Chapter 18
Advances in Date Palm (*Phoenix dactylifera* L.) Breeding

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Abstract Date palm is one of the oldest cultivated plants, grown in the arid and semi-arid regions of the world. The date fruit serves as a vital worldwide component of the human diet and a staple food for millions of people. Unfortunately, various abiotic and biotic stresses along with agronomic constraints are hindering date productivity. Those date cultivars adapted to stress conditions have low fruit production. Conventional breeding, depending on crosses and backcrosses, is a time-consuming process. The applied research carried out on date palm is limited, still there is enormous potential to improve date palm breeding methods. Advanced biotechnology creates unparalleled opportunities to develop new varieties with quality fruit, increased fruit yield and resistance to pests and pathogens. It also minimizes the application of potentially-harmful fungicides and pesticides and increases crop productivity. This chapter provides current and innovative information about date palm progress in terms of distribution, production, marketing strategy, current achievements, limitations and challenges facing date palm breeding. It also focuses on recent advances in tissue culture, genetic transformation and molecular breeding to improve the productivity and quality of the date.

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18.1 Introduction

Date palm (*Phoenix dactylifera* L.) belongs to the family Arecaceae (Palmae) and is one of mankind's oldest cultivated plants. Based on archeological evidence, Iraq is considered as the center of origin (Johnson et al. 2013). The majority of the date palm groves are distributed in the region of the Middle Eastern countries where the date is an important commercial crop with high cultural and religious value. Date fruits harbor nutritional sources such as essential minerals, antioxidants, vitamins, amino acids and phenolic compounds (Kamal-Eldin and Ghnimi 2018; Vayalil 2012). The production and utilization of date fruits vary from one country to another due to the influence of climatic conditions, cultivation practices and farm management. The date fruit has a significant international market from which any country can build a strong economy (El Hadrami et al. 2011a).

Most of the date palm growing countries practice conventional breeding methods, and they are facing problems related to germplasm identification, sex determination, agronomic traits, abiotic stress and biotic stress (Jain et al. 2011). The major date palm growing countries are located in the desert, and experience harsh environmental conditions or abiotic stresses, such as high temperature, drought and salinity (Shabani et al. 2012). These abiotic stresses affect the physiology and biochemical process of the date tree and ultimately productivity. Bayoud disease caused by *Fusarium oxysporum* f. sp. *albedinis* and red palm weevil (*Rhynchophorus ferrugineus* Oliv.) pest are the two deadliest threats to the date palm. The application of fungicides and pesticides is ineffective in controlling both maladies. To overcome these constraints, along with the conventional breeding, advanced biotechnological tools need to be applied.

Recent plant biotechnological tools such as plant tissue culture, protoplast culture, embryo rescue, in vitro mutagenesis, cryopreservation, genetic engineering, marker-assisted selection and genomics represent new means to create date fruits with desired traits. The present chapter deals with the distribution, production, marketing strategy, current achievements and challenges facing date palm breeding. It also reports on the latest applications of tissue culture, genetic engineering and molecular breeding towards the improvement of productivity and quality as well as tolerance to abiotic stresses and disease/pest resistance.

18.1.1 Botanical Overview

The scientific name *Phoenix dactylifera* is derived from the word *phoenix*, a long-lived bird from Greek mythology and *dactylos* meaning *finger* which refers to the shape of the fruit. The date palm is dioecious in nature, where separate trees bear male and female organs. The mature date palm reaches up to 30 m height, branches are absent on the trunk and it is the tallest among the Phoenix species. The plant possesses a well-developed root system and also one terminal shoot apex responsible for linear growth. The leaves are arranged alternatively, pinnate and erect with a number of stiff leaflets. An adult date tree contains about 100–120 leaves of about 3–6 m in length and forms the terminal rosette crown. In a year a plant produces 12 leaves with a life span of each leaf of 3–7 years. The leaf is very tough and needle-like, adaptations which may be to protect the growing tip against grazing animals during its early years of development and under drastic environmental conditions. Auxiliary buds are produced from the leaf axil that may be vegetative, floral or intermediate (Bouguedoura 1991; Bouguedoura et al. 1990). During the early years of growth, auxiliary buds can form shoots, called *suckers* or *offshoots*, and produce inflorescences. The appearance of fruiting is from auxiliary buds within the terminal rosette in the form of branched clusters. Male and female flowers are found on separate palms and only female trees bear fruits. The flowers are white in color and small in size, richly branched, the spadix surrounded by a large single spathe. The calyx and petals are three-toothed and cup-shaped. (El Hadrami and Al-Khayri 2012). Flowers consist of three ovaries out of which only one develops into a fruit. Six stamens have a linear dorsifixed anther arrangement. Pollination is anemophilous and artificial pollination is recommended to achieve high productivity. A fully productive date palm tree can yield more than 100 kg of fruit annually, productivity depending on the cultivar, environmental conditions and cultivation practices. There are four stages of fruit development viz. Kimri, Khalal, Rutab and Tamar stages (the names of Arabic origin) containing average moisture contents of about 80, 60, 40 and 20%, respectively (Al-Shahib and Marshall 2002; Fayadh and Al-Showiman 1990). The date fruit is usually cylindrical, with a single elongated seed, variable in size, 2.5–7.5 cm long × 4 cm wide with a fleshy sweet mesocarp covered with a thin epicarp, yellowish to reddish brown in color (Purseglove 1972; Saker 2011). A date palm plantation has an economic life of about 50 years, subsequently yield decreases (Johnson et al. 2015).

18.1.2 Distribution and Biodiversity

Date palm is believed to be the oldest cultivated tree. The earliest records go back 7000 years in Iran, Egypt and Pakistan, while early cultivation was found around 3700 B.C. (Munier 1973) in the area between the Euphrates and the Nile rivers. From these regions date palm spread to the rest of the world mainly between 9–39° north latitude of arid and semiarid regions with suitable climatic conditions. The geographical distribution of date palm was driven by a number of factors such as zoochory, ornithochory and anthropochory. The center of origin of date palm is still debatable although available evidence suggests it originated from the region of Mesopotamia, modern Iraq (Johnson et al. 2013; Wrigley 1995). In Dalma, an Abu

Fig. 18.1 Date palm distribution in the world. *Source of blank map* http://onlinemaps.blogspot.com/2011_11_01_archive.html

Dhabi island, the oldest ^{14}C-dated seeds were found. The two oldest seeds, dated to 5110 B.C. and 4670 B.C., were found in 1998 (El Hadrami et al. 2011a). It has been reported that ancient date seeds over 2000 years old were found in the Judean Dead Sea region during an excavation, and the seeds were tested for viability, germinated and produced a date palm similar to that of modern date seedlings (Ben-Yehoshua and Ben-Yehoshua 2012; Sallon et al. 2008).

At present, date palms are abundant in many Old World arid regions, and to a lesser extent in temperate zones. The major date production is in Middle Eastern countries such as Iraq, Iran, Saudi Arabia, United Arab Emirates, and also in African countries where the plant grows in drier regions (Fig. 18.1). There are hundreds of different date cultivars grown worldwide with different colors, tastes, texture and sizes due to varietal diversity and growth conditions. Date culture apparently spread throughout the Arabian Peninsula, North Africa and the Middle East from its center of origin. The expansion of Islam extended the cultivation of date palm and it reached to Spain and Pakistan (Chao and Krueger 2007). Based on rich historical evidence and documents it has been revealed that that the introduction of date seeds to America was from Spain. The Spanish were the first to introduce date palms outside the Arabian Peninsula and North African countries; beginning in the late nineteenth century offshoots carried them directly to the USA (Nixon 1951; Rivera et al. 2013). In the past century or so, a number of elite cultivars have been traded throughout the world for modern date palm plantations (Johnson et al. 2015).

18.1.3 World Production and Economics

The date palm is an important crop, not only because of its history as one of the oldest crops of human civilization; it is an economically-important crop grown widely. World annual production of dates amounted to 6.4 million mt in 2003 (El Hadrami and El Hadrami 2009), over 6.8 million mt in 2010 (FAOSTAT 2010) and according to the FAOSTAT (2012) the world date fruit production reached over 7.5 million mt in 2012. The production of each country is listed in Table 18.1 in descending order of production magnitude. According to this, Egypt, Saudi Arabia and Iran are the top three date producing countries. About 100 million date palms are present in the world's estimated area of 1 million ha, out of these 60% is present in North Africa and the Middle East, where the date is of cultural and religious importance to the people in these regions (Wakil et al. 2015). Date production offers employment and it is estimated around 50 million people are benefitted, out of which 35% are located in the southern Mediterranean countries (El Hadrami and El Hadrami 2009). Date fruit is not only a staple food of the local population of the many countries, it also yields significant contributions towards the economic status of those countries (Chao and Krueger 2007). The production of dates and their annual trade market value is estimated at almost 2 billion USD (El Hadrami et al. 2011a). India is the world's largest importer of dates with over 250,000 mt and an expenditure of 70 million USD per year. Iran became the leading exporter with over 240,000 mt, generating annual revenues of over 80 million USD. Medjool is the most popular and appreciated cultivar and Deglet Noor is the most exported cultivar (Al-Khayri et al. 2015a, b).

18.1.4 Nutritional and Medicinal Benefits

Since time immemorial, date fruits have comprised a vital component of the human diet and a staple food for millions of people around the world. As a part of regular diet, dates are also consumed by people as a religious practice and as nutritional therapy in different traditions around the world. Date fruit is ascribed to have a number of medicinal properties (Vayalil 2012). Dates are a high energy source in the form of carbohydrates (fructose and glucose) constituting about 70–80%. These carbohydrates are quickly assimilating by the human body (Al-Farsi et al. 2005a; Saafi et al. 2008). Dates are also a good source of essential minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, selenium and manganese which enhance their nutritional values (Al-Farsi et al. 2005a; Al-Shahib and Marshall 2002, 2003; Elleuch et al. 2008). The nutritional value of date cvs. Deglet Noor and Medjool are listed in the Table 18.2. Studies have revealed that aqueous extracts of date have a potential antioxidant and antimutagenic activity (Al-Farsi et al. 2005b, 2007; Biglari et al. 2008; Saafi et al. 2009; Vayalil 2002). The antioxidant activity is due to the presence of wide range of phenolic compounds present in the fruits, viz. gallic, protocatechuic, *p* hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic and

Table 18.1 World production of date fruit

Ranking	Country	Production (mt)
1	Egypt	1,470,000
2	Iran	1,066,000
3	Saudi Arabia	1,050,000
4	Algeria	789,357
5	Iraq	650,000
6	Pakistan	600,000
7	Sudan (former)	433,500
8	Oman	270,000
9	United Arab Emirates	250,000
10	Tunisia	190,000
11	Libya	170,000
12	Morocco	113,397
13	Yemen	55,181
14	Israel	42,866
15	Kuwait	34,600
16	USA	28,213
17	Mauritania	22,000
18	Qatar	21,843
19	Chad	20,000
20	Niger	17,000
21	Bahrain	15,000
22	Somalia	13,000
23	Jordan	10,417
24	Mexico	6012
25	Spain	4000
26	Syria	3986
27	Palestine	3600
28	Benin	1300
29	Kenya	1100
30	Cameroon	600
31	Namibia	400
32	Peru	400
33	Djibouti	86

