Emir. J. Food Agric. 2012. 24 (5): 400-407
http://ejfa.info/

REVIEW ARTICLE

In vitro mutagenesis for improving date palm (*Phoenix dactylifera* L.)

S. Mohan Jain*

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

Abstract

Genetic variability is needed for the improvement of crops, which can be either spontaneous or induced by mutagen treatments. The frequency rate of spontaneous mutations is very low and can't be used for plant breeding in developing new cultivars. Mutation breeding has been quite successful for producing new mutant cultivars with desirable traits in both seed and vegetative propagated crops (http://www-mvd.iaea.org). Somatic embryogenic cell cultures of date palm were irradiated with gamma radiation, and regenerated plants were transferred in the greenhouse and treated with Bayoud toxin, isolated from the causal fungus *Fusarium oxysporum* f. sp. *albedinis*. Several selected putative mutants tolerant to Bayoud disease were initially maintained in the greenhouse and finally transferred in the field for further evaluation. These plants have maintained tolerance to Bayoud disease under field conditions.

Key words: Bayoud, Gamma radiation, Mutatgen, Mutation breeding, Somatic embryogenesis

Introduction

The unique characteristic of date palm (*Phoenix dactylifera* L.) is a life-line to people living in Sahara and sub-Sahara regions. It is considered as one of the most ancient plants which was cultivated in Mesopotamia some 4,000 years ago (Omar and Hameed, 2006; Dakheel, 2003). Perhaps date palms 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 global warming, and that is why it is highly desirable to pay more attention to the genetic improvement of date palm cultivars that could withstand natural calamities without compromising the yield and quality. Dakheel (2003) showed a unique date palm production system under harsh climatic conditions: high resilience and tolerance to environmental stresses-high temperature and radiation, low soil and atmospheric moisture, extended periods of drought, high salinity levels, and large diurnal and seasonal fluctuations.

Received 2 April 2012; Revised 12 May 2012; Accepted 15 May 2012

*Corresponding Author

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

Email: mohan.jain@helsinki.fi

Date palm belongs to the monocot family Arecaceae and is an arborescent, dioecious tall evergreen and highly heterozygous plant (Jain, 2007). Date fruits are a most important source of human nutrition as well as an export item for many date palm-growing countries. The annual world production of dates in 2010 was approximately 7.9 million mt according to FAO (http://faostat.fao.org). The major bulk of date palm production, about 68% of the total world production, comes from Egypt, Saudi Arabia, Iran, United Arab Emirates, Pakistan, Algeria and Iraq. In Saudi Arabia, over 200 date palm cultivars are grown and produce 1,078,000 mt of dates, which is about 14% of the total world date production.

Date palm fruits are a source of nutrition to people living under harsh climatic conditions; and are a rich source of sweeteners, glucose, and fructose (Al-Eid, 2006). Date syrup is composed mainly of reducing sugars; glucose and fructose as major source of the sugar fraction. (Al-Ghamadi and Al-Kahatani, 1993a,b,c). Dates could be considered as an ideal food which provides a wide range of essential nutrients and potential health benefits.

Major Diseases and Pests

Date palm suffers from several diseases and insect pests leading to severe economic loss to the growers. There are about 25 diseases and disorders affecting date palm worldwide. Among them, 14 are caused by fungi (Karampour and Pejman, 2007). Date bunch fading disorder (DBF) is the most harmful phenomenon that damages both the

quality and quantity of date yield. In Iran, this disorder has caused wilting and drying of bunches and finally severe defoliation of date palm fruits during the last five years (Karampour and Pejman, 2007). In Egypt, 21 fungal species belonging to 15 genera were isolated from diseased date palm samples collected from different Egyptian localities (El-Deeb et al., 2007).

