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Complementary Strategy For Conservation Of Date Palm Germplasm

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ABSTRACT

Conservation of date palm genetic resources has become an important issue regarding the development of date production and its importance for food security in many countries. The conservation of date palm varieties in their original habitats considered one of the important aspects for maintenance of its genetic resources. Otherwise, several field genebanks involved collecting propagation materials and growing them in specific locations have been established for base and active collections. The main disadvantages of the two methods are pests, diseases, natural disasters and vandalism. The conventional method used to conserve genetic resources of date palm has been supplemented in recent years by rapid developments in plant biotechnology. *In vitro* techniques developed for storage of date palm plant materials fall under two categories: (1) slow growth procedures, where germplasm accessions are kept as sterile plant tissues or plantlets on nutrient gels; and (2) cryopreservation where plant material is stored in liquid nitrogen. Slow growth procedures provide short- and medium-term storage options, while cryopreservation enables long-term storage of the plant material. As progress in biotechnology advances, DNA storage is regarded as one of the emerging *ex-situ* techniques for germplasm conservation. A DNA bank is a particular type of plant genetic resource bank that preserves and distributes the DNA molecules and provides associated information. Increased application of molecular tools are further facilitate the use of such germplasm in date palm breeding efforts and add new value to the existing collections. All the conservation approaches and methods mentioned above have advantages and disadvantages and a complementary approach to safely conserve the genetic diversity of date palm is strongly recommended. This article presents the different *in-situ* and *ex-situ* methods as well as the modern technologies used to conserve date palm germplsm.

Key word: Date palm, conservation, *in situ*, *in vitro*, field genebank

Introduction

The date palm, *Phoenix dactylifera* L., is one of the most economically important fruit tree grown in the Middle East and North Africa. About 75% of the world production of dates is in Arab countries for 2008; half of these is in Gulf area (including Iraq and Yemen) and the other half is in North Africa countries (including Sudan) (FAOSTAT, 2009).The tremendous advantages of the tree are its resilience, its requirement for limited inputs, its long-term productivity and its multiple purpose attributes (Bircher, 1990). The fruit is highly nutritious and rich in source of sugar, minerals, and vitamins and it is considered the most important economical product of date palm tree. Furthermore, all the plant parts of the date palm tree have integrated in traditional or industrial applications (Barreveld, 1993). In addition, palm tree tolerates adverse environmental conditions and it is important in reducing desertification. Moreover, it provides protection to under-crops from the harshness of the climate (heat, wind and even cold weather), and reduces damage caused by sand storms and wind erosion. So, palm tree is an excellent candidate for cultivation in arid and semi arid regions of the world. There are >2,000 date varieties with differences in color, flavor, shape, size and ripening time (Al-Farsi and Lee, 2008). Date palm cultivars are grouped into three classes: soft, semidry or dry, based on upon the texture of the fruit under normal ripening conditions. World production of dates was about 7 million tones and the top 10 producing countries are Egypt, Saudi Arabia, Iran, United Arab Emirates Pakistan, Algeria, Sudan, Oman, Libya, and Tunisia (Kader and Hussein, 2009).

The date palm is considered a renewable natural resource because it can be replaced in a relatively short period of time or used through conservation efforts without depletion (University of Delaware, 2004). Countries which hold significant amounts of genetic diversity of date palm have a great responsibility to conserve and safe guard date palm germplasm to utilize for genetic improvement and development of crop cultivars for domestic and foreign markets. There are various techniques for conserving plant germplasm, each with their own advantages and disadvantages. Since date palm is a dioecious and heterozygous fruit tree, and for commercial purposes most often vegetatively propagated through offshoots, its germplasm cannot be stored or handled easily by conventional means. At the present, the most common method used to preserve the genetic resources of date palm is as whole plants on- farm (Bettencourt *et al*., 1992). While *in situ* conservation is essential to maintain the evolution of the species and allow new diversity to be created through natural selection processes, it presents many disadvantages for conservation. Field genebank provides ready access to conserved material

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for research as well as for use. There are, however, several problems with the field genebank. The collections are exposed to natural disasters and attacks by pests and pathogens. Moreover, labor cost and the requirements for technical personal are very high. In addition, distribution and exchange from field genebank is difficult because of the vegetative nature of the material and the greater risk of disease transfer.

