KEYWORDS

Biodiversity
Biotechnology
Endangered plants
Ex situ
Genetic diversity
In situ
Plant Tissue Culture
In Vitro Gene Bank

ABSTRACT

Biological diversity provides the variety of life on the Earth and can be defined as the variability among and between the living organisms and species of surrounding ecosystems and ecological complexes of their life support. It has been estimated that one third of the global plant species are threatened in different level according to the International Union of Conservation of Nature (IUCN). The major threat to rapid loss and extinction of genetic diversity due to habitat destruction, pollution, climate change, invasion of exotic species, human population pressure, ever increasing agricultural pressure and practices, life style change etc. are well-known. Biodiversity conservation is a global concern. All member states of the Convention on Biological Diversity (CBD) took measure to preserve both native and agricultural biodiversity. The global concern of biodiversity conservation initiated either by in situ or ex situ methods. In situ methods protect both plants and their natural habitat. On the other hand, ex situ methods involves preservation and maintenance of plant species or plant parts (such as seeds, cuttings, rhizomes, tubers etc.) outside their natural habitat for the purpose of developing seed banks or more preciously gene banks following classical / advanced methods of plant propagation. Classical methods of plant propagations have certain limitations in terms of rapid production of plants or plant propagules and their long term conservation. So, the biotechnological methods such as plant tissue culture, plant cell culture, anther culture, embryo culture etc. are quite applicable and useful techniques for ex situ conservation. On the other hand, the production of superior quality seeds has enhanced by the application of plant biotechnology. So, plant biotechnology offers new means of improving biodiversity conservation rather than threatening biodiversity in various ways.

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1 Introduction

Biodiversity is the mass of different living beings in a particular ecosystem or on the whole earth. It exists in three different levels: genes, species, and ecosystems. Each of the components has its own composition, structure and function (Redford & Richter, 2001; Noss, 2005). Biodiversity provides the basis for ecosystems and their services, upon which all people fundamentally depend (Cardinale et al., 2012). Biodiversity is considered as the base of agriculture, source of all recent crops and domestic livestock species since the beginning of human civilization. Similarly, the origin of biotechnology is very deep rooted in the human history from the starting of domestication of wild plants and animals to recent time. Genetic manipulation by classical methods of plant breeding and selection of superior and new varieties started since prehistoric time. Similarly, biotechnology has been used to improve and enhance crop productivity, as well as to conserve, evaluate and utilize the various aspects of biodiversity (Brink et al., 1999; Wolfe, 2000).

The major causes of loss of biodiversity is due to human activities such as habitat destruction, pollution, climate change, invasion of exotic species, human population pressure, agricultural practices, lifestyle change etc. (Opdam & Wascher 2004). The United Nations Food and Agricultural Organization (FAO) assuming that global population is approaching towards 9.1 billion in 2050 and there is a need of 70% increase in food production (Godfray et al., 2010). In this current situation, undertaking of effective as well as productive agricultural land uses has raised a substantial challenge of keeping biodiversity (Tscharntke et al., 2012). At the same time, FAO advised to conserve plant genetic biodiversity as it is essential for future food security. As the reduction in crop diversity is causing a potential threat to food security in global food supplies is an important issue (Khourya et al., 2014). Hence, the International Treaty on Plant Genetic Resources for Food and Agriculture came into exist in 2001 to recognize farmers contribution to the diversity of crops, establish mechanism to access plant genetic materials and share benefits of developing genetic materials (FAO, 2014).

It has been estimated that one third of the global plant species are threatened in different level according to International Union of Conservation of Nature (IUCN, 2013). The increasing awareness and great concern about global biodiversity conservation, the United Nations (UN) declared the current decade (2011-2020) as the “Decade of Biodiversity” and has set 2020 as the target for restoring at least 15% of degraded ecosystems as well as conserving 17 and 10% of terrestrial and inland water and marine and coastal areas respectively (Tscharntke et al., 2012; SCBD, 2014). Global concern about the loss of valuable genetic resources stimulated many new programs for the conservation, protection and management of natural resources and wildlife. Conservation of biodiversity means protection of valuable natural resources for future generations as well as well being of eco-system function. Within past few decades, several conservation strategies have been developed mainly in the methods of in situ and ex situ conservation policy. Biodiversity conservation is based mainly on in situ conservation where habitats, species and ecosystems are naturally present and preserved in natural condition without any changes. Genetic variation is necessary for long term survival of plant species in natural habitat (Lande, 1988). The genetic diversity can be maintained by introducing new individuals to increases population size to minimize inbreeding depression, genetic drift and extinction risk (Nybom, 2004). The gradual declining of plant species is the common phenomena of in situ preservation method, due to natural habitat loss, fragmentation, modification even in reserve area with time (Fahrig, 1997).

