Chapter 6
Breeding Date Palm

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6.1 Introduction

Date palm, *Phoenix dactylifera* L., is a perennial long-lived dioecious monocotyledon of great socio-economic importance especially in North Africa and the Middle East. These countries grow 62 million of the 105 million trees available worldwide on an area of over a million hectares (Fig. 6.1; Table 6.1). These 'trees' are cultivated not only for their valuable fruits (dates), but also for producing fuel, fibre and as shelter for ground crops. Production of dates is of approximately 6.5 million metric tons around the world (Table 6.2) and generates an important commercial activity. Countries such as Egypt, Islamic Republic of Iran and Saudi Arabia represent the top three producers worldwide. Furthermore, in the areas where it is cultivated, the date palm contributes to the creation of a micro-climate that enables agricultural development of other species.

6.1.1 History, Botany and Ecology

Date palm is considered as one of the oldest fruit trees domesticated by man and is mentioned in the Qur'an ('Shake the trunk of the palm tree towards thee: it will drop fresh, ripe dates upon thee. Eat then drink, and let thine eye be gladdened'. Qur'an 19: 25–26) as well as in the Bible ('The next day the great crowd that had come to the festival heard that Jesus was coming to Jerusalem. So they took branches of palm trees and went out to meet him, shouting "Hosanna! Blessed is the one who comes in the name of the Lord – the King of Israel!"' St. John 12: 12–13). The representations of this tree appear in hieroglyphic engravings of old Egypt like in the writings of Neolithic civilizations of Mesopotamia. Probably the earliest wild findings of date palm were recorded around 5000–6000 B.C. from Iran, Egypt and

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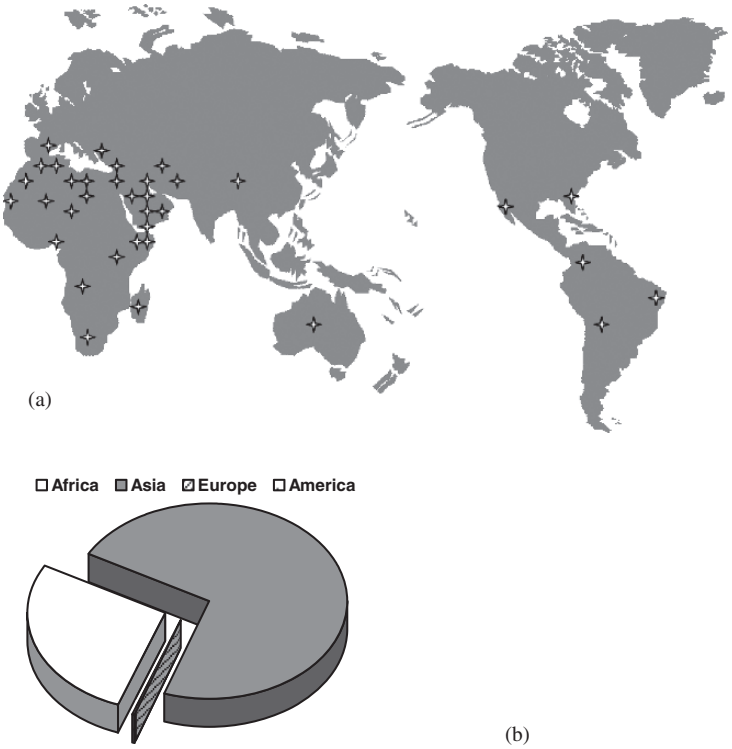

Fig. 6.1 Geographical repartition of date palm growing areas (a) and the relative groves surface per continent in Ha (2004) (b)

Pakistan while the earliest cultivations were found around 4000 B.C. from Eridu and Lower Mesopotamia. It had also been mentioned in Akkadian and Sumerian cuneiform sources dated 2500 B.C. and later. Date palm had been recorded in old history at the areas extending from the Indus valley (now Pakistan) to Mesopotamia in the Tigris/Euphrates valleys (now Iraq), the Nile valley, Southern Persia to the Eastern Mediterranean. Its centre of origin is still uncertain even if there are several claims that date palm originated from Babel in Iraq, from Dareen or Hofuf in Saudi Arabia or Harqan and also from an island on the Arabian Gulf in Bahrain. The oldest radiocarbon dated discovery of date seeds was on Dalma island, part of the Abu Dhabi Islands group. Two seeds were found in 1998, the oldest was 5110 B.C. and the other, 4670 B.C. It had been then introduced into Spain by the Moors and by the Spanish into the Americas. *P. dactylifera* is now found in tropical and subtropical regions all over the world as well as in temperate and arid regions in USA, Australia, southern Spain and the Mediterranean coast of Africa and West Asia.

The wild stock for the first domesticated variety was thought to originate in the southern near east of the fertile crescent (Zohary and Spiegel-Roy 1975). However, there are many other *Phoenix* species that have probably hybridised with the

Table 6.1 Expansion of the surface area of date palm groves around the globe over the last six years (FAO Stat 2004)

	Area occupied by date palm trees (Ha)					
	Years					
	1999	2000	2001	2002	2003	2004
Africa	268, 155	265, 702	304, 772	319, 379	334, 300	339, 305
Algeria	100, 120	100, 120	120, 036	135, 059	135, 000	135, 000
Benin	360	360	360	360	360	360
Cameroon	95	100	105	110	110	115
Chad	7, 600	7, 600	7, 600	7, 600	7, 600	7, 600
Egypt	28, 195	28, 982	29, 461	29, 620	29, 600	29, 600
Kenya	345	330	330	330	330	330
Libyan Arab Jamahiriya	23, 000	24, 000	28, 000	28, 000	28, 000	28, 000
Mauritania	5, 000	5, 000	8, 000	8, 000	8, 000	8, 000
Morocco	44, 200	30, 400	33, 600	33, 000	48, 000	48, 000
Niger	2, 200	2, 200	2, 300	2, 300	2, 300	2, 300
Sudan	26, 040	35, 000	35, 000	35, 000	35, 000	35, 000
Tunisia	31, 000	31, 610	39, 980	40, 000	40, 000	45, 000
Asia	639, 908	666, 025	660, 600	662, 575	665, 600	886, 430
Bahrain	830	823	823	1, 670	1, 670	1, 650
China	6, 000	6, 000	6, 000	6, 000	6, 500	7, 500
Islamic Republic of Iran	177, 272	184, 725	183, 269	184, 000	184, 000	185, 000
Israel	1, 301	2, 070	2, 170	2, 600	2, 600	2, 600
Jordan	251	264	264	346	554	550
Kuwait	1, 050	1, 350	1, 350	1, 350	1, 350	1, 400
Oman	35, 500	35, 508	33, 919	33, 869	33, 848	34, 000
Pakistan	76, 900	78, 590	78, 469	77, 900	78, 000	80, 000
Palestine	346	368	378	424	476	480
Qatar	1, 366	1, 343	1, 516	1, 463	1, 500	1, 500
Saudi Arabia	141, 750	142, 450	139, 099	139, 979	141, 421	145, 000
Syrian Arab Republic	1, 000	1, 009	1, 037	433	900	900
Turkey	3, 850	3, 440	3, 850	3, 850	3, 850	3, 850
United Arab Emirates	170, 330	185, 330	185, 330	185, 329	185, 330	186, 000
Yemen	22, 162	22, 755	23, 126	23, 362	23, 601	23, 600
Europe	525	754	856	764	856	856
Spain	525	754	856	764	856	856
America	2668	2915	2993	2787	3090	2997
United States of America	1, 983	1, 943	1, 983	1, 942	2, 104	2, 000
Mexico	617	894	926	759	900	900
Peru	68	78	84	86	86	97
World	911, 256	935, 396	969, 221	985, 505	1, 003, 846	1, 229, 588