As progress in biotechnology, several techniques to conserve vegetatively propagated species have recently been developed and some are undergoing rigorous testing. Tissue culture in combination with molecular biology techniques are of great interest for collecting, characterization, multiplication and storage of such germplasm. Miniaturization of explants allows reduction in space requirements and consequently labor cost for the maintenance of germplasm collections (Bekheet, 2011). In this respect, different *in vitro* techniques to preserve date palm cultures have been recognized (Mater, 1987; MyCock *et al*. 1997; Bekheet *et al*., 2001, 2007). Moreover, biochemical and molecular markers have successfully used for detection of genetic variation of date palm tissue cultures (Saker *et al*., 2000; Al-Khateeb *et al*., 2007; Othmani *et al*., 2009). Recently, DNA storage considered one of the emerging *ex situ* techniques for germplasm conservation. DNA from the nucleus, mitochondrion and chloroplasts are now routinely extracted and immobilized into nitro-cellulose sheets where the DNA can be probed with numerous cloned genes. Despite its economic importance, there are very few DNA sequences of date palm found in the Genbank databases (Zhang *et al*., 2011). Establishment of date palm DNA Bank Network will enhance taxonomic, systematic, genetic, conservation and evolutionary studies. Because each conservation approach has distinct advantages and disadvantages, a complementary conservation strategy is necessary to ensure optimum sustainable use of genetic diversity of date palm. Moreover, the collected and conserved date palm genetic resources should be readily available to researchers and other interested parties; with detailed characterization, evaluation, and utilization as well as proper documentation. This article discusses the traditional and advanced aspects can be used for conservation of date palm germplasm.

1- Need for conservation of date palm germplasm:

The geographic distribution of date palm covers a wide range of environmental conditions. For instance, it grows and flourishes from 392 m below to 1 500 m above sea level with an altitude range of 1 892 m (Zaid and de Wet, 2002). There are many factors threatening the genetic diversity and species of date palm including natural and man-made factors. Environmental changes include drought and floods, as well as seasonal fluctuations in temperature and rainfall. Such changes could result in genetic erosion due to crop failure and loss of varieties. Millions of date palm trees were lost within one decade in North Africa due to such natural disasters (Baaziz *et al*. 2000). Moreover, biotic factors such as pests and diseases can attack date palms resulting in negative impacts on the genetic variability within species (Bendiab *et al*. 1993). In this regard, the principal constraints limiting date palm culture in the Maghreb countries (Morocco, Algeria, Tunisia) are drought, salinity, deserts, development senescent date palm trees and bayoud disease. Bayoud destroyed the world's most renowned date palm varieties that are susceptible to the disease and particularly those which produce high quality and quantity fruit (Medjool, Deglet Nour, Bou Fegouss) (Zaid *et al*., 1999). Moreover, in the Gulf countries and Egypt, the Red Palm Weevil has recently become one of the major date palm pests causing severe damage in date palm orchards where whole plantations had to be cut down due to palm weevil infestation. Otherwise, land use changes which include construction and building of roads, factories, canals, dams and new residential areas are other factors. In this respect, in Egypt, date palm tree numbers decreased from 2.5 million to just over 1 million in the Aswan area due to the building of the High Dam (Hussein *et al*., 1993).In specific cases, wars have brought about tremendous degradation in date palm orchards. For instance, Iraq used to be a major producer of dates, but in recent years production and exports have been curtailed (El-Juhany, 2010). On the other hand, date palm orchards in North Africa are aging; almost one-third of productive date palm trees in Algeria are beyond the limits of their productive years, and almost half of the Tunisian productive date palms are more than 50 years old (Jaradat, 2011).

So, these facts suggest that large numbers of traditional cultivars in many countries may be diminishing, and it has been suggested that urgent conservation efforts are needed worldwide. The conservation of date palm genetic resources is important not only to preserve the good quality cultivars but also to ensure future access to valuable genes for plant improvement programs. The date palm is a usually vegetatively propagated plant from offshoots because seeds do not ensure true-to-type palms. Vegetatively propagated material of date palm, either as whole plants or offshoots, represents the highest risk category involving potential spread of different stages of all types of pests such as insects, mites, fungi and bacteria. Tissue culture offers a great potential for conservation of germplasm of such vegetatively-propagated crops. Regeneration and successful propagation of genetically-stable plantlets from cultures are prerequisites for *in vitro* conservation methods. Two types of *in vitro* genebanks for conservation have been reported: (a) slow growth, and (b) cryopreservation (Withers and Williams, 1985). Moreover, the progress in molecular techniques and genome analysis, led to the establishment of DNA libraries, which store total genomic information of germplasm. The advantage of this technique is that it is efficient and simple and takes up very little space. Strategies have to be developed on how to use the material

stored in the form of DNA. Therefore, it is vital to establish complementary genebank and more global efforts should be made to support and maintain the existing traditional oases and the date palm genetic diversity that they contain. Date palm genetic resources can include genotypes or populations of the plants, representing cultivars, genetic stocks, wild species, etc. All this would require essentially five steps: 1) exploration, collecting and assembly; 2) conservation and distribution; 3) characterization and evaluation; 4) documentation and 5) use.

