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REVIEW

Biotechnological approaches for conservation and improvement of rare and endangered plants of Saudi Arabia

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Abstract Genetic variation is believed to be a prerequisite for the short-and long-term survival of the plant species in their natural habitat. It depends on many environmental factors which determine the number of alleles on various loci in the genome. Therefore, it is important to understand the genetic composition and structure of the rare and endangered plant species from their natural habitat to develop successful management strategies for their conservation. However, rare and endangered plant species have low genetic diversity due to which their survival rate is decreasing in the wilds. The evaluation of genetic diversity of such species is very important for their conservation and gene manipulation. However, plant species can be conserved by *in situ* and *in vitro* methods and each has advantages and disadvantages. DNA banking can be considered as a means of complimentary method for the conservation of plant species by preserving their genomic DNA at low temperatures. Such approach of preservation of biological information provides opportunity for researchers to search novel genes and its products. Therefore, in this review we are describing some potential biotechnological approaches for the conservation and further manipulation of these rare and endangered plant species to enhance their yield and quality traits.

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Contents

1. Introduction

The Arabian Peninsula is one of the richest biodiversity area which comprises medicinal herbs, shrubs and trees. The medicinal plants of the Kingdom have potential diversity (Rahman et al., 2004), their usage to cure numerous human ailments is ancient and still available among the tribal, local people and traditional healers. The four fifths of the Arabian Peninsula are found in 225,000 km^{-2} area of Saudi Arabia which is a rich area of medicinal herbs, and the use of these in folk medicine has existed since time immemorial.

The flora of Saudi Arabia is suffering from various threats such as inconsistent weather, altered soil pH and composition, heavy metals stress, drought, salinity, extreme temperatures, altitudes and increased habitat fragmentation. Due to these reasons, their conservation and further improvement through biotechnological approaches are unavoidable. Their conservation became part of the governmental policy as early as in 1970s, and the Kingdom of Saudi Arabia joined the Convention on Biological diversity (CBD) in 2001. The CBD highlights three objectives: conservation of biological diversity, sustainable use of its components, and equitable sharing of benefits arising from the use of genetic resources. There are some plant genetic resources (PGR) in the Kingdom of Saudi Arabia in which the terrestrial ecosystems have small numbers of species with little inbuilt redundancy. The government of Saudi Arabia has introduced a number of policies for promoting natural resource management aimed at protecting the environment and its biodiversity and using the natural resources in a framework that allows the benefits to be shared among all stakeholders. However, the flora of Saudi Arabia is the admixture of the elements of Asia, Africa and Mediterranean region. The Southwestern and most of the Western coastal plains of this country fall under the Somalia-Masai phytogeographical region (Chaudhury and Al-Jowaid, 1999) and some places such as Asir, Hijaz and the Western mountainous area have more diversity which may be due to greater rainfall and altitude (Collenette, 1998). The natural habitat of Saudi Arabia has endemic (242) plants, rare and endangered (600) out of 2250 which need conservation and sustainable development (Collenette, 1998; NCWCD, 1998; Al-Farhan, 2000). Diversity in vegetation is generated due to the impact of various physiographic factors coupled with diverse climatic variations. The few species survive successfully in *ex situ* conservation; *in situ* conservation is difficult for most of the plant species due to habitat fragmentation, invasion of alien species as well as changes in climate. However, environmental factors are responsible for genetic diversity in a natural habitat therefore; *in situ* conservation is the best option for their conservation. There are many advantages of such conservation including genetic exchange through random mating and recombination, accumulation of beneficial mutation and transfer among the populations, competition among populations which helps for their better adaptation and greater genetic diversity (Engles, 2001; Vijayan et al., 2011). Various strategies such as botanical gardens, GenBank and seed banks should be implemented for the conservation of critically endangered species. The rare and endangered plants which may have potential genes should be conserved for future research in the Kingdom. As a prelude to conservation of various rare and endangered plant species from their natural habitat, identification of their

genetic diversity and phylogenetic relationship using the molecular tools is indispensible.