While, ex situ conservation involves preservation and maintenance of living samples outside their natural habitat using several techniques such as in botanical gardens, seed banks, gene banks etc. and is able to solve some problems related to in situ preservation techniques. Different modified techniques have applied to preserve different categories of plant species by using both in situ and ex situ methods of conservation as they are complementary to each other (Khan et al. 2012). The maintenance and analysis of genetic diversity are important factors in the preservation of rare and endangered plant species either by in situ or ex situ methods. The conservation of plants, seed, pollen, vegetative propagules, tissue or cell by using plant tissue culture techniques, which is highly accepted biotechnological approaches for conservation of rare and endangered plant species (Paunescu, 2009). Moreover, various bio-techniques are not only offer the possibilities of faster multiplication of clones of endangered plant species for conservation of genotypes, but also conserve genetic material, provide the power of modification at genetic level by changing their expression level (Verpoorte & Memelink, 2002). Biotechnological methods are reliable and can provide continuously safe, higher quality natural products for food, pharmaceuticals and cosmetic industries, similarly, they are applicable in preserving biodiversity in several ways (Nalawade et al., 2003; Julsing et
al., 2007). Several biotechnological approaches are important factors to conserve, analyze and detect genetic diversity of rare and endangered plants such as different molecular marker techniques starting from biochemical, physiological and DNA based markers (Khan et al., 2012). The present review will discuss the global impact of biodiversity, factors of biodiversity loss and use of biotechnology to conserve, maintain and enhance biodiversity.

2 Global Impact of Biodiversity

Biodiversity is needed for human survival either in direct or indirect way. The direct use includes things like food, fibers, medicines and biological use, while the indirect uses include ecosystem services such as atmospheric regulation, water and nutrient cycling and industrial raw materials etc (Fahrig 1997; Redford & Richter 2001). Biodiversity plays effective role in providing various genetic resources of agriculture which is important for the biological basis for world food security and support of human livings. Preservation of genetic resources of plants of different categories is central not only for global economy but also to maintain ecological balance. In the field of agriculture, the genetic diversity of each type of crop (varieties, cultivars etc.) has considerable importance in crop improvement programs. Various programs pay attention to conserve biodiversity for food and agriculture at national and international levels (Campbell et al., 2010). The germplasm of a large number of primitive and wild cultivars of several plants constitute a pool of genetic diversity to support future crop improvement programs (UNEP 2008). A wide range of genetic diversity can be introduced in plants either by classical or advanced technology against the major threats of plant survival such as disease, pests, environmental stresses etc. (Cadotte et al., 2008; Corlett & Primack, 2008).

The biodiversity of organisms has great impact on the functioning of the natural ecosystem services and ecological processes for the total function of humankind. Ecosystem services provide benefits in different ways those are involved in the production of renewable resources such as food, wood, increased carbon sequestration, photosynthesis, recycling of nutrients, air and water purification, pollination, prevention of soil erosion etc, and regulating services are those that minimize environmental changes such as climatic change, controlling pest/disease (Tscharntka et al., 2012). Different ecological services have significant contribution in the world’s economy both in industrial and agricultural development either directly or indirectly. Several industrial products such as oil, rubber, fiber, building material, timber, wood, paper etc. are widely obtained from biological resources. Being a member of ecosystem, microorganisms play a dynamic role in food processing mechanism in food industry (Cardinale et al., 2012).

Biodiversity plays a significant role in human health as plants are great source of medicines, especially traditional medicines, which are useful in the treatment of various diseases are well recognized (Alves & Rosa, 2007). Traditional Chinese medicine, Indian ayurveda and Arabic unani and ethnomedicine contain thousands of compounds isolated from leaves, herbs, roots, bark etc. Except these traditional medicines, large number recent days advanced technology based pharmaceutical products are also obtained from plants. Both developing and developed countries are isolating different drugs following either classical or advanced ways. Almost 80% of the world population depends on medicines those are obtained from natural resources either in direct or indirect ways (WHO, 2002). To add on, nearly 25% of all commercial medical drugs in developed countries are based on plants and plant derivatives. This dependency may reach as much as 75% in case of developing countries (Principe, 1991).