2- In-situ conservation of date palm germplasm:

In-situ conservation is one of two possible strategies to conserve plant genetic resources. *In situ* conservation on-farm refers to the continuous cultivation and management of a diverse set of populations by farmers in the agroecosystems where a crop has evolved. There are significant advantages to *in situ* conservation. One is its conservation of both genetic material and the processes that give rise to diversity. The long-term sustainability of breeding efforts may depend on the continued availability of the genetic variation that can be maintained and developed in farmer's fields. The objectives that may shape an on-farm conservation programme are: 1) conservation the processes of evolution and adaptation of crops to their environments, 2) conservation diversity at different levels – ecosystem, species, within species, 3) maintain or increase farmers' control over and access to crop genetic resources, 4) integrating farmers into the national plant genetic resources conservation system and 5) improving the livelihoods of resource-poor farmers. The conservation of date palm varieties and cultivars in their natural surroundings and original habitats considered one of the important aspects for maintenance of its genetic resources. As date palm is adapted to areas with long, very hot summers with little rain and low humidity, its origins were found in oases and river valleys in the arid sub-tropical deserts of the Middle East (Krueger, 2011). Thousands of date palm cultivars exist in different growing countries. These cultivars have been developed by continuous selection performed by date palm growers all over the world mainly to improve crop yield and quality. In this connection, sate palm counts as high as 5000 cultivars with differences in color, flavor, shape, size and ripening time all around the world (Jaradat and Zaid 2004). Countries in North Africa and the Arabian Peninsula are both part of the center of origin and constitute a major part of the center of diversity of date palms'; they are the home to the world's highest production and consumption of dates. Due to economic and social factors, the diversity of date palm groves of oasis is declining. Otherwise, desertification and soil salinization are other factors threatening the natural habitat of date palms. Salt water intrusion in certain parts of the Arabian Gulf has caused the loss of entire date palm groves. So, the genetic diversity can be lost due to these factors if they result in the loss of local varieties having specific genetic constitutions.

Traditional oases have vital role in the maintenance of date palm genetic diversity. Support for the traditional oases must balance the need for preservation of their positive aspects with improvement of some of their less desirable characteristics. Improvement of agricultural practices, salinity and diseases control in addition of irrigation water are considered the most important issues of date palm cultivation in its nature growing regions. Moreover, farmers should be encouraged to replant date palms with locally produced offshoots or seedlings. On the other hand, documentation of on-farm growing varieties considered one of the important aspects for *in-situ* conservation of date palm. Documenting the amount and distribution of genetic diversity on-farm requires information on the genetic identity of farmer-named varieties, the genetic structure of populations, and the pattern of farmer-named variety occurrence. In this connection, documentation for on-farm conservation may be particularly effective when it involves farming communities (Jarvis *et al.,* 2000).

Date palm field genebank:

There are two approaches of plant conservation, *ex situ* and *in situ*. *Ex situ* conservation approach generally comprises the following methods: seed storage, field genebanks, *in vitro* storage, DNA storage and botanical gardens. Conservation of plant diversity using reserves/protected areas, on-farm and home gardens are considered as *in situ* conservation approach. *Ex situ* conservation has several important advantages for plant genetic resources conservationists. It is relatively easy to identify the genetic diversity conserved in a genebank or botanical garden, as the material is usually fully documented for the use of plant breeders and other scientists. Moreover, the genetic diversity maintained by these methods is directly controllable: as long as accessions are kept in suitable conditions and regenerated periodically, the likelihood of losing material is relatively low. Conservation in field genebank is necessary to bring genotypes from an environment in which they are adapted to one in which they may not be. For field collection, both random and non-random sampling may be used while collecting the samples. Non-random sample will select only those with clear morphological characters leaving those associated with disease resistance and other physiological characteristics (Hawkes, 1987). Collecting involves gathering samples from populations in the field or natural habitats for conservation and subsequent use and the information about the collection should be recorded. The suggested basic data are title of the expedition, an identification of the plant, the collector, date of collection, collection site, status of material, frequency,