2. Factors affecting genetic diversity

Study on genetic diversity is very important for rare and endangered plant species from their natural habitat. It will be helpful in formulating plans for management for preserving their genetic diversity and ensuring their long term survival. The natural habitat of a plant species determines its genetic diversity which is important for long-term survival and evolution under abiotic and biotic factors. Since, genetic diversity is influenced by numerous geographical factors due to which it constitutes subspecies, races or ecotypes in the nature (Sreekumar and Renuka, 2006). In addition, it is also affected by number of individuals and population size of a plant species. The species evolution in the natural habitat is affected by distribution of plant species. Genetic variation is necessary for short- and long-term survival of plant species in the wild conditions (Schonewald-Cox et al., 1983; Lande, 1988). The conservation of wild species is very important for their preservation of genetic diversity because their extinction rate is increasing directly and indirectly in response to anthropogenic activities and also by environmental factors.

Analysis of genetic structure of rare and endangered plant species is necessary which will help in their preservation either by *ex situ* or *in situ*. *In situ* conservation of plant species needs more attention to restore the suitable habitat based on the biological characteristics and ecological traits according to the natural habitat and to restore the efficient population size to minimize the loss of the genetic variation. To maintain the genetic diversity of a population, it is necessary to increase the population size by introduction of new populations in the place of those species which have been lost from the community. However, the natural habitat of a plant species is disturbed by numerous environmental factors which may lead to declination of the genetic diversity. Thus, loss in genetic diversity, inbreeding and extinction risk is resulted from the reduction in population size of a species (Frankham et al., 2002). The demographic history, geographic distribution range, mating systems, pollen and seed dispersal and successional stages are some of the factors that affect the genetic variation patterns among the plant populations (Hamrick and Godt, 1989; Nybom and Bartish, 2000; Nybom, 2004). Identification of genetic diversity within and among widespread, restricted and endangered congeneric species is necessary, prior to their conservation (Gitzendanner and Soltis, 2000) *in vivo* as well as *in vitro*. The sufficient genetic variability within and among populations of endangered species is brought through gene flow which is necessary for their long-term survival and evolution, and further, new selection pressure is resulted by environmental changes (Barrett and Kohn, 1991). The reduction in genetic diversity is resulted from the loss of alleles which may be due to the reduction in population sizes as genetic drift can induce a reduction in genetic variation and thus affects the survival of natural population (Reed and Frankham, 2003). The gene flow may occur among populations due to geographical proximity and ecological similarities, however, low migration levels could cause a reduction in genetic variation and bring about some natural selection pressure. Single marker is not sufficient for the evaluation of genetic diversity however; other markers should be employed for accuracy of the results. Assessment of genetic diversity would be more accurate if morphological, biochemical, phytochemical and DNA based markers are employed in the study.

3. Biotechnological approaches

A number of biotechnological approaches are being used for the conservation and improvement of plant species for desired traits and each of them has some advantages and disadvantages. Some important techniques which have great potential in the determination of genetic diversity have been used in many plant species including rare and endangered species.

3.1. Molecular marker technology

3.1.1. Biochemical marker

Different variants of the same enzymes having identical or similar functions are known as isozymes (or isoenzymes). They are powerful tools for the study of genetic variability within and between populations of plants and animals. Isozymes have been used in taxonomy, genetic, evolutionary and ecological studies, identification of cultivars and lines (Peirce and Brewbaker, 1973; Mondini et al., 2009). They are especially useful when several taxa, accessions and individuals are to be compared, as the assumption of homology is more accurate than with some DNA markers. Isozyme electrophoresis has been successfully used to identify clones and to examine the clonal structure of plant populations (Johanson et al., 1996; Lehmann, 1997).

3.1.2. Phytochemical markers

The discovery of novel compounds (phytochemicals) from wild plant species is an achievement toward the enhancement of the eradication of the human diseases. With the advancement of modern techniques such as mass spectrometry (MS) and nuclear magnetic resonance spectrometry (NMR) combined with separation techniques facilitated the identification and structural elucidation of molecules. These phytochemical analyses are valuable tools for taxonomic differentiation within species or for evaluating the effect of environmental factors (Hawkes, 1992). Metabolomics has been used to distinguish individual species within a genus (Heinrich, 2008; Tianniam et al., 2010), and also to discriminate the regional origins of particular species (Kang et al., 2008). Variation in biosynthesis of these metabolites could be a result from both genetic and environmental factors, which play important roles in the development of phenotypic variations in plants.

