Emir. J. Food Agric. 2012. 24 (5): 386-399
http://ejfa.info/

REVIEW ARTICLE

Date palm biotechnology: Current status and prospective - an overview

S. Mohan Jain*

Department of Agricultural Sciences, University of Helsinki, PL-27, Helsinki, Finland

Abstract

The date palm is one of the most ancient plants, grows in the regions of Middle East, North Africa, South Sahel, East and South Africa. Its sexually propagation hampers propagation of true-to-type genotypes due to heterozygosity. The vegetative propagation is carried out with the off shoots, produced from axillary buds situated at the base of the trunk during the juvenile life of palm tree. Offshoot production is slow; their numbers are limited, laborious and can't meet the rapidly growing demand of varieties. To speed up the date palm genetic improvement, *in vitro* culture techniques could be handy; however, genotype influence limits the effective use. Bioreactor is being used for large-scale production of somatic embryos. Somaclonal variation is common among *in vitro*-derived date palm plants. However, it could broaden genetic variability together with mutagenesis; molecular markers AFLP used to identify variability and to select useful variants. Dwarf date palm hybrid was developed by embryo rescue by interspecific hybridization of *Phoenix dactylifera* and *P. pusilla*. *In vitro* germplasm conservation is done by cryopreservation for long-term storage. Alternatively, *in vitro* shoot cultures and plantlets are stored at 4°C for short term-storage. Micro-calli is produced from date palm protoplasts; *Agrobacterium*-mediated transformation succeeded in GUS gene expression in callus. Date palm genomics can distinguish multiple varieties and a specific region of the genome linked to gender.

Key words: *Agrobacterium*-mediated transformation, Somaclonal variation, Genomics, Cryopreservation, Embryo rescue, Mutagenesis, Bioreactor

Introduction

The unique characteristics of date palm can be truly called 'tree of life' and is considered as one of the most ancient plant, and is distributed throughout the Middle east, North Africa, South Sahel, areas of East and South Africa, and even certain parts of Europe and USA. It makes a significant contribution towards the creation of equable microclimates within oasis ecosystems and thus enabling sustainable agricultural development in saline and drought affected areas. The rich fruit plays an important role in the nutrition of human population, and also several products are made that generate employment and thus influence socio economic aspect of people. Therefore it is widely acknowledged sustainability value in social, economic and ecological terms. Moreover, this crop has a great potential as a source of renewable energy, an alternate source to the fossil energy, by producing bio-fuel since its fruits high in carbohydrates, 44-88% total sugars.

Sexual propagation is widely used for date palm propagation. However this method can't be used commercially for propagating the cultivars of interest in a true-to-type manner. Interspecific hybridization between the date palm (*Phoenix dactylifera*) and the dwarf date palm (*P. pusilla*) has been successfully carried out, aimed at the development of short hybrid date palms (Sudhersan et al., 2009). Heterozygosity in date palm is related to the dioecious nature. Half of the date palm progeny is generally male and they don't produce fruits, and also large variation can occur in the progeny. There is no known method for sexing date palm at an early stage of tree development and that makes hard to eliminate non-productive male trees in the nursery before planting in the field. Another drawback of seed propagation is that the growth and maturation of seedlings is extremely slow. A date palm seedling may take 8-10 years or more before fruiting occurs. It is not surprising that little work has been done on date palm genetic improvement for developing new cultivars by traditional approaches. Therefore to speed up the date palm breeding programmes, particularly the areas where date palm is threatened by red weevil, devastating diseases like Bayoud and Brittle Leaf;

Received 1 February 2012; Revised 28 March 2012; Accepted 4 April 2012

*Corresponding Author

S. Mohan Jain
Department of Agricultural Sciences, University of Helsinki, PL-27, Helsinki, Finland

Email: mohan.jain@helsinki.fi

as a source of bio-fuel, biotechnology would be of great help in overcoming these problems (Jain et al., 2011).

Problems facing date palm genetic improvement

The date palm cultivation encounters several constraints mainly due to its development under harsh desert conditions, *e.g.* water shortages, high temperature and irregular supply of amendments. Date palm also faces many biotic constraints, especially Bayoud disease caused by *Fusarium oxysporum* f. sp. *albedinis* (Figure 1) (Carpenter and Klotz, 1966; Djerbi, 1988).

Figure 1. Bayoud disease caused by *Fusarium oxysporum* f. sp. *albedinis*.

This disease is the most devastating to the date palm cultivation and was first described in southern Moroccan groves. Currently, it continues to spread across North African countries, especially in Morocco and Algeria where more than 12 million date palm trees have been destroyed so far. No effective means is known to control this disease and only a few cultivars with poor-quality fruits, unfortunately, are known to be resistant to Bayoud (El Hadrami et al., 1998). Therefore, proper date palm cultivation requires, disease resistant cultivars, pruning, pollination, fruit thinning, bunch removal and fruit harvesting, are highly essential for good quality fruit production. The cost of date production increases when the trees grow taller, due to the high labour cost in many producing countries. Mechanization is also expensive and unjustifiable in the case of small growers. Frequent climbing for fruit harvesting is highly dangerous in the case of taller old trees. Tree height is one of the major constraints to good quality date production. In order to reduce tree height and to develop dwarf date palms, a related dwarf palm species, *Phoenix pusilla*, was crossed with selected female date palm cultivars (Sudhersan et al., 2009).

Red palm weevil (RPW) (Figure 2) is a major pest in date palm growing countries in the Near East including the United Arab Emirates (UAE), Iran, Egypt and others (Jain et al., 2011). It appeared for the first time in the Middle East in 1985. It is a great cause of concern to the date palm growers in these countries. The control of RPW is mainly done by applying chemical insecticides through direct injection into the trunk of the date palm tree or by fumigation. Pheromone traps are also commonly used to control RPW, which still requires more refinement for more effectiveness to control this pest. Baculoviruses could be another way to control RPW, especially genetically engineered ones inserted with a set of genes dealing with neuro toxin, light-emission (fire fly gene), and heat tolerance. Another approach would be to express *Bacillus thuringiensis* (*Bt*) crystal insecticidal protein genes, to address problems related to insect pests (Sharma et al., 2002) and chitinase (to address problems related to basal stem rot).

Figure 2. Red palm weevil (*Rhynchophorus ferrugineus*).

