource and fair and equitable sharing of benefits accruing from use of these resources. This body is helped at the state level by the State Biodiversity Board (SBBs) which performs similar functions following guidelines laid down by the Central Government, on issues similar as above. These bodies perform several regulatory functions pertaining to commercial utilization of any biological resource by Indians. At the local level, the Biodiversity

Management Committees (BMCs) look after conservation, sustainable use and documentation of biological diversity. Activities such as conservation of land races, folk varieties and cultivators, preservation of habitats, domesticated stocks and breeds of animals and micro-organisms and documentation of knowledge relating to biological diversity fall under the purview of these bodies delivered through a structure that comprises the Authority, Secretariat, SBBs, BMCs and Expert Committees. So far SBBs have been created in 29 states and NBA operates in states where it has not been established. Around 62,500 BMCs are also operational at the local level¹⁴.

Following a permit made available to the Access and Benefit-sharing, India became the first country to clear on 1 October 2015, the internationally recognized certificate of compliance. The permit was issued by India's National Biodiversity Authority. This certificate was used by a researcher affiliated with the University of Kent in the United Kingdom to access the ethno-medicinal knowledge of the Siddi community from Gujarat and share the benefits arising from this knowledge with the community.

In today's situation where the benefits accruing from the results of modern biotechnology are seen with a sense of doubt, it is encouraging to learn that this science has provided us with multiple options to improve our knowledge about plant systems which when combined with traditional knowledge can help us conserve the plant genetic resources and biodiversity in general to meet the challenges of curbing hunger and malnutrition for the ever increasing world population under climate change scenario.

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