

# Biotechnological Tools for Conservation of Bioresources

R.K. Patidar\*, Debashish Sen, K.M. Singh and R.C. Shakywar

College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh, India.

**Email:** raghubirpatidar@gmail.com

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## Abstract

The rate of loss of natural habitats including forest and bioresources are not only a serious but a complex issue due to several reasons viz. deforestation, agricultural land degradation, ignorance of people, escalating population density, immigration of people toward urban areas etc. On the other hand forest and bioresources offer a variety of habitats for plants, animals and microorganisms. Therefore, conservation and sustainable use of bioresources is the need of the hour. The preference of conservation methods and technologies depends upon the prevailing objective, conservation efforts, breeding methods, adoption and behaviour of the species in question as well as available resources including funds, infrastructure, trained personal, and technologies. The biotechnology implies an approach of creation, invention and innovation. Biotechnological tools can be used to improve and conserve agriculture, horticulture, animals, medicine and environment. In the present article, conservation of bioresources has been highlighted covering multifaceted tools of biotechnology viz. plant genetic resources, micropropagation, *in vitro* conservation, cryopreservation, tissue culture, molecular markers, somatic hybridization and genetic engineering.

## Highlights

- Biotechnological tools play gigantic role for genetic improvement of bioresources
- Bioresources can easily be identified and made available through conservation of genetic resources
- Genetically engineered crop-based agriculture can potentially conserve the bioresources

**Keywords:** Bioresources, biotechnological tools, conservation

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Bioresources means resources from biological origin or the total biological variation manifested as individuals such as animals, plants and their gene pools which can be taken by man for use in drug, food, live stocked, construction materials for shelter, environment protection etc. It is also used in the development of improved crops and animals for higher yields and tolerance to biotic and abiotic stresses

(Eneobong, 1997). The loss of bioresources due to developmental activities such as hydroelectric projects, road laying, urbanization and changes in agricultural practices. Over-grazing and changes in land-use pattern are taking heavy toll on biodiversity available in the wild species. Globalization and market demand are also contributing indirectly to the loss of biodiversity, particularly of minor and neglected crops (Kameswara, 2004).



The most important point to remember is once these species are vanished, that knowledge along with the potential benefits is also lost. In other words, once these genes are lost, there is absolutely no chance of bringing them back at any cost. Another major concern is the genetic erosion of important plant species that used to be the part of our ecosystem (O'Neill *et al.*, 2001). "Habitats Directive" (2004), of the European Union aims to contribute towards ensuring bio-diversity through the conservation of natural habitats and of wild fauna and flora in the European territory of the member states to which the treaty applies, through a coherent European ecological network of special areas of conservation. An ecological network of special protected areas, known as "Natura 2000", is being set up for this purpose.

According to the summary report of the World Commission on Forests and Sustainable Development, about 15 million hectares of productive forests are being cleared every year. The structural integrity of much of the forest that remains has deteriorated. Forests have virtually disappeared in 25 countries; 18 have lost more than 95% of their forests and another 11 have lost 90%. The highest current estimate of the world's remaining forests areas is about 3.6 billion hectares from an originally forest area of more than 6.0 billion hectares (WCFSD, 1999). After considering the interpretational changes, the actual loss in Indian forest cover between two assessment periods i.e. 2009-2011 works out to 367 km<sup>2</sup> (Indian State of Forest Report, 2011). Forest biodiversity is under serious threat due to both habitat loss and degradation of forest ecosystems, as confirmed by the key studies such as the State of World's Forests (FAO, 2007).

The concept of genetic conservation is the knowledge and methodologies necessary for conception, assessment and development of sustainable technologies. This can be attained economically through ecologically sound biotechnological tools namely plant genetic resources, micropropagation, *in vitro* conservation, cryopreservation, tissue culture, molecular markers, somatic hybridization and genetic engineering.

### Plant Genetic Resources

Plant genetic resources for food and agriculture (PGRFA) are the basis of global food security. They comprise diversity of genetic material contained in traditional varieties, modern cultivars and their wild relatives as well as other wild

species. Loss of plant genetic resources has serious implications for food security in the long term. The full spectrum of PGR consists of diverse type of collections such as those derived from centres of diversity, centres of domestication and from breeding programmes. Advances in biotechnology, especially in the area of *in vitro* culture techniques and molecular biology provide some important tools for improved conservation and management of plant genetic resources (Kameswara, 2004).

Natural products have been our successful sources of medicines. Each plant is like a factory, capable of synthesizing unlimited number of highly complex and unusual chemical substances whose structures could otherwise escape forever, those can be conserved (Kinghorn and Soejarto, 2002). There are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently use in the world, while several other drugs are simple synthetic modifications of the natural products (Farooqi and Sreeramu, 2001). Important features of an *ex situ* conservation programme reported by Amaral and Yanchuk (2004) are (a) Acts as a backup measure to *in-situ* conservation (b) Ensures that the wide range of diversity in a species is conserved (c) Manages the regeneration of the species outside the natural range.

