Chapter 6
Date Palm Status and Perspective in Tunisia

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Abstract Date palm is one of the most important fruit trees grown in southern Tunisia and represents a good cash crop for many farmers. Here, we provide an overview of the Tunisian date palm status. In fact, this important subtropical fruit crop is currently in danger due to several constraints such as anthropogenic spread of disease, water shortages, salinization, and irregular climatic conditions. In addition, Tunisian date palm is threatened by genetic erosion as a consequence of the predominance of the elite cultivar Deglet Noor in modern plantations and the disappearance of many cultivars with medium and low fruit qualities. A series of successful experiments were widely applied for micropropagation of endangered cultivars. Many exhaustive resource inventory programs of date palms have been done in Tunisia. Tunisian genetic diversity was studied by using morphological and molecular markers; some are related to agronomic traits. Tunisian date production increased during recent decades and represents more than 18 % of the national tree production. Tons of cull dates are rejected by processing industries, and attempts to develop new products are supported by research programs. However, the major products are currently destined for exportation because of limited local marketing and the dietary habits of the Tunisian people.

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6.1 Introduction

The date palm (*Phoenix dactylifera* L.) was introduced in Tunisia by the Phoenicians before the Roman occupation (Hodgson 1932). However, the Romans did not give much importance to date palm cultivation in comparison with other crops such as olives and vegetables. The development of date palm cultivation occurred after the settlement of southern Tunisia by Arabs, and this was observed especially in the region of Jerid. The date palm has played an important role in establishing oases and caravan trade routes. Gradually, the oases became more organized, which is reflected in the thirteenth century, when Ibn Chabbat (1221–1285) conceived of a judicious system of sharing water from sources in the oasis of Tozeur (Jerid region). In addition, in the fourteenth century, Tozeur's agriculture became sophisticated and this stimulated a demographic explosion (Rouissi 1969). In fact, many date palm cultivars grown in this area were of seedling origin or brought by travelers and pilgrims especially from the East. However, Deglet Noor cv. was introduced into Tunisia four centuries ago from Oued Souf, in western Algeria (Kearney 1906), and became the most appreciated cultivar of the farmers. After this period, high taxes imposed by the government and external attacks caused serious problems in these oases (Rouissi 1969). In consequence, many oases, especially Nafta, were destroyed and it took up to a century to rebuild them. In the nineteenth century, the oasis economy changed again after the decline of the caravan trade. Indeed, the French occupation of Algeria disrupted the human migrations between Algeria and the south of Tunisia. Consequently, the oasis plantations disappeared gradually.

At the beginning of the twentieth century, many water sources were developed by the indigenous people and nomads who became founders of oases. The French protectorate, with security objectives, contributed by creating small areas scattered around water sources especially in the Nefzaoua. Kearney (1906) reported that there were around 1.3 million date palms in Tunisia with 30,000–50,000 of Deglet Noor cv. Thereafter, the number increased and the oasis area was 7,300 ha with 2.65 million date palm trees (Hodgson 1932), and the most productive harvest was in 1920–1930 with 41,500 mt of dates.

After independence, many strategies have been tried, aimed at modernizing oases and especially to reduce water stress. Indeed, the state sold the nationalized ex-settlers' land to the famous STIL society (Société Tunisienne des Industries Laitières) which provided enormous potential for the export development of Deglet Noor dates (Hajji 1997). This economic progress prompted the authorities to create new modern oases at the end of the 1980s such as in the region of Rjim Maatoug (Nefzaoua) where more than 2,000 ha was established by the national army. To the present, 6,000 people have been settled in this desert region. In 2011, there were more than five million date palms growing in southern Tunisia on more than 40,000 ha of oases (Fig. 6.1). Tunisia's contribution to world date production has become important; it has increased significantly in recent decades. In fact, 190,000 mt were produced in 2011, 76 % represented by cv. Deglet Noor (GIF 2011).

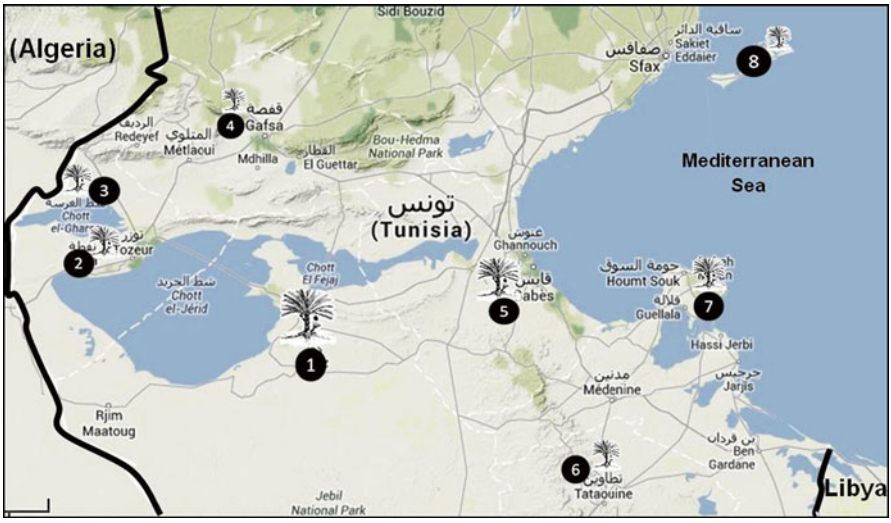

Fig. 6.1 Sites of Tunisian oases. *1* Nefzaoua, *2* Jerid, *3* Chebika and Tamerza, *4* Gafsa, *5* Gabès, *6* Tataouine, *7* Jerba, and *8* Kerkena

The date palm now is an important income source for farmers. In many cases, and principally in Nefzaoua, a date palm can provide a total of USD 100 per year worth of dates. This has increased farm income and encouraged the creation of new plantings. In addition many farmers have introduced intercropping with olives, pomegranates, grape vines, cucurbits, and forage crops which are the most adapted to Tunisian oases. In addition, since 2011 many agricultural cooperatives have been established, and they have mounted programs of oasis development like the introduction of new crops to optimize water use. Despite the fact that these attempts are only beginning, they can produce high-quality potatoes and farmers are becoming more convinced of the value of these changes.

Besides the operations under state control, a large expansion was carried out by private enterprise mainly in Nefzaoua where many date groves were created with unauthorized well drilling and based on the monoculture of Deglet Noor cv. Today, these *illegal* areas represent one-half of the date palm groves in this region. Consequently, the availability of water has become a serious threat to the sustainability of some oasis zones. This situation is aggravated by the threat of destructive diseases such as bayoud, caused by the fungus *Fusarium oxysporum* f. sp. *albedinis* (Baaziz et al. 2000; Louvet and Toutain 1973). It is estimated that bayoud disease killed more than 13 million susceptible trees in Morocco and Algeria in less than a century (Oihabi 2000a, b). Deglet Noor is very sensitive to this fungus which exacerbates the overall threat to Tunisian date palms by genetic erosion. In Tunisia, bayoud disease has not been reported, but other diseases are known, including unidentified diseases and those associated with the presence of certain fungi and insects.

Tunisian oases have important productive, ecological, economic, and social potentialities. This chapter focuses on the date palm status in Tunisia and its perspectives. The vulnerability and challenges facing oasis areas related to water resources and biotic pressures were studied. In addition, Tunisian strategies to preserve and restore oasis/date palm heritage are described and analyzed.

6.2 Cultivation Practices

6.2.1 Propagation

Propagation by seed is occasionally practiced in Tunisia leading to date palms with very low productivity; the males are often kept for pollination. Commonly, propagation is by offshoots and the main interest is on the Deglet Noor cultivar. The best planting time is in May/June and the planting density is usually 100 trees/ha. Offshoots are planted in a hole of 1 m^3 filled with pure soil.

Serious problems should be solved for date palm propagation, expansion of oases, and improvement of disseminated plant materials. The main problem is the smuggling of offshoots from neighboring oases or countries which may be infested with diseases or insects. A source of healthy offshoots with high growth vigor and fruit production is necessary.