Date palm propagation methods

Available techniques of rapid multiplication of date palm have contributed immensely to meet the increased demand of date palm fruits worldwide (Jain et al., 2011). Traditionally, date palm is propagated by both sexually through seeds and

vegetatively by off shoots that produced from axillary buds situated on the base of the trunk during the juvenile phase in date palm tree. It is quite slow for off shoots to develop and that hampers vegetative propagation of date palm trees. So far, there is no available technique to speed up in increasing the off shoot numbers as well as reduce the time in developing them. The use of off shoots preserve true-to-type character of multiplied genotypes. Moreover, sexual propagation of date palm is unsuitable for commercial production/propagation of true-to-type value-added genotypes. It is due to heterozygous nature of date palm seedlings and their dioecious nature (Jain, 2007a). In addition, half of this progeny is composed of male trees which aren't distinguished before flowering stage. The female plants produce variable fruits and generally of inferior quality (Eke et al., 2005). Furthermore, seed propagation method has another limitation that the growth and maturation of seedlings is extremely low, and therefore, date palm seedling may begin to fruit after 8-10 years of plantation. Although offshoot propagation is a true-to-type technique, it is not commercially practical for the following reasons:

- Offshoot production is limited to a relatively short vegetative phase of about 10 to 15 years;
- Only a limited number of offshoots are produced during this phase (20 to 30 offshoots, depending on variety);
- Some varieties produce more offshoots than others (some do not produce offshoots at all);
- Offshoot survival rate is low;
- The use of offshoots enhances the spread of date palm diseases and pests;
- Offshoot propagation is difficult, laborious, and therefore expensive.

In vitro propagation of date palm

The use of *in vitro* techniques such as somatic embryogenesis and organogenesis is highly suitable for large-scale plant multiplication of vegetatively propagated crops. The success of these techniques is highly genotypic dependent, however, have successfully been applied for plant propagation in wide ranging crops including date palm (Jain, 2007a). Micropropagation via direct organogenesis is widely used for rapid clonal propagation of elite genetic material of date palm (Khierallah and Bader, 2007). The performance of micropropagated date palm seems to be better than conventionally grown plants in terms of yield, early flowering time, and quite uniform in fruit quality and physical properties. Aaouine (2003) reported plant regeneration from 30 genotypes of date palm via direct shoot organogenesis. The major concern with this approach is somaclonal variation that is dependent on various factors including genotype, explants, plant growth regulators (Jain, 2001). Moreover, it is highly desirable to maintain genetic fidelity of regenerated plants, which can be studied by various molecular markers Micropropagation has an advantage of using low concentrations of plant growth regulators, consequently callus phase is avoided. Direct regeneration of vegetative buds minimizes the risk of somaclonal variation among regenerants. The duration of culture period is limited by frequent subcultures for maintaining and providing shoot cultures for plantlet production. However, the highest number of subcultures must be determined before starting the fresh cultures from the mother plants. This is done to prevent or reduce somaclonal variation. Currently, only a few laboratories use this technique to produce commercially *in vitro* date palm plants, mainly in Morocco, Saudi Arabia and United Arab Emirates. Micropropagation technique has been used commercially in selected date palm cultivars (Jain, 2006) described advantages and limitations of date palm micropropagation; major advantages are year round availability of plants, quality control, rapid production of plants of elite cultivars, and cold storage of elite genetic material.

Embryo rescue

Embryo rescue technique is carried out by the removal of a zygotic embryo from the seed and planting in a sterile nutrient culture medium. Embryo culture has several potential applications in agricultural crop improvement research programs. This technique has been used in several crops to produce new hybrids e.g. triticale; used for haploid production by making intergeneric and interspecific crosses, e.g. wheat and oat, *Hordeum vulgare* and *H. bulbosum*. It is used to save embryos that fail to develop naturally in interspecific or intergeneric hybridization where defective endosperms are common (Hodel, 1977). Embryo culture may also be used to reduce lengthy dormancy periods or with seeds difficult to germinate due to physical or physiological factors. Excised embryos cultured *in vitro*, under suitable basal nutrient culture media, usually germinate immediately. Embryo culture also can be useful in seedling developmental studies. Sudhersan et al. (2009) were successful in reducing the date palm height by embryo rescue of a cross between a dwarf palm species *Phoenix pusilla* and cultivated selected *P. dactylifera* cultivars (Sudhersan et al., 2009). This is the first

report on reducing the plant height in date palm by embryo rescue, and opens the way to genetically improve date palm in a short time.

Protoplasts

Date palm biotechnology is routinely being used in tissue, organ and cell culture for large-scale plant production and multiplication. Protoplast technique is yet to reach a stage of being used routinely in date palm genetic improvement, especially for somatic cell hybridization. The protoplasts are free of cell wall, consisting of cytoplasm bounded by the plasma membrane. The availability of commercial enzymes enables the production of large numbers of uniform protoplasts. Regeneration of fertile plants from isolated protoplasts was reported in tobacco (*Nicotiana tabacum*) for the first time by Nagata and Takebe (1971) and Takebe et al. (1971). The current status of protoplast plant regeneration has been reported for more than 400 plant species, (Davey et al., 2005).

There are very few reports on date palm protoplast work. Chabane et al. (2007) reported callus formation from protoplasts in cvs. Deglet Noor and Takerboucht. Similarly, Rizkalla et al. (2007) succeeded in inducing callus from protoplasts in Barhee and Zaghloul cvs. So far, critical steps of plant regeneration from recalcitrant date palm protoplasts have been accomplished. For example callus formation was achieved in commercial cvs. Deglet Noor, Takerboucht, Barhee and Zaghloul. The use of feeder layer was the main factor for inducing cell divisions as well as subsequent microcallus and callus formation. However, plant regeneration from protoplast callus has yet to be accomplished before this technology can further be used for producing somatic hybrids. Another major application of protoplast technique is to genetic transformation of date palm by introducing useful genes, e.g. disease resistant, fruit quality, plant height and others. This approach would enable (1) the selection of resistant cultivars and cultivars with excellent fruit quality through field trials, (2) and then combining both traits in one cultivar through conventional crossbreeding or somatic hybridization. Also resistance genes can be taken from a cultivar or species with high resistance level to a particular disease through asymmetric somatic cell hybridization, partial genome transfer from donor to the recipient parent. By this approach, virus resistant plants have been produced by fusing protoplasts of *Solanum brevidens* and *S. tuberosum* (Valkonen et al., 1994); herbicide resistance in *Solanum nigrum* and *S. tuberosum*, and *S. nigrum* and *Lycopersicon esculentum* (Binding et al., 1982; Jain et al., 1988).

Somatic embryogenesis

Somatic embryogenesis has tremendous potential for rapid large-scale plant production. In date palm, this technology can be used for the large-scale propagation, thereby opening the way for the production of artificial seeds. Somatic embryogenesis of date palm has been quite successful in plant regeneration (Fki et al., 2003; Al-Khayri, 2005). The most frequently used explants of date palm are apical shoot tips and lateral buds for successful plant regeneration (Jain, 2007a). However, it should be noted that factors controlling callus induction are so numerous that's why other optimizations are still to be done to improve the quality of the embryogenic calli and to increase the frequency of callus induction from diverse explants. Both abnormal somatic embryo differentiation and somaclonal variation were associated with the utilization of high concentrations of 2,4-D. Reducing its concentration significantly had minimized the number of abnormal somatic embryos and somaclons (Fki, 2005). Smith and Aynsley (1995) studied field performance of tissue culture-derived date palm clonally produced by somatic embryogenesis, and the results demonstrated that these plants started bearing fruits within 4 years from field planting of small plants with leaf length 100 cm and 1.5 cm diameter at the base. The main advantages of somatic embryogenesis are ideal for cryopreservation, cost effective for large-scale propagation, and embryo production in a bioreactor (Table 1).