provenance (field, farm store), and others which are varied from species to species. In this connection, molecular techniques proved useful in a number of ways to improve the conservation and management of plant genetic resources. Molecular markers have been applied to study genetic diversity from natural populations and formulate efficient sampling strategies to capture maximum variation for genetic resources conservation (Rao, 2004). In the field genebank, a significant acreage of land is required if it is to contain adequate samples of the genetic variability of the species. The field planting should be at a spacing that allows adequate room for tree growth. Otherwise, the crops in the field or nursery require proper husbandry, which includes adequate nutrition, pest and disease control and irrigation (Yap and Saad, 2001).The most problems and challenges in managing field genebank are diseases and environmental disasters. At all stages of the crop growth are susceptible to pests. Prevention is an important step in controlling infestations and some phytosanitary measures should be practiced. Environmental disasters can cause serious damage to the field genebanks. Flooding, drought lightning, strong winds and thunderstorm and haze are amongst the common environmental hazards that can destroy and damage the established plants in the genebanks (Aman, 2001). For date palm field genebank, the accessions should be established with multiple inventory items (i.e., several plants of each type). It is suggested that initially two or three plants of each type be established. The need for offshoots for research and other purposes may mean that some or all accessions need to have additional trees established. This is particularly true for varieties that produce few offshoots, such as Barhee. A suggested spacing that works well is 9 × 9 m. This spacing allows 125 trees per hectare (Krueger, 2011). In concern of formal date palm field genebank, in 1992, the USDA-ARS National Germplasm Repository for Citrus and Dates (NGRCD) began to establish a back up collection of date palm germplasm at the University of California Coachella Valley Agricultural Research Station (CVARS) in Thermal, California, a more suitable area for dates. As the CVARS planting becomes established, it will become the basic field genebank. In this respect, Bettencourt *et al*. (1992) list 15 formal date palm genebanks, the largest of which were found in Algeria, India, Iraq, Nigeria and the United States. Except possibly for the Nigerian collections, most date palm accessions maintained in genebanks appear to be elite cultivars or breeding lines, so the genetic diversity is probably rather low. Recently, Al-Ghamdi (2001**)** reports a date palm germplasm bank at King Faisal University, Saudi Arabia, having 18 elite varieties produced by tissue culture in various laboratories around the world. A larger genebank has reportedly been established at the campus of Al Qasim University in central Saudi Arabia (Krueger, 2011). In Bahla, Sultante of Oman, there is a collection of 167 pistillate date palm varieties (including 11 important cultivars) and 19 staminate varieties. Currently there are 7.8 million date palms cultivated in various part of the Sultanate of Oman of which there are approximately 180 female and 48 male cultivated varieties. The date palm genebank of the Ministry of Agriculture (MA) contains 166 female and 21 male cultivars, of which 81 produce yellow fruits, 24 produce red fruits, and the remaining produce various other fruit colors (Al-Yahyai and Al-Khanjari, 2008). Moreover, molecular and morphological characterization and registration of the Omani date palm germplasm as well as genome mapping are carrying out. Otherwise, There is also the possibility of the development of a germplasm repository of sorts at the Date Palm Development Center in Al-Ain, UAE (Krueger, 2011). In Egypt, a total of cultivated 52 date palm cultivars were collected, represent dry, semi-dry and soft date palm cultivars (Rizk and El Sharabasy, 2006**)**. For characterization and management of date palm a system of descriptors is recognized. It is contains standard passport data (17 traits), details of eco-geography (13 traits), ethnobotany (15 traits), management (18 traits), and characterization (58 vegetation traits, 27 fruit traits, 22 seed traits & 41 inflorescence traits) and evaluation activities (113 traits). Trait data were collected both from trees growing in their natural habitats as well as from trees have deposited on farms of the Central Laboratory of Date Palm Research & Development.

4- In vitro preservation of date palm germplasm:

The conventional methods used to conserve plant genetic resources have been supplemented in recent years by rapid developments in plant biotechnology. Some of the recent developments include *in vitro* conservation techniques developed for preservation of plant tissue cultures. *In vitro* conservation involves the maintenance of explants in a sterile, pathogen-free environment. It is widely used for the conservation of species which produce recalcitrant seeds, no seeds at all, or for material which is propagated vegetatively to maintain particular genotypes (Engelmann, 1997). For some species, the *in vitro* preservation is the only option available for conservation of their germplasms. In this respect, different *in vitro* conservation methods are employed depending on the storage duration required. There are two main types of *in vitro* storage of plant germplasm. First is the slow growth which achieved by modifying the culture medium or reducing temperature requirements. Second is cryopreservation which understood as storage between -79 and -196 ˚C, the low extreme being the temperature of liquid nitrogen. On the other hand, virtually any part of the plant could be used as explants in establishing cultures for storage, although the best results have been obtained using apical meristems, axillary buds, embryos and gametes. For a number of plant species, these alternative methods have been fully developed so that they are effectively used (Table 1). The *in vitro* genebank comprises two components: 1) the *in vitro* base genebank and 2) the *in vitro* active genebank. The attributes of the *in vitro* base

genebank are germplasm is preserved for the long-term and not normally distributed directly to users. The *in vitro* active genebank holds accessions that are immediately available for multiplication and distribution (Genebank Standards, 1994).

Since date palm is a dioecious and heterozygous fruit tree, and for commercial purposes most often vegetatively propagated through offshoots, it is difficult to store or handle it's germplasm by conventional means. Tissue culture techniques have great potential for the collecting, multiplication and storage of date palm germplasm. In this respect, date palm germplasm have been preserved *in vitro* in the form of shoot tips (Bekheet *et al*., 2001), somatic embryos (MyCock *et al*., 1997; Bekheet *et al*., 2005), pollen (Tisserat *et al*., 1985; Mortazavi *et al*., 2010), and callus cultures (Mater, 1987; Subaih *et al*., 2007).The miniaturization of explants allows reduction in space requirements. Moreover, disease-free stock is simplifying quarantine procedures for the regional and international exchange of germplasm. In this context, large scaling-up production systems of date palm micropropgation can be used effectively for both *in vitro* preservation and exchange of germplasm (Fig. 1).

Table 1: Some plant species where *in vitro* preservation has worked out.