3.1.3. DNA based markers

DNA based markers either PCR based or non-PCR based provide genetic structure of the plant species and have been used in various fields such as embryology, genetic engineering, physiology, taxonomy etc. These markers are helpful in the identification of plant species and their cultivars to clarify errors occurred with the herbs in the local markets (Kiran et al., 2010). However, molecular linkage maps are being used successfully in many crop species for their improvement by locating the potential genes which control important traits in the plants. Some DNA markers assessed genetic diversity based on polymorphism. The polymorphism may be defined as simultaneous occurrence of multiple phenotypic forms of a trait attributable

to the alleles of a single gene or the homolog of a single chromosome within or between populations. Various loci are amplified with the use of various markers and help in the evaluation of genetic diversity about the concerned plant species. The potential markers which are being used in the evaluation of genetic diversity for a plant genome are given below.

3.1.3.1. Random amplified polymorphic DNA (RAPD). RAPDs are very quick and easy to develop due to the arbitrary sequence of the primers. It resolves most of the technical obstacle owing to its cost effective and easy to perform (Welsh and McClelland, 1990; Williams et al., 1990). Therefore, RAPDs have been extensively used in assessing genetic relationship among various accessions of different plant species (Khan et al., 2009a,b, 2010, 2011a,b).

3.1.3.2. Sequence characterized amplified region (SCAR). The RAPD marker(s) can be converted into sequence characterized amplified regions (SCAR) (Paran and Michelmore, 1993), which is monolocus and more specific. These markers are reproducible and less sensitive to reaction conditions for amplification. These markers are based on sequencing of the unique band. The unique band is selected for the designing of longer primers that specifically binds to same loci of exact molecular weight as amplified in the PCR (Kiran et al., 2010; Al-Qurainy et al., 2011b).

3.1.3.3. Simple sequence repeat (SSR). These are tandemly repeated DNA sequences that occur throughout the eukaryotic genome. The length variation in this marker is due to the DNA polymerase slippage during the replication of the SSR region (Levinson and Gutman, 1987). The frequency of this marker in the plant genome is estimated as one in every 6–7 kb (Cardle et al., 2000) thus, these markers show a high level of allelic diversity. The allelic polymorphism and co-dominant nature revealed by SSR markers have provided detailed information on genetic structure and gene flow in natural plant populations. The genomic library or SSR enriched library is important in searching the SSRs in eukaryotic genomes for which public DNA sequence data is lacking. SSR marker has been used for the assessment of genetic diversity in a numerous crops such as *Psathyrostachys huashanica, Zea maize, Apium graveolens*, *Prunus domestica* L., cherry plum (*P. cerasifera* Ehrh.) and sloe (*P. spinosa* L.) (Liu et al., 2010, 2010a; Wang et al., 2011; Horvath et al., 2011). Despite the advantages of these markers, their development is time consuming for a plant species for which little DNA sequence information is available in the public database.

3.1.3.4. Inter simple sequence repeat (ISSR). ISSR marker is used for the evaluation of genetic diversity in the plant species where the sequence information is limited (Zietkiewicz et al., 1994; Al-Qurainy, 2010). The nucleotide repeats (inter simple sequence repeats) are distributed throughout the genome and has potential discriminatory power which has been seen in the clonal plant species for the assessment of genetic diversity (Camacho and Liston, 2001; Wang et al., 2004). The different plant species and their populations such as *Monimopetalum chinens*, *Gynostemma pentaphyllum* and *Heptacodium miconioides* which are endangered species of China were assessed by ISSR marker (Xie et al., 2005; Wang et al., 2008; Jin and Li, 2007). The critically endangered tree *Dimorphandra wilsoni* and widely distributed species *D. mollis* have been used for the identification of genetic diversity and structure for their conservation (Viana e Souza and Lovato, 2010; Vicente et al., 2011). ISSRs markers are quick, easy to handle and more reproducible than RAPDs markers (Wu et al., 2010).