6.2.2 Irrigation

The southern Tunisian oases are supplied with water from two principal aquifers, the Continental Intercalary (CI) and Complex Terminal (CT), which extend over 80,000 km^2 and are exploited with more than 1,200 well drillings (OSS 2009). The CT aquifer depth range is 30–500 m while the CI varies from 60 to 2,800 m (Omrani and Burger 2010). A recent inventory revealed 3,069 illegal drilling sites from the CT in the Nefzaoua region alone (MEDD 2010). Groundwater in Nefzaoua is increasingly overexploited, essentially during the summer period, and the area is threatened by salinization. The situation became aggravated in the Nefzaoua oases where the exploitation of the CT in 2011 exceeded by 166 % the allowable quantity (ODS 2011). Flood irrigation is the method most widely used in Tunisian date palm culture. This traditional irrigation system wastes large quantities of water through evaporation loses, and farmers are being required to adopt modern irrigation methods.

Ghazouani et al. (2009) summarized the irrigation constraints cited by many farmers: (a) waterlogging, (b) hot irrigation water, and (c) low frequency of delivery water. That is why the knowledge of experts and farmers should be combined to allow a critical evaluation to resolve these problems. In this regard, a comparative

study was done to determine the optimum water amount to be applied according to plantation density (Omrani and Burger 2010). Three palm tree densities were tested: 64, 100, and 156 tree/ha. The best water efficiency in 2009 was obtained with water application of 8,500 m^3/ha/year for all date palm tree densities (Omrani and Burger 2010). However, when the experiment was repeated for 3 years (2009, 2010, and 2011), the results were different. Results showed that the most productive water volume was 17,000 m^3/ha/year at a density of 100 tree/ha and 20,000 m^3/ha/year for the other two densities. In the past 2 years, many farmers have begun to practice drip irrigation. This irrigation method was introduced and developed by the farmers for more efficient use of water; only the amount of water needed by the palm is applied. However, this method is very expensive for farmers when no grants are available from the government.

6.2.3 Fertilization

Soil fertility decline can be corrected by adequate application of mineral fertilizers, especially manure. In 2009, 660 mt of phosphate fertilizers, mainly diammonium phosphate (DAP), 613 mt of ammonium nitrate, and 116,000 mt of manure were used by farmers (MEDD 2010). For both mineral fertilizers, the average amount applied per hectare was around 16 kg, less than 150 g per date palm. Concerning manure, the average is about 20 kg per date palm tree. For the oases which suffer from soil fatigue, farmers bring in sand to renovate the field.

6.2.4 Pollination

March, April, and May is the normal pollination period in Tunisia. Farmers collect male spathes after they burst and insert, by hand, male inflorescence flowers into the female spathes. The number of flowers inserted depends on the female cultivar and the pollen quality. The selection of pollen is done by the farmers who prefer male inflorescences with a high pollen density. Pollination is usually done on a good sunny day as soon as the first break in the female spathe is observed.

Date palm pollination is manual and no mechanical method is employed. Short-term pollen storage or the practice of storing pollen from 1 year to another is practiced to pollinate early cultivars in which the female spathes open before the male spathes.

Bchini (2006) selected traits of male date palm trees which have a pollen effect (metaxenia) on maturation, early flowering, and fruit size in Deglet Noor cv. He concluded that pollen derived from early-bearing cultivars is favorable for earliness and pollen from a male seedling of a cultivar bearing large fruits is favorable for fruit size.

6.2.5 *Bunch and Fruit Management*

Fruit and bunch thinning is mainly practiced in Deglet Noor cultivar, but rarely in others. The aim of these practices is to enhance fruit size and consequently satisfy market preference, reduce damage due to humidity by a greater air circulation around the fruits while on the bunch, and reduce incidence of blacknose (darkening and shriveling). Tunisian farmers realize date-fruit thinning by reducing:

(a) The number of bunches per palm to keep a proper balance between the number of leaves and fruit bunches; a ratio of eight to ten leaves per bunch should be maintained, which guarantees good production the following year.
(b) The number of fruits per strand (at pollination): the lower third of the female inflorescence is removed.
(c) The number of strands per bunch (12–14 weeks after pollination): the lower third or slightly more of the bunch is removed mostly from the center.

In Tunisia, the Deglet Noor ripening season coincides with the rainy season and the rains can cause severe loss of fruit. Farmers use plastic bags to protect bunches against rain and humidity. Protection is applied at the early kimri stage. Over the last 5 years, farmers have used a new bag promoted by CRRAO (Center for Date Palm Research) Degache to cover the fruit bunches. This bag protects the fruits against both rain and insects; it has two parts: the upper part protects from rain and moisture and the lower is an insect net especially against the carob moth. Synchronized with bunch bagging, farmers usually carry out leaf pruning. Leaves are removed when they became dry. In the coastal oases, farmers practice thorn removal to facilitate pollination and handling of fruit bunches.

6.2.6 *Date Palm Diseases and Pests in Tunisia*

In southern Tunisia, particularly in the Jerid and Nefzaoua zones of high date production, the date palm is often prone to various insect attacks. The major pests found in Tunisia and their methods of control are described below.

6.2.6.1 *Ectomyelois ceratoniae* Zell.

The larva of the carob moth, *Ectomyelois ceratoniae*, infests the dates internally not only in the field before harvest but also during storage (Triki et al. 2003) (Fig. 6.2). It is a major constraint for date exports, especially for Deglet Noor fruit. To control this pest, farmers usually protect the fruit bunches and try to keep the plantation clean. Rarely the date traders fumigate harvested and stored dates. Many procedures should be taken to decrease the incidence of carob moth attack by the use of pheromone traps or by spraying the infested fruits with the biological pesticide *Bacillus thuringiensis* (Djerbi 1995). A recent study by Jemni et al. (2014) tested a treatment