Bayoud disease is a serious threat to date palm plantations in North African Saharan and sub-Saharan regions; it is caused by a soil-borne fungus *Fusarium oxysporum f.* sp. *albedinis* (FOA). It was first observed in the Draa Valley, Morocco in 1870, and from there reached the Algerian Central oasis around 1889, and it rapidly advanced into new areas. The disease has destroyed more than 10 million palm trees in Morocco and nearly 3 million in Algeria. The rate of destruction by Bayoud disease is thus estimated at 5 percent per year (Oihabi, 2003). Presently, the disease is known to occur in Morocco, Algeria and Mauritania. Tunisia takes very drastic quarantine measures and strict surveillance to prevent the introduction of the disease from Algeria.

Quenzar et al. (2001) identified two circular plasmid-like DNAs (S and R plasmids) in the mitochondria of date palm. By employing a PCR-based approach, they showed that the presence of R plasmid and absence of S plasmid can be considered as a reliable molecular marker of Bayoud disease resistance. The study revealed that the presence of S plasmid and the absence of R plasmid are correlated to the susceptibility to Bayoud disease. This diagnostic molecular tool would ultimately become a simple, reliable, rapid and efficient approach to identify Bayoud resistant and susceptible genotypes from the large pool of date palm lines.

Red palm weevil (RPW) is a major pest in date palm growing countries in the Near East including the United Arab Emirates, Iran, Egypt and others (Oihabi, 2003). 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 tree or by fumigation. Pheromone traps are also commonly used to control RPW, which still requires more refinement for greater 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 neurotoxin, light-emission (firefly gene), and heat tolerance.

In Vitro Culture of Date Palm

The date palm is well known to propagate both sexually through seeds and vegetatively by offshoots that are produced from axillary buds situated on the base of the trunk during the juvenile life of the palm tree. Seed propagation of date palm is not appropriate for commercial production due to the heterozygous characteristics of seedlings, which is related to the dioecious nature of the date palm; half of the progeny are generally male and don't produce fruit, and also large phenotypic variation can occur in the progeny (Jain, 2006). Currently, there is no known method for sex determination of date palm at the early stage of tree development, making it rather difficult to discriminate between productive and non-productive male trees in the nursery before transplanting them to the field. Furthermore, the seed propagation method has another limitation in that the growth and maturation of seedlings is extremely low, and therefore, date palm seedling may not begin to fruit until 8-10 years of age. The ideal way would be to look for molecular markers for sex determination on the line of work done in papaya (Deputy et al., 2002).

Offshoot production is slow; their numbers are limited, laborious and can't meet the rapidly growing demand of cultivars. Normally offshoot numbers vary from 10-30, depending on the genotype, and are produced only within a certain period time in the mother palm's life (Jain, 2007). There are no field-based methods yet available for increasing the number of offshoots per plants. There are not many commercial tissue culture laboratories worldwide micropropagating date palm for large-scale plant production (for more information see: Al Kaabi and Zaid, 2003).

In vitro culture techniques such as somatic embryogenesis and organogenesis have been effectively used for large-scale plant multiplication of horticultural crops and forest trees (Jain and Ishii, 2003; Jain and Gupta, 2005; Jain and Hagmann, 2007). Plant multiplication via organogenesis is routinely followed in commercial laboratories worldwide especially in ornamental plant industries and also to some extent in fruits and cash crops like coffee, sugarcane, etc. The cost of plant production is generally high due to labor and energy costs that reduce the profit margin. Most of the commercial date palm micropropagation laboratories are operating in low labor cost countries. The performance of *in vitro* propagated plantlets seems to be better in terms of yield and early flowering. Al-Ghamadi and Al-Kahatani (1993a,b,c) made a detailed comparative analysis of fruit quality of micropropagated and conventionally

propagated plants and found no major variation affecting the fruit quality and properties. The results clearly indicate that *in vitro*-grown date palm are quite uniform in terms of fruit quality and physical properties. Smith and Aynsley (1995) reported the results of field performance of tissue culture-derived date palm clonally produced by somatic embryogenesis. These plants started bearing fruits within 4 years from field planting of small plants with a leaf length of 100 cm and 1.5 cm diameter at the base. Fruit from the tissue culture-derived plants of cv. Berhi was indistinguishable from the fruits of plants originated from suckers (offshoots). These results certainly justify the commercial scale of micropropagation procedures of somatic embryogenesis to provide rapid, cost-effective means of obtaining elite date palm planting material. However, this approach has a major bottleneck in that plant multiplication rate is highly genotype dependent, and may require modification of the culture medium depending on the genotype. For more information, see Jain (2006), who described advantages of date palm micropropagation and its limitations. Some of the major advantages of micropropagation are year round availability of plants, quality control, rapid production of plants of elite cultivars and cold storage of elite genetic material.