For instance, about 200–250 herbal species used for treating human illnesses are traded in the Mediterranean region (Lev & Amar, 2002; Said et al., 2002). The greater diversity of life increasing the possibility of discovering new medicines and fostering economic development, as almost every plant species are of potential use of some commercial or medical value (Opdam & Wascher, 2004). According to this view, it is therefore very important to conserve all living species. Biodiversity not only supply essential materials for human requirements, it also provide non-material benefits like spiritual inspiration and visual values, thoughtful artistic mentality, cultural diversity, aesthetic and natural bonding to develop, nourish, enjoy, cultivate and express the internal quality of human being. The beauty inherent in biodiversity is a great source of pleasure. Although this aesthetic value is impossible to quantify, but it remains as source of fundamental development of human being (Sarkar & Frank, 2012).

3 Degradation of Biodiversity

The Earth’s environment is changing on all scales from local to global, in a large measure due to human activities. The rapid increase in human populations and their increasing needs has caused a major threat to biodiversity and wildlife in recent time. In the last 50 years, there is a significant shift in ever increasing population pressure on earth, life style change, urbanization, extensive agriculture, overexploitation and unsustainable utilization of natural resources and important plant species, introduction of alien species resulted in unprecedented decline in biodiversity and ecosystem services (Fahrig, 1997). The most important causes of biodiversity decline are habitat loss, fragmentation and habitat isolation (Millennium Ecosystem Assessment, 2005). For instance, the Mesopotamian marshes of Iraq lost more than 90% of their original area due to water diversion projects during the 1990s (SCBD, 2010). The increasing rate of manmade activities augmented greenhouse gasses and working as key factor in current global warming around 3-5°C in the next 100 years has predicted by using several computer models (Giam et al., 2010). The relation of global warming and climate change and loss of biodiversity have indivisibly linked with associated ecosystem services, still there is no ecosystem management plan has been devised that can assist living organisms in
mitigating and adapting to climate change (Schroth & McNeely, 2011). Climate change and global warming affect the habitat change of plants, animals, microorganisms and at the same time affect species at cellular level that may lead to the alteration at genetic makeup of individual cell. Moreover, the issue of land use and climate changes has great impact on worldwide loss of biodiversity (Corlett & Primack, 2008). Several studies suggest extensive and rapid land use due to human activities and climate change are the main indicators of increasing species extinction from natural habitat. Climate changes affecting plant physiological processes which can cause shrinking of plant species area wise locally then globally as a whole. The rate of species loss in recent decades is 100-1,000 times faster than the natural rate and 60% of the ecosystems products and services on which we as a society rely are in degraded or in decline condition (SCBD, 2014).

4 Conservation of Biodiversity

The concept of conservation biology matured in the mid-20th century as ecologists, naturalists, and other scientists worked together to address the declining of global biodiversity as the major issue. The conservation of biodiversity was recognized in multiple levels of interactions between the international communities, those led the United Nations to process and action in the development of the convention of biological diversity (CBD, 1992). The international understanding developed to adopt and recognize the conservation of biodiversity as a global issue and legal binding of sustainable use of biological resources. The conservation ethic promote the management of natural resources for the purpose of sustaining biodiversity in species, ecosystems, evolutionary process, human culture and society (Soule, 1985). However, conservation biology reformed around strategic plans with time to protect regional biodiversity with specific issues in the later time (Margules & Pressey, 2000). At the same time, priority has given to the strategic conservation plans to uses public policy in local, regional and global scales of communities, ecosystems, and cultures (Gascon et al., 2007). Action plans identified the ways of sustaining human well-being, employing natural capital, market capital, and ecosystem services for the survival of mankind as in recent years, while increasing loss of biodiversity has posed a serious threat to the survival of human being (Corlett & Primack, 2008).