Table 6.2 Annual productions of dates, during the last six years (FAO Stat, 2004)

	Dates production in metric tons (Mt)					
Country	1999	2000	2001	2002	2003	2004
Africa	1, 920, 837	2, 063, 658	2, 187, 137	2, 148, 355	2, 153, 355	236,275
Algeria	427, 583	365, 616	437, 332	437, 000	437, 000	450,000
Benin	1, 000	1, 000	1, 000	1, 000	1, 000	1,000
Cameroon	320	340	360	380	380	390
Chad	18, 000	18, 000	18, 000	18, 000	18, 000	18,000
Djibouti	70	72	75	75	75	75
Egypt	905, 953	1, 006, 710	1, 113, 270	1, 115, 000	1, 115, 000	1,100,000
Kenya	1, 100	1, 000	1, 000	1, 000	1, 000	1,000
Libyan Arab Jamahiriya	114, 150	120, 000	140, 000	140, 000	140, 000	140,000
Mauritania	20, 000	22, 000	20, 000	24, 000	24, 000	24,000
Morocco	72, 561	74, 000	32, 400	33, 200	33, 200	54,110
Niger	7, 600	7, 600	7, 700	7, 700	7, 700	7,700
Somalia	9, 500	10, 000	11, 000	11, 000	11, 000	n.d.
Sudan	240, 000	332, 320	300, 000	250, 000	250, 000	330,000
Tunisia	103, 000	105, 000	105, 000	110, 000	115, 000	110,000
Asia	3, 669, 014	4, 081, 424	4, 247, 333	4, 220, 449	4, 226, 449	3,589,211
Bahrain	16, 774	16, 508	16, 508	16, 508	16, 508	17,000
China	115, 000	125, 000	117, 000	115, 000	120, 000	125,000
Islamic Republic of Iran	908, 340	869, 573	874, 986	875, 000	875, 000	880,000
Iraq	438, 000	600, 000	650, 000	650, 000	650, 000	n.d
Israel	10, 900	11, 732	9, 163	9, 200	9, 200	10,000
Jordan	1, 104	1, 320	1, 420	2, 110	2, 110	1,900
Kuwait	7, 894	10, 155	10, 376	10, 376	10, 376	10,500
Oman	282, 000	280, 030	298, 006	238, 611	238, 611	238,611
Pakistan	579, 880	612, 482	630, 281	650, 000	650, 000	650,000
Palestine	3, 852	3, 819	5, 051	5, 127	5, 127	5,500
Qatar	16, 389	16, 116	14, 230	16, 500	16, 500	16,500
Saudi Arabia	712, 000	735, 000	818, 000	829, 000	830, 000	830,000
Syrian Arab Republic	3, 000	3, 051	3, 921	1, 453	1, 453	1,500
Turkey	9, 400	9, 200	9, 200	9, 200	9, 200	9,400
United Arab Emirates	535, 964	757, 601	757, 601	760, 000	760, 000	760,000
Yemen	28, 517	29, 837	31, 590	32, 364	32, 364	33,300
Europe	7, 565	10, 717	11, 000	11, 000	11, 000	3,732
Spain	7, 565	10, 717	11, 000	11, 000	11, 000	3,732
Americas	23, 022	19, 949	22, 375	25, 374	21, 422	21,850
United States of America	20, 140	15, 785	17, 872	21, 954	18, 000	18,000
Mexico	2, 579	3, 965	4, 309	3, 172	3, 172	3,600
Peru	303	199	194	248	250	250
World	5, 620, 438	6, 175, 748	6, 467, 845	6, 405, 178	6, 412, 226	6,772,068

n.d.: not determined.

domesticated variety and had led to the current cultivars. The earliest cultivation of date palm had been recorded in 3700 B.C. (Munier 1973) in the area between the Euphrates and the Nile rivers. Then it was extended to other areas of the globe where the climate requirements of the plant are suitable, situated mainly between the parallel 9° and 39° North latitude (Munier 1973; Fig. 6.1) especially in dry and semi-arid regions. Hundreds of date palm cultivars are grown worldwide. Their fruits have different colours, flavours, sweetness, acidity and textures. The most popular and appreciated variety of dates in the world is mainly Majhool (originating from Morocco) and the most exported variety is Deglet Nour from Algeria and Tunisia.

P. dactilyfera belongs to *Arecaceae* (*Palmaceae*) family rich with over 200 genera and more than 2,500 species (Corner 1966) including *P. canariensis* (Canary island palm), *P. reclinata* (Senegal date palm) and *P. sylvestris* (Indian sugar date palm). The scientific name was derived from 'Phoenix', the legendary bird of old Greece, and 'dactylos' meaning 'finger' taking into account the shape of the fruit. Date palm is a dioeciously species where the male and female organs are carried by separate trees. It is the tallest tree among all the Phoenix species and the non-branching trunk can grow, under some conditions, higher than 30 m (Fig. 6.2). The plant has one terminal shoot apex that ensures the growth lengthwise. The root system of a date palm is highly developed. The leaves are large 4–5 m, long 4–8 m, alternates, pinnate, ground upward in a spiral pattern on the trunk and sheathing in dense terminal rosettes or crown of 100–120 leaves. The ends of the leaf are needle sharp, which seems to be an adaptation to protect the growth tip from grazing animals. Each leaf has an auxiliary bud that may be vegetative, floral or intermediate (Bouguedoura et al. 1990; Bouguedoura 1991). Auxiliary buds can form shoots commonly called offshoots or suckers during the juvenile life of the date palm and can carry inflorescences to maturity. The fruiting apparel emerges from auxiliary buds as clusters at the top of the tree among the terminal rosettes. Male and female flowers are issued on separate trees taking into account the dioeciously character of the species. Flowers are small and white on a richly branched spadix surrounded by a solitary, large spathe. The calyxes are cup-shaped, three-toothed while the petals are three-toothed, twice longer than the calyx in female flowers. The ovaries are three in general, but only one can develop into a fruit. The stamens are six with linear dorsifixed anthers. Pollination is generally wind-borne and artificial pollination of pistillate trees by placing cut portions of the male flower spikes on the receptive female inflorescence is, nevertheless, usually practiced and recommended to ensure high productivity. A fully productive date palm tree can support up to 10 clusters, which can carry more than 100 kg of fruits. Single fruit date or 'T'mar' in Arabic is usually cylindrical, occasionally rounded or ovoid, a drupe single seeded of 2.5–7.5 cm long × 4 cm large with fleshy, sugary pericarp, yellowish to reddish brown (Tackholm and Drar 1973; Purseglove 1972).