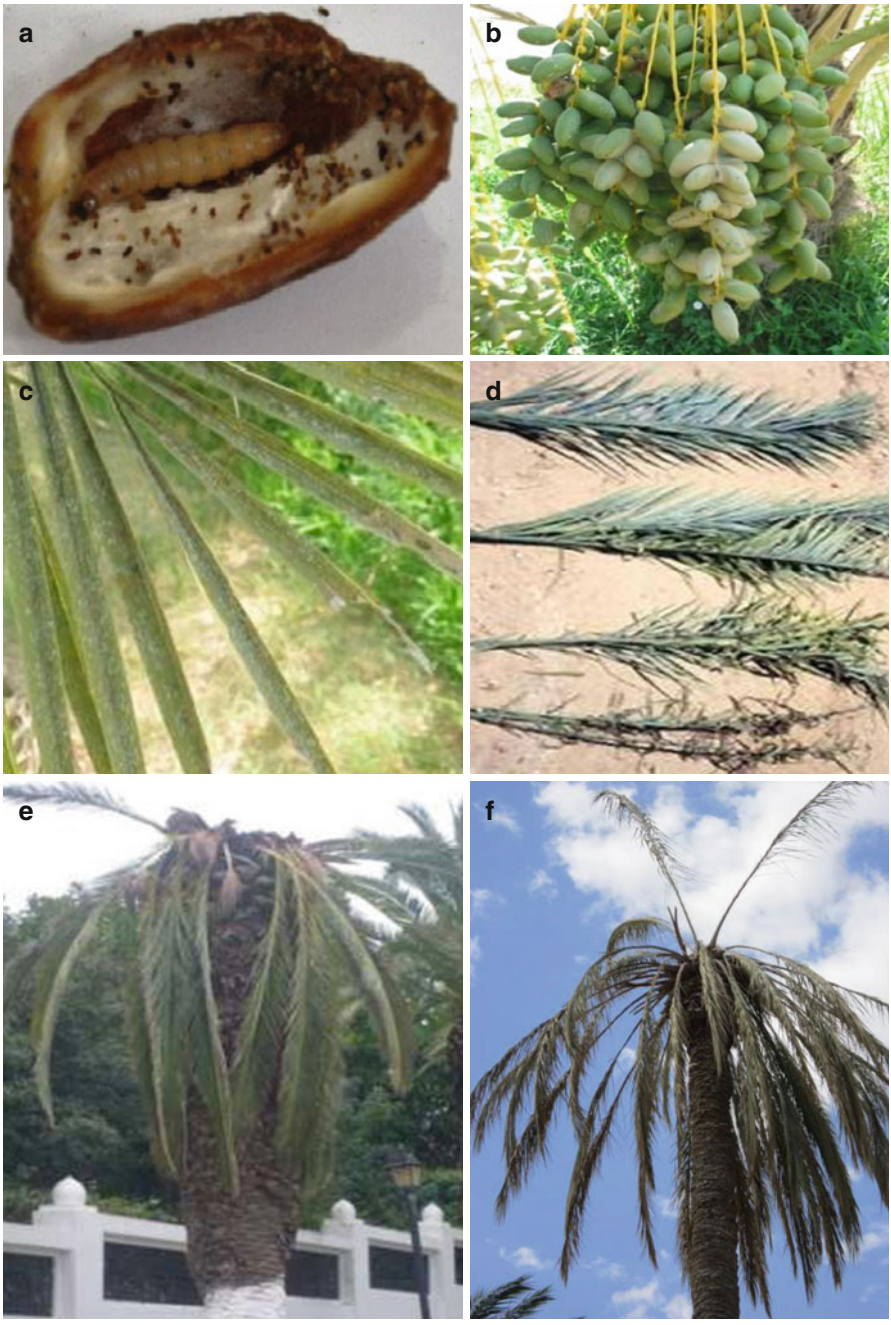

Fig. 6.2 Major pest in Tunisian oases. (**a**) *Ectomyelois ceratoniae* Zell. (**b**) Date palm fruit attacked by *Oligonychus afrasiaticus* McGr. (**c**) *Parlatoria blanchardii*, white scale (photo by S. Touil). (**d**) Brittle leaf disease (photo by A. Namsi). (**e**) *Phoenix canariensis* attacked by the RPW in the city of Carthage, Tunis (photo by S. Touil). (**f**) *Phoenix dactylifera* attacked by *Phytophthora* sp. (photo by H. Hamza)

of Deglet Noor dates with NaClO, UV-C, O_3, and EW and showed a positive effect for lowering their natural infestation by carob moth as well as microbial growth after 30 days of storage at 20 °C. In particular, UV-C and EW were the most effective against moth without adverse effects.

6.2.6.2 *Oligonychus afrasiaticus* McGr.

The date palm spider mite, *Oligonychus afrasiaticus*, causes serious damage to fruits (Dhouibi 1991; Khoualdia et al. 1997) (Fig. 6.2). In Tunisia, the infestation time varies from year to year, ranging from the first to the third week of July (Ben Chaaban et al. 2011). To decrease pest damage, Palevsky et al. (2004) proposed several control techniques that should be practiced: (a) reduction of the overwintering populations, (b) application of physical barriers to prevent airborne mites from reaching fruit bunches, (c) choice of a properly timed seasonal acaricide application, and (d) postharvest rehydration treatment.

6.2.6.3 *Parlatoria blanchardii* Targ.

Four generations of white scale insect, *Parlatoria blanchardii*, per year have been detected on date palms in southwestern Tunisia (Khoualdia et al. 1993). The most injurious is the spring generation. All green parts of the trees are damaged, with infestation higher on the palms at the base of the crown than in the center (Fig. 6.2). Variability in cultivar resistance to the white scale is underscored. Indeed, Kentichi cv. is significantly more resistant than Deglet Noor, Aligue, or Khouat allig (Khoualdia et al. 1993). In Tunisia, biological control against white scale was tried by the introduction of an exotic predator in the Segdoud region (oases of Gafsa). The exotic ladybird beetle, *Chilocorus bipustulatus* L. var. *iranensis*, is giving promising results (Khoualdia et al. 1997).

6.2.6.4 *Oryctes agamemnon* Burm.

Oryctes agamemnon, the Arabian rhinoceros beetle, was accidently introduced in the late 1970s into the date palm plantations of Mrah Lahouar in the Jerid region by offshoots brought in from the Persian Gulf region, where the pest originates (Baraud 1985). Ten years later, the beetle infected Rjim Maatoug oases in the Nefzaoua region. Gradually, this insect affects more date palms especially Deglet Noor cv. The symptoms appear only at very advanced stages and threaten sudden death of the tree (Soltani 2004). The only practical preventive method to reduce insect populations is their collection at the adult or larval stages. Because of the large infested area, the collection takes a long time and requires a sophisticated light trap. In addition, it should be done before the reproductive period of the beetle, a practice which has not been followed (Soltani 2010).

6.2.6.5 Brittle Leaf Disease

Brittle leaf disease (BLD) has been reported from Tunisia since the 1980s. Namsi et al. (2007) reported that there are 40,000 trees affected by this disease. It has been observed on most Tunisian cultivars including Deglet Noor, Tozerzaid, Akhouat Alig, Ammy, and Kinta, as well as on seedling trees and pollinator trees (Fig. 6.2). Kentichi cv. seems to be relatively tolerant. No data are available on other possible hosts, for example, on ornamental palms. BLD does not involve a pathogen but it is related to manganese (Mn) deficiency in the palm trees (Namsi et al. 2007). Currently, a tentative treatment is being done on affected date palm by adjusting the Mn quantities by trunk injection (Namsi Ahmed, personal communication, 2013).

6.2.6.6 *Rhynchophorus ferrugineus* Oliv.

Red palm weevil (RPW), *Rhynchophorus ferrugineus*, originates from Asia, where it is a serious pest of coconuts (Rahalkar et al. 1985). RPW is a polyphagous insect (Murphy and Briscoe 1999) which can infect many species of fruit and ornamental palm trees.

Following a notification in December 2011 from the city of Carthage (Fig. 6.2), Tunis, by the General Directorate of Protection and Quality Control of Agricultural Products, quarantine specialists visited the site where RPW was observed in order to establish a diagnostic (Chebbi 2011). The first observation noted tens of ornamental Canary Islands palms (*Phoenix canariensis*) exhibiting dried leaves. The preventative measures recommended were not followed quickly enough and the attack expanded progressively in area. Programs should be installed as soon as possible before this problem affects date palms.

6.2.6.7 *Phytophthora* sp.

Infection with water mold, *Phytophthora* sp., causes *belâat* disease and the death of young fronds along with an odor of acetic and butyric fermentation (Zaid et al. 2002). Usually, this disease is rare in Tunisia, but in the spring of 2012 and 2013, it was noted in many oases especially those in the Nefzaoua area (Fig. 6.2). Keeping date plantations clean is recommended to prevent attacks of water mold. Spraying with maneb or Bordeaux mixture at the rate of 8 l/palm can control the disease during early stages (Zaid et al. 2002).

6.2.6.8 *Ommatissus binotatus* var. *lybicus*, De Berg.

Ommatissus binotatus var. *lybicus*, the dubas bug, is a newly detected insect pest found for the first time in the Tamerza oasis in 2010. Thereafter, it was recorded in different areas of the Jerid oasis such as Chebika, Tozeur, Hezwa, and Nafta (Zouba and Raeesi 2010a). Biological and chemical controls are effective (Aldryhim 2008).

6.2.6.9 *Raoiella indica* Hirst

Red palm mite (*Raoiella indica*) is another newly discovered insect pest detected for the first time in Tunisia in July 2010 on Deglet Noor cv. It was observed initially in the old oasis of Nefzaoua and subsequently in different areas of the Jerid oasis such as Tozeur, Degache, and Nafta (Zouba and Raeesi 2010b).

6.3 Genetic Resources and Conservation

The date palm is widespread in the southern Tunisia, primarily in two main regions: continental and littoral oases (Fig. 6.1). Dominant are the continental oasis with 33,723 ha or 82 % of the total oases (Table 6.1). These oases contain more than 89 % of the total number of date palms in the country and contribute up to 85 % of the national date production (Sghaier 2010).

The continental oases are divided into Saharan and mountain oases. The Saharan oases are located in Jerid and Nefzaoua. The mountain oases are situated in Tamerza, Chebika, and Gafsa regions and are characterized by the predominance of common Tunisian cultivars.