Source FAOSTAT (2012)

o-coumaric acid as well as sinapic acids, flavonoids and procyanidins (Al-Farsi et al. 2005b; Biglari et al. 2008; El Hadrami et al. 2011b; Hong et al. 2006; Jaiti et al. 2009; Mansouri et al. 2005). Flavonoids such as luteolin, quercetin and kaempferol, as well as more or less polymerized proanthocyanidins are also found in abundant quantities in fresh dates (El Hadrami and Al-Khayri 2012). Dates also comprise a good source of vitamins, especially β-carotene (vitamin A) thiamine (B1), riboflavin (B2), niacin, ascorbic acid (C) and folic acid (folacin) (Al-Shahib and Marshall 2002; El Hadrami and El Hadrami 2009; El Hadrami et al. 2011b). Dates provide essential amino acids which cannot be synthesized by the human body. Assirey (2015) reported the presence of amino acids in date extract viz. aspartic acid, proline, glycine, histidine, valine, leucine, arginine, threonine, serine, methionine, isoleucine, tyrosine, phenylalanine, lysine and alanine. Dates are also the source of a number of secondary metabolites such as carotenoids, polyphenols especially phenolic acids isoflavons, lignans, and flavonoids, tannins and sterols Dates are also a rich source of low glycemic index sugar fructose (Al-Farsi and Lee 2008; Miller et al. 2003; Vayalil 2012).

Indian and Middle Eastern traditional medicine, Ayurveda and folklore, respectively, consider the date fruits to have a number of medicinal properties (Puri 2002). Dates are purported to act as a demulcent, aphrodisiac, nutrient, emetic, laxative, expectorant and are widely used to treat tuberculosis, gastroenteritis, coughs, respiratory diseases, asthma, diabetes, hypertension, various infectious diseases and cancer (Puri 2002; Tahraoui et al. 2007). Dates are claimed to have potential use to treat cardiovascular, cerebrovascular diseases and bacterial and fungal infections (Vayalil 2012). Mineral compounds such as potassium, calcium and phosphorus are essential for metabolic activity in the human body, for the healthy development of bones and energy metabolism. Calcium and magnesium play an important role in the production of red blood cell iron; to treat hypertension, high content of potassium and low sodium, all of the above compounds are available in date fruits (Assirey 2015). Low glycemic index diet improves the level of blood glucose level, reduces insulin demand and reduction in the level of blood lipids which manage and play important roles in the control of a number of chronic diseases (Augustin et al. 2002; Brand-Miller 2003; Jenkins et al. 2002; Ludwig 2002).

Phenolic compounds such as phenolic acids, hydroxycinnamates and flavonoids, including tannins present in dates, are known for their beneficial effects on human health as well as acting against oxidation-linked chronic or degenerative illnesses such as cancer and cardiovascular diseases. Phenolics are well-known for inhibiting pathogens and parasites. The antioxidant metabolites contained in dates seem to be linked to the role of these phytochemicals in maintaining specific cellular homeostasis and contributing in a preventive manner to beneficial effects across diverse biological systems and cell types (Vayalil 2012). Phenolics such as caffeic and ferulic acids, highly present in dates, are known to react with nitrite and inhibit the in vivo formation of nitrosamine, hence inhibiting skin tumors (Kaul and Khanduja 1998). Phenolics contained in dates may also be able to reduce blood pressure and have antithrombotic and anti-inflammatory effects (Gerritsen et al. 1995; Muldoon and Kritchvesky 1996). In addition, phenolics are known to inhibit α-amylase and α-glucosidase activities

Table 18.2 Nutritional value of dates cvs. Deglet Noor and Medjool (nutrient values and weights are for edible portion)

Nutrient	Unit	Value per 100 g	
		Deglet Noor cv.	Medjool cv.
Proximates			
Water	g	20.53	21.32
Energy	kcal	282	277
Protein	g	2.45	1.81
Total lipid (fat)	g	0.39	0.15
Carbohydrate	g	75.03	74.97
Fiber, total dietary	g	8.0	6.7
Sugars, total	g	63.35	66.47
Minerals			
Calcium, Ca	mg	39	64
Iron, Fe	mg	1.02	0.90
Magnesium, Mg	mg	43	54
Phosphorus, P	mg	62	62
Potassium, K	mg	656	696
Sodium, Na	mg	2	1
Zinc, Zn	mg	0.29	0.44
Vitamins			
Vitamin. C, ascorbic acid	mg	0.4	0.0
Thiamin	mg	0.052	0.050
Riboflavin	mg	0.066	0.060
Niacin	mg	1.274	1.610
Vitamin B_6	mg	0.165	0.249
Folate, DFE	μg	19	15
Vitamin A, RAE	μg	0	7
Vitamin A, IU	IU	10	149
Vitamin E	mg	0.05	–
Vitamin K	μg	2.7	2.7
Lipids			
Fatty acids, total saturated	g	0.032	–
Fatty acids, total monounsaturated	g	0.036	–
Fatty acids, total polyunsaturated	g	0.019	–
Fatty acids, total trans	g	0	0

Source United States Department of Agriculture (2016) causing the postprandial increase in blood glucose level, often manifested in type-II diabetes (Andlauer and Furst 2003; McCue and Shetty 2004; Vayalil 2012).

Phytosterols are beneficial to human health and are thought to be involved in lowering the blood levels of low-density lipoprotein LDL, the so-called *bad* cholesterol. Phytosterols are also involved in blocking the absorption of dietary cholesterol into the bloodstream and in inhibiting the reabsorption of cholesterol from bile acids in the digestive process, thus reducing the amount of cholesterol returned to the bloodstream. Other health benefits may include positive effects for patients with autoimmune diseases. For instance, phytosterols were shown to reduce inflammation and suppress over-reactive immune systems in patients suffering from rheumatoid arthritis. In addition, the use of concentrated extracts of phytosterols has been shown to be effective against certain forms of cancer (i.e. prostate) and to enhance the insulin production of pancreatic cells (El Hadrami et al. 2011b).

Alpha-tocopherol (vitamin E) is often used orally to treat deficiencies, and in preventing cardiovascular diseases, diabetes and its complications, and benign prostatic hyperplasia. This vitamin is also administered against angina, thrombophlebitis, intermittent claudication, hypertension, and to prevent ischemia-reperfusion injury after coronary artery bypass surgery. Alpha-tocopherol is claimed to reduce the risks of various cancers, Alzheimer's and Parkinson's diseases and other dementias. Vitamin E is also used against allergies, asthma and other respiratory problems, as well as digestive or circulatory diseases. Additionally, vitamin E is used topically against dermatitis, aging skin, granuloma annulare and in preventing skin ulceration as a consequence of hemotherapeutic drug use (El Hadrami et al. 2011b).

18.1.5 Uses of Different Date Palm Tree Parts

Date palm is a socioeconomically and traditionally important crop; apart from the nutritious fruit, the rest of the tree parts have also been used for centuries and are a resource of the rural economy. In its entirety, the tree provides good habitat, food, shade and shelter from the extreme desert conditions. The canopy provides abundant shade and one can successfully grow vegetables, and other field crops beneath it. Other parts of the palms such as seeds, leaves, trunk and sap are used for hundreds of different purposes. In some countries, roasted and ground seed powder of the dates is used as an additive to coffee and flour (El Hadrami and Al-Khayri 2012). Soaked seeds are used as a feed for camels, horses, sheep, goats and dried powdered seeds mixed with chicken feed. The leaf and leaflets are used for making handicrafts such as beds, brooms, furniture, mats, hats, trays and baskets (Al-Khayri et al. 2015b). Thus it promotes cottage industries, and provides many employment opportunities in rural areas. The high fiber leaf material is used to make rope and twine; also, the woody midrib is used to make traditional fishing cages. The trunk or stem of the date tree is used to construct roof coverings for houses, wooden boats and timber for wood industry (Al-Khayri et al. 2015a). As well, the various tissues of leaves serve as raw material for making paper and fibers. Both trunk and leaves also serve as a good source of fuel. Tapping of the date tree provides sap, which is a good source for palm syrup, palm sugar and *jaggery*. Tapping is practiced in many countries in North and West Africa and also in India. The sweet sap is an excellent source for making sweet and alcoholic beverages; in India date palm wine is called *neera* and is commonly consumed in rural areas.

18.2 Current Challenges

World date palm cultivation is concentrated in the Middle East, North and East African countries. Egypt, Iran, Saudi Arabia, Algeria and Iraq are the top five date-producing countries of the world. In recent years there has been a decline in the diversity of date palm groves in most of the date palm growing countries due to the extensive exploitation for both economic and social factors. The production and utilization of the date fruits also varies from country to country due to the influence of current environmental conditions and agricultural priority of the countries. There are a number of constraints to the production of date palm, which can be summarized as follows: poor cultivation practices (monoculture), major pests and diseases (red palm weevil and bayoud disease), quality of soil and water (salinity and drought), poor harvest and postharvest practices, low quality of processing facilities, scarcity in technical support and extension services, an insufficient number of agricultural laborers, poor marketing strategies on a national and international level, inadequate rapid modes of transportation, limited research funds, lack of adequate advanced research infrastructure and lack of efficiency experts (Al-Khayri et al. 2015a, b; El-Juhany 2010).

18.2.1 Cultivation

Date palm cultivation has been recorded from 7000 years ago. At Mohenjo Daro in the Indus River Valley and at the site of the temple of the Moon god in Ur of lower Mesopotamia 5000- and 4500-year-old-date seeds were collected, respectively. Cultivation practices vary from one region to another. Egyptian farmers have very long experience in irrigation methods and have a traditional irrigation system in the date fields of dividing them into 10 × 10 m basins. The basin supplies uniform water amounts to the date field. To improve yield and fruit quality, fertilizer application is most important; the application of chemical fertilizer is most common (Osman 2009). Farmers are showing interest in the application of organic manure to increase date palm yield and fruit color, either alone or in combination with chemical fertilizer (Kassem and Marzouk 2010). To control pests and diseases a number of steps are taken, including destruction of infected plants along with the use of chemicals and biocontrol methods.

In Iran, farmers are following traditional practices with dates cultivated along with the other crops like citrus. Growers artificially pollinate the flowers; the days of pollination depend on the elite cultivars used (Al-Khayri et al. 2015b). Flood irrigation is the primary practice in Saudi Arabia; modern irrigation methods like drip and sprinkler methods are also used by the most farmers (Fig. 18.2). The time of application of fertilizer varies among growers in Saudi Arabia. Farmers consider manure to be superior to inorganic fertilizers. For pollination, male seedling date palms are used. To control red palm weevil, both insecticide treatment by stem injection and pheromone technology in date plantations are used (Al-Khayri et al. 2015b). Currently there is a remarkable potential to increase fruit yield and quality by adopting best-practices technology in cultivation.