In vitro plant regeneration

Date palm tissue culture work has revolved around somatic embryogenesis (Fki et al., 2003; Al-Khayri, 2005) and organogenesis for plant regeneration (Khierallah and Bader, 2007; Al-Khayri, 2007). Aaouine (2003) reported plant regeneration from 30 genotypes of date palm via direct shoot organogenesis.. Furthermore, the initiation period for somatic embryogenesis induction is 4-6 months as compared to 8-10 months for organogenesis; and the total time from the induction phase to plant marketing is 40-44 months via somatic embryogenesis vs. 60 months via organogenesis (Aaouine, 2003).

Murashige and Skoog (1962) is the most commonly used culture medium for both somatic embryogenesis and organogenesis of date palm, which is also modified depending on the genotype or cultivar (Jain, 2006). Young offshoots from 2-3-year-old date palm cv. Maktoon were used for direct shoot induction after they were sterilized with commercial bleach and rinsed with sterile distilled water (Khierallah and Bader, 2007).

The somatic embryogenesis approach for date palm plant regeneration seems to be more effective for clonal propagation. Fki et al. (2003) improved somatic embryogenesis protocol of date palm cv. Deglet Noor for large-scale clonal propagation. Initially, embryogenic callus cultures were initiated from both leaf and inflorescence explants (Figure 1) on MS medium containing 0.5 and 10 mg/l 2, 4-D . These cultures were used to develop highly proliferating cell suspension cultures in liquid medium supplemented with 1 mg/l 2, 4-D. Somatic embryos were initiated from actively growing cell suspension, and finally somatic embryos were germinated and regenerated into plantlets. Abul-Soad et al. (2008) induced somatic embryogenesis of female inflorescence explants of date palm.

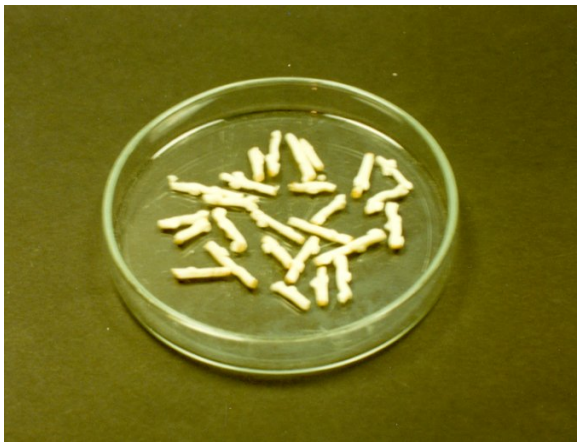

Figure 1. Date palm inflorescence for the induction of somatic embryogenesis.

The overall production of somatic embryos reached 10,000 units per liter per month. The partial desiccation of the mature somatic embryos significantly improved somatic embryo germination rate from 25 to 80%. The cutting back of cotyledon leaf was stimulatory to the germination rate. Furthermore, flow cytometry analysis showed no variation in ploidy level of somatic seedlings. Several research groups have modified the culture medium composition by adding vitamins, adenine sulfate, thiamine, glycine, glutamine, myo-inositol and activated charcoal (Al-Khayri, 2005). The role of vitamins in date palm tissue culture is not known. For more details see Jain (2006) and Al-Khayri 2005).