In addition, further studies are still to be done to find other biochemical and new molecular markers of embryogenesis in date palm. Most of the methods used to assess somaclonal variations have limitations: cytogenetically analysis cannot reveal alteration in specific genes, isozyme markers are subject to ontogenic variation, and molecular markers investigate only a small part of the genome. Hence, field performance analyses remain the most reliable strategy to assess genetic integrity in date palm. Studies related to the cryopreservation of date palm embryogenic cultures are scarce that's why developing innovative procedures will be beneficial for date palm genetic resources preservation and a fabulous support for commercial propagation laboratories. The preliminary studies revealed that embryogenic cultures constitute an adequate plant material for further experiments on mutation induction for useful mutants selection, transfer of genes and isolation of regenerable protoplasts.

Table 1. Advantages and disadvantages of somatic embryogenesis (Jain, 2007b).

Advantages	Disadvantages
Cost effective clonal propagation	Low number of field plantable plantlets
Both shoot and root meristem development in the same step of the process	Highly genotypic dependent
Quick and easy to scale-up in liquid cultures, e.g. bioreactors	Inability to produce somatic embryos from mature seeds in many plant species
Long-term storage via cryopreservation	Gradual fluctuation and eventual decline in embryogenic culture productivity
Establishment of gene bank	Somatic embryogenic cultures from seeds or seedlings have unproven genetic value
Production of somatic seeds by encapsulation of mature somatic embryos	Long life cycle may show genetic variability or new mutations at the later stage of development
Somatic seedlings may be rejuvenated	
Genetic transformation	
Automation of somatic embryo production	
Somatic seedlings are virus-free	
Mutation induction	

Finally, there are still a number of problems such as abnormal somatic embryos differentiation, endophytic bacteria proliferation in *in vitro* culture and somaclonal variation, needing further extensive research to be totally solved. Concerning the endophytic bacterial contamination, only juvenile explants could be used to establish clean *in vitro* tissue culture since antibiotics such as cefotaxim have only a bacteriostatic effect. Immaturity of vascular tissue in these explants may explain the absence of this kind of contaminants in such explants (Fki, 2005).

Genetic diversity conservation

Plant genetic diversity is highly essential for the genetic improvement of crops for sustainable agriculture and its gradual loss is as a consequence to rapid human population growth, industrialization, deforestation, and natural calamities (Jain, 2010a,b; 2011a,b). In the future, the impact of climate change may have an adverse impact on sustainable date palm productions as well other crops. The conservation, distribution and proper utilization of plant genetic diversity/resources have become necessary for the development and improvement of date palm cultivars for sustainable crop production by the establishment of gene/germplasm bank both nationally and internationally. The Gene bank should encourage researchers to survey and monitor the genetic diversity of natural populations and landraces on farmer's fields. *In vitro* conservation techniques, cryopreservation or cryo-storage and cold storage, are excellent system for genetic resources conservation of forest trees and horticultural crops. Cold-storage approach has disadvantage of frequent subculture and that may run into a risk of contamination and somaclonal variation. Cryo-storage has an advantage of long-term storage without going through frequent subcultures and somaclonal variation. For this, *in vitro* cultures are suitable, e.g. somatic embryos/ cell suspension, callus, and should be able to regenerate plants with minimal somaclonal variation. In date palm, the most common *in vitro* culture approach has been somatic embryogenesis, which is very much dependent on genotype and culture medium for plant multiplication, even though there is a risk of genetic variability among regenerated plants For the first time, cryo-storage of date palm somatic embryos was done in Tunisia, FAO/IAEA project, and plant regeneration is yet to be accomplished. In Asia, National Bureau of Plant Genetic Resources (NBPGRI, India) is the biggest germplasm bank, and conserves mainly local germplasm seed and vegetative propagated crops and introduces new crops as well.

***In vitro* conservation and cryopreservation of germplasm**

The purpose of date palm genetic material conservation is to protect from deforestation, man-made environmental pollution, and natural calamities such as hurricane, floods, drought, fire etc. In Grenada, hurricane Ivan and Emily in 2004 and 2005 damaged 90% nutmeg and other spice trees, and resulted in loss of agriculture production, elite germplasm, and exports. The basic requirement of *in vitro* conservation and cryopreservation of genetic resources is the reliable plant regeneration from *in vitro* explants and large-scale disease-free plant multiplication. In failing to plant regeneration, this

technique may is useless to storing *in vitro* cultures. Most common *in vitro* cultures are being used such as shoot tips, callus, cell suspension, microspore, and somatic embryos. At low temperature, 0-5^{O}C, growth of stored shoot cultures is slowed down and that reduces the number of subcultures on the fresh culture media without influencing the genetic stability of cultures. It allows store cultures for several years as long as over 10 years depending on plant type. However, rooted shoots enhances storage time much longer, e.g. in strawberry shoot cultures that developed excellent roots could be stored for three years without change of culture medium under low light intensity and 4°C (S. M. Jain personnel communication). The growth rate can also be reduced by increasing sucrose concentration or addition of mannitol or sorbitol in the culture medium. Bekheet et al. (2001) were successful in the conservation of *in vitro* tissues including shoot buds and callus cultures of date palm var. Zaghloul by slow growth method for 12 months at 5^{o}C in the darkness. *In vitro* conservation has many advantages: disease-free planting material, high plant multiplication rate, all year round plant supply to the growers, potential of producing low cost planting material, and maintain the genetic fidelity verified with molecular markers. The major disadvantages of *in vitro* conservation are: loss of genetic material by contamination due to bacteria, fungi, virus and mites; subcultures on the fresh culture medium; labour intensive; destruction of stored genetic material due to fire or earth quake; and power supply interruptions. Therefore, utmost precaution should be taken to use healthy plant tissues for storage, and also test for virus-free material especially for example in cassava, strawberry and so on before initiating *in vitro* cultures for storage.