18.2.2 Marketing

The global production of date fruit exceeds 7.5 million mt. Dried date fruits have a unique marketing nature for they can be stored in normal environmental conditions at low cost for a long duration. Dates are considered to be the most suitable fruit to export, with the annual export in the world market of about 420,000 mt; Egypt, Iran, Saudi Arabia, Algeria and Iraq are the leading exporters (FAOSTAT 2005). In Egypt, the market strategy is not well developed as a long chain of intermediaries exist between producer and consumer. There are three kinds of markets in Egypt viz. farmers' markets, assembly markets and wholesale markets. Depending on the quality of the date, the export price in 2004 was fixed at USD 500–1500 per mt (FAOSTAT 2005). Hanyany and Siwy cvs. are the most important Egyptian exports. Iran has annual production of 1 million mt of date fruits, of which around 55–60% is consumed domestically. The exporting percentage is 12–16% of the total production and 24–33% are wasted. In the market, quality stands first; the select cultivars should possess good taste, size, color, texture, freshness, be undamaged and free of insects.

The major cultivars of Saudi Arabia are Ajwa, Khalas, Anbara, Barhee, Sheshy and Ruzeiz and the country is giving more attention to cultivation and field production to increase their export quantity (Aleid et al. 2015). Saudi Arabian dates are exported to India, France and Germany. The comparison of export percentage of the domestic production and global exports is 6.8 and 8.7%, respectively, and the average unit price of the export is USD 1065 per mt (El-Habba and Al-Mulhim 2013). Saudi Arabia has quality dates for export to the international market, thanks to the presence of advanced date palm plantations which control the quality of dates, the presence of elite cultivars which meet the international market needs, companies with advanced technologies to launch innovative products, upgraded packing systems to the international market and finally the date is consider as a holy fruit among Muslim populations worldwide (Al-Shreed et al. 2012). Some of the important technical aspects should be taken into account in exporting dates viz. types of the dates: dates with strong physical and chemical properties are suitable for export, the size and quality of the date package, specifications required by the importing countries and mode of transport.

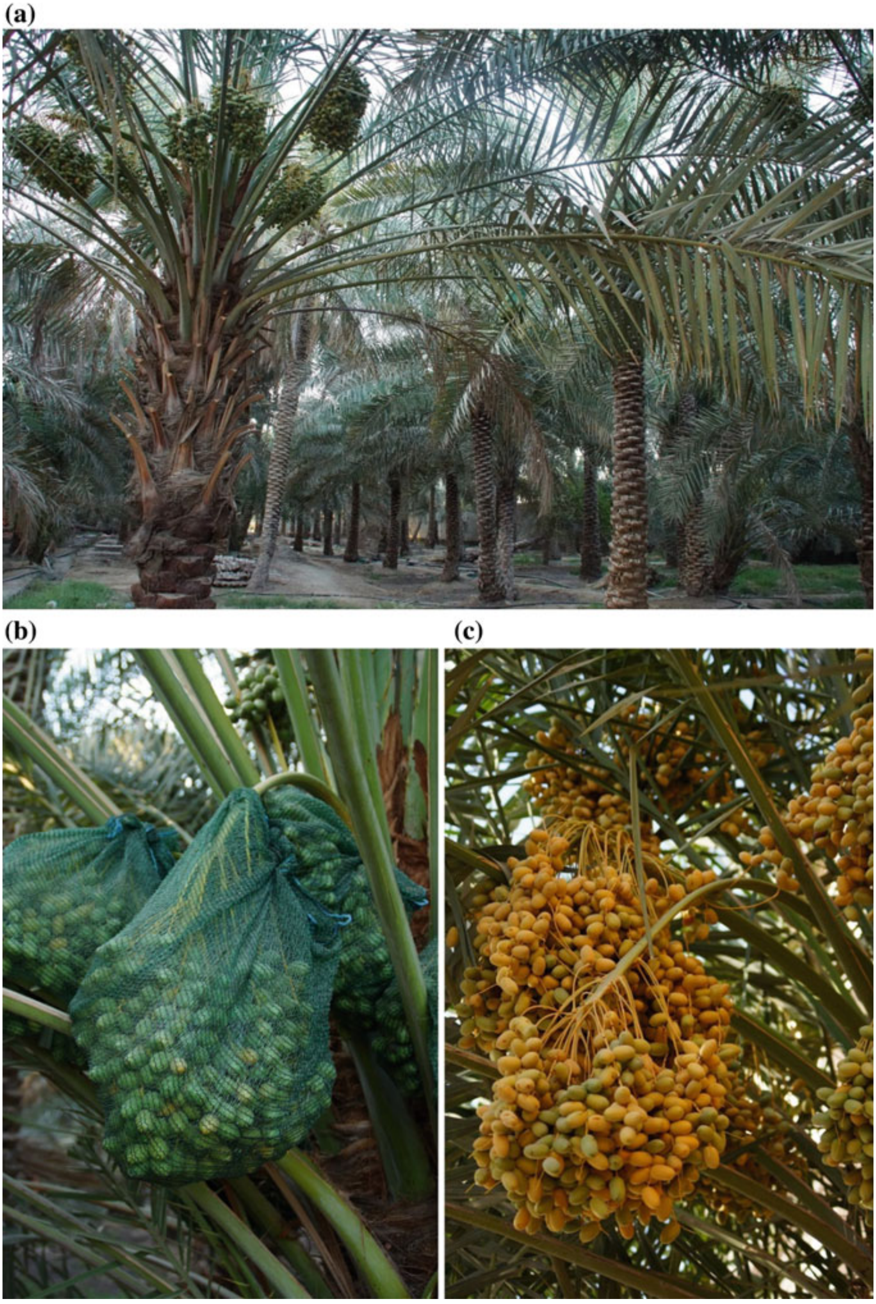

Fig. 18.2 Date palm grove. **a** Date palm plantation at Al-Hassa region of Saudi Arabia, **b** Bagged fruits at Khalal stage, **c** Rutab stage. *Photos* Courtesy of Hussain A. Alwael

18.2.3 Processing

On national as well as international markets, dates processed and properly packed have their own potential demand. Processing procedures include fumigating, sorting, washing, grading, glazing, weighing and packaging (Al-Khayri et al. 2015b). Fumigation is the primary step to disinfect the fruits. In the date industry huge losses occur due to insect attack insect and fumigation is the one method to avoid insect-caused losses. Chemicals such as phosphine and carbon dioxide are used as effective fumigants; in some cases heat treatment is also used as an alternative to date disinfection. Sorting of dates starts as soon as the harvest is over and is done manually by removing the damaged fruits and other impurities. Washing is the general procedure to remove dust, dirt and debris from the fruit. Washing with a sanitizer is important to reduce the microbial count and to avoid cross-contamination, to maintain the quality as well as shelf-life of the fruit. Generally, two types of washing systems are used in a hygienic environment, sprinklers and circular washers combined with a drier using the hot air blower system. Temperatures ranging from 55–65 °C are used to dry the date, which is considered a partial pasteurization to control the activity of enzymes and microorganisms. The final step is packing and it is a crucial process because it must meet national and international standards. Nowadays vacuum packaging technologies are applied for lengthy storage of dates using plastic coverings or polyethylene-polyamide bags (Mohsen et al. 2003). In the date industry, the processing factories produce a number of value-added products such as jelly, paste, jam and syrup. Date pastes are used in the baking industry, in pastries, pickles, chutney, candy, biscuits, cake, bread and chocolate; it is also used in the preparation of animal feed, alcohol, vinegar and citric acid production (Al-Khayri et al. 2015a, b). Date syrup serves as a sweetener in traditional foodstuffs. In addition, date extracts are used in pharmaceutical, cosmetic and medicinal products.

18.3 Conventional Breeding

Plant breeding is the branch of agriculture wherein desired characters are generated by altering the plant traits. The practice of agriculture originated 8000–10,000 years ago, when farmers simply selected the best-looking crop plant with desired characters; over generations, valuable traits were accumulated. Plant breeding focuses on improvement of present cultivated crops by developing new varieties that are higher-yielding, possess highly-valued agronomical traits, such as fruit quality, disease resistance and can tolerate extreme environmental conditions. Plant breeding success depends on the collection and selection of germplasm, evaluation of the crop for desired characters, multiplication of germplasm and release and distribution of new varieties. Date palm is one of the oldest cultivated crop and up to now most countries have followed conventional breeding methods. Date palm breeding objectives mostly depend on regional problems such as abiotic and biotic stresses (Jain et al. 2011).

18.3.1 Breeding Achievements

The combination of conventional breeding and applied biotechnological tools will introduce genetic variations and produce fast-propagating new genotypes with superior fruit quality, and resistance to biotic and abiotic stresses. In date-palm breeding, one of the major objective is reduction of plant height. Use of tissue culture techniques and conventional breeding in Kuwait have produced a cross between tall female cvs. and a dwarf palm (*Phoenix pusilla*), which has set seedless fruit (Sudhersan et al. 2009). From these, embryos were rescued and cultured in vitro to regenerate a plant. The plants are acclimatized and transferred into the field and progeny selected and tested for their inheritance of dwarfism. The effective identification of the genotype is becoming possible by using biochemical and molecular markers (Al-Faifi et al. 2016; Bendiab et al. 1993; Qacif et al. 2007; Yusuf et al. 2015;). The identification of date-palm gender is not possible before the onset of fruiting, which takes 5–7 years; scientists have made a series of attempts to identify the sex of date palm using sex specific DNA markers and in some cultivars they have succeeded (Heikrujam et al. 2015; Maryam et al. 2016; Milewicz and Sawicki 2013).

Bayoud is the one of the deadliest diseases of date palm; Morocco and Algeria have experienced great losses due to this disease (Sedra and Lazrek 2011). The disease is caused by the soil fungus *Fusarium oxysporum* f. sp. *albedinis*. Studies suggest that the fungus penetrates through the roots and then enters the vascular system, causing leaf dryness and bleaching; the name bayoud means *white*. When the fungus reaches the terminal bud the plant dies (Matheron and Benbadis 1985). Various control measures have been undertaken against bayoud disease such as agriculture practices, biological and chemical applications, but use of resistant cultivars has emerged as the most promising and effective. Several breeding programs and research groups are working to identify suitable resistant cultivars to bayoud which also produce high-quality fruits, in regions of Morocco and Algeria (Fernandez et al. 1998; Louvet and Toutain 1973; Saaidi 1992). Taqerbucht cv. was found to be the most effective against bayoud in the oases of Tidikelt and Salah, Algeria (Al-Khayri et al. 2015a). Conventional breeding and application of advanced biotechnological tools will provide future solutions to the problems of date palm cultivation (Sedra 2011a).