5 Techniques for Plant Conservation

Different level of genetic diversity helps plant species to survive in their natural habitat, so conservation of plants in their natural habitat play important role. According to the guidelines of International Union for Conservation of Nature, both in situ and ex situ methods are well known and applied for the conservation of Red listed plants (IUCN, 2013). The choices of one or the other technique, or a combination of both, depend on the particular case and both are complement to each other based on the adoption of conservation policy. In situ conservation method is the most valuable method, as it is natural and totally depends on the plants in their living forms in their natural habitat and allowing natural evolution (Ashmore et al., 2011). So, in situ conservation involves the maintenance and protection of natural habitats, while ex situ conservation involves the conservation or propagation of a species variety, clone or genetic material of plant species either in botanical garden, or in the process of seed banks or using some semi-natural habitat environment. Being a naturally adopted process of conservation, in situ conservation faced several problems due to restricted and fragmented habitat, climate change, unsustainable use of plant resources, attack of pathogenic organisms and invasive species in natural environment while ex situ method is more effective and more scientific because of its way of conservation which is governable with rules, regulation and adopted methodology in artificial way (Al-Elwaawi 2003; Reed et al., 2011). Although it hampers plant evolution, the principle of any applied technology for germplasm conservation should have to preserve the maximum possible genetic diversity of a particular plant or genetic stock for future use. Environmental factors are the cause for genetic diversity in a natural habitat, in that case, in situ is the best alternative. Diversity in plants exists at generic, specific and individuals while specific genetic stocks can be develop and maintain through breeding, mutation, plant tissue culture and applying transgenic techniques to fulfill specific objectives through ex situ conservation methods (Brink et al., 1999). In situ conservation maintains and manages existing genetic diversity and viable populations of wild taxa in order to maintain biological interactions, ecological processes and functions under natural conditions (Ashmore, 1997). The ex situ method follows the propagation of plant species, varieties, specific clones either by classical method using seed, cuttings, rhizomes, corms or by using biotechnological methods such cell, tissue, organ culture, micropagation techniques, cryopreservation, germplasm banking, gene banking, applied advanced research to introduce new genetic modification in the existing population, reinforcement of existing population and reintroduction into the wild controlled environments (Schemske et al., 1994). The main objective of germplasm conservation is to maintain constant preservation of germplasm as it can be available at any time. In nature, propagation by seed germination is usual, but sometimes seeds fail to germinate due or incomplete and rudimentary embryos or environmental reasons (Hamrick & Godt, 1989). In general, most of the orthodox seeds of different plant species can be dehydrated down to low water content and thus can be stored at low temperature for long time without the loss of seed viability (Dussert et al., 1997). There are abundant forest tree species, especially tropical forest tress, producing recalcitrant seeds; those seeds cannot be dried to sufficiently low moisture level to allow their storage at low temperatures (Roberts, 1973). Moreover, there are large number of plants produce very few rudimentary and incomplete, heterozygous seeds and their conservation in seed forms are problematic, those are categorized as intermediate plants (Ellis, 1991). Conservation of these plant species by traditional ex situ procedure has several drawbacks, which limit its efficacy and threaten the safety of the growing plants genetic resources in the field.
Moreover, conservation of rare and endangered plant species due to their high rate of threats in natural environment need to be conserved following advanced biotechnological methods of \textit{ex situ} conservation process was highlighted after the world biodiversity declined in unprecedented rate. These conventional methods of seed and clonal propagation of plant genetic resources in \textit{ex situ} conservation process was replaced by \textit{in vitro} cultural methods which are more promising and advantageous of regenerating large numbers of plant propagules using minimum of start-up plant material (Sarasana et al., 2006; Rowntowne, 2006).

5.1 Biotechnology

Biotechnology has been defined as any technique that uses living organisms, or substances to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses. It consists of a gradient of technologies, ranging from the long-established and widely used techniques of traditional biotechnology to novel and advanced biotechniques of cell and tissue-culture methods and transgenic methodology. The beneficial impact of plant biotechnology on improvement and production of economically important crops are well established, while it has great impact in conservation of biodiversity too. Advances in biotechnology, especially in vitro culture and molecular biology especially transgenic technology lead to the production of a new category of germplasms, cell lines with special attributes and genetically transformed material (Engelmann, 1991). \textit{In vitro} culture and collection of germplasms by rapid, medium and slow growing multiplication processes, slow growth storage, cryopreservation have great applicability to reduce the risk of loss of plant genetic resources those are venerable in general growing and storage conditions (Pence et al., 2002). The huge loss and degradation of plant genetic resources were partially assessed, conserved and managed by adopting advanced \textit{ex situ} techniques of advanced biotechnological methods especially \textit{in vitro} culture and several techniques of molecular biology for study and analysis purpose of genetic diversity (Ashmone, 1997; Sarasana et al., 2006; Paunescu, 2009).