Cryopreservation

Cryo-storage or cryopreservation is widely used for long-term storage of *in vitro* cultures of genetic material under ultra-low temperatures, usually at -196^{o}C in the liquid nitrogen (Subaith et al., 2007; Bekheet et al., 2007). This method preserves contamination-free material and prevents somaclonal variation. Since date palm *in vitro* culture has been worked out for plant regeneration, several groups have been engaged in cryo-storage of date palm tissues such as shoot tips, nodular cultures, callus, and somatic embryogenic cultures (Bekheet et al., 2007). Cryoprotectant treatment is given before plunging the tissue in the liquid nitrogen for preventing ice crystal formation in the tissue in order to avoid any damage to the tissue that may adversely affect plant regeneration upon thawing of cryo-stored material. The common cryoprotects are polyehthylglycol (PEG), glucose, and dimethylsulfoxide (DMSO). In date palm, somatic embryo growth remains normal when treated with cryo-protectant mixture of glycerol and sucrose. The growth rate or germination rate of somatic embryos should remain normal after the cryopreservation and that would reflect any adverse impact of various treatments during the following the protocol.

Cryo-therapy for virus elimination

Cryopreservation has application for the elimination of viruses, which is also termed as cryo-therapy. Several viruses have been eliminated from various plants such as cucumber mosaic virus and banana streak virus from banana (Helliot et al., 2002), grape virus A (GVA) *in vit*ro-grown shoot tips of *Vitis vinifera* L. (Wang et al., 2003), potato leafroll virus (PLRV) and potato virus Y (PVY) from potato shoot tips (Wang et al., 2006). The cryopreservation method allows only the survival of small areas of cells located in the meristematic dome and at the base of the primordial (Helliot et al., 2002). Therefore, cryo-therapy would be an alternative efficient procedure to eliminate viruses to producing virus-free plant material and simultaneously long-term storage of genetic material.

Mutation breeding

The exploitation of genetic variability is essential for the development of new cultivars. Genetic variability can be induced by chemical and physical mutagens, T-DNA insertional mutagenesis, and tissue culture-derived variation or somaclonal variation. The most common physical mutagen used is gamma radiation. In this review, we will stick to physical mutagens only. Induced mutations are random changes in the nuclear DNA or cytoplasmic organ, resulting in chromosomal or genomic mutations that enable plant breeders to select useful mutants such as disease resistant, high yield etc. First of all, gamma irradiation breaks DNA into small fragments and secondly DNA starts repair mechanism. During this 2nd step, new variations develop or mutations occur. In date palm, there is hardly any work done on mutation induction, except that of FAO/IAEA Coordinated Research Project on development of Bayoud disease resistant date palm mutant varieties in North Africa (Jain, 2002, 2005, 2006). Mutation induction in date palm is feasible now due to a reliable plant regeneration system via somatic embryogenesis and organogenesis. Somatic embryogenesis system is more preferable approach due to single cell origin of somatic embryos and that

prevents or reduces the occurrence of chimeras. Moreover, mutant somatic embryos are germinated into direct plantlets in a single step, avoiding laborious rooting step. The irradiation of multicellular structures, e.g. seed, meristem tissue or offshoots, may result in chimeras in regenerated plants, and that would require a lot of extra work to dissociate chimeras by plant multiplication up to M1V4 generation (Jain, 2007a,b).

Mutant isolation

Mutant isolation can be done in two ways either in a single step or stepwise selection. In the first approach, irradiated cells are put under very high selection pressure for the isolation of mutant cell clumps/lines. The initial selection pressure should be as high as high LD_{75}. Remove isolated mutant cells and transfer them on the fresh culture medium with reduced selection pressure allowing them to recover from the initial selection pressure for about one week. The selected lines are put for shoot and root differentiation. Before selected mutant lines are put for shoot differentiation, they should be grown for 2 generations devoid of selection pressure and put them back again to the selection pressure. This step is done to make sure that the selected mutant lines are stable due to genetic changes rather than due to epigenetic changes. In the second approach, the selection pressure is reduced stepwise, from high to low concentration. All other steps are more or less similar to the first approach.

In vitro selection of mutants, normally type of the selection pressure varies, *e.g.* salt concentration, fungal toxin, polyethyl glycol (PEG), herbicide etc. For appropriate selection pressure, it is better to determine LD_{50} dose (Jain et al., 2010).

The third option is to select mutants at the whole plantlet level, *e.g.* by spraying herbicide or water withholding for drought tolerant selection, fungal toxin spraying or injection. In date palm, Bayoud disease resistant mutant plants were selected in the greenhouse by treating them with isolated toxin from *Fusarium oxysporum* f. sp. *albedinis* fungus causal agent (Jain, 2006). These plants are already in the field for the last four years. So far, they are doing just fine.

Somaclonal variation

Somaclonal variation is well suited to date palm genetic improvement by using selected somaclones with traits such as abiotic and biotic tolerance, high quality and other agronomic traits (Jain, 2001; El Hadrami and El Hadrami, 2009). It has a real advantage in widening the genetic basis of this species, relying more or less solely on vegetative propagation. Variation in the somaclones has often been associated with changes in chromosome numbers and/or structure, punctual mutations or DNA methylation or other epigenetic events (Jain et al., 1998; Brar and Jain, 1998). Somaclonal variation is undesirable from an industrial production stand point of view but may provide an enrichment of the genes pool. Its frequency depends, among others, on the genotype and the length of the proliferation process. Jain (2006) reported that rapid shoot proliferation can be achieved from various parts of the plant including shoot tips, stem cuttings, auxiliary buds and roots. He also pointed out that the selection of the genotype and the number of sub-culture cycles help limit the appearance of somaclones after the step of plant regeneration. Many off-type plants and abnormal dwarf phenotypes with low fruit sets as well as vitiated multi-carpel fruits (Fig.3) are observed among the *in vitro*-propagated date palm tree population. These phenotypes are not always detectable at seedling stages and often become apparent a few years after planting. However, the technological advances and the development of molecular markers have made it possible, in recent years, to early and accurately detect these variants and eliminate them for the mass production (Saker et al., 2000). These off-types and somaclones can be further investigated to enrich the genetic pool.