Advantages of Somatic embryogenesis

- Somatic embryos originate from a single cell and minimize or eliminate chimera depending on the plant species

- Somatic embryo cell suspension is ideal for mutation induction due to production of direct mutant somatic embryos
- Somatic embryos behave like a zygotic embryo in germination
- Single somatic embryo can be encapsulated to develop into a somatic seed that could germinate like a normal seed. This aspect still requires further research for use at a commercial scale
- Most suitable approach in woody species for plant regeneration
- Somatic embryos can be produced in a bioreactor which could be automated for large-scale production of somatic embryos
- Somatic embryos are suitable for long term storage by cryopreservation

Disadvantages of somatic embryogenesis

- Somatic embryogenesis is highly genotypic dependent and therefore culture medium modification may be needed for different genotype.
- Germination rate of somatic embryos is very poor in most of the crops
- Somatic embryogenic cultures can lose their property if they are not sub-cultured regularly on the fresh culture medium, and that raises the chance of getting genetic variability.

Mutation Induction

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 paper, we will focus on 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 resistance, high yield, etc. First of all, gamma irradiation breaks DNA into small fragments and secondly DNA starts a repair mechanism. During this second 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 cultivars in North Africa (Jain 2005, 2006). Mutation induction in date palm is feasible now due to a reliable plant regeneration system via somatic embryogenesis and organogenesis. The somatic embryogenesis system is the more preferable approach due to single cell origin of somatic embryos that prevents or reduces the occurrence of chimeras. Moreover, mutant somatic embryos are germinated into direct plantlets in a single step, avoiding the 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, 2007).

Mutagen selection and treatment

Mutagens are mainly classified in two types: a) chemical and b) physical (ionizing radiations) (Jain, 2007). The main aim is to develop efficient methods for inducing the highest rate of gene mutations with the lowest chromosomal or physiological damage to the mutagen treated plant material. The mutagen selection is very much dependent on the plant material, e.g. organs (shoot tips, meristems, axillary buds) or cell suspension or protoplasts. *In vitro* mutagenesis, protocols for preparing cultures are similar to micropropagation for *in vitro* mutation breeding (Predrieri, 2001).

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}. Then isolated mutant cells are removed and transferred to fresh culture medium with reduced selection pressure allowing them to recover from the initial selection pressure for about 1 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 two generations devoid of selection pressure and then put them back again under the selection pressure. This step is done to make sure that the selected mutant lines are stable due to genetic rather than 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.

The other option is to select mutants at the whole plantlet level, e.g. by spraying herbicide or withholding water 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 have already been in the field for four years and have not shown any sign of disease.

Chemical mutagen treatment

In this section, we will describe the ethyl ethane sulfonate (EMS) mutagen treatment, which is most commonly used for mutation induction. Similar steps can be used for other chemical mutagen with some minor modification in the protocol.

The doses flanking the LD_{50} value shall be selected for generating the mutant populations for evaluation. The quantity of callus and cells (from suspensions) and the number of cultures to be treated need to be judged from their growth rates and regeneration potential. Use high cell density for mutagen treatment and select growing cell clumps after plating of mutagen treated cell suspension on a solidified culture medium. Thus all dead cells are eliminated. The larger the surviving population of cells the better it is for regeneration of large number of plants, which is ideal for selection of mutant lines. Given the low differentiation levels during the *in vitro* phase, cultures need to be critically and regularly observed for variations. The response of the cultures (in terms of media color change, alterations in growth rate, somatic embryo germination rate, plantlet morphology, etc.) to the mutagenic treatments shall be recorded at every stage.