In Nefzaoua, certain cultivars are ubiquitous in every oasis such as Deglet Noor, Alig, Bisr Helou, Horra, and Kinta (Fig. 6.3 and Table 6.2). But, other cultivars are rarely present (Hamza et al. 2006). The distribution of cultivars shows the dominance of the Deglet Noor cv. with 82 %. In second place is Alig cv. with 6.35 %. The latter has a fairly significant value in the local market and it is well known for its stress tolerance. The cultivation of male date palms, *dhokkars*, depends on the number of female palm of the plot. In the Jerid oases, the cultivar structure is almost the same. Deglet Noor cv. is about 60 % (MEDD 2010) and the Kentichi cv. is specific to the region, and it is more ubiquitous than the other common cultivars with 2.66 %.

Littoral oases are mainly in the region of Gabès (Fig. 6.1) and occupy 7,080 ha or 17 % of the total (Table 6.1) and contain 10 % of the total number of palms in Tunisia. The date palm cultivation patterns are different; there are other cultivars like Bouhattem, Garn Ghazel, Eguiwa, Lemsi, and Arichti (Ben Salah 2012) (Table 6.2).

Tunisian oases are also classified into two types: traditional and modern. Traditional oases occupy an area of 15,051 ha, or 37 % of the total. They contain 46 % of the palm trees; the majority are made up of common cultivars planted at a relatively high density of 166 trees per ha. These oases are characterized by small

Table 6.1 Types of Tunisian oases regarding their geographical origin

Oases	Location	Area (ha)	Percentage
Continental Saharan oases	Nefzaoua	22,980	56
	Jerid	8,363	20
Continental mountain oases	Gafsa	2,380	6
Littoral oases	Gabès	7,080	17
Total		40,803	100

Source: Sghaier (2010)

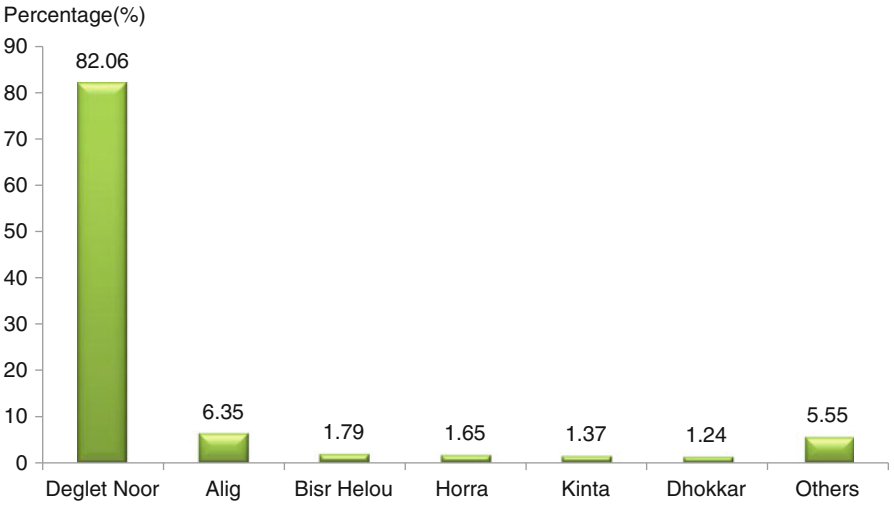

Fig. 6.3 Structure of date palm cultivars in Nefzaoua (Source: Hamza et al. 2006)

Table 6.2 Some characteristics of the main varieties in Tunisian oases

Variety	Distribution	Precocity	Fruit color at tamar stage	Fruit consistency	Seed weight/ fruit (%)
Deglet Noor	Continental oases	Late	Amber	Semisoft	9.7
Alig	All oases	Late	Dark brown	Semidry	9.9
Bisr Helou	All oases	Season	Light brown	Dry	17
Kinta	All oases	Season	Amber	Dry	13.6
Kentichi	Jerid	Late	Reddish	Dry	16.33
Bouhattem	Littoral oases	Season	Dark brown	Soft	14
Lemsi	Littoral oases	Season	Dark brown	Soft	14
Arichti	All oases	Late	Dark brown	Dry	12

Source: Ferchichi and Hamza (2008), Rhouma (2005)

farm size, resulting from property subdivision through inheritance. Modern oases occupy 25,752 ha (63 %) of the total area of the oases. They are characterized by larger farms and a lower planting density, fewer than 115 palm trees/ha with a high proportion of Deglet Noor cv.

In Nefzaoua, where many modern oases have been established, a correlation can be noted between the age of the oasis and the percentage of Deglet Noor (Fig. 6.4), the modern extensions showing a trend toward monoculture (Hamza et al. 2006). However, the hectarage of other date farms is decreasing and some disappearing in spite of their important nutritional and economic value. It is necessary to elaborate a strategy of preservation to protect production, adaptation, and resistance of the genetic inheritance. For this purpose, conservation plots were created in order to preserve the threatened cultivars. Four stations, located in Degache, Ibn Chabbat I, Ibn Chabbat II, and Atilet, are maintaining live specimens of 100 endangered cultivars.

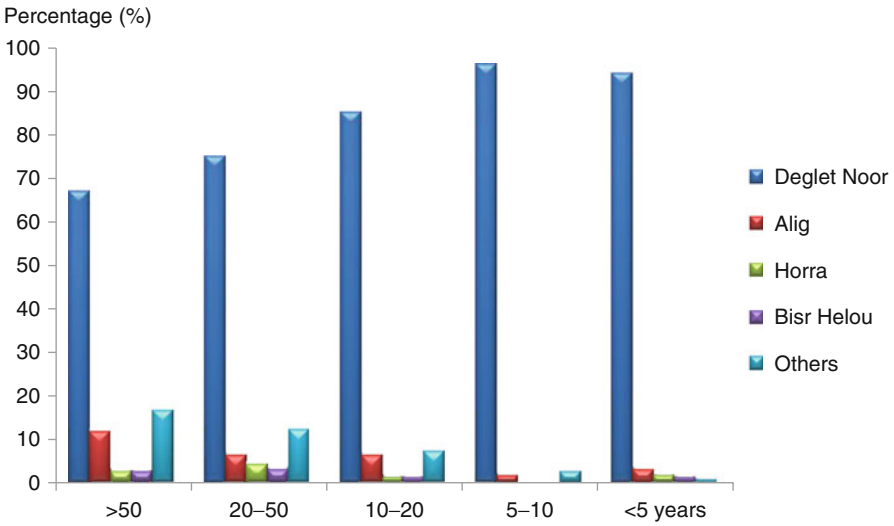

Fig. 6.4 Percentage of variation of date palm cultivars related to the oasis age (Source: Hamza et al. 2006)

6.4 Plant Tissue Culture

Many date palm cultivars are characterized by their good fruit quality but also their rarity. In vitro culture techniques contribute to the preservation of such endangered genotypes. In Tunisia, in vitro culture investigations are very highly developed and perfected. Two Tunisian laboratories are leading this effort: the Laboratory of Plant Biotechnology, Faculty of Sfax, and the Laboratory of In Vitro Culture, CRRAO Degueche.

Several plant tissue culture methods have been successfully adapted to a number of Tunisian cultivars such as Deglet Bey, Boufeggous, Gondi, and Cheddakh. To the present, Tunisian laboratories have produced up to 1,600 of plantlets by tissue culture. Different approaches have been established for the micropropagation but the most used is somatic embryogenesis. It offers a higher potential for mass propagation, allows for rapid propagation, and preserves date palm genetic resources (Fki et al. 2011a; Othmani et al. 2010). An improvement in somatic embryogenesis production of Deglet Noor cv. was made by Sghaier et al. (2008). A detailed discussion of the date palm somatic embryogenesis process and the influencing factors was recently provided by Fki et al. (2011b). Tunisian investigations by Drira and Benbadis (1985) and Fki et al. (2003) showed that callogenic capacity of inflorescences is higher than that of leaves. Furthermore, Fki et al. (2003) reported that the 2,4-D auxin is the most popular for callogenesis. The same authors in 2011 used reduced amounts of 2,4-D (0.2 mg l^{-1}) to establish adventitious bud growth of the introduced cultivar Barhi, from juvenile leaves, and this contributed to limiting the risk of somaclonal variation.