5.2 Plant Tissue culture

Plant tissue culture (PTC) is a quick, season independent and efficient \textit{in vitro} technique to propagate plants under sterile micro environment. It is very effective method of cloning of plant material and to develop disease free clean plant stock. PTC is a sun-rise technology and working as a catalyst of agricultural and industrial development. Any plant cell has the power of cellular totipotency to be differentiated into whole plant in the process of plant tissue culture methodology. There are different types of culture methods using different organs (Chawla, 2009). The technique of different types of culture is applied with several objectives, the most important one is the enhancement of plant production rate by quick regeneration of plants in the absence of seed, or otherwise by using the seeds which have very low chances of germination capability (Ellis, 1991; Abo El-Nil 1997). Different techniques in PTC may offer certain advantages over traditional methods of propagation for assembly, proliferation, preservation and storage of plant genetic resources (Bunn et al., 2007). The success of plant tissue culture depends on the success of shoot regeneration in a rapid and reproducible way. It has great importance in the crop improvement program which is facing the increasing depletion of natural resources. Moreover, tissue culture techniques can be applied in germplasm conservation of medicinally important plants those are of important source pharmaceutical compounds. It can be applied for regenerating different clean disease free stock of plants in the field of agriculture, horticulture, floriculture and pharmaceutical industry (Fischer et al., 2004). Tissue culture is a useful technique to preserve somatic embryos which can be applied in the medium and long-term conservation process. In the medium–term conservation, there is a need to lengthen the period between subcultures by reducing growth rate. PTC is also a great source of creating variations through the development, selection and isolation somaclones commonly known as somaclonal variations. Thus biotechnology lead to the production of a new category of germplasms, clones of special category, elite cell lines and genetically transformed material with desired traits. The cultivation and conservation of the new germplasms in the changed environmental situation can be able to add some specific impact in changed environmental situations.

Rapid and mass propagation of plant species and their long-term germplasm storage can be achieved in a small space within short time period, with no damage to the existing population using PTC techniques. Plant material can be produced throughout the year without any seasonal limitation. Large numbers of uniform and disease-free, virus free plants can be produced from very small portions of the mother plant due to the aseptic nature of tissue culture technique. The sterile nature of \textit{in vitro} cultures facilitates the exchange of germplasm or plant materials even at international level (Sharma & Sharma, 2013). Genetic resources of recalcitrant seeds which are difficult to germinate, vegetatively propagated plants, rare, threatened plant species, elite crop varieties and some genetically modified plant materials can be efficiently multiply and store in long term basis by using \textit{in vitro} techniques (Lidder & Sonnino, 2012).

5.3 Micropropagation and Cloning

\textit{In vitro} clonal propagation method is commonly known as micropropagation which helps to produce mass production of plant propagules from any plant part or cell. Micropropagated propagules are used to raise and multiply the stock plant material in micro environment. Micropropagation and cloning of plant tissue based on different explants is commonly used to conserve different endangered plants. In vitro propagation of plants possesses huge potential in production of high quality based medicines at the same time conservation of medicinal plants. The \textit{in vitro} plant regeneration/ micropropagation program involved in several steps starting from initial shoot development either directly from nodal part of explants or...
through indirect way of callus mediated de-differentiation of shoot initials. Next, the shoot initials go for elongation and developmental stage where small plantlets with well developed shoots and root system will be generated for transplant in soil (Castellanos et al., 2008; Sadeq et al., 2014a). Micropropagation technique assists in the rapid, season independent, continuous propagation, maintenance and storage of rare and endangered plants by using any plant parts as explant source (Sarasan et al., 2006; Chandra et al., 2010). Several medicinally important plants are micropropagated using several explants are listed in Table 1.