- Prepare a fine cell suspension, clump size 3-4 cells, in the liquid medium from a fragile callus. Determine the doubling time of cells, growth and stationary phase.
- Carefully add freshly prepared EMS (0.5-1.0%) solution in actively growing cell suspension in the growth phase stage
- Use high cell density 100,000 cells/ml
- Shake them while mutagen treatment is on, 100 rpm on a horizontal shaker, for 6 hrs. Treatment time can be modified for other crops.
- Transfer cells in centrifuge tubes and centrifuge at 5,000 or 6,000 rpm for 5 min
- Remove the pellet, add fresh culture medium, and centrifuge at 5000 or 6000 rpm and repeat this process 5-6 times for removing mutagens. Utmost care should be taken to dispose of mutagens while washing the cells.
- Spread uniformly mutagen treated cells on the upper surface of agar culture medium containing plant growth regulators, and allow them to stand for 96 hrs to recover from mutagen treatment shock. We can also put a sterile filter paper on the upper surface of the agar medium and transfer cells.
- Put mutagenized cell population under the selection pressure, e.g. fungal toxin for the selection of disease resistant lines. In case of strawberry, crude extract of a fungus *Phytophthora cactorum* which is a causal agent of fruit rot disease was used. Filter sterile crude fungal extract is directly added in the culture medium. Grow mutagenized cell population on this medium and select surviving clump of cells and transfer to the fresh culture medium containing crude extract.
- The surviving tissue should be transferred onto the fresh shoot regeneration medium for callus production or direct shoot formation depending on plant or genotype. Keep the selection pressure during shoot differentiation.
- Harden *in vitro* plantlets in the greenhouse under 70-90% relative humidity
- Perform initial screening of plantlets, 100-200 at one time, in the greenhouse by inoculating each plant with fungal crude extract
- Field testing in “hot spot” areas under field conditions.

Physical mutagen treatment

Cell suspension

- Prepare and use actively growing cell suspension, as described in section 3.1.
- Spread cells uniformly on filter paper and place them on a solidified culture medium in a Petri dish and seal it with parafilm. The advantage of using filter paper is that cells are uniformly spread and easy to transfer on the fresh culture medium.
- Irradiate cells with an optimal dose, LD50, of gamma rays or any other physical mutagen
- Transfer irradiated cells onto the fresh culture medium in order to recover from irradiation shock for 96 h.
- Follow steps as described in section 3.1.

Determination of radiosensitive dose

All plants show variation in radiosensitivity and therefore it is important to make a radiosensitive curve to determine LD_{50} dose for mutation induction. High radiation doses are detrimental to the plant genome and cause heavy damage to DNA. This leads to large number of mutations, which are mostly undesirable, cumbersome to identify any useful mutants, and handling of mutant population becomes more difficult. In some crop plants, low radiation dose promotes shoot growth, e.g. in citrus

30 Gy dose stimulates shoot growth (Jain, personal communication) and 10 Gy maintains the somatic embryogenesis nature of date palm for up to 3 years (Drira, personal communication). In date palm 20-30 Gy was used for mutation induction depending on the genotype used. For Deglet Noor date palm cv., LD_{50} of somatic embryogenic cell suspension cultures were 20 Gy and used for mutation induction. Actively growing cell suspension in the growth phase was transferred on the filter paper in the Petri dish. Cell clumps were uniformly spread on the filter paper and the dishes were sealed with Para film. Cells were irradiated with different gamma radiation doses and they were transferred for overnight to the fresh solid culture medium for recovery from the radiation treatment. The irradiated cells were transferred in the liquid medium, and distributed in 50 ml flasks containing 30 ml liquid medium. The cell viability test was made with FDA staining to determine the cell survival rate after the different radiation dose treatments. The number of surviving cells was calculated on the basis of cells per ml per radiation dose. After this step, in each flask, 100,000 cells per ml were added as starting material and the cell growth was determined after 1 week. The number of cells per ml per radiation dose treatment was counted with a haemocytometer. These results established the radiosensitive curve and determined LD_{50} radiation dose for mutation induction.

Post mutagenesis handling

The aspect of *in vitro* mutagenesis is crucial for handling mutated cell population and identification of mutants. The number of subcultures depends primarily on the crop or genotype LD_{50} dose, and other factors such as proliferation rate, number of plants to be field evaluated, etc. For example, *in vitro* selected banana putative mutant lines showing resistance to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (FOC), multiplied in large numbers by micropropagation up to M1V4 generation, in the range of 500-1000 shoots, which are further rooted to develop into well-developed plantlets for greenhouse evaluation before field trials. *In vitro* shoots can be directly rooted in the greenhouse under 70-90% humidity and avoid one step of *in vitro* rooting. The number of plants is very much dependent on the greenhouse facilities for handling large number of plants at one time.