The plant Genetic resources (PGR) are vulnerable to losses due to introduction of new crop varieties in agriculture, growing urbanization, natural hazards, etc. Over the years, genebanks have been established in a number of countries and the number of accessions conserved in about 1400 genebanks now exceeds six million (FAO, 1998). Several landraces of some of the crops are conserved in the ICRISAT genebank at Patancheru, India have now disappeared from their natural habitats in Africa and Asia. The results of joint evaluations have led to better understanding of the germplasm material conserved at ICRISAT gene bank by the NARS scientists. Germplasm sets were evaluated for agronomic performance in India, Nepal, Thailand, Indonesia, Ethiopia and Kenya in collaboration with the National Bureau of Plant Genetic Resources (NBPGR), New Delhi and NARS (National Agricultural Research Systems). It is necessary to set standards based on current scientific knowledge and available technologies for the proper handling and storage of seeds in genebanks that will ensure their conservation over the longest possible time, without the need for frequent costly regeneration.



## Micropropagation

The art and science of plant multiplication *in vitro* known as micropropagation. The simplest type of *in vitro* plant propagation is the stimulation of axillary bud development. One of the most exciting and important aspects of *in vitro* cell and tissue culture is the capability to regenerate and propagate plants from cultured cells and tissues. Usually derived from meristem (or vegetative buds) without a callus stage, tends to reduce or eliminate somaclonal variation, resulting in true clones (Table 1). Axillary bud proliferation and culture of individual nodes are the techniques most widely used in commercial micropropagation and which show the least variation among the propagated plants.

Plant is our natural wealth and its conservation is important for economic, ecological, scientific, medicinal and ethical reasons. Therefore, there is a great need to conserve forest ecosystems by biotechnology. In recent years, *in vitro* approaches have been used as an efficient tool for micropropagation of trees and it proved that tissue culture technology is suitable for large-scale propagation of trees in short time (Pena and Seguin, 2001). Plants containing beneficial and medicinal properties have been known and used as sources of food, fodder, oils, medicines, fuel, wood, fibers and timber by increasing population growth. Due to increased demand for pulp, paper, construction materials, farmlands and fuel, status of woody trees especially forest trees are greatly affected (Giri *et al.*, 2004). Propagation of woody trees through tissue culture has many advantages over conventional propagation method like fast multiplication of the important genotypes, quick release of improved cultivars, production of disease-free plants, season-independent production of plants, germplasm conservation and facilitating their easy exchange (Asthana

*et al.*, 2011). The micropropagation is to reproduce abundant of clonal population very rapidly. More than 1000 plants species have been micropropagated, including more than 100 forest species (FAO 2001).

## In Vitro Conservation

Today, the conservation of germplasm can take advantage of innovative techniques which allow *in vitro* preservation. *In vitro* conservation refers to the techniques enabling the slow growth storage of shoot cultures in aseptic conditions by reducing the frequency of periodic subculturing, without affecting the viability, regrowth of shoot cultures and the consequent risks of contamination. In live gene bank, there are lot of problems to maintain the plant species, it requires lot of land, labour, money, documentation etc. and often damaged by biotic and abiotic stress agents. On the other hand germplasm collection required little space to maintain thousand of genotypes during storage, movement and exchange of germplasm. It is very much useful in vegetative propagated crops like banana, yam, cassava etc.

## 3. Cryopreservation

Cryopreservation (Gr. *Kryos* means frost) refers to "preservation in the frozen state". It means storage at very low temperature such as over solid carbon dioxide (-79°C), in deep freezers (-80°C), in vapour phase nitrogen (-150°C) or in liquid nitrogen (-196°C). The plant material is generally preserved and maintained in liquid nitrogen. The conventional methods of storage fail to prevent from losses due to pathogens and pests, climatic disorders, natural disorders and political and economic causes. However, the conventional methods could not save the viability of short lived seeds of economic plants, for example, oil palm (*Elaeis*

**Table 1:** List of the plants with virus eliminated by meristem culture

| Sl. No. | Plant species                | Virus eliminated                              |
|---------|------------------------------|---|
| 1.      | <i>Solanum tuberosum</i>     | Leaf roll, potato virus- A, X, Y, S           |
| 2.      | <i>Nicotiana tabacum</i>     | Tobacco mosaic virus                          |
| 3.      | <i>Saccharum officinarum</i> | Mosaic virus                                  |
| 4.      | <i>Allium sativum</i>        | Mosaic virus                                  |
| 5.      | <i>Brassica oleracea</i>     | Cauliflower Mosaic virus, turnip mosaic virus |
| 6.      | <i>Armoracia rusticana</i>   | Turnip Mosaic virus                           |
| 7.      | <i>Musa paradisiaca</i>      | Cucumber Mosaic virus                         |
| 8.      | <i>Hycinthus spp</i>         | Hycinthus Mosaic virus                        |
| 9.      | <i>Dahlia spp</i>            | Dahlia Mosaic virus                           |
| 10.     | <i>Petunia spp</i>           | Tobacco mosaic virus                          |
| 11.     | <i>Iris spp</i>              | Iris Mosaic virus                             |



*guineensis*), rubber (*Hevea brasiliensis*), *Citrus* sp. and *Coffee* sp (Table 2). Therefore all cryopreserved collections must confirm three core responsibilities (Day, J. G. and Harding, K. 2008; Stacey and Day, 2007) such as 1. Purity: freedom from contaminating organisms 2. Authenticity: correct identity 3. Stability: including functionality. Steps involve in cryopreservation are:

**(a) Selection of Materials:** Young, meristematic, highly cytoplasmic and small cells which are non-vacuolated thin walled and in small aggregates, are good materials to be selected for this purpose. Different types of explants which are used in cryopreservation are apical meristem and plant organs, ovules, anther/pollen, embryos, protoplasts etc.