Production of dates around the world, according to FAO statistics, had peaked in 2002–2003 at 6,405,178 and 6,412,226 metric tons (Mt), respectively (Table 6.2). The world's largest producer over the past two years is Egypt with 1,115,000 mt followed by Islamic Republic of Iran (875,000 mt), Saudi Arabia (830,000 mt), United

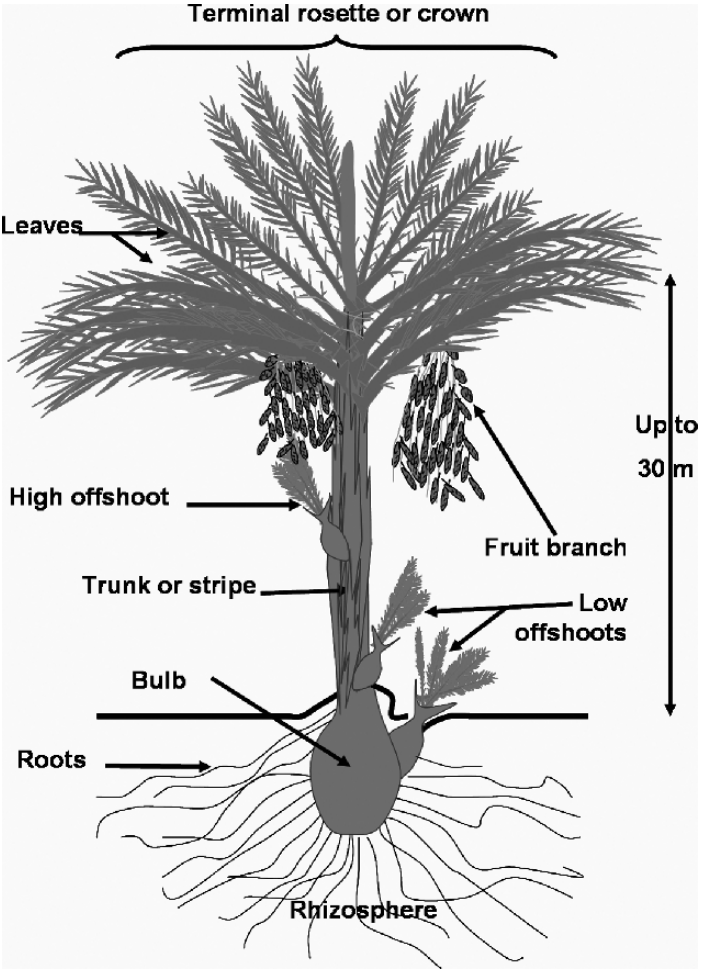

Fig. 6.2 Diagram representing the vegetative apparel of date palm

Arab Emirates (760,000 mt), Pakistan and Iraq (650,000 mt), Algeria (437,000 mt), Sudan and Oman (around 240,000 mt). Other significant producing countries are Libya, China, Tunisia, Morocco, Yemen, Mauritania, USA, Bahrain, Qatar, Spain and Kuwait.

The species is commonly propagated by offshoots and by seeds. Offshoots have a slow development and a healthy selected tree can produce from 0 to 3 offshoots per year and, in general, not more than 10–40 during its lifetime depending on the cultivar and the environmental conditions. Date palm normally begins to bear fruit within an average of 5–8 years after planting the offshoots; they reach their maturity at around 30 years and their decline begins after over 100 years of cultivation. Seed germination is the easiest but seedlings may take up to 10 years before flowering and fruiting occur.

Table 6.3 Comparison of propagation techniques

	Advantages	Disadvantages
Seeds	Easy and quick propagation Useful under-groves conditions Useful for breeding purposes Economic for selecting clones	Space, time and resources-consuming Produces heterogeneous population (a) Sex and pollen quality uncertain (b) Female plants attain maturity late Poor quality fruits
Offshoots	Produce individual 'true to mother' Bear fruits 2–3 years early	Space, time and resources-consuming Limited number of offshoots (c)
In vitro	Rapid propagation No seasonal effect on the plants Genetical uniformity ensured Exchange without risk of diseases and pests spread Economically reliable when large production is required.	Success in induction and maintaining callus nominal

The progeny derived from seeds are heterozygote and do not carry the same mother characters. Nevertheless, such propriety of sexual propagation is intended to create new genotypes and provides a basis for the selection of elite trees. In vitro propagation from tissue cultures is an alternative method for the mass propagation of date palm even though many difficulties have to be encountered in order to achieve that objective (Table 6.3). One of the limits in the mass production of plants through tissue culture techniques is undesirable plant off-types of a poor quality that can cause severe losses in the production and consequently affect the attributes of the whole process (Karp 1993; Cassells et al. 1999). Off-type production in plant tissue culture can result from stressful processes (Phillips et al. 1994; Skirvin et al. 1994) and can be searched in some cases for inducing genetic variability.

A seedling population shall have either of three attributes: (a) heterogeneous population: Mixture of individuals, often with a low production potential, poor fruit quality and late harvesting time (b) Sex, fruit and pollen quality of progeny cannot be determined before the first flowering about seven years after planting due to dioecious nature of the species (c) Offshoots are mainly produced in a limited number (20–30 per palm) during the early life of the plant (10–15 years from the planting) depending on the variety and on prior addition of fertiliser, irrigation and earthing around the trunks.

P. dactylifera is a widely distributed species covering an extensive geographic, soil and climatic areas. Almost 100 million date palm trees exist in the world with the vast majority located in the Middle East, North Africa and to a small extent in California and Mexico. The common requirement between all the date palm growing areas is the high temperature (35 °C, the optimum temperature for pollen germination) and the low relative humidity of the air necessary for fruit setting and ripening. Such desert tree requires large quantities of water ('even if its head is in fire its foot is still in water!'). Date palm grows in nearly rainless regions between 9 and 39° North Latitude, which is represented by the Sahara and Southern fringe of

the Near East (Arabia Peninsula, South of Iraq, Jordan). Both wild and domesticated cohabiting trees are morphologically and ecologically similar. Wild dates are of a small shape and non-edible compared to those coming from domesticated trees. Cross hybridisation among the two types of trees may still present in some regions, making the distinction of both types quite difficult.

Date palm is a high salt-tolerant tree and may harbour a good production even under 3,000 ppm of salty water. Some varieties had exhibited a high tolerance of total dissolved salt (22,000 ppm) but their productivity had been affected (unpublished data).