18.3.2 Breeding Limitations

Date-palm breeding has various limitations due to environmental conditions of the desert, where the plant has to survive under extreme drought and salt stress. Date cultivars adapted to these stresses, however, have low-quality fruit production. Conventional breeding of date palm which depends on crosses and backcrosses, is time-consuming, requiring 30 years to complete 3 backcrosses due the palm's long life-cycle. Another constraint is gender identification, which is not possible before the onset of fruiting because date palm reaches sexual maturity 5–7 years after planting. Bayoud is one of the most devastating biotic constraint initially reported in Morocco, and has spread throughout the North African countries with the destruction so far of an estimated 12 million date palms. The rise of a new disease *maladie des feuilles cassantes* or *brittle leaf disease* in Tunisia (Triki et al. 2003) where in a short time span 40,000 date trees were destroyed due to wilting; the cause of this disease remains unknown. The large number of senescent date groves and replacement of these trees through natural offshoots propagation is becoming one of the major challenges because of an insufficient supply of offshoots of good quality and at a low price (Al-Sakran and Muneer 2006). Date palm farmers are using traditional and undeveloped agricultural practices from generation to generation. There is an almost total lack of operational field stations to guide the farmers with proper cultivation methods to improve date production. A substandard level of crop and farm management, scarcity of water, lack of knowledge about advanced breeding techniques, low mechanization, traditional irrigation system, overuse of water resulting in the falling of the water table, the shortage of well-qualified and highly-trained national staff and laborers and the lack of national programs in rural areas regarding the promotion of date palm culture are some of the limitations in the date palm breeding (Al-Khayri et al. 2015a).

18.4 Tissue Culture Approaches

Tissue culture refers to the regeneration of a whole plant from cells, tissues or organs under an aseptic culture medium supplemented with macroelements, microelements as nutrients, sucrose as a source of energy and vitamins, carried out in a controlled environment at an optimal level of temperature, humidity and illumination. Tissue culture produces large numbers of homogenous plantlets in a short time, which are genetically identical and true-to-type, as well as virus, pest and disease free. With tissue culture, the plant source is not seasonal and the exchange of these plant materials is easy and safe (Jain et al. 2011). Plant tissue culture techniques such as somatic hybridization, embryo rescue, in vitro fertilization and in vitro flowering provide a solid platform for plant breeders to understand underlying genetic principles. Traditional means of date palm propagation include sexually through seeds and vegetatively by offshoots. Tisserat (1979) first reported the propagation of date palm by somatic embryogenesis from offshoots; later, several research groups and scientists worked on the standardization of date palm regeneration of different elite cultivars using different tissue culture techniques. Rapid multiplication of date palm through tissue culture techniques has greatly contributed to meeting world demand for planting material (Jain et al. 2011).

18.4.1 *Advances in Micropropagation*

Over the last four decades, Murashige and Skoog (1962) medium has been used as the standard culture medium to carry out tissue-culture experiments in date palm (Bhansali 2010). Date palm micropropagation has been achieved from shoot tips, zygotic embryos, lateral buds, leaves and inflorescences (Abul-Soad et al. 2008; Al-Khayri 2005, 2007; Mazri et al. 2017). Somatic embryogenesis and direct organogenesis are the two main methods of date palm micropropagation adopted by most researchers (Al-Khayri and Naik 2017; Bhansali 2010).

18.4.1.1 Somatic Embryogenesis

Somatic embryogenesis is considered to be one of the most efficient regeneration process for date palm micropropagation (Fig. 18.3). A number of research studies were carried out to standardize the protocol for somatic embryogenesis of date palm through altering the culture medium ingredients using amino acids (Abdel-Rahim et al. 1998; El-Shiaty et al. 2004; Zouine and El Hadrami 2007), auxins and cytokinins (Al-Khayri 2003; Eshraghi et al. 2005; Roshanfekrrad et al. 2017; Zouine and El Hadrami 2007), abscisic acid (Alwael et al. 2017; Sghaier et al. 2009, 2010; Zouine et al. 2005), N-phenyl N′-1,2,3-thidiazol-5-ylurea (TDZ) for direct somatic embryo (Sidky and Zaid 2011), physical status (Fki et al. 2003; Taha et al. 2001), sucrose (Sghaier et al. 2010; Taha et al. 2001), silver nitrate as an inhibitor of ethylene (Al-Khayri and Al-Bahrany 2001, 2004a, b), biotin and thiamine (Al-Khayri 2001; El-Shiaty, et al. 2004), basal salts formulation and strength (Al-Khayri 2003, 2011; Taha et al. 2001) and organic additives (Al-Khayri 2010; El-Dawayati et al. 2018; Khierallah and Hussein 2013). In most of the reports, activated charcoal is used as an agent to absorb the phenolic compounds from the embryogenic culture media (Al-Khayri 2010, 2012; El Hadrami 1995; Naik and Al-Khayri 2016). Othmani et al. (2009a, b) reported significant improvement of plant regeneration via partial desiccation of somatic embryos and showed improved differentiation of embryogenic callus via embryogenic suspension culture of elite date cv. Deglet Bey.

Callus culture (somatic embryogenesis) combined with cell suspension culture provides a new pathway for genetic improvement of date palm based on in vitro selection studies and mutagenesis (Fig. 18.4) (Abohatem et al. 2011; Al-Khayri 2012; Jain 2007; Sidky and Gadalla 2013). Recently, Naik and Al-Khayri (2016) reported the protocol procedure of induction of callus from date palm shoot-tip explants, cell-suspension culture, somatic-embryo development and plant regeneration. Fki et al. (2011) and Al-Khayri (2013) reviewed the effects of different factors with respect to date palm somatic embryogenesis. These studies help in understanding the effects of different medium components and culture conditions leading to enhanced plant regeneration. On the other hand, the standardization of callus induction and somatic embryogenesis still continues because there are a number of factors which control callus induction.

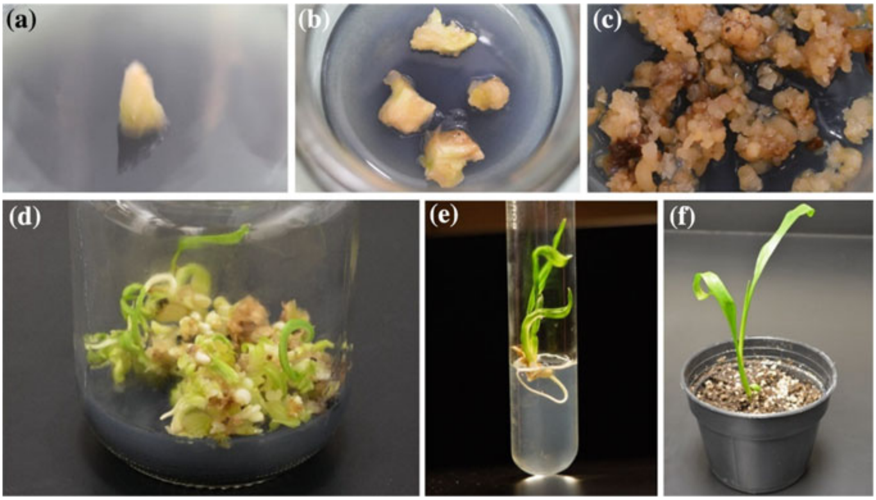

Fig. 18.3 Stages of date palm tissue culture. **a** Culture initiation, **b** Callus induction, **c** Callus proliferation, **d** Embryo formation, **e** Rooting, **f** Transplanted plant

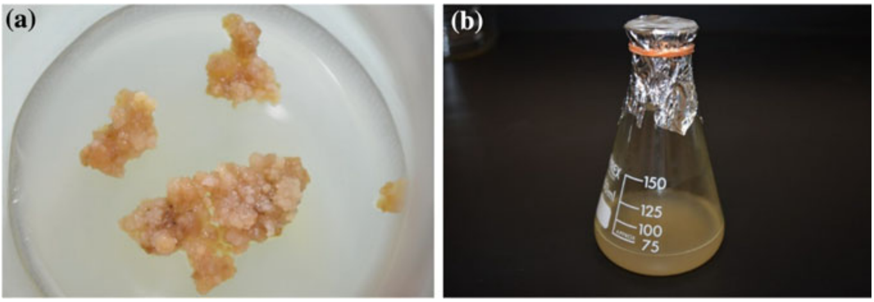

Fig. 18.4 Date palm suspension culture. **a** Actively growing callus, **b** Suspension culture

18.4.1.2 Organogenesis

In plant tissue culture, organogenesis refers to the initiation and development of organ tissue and finally leads to the regeneration of a plant, avoiding the callus stage from the cultured explants. Organogenesis maintains the consistency in the in vitro cultured plants and also minimizes the risk of genetic variation. The application of direct organogenesis is widely adopted in elite genetic material for rapid clonal propagation (Khierallah and Bader 2007). There are four steps in the organogenesis technique: vegetative buds, bud multiplication, shoot elongation and rooting. The initial step is crucial and determines the outcome of the culture. A number of researchers have performed experiments on organogenesis of date palm (Bekheet 2013; Jazinizadeh et al. 2015; Khan and Bi 2012; Khierallah and Bader 2007). Aaouine (2003) reported direct-shoot organogenesis and plant regeneration from 30 genotypes of date palm.

Direct organogenesis shoot tips of Zaghloul cv. were achieved by Bekheet (2013). Jazinizadeh et al. (2015) investigated the direct organogenesis of cv. Barhee by using different media, sucrose levels and hormone concentrations. Currently Morocco, Saudi Arabia and United Arab Emirates are employing micropropagation techniques as a tool to produce commercially-important date varieties.

18.4.1.3 Protoplasts

Protoplast consists of cytoplasm surrounded by the plasma membrane, but without a cell wall. To the present, more than 400 plant species have been regenerated through protoplast culture (Davey et al. 2005). The protoplast-culture technique in date palm is still in its juvenile stage. Initial reports of protoplast isolation and cultures were reported by Chabane et al. (2007) and Rizkalla et al. (2007). Chabane et al. (2007) reported the isolation of protoplast from young leaves of offshoots and embryogenic calli and subsequently achieved callus formation in cvs. Deglet Noor and Takerboucht. Rizkalla et al. (2007) isolated the protoplast from young leaves of offshoots in cvs. Barhee and Zaghloul and succeeded in initiating callus growth. Chabane et al. (2010), as part of extended research, succeeded in producing embryos from protoplast culture. Yatta et al. (2013) isolated the protoplast from cell suspension cultures of Algerian date palm cvs. Takerbucht and Tegaza. Recently Titouh et al. (2015) isolated the protoplast from embryogenic callus of date cvs. Deglet Noor, Akerbouch, and Degla Beida, and also induced callus growth. However, efforts are still ongoing to regenerate the plant from protoplast callus. The protoplast technique has the potential for a number of applications including somatic hybridization, introduction of desired genes through genetic transformation of date palm (disease resistance, plant height and improved fruit quality).