**(b) Addition of Cryoprotectors/Cryoprotectant:** Cryoprotectors are the chemicals which decrease cryodestruction. Dimethyl sulfoxide (DMSO), sucrose, glycerol and proline are most frequently used cryoprotectants. DMSO has proved an excellent cryoprotectant.

**(c) Freezing:** Freezing should be done in such a way that it does not cause intracellular freezing and crystal formation. The following types of freezing can be done:

**(i) Rapid freezing:** After placing the plant material, the cryovials are put into liquid nitrogen which causes a decrease in temperature. Freezing is done quickly so that there should be least change or development of intracellular crystals. Ultra cooling prevents ice crystals. To achieve this objective, dry ice (Co<sub>2</sub>) can be used instead of nitrogen. Rapid freezing of several plant materials has been done, for example, somatic embryos and shoot tips of *Brassica napus*, strawberry, potato, etc. **(ii) Slow freezing:** In this method the rate of freezing is slow i.e. 0.1 -10° C per minute. Therefore, extracellular ice crystals are formed but not intracellular crystals. Meristems of potato, cassava, strawberry, etc. have successfully been cryopreserved. **(iii)**

**Stepwise freezing:** In this method temperature gets lowered by -20 to -40°C. It allows protective freezing of the cells. Further, freezing is stopped for 30 minutes. Thereafter, it is rapidly freezed in liquid nitrogen to get -196°C. By doing such stepwise decline in temperature, formation of big crystal is increased and good results are obtained. Excellent results have been obtained with suspension cultures and strawberry by adopting this method.

**(d) Storage in Liquid Nitrogen:** This can be simply achieved with the help of liquid nitrogen, which keeps the temperature -196°C.

**(e) Thawing:** Thawing is the process of releasing the vials containing cultures from the frozen state to elevate the temperature between 35 and 45°C. It should be done quickly but without overheating. As soon as the last ice crystals disappear, the vials are transferred into a water bath maintained at 20-25°C.

**(f) Washing and Reculturing:** Washing of plant materials is done only to remove the toxic cryoprotectants. If non-toxic cryoprotectants are used, the cultures should not be washed, but simply recultured.

**(g) Regeneration of Plantlets:** The viable cells are cultured on non-specific growth media to regenerate into plantlets.

#### 4. Tissue Culture

Tissue culture is the *in vitro* cultivation of plant cells (protoplasts, anthers, microspores, ovules and embryos) in an unorganized mass. The technique was developed initially to demonstrate the totipotency of plant cells. It is used to propagate plants under sterile conditions or in a controlled environment, often to produce mass clonal propagation plants, to create genetic variability, increase the number of desirable germplasms, incorporate specific traits and eradicate pathogens from clones as well as utilized

**Table 2:** List of cryopreserved plants in various forms

| Sl. No. | Plant materials  | Plants species  |
|---------|------------------|---|
| 1.      | Cell suspensions | <i>Oryza sativa</i> , <i>Glycine max</i> , <i>Nicotiana tabacum</i> , <i>Zea mays</i> , <i>Capsicum annum</i> |
| 2.      | Callus           | <i>Oryza sativa</i> , <i>Capsicum annum</i> , <i>Saccharum spp.</i>   |
| 3.      | Protoplast       | <i>Zea mays</i> , <i>Nicotiana tabacum</i>  |
| 4.      | Meristems        | <i>Solanum tuberosum</i> , <i>Cicer arietinum</i>   |
| 5.      | Zygotic embryos  | <i>Zea mays</i> , <i>Hordium vulgare</i> , <i>Manihot esculentum</i>  |
| 6.      | Somatic embryos  | <i>Citrus sinensis</i> , <i>Daucus carota</i> , <i>Coffea arabica</i>   |
| 7.      | Pollen embryos   | <i>Nicotiana tabacum</i> , <i>Atropa belladonna</i> , <i>Citrus spp.</i>                                      |

**Table 3:** List of secondary metabolites obtained from plant tissue cultures along with their applications

| Sl. no. | Product                              | Plant species                                     | Applications                     |
|---------|--------------------------------------|---|----------------------------------|
| 1.      | Shikonine                            | <i>Lithospermum erythrorhizon</i>                 | Dye, pharmaceutical              |
| 2.      | Codeine, morphine                    | <i>Papaver somniferum</i>                         | Analgistic                       |
| 3.      | Quinine                              | <i>Cinchona officinalis</i>                       | Antimalarial                     |
| 4.      | Atropine                             | <i>Atropa belladonna</i>                          | Muscles relaxant                 |
| 5.      | Digoxin                              | <i>Digitalis lanata</i>                           | Cardiovascular disorder          |
| 6.      | Reserpine                            | <i>Rauwolfia serpentina</i>                       | Hypotensive                      |
| 7.      | Vanillin                             | <i>Vanilla spp.</i>                               | Vanilla                          |
| 8.      | Jasmine                              | <i>Jasmiun spp.</i>                               | Perfume                          |
| 9.      | Vinblastine, ajmalicine, vincristine | <i>Catharanthus roseus</i>                        | Anticancer                       |
| 10.     | Pyrethrins                           | <i>Tagetes erecta</i> / <i>Chrysanthemum spp.</i> | Insecticide                      |
| 11.     | Rotenoids                            | <i>Derris elliptica</i> / <i>Tephrosia spp.</i>   | Insecticide                      |
| 12.     | Nicotine                             | <i>Nicotiana tabacum</i>                          | Insecticide                      |
| 13.     | Saffron                              | <i>Crocus sativus</i>                             | Food colour and flavouring agent |
| 14.     | Stevioside                           | <i>Stevia rabaudiana</i>                          | Sweetener                        |
| 15.     | Rosamarinic                          | <i>Coleus blunei</i>                              | Antioxidant                      |
| 16.     | Berberine                            | <i>Coptis japonica</i>                            | Antibacterial                    |
| 17.     | Sarcoplasmine                        | <i>Datura stramonium</i>                          | Treatment of nausea              |