Plant species	Technique	Explant	References
Asparagus offiicinalis	Cryoprservation Cryoprservation Slow growth	- Axillary buds. - Embryogenic callus, node segments. - Axillary buds & callus cultures	Uragami *et al*., (1990). Uragami (1991). Bekheet (2000).
Musa spp	Cryoprservation	Meristem cultures	Panis *et al*., (1996).
Malus pumila	Slow growth	Shoot tips.	Hao and Deng (2003).
Rubus idaeus	Cryoprservation	Shoot tips.	Wang *et al*. (2005).
Theobroma cacao	Cryoprservation	Somatic embryos.	Fang *et al*. (2004).
Coffea arabica	Cryoprservation	Somatic embryos.	MyCock *et al*. (1995)
Manihot esculenta	Cryoprservation	Somatic embryos.	Stewart *et al*., (2001).

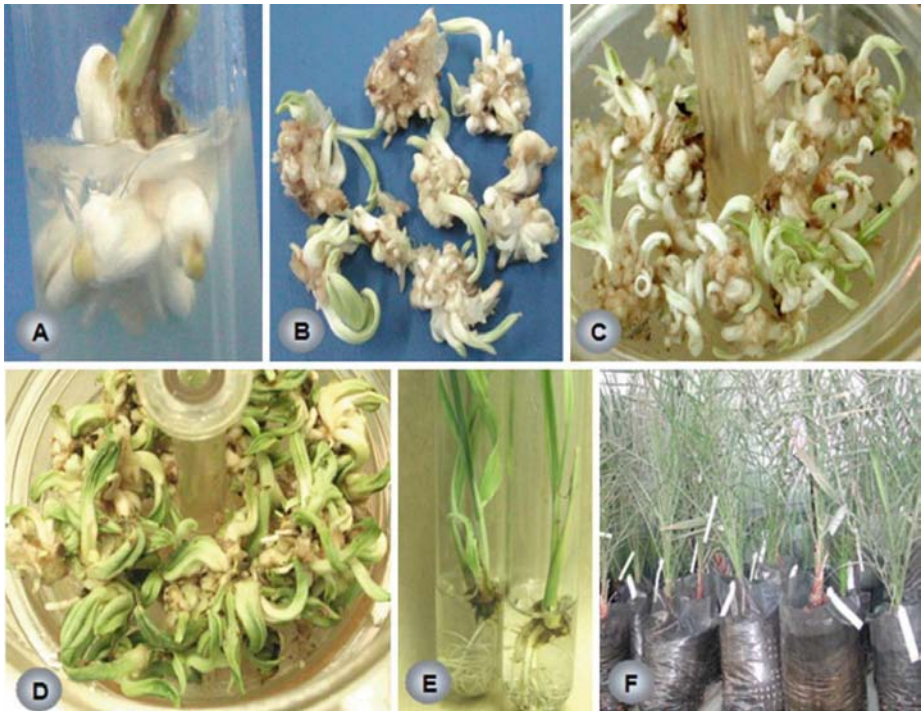

Fig. 1: Developmental stages of shoot buds. (a), evolution of shoot clusters (b) induction of *de novo* shoots after 2 weeks, (c) Shoots development after 4 weeks of culture, (d) vigorous shoots obtained after 6 weeks of culture, (e) rooting of 2 shoots after 1 month of culture on rooting medium and (f) potted plants 18 months after transfer to a greenhouse. *Source*: Othmani *et al*. (2011).

4.1. Preservation by slow growth:

The slow growth method for an active germplasm collection aims to minimize cell division and growth to increase longevity without genetic changes. Slow growth procedures allow clonal plant material to be held for 1–5 years under tissue culture conditions with periodic sub-culturing, depending on species. Storage under low temperature is one of the major tissue culture techniques used for preservation of plant genetic resources by

slow growth (Moges *et al*., 2003). Under such condition, accumulation of unsaturated lipids on the cell membrane would cause cell membrane thickening and retard cell division and elongation (Engelmann, 1997). In cold storage, temperature varies depending on the origin of stored species. Temperate species may be stored at 4°C, whereas tropical plants are require temperatures in the range of 15–20°C. Otherwise, plant species differ in their requirement for light or dark during storage. Most cold preservation protocols were performed under either low light intensity or complete darkness **(**Wang and Charles, 1991**)**. Otherwise, the addition of osmotica to the culture media has been proved to be efficient in reducing growth and increasing the storage life of many *in vitro* grown tissues of different plant species. In this respect, mannitol, sucrose and sorbitol were reported to be good materials to lengthen the storage life of *in vitro* grown cultures (Shibli *et al*., 1992**)**. Alternative techniques include modifications of the gaseous environment of cultures, and desiccation and/or encapsulation of explants. The advantages of these methods are that culture deterioration can be detected visually and therefore loss of viability can be avoided (Ng and Ng. 1991). In this context, oil palm polyembrogenic cultures were conserved at room temperature in a controlled atmosphere with 1% oxygen (Engelmann, 1990). Slow growth is achieved in date palm for short and mid-term preservation (Bekheet *et al*., 2001).They described a method for preservation of Egyptian date palm tissue cultures (shoot buds and callus cultures) at 5 ºC in the dark conditions. Moreover, preservation of date palm germplasm via artificial seeds was recognized by Bekheet *et al*. (2005). Somatic embryos proliferated *in vitro* from shoot-tip cultures were encased in sodium alginate capsules and stored for 12 months. Recently, Fki *et al*. (2011**)** developed applicable methods for somatic embryogenesis and synthetic seeds (Fig. 2) of date palm.