6.1.2 Socio-Economic Importance

The importance of date palm becomes, first, not only from its history as an inherited species for which cultivation was practiced by the oldest human civilizations, but also from its large ecological amplitude (highly adapted to arid conditions where the annual precipitation rarely exceeds 250 mm with a strong summer heat of about '50 °C' and cold winters of about '−10 °C'). Moreover, over 183,000 tons of dates with a trade value of about US $190,000 are marketed each year (Greiner 1996; Greiner 1998). Currently, date palm production bestows jobs for a population estimated at 50 million people. Thirty five percent of this manpower is localized in the

Table 6.4 Chemical constituents of dates

	Quantities per 100 g of dates	
Nutrients	Source 1	Source 2
Carbohydrates (g)	75.8	71.30
Fat (g)	0.4	0.45
Proteins (g)	2.5	1.97
Fibre (g)	3.9	n.d.
Ash (g)	2.1	1.58
H_2O (g)	15.3	22.5
Sodium (mg)	37.0	3.0
Potassium (mg)	680.0	652.0
Calcium (mg)	120.0	32.0
Phosphorus (mg)	50.0	40.0
Iron (mg)	7.3	1.15
Vitamins		
equivalentβ-carotene (μg)	26.0	50.0(UI Vitamin A)
thiamine (mg)	0.01	0.09
riboflavin (mg)	0.02	0.10
niacin (mg)	0.9	2.20
ascorbic acid (mg)	3.0	n.d.
Energy (Cal)	317.0	297.13

[1]Council of Scientific and Industrial Research, C.S.I.R. 1948–1976. The wealth of India. 11 vols. New Delhi. Fat (8% lauric, 4% myristic, 25% palmitic, 10% stearic, 45% oleic and 10% linoleic. Trace of capric and caprylic acids were found as well).
[2]USDA Handbook 8. n.d.: not determined.

Table 6.5 Organic and inorganic constituents among different cultivars

	Date Palm cultivars								
	Medjoul	Hayani	Deglet Nour	Zahidi	Amari	Halawi	Deri	Hadrawi	Barhi
Carbohydrates (g)	64	34	64	64	64	64	64	64	34
Fat (g)	0.4	0.1	0.4	0.4	0.4	0.4	0.4	0.4	0.1
Proteins (g)	2	1	2	2	2	2	2	2	1
Fibre (g)	6	5	6	6	6	6	6	6	5
Sodium (mg)	3	2	3	3	3	3	3	3	2
Potassium (mg)	650	320	650	650	650	650	650	650	320
Magnesium (mg)	35	15	35	35	35	35	35	35	15
Iron (mg)	2	2	2	2	2	2	2	2	2
Energy (Cal)	268	140	268	268	268	268	268	268	140

Source: http://www.hadiklaim.com/dates_values.asp

southern Mediterranean countries (Ferry 1996). In the majority of these countries, particularly Morocco, date palm creates a micro-climate in the vast desert allowing the settlement of several subjacent cultures, which constitutes the principal subsistence cropping for a considerable human population and their animals. Dates are very rich in carbohydrate, minerals and vitamins (Tables 6.4, 6.5), which can give the necessary metabolites to humans far away from the luxury of stores (Duke 1983). Dates are low in fat and high in carbohydrates, fibres, potassium and vitamins (Tables 6.4, 6.5). They can be dried on stalks or spread out on mats. Date products include syrup, "dibs" (an indigenous honey made from the juice), jam, chutney, vinegar and fermented date juice.

The date groves provide shade for a variety of other crops, such as cotton, maize, citrus fruits, pomegranates, alfalfa, vegetables, mango and cereals. Various trees are also often found between the date palm trees. The leaves are often dried, dyed and then plaited into mats, hats, trays or baskets; they can also be used to attach various materials and to make cords; the wooden midribs are used for roofing. In the past, the midribs were also used to make the dome-shaped fishing cages, now made of metal. The palm trunks provide a sturdy building material or used in the construction of fishing boats in some countries.

6.2 Constraints and Challenges

6.2.1 Constraints

Date palm culture face many constraints that are mainly due to its development under hostile desert conditions, which requires high tolerance to drought and salt stresses and a growth without regular amendment supply. Adaptation of different date palm genotypes to such conditions and variation in yield might occur. Date palm also face many biotic constraints especially *bayoud* caused by *Fusarium oxysporum* f. sp. *albedinis* (Malençon 1934; Carpenter and Klotz 1966; Louvet and

Toutain 1973; Laville 1973; Djerbi 1988). This vascular fusariosis is the most devastating disease of the date palm trees. It has been described, first, in the south of Moroccan groves. Currently, it is still spreading through North African countries especially in Morocco and Algeria where more than 12 million date palm trees have been destroyed so far. No efficient mean is known to control this disease and only few cultivars with a poor quality of dates, unfortunately, are known to be resistant to bayoud (El Hadrami et al. 1998). Recently, a new date palm disease called 'maladie des feuilles cassantes' or brittle leaf disease was recently discovered in Tunisia (Triki et al. 2003), where more than 40,000 trees were destroyed within a short period of time. However, the cause of such wilting is still undetermined. Another constraint that appears on top of the list for date palm is the fact that current trees in different oases' groves are getting old and their replacement through natural offshoots propagation is not sufficient to maintain such perennial crop. The lack of national programmes aimed at the promotion of date palm culture in the rural communities increases the impact of this problem and leads the farmer to change this culture to another to ensure their agricultural incomes.

6.2.2 Breeding Challenges

Taking into account the date palm constraints many challenges are directed towards the breeding programme. These challenges vary between countries and can be classified into three levels as short-, mid- and long-term challenges depending on the objectives. The short-term challenge is to maintain the genetic diversity within groves and to reduce the use of monovarietal cultures, which leads to the impoverishment of the genetic pool. Another short-term objective is to replace and provide oases' groves with juvenile material in order to ensure the perennial aspect of the date palm culture. The mid-term challenge represents the core of the breeding programme wherein resistances to different biotic and abiotic stresses are pursued and the 'date palm complex system' is studied. As for the long-term objective, the creation of new cultivars using conventional and/or non-conventional approaches would lead hopefully to resolve some of the date palm constraints especially to "eradicate" or at least reduce the impact of the bayoud disease.

Facing these challenges, one can notice that only a little has been done with regard to the breeding programme of this species. Several attempts have been conducted in the past but they have led to only a few achievements due to the difficulty encountered such as the slow plant growth and its dioecious character.

6.2.2.1 Genetic Resources and Yield Variation

The genetic pool in terms of date palm varieties is rich but not well known and characterised depending on the country (about 5,000 varieties claimed). Most of these varieties are not grown any more in the groves because high productive and homogeneous individuals were preferred to them. Many concerns have been raised

against the impoverishment that the date palm groves are encountering since the last three decades.

The establishment of new and industrial plantations of date palm around the world will certainly lead to an impoverishment of the genetic pool. These plantations have been established taking into account the dates trade outcomes and the adaptation of some varieties to several conditions depending on the country. In Tunisia for instance, the cultivation of the best trade-marketable producing-date variety Deglet Nour may represent more than 65% of the plantations. Similar trend of plantation of the variety are reported in Algeria. In the south of Morocco, Boufeggous and Jihel are the two varieties most appreciated in the Valley of Draa, while Mejhoul is the best variety adapted to the conditions of Tafilalet region. These practices of monovarietal cultures are very treacherous because they could lead within a short period of time to a high reduction of the date palm biodiversity.