5.4 Somatic Embryogenesis and Organogenesis

The development of somatic embryo by the differentiation of a single somatic cell or tissue to regenerate large number of plants at the same time is very commonly used technique of somatic embryogenesis in plant tissue culture. Somatic embryogenesis and organ development through organogenesis from various culture of explants are the most commonly used technique applied to regenerate several endangered plants for the purpose of conservation (Sadeq et al., 2014a). Culture of explant in suitable culture media helps to regenerate whole plants either by following direct or indirect way of somatic embryogenesis. In case of direct way of somatic embryogenesis, plants directly develop from explants without any intervening step of callus induction (mass of unorganized cells) and dedifferentiation of callus towards organized growth of plants as found in indirect way of somatic embryogenesis Induction of somatic embryos from the cultured explants in specialized culture media and their germination into whole plantlet following several steps where different plant growth regulators play important role (Sadeq et al., 2014b). It has great application in the rapid multiplication of important medicinal plants those has considered as endangered. Table 2 presents a list of endangered plants regenerated through somatic embryogenesis. Generally, the cultured cells or tissue or regenerated shoots can be maintained by serial subcultures at 4-8 week intervals for unlimited time period and plants can be regenerated at any time to transfer in the suitable field condition for conserving plant genetic resources. The culture of germplasm by unlimited cultures has some disadvantages such as risk of sudden loss of material due to human error or infection, or genetic instability; this problem can be rectified by restricting growth rate under culture condition.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Importance</th>
<th>Use</th>
<th>Plant part</th>
<th>Multiplication</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera</td>
<td>Anti-inflammatory</td>
<td>Cosmetics, health benefit</td>
<td>Leaf</td>
<td>Micropropagation</td>
<td>Hashem &amp; Kaviani, 2010</td>
</tr>
<tr>
<td>Asparagus adscendens</td>
<td>Diarrhoea, asthma and fatigue.</td>
<td>Therapeutic</td>
<td>All parts</td>
<td>Micropropagation</td>
<td>Mehta &amp; Subramanian, 2005</td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>Terpinoids</td>
<td>Antioxidant, antidepressant medicines</td>
<td>Leaf</td>
<td>Micropropagation of whole plant</td>
<td>Thangapandian et al., 2012</td>
</tr>
<tr>
<td>Cunila galioides</td>
<td>Volatile oils</td>
<td>Therapeutic</td>
<td>Leaf and flower</td>
<td>Micropropagation of plant</td>
<td>Fracaro &amp; Echeverrigaray, 2001</td>
</tr>
<tr>
<td>Liriope platyphylla</td>
<td>Steroids</td>
<td>Herbal</td>
<td>Shoot meristem</td>
<td>Plant regeneration and micropropagation</td>
<td>Park et al., 2011</td>
</tr>
<tr>
<td>Melissa officinalis</td>
<td>Essential oils, terpinoids, polyphenol compounds</td>
<td>Therapeutic</td>
<td>Whole plant part</td>
<td>Micropropagation of plant</td>
<td>Ghiorghita et al., 2005</td>
</tr>
<tr>
<td>Olea europaea</td>
<td>Phenolic compounds</td>
<td>Antioxidants, Health benefit</td>
<td>Fruit</td>
<td>In vitro cultivation and plant regeneration</td>
<td>Rkhis et al., 2006</td>
</tr>
<tr>
<td>Pelargonium radula</td>
<td>Terpenoids</td>
<td>Biopesticide antimicrobial</td>
<td>Leaf</td>
<td>Micropropagation</td>
<td>Zuraida et al., 2013</td>
</tr>
<tr>
<td>Phylanthus amarus</td>
<td>Alkaloids</td>
<td>Herbal and traditional medicines</td>
<td>Shoot part</td>
<td>Shoot multiplication</td>
<td>Xavier et al., 2012</td>
</tr>
<tr>
<td>Psoralea corylifolia</td>
<td>Anti-inflammatory Skin diseases</td>
<td>Therapeutic such as Leucoderma, leprosy, psoriasis etc.</td>
<td>From Seeds to all parts</td>
<td>Micropropagation</td>
<td>Baskaran &amp; Jayabal, 2008</td>
</tr>
<tr>
<td>Pteris viitata</td>
<td>Phosphate transporter</td>
<td>Arsenic detoxification</td>
<td>Whole plant</td>
<td>Micropropagation</td>
<td>Shukla &amp; Khare, 2012</td>
</tr>
</tbody>
</table>