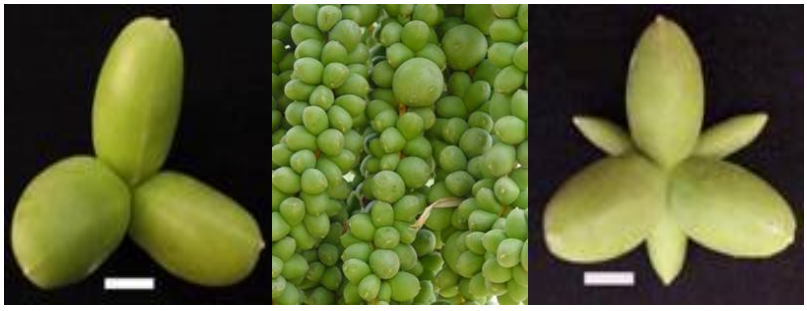

Figure 3. Somaclonal variation in multicarpel fruits of dates.
(Photos are provided by Dr. Nasser S. Alkhalifah, Riyadh, Saudi Arabia)

In vitro-selection represents useful biotechnology tools in date palm breeding for tolerance to biotic and abiotic stresses *i.e.*, drought, salinity, and diseases and pests (Jain et al., 2011). These techniques also offer an improvement of the value-added of the new genotypes with traits such as an increase in the number and/or size of fruits or their texture or taste, or a modification in flower structure (Witjaksono, 2003). By applying specific selective agents or providing particular conditions to *in vitro*-propagated tissues, somaclones with desired traits can be produced at a high frequency. Causes of somaclonal variation during the multiplication are diverse and tightly dependent upon the genotype, its level of ploidy, the growth conditions and duration of selection (Maluszynski and Kasha, 2002). Studies of the determinants of such a variation revealed that it can be due to changes at the gene level through genetic events such as duplication, translocation, mutation by insertion or deletion of transposable elements, or methylation. It can also occur at the chromosome level through instability, inversion, and transient or permanent ploidy changes (Kumar and Mathur, 2004). These phenomena often lead to irreversible pleiotropic and epigenetic events and the production of variants called chimera.

Genetic transformation

The global population growth rate is alarming and the situation demands to enhance food production to feed new mouths by developing new tools for plant breeders. Since date palm is more or less like a food crop and feeds people and serves as nutrition security, genetically engineered date palm would be able to generate disease and pest resistant plants by over expression of bio pesticide and antifungal. Growing of such palms will significantly reduce the hundreds of tons of pesticide applied yearly risking human health and degradation of the ecosystem. Genetic engineering would assist in reducing time scale in developing new cultivars; only when precisely single trait genes to be expressed without altering the remaining genetic makeup. However, genetically modified (GM) crops have yet to win the confidence of the consumer worldwide even though growing area of GM crops is expanding.

A large number of plant species have subsequently been genetically transformed, primarily using two different strategies for DNA delivery into totipotent cells; T-DNA delivery with *Agrobacterium tumefaciens* (Horsch et al., 1984) and direct gene transfer with particle bombardment. Generally, *Agrobacterium*-mediated transformation has several advantages over particle bombardment method e.g. integration of a well-defined DNA sequence, typically low copy number and preferential integration into actively transcribed chromosomal regions (Gheysen et al., 1998). Many approaches have been pursued in order to improve the efficiency of *Agrobacterium*-mediated transformation in recalcitrant monocot plant species, e.g. use of hypervirulent *Agrobacterium* strains, use of particular combinations of *Agrobacterium* and plasmids, optimization of co-culture media and conditions that increase the interaction of *Agrobacterium* with the plant cell (Cheng et al., 2004; Kumlehn et al., 2006). For date palm, *Agrobacterium*-mediated transformation used GUS (β-glucuronidase) as a reporter gene, which is easy to assay. So far, no conclusive report is available on the expression of economically-important genes in date palm to the present. The first report on successful infection of date palm embryogenic callus with *Agrobacterium,* and that led to the development of its gene transfer system (Saker et al., 2009). It involves callus production from shoot tip explants on callus induction medium (CIM) containing MS salts, B5 vitamins, 30 g/l sucrose, 10 mg/l 2, 4-D, 3 mg/l 2ip, 170 mg/l KH_2PO_4 and 3 g/l activated charcoal, followed by mass propagation of the proliferated microcalli on MS medium supplemented with 0.4 mg/l NAA and 0.1 mg/l 2ip. Factors influencing transient expression of the GUS gene were evaluated following the infection of embryogenic callus; results indicated that high bacterial density (OD_{600} 1-1.5) and prolonged infection (2 hrs) gave the highest percentage of GUS-expressing calli concluding date palm gene transfer achievable. Alternatively, direct gene transfer in date palm cells was optimized by particle bombardment method (Habashi et al., 2008; Saker, 2006, 2007). A construct harbouring a cholesterol oxidase gene, which renders plants resistance to insect attack, was introduced into embryogenic date palm callus using PDS1000/He particle bombardment system. Three calli out of 200 putative transformed microcalli gave positive GUS expression after bombarded with DNA-coated particles, gave positive GUS expression. The successful integration of GUS gene in GUS positive clones was verified by PCR. The reported system involves the establishment of embryogenic callus cultures from shoot tip explants, followed by shooting of the embryogenic callus with DNA coated particles under optimized physical conditions. The most effective physical factors

influencing gene delivery using a bio-listic gun were flight distance of micro-projectiles and their size and applied pressure, cell and tissue type dependent (Iida et al., 1990).

Molecular markers

Molecular markers are an increasingly important resource for all crops. DNA markers, especially those based on simple sequence repeats and single nucleotide polymorphisms, are playing an increasingly important role in plant variety identification, germplasm resource collection and breeding activities. In general, the molecular marker resources for date palm are somewhat limited. However, most of the available DNA marker types have been used on some material, mostly to cluster date palm varieties into related groups. The most profound effect on the development of the DNA marker resources for date palm is the newly available shotgun sequence. Mining this sequence database and the steady lowering of the costs of high throughput sequencing will increase rapidly the molecular marker resources and their application to date palm over the next few years

It is clear that the date palm genome is structured similarly to that of other characterized plants. Therefore all the tools that have been developed for using DNA markers are available. Preliminary studies have demonstrated that population structures and lineage relationships can be identified with the current crop of DNA markers. The availability of the complete genome sequence will facilitate the development of suitable marker among different marker types. The development of a series of sequenced tagged sites (probably based in SSRs) will supply resources needed for the screening of collections to reduce the number of samples kept in germplasm banks. They will also add impetus to identifying markers linked to the various disease-resistant genes. With the steady increase in the sequencing resources, SNPs will also become more useful but the relative costs of SNP and SSR analyses may well determine which of the two-marker systems becomes most widely used. It is undoubted that the collection of many high polymorphism information content SSR primer pairs and validated SNPs will provide the tools for phylogenetic analyses as well as germplasm conservation. However, once genomic regions associated with important characteristics such as disease resistance, taste and post-harvest stability, the sequencing of these regions and the identification of the actual bases for these characteristics can be incorporated into the breeding and improvement programs. The identification of off-types arising in tissue culture propagation and the complete genome sequencing of normal and off-type individuals will lead to the identification of both markers for assessing off-type individuals in the regenerated plants as well as the 'mutations' responsible for these off phenotypes. Therefore these molecular markers and the tools developed through their use will facilitate the improvements in available germplasm for increasing the area under date palm cultivation as well as for the overall improvement of the plant material available to growers.