6.2.2.2 Breeding Objectives

Date palm breeding is mostly based on conventional methods. Advances in selection for important agronomical traits, such as fruit quality or yield, and disease resistance are difficult due to the time-consuming generation of new individuals of the species. Moreover, the identification of trees is not usually possible before the onset of fruiting, which takes 3–5 years after the planting. Varieties description is mainly based on morphological markers, such as those of the fruit, which are complex and greatly submitted to the effect of the environment (Sedra et al. 1998). During the last two decades, biochemical markers such isozymes and proteins have developed to ensure an effective genotypic identification (Bendiab et al. 1993; Bennaceur et al. 1991; Fakir et al. 1992). However, these markers encounter many limits due to their sensitivity and willingness of detecting genomic variation. Recently, amplified fragment length polymorphism (AFLP) have been shown to be advantageous markers in terms of studies of the date palm genetic diversity, identification of genotypes (Al Khalifah and Askari 2003) and diagnostic of the pollinisation patterns among and within groves. The same markers are also used during the process of the clone's micro-propagation in vitro in order to differentiate between genotypes and chase the genetic stability of their derived clones. For this purpose, the uses of molecular marker has become an advantage as compared to the traditional way based on morphometry and/or cytogenesis approaches. On the other hand, genetic engineering and molecular markers (Zietkiewicz et al. 1994; Zehdi et al. 2004), although they are of utter significance, have not been yet successfully used in date palm breeding due to long period required for regenerating the crop.

Several investigations aimed at the study of bayoud resistance characteristics have been carried out by many authors (Sedra 1995; El Hadrami et al. 1997; El Hassni et al. 2004) as one of the major component of the IPM strategy that aims to overcome this disease. Several breeding programmes of date palm have been instigated in Morocco (Louvet and Toutain 1973, Saaidi 1979, 1992) and Algeria (Fernandez et al. 1998). Their objectives were to identify date palm genotypes among the Moroccan and Algerian groves with a high or a suitable resistance level

to bayoud and high quality of dates that might constitute parents for the breeding programmes. After selecting the wild genotypes with the suitable characteristics of either resistance or fruits quality, male flower spikes were collected from trees showing high degree of resistance to bayoud or an effect on the fruit set and placed on the receptive female inflorescence known to be of a high quality of fruits or resistant to bayoud, respectively. These cross hybridisations have led to generate new genotypes that have been introduced under greenhouse and in vitro for multi-annual tests for the resistance after field checking of the quality of the fruits. Other attempts at production of new genotypes with resistance to bayoud have been conducted in USA, Algeria and Morocco, but so far did not lead to the expected results (Bouguedoura 1991) due to the difficulties encountered in working with date palm, requiring a long time for regenerating F_1 and F_2 progenies and for back-crossing them (up to 30 years approximately). It is therefore straightforward understandable why only a little has been achieved in the conventional date palm breeding programme.

Similarly to the breeding for resistance to the bayoud, and to the best of our knowledge, nothing has been described concerning the breeding for other abiotic constraints such as drought and salinity mainly due to the same difficulties.

Tremendous advances have been made with regards to the study of date palm genetics. The determination of the number of chromosome has been investigated by many authors who have reported different chromosome numbers. In 1910, Nemec had reported for this diploid species $2n = 28$ while he was working with young developing embryos of a non-specified cultivar. Later, other authors have stated that the chromosomes number is $2n$=32 or 36 (Beal 1937; Al Salih and Al Rawi 1987; Al Salih et al. 1987; Al-Salih and Al-Jarrah 1987; Ibrahim et al. 1998) or 26 (Loutfi 1999). These differences in the evaluation of chromosome numbers are most likely due to difficulties in obtaining soft tissues in mitosis especially from adult trees where the chromosomes are small and numerous. The development of a cytological method based on chromocyanin staining (Siljak-Yakovlev et al. 1996) has also shown the occurrence of sexual chromosomes carrying distinctive nucleolar heterochromatin. On the other hand, studying the genomic size of date palm using flux cytometry and Arabidopsis as a standard, Ouenzar et al. (2001) had estimated the 2X DNA to 490 Mbp (0.51 pg). Comparing this result to other findings on monocots and perennial plants, it seems that date palm has a small genome.

So much needs to be known about date palm in terms of molecular genetics in order to better understand the genetic diversity of the species. Up-to-date, no hands-on molecular method is available for distinguishing date palm female producing trees from males before the first flowering about 5 years after planting. Also, it is difficult to identify female clones according to their morphological characteristics other than at the fruiting time.

At present, about 5,000 cultivars have been identified for date palm within the 34 producing countries. They are fundamentally distinguished on the basis of their fruit characteristics, which are heterogeneous and environmentally dependant. It is, hence, necessary to develop efficient systems based on neutral molecular markers that might be useful in determining the sex within progenies as well as the traits controlling fruits quality and resistance/tolerance genes to bayoud and other biotic

and abiotic stresses. Many efforts have been directed towards achieving this objective and numerous isoenzymatic systems have been tentatively used to characterise date palm cultivars (Torres and Tisserat 1980; Stegemann et al. 1987; Baaziz and Saaidi 1988, Chandra-sekhar and de Mason 1988; Bennaceur et al. 1991). Many of these studies have used either fruits (Stegemann et al. 1987), seeds (Chandra-sekhar and de Mason 1988) or leaves belonging to seedlings of known parents (Torres and Tisserat 1980, Bendiab et al. 1993) or adult plants (Baaziz and Saaidi 1988; Bennaceur et al. 1991). More recently, molecular markers such as RFLP, RAPD, mitochondrial minicircular plasmid-like DNAs and AFLP have been developed in order to identify and/or characterise date palm cultivar of either known or unknown genotypes or to establish phylogenetic relationships between genotypes carrying fruits of a good quality (Ait-Chitt et al. 1993; Benslimane et al. 1994; Cornicquel and Mercier 1997; Bouachrine 1997; Sedra et al. 1998; Ben Abdallah et al. 2000; Trifi et al. 2000; Trifi 2001; Al Khalifah and Askari 2003). On the other hand, research on the biochemical dissection of the mechanism of resistance to bayoud or to other abiotic stresses are still ongoing and several biomarkers of phenolic nature or different matters of antifungal compounds have been identified (Ziouti et al. 1996; El Hadrami et al. 1997; Ramos et al. 1997).

6.3 Progress in Biotechnologies

Both conventional and non-conventional breeding programmes of date palm rely at certain time points on biotechnologies and specifically the use of tissue culture for transformation and/or regeneration of plants. Many advances have been made in this field of investigation regarding the determination of condition factors and stimuli that control the date palm tissue plasticity and totipotency. These two notions, as in many other systems, are still empirical making the identification of culture conditions and stimuli extremely difficult to gather. The breeding programmes will be more appreciated when the in vitro regeneration of date palm can be controlled.

6.3.1 Date Palm Tissue Culture

Date palm is among the small number of crops where in vitro techniques including organogenesis and somatic embryogenesis have completely or partially replaced traditional vegetative propagation practices depending on the country. Many date palm tissues such as leaves, apical dome, shoot tips, lateral buds and roots have been proven to be useful as explants for initiating tissue culture (Zaid and Tisserat 1983). Their plasticity, which allow them to change their metabolism, growth and development to best suit a specific environment controlled by specific stimuli, subsequently leads to the regeneration of whole plants. However, with this monocotyledon species, many attempts of tissue culturing were facing a browning (El Bellaj

and El Hadrami 2004; El Hadrami 1995) followed by the rapid death of the tissues depending on the cultivar.