Table 1 List of several micropropagated medicinally important plants.
### Table 2 List of endangered and threatened plants regenerated via somatic embryogenesis and organogenesis by the process of plant tissue culture.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Plant type</th>
<th>Importance</th>
<th>Explant used</th>
<th>Multiplication</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisia vulgaris</td>
<td>Restricted</td>
<td>Medicinal</td>
<td>Leaf</td>
<td>Organogenesis</td>
<td>Borzabad et al., 2010</td>
</tr>
<tr>
<td>Baliospermum montanum</td>
<td>Threatened</td>
<td>Medicinal</td>
<td>Nodal bud</td>
<td>Shoot differentiation</td>
<td>Sasikumar et al., 2009</td>
</tr>
<tr>
<td>Calligonum comosum</td>
<td>Endangered</td>
<td>Medicinal</td>
<td>Node</td>
<td>Shoot organogenesis and somatic organogenesis</td>
<td>Sadeq et al., 2014b</td>
</tr>
<tr>
<td>Eleutherococcus senticosus</td>
<td>Endangered</td>
<td>Medicinal</td>
<td>hypocotyl explants</td>
<td>Somatic embryogenesis, plant regeneration</td>
<td>Choi et al., 1999</td>
</tr>
<tr>
<td>Hedychium coronarium</td>
<td>Endangered</td>
<td>Medicinal</td>
<td>Rhizome</td>
<td>Somatic embryogenesis</td>
<td>Verma &amp; Bansal, 2012</td>
</tr>
<tr>
<td>Heliotropium kotschyi</td>
<td>Endangered</td>
<td>Medicinal</td>
<td>Node</td>
<td>Shoot organogenesis</td>
<td>Sadeq et al., 2014a</td>
</tr>
<tr>
<td>Lilium ledebourii</td>
<td>Endangered</td>
<td>Medicinal</td>
<td>Bulb scale</td>
<td>Somatic embryogenesis and plant regeneration</td>
<td>Bakhshaie et al., 2010</td>
</tr>
<tr>
<td>Psoralea corylifolia</td>
<td>Endangered</td>
<td>Medicinal</td>
<td>hypocotyl segments</td>
<td>Somatic embryogenesis</td>
<td>Sahrawat &amp; Chand, 2001</td>
</tr>
<tr>
<td>Rauvolfia serpentina</td>
<td>Endangered</td>
<td>Medicinal</td>
<td>Leaf</td>
<td>Somatic embryogenesis and plant regeneration</td>
<td>Singh et al., 2009</td>
</tr>
<tr>
<td>Turbinicarpus pseudomacrochele</td>
<td>Endangered</td>
<td>Medicinal</td>
<td>Medullar tissue discs</td>
<td>Somatic embryogenesis and plant regeneration</td>
<td>Munoz &amp; Garay, 1996</td>
</tr>
<tr>
<td>Woodfordia fruticosa</td>
<td>Rare</td>
<td>Medicinal</td>
<td>Shoot cuttings</td>
<td>Organogenesis</td>
<td>Krishnan &amp; Seeni, 1994</td>
</tr>
</tbody>
</table>

#### 5.5 Growth Restriction

The practical of a plant tissue culture method in germplasm conservation is to reduce the frequency of subculture during the process of culture. Development and use of several slow growth culture situations in modified culture media help to reduce the subculture frequency (Bunn et al., 2007). The addition and alteration of growth regulators, modification of salt concentration of culture media, high or low concentration of sugars in the growth media, addition of osmotically active compounds and sometime modulation of some external factor such as low temperature are used in slow growth strategy (Reed & Chang, 1997). Tropical species are mostly cold sensitive and have to store at high temperature. In vitro cultivation of Musa sps. can be stored up to 15 months at 15°C (Banerjee & de Langhe, 1985). While other tropical species such as cassava are more cold sensitive, so their shoots can be stored at temperatures higher than 20°C (Roca et al., 1984). Various parameters influence the efficiency of in vitro slow growth including type of explants, their physiological state of culture entering, type of culture vessel, volume and type of closures. In vitro slow growth storage technique is routinely used for medium-term conservation of numerous species both from tropical and temperate origin and endangered species. However, in vitro conservation process for medium and long term storage still need customization of genetic stability with time and one single protocol for conserving genetic diversity of the cultured materials are not applicable. Sometimes genetic erosion during the program of storage condition may hamper the total process, so special guidelines for in vitro germplasm collection and storage of genetic material in gene bank and botanical garden establishment and management is very important (Pence et al., 2002).