Fig. 2: Mass production of somatic embryos (A) and synthetic seeds (B) of date palm. *Sourc*e: Fki *et al*., (2011)

4.2. Cryopreservation:

Collected plants are normally stored either in active gene banks containing material that is kept ready for distribution, evaluation or exchange; or as base collections containing duplicates that are kept for future use (long term) or "emergency" material in case of loss from the active gene banks. One of the principle long-term *in vitro* conservation methods is cryostorage. Cryopreservation generally refers to storage between -79°C and -196°C in liquid nitrogen. The major advantage of cryostorage is that both metabolic processes and biological deterioration are considerably slowed or even halted. So, cryopreserved material remains genetically stable, thus affording an advantage over the other conservation methods. Successful cryopreservation requires the optimization of numerous variables including the size of specimen, the correct type and concentration of cryoprotectant, sample water content and rate of freezing and thawing. Otherwise, the capacity to survive storage in liquid nitrogen is dependent upon many factors including genotype, physiological status and pre- and post- freezing manipulations. On the other hand, although cryopreservation has many advantages, freezing and thawing injuries related to membrane structure and function that would result in low survival percentages are still the major limiting factors (Ashmore, 1997). The cryopreservation procedure comprises a number of steps including preculture in media with osmotically active compounds, treatment with cryoprotective agents, cooling and storage at -196°C, thawing, post-thaw treatments and recovery of growth (Moges *et al*., 2003). The techniques for cryopreservation currently in use are quite varied and include the older classical techniques based on freeze-induced dehydration of cells as well as newer techniques based on vitrification. In general, cryopreservation is well established for vegetatively propagated species. However, it is muchless advanced for recalcitrant seed species due to some of their characteristics, including their very high sensitivity to desiccation,

structural complexity and heterogeneity in terms of developmental stage and water content at maturity. Earlier reports have described storage methods of date palm tissue cultures by cryopreservation (Tisserat, 1981; Finkle *et al.* 1982; Ulrich *et al*., 1982**)**. Later on, Mater, (1987) reported that freezing at -25 ºC for 4 months did not affect the potential of embryogenesis induction from callus although growth during the first two months of culture was inhibited. MyCock *et al*. (1995) developed date palm plantlets using somatic tissue previously frozen for several months in LN. Recently, an applicable cryopreservation method has been developed for date palm by Bekheet *et al*. (2007). Undifferentiated tissue cultures (nodular cultures) were successfully cryopreserved by freezing methods; subsequently the plantlets were regenerated. Among different types of sugars used as osmotic agents in preculture medium, sucrose was the best for the survival of cryopreserved date palm tissue cultures. Otherwise, cryopreservation of date palm embryogenic callus via encapsulation-dehydration vitrification and encapsulation-vitrification was achieved by Subaih *et al*. (2007). More recently, cryopreservation procedures of cell suspension of date palm cv. Khalas were optimized by Al-Bahrany and Al-Khayri (2012). The highest colony formation, greatest callus growth and highest embryos number was recovered from cell cryoprotected in 10% DMSO supplemented with 0.75 M sucrose. A number of steps have been followed for cryopreservation, which include: preculture in media with osmotically or vetrification compounds, treatment with cooling and storage at -196°C, thawing, post-thaw treatments and recovery of growth.

5. Date palm DNA banking:

The tools of modern biotechnology are being increasingly applied for plant diversity characterization and undoubtedly they have a major role in assisting plant conservation programmes. In this respect, DNA technology is rapidly increasing in terms of importance. With the development of PCR (polymerase chain reaction) one can now routinely amplify specific oligonucleotides or genes from the entire mixture of genomic DNA. These advances have led to the formation of an international network of DNA repositories for the storage of genomic DNA (Adams, 1997). DNA banks enhance DNA barcoding projects by providing: access to DNA, high quality, long-term storage of DNA and complete documentation. Storage of DNA is, in principle, simple to carry out and widely applicable. The storage of DNA seems to be relatively easy and cheap. The stored materials can be utilized as research resources or for reference in future. For many species that are difficult to conserve by conventional means (either as seeds or vegetatively) or that are highly threatened in the wild, DNA storage may provide the ultimate way to conserve the genetic diversity of these species and their populations in the short term, until effective methods can be developed. In this respect, Adams and Adams (1991) recommended the following functions of DNA Bank-Net: 1) collection of plant material, 2) DNA extraction, 3) long term preservation of DNA in liquid nitrogen, 4) DNA analysis/gene replication and 5) distribution of DNA (genes, gene segments, oligonucleoties etc.). The disadvantage of this technique that is it does not allow the regeneration of entire plants.