In addition, Othmani et al. (2009) successfully obtained in vitro regeneration of the elite date palm cultivar Deglet Bey (Mnakher) through both somatic embryogenesis (Fig. 6.5) and direct shoot formation (Fig. 6.6) from young leaf explants cultured on MS agar-solidified medium supplemented with 10 mg l^{-1} 2,4-dichlorophenoxyacetic

Fig. 6.5 Induction of somatic embryogenesis and plant regeneration from leaf explants of date palm cv. Deglet Noor. (**a**) Primary callus formation after 6 months of culture. (**b**) Induction of an embryogenic callus from primary callus after 10 months of culture. (**c**) Differentiation of somatic embryos from embryogenic callus. (**d**) Maturation of somatic embryos after 1 month of transfer on MS medium deprived of plant growth regulators. (**e**) Plantlet derived from the conversion of a matured somatic embryo. (**f**) Potted plant after 1 year of transfer to the greenhouse. (**g**) Plant after 2 years of transfer to the greenhouse (Photos by A. Othmani)

Fig. 6.6 Induction of shoot organogenesis and plant regeneration from leaf explants of date palm cv. Deglet Noor. (**a**) Induction of primary shoots from a leaf explant after 10 months of culture. (**b**) Initiation of shoot multiplication after transfer of explants on MS medium supplemented with NAA and BAP. (**c**) Shoot cluster in active multiplication phase. (**d**) Initiation of elongation of shoots. (**e**) Induction of roots from elongated shoots. (**f**) Plantlet obtained from the conversion of an elongated shoot after 1 month of culture on MS medium without plant growth regulators. (**g**) Potted plants after 3 months of transfer into the greenhouse (Photos by A. Othmani)

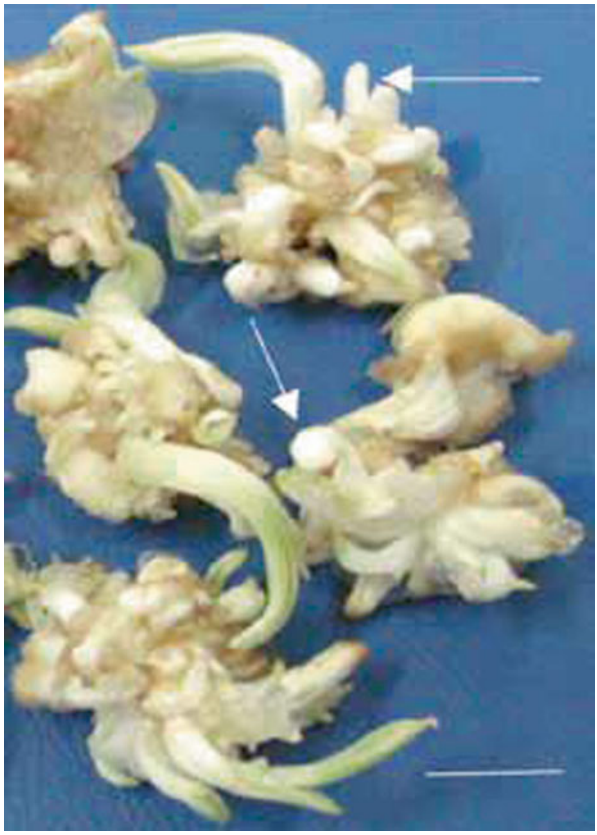

Fig. 6.7 Multiple-shoot induction (*arrows*) from shoots obtained on agar-solidified medium that were cultured for 2 weeks in the TIB system containing proliferation medium. The immersion frequency was 5 min every 8 h. *Scale bar*: 0.5 mm (Othmani et al. 2009)

acid for 8 months. They have developed somatic embryogenic suspensions to improve differentiation of embryogenic callus and culture of shoots in a temporary immersion bioreactor (TIB) to enhance proliferation of regenerated shoots. The principles, advantages, and disadvantages of TIB were recently described in detail by Othmani et al. (2011). The immersion frequency tested so far appears to be suboptimal for date palm callus growth and could be optimized by testing different durations. These authors reported that the embryogenic calli of date palm cv. Deglet Bey turned brown and died using a RITA® bioreactor with immersion frequency of 5 min every 8 h. They found that the temporary immersion system was better than the solid medium only for shoot proliferation (Fig. 6.7). Despite many promising results, culturing in bioreactors proves to be more complicated than culturing on agar-solidified media in terms of hyperhydricity and contamination risks. As for the expression of exogenous contamination, often it can be controlled by good sterile techniques; however, endogenous contamination cannot be easily controlled in repeated subcultures. The contamination problem can be controlled using embryogenic callus and shoot clusters pretested to be free from endophytic bacteria. Nevertheless, subculture of affected plant material in antibiotic-amended liquid medium did not avoid this problem. The antibiotics tested were cefotaxime (250 mg l^{-1}) and streptomycin (500 mg l^{-1}).

To preserve contamination-free material and prevent somaclonal variation, cryostorage of date palm somatic embryos was done in Tunisia. For the purpose of creating a cryobank of proliferating tissues, Fki et al. (2013) tested the possibility of generating and cryopreserving highly proliferating meristems of the Kheneizi cultivar. These investigations showed that regeneration rates using standard vitrification, droplet-vitrification, and encapsulation-vitrification protocols reached 26.7, 60.0, and 40.0 %, respectively. Only explants smaller than 3 mm in diameter were found to survive cryogenic treatments. In addition, sucrose pre-culture, cold hardening, and loading solution pretreatments showed significant effects on regeneration rates. Moreover, our results indicate that both sucrose pre-culture and cold acclimation of explants increased proline content.

On the other hand, Masmoudi-Allouche et al. (2009, 2010) achieved in vitro hermaphrodism induction in date palm female flowers which opens opportunities for the investigation of an in vitro self-fertilization process. In fact, several date palm cultivars were the objective of sex modification under these particular tissue culture conditions. Masmoudi-Allouche et al. (2009) used an appropriate hormonal treatment based on the addition of IBA (indole-3-butyric acid) and BAP (6-benzylaminopurine) at different concentrations. Further investigations by Masmoudi-Allouche et al. (2011) were done to search for putative variations that may have occurred on the initial genome due to the application of plant growth regulators. The results revealed that hormonal treatment entailed no detectable genetic variation in the treated date palm flowers.

Date palm tissue culture techniques should be adopted as a central part of innovation. The creation of new pest-resistant cultivars is very important. In fact, several experiments have been started by the Laboratory of Plant Biotechnology, Faculty of Sfax, in collaboration with the International Atomic Energy Agency (IAEA) to select cultivars resistant to bayoud disease (FAO/IAEA 2001). Selection was based on the resistance against the active toxins of the pathogen applied to irradiated in vitro cultures. The isolation of bayoud from leaves and the production of toxin were achieved in Morocco.

6.5 Cultivar Identification

An exhaustive resource inventory program of date palms has been done in Tunisia. A number of methods have been used to analyze genetic diversity in germplasm accessions, breeding lines, and populations. These methods have relied mainly on two genetic markers: morphological and molecular.

6.5.1 Morphological Characterization

As to morphological markers, the most common characters used to identify different cultivars in date palm are the phenotypic expression of leaves, spines, and fruit characters. Several studies have described the importance of morphological traits in

identifying Tunisian date palm cultivars (Ben Salah 1993; Ben Salah and Hellali 2004; Rhouma 1994, 2005). Despite these descriptions, it remains very difficult to identify cultivars, especially outside the fruiting period. In fact, owing to the great adaptive flexibility of this species, many farmers cannot recognize cultivars outside their oasis farm. Indeed, the majority of morphological characters are an adaptive response to the environment. Hamza et al. (2009) sifted through morphological characters that are not controlled by edaphic or climatic factors. These characters were selected on the principle of their low environmental plasticity and strong genetic control, important in cultivar identification, and also they have an intra-genotype measure reproducibility (Hamza et al. 2009, 2011). Nine characters were selected. Six characters are vegetative: spiny midrib part length (%), apical divergence angle (°), maximal pinna width at the top leaf (cm), solitary spine number (%), spine length in the middle (cm), and maximal spine angle (°). Three reproductive characters describe the inflorescences and fruit: bunch length without spikelet (%), spikelet length without fruits (%), and fruit internal cavity ratio.