Traditional and modern genetic improvement in date palm need extended time periods and considerable funds. Therefore, they can be assisted by molecular markers that give better and more efficient research strategies. Data based on molecular markers such Random Amplified Polymorphic DNA (RAPDs), have been developed to molecularly characterize date-palm genotypes of cultivars and to examine their phylogenetic relationships (Trifi et al., 2000). Earlier results showed the use of molecular markers as tools to evaluate genetic diversity and genotyping of date-palm cultivars (Jain et al., 2011). Based on statistical analysis, Sedra (2007c) reported certain informative molecular markers which are associated with specific phonological characters in date palm. Previous study of date-palm mitochondrial DNA gave evidence of two plasmid-like DNAs that seem to be linked to Bayoud disease resistance (Benslimane et al., 1996) but these markers cannot distinguish both cultivars studied (Trifi, 2001). Each marker corresponds to one part of date palm DNA and the genome has the size estimated to 1.7 pg and it is constituted of more than $١٠^{١٢}$ nucleic bases. These data seem to suggest that the higher the number of markers used the greater the probability to achieve more precise results. Trifi group, Tunisia used several hundred RAPD and inter-simple sequences repeats (ISSR) primers and identified several markers to distinguish partially or totally between resistant and susceptible cultivars of date palm. The difficulty and relatively weak efficiency were probably due to the nature of the genetic status of resistance.

Genomics

Genomics is carried out to study the whole genome of an organism, which is the sum total of DNA molecules harbouring all genes of an organism. This type of work is performed to study all the genes of a given cell, tissue and organism;

DNA (genome) as well as RNA (transcriptome), and protein (proteome) in the context of a regulatory network as well across taxa (evolution). The field includes intensive efforts to determine the entire DNA sequence of various organisms and to construct a genetic map, using large-scale sequencing technology, to generate massive, adequate and high-quality data, by using bioinformatics tools for assembly, annotation and in-depth analysis. A major branch of genomics is still focused on sequencing the genomes of various species, but the knowledge of full genomes has created the possibility for the field of transciptomics, proteomics, bioinformatics, function genomics, metagenomics and system biology.

A team from Weil Cornell Medical College in Qatar tried to sequence the entire date palm genome using Solexa (illumine) sequencer based on a shotgun method. They announced the finished draft map in 2009 and released the sequence data subsequently: (http://qatar-weill.cornell.edu/ research/datepalm Genome/index.html). According to their analyses, the genome assembly has a predicted genome size of ~550Mbp. The following are genome parameters of their draft sequence assembly:

- 45,000 scaffolds greater than 2kb
- Scaffold N50 is 4250bp
- 850,000 novel high quality SNPs between parental alleles
- GC content of the nuclear genome is 37%
- 302Mb of assembled sequence with 18.5Mb of ordered gaps
- Unique sequence is 292Mb at the 24-mer level

The date palm genomic project (DPGP) is being carried out at the King Abdulaziz City for Science and Technology (KACST) jointly with the Beijing Institute of Genomics, Chinese Academy of Science (BIG/CAS). The objectives are bioinformatics, genetics, biochemistry, transcriptomes and post-genomics. Data have been generated by using second-generation sequencers and sequence assembling has been working on most likely in a complex process where different types of data are integrated to ensure both quality and contiguity.

The first phase of the DPGP is focused on genomics and bioinformatics that pave the way for genetic and biochemical studies.

- The specific aims of the DPGP are: a working draft with sequence coverage; 10x from 454 and 50x from SOLiD; a complete map will be built with end-sequences from BACs and Fosmids; a genome diversity map built with shotgun sequencing of 30 cultivars; each with 30x of SOLiD reads; the date palm transcriptomes: full-length cDNA, over 30,000 unigenes; and expression profiles for leaves, roots, and flowers (~50 tissue samples).
- They have already preliminary data on genome sequencing and assembly, chloroplast genome sequencing and transcriptomics.

Conclusions and prospects

Date palm is life-line of people living in Sahara and sub-Sahara regions and also an important source of income in Near Eastern countries. Most of the date palm trees are very old, as old as 70-100 years and perhaps are becoming more vulnerable to various diseases and pests. One of the reasons could be due to global warming or global climatic changes. An increase in global temperature would bring new pests and disease and get rid of some existing types. Since date palm has a long life cycle, it could become more vulnerable to the global warming, and that is why it is highly desirable to pay more attention to the genetic improvement of date palm varieties that could with stand natural calamities without compromising the yield and quality. The use of chemical insecticide and pesticides is very common to control diseases and pests of date palm. These practices could become deadly health hazard to human health and that may also curtail their export market. Innovative techniques are needed to apply for the control of disease and pests, and that is where genetic modifications of organisms would be of highly effective. Genetic engineering of baculoviruses may be of great help in controlling the RPW by inserting a set of genes including neuro toxin (gene from scorpion or snake), light-emitting (fire-fly), and heat tolerance (bacterial gene). The engineered baculoviruses would multiply inside the insects and kill them instaneously. One could monitor the rate of viral multiplication inside the insect by light meter. Insertion of *Bt* gene in date palm won't be the right approach due to long life cycle of date palm and it would be rather difficult to predict the behaviour of transgene in the long run. Moreover, food safety regulations don't permit to insert *Bt* gene in food crops.

The progress of *in vitro* culture techniques has enabled date palm micro propagation more as a routine technique for large-scale plant production in many countries. The influence of genotype has handicapped micro propagation of different commercially valuable date palm varieties. This area needs serious attention by modifying the

culture medium well suited for several date palm cultivars. This type of work perhaps may require more empirical work in order to modify the composition of the culture medium. Now the question arises how well molecular approach would assist plant tissue culturists to modify the culture medium and growing conditions or the selection of appropriate explants or pre-conditioning of explants. To answer these questions, plenty of work is foreseen and in other words this area of research is 'virgin'.

The date palm shoot multiplication rate could be improved by using liquid culture system or 'bioreactor'. Few groups have started working on liquid culture for in vitro propagation of date palm. RITA bioreactor, based on temporary immersion system, should be tried in date palm shoot multiplication and somatic embryo production. Micro propagation via organogenesis or direct shoot formation is extensive labour-oriented. Somatic embryogenesis may reduce labour cost and also asset in developing automated somatic embryo production. However, genetic fidelity of micro propagated plants should be maintained with minimal somaclonal variation, otherwise there will be severe economic loses to the growers. Molecular marker analysis would be an ideal approach to identify genetic variability at the early stage of plant development. It would be difficult to identify point mutations or any genetic change at the early stage of plant development because it may not express phenotypically and may express at the later stage of plant development. This scenario occurred in oil palm tissue culture-derived plants in Malaysia and the oil palm industry lost millions of US dollars.

Haploid production in date palm has not yet been accomplished. Inflorescence culture will be one way to induce haploid somatic embryo production. Fki et al. (2003) induced callus from immature inflorescence of date palm var. Deglet Nour, and the calli originated from the proliferation of floral primordia showed embryogenic potential. The capacity of inflorescence to form callus was much higher than cultured leaves. They did not determine the ploidy level of callus and regenerated plants from inflorescence-derived callus. In the future, the success of this type of work would revolutionise date palm genetic improvement program as well as molecular genetics for useful gene identification.