DNA Bank revealed that long-term storage of DNA samples in buffer should be carried out at -80°C or below. The two general rules to prevent DNA damage during storage are “low temperatureand “as dry as possible". Since secondary compounds and heavy metal ions can result in highly reactive intermediates causing all sorts of DNA damage, high purity of extracted DNA must be ensured. The impact of fast and careful tissue fixation and tissue preparation prior to DNA extraction to receive high quality DNA has yet been underestimated. In this respect, the characteristics of four commercial dry storage systems at ambient temperature are presented **(**Table 2**)**.To extract high amounts of DNA; young, fresh, and healthy leaf tissue should preferably be sampled. The tissue material should be taken using a tweezer. Sample bags should at least be labeled by the collector’s number and might have a unique tissue barcode and/or the species name too. DNA (molecular weight and authenticity of sequences) is the major determinant of its value in genomic surveys (Walters and Hanner, 2006). Otherwise, preservation stresses (drying, freezing or time) cause some damage to DNA and storage temperature has weak influence of on DNA quality. But storage in water led to complete degradation at higher temperatures **(**Zetzsche and Gemeinholzer, 2009).

DNA attributes are be used for variety identification, source of information of date palm genebank and for studying the genetic diversity of cultivars. Recent advances in DNA sequencing technology and reduction in the cost of sequencing reagents have brought remarkable progress in genome analysis of date palm. To address this issue, researchers began an ambitious DNA sequencing programme to identify date palm genes that are expressed during important development stages. It was found that date palm genome is composed of 18 chromosome pairs, and the estimated size is 350 Mb (Al-Dous *et al*., 2011). Moreover, the genome size of date palm is estimated to be 1.2 billion base pairs, based on flowcytometry (Zhang *et al*., 2011). In this respect, complete sequence of the date palm chloroplast genomefrom an elite cultivar Khalas (Al-Hssa Oasis, Saudi Arabia), based on pyrosequencing has been reported by Yang *et al*. (2010). Effective methods for isolation and sequencing, handling and preservation of date palm DNA from collection materials will be useful as DNA banking in a complementary strategy for conservation of date palm germplasm.

Table 2: Comparison of products for long-term DNA storage using four commercial dry storage systems at ambient temperature.

Characteristics	Storage system			
	GenPlate™ (FTA paper)	GenTegra™	SampleMatrix™ QIAsafe™	DNAshell™
Principle	DNA binding to modified cellulose matix	DNA binding to mineral matrix	DNA protection by trehalose glass	DNA in glass insert, inert gas in air-tight, metal capsule
DNA recovery	Eluation with special solution	Rehydration	Rehydration	Rehydration
DNA integrity	Short-term to long-term	> 10 (29) years	Short-term to long-term	Short-term to very long-term
Limitations	Oyxgen, DNA aggregation, irradiation	DNA aggregation, irradiation	oxygen, meta-bolizable, recyclable	non recyclable
Costs	Low	Medium	Medium	High

Source: Zetzsche and Gemeinholzer (2009).

6- Exchange and use of date palm germplasm:

The ability to exchange healthy plant germplasm is fundamental to effective conservation and use genetic resources. Two ways have been used for exchange and distribution of plant material: a) with non aseptic plant material (tubers, seeds, nodal cuttings, corm and offshoots), b) with plant material in aseptic conditions (micro-nodal cuttings, micro-tubers, apices, zygotic or somatic embryos, and callus and cells suspension). Recently a new method (encapsulation technique, with the embedment of tissue culture in alginate beads) has been developed for a concept of plant material transfer (Hasan and Takagi, 1995). *In vitro* exchange of plant germplasmhas been used for a number of years by institutes, including several International Agricultural Research Centers, to distribute germplasm efficiently and securely. Practical procedures for culture packaging, handling before and after transit, certification and *in vivo* transfer are well established and success rates are high. In date palm, the limited and variable number of the produced offshoots is hindered germplasm exchange in form of vegetative materials. Moreover, although, phytosanitary regulations are incorporated as part of the date palm genebank activities, exchange and distribution are difficult because of the vegetative nature of the material and the greater risk of disease transfer. This explain, the low number of date palm field genebanks (Krueger, 2011). Many countries restrict the introduction of propagative material due to the potential to introduce devastating exotic pests or diseases. Exchange of date palm germplasm must be accomplished in a manner that minimizes these risks and as such would involve some sort of quarantine and pathogen-testing mechanism.