Similar to other plant systems, initiating in vitro tissue cultures of date palm requires to meet both chemical and physical needs by providing the culture vessel with adequate growth medium and external environment (quality and duration of light, temperature, pH, gaseous environment, osmotic pressure, etc.). In the date palm case, adding some antioxidants into the MS medium (Murashige and Skoog 1962) is critical because of the ability of the tissues to rapidly get oxidized. Activated charcoal has been reported in many studies as an agent that minimise the effect of tissue browning under induction conditions for embryogenic cultures (Tisserat 1979; Sharma et al. 1984; Bhaskaran and Smith 1992; El Hadrami 1995; Loutfi 1999). Other antioxidants such as polyvinylpyrrolidon (PVP) and ascorbic acid have also been used (Poulain et al. 1979; Beauchesne et al. 1986). Also, depending on the type of culture, carbon sources other than sucrose might be worth the use to initiate or propagate date palm tissue cultures (Zouine and El Hadrami 2004).

Culture media used for the in vitro cultivation of date palm require the use of the macroelements and the micro-elements described by several authors (Tisserat 1979; El Hadrami 1995, 1998; El Bellaj 2000; Fki 2005). Other organic supplements such as thiamine and carbon source might need to be added in a specific form to achieve the process of generating plantlets.

Plant tissue culture, including that of date palm, requires a balance of plant growth regulators during their different steps in vitro. The type, amount and ratios of the growth regulators are a critical point in generating plantelets from date palm tissue culture. Depending on the step of induction, multiplication or organogenesis,growth regulators ratio such as the ratio of auxin to cytokinin are to be adjusted. Intermediate ratios of auxin to cytokinin are usually used to initiate formation of calli from date palm while low ratios are preferably used during the shoot regeneration step.

6.3.2 In Vitro Culture Methods of Date Palm

Tissue culture such as callus, cell-suspension cultures, root cultures, shoot tip and meristem, embryo or micro-spore cultures have been developed for date palm with more or less success in generating plantlets (El Bellaj 2000; Fki 2005; Fki et al. 2003).

6.3.2.1 Organogenesis

The regeneration of date palm plantlets through organogenesis involves the achievement of several steps that are more or less critical: (i) meristem induction; (ii) shoot multiplication; (iii) shoot elongation; and (iv) acclimatisation.

Organogenic cultures are usually induced from the internal basic side of young leaves carried by the offshoots. Induction generally requires no light in order to minimise the accumulation of phenolics and tissue browning as well as the stimu-

lation of cell division. This step takes four to six months under the aforementioned normal conditions. It is mediated by the interaction of several factors including the composition of the culture medium, the genotype and the time taken between the offshoot collection from the mother plant and its introduction in vitro.

The induction medium consists, in general, of regular MS medium amended with one of the following combinations of plant growth regulators that provide optimum conditions for induction of organogenic cultures: (i) 5.4 μM NAA (1-naphthaleneacetamide), 4.9 μM IBA (indolebutyric acid), 5–27 μM NOA (2-naphthalenyloxyacetic acid) and (ii) 0.5 μM 2iP (*N*-(3-methyl-2-butenyl)-1*H*-purin-6-amine) or 5.4 μM NAA, 5.7 μM IAA (indol-3-ylacetic acid), 5–27 μM NOA, 0.5–14.8 μM 2iP (Poulain et al. 1979). Even under the same culture conditions, there might still be significant variation observed in terms of frequency of induction among cultivars. On the extreme end, each date palm genotype might require a specific culture medium as shown by several authors. In addition, explants from offshoots and inflorescences can develop roots much earlier than form shoots leading to an inhibition of a further caulogenesis (Loutfi 1999). Meanwhile, groups of cultivars have been distinguished to have the same behaviour in terms of percentage of shoot and callus formation (Loutfi and Chlyah 1998).

The multiplication of shoots normally requires plant proliferation medium in which the auxin to cytokinin ratio is higher than 1. Upon such a proliferation medium, the multiplied shoots bear a resemblance to rosettes. Physiological disorders such as hyperhydricity are often observed in these cultures, but factors affecting their regulation have not yet been clearly identified. Preliminary studies have indicated that the high levels of ammonium nitrate could enhance the rapid growth and the hyperhydricity of date palm cultures.

Elongation of offshoots is frequently obtained after a transfer of the shoot buds into a growth medium with a high auxin to cytokinin ratio (Beauchesne et al. 1986; Loutfi and Chlyah 1998). Approximately 1–2 years after induction, date palm plantlets can be regenerated and transferred to the greenhouse for evaluation/screening. Roots formation occur easily during the final stages of in vitro culture.

6.3.2.2 Somatic Embryogenesis

Embryo-like structures of date palm derived from somatic (asexual) embryogenesis are able to develop into a whole plant in a similar way as zygotic embryos. Till date, two consecutive steps have been known to generate date palm plantlets through somatic embyogenesis (Poulain et al. 1979; Reynolds and Murashige 1979; Tisserat 1979). Poulain et al. (1979) have firstly described the initiation of vegetative buds from date palm offshoots heart. Later on during the same year, Reynolds and Murashige (1979) then Tisserat (1979) have described the induction and regeneration of somatic embryos from these cultures and utilized other various tissues as explants. Tisserat (1979) used plant growth medium supplemented with activated charcoal and 450.5 μM of 2,4-D (2,4-dichlorophenoxyacetic acid) that is higher than the most commonly used concentrations. This technique is being successfully

used for large-scale micro-propagation of date palm by somatic embryogenesis in numerous commercial laboratories. More recently, little improvements have been reported regarding the use of somatic embryogenesis in date palm system (Zaid and Tisserat 1983; Daguin and Letouzé 1988; Bhaskaran and Smith 1992; El Hadrami 1995; Sharma et al. 1996; Loutfi 1999; El Bellaj 2000; Zouine and El Hadrami 2004; Al-Khayri 2005).