Somatic embryogenic cell suspension is an excellent system for mutation induction and isolates useful mutants of date palm. Direct mutant somatic embryos can be produced and germinated into mutant somatic seedlings. These mutant seedlings can further be micro propagated for large-scale production. The utmost care should be taken while handling somatic embryogenic cultures, and in failing to do, the chances getting somaclonal variation becomes very high. This approach is an excellent example of combining mutagenesis and biotechnology for date palm improvement. Transgenic date palm is long way to go before consumers accept to consume them and consequently export market will also be lost. Therefore, transgenic approach to modify date palm should be followed with a great caution, even though it has a great potential to overcome several of its problems.

References

Aaouine, M. 2003. Date palm large-scale propagation through tissue culture techniques. In: The date palm from traditional resource to green wealth. Emirates Centre for Strategic Studies and Research, pp. 79-86. Abu Dhabi, United Arab Emirates.

Al-Khayri, J. M. 2005. Date palm *Phoenix dactylifera* L. In: Jain, S. M. and P. K. Gupta (Eds.), pp. 309-319. Protocols for somatic embryogenesis in woody plants. Springer, Netherlands.

Bekheet, S. A. H. S. Taha and M. M. Saker. 2001. Factors affecting *in vitro* multiplication of date palm. Biol. Plant 44:431-433.

Bekheet, S. A., H. S. Taha, M. E. Solliman and N. A. Hassan. 2007. Cryopreservation of date palm (*Phoenix dactylifera* L.) cultured *in vitro*. Acta Hort. 736:283-291.

Benslimane, A. A., C. Hartmann, B. Ouenza and A. Rode. 1996 Intramolecular recombination of a mitochondrial minicircular plasmide-like DNA of a date-palm mediated by a set of short direct repeat sequences. Curr. Genet 29: 591-593.

Binding, H., S. M. Jain, J. Finger, G. Mordhorst, R. Nehls and J. Gressel. 1982. Somatic hybridization of an atrazine resistant biotype of *Solanum nigrum* and *S. tuberosum*. I. Clonal variation in morphology and in atrazine sensitivity. Theor. Appl. Genet. 63: 273-277.

Brar, D. S. and S. M. Jain. 1998. Somaclonal variation:mechanism and applications in crop improvement. In: S.M. Jain. D.S. Brar and

B.S. Ahloowalia BS (Eds.). pp. 15-38. Somaclonal variation and induced mutations in crop improvement. Kluwer Academic Publisher, Netherlands.

Carpenter, J. B. and L. J. Klotz. 1966. Diseases of the date palm. Date Grow Inst. Rep. 43:15-21.

Chabane, D., A. Assani, N. Bouguedoura et al. 2007. Induction of callus formation from difficile date palm protoplasts by means of nurse culture. C. R. Biologies 330:392-401.

Cheng, M., B. A. Lowe, T. M. Spencer et al. 2004. Factors influencing *Agrobacterium*-mediated transformation of monocotyledonous species. *In Vitro* Cell Dev. Biol. Plant. 40:31-45.

Davey, M. R., P. Anthony, J. B. Power and K. C. Lowe. 2005. Plant protoplast technology: current status. Acta Phys. Plant. 27:117-129.

Djerbi, M. 1988. Les maladies du palmier dattier. Projet régional de lutte contre le Bayoud, FAO, Alger.

El Hadrami, I. and A. El Hadrami. 2009. Breeding date palm. In: Jain, S. M. and P. M. Priyadarshan (Eds.) pp. 191-216. Breeding plantation tree crops, Springer, New York.

El-Hadrami, I., M. El-Bellaj, A. El-Idrissi, et al. 1998. Plant biotechnology and breeding of the date palm (*Phoenix dactylifera* L.), a mainstay of Moroccan oasis agriculture. Cah. Agr. 7:463-468.

Eke, C. R. and O. Akomeah and P. Asemota. 2005. Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) from apical meristem tissues from 'Zebia' and 'Loko' landraces. Afric. J. Biotech. 42:244-246.

Fki, L., R. Masmoudi, N. Drira and A. Rival. 2003. An optimised protocol for plant regeneration from embryogenic suspension cultures of date palm (*Phoenix dactylifera* L.) cv. Deglet Nour. Plant Cell Rep. 21:517-524.

Fki, L. 2005. Application des suspensions cellulaires embryogenes au clonage et à l'amélioration *in vitro* du Palmier dattier. Thèse de doctorat, Faculté des Sciences de Sfax-Tunisie.

Gheysen, G., G. Angenon and M. Van Montagu. 1998. *Agrobacterium*-mediated plant transformation: a scientifically intriguing story with significant applications. In: Lindsey, K. (Ed.), pp. 1-33. Transgenic plant research. Harwood Academic, Amsterdam.

Habashi, A. A., M. Kaviani, A. Mousavi, S. Khoshkam. 2008. Transient expression of β-glucuronidase reporter gene in date palm (*Phoenix dactylifera* L.) embryogenic calli and somatic embryos via microprojectile bombardment. J. Food Agric. Environ. 6:160-163.

Helliot, B.B., B. Panis, Y. Pumay, R. Swennen and P. Lepoivre. 2002. Cryopreservation for the elimination of cucumber mosaic and banana streak viruses from banana (*Musa* spp.). Plant Cell Rept. 20: 1117-1122.

Hodel, D. 1977. Notes on embryo culture of palms. Principes 21:103-108.

Horsch, R. B., R. T. Fraley, S. G. Rogers et al. 1984. Inheritance of functional foreign genes in plants. Sci. 223:496-498.

Iida,, A., M. Seki, M. Kamada et al. 1990. Gene transfer into cultured plant cells by DNA-coated gold particles accelerated by a pneumatic particle gun. Theo. Appl. Genet. 80:813-816.

Jain, S. M. 2001. Tissue culture-derived variation in crop improvement Euphytica 118:153-166.

Jain, S. M. 2002. A review of induction of mutations in fruits of tropical and subtropical regions. Acta Hort. 575:295-302.

Jain, S. M. 2006. Radiation-induced mutations for developing Bayoud disease resistant date palm in North Africa. Proc. Intern. Workshop on True-to-Typeness of Date Palm Tissue Culture-Derived Plants, Morocco, 23-25, 2005. pp 31-41. UAE University, Date Palm Global Network, Al Ain, United Arab Emirates.

Jain, S. M. 2007a. Recent advances in date palm tissue culture and mutagenesis. Acta Hort. 736:205-211.

Jain, S. M. 2007b. Biotechnology and mutagenesis in genetic improvement of cassava (*Manihot esculenta*). Gene Conserve 6(23):329-343.