secondary metabolites obtained from plant tissue cultures (Table 3). Brazilian strawberry crop is cultivated from tissue culture plant material using somatic embryogenesis (Smykal *et al.*, 2007). This technique makes it possible to produce a great number of clones, free of pathogenic fungi and bacteria (Siragusa *et al.*, 2007). In the world of growing population and dwindling nonrenewable resources, the demand for food security, wood and wood products is expected to rise over the next several decades. Definitely tissue culture techniques may provide new dimensions for tree improvement programmes.

### 5. Molecular Marker

Molecular markers have highly polymorphic nature, show co-dominant inheritance, occur frequently in genome, unbiased to environmental conditions or management practices and easily available, highly reproducible and allow easy exchange of data between laboratories (Joshi *et al.*, 2004). According to general guidelines for methodologies on research and evaluation of traditional medicines by the WHO, first step in assuring quality, safety, and efficacy of traditional medicines is correct identification and this can be done very successfully with the application of molecular markers. DNA-based molecular markers have been used extensively for a wide range of applications in food crops and horticultural plants. These applications include study of genetic variation, cultivar identification, cross-breeding studies, identification of disease-resistant genes, identification of quantitative-trait loci, diversity analysis of

exotic germplasm, sex identification of dioecious plants, phylogenetic analysis, etc. Recently, the application of DNA-based molecular markers is being explored in the field of nutraceuticals (Wang, *et al.*, 2001 and Tusa *et al.*, 2002).

Maiti *et al.* (2006) studied genotypic variability in salinity tolerance of rice hybrids and their parents and thereby giving opportunity to the breeders for genetic improvement for salinity tolerance. The scope for enhancing salt tolerance in maize through selection and breeding on the basis of root length was found by Khan *et al.*, 2003. Genotypic variability of salinity tolerance is observed and some hybrids were selected for salinity tolerance lines (Maiti, *et al.*, 2009, 2010). Salicylic Acid could be used as a potential growth regulator to improve salt tolerance in plants (Hussain *et al.*, 2010). Drought tolerant inbred lines showed distinct root system than sensitive lines by presenting larger root length, surface area, volume and greater contribution of roots to total root length (Fernando Rodrigo *et al.*, 2008). Scientists found that the terminal drought tolerant lines have a relatively more profuse rooting in the deeper layers than the sensitive lines (Vadez *et al.*, 2005). The heat stress considerably reduced anther dehiscence and pollen fertility rate in sensitive lines whereas, its effects were much smaller in tolerant (Yun-Ying Cao *et al.*, 2008). There are different types of widely used marker genes obtained from various sources and used for antibiotic resistance, herbicide resistance, etc. (Table 4).

**Table 4:** List of selected marker genes, their sources and substrates used for selection

| Selected marker gene(encoded enzyme)     | Abbreviation | Source of gene  | Substrate(s) used for selection            |
|--|--------------|---|--|
| Antibiotic resistance                    |              |   |  |
| Neomycin phosphotransferase II           | nptII        | <i>E. coli</i>  | Kanamycine, geneticin (G418)               |
| Neomycin phosphotransferase III          | nptIII       | <i>Streptococcus faecalis</i>                         | Kanamycine, geneticin (G418)               |
| Hygromycin phosphotransferase            | hpt/hyg      | <i>E. coli</i>  | Hygromycin                                 |
| Bleomycin resistance                     | ble          | <i>E. coli</i>  | Bleomycin                                  |
| Aminoglycoside adenyltransferase         | aadA         | Shigella  | Streptomycin, spectinomycin                |
| Antimetabolites markers                  |              |   |  |
| Dihydrofolate reductase                  | dhfr         | Mouse   | Methotrexate                               |
| Dihydropteroate synthase                 | dhps         | <i>E. coli</i>  | Sulphonamides                              |
| Herbicide resistance                     |              |   |  |
| Phosphinothricin acetyltransferase       | bar/pat      | <i>Streptomyces hygrosopicus/S. Viridochromogenes</i> | Glufosinate, L-Phosphinothricin, biolophos |
| Enolpyruvyl shikimate phosphate synthase | epsps/aroA   | <i>Agrobacterium sp/Petunia hybrid</i>                | Glyphosate                                 |
| Acetolactase synthase                    | als          | <i>Arabidopsis sp/maize/tobacco</i>                   | Sulfonylureas                              |
| Glyphosate oxidoreductase                | gox          | Achromobacter LBAA                                    | Glyphosate                                 |
| Bromoxynil nitrilase                     | bxn          | <i>Klebsiella pneumonia</i>                           | Bromoxynil                                 |
| Others                                   |              |   |  |
| Â-glucuronidase                          | gus/uidA     | <i>E. coli</i>  | Cutokinin glucuronide                      |
| Xylose isomerase                         | xylA         | Thermoanaerobacterium thermosulfurogenes              | Xylose                                     |
| Mannose 6-phosphate isomerase            | pmi/manA     | <i>E. coli</i>  | Mannose                                    |
| Bataine aldehydes dehydrogenase          | bath         | Spinach   | Bataine aldehydes                          |