Critical factors controlling the establishment of date palm embryogenic cultures include explant type, genotype and plant growth regulators. Either offshoots or flower buds were successfully used as explants to regenerate embryogenic cultures. Optimal results were obtained using 2,4-D (2,4-dichlorophenoxy-acetic acid) as growth regulator even though other auxins such as picloram, 2,4,5-T (2,4,5-Trichlorophenoxyacetic acid), NAA naphthaleneacetic acid, IAA (indolacetic acid) and NOAA (naphthoxyphenoxyacetic acid) have led to more or less inducible embryogenic callus cultures. Early studies had suggested that the use of higher concentrations of 2,4-D (450.5–901 μM) in the presence of activated charcoal are necessary for the induction of embryogenic cultures, while more recently it has been demonstrated that lower concentrations of 2,4-D are enough to achieve that goal (El Hadrami 1995; El Hadrami and Baaziz 1995; El Hadrami et al. 1995). The induction medium consists of a semi-solid modified MS containing de Fossard vitamins (deFrossard 1976), 22.6 μM 2,4-D, 22.2 μM benzyladenine (BA) and 150 $mg.l^{-1}$ of activated charcoal (El Hadrami 1995; El Bellaj 2000; Zouine et al. 2005). The explants are placed in the dark for 4 months under $26 \pm 2\,^{\circ}C$ and sub-cultured on the same medium every 4–5 weeks. On the other hand, differences were observed among date palm genotypes in terms of embryogenic potential (El Hadrami 1995; Loutfi 1999; El Bellaj 2000), which is ascribed to the biochemical and histological changes occurring in the cultures (El Hadrami 1995; El Hadrami and Baaziz 1995; Baaziz et al. 1994). Those changes include a higher variation in terms of phenolics contents, proteins and peroxidases activities. Date palm embryogenic cultures are generally characterised by an accumulation of flavonoids that would indicate an acquisition of an embryogenic potential. Meanwhile, an increase of the activity of some isoforms of peroxidases can be also observed (El Hadrami 1995; El Hadrami and Baaziz 1995; Baaziz et al. 1994; Fki 2005) leading to many tissue browning effect. In many cases, tissue browning for embryogenic cultures can be avoided by adding activated charcoal or other antioxidants such as PVP and ascorbic acid (Tisserat 1979; Sharma et al. 1984; Bhaskaran and Smith 1992; El Hadrami et al. 1995; Loutfi 1999; Poulain et al. 1979; Beauchesne et al. 1986).

Maintaining embryogenic cultures can be achieved on the same semi-solid modified MS medium used for the induction and containing de Fossard vitamins (deFrossard 1976), 22.6 μM 2,4-D, 22.2 μM BA, and 150 $mg.l^{-1}$ of activated charcoal (El Hadrami 1995; El Bellaj 2000; Zouine et al. 2005). Other culture mediums also allow a rapid proliferation of embryogenic-suspension cultures (Sharma et al. 1986; Daguin and Letouzé 1988; Bhaskaran and Smith 1992; El Bellaj 2000; Fki 2005). The growth medium for suspension cultures consists of MS medium containing 0.45 μM BAP (Benzylaminopurine), 2.22 μM, 2,4-D, 100 $mg.l^{-1}$ glutamine and 10^{-7} M abscissic acid. About 0.5–1 g FW transferred into 50 ml Erlenmeyer

flask containing 20–25 ml of medium are enough to induce a new embryogenic-suspension cultures. Suspension cultures are maintained at 100 rpm in the dark at 26 °C. The suspensions are sub-cultured each 15 days upon the same medium. Other protocols have been recently described concerning the establishment of the embryogenic suspension cultures in date palm (Fki 2005).

Somatic embryos development involves the use of modified semi-solid MS medium supplemented either with 2.3 μM 2,4-D and 0.44 μM BA or without any phytohormones (El Bellaj 2000; Zouine and El Hadrami 2004; Fki 2005).

Mature somatic embryos can germinate on medium without plant growth regulators. However, the percentages of recovery obtained are lower and does not meet the expectations and much progress in this area of research are still ongoing (Zouine et al. 2005; Fki 2005).

Both organogenesis and somatic embryogenesis are being used to produce large numbers of date palm plants on a commercial scale. Each technique has certain limiting factors while having its particular advantages. The main limits of plant regeneration from both pathways are the tissue browning, the late response of explants and endophytic contaminations. Callus and morphogenic cultures of date palm have been induced from different explants, including zygotic embryos, roots (Eewens 1978; Sharma et al. 1980), young leaves (Sharma et al. 1984), shoot tips (Zaid and Tisserat 1983; Gabr and Tisserat 1985), fragments of stems excised from seedlings, bases of young leaves obtained from the hearts of offshoots (Beauchesne et al. 1986; El Hadrami 1995), fragments of young inflorescences (Drira and Benbadis 1985; Bhaskaran and Smith 1992; Loutfi and Chlyah 1998) and indeterminate auxiliary buds (Bouguedoura et al. 1990). The most commonly used explants consists of segments taken from the hearts of offshoots and that often contain auxiliary buds (Poulain et al. 1979; Beauchesne et al. 1986, Sharma et al. 1986; El Hadrami et al. 1995; Veramendi and Navarro 1996). Floral segments have also been frequently used as well (Drira and Benbadis 1985; Bhaskaran and Smith 1992; Loutfi and Chlyah 1998; Loutfi 1999; Fki 2005). Endophytic microbial contamination is a major problem and consists primarily of *Bacillus* spp. Antibiotics such as Gentamycin were tentatively used by some authors with uneven success (Cherkaoui 1997).

6.3.2.3 Haploid Recovery from Anthers or Ovules

Studies dedicated to anther and ovule cultures recovery are scarce for date palm. Attempts under various conditions have led to cell divisions and to the formation of globular embryoids from uninucleate micro-spores. For some of the successive attempts, cold treatment combined to the use of two auxins and one cytokinin have been proven to be the key elements to generate embryoids (Bouguedoura 1991; Chaibi et al. 2002) that unfortunately were unable to develop. Investigation of different treatments and various exogenous factors had remained without any significant positive effect unless there was a formation of the weak calli surviving only during a short period of time. The main difficulties encountered in such studies are related to the short-time flowering period that does not allow usually having enough

fresh anthers with uninucleate micro-spores. Furthermore, date palm male anthers typically turned brown and died a few weeks after their culture. Chaibi et al. (2002) reported the definition of the most suitable stage for anthers to be treated with a thermal choc treatment at 37–38 °C prior to their in vitro culture. These authors reported also the use of MS medium amended with 2,4-D, 2-isopentenylaminopurin (2-iP) and activated charcoal to prevent the tissue browning, which had allowed them to observe an increase in the percentage of micro-spore division.

Some haploid recovery attempts have concerned also date palm unfertilised ovules. Due to the small size of these ovules, browning and necrosis were the main limits encountered by these cultures. Although the carpels enlarged and became quite prominent when cultured, the use of activated charcoal is required to ensure them a much longer survival and roots or callus formations (Bouguedoura 1991). Up-to-date, the best results ever obtained were from flowers taken from closed spaths and in which the embryo sacs were formed that contained undifferentiated cells.

6.3.2.4 Genetic Manipulations of Protoplasts or Cell Suspension Cultures

Till the time of writing this chapter, no study or report have been published regarding date palm protoplast isolation and culture even as several attempts were carried out in many laboratories around the world (Algeria, Tunisia, USA). Serious difficulties were encountered to overcome the browning and rapid death of protoplasts. Few reports describe the use of cell suspensions as a tool of genetic manipulation of date palm. It has also been shown that embryogenic cells could be irradiated and submitted to toxins coming from *Fusarium oxysporum* f.sp. *albedinis*, the agent causing the Bayoud, to screen resistant individuals (Fki 2005).

Date palm as a fruit tree could be irradiated in vitro as micro-cultures (Ahloowalia and Maluszynski 2001). Induced mutation in this system would be an effective way of introducing variability within the wild and the bred stock as reported for other crops (Maluszynski et al. 1995; Szarejko et al. 1995; Jain 2006). This approach can be used in conjunction with the regeneration of the material either through somatic embryogenesis or organogenesis. Somatic embryos often start off a single cell, which makes them an ultimate candidate for applying induced mutagenesis with less chimerism (Jain 2002; Ahloowalia 1997). However, somatic embryos germinate at a very poor rate that make them worthless for a large-scale multiplication based on mutation induction (Jain 2002).