3.1.3.5. Restriction fragment length polymorphism (RFLP). The principle of RFLP is based on the variation in the restriction site of the enzyme which produces different restriction fragments on digestion. Being a co-dominant marker, it can detect coupling phase of DNA molecules as DNA fragments from all homologous chromosomes are detected. Due to highly specific, however, it is quite tedious and expensive since it requires large amount of purified DNA and an expertise of handling the radioactive substances. Genetic and chemical diversity of *Eleutherococcus senticosus* has been assessed by PCR-RFLP based on chloroplast *trnK* intron sequence (Zhu et al., 2011).

3.1.3.6. Amplified fragment length polymorphism (AFLP). This technique is particularly useful for evaluating genetic diversity in those plant species where prior genetic information is not available. It is the modified form of the RFLP and uses PCR for the amplification of the restriction fragments generated on digestion of genomic DNA with restriction endonucleases. More variation in the restriction site, more polymorphism would be generated. However, genetic diversity is very important for the survival of the plant species in their natural habitat which is evaluated by AFLP marker in many plant species such as *Hibiscus rosa-sinensis* (Braglia et al., 2010), *Ocimum* spp. (Moghaddam et al., 2011), *Punica granatum* L. (Moslemi et al., 2010), Thai bananas (Wongniam et al., 2010), *Aegiceras corniculatum* (Deng et al., 2009).

3.1.3.7. Selective amplification of microsatellite polymorphic loci (SAMPL). It is the modified form of the AFLP technique and has been used for the analysis of highly variable microsatellite regions of eukaryotic genomes. SAMPL has been reported to be more powerful than AFLPs in discriminating between closely related individuals in several plant complexes (Singh et al., 2002), assessing genetic diversity in *Terminalia arjuna* (Sarwat et al., 2011), *Garnacha Grapevine* (Meneghetti et al., 2011) and *Origanum vulgare* (Azizi et al., 2009). In another study, SAMPL was found to be more efficient than AFLP in differentiating closely related accessions of *Azadiracta indica* (Singh et al., 2002).

3.1.3.8. Single nucleotide polymorphism (SNP). Single nucleotide polymorphisms (SNPs) are the most abundant sequence variations in the plant genome which are widely used as molecular genetic markers in cultivar identification, genetic diversity studies and linkage mapping, characterization and phylogenetic studies, marker-assisted breeding, positional cloning and genetic comparative mapping (Dong et al., 2010; Troggio et al., 2007). These markers occur in unlimited numbers, and such differences in nucleotide sequences between individuals in single copy DNA are potentially useful markers. Genetic diversity was assessed in wheat accessions using SNPs marker based on stress responsive gene TaSnRK2.7-B (Zhang et al., 2011).

3.1.3.9. Expressed sequence tagged (EST). ESTs are used for full genome sequencing and mapping programs underway a number of organisms for identifying active genes thus helping

in identification of diagnostic markers. Moreover, an EST that appears to be unique assists to isolate new genes of beneficial agronomic traits. Once generated, they are useful in cloning specific genes of interest and synteny mapping of functional genes in various related organisms. EST-SSR marker has been used for the assessment of genetic diversity in Chinese orchid cultivars (Huang et al., 2010), Chinese wild almond and *Amygdalus nana* (Tahan et al., 2009), *Soja* (Liu et al., 2010b), Listada de Gandía (Munoz-Falcon et al., 2011) and *Dactylis glomerata* L. (Xie et al., 2010).

3.1.3.10. Single strand conformation polymorphism (SSCP). This technique is used for the identification of point mutation and also for polymorphism. The heterozygosity is easily detected by this technique as the mobility of the single stranded DNA changes with change in its GC content due to its conformational changes. In plants, however, it is not well developed although its application can be exploited in discriminating progenies, once suitable primers are designed for agronomically important traits.