6.5.2 Molecular Characterization

Sakka et al. (2004) used chloroplast DNA to identify Tunisian cultivars following the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). These markers have the advantage of being codominant and can scan all the DNA extract. The results are highly reproducible and great diversity was observed as a function of the enzyme and probe used. However, the RFLP technique requires a large amount of DNA and markers used for nuclear DNA are limited (Dowling et al. 1996). To detect variability, it is necessary to use more enzymes which make analysis expensive. Some enzymes have more restriction sites which makes the results dependent on gene activation. The DNA fragment migration on gel is logarithmic, making it difficult to detect fragments of larger sizes.

Random amplification polymorphism DNA (RAPD) is widely used in the identification of date palm cultivars in Tunisia and to study their phylogenetic relationships (Ben Abdallah et al. 2000; Trifi et al. 2000). This technique is important in identifying the genetic fingerprints of date palm cultivars and in the early detection of genotypes. The advantage of RAPD is its simplicity as it does not require a large amount of DNA. However, Benkhalifa (1999) reported that RAPD shows a low rate of polymorphism. Moreover, it is widely criticized for the reproducibility, the structure of the primers, the dominance of markers, and the independence of loci.

Inter-simple sequence repeat (ISSR) markers were used to assess the polymorphism in Tunisian cultivars (Zehdi et al. 2002; Zehdi-Azouzi et al. 2011). Among 12 ISSR primers used in the study, only 7 generated polymorphic fragments. In addition, using other ISSR primers (Fig. 6.8), Hamza et al. (2012) detected genetic differentiation between Tunisian subpopulations in terms of fruit consistency, and soft fruit cultivars were significantly differentiated from dry fruit cultivars. The ISSR markers have the advantage of being highly polymorphic. Thus, it has been reported that these markers are more variable than RFLP and RAPD techniques (Nagaoka and Ogihara 1997). Moreover, they are more reproducible than those

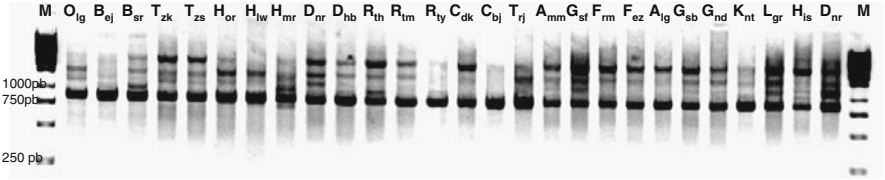

Fig. 6.8 Banding patterns in different date palm cultivars by ISSR primer D12. *M* 1 Kb ladder, O_{lg} Om Leghlez, B_{ej} Bejjou, B_{sr} Bisr Helou, T_{zk} Tezerzayet Kahla, T_{zs} Tezerzayet Safra, H_{or} Horra, H_{lw} Halwa, H_{mr} Hamra, D_{nr} Deglet Noor, D_{hb} Dhahbi, R_{th} Rtob Houdh, R_{tm} Rotbayet Elmansoura, R_{ty} Rotbayet Yagouta, C_{dk} Cheddakh, C_{bj} Choddekh Ben Jbir, T_{rj} Tronja, A_{mm} Ammary, G_{sf} Ghars Souf, F_{rm} Fermla, F_{ez} Fezzani, A_{lg} Alig, G_{sb} Gosbi, G_{nd} Gondi, K_{nt} Kentichi, L_{gr} Loghrabi, H_{is} Hissa (Hamza et al. 2012)

generated by the RAPD technique (Nagaoka and Ogihara 1997). However, ISSR markers have drawbacks such as sensitivity to the variation of the amount of template DNA as well as the annealing temperature, which must be carefully developed (Bornet and Branchard 2001).

The random amplified microsatellite polymorphism (RAMPO) technique is a modification of the RAPD to avoid the lack of reproducibility and dominance. Rhouma (2008) found a high genetic diversity in Tunisian cultivars compared with the results of RAPD and ISSR.

Rhouma (2008) used six amplified fragment length polymorphism (AFLP) primer combinations to characterize Tunisian cultivars, and a total of 428 polymorphic bands were generated. AFLP markers have the disadvantage of the need for high technical expertise and high-quality DNA.

The specific simple sequence repeat (SSR) date palm primers were developed by Billotte et al. (2004). Zehdi et al. (2004) did genotyping of many Tunisian cultivars, and the comparison of theoretical geographic populations showed that each Tunisian oasis constitutes a single population. The identity probability of cultivars showed that the use of only three microsatellite markers can discriminate a cultivar from the rest of the population. Key cultivar identification was well established by Hamza et al. (2010) and Zehdi et al. (2004). In addition, the SSR markers have been used to study certain agronomic traits in Tunisia such as maturity period and fruit consistency (Hamza et al. 2011). The results detected genetic differentiation between subpopulations in terms of fruit consistency. The cultivar subpopulations with semisoft dates showed a significant genetic differentiation, which places them between soft and semidry date cultivars. This suggests that the continental date palm oases could be a set of populations with a different origin.

6.6 Cultivar Description

More than 200 named cultivars are grown in Tunisian oases (Ferchichi and Hamza 2008; Rhouma 1994, 2005). These cultivars exhibit differences in ripening time; three different classes are identified in that regard: early, mid-season,

and late. Fruit consistency is the most important parameter that usually determines fruit quality and commercial interest in them. Traditionally, this trait was segregated into three different fruit classes: soft, semidry, and dry (Munier 1973). New research has established semidry fruit cultivar heterogeneity. As a consequence, semidry has been subdivided into two new clusters: semisoft and semidry with supportive morphological and genetic distinction (Hamza et al. 2009, 2011, 2012).

The application of the selected morphological characters on cultivars of continental oases (Hamza et al. 2009, 2011) showed a high correlation with maturity period and fruit consistency. In fact, the percentage of spiny midrib parts for early and soft cultivars was significantly smaller than for the others, and these cultivars also showed the highest percentages of solitary spines (Fig. 6.9). In addition, the percentage of bunch lengths without spikelets and the percentage of fructified spikelet lengths for the early cultivars were, respectively, the lowest and the highest in comparison with the mid-season and late-maturing cultivars.

6.7 Date Production and Marketing

The date harvest in Tunisia starts at the end of July for early-maturing cultivars and ends in the middle of December for the late cultivars. Earlier cultivars produce soft dates that must be transported in the early morning to local markets for consumption. Late-maturing cultivars like Deglet Noor have a long-season market. These cultivars are subjected to postharvest practices in order to prepare them for marketing.

Tunisian date production increased from 118,000 mt in 1999 to 190,000 mt in 2011. Concerning Deglet Noor cv., the production increased from 78,200 mt in 1999 to 137,000 mt in 2011. This increase was about 61 % for all date production and 76 % for Deglet Noor. The Nefzaoua region is the most important, accounting for about 59 % of the national date production (ODS 2011).

During the last quarter of the twentieth century, world date production has been allocated on average at a rate of 75 % for local consumption and 25 % for export. The latter began to increase significantly over the past decade, averaging a third of the total production (Fig. 6.10). At the same time, prices of Deglet Noor cv. dates have changed significantly. In fact, the farm gate price has increased from USD 604/mt in 1999 to USD 927/mt in 2008, while the price for local consumption increased from USD 1,327 to USD 2,039/mt during the same period. The export price was USD 1,434/mt in 1999 and climbed to USD 1,954/mt in 2008.

Date exports are an important component of the national economy. Average annual exports for the period 2002–2006 were approximately 41,000 mt, equivalent in value to USD 69.3 million per year. It should be noted that date exports for the 2008/2009 season exceeded 60,000 mt with a value of USD 140 million; those levels place Tunisia as the fourth largest world exporter of dates in quantity and the first in value.