18.4.2 In Vitro Embryo Rescue

Embryo rescue is the one of the oldest and most successful of in vitro culture techniques, with the objective to encourage development of an immature or weak embryo into a complete plant. This technique has a number of key applications in the field of crop improvement. Embryo rescue, also referred to as embryo culture, initially began with the crucifers (Hanning 1904). Rescued embryos converted directly into seedlings resulted in the reduction of the time-span for the next generation (Lulsdorf et al. 2014). It also contributed to the introduction of novel genetic variability (Mujeeb-Kazi 2003). This technique has been successfully applied in several crops to produce interspecific hybrids such as *Helianthus annuus* × *H. argophyllus* (Sauca and Lazar 2011), *Allium cepa* × *A. roylei* (Chuda and Adamus 2012), *Triticum durum* × *Aegilops tauschii* (Trethowan 2014) and *Eruca sativa* × *Brassica campestris* (Agnihotri et al. 1990).

Before the 1970s, it was very difficult to obtain genetically superior high-yielding date palms for large-scale plantation to expand the date industry. Using in vitro culture technology solved this problem. The recent development in the date palm mass clonal propagation is based on the embryo rescue technique (Sudhersan and Al-Shayji 2011). Date palm zygotic embryo culture studies were started about four decades ago and in vitro germination of zygotic embryos plantlets was achieved (Tisserat 1979; Zaid 1987). The medium supplemented with 2,4-D and hormone-free medium where the callus regeneration followed by asexual somatic embryogenesis from zygotic embryos and finally date palm plantlet regeneration was reported by Tisserat (1979). By using the embryo rescue technique, the successful cross between the dwarf palm species *Phoenix pusilla* and cultivated selected *P. dactylifera* cultivars resulting in the reduction of date palm height (Sudhersan et al. 2009). This is the first ever report to produce a shorter date palm using embryo rescue. This opens a new avenue to improve the date palm genetically in a short time span.

18.4.3 Somaclonal Variation

Somaclonal variation is the variation observed in the phenotype and genotype of a plant regenerated from tissue culture. The variation is due to the changes in the chloroplast, mitochondrial or nuclear genomes of the regenerated plants. These variations may carry some desirable characters of agronomic and commercial interest and ultimately lead to new varieties (El Hadrami et al. 2011c). The concept of somaclonal variation became known in the early 1980s, with cell culture described as having variation (Larkin and Scowcroft 1981). In the micropropagated plants, somaclonal variations is quite common and is observed in many plant species; through the optimal in vitro culture techniques it can be controlled. On the other hand, somatic variation can be induced by manipulating the factors which control the cell cycle during micropropagation, which includes the source of the explant, age of explant, culture age, subculture number and growth supplement. Some other sources which have the capability to induce variations include mutagens are microbial or synthetic toxins, sulfonate, X-rays and gamma rays (Co-60). An efficient in vitro selection is possible through induction of mutation which creates a wide range of variation and also a broad genetic base (El Hadrami et al. 2011c). Somaclonal variation also provides opportunities such as production of synthetic seeds, mass propagation and cryopreservation systems. Date palm biotechnology and breeding are in need of somatic variation, in which they produce the somatic cells capable of introducing new genotypes with tolerance to biotic and abiotic stresses, disease resistant and improved fruit quality (Ahloowalia and Maluszynski 2001; El Hadrami and El Hadrami 2009; Jain 2001). Saker et al. (2000) reported that the frequency of somaclonal variations was age-dependent in date palm, and also analyzed the somaclonal variation in plant tissue cultured derived plants through DNA fingerprinting. Somaclonal variations are also observed in tissue cultured derived date cvs. Barhee and Khalas which exhibit abnormal flower development and dwarfism (Al-Kaabi et al. 2007; Zaid and Al-Kaabi 2003).

18.4.4 In Vitro Mutagenesis

Induced mutation is achieved by treating plant parts or seeds with chemical or physical mutagens and then selecting for desirable changes to mimic spontaneous mutations and artificially change the genetic architecture. The application of physical and chemical mutagens for crop improvement over the past 80 years has increased crop biodiversity and productivity in different parts of the world. Mutation induction has become a proven way to create variation in a crop cultivar and induce desired traits not found in nature or those lost during evolution. The created variability is limited and may add a change that was almost found in the species, unlike transgenic variability that inserts foreign DNA material and faces a major limiting factor of silencing of the transgene (Mondal et al. 1997). Starting in the 1970s, the International Atomic Energy Agency (IAEA) and Food and Agriculture Organization (FAO) jointly sponsored research on mutation induction to enhance genetic improvement of food and industrial crops to breed new improved varieties. The number of officially released crop mutant varieties exceeds 2252 (Maluszynski et al. 2000). China (26.8%) contributed the most mutant varieties, followed by India (11.5%), USSR/Russia (9.3%), the Netherlands (7.8%), USA (5.7%) and Japan (5.3%). Tissue culture induced variations are most effective and are successfully associated with cellular level selection and easy handling of large populations for screening (Suprasanna et al. 2009). The use of chemical mutagens as compared to physical mutagens is less practical and radiation-induced mutation alone accounts for up to 90% of in vitro mutant varieties (Ahloowalia and Maluszynski 2001).

In date palm, little work has been done on mutation induction, apart from that of the FAO/IAEA Coordinated Research Project on the development of bayoud-disease resistant date palm mutant cultivars in North Africa (Jain 2005, 2006). Gamma radiation has been used to induce mutants resistant to the bayoud disease based on in vitro selection in the presence of toxin extracted for *Fusarium oxysporum* f. sp. *albedinis* culture (Sedra and Lazerk 2011). 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 most preferable approach due to the single cell origin of somatic embryos, which 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 requiring significant extra work to dissociate chimeras by plant multiplication up to M1V4 generation (Jain 2007). The effect of X-ray on physiological and biochemical aspects such as DNA content, ions, photosynthetic pigments, proline content, growth and water content were estimated in date palm seedlings by Al-Enezi and Al-Khayri (2012a, b). This information will facilitate the developing strategy for date palm genetic improvement through mutation techniques.

18.4.5 Tolerance to Abiotic Stress

Date palm growth and productivity is affected adversely by extreme environmental factors, such as high temperature, high water or soil salinity and drought (Al Busaidi and Farag 2015). Tissue culture is one of the best tools to understand the effect of these abiotic stresses on physiological aspects of in vitro cultures. Presently, further research relevant to in vitro section is necessary to harness the full benefit of this approach. Deriving reliable abiotic stress-resistant date palm is of particular importance to combat the effects of global climate changes (El Hadrami et al. 2011d; Shabani et al. 2012). Several researchers have attempted to understand the behavior of date palm in vitro culture by creating artificial stress conditions in the culture media (Abass 2016; Al-Bahrany and Al-Khayri 2012; Al-Khayri and Al-Bahrany 2004a, b; Al-Khayri and Ibraheem 2014; Al-Khayri et al. 2017; Al Mansoori and Eldeen 2007; Bekheet 2015; Jasim et al. 2010). Al-Khayri and Al-Bahrany (2004a, b) selected polyethylene glycol (PEG) as a drought agent, and examined the effect of PEG on callus culture of cvs. Barhee and Hilali; they found a reduction in the relative growth rate, callus fresh mass, water content, index of tolerance and elevation in the endogenous free proline content as the PEG concentration increased. Similar results were observed by Bekheet (2015) where the growth parameters such as survival, fresh mass and osmotic tolerance ratio decreased with increased mannitol or PEG concentration in the culture medium.

In a salinity test Al Mansoori and Eldeen (2007) found that when the callus culture was treated with NaCl it retarded growth of callus and zygotic immature embryos, and complete reduction of growth was observed at 3.0% NaCl. Jasim et al. (2010) also observed similar results where the treatment of NaCl leads to a negative pathway in the growth of date palm callus and somatic embryos. The experiment carried out by Al-Bahrany and Al-Khayri (2012) revealed that the exposure of $CaCl_2$, KCl and NaCl to the cell suspension media leads in the negative growth of callus, callus water content and Na^+/K^+ ratio. The supplementation of NaCl to the media resulted in a significant reduction on the percentages of somatic embryo germination, and also affects germination time (Al-Zubaydi et al. 2013; Ibraheem et al. 2012). Al-Mulla et al. (2013) examined salinity stress on tissue-cultured date palms maintained under greenhouse conditions. Al-Ahamad, Nabusaif, Barhee, Khalas and Kasab cvs. were treated with different salinity levels; after 1 year they found cvs. Khalas, Kasab and Barhee tolerated salinity to the level of 10 dS/m; whereas after 2 years they observed Khalas tolerated up to 20 dS/m. They also noted average heights of the palms and the number of leaves, which were inversely related to increasing salinity levels.

18.4.6 Date Palm Germplasm Conservation

The primary goal of the date palm breeder should be to construct a germplasm collection, in vitro conservation and preservation of the germplasm for the long-term without any alteration. Due to human activities and urbanization, the genetic diversity of the date palm is being reduced; other important reasons are a lack of suitable arable land, extreme climatic conditions, monoculture and diseases. To maintain date palm germplasm biodiversity and successful utilization of genetic resources requires suitable conservation methods. There are two major germplasm conservation methods: in situ and ex situ. From the experience gained so far it is concluded that germplasm diversity can be maintained by applying diverse conservation methods.

18.4.6.1 In Situ/Ex Situ Conservation

In situ conservation refers to the maintenance of genetic integrity of germplasm samples within their natural environment. Farmers play an important role in the conservation of the date-palm germplasm by cultivating different varieties in their traditional groves. Little attention has been paid to in situ conservation of wild relatives of the date palm, and it is not included in the movement of in situ conservation of wild crop relatives or in any global review of their status (Meilleur and Hodgkin 2004). Due to the loss of genetic diversity and an accelerating rate of species extinction, wild crop relatives were identified in the 1980s as a target for conservation (IPGRI 2005). The conservation of germplasm is not feasible using only the in situ method; ex situ conservation is also necessary to maintain the integrity of the genetic resources (Singh et al. 2012). Ex situ conservation is concerned with the maintenance of genetic integrity of the germplasm samples outside their natural habitat. Ex situ collections serve researchers, allowing access to disease-free germplasm. It is also necessary for characterization and evaluation of germplasm, and their utilization in breeding programs and other research activities. Although ex situ conservation of date palm germplasm has increased, it is comparably less than in other crops, due to the relatively narrow base of genetic diversity. Ex situ accessions in the gene banks of five plants (rice, barley, wheat, maize and beans) represent one third of the total collections worldwide (Lanteri and Barcaccia 2005). There are only few date palm gene banks known; the major ones are in Algeria, India, Iraq, Nigeria and the United States (Krueger 2011).