3.1.3.11. Cleaved amplified polymorphic sequence (CAPS). CAPS markers are based on STSs derived from ESTs marker and also known as PCR-RFLP. Unlike RAPD markers, the CAPS marker is a PCR based co-dominant marker which is reproducible and easier to manipulate in marker assisted selection (MAS) (Caranta et al., 1999). Generally, CAPS marker is a PCR-based marker in which a restriction site is present in only one of the two amplified sequences and this difference can be due to a SNP marker (Nemri et al., 2007). Thus, CAPS markers are required for genotyping, positional or map based cloning and molecular identification studies (Spaniolas et al., 2006). The CAPS marker has been successfully used for the cultivar identification of strawberry, which is a vegetatively propagated polyploid plant like the sweet potato (Kunihisa et al., 2003, 2005). Similarly, citrus and sweet potato cultivars have been identified by the CAPS marker (Amar et al., 2011; Tanaka et al., 2010).

3.2. Additional markers

3.2.1. Internal transcribed spacer (ITS) sequences

ITS markers are used for the identification of plant species based on the sequence variation. These sequences are varied from species to species in the plant Kingdom and have different rate of evolution, therefore they become favored markers in evolutionary studies in different taxonomic levels (Gulbitti-Onarici et al., 2009). The nrDNA region has frequent insertions/deletions which can be phylogenetically informative (Baldwin et al., 1995). Internal transcribed spacer sequences (ITS) of nrDNA have been widely used for resolving phylogenetic relationships in many plant species (Gulbitti-Onarici et al., 2009; Choo et al., 2009; Al-Qurainy et al., 2011), molecular authentication of herbal materials (Zhang et al., 2007), genetic diversity assessment (Mondini et al., 2009), intra-specific variation study (Haque et al., 2009), and DNA barcoding (Zuo et al., 2010).

3.2.2. Chloroplast spacer sequences

The chloroplast (cp) genome of most angiosperm is circular and has size from 120 to 160 kb in length. A total of 132 cp genome has been sequenced up to now and out of them 22 are from monocot, and date palm. Khalas cultivar is the first member of Arecaceae family which has been sequenced (Jansen et al., 2005; Yang et al., 2010).The sequencing based DNA barcode generated from nuclear and/or chloroplast DNA has many advantages over polymorphic markers. The barcoding approach also has great potential for identifying plants and their adulterants easily (Jaakola et al., 2010; Yao et al., 2009; Al-Qurainy et al., 2011a). The genetic differentiation in the populations of *Notopterigium forbesii* was examined using chloroplast *trnH-psbA* intergenic spacer sequences (Zhou et al., 2010) and found low level of differentiation due to abundance of ancestral haplotype sharing and the high number of private haplotype.

3.2.3. DNA microarray

To date, the main applications of microarrays are to monitoring simultaneous gene expression, DNA variation analyses for the identification and genotyping of mutations. DNA microarray uses gene chips or microarrays to examine a multitude of loci at one time. To decrease complexity, cDNA or RNA is hybridized. The DNA on slide is probed with a fluorescently labeled probe and then scanned for polymorphisms. This technique is robust and high-throughput while eliminating the need for either agarose or polyacrylamide gels.