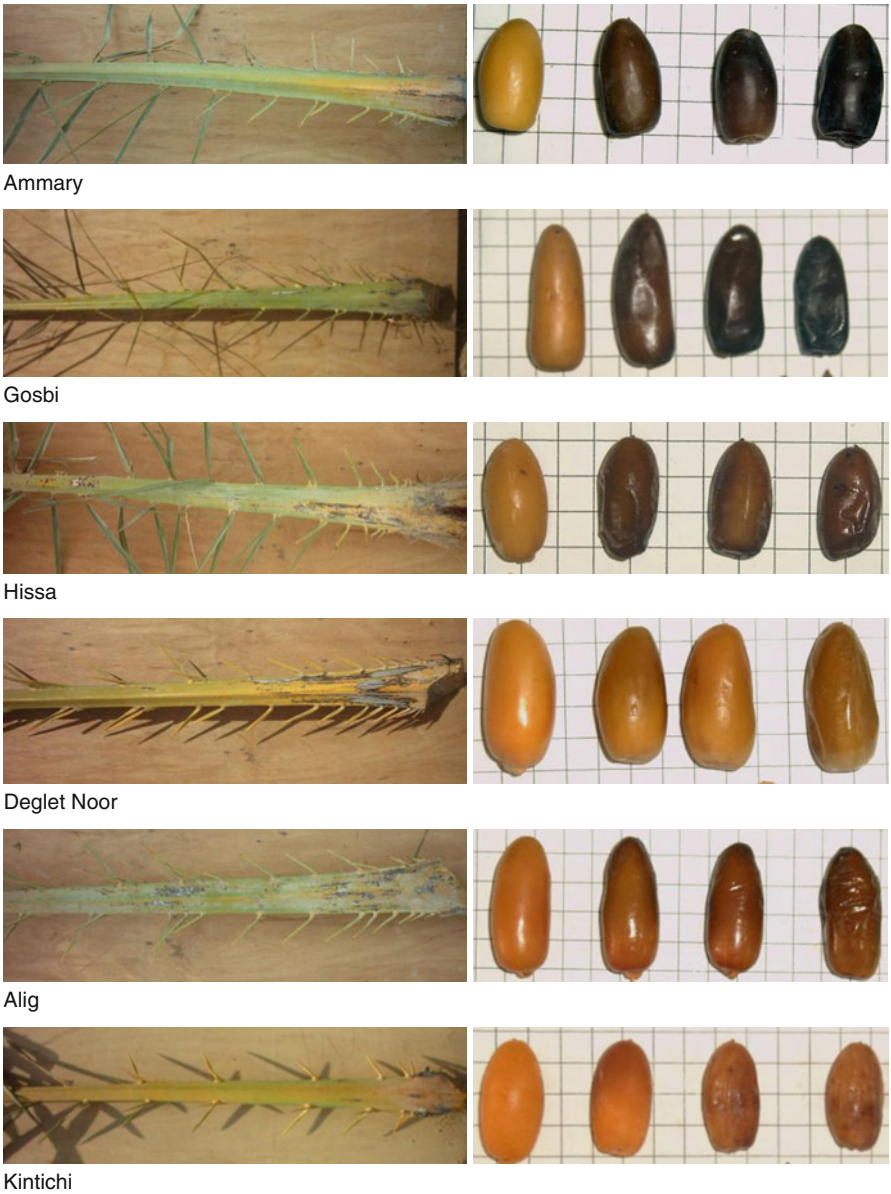

Fig. 6.9 Spiny midrib parts and fruits of Tunisian date palm cultivars. Early cultivars with soft dates (cvs. Ammary, Gosbi, and Hissa) and late cultivars with semisoft dates (cv. Deglet Noor), semidry dates (cv. Alig), and dry fruit (cv. Kentichi) (Photos by H. Hamza)

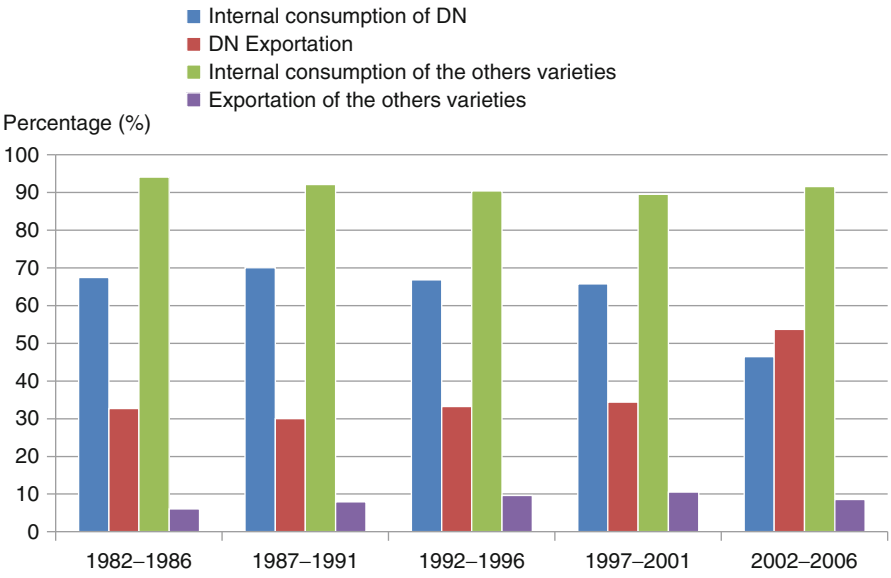

Fig. 6.10 Date palm production, internal consumption, and exportation (DN: Deglet Noor) as compared to the total of other cultivars (Source: Annuaires Statistiques Agricoles; Ministère de l'Agriculture 2010)

6.8 Processing and Novel Products

The main activity of date processing stations is date packaging and export. However, tons of cull dates are unfit for human consumption because of poor quality, dryness, or contamination. These culls are rejected by date processors but a small proportion is recycled as plant fertilizer or goes to animal feed. Cull dates are a rich source of carbohydrates essentially sugars and dietary fiber. Due to their richness, some studies have been carried out to valorize these dates and to develop new products as summarized in Fig. 6.11. In Tunisia, several research programs were established for this purpose, focused on:

(a) Date juice: dates are pitted and ground then the mix solubilized in hot water. After that, filtration is necessary to clarify the juice. The latter should be stored at a cold temperature. Chaira et al. (2009) perfected a cooking method for date palm juice. The application of a temperature of 80 °C for 90 min on pulpy cultivars gave the best results. It produces an energy juice drink rich in total solids, of low acidity, and with a high power to scavenge superoxide free radicals.