18.4.6.2 Cold Storage

Cold storage is the one of the most appropriate and cost-effective, as well as having the highest potential of in vitro culture methods of germplasm conservation, which includes date palm, vegetatively-propagated crops and other horticultural crops (Al-Khayri et al. 2015a; Bekheet et al. 2001; Jain 2010; Othmani et al. 2009c). Cold stor-

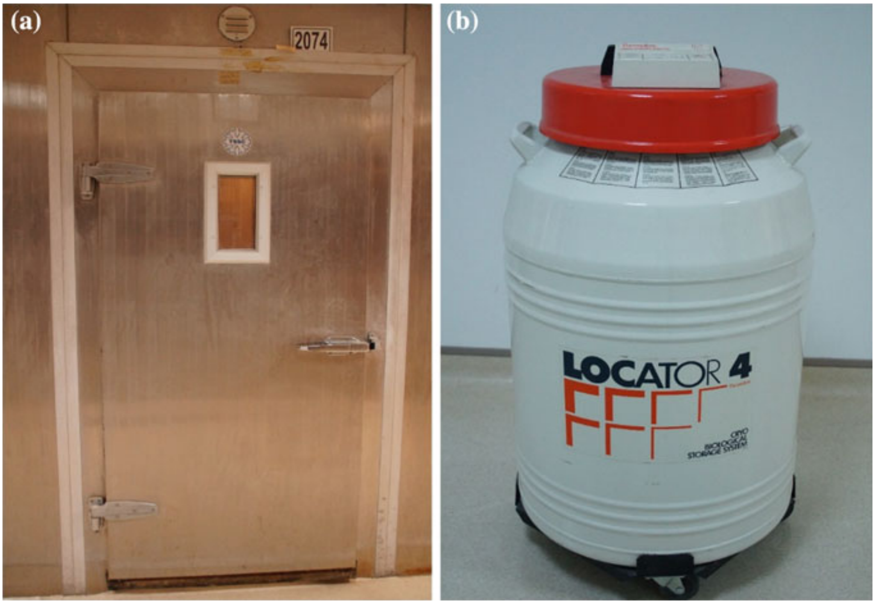

Fig. 18.5 Date palm germplasm conservation. **a** Cold storage room, **b** Cryogenic container

age temperatures are set according to the nature and origin of the stored germplasm (Fig. 18.5a). Tropical origin germplasm needs to be stored at 15–20 °C, while temperate region species require 4 °C. Due to cold storage, subculture periods in many species are reported to extend for 1–4 years (Bekheet 2011). Various studies have reported that the plants recovered from cold storage aim to minimize cell division and growth to increase longevity without any genetic alteration. (Hao et al. 2004; Renau-Morata et al. 2006). This method provides prolonged storage facility and also reduces maintenance costs by lengthening the time between transfers. The Egyptian cold storage facility of the National Gene Bank (NGB) contains more than 30,000 accessions (NGB 2007).

18.4.6.3 Cryopreservation

Cryopreservation is the process where in vitro cultured genetic materials such as shoot tips, callus, cell suspension, microspore and somatic embryos are stored long-term under ultra-low temperatures, usually at –196 °C in liquid nitrogen (Fig. 18.5b). This method is aimed at the preservation of contamination-free genetic material and to avoid somaclonal variation. A reliable plant regeneration method from the in vitro explants and a suitable large-scale disease-free plant multiplication is the basic requirement of cryopreservation of genetic material and in vitro conservation. Several research groups have standardized the date palm in vitro culture for plant regeneration and initiated the cryopreservation of date palm tissues viz. shoot tips, callus, nodular cultures and somatic embryos (Bekheet et al. 2007). The first attempt at date palm cryopreservation was made in the late 1970s (Finkle et al. 1979; Ulrich et al. 1979) and later Tisserat et al. (1985) reported cryopreserved pollen did not alter the fruit yield of 10-year-old Deglet Noor cv. date palms. To avoid ice-crystal formation and damage to cells or tissue from the liquid nitrogen, cryoprotectant treatment is given to cells or tissue to perform their normal functions after thawing. Some of the cryoprotectants used are polyethylene glycol (PEG), glucose, sucrose, glycerol and dimethylsulfoxide (DMSO).

Date palm embryogenic calli were subjected to cryogenic treatments and stored in liquid nitrogen at −196 °C for several months. Regenerated date plantlets were recorded after the calli was revived from 4–8 weeks of latency (Tisserat 1982). Mater (1987) achieved the successful cryopreservation of date palm callus after 4 months at –25 °C by treating it with a mixture of PEG, glucose and DMSO. When the callus was thawed, it resulted in the initial slowdown of callus growth for 2 months, but did not affect the callus becoming embryogenic. MyCock et al. (1997) observed the normal growth and development when late globular and early torpedo stage date palm embryos were pre-treated with a glycerol and sucrose cryoprotectant mixture, and maintained the water content of 0.4–0.7 g/g. Kristina and Towill (1993) reported the cryopreservation protocol for handling pollen. Bekheet et al. (2002) developed the method to preserve the tissue cultures for long-term use in vitro shoot bud and callus cultures. A relatively high percent of cultures remained viable even after the 12 months treatment at 5 °C. Al-Bahrany and Al-Khayri (2012) developed the suitable cryoprotectant solution for date palm cell suspension of Khalas, a popular commercial cultivar in Saudi Arabia. The findings summarized above provide the basic knowledge to conserve the date palm through a cryopreservation gene bank.

18.5 Genetic Engineering

Advanced biotechnology provides a number of tools to ameliorate the effects of salinity, drought, extreme heat, insect attacks, diseases and productivity. These technologies can minimize the use of potentially-harmful chemicals currently used to enhance the agriculture productivity (Saker 2011). Genetic engineering, or genetic transformation, is one such tool which has the capability to solve most breeding problems. The simple meaning of transformation is change, but in plant biotechnology the definition connotes the process of introduction of a desired gene into a target plant cell, bringing about a permanent alteration in the genetic constituency of the cell and its derivatives. Some crops are hard to transform and there is great need for the application of optimal procedures to those crops (Finer 2010). Horsch et al. (1985) first reported the transformation procedure from plant cell to the whole plant. Later, this finding transformed plant science by creating genetically-modified crops. In the history of transformation several different methods have been widely employed, among them *Agrobacterium* and biolistic-mediated genetic transformation. A number of transformation protocols have been reported for both dicots and monocots including oil palm via embryogenic and organogenic regeneration processes (Bhore and Shah 2012; Chavarri et al. 2010; Gong and Liu 2013; Kadir 2008; Mahatre 2013; Tripathi et al. 2012)

18.5.1 Current Status

The history of date palm biotechnological research and its progress can be divided into four stages. First is plant tissue culture where embryogenesis and organogenesis are the two major steps in micropropagation. The application of DNA markers and fingerprinting in date palm is the second stage where the analysis of germplasm diversity, phylogenetic relationships, disease resistance genes, genetic analysis of tissue culture-derived date palm offshoots and sex identification were carried out (Saker 2011). Stage three is genetic engineering or transformation, where only scant published data are available regarding the *Agrobacterium* or biolistic-mediated transformation of date palm (Aslam et al. 2015; Habashi et al. 2008; Mousavi et al. 2009, 2014; Saker et al. 2007, 2009). The evidence available regarding transformation is still at a rudimentary stage; there is the need of a rapid advance to promote transformation. The fourth stage is genomic analysis; many research groups have worked on the sequencing of the genome and genetic mapping and achieved success to a small extent (Al-Dous et al. 2011; Al-Mssallem et al. 2013; Hazzouri et al. 2015; Mathew et al. 2014). Research in genomic analysis of the date palm is in a rapid mode as compared to transformation studies. Habashi et al. (2008) reported DNA delivery by microprojectile bombardment to date palm embryogenic calli and somatic embryos. The study was to optimize the different parameters such as osmotic conditioning of the explants before and after bombardment, explant types and acceleration pressure, bombardment distance and gold particle size. Similarly, Mousavi et al. (2009) established an efficient genetic transformation system in embryogenic callus of date palm through particle bombardment and physical, biological and DNA parameters. A successful gene delivery in the somatic embryos of Estamaran cv. was performed by Mousavi et al. (2014) using the particle bombardment technique. Saker et al. (2009) successfully established the *Agrobacterium*-mediated transformation of the GUS gene in embryogenic callus of date palm. Recently Aslam et al. (2015) reported the efficient *Agrobacterium*-mediated genetic transformation system in cv. Khalasa, using matured somatic embryos; the protocol also involves the rapid regeneration of somatic embryos. These available data are highly valuable and will help in future improvement of date palm productivity.

18.5.2 Opportunities for Trait Improvements

Generally, date palm is grown under adverse climatic conditions and the plants experience extreme heat shock, prolonged periods of drought and high soil and/or water salinity (Shabani et al. 2012) which adversely affect physiological and biochemical processes. High salt concentration in soil creates osmotic stress and makes it harder for roots to absorb water and nutrients from the soil, and ultimately leads to stunted growth (Munns and Tester 2008). It also affects cell growth and toxicity resulting from extreme salinity leading to cellular damage (Abass 2016). Finally, the productivity of the date is affected, so there is a need to develop new date cultivars with resistance to abiotic stresses. Bayoud is a fungal disease caused by *Fusarium oxysporum* f. sp. *albedinis* one of the deadliest diseases in date palm; this disease has made the cv. Medjool nearly extinct in North Africa (Al-Khayri et al. 2015a). The fungus is soil-borne, so chemical fungicides are ineffective to control the disease. The red palm weevil, *Rhynchophorus ferrugineus* Oliv., is one the most destructive pests attacking the date palm. Application of pesticide likewise is ineffective to manage the pests which inhabit the trunk of the tree (Saker 2011). Production of disease resistance to bayoud and red palm weevil pest resistance through conventional breeding is a time-consuming and tedious process. There is enormous scope in the transformation technology to introduce disease-resistant traits in the date plant. Date fruits face a storage problem, as the shelf-life of the date declines at normal environmental conditions, affecting the date-marketing potential internationally (Al-Khayri et al. 2015b). There is need to improve date cultivars to give them moderate shelf-life. Date fruits can be stored in freezers for up to one year, but that alternative is impractical in many date-growing areas, especially those which are remote and lack a reliable electricity supply. There is a remarkable opportunity for the improvement of date palm productivity through advanced plant biotechnological applications, and the genetic engineering is the most important one.

18.6 Molecular Breeding

Molecular breeding is the application of molecular biology and DNA-based analysis of genome polymorphism in plant-breeding programs. Molecular breeding includes quantitative trait loci (QTL) mapping, marker-assisted selection, genomics selection and genetic engineering. Currently, molecular markers are gaining in popularity as major resources in all type of crops. DNA markers are playing an important role in the identification of plant cultivars, germplasm diversity, phylogenetic relationships, disease resistance genes and other breeding activities (Elsafy et al. 2016; Mirbahar et al. 2014; Pintaud et al. 2010; Soliman et al. 2003; Trifi et al. 2000).

18.6.1 Genetic Diversity Analysis with Molecular Markers

Currently a number of molecular markers are available for cultivar identification in addition to markers to identify specific traits including resistance to bayoud disease (Sedra 2011b). The most widely used and important DNA markers are random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), inter simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP) and microsatellite markers containing simple sequence repeats (SSRs). Date palm has a wide range of genetic diversity, this fact opens an avenue to explore the identification of cultivars and their genetic relationships.