Induced mutagenesis use either ionizing radiation such as X- or γ-rays and neutrons or chemical mutagens for inducing variation (Ahloowalia and Maluszynski 2001). Combined to various in vitro cultures induced mutagenesis represents the simplest, fastest and highly efficient method for improving crops. It can result in the development of mutant cultivars able to exhibit resistances to biotic or abiotic stresses, to produce a desirable quality and/or an improved quantity of fruits as well as specific morphological features (Jain 2002). Moreover, in vitro techniques nowadays allow the induction of mutations in a large number of propagules within a reduced working space. Several cycles of sub-culture carried out over a short period

of time can be sufficient to screen for the true and stable mutations. Many breeding programmes around the world use this technology to induce variation within the stock. Maluszynski et al. (1992) and Ahloowalia and Maluszynski (2003) reported that more than 1,800 cultivars out of the collection maintained by the FAO/IAEA division of the Nuclear Techniques in Agricultures are/were either direct mutants or derived from crosses involving individuals subjected to induced mutagenesis, and those cultivars have been released in more than 50 countries. The improvement of in vitro techniques for date palm has made it possible to irradiate these cultures in a large scale and to maintain them within the same collection (Jain 2006). Moreover, recombinant DNA research and the use of the model species *Arabidopsis thaliana* have been active during the last two decades in providing labeled probes such as RFLP, micro-satellite based DNA fingerprinting, developed for cloning and mapping plant genes or transgenesis that are able to trace such modifications within the genome. Nowadays, it is routine to identify and analyse mutants using DNA fingerprinting or mapping genetic alterations using PCR based markers, such as RAPD, AFLP, SSR, SNP... and thus tagging mutants (Beetham et al. 1999; Zhu et al. 1999). Stable mutations can be then linked to noticeable changes in the DNA sequence of specific plant traits, then mapped and located on the chromosomes before being analysed by functional genomics and transferred into a desirable background variety. In turn, breeding processes can be accelerated to lead to new varieties of crops enhanced for either their yield or quality or resistance to biotic or abiotic stresses.

6.3.2.5 Cryopreservation

The first attempts at freeze preservation of date palm go back to the late 1970s when Finkle et al. (1979) and Ulrich et al. (1979) investigated this possibility. Later on in the mid-1980s, Tisserat et al. (1985) reported the cryopreservation of pollen dusted on freshly opened spathes of 10-year-old Deglet Noor palm tree. The freezing of pollen had no effect on the fruit yield and developments as compared to the non-frozen pollen.

Bangniol et al. (1992) suggested that for cryopreservation of date palm, gradients may be exhibited both for outflow of water and the penetration of the cryoprotectants. MyCock et al. (1997) has reported that late globular and early torpedo stage date palm embryos can continue their normal growth and development after cryopreservation. To do so, embryos have to be pre-treated with a cryoprotectant mixture of glycerol and sucrose, then dried to a water content of 0.4–0.7 g/g. Prior to that, Mater (1987) had reported that callus of date palm could be treated with a cryopreservation for 4 months at −25 °C within a mixture of PEG (polyethylene glycol), glucose and DMSO (dimethyl sulfoxide). The freezing under these conditions did not affect the potential of the calli to be embryogenic once they were unfrozen; however, their growth was slowed down during the first 2 months of culture. Some results on the same subject have been recently reported (Fki 2005) showing the feasability of this technique.

The main goal of date palm pathologist, breeders and agronomists is to construct a *germplasm* collection, to be able to retrieve it using easy and versatile tools and preserve it long-term without inducing any variability. Embryogenic date palm calli were subjected to cryogenic treatments and stored in liquid nitrogen at −196 °C for several months. In some cases, calli was invigorated after a quiescent period of 4–8 weeks and had regenerated plantlets (Tisserat 1982). To control the conformity of this technique, the polymorphism observed based on five enzymatic systems (alcohol dehydrogenase, esterase, peroxidases, phosphoglucomutase and phosphoglucoisomerase) was analysed in the leaves of the regenerated plantlets. Isozyme patterns observed for regenerated plants from frozen calli were similar to those regenerated from unfrozen calli.

Other studies had reported a protocol for pollen-handling as well as the characteristics for its hydration/dehydration in preparation for long-term storage (Kristina and Towill, 1993). More recently, a long-term method for preserving date palm tissue cultures was reported using in vitro shoot bud and callus cultures (Bekheet et al. 2002). After 12 months of incubation at +5 °C in the dark, a relatively high percent of cultures had remained viable. Other studies are ongoing to test more long-term cryogenic storage with different date palm tissues.

6.4 Conclusions

Tremendous progress has been made during the last few years regarding the in vitro regeneration of date palm from zygotic or somatic explants through either organogenesis or somatic embryogenesis. This progress has made it possible to afford large production of vitroplants even though the regeneration process is still to be shortened to meet the needs of the industry. Many of the physiological mechanisms controlling various stages of in vitro development of tissues remain non-elucidated and numerous studies are carried out in different laboratories to understand them better. Also, it is necessary to develop reliable molecular markers that are able to trace and discriminate genotypes as well as their sex within a progeny. The breakthrough made in terms of bioreactor technology development, a process by which somatic embryogenesis will reach the industrial level, seems very promising. In a such process, cotyledonary stage embryos grown under high photosynthetic photon flux (PPF) to allow them to become photoautotrophic or nodular callus could be transferred into different types of culture systems, such as Magenta vessel, RITA-bioreactor (modified system to improve air exchange), temporary (root zone) immersion bioreactor system (TRI-bioreactor) with forced ventilation, to achieve large-scale embryo-to-plantlet conversion. Even if there is still a need for speeding up these systems of culturing plant in vitro, the potential success of such techniques in multiplying a few crops such as coffee or strawberries (Hanhivena et al. 2005; Afreen et al. 2002) has opened a new era of a rapid and economical way of developing new cultivars massively and plan for a large commercialisation. One would hope to see these techniques applied to the multiplication of date palm in the near

future since many advantages are to be expected. Clonally-propagated plants can produce high quality and uniform seeds; improve the breeding process as well as the vigour and quality of the progenies. In addition, clonal propagation can produce disease-free *germplasm* proper for international exchange, decreasing thus the labor, production and testing expenses of hybrid seeds. One would besides wish for a settlement of a date palm breeding programme based on molecular maker assisted selection in the next few years. The most suitable markers, out of those discriminating the sex within a progeny, would be markers for the productivity of date palm trees (yield and quality of dates) and for the resistance/tolerance to bayoud and other biotic and abiotic constraints. Also, it is necessary to be aware of the danger from increasing productivity using monovarietal plantations. One should keep in mind that the sustainability of date palm groves relies on the preservation of the biodiversity of the crop within the groves. Management of date palm cropping should hence be planned in a monoculture composed of various cultivars rather than a perpetuation of hectares covered by monoline cultures.

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