Biotechnology has made significant contributions to improve conservation and use of date palm genetic resources. *In vitro* techniques offer the potential for the improvement of procedures in several areas including transfer, indexing and contained quarantine. Exchange of date palm germplasmas using *in vitro* cultures offers considerable advantages like reduced volume and weight as well as the improved health status of the cultures (Bekheet, 2011). There are two main considerations that determine the success of *in vitro* exchange, namely practical points relating to the culture and transportation processes, and phytopathological points relating to the disease status of the cultured material and quarantine requirements. Otherwise, date palm DNA Bank can provide high quality, long-term storage of DNA material on which molecular studies have been performed. Moreover, complete on-line documentation of each sample, including the provenance of the original material, the place of voucher deposit, information about DNA quality and extraction methodology will provide an international information service. Complementary conservation strategy of date palm can be defined as the combination of different conservation actions, which together lead to an optimum sustainable use of its genetic diversity existing in a target genepool, in the present and future. The application of complementary conservation strategy should include scientific and institutional linkage between *in situ* and *ex situ* conservation. Moreover, collaboration within countries or regions should be established involving different ministries, or institutions which have not previously been brought together (Agriculture, Environment, Science and Technology, Planning etc.). In this connection, Dulloo *et al*. (2005) provides a framework and decision-making guideline for developing a complementary conservation strategy for coconuts that can be used as a model to develop complementary conservation strategies for other crops or species. Conservation approaches can be used for complementary conservation strategies of date palm genetic resources are summarized in Figure (3).

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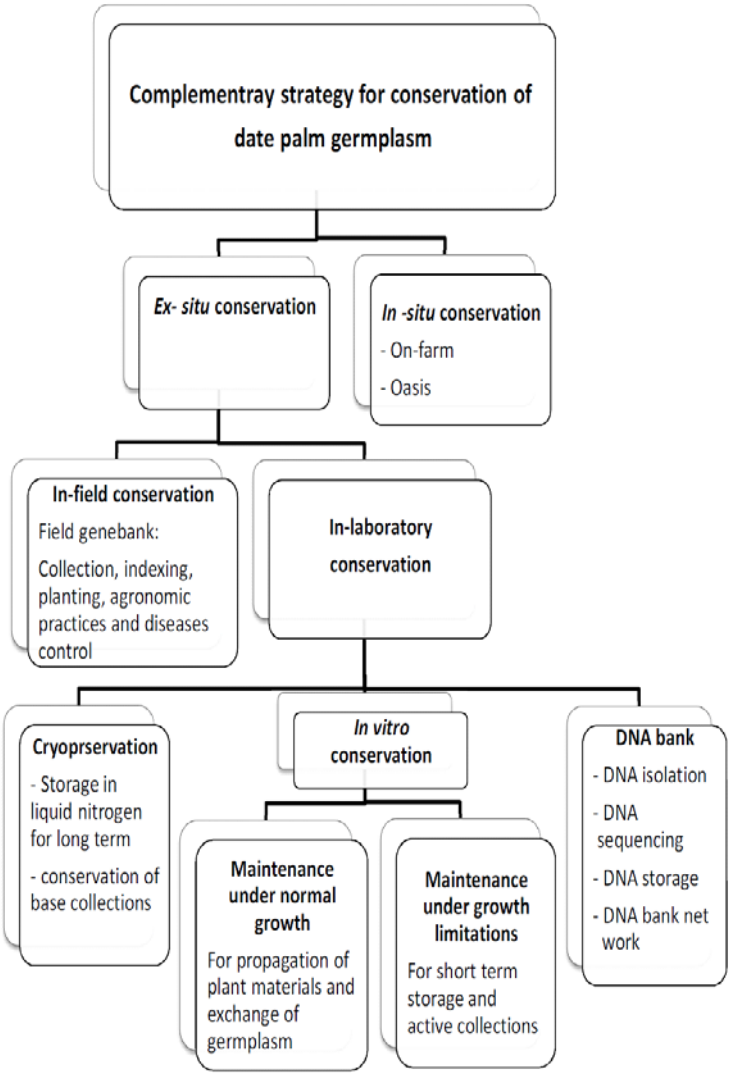

Fig. 3: Different methods used for preservation of date palm germplasm.

Conclusion:

An appropriate conservation strategy for date palm germplasm requires a holistic approach, combining the different *ex situ* and *in-situ* conservation techniques available in a complementary manner. In this respect, a number of different issues including consideration of biological characteristics, identification of conservation objectives, methodologies available, socio-economic factors and organizational and funding issues, need to be taken into account. Although field genebanks provide easy access to conserved material for use, they run the risk of destruction by natural calamities pests and diseases. For this reason, safety duplicates of the living collections should be established using alternate strategies of conservation and it is in this area that biotechnology contributed significantly by providing complementary *in vitro* conservation options through tissue culture techniques. *In vitro* conservation offers several distinct advantages. For example, the material can be maintained in a pathogen-tested state, thereby facilitating safer distribution. Further, the cultures are not subjected to environmental disturbances. Moreover, DNA extracts, DNA or RNA sequences are considered as genetic resources and they are now routinely extracted and conserved in DNA banks. Genetic resource collections in the form of frozen tissues, purified DNA samples, frozen viable cell cultures and derivatives such as RNA, cDNA and genomic libraries all represent valuable components of comprehensive storage strategy. So, a complementary strategy for conservation of date palm genetic diversity should employ a combination of methods including nature reserves, genebanks, and others, as no single method can conserve all the diversity. In this respect, three items are necessary to initiate such strategy: 1) strengthening national conservation programmes, 2) contributing to international collaboration and 3) improving methodologies and technologies for conservation.

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