A number of research teams using molecular markers have made important contributions to the identification of date palm cultivars and their genetic diversity. Cao and Chao (2002) carried out a study to identify the date cultivars grown in California using AFLP analysis with near infrared fluorescence labelled primers. They collected 21 date cultivars and 4 sets of primers were used to detect polymorphism. An unweighted pair group method with arithmetic mean (UPGMA) cluster analysis showed 328 polymorphic bands, by which a majority of the cultivars were divided into two main groups. Diaz et al. (2003) identified date cultivars using the AFLP technique, also with fluorescence labelled primers. An automated DNA sequencer with the analysis fragment option was used to detect amplified fragments on capillary gel electrophoresis. A total of 310 AFLP fragments were derived from 5 primer combinations. Adawy et al. (2005) used 16 AFLP primer combinations to study the genomic diversity of 14 date palm accessions from 6 Egyptian cultivars (Bertmoda, Fraihy, Gandila, Malkaby, Sakkoty and Siwi) collected from different locations. The result showed 657 amplicons with 45.8% of polymorphism. AFLP results exhibited a high sum effective number of alleles (205.7) compared to those obtained by RAPD (45.1) and ISSR (17.8). Heterozygosity in AFLP was 0.39 when compared to 0.36 and 0.35 in RAPD and ISSR, respectively. Also, the marker index in AFLP was greater than that of RAPD and ISSR. These indicate that AFLP is more suitable in determining polymorphism than RAPD and ISSR. Haider et al. (2012) assessed the genetic polymorphism of 23 date palm cultivars in Syria (18 female and 5 male) using RAPD and ISSR markers. RAPD and ISSR markers exhibited average polymorphism of 58.5 and 50.6%, respectively.

Marsafari and Mehrabi (2013) collected 15 native cultivars of date palm from south and southwest Iran to study the molecular identification and genetic diversity by using RAPD and ISSR. Five genotypes were selected from each cultivar, 10 RAPD and 14 ISSR primers were used in the experiment. RAPD primers generated 132 amplicons with 92.4% polymorphism, on the other hand, ISSR exhibited 162 amplicons with 95.67% polymorphism. Dice coefficients were used to analyze the genetic similarity among cultivars and 94.1 and 99.4% genetic similarity was observed in RAPD and ISSR, respectively. Srivashtav et al. (2013) analyzed the genetic diversity of 8 date palm genotypes grown in the Kutch region of India. The 8 RAPD primers tested yielded 88 bands, out of which 35 were polymorphic, with an average of 2.69 polymorphic fragments per primer. From the 2 ISSR primers 13 bands were produced from which 3 were polymorphic. UPGMA cluster analysis revealed the dendrogram data obtained from the RAPD and RAPD in combination with ISSR were the same. Khierallah et al. (2014) reported the genetic characterization of 17 date cultivars (10 female and 7 male) from Iraq using 30 RAPD universal primers and 12 ISSR primers. From RAPD analysis 86 polymorphic bands were detected and 85 bands from ISSR. Combined data of both the markers yielded a total of 2530 scorable bands with an average of 72.29 fragments per primer. UPGMA analysis of the same divided the date palm cultivars into two major clusters depending of their origin and sex. Sabir et al. (2014a) explored the genetic relationship among 10 Saudi Arabian cultivars using AFLP and ISSR markers. A total of 6 AFLP and 13 ISSR primers were tested, out of which an average polymorphism of 76% was observed in AFLPs and 85% from ISSRs.

A collaborative Italian and Libyan research group completed the genetic characterization of 377 female date palm trees representing 18 Libyan cultivars having common genotypes from the Al Jufrah Oasis, using 16 highly polymorphic microsatellite markers (SSR). They scored the total number of 110 alleles with an average of 6.88 alleles per locus, which indicated the existence of a high level of polymorphism (Racchi et al. 2014). The date palms of Nigeria and neighboring countries exhibit gene recombinations due to introduced cultivars, resulting in the creation of considerable genetic variation. To identify the Nigerian germplasm and their genetic relationship they were studied by using SSR as a tool kit. A group of researcher conducted a study of the genetic diversity among 10 Nigerian and 4 Saudi Arabian cultivars using SSR markers. Of the 14 cultivars, 6 SSR markers were tested against these cultivars and high genetic diversity was observed with 83.3% polymorphism (Yusuf et al. 2015). Al-Faifi et al. (2016) collected 32 Saudi Arabian date palm cultivars from different geographical regions of the country, and screened 93 SSR markers to assess the genetic relationship and polymorphism. Results revealed 71% of genomic SSRs were dinucleotides, of 93 SSR markers a total of 91 alleles were generated from 22 primers with 4.14 alleles per locus and 100% polymorphism. Principal coordinate analysis (PCoA) displayed the cultivars into different groups according to their region of cultivation, class of maturity and fruit color. Recently Elsafy et al. (2016) determined the genetic diversity of 89 female date palms, representing 18 cultivar groups from Sudan using 10 loci of SSR primers. The SSR primers showed high polymorphism with 12.6 alleles per locus. From these reports SSR markers can be considered as superior to RAPDs, ISSRs and AFLPs because of their co-dominant nature, high polymorphism and high reproducibility.

18.6.2 Sex Determination

The date palm is dioecious, making it impossible for farmers to identify male and female trees at the seedling or early growth stage. If it were possible to identify the sex at an early stage, the farmer could cultivate large numbers of female plants with but a few superior male plants for pollination. To reach maturity a date palm requires 5–7 years (Shaheen 1990), which is the significant limiting factor of traditional genetic improvement programs. The palm's heterozygous nature and long life span make it difficult to select superior characters over existed improved cultivars. The genetics of sex determination is not yet fully understood. The initiation and development of sex organs are key incidents in plant morphogenesis. Environmental factors such as illumination, temperature, soil nutrients and as well as the application of hormones may influence or alter the sex expression of plants (Heikrujam et al. 2015).

18.6.2.1 Morphological Markers

Morphological features of plants are the root, stem, leaf, flower, height, fruit traits like size, shape, color, taste, and other visible parts. Traditionally, morphological characters have been used to analyze the diversity of various crop species. In general, traits used for identification are affected by environmental conditions and are laborious to study and the method is applicable only at plant maturity and not at a juvenile stage (Khosla and Kumari 2015). Date palm genotype identification normally is based on fruit characters and vegetative morphology of female cultivars. Other characters to identify a date genotype include the phenotypic expression of leaves and spines. Al-Khalifah et al. (2012) characterized 14 elite date cultivars from Saudi Arabia using morphological characters. Recently Soliman et al. (2013) evaluated male date palms originating from various elite cultivars at the Research and Agriculture Experiment Station, Dirab, King Saud University, Riyadh, based on the leaf morphology characters. There are a number of reports that the various qualitative and quantitative morphological the leaf and leaflet traits selected to identify the elite cultivars are stable and did not exhibit any deviation in response to any environmental factors. One can select those morphological traits to describe and identify date cultivars at any growth stage (Ahmed et al. 2011; Hammadi et al. 2009).

18.6.2.2 Biochemical Markers

Biochemical markers exist to differentiate between male and female date palms. Peroxidase can be used as a biochemical marker, which expresses more in the reproductive organs; based on the diversity of the peroxidases in male and female inflorescences one can develop a gender-specific marker. Qacif et al. (2007) reported the presence of low peroxidase activity in male flowers and a single major isoperoxidase band with Rf 0.35 but it showed two major isoperoxidase in rachillae with Rf 0.32 and 0.35 in the female inflorescence. Furthermore, Bekheet et al. (2008) observed a higher level of peroxidase activity in adult and offshoot females than in male date palms. Similarly, glutamate oxaloacetate activity was found at a maximum in females (249 units/g fresh weight) when compared to males (190 units/g fresh weight). But in contrast, acid phosphatase activity was shown to be lower in females but observed as optimum in males. Sonia et al. (2013) extracted the leaf protein of Deglet Noor cv. and used it to identify gender. Only one distinguished spot was observed in the male gender, which indicates the protein that is specifically accumulated in the male tree. Later on the validity of the polypeptide as a gender biomarker was confirmed in other cultivars such as Aligue, Kenta, Kentichi and Khouet Aligue. These biomarkers are helpful in quick identification of date palm gender in immature trees.

18.6.2.3 Molecular Markers

Sex identification leads to physical separation of male and female gametes into two different individuals of a species. In recent years, biotechnology has emerged as a prime tool in plant breeding and can be useful in the early detection of sex and to help breeders and producers to improve the qualities of the date palm. Over the past decade, a serious effort has been made to understand the genetic basis of sex determination in date palms and to develop methods to identify sex at an early stage of development by using molecular markers. Molecular biology techniques such as RAPD, RFLP, ISSR, and microsatellite markers containing SSRs can be used to identify sex-specific DNA markers (Al-Khalifah et al. 2006; Bekheet and Hanafy 2011; Bekheet et al. 2008; Elmeer and Mattat 2012; Heikrujam et al. 2015; Maryam et al. 2016; Milewicz and Sawicki 2013; Soliman et al. 2003; Younis et al. 2008).

A series of attempts have been made to identify the genetic difference between male and female date palms. Ben-Abdallah et al. (2000) extracted genomic DNA from the leaves of Deglet Noor, Allig, Kentich, Menakher female cultivars, a male genotype pollinator T23 and an F1 hybrid. Out of 53 RAPD primers, 11 primers gave reproducible polymorphic bands. Therefore, RAPD primers can successfully be used to differentiate female and male cultivars, along with F1 hybrids (Fig. 18.6). The genetic material of 4 female date palms (Zaghloul, Amhat, Samany and Siw) and 4 unknown male Egyptian trees were tested with RAPD. The banding profile revealed that the unknown male trees are genetically closely related (87.5–98.9%) to the 4 female date palm (Soliman et al. 2003). RAPD analysis of genomic DNA and RNA extract of 4 known females and 3 unknown males of Egyptian date palm provided the rapid and effective detection of genetic relationship and similarities between the tested date palms (Ahmed et al. 2006). Al-Khalifah et al. (2006) reported the RAPD analysis of early detection of cultivars and genetic diversity among male and female palms (Barhee and Sukkary cvs.), date palm genotypes of different origin, offshoot-derived, seed-derived plants and 2 in vitro cultures of Barhee and Sukkary. The results expressed 73.6% of genetic similarity between the offshoot-derived male and female plants of Barhee, with 43.1% similarity between male and female plants of Sukkary. In seedlings, 87.2% male and female plants of Barhee are alike and 62.3% Sukkary were genetically similar. Early identification of sex in the in vitro differentiated cultures of date palm embryos were also analyzed by using RAPD markers (Bekheet et al. 2008). Younis et al. (2008) developed a gender specific ISSR marker with a different fragment size specific to male individuals. Recently Al-Ameri et al. (2016a) screened the ISSR primer as a putative sex-marker to identify the male and female date palms. Where they found two primers, primer IS A02 with a band size 390 bp are clearly visible in all female plants and are absent in all males, in contrast to another primer IS A71 with a band size 380 bp were observed in all male and was absent in all the female plants. From this evidence, these markers can be used for early detection of the sex in the date palms. Dhawan et al. (2013) screened 100 RAPD primers and developed sequence characterized amplified region (SCAR) markers specific to identify the male date palm (Fig. 18.7). Al-Ameri et al. (2016b) developed the SCAR marker and validated it independently on the male and female