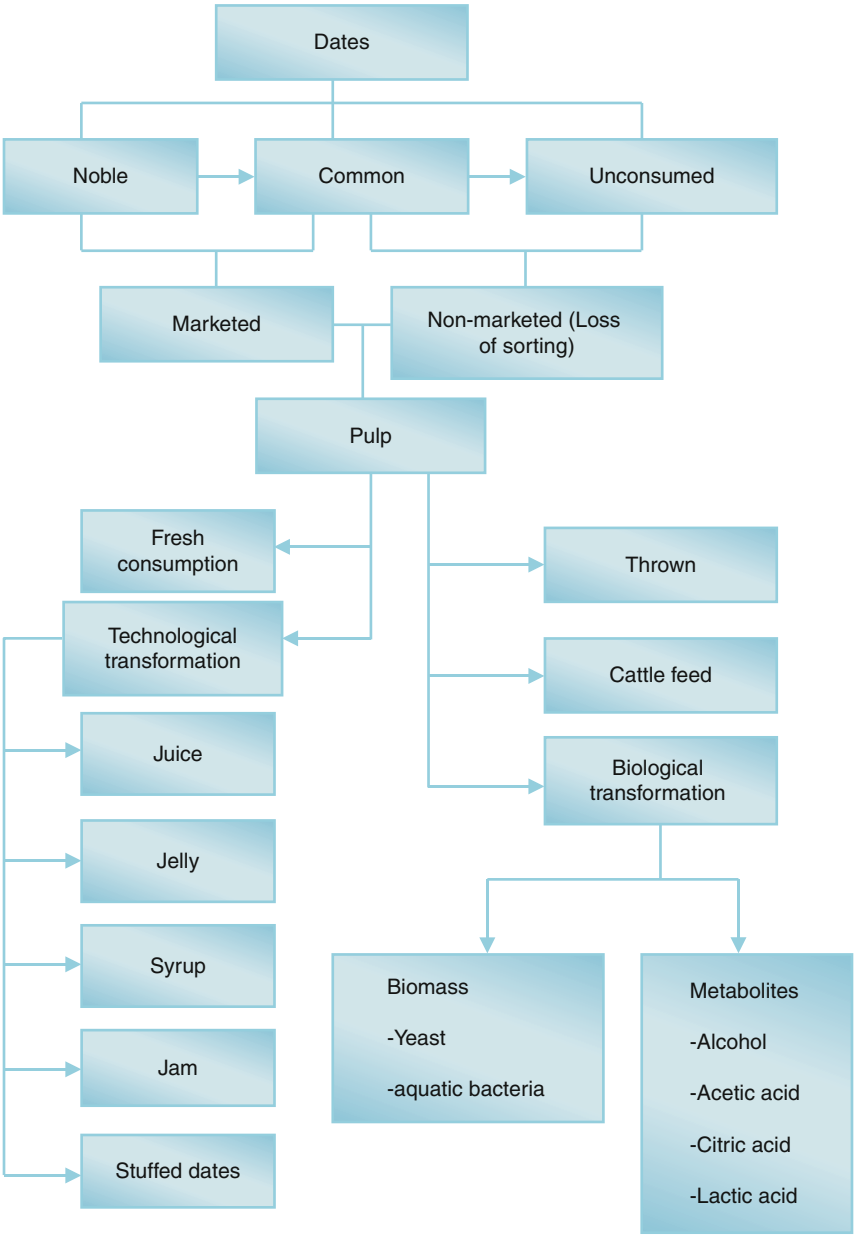

Fig. 6.11 Possible ways of date-pulp valorization (Besbes et al. 2006)

(b) Date syrup: date syrup, also called *rub al tamr*, is produced in southern Tunisia from certain cultivars. Extraction of date syrup is carried out as follows: date paste is mixed with water and boiled at 100 °C. The juice produced is passed through a cloth filter. To prepare date syrup, juice is concentrated at 100 °C. The date syrup is consumed directly or used as an ingredient in certain foods such as ice cream, beverages, confectionery, bakery goods, sesame paste/date syrup blends, jams, and butters. Date syrup is a high-energy food rich in carbohydrates and a good source of minerals; it also contains a very complex mixture of other saccharides, amino and organic acids, polyphenols, and carotenoids. These techniques have produced a high-commercial-value syrup by enzymatic treatment. This method has enhanced the total soluble solids and decreases the turbidity by using pectinase and cellulase. In addition enzymatic treatment improves the syrup clarity which becomes lighter. This new syrup was very appreciated by consumers at a large taste evaluation. In addition, results showed that the date syrup prepared after extraction with pectinase and cellulase mixture gave the lowest phenolic, flavonoid contents and antioxidant activity (Abbès et al. 2011, 2013).

(c) Date jam: date jam is unknown on the market of Tunisia despite the effort of some manufacturers trying to produce it. According to Besbes et al. (2009), jam is prepared by boiling ground fruit flesh with sucrose in water. The mix is cooked to about 65 °Brix. A significant effect of the date cultivar used was noted on the composition and physical characteristics of date jams (Besbes et al. 2009). Indeed, Alig cv. jam was richer in reducing sugars and was characterized by its greater firmness and water-retention capacity. Alig and Kentichi cv. jams presented a higher overall acceptability in comparison with quince and did not show any significant difference from Deglet Noor jam.

(d) Dietary fiber: fiber is extracted from date flesh by hot water and recuperated by centrifugation after dissolving the sugars (Elleuch et al. 2008). Extraction of dietary fiber (Fig. 6.12) was studied at different temperatures (40, 50, and 60 °C). A significant decrease in water-holding capacity, swelling capacity, and emulsifying capacity was noted at 60 °C (Borchani et al. 2012).

(e) Biomass production: according to the richness of date juice in simple sugar, it constitutes a favorable medium for biomass such as the production of biopesticide bacteria. To do this, an experimental design was devised. Indeed, given the low protein content of date juice, the addition of protein is necessary. Optimizing the composition date juice and fermentation parameters was performed. The main results obtained are the concentration of sugar in media and the concentration of protein added; the pH of media and temperature of fermentation have a significant effect on the yield and on the productivity of these biopesticide bacteria (Jemni et al. 2010).

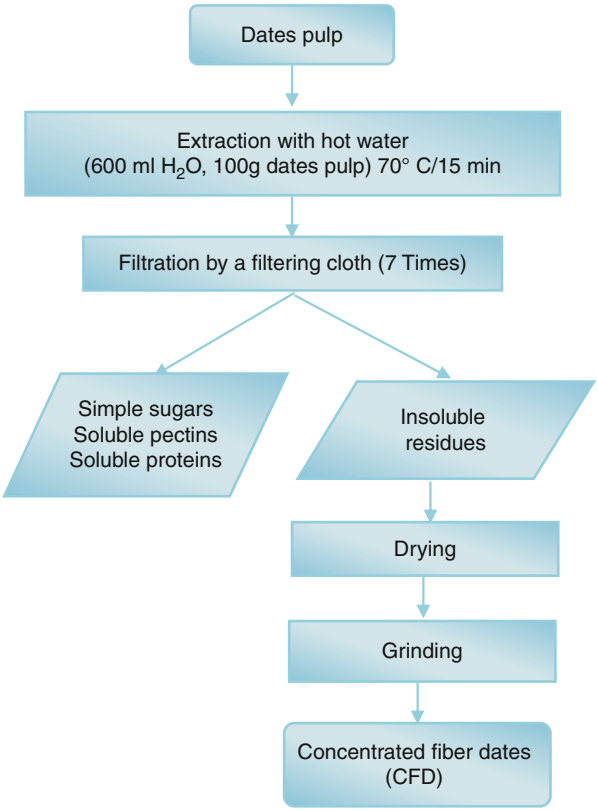

Fig. 6.12 Diagram of preparation of date-pulp fiber (Borchani et al. 2010)

(f) Metabolite production: the date juice has been used for the production of some metabolites such as vinegar which is obtained by double fermentation involving alcoholic and acetic acid at temperatures of 27–30 °C (Besbes et al. 2006) and curdlan production with a yield of 22.83 g/l (Ben Salah et al. 2011). The purified date by-product curdlan (DBP-curdlan) had a molecular weight of 180 kDa. Moreover, bioethanol, with a concentration of 25 %, was produced in Tunisia by fermentation of date juice by the yeast *Saccharomyces cerevisiae* using a sugar concentration of 200 g/l at 30 °C and natural pH (Louhichi et al. 2013).

There are many industries in Tunisia trying to produce some of these secondary products in addition to their main activity of processing dates. Syrup is the major product because of its ease of production. Projects to valorize dates are of particular importance for the national economy. The products have high added value and levels of integration and are important in terms of the extent of investment and job creation. However, marketing and the dietary habits of Tunisians are the main problems limiting the adoption of these products by consumers. Therefore, the major by-products currently are targeted for export and not for local markets. To promote

the domestic market for date by-products, government grants to publicize and promote the products and to assist manufacturers would be a positive step.

6.9 Conclusions and Recommendations

At present, Tunisian oases enjoy an economic and social stability. However, they are threatened by unsustainability aggravated by climate change, overexploitation of water resources, and an increasing trend toward Deglet Noor cv. monoculture. Current research on oasis agriculture does not adequately address priority areas such as water needs and protection of date palms against diseases and pests which present the risk of biodiversity loss. Given these problems, comprehensive programs must be mobilized, to include: (a) halting of date-cultivation expansion in areas where water resources are overexploited; (b) enhanced proficient use of water resources; (c) improved understanding of the irrigation water needs of specific oasis crops; (d) collection, evaluation, and conservation of plant genetic resources; (e) improved opportunities for in vitro techniques in order to contribute to the propagation of cultivars under threat of extinction; and (f) strengthening of the role of general date palm research. Dates have always suffered from postharvest losses due to deformation, microorganism contamination, and carob moth and other insect infestations. Fumigation by methyl bromide is an effective method of protecting dates from the moth but its use will be prohibited in 2015. As a consequence, it is necessary to search for alternatives treatments. Valorization of lost biomass in the food industry and in bioenergy is also a significant issue which requires in-depth research.

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