Random Fragment Length Polymorphism (RFLP) occurs when the length of a detected fragment varies between individuals. Each fragment length is considered an allele, and can be used in genetic analysis (Joshi *et al.*, 2004). Interspecies variation has been studied using RFLP and RAPD (Random Amplification Polymorphic DNA) in different genera such as *Glycerrhiza* (Nakai *et al.*, 1996), Echinacea (Barth *et al.*, 2002) and *Arabidopsis* (Kapteyn *et al.*, 2002). RAPD-based molecular markers have been found to be useful in differentiating different accessions of *Taxus wallichiana* (Tava, 2002), neem (Khanuja, 2002), *Juniperus communis* L. (Tava, 2002), *Codonopsis pilosula* (Farooqui *et al.*, 1998), *Allium schoenoprasum* L. (Zhang *et al.*, 2003) and *Andrographis paniculata* (Zhang *et al.*, 2003) collected from different geographical regions. Genetic variation within *Brassica campestris* cultivars has been studied using AFLP and RAPD markers (Singh *et al.*, 1999). Varietals identification and genetic purity test in pepper and *Capsicum annuum* were carried out using RAPD markers. RFLP technique was used for interspecific genetic variation within the genus *Capsicum* and also for DNA fingerprinting of pepper cultivars (Hosokawa, *et al.*, 2000).

## 7. Somatic Hybridization Using Protoplasts

Protoplasts can be induced to fuse (complete/partial) genome with one another using cell wall degrading enzymes through electrofusion and Polyethylene glycol (PEG) techniques to overcome the prezygotic incompatibilities in crossing. An efficient plant regeneration system from protoplast has proved a very useful technique for crop genetic improvement programs, a prerequisite for somatic hybridization and genetic transformation by direct DNA uptake (Chen *et al.*, 2004; Veera *et al.*, 2009; Sheng *et al.*, 2011) (Table 5). The most widespread use of protoplasts involves somatic hybridisation experiments either to overcome barriers in sexual crosses or modify cytoplasmic traits by altering organelle populations (Glimelius, 1999). The genus *Brassica* includes a wide range of crop species with great economic value worldwide. Therefore, they attract not only breeders using conventional methods but also those concerned with biotechnological methods (Klýmá *et al.*, 2009). Since the early days of somatic hybridization, many intergeneric somatic hybrids in *Brassica sp.* have been developed through symmetric fusion, asymmetric fusion and microfusion (Arumugam *et al.*, 2000; Hu *et al.*, 2002a, b; Chen *et al.*, 2007; Tu *et al.*, 2008; Sheng *et al.*, 2008; Du *et al.*, 2009).

**Table 5:** Selected examples of plant species regenerated from protoplasts

| Sl. No. | Category      | Plant species   |
|---------|---------------|---|
| 1.      | Cereals       | <i>Oryza sativa</i> , <i>Zea mays</i> , <i>Hordeum vulgare</i>            |
| 2.      | Vegetables    | <i>Cucumis sativus</i> , <i>Brassica oleracea</i> , <i>Capsicum annum</i> |
| 3.      | Woody trees   | <i>Larix eurolepis</i> , <i>Coffea canephora</i> , <i>Prunus avium</i>    |
| 4.      | Ornamentals   | <i>Rosa spp</i> , <i>Chrysanthemum sp</i> , <i>Pelargonium spp</i>        |
| 5.      | Tuber & roots | <i>Beta vulgaris</i> , <i>Ipomoca batatas</i>                             |
| 6.      | Oil crops     | <i>Helianthus annuces</i> , <i>Brassica napus</i>                         |
| 7.      | Legumes       | <i>Glycine max</i>  |

### Genetic Engineering

The present techniques for genetic material transfer are based on the natural process of transformation. They are mainly recombinant DNA technology plus tissue culture,

aided by several molecular biology tools such micro-injection, electroporation, agrobacterium e.g. agrobacterium-mediated transfer which is quite successful for dicots but not monocots (Eneobong, 2003) (Table 6).

**Table 6:** List of some transgenic plant

| Crops  | Trade name         | Bt protein | Resistance to insect             |
|--------|--------------------|------------|----------------------------------|
| Cotton | Bollgard           | Cry 1Ac    | Cotton bollworm, Tobacco budworm |
| Maize  | YieldGard Knockout | Cry 1Ab    | European corn borer              |
| Maize  | Starlink           | Cry 9c     | European corn borer              |
| Maize  | Herculex I         | Cry 1f     | European corn borer              |
| Maize  | Bt-Xtra            | Cry 1Ac    | European corn borer              |
| Potato | New-leaf           | Cry 3A     | Colorado beetle                  |