Jain S. M. 2011a. Date palm genetic diversity conservation of for sustainable production, Acta Hort. 882:785-791.

Jain, S. M. 20011b. Prospects of *in vitro* conservation of date palm genetic diversity for

sustainable production. Emirates J. Food Agric. 23 (2):110-119.

Jain, S. M. 2010a. Mutagenesis in crop improvement under the climate change. Romania Biotech. Letters 15 (2), supplement, 88-106.

Jain, S. M. 2010b. In vitro mutagenesis for banana (*Mus*a spp.) improvement. Acta Hort. 879:605-614.

Jain, S. M., E. A. Shahin and Sam Sun. 1988. Interspecific protoplast fusion for the transfer of atrazine resistance from *Solanum nigrum* to tomato (*Lycopersicon esculentum* L.). Plant Cell, Tiss. Org. Cult. 12:189-192.

Jain, S. M., S. J. Ochatt, Y. M. Kulkarni and S. Predieri. 2010. In vitro culture for mutant development. Acta Hort. 865:59-68.

Jain, S. M. 2005 Major mutation-assisted plant breeding programmes supported by FAO/IAEA. Plant Cell Tiss. Org. Cult. 82:113-121.

Jain, S. M., D. S. Brar and B. S. Ahloowalia (Eds.) 1998. Somaclonal Variation and Induced Mutations in Crop Improvement. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Jain, S. M., J. M. Al-Khayri and D. V. Johnson (Eds.). 2011. Date palm Biotechnology, Springer.

Khierallah, H. S. M. and S. M. Bader. 2007. Micropropagation of date palm (*Phoenix dactylerefa* L.) var. Maktoom through organogenesis. Acta Hort. 736:213-223.

Kumar, P. S. and V. L. Mathur. 2004. Chromosomal instability is callus culture of *Pisum sativum*. Plant Cell Tiss. Org. Cult. 78:267-271.

Kumlehn, J., L. Serazetdinova and G. Hensel. 2006. Genetic transformation of barley (*Hordeum vulgare* L.) via infection of androgenetic pollen cultures with *Agrobacterium tumefaciens*. Plant Biotech. J. 4:251-261.

Maluszynski, M. and K. J. Kasha. 2002. Mutations, in Vitro and molecular techniques for environmentally sustainable crop improvement. Kluwer Academic Publishers, Dordrecht, 246p.

Nagata, T. and I. Takebe. 1971. Plating of isolated tobacco mesophyll protoplasts on agar medium. Planta 99:12-20.

Rizkalla, A. A., A. M. Badr-Elden and A. A. Nower. 2007. Protoplast isolation, salt stress and callus formation of two date palm genotypes. J. Appl. Sci. Res. 3(10):1186-1194.

Saker, M., M. Bekheet and H.S. Taha et al. 2000. Detection of seasonal variations in tissue culture derived date palm plants using isozyme analysis and RAPD fingerprints. Biol. Plant. 43:347-351.

Saker, M., S. S. Adawy, A. A. Mohamed and H. A.El-Itriby. 2006. Monitoring of cultivar identity in tissue culture-derived date palms using RAPD and AFLP analysis. Biol. Plant. 50: 198-204.

Saker, M., M. A. Allam, A. H. Goma et al. 2007. Optimization of some factors affecting genetic transformation of semi-dry Egyptian date palm cultivar (Sewi) using particle bombardment. J. Genet. Eng. Biotech. 5:1-6.

Saker, M., H. Ghareeb and J. Kumlehn. 2009. Factors influencing transient expression of *Agrobacterium*-mediated transformation of GUS gene in embryogenic callus of date palm. Adv. Hort. Sci. 23:150-157.

Sedra, M. Y. H. 2007. Selection of Morphological Characteristics and Molecular Markers and their Use for Identification and Distinguishing between Date Palm Varieties and the Plants Issued from Tissue Culture. Proceeding of the Fourth Symposium on Date Palm King Faisal University, Hofuf, 5-8 May 2007, Kingdom of Saudi Arabia.

Sharma, H. C., J. H. Crouch and K. K. Sharma et al. 2002. Applications of biotechnology for crop improvement: prospects and constraints. Plant Sci. 63:381-395.

Smith, R. J. and J. S. Aynsley. 1995. Field performance of tissue cultured date palm (*Phoenix dactylifera* L.) clonally produced by somatic embryogenesis. Prin 39:47-52.

Subaith, W. S., M. A. Shatnawi, and R. A. Shibli. 2007. Cryopreservation of date palm (*Phoenix dactylifera*) embryogenic callus by encapsulation-dehydration, vitrification and encapsulation-vitrification. Jordan J. Agric. Sci. 3:156-170.

Sudhersan, C., Y. Al-Shayji and Y. Jibi and S. Manuel. 2009. Date palm crop improvement via T x D hybridization integrated with *in vitro* culture technique. Acta Hort. 829:219-224.

Takebe, I., G. Labib and G. Melchers. 1971. Regeneration of whole plants from isolated mesophyll protoplast of tobacco. Naturwissenschaften 58:318-320.

Trifi, M., A. Rhouma, M. Marrakchi. 2000. Phylogenetic relationships in Tunisian date-palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. Agron. 20:665-671.

Trifi, M. 2001. Polymorphisme et typage moléculaire de variétés tunisiennes de palmier dattier (*Phoenix dactylifera* L.): relation avec la résistance au bayoud. Thèse de Doctorat d'Etat, Université de Tunis El Manar, Faculté des Sciences de Tunis, Tunisie, p.141.

Valkonen, J. P. T., Y. S. Xu, S. Pulli, E. Pehu and Y. M. Rokka. 1994. Transfer of resistance to potato leafroll virus, potato virus Y and potato virus X from *Solanum brevidens* to S. *tuberosum* through symmetric and designed asymmetric somatic hybridization. Ann. Appl. Biol. 124:353-362.

Wang, Q., M. Mawassi, P. Li, R. Gafiny, I. Sela and E. Tanne. 2003. Elimination of grapevine virus A (GVA) by cryopreservation of *in vitro*-grown shoot tips of the *Vitis vinifera*. L. Plant Sci. 165: 321-327.

Wang, Q., Y. Liu, Y. Xie and M. You. 2006. Cryotherapy of potato shoot tips for efficient elimination of potato leafroll virus (PLRV) and potato virus Y (PY). Potato Res. 49:119-129.

Witjaksono, W. 2003. Peran bioteknologi dalam pemuliaan tanaman buah tropika. Seminar Nasional Peran Bioteknologi dalam Pengembangan Buah Tropika. Kementerian Riset dan Teknologi RI & Pusat Kajian Buah Buahan Tropika, IPB. Bogor, 9 Mei 2003.