3.3. Plant DNA bank

Plant DNA bank has emerged as new resources with great potential for characterization and utilization of biodiversity worldwide. It can be used for plant breeding, biotechnology and biodiversity evaluation (Hodkinson et al., 2007). In the Kingdom of Saudi Arabia, plant biodiversity of major and minor crops is under serious threat from various factors such as high- and low-temperature, altitudes, salinity, habitat fragmentation, exposure to pollution, drought etc. The DNA collections have become important resources in worldwide and effort to address the biodiversity crisis, manage the world's genetic resources and maximize their potential. However, analysis of DNA sequence is a powerful method by which the identification and delimitation of species, higher taxa can be done via DNA taxonomy and DNA barcoding. There is an urgent need in Saudi Arabia for more formal co-operation and communication between existing research institutes in the world, so that experiences and expertise can be shared. Such DNA bank in the Kingdom of Saudi Arabia would be of many advantages for the plant molecular biology researchers working on phylogeny, gene identification and its manipulation for agronomic traits. The database is indispensable for the DNA bank which is pivotal for the efficient utilization of the germplasm. Such DNA database is available from fully developed DNA bank worldwide and now Kingdom of Saudi Arabia is aware to establish plant DNA bank for conservation of vast diversity of flora. The databases concerned to DNA bank have numerous information regarding plant family, genus, author of DNA extraction, and type of DNA product or marker generated from the DNA which will be available via the Worldwide-Web for easy access. The recipient may utilize the material for breeding, research and training but cannot claim ownership over the material, or exchange it with others, without a legally binding written agreement. The establishment of DNA bank in the Kingdom of Saudi Arabia will facilitate the screening of plant by making DNA from large numbers of plant species available widely. To access and benefit sharing nationally and internationally, DNA bank requires the development of appropriate policies. Members of the organization on DNA

banking network often meet to share technical knowledge among themselves on cryo storage of DNA and DNA-rich materials (Adams and Adams, 1992, 1998). Once DNA bank is being developed in the Kingdom of Saudi Arabia, we will able to construct genomic library, cloning of novel genes and molecular markers besides intact DNA. Due to inconsistent environmental conditions, therefore, there is an immediate requirement to establish a plant DNA bank in the Kingdom of Saudi Arabia to speed up the research in the field of plant science.

3.4. Unconventional biotechnological methods

3.4.1. Micropropagation

Plant micro-propagation is a biotechnological approach for the conservation of plant species and even for recalcitrant seeds of economically important crops. It may help in the production of virus free plants and is successful for those plant species which have difficulties in propagation using conventional methods. It is an *in situ* conservation of plant species having special phenotypic characters by introduction of regenerated plantlets directly into its natural habitat (Juliani et al., 2010). Nowadays, the trend in plant conservation is to combine *ex situ* and *in situ* species within an integrated conservation program including the collaboration among scientific or plant breeding organizations (Akeroyd, 2006). In a short period of time and limited space, it allows the production of numerous plant species. It is exploited for the production of pathogen-free cloned plants for agriculture and forestry usages. From conservation point of view, the regenerated plantlets should have minimum somatic variations through micro-propagation method by reducing the number of sub-culturing and axillary bud or shoot tip culture. During *in vitro* culture, since cells receive sequential stimuli for proper growth and finally plantlets formation in a large number. However, in a gene pool of the cells there may be insertion, deletion or substitution which leads to the formation of somatic variations. The selection of the explants is very important for the successful micro-propagation which decides the final fate of the developed plantlets via callus or without formation of calluses. The micropropagation of *Lavandula pedunculata* was done for essential oil production (Zuzarte et al., 2010) without affecting natural resources. Generally, somaclonal variations are produced during calluses formation or from suspension cell culture which reduces the economic value of the regenerated plants (Borse et al., 2011). Somaclonal variation may be genetic (heritable) or epigenetic (due to gene silencing or gene inactivation) which may be produced from point mutation, gene duplication, changes in number and the structure of the chromosome and movement of transposable element (Jain, 2001).

3.4.2. Protoplast culture

It is a physical phenomenon and during fusion two or more protoplasts come in contact and adhere with one another either spontaneously or in the presence of fusion inducing agents. Some useful genes such as disease resistance, nitrogen fixation, rapid growth and more product formation rate, protein quality, frost hardiness, drought resistance, herbicide resistance, heat and cold resistances are possible to transfer from one species to another. Thus, such biotechnological approach has been used to combine genes from different organisms to create strains with desired properties. Para-sexual hybrid protoplasts can be obtained from two genetically different protoplasts isolated from the somatic cells and are experimentally fused to each other.