**Table 7:** List of transgenic crop plants (GM crops approved in USA) for commercial use

| Crop plants           | Genetically altered trait | Product name   |
|-----------------------|---------------------------|----------------|
| Cotton                | Insect resistance         | Bollgard       |
|                       | Glyphosate resistance     | Roundup ready  |
|                       | Bromoxynil resistance     | BXN            |
|                       | Sulfonylurea resistance   | -              |
| Maize                 | Insect resistance         | Yield Guard    |
|                       | Insect resistance         | Maximizer      |
|                       | Glyphosate resistance     | Roundup ready  |
|                       | Glyphosate resistance     | Liberty link   |
| Rice                  | Vitamin A enrichment      | Golden Rice    |
| Tomato                | Delayed ripening          | Flavr Savr     |
|                       | Delayed ripening          | Endless Summer |
|                       | Virus resistance          | -              |
| Soybean               | Glyphosate resistance     | Roundup ready  |
| Potato                | Insect resistance         | Newleaf        |
|                       | Modified starch           | -              |
| Oilseed rape (canola) | Glufosinate resistance    | Innovator      |
|                       | Glyphosate resistance     | Roundup ready  |
|                       | High lauric acid          | Laurical       |
|                       | male sterility hybrid     | -              |
| Squash                | Virus resistance          | Freedom II     |
| Tobacco               | Virus resistance          | -              |
| Capsicum              | Virus resistance          | -              |
| Carnation             | Modified flower color     | -              |



Management practices used to control the disease are varied, for example, chemical control

with fungicides (Nel *et al.*, 2003), modification of plants to obtain organisms with improved genetic capabilities carried out by plant breeding and by integrating foreign DNA into plant genomes to produce transgenic plants (Hwang and Ko, 2004) (Table 7).

The bar-code of life concept is evolving in bar-code as they diversify in terms of their roles and operational procedures (Williamson and Day, 2007). Cryopreserved collections usually form the basis of the bar-code (Day *et al.*, 2008; Day and Stacey, 2007) and provide cryobanks with several distinct roles in delivering documented and authenticated cultures and cell lines for use in medicine, bio-resources, environmental industries, strains and cultures for biological assays to ratify their use as authenticated materials in research publications, type strains for taxonomic studies and repositories for conserving biodiversity.

## Conclusion

Bioresources are the different forms of living organisms those have potential to generate wealth and improved the lives. Conservation of bioresources creates innovative mechanisms for sustainable development that encompasses the interface between health and the environment. Therefore, exploration, conservation and preservation of bioresources are the centre of attention around the world. On the contrary, the latest advancement in biotechnology play an important role to create awareness, conservation and sustainable utilization of immense biodiversity. Biotechnology tools perform an significant role in creating effective *ex-situ* and *in-situ* conservation strategies, groupings of bioresources through molecular lineages, identify useful genes through gene maps and develop a genetically modify bioresources.

## References

Amaral, W., and Yanchuk, A. 2004. Integrated Approaches for Ex-situ Conservation and Use of Forest Genetic Diversity. Chapter 1. In: FAO, FLD, International Plant Genetic Resources Institute, Rome, Italy.

Arumugam, N., Mukhopadhyay, A., Gupta, N., Sodhi, Y. S., Verma, J. K., Pental, D., and Pradhan, A. K. 2000. Somatic cell hybridization of 'oxy' CMS Brassica juncea (AABB) with B. oleracea (CC) for correction of chlorosis and transfer of novel organelle combinations to allotetraploid Brassicas. *Advances in Plant Sciences* **100**:1043–1049.

Asthana, P., Jaiswal, V. S., and Jaiswal, U. 2011. Micropropagation of *Sapindus trifoliatus* L. and assessment of genetic variability of micropropagated plants using RAPD analysis. *Acta Physiologiae Planta* **33**:1821–1829.

Barth, S., Melchinger, A. E., and Lubberstedt, T. 2002. Genetic diversity in *Arabidopsis thaliana* L. Heynh. investigated by cleaved amplified polymorphic sequence (CAPS) and inter-simple sequence repeat (ISSR) markers. *Molecular Ecology* **11**(3): 495–505.

Chen, F. H., Wang, H., and Li, Z.Y. 2007. Production and genetic analysis of partial hybrids in intertribal crosses between Brassica species (*B. Rapa*, *B. napus*) and *Capsella bursa-pastoris*. *Plant Cell Reporter* **26**:1791–1800

Chen, L. P., Zhang, M. F., Xiao, Q.B., Wu, J. G., and Hirata, Y. 2004. Plant regeneration from hypocotyl protoplasts of red cabbage (*Brassica oleracea*) by using nurse cultures. *Plant Cell Tissue Organ Culture* **77**:133–138

Day, J. G., and Harding, K. 2008. Cryopreservation protocols for algae in working laboratories. In: *Plant Cryopreservation: A Practical Guide*: 95–116. Chapt. 6. Reed, B. M. Ed., Springer, New York, USA.

Du X. Z., Ge, X. H., Yao, X. C., Zhao, Z. G., and Li, Z. Y. 2009. Production and cytogenetic characterization of intertribal somatic hybrids between *Brassica napus* and *Isatis indigotica* and backcrossing progenies. *Plant Cell Reporter* **28**:1105–1113.

Eneobong, E. E. 1997. Biotechnological techniques for the conservation and use of plant genetic resources: 72-75. In EE Eneobong, ed. Biological conservation for sustainable agricultural production, Federal University of Agriculture, Umudike, Nigeria.