#### 5.6 Cryopreservation

Cryopreservation is one of the biotechnological method of ex situ plant conservation and applicable for long term storage of plant genetic material. Cryopreservation is extremely helpful method to conserve rare, endangered, threatened plant species (Dussert et al., 1997; Zhao et al., 2008; Paunescu, 2009). Moreover, for long-term storage of cultured plant materials require the use of ultra-cold storage methods. This section highlights the requirements for laboratory facilities and the basic techniques. Moreover, most of the experimental systems used in cryopreservation (cell suspensions, calli, shoot tips, embryos etc.) contain high amount of cellular water and are thus very sensitive to freezing injury. So, artificial dehydration procedure is important step although classical techniques...
involve freeze induced dehydration, while new techniques are based on vitrification (Zhao et al., 2008). The principle is bringing the plant cell and tissue cultures to a non-dividing or zero metabolism stage by subjecting them to supraoptimal temperature in the presence or absence of cryoprotectants. Classical freezing includes the successive steps such as pregrowth of samples, cryoprotection, slow cooling (0.5-2°C / min) to a determined prefreezing temperature (usually around -40°C), rapid immersion of samples in liquid nitrogen (LN), storage, rapid thawing and recovery. It needs technical skill as it has several steps such as freezing, storage, thawing, reculture of living plant cells during cryopreservation against cryogenic injuries. Maintenance of cryogenic cultures in LN at -196°C or in the vapor phase of LN at -135°C is in such a way that the viability of stored tissues is retained following re-warming. Various cryopreservation methods are being used for various plant species such as vitrification, encapsulation–dehydration and encapsulation vitrification (Stacey & Day, 2007). The genetic stability can be maintained during cryopreservation that has been proved by molecular marker study (Liu et al., 2008). The cryopreserved tissue has considered as safer, clean, disease free genetic stock for international exchange (Feng et al., 2011).

5.7 In Vitro Gene Bank

The maximum possible genetic diversity of the particular genetic stock can be maintained using in vitro gene bank technique. Several International Organizations are engaged potentially to conserve disease free, clean and elite class of genetic stock and using this process mainly following slow growth, in vitro techniques, cryopreservation of several stocks together with routine analysis of genetic diversity. The International Plant Genetic Resources Institute (IPGRI) as well as the Consultative Group on International Agricultural Research (CGIAR) Centers like the International Center for Agricultural Research in the Dry Areas (ICARDA) is heavily involved in the conservation of rare and endangered plant species by maintaining in vitro gene bank (Reed et al., 2004; ICARDA, 2014).

Several DNA marker based techniques are generally useful methods in monitoring variability in rare and endangered plant species. Another important fact is the simple and uniformly available facility in the germplasm conservation program is very important factor in monitoring gene bank at the organizational level. Regular visual examination of tissue culture derived plants growing in field or green house condition must be conducted to identify any morphological changes or to identify any somaclonal variations among them. Selection and preservation of important desirable somaclonal variations can be an important source of genetic variability among the growing population.

In vitro derived genetic variation in somaclones are of important source of genetic variability if it persists generation after generation as genetic variant. A large number of biotechnological approaches can be of useful technique in determining the genetic variability among the germplasms. The variability can be determined either using biomolecular markers or by using DNA based markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence characterized amplified regions (SCAR), simple sequence repeat (SSR), inter simple sequence repeat (ISSR), those are either PCR based or non-PCR based techniques (Glaszmann et al., 1987; Khan et al., 2012). Determination of storage conditions, provision of inventory, evaluation of viability and verification of genetic stability are very important components of gene bank structure. DNA banking can be considered as a means of complimentary method for the conservation of plant species together with conventional ex situ approaches in preserving biodiversity.

6 Conclusions

Biodiversity is the very basis of human survival and economic development. Ever increasing loss of biodiversity has posed a serious threat to the survival of mankind. Worldwide one third of the plant species are threatened due to several reasons. As the conservation of biodiversity is a global concern, several strategies has adopted in understanding and conserving plant diversity throughout the world. Both ex situ and in situ methods of biodiversity conservation are equally important. The review presents the use of biotechniques in improving ex situ conservation process to maintain biodiversity. It is now well recognized that an appropriate conservation strategy for a particular genotype requires combining approach of ex situ and in situ techniques according to the need of the program. As it is known fact that genetic variability is the main perquisite of the survival of any plant species in their natural habitat, so study of genetic diversity in conserved germplasm is important and application of different biotechnological process playing a promising role. In vitro plant propagation is a helpful technique in the conservation of genetic diversity of all types of plants (including rare, threatened and endangered plants) in a rapid and reliable way by maintaining the same clone or stock of plant material. It is just an efficient alternative method of plant propagation technique. It goes well to the natural process of plant conservation to restore the declined ecosystem with tremendous applicability.

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