3.4.3. Somatic embryogenesis

The development of somatic embryo from a single or multicells is known as somatic embryogenesis. Plant genetic transformation and gene cloning depend on the plant tissue culture practices for their regeneration on suitable media. However, somatic embryos have been used as the starting material for all transformation methods and can be obtained from reproductive explants including ovaries, stigmas, anthers, and whole flowers (Kikkert et al., 2005; Morgana et al., 2004; Gambino et al., 2007; Prado et al., 2010). Plant propagation through somatic embryogenesis helps to obtain a large number of plants irrespective of seasons and provides several advantages over traditional methods. The developed plantlets through somatic embryogenesis (SE) are to be elite clones whereas zygotic embryo, are unable to produce elite clones. The main advantage of SE is the production of numerous plantlets from a single cell which provides an option for their screening and evaluation. However, the regeneration of plantlets depends on the type of explants, combination and concentrations of growth regulators and medium used at different stages of culture. Haploid plantlets can be developed through this method which has potential application in the mutation research. Earlier, plant breeders have many difficulties in the production of homozygous line through selfing but biotechnological tools have an important contribution in the production of homozygous line from gametic embryogenesis (Germana et al., 2011). The direct embryogenesis is the preferable choice for embryos development; because it avoids the dedifferentiated callus phase and thus produced plantlets are genetically stable.

3.4.4. Organogenesis

The *de novo* organ synthesis such as buds, roots and shoots from cultured tissues is called organogenesis. A high cytokinin to auxin ratio induces *in vitro* shoot organogenesis, while high auxin to cytokinin induces production of roots. Recently, new insights into cytokinin and auxin interactions during plant organogenesis have been reported, demonstrating a link between the distribution of these hormones and *de novo* organogenesis Benkova et al., 2009; Pernisova et al., 2009). Plant mature cells have the capability to reverse their state of differentiation and produce new organs under cultured conditions. *In vitro* organogenesis, dedifferentiation and redifferentiation phases are commonly characterized. The balance of exogenous auxin and cytokinin in the medium is essential for *de novo* organogenesis (Skoog and Miller, 1957). Three phases are characterized for the organogenesis; first explants respond to phytohormone, second quiescent cells re-enter in the cell cycle and cell fate is determined to form specific organ primordia, which is the key step during *de novo* organogenesis and in the final stage, the morphogenesis of *in vitro* organs. Thus, organogenesis entails the regulation of cell division, cell expansion, cell and tissue type differentiations, and patterning of the organ as a whole.

3.4.5. Micrografting

The rejuvenation of the mature scion shoot tip onto the juvenile root stock is called micro-grafting and it was studied *in vivo* as well as *in vitro*. This method was developed (Jonard,

1986) for the production of disease-free scions, the rejuvenation and/or the invigoration of mature shoot materials, the enhanced potential for true to type cloning mature plants, and the study of graft unions (Onay et al., 2003). The kinnow mandarian was regenerated virus free using *in vitro* micrografting (Singh et al., 2008). There is one limitation in the production of micrografted plantlets as browning of cut surfaces due to oxidation of phenolic compounds. However, this problem may be removed by dipping the shoot scion in the MS medium.

3.4.6. Cryopreservation

It is important for long-term preservation of clonally propagated plant species which produce recalcitrant seeds and cannot be readily conserved by conventional methods through seed preservation (Benson, 2004). Now it is applied to a diverse range of plant species and their tissues (Reed, 2008). These species are unable to produce true seeds but can propagate through grafting or cutting. Various cryopreservation methods are being used for various plant species such as vitrification, encapsulation–dehydration and encapsulation–vitrification (Kami et al., 2009). Before cryopreservation of plant material, dehydration is necessary to protect the cells from lethal intracellular freezing and, dehydration can occur during rapid cooling in liquid nitrogen (Popova et al., 2010; Chen et al., 2011). The cooling rate is also an important factor for successful cryopreservation because ultra freezing rate helps to avoid intracellular freezing. Sometimes genetic variations are generated in the cryopreserved material due to the free radical generation and it is presumed due to dehydration process (Sanchez et al., 2008; Martin et al., 2011). There are some limitations of this method for the preservation of such plant species. For instance, during the preservation of the plant tissues, methylation starts in the cell and it occurs in the plant mainly on cytosine (CpG and CpXpG) and forming 5-methyl-cytosine. Undoubtedly, genetic/physiological factors determine outcomes of the cryopreservation for which a better understanding for their predisposing actions would benefit cryopreservation protocol development and germplasm responses following recovery (Johnston et al., 2009). DNA methylation does not change the gene sequence and its function but its level of expression is changed. The genetic stability is maintained during cryopreservation and has been proved by molecular markers study (Liu et al., 2008a). The cryopreserved tissue may also be considered to be safer for exchange of germplasm between country and regions. It also helps in eradication of virus and phytoplasm from plant tissues, and making them free from pathogen (Feng et al., 2011).