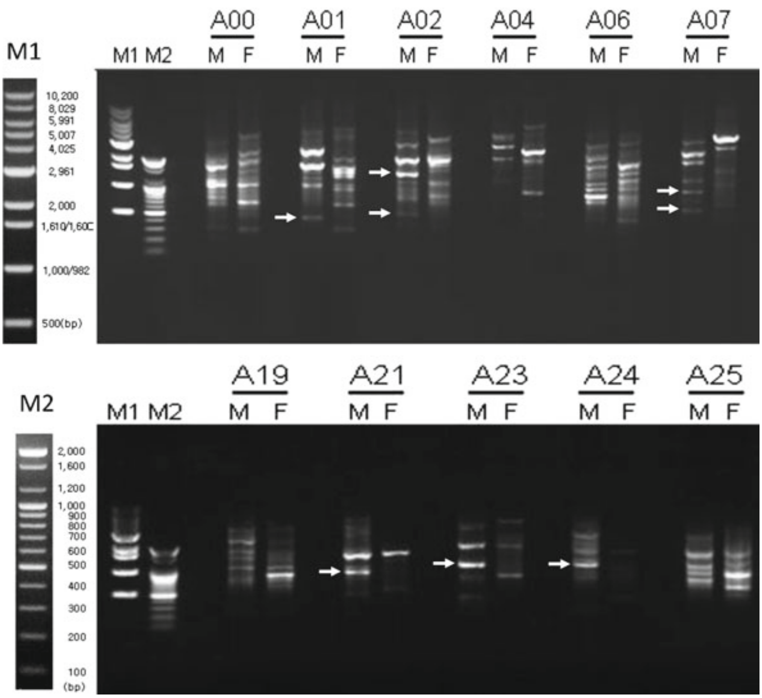

Fig. 18.6 Sex determination through RAPD analysis: M1 is 1 KB ladder, M2 is 100 bp ladder. DNA polymorphisms of (M) male and (F) female date cultivars amplified with Bex RAPD primers A00, A01, A 02, A 04, A06, A07, A19, A21, A23, A24 and A25. Arrows indicating the selected fragments for further analysis *Source* Ageez and Madboly (2011)

date trees at flowering stage. The specific and unique band was amplified in the male but was absent in the female samples.

Elmeer and Mattat (2012) used microsatellites (SSR) to determine the sex of immature date palms. Among the tested SSR markers, primer mPdCIR048 reoccurred 4 times in the 12 individual male samples but was absent in the 117 female samples, and hence a promising candidate marker to detect male sex in date palm was discovered. Maryam et al. (2016) reported the analysis of SSR markers to detect the sex in 1-year-old date palm seedlings. In the results the primer mpdCIR48, with a specific locus 250/250 bp occurred in all male samples but was absent in the female, another primer DP-168, a locus of 300/310 bp observed in 5 male date palm samples, which indicates the promising candidate markers involved in the early detection of sex in date palm seedlings.

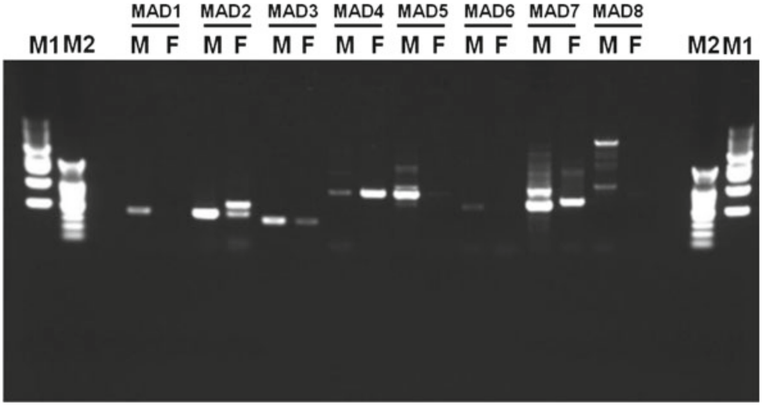

Fig. 18.7 SCAR analysis with the male-specific primers: M1 is 1 KB ladder, M2 is 100 bp ladder. DNA from ten male and ten female date plants were used. *Source* Ageez and Madboly (2011)

18.6.3 Genomics

Genomics deals with the sequencing and analysis of the genome of an organism, or is defined as the sum of total DNA content of all genes of an organism. Genomics has several applications in determining genetic diversity of plant/animal species, disease research, evolutionary processes and synthetic biology. Sequencing of the whole genome of any plant species helps in the development of new cultivars with desirable traits. Recently, the date palm genome was sequenced and its genetic map developed (Al-Dous et al. 2011; Al-Mssallem et al. 2013; Hazzouri et al. 2015; Mathew et al. 2014; Sabir et al. 2014b) but these studies are limited and there is still enormous possibility to improve date palm genomics.

To expand the knowledge about date palm genomics, a number of projects have been conducted worldwide. In Qatar, a research team from Weil Cornell Medical College, genomics laboratory sequenced the whole date palm genome using the next-generation sequencing approach, the Solexa (illumine) sequencer based on a shotgun sequencing method. The date palm genome was made available in 2009 (https://qatar-weill.cornell.edu/research-labs-and-programs/date-palm-research-program/date-palm-genome-data). The genome size is 500 Mbp. King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia in 2008 conducted the Date Palm Genome Project (DPGP) in association with Beijing Institute of Genomics, Chinese Academy of Science (BIG/CAS). The main objectives of the project included date palm genome sequencing and sequencing of chloroplast, mitochondrial and nuclear genomes (Zhang et al. 2011). From the DPGP they developed a genome diversity map for 30 cultivars using the shotgun-sequencing method. The transcriptome study resulted in the more than 30,000 unigenes, full-length cDNA and the expression profile for nearly 50 tissue samples of roots, leaves and flowers.

Yang et al. (2010) sequenced and assembled the date palm chloroplast genome using pyrosequencing technology. The size of the chloroplast genome was found to be 158,462 bp double-stranded circular DNA molecules. The mitochondrial genome of date palm, cv. Khalas, was assembled by Fang et al. (2012) using pyrosequencing and ligation-based sequencing. They found the date palm mitochondrial genome size of 715,001 bp with circular DNA molecules. Mitochondrial gene expression levels were studied in the female flower, male flower, bud and root based on the transcriptomic data and were compared to tissue of the leaf, seed and fruit. In Saudi Arabia, Al-Mssallem et al. (2013) reported the successful sequencing of the nuclear genome of date palm cv. Khalas using pyrosequencing. Based on the results the genomic size is 605.4 Mb which consists of about 90% of the genome and 96% of its genes. Furthermore, Sabir et al. (2014b) sequenced the mitochondrial and plastid genomes of nine Saudi Arabian cultivars using Illumina HiSeq 2000 platform. The published date palm mitochondrial and plastid reference genomes are compared with the sequences of each cultivar, and SNPs were identified. Out of nine cultivars, eight were identified as cultivar-specific SNPs. The first genetic map for date palm and identification of a putative sex chromosome was reported by Mathew et al. (2014). They developed the genetic map from the date palm cv. Khalas, with the draft genome. From this draft genome, for the first time, 19% of sequence scaffolds were placed onto linkage groups. The analysis results gave ~1.9 cM/Mb on the map. The comparison of linkage groups in the date palm showed remarkable long-range synteny to oil palm.

Date palms growing under adverse climatic conditions have evolved in response to stress conditions. The date palm is not a halophytic species, but it has the capacity to withstand extreme drought, heat and salinity-stress conditions. The salinity tolerance of the date palm varies from one cultivar to another (Alrasbi et al. 2010; Ramoliya and Pandey 2003). This nature of the date palm yielded a valuable genome for identifying tolerance genes. Little research information is available on the expression analysis of date palm in response to abiotic stress (Bourgis et al. 2011; Zhang et al. 2012). To understand the mechanisms underlying date palm adaptation to salinity, more knowledge about physiological and molecular studies are required. The combination of genomics and transcriptomics can fulfill the above need. Radwan et al. (2015) subjected the young roots of Deglet Beida cv. to salt-stress treatment, applying the RNA-Seq expression profiling and transmission electron microscopy (TEM) analysis. There they found that regulatory genes belong to DNA/RNA, protein, membrane and signaling functional categories play functional roles in tolerance to salt stress. It also revealed certain key genes are involved in sodium uptake and transport are down-regulated, which means slowing down the up-take mechanism and salt solute transport within plant tissues. This information can be used to improve abiotic stress in sensitive cultivars of date palm. Recently, Yaish et al. (2015) conducted experiments on date palm cv. Khalas and revealed some miRNAs in date palm expressed differential regulation under salinity, and identified the miRNAs and their targets which have the ability play a critical role in adverse environmental conditions such as extreme temperature, drought and salinity.

18.7 Conclusions and Prospects

Date palm is one of the most important tree crop species in the world, especially in the Middle Eastern countries which practice conventional breeding methods that are time-consuming and laborious. The combined application of conventional breeding and applied biotechnological tools can generate genetic variations and produce fast-propagating new genotypes with superior fruit quality, and resistance to biotic and abiotic stresses. Well-established plant tissue culture systems can be used for the rapid propagation of elite cultivars, to establish germplasm banks and for mutagenic studies in vitro to isolate useful mutants. The successful application of protoplast techniques can be utilized in somatic hybridization and introduction of desired genes through genetic transformation of date palm. Available evidence regarding genetic transformation is still at a rudimentary stage and there is need of a rapid pace to promote transformation studies. DNA markers are playing an important role in the identification of plant cultivars, germplasm diversity, phylogenetic relationships and other breeding activities. The dioecious nature of date palm makes it difficult for farmers to identify male and female palms at the seedling or early growth stage. If it is possible to identify the sex at an early stage, farmers can cultivate a large number of female plants with a small number of superior male plants for pollination. Researchers are continuing their efforts to determine the sex at the seedling stage by using sex-specific DNA markers. To understand the mechanisms underlying date palm adaptation to abiotic and biotic stresses, more knowledge about physiological and molecular studies is required. The identification and sequencing of the genomic region related to disease resistance, quality and productivity are necessary for breeding and improvement programs. The application of molecular breeding, genomics, proteomics and transcriptomic studies are needed to meet the above objectives.

Appendix 1

Research institutes concerned with date palm.

Appendix 2

Genetic resources of date palm.

This information is available in the 2-volume book Date Palm Genetic Resources and Utilization (Al-Khayri et al. 2015a, b).

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