Eneobong, E. E. 2003. Current issues in agricultural biotechnology. Quarterly public lecture Nigerian Academy of Science.

FAO, 1998. The state of *ex situ* conservation. In The state of world plant genetic resources for food and agriculture. Rome, Italy. : 90. Food and Agriculture Organization of the United Nations.

FAO, 2001. Biotechnologies and tree improvement (review of the current status of biotechnology). Biotechnologies and tree improvement (Review of the current of biotechnology and applications in tree developments). [www.fao.org/DOCREP/003/X3910E/X3910E08.htm](http://www.fao.org/DOCREP/003/X3910E/X3910E08.htm).

FAO, 2007. *State of the Worlds' Forests*. : 154. FAO, Rome, Italy.

Farooqi, A. A., and Sreeramu, B. S. 2001. Cultivation of Medicinal and Aromatic Crops. University Press (India) Ltd., Hyderabad, India.

Farooqui, N., Ranade, S. A., and Sane, P.V. 1998. RAPD profile variation amongst provenances of neem. *Molecular Biology International* **45**: 931-939.

Fernando Rodrigo De, O. C., Frederico Ozanan, M. D., Antonio Carlos D. O., Angela Maria S., and Epaulo Cesar, M. 2008. Morphological attributes of root system of maize genotypes contrasting in drought tolerance due to phosphorus stress. *Revista Brasileira de Milho e Sorgo* **7**(2): 113-127.

Giri, C. C., Shyamkumar, B., and Anjaneyulu, C. 2004. Progress in tissue culture, genetic transformation and applications of biotechnology to trees: an overview *Trees Structure Function* **18**: 115–135.

Glimelius, K. 1999. Somatic hybridization. In: Go´mez-Campo C (ed.) *Biology of Brassica coenospecies*. Elsevier, New York,