3.5. Genetic manipulation for improvement of yield and quality traits

3.5.1. Agrobacterium tumefaciens-mediated transformation

Biotechnological tools are important for selecting, analyzing, multiplying and improving the plants (Khan et al., 2009a,b). Over the years, several techniques have been adopted for enhancing bioactive molecules in medicinal plants (Khan et al., 2009a,b). Combinatorial biosynthesis is a tool to combine genes from different organisms to produce bioactive compounds in plants. The basic concept of this approach is combining the metabolic pathways in different organisms on genetic level (Horinouchi, 2009). *Agrobacterium rhizogenes* possesses infecting plasmids called root-inducing plasmid or Ri plasmid with T-DNA. The plasmid infects plant cell and induces the production of root-like hairy structures called ''hairy root disease''. The neoplastic (cancerous) roots produced upon infection have genetic and biochemical stability and a fast growth rate resulting in a large mass/medium ratio in a hormone free-media (Sivakumar, 2006; Srivastava and Srivastava, 2007). These genetically transformed root cultures can produce secondary metabolites in large amounts comparable with those in intact plants, and the transformed root lines can be a promising source for the constant and standardized production of secondary metabolites.

3.5.2. RNAi technology

The RNA interference (RNAi) technique can lower the enzyme activities, which can help to suppress the formation of unwanted natural compounds or channelize the metabolites into pathways leading to desired products. To date RNAi is a potential technique and is being used for the production of medicinal plants with potential traits for a particular marker compound which is needed for eradication of diseases. RNA interference (RNAi) technology has provided an alternative to block the activity of such enzymes that are not only encoded each by a multigene family but are also expressed across a number of tissues and developmental stages.

3.5.3. Molecular pharming for high production of bioactive compounds

Production of potential pharmaceutical compounds using plant transformation strategies and transient expression system such as agro-infilteration, virus infection, magnifection (Obembe et al., 2011) is known as molecular pharming or bio-pharming. Thus, it focuses mainly on the biosynthesis of proteins and secondary metabolites which are very useful for humans but they are costly in the market. Earlier, molecular pharming was used on transgenic animals for the production of high valued compounds which are being used at large scale for industrial purpose. Plants can act as factories for the biosynthesis of drugs or compounds which are very costly in the markets. The biosynthesis of compounds in plant based systems has many advantages over others. There are various bio-products such as vaccine, antigen, antibody, therapeutical and nutraceutical proteins, non-pharmaceutical plant derived proteins are being produced using molecular pharming (Obembe et al., 2011).

3.6. Conclusion and future prospects

Biotechnological tools are necessary for the conservation and improvement of the plant species mainly rare and endangered which have importance economically and medicinally. Genetic diversity is important for the survival of the plant species in their natural habitat. However, loss in genetic diversity of the plant may lead to decline its ability to cope with changing environment and demographic fluctuations both in short- and long-term. Once plant species are disappeared from their natural habitat, they cannot be regenerated again which is very difficult to reestablish their previous rich diversity. Therefore, these tools should be applied for the conservation and improvement of the various plant species in a number of ways to broaden the genetic diversity of the rare and endangered plants of Saudi Arabia. DNA banking is also a potential

method for the conservation of biological information by preserving the genomic DNA at low temperatures and has been established in few countries. The DNA isolation is easy and can be used extensively for the characterization and utilization of biodiversity. The implementation of such biotechnological tools on rare and endangered plant species of Saud Arabia may help in revival of their previous genes and their products which have been disappeared or inactivated in natural habitat.

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