Greenhouse weaning

The putative multiplied mutant plantlets may respond differentially to greenhouse hardening and field transfer. Considerable mortality can be expected at both these stages. Hence, only experienced staff should do the greenhouse hardening and subsequent field transfer. This step requires 70-90% relative humidity in the greenhouse or plastic house otherwise *in vitro* plants would wither and die. Normally *in vitro* culture conditions, the relative humidity is over 90%. Greenhouse-hardened plants may be field evaluated following standard procedures depending on the trait to be evaluated either in a single or multi-location trials.

Greenhouse screening

Well adapted *in vitro* mutant plantlets are subjected to the selection pressure for the selection of solid mutants before transfer to the field evaluation. The selection pressure could be applied 2-3 times to make sure that the selected mutant lines are solid mutants.

For example in banana, the selection of mutant plants against *Fusarium oxysporum* f. sp. *cubense* (FOC) is done by dipping roots of 500 plants into conidial suspension (500,000 conidia per ml) of the fungus for 20 min and transferring them to sterile perlite. The selected plants could well undergo one round of selection before transfer them to the "hot spot" in the field. In date palm, mutant plants were treated with *Fusarium* toxin and selected 10 mutant plants in the greenhouse, which later on transferred in the field (Figure 2). These mutants showed tolerance similar to the check resistant cv. Saver Layalet from Morocco.

Figure 2. Mutants plant are selected after infection with Bayoud toxin.

Field trials

The selected mutant plants are directly transferred to the "hot spot" in the field depending on the selected trait, e.g. salinity, drought, acidity, soil infected with fungal disease etc. The performance of the mutant plants is evaluated on the basis of survival rate and other agronomical

aspects including yield. In date palm, Bayoud tolerant 10 mutant plants were transferred for the field evaluation, and did not show any sign of disease (Figure 3).

Figure 3. *In vitro* derived plants are growing in Zagora a Bayoud prone area for further testing.

Conclusions

Somatic embryogenic cell suspension is an excellent system for mutation induction and isolates, providing useful mutants of date palm. Direct mutant somatic embryos can be produced and germinated into mutant somatic seedlings. These mutant seedlings can further be micropropagated for large-scale production. The utmost care should be taken while handling somatic embryogenic cultures, and, failing to do, the chances of getting somaclonal variation becomes very high. This approach is an excellent example of combining mutagenesis and biotechnology for date palm improvement. Transgenic date palms have a long way to go before consumers accept to consume them and consequently the export market will also be lost. Therefore, the transgenic approach to modify date palm should be followed with great caution, even though it has a great potential to overcome several problems. There is a complete lack of date palm molecular biological research to address several issues facing date palm genetic improvement. Molecular marker-assisted selection and breeding need serious attention by identifying trait specific genes from natural or induced genetic variability. Functional genomic date palm breeding would probably become a reality in time to come and that would speed up genetic improvement of date palm.

References

Abul-Soad, A. A., G. S. Markhand and S. A. Shah. 2008. Effect of Naphthaleneacetic acid and Indole-3-acetic acid on somatic embryogenesis of female inflorescence explants of date palm (*Phoenix dactylifera* L.) Aseel cv. pp. 222-231. In: The 3rd International Conference on Date Palm. 25-27 April, Faculty of Agric. and Env. Sci., Suez Canal University, North Sinai, Egypt.

Al-Eid, S. M. 2006. Chromatographic separation of fructose from date syrup. Int. J. Food Sci. Nutr. 57:83-96.

Al-Ghamdi, A. S. and M. S. Al-Kahtani. 1996a. True-to-type date palm (*Phoenix dactylifera* L.) produced through tissue culture. 5. Fruit chemical properties. pp. 55-67. In: Proc. 3rd Symposium on the Date Palm. Vol. 1. Mars Publishing, Riyadh, Saudi Arabia.

Al-Ghamdi, A. S. and M. S. Al-Kahtani. 1996b. True-to-type date palm (*Phoenix dactylifera* L.) produced through tissue culture. 6. Fruit sugar content. pp. 67-78. In: Proc. 3rd Symposium on the Date Palm. Vol. 1. Mars Publishing, Riyadh, Saudi Arabia,

Al-Ghamdi, A. S. and M.S. Al-Kahtani. 1996c. True-to-type date palm (*Phoenix dactylifera* L.) produced through tissue culture techniques. 7. Mineral content. pp. 81-90. In: Proc. 3rd Symposium on the Date Palm. Vol. 1. Mars Publishing, Riyadh, Saudi Arabia.

Al-Khayri, J. M. 2005. Date palm, *Phoenix dactylifera* L. In: Jain, S.M. and P.K. Gupta (Eds.) pp. 309-320. Protocols for Somatic Embryogenesis in Woody Trees, Springer, Dordrecht.

Al-Khayri, J. M. 2007. Protocol for micropropagation of date palm, *Phoenix dactylifera*. pp. 509-526. In: Jain, S.M and H. Hagman (Eds.) Protocols for Micropropagation of Woody Trees and Fruits. Springer, Dordrecht.

Around, M. 2003. Date palm large-scale propagation through tissue culture techniques. pp 79-86. In: The Date Palm from Traditional Resource to Green Wealth. Emirates Centre for strategic studies and research, Abu Dhabi, UAE.

Dakheel, A. 2003. Date palm and biosaline agriculture in the United Arab Emirates. pp. 199-211. In: The Date Palm from Traditional Resource to Green Wealth. Emirates Centre for strategic studies and research, Abu Dhabi, United Arab Emirates.

Deputy, J.C., R. Ming, H. Ma., Z. Liu, M.M. Fitch, M. Wang, R. Manshardt, and J.I. Stiles. 2002. Molecular markers for sex determination in papaya (*Carica papaya* L.). Theor. Appl. Genet. 106:107-111.

El-Deeb, H. M., S. M. Lashin and Y. A. Arab. 2007. Distribution and pathogenesis of date palm fungi in Egypt. Acta Hort. 736:421-429.

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.

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. 2006. Radiation-induced mutations for developing Bayoud disease resistant date palm in North Africa. pp 31-41. In: Zaid, A. (Ed.) Proceedings of the International Workshop on True-to-typeness of Date Palm Tissue Cultured-derived Plants. Plant tissue culture laboratory, UAE University, Al Ain, UAE.

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

Jain, S. M., and K. Ishii (Eds.) 2003. Micropropagation of Woody Trees and Fruits. Springer, Dordrecht.

Jain, S. M. and P. K. Gupta (Eds.) 2005. Protocols for Somatic Embryogenesis in Woody Trees. Springer, Dordrecht.

Jain, S. M. and H. Haggman (Eds.) 2007. Protocols for Micropropagation of Woody Trees and Fruits. Springer, Dordrecht.

Karampour, F. and H. Pejman, 2007. Study on possible influence of pathogenic fungi on date bunch fading disorder in Iran. Acta Hort. 736:431-439.

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

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Phys. Plant. 15:473-497.

Oihabi, A. 2003. Major pests of the date palm. pp 143-147. In: The Date Palm from Traditional Resource to Green Wealth. Emirates Centre for Strategic Studies and Research, Abu Dhabi, UAE.

Omar, M. S. and M. K. Hameed, M. K. 2006. *In vitro* propagation of date palm. In: Zaid, A. (Ed.) pp. 86-92. Proceedings of the International Workshop on True-to-typeness of Date Palm Tissue Cultured-derived Plants. Plant Tissue Culture Laboratory, UAE University, Al Ain, UAE,

Predieri, S. 2001. Mutation induction and tissue culture in improving fruits. Plant Cell Tiss. Org. Cult. 64:185-210.

Quenzar, B., M. Trifi, B. Bouachrine, C. Hartman, M. Marrakchi, A.A. Benslimane and A. Rode. 2001. A mitochondrial molecular marker of resistance to Bayoud disease in date palm. Theor. Appl. Gen. 103:366-370.