pp 107–148

- Hosokawa, K., Minami, M., Kawahara, K., Nakamura, I., and Shibata, T. 2000. Discrimination among Three Species of Medicinal *Scutellaria* Plants using RAPD Markers. *Planta Medecine* **66**(3): 270–272.
- Hu, Q., Andersen, S. B., Dixelius, C., and Hansen, L. N. 2002a. Production of fertile intergeneric somatic hybrids between *Brassica napus* and *Sinapis arvensis* for the enrichment of the rapeseed gene pool. *Plant Cell Reporter* **21**:147–152.
- Hu, Q., Hansen, L. N., Laursen, J., Dixelius, C., and Andersen, S. B. 2002b. Intergeneric hybrids between *Brassica napus* and *Orychophragmus violaceus* containing traits of agronomic importance for oilseed rape breeding. *Theoretical Applied Genetic* **105**:834–840.
- Hussain, K., Nawaz, K., Majeed, A., Khan, F., Lin, F., Ghani, A., Raza, G., Afghan, S., Zia-ul-Hussnain, S., Ali, K., and Shahzad, A. 2010. Alleviation of salinity effects by exogenous applications of salicylic acid in pearl millet (*Pennisetum glaucum* (L.) R. Br.) seedlings. *African Journal of Biotechnology* **9**(50): 8602-8607.
- Hwang, S.C., and Ko, W. H. 2004. Cavendish banana cultivars resistant to fusarium wilt acquired through somaclonal variation in Taiwan. *Plant Disease* **88**: 580–588.
- Indian State of Forest Report. 2011. Forest survey of India : 19-21.
- Joshi, K., Chavan, P., Warude, D., and Patwardhan, B. 2004. Molecular markers in herbal drug technology. *Current Science* **87** (2): 159-165.
- Kameswara Rao, N. 2004. Plant genetic resources: Advancing conservation and use through biotechnology. *African Journal of Biotechnology* **3** (2): 136-145
- Kapteyn, J., Goldbrough, B., and Simon, E. 2002. Genetic relationships and diversity of commercially relevant *Echinacea* species. *Theoretical Applied Genetics* **105**: 369–376.
- Khan, A. A., Rao, S. A., and McNeilly, T. 2003. Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). *Euphytica* **131**: 81-89.
- Kinghorn, A. D., and Soejarto, D. D. 2002. Discovery of terpenoids and phenolics sweeteners from plants. *Pure Applied Chemistry* **74**(7):1169-1179.
- Klý' ma, M., Abraha, E., Vyvadilova, M., and Bechyn, M. 2009. Protoplast culture and fusion between *Brassica carinata* and *Brassica napus*. *Agricultura Tropica Et subtropica* **42**: 34–45.
- Maiti, R. K. 2010. A Novel Technique for Evaluating and Selecting Crop Cultivars for Salinity Tolerance: its Progress. *International Journal of Bioresource and Stress Management* **1**(1): 51-53.
- Maiti, R. K., Pawar, R., Rodriguez, H. G., Rajkumar, P., and Vidyasagar, Macro Vinicio Gomez meza 2009. Salt tolerance of pipeline Bt-cotton (*Gossypium hirsutum*) hybrids subjected to NaCl stress. *International Journal of Agriculture Environment and Biotechnology* **2**(2): 125-132.
- Maiti, R. K., Vidyasagar, P., and Banerjee, P. P. 2006. Salinity tolerance in rice (*Oryza sativa* L.) hybrids and their parents at emergence and seedling stage. *Crop Research* **31**: 427-433.
- Nakai, R., Shoyama, Y., and Shiraishi, S. 1996. Genetic characterization of *Epimedium* species using random amplified polymorphic DNA (RAPD) and PCR-restriction fragment length polymorphism (RFLP) diagnosis. *Biology Pharmacy. Bulletin* **19**: 67–70.
- Nel, B., Viljoen, A., and Stein berg, C. 2003. Evaluation of chemical substances for the management and control of Fusarium wilt of banana. In: Picq, Claudine, Vezina, Anne (Eds.), 20d International Symposium on Fusarium Wilt on Banana. Brazil, O'Neill, G. A., Dawson, I., Sotelo-Montes, C., Guarino, L., and Weber. J. C. 2001. Strategies for genetic conservation of trees in the Peruvian Amazon. *Biodiversity and Conservation* **10**: 837–850.
- Pena, L., and Seguin, A. 2001. Recent advances in the genetic transformation of trees. *Trends Biotechnol* **19**(12): 500–506.
- Sheng, X. G., Liu, F., Zhu, Y., Zhao, H., Zhang, L., and Chen, B. 2008. Production and analysis of intergeneric somatic hybrids between *Brassica oleracea* and *Matthiola incana*. *Plant Cell Tiss Organ Culture* **92**: 55–62.
- Sheng, X. G., Zhao, Z. Q., Yu, H. F., Wang, J.S., Zhang, X. H., and Gu, H. H. 2011. Protoplast isolation and plant regeneration of different doubled haploid lines of cauliflower (*Brassica oleracea* var. *botrytis*). *Plant Cell Tissue Organ Culture* **107**:513–520.
- Singh, B. M., Sharma, K. D., Katoch, M., Guleria, S., and Sharma, T. R. 1999. *PGR Newsletter* **124**: 57–61.
- Siragusa, M., Carra, A., Salvia, L., Puglia, A.M., De Pasquale, F., and Carimi, F. 2007. Genetic instability in calamondin (*Citrus madurensis* Lour) plants derived from somatic embryogenesis induced by diphenylurea derivatives. *Plant Cell Reporter* **26**: 1289–1296.
- Smy'kal, P., Valledor, L., Rodry' guez, R., and Griga, M. 2007. Assessment of genetic and epigenetic stability in long-term *in vitro* shoot culture of pea (*Pisum sativum* L.). *Plant Cell Reporter* **26**: 1985–1998.
- Stacey, G. N., and Day, J. G. 2007. Long-term ex situ conservation of biological resources and the role of biological resource centres. In: *Methods in Molecular Biology* Vol. **38** Cryopreservation and Freeze Drying Protocols. pp. 1–14. Day, J. G. and Stacey, G. Eds., Humana Press, 2nd Edition. Totowa Inc., New Jersey, USA.
- Tu, Y.Q., Sun, J., Liu, Y., Ge, XH., Zhao, Z. G., Yao, X. C., and Li, Z. Y. 2008. Production and characterization of intertribal somatic hybrids of *Raphanus sativus* and *Brassica rapa* with dye and medicinal plant *Isatis indigotica*. *Plant Cell Reporter* **27**: 873–883
- Tusa, N., Abbet, L., Ferrante, S., Lucreti, S., and Scarano, M. T. 2002. Identification of zygotic and nucellar seedlings in Citrus interploidy crosses by means of isozymes, flow cytometry and ISSR-PCR. *Molecular Biology Letter* **7**: 703–708.
- Vadez, V., Kashiwagi, J., Krishnamurthy, L., Serraj, R., Sharma, K. K., Devi, J., Bhatnagar-Mathur, P., Hoisington, D., Chandra, S., Gaur, P. M., Nigam, S.N., Rupakula, A., Upadhyaya, H. D., Hash, C. T., and Rizvi, S. M. H. 2005. Recent advances in drought research at ICRISAT: Using root traits and rd29a:DREB1A to increase water use and water use efficiency in drought-prone areas. Poster presented at the International drought II Conference, Rome.
- Veera, R.N., Gregory, D. N., Philip, J. D., and Trevor, W. S. 2009. Regeneration from leaf explants and protoplasts of *Brassica oleracea* var. *botrytis* (cauliflower). *Scientia Horticulture* **119**: 330–334.



- Wang, J., Ha, W.Y., Ngan, F.N., But, P. P. H., and Shaw, P. C. 2001. Application of Sequence Characterized Amplified Region (SCAR) Analysis to Authenticate *Panax Species* and their Adulterants. *Planta Medica* **67**: 781–783.
- Williamson, P., and Day, J. G. 2007. The problem with protists: is barcoding the answer? *The Biologist* **54**: 86–90.
- World Commission on Forests and Sustainable Development (WCFSD) 1999. Summary Report. International Institute for Sustainable Development (IISD). Winnipeg, Manitoba, Canada.
- Yun-Ying, C., Hua, D., Li-Nian, Y., Zhi-Qing, W., and Jian-Chang, Y. 2008. Effect of Heat Stress during Meiosis on Grain Yield of Rice Cultivars Differing in Heat Tolerance and its Physiological Mechanism. *Acta Agronomica Sinica* **34** (12): 2134-2142.
- Zhang Y. B., Wang, J., Wang, Z. T., But, P. P. H., and Shaw, P. C. 2003. DNA Microarray for Identification of the Herb of *Dendrobium* Species from Chinese Medicinal Formulations. *Planta Medica* **69** (12